TRANSLATIONAL REGULATION IN THE CHLOROPLAST

Gene expression is potentially amenable to regulation at different points along the linear path from a gene to a functional protein. To control gene expression the regulatory event has to occur at a limiting rate. Chloroplast gene expression has been shown to be primarily regulated by posttranscriptional events, including transcript stability, translation, protein turnover, and protein activity. An increasing body of data has demonstrated the significant role of translational control in regulation of gene expression in mature chloroplasts (for review, see Gillham et al., 1994; Mayfield et al., 1995; Rochaix, 1996).

What might be the special requirements in the mature chloroplast that made translational control a favored mechanism for regulating gene expression? Translational regulation provides a capacity to rapidly induce or reduce massive levels of protein synthesis from an existing pool of transcripts. In contrast, transcriptional regulation is relatively slow to induce protein synthesis and has to be accompanied by mRNA instability to diminish translation in a short time. Is there a requirement for rapid adjustment of gene expression in mature chloroplasts? Light, the primary energy source of plants, is captured in the chloroplast and converted into chemical energy by a set of protein complexes localized in the thylakoid membranes. The chemical energy produced by the thylakoid protein complexes in the form of ATP and reducing power drives the different stromal reactions, such as fixation of carbon, fixation of nitrogen, biosynthesis of amino acids, and biosynthesis of lipids. Consequently, the availability and intensity of light have pronounced effects on chloroplast metabolism and hence on chloroplast gene expression. In addition, light absorbance and conversion into beneficial chemical energy is accompanied by deleterious side effects that result in protein turnover that parallels light intensity (Barber and Anderson, 1992). Light intensity and availability are not controlled by plants, and fluctuate during the course of the day and from day to day. Therefore, dynamic adjustment of gene expression is required.

The chloroplast, which probably evolved from endosymbiotic photosynthetic cyanobacteria (Whatley, 1993), lost most of its self-encoded functions to the nuclear genome during its evolution and underwent a shift from the mostly, but not entirely (Gold, 1988), transcriptional regulation observed in prokaryotes. The mitochondrion, the other semiautonomous prokaryotic-like organelle, shows evolutionary parallelism in its evolution to the chloroplast, including the transfer of many of its self-encoded functions to the nucleus and an increase in translationally regulated gene expression (Fox, 1996). It is possible that the similar situation of the chloroplast and mitochondrion, as semiautonomous endosymbiotic organelles, dictates this common path of evolution.

REGULATION OF TRANSCRIPT TRANSLATABILITY

A transcript may have to undergo both nonreversible modifications, such as editing, splicing, and processing, and reversible modifications, such as association with specific proteins, to be competent for translation. Regulation of transcript translatability by irreversible modifications may allow rapid induction of protein synthesis but should be coupled with decreased mRNA stability to enable rapid decline of translation. Conversely, regulation by reversible modifications can be used for rapid and massive changes of both induction and repression of translation. There is evidence for both types of modifications in the chloroplast. Editing of an ACG codon to the initiator codon AUG creates translatable \( psbL \) mRNA in tobacco \((Nicotiana tabacum L.)\) plastids. This editing activity appears to be mediated by a \( psbL \)-specific factor that is present in limiting amounts, suggesting that its activity controls the translatability of \( psbL \) mRNA (Chaudhuri et al., 1995). Processing of \( petD \) mRNA in maize requires a specific factor \( crpI \) whose absence results in lack of the monocistronic form of \( petD \) and a large decrease in translation of \( petD \) mRNA. This implies that \( crpI \) may affect the translatability of \( petD \) by controlling its processing (Barkan et al., 1994). Alternative processing of the 5'-UTR of \( rbcl \) transcript in barley was proposed to modulate the translatability of \( rbcl \) in response to exogenous methyl jasmonate (Reinbothe et al., 1993).

Regulation of message translatability by specific protein-mRNA interactions has been implicated mostly by genetic analysis in the unicellular green algae \( Chlamydomonas reinhardtii \). Several nuclear mutations in \( C. reinhardtii \) and \( Arabidopsis thaliana \) have identified gene products that are required for the translation of chloroplast mRNAs (Meurer et
ottic translation (Table I). In eukaryotes the m7G-capped mRNA differs significantly between prokaryotic and eukaryotic translation. The efficiency of the recognition by the initiation complex. The scanning for the correct initiator codon. The sequence surrounding the initiator codon determines the efficiency of the recognition by the initiation complex. The scanning process is dependent on the initiation complex.

THE ROLE OF THE 5'-UTR IN INITIATION OF TRANSLATION IN PROKARYOTES AND EUKARYOTES

Ribosomes must recognize and bind the initiator codon of the correct ORF to initiate translation. Several steps preceding and facilitating this event begin with the binding of the translation-initiation complex containing the small ribosomal subunit and several initiation factors to the 5'-UTR of a specific chloroplast mRNA and activate the translation of the downstream ORF (Fig. 1). As seen above, many of the nonreversible and reversible modifications that affect transcript translatability affect or interact with the 5'-UTR of the transcript. The biological role of the 5'-UTR has to be discussed to better understand the mechanisms by which translation is regulated.

INITIATION OF TRANSLATION IN THE CHLOROPLAST

The elucidation of the mechanism of initiation of translation in the chloroplast is crucial for an understanding of regulation of protein synthesis in the chloroplast. Unfortunately, the information provided up to now has been inconclusive and may support the hypothesis that there is more than one pathway for initiating translation functions in the chloroplast. The major constituents of the translation machinery in chloroplasts resemble their prokaryotic counterparts, but several differences can be found as well. Similar to prokaryotes, the chloroplast contains 70S-like ribosomes, chloroplast transcripts are not m7G-capped at their 5' end, and chloroplast transcripts can be transcribed as polycistronic units. However, a close inspection of chloroplast ribosomes has suggested that they have deviated from the bacterial ribosomes (Harris et al., 1994). It is conceivable that the chloroplast ribosomes may have undergone these changes to accommodate the translational regulatory pathways that are unique to chloroplasts.

What is the binding site of the chloroplast translation initiation complex? Studies of expression of polycistronic transcripts have indicated that internal ORFs can be translated in polycistronic transcripts (Barkan, 1988), suggesting that, similar to prokaryotes, the chloroplast translation initiation complex binds internally to the chloroplast transcript and not to its 5' terminus. Analysis of the 5'-UTR of chloroplast mRNAs has demonstrated that only about 40% of the chloroplast mRNAs contain SD-like sequences in close proximity to the initiator codon, and that the spacing between the SD-like domains and the initiator codon is not as strict as in the prokaryotes (Harris et al., 1994). Determination of the functionality of the SD-like domains by site-directed mutations analysis has yielded inconclusive

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Figure 1. Model of function of nuclear-encoded factors involved in gene-specific translational regulation in the chloroplast.
results. Deletion of the SD-like domain in psbA mRNA in C. reinhardtii diminished translation and accumulation of the D1 protein, concomitant with decreased stability of psbA mRNA in vivo (Mayfield et al., 1994). In contrast, base changes of this site in a chimeric mRNA composed of plast ribosomes. Conversely, in system. However, mutations of additional sites that do not affect its translation in the chloroplast in vitro translation results. Deletion of the SD-like domain in plast ribosomes. Conversely, in a message that does not contain an SD-like sequence located in close proximity to the initiator codon, their patterns of association differed (Kim and Mullet, 1994). These results suggest that binding of the initiation complex is mediated by the SD-like sequence in messages in which the SD-like sequence is located in close proximity to the initiator codon, and that additional factors unique to the chloroplast are required in the remainder of chloroplast messages. The identity of the cis-elements and trans-acting factors required for binding of the initiation complex to chloroplast mRNAs that do not contain an SD-like sequence in close proximity to the initiator codon is yet to be determined. Finding of additional 5'-UTR cis-elements that are required for translation may help to identify these alternative binding sites for the chloroplast initiation complex and elucidate their mode of function.

**ALTERNATIVE TRANSLATIONAL cis-ELEMENTS IN THE 5'-UTR OF CHLOROPLAST mRNAs**

The absence of SD-RBS in the large fraction of chloroplast mRNAs suggests that binding of the initiation complex to the mRNA is mediated by an alternative type of cap-independent-binding domain. Since sequence complementarity with a small ribosomal subunit may not be required for binding, the binding of the initiation complex could be facilitated by proteins interacting with this domain. The finding of upstream 5'-UTR cis-elements that are required for translation may identify these putative initiation complex binding domains. In vivo expression of truncated forms of the 363-nucleotide-long 5'-UTR of petD in C. reinhardtii has demonstrated the presence of two such domains. One is located from 210 to 161 and the other from 51 to 33 nucleotides upstream from the initiator codon (Sakamoto et al., 1994). Mutation analysis of the 5'-UTR of tobacco psbA mRNA fused to a reporter ORF has identified an additional translationally active cis-element that does not resemble SD-RBS and interacts with specific proteins (Hirose and Sugiura, 1996). Genetic analyses in C. reinhardtii have identified a 97-nucleotide region located 236 nucleotides upstream of the initiator codon of psbC mRNA (Zerges et al., 1997) and a 130-nucleotide region located 12 nucleotides upstream of psbA mRNA (Stampacchia et al., 1997); both have the capacity to fold into stem-loop structures. The interaction of each of these cis-elements with its mRNA-specific nuclear-encoded factor is crucial for mRNA translation. This is independent of which ORF is fused downstream of the 5'-UTR, suggesting that initiation is the checkpoint for regulating translation.

Mutations predicted to decrease structural stability of the 5'-UTR cis-elements of psbC and psaB mRNAs increased the translatability of the mRNA in the absence of a functional, interacting, nuclear-encoded factor (Stampacchia et al., 1997; Zerges et al., 1997). Mutations that increased the structural stability of the 97-nucleotide cis-element decreased the level of translation of psbC mRNA. Taken together, these results suggest that nuclear-encoded proteins interact with the alternative cis-elements in an mRNA-specific manner to control the initiation of translation of the downstream ORF, and that the structure of these cis-elements is crucial for their translational regulatory function. Strengthening this hypothesis is the finding that both the association of chloroplast psbA mRNA with ribosomes and its association with a 5'-UTR-binding protein complex are aberrant in the presence of the nuclear mutation F35, which reduces translation of psbA mRNA (Yohn et al., 1996).
5'-UTR-BINDING PROTEINS

The expanding number of nuclear mutations, each responsible for the lack of translation of a unique chloroplast mRNA, identifies several different factors, with each regulating the synthesis of one protein in the chloroplast. A simple model would predict that one nuclear-encoded protein, unique to one chloroplast mRNA, is interacting with the 5'-UTR and regulating the translation of downstream ORF (Fig. 1). However, genetic studies of chloroplasts and mitochondria have suggested that more than one protein is required for the regulation of a single organellar mRNA (Zerges et al., 1997). Comparisons of 5'-UTR interacting proteins of rps12, rbcL, and atpB have detected at least seven binding proteins of 15, 36, 38, 47, 56, 62, and 81 kDa. The 38-, 47-, and 81-kDa proteins were shown to associate with all tested 5'-UTRs, and multiple forms of the 47- and 81-kDa proteins have been identified (Hauser et al., 1996). These results, obtained by UV cross-linking, which mostly detects proteins that have direct contact with the RNA, show that several proteins may associate with the 5'-UTR of chloroplast mRNAs. This requires the determination of the significance of each protein to translational regulation. The level of the 36-kDa protein was diminished in cells that preferentially translate chloroplast-encoded ribosomal proteins, suggesting that it may be required for translation of a class of mRNAs encoding photosynthetic proteins (Hauser et al., 1996). Binding of a 46-kDa protein to the 5'-UTR of psbC was detected only in cells carrying the nuclear mutation F64, which is responsible for the translational arrest of the chloroplast psbC mRNA. This implies that the binding of 46-kDa protein may inhibit translation of psbC (Zerges and Rochaix, 1994).

RNA-affinity chromatography, capable of isolating both proteins that bind directly to the RNA and proteins that are associated through protein-protein interactions, was used to identify a set of four proteins (38, 47, 55, and 60 kDa) specific to the 5'-UTR of psbA. The binding activity of the 38- and 47-kDa proteins to the RNA was lower in protein lysates isolated from dark-grown cells than from light-grown cells, yet the amount of the 47-kDa protein did not vary between light and dark (Danon and Mayfield, 1991). Lower binding activity of these proteins was also detected in cells bearing the nuclear mutation F35, which causes diminished translation of psbA (Yohn et al., 1996), as well as in cells aberrant in photosynthetic electron transport (Danon and Mayfield, 1994a). These results and the modulation of RNA-binding activity of these proteins in vitro in response to redox and ADP concentration indicate that the capacity to respond to chloroplast-generated signals is intrinsic to the psbA 5'-UTR-binding proteins (Danon and Mayfield, 1994a, 1994b). These results suggest that variation in illumination controls the activity of psbA 5'-UTR-binding proteins, which in turn controls the translation of psbA mRNA. The capacity of these proteins to alter their RNA-binding activity with no apparent change in their amount in vivo and in vitro implies that these proteins may function semiautomously, altering chloroplast gene expression by responding locally to chloroplast-generated signals.

**MODELS OF FUNCTION OF THE ALTERNATIVE TRANSLATIONAL cis-ELEMENTS AND INTERACTING PROTEINS**

The proposed function of the alternative translational cis-elements is summarized in Figure 2. Two major steps must be facilitated for initiation to occur. The first (Fig. 2A) is the association of the initiation complex with the 5'-UTR, and the second (Fig. 2B) is the recognition of the correct initiator codon. The 5'-UTR cis-element can fold into a stem-loop structure that is required for the translational function of the cis-element. A protein complex comprised of nuclear-encoded proteins interacts specifically with its unique cis-element. The association of the protein complex and the RNA is controlled by modulating its RNA-binding activity, which is sensitive to chloroplast-generated signals, such as redox and ADP concentrations. The binding of the protein complex to the cis-element mediates the association of the mRNA and the small ribosomal subunit, and regul
lates initiation of translation. Because of the relaxed spacing between the alternative cis-elements and the initiator codon, it is not clear how binding of the initiation complex to the putative RBSs may facilitate initiator codon recognition.

Two main alternatives can be predicted. The first is B1 in Figure 2. Recognition of the initiator codon is mediated in a manner similar to euukaryotic initiation of translation by scanning of the 5'-UTR by the initiation complex and recognition of these sequences surrounding the correct initiator codon. This alternative predicts that translation initiation should be sensitive to the insertion of short ORFs upstream of the correct initiator codon. The step of scanning in this model requires helicase activity, which has yet to be identified in the chloroplast. The second is B2 in Figure 2. According to the second alternative, the structure of the 5'-UTR adjongs the upstream cis-elements and the initiator codon such that the association of the small ribosomal subunit with the translational regulatory protein complex, bound to the upstream cis-element, leads to the recognition of the 5'-UTR. Because of the primary role of the structure of the 5'-UTR, this model predicts that initiation of translation will be highly sensitive to deletions in the 5'-UTR.

**PERSPECTIVES**

Regulation of chloroplast gene expression was predicted to mimic regulation in prokaryotes because of its presumed prokaryotic origin. However, it is becoming clear that during chloroplast evolution it has undergone major changes in its genome composition and regulation. One of the consequences of the transfer of the large fraction of the chloroplast genome to the nucleus is the requirement for coordination of the nuclear and chloroplast gene expression. This coordination is especially crucial for regulating the amounts of chloroplast protein complexes that are comprised of both nuclear- and chloroplast-encoded subunits. The coordination of nucleus and chloroplast gene expression may require a communication capacity (signal transduction) that would mirror the demand for alteration in gene expression in one compartment to the other, and regulatory factors to control the expression of the other gene compartment. The nuclear-encoded factors, identified by genetic analysis as being required for regulation of chloroplast gene expression, are ideal candidates for factors participating in the nuclear control over the chloroplast genome. The nature of these regulatory factors is particularly intriguing, as they interface between the euukaryotic nuclear genome and the prokaryotic-like chloroplast genome. It is becoming apparent that regulation of chloroplast gene expression has deviated from the mostly transcriptional regulated gene expression in prokaryotes. However, the mechanism of the particular posttranscriptional steps of chloroplast gene expression such as initiation of translation are far from being understood. The elucidation of the nature and mode of function of the nuclear encoded regulatory factors should help to unravel both the mechanisms of postranscriptional regulation in the chloroplast and the pathways by which the nuclear genome controls chloroplast gene expression.

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**LITERATURE CITED**


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Chen X, Kindle K, Stern D (1995) The initiation codon determines the efficiency but not the site of initiation and that insertion of a stop codon immediately upstream of the initiator codon did not compromise translation (Chen et al., 1995). According to this model, regulation of initiation could be mediated by either the activity of the translational regulatory protein complex or by changes in the structure of the 5'-UTR. Because of the primary role of the structure of the 5'-UTR, this model predicts that initiation of translation will be highly sensitive to deletions in the 5'-UTR.