Chloroplast Ribonucleoproteins Function as a Stabilizing Factor of Ribosome-free mRNAs in the Stroma*

Received for publication, September 27, 2000
Published, JBC Papers in Press, October 18, 2000, DOI 10.1074/jbc.M008817200

Takahiro Nakamura‡, Masaru Ohta‡‡, Masahiro Sugiru‡, and Mamoru Sugita‡‡‡

From the ‡Center for Gene Research, and the ‡‡Graduate School of Human Informatics, Nagoya University, Nagoya 464-8601, Japan

Post-transcriptional RNA processing is an important step in the regulation of chloroplast gene expression, and a number of chloroplast ribonucleoproteins (cpRNPs) are likely to be involved in this process. The major tobacco cpRNPs are composed of five species: cp28, cp29A, cp29B, cp31, and cp33 and these are divided into three groups (I, II, and III). By immunoprecipitation, gel filtration, and Western blot analysis, we demonstrated that these cpRNPs are abundant stromal proteins that exist as complexes with ribosome-free mRNAs. Many ribosome-free psbA mRNAs coprecipitate with cpRNPs, indicating that the majority of stromal psbA mRNAs are associated with cpRNPs. In addition, in vitro mRNA degradation assay indicated that exogenous psbA mRNA is more rapidly degraded in cpRNP-depleted extracts than in nondepleted extracts. When the depleted extract was reconstituted with recombinant cpRNPs, the psbA mRNA in the extract was protected from degradation to a similar extent as the psbA mRNA in the nondepleted extract. Moreover, restoration of the stabilizing activity varied following addition of individual group-specific cpRNPs alone or in combination. When the five cpRNPs were supplemented in the depleted extract, full activity was restored. We propose that these cpRNPs act as stabilizing factors for nonribosome-bound mRNAs in the stroma.

Chloroplasts contain their own genes, and chloroplast gene expression is regulated at both the transcriptional and posttranscriptional level (1–3). Quantitative analysis of spinach (4) and barley (5, 6) has revealed that the mRNA levels of several protein-encoding genes in chloroplasts can increase dramatically (from 20- to ~1,000-fold) during plastid differentiation and chloroplast development. The increased abundance of these mRNAs cannot, however, account for the relative transcription rate of these genes (4).

The half-lives of chloroplast mRNAs have been shown to range from 6 h for the mRNA encoding the 83-kDa chlorophyll a apoprotein of photosystem I gene (psaA) to over 40 h for the mRNA encoding the D1 protein of photosystem II (psbA). 1 Refs.

† This work was supported by Grant-in-aid for Scientific Research in Priority Areas No. 0927103 (to M. S.) from the Ministry of Education, Science, Sports, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ Present Address: Plantech Research Inst., Research Center, 1000 Kamoshida-cho, Aoba-ku, Yokohama 227-0033, Japan.
§ To whom correspondence should be addressed. Tel/Fax: 81 52 789 4779; E-mail: sugita@info.human.nagoya-u.ac.jp.
¶ The abbreviations used are: psbA, gene encoding D1 protein of photosystem II; IR, inverted repeat; UTR, untranslated region; cpRNP, chloroplast ribonucleoprotein; PAGE, polyacrylamide gel electrophore-
7 and 8). Moreover, the stability of chloroplast mRNAs has been shown to change in response to chloroplast development and varying light conditions. This suggests that the differential accumulation of chloroplast mRNA is regulated primarily at the post-transcriptional level, with mRNA stability then contributing to the mRNA steady-state level. The mechanism underlying mRNA stability, however, is poorly understood.

Like Escherichia coli mRNAs, most chloroplast mRNAs contain an inverted repeat (IR) sequence in their 3′-untranslated region (UTR) that can fold into a stable stem-loop structure. This structure has been shown to be important in determining mRNA stability both in vitro (9–11), and in vivo (11, 12). Several chloroplast proteins, detected by UV cross-linking (13–17) and gel-shift assays (18–20), have been found to bind to the 5′- or 3′-UTRs of mRNAs. These chloroplast proteins could be gene-specific mRNA-binding proteins. In addition, numerous nuclear mutants of Chlamydomonas reinhardtii (17, 21), maize (22), barley (23, 24), and Arabidopsis thaliana (25), have been identified, which fail to accumulate individual chloroplast-encoded mRNAs (or precursor (pre)-mRNAs) despite having normal transcription rates.

We previously isolated five nuclear-encoded chloroplast ribonucleoproteins (cpRNPs) from tobacco, which we named cp28, cp29A, cp29B, cp31, and cp33 according to their sizes in kDa (26, 27). Based on phylogenetic comparison to the cpRNPs from A. thaliana, tobacco cpRNPs can be classified into three groups: cp29A and cp29B in group I, cp28 and cp31 in group II, and cp33 in group III (28). Tobacco cpRNPs have two consensus sequence-type RNA-binding domains and an acidic N-terminal domain. Similar proteins and genes encoding cpRNP homologs have also been found in a variety of other plant species (29) including spinach (30), A. thaliana (28), maize (31), and barley (32).

In vitro, tobacco cpRNPs have a strong affinity for RNA homopolymers (poly(G) and poly(U)) rather than single-stranded or double-stranded DNA (33, 34). After UV cross-linking chloroplast proteins with several mRNA probes, a subset of proteins of around 30 kDa can usually be detected in the chloroplasts of land plants (15, 16) and green algae (17). This suggests that cpRNPs bind nonspecifically to chloroplast RNAs. Spinach 28RNP, a similar protein to tobacco cp28 and cp31, was reported to be required for the formation of the 3′-end of several mRNAs in vitro (30, 35). Further studies have shown this protein directs correct processing of the 3′-end pre-mRNA by the high molecular weight complex in vitro (36). We recently found that tobacco cpRNPs in vivo bind not only to mRNAs (and pre-mRNAs) but also to intron-containing pre-mRNAs (37). This suggests that cpRNPs are involved in RNA

* The abbreviations used are: psbA, gene encoding D1 protein of photosystem II; IR, inverted repeat; UTR, untranslated region; cpRNP, chloroplast ribonucleoprotein; PAGE, polyacrylamide gel electrophore-
processing rather than in the 3'-end formation of pre-mRNAs.

Despite extensive biochemical analysis of cpRNPs in vitro, little is known about their physiological function in vivo. To investigate the function of cpRNPs in RNA processing, we quantified cpRNPs and psbA mRNA levels in tobacco chloroplasts. We found cpRNPs were surprisingly abundant in the stroma, and the majority of these proteins exist as 30- to 600-kDa complexes with chloroplast RNAs that contribute to the stability of stromal mRNAs.

**EXPERIMENTAL PROCEDURES**

**Preparation of Intact Chloroplasts and Stromal Extracts—** Intact chloroplasts were isolated from the green leaves (5–8 cm) of tobacco plants (*Nicotiana tabacum* var. Bright Yellow 4) as described previously (26). The chloroplasts were lysed in extraction buffer (50 mM Tris-HCl, pH 8.0, 50 mM KCl, 2 mM dithiothreitol, 10 mM MgCl₂, and 500 units of RNase inhibitor from Takara Shuzo) for 15 min at 4 °C. Stromal extracts were obtained by centrifuging the lysate at 14,000 g for 10 min, and filtering the supernatant through a Millipore filter (0.22-μm pore size). The protein concentration of the extracts was determined using a Bio-Rad protein assay kit (Bio-Rad).

**Detection of cpRNPs—** The number of isolated intact chloroplasts was counted in a hemocytometer by light microscopy. A series of dilute chloroplast suspensions (containing 10⁵–10⁶ chloroplasts) was prepared. Total protein was extracted from the suspensions by the addition of SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% 2-mercaptoethanol, 10% glycerol, 0.004% bromophenol blue). The protein extracts and recombinant cpRNPs, re-cp28, re-cp29A, and re-cp33 (1, 10, and 100 ng; Ref. 37) were separated by SDS-PAGE (15% polyacrylamide gel) and then transferred onto polyvinylidene difluoride membranes (Problott, PerkinElmer Life Sciences). The cpRNPs on the membrane were detected using antibodies against re-cp28, re-cp29A, re-cp31, or re-cp33 (37) and the ECL Western blotting analysis system (Amersham Pharmacia Biotech). The intensity of the membrane signals was quantified using a Fluor-S multi-imager (Pharmacia Biotech). Autograph cpRNPs in the extract were detected to be ~30 kDa whereas the recombinant cpRNPs fused with maltose-binding protein was 70 kDa (37). The antibodies used in this study cross-react with both cpRNP itself and with the maltose-binding protein of recombinant cpRNPs. By comparing the intensity of authentic cpRNPs with that of recombinant proteins having different molecular weights, we calculated the amount of cpRNPs in chloroplasts using appropriate corrections.

**Gel Filtration of the Stromal Extract—** The stromal extract (1 mg protein) was applied to a Superdex 200PC 3.2/30 column using the SMART system (Amersham Pharmacia Biotech) and 50 μl/min extraction buffer. Ten minutes after sample injection, 100 μl of each of the 21 fractions was collected and subjected to Western blot analysis using recombinant cpRNPs antibodies. The size of the proteins within each fraction of the extract was determined by comparison with the LMW (low) and HMW (high) molecular mass calibration markers (Amersham Pharmacia Biotech).

**Immunoprecipitation—** Protein A-Sepharose (PAS) resin (0.3 mg, Amersham Pharmacia Biotech) was suspended in 500 μl of extraction buffer and was incubated with each antibody for 1 h at 4 °C. After washing five times with 1 ml of extraction buffer, the antibody-PAS resin was incubated with the stromal extract at 4 °C for 20 min, followed by centrifugation (10,000 × g for 30 s). The supernatant was collected and is labeled *sup* in Fig. 3. The precipitated resin was washed with 5 ml of extraction buffer and then collected and labeled *ppt* in Fig. 3. The supernatants and precipitates were used for RNA or protein extraction.

**Nucleic Acid Isolation and Northern Blot Analysis—** Total nuclear acids were isolated from the precipitate by phenol/chloroform extraction (38). RNA electrophoresis and Northern blotting onto a nylon membrane (Hybond-N+, Amersham Pharmacia Biotech) were carried out as described by Li and Sugita (26). A 3-kb 23 S RNA gene-specific probe and 1.5-kb psbA probe were prepared from plasmids p23S and psbA-F (37), respectively, and were 32P-labeled using a random primer labeling kit (Takara Shuzo). The amount of cpRNPs in a series of dilute chloroplast suspensions was determined by Western blot analysis. For preparation of the reconstituted extract, individual recombinant cpRNPs (3 μg each of group I re-cp29A and re-cp29B, 1.5 μg each of group II re-cp28 and re-cp31, or 0.15 μg of group III re-cp33) were added to 300 μl of cpRNP-depleted extract (1.5 μg protein). Some samples of 32P-labeled full-length psbA mRNA (1,589 bases) or shorter psbA mRNA lacking 3'-UTR (1,454 bases) were produced from *BamHII* or *HhaI*-digested psbA-F, respectively, using the T7 RNA polymerase in MEGA Script (Ambion). The labeled RNA (final concentration of 25 ng/ml) was added to six tubes, each containing 30 μl of the prepared extract and was incubated at 37 °C. At the indicated time the incubation was stopped, and total RNA was extracted from the samples with phenol/chloroform (38). In further reconstituted assays, individual group-specific recombinant cpRNPs alone or combinations of group-specific ones were added to the cpRNP-depleted extract and incubated at 37 °C for 3 min. The extracted RNAs were separated by electrophoresis through a formaldehyde/agarose gel and then transferred onto a Hybond-N+ membrane. The membrane was analyzed using a BAS2000 Fuji Imagining analyzer (Fuji Photo Film, Japan). For the UV cross-linking assay, 30 μl of each stromal extract was incubated with 32P-labeled mRNA for 1 min and then UV irradiated (360 mJ/cm²) in a UV cross-linker (Funa, FS-1500). Subsequent digestion with RNase A (at a final concentration of 75 μg/ml) was performed as described by Vera and Sugita (39).

**RESULTS**

**cpRNPs Are Abundant Stromal Proteins—** The amount of cpRNPs in a series of dilute chloroplast suspensions was determined by Western blot analysis. By comparing the intensity of the five cpRNP protein bands with the intensity of the control recombinant cpRNPs bands, 10⁶ chloroplasts were expected to contain ~20 ng of cp29A, 10 ng of cp28, and 2 ng of cp33 (Fig. 1A). This is equivalent to 10⁵ molecules of cp29A, 51,000 molecules of cp28, and 8,000 molecules of cp33 per chloroplast. The levels of cp29A and cp29B were similar, and the cp28 and cp31 levels were equivalent to the cpRNP levels previously estimated by single-stranded DNA column chromatography (26, 27). These results indicate that tobacco cpRNPs accumulate at high levels in the chloroplasts of green leaves. In comparison, 10⁶ chloroplasts have been shown to contain 200 ng of the large subunit (LS) of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Fig. 1B). This is equivalent to 2.2 × 10⁶ molecules of LS per chloroplast. RuBisCO holoenzyme is composed of eight each of the large and small subunits and is thus calculated to be 2.8 × 10⁷ molecules of RuBisCO holoenzyme per chloroplast. This is comparable with 5.8 × 10⁶ RuBisCO molecules per chloroplast in barley (40).

To compare the relative amount of cpRNP protein and mRNA in chloroplasts, we quantified the steady-state level of psbA mRNA by Northern blot analysis (Fig. 1C). Using in vitro synthesized psbA mRNA (1–500 ng) as the control, we detected 120 ng of psbA mRNA per 10⁷ chloroplasts. This is the equivalent of ~14,000 psbA mRNA molecules per chloroplast. cpRNPs Form Complexes with RNA—In a previous analysis of the sedimentation profiles of stromal extract cpRNPs by sucrose density gradient centrifugation, we showed that most cp28 and cp31 sediments lie between the top of the gradient and the 18 S RuBisCO holoenzyme (41). Moreover, we have recently observed that stromal mRNAs and intron-containing pre-tRNAs coprecipitate with cpRNPs (37). These observations suggest that cpRNPs may form complexes with RNAs. If cpRNPs-RNA complexes do exist in the stroma, their size may be expected to range from a minimum of ~30 kDa, up to 550 kDa (the size of the RuBisCO holoenzyme).
To determine the size of these proposed cpRNP-RNA complexes, tobacco stromal extracts were separated by size exclusion chromatography through a Superdex 200PC column (Fig. 2). This column separates proteins in the size range of 10–600 kDa. The 30 S ribosomal subunits (900 kDa) and 70 S ribosomes (2,500 kDa) could be excluded from the column and were collected in void fractions 1–6 (Fig. 2B). The cpRNPs were distributed over a wide range of fractions from 30–600 kDa, and cp29A, cp29B (group I), and cp33 (group III) were also detected in fraction 6 (>600 kDa) (Fig. 2A, frac. no.). The broad cpRNP peaks could not be attributed to overloading the column, because a reduction in the amount of extract loaded did not change the separation profiles (data not shown). The size of the cp28 complexes ranged from 30–600 kDa (fractions 7–14), and the cp31 complexes ranged in size from 30–400 kDa (fractions 8–13). This implies that most of the cpRNPs do not cofractionate with ribosomes.

When the stromal extracts were treated with RNase A prior to size fractionation, the peaks for cp29A, cp29B, and cp28 shifted dramatically to 30 kDa (fraction 12) at the same position as recombinant cp28 (Fig. 2C, his-cp28). This confirms that the proteins detected at 30 kDa are RNA-free cpRNPs. By contrast, the peaks of cp31 and cp33 were detected at about 50 kDa (fraction 11). RNase treatment of the extracts did not change the protein profiles of stromal extracts (data not shown). Overall, these results suggest that cpRNPs form a stable RNA-protein high molecular weight complex with non-ribosome-bound RNAs, with cp28, cp29A, and cp29B interacting as monomers and cp31 and cp33 interacting as oligomers.

Most of the Stromal psbA mRNA Binds with cpRNPs—To test whether the cpRNPs are associated with ribosome-free stromal mRNAs, the stromal extracts were subjected to immunoprecipitation using antibodies against group I cpRNP (cp29A), II (cp28 and cp31), and III (cp33), respectively. The amount of mRNA was then determined by Northern blot analysis using gene-specific probes. Approximately 90% of the stromal psbA mRNA coprecipitated with group I and II cpRNPs (Fig. 3), whereas psbA mRNA coprecipitated less with group III cpRNP (cp33) than with group I and II proteins. This indicates that most of the psbA mRNAs are always associated with group I and II cpRNPs but not with group III cpRNP (cp33). Distribution of mRNA into the supernatant and pellet coincides with that of group I and II cpRNPs (Fig. 3). By contrast, the majority of 23 S ribosomal RNA did not coprecipitate with any group of cpRNPs and remains in the supernatant (Fig. 3). This result confirms that most of the mRNA associated with cpRNPs is likely to be ribosome-free. The anti-β-gal antibody did not coprecipitate with psbA mRNA to such a high degree. These observations support previous studies that have shown that most of the stromal psbA mRNA in barley is ribosome-free (42).

cpRNPs Stabilize psbA mRNA—To examine the possibility that cpRNPs bind to ribosome-free RNA to protect the RNA from degradation, the effect of cpRNPs on RNA degradation was analyzed using three different stromal extracts: an extract...
treated with unrelated serum (control ex), a cpRNP-depleted extract (dep-ex), and a depleted extract supplemented with recombinant cpRNPs (dep-ex + cpRNPs) (Fig. 4). Based on quantification of cpRNPs in the stromal extract (Fig. 1), 3 μg each of cp29A and B, 1.5 μg each of cp28 and cp31, and 0.15 μg of cp33 were supplemented to the depleted extract. Western blot analysis verified that prior to mRNA incubation the depleted extract sample was completely depleted of all five cpRNPs, and appropriate amounts of recombinant proteins were supplemented to the depleted extract (Fig. 4C). The in vitro-synthesized psbA mRNA was then incubated with each extract, and its degradation was monitored (Fig. 4, A and B). The half-life of the full-length psbA mRNA was 6 min in the control extract and 2 min in the depleted extract. Thus, the half-life of the psbA mRNA in the depleted extract was 3-fold shorter than in the control extract. Supplementing the depleted extract with five recombinant cpRNPs, however, lowered the degradation rate back to the level of the control extract. This result was the same as that of an in vitro assay using a shorter psbA mRNA, which lacks IR-containing 3'-UTR (data not shown).

The protein bands corresponding to the endogenous cpRNPs and the recombinant cpRNPs were detected by UV cross-linking and a 32P-labeled psbA mRNA probe. The cpRNPs interacted directly with the exogenous psbA mRNA probe (Fig. 4C). Overall, the results of this experiment suggest that binding of all or some cpRNPs to mRNA protects the RNA from degradation.

**Different Contributions of Individual Group cpRNPs to mRNA Stability**—To investigate different effects of individual cpRNPs on mRNA stability, we carried out further in vitro mRNA degradation assays using reconstituted stromal extracts. As shown in Fig. 5, depletion of all three groups of cpRNPs reduced mRNA stability levels to 55% of the control extract. When either of three groups of recombinant cpRNP was supplemented to cpRNPs-depleted extract (Fig. 5, control), mRNA stability was increased. Supplement of the group III cpRNP (cp33) resulted in a drastic increase in mRNA stability rather than group I and II proteins. When the two groups of cpRNPs, in any combination, were supplemented to the depleted extract, mRNA stability was further increased to levels reaching 80–90% of the control extract. These observations indicate that all three groups of cpRNPs are cooperatively involved in stability of psbA mRNA, possibly via individual group-specific cpRNPs. In addition, group III protein (cp33) exhibited the most effective influence on mRNA stability. Supplementation of three groups resulted in full restoration of mRNA stability. This agrees with the previous experiment (Fig. 4).

**DISCUSSION**

The present study has shown that the five cpRNPs are abundant (~3 × 10^6 molecules) and accumulate at one-tenth of the level of RuBisCO in tobacco chloroplasts. Their amounts are apparently greater than total molecules of chloroplast mRNAs, including the most abundant psbA mRNA (~14,000 molecules), and perhaps greater than ribosomes. For instance, Rapp et al. (6) estimated that each chloroplast of dark-grown barley seedlings has 1.4 × 10^5 molecules of 16 S rRNA.

We used size fractionation and immunological tools to show that the cpRNPs range in size from 30 to 600 kDa (cp28 and cp31), or to larger (cp29A, cp29B, and cp33) and that most of the cpRNPs bind to ribosome-free RNAs. This size distribution probably reflects the binding of either single or multiple cpRNPs to various RNA species in the stroma. The cpRNPs appear to be part of a higher molecular weight complex that is associated with RNA. RNase treatment of the complex shifts it to a smaller size ~30 kDa. Interestingly, the gel filtration results also suggest that cp28, cp29A, and cp29B interact with RNAs as monomers, whereas cp31 and cp33 may interact as oligomers (~50 kDa). This suggests that the presence of two
distinct forms of cpRNPs may reflect different function(s) for different cpRNPs.

In the present study, the important finding was that cpRNPs contribute to RNA stabilization via direct binding to target RNAs. Chloroplast extracts depleted of all five cpRNPs degraded exogenous psbA mRNA faster than did nondepleted extracts. The IRs of psbA mRNA have previously been shown to act as cis-elements for RNA stability in spinach (9) and C. reinhardtii (11). In this study, however, the rapid degradation of IR-containing exogenous psbA mRNA suggests that the IR of psbA mRNA may only contribute in part to mRNA stability in vitro. Numerous ribonuclease activities have been reported in chloroplasts (36, 43–47). Klaff (48) reported that degradation of psbA mRNA is initiated by endonucleolytic cleavage of psbA mRNA. Once the mRNA has been cleaved internally, the RNA fragments are then efficiently polyadenylated and exonucleolytically degraded (49, 50). The cpRNPs probably bind to internal sequence(s) targeted for cleavage by endoribonucleases, thereby protecting these sequences from degradation. Although cp33 exists at a 10-fold lower level than other cpRNPs, it demonstrated a significant effect on mRNA stabilization rather than the more abundant group I and II cpRNPs. This implies that cp33 is involved, directly or indirectly, in the stability of mRNAs or pre-RNAs. Alternatively, one possibility is that cp33 may bind initially to mRNAs, and thereby recruit other cpRNPs or unknown components to facilitate the formation of stable cpRNP and mRNA complexes.

Our previous work has clearly shown that several mRNAs (psbA, petD, and rbcL) encoding photosynthetic components and intron-containing pre-tRNAs coprecipitate predominantly with group I and II cpRNPs (37). This suggests that group I and II cpRNPs are involved mainly in the stability of mRNA and/or splicing of pre-tRNAs. Moreover, using an in vitro RNA editing system developed from tobacco chloroplasts, we have observed that only cp31 is required for RNA editing (C→U conversion) of psbL mRNA that encodes the L-protein of photosystem II. These results indicate that each cpRNP contributes to a differing extent to RNA stability, RNA cleavage, RNA editing, or RNA splicing. It is likely that tobacco cpRNPs are general RNA-binding proteins, like nuclear-localized heterogeneous ribonucleoprotein (hnRNP). Both cpRNPs and hnRNPs have strong affinities for poly(G), poly(U), and single-stranded DNA (33, 34, 51), and both are abundant proteins within the chloroplast and nucleus, respectively. In analogy to the function of hnRNP, cpRNPs play a role in various RNA processing before initiation of translation of mature mRNAs. Transcription is believed to occur in nucleoids that are composed of chloroplast DNA and several proteins (52, 53). It is interesting to note that cpRNPs are also detected in tobacco chloroplast nucleoids. This suggests that cpRNPs bind to nascent RNAs in the nucleoids.

From the overall results of the present study, we propose a model for the possible role of cpRNPs. The cpRNPs associate with nascent RNAs or pre-RNAs immediately after transcription in the nucleoids, and form RNA-protein complexes in the stroma. These cpRNP-RNA complexes confer stability and ribonuclease resistance to the RNAs. The complexes also act as a scaffold for the specific catalytic machinery involved in RNA maturation, RNA splicing of intron-containing pre-tRNAs, or RNA editing. When the cpRNPs dissociate from fully processed and mature RNAs, ribosomes then attach to the mRNAs for translation.

The cp31 and cp33 proteins have 64 and 42 residues, respectively, of auxiliary domains in their N terminus with 43% acidic residues (26). The N-terminal regions of these proteins may be functionally significant, because the acidic region is required for protein-protein interaction (54). The N-terminal acidic regions of some cpRNPs are efficiently phosphorylated in organello in a light-dependent manner, and association of cpRNPs with RNAs and their dissociation from RNAs may be regulated by phosphorylation in tobacco and spinach (55). To clarify this possibility, further biochemical and molecular analyses need to be carried out.

Acknowledgments—We thank T. Hirose and M. Mutsuda for valuable discussions and G. Schuster for critical reading of the manuscript.

REFERENCES

27. Ye, L., Li, Y., Fukami-Kobayashi, K., Gu, M., Konishi, T., Watanabe, A., and

\(^{2}\) T. Hirose and M. Sugiura, unpublished results.

\(^{3}\) A. Sakai and M. Sugiita, unpublished results.