The Protein Disulfide Isomerase-like RB60 Is Partitioned between Stroma and Thylakoids in *Chlamydomonas reinhardtii* Chloroplasts*

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Tova Trebitsh‡§, Eti Meiri‡¶, Oren Ostersetzer‖, Zach Adam, and Avi Dannon‡‡

From the ‡Department of Plant Sciences, Weizmann Institute of Science and the ‡Department of Agricultural Botany, The Hebrew University of Jerusalem, P. O. Box 12, Rehovot 76100, Israel

Translation of *psbA* mRNA in *Chlamydomonas reinhardtii* chloroplasts is regulated by a redox signal(s). RB60 is a member of a protein complex that binds with high affinity to the 5′-untranslated region of *psbA* mRNA. RB60 has been suggested to act as a redox-sensor subunit of the protein complex regulating translation of chloroplast *psbA* mRNA. Surprisingly, cloning of RB60 identified high homology to the endoplasmic reticulum-localized protein disulfide isomerase, including an endoplasmic reticulum-retention signal at its carboxyl terminus. Here we show, by *in vitro* import studies, that the recombinant RB60 is imported into isolated chloroplasts of *C. reinhardtii* and pea in a transit peptide-dependent manner. Subfractionation of *C. reinhardtii* chloroplasts revealed that the native RB60 is partitioned between the stroma and the thylakoids. The nature of association of native RB60, and imported recombinant RB60, with thylakoids is similar and suggests that RB60 is tightly bound to thylakoids. The targeting characteristics of RB60 and the potential implications of the association of RB60 with thylakoids are discussed.

The chloroplast contains a small circular genome that encodes about 5–10% of the chloroplast proteins (1). The rest are encoded by the cell nucleus. This two-compartment gene organization dictates a close coordination between nuclear and organelar gene expression (2–4). Chloroplast mRNAs accumulate in fully developed chloroplasts to relatively high levels in both light- and dark-grown plants and algae. Translation of these mRNAs occurs at a much higher rate during the light-growth phase, thus identifying translation as a key regulatory point (reviewed in Refs. 2 and 4–6). The molecular basis of light-regulated translation in the chloroplast has been shown to be dependent on the function of a growing list of nucleus-encoded proteins (7–14). These factors are thought to mediate translational regulation by interacting with the 5′-untranslated region (5′-UTR) of chloroplast mRNAs (11, 12, 15–17). Nucleus-encoded chloroplast proteins are typically directed to the chloroplast by a transit peptide located at the amino terminus of the protein (18).

A set of mRNA-binding proteins that bind to the chloroplastic *psbA* mRNA 5′-UTR with high affinity and specificity has been identified and purified from *Chlamydomonas reinhardtii* cells (19). *psbA* mRNA 5′-UTR-binding proteins are composed of four major proteins, RB38, RB47, RB55, and RB60. These form a complex (*psbA* 5′-PC) that appears to bind the mRNA via the RB47 protein. The level of binding of *psbA* 5′-PC to the mRNA parallels the level of *psbA* mRNA translation and its association with polyribosomes in light- and dark-grown wild-type *C. reinhardtii* (19). Moreover, several nuclear mutants have been isolated in which the loss of RB47 is accompanied by the absence of D1 synthesis due to a block in the association of *psbA* mRNA with polyribosomes (10, 20). This suggests that light regulates polyribosome association and translation of *psbA* mRNA by modulating the binding of *psbA* 5′-PC to the 5′-UTR. Cloning of RB47 revealed its high homology with poly(A)-binding proteins (10). Characterization of the intrachloroplast localization of RB47 showed that it is associated with thylakoids in the *C. reinhardtii* chloroplast (21).

RB60 has been implicated as a regulatory subunit of *psbA* 5′-PC which is subject to light control via phosphorylation and redox signals in the chloroplast (22, 23). Cloning of RB60 identified high homology to protein disulfide isomerase (PDI) (24). PDI-like proteins typically catalyze the formation, reduction, and isomerization of disulfide bonds during protein folding in the endoplasmic reticulum (ER). However, in addition to their enzymatic role, PDI-like proteins have also been found to be indispensable subunits in protein complexes such as prolyl hydroxylase and triacylglycerol transfer protein (25). Furthermore, PDI-like proteins have recently been implicated in the regulation of E2A transcription factor (26) and the shedding of L-selecin (27). PDIs are most abundant in the lumen of the ER. They are directed to the ER by a signal peptide at the amino terminus and then are retained there by virtue of a second signal, -(K/H)DEL, at the carboxyl terminus (25, 28). The open reading frame of the recombinant RB60 (rRB60) contains an amino-terminal extension, the targeting identity of which has yet to be determined. Notably, despite the implicated function of rRB60 in the chloroplast, its open reading frame contains a carboxyl-terminal signal for ER retention (24).

Therefore, we first set out to determine whether the cloned rRB60 gene product is targeted to chloroplasts, and thereafter to study the subchloroplast localization of the native RB60. We show that rRB60 is imported into isolated *C. reinhardtii* and pea chloroplasts in a transit peptide-dependent fashion. Sub-
Fractionation of chloroplasts showed that whereas a portion of the native RB60 is present in the stroma, it is also found tightly bound to thylakoids. Moreover, following uptake by chloroplasts, the recombinant rRB60 associates with thylakoids in a manner similar to the native RB60. The association of RB60 to thylakoids was resistant to EDTA and RNase treatments, indicating that it is probably not mediated by binding to poly-some-associated pshA mRNA.

**Experimental Procedures**

Preparation of Intact Chloroplasts—C. reinhardtii cu15 cells were grown in Tris/acetate/phosphate medium (29), under a 12-h light/12-h dark period at 25 °C, to a density of ~1 × 10⁶ cells/ml. Intact chloroplasts were collected from the 45/70% interface of discontinuous Percoll gradient according to a protocol based on Goldschmidt-Clermont et al. (30) and Belknap (31).

Pea seedlings (Pisum sativum var. Alaska) were grown under standard conditions (32). Intact chloroplasts were isolated on Percoll gradients as described (32, 33).

Chlorophyll concentration was determined spectrophotometrically according to Arnon (34).

In Vitro Import Assays—In vitro protein synthesis reactions were performed with a T7, TNT-coupled reticulocyte lysate system according to manufacturer’s instructions (Promega) using 2 µg of RNA from RB60, Δ28RB60, LHCl, or SSU, or pea PPO cDNA containing plasmid constructs. Translation products were fractionated by SDS-PAGE, and protein incorporation of [35S]methionine was determined by trichloroacetic acid precipitation. Import into intact chloroplasts was performed as described previously (32, 35). The import assay was conducted for 30 min at 25 °C in the light in the presence of 10 mM ATP, unless otherwise indicated. Competition import assays were performed in the presence of nonlabeled pea OEE1 protein, which was expressed and purified as described by Betts et al. (36). Following import, chloroplasts were pelleted by centrifugation and resuspended in HS buffer (50 mM Hepes-KOH, pH 8, 0.33 M sorbitol). Import into intact chloroplasts was performed as described previously (32, 35).

Localization of Imported rRB60 and Native RB60—To localize RB60, we used isolated chloroplasts (10 µg of chlorophyll) or isolated chloroplasts containing imported radioactive rRB60 that were re-isolated using a 40% Percoll cushion and washed with HS buffer containing 5 mM EDTA. Stromal and thylakoid fractions were obtained by freezing and thawing, followed by a 1-min centrifugation at 15,000 × g at 4 °C (37). The supernatant fractionated by SDS-PAGE on 12% (w/v) polyacrylamide gels (38). Proteins were electroblotted onto nitrocellulose membranes (Schleicher & Schuell). Imported radiolabeled proteins were detected by autoradiography. Molecular masses of reference proteins (kDa) are shown on the left.

**Results**

rRB60 Is Imported into C. reinhardtii Chloroplasts—The targeting of proteins synthesized by cytoplasmic ribosomes to the chloroplast is typically determined by an amino-terminal transit peptide. The amino-terminal sequence of RB60, preceding the conserved sequence of PDIs, is quite different from other PDIs in both length and composition (Fig. 1) and contains a putative cleavage sequence in position 26–28 (39). To test whether the amino-terminal sequence of rRB60 could direct import into chloroplasts, radiolabeled rRB60 was synthesized in vitro and incubated with C. reinhardtii chloroplasts isolated by Percoll step gradient. Since in contrast to import by the ER, protein uptake by chloroplasts occurs post-translationally, we added the radiolabeled rRB60 only after termination of in vitro translation. Following incubation, protein that had not entered the chloroplast was degraded by treatment with the protease thermolysin. Intact chloroplasts were then repurified on a Percoll cushion and lysed with denaturing buffer, and the protein extracts were fractionated by SDS-gel electrophoresis. As seen in Fig. 2, rRB60 was imported into the chloroplasts and was protected from protease treatment (Fig. 2, lane 3), similar to a control import reaction containing in vitro synthesized chloroplast LHClI protein (Fig. 2, lane 11). Disruption of chloroplast membranes by treatment with nonionic detergent resulted in the degradation of both imported rRB60 (Fig. 2, lane 4) and LHClI proteins (Fig. 2, lane 12). These results verified the effectiveness of the protease treatment and that the radiolabeled protein was indeed protected from degradation by being taken up into chloroplasts.

To assay whether the import into chloroplasts was determined by its amino-terminal sequence, a leaderless version of rRB60 (lacking the first 28 amino acids, Δ28RB60) was prepared and subjected to chloroplast import assays. The leaderless rRB60 was not imported by isolated chloroplasts (Fig. 2, lane 7), corroborating the proposed function of the amino-terminal sequence of RB60 as a chloroplast transit peptide required for import into chloroplasts. The mobility of the chloroplast-imported rRB60 was similar to that of its in vitro translated precursor (Fig. 2, lanes 1 and 3), and it corresponded to the mobility of the native RB60 in immunoblot assays (data not shown). In contrast, the mobility of the Δ28RB60 was slightly higher (Fig. 2, lane 5), suggesting that the transit

**Fig. 1.** Multiple sequence alignment of the amino terminus of C. reinhardtii RB60 and related protein disulfide isomerases from mammals, plants, and yeast. Multiple alignment of the polypeptides was generated using ClustalW. Homologous amino acids are shaded. The putative cleavage site for RB60 (marked by an arrow) was identified using the computer program of Nielsen et al. (39).

**Fig. 2.** rRB60 is imported into isolated C. reinhardtii chloroplasts. Import into C. reinhardtii chloroplasts was performed with in vitro synthesized, 35S-labeled RB60 (lanes 1–4), Δ28RB60 (a deletion spanning amino acids 1–28) (lanes 5–8), and LHClI (lanes 9–12) recombinant proteins. 2% of the input translation products of RB60 (lane 1), Δ28LRB60 (lane 5), and LHClI (lane 9) is presented. Import into chloroplasts (lanes 2, 6, and 10) was protected from thermolysin (0.3 mg/ml) degradation (Therm, lanes 3, 7, and 11). Treatment with 1% Triton X-100 (Trition, lanes 4, 8, and 12) ensured that the thermolysin-treated proteins were indeed taken up by chloroplasts. Proteins were fractionated by SDS-PAGE and electroblotted onto nitrocellulose membrane. Radiolabeled proteins were detected by autoradiography. Molecular masses of reference proteins (kDa) are shown on the left.
leaves (blot analysis of BiP, an ER protein. Total proteins extract of tobacco
D of 35S-labeled recombinant proteins, import into pea chloroplasts, and rRB60 recombinant proteins into pea chloroplasts.

precursor pea OEE1 protein, as indicated
plasts was performed as in Fig. 2,
imported Ps-PPO and Cr-SSU proteins (Fig. 3
7S labeled pea OEE1 protein. Thermolysin-protected import into chloro-
pea OEE1 (52) (lane 1, lanes 3 and 7). Disruption of chloroplast membranes by treating
with nonionic detergent resulted in degradation of the imported Ps-PPO and Cr-SSU proteins (Fig. 3A, lanes 4 and 8).

Fig. 3. rRB60 is imported into isolated pea chloroplasts. A, autoradiogram showing import of control proteins into pea chloroplasts. Ps-PPO denotes pea polyphenol oxidase; Cr-SSU denotes small subunit of Rubisco of C. reinhardtii. B, autoradiogram showing import of C. reinhardtii rRB60 recombinant proteins into pea chloroplasts. Δ28RB60 is a deletion of amino acids 1–28 of RB60. In vitro synthesis of 35S-labeled recombinant proteins, import into pea chloroplasts, and detection of the imported proteins were performed as described in Fig. 2. C, autoradiogram of ATP-dependent import of recombinant RB60 into pea chloroplasts. Thermolysin-protected import into pea chloroplasts was performed as in Fig. 2, lane 3, except that import was performed in the absence (lane 1) or in the presence (lane 2) of 10 mM Mg·ATP. D, autoradiogram showing competition by import of nonradio-
labeled pea OEE1 protein. Thermolysin-protected import into chloro-
plasts was performed as in Fig. 2, lane 3. Import reactions were per-
fomed in the presence of increased amounts (nanomoles) of the
precursor pea OEE1 protein, as indicated above each lane. E, immuno-
blot analysis of BiP, an ER protein. Total proteins extract of tobacco leaves (Tobacco, lane 1), pea leaves (Pea Total, lane 2), and isolated pea chloroplasts (Pea Chlps, lane 3) or cells of C. reinhardtii (Cr Total, lane 4) and isolated C. reinhardtii chloroplasts (Cr Chlps, lane 5) were fractionated by SDS-PAGE, electrophoresed onto nitrocellulose membrane, and decorated with antibodies specific to tobacco BiP (41) and pea OEE1 (52) (lanes 1–3) and antibodies specific to yeast BiP and C. reinhardtii OEE2 (lanes 4 and 5).

The Targeting of rRB60 Is Conserved in Chloroplasts of Higher Plants—Next, we assayed whether rRB60 would also be taken up by the well established system of highly purified pea chloroplasts. We chose pea polyphenol oxidase (Ps-PPO) as a control for the import activity of the isolated pea chloroplasts and C. reinhardtii small subunit of ribulose-1,5-bisphosphate carboxylase (Cr-SSU) as a control for import of a heterologous protein. All proteins were incubated with chloroplasts post-in vitro translation. As seen in the autoradiogram in Fig. 3A, both pea PPO and C. reinhardtii SSU were imported into the chloroplasts and were protected from protease treatment (Fig. 3A, lanes 3 and 7). To reaffirm the import of rRB60 into chloroplasts, we tested whether its import exhibits additional characteristics typical of chloroplast import. The findings that the uptake of rRB60 was enhanced in the presence of ATP (Fig. 3C) and was competed by the pea chloroplast protein OEE1 (Fig. 3D) corroborate this. To rule out the possibility of contaminating ER in the import reactions, we assayed the purity of the isolated chloroplasts. Fig. 3E shows that antibodies raised against the tobacco ER protein BiP (41) reacted against pea BiP in total protein extract of leaves but not of isolated chloroplasts. Similar results were obtained for C. reinhardtii chloroplasts using antibodies raised against the yeast BiP (Fig. 3E). This indicates that the isolated chloroplasts used were devoid of ER and that rRB60 was taken up by the chloroplasts. The import of rRB60 into both C. reinhardtii and pea chloroplasts suggests that it is directed to
chloroplasts in vivo and that this capacity is conserved in higher plants.

**RB60 Is Partitioned between Stroma and Thylakoids—** Import to *C. reinhardtii* and pea chloroplasts, in a transit peptide-dependent manner, indicated that the nucleus-encoded RB60 contains a chloroplast targeting signal. To identify the subchloroplast localization of native RB60, we lysed isolated *C. reinhardtii* chloroplasts, and we separated the thylakoid fraction from the supernatant containing the stroma proteins by centrifugation. The purity of the stromal fraction was determined by the absence of thylakoid-associated OEE2 and CF1 proteins, and the purity of the thylakoid fraction was verified by the lack of stromal ClpC protein (Fig. 4A). RB60 was present in both the stroma and thylakoid fractions of *C. reinhardtii* chloroplasts (Fig. 4A, lanes 2 and 3). The proportion of RB60 in these fractions fluctuated in several replications of this assay (data not shown). However, we routinely observed about 50% of the pool of *C. reinhardtii* chloroplast RB60 to be associated with thylakoids.

To check whether following import rRB60 is also directed toward the thylakoid membranes, we assayed the association of imported rRB60 with thylakoids. Both *C. reinhardtii* and pea chloroplasts containing imported radiolabeled rRB60 were disrupted and fractionated as in Fig. 4A. Most of the imported rRB60 was found associated with thylakoids of *C. reinhardtii* chloroplasts, whereas the proportion of soluble rRB60 was higher in pea chloroplasts (Fig. 4B). Together, these results suggest that at least some of the pool of chloroplast RB60 is associated with thylakoids.

**The Nature of the Association of RB60 with Thylakoids—** Next, we studied the nature of the association between native RB60 and *C. reinhardtii* thylakoids by washing purified thylakoids with high salt (1 M NaCl) with or without 0.05% Triton X-100 or with alkali buffer (0.1 M Na₂CO₃, pH 11), or by treating with the protease thermolysin (0.1 mg/ml) (Fig. 5). Thylakoid membranes were then isolated by centrifugation. The purity of the stromal fraction was performed with antibodies against *C. reinhardtii* RB60, OEE2, and CF1.

**Fig. 5. Association of native RB60 with *C. reinhardtii* thylakoid membranes.** Thylakoid membranes (obtained as in Fig. 4A) were washed with buffers containing 1 M NaCl, 1 M NaCl, and 0.05% Triton X-100 or 0.1 M Na₂CO₃, pH 11, or subjected to a protease treatment (thermolysin (Therm), 0.1 mg/ml) and each separated into membrane-associated (P) and soluble (S) fractions. Immunoblot analysis of each fraction was performed with antibodies against *C. reinhardtii* RB60, OEE2, and CF1.

RB60 was first isolated as a component of a protein complex (psbA 5'-PC) which assembles with high affinity on the 5'-UTR of *psbA* mRNA (19). Because translation of *psbA* mRNA is by thylakoid-bound polyribosomes (42, 43), RB60 may associate with thylakoids by binding to the 5'-UTR of polyribosomes-associated *psbA* mRNA. The resistance of thylakoid-associated RB60 to reduction by DTT did not corroborate this assumption. RB60 was initially isolated and characterized as a component of a protein complex showing high affinity to the 5'-UTR of *psbA* mRNA (19). Because translation of *psbA* mRNA is by thylakoid-bound polyribosomes (42, 43), RB60 may associate with thylakoids by binding to the 5'-UTR of polyribosomes-associated *psbA* mRNA. The resistance of thylakoid-associated RB60 to reduction by DTT did not corroborate this assumption. RB60 was first isolated as a component of a protein complex (psbA 5'-PC) which assembles with high affinity on the 5'-UTR of *C. reinhardtii* chloroplast *psbA* mRNA (19). Consistent with the predicted location of RB60, antisera raised against RB60 cross-reacted with a single protein in *C. reinhardtii* chloroplasts (23). However, the cloning of rRB60 depicted a PDI-like protein containing a putative amino-terminal leader sequence.
whose targeting information was unknown, and an ER-retention signal, KDEL, at the carboxyl-end of the protein (24). This prompted us to investigate the authenticity of the recombinant rRB60 by testing whether its amino terminus can direct import by chloroplasts. We showed that rRB60 is imported into both C. reinhardtii and pea chloroplasts in a transit peptide-dependent process (Figs. 2 and 3), indicating that RB60 is directed by its amino-terminal sequence to chloroplasts in vivo. The import of rRB60 displayed several characteristics typical of chloroplast import, such as post-translational import, ATP-dependent import, and sensitivity to a competing import of a nonlabeled chloroplast protein (Fig. 3 and Fig. 4, B and C). The authenticity of rRB60 was further substantiated by the similar intra-chloroplast localization of the native RB60 and the imported rRB60 (Fig. 4). The reason for the presence of a KDEL signal in the carboxyl terminus of RB60 is yet unknown. It may be cryptic and may represent a potential signature of the evolution of RB60 from an ancestral PDI gene. Alternatively, RB60 may have dual functions as follows: one in the chloroplast and a second in the ER. Such polytopic proteins, present in both the mitochondrion and an additional cell compartment, have been described (44). Polytopic targeting of mitochondrial proteins has been suggested to arise either from export mechanisms from the mitochondria (44) or, alternatively, via dual targeting by a unique amino terminus signal peptide (45). We are currently studying whether RB60 is also an ER protein.

In the chloroplast, RB60 is partitioned between the stroma and the thylakoids (Figs. 4–6). A second component of psbA 5’-UTR, the RB47 protein, has also been shown to be tightly bound to thylakoids (21). The finding of RB60 and RB47 as membrane-associated proteins suggests a regulatory membrane-associated step in the expression of psbA mRNA. Translation of chloroplast mRNAs encoding integral thylakoid proteins is via membrane-bound polyribosomes (42, 43). Therefore, it is possible that RB60 attaches to thylakoids by binding to the 5’-UTR of polysome-associated psbA mRNA. However, the association of RB60 with thylakoids is resistant to washes containing EDTA and treatment with RNase A, suggesting that the binding is not mediated by membrane-bound ribosomes. This, however, does not rule out the possibility that some amount of RB60 was associated with psbA mRNA and was washed away from the thylakoids in our Mg²⁺-free washes.

An alternative explanation for the membrane association of 5’-UTR-interacting proteins has been invoked by genetic studies showing many parallels in translational regulation in C. reinhardtii chloroplast and Saccharomyces cerevisiae mitochondrion (2, 3, 46). In the latter, the cox3 mRNA translation activator proteins, PET54, PET122, PET494, form a complex that has the capacity for three-way interaction with the 5’-UTR, the small ribosomal subunit, and the inner mitochondrial membrane (47). This three-way association suggests that the yeast translational activators tether the mitochondrion mRNA to the inner membrane (46). Similar function has been proposed for the proteins binding the 5’-UTR of chloroplast mRNAs, encoding thylakoid membrane proteins (2, 3). The detection of tight binding of RB60 (Figs. 4–6) and RB47 (21) to thylakoids corroborates this model. Furthermore, the characterization of RB60 as a peripheral protein localized on the stromal face of the thylakoids (Fig. 5) is consistent with this proposed function of 5’-UTR-binding proteins in the chloroplast. However, thylakoid association is not common to all translational regulators: CRP1, a regulator of petA and petD mRNAs in maize, was found to be part of a soluble high molecular weight complex and not to be associated with chloroplast membranes (14).

The finding of specific oxidizing activity of RB60 (23) suggests an additional reason for the RB60-thylakoid association. Recently, a novel yeast protein, ERO1, whose function is to oxidize PDI, has been identified and shown to be membrane-localized (48, 49). Likewise, in Escherichia coli, the source of oxidizing equivalents necessary for protein disulfide catalysis has been shown to be membranal electron transport (50, 51). By analogy with these studies, one may hypothesize that the photosynthetic electron transport of the thylakoid is the source of oxidizing equivalents for RB60. If so, this might necessitate the close association of RB60 with thylakoids.

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REFERENCES

1. Sugiyra, M., Hirose, T., and Sugita, M. (1998) Annu. Rev. Genet. 32, 437–459
2. Gillham, N. W., Boynton, J. E., and Hauser, C. R. (1994) Annu. Rev. Genet. 28, 71–93
3. Rochaix, J.-D. (1996) Plant Mol. Biol. 32, 327–341
4. Mayfield, S. P., Yohn, C. B., Cohen, A., and Danon, A. (1995) Annu. Rev. Plant Physiol. Plant Mol. Biol. 46, 147–166
5. Danon, A. (1997) Plant Physiol. 115, 1293–1298
6. Stern, D. B., Higgs, D. C., and Yang, J. J. (1997) Trends Plant Sci. 2, 308–315
7. Jensen, K. H., Herrin, D. L., Plummer, F. G., and Schmidt, G. W. (1986) J. Cell Biol. 103, 4515–4527
8. Kuchka, M. R., Mayfield, S. P., and Rochaix, J.-D. (1988) EMBO J. 7, 319–324
9. Girard-Bascou, J., Pierre, Y., and Drapier, J. (1992) Curr. Genet. 22, 47–52
10. Yohn, C. B., Cohen, A., Danon, A., and Mayfield, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2238–2243
11. Zerges, W., Girard-Bascou, J., and Rochaix, J.-D. (1997) Mol. Cell. Biol. 17, 3440–3448
12. Stampachia, O., Girard-Bascou, J., Zanasco, J. L., Zerges, W., Benounn, P., and Rochaix, J.-D. (1997) Plant Cell 9, 773–782
13. McCormac, D. J., and Barkan, A. (1999) Plant Cell 11, 1709–1716
14. Fisk, D. G., Walker, M. B., and Barkan, A. (1999) Plant Cell 11, 1621–1630
15. Zerges, W., and Rochaix, J.-D. (1994) Mol. Cell. Biol. 14, 5268–5277
16. Sakamoto, W., Chen, X., Kindle, K. L., and Stern, D. B. (1994) Plant J. 6, 503–512
17. Mayfield, S. P., Cohen, A., Danon, A., and Yohn, C. B. (1994) J. Cell Biol. 127, 1537–1545
18. Keesstra, K., and Cline, K. (1999) Plant J. 11, 557–570
19. Danon, A., and Mayfield, S. P. (1993) EMBO J. 12, 3993–4001
20. Yohn, C. B., Cohen, A., Danon, A., and Mayfield, S. P. (1996) Mol. Cell. Biol. 16, 3560–3566
21. Zerges, W., and Rochaix, J.-D. (1998) J. Cell Biol. 140, 101–110
22. Zerges, W., and Mayfield, S. P. (1994) EMBO J. 13, 2227–2235
23. Trebitsh, T., Levitan, A., Sofer, A., and Danon, A. (2000) Mol. Cell. Biol. 20, 1116–1123
24. Kim, J., and Mayfield, S. P. (1999) Science 278, 1954–1957
25. Freedman, R. B., Hirst, T. R., and Tuite, M. F. (1994) Trends Biochem. Sci. 19, 331–336
26. Markus, M., and Benezra, R. (1999) J. Biol. Chem. 274, 1040–1049
27. Bennett, T. A., Edwards, B. S., Sklar, L. A., and Rogelj, S. (2000) J. Immunol. 164, 4120–4128
28. Pelham, H. R. (1990) Trends Biochem. Sci. 15, 483–486

FIG. 7. Thylakoid association of native RB60 is not mediated by ribosome or mixed disulfide interactions. Thylakoid membranes (T, lane 1) and washes (lanes 2–5) were performed as in Fig. 4 except that wash solutions included 10 mM DTT (lanes 2 and 3) or 10 mM EDTA (EDTA, lanes 4 and 5 producing membrane-associated (P) and soluble (S) fractions. Immunoblot analysis of each fraction was performed with antibodies against C. reinhardtii RB60, OEE2, and CF1.
29. Gorman, D. S., and Levine, R. P. (1965) Proc. Natl. Acad. Sci. U. S. A. 54, 1665–1669
30. Goldschmidt-Clermont, M., Malnoë, P., and Rochaix, J.-D. (1989) Plant Physiol. 89, 15–18
31. Belknap, W. R. (1983) Plant Physiol. 72, 1130–1132
32. Koussevitzky, S., Ne’eman, E., Sommer, A., Steffens, J. C., and Harel, E. (1998) J. Biol. Chem. 273, 27064–27069
33. Cline, K. (1986) J. Biol. Chem. 261, 14804–14810
34. Arnon, D. (1949) Plant Physiol. 24, 1–15
35. Adam, Z., and Hoffman, N. E. (1993) Plant Physiol. 102, 35–43
36. Betts, S. D., Hachigian, T. M., Pichersky, E., and Yocum, C. F. (1994) Plant Mol. Biol. 26, 117–130
37. Ostersetzer, O., Tabah, S., Yarden, O., Shapira, R., and Adam, Z. (1996) Eur. J. Biochem. 236, 932–936
38. Laemmli, U. K. (1970) Nature 227, 680–685
39. Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G. (1997) Protein Eng. 10, 1–6
40. Snyder, S., and Kohorn, B. D. (1999) J. Biol. Chem. 274, 9137–9140
41. Pedrazzini, E., Giovinazzo, G., Bellini, R., Ceriotti, A., and Vitale, A. (1994) Plant J. 5, 103–110
42. Harris, E. H., Boynton, J. E., and Gillham, N. W. (1994) Microbiol. Rev. 58, 700–754
43. Minami, E., and Watanabe, A. (1984) Arch. Biochem. Biophys. 235, 562–570
44. Soltys, B. J., and Gupta, R. S. (1999) Trends Biochem. Sci. 24, 174–177
45. Bhagwat, S. V., Biswas, G., Anandatheerthavarada, H. K., Addya, S., Pandak, W., and Avadhani, N. G. (1999) J. Biol. Chem. 274, 24014–24022
46. Fox, T. (1996) Experientia (Basel) 52, 1130–1135
47. Brown, N. G., Costanzo, M. C., and Fox, T. D. (1994) Mol. Cell. Biol. 14, 1045–1053
48. Pollard, M. G., Travers, K. J., and Weissman, J. S. (1998) Mol. Cell 1, 171–182
49. Frand, A. R., and Kaiser, C. A. (1998) Mol. Cell 1, 161–170
50. Bader, M., Muse, W., Bailou, D. P., Gassner, C., and Bardwell, J. C. (1999) Cell 98, 217–227
51. Kobayashi, T., and Ito, K. (1999) EMBO J. 18, 1192–1198
52. Itzhaki, H., Naveh, L., Lindahl, M., Cook, M., and Adam, Z. (1998) J. Biol. Chem. 273, 7984–7988
53. Ostersetzer, O., and Adam, Z. (1996) Plant Mol. Biol. 31, 673–676
54. Avital, S., and Gromet-Elhanan, Z. (1991) J. Biol. Chem. 266, 7067–7072