Nuclear–chloroplast signalling

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Chloroplast development and function relies both on structural and on regulatory factors encoded within the nucleus. Recent work has lead to the identification of several nuclear encoded genes that participate in a wide array of chloroplast functions. Characterization of these genes has increased our understanding of the signalling between these two compartments. Accumulating evidence shows that a variety of molecular mechanisms are used for intercompartmental communication and for regulating co-ordinated chloroplast protein expression.

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Abbreviations
IR inverted repeats
NEP nuclear encoded RNA polymerase
PEP plastid encoded RNA polymerase
SD Shine–Dalgarno
UTR untranslated region

Introduction

Chloroplasts are thought to have arisen by endocytobiosis of a photosynthetic unicellular prokaryote into a eukaryotic host [1]. Integration of the endosymbiont genome with that of the host involved translocation of genes from the plastid to the host nucleus. This gene transfer required the emergence of new regulatory interactions in order to maintain a co-ordinate expression of proteins functioning within the plastid. The amount of gene transfer into the nucleus varies among different species, suggesting that gene transfer from the plastid to the nucleus is an ongoing process. It is not obvious why certain genes are maintained within the plastid genome, but one possibility might be to avoid transporting highly hydrophobic proteins, containing multiple transmembrane helices, across the thylakoid envelope membranes [2]. Functional harmony between the nucleus and the chloroplast is maintained by a number of regulatory activities that respond to stimuli and signals perceived or generated in one or the other compartments.

The photosynthetic apparatus constitutes the major protein component of the chloroplast. Of the several hundred proteins participating in photosynthesis, less than one hundred are encoded by the chloroplast genome — the remaining are nuclear encoded. Along with functional roles in photosynthetic complexes, nuclear encoded proteins are involved in regulating many chloroplast processes including transcription, mRNA processing, translation, protein targeting and protein turnover. Work during the past year has added new insights to the roles played by nuclear encoded factors in controlling chloroplast functions. This review will focus on the processes of chloroplast development and differentiation, and on plastid protein expression and targeting, highlighting several regulatory aspects of the interaction between the nucleus and chloroplast involved in these key processes.

Plastid gene expression

Expression of chloroplast proteins is primarily regulated post-transcriptionally. A number of nuclear encoded factors have been isolated that are required for plastid gene expression. We will discuss the identification of specific genes and the roles they play in plastid gene expression.

Transcriptional activation of plastid genes

Transcription in the chloroplast resembles that of prokaryotes, particularly in the use of consensus promoter elements. Plastid genomes are transcribed by two different RNA polymerases: a nuclear encoded RNA polymerase (NEP) that transcribes genes required for transcription and translation, and a plastid encoded RNA polymerase (PEP), that transcribes the photosynthetic genes [3]. Use of ribosome deficient mutants, such as *iojap* [4] and *in vitro* transcription systems [5], enabled systematic dissection of the NEP promoters and demonstrated that a single short sequence element (a CRT motif) is enough for transcriptional initiation. Transcription from PEP promoter requires both a –10 and a –35 element, which are similar to elements found in the same position of bacterial genes [6].

Transcription of both polymerases (PEP and NEP) may be enhanced by σ-like factors, and by gene specific DNA binding proteins acting as activators or repressors. At least two nuclear encoded σ factors have been identified for PEP in the red alga *Cyanidium* (*sigB* and *sigC* [7]) and in *Zea mays* (*sig1* and *sig2* [8•]). A putative σ-like factor, *Os-sigA* was isolated from *Oryza sativa* [9•], and shown to increase in abundance during light growth suggesting that this inducible σ factor may contribute to light dependent transcriptional regulation of plastid genes. Although the correlation between transcription of chloroplast genes and protein expression is generally poor, a recently identified nuclear gene encoding the plastid ribosomal protein (RPL4) has been shown to co-purify with plastid RNA polymerase and transcription factor CDF2, suggesting a possible role in co-ordinating plastid transcription and translation [10].

RNA processing

Many chloroplast genes are transcribed as polycistrionic mRNAs and thus require intra and intermolecular splicing and processing to form mature transcripts. Introns belonging to both group I, where the splicing is initiated

by an activated G residue that attacks and breaks the phosphodiester bond at the 5′ splice site, and group II, where the splicing is initiated by a specially reactive A residue in the intron sequence that attacks the 5′ splice site, forming a lariat intermediate, have been characterized in plastid genes. Although little is known about the splicing machinery of the plastid introns, nuclear mutants such as *ac20*, *crs1* and *crs2* [11,12] have indicated an essential, or at least a direct, role for nuclear factors in mRNA splicing. It has been shown that RNA editing is involved in producing a functional mRNA. The high specificity of RNA editing relies on *cis* acting and *trans* acting factors, some of which are nuclear encoded. Inhibition of chloroplast translation blocks RNA editing, suggesting that chloroplast translation products serve as auxiliary factors, perhaps mediating accessibility of the substrate site during editing [13].

The 5′ untranslated region (UTR) of psbA has been shown to be processed [14•] by the removal of 54 nucleotides including a stem-loop structure. Chloroplast or nuclear mutations blocking psbA translation reveal a correlation between processing and ribosome association. Loss of the ribosome binding site by mutation in the 5′ UTR of the message, or mutations blocking ribosome association both result in the absence of mRNA processing and translation of the *psbA* mRNA. These data suggest that 5′ mRNA processing of this mRNA may be a consequence of translation but not necessarily a pre-requisite for it.

Shine–Dalgarno sequences

Prokaryotic-like Shine–Dalgarno (SD) sequences have been identified in a number of chloroplast transcripts, but not in all. The idea that SD sequences are required for translation has, therefore, been controversial. The 5′ UTR of many chloroplast messages contain putative SD sequences, but few of these have the prokaryotic location in relation to the initiation codon, which must be within ten nucleotides. Fargo *et al.* [15•] analyzed the function of potential SD sequences of four transcripts — *atpB*, *atpE*, *rps4* and *rps7* — by replacement mutagenesis in *Chlamydomonas* and *E. coli* and showed no effect on the expression of these genes, leading them to propose a SD independent mechanism for plastid translation. However, studies on SD-like sequences of tobacco *rps14* [16] and *Chlamydomonas*, *psbA* mRNA [14•] show a requirement of SD sequences for translation. In *Chlamydomonas*, the putative SD sequence in the *psbA* mRNA is 27 nucleotides upstream of the initiation codon, and deletion of this sequence results in a loss of ribosome association and also in a decrease in message stability — both strongly suggestive of an authentic SD sequence. The structural changes wrought on the 5′ UTR by manipulation of any sequence, including the SD sequences, makes interpretation of the above results complicated. The existing evidence supports the presence of SD sequences, with non-prokaryotic spacing, for some chloroplast messages, in others translation initiation may result from mechanism independent of SD sequences. However, these results present evidence for translation initiation of some chloroplast messages from SD sequences with non-prokaryotic spacing, while others show translation initiation by mechanisms independent of SD sequences.

mRNA stability

Many plastid RNAs have inverted repeats (IR) in the 3′ UTR that are capable of forming hairpin structures required for correct 3′ end formation [17]. In *Chlamydomonas*, deletion of the IR in the 3′ UTR of the *atpB* gene reduces accumulation of the transcript [18•], suggesting that the IR plays some role in stabilizing the transcripts [18•]. Nuclear encoded proteins binding to this 3′ UTR have been identified. Although their precise role is yet to be elucidated, the evidence provided by Rott *et al.* [18•] suggests that the binding of these proteins to the 3′ UTR may influence the efficiency of endonucleolytic cleavage or the exonucleolytic trimming in proper 3′ end formation. Some of these 3′ UTR binding proteins also appear to interact with the 5′ UTR or 5′ UTR binding proteins to control RNA degradation [19], suggesting a connection between RNA processing, stability and degradation [2,20]. Several nuclear mutants have been identified that affect stability of specific RNA transcripts. A nuclear mutant of *Chlamydomonas reinhardtii*, *mcd-1*, shows degradation of *petD* mRNA by a 5′ to 3′ exoribonuclease activity, providing evidence that the nuclear gene product, MCD, protects RNA from degradation by interacting with the 5′ UTR [21•]. MCD may provide a specific mechanism protecting the mRNA or it may have another primary role in mRNA translation or processing. It is not yet clear which of these roles MCD plays in influencing the stability of the transcript.

Translation

Many factors required for translation of chloroplast mRNAs are nuclear encoded. Genetic analysis of *Chlamydomonas* has revealed a class of nuclear genes that are required for translation of specific chloroplast mRNAs. Many of these factors directly interact with the 5′ UTR of specific mRNAs. The 5′ UTRs of chloroplast mRNAs tend to be A/U-rich and often contain predicted stem-loop structures. Sequences upstream of the chloroplast initiation codon have been proposed to affect translation, containing binding sites for activators and repressors [22].

Studies on photosynthetic mutants of *Chlamydomonas* suggest that some of the nuclear encoded factors are message specific while others may be class specific. Using complementation rescue the gene disrupted in the mutant *ac115* has been characterized [23], and shown to encode a novel protein that has been proposed to play a role in stabilizing intermediates of the D2 translation product. Another novel nuclear gene, *crp1*, the disruption of which blocks translation and mRNA processing of the *petA* mRNAs in maize has been cloned [24•]. This protein shows similarity to yeast proteins involved in translation of mitochondrial mRNAs. The

yeast protein is required for association of the mRNA with membrane bound polysomes. Many proteins that bind to the 5′ UTRs of chloroplast transcripts, like *psbA*, *psbC* and *psbD* have been shown to be at least partially membrane associated, indicating a potential role in the proper localization of these messages to the thylakoid membrane. Of the set of proteins that associate with the *psbC* mRNA, the 46 kDa protein has been shown to bind an A/U rich region in the 5′ UTR. The binding of this 46 kDa protein is light dependent and can be inhibited by ADP [25].

A complex of proteins has been identified that binds to the 5′ UTR of the *psbA* mRNA. Of these proteins (RB60, RB55, RB47 and RB38), the 47 kDa protein shows homology to poly(A) binding proteins (PABP) [26•]. Although chloroplast transcripts generally lack poly (A) tails, the *psbA* mRNA contains an A-rich 5′ UTR that has been identified as the binding site of the chloroplast poly(A) binding protein (cPABP). Identification of two nuclear mutants deficient in *psbA* translation that also lack the PABP (RB47) suggests that this protein is necessary for translation of the *psbA* transcript [27•]. Modulation of complex binding to the *psbA* mRNA mediated through cPABP binding shows that ADP-dependent kinase or oxidizing conditions can abolish binding of the complex. Redox potential generated by photosynthetic activity has been proposed to regulate this complex binding through a chloroplast localized protein disulfide isomerase (cPDI). On the basis of the above data, we suggest a model where chloroplast redox potential regulates binding of message-specific translational activator proteins, including RB47, using a redox potential generated by photosynthesis and transduced through the cPDI.

Autoregulation of cytochrome *f* translation has been shown to occur by an interaction between the 5′ UTR of the *petA* mRNA and the carboxy-terminal domain of the unassembled protein [28••]. Cytochrome *f* that is not incorporated into the cytochrome *b* 6/*f* complex attenuates translation of *petA* mRNA by interacting with the 5′ UTR, directly or indirectly. Such attenuation of subunits of a protein complex in the absence of other subunits of the complex is described as control by epistasy of synthesis (CES). The properties of CES have been suggested as a way to regulate assembly of multi-subunit complexes within the chloroplast [28••]. While a mechanism for translational regulation has not yet completely emerged, the data presented above suggest that this regulation occurs by the interaction of nuclear encoded factors with the 5′ UTR of chloroplast mRNAs. These translation initiation factors in many cases appear to be mRNA specific, although the underlying mechanism by which they activate translation may be common to many chloroplast mRNAs.

Protein targeting to the chloroplast

Chloroplast proteins encoded by the nucleus are synthesized with an amino-terminal targeting sequence, rich in hydrophobic residues, that facilitates transport of these proteins into the chloroplast and then to their site of function. All the chloroplast proteins enter plastids through the general import pathway [29]. Several components that constitute the translocation machinery have been identified [30]. Toc 33, a small GTP binding protein is the latest of a family of outer envelope (Toc) proteins identified. *Arabidopsis* mutants with defective Toc33 show defective protein import [31•]. Tic 22, a peripheral membrane protein belonging to the family of inner envelope (Tic) proteins, has been proposed to connect inner and outer membrane protein complexes [32]. Proteins destined for the thylakoid membranes are transported into or across the thylakoid membranes by different pathways: Δ pH, thylakoid secretory (Sec) pathway, chloroplast signal recognition particle (cpSRP), and by spontaneous insertion [33,34]. Components involved in these different mechanisms are being characterized.

The soluble protein cpSecA is involved in translocation of a number of nuclear encoded lumenal proteins through the thylakoid membrane. Analysis of maize mutants, defective in cpSecY, a chloroplast localized component of the translocon, shows significant reduction in thylakoid membrane accumulation [35]. The severe reduction in chloroplast translation in these mutants suggests that cpSecY function goes beyond its involvement with the SecA dependent protein translocation. Roy and Barkan [35] proposed that cpSecY plays a role in the interaction between chloroplast translation and membrane biogenesis.

The chloroplast homolog of SRP, cpSRP54, forms a transit complex with cpSRP43 and LHCP. The formation of this complex is essential for translocation and integration of LHCP into the thylakoid membrane [36•,37,38]. Nilsson *et al.* [39•] show that cpSRP54 interacts tightly and specifically with ribosome nascent chain complexes of D1 protein, implicating a role for cpSRP54 in D1 biogenesis. An *Arabidopsis* mutant, *chaos*, has been identified as a mutation in the gene encoding cpSRP43. The *chaos* mutant phenotype differs distinctly from mutants of cpSRP54, suggesting that the functions of the two proteins do not entirely overlap [40•]. Pilgrim *et al.* [41] have shown that a chloroplast homolog of SRP54 (cpSRP54) plays an important role in chloroplast biogenesis [41]. Mutants with reduced levels of cpSRP54 display delayed maturation of proplastids and aberrant light induced movement. Analysis of these mutants suggests a role for cpSRP54 in the biogenesis of several proteins, co-translationally and post-translationally. *Arabidopsis* mutants that lack cpSRP54 have been shown to have defective plastid biogenesis [41], whereas *chaos* shows only defects in LHCP targeting. FtsY, a bacterial homologue of the SRP receptor protein has been identified from *Arabidopsis*, and shown to play a role in the SRP pathway [42].

Maize mutants of *hcf 106* are defective in the Δ pH pathway. *Hcf106* encodes a membrane protein that may play a

critical role in this pathway [43]. Another gene, *tha4*, that has similarity to *hcf106* in topology and structure has been identified from maize [35], suggesting these two gene products may function in a common pathway. Whether these different translocation pathways converge is a question that awaits additional evidence. The necessity for different pathways for protein targeting into thylakoid membranes may be specified by the nature and sub organelle location of the transported proteins.

Nuclear control of chloroplast biogenesis

In addition to regulating chloroplast specific processes, nuclear genes have also been shown to co-ordinate chloroplast and nuclear gene expression. In many plants, differentiation of proplastids or etioplasts into chloroplasts occurs only upon perception of a light signal. This light induced development involves rapid accumulation of chlorophyll, photosynthetic membranes, and the associated photosynthetic proteins. This transition is mediated by two classes of photoreceptors, phytochromes and cryptochromes. Mechanisms by which plants perceive and transduce light signals have been extensively studied (reviewed in [44]). Mutational analysis has lead to the identification of nuclear encoded regulatory elements that affect biogenesis of the chloroplast in a variety of ways. The DET, COP (both *Arabidopsis*) and RegA (*Volvox*) proteins have been shown to repress transcription of genes required for plastid biogenesis [45–47]. Another class of mutants has been described (CAB underexpression or *cue*, reviewed in [48••]) that show defects in chloroplast development and light induction of nuclear encoded *cab* mRNA transcription, providing additional evidence that photoreceptor signal transduction pathways and plastid signalling pathways share common factors in an intricate network.

Genetic analysis in *Arabidopsis* has identified a number of genes which influence chloroplast development. These mutants include those with defects in differentiation and greening (*dag*), chloroplast and leaf development (*dcl*) and chlorophyll accumulation (pale cress, *pac*). Each of these mutants suggests that the nucleus can sense the physiological status of the developing plastid and respond accordingly. PAC is a nuclear encoded protein involved in processing of chloroplast mRNAs [49•]. Nuclear gene expression is unaffected in *pac* mutants, whereas chloroplast transcripts such as *psbA-D, petB*, and several genes from the *ndh* cluster, are dramatically reduced in abundance indicating that PAC may function in the recognition and processing of maturation signals found in chloroplast mRNAs. A number of mutants with defects in pigment biosynthesis have been shown to have pleiotropic effects on chloroplast biogenesis [50]. Disruption of the *im* gene in *Arabidopsis* results in a variegation mutant (*IMMUTANS* [51•]), which is similar to the previously characterized mutants *iojap* and *albostrians*. The IM protein product has an alternative oxidase activity that indicates a role as a phytoene desaturase, an enzyme critical for preventing photo-oxidative damage during early chloroplast biogenesis.

Chloroplast effects on nuclear gene expression

Expression of a number of nuclear genes is influenced by the state of plastid development. Studies on *IMMUTANS* have shown that disruption of carotenoid biosynthesis leads to photo-oxidation, which in turn inhibits expression of a set of nuclear encoded photosynthesis related proteins [52•]. This effect is most noticeable on the expression of the light harvesting chlorophyll *a*/*b* (CAB) proteins. Although such plastid effects on nuclear gene expression are well established, the specifics of this interaction have not been elucidated. The accepted opinion is that this regulation is at least partly due to changes in nuclear gene transcription. There is evidence for both positive and negative plastid signalling to the nucleus, but the nature of the signal remains elusive. In *Chlamydomonas*, at least some of the properties of the plastid signal can be achieved by the addition of chlorophyll precursors to cells lacking chlorophyll biosynthesis [53]. The plastid signals in higher plants have been speculated to be metabolites, secondary messengers or macromolecules. Although the nature of the signal is not known, analysis of *cue* (described in the previous section) mutants lead to the proposal that the plastid signal follows at least part of the pathway through which phytochrome mediated signalling operates [48••].

Conclusions

Regulation of gene expression in the chloroplast involves a complex coordination between the nucleus and the chloroplast. The available data suggest that communication between these two compartments occurs primarily to regulate photosynthetic and photomorphogenetic activities. The understanding of nuclear chloroplast signalling is still in its early stages. The discoveries of the past year have established that a large number of nuclear factors are required for expression of chloroplast genes and that chloroplast biogenesis and function can have profound effects on nuclear gene expression and plant development.

Thus plastid development has an effect on nuclear gene expression and nuclear genes are required for plastid development and functioning. It appears that the nuclear genome sets a program of development, where expression of specific nuclear genes is required for the formation of a fully functional plastid. This overall program is fine-tuned by environmental signals and by the interactions of the developing plastid with the nucleocytoplasmic compartment. Questions regarding the identification of the signal from the plastid to the nucleus, molecular mechanisms used to regulate gene expression in the plastid, and signal transduction pathways that influence photomorphogenetic development, await to be addressed. The progress in identification and characterization of nuclear mutants and novel genes involved in these various processes suggests that some of these answers, however, may be soon at hand.

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A homologous translation system has been used to investigate the mechanism of targeting and assembly for chloroplast D1 protein. Cross linking studies showed that cpSRP54 interacts only when D1 nascent chain is still attached to the ribosome, implicating a role for cpSRP54 in D1 biogenesis.

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Transposon tagging was used to isolate a recessive mutation in a protein designated as cpSRP43 involved in the SRP pathway. The function of cpSRP43 is distinct from cpSRP54 as mutants deficient in either of these components show different phenotypes. The function of cpSRP43 has been proposed to be limited to thylakoid membrane targeting, whereas cpSRP54 has been shown to be involved in additional processes along with protein targeting to the thylakoid membranes.

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