Proc. Natl. Acad. Sci. USA
Vol. 78, No. 12, pp. 7304–7308, December 1981
Biochemistry

Cloned DNA sequences complementary to mRNAs encoding precursors to the small subunit of ribulose-1,5-bisphosphate carboxylase and a chlorophyll a/b binding polypeptide

(chloroplast protein/leaf cytoplasmic mRNA/cDNA synthesis/hybridization selection/chloroplast uptake and processing)

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Communicated by Philip Siekevitz, August 3, 1981

ABSTRACT **Double-stranded cDNA was synthesized from pea poly(A)-containing mRNA and inserted into the *Pst* I site of the bacterial plasmid pBR322 by the addition of synthetic oligonucleotide linkers. Bacterial colonies containing recombinant plasmids were detected by hybridization to partially purified mRNAs and further characterized by cell-free translation of hybridization-selected mRNAs. To confirm the identity of cDNA clones encoding chloroplast polypeptides, we incubated translation products derived from complementary mRNAs with intact chloroplasts *in vitro*. After uptake, precursor polypeptides were converted to their mature size and identified by fractionation of the chloroplast stroma and thylakoid membranes. By using these procedures, we have isolated and characterized cDNA clones encoding the two major cytoplasmically synthesized chloroplast proteins: the small subunit of ribulose-1,5-bisphosphate carboxylase and a constituent polypeptide (polypeptide 15) of the light-harvesting chlorophyll a/b–protein complex. Similarly, a third cDNA clone was isolated and shown to encode a 22,000-dalton thylakoid membrane polypeptide.**

The major products of cytoplasmic protein synthesis in pea leaves correspond to polypeptide components of two chloroplast proteins. These polypeptides are the small subunit of ribulose-1,5-bisphosphate (Rbu-1,5-P_2) carboxylase and the constituent polypeptides of the light-harvesting chlorophyll a/b binding protein. Both the small subunit (1–3) and the chlorophyll a/b polypeptides (4, 5) are synthesized, on free cytoplasmic polyribosomes, as soluble precursors that function in the posttranslational transport of the polypeptides from their site of synthesis into chloroplasts (2, 3). From studies on the mode of inheritance of peptide variants (6, 7) and from hybridization studies (8), it appears that these cytoplasmically synthesized polypeptides are encoded by nuclear genes. In contrast, the large subunit of Rbu-1,5-P_2 carboxylase is translated on chloroplast ribosomes (9) and is encoded by the chloroplast genome (10).

The selective expression of the genes encoding these cytoplasmically synthesized polypeptides is exhibited in a number of ways. Light induces the synthesis of both the small subunit of Rbu-1,5-P_2 carboxylase and the chlorophyll a/b polypeptides and this expression is apparently regulated at the level of mRNA synthesis (5, 11). In C4 plants, the small subunit of Rbu-1,5-P_2 carboxylase is synthesized in bundle sheath cells but not in mesophyll cells (12, 13) whereas the chlorophyll a/b polypeptides are made in mesophyll cells but in reduced amounts in bundle sheath cells (14). In these examples, the expression of the chloroplast gene encoding the large subunit of Rbu-1,5-P_2 carboxylase is coordinately regulated in a manner similar to that of the nuclear gene encoding the small subunit. RNA·DNA hybridization studies showed that the nuclear gene encoding the small subunit of Rbu-1,5-P_2 carboxylase is present in single, or close to single, copies per haploid genome (8). In contrast, the circular DNA molecules encoding the large subunit of Rbu-1,5-P_2 carboxylase are present in 15–30 copies per chloroplast (15) and leaf cells may contain several hundred chloroplasts (16). Consequently, the gene for the large subunit of Rbu-1,5-P_2 carboxylase may be reiterated several thousand-fold per cell.

We are interested in examining the factors involved in the selective expression of nuclear genes encoding cytoplasmically synthesized leaf polypeptides. In addition, we are interested in how the leaf cell coordinates the expression of the nuclear and chloroplast genomes. This latter question has added interest in view of the vastly different copy numbers of these two genomes. To study these questions, it is necessary to have molecular hybridization probes that can be used for the quantitation, isolation, and characterization of RNA and DNA sequences encoding the polypeptides of interest. We report here the characterization of cloned DNA sequences complementary to pea leaf mRNAs encoding precursors to the small subunit of Rbu-1,5-P_2 carboxylase and a chlorophyll a/b binding polypeptide. In a previous study, Bedbrook *et al.* (11) have reported the isolation and characterization of a cDNA clone encoding the small subunit of Rbu-1,5-P_2 carboxylase from pea.

EXPERIMENTAL PROCEDURE

Materials. Reverse transcriptase (RNA-dependent DNA nucleotidyltransferase) was supplied by Dr. G. W. Beard (Life Sciences, St. Petersburg, FL); restriction enzymes and T4 DNA ligase were from Bethesda Research Laboratories (Rockville, MD); nuclease S1 was from Sigma; DNA polymerase, T4 polynucleotide kinase, and calf intestinal alkaline phosphatase were from Boehringer Mannheim; cesium chloride was technical grade from Kawecki Berylco (Reading, PA); nitrocellulose was from Schleicher & Schuell; *N*-(nitrobenzyloxymethyl)-pyridinium chloride was from British Drug House (Poole, England); oligo(dT)$_{12-18}$ and *Pst* I linkers were from Collaborative Research (Waltham, MA); ^{32}P-labeled nucleotide triphosphates were from Amersham; and [^{35}S]methionine was from New England Nuclear.

RNA Extraction and Fractionation. Poly(A)$^+$ mRNA was prepared by poly(U)-Sepharose chromatography of cytoplasmic RNA from pea leaves (1). The mRNA was fractionated by centrifugation through sucrose gradients (8) and translation prod-

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Abbreviations: Rbu-1,5-P_2, ribulose-1,5-bisphosphate; CPII, chlorophyll a/b light-harvesting complex.

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ucts were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (17).

Double-Stranded cDNA Synthesis. mRNA (6 μg/60 μl of reaction mixture) was transcribed with reverse transcriptase (41 units) in 50 mM Tris·HCl, pH 8.5 at 25°C/90 mM KCl/10 mM $Mg(OAc)_2$/10 mM dithiothreitol/50 μM dNTP (dGTP, dATP, dTTP, and dCTP) containing 100 μCi (1 Ci = 3.7×10^{10} becquerels) of [α-^{32}P]dCTP and 4.8 μg of oligo(dT). Transcription was for 1 hr at 42°C and the resulting RNA·DNA hybrid was denatured by heating to 100°C for 2 min. For second-strand synthesis, the above reaction mixture was 200 μl of 50 mM Tris·HCl, pH 8.5 at 25°C/90 mM KCl/10 mM $Mg(OAc)_2$/10 mM dithiothreitol/500 μM dNTP containing 366 units of reverse transcriptase. Incubation was for 2 hr at 42°C. Nuclease S1 was used for the removal of single-stranded DNA sequences. To 180 μl of the above reaction mixture was added 720 μl of S1 buffer [0.3 mM NaCl/30 mM $Zn(OAc)_2$] and 5 μl (135 units) of nuclease S1. Incubation was for 30 min at 37°C and then 100 μl of 0.1 M Tris·HCl, pH 9.0 at 25°C, and 10 μl of 0.4 M EDTA, pH 8.0, was added. The reaction mixture was extracted with an equal volume of phenol and then twice with chloroform and then the DNA was recovered by ethanol precipitation.

Insertion of cDNA into pBR322. *Pst* I linkers were added to the double-stranded cDNA. The linkers (1.6 μg) were first phosphorylated at the 5′ end by incubating for 1 hr at 37°C in 10 μl of 60 mM Tris·HCl, pH 7.5 at 25°C/8 mM $mg(OAc)_2$/10 mM dithiothreitol/60 μM [α-^{32}P]ATP (200 μCi) containing bovine serum albumin at 100 μg/ml and 5 units of polynucleotide kinase. After incubation, the volume was increased to 20 μl such that all reaction components were at the same initial concentration, except that ATP was 1.5 mM and 5 additional units of kinase were added. This mixture was incubated for 1 hr at 37°C and then for 5 min at 65°C. Prior to the ligation of linkers, the double-stranded cDNA was treated with DNA polymerase I (18). The cDNA (0.5 μg) was incubated in 20 μl of 60 mM Tris·HCl, pH 7.5 at 25°C/8 mM $Mg(OAc)_2$/5 mM dithiothreitol/1 mM ATP/0.2 mM dNTP (dCTP, dGTP, dATP, and dTPP) containing bovine serum albumin at 100 μg/ml and 1 unit of DNA polymerase I. Incubation was for 10 min at 20°C and then the volume was increased to 30 μl by the addition of 2 μl of phosphorylated linkers (0.16 μg), 3 μl of T4 DNA ligase (12 units), and Tris·HCl, pH 7.5 at 25°C, $Mg(OAc)_2$, and dithiothreitol to maintain their initial concentrations. This mixture was incubated for 2 hr at 20°C. To cleave the ligated synthetic linkers, the reaction mixture was adjusted to 50 mM $(NH_4)_2SO_4$/20 mM Tris·HCl, pH 7.5 at 25°C/10 mM $Mg(OAc)_2$ in a final vol of 150 μl. The mixture was heated to 65°C for 5 min and then incubated at 37°C for 4 hr with 50 units of *Pst* I. After extraction with first phenol and then chloroform, the mixture was chromatographed on Sephacryl S-200 to separate the DNA from the linkers and ATP. The DNA was recovered from the column eluates by ethanol precipitation. Prior to the insertion of cDNA into pBR322, the plasmid was cleaved with *Pst* I and then treated with calf intestinal alkaline phosphatase (18, 19). The plasmid (1 μg) was then incubated for 2 hr at 15°C with 0.1 μg of cDNA in 36 μl of 60 mM Tris·HCl, pH 7.5 at 25°C/8 mM $Mg(OAc)_2$/5 mM dithiothreitol/1 mM ATP containing 2.5 units of T4 ligase. This ligation mix was used directly for transformation.

Transformation and Colony Selection. *Escherichia coli* C600 was treated with $CaCl_2$ and then transformed with recombinant plasmids (20). Tetracycline-resistant colonies were picked and screened for ampicillin sensitivity. Cleared lysates containing plasmid DNA were prepared from 1-ml cultures (21) and, after ethanol precipitation, one-half of the resolubilized lysate was spotted onto 5 × 10 cm nitrocellulose filters that were then heated at reduced pressure for 2 hr at 80°C. These filters, containing 50 lysates per filter, were used for hybridization to 5′-end-labeled mRNA (22). The labeled RNA was prepared from fractionated mRNA (3 μg; fractions 9 and 14 of Fig. 1). Hybridization was for 48 hr at 50°C in 50% deionized formamide/0.60 M NaCl/0.060 M Na_3 citrate, pH 7.0/20 mM sodium phosphate, pH 7.0, containing 50 μg of *E. coli* tRNA per ml, poly(A) at 20 μg/ml and 10^5 Cherenkov cpm of [^{32}P]RNA per filter. After hybridization, the filters were washed with hybridization buffer for two 30-min periods at 50°C and then with 0.30 M NaCl/0.030 M Na_3 citrate, pH 7.0, for two 30-min periods at room temperature. The washed filters were exposed for 24–48 hr at −80°C to Cronex 2DC x-ray film with Cronex Lightning Plus intensifying screens.

Hybridization and Elution of Specific RNA from DNA Filters. Plasmid DNA was prepared from alkaline $NaDodSO_4$ lysates of 1-liter bacterial cultures (21) and purified by isopycnic centrifugation in CsCl/ethidium bromide gradients. Diazobenzyloxymethyl-paper discs (1 cm) were prepared according to the method of Alwine *et al.* (23). Supercoiled plasmid DNA (50–100 μg) was denatured and fragmented by boiling for 5 min in 0.33 M NaOH. After neutralization and ethanol precipitation, the plasmid DNA was dissolved in 20 mM sodium phosphate, pH 6.0/80% dimethyl sulfoxide and added to freshly prepared diazobenzyloxymethyl-paper discs. After washing, the DNA filters were stored at 4°C in 50% formamide/10 mM Tris·HCl, pH 8.0. For hybridization of mRNA to DNA filters (23), poly(A)$^+$ mRNA (8 μg per filter) was incubated with one to six filters in 100–200 μl of 0.4 M NaCl/50% deionized formamide/0.2% $NaDodSO_4$/1 mM EDTA/10 mM 1,4-piperazinediethanesulfonic acid, pH 6.4, containing wheat germ tRNA at 360 μg/ml. Hybridization was initiated at 55°C and then the temperature was slowly decreased to 45°C over a period of 5 hr (cf. ref. 24). The filters were washed five times for 5 min each with hybridization solution at 37°C, for two 5-min periods at 25°C with 0.30 M NaCl/0.030 M Na_3 citrate, pH 7.0/0.5% $NaDodSO_4$, and once each with 0.15 M NaCl/0.015 M Na_3 citrate, pH 7.0/0.2% $NaDodSO_4$ and 2 mM EDTA, pH 8.0. RNA was eluted by boiling the filter twice for 1 min each with 100 μl of water and quickly cooling the eluates in dry ice/ethanol. The eluted RNA was pooled and ethanol precipitated with 6 μg of wheat germ tRNA as carrier and translated in a wheat germ cell-free system (25). Samples were analyzed on $NaDodSO_4$/polyacrylamide gels containing 7.5–15% acrylamide gradients (17). Radioactive polypeptides were detected by fluorography (26).

***In Vitro* Chloroplast Uptake Assays.** Translation products (20 μl) were incubated with intact pea chloroplasts (100 μg of chlorophyll) in 50 mM Hepes/KOH, pH 8.0/8.3 mM methionine/0.33 M sorbitol. After incubation at 25°C for 1 hr in the light, chloroplasts were pelleted and treated with proteases, and thylakoid membranes were separated from stromal protein as described (25). Stromal proteins were fractionated by sedimentation through 10–30% sucrose gradients. Samples were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis in 8 M urea gels having an acrylamide gradient of 12–18% (27). For the isolation of the chlorophyll a/b light-harvesting complex (CPII), thylakoid membranes were solubilized in 2% $LiDodSO_4$/60 mM Na_2CO_3/60 mM dithiothreitol/12% sucrose at 4°C and subjected to electrophoresis in 0.9 mM $MgCl_2$/9% polyacrylamide gels at 4°C (4). After electrophoresis, gels were stained, destained, and fluorographed.

RESULTS

Construction of cDNA Clones. Cytoplasmic poly(A)$^+$ mRNA from pea leaves was fractionated by centrifugation through a sucrose gradient. From this gradient, fractions were obtained that were enriched in mRNAs for either the small subunit of

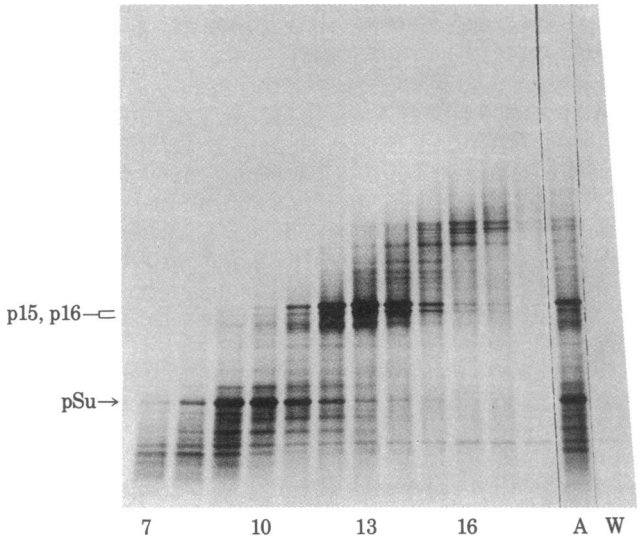

FIG. 1. Translation of fractionated pea leaf mRNA. Pea leaf cytoplasmic poly(A)$^+$ mRNA was fractionated by centrifugation through 5–20% sucrose gradients. mRNA in each fraction was translated in a wheat germ extract. Translation products derived from mRNA in fractions 7–18, unfractionated poly(A)$^+$ mRNA (A), and the wheat germ extract (W) were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and detected by autoradiography. p15, p16, and pSu, precursors to polypeptides 15 and 16 and to the small subunit of Rbu-1,5-P_2 carboxylase, respectively.

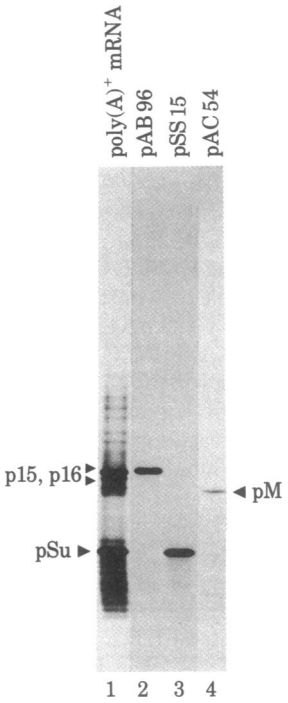

FIG. 2. Translation products derived from mRNAs purified by hybridization selection to recombinant plasmid DNAs. Polypeptides were fractionated by $NaDodSO_4$/polyacrylamide gel electrophoresis and detected by fluorography. The polypeptides were derived from translation of cytoplasmic poly(A)$^+$ mRNA or mRNAs complementary to plasmids pAB96, pSS15, and pAC54. pM, unidentified translation product.

Rbu-1,5-P_2 carboxylase or the chlorophyll a/b binding polypeptides (Fig. 1). Both unfractionated and fractionated mRNAs were used for the synthesis of cDNAs. Reverse transcriptase was used for the synthesis of both the first and second cDNA strands. We have discussed details of this reaction elsewhere (28).

For insertion of the cDNA into the plasmid pBR322, *Pst* linkers were ligated to the double-stranded cDNA. Recombination of the cDNAs and the linearized plasmid was achieved with T4 DNA ligase. The recombinant plasmids were used to transform *E. coli* C600. Tetracycline-resistant colonies were picked from the transformation mixture and, from these, ampicillin-sensitive colonies were selected.

Colony Selection. Bacterial colonies containing cDNA sequences complementary to the major pea leaf mRNAs were detected by filter hybridization of cleared lysates to labeled fractionated mRNAs. Colonies were selected on the basis of differential hybridization to mRNAs enriched in sequences encoding precursor polypeptides to the small subunit of Rbu-1,5-P_2 carboxylase and the chlorophyll a/b binding polypeptides (fractions 9 and 14, respectively, of Fig. 1). Selected colonies were further analyzed for the ability of their plasmid DNA to hybridize complementary mRNAs, which were then assayed by cell-free translation (Fig. 2). By this procedure, plasmid DNA from clone pAB96 was shown to hybridize to mRNA encoding a polypeptide comigrating with the precursor (p15) to a chlorophyll a/b binding polypeptide (Fig. 2, lane 2). In pea, CPII contains at least two immunologically related polypeptides of 28,000 and 27,000 daltons (designated 15 and 16, respectively); both are synthesized as larger forms, p15 (33,000 daltons) and p16 (32,000 daltons), in cell-free translation systems (4). Under the hybridization conditions used in our experiments, plasmid DNA from clone pAB96 selectively hybridizes mRNA encoding the precursor (p15) to polypeptide 15.

A second clone, pSS15, hybridizes to a mRNA that, on translation, yields a protein that comigrates with the precursor polypeptide (pSu) to the small subunit of Rbu-1,5-P_2 carboxylase (Fig. 2, lane 3). A third clone, pAC54, hybridizes to a mRNA encoding an as yet unidentified translation product (pM) of 30,000 daltons (Fig. 2, lane 4).

Chloroplast Uptake and Processing. To confirm the identity of recombinant plasmids containing sequences complementary to mRNAs encoding cytoplasmically synthesized chloroplast polypeptides, we used the fact that >100 stromal polypeptides and 20–30 thylakoid membrane polypeptides can be taken up into intact chloroplasts when the latter are incubated with cell-free translation products under optimal conditions in the light (25). Thus, by incubating with intact chloroplasts translation products derived from any mRNA purified by hybridization to recombinant plasmid DNA, we can identify precursor forms of chloroplast proteins. After uptake, chloroplasts can be lysed and sedimented at 30,000 × *g* to separate stromal polypeptides from thylakoid membrane proteins. By analysis of the radioactive polypeptides present in these two fractions, we can determine further the subchloroplast location of the processed polypeptides.

We have shown by such an analysis that the translation products encoded by mRNA complementary to cDNA sequences from clones pSS15, pAB96, and pAC54 represent cytoplasmically synthesized precursors for chloroplast proteins (Fig. 3). Thus, the identity of the precursor polypeptide pSu is confirmed by the fact that, when it is incubated with intact chloroplasts, it is taken up and processed to a stromal polypeptide comigrating with the mature small subunit of Rbu-1,5-P_2 carboxylase (Fig. 3, lanes 3 and 4). Further evidence for this conclusion is obtained by the finding that >90% of the newly transported small subunit is found in association with the Rbu-1,5-P_2 carboxylase large subunit when the 18S holoenzyme is resolved by sedimentation of the chloroplast stroma through 10–30% sucrose gradients (3) (Fig. 4*A*).

In a similar experiment, when the translation product encoded by mRNA complementary to pAB96 sequences is incubated with intact chloroplasts, the major radioactive polypep-

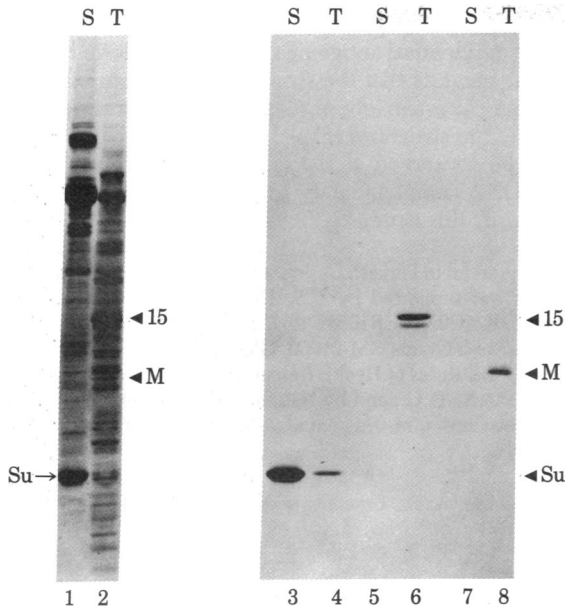

FIG. 3. Chloroplast uptake of translation products derived from hybridization-selected mRNAs. Polypeptides were fractionated by $NaDodSO_4$/polyacrylamide gel electrophoresis. Lanes 1 and 2, chloroplast stromal (S) and thylakoid (T) polypeptides were detected by staining. Lanes 3–8, imported translation products were detected by fluorography. Translation products were derived from hybridization-selected mRNAs complementary to plasmids pSS15 (lanes 3 and 4), pAB96 (lanes 5 and 6) and pAC54 (lanes 7 and 8). 15, polypeptide 15; M, unidentified product; Su, small subunit of Rbu-1,5-P_2 carboxylase.

tide transported into the chloroplasts can be found exclusively in the membrane fraction comigrating with the mature 28,000-dalton chlorophyll a/b binding polypeptide 15 (Fig. 3, lanes 5 and 6). Since plasmid DNA from clone pAB96 selectively hybridizes to mRNA encoding p15, it is likely that the faster migrating species results from some proteolysis of polypeptide 15 after chloroplast lysis. To confirm that the newly transported polypeptide was indeed a constituent of the chlorophyll a/b–protein complex, thylakoid membranes were solubilized with $LiDodSO_4$ and the CPII complex was isolated by polyacrylamide gel electrophoresis at 4°C. Fluorography of the gel shows that ≈30% of the newly transported polypeptide has assembled into the chlorophyll a/b–protein complex (Fig. 4*B*, lane 4). On heating, the chlorophyll–protein complex is dissociated, resulting in the release of radioactive polypeptide 15 (Fig. 4*B*, lane 3).

In addition to identifying the polypeptides encoded by mRNA complementary to cDNA sequences of plasmids pSS15 and pAB96, we characterized a chloroplast polypeptide encoded by mRNA complementary to clone pAC54. When the 30,000-dalton translation product (pM), derived from mRNA purified by hybridization to plasmid pAC54, was incubated with intact chloroplasts as described above, it was taken up by chloroplasts and processed to a 22,000-dalton membrane protein (Fig. 3, lanes 7 and 8). Further fractionation of thylakoid membranes will be required to determine the function of this protein.

Size of Cloned cDNAs and Complementary mRNAs. Isolated plasmid DNA was cleaved with *Pst* I and the resulting fragments were fractionated by agarose gel electrophoresis (Fig. 5*A*). Plasmids pSS15 and pAB96 yielded inserts of 740 and 850 nucleotides, respectively. By agarose gel electrophoresis under denaturing conditions (29), the complementary mRNAs, encoding the small subunit of Rbu-1,5-P_2 carboxylase and the chlorophyll a/b binding polypeptide, were estimated to be 850 and 1100 nucleotides, respectively (Fig. 5*B*). From these results, it is clear that neither cloned cDNA is full length although the

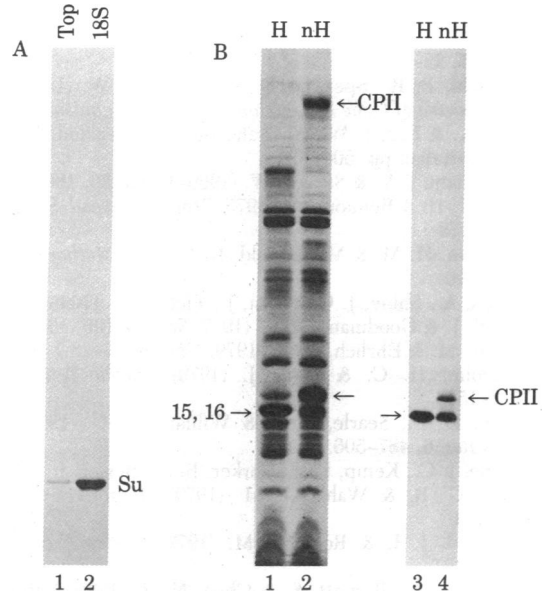

FIG. 4. Assembly of polypeptides transported into isolated chloroplasts. (*A*) Polypeptides were separated on sucrose gradients and then the top and 18S fractions were examined by $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography. (*B*) Assembly of polypeptide 15 into CPII was followed by $LiDodSO_4$/polyacrylamide gel electrophoresis of heated (H) and not heated (nH) thylakoid membranes. The gel was stained (lanes 1 and 2) and then fluorographed (lanes 3 and 4). 15 and 16, polypeptides 15 and 16; Su, small subunit of Rbu-1,5-P_2 carboxylase.

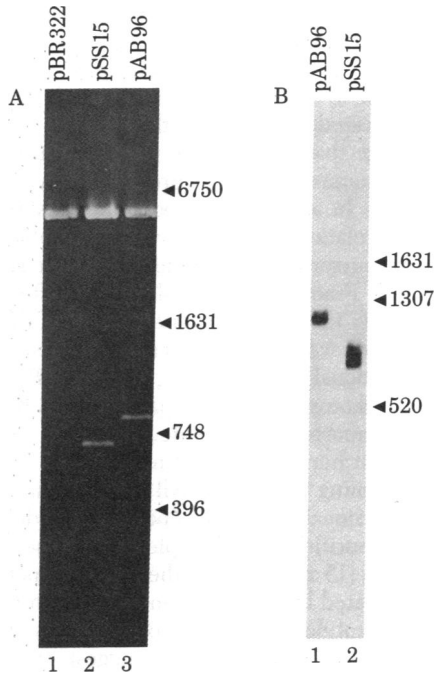

FIG. 5. Gel electrophoresis of cloned cDNAs and complementary mRNAs. (*A*) Plasmid DNA was cleaved with *Pst* I and fractionated by electrophoresis through 0.8% agarose gels. The gel was stained with ethidium bromide and photographed under UV light. (*B*) Pea leaf cytoplasmic poly(A)$^+$ mRNA was denatured with formamide/formaldehyde, fractionated by electrophoresis through 1.25% agarose gels, and then transferred to nitrocellulose (29). Hybridization was performed with nick-translated plasmids pSS15 or pAB96 and hybrids were detected by autoradiography. Molecular lengths were determined by comparison with DNA cleavage fragments of known size.

small subunit clone is close. Sequence studies on pSS15 have confirmed this conclusion; the cloned cDNA contains all the 3'-noncoding sequences, all the coding sequences for the mature small subunit, and 100 nucleotides of the coding region for the transit sequence (G. Coruzzi, personal communication). The availability of this cloned cDNA has enabled us to compare with, and extend, the sequence studies recently reported by Bedbrook *et al.* (11).

DISCUSSION

We have isolated three cloned cDNA sequences complementary to pea leaf poly(A)$^+$ mRNA. Two of these cloned DNAs correspond to sequences encoding the major cytoplasmically synthesized leaf proteins; these are the small subunit of Rbu-1,5-P_2 carboxylase and one of the constituent polypeptides of the light-harvesting chlorophyll a/b–protein complex. The identity of these cloned DNAs was suggested by NaDodSO$_4$/polyacrylamide gel electrophoresis of translation products derived from complementary mRNAs and confirmed by uptake and processing of the translation products with isolated chloroplasts. The precursor of the small subunit of Rbu-1,5-P_2 carboxylase is taken up and processed, by isolated chloroplasts, to yield the mature small subunit, which in turn assembles with the large subunit to form the soluble native Rbu-1,5-P_2 carboxylase. Similarly, the precursor for the chlorophyll a/b binding polypeptide (p15) is processed by isolated chloroplasts to yield polypeptide 15, which integrates into thylakoid membranes and becomes associated with chlorophyll a/b and polypeptide 16 to form the CPII light-harvesting complex. The third cloned cDNA encodes a polypeptide (pM) of 30,000 daltons. This polypeptide is taken up by isolated chloroplasts and processed to a 22,000-dalton thylakoid membrane polypeptide.

We have described here an efficient procedure for screening cDNA clones encoding chloroplast polypeptides. By allowing translation products derived from mRNAs complementary to recombinant plasmid DNA to be taken up by chloroplasts *in vitro*, it is possible to distinguish chloroplast polypeptides from those that are located elsewhere in the leaf cell. This method has the advantage that it avoids the use of monospecific antibodies and immunoprecipitation of precursor polypeptides synthesized *in vitro*. In addition, it is possible to establish a precursor–product relationship for a given polypeptide in which the selected precursor is converted to the mature size by chloroplasts *in vitro*. Fractionation of chloroplasts after uptake allows us to identify the subchloroplast location of the imported polypeptide. Further fractionation of chloroplast stroma or thylakoids into functional complexes (e.g., Rbu-1,5-P_2 carboxylase holoenzyme or photosystem I or II particles) can then be used to identify the imported polypeptide.

Also of interest here is the exact number and nature of the polypeptides forming the chlorophyll a/b–protein (CPII) complex. One-dimensional NaDodSO$_4$/polyacrylamide gel electrophoresis of the purified CPII complex shows the presence of two polypeptides (15 and 16). That these are distinct translation products is indicated by cell-free translation, which yields two precursor polypeptides (p15 and p16), and close examination of the autoradiograph shown in Fig. 1 suggests that these polypeptides are the product of distinct mRNAs. This is concluded from the observation that the relative amounts of the two polypeptides change according to the gradient fraction from which the mRNA is derived, the mRNA encoding p15 sedimenting more slowly than the mRNA encoding p16. Consistent with the conclusion that polypeptides 15 and 16 are translation products of distinct mRNAs is the observation (Fig. 2), that, under the hybridization conditions used here, plasmid pAB96 hybridizes to mRNA encoding p15 but not to mRNA encoding p16. This was not necessarily the expected result, since it has been shown previously that polypeptides 15 and 16 are structurally related, as they share identical antigenic sites (4). The above hybridization result suggests that the structural relationship is a rather limited one. Alternatively, through the degeneracy of the genetic code, the structural relatedness at the polypeptide level may not be conserved at the genetic level. The isolation of cloned cDNA complementary to p16, and sequence studies, should clarify this issue.

We are grateful to Parkash Cashmore for skilled technical assistance. This work was supported by U.S. Department of Energy Research Contract DE-AC02-80ER10581 to A.R.C.; by National Institutes of Health Research Grants GM-21060, GM-25114, and PHS PR07065-15; by National Institutes of Health Research Career Development Award GM-00223 to N.-H.C.; and by National Institutes of Health Postdoctoral Fellowships GM-07446 and GM-06678 to R.B and S.G.B., respectively.

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