ANTIOXIDANTS & REDOX SIGNALING
Volume 5, Number 1, 2003
© Mary Ann Liebert, Inc.

Forum Review

Redox Control of Posttranscriptional Processes in the Chloroplast

DWIGHT BARNES and STEPHEN P. MAYFIELD

ABSTRACT

The ability to couple photosynthetic electron transport and redox poise to plastid gene expression enables plants to respond to environmental conditions and coordinate nuclear and chloroplast activities in order to maintain photosynthetic efficiency. The plastid redox regulatory system serves as a paradigm for understanding redox-regulated gene expression. In this review, we will focus on posttranscriptional events of redox-regulated gene expression in the chloroplast. As redox regulation of enzymatic activities in the chloroplast will be covered in other reviews in this volume, as will discussions on the redox regulation of chloroplast transcription, we will concentrate on the available evidence for redox regulation of chloroplast translation, and mRNA splicing and turnover. *Antioxid. Redox Signal.* 5, 89–94.

INTRODUCTION

CHLOROPLASTS are thought to have arisen by endocytobiosis of a photosynthetic unicellular prokaryote into a eukaryotic host with a subsequent translocation of genes from the plastid to the host nucleus (19). This gene transfer required the emergence of new regulatory interactions to maintain a coordinate expression of proteins functioning in the plastid. Signaling networks that involve a mutual exchange of information between the nucleocytoplasmic and chloroplast compartments have evolved to ensure stoichiometric accumulation of proteins derived from the two genomes. Regulatory connections responding to light stimuli perceived in the chloroplast have been identified that link photosynthetic electron transport to gene expression. The light signal, transduced via the photosynthetic electron transport chain, is used to regulate both transcriptional and posttranscriptional processes within plastids, as well as the expression of plastid-localized proteins encoded in the nucleus. Genetic and biochemical analysis has identified plastoquinone and the ferredoxin/thioredoxin system as redox-active signaling components (2, 6, 12, 26).

POSTTRANSCRIPTIONAL REGULATION OF CHLOROPLAST GENE EXPRESSION

Plant development and gene expression are tied closely to environmental factors, and it has been long known that light is capable of activating the transcription of many nuclear genes encoding photosynthetic proteins (34). Plastid gene expression also responds to light exposure (13), and expression of plastid-encoded photosynthetic proteins is precisely coordinated with that of their nuclear encoded partners (34). Unlike nuclear encoded photosynthetic proteins, many chloroplast mRNAs accumulate to relatively high levels in the dark, and do not show the dramatic change in levels following light exposure that is observed for many cytoplasmic mRNAs (18). These observations implicate posttranscriptional processes as the primary component of light-activated increases in chloroplast protein synthesis, and suggest that translational regulation may be a key component of light-regulated gene expression in plants (28, 33). Thus, the coordinate expression of photosynthetic proteins is primarily achieved by the transcriptional activation of nuclear genes and the translational activation of plastid genes. However, it should be pointed out

Department of Cell Biology, The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA.

that both chloroplast transcription and mRNA stability are enhanced during light-induced greening (8, 10, 21), and the dynamic interactions between transcription, translation, and mRNA stability are only now being studied.

IDENTIFICATION OF mRNA SPECIFIC BINDING PROTEINS ASSOCIATED WITH CHLOROPLAST TRANSLATION

Once translational regulation had been identified as a key component of chloroplast gene expression, genetic and biochemical studies were undertaken to identify the regulatory components of this process. Biochemically, several proteins that bind the 5′ and 3′ untranslated regions (UTRs) of chloroplast mRNAs have been identified (5, 28, 37), and genetic studies have described a number of factors as potential gene-specific translational activators (9, 20, 29, 32, 38, 40, 41). Based on these studies, both RNA elements and corresponding RNA binding proteins were identified as components of plastid translational regulation (39).

A set of mRNA-binding proteins (38, 47, 55, and 60 kDa) that bind the *psb*A 5′ UTR with high affinity and specificity were purified from *C. reinhardtii* chloroplasts using RNA affinity chromatography (1). Binding of these proteins to the *psb*A 5′ UTR correlates with light-regulated translational activation of this message. A similar set of proteins have been described for the *psb*D mRNA from *C. reinhardtii* (25). Subsequently, nuclear mutants have been characterized in which the loss or deficiency of one of these proteins has been shown to affect the translation of the chloroplast *psb*A or *psb*D mRNAs (23, 37, 38).

More recent studies have shown that polyribosome association and translation of the *psb*A mRNA requires binding of the oligomeric protein complex to the 5′ UTR through RB47 (RNA binding protein of 47 kDa). This protein is homologous to poly(A)-binding proteins (38) and contains the classic RNA recognition motifs found in many RNA binding proteins. Poly(A)-binding proteins have been shown to function in translation initiation of cytoplasmic mRNAs, and a similar role has been proposed for RB47 in *psb*A mRNA translation in plastids (38).

TRANSLATIONAL CONTROL BY A REDOX–RESPONSIVE *PSB*A mRNA BINDING COMPLEX

Cells grown in the dark contain relatively high levels of the *psb*A mRNA binding proteins, but have low RNA binding activity for the message. Shifting cells to light results in a dramatic increase in *psb*A mRNA binding activity, with only a slight increase in the levels of the *psb*A mRNA binding proteins (1), suggesting that RNA binding proteins are activated in a light-dependent manner. A similar modulation of RNA binding activity for the *psb*A mRNA could be achieved *in vitro* using purified *psb*A mRNA binding proteins and oxidizing and reducing equivalents (3). Incubation of proteins with the oxidant dithionitrobenzoic acid completely abolished RNA binding. Incubating oxidized proteins with dithiothreitol, a dithiol reductant, restored RNA binding activity (3), and binding activity was also efficiently restored by incubation with reduced thioredoxin. Treatment of oxidized proteins with a monovalent reducing agent (β-mercaptoethanol) did not restore RNA binding activity, suggesting that vicinal dithiols, and not just reducing potential, were required for activation of RNA binding. These data demonstrated redox regulation of *psb*A RNP (RNA binding protein) complex binding *in vitro*, and suggested that thioredoxin might be the *in vivo* redox regulator of *psb*A translation (3). Indeed, a *Chlamydomonas* mutant that lacks photosystem I, the primary reducer of ferredoxin and hence thioredoxin, resulted in reduced *psb*A RNA binding activity and reduced *psb*A translation (3).

The observation that RB60 (RNA binding protein of 60 kDa), a member of the *psb*A mRNA binding complex, was a protein disulfide isomerase (14) suggested that RB60 might function in the redox cascade as a primary regulator of *psb*A RNA binding activity. Using purified recombinant protein, RB60 was shown to be capable of activating the RNA binding activity of RB47 using reducing equivalents (14). Conversely, RB60 was found to be capable of utilizing oxidizing equivalents to oxidize RB47 and cause a loss of RNA binding activity *in vitro* (14). These data suggest that RB60 could act as a switch to turn *psb*A RNA binding activity on or off dependent upon the plastid redox state. The regulatory residues for activation of RNA binding activity in RB47 were found to reside at cysteine residues located in RNA binding domains two and three (7). Changing these cysteine residues to serines resulted in the formation of an RB47 protein that was insensitive to redox regulation, remaining fully active in the presence of oxidizing equivalents (7).

Recently, Trebitsh and Danon (35) have identified a second redox regulatory component of *psb*A translation. Under this second system, which they term priming, the redox state of the plastoquinone pool serves as a signal for translation initiation. The components of this priming system have yet to be identified, but the authors suggest that this system may act through the plastoquinone regulated kinase, the same system identified as a regulator of plastid gene transcription (27). Together, these two systems are proposed to act as modulators of plastid translation, first by a priming signal initiated by the plastoquinone redox state, and then by a thiol signal mediated by the redox state of RB60 and RB47 (36). This direct link between light and the translation of the D1 protein provides the cell with a mechanism to coordinate the various components of photosynthesis to fluctuating light levels and replace photooxidized reaction center proteins at a rate comparable to that of photosynthesis (35).

Translational regulation of the chloroplast large subunit (LSU) of Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) has also been identified. LSU translation follows a unique and opposite pattern in response to light intensity than that observed for the D1 polypeptide. LSU synthesis is down-regulated dramatically upon shifting *C. reinhardtii* cells from low light to higher light intensities, whereas D1 is up-regulated (11). This change in LSU and D1 translation could not be correlated to changes in steady-state levels of their corresponding mRNAs (31). Moreover, the authors observed a sustained increase in the reduction state of bound plastoquinone, a decline in photosynthetic efficiency, and a delay in cell division during high-light growth. Subsequent work suggested that modulation by glutathione redox potential, in response to the levels of reactive oxygen species generated by excess light energy, controls LSU translation (11). The authors speculated that translational

arrest of LSU might result from changes, during photoacclimation, in the sulfhydryl status (oxidation) of one or more components of the *rbc*L 5′ UTR binding complex.

REDOX CONTROL OF TRANSLATION ELONGATION

Evidence is also mounting that other steps in chloroplast gene expression are subjected to photosynthetic redox control, including translation elongation, RNA degradation, and RNA splicing.

Superimposed on modulation of translation initiation by redox-regulated factors is the light-regulated elongation of nascent translation products. In barley, ribosomes were shown to pause at specific sites during elongation of the D1 polypeptide, which was attributed to a requirement for adding components to the newly synthesized apoprotein (15). Subsequent studies by Kuroda *et al.* in isolated pea chloroplasts identified the regulator of pausing as a redox-reactive factor that arises from photosynthetic electron transport (16). Electron transport uncouplers were used to release the proton gradient generated by photosynthetic electron transport, resulting in the inhibition of light-dependent translation elongation (22). From

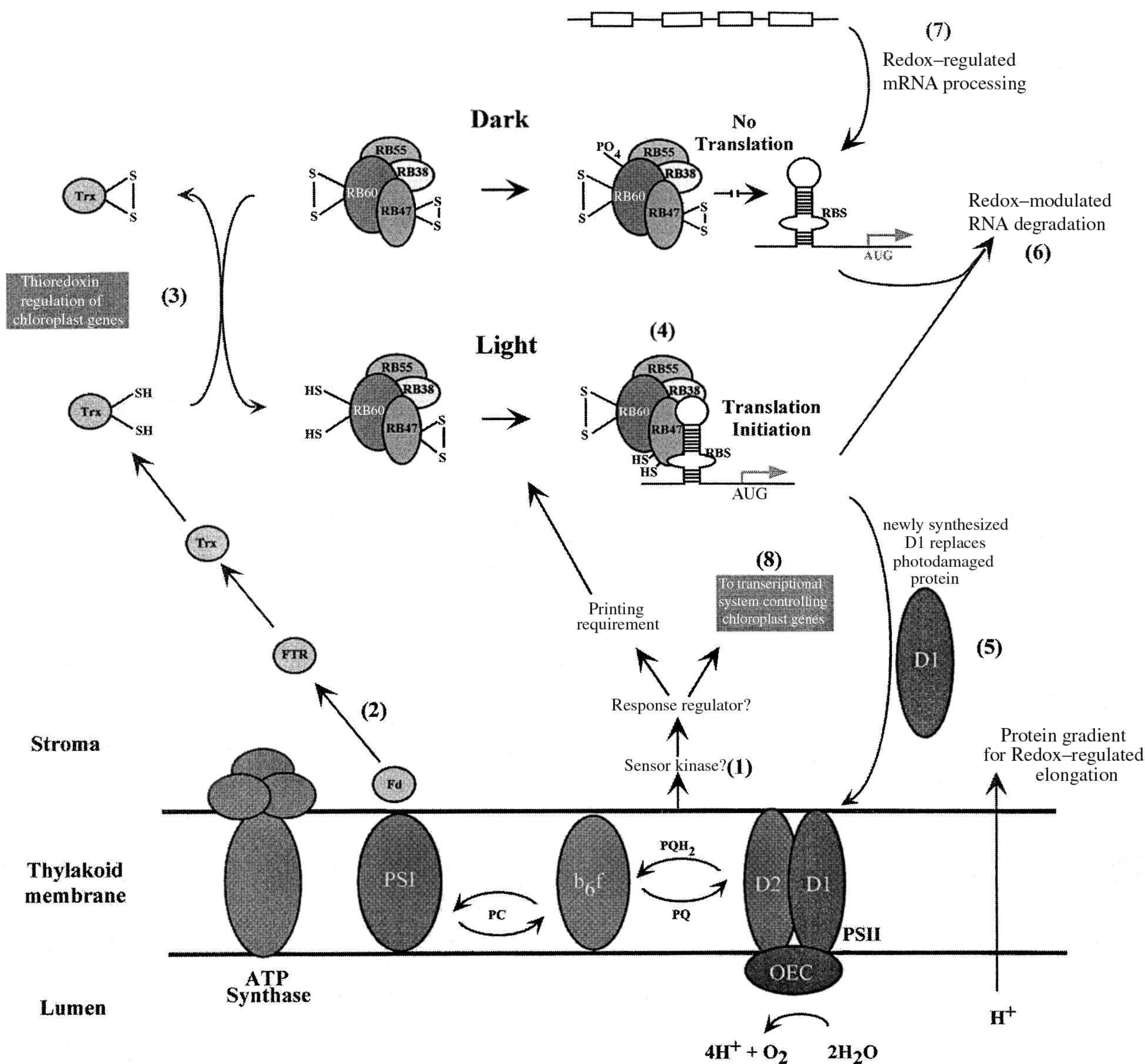

FIG. 1. Redox effects on posttranscriptional processes in the chloroplast; mechanism for light-activated translation of the *psb*A mRNA. A priming signal initiated by the plastoquinone redox state (**1**) and a thiol signal initiated by reduction of ferredoxin (**2**) are required for *psb*A translation. Reducing potential generated by the light reactions of photosynthesis is transferred to RB60 via thioredoxin (**3**) to activate the RNA binding activity of RB47 (**4**). Binding of this complex is required for translation initiation. Other steps in chloroplast gene expression that are subject to photosynthetic redox control include translation elongation (**5**), RNA degradation (**6**), RNA processing (**7**), and transcriptional activation (**8**). AUG, initiation codon; Fd, ferredoxin; FTR, ferredoxin:thioredoxin reductase; OEC, oxygen evolving complex; PC, plastocyanin; PQ, plastoquinone; RBS, ribosome binding site; Trx, thioredoxin.

these studies, the authors propose that light-activated translation elongation in chloroplasts is dependent on the formation of a proton gradient across the thylakoid membrane. It will be interesting to see which component(s) function to couple elongation and electron transport in this process.

REDOX EFFECTS ON RNA PROCESSING AND RNA DEGRADATION

A second aspect of plastid gene expression that has recently been identified to be under redox control is mRNA splicing. Deshpande and co-authors reported that light stimulates pre-RNA intron splicing in the *C. reinhardtii psb*A mRNA, and showed that this splicing is dependent upon photosynthetic electron transport (4). The light-induced increase in *psb*A pre-RNA splicing could be abolished by the addition of photosynthetic electron transport inhibitors, but not by an ATP synthesis inhibitor. In addition, a photosystem I-deficient mutant did not show light-activated pre-RNA splicing, and complementation of the mutant to restore photoautrophy restored light-responsive pre-RNA processing as well.

A signal pathway similar to the light regulation of *psb*A translation via redox control may also have a role in stabilizing transcripts in *C. reinhardtii* chloroplasts by regulating the interaction of proteins and sequence elements located within their 5′ UTRs (30). A chimeric β-glucuronidase (GUS) reporter gene under the control of the *rbc*L promoter was introduced into *C. reinhardtii* chloroplasts (30). Inhibition of photosynthetic electron transport with 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) prevented the light-dependent degradation of the reporter gene transcript (30). Furthermore, the addition of oxidizing agents resulted in the delay of degradation, whereas the addition of reducing agents induced the degradation of GUS transcripts in the dark. Taken together, these data suggest that redox carriers mediate the regulation of transcript stability, linking photosynthetic electron transport to the RNA degradation machinery.

Recently, a chloroplast endoribonuclease, p54, was identified and subsequently shown to be involved in 3′-end processing, possibly playing a role in RNA stability (17, 24). The activity of this protein was shown to be regulated in an opposite fashion to that of RB60, in which phosphorylation or oxidation activates the protein and dephosphorylation or reducing equivalents inhibit its activity. Thus, phosphorylation and sulfhydryl status potentially function in RNA degradation, providing an additional level of redox control in plastid gene expression.

CONCLUSIONS

Light is one of the most important external signals influencing plastid gene expression and plant development. As shown in Fig. 1, redox potential generated by the light reactions of photosynthesis acts as a signal for plastid gene expression, coupling photosynthetic electron transport to various molecular processes in chloroplasts, including transcription, translation, and mRNA turnover.

The light-activated translation of the *C. reinhardtii psb*A mRNA is a well characterized system whereby a redox signal carried from the photosynthetic electron transport chain is transduced to an RNP complex. The redox state of the plastid induces a change in the sulfhydryl status of the RNP complex, thereby allowing binding to the *psb*A mRNA and formation of a translation initiation complex. As discussed in this review, similar pathways exist in other light-modulated chloroplast processes that are affected by redox poise, albeit via a different set of interactive proteins.

Thus, it appears that redox control of processes involved in transcriptional and posttranscriptional chloroplast gene expression function within a global regulatory network directing plastid metabolism and development. Elucidating signaling mechanisms that connect photosynthetic electron transport with plastid gene expression, and characterization of the components involved in these various processes, will be required for a comprehensive understanding of plant gene expression.

ACKNOWLEDGMENTS

We thank Aravind Somanchi for a critique of the manuscript. This work was supported by funds from the U.S. Department of Energy (93ER70116) and the National Institutes of Health (GM54659) to S.P.M.

ABBREVIATIONS

GUS, β-glucuronidase; LSU, large subunit; RB47, RNA binding protein of 47 kDa; RB60, RNA binding protein of 60 kDa; RNP, RNA/protein complex; UTR, untranslanted region.

REFERENCES

1. Danon A and Mayfield SP. Light regulated translational activators: identification of chloroplast gene specific mRNA binding proteins. *EMBO J* 10: 3993–4001, 1991.
2. Danon A and Mayfield SP. ADP-dependent phosphorylation regulates RNA-binding in vitro: implications in light-modulated translation. *EMBO J* 13: 2227–2235, 1994.
3. Danon A and Mayfield SP. Light-regulated translation of chloroplast messenger RNAs through redox potential. *Science* 266: 1717–1719, 1994.
4. Deshpande NN, Bao Y, and Herrin DL. Evidence for light/redox-regulated splicing of psbA pre-RNAs in *Chlamydomonas* chloroplasts. *RNA* 3: 37–48, 1997.
5. Drager RG, Girard-Bascou J, Choquet Y, Kinde KL, and Stern DB. In vivo evidence for 5′→3′ exoribonuclease degradation of an unstable chloroplast mRNA. *Plant J* 13: 85–96, 1998.
6. Escoubas JM, Lomas M, LaRoche J, and Falkowski PG. Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. *Proc Natl Acad Sci U S A* 92: 10237–10241, 1995.

7. Fong CL, Lentz A, and Mayfield SP. Disulfide bond formation between RNA binding domains is used to regulate mRNA binding activity of the chloroplast poly(A)-binding protein. *J Biol Chem* 275: 8275–8278, 2000.
8. Gillham NW, Boynton JE, and Hauser CR. Translational regulation of gene expression in chloroplasts and mitochondria. *Annu Rev Genet* 28: 71–93, 1994.
9. Girard-Bascou J, Pierre Y, and Drapier D. A nuclear mutation affects the synthesis of the chloroplast *psbA* gene production *Chlamydomonas reinhardtii. Curr Genet* 22: 47–52, 1992.
10. Gruissem W and Tonkyn J. Control mechanisms of plastid gene expression. *CRC Crit Rev Plant Sci* 12: 19–55, 1993.
11. Irihimovitch V and Shapira M. Glutathione redox potential modulated by reactive oxygen species regulates translation of Rubisco large subunit in the chloroplast. *J Biol Chem* 275: 16289–16295, 2000.
12. Karpinski S, Escobar C, Karpinska B, Creissen G, and Mullineaux PM. Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. *Plant Cell* 9: 627–640, 1997.
13. Kettunen R, Pursiheimo S, Rintamaki E, Van Wijk KJ, and Aro E-M. Transcriptional and translational adjustments of *psbA* gene expression in mature chloroplasts during photoinhibition and subsequent repair of photosystem II. *Eur J Biochem* 247: 441–448, 1997.
14. Kim J and Mayfield SP. Protein disulfide isomerase as a regulator of chloroplast translational activation. *Science* 278: 1954–1957, 1997.
15. Kim J, Klein PG, and Mullet JE. Ribosomes pause at specific sites during synthesis of membrane-bound chloroplast reaction center protein D1. *J Biol Chem* 266: 14931–14938, 1991.
16. Kuroda H, Kobashi K, Kaseyama H, and Satoh K. Possible involvement of a low redox potential component(s) downstream of photosystem I in the translational regulation of the D1 subunit of the photosystem II reaction center in isolated pea chloroplasts. *Plant Cell Physiol* 37: 754–761, 1996.
17. Liere K and Link G. Chloroplast endoribonuclease p54 involved in RNA 3′-end processing is regulated by phosphorylation and redox state. *Nucleic Acids Res* 25: 2403–2408, 1997.
18. Malnoe P, Mayfield SP, and Rochaix J-D. Comparative analysis of the biogenesis of photosystem II in the wildtype and y-1 mutant of *Chlamydomonas reinhardtii. J Cell Biol* 106: 609–616, 1988.
19. Martin W and Herrmann RG. Gene transfer from organelles to the nucleus: how much, what happens, and why? *Plant Physiol* 118: 9–17, 1998.
20. Mayfield SP, Cohen A, Danon A, and Yohn CB. Translation of the psbA mRNA of *Chlamydomonas reinhardtii* requires a structured RNA element contained within the 5′ untranslated region. *J Cell Biol* 127(6 Pt 1): 1537–1545, 1994.
21. Mayfield SP, Yohn CB, Cohen A, and Danon A. Regulation of chloroplast gene expression. *Annu Rev Plant Physiol Plant Mol Biol* 46: 147–166, 1995.
22. Muhlbauer SK and Eichacker LA. Light-dependent formation of the photosynthetic proton gradient regulates translation elongation in chloroplasts. *J Biol Chem* 273: 20935–20940, 1998.
23. Nickelsen J. Mutations at three different nuclear loci of *Chlamydomonas* suppress a defect in chloroplast *psbD* mRNA accumulation. *Curr Genet* 37: 136–142, 2000.
24. Nickelsen J and Link G. The 54 kDa RNA-binding protein from mustard chloroplasts mediates endonucleolytic transcript 3′ end formation in vitro. *Plant J* 3: 537–544, 1993.
25. Nickelsen J, van Dillewijn J, Rahire M, and Rochaix JD. Determinants for stability of the chloroplast *psbD* RNA are located within its short leader region in *Chlamydomonas reinhardtii. EMBO J* 13: 3182–3191, 1994.
26. Pfannschmidt T, Nilsson A, Tulberg A, Link G, and Allen JF. Direct transcriptional control of the chloroplast genes *psbA* and *psaAB* adjusts photosynthesis to light energy distribution in plants. *IUBMB Life* 48: 271–276, 1999.
27. Pfannschmidt T, Schütze K, Brost M, and Oelmüller R. A novel mechanism of nuclear photosynthesis gene regulation by redox signals from the chloroplast during photosystem stoichiometry adjustment. *J Biol Chem* 276: 36125–36130, 2001.
28. Rochaix JD. Post-transcriptional regulation of chloroplast gene expression in *Chlamydomonas reinhardtii. Plant Mol Biol* 32: 327–341, 1996.
29. Sakamoto W, Chen X, Kindle KL, and Stern DB. Function of the *Chlamydomonas reinhardtii petd* 5′ untranslated region in regulating the accumulation of subunit IV of the cytochrome b6/f complex. *Plant J* 6: 503–512, 1994.
30. Salvador ML and Klein U. The redox state regulates RNA degradation in the chloroplast of *Chlamydomonas reinhardtii. Plant Physiol* 121: 1367–1374, 1999.
31. Shapira M, Lers A, Heifetz PB, Irihimovitz V, Osmond CB, Gillham NW, and Boynton JE. Differential regulation of chloroplast gene expression in *Chlamydomonas reinhardtii* during photoacclimation: light stress transiently suppresses synthesis of the Rubisco LSU protein while enhancing synthesis of the PS II D1 protein. *Plant Mol Biol* 33: 1001–1011, 1997.
32. Stampacchia O, Girard-Bascou J, Zanasco JL, Zerges W, Bennoun P, and Rochaix JD. A nuclear-encoded function essential for translation of the chloroplast *psaB* mRNA in chlamydomonas. *Plant Cell* 9: 773–782, 1997.
33. Sugita M and Sugiura M. Regulation of gene expression in chloroplasts of higher plants. *Plant Mol Biol* 32: 315–326, 1996.
34. Surpin M and Chory J. The co-ordination of nuclear and organellar genome expression in eukaryotic cells. *Essays Biochem* 32: 113–125, 1997.
35. Trebitsh T and Danon A. Translation of chloroplast *psbA* mRNA is regulated by signals initiated by both photosystems II and I. *Proc Natl Acad Sci U S A* 98: 12289–12294, 2001.
36. Trebitsh T, Levitan A, Sofer A, and Danon A. Translation of chloroplast *psbA* mRNA is modulated in the light by counteracting oxidizing and reducing activities. *Mol Cell Biol* 20: 1116–1123, 2000.
37. Yohn CB, Cohen A, Danon A, and Mayfield SP. Altered mRNA binding activity and decreased translational initiation in a nuclear mutant lacking translation of the

chloroplast *psbA* mRNA. *Mol Cell Biol* 16: 3560–3566, 1996.

38. Yohn CB, Cohen A, Danon A, and Mayfield SP. A poly(A) binding protein functions in the chloroplast as a message-specific translation factor. *Proc Natl Acad Sci U S A* 95: 2238–2243, 1998.
39. Zerges W. Translation in chloroplasts. *Biochimie* 82: 583–601, 2000.
40. Zerges W, and Rochaix JD. The 5′ leader of a chloroplast mRNA mediates the translational requirements for two nucleus-encoded functions in *Chlamydomonas reinhardtii*. *Mol Cell Biol* 14: 5268–5277, 1994.
41. Zerges W, Girard-Bascou J, and Rochaix JD. Translation of the chloroplast *psbC* mRNA is controlled by interactions between its 5′ leader and the nuclear loci TBC1 and TBC3 in *Chlamydomonas reinhardtii*. *Mol Cell Biol* 17: 3440–3448, 1997.

Address reprint requests to:
Professor Stephen P. Mayfield
Department of Cell Biology
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, CA 92037, U.S.A.

E-mail: mayfield@scripps.edu

Received for publication December 5, 2001; accepted April 15, 2002.