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REVIEW

Chloroplast translation regulation

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Abstract Chloroplast gene expression is primarily controlled during the translation of plastid mRNAs. Translation is regulated in response to a variety of biotic and abiotic factors, and requires a coordinate expression with the nuclear genome. The translational apparatus of chloroplasts is related to that of bacteria, but has adopted novel mechanisms in order to execute the specific roles that this organelle performs within a eukaryotic cell. Accordingly, plastid ribosomes contain a number of chloroplast-unique proteins and domains that may function in translational regulation. Chloroplast translation regulation involves cis-acting RNA elements (located in the mRNA 5′ UTR) as well as a set of corresponding trans-acting protein factors. While regulation of chloroplast translation is primarily controlled at the initiation steps through these RNA-protein interactions, elongation steps are also targets for modulating chloroplast gene expression. Translation of chloroplast mRNAs is regulated in response to light, and the molecular mechanisms underlying this response involve changes in the redox state of key elements related to the photosynthetic electron chain, fluctuations of the ADP/ATP ratio and the generation of a proton gradient. Photosynthetic complexes also experience assembly-related autoinhibition of translation to coordinate the expression of different subunits of the same complex. Finally, the localization of all these molecular events among the different chloroplast subcompartments appear to be a crucial component of the regulatory mechanisms of chloroplast gene expression.

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Abbreviations

ROS	Radical oxygen species
NADH dh	NADH dehydrogenase
nt	Nucleotides
PDI	Protein disulfide isomerase

Introduction

Chloroplasts are responsible for the photosynthetic activity of plants and algae and participate in other important processes, such as amino acid and fatty acid biosynthesis. Plastids evolved from a cyanobacterium-like ancestor after endosymbiosis into a eukaryotic host cell followed by a massive transfer of genetic material to the nucleus. Present chloroplasts persist in retaining their own circular genomes with a set of 100–200 genes encoding rRNA, tRNA, and 80–150 proteins. Plastid genes encode components of the chloroplast transcription and translation machinery (RNA polymerase, ribosomal proteins and initiation factors), structural proteins of the photosynthetic electron chain (photosystems I and II, cytochrome b_6/f complex), subunits of the ATP synthase, NADH dehydrogenase and the large subunit of Rubisco. The expression of these essential components is primarily regulated at post-transcriptional steps, including mRNA processing, stabilization, and translation, but still relatively little is known about the

molecular mechanisms that regulate protein expression in this organelle (reviewed in Danon 1997; Monde et al. 2000; Zerges 2000; Nickelsen 2003; Herrin and Nickelsen 2004).

Light is one of the most important signals controlling protein expression in chloroplasts, inducing changes in the redox state of components of the photosynthetic electron chain (e.g., plastoquinone, ferredoxin), which propagate to other redox-sensitive elements within the chloroplast, like thiorredoxin and glutathione. These factors, along with the generation of a proton gradient accross the thylakoid membrane, and the subsequent increase in the ATP/ADP ratio in the chloroplast stroma have been proposed to mediate light-dependent chloroplast gene expression at several steps including transcription, mRNA stability, and translation (Salvador and Klein 1999; Pfannschmidt 2003). Moreover, during transition states, the relative affinity of the light-harvesting complex for photosystems I and II is regulated by phosphorylation, which in turn is regulated by the redox state of the plastoquinone pool (Allen and Forsberg 2001; Allen 2005).

Within the chloroplast, both nuclear- and plastid-encoded proteins are found in the same multisubunit complexes, requiring a tight coordination of gene expression from both compartments. The nucleus-to-chloroplast information flow is performed through a set of nuclear-encoded protein factors that regulate both transcriptional and post-transcriptional steps in chloroplast gene expression including RNA splicing, editing, processing, and translation (Monde et al. 2000; Zerges 2000; Nickelsen 2003; Herrin and Nickelsen 2004; Shikanai 2006). The retrograde information flow from chloroplasts to the nucleus is mediated by different signaling pathways, one of which is triggered by the accumulation of Mg-protoporphyrinIX (Strand et al. 2003) while others are directed by the redox state of electron transport chain components as well as photosynthetic products (Fey et al. 2005).

The bacterial origin of the chloroplast and the fact this organelle shares many characteristics with present prokaryotes led to the general belief that translation in chloroplasts occurs through the same mechanisms as in bacteria. Further investigation revealed that the chloroplast translation machinery displays distinctive features possibly developed for the highly specialized roles that this organelle acquired during evolution. These data suggest that the basic process of translation in the chloroplast is distinct from bacteria or other eukaryotic cellular compartments. Additionally, the potential use of green algae to express proteins with biotechnological interest at low cost in the chloroplast raises interest in understanding the mechanisms that regulate translation in this organelle (Mayfield and Franklin 2005; Mayfield et al. 2003).

Chloroplast translation machinery

Ribosomal proteins and RNA

Chloroplasts contain 70S-type ribosomes similar to prokaryotes and different from their cytosolic counterparts, 80S ribosomes. The complete set of chloroplast ribosomal proteins from a higher plant (spinach) and a green alga (*Chlamydomonas reinhardtii*) has been characterized by genetic and proteomic approaches (Yamaguchi et al. 2000; Yamaguchi and Subramanian 2000; Yamaguchi et al. 2002; Yamaguchi et al. 2003; Beligni et al. 2004a). These studies revealed that, in addition to having a majority of proteins with similarities to bacteria, both the large and the small subunit of the ribosome from spinach chloroplasts also include plastid specific ribosomal proteins (PSRP-1 to PSRP-6) with no homologs in the bacterial ribosome. *Chlamydomonas reinhardtii* ribosomes have a similar set of PSRPs, except lacking PSRP-2 and PSRP-5, and additionally contain PSRP-7, a protein with two S1 domains that has been found in other vascular plants (*Arabidopsis thaliana, Oriza sativa*). PSRP-7 is synthesized as a polyprotein (PETs) together with elongation factor Ts, which is post-translationally processed to render two independent products. Interestingly, the full-length PETs precursor has been found on ribosomes (together with mature PSRP-7) and, at minor levels, in polysomes (Beligni et al. 2004b).

Many of the chloroplast ribosomal proteins are larger than their bacterial orthologues, mainly due to short insertions or extensions at their N- or C-termini. Especially remarkable are three proteins (S2, S3, and S5) from the *C. reinhardtii* ribosome, which contain large additional domains representing 58%, 68%, and 75% of the total mass of each polypeptide, respectively. Comparison with the 30S ribosome structure obtained from *Thermus thermophilus* indicates that these additional domains in plastid ribosomes would be located adjacent to each other, on the solvent-exposed side of the small ribosomal subunit (Yamaguchi et al. 2002). The structure of the chloroplast ribosome from *C. reinhardtii* has recently been solved, using cryoelectron microscopy and single particle reconstruction, and will be available soon (Manuell, in preparation). It has been proposed that PSRPs and the additional domains in S2, S3, and S5 may have a role in regulating the specialized responses involved in chloroplast gene expression (e.g., light-dependent, redox-regulated translation). In contrast to these major changes in protein composition between chloroplast and bacterial ribosomes, only minor differences occur in the large and small subunit rRNAs.

The assembled ribosome from *C. reinhardtii*, but not either of the subunits, contains two additional proteins, RAP38 and RAP41. These proteins share sequence

similarity with spinach CSP41, a chloroplast RNA-binding protein with endoribonuclease activity involved in mRNA turnover (Bollenbach et al. 2003). Homologs of RAP38 and RAP41 have also been found in the genome of *A. thaliana* and a recent study proposes an additional role for these proteins in rRNA maturation (Beligni, in preparation).

Translation factors and general regulatory mechanisms

Orthologues for all the general translation factors required for initiation (IF1, IF2, IF3), elongation (EF-Tu, EF-Ts, EF-G) and release/recycling (RF1, RF2, RF3/RRF) from the photosynthetic bacteria *Synechocystis* sp. PCC 6803 have been found in the *C. reinhardtii* genome (Beligni et al. 2004a). A peculiar feature of the spinach chloroplast ribosome is that RRF is strongly associated with the 70S complex (Yamaguchi and Subramanian 2000). More revealing is the fact that some of the ribosomal proteins and elongation factors from spinach and *C. reinhardtii* have been found to interact electrostatically with thioredoxin (Balmer et al. 2004; Lemaire et al. 2004), suggesting a possible role of these factors in light/redox regulation of translation. Indeed, the specific activity of EF-G is light stimulated in pea chloroplasts (Akkaya and Breitenberger 1992). The expression of EF-Tu is also induced by light at both transcript and protein levels in pea chloroplasts (Singh et al. 2004) and similar results have been reported for most of the initiation and elongation factors in chloroplasts from *Euglena graciilis* (Breitenberger et al. 1979; Fox et al. 1980; Gold and Spremulli 1985; Sreedharan et al. 1985; Kraus and Spremulli 1986). Abiotic stresses may also impact translation through these general factors, since EF-Tu expression is down regulated in response to salinity and abscisic acid, but up-regulated in response to low temperature and salicylic acid treatment in pea chloroplasts (Singh et al. 2004). Analysis of the phosphoproteome of *C. reinhardtii* revealed that EF-Tu, RRF, and RAP41 (see above) may be targets for protein kinases (Wagner et al. 2006) and thus, phosphorylation may represent another mechanism for translational regulation in chloroplast.

Shine–Dalgarno interactions

In prokaryotes, binding of the ribosome to an mRNA is mediated by complementary base-pairing of the 3′ terminus of the 16S rRNA with a Shine–Dalgarno (SD) sequence, located in the 5′ untranslated region (UTR) of the mRNA. The localization of the SD sequence, between 4 and 12 nucleotides upstream from the start codon, places the ribosome in the correct position for the initiation of translation (McCarthy and Gualerzi 1990). In contrast, in eukaryotes, cytosolic translation initiation proceeds through different pathways. The main mechanism relies on the binding of eIF-4E to the 7-methylguanosine cap structure on the 5′ end of the mRNA, followed by the formation of a complex with other initiation factors and the ribosomal small subunit. A helicase activity intrinsic to this complex allows the ribosome to scan the mRNA, 5′ to 3′, in search of the start codon in an ATP-dependent process (Algire and Lorsch 2006). An alternate pathway in eukaryotic cytosolic mRNAs relies on the recruitment of the ribosome by internal ribosome entry sites (IRESes). IRESes form complicated secondary structures in the 5′ UTR of the mRNA that allow for association with the small ribosomal subunit in the absence of some initiation factors (Komar and Hatzoglou 2005). Recently, a mRNA-rRNA base-pairing mechanism for translation has been described in eukaryotes (Dresios et al. 2006). In this case, a 9-nucleotide sequence located at –25 nt from the start codon in the 5′ leader of the mouse Gtx homeodomain mRNA facilitates translation initiation by base pairing to 18S rRNA.

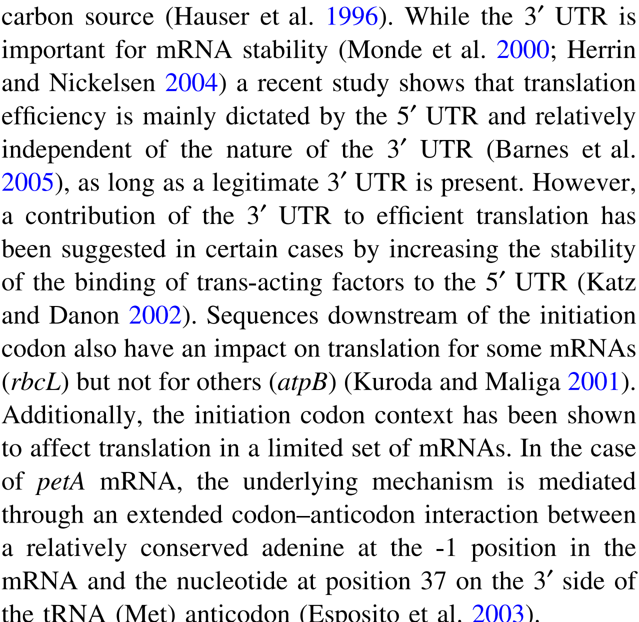
The mechanism for the correct positioning of the chloroplast ribosome on the start codon of mRNAs is not understood yet. Chloroplast mRNAs are not capped, but predicted secondary structures are found in many 5′ UTRs. The 3′ end of the 16S rRNA sequence is highly conserved between chloroplasts and prokaryotes and SD-like sequences are found in about 90% of chloroplast 5′ UTRs (Hirose and Sugiura 1996). Identification of SD-like sequences in the 5′ UTR of chloroplast mRNAs is difficult because the location of these elements is much more variable than in *E. coli*. Moreover, it is possible that the sequences used for rRNA pairing in chloroplast mRNAs are complementary to other regions of the 16S rRNA, thus differing from the typical SD sequence (GGAGG). This seems the case for the 5′ UTR of *psbA* in tobacco, where mutagenesis studies have revealed that two RNA elements (AAG and UGAUGAU) complementary to the 3′ end of 16S rRNA and located at –9 and –22 nt from the start codon, respectively, are required for translation in an *in vitro* system. In contrast, a SD-like sequence (GGAG) located at –33 nt from the AUG codon was dispensable in the same system (Hirose and Sugiura 1996). Conversely, in the 5′ UTR of *psbA* from *C. reinhardtii*, the SD-like sequence is located at –25 nt, and the deletion of this element results in complete inhibition of D1 translation in vivo (Mayfield et al. 1994). SD-like sequences in other 5′ UTRs have been shown to be important for translation in some genes (*rbcL, atpE, rps14* from tobacco), but not in others (*rps12, petB* from tobacco and *atpB, atpE, rps4, rps7, petD* from *C. reinhardtii)* (Sakamoto et al. 1994; Fargo et al. 1998; Hirose et al. 1998; Nickelsen et al. 1999; Hirose and Sugiura 2004a, b). In one case (*rps2*), a SD-like sequence, located at –8 to –5 nt from the start codon, was shown to

actually be a negative regulatory element (Plader and Sugiura 2003). Thus, it should be considered that SD-like elements may not be required for translation of all chloroplast mRNAs and that other pathways for translation initiation may exist (e.g., via interaction of specific sequences or RNA secondary structures with trans-acting factors which would subsequently recruit the ribosome).

The functionality of chloroplast SD-like sequences as base-pairing elements to rRNA has been, for a long time, the focus of a debate that is not yet definitely resolved (reviewed in Zerges 2000). In some cases, where the SD-like sequence is located far from the start codon, this element may be necessary but not sufficient for correct positioning of the ribosome. Many RNA-binding factors have been identified as essential for the translation of chloroplast mRNAs. Some of these proteins have been described to bind to different regions of the 5′ UTR (see below). One could speculate that interaction between RNA-binding factors or between these proteins and ribosomal subunits may change the tertiary structure of the mRNA in order to position the AUG correctly on the small subunit of the ribosome. Thus, SD-like sequences may be used as metastable anchors for the ribosome to the mRNA, whereas the right positioning would be achieved through interaction with other trans-acting factors. As mentioned in previous reviews, definite proof for the role of SD elements in chloroplast mRNAs should come from similar experiments to those performed in prokaryotes and mammals, i.e. through compensatory mutations in the 16S rRNA which restore base-pairing and translation of genes containing mutated SD sequences.

Translation initiation in the chloroplast: specific regulation through cis elements and trans factors

The regulated expression of diverse chloroplast genes is mediated by the (direct or indirect) interaction of nuclear-encoded factors with the 5′ UTR of the plastid mRNAs. Many nuclear genes that impact chloroplast translation have the property of affecting one individual mRNA specifically. However, other nuclear factors with a broad range of targets have also been described in vascular plants, and in fewer cases in *C. reinhardtii*. Whether these findings are representative of the real situation or are biased to some extent by the screening strategies employed, remains to be resolved (Barkan and Goldschmidt-Clermont 2000). Initial studies identified a set of 5′ UTR binding proteins from *C. reinhardtii* that included factors interacting with a broad range of chloroplast mRNAs, as well as others more specific for a particular mRNA or group of mRNAs. The RNA-binding pattern of some of these proteins was affected by environmental factors such as light or the



carbon source (Hauser et al. 1996). While the 3′ UTR is important for mRNA stability (Monde et al. 2000; Herrin and Nickelsen 2004) a recent study shows that translation efficiency is mainly dictated by the 5′ UTR and relatively independent of the nature of the 3′ UTR (Barnes et al. 2005), as long as a legitimate 3′ UTR is present. However, a contribution of the 3′ UTR to efficient translation has been suggested in certain cases by increasing the stability of the binding of trans-acting factors to the 5′ UTR (Katz and Danon 2002). Sequences downstream of the initiation codon also have an impact on translation for some mRNAs (*rbcL*) but not for others (*atpB*) (Kuroda and Maliga 2001). Additionally, the initiation codon context has been shown to affect translation in a limited set of mRNAs. In the case of *petA* mRNA, the underlying mechanism is mediated through an extended codon–anticodon interaction between a relatively conserved adenine at the -1 position in the mRNA and the nucleotide at position 37 on the 3′ side of the tRNA (Met) anticodon (Esposito et al. 2003).

Table 1 summarizes the chloroplast mRNAs for which interacting nuclear-encoded factors affecting translation are known, and shows schematically those cis-acting elements within the 5′ UTR that have been described to be important for translation or mRNA stability. Some of these cases will be discussed below in detail.

Translation of *psbA* (D1 protein)

The most striking example of light-induced translation is the case of *psbA*, encoding the D1 protein that, together with D2, forms the core of the reaction center of photosystem (PS) II. D1 translation is up-regulated 50- to 100-fold during dark to light shifts and both initiation and elongation are impacted (see below). Regulation of translation initiation of *psbA* mRNA has been proposed to rely on the binding of a complex of four proteins (RB47, RB38, RB60 and RB55) to the 5′ UTR of the mRNA in *C. reinhardtii*. Both RB38 and RB47 are RNA-binding proteins, the RNA affinity of RB38 being independent of the redox environment (Barnes et al. 2004) whereas RB47 interaction with the *psbA* 5′ UTR is strongly enhanced under reducing conditions *in vitro* (Kim and Mayfield 1997). The requirement of RB47 for *psbA* translation has also been shown in vivo by the analysis of mutants lacking this factor (Yohn et al. 1998a). RB60 is a protein disulfide isomerase homolog that regulates the redox status of RB47. This interaction has been shown by the ability of RB60 to modulate the RNA affinity of RB47 in vitro (Kim and Mayfield 1997), and by the detection of a disulfide intermediate between RB60 and RB47 (Alergand et al. 2006). Studies performed with isolated chloroplasts revealed that light initiates an oxidative signal that decreases the pool of reduced RB60. However, increasing light intensities cause

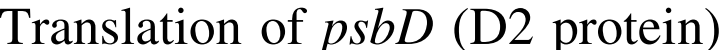
Table 1 Nuclear-encoded factors and cis elements involved in translation

complex	protein	gene	5´-UTR mRNA	TRANS factors	Species	References
PSII	D1	*psbA*	I II long: -90; short: -36	- F35 - Tba1 - RB47/RB38/ RB60/RB55 complex (I and II)	*C. r.*	Girard-Bascou (1992), Yohn (1996), Kim (1997), Bruick (1998), Somanchi (2005)
	D2	*psbD*	Urich I II III long: -74; short: -47	- Nac1 - Acc-115 - Nac2 (I) - RBP40 (II)	*C. r.*	Wu (1995), Nickelsen (1999), Klinkert (2006)
	P6 (CP43)	*psbC*	I II III IV V SD	- TBC1 or F34 (II, III, IV, SD) - TBC2 or F64 (IV, II or V) - TBC3 (I, SD)	*C. r.*	Zerges (2003)
	CP47	*psbB*	long: -147; short: -35	- Mbb1	*C. r.*	Vaistij (2000a), Vaistij (2000b)
				- HCF107	*A. t.*	Sane (2005)
	P10	*psbH*		- HCF107	*A. t.*	Sane (2005)
PSI	PsaB	*psaB*		- Tab1 (F15) - Tab2 (F14)	*C. r.*	Stampachia (1997), Rochaix (2004)
	PsaC	*psaC*		- Crp1	*Z. m.*	Schmitz-Linneweber (2005)
Cyt b6/f	Cyt f	*petA*		- Sim30 - Mca1 [stability] - Tca1	*C. r.*	Chen (1997), Wostrikoff (2001)
				- Crp1	*Z. m.*	Fisk (1999)
	SUIV	*petD*	I II III	- Sim30 - Mcd1 (I)	*C. sp*	Sakamoto (1994), Chen (1997), Drager (1998), Higgs (1999), Kramzar (2006)
				- Crp1	*Z. m.*	Fisk (1999)
ATP synthase	α subunit	*atpA*		- F54	*C. r.*	Drapier (1992)
	CF_0-IV subunit	*atpI*	I II	Unidentified protein complexes: - X (I) - Y (II)	*Sp. o.*	Merhige (2005)
	βsubunit	*atpB*	U rich [I]	- p50 (I)	*N. t.*	Hirose (2004b)
				- Atp1	*Z. m.*	McCormac (1999)
NAD(P)H dh		*ndhD*		- CRR4 [editing]	*A. t.*	Kotera (2005), Okuda (2006)

The 5´ UTRs of some mRNAs are represented schematically. Predicted secondary structures are shown with the shape of a stem-loop and elements relevant for translation and/or mRNA stabiliy are indicated with grey boxes. In the case of *psbC*, the stripped boxes correspond to elements partially required for translation, compared to the full boxes that are essential for translation. Black boxes represent SD-like sequences and the initiation codon is shown with a star. Arrows indicate processing sites with the numbers beneath representing the size in nucleotides of the long and short versions of the resulting 5´ UTRs . For some nuclear factors the interacting cis element experimentally predicted within the 5´ UTR is indicated in brackets with a roman number. Species names are abbreviated (*C.r.: C. reinhardtii; Z.m.: Zea mays; A.t.. Arabidopsis thaliana; C. sp; Chlamydomonas sp.; Sp.o.: Spinacea oleracea*)

an induction in *psbA* translation which parallels an increase in the pool of reduced RB60, probably transduced from photosystem I by the ferredoxin–thiorredoxin system (Trebitsh et al. 2000). Recent work showed that oxidation of at least one of the two fast-reacting cysteines of RB47 (most likely Cys 143 or Cys 259) is sensitive to pH conditions, suggesting that light-induced changes in the stromal pH may contribute to the regulation of *psbA* translation (Alergand et al. 2006). Redox-dependent RNA binding of RB47 is also modulated by Tba1, a protein with homology to oxidoreductases that could be incorporated into the previous model for the light-dependent translation activation of D1 (Somanchi et al. 2005). At another level of regulation, a serine/threonine protein phosphotransferase associated with the *psbA* 5′ UTR binding complex (RB47/RB38/RB60/RB55) is able to inactivate the RNA-binding properties of the complex through the ADP-dependent phosphorylation of RB60. This inactivation requires high ADP levels and thus, attenuation of translation in the dark may be achieved by the concomitant increase of the ADP/ATP ratio (Danon and Mayfield 1994). The photosynthetic electron transfer chain has been proposed to control *psbA* translation via two pathways (Trebitsh and Danon 2001). One originates from PSI and is transduced as a thiol-mediated signal, through ferredoxin and thioredoxin, probably to the *psbA* 5′ UTR binding complex. The other pathway, termed priming, is a pre-requisite to the thiol-mediated pathway and involves the reduction of the plastoquinone pool. It is tempting to speculate that this priming signal activates the dephosphorylation of RB60 in response to light, rendering this factor competent for the redox modulation of RB47. A model describing all these regulatory steps is shown in Fig. 1a.

A predicted stem-loop structure and the contiguous SD-like sequence in the 5′ UTR of *psbA* have been shown to be relevant for i*n vivo* D1 translation in *C. reinhardtii* (Mayfield et al. 1994). In tobacco, light-dependent and tissue-specific accumulation of D1 is also translationally regulated by elements within the *psbA* 5′ UTR (Staub and Maliga 1993), although the structure of these cis elements (Hirose and Sugiura 1996) may differ from that of *C. reinhardtii*. Redox-dependent binding of trans factors to the *psbA* 5′ UTR has been reported also in *A. thaliana*, where oxidative treatments to chloroplast extracts abolished the in vitro association of a 43 kDa and a 30 kDa protein to the 5′ UTR of *psbA* (Shen et al. 2001). The same authors isolated cDNAs from *A. thaliana* that are homologs to *C. reinhardtii* RB60 and RB47 and have putative chloroplast targeting signals, but with a different size in the deduced protein sequence (see discussion in (Shen et al. 2001)). Thus, a similar system to that described for *C. reinhardtii* activating D1 translation in response to light may operate in vascular plants.



Translation of *psbD* (D2 protein)

Translation of the D2 protein, the molecular partner of D1 in the PSII reaction center, is also up-regulated by light (Malnoe et al. 1988) but the mechanism for this control is less understood than that of *psbA*. Both initiation and post-initiation steps (see below) are under the regulation of nuclear proteins. A model has recently been proposed to explain the role of the previously described essential factors (Nac2 and RBP40) in the activation of *psbD* translation initiation (Klinkert et al. 2006). Nac2 is a tetraricopeptide repeat protein that is part of a 600 kDa complex localized in the stromal compartment (Boudreau et al. 2000) and interacts with an RNA element within the 5′UTR of *psbD*, called PRB2 (Nickelsen et al. 1999). This binding confers protection against RNA exonucleases and guides RBP40 to a U-rich site immediately downstream of PRB2 (Ossenbuhl and Nickelsen 2000). After recruitment of RBP40, Nac2 is released from the mRNA. Deletion of the U-rich element results in a translational defect, which is overcome by suppressor mutations affecting an RNA secondary structure that occludes the initiation codon. These results suggest that at least one of the roles of RBP40 binding is to destabilize this stem-loop element, facilitating the access of the ribosomal small subunit to the initiation codon (Fig. 1b).

Translation of *psbC* (P6 or CP43 protein)

tbc1, *tbc2,* and *tbc3* are the three nuclear loci that have been identified to affect *psbC* translation in *C. reinhardtii*. *tbc1* and *tbc2* mutants are unable to translate *psbC* (Rochaix et al. 1989) whereas the *tbc3* mutation partially reverses the translational block caused by the *tbc1* mutation or the deletion of a specific element in the 5′ UTR of *psbC* (Zerges et al. 1997).The Tbc2 protein is associated with a 400 kDa complex within the chloroplast stroma and shares partial homology to Crp1, a protein reported in *Zea mays* to be involved in the processing and translation of the chloroplast *petA* and *petD* mRNAs (Auchincloss et al. 2002). The current model proposes that *psbC* translation requires the interaction of Tbc1 with a predicted secondary structure (positions 223–320) in the 5′ UTR of *psbC* (element II in Table 1) and sequences immediately downstream of the initiation codon. Tbc3 may cooperate with Tbc1, probably targeting the sequences in the 95–222 region (element I in Table 1) and the SD-like sequence. Since Tbc3 was identified as a suppressor mutation, this could also be the result of a new RNA-binding affinity conferred to a protein that in the wild-type strain interacts elsewhere and therefore indirectly compensates for the Tbc1 defect. Tbc2 appears to function in a different pathway to Tbc1 and Tbc3, but

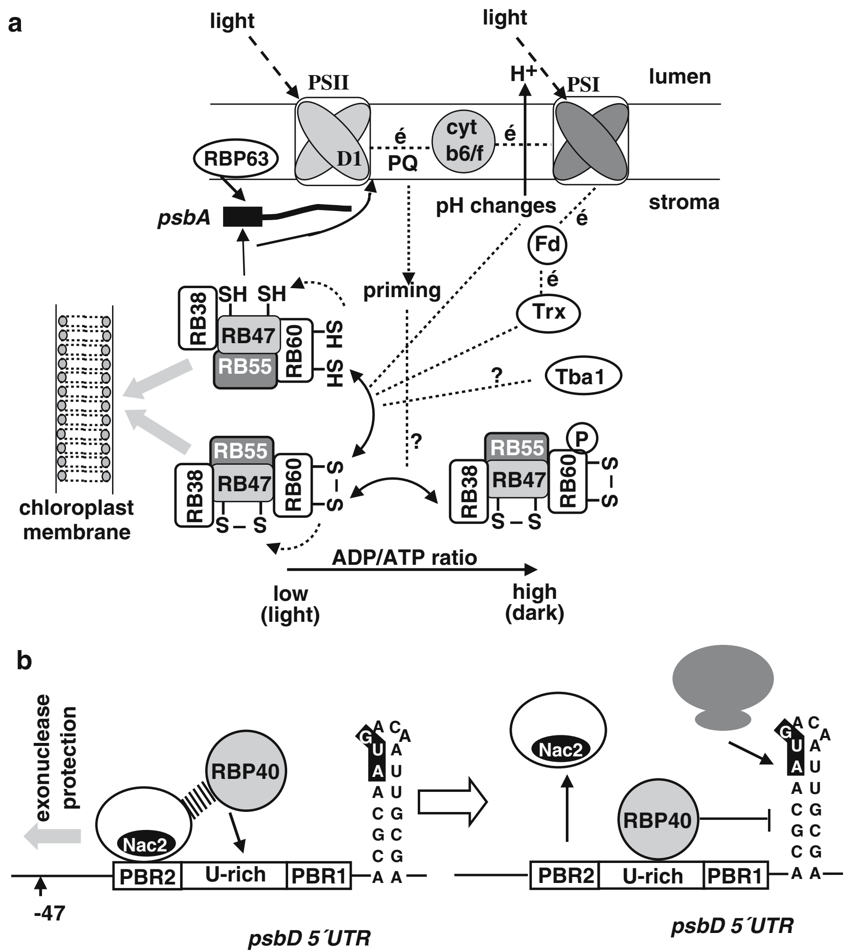


Fig. 1 Models for translation initiation regulation (see text for details). (**a**) Model for light activated translation of the *psbA* mRNA. Light generated reducing equivalents from photosynthesis is used to activate binding of a set of proteins (RB38/RB47/RB55/RB60) to the *psbA* mRNA by reduction of a critical cysteine pair in RB47 that is modulated by the PDI-like RB60. Binding of this set of proteins results in recruitment of the *psbA* mRNA onto the ribosome for active translation. Changes in ADP/ATP ratio can inactivate the binding complex by phosphorylation of RB60, and changes in the stromal pH may also modulate the redox potentials of the intervening cysteine residues. Additional redox-sensitive proteins, like Tba1, may function in the activation of translation by interacting with the RNA-binding complex. RB60 and RB47 are associated with chloroplast membranes and may mediate the targeting of *psbA* mRNA to membranes for cotranslational insertion of D1 in the lipid bilayer. RBP63, a protein localized to thylakoid membranes, also binds the 5′ UTR of *psbA* and therefore may help to recruit *psbA* mRNA to this chloroplast subcompartment. (**b**) Model for *psbD* translational activation. Similar to *psbA* activation, a number of *psbD* mRNA specific binding proteins have been identified that are required for *psbD* translation. Nac2 binding to the 5′ UTR of the *psbD* mRNA protects it against exonuclease degradation and recruits the RBP40 factor, which in turn facilitates ribosome association by destabilizing a secondary structure that partially blocks the initiation codon. Although it is unknown if these proteins are activated by change in the redox potential, *psbD* is light activated in a similar manner as *psbA* translation, and these factors could function in such regulation

both routes are required for *psbC* translation (Zerges et al. 2003).

Translation of *psaB*

Two nuclear-encoded factors from *C. reinhardtii*, Tab1 and Tab2, are necessary for the translation of *psaB*, which encodes one of the subunits of the reaction center of PSI. The ultimate target site for these trans factors is localized within the *psaB* 5′ UTR, as indicated from the fact that *Tab1* and *Tab2* mutants (initially named F15 and F14, respectively) are deficient in the expression of a reporter gene under the control of the *psaB* 5′ UTR (Stampacchia et al. 1997; Dauvillee et al. 2003). A suppressor mutation of F15 was localized in a predicted stem-loop element occluding a SD-like element in the *psaB* 5′ UTR, and this suggested the possibility that Tab1 function may be required for opening the stem to allow for translation (Stampacchia et al. 1997). Tab1 contains five putative trans-membrane regions, whereas Tab2 is localized in the chloroplast stroma as part of a high molecular weight complex that also contains *psaB* mRNA. It has been

suggested that Tab2 could act early in the translation of *psaB*, perhaps by binding to the 5′ UTR of its mRNA and targeting the mRNA to the thylakoid membrane, where it could interact with other factors, such as Tab1 (Rochaix et al. 2004).

mRNA processing and translation

The 5′ UTR of many chloroplast mRNAs is processed to one or more shorter versions of specific sizes by mechanisms that may involve both endonucleolytic and exonucleolytic activities. The extent of this processing event is variable depending on each mRNA and, in some cases, on the environmental conditions. Thus, the 5′ UTRs of *psbA* are predominantly cleaved to the shorter version in *C. reinhardtii* (Bruick and Mayfield 1998) (see Table 1) while the longer version is more abundant for the *psbA* 5′ UTR from *A. thaliana* (Shen et al. 2001). Diverse experimental evidence suggests a relation between the processing of certain 5′ UTR and the translation efficiency of the corresponding coding sequence. Treatment of barley leaves with methyl jasmonate induced a shift of the 5′ end of the *rbcL* transcripts from the naturally occurring position –59 (relative to the translation initiation site) to –94. This shift correlated with a decline in the in vitro-translatable *rbcL* mRNA, suggesting that the processing to the shorter form was required for efficient translation (Reinbothe et al. 1993). Similarly, light stress in *C. reinhardtii* transiently suppresses the synthesis of Rubisco large subunit (LSU) coincidently with a temporary change in the ratio of the two forms of this transcript (–93 and –168), with the shorter form being more abundant when LSU is actively translated (Shapira et al. 1997). The 5′ UTRs of *psbB* and *psbD* from *C. reinhardtii* are also cleaved to shorter forms (see Table I) to a high extent in the wild-type strain. Mutation of the trans factors Mbb1 and Nac2 abolishes the processing of those 5′ UTRs, respectively, and is accompanied by a loss of translation of the corresponding protein. This could be interpreted either as a requirement of the processing for translation to proceed, or as an involvement of the trans factor in both the cleavage of the 5′ UTR and in some essential step of translation (Nickelsen et al. 1999; Vaistij et al. 2000a). In any case, a molecular link between processing and translation seems to be implicated for these mRNAs. A similar relation between mRNA stability and translation has been proposed in the case of the Mcd1 factor from *C. reinhardtii*, which is essential for *petD* mRNA stability, but that may also be involved in translation (Drager et al. 1999). The processing of the 5′ UTR of *psbA* to the shorter version in *C. reinhardtii* is almost complete regardless of the light conditions, although *psbA* translation is light-regulated. However, processing is completely abolished when the SD-like sequence in the 5′ UTR is mutated or in several nuclear mutants that fail to translate D1 protein (Bruick and Mayfield 1998). Thus, it was hypothesized that in this case processing may occur concomitantly with ribosome binding to the 5′ UTR or during the initial translation steps (Bruick and Mayfield 1998). A recent study using a new in vitro translation system indicates that the role of 5′ UTR processing in regulating the expression of chloroplast proteins differs between mRNAs (Yukawa et al. 2007). Using this tobacco system, the authors found that the rates of translation of unprocessed and processed *atpH* and *rbcL* 5′ UTRs were similar, whereas translation of mRNAs with processed *atpB* and *psbB* 5′ UTRs was more efficient than those with the unprocessed 5′ UTR. Additionally, 3′ UTR processing has been reported to be important for translation, since correctly 3′ processed mRNAs are preferentially associated to polysomes (Rott et al. 1998).

Many chloroplast mRNAs are transcribed as polycistronic transcripts, which are subsequently cleaved to render monocistronic or dicistronic mRNAs. TPR-like proteins have been involved in many of these processing events, and their relation to translation is the focus of many recent studies. In maize, *petD* mRNA, encoding subunit IV (SUIV) from the cytochrome b_6/f complex, is transcribed as part of the polycistronic cluster *psbB-psbH-petB-petD*, which is subsequently processed to monocistronic mRNAs. The nuclear mutant *crp1* is deficient in *petD* and *petA* translation, and this is accompanied by a failure to produce a monocistronic *petD* mRNA. The inefficient processing may explain the defect in translation of *petD* due to the masking of the initiation codon inside a stable hairpin loop element predicted to be found in the polycistronic mRNA, but not in the monocistronic form (Barkan et al. 1994). However, the reduced expression of *petA* in the same mutant must be due to a translation deficiency, since *petA* mRNA is found at normal size and abundance. Thus, the Crp1 protein may have a dual role in processing and translation of two different mRNAs. Alternatively, *petA* translation, encoding cytochrome f, may require the presence of SUIV, as has been shown in *C. reinhardtii* (see below), but other evidence indicates that in vascular plants, like tobacco, *petA* translation can proceed in the absence of *petD* expression (see discussion in Fisk et al. 1999). Crp1 is a stromal protein that is found in a stable complex of about 300 kDa and contains a pentatricopeptide (PPR)-like motif. The PPR motif builds an RNA-binding domain in a group of nuclear-encoded proteins affecting chloroplast RNA metabolism (Nakamura et al. 2004). In Crp1 this motif may mediate the formation of the 300 kDa complex or might be involved in transient interactions with the mRNA or other translation factors. Recently, RNA immunoprecipitation and microarray analysis showed that

Crp1 associates with the 5′ UTR of *petA* and *psaC*, suggesting that this PPR protein may influence gene expression through direct interaction with specific mRNAs in vivo (Schmitz-Linneweber et al. 2005).

Other proteins from the helical repeat family have been reported to impact translation and processing of polycistronic mRNAs in chloroplasts from different organisms. In *C. reinhardtii*, expression of the *psbB-psbT-psbH* cluster is affected by the Mbb1 protein, which contains ten tandem repeats of a tetratricopeptide (TPR)-like motif (Vaistij et al. 2000b). Mbb1 is localized in the chloroplast stroma as part of a 300 kDa complex. This complex has been proposed to be involved in *psbB* processing, stability and/or translation. In *A. thaliana*, the *HCF107* gene has been shown to be involved in the processing/stability and/or translation of *psbH* and in the translation of *psbB*, both genes from the same operon (*psbB-psbT-psbH-petB-petD*). HCF107 contains 11 TPR-like motifs (designated RNA-TPR) tandemly arranged (Sane et al. 2005). The bulk of this protein is present in a 100–240 kDa complex, but a significant proportion is also associated to a 600–800 kDa complex. Unlike the previously discussed examples, HCF107 is localized to the plastid membranes.

Finally, in vascular plants *psaC* and *ndhD* are cotranscribed as a dicistronic precursor that is cleaved to render two mature mRNAs. This processing is required for translation of both *psaC* and *ndhD* as revealed from in vitro studies and it was proposed that a predicted inhibitory secondary structure involving the intercistronic region was responsible for the translational block (Hirose and Sugiura 1997). Such a negative element might contain an intramolecular base-pairing between a sequence in the *psaC* coding region and a complementary sequence in the *ndhD* 5′ UTR. Moreover, *ndhD* translation requires the editing of the initiation codon from ACG to AUG. The extent of editing at this site is regulated and depends on developmental and light conditions. A pentatricopeptide repeat protein from *A. thaliana*, Crr4, has been identified as a component in this editing event (Kotera et al. 2005; Okuda et al. 2006). Crr4 seems to act as a site recognition factor binding to two RNA regions around the editing site and possibly cooperating with an unidentified RNA editing enzyme.

Autoregulation of translation

The rate of synthesis of some chloroplast proteins is under the control of an assembly-dependent regulatory process, named ‘control by epistasy of synthesis’ (CES process). The mechanism underlying this process is a negative feed back of the unassembled protein on translation of its own mRNA, and several cases have been reported. Cytochrome f is encoded by the *petA* gene and forms part of the cytochrome b_6/f complex, together with the SUIV protein and cytochrome b_6. When any of the components of this complex are absent, translation of cytochrome f is seriously compromised, but this negative effect can be bypassed if the C-terminal domain of cytochrome f is absent. As this domain is shielded upon the formation of the cytochrome b_6/f complex, it was proposed that the steady state fraction of unassembled cytochrome f would inhibit its own translation through this exposed domain (Fig. 2b, left panel) (Choquet et al. 1998). Since no RNA-binding domains can be identified in the C-terminus of cytochrome f, an indirect interaction between this motif and the 5′ UTR of *petA* was hypothesized. According to this model, a ternary factor essential for cytochrome f translation would be trapped by the C-terminal motif, and released after assembly of the cytochrome b_6/f complex (Fig. 2a). More recent experiments suggested that TCA1, a nuclear-encoded translational activator specific for *petA* mRNA may act as this potential ternary factor (Wostrikoff et al. 2001). A similar mechanism has been proposed to act in the biogenesis of PSI and PSII. In the first case (Fig. 2b, middle panel), PSI assembly is initiated by the CP1 core complex (PsaA/PsaB), to which PsaC and other subunits are added in

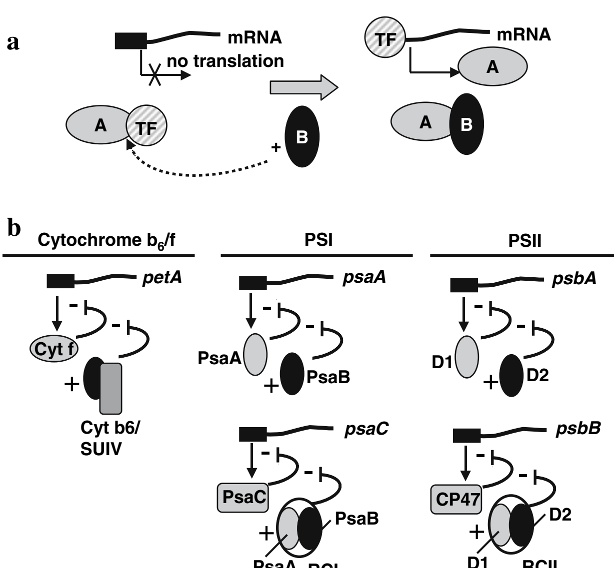


Fig. 2 Models for the autoregulation of translation (see text for details). (**a**) Model for the mechanism of translation auto-repression. One of the proteins of a multisubunit complex (A), in the absence of its partner (B), represses its own translation by sequestering a ternary factor (TF) that is required for translation initiation. When B is present and binds A, the ternary factor is released, free to activate the translation of A. (**b**) Diverse examples of the auto-repression model. During the assembly of the photosynthetic complexes cytochrome b_6/f, PSI and PSII, different steps are regulated by auto-repression of one of the subunits, which is released upon the binding (+) of its partners in the complex

sequential steps. Unassembled PsaA protein represses its translation until it is sequestered by PsaB, and in turn, PsaC exerts a similar autoinhibition unless the CP1 core complex is present (Wostrikoff et al. 2004). Similarly, during PSII biogenesis, *psbA* translation (encoding the D1 protein) would be downregulated by free D1 when D2 is absent (Fig. 2c, right panel). In subsequent steps the D1/D2 core complex would bind other subunits to form the reaction center II (RCII). Recruitment of the antenna subunit CP47 by RCII would release a second auto-repression event on *psbB* (encoding CP47) (Minai et al. 2006). In all these cases, translation autoregulation seems to act at the initiation level, since this negative feed back was also observed with a reporter gene placed under the control of the corresponding 5′ UTRs (*petA*, *psaA*, *psaC*, *psbA,* or *psbB*). A similar model may operate in the translation of *atpA*, encoding the α-subunit of the ATP synthase, which requires the presence of the β-subunit of the same complex (Drapier et al. 1992).

Autoattenuation mechanisms operate at least at three different levels for the translation of *rbcL*, encoding the large subunit of Rubisco. First, a CES-like process may be involved, since decreased levels of the nuclear-encoded small subunit of Rubisco hamper the association of *rbcL* mRNA with polysomes and reduce the accumulation of large subunit (Rodermel et al. 1996). Thus, in this case the unassembled protein may be responsible for repressing its own translation. Second, auto-repression was suggested as a possible mechanism for the observed decrease in Rubisco translation during high light conditions that generate a higher concentration of radical oxygen species (ROS) and shift the glutathione pool toward its oxidized form. It has been shown that Rubisco large subunit experiences a conformational change upon oxidation that exposes an RNA recognition motif (RRM), enabling the protein to bind RNA non-specifically (Yosef et al. 2004). The structure of the N-terminal domain of Rubisco containing the RRM is highly conserved in photosynthetic organisms (Cohen et al. 2006). In this case, translational arrest most likely occurs at a post-initiation stage because *rbcL* RNA shifts toward fractions that contain smaller polysomes and monosomes during oxidative stress (Cohen et al. 2005). The proposed mechanism involves ribosome stalling due to Rubisco binding (in its oxidized form) to the *rbcL* mRNA, thus preventing the assembly of additional ribosomes. Finally, Brutnell and co-workers hypothesized that aggregates of the Rubisco large subunit may attenuate *rbcL* translation in the maize *Bsd2* mutant by ribosome pausing. The BSD2 protein contains a J-domain motif, similar to that of the DnaJ protein, which is involved in maintaining nascent peptides in an unfolded state prior to completing chaperone-assisted folding by the DnaK system. In the *Bsd2* mutant there is no Rubisco accumulation but most *rbcL* transcripts are associated with polysomes. This is accompanied by an ectopic expression of *rbcL* mRNAs in mesophyll cells and by aberrantly high levels of the transcript in dark-grown tissue. The authors proposed that the lack of the Bsd2 protein, which would assist the protein folding in wild-type plants, may cause the Rubisco large sunbunit to aggregate, resulting in ribosome stalling on the *rbcL* transcripts and a subsequent mRNA protection from degradation (Brutnell et al. 1999).

Regulation of chloroplast translation during elongation

Although the dominant mechanisms of translational regulation appear to operate during initiation, the expression of several genes is also regulated during the elongation steps. Ribosomal pausing has been observed during D1 translation at distinct sites, and this behavior has been postulated to facilitate co-translational protein folding, chlorophyll binding, and assembly of PSII. During this process, D1 translation intermediates may be stabilized by the nuclear-encoded factor Vir-115 (Kim et al. 1994). Similarly, translation of the D2 protein is regulated at the elongation level by the nuclear gene *ac115*. Absence of Ac115 protein abolishes D2 synthesis, but does not block the association of *psbD* mRNA with polysomes. The Ac115 factor is a protein of 113 amino acids with a hydrophobic and cysteine rich domain in the C-termini that is predicted to be inserted in the thylakoid membrane or may mediate protein/protein interactions (Rattanachaikunsopon et al. 1999). Acc115 has been proposed to stabilize D2 intermediates during translation and facilitate its folding and binding to cofactors or, alternatively, to direct D2 to the thylakoid membranes perhaps through interaction with other factors.

In isolated chloroplasts from barley or spinach, D1 elongation is stimulated by light and the mechanism for such induction involves a redox pathway derived from PSI (Zhang et al. 2000). D1 elongation is also regulated by the formation of a proton gradient through the thylakoid membrane (Muhlbauer and Eichacker 1998; Zhang et al. 2000) and is tightly coupled with the assembly and membrane insertion steps. D1 membrane insertion is probably regulated by the transmembrane proton gradient, as is the case of co-translational insertion of proteins in *E. coli* periplasmic membranes through the SecY/SecE translocon. Chloroplast homologs of the bacterial SecY and SecE are present in the thylakoid membrane (Laidler et al. 1995; Schuenemann et al. 1999), and a transient interaction between D1 elongation intermediates and cpSecY has been detected (Zhang et al. 2001). Rubisco elongation rates have also been shown to be up-regulated under (low) light exposure in barley seedlings and isolated chloroplasts (Muhlbauer and Eichacker 1998; Kim and Mullet 2003).

Toeprinting assays showed that, in the dark, read-out of ribosomes from translation initiation complexes into elongating ribosomes was inhibited, and that this arrest was released upon illumination. However, as previously mentioned, bright light conditions that generate ROS have the opposite effect on Rubisco translation (Shapira et al. 1997; Cohen et al. 2005). The regulation of elongation factors by light and other abiotic factors may explain a general control at the elongation steps during chloroplast translation, while other factors may be required for the specific induction of D1 or Rubisco elongation.

A mechanism for translational regulation through transcriptional control has also been proposed. Since some plastid-encoded tRNAs limit protein synthesis during chloroplast development, transcription of these tRNAs could be relevant to translational rates under these conditions. In vascular plants, the plastid-encoded RNA polymerase is modulated by nuclear-encoded sigma factors. One of these sigma subunits in *A. thaliana*, SIG2, is essential for the transcription of at least seven tRNAs, including tRNA-Glu. In the chloroplasts of plants and algae, glutamyl-tRNA-Glu is used as the initial substrate in the synthesis of ALA, the rate-limiting step for chlorophyll formation. An interesting hypothesis proposes that SIG2 may be involved in coupling translation and pigment synthesis in chloroplasts, controlling the supply of tRNA-Glu for both pathways (Kanamaru and Tanaka 2004).

Localization of translation

The fact that the genes encoding most of the integral membrane proteins in the chloroplast have been retained in the plastid genome has been proposed to facilitate cotranslational insertion into the lipid bilayer, implying that the corresponding mRNAs would somehow be targeted to the membrane. Moreover, soluble proteins like the large subunit of Rubisco (Muhlbauer and Eichacker 1999) and elongation factor EF-Tu (Breidenbach et al. 1990) have been reported to be synthesized by membrane-bound polysomes. In the case of Rubisco translation, such targeting may be required for the proposed regulatory role of the thylakoid membrane proton gradient in gene expression (Muhlbauer and Eichacker 1998; Muhlbauer and Eichacker 1999). In-depth analysis of the thylakoid membrane proteome from *A. thaliana* has revealed the presence of 16 proteins involved in translation, including ribosomal proteins (putative) RNA-binding proteins, three PSRPs (PSRP-2, PSRP-3, and PSRP-5), and the ribosome recycling factor, demonstrating that the thylakoid surface is an important site for protein synthesis (Friso et al. 2004). Three models have been considered regarding the specific membrane fraction where translation occurs, with experimental evidence supporting either the thylakoid membrane, the inner membrane of the chloroplast envelope or the ends of thylakoid grana (reviewed in Zerges 2000). A membranous subcompartment called ‘‘low density membranes’’ (LDM) may have some relevance to these models, since they were shown to be associated with a set of light-activated RNA-binding proteins (see below). These LDMs are similar in buoyant density and acyl lipid composition to the chloroplast inner envelope membrane and are physically associated with thylakoid membranes (Zerges and Rochaix 1998). Since this class of LDMs only separates from the thylakoid fraction in the absence of magnesium, many studies that ascribe protein binding to thylakoids (with magnesium ions being present in these preparations) may actually reflect an association to LDMs.

The mechanism for protein targeting to any of those membrane fractions has yet to be established, but current data have involved both the chloroplast signal recognition particle (cpSRP) and membrane-associated translation factors with RNA-binding activity. The SRP in eukaryotes is a ribonucleoprotein complex responsible for targeting of proteins to the endoplasmic reticulum membrane. In vascular plants, two different cpSRP-dependent pathways have been identified, one post-translational for proteins imported into the chloroplast, and the other co-translational for plastid-encoded proteins (Groves et al. 2001; Rosenblad and Samuelsson 2004). In fact, a transient interaction between cpSRP54 and elongating D1 protein was observed using an *in vitro* chloroplast translation system (Nilsson and van Wijk 2002). Additionally, the finding of RNA-binding proteins (RBPs) in membranous fractions led to a hypothesis in which these proteins may localize different mRNAs to be translated in specific chloroplast membranes. This is the case of a set of RBPs of 30–32, 46, 47, 60, and 80 kDa that were found associated with LDMs from *C. reinhardtii*. Interestingly enough, RNA-binding activity of these proteins is significantly enhanced in light-grown cells. The 46 and 47 kDa proteins have a general affinity for sequences rich in A and U and are inhibited by ADP in vitro, suggesting that modulation of the ADP pools could mediate the light activation of these proteins (Zerges et al. 2002). RB47, an RBP protein proposed to mediate the light regulation of *psbA* (see above), also cofractionated with LDMs as indicated by immunoblot assays (Zerges and Rochaix 1998). Despite some similarities between RB47 and the 47 kDa RBP (light induction, in vitro ADP inhibition and cofractionation in LDMs), their RNA affinities differ, since 47 kDa RBP binding is competed by either poly-A or poly-U, whereas only poly-A can compete in the case of RB47 binding (Yohn et al. 1998b; Barnes et al. 2004). Thus, the 46 kDa, 47 kDa, and RB47 may have a similar role in chloroplast translation but affecting a different spectrum of mRNAs. The 46 and 47 kDa RBPs are

inhibited in vitro by intermediates in the chlorophyll biosynthetic pathway, suggesting that these RBPs may participate in the coordination of the photosynthetic rate, chlorophyll synthesis and protein expression (Zerges et al. 2002). Another protein proposed to be involved in the light regulation of *psbA* translation, the protein disulfide isomerase-like RB60, is partitioned between the stroma and a chloroplast membrane fraction (Trebitsh et al. 2001). As previously mentioned, the HCF107 factor from *A. thaliana*, affecting the expression of *psbB* and *psbH*, is also associated with membranes. A chloroplast RBP named RBP63 that specifically binds the 5′ UTR of *psbA* is part of a 700 kDa complex localized in the stromal thylakoid membranes. Since the RNA binding of this protein is dependent on an A-rich region previously shown to be required for D1 translation, RBP63 has been suggested to be involved in the translational control of *psbA* (Ossenbuhl et al. 2002). Altogether, these data provide more evidence that regulatory steps in the translation of chloroplast mRNAs proceed in association with chloroplast membranes.

A model for chloroplast translation

In bacteria, negative regulation of translation is a dominant mechanism used to regulate protein synthesis. In other words, most mRNAs are translated relatively efficiently unless a specific signal releases a repressor trans-acting factor or causes a secondary structure change in the mRNA that sequesters the SD sequence. In the chloroplast, however, positive regulators of translation are more commonly used. This implies that most mRNAs are translated very inefficiently or not at all until specific signals induce their translation. These signals may be in response to light or other biotic/abiotic factors, may be sensitive to autoinhibition, and in some well-studied cases induce the assembly of protein translation factors onto the mRNA that yield it translationally competent. From experiments with reporter genes it is clear that the 5′ UTR of many chloroplast messages is sufficient to confer such regulated translation.

The presence of chloroplast-unique proteins on an otherwise bacterial-type ribosome in the chloroplast has been documented in both a land plant and in algae. Some of these proteins are shared between the two species, while others may be specific to each organism. Regardless, it is likely that these unique protein components of the chloroplast ribosome are centrally involved in the positive regulation of translation in the chloroplast. Recent structural study on the structure of the chloroplast ribosome has revealed that in *C. reinhardtii* these chloroplast-unique proteins form discrete structures on the small subunit of the ribosome (Manuell, in preparation). These structures are located at prime positions to interact with initiation complexes during translation initiation.

Identification of bacterial-type initiation, elongation, and termination factors suggests that once an mRNA has been positioned properly for initiation, translation proceeds in a relatively bacterial-type manner. There is also some evidence for pausing during the translation of certain chloroplast mRNAs (as for *psbA*, discussed above), and chloroplast-unique portions of the ribosome may also be involved in this phenomena.

Overall, translation regulation in chloroplasts comprises a finely tuned cooperation of nuclear and chloroplast elements, with multiple signals and regulatory steps potentially affecting the expression of any single plastid mRNA. In order to fully understand the mechanisms of translation regulation a prominent issue will be to determine the subcompartmentalization of the different regulatory components within the chloroplast. For this purpose, a thorough subfractionation of the chloroplast membranes will be compelling in future experiments. Moreover, a better understanding of the molecular nature of the regulatory pathways of translation, including their interactions and timing, will be key to determine the governing steps in chloroplast gene expression. Such knowledge will contribute to our overall understanding of translation as well as to the success of the chloroplast as an expression platform for proteins of pharmaceutical interest.

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