Plant Physiol. (1981) 67, 1090–1096
0032-0889/81/67/1090/07/$00.50/0

Synthesis of Chloroplast Proteins during Germination and Early Development of Cucumber

Received for publication May 27, 1980 and in revised form November 7, 1980

RICHARD WALDEN[1, 2] AND CHRISTOPHER J. LEAVER
Department of Botany, University of Edinburgh, Edinburgh, Scotland, United Kingdom

ABSTRACT

Cell-free protein synthesizing systems have been used to study the developmental changes in the synthesis of chloroplast proteins in the cotyledons of cucumber seedlings grown in the light or in the dark. *Escherichia coli* and wheat germ *in vitro* protein synthesizing systems have been used to assay the changes in the levels of the mRNA's coding for ribulose 1,5-bisphosphate carboxylase (RuBPCase). The large subunit of cucumber RuBPCase has been identified among the translation products of the *E. coli* system. The wheat germ system translates the cucumber mRNA coding for the small subunit of RuBPCase to produce a 25,000 molecular weight precursor polypeptide. Plastids isolated from light-grown cotyledons were used to study developmental changes in their capacity to synthesize protein. The data obtained indicate that in the light there is an initial 48-hour period of accumulation of the mRNA's coding for the large and small subunits of RuBPCase, coupled with an increase in the capacity of the isolated plastids to synthesize protein. This is followed by a decline. This decline is not reflected in the accumulation of RuBPCase in the cotyledons which remains constant over the period of study.

The cotyledons of seeds which exhibit epigeal germination provide a useful system to study plastid development. As part of a program to study germination and development of cucumber (*Cucumis sativus*) seedlings (4) we became interested in the acquisition of photosynthetic competence by the cotyledons. During seedling development, the initial source of energy for the seedling is provided by the metabolism of stored lipid (4). However, once the cotyledons emerge above the soil there is a decline in the activities of the enzymes that are involved in lipid metabolism. This decline coincides with increases in the levels of phototrophic indicators such as Chl and RuBPCase[3] (4). Our aim was to study the control mechanisms involved in the synthesis of chloroplast proteins during the transition of the cotyledon from growth on endogenous reserves to growth on newly fixed carbon.

In this paper we describe the use of cell-free protein synthesizing systems to study developmentally related changes in the levels of translatable mRNAs which code for RuBPCase within the cotyledons of cucumber seedlings. Previous workers have shown that *in vitro* translation systems can be used to translate the mRNAs coding for the LSu and SSu of RuBPCase. The *Escherichia coli* system has been used to translate the chloroplast mRNA coding for LSu (14), whereas the wheat germ system has been used to translate the cytoplasmic mRNA coding for the pSSu of RuBPCase (10). It has been shown that the pSSu synthesized *in vitro* can be transported into isolated chloroplasts and processed into the mature SSu of RuBPCase (9, 31). Using cucumber total RNA to program an *E. coli* and a wheat germ translation system we have identified cucumber LSu and pSSu, respectively, among the translation products by immunoprecipitation. We have compared the developmental changes in the levels of mRNAs coding for RuBPCase, as assayed *in vitro*, not only with the changes in the amounts of RuBPCase protein observed in the cotyledons but also with the ability of plastids isolated from the cotyledons at different stages of light-grown seedling development to synthesize protein. A preliminary report of this work has been presented (33).

MATERIALS AND METHODS

Growth Conditions and Assays. The growth conditions of *Cucumis sativus* L. var. "Long Green Ridge" have been described (4). Seeds were either kept in continuous darkness (dark-grown) or illuminated for 12 h/day with a mixture of fluorescent and incandescent lamps at an approximate intensity of 6,500 lux (light-grown). The determination of the amounts of RuBPCase protein in the cotyledons was carried out by the electrophoretic fractionation of a preparation of total cucumber protein using 5% nondenaturing polyacrylamide gels and the RNA contents were determined by the perchloric acid technique (4). Determination of the amounts of Chl was carried out by the method of Arnon (2).

Total RNA Preparation. Cotyledonary RNA was extracted at 4 C by the phenol procedure of Leaver and Ingle (18). Fractionation into poly(A)$^+$ and poly (A)$^-$ RNA was carried out by chromatography on oligo(dT)-cellulose (Collaborative Research Type T2) by the method of Aviv and Leder (3).

Chloroplast RNA Preparation. All operations were carried out at 4 C. Chilled tissue was homogenized in four volumes of semi-frozen 0.33 M sorbitol, 50 mM Tris-HCl (pH 7.0), 5 mM $MgCl_2$, and 1 mM NaCl with a Willem's Polytron (Northern Media Supply Ltd, Hull, U.K.) with two 4-s bursts at half speed. The macerate was squeezed through two layers of muslin and filtered through eight layers of muslin into 50-ml plastic centrifuge tubes and centrifuged from rest to 4,000*g* in approximately 90 s. The supernatant was removed and the pellet gently resuspended in the homogenization buffer with a paint brush and recentrifuged. The chloroplasts were washed three times by this procedure. Chloroplasts were lysed by resuspension in homogenization buffer lacking sorbitol. To the resulting suspension was added an equal volume of 100 mM Tris-HCl (pH 8.5), 6% (w/v) *p*-aminosalicylic acid, and 1% (w/v) tri-propylnaphthalenesulfonic acid and the nucleic acid extracted by the method of Leaver and Ingle (18).

***In Vitro* Protein Synthesis.** The *E. coli* cell-free translation system was prepared from the CSH 73 strain of *E. coli* (23) as described by Modolell (24). Each incubation contained in a final

[1] Recipient of a Science Research Council postgraduate studentship.

[2] Present address: Department of Biology C-016, University of California, San Diego, La Jolla, CA 92096.

[3] Abbreviations: RuBPC, ribulose bisphosphate carboxylase; SSu, small subunit, LSu, large subunit; pSSu, precursor of small subunit; poly(A)$^+$, poly A-containing RNA; poly(A)$^-$, poly A-lacking RNA; GTP, guanosine triphosphate; CCCP, carbonyl cyanide *m*-chlorophenylhydrozone.

volume of 50 μl: 2 A_{260} units of *E. coli* extract, 50 mM Tris-HCl (pH 8.0), 75 mM NH_4 acetate, 10 mM Mg acetate, 10 mM K acetate, 10 mM P-enolpyruvate, 6 mM ATP, 1 mM GTP, 0.48 units pyruvate kinase, 2 mM DTT, 160 mM calcium leucovorin, 25 μM of each amino acid except methionine, 5 μCi [^{35}S]methionine (Radiochemical Center, Amersham U.K.) and the quantities of each RNA as described in the text. Incubations were carried out at 37 C for 20 min and except when immunoprecipitations were being carried out were terminated by the addition of acetone to a final concentration of 80%.

The wheat germ translation system was prepared from wheat germ (General Mills) by the method of Marcu and Dudock (21). Each incubation was carried out in a final volume of 50 μl containing: 1.4 A_{260} units of wheat germ extract, 28 mM Hepes-NaOH (pH 7.8), 104 mM K acetate, 2.25 mM Mg acetate, 0.25 mM spermidine, 1 mM ATP, 50 μM GTP, 8 mM creatine phosphate, 5 mg creatine phosphokinase, 2 mM DTT, 25 μM of each amino acid except methionine, 5 μCi [^{35}S]methionine, and the amounts of RNA as described in the text. Incubations were carried out at 25 C for 90 min and except when immunoprecipitations were being carried out were terminated by the addition of acetone to a final concentration of 80%.

Protein synthesis in isolated plastids was carried out essentially as described by Siddell and Ellis (29). All materials were sterilized by autoclaving or by washing in methanol and rinsing in sterilized H_2O. All buffers were sterilized by filtration. Plastids were prepared and incubated by the method of Siddell and Ellis (29). Incubations were carried out in a final volume of 300 μl containing 10 μCi[^{35}S]methionine (1,005–1,400 Ci/mmol). For each day of development, plastids were isolated from 5 g of fresh tissue. The plastid protein concentration of each incubation was between 1.6 and 2.2 mg. Protein concentrations were estimated by the procedure of Lowry *et al.* (19) after initial precipitation and washing of the protein with 5% (w/v) trichloroacetic acid. After a 90-min incubation the incorporation was terminated by the addition of acetone to a final concentration of 80%.

With each experimental system the amount of radioactivity incorporated into protein was estimated by the method of Mans and Novelli (20).

Analysis of *in Vitro* Translation Products. Acetone precipitates were collected by centrifugation at 12,000*g* for 30 s and precipitates were resuspended in 60 mM Tris-HCl (pH 6.8), 2% (w/v) SDS, 5% β-mercaptoethanol, 10% (w/v) sucrose, 0.001% bromophenol blue and fractionated by electrophoresis on 15 or 20% SDS-polyacrylamide gels as described by Laemmli (17). Labeled products were detected by exposure of Regulix BB5 x-ray film to the dried gels.

Immunoprecipitation. Immunoprecipitations were carried out with antisera raised against complete spinach RuBPCase (gift of W. Bottomley) or raised against gel purified LSu or SSu of cucumber RuBPCase as described (27). Immunoprecipitations were carried out by using complete incubation mixtures of the *E. coli* system or the ribosomal supernatants from the wheat germ system. In the latter case this was prepared by the centrifugation of the incubation mixture at 105,000*g* for 60 min, and aliquots of the supernatant were used for immunoprecipitation. A suitable amount of antisera, estimated by titration experiments, was added to 50 μl of the incubation mixture or the ribosomal supernatants and the volume was made up to 300 μl by the addition of 10 mM Tris-HCl (pH 7.5), 5 mM EDTA, 1% (w/v) Triton X-100, and 500 mM NaCl. Immunoprecipitations were carried out at 27 C for 1 h then at 4 C for 16 h. The immunoprecipitates were collected by the method of Kessler (16) by using *Staphylococcus aureus*—bound protein A as described (27).

Estimation of Radioactivity Present in Polyacrylamide-Gel Slices. The relevant labeled polypeptide was located by autoradiography and excised from the SDS-polyacrylamide gel. Each gel slice was dissolved in 0.6 ml of 100 volume H_2O_2 in a scintillation vial. The vials were placed in an oven at 80 C for 3 h. The vials were then cooled and 5 ml of a 1:2 mixture of Triton X-100:toluene containing 0.4% (w/v) butylphenolbiphenyloxadiazone added and the radioactivity estimated by scintillation counting.

RESULTS

Data concerning the developmental changes in cotyledonary protein, RNA, and enzyme activities in cucumber seedlings have been presented (4). Of particular interest to us was the accumulation of RuBPCase (Fig. 1). In seedlings grown under a 12-h photoperiod, significant RuBPCase synthesis is detected between days 2 and 3, approximately 24 h before Chl (and associated membrane polypeptides). Unlike RuBPCase, the synthesis of Chl is light-dependent and is initiated when the cotyledons emerge above the soil on day 4. The initial rate of accumulation of RuBPCase is similar in light- and dark-grown tissue. However, after day 4 in the dark the rate of RuBPCase accumulation declines, whereas in the light the rate of accumulation is maintained. This is in agreement with the finding that light is not required for the accumulation of RuBPCase in the cotyledons of mustard and castor bean seedlings (11, 25). During the time period under study, the cell number of the cotyledons remains constant; (4) hence, all data presented here, expressed on a per cotyledon basis, can in effect be extrapolated to a per cell basis.

Synthesis of Chloroplast Proteins *in Vitro*. In the absence of cDNA probes to assay for specific mRNAs by hybridization, we sought to assay levels of mRNAs by quantitative translation by using cell-free protein synthesizing systems. We used an *E. coli* system to translate preferentially chloroplast mRNAs and a wheat germ system optimized to translate cytoplasmic mRNAs.

When the optimized *E. coli* system is programmed with RNA extracted from the cotyledons of light-grown cucumber seedlings and the [^{35}S]methionine-labeled translation products fractionated by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography, a range of polypeptide products are seen (Fig. 2). The profile of the translation products programmed by total and chloroplast RNA are essentially the same. This lends support to the proposal that the *E. coli* system, when programmed with total RNA extracted from a higher plant, preferentially translates chloroplast RNA (7). Among the translation products programmed by total and chloroplast RNA is a 54,000 mol wt polypeptide which coelectrophoreses with purified LSu of Ru-

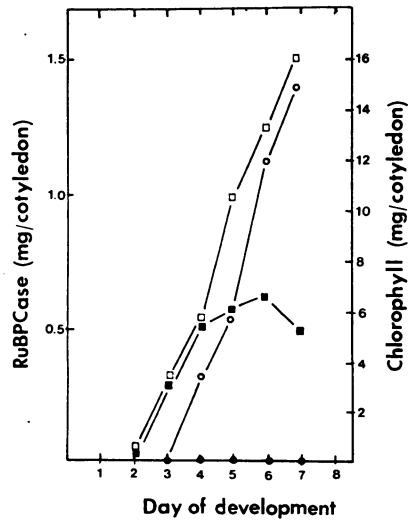

FIG. 1. Developmental changes in RuBPCase protein and Chl in cotyledons of light-grown (○, □) and dark-grown (●, ■) cucumber seedlings. Amounts of RuBPCase (□——□) and amounts of Chl (○——○).

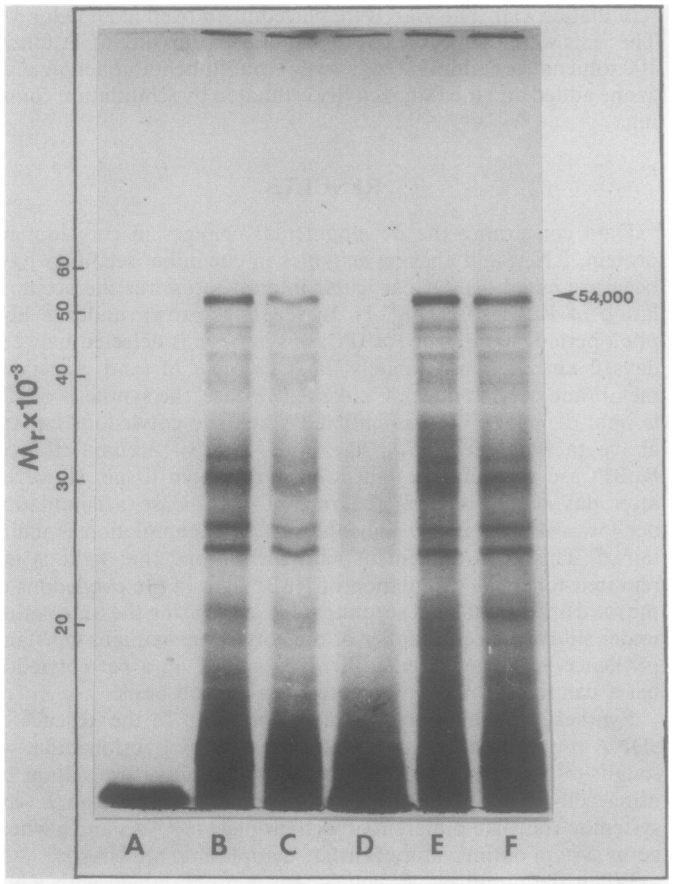

FIG. 2. Autoradiographs of the SDS-polyacrylamide-gel profiles of the translation products of the *E. coli* system programmed with 5-day light-grown cucumber cotyledon RNA. (A) minus RNA; (B) and (F) total RNA, 28.8 μg; (C) chloroplast RNA, 28.0 μg; (D) poly(A)$^+$ RNA, 1.0 μg; (E) poly(A)$^-$ RNA 29.0 μg.

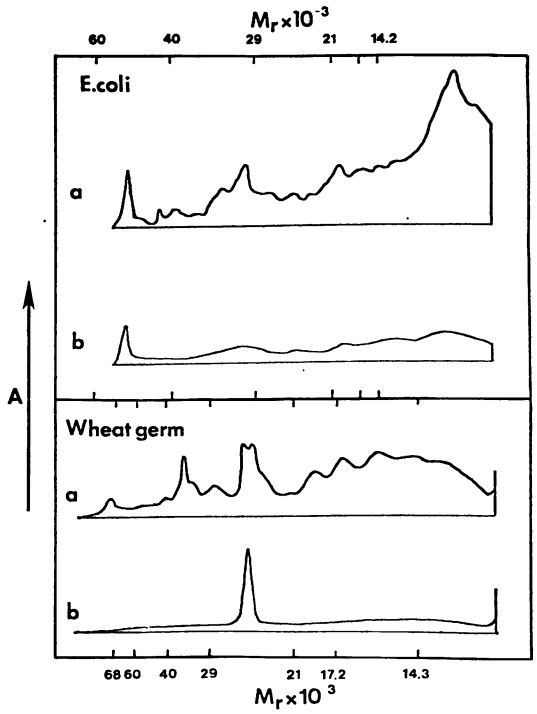

FIG. 3. Densitometer scans of the autoradiographs of the SDS-polyacrylamide-gel profiles of the immunoprecipitates obtained from the *E. coli* and wheat germ translation systems programmed with 5-day light-grown cucumber cotyledon RNA by using antisera raised against spinach RuBPCase. Electrophoresis was carried out on 15 and 20% polyacrylamide gels, respectively, for the *E. coli* and wheat germ translation products. In both cases (a) complete translation products (b) immunoprecipitates.

BPCase. This polypeptide is absent from the translation product profile programmed by poly(A)$^+$ RNA prepared by fractionation of total RNA on an oligo (dT) cellulose column. Previous workers have shown that the mRNA coding for the LSu of RuBPCase does not contain a poly(A) sequence (34). This suggested that the 54,000 mol wt polypeptide synthesized *in vitro* was the cucumber LSu. Confirmation of this was provided by immunoprecipitation. The 54,000 mol wt polypeptide was immunoprecipitated by antisera raised against either complete spinach RuBPCase or gel-purified cucumber LSu (Fig. 3).

The translation product profile of the wheat germ system programmed with cucumber RNA is different from that of the *E. coli* system programmed with the same RNA (Fig. 4). Under the conditions used, chloroplast RNA does not serve as an efficient template for the wheat germ system. This result is in agreement with those of previous workers (7) who programmed an *E. coli* and wheat germ system with spinach RNA and suggests that the wheat germ system preferentially translates cytoplasmic mRNAs. We have previously identified among the translation products of the wheat germ system programmed by cucumber RNA the enzymes involved with lipid metabolism, namely isocitrate lyase, catalase, malate dehydrogenase, and malate synthase (27). When the system is programmed with total, poly(A)$^+$ and poly(A)$^-$ RNA the translation product profiles are essentially similar.

Other workers have shown that the wheat germ system translated the mRNA coding for the SSu of RuBPCase as a precursor polypeptide of approximately 20,000 mol wt (10). Therefore, we sought to identify the pSSu coded for by cucumber RNA. However, no major polypeptides synthesized in the wheat germ system under the direction of cucumber RNA were of 20,000 mol wt (Fig. 4). To identify the cucumber pSSu, antisera raised against either complete spinach RuBPCase or gel-purified cucumber SSu were added to the translation products of the wheat germ system. A single polypeptide of approximately 25,000 mol wt, which is a major translation product of the wheat germ system when programmed with cucumber RNA, was immunoprecipitated (Fig. 3).

To investigate the developmental changes in the capacity of the plastids to synthesize proteins, the experimental system of Siddell and Ellis (29) was adopted. This involved the incubation of a plastid preparation from the cotyledons of light-grown seedlings with [^{35}S]methionine. We were unsuccessful in our attempts to isolate plastids from dark-grown tissue which were able to incorporate radioactivity into protein. An example of the characteristics of protein synthesis in plastids isolated from cucumber cotyledons is shown in Table I. Protein synthesis in isolated plastids can be driven by light energy or by addition of an exogenous source of ATP. The ability of ATP to replace light as a source of energy has been observed by previous workers (6, 8), and has lead to the suggestion that this may be due to the incorporation of radioactivity by lysed plastids. That this is the case can be excluded by the observation that incorporation of radioactivity is largely insensitive to ribonuclease, plastid intactness is thought to be directly related to the sensitivity of protein synthesis to ribonuclease (6). Incorporation of radioactivity is inhibited by D-threochloramphenicol but not by cycloheximide, indicating that protein synthesis is taking place on 70S ribosomes. CCCP, a commonly used inhibitor of photophosphorylation inhibits the incorporation by 50%. Ellis and co-workers have found that CCCP totally inhibits protein synthesis in isolated chloroplasts, whereas with isolated etioplasts the inhibition was only 22% (6, 29). The intermediate effect of CCCP observed here is thought to be due to the developmental age of the plastids used. The amount of radioactivity

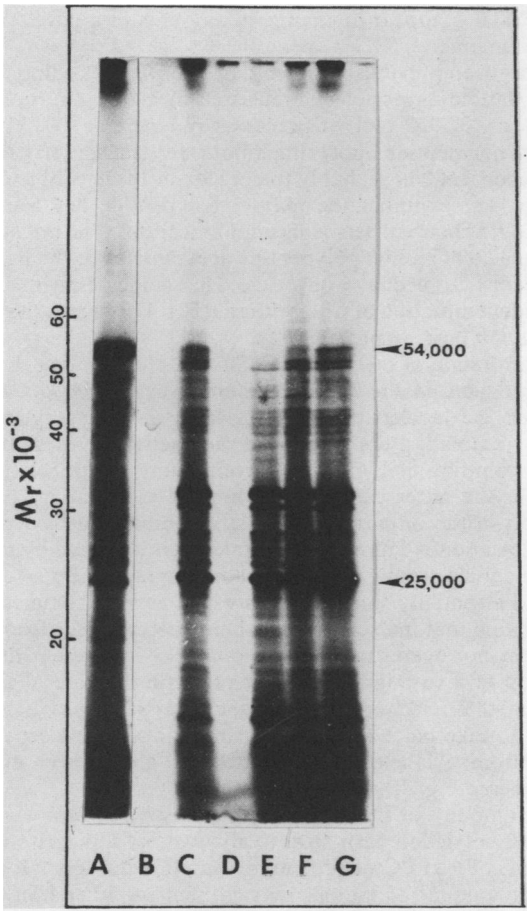

FIG. 4. Autoradiographs of the SDS-polyacrylamide-gel profiles of the translation products of the *E. coli* and wheat germ systems programmed with 5-day light-grown cucumber cotyledon RNA. (A) *E. coli* system, total RNA, 28.8 μg; (B) to (G) wheat germ system; (B) minus RNA; (C) and (G) total RNA, 28.8 μg; (D) chloroplast RNA, 28.0 μg; (E) poly(A)$^+$ RNA, 0.5 μg; (F) poly(A)$^-$ RNA 29.5 μg.

Table I. *The Effect of the Energy Source and Inhibitors on the Incorporation of [^{35}S]Methionine into Protein by Chloroplasts Isolated from Day 6 Cucumber Cotyledons*

Chloroplasts were isolated from cucumber cotyledons from day 6 of light-grown seedling development and incubated as described. In each case where ATP was used the final concentration was 2 mM. Incorporation by the light driven system is called 100%.

Energy Source	Treatment	Incorporation
		%
Light	Complete	100
None	Complete	8
Light + ATP	Complete	126
ATP	Complete	100
Light	+ D-Threochloramphenicol (50 μg/ml)	27
None	+ D-Threochloramphenicol (50 μg/ml)	6
Light	+ Cycloheximide (12.5 μg/ml)	100
None	+ Cycloheximide (12.5 μg/ml)	6
Light	+ Ribonuclease (33 μg/ml)	80
Light	+ CCCP (5 μM)	50
Light + ATP	+ CCCP (5 μM)	70
None	+ Na acetate (20 mM)	7

incorporated in the dark with the addition of Na acetate allowed us to assess the amount of bacterial contamination of the plastid preparation, and indicates that it is very low.

The proteins synthesized in plastids isolated from the cotyledons of light-grown cucumber seedlings resemble those described by other workers using chloroplasts isolated from pea, spinach, or maize (12, 13, 22) (Fig. 5). Provisional identification of the major labeled polypeptides was provided by coelectrophoresis with purified proteins, including LSu of RuBPCase and the α- and β-subunits of chloroplast ATPase (59,000 and 56,000 mol wt, respectively).

Developmental Changes in the Synthesis of Chloroplast Proteins during Seedling Development. Quantitation of a specific translatable mRNA requires the quantitative translation of the mRNA *in vitro*. With both *E. coli* and wheat germ system, it was found that under the conditions used, with increasing amounts of total cotyledon RNA up to 20 μg RNA/50 μl incubation, the overall incorporation of radioactivity into protein was linear, and the synthesis of both LSu and pSSu bore a linear relationship to total protein synthesis. Thus, it was possible to use the *in vitro* systems to assay both LSu and pSSu mRNAs by the quantitation of the amount of LSu and pSSu synthesized *in vitro*. In experiments to investigate developmental changes in the amounts of these mRNAs, the *in vitro* translation systems were programmed with 15 μg of total RNA extracted from the cotyledons of seedlings of different ages, grown under either a 12-h photoperiod or in darkness (Figs. 6 and 7).

Three points emerge from the analysis of the translation products of both the *E. coli* and wheat germ systems programmed with equal amounts of cotyledonary RNA extracted from light- and dark-grown seedlings. First, there are developmentally related

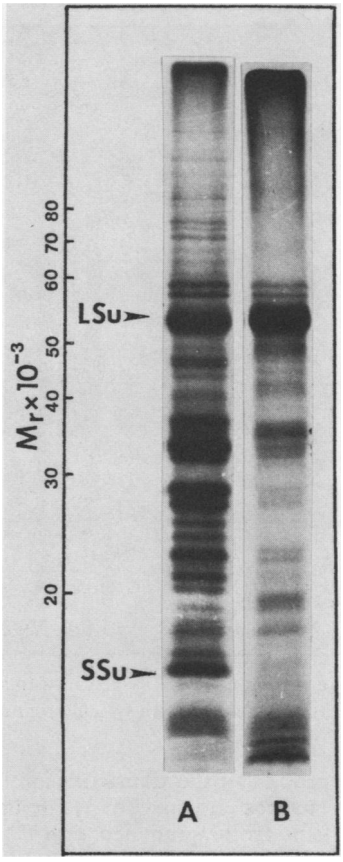

FIG. 5. SDS-polyacrylamide-gel profile of the translation products of isolated plastids. (A) stained gel of plastid proteins; (B) autoradiograph of (A).

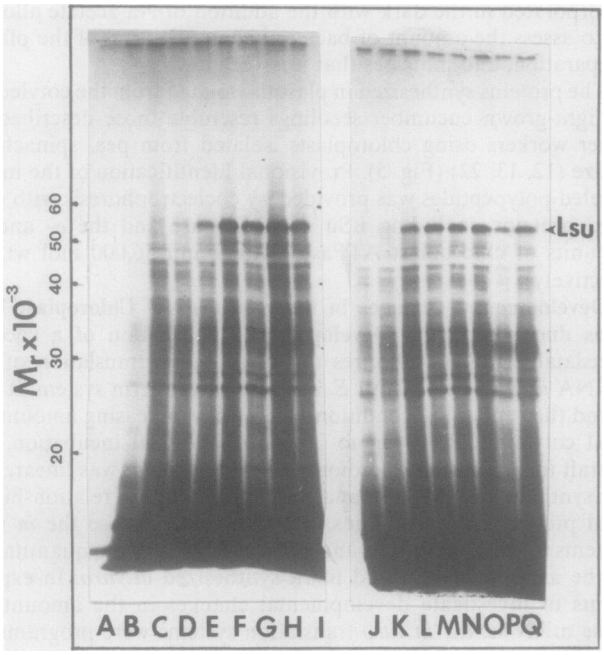

FIG. 6. Autoradiograph of the SDS-polyacrylamide-gel profile of the translation products of the *E. coli* system programmed with cucumber cotyledon RNA extracted from seedlings of different stages of light-and dark-grown development. (A) minus RNA; (B) to (I) RNA extracted from the cotyledons of light-grown seedlings from day 0 to day 7; (J) to (Q) RNA extracted from the cotyledons of dark-grown seedlings from day 1 to day 8.

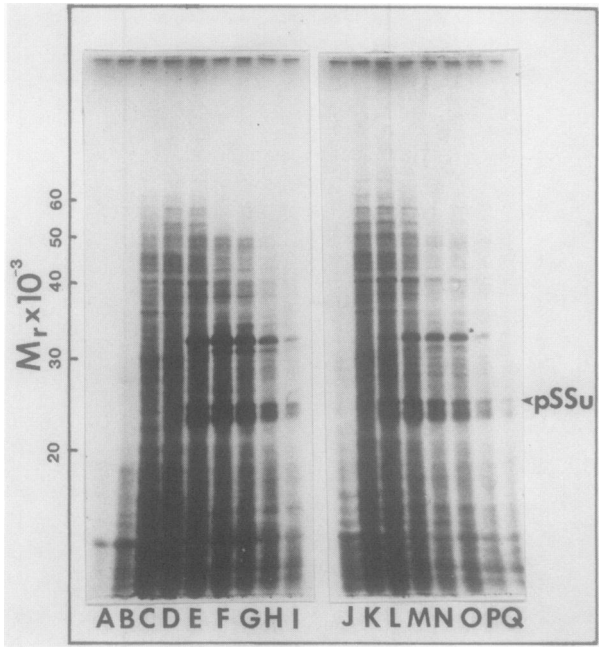

FIG. 7. Autoradiograph of the SDS-polyacrylamide gel profile of the translation products of the wheat germ system programmed with the same RNA as described in Figure 6.

changes in the profiles of the translation products. Second, the developmental changes are essentially similar irrespective of whether the systems are programmed with RNA extracted from light- or dark-grown tissue. Third, the translatable mRNAs coding for the LSu and pSSu of RuBPCase first appear at approximately day 2, and thereafter their levels increase. Among the translation products of both systems, a number of unidentified labeled polypeptides also appear to change in amounts in a developmentally related manner.

It has been previously shown that the mRNA coding for barley Chl *a/b* binding protein is translated in a wheat germ system to produce a 32,000 mol wt precursor polypeptide (1). The 32,000 mol wt polypeptide appearing among the translation products of cucumber RNA is probably the precursor of the Chl *a/b* binding protein. In cucumber the mature polypeptide has a mol wt of 29,000 (J. Schouten, personal communication). The mRNA coding for the 32,000 mol wt polypeptide accumulates in the light-grown cotyledons from day 4 onwards. This coincides with the light-dependent initiation of Chl synthesis (Fig. 1) and also the synthesis of Chl *a/b* binding protein (32).

Quantitation of the levels of LSu and pSSu among the translation products of the *in vitro* systems was carried out either by excising the labeled polypeptides from the polyacrylamide gels and by estimating the amount of radioactivity present by scintillation counting or by immunoprecipitating the translation products. In the latter case, the amount of radioactivity present in aliquots of the immunoprecipitate was estimated by scintillation counting and the immunoprecipitates were analyzed by polyacrylamide gel electrophoresis and autoradiography. Both techniques yielded essentially similar results which gave us some confidence in thinking that in excising the labeled polypeptides from the gels we were not overestimating the radioactivity present due to the excision of a co-migrating polypeptide (in the case of the wheat germ system, electrophoresis of the translation products was allowed to take place for a longer time for complete separation of pSSu from a labeled polypeptide of slightly lower molecular weight [see Fig. 3]).

The amounts of LSu and pSSu synthesized *in vitro* were plotted on a per cotyledon basis so as to allow direct comparison with the amounts of RuBPCase protein measured in the cotyledons. To do this the amount of radioactivity present in either LSu or pSSu, estimated on a per μg RNA incubated basis, was multiplied by the amount of RNA present in the cotyledon for the particular day in question (4).

The translatable mRNAs for both LSu and pSSu appear in the light- and dark-grown tissue between days 2 and 3 which is at about the same time as the appearance of RuBPCase (Fig. 8). Thus, the initial accumulation of the mRNA coding for LSu and pSSu is light-independent. The amounts of LSu and pSSu mRNA increase in the light- and dark-grown cotyledons until day 4 and over this period appear to be positively related to the amounts of

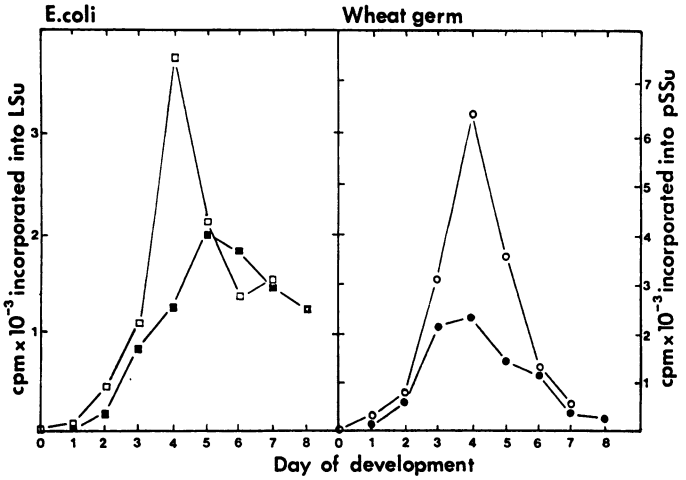

FIG. 8. Quantitation of LSu and pSSu synthesized by the *E. coli* and wheat germ systems programmed with cucumber cotyledon RNA extracted from the seedlings of different stages of light- and dark-grown development. Data on a per cotyledon basis.

RuBPCase protein synthesized within the cotyledons. At later stages of development, the amount of LSu and pSSu mRNA as assayed *in vitro* appears to decline sharply in the light-grown but much more slowly in the dark-grown tissue. While the levels of the mRNAs appear to decline in the light-grown cotyledons, the rate of accumulation of RuBPCase in the cotyledons is maintained.

To investigate the protein synthetic capacity of the plastids themselves these were isolated from the cotyledons on different days of light-grown seedling development and were incubated with [^{35}S]methionine. To allow direct comparison with the other data shown here the results are expressed on a per cotyledon basis. To do this the amount of radioactivity incorporated by the plastid extract, prepared from 5 g of tissue, was divided by the number of cotyledons in 5 g of tissue.

With light or ATP as a source of energy the temporal pattern of the development of the protein synthetic capacity of the plastids is similar (Fig. 9). However, ATP alone up to day 5 does not effectively replace light as a source of energy. After this time light can be replaced by ATP. This may be due to subtle changes in the permeability of the plastid membranes to ATP or the energy requirements of plastid protein synthesis. There appears to be a peak of protein synthetic activity between days 4 and 5 which is followed by a dramatic decline. Analysis of the translation products of the isolated plastids shows little evidence to suggest differential translation of specific mRNAs (data not shown).

CONCLUSIONS

The data presented here show that once optimized to produce quantitative translation, cell-free translation systems can be used to study developmentally related changes in the amounts of those mRNAs coding for chloroplast proteins. Previously, *in vitro* systems have been used to correlate the accumulation of an mRNA in greening maize and *Spirodella* with increased synthesis of a 32,000 mol wt chloroplast membrane polypeptide (5, 26). Recently, changes in the synthesis of the LSu of RuBPCase and the 32,000 mol wt chloroplast membrane polypeptide in developing spinach leaves have been shown to be reflected in the amounts of mRNA coding for these polypeptides assayed in a reticulocyte lyase system (30).

One of the most significant points raised by this study is that the wheat germ system translates cucumber cotyledon SSu mRNA to produce a precursor polypeptide of approximately 25,000 mol wt, 11,000 daltons larger than the mature protein. Several groups of workers have shown that the cytoplasmic mRNAs coding for SSu (10) and other chloroplast proteins such as Chl *a/b* binding protein (1) and ferredoxin(15) are translated in a wheat germ system to produce precursor polypeptides 4,000 to 5,000 daltons larger than the mature protein. It has been shown that the increased size of the precursor to *Chlamydomonas* SSu can be accounted for by a 44 amino acid residue-long extension at the NH_2 terminus of the polypeptide (28). The reason for the relatively large pSSu of cucumber remains to be explained. The mRNA coding for the 25,000 mol wt polypeptide is not unique to the cotyledons. Experiments have shown that the wheat germ translation products programmed by RNA extracted from cucumber cotyledons and leaves are essentially the same, the 25,000 mol wt polypeptide being present in both product profiles (Dosser and Walden, unpublished results).

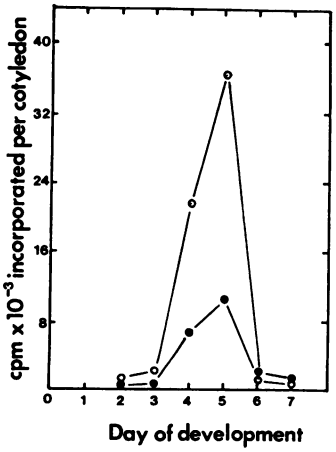

FIG. 9. Incorporation of [^{35}S]methionine into protein by plastids isolated from cucumber cotyledons of differing stages of light-grown seedling development. Between 1.6 and 2.2 mg of plastid protein was incubated in a final volume of 300 μl containing 10 μCi [^{35}S]methionine (1005–1400 Ci/mmol) with either light (○) or ATP (●) as an energy source. Data plotted on a per cotyledon basis.

The developmental changes in the translation product profiles of the cell-free systems, programmed by equal amounts of RNA extracted from light- and dark-grown cucumber cotyledons are similar. This suggests that although the amounts of RNA present in light- and dark-grown tissue are different (4) the relative amounts of individual mRNAs are similar. The only major exception to this appears to be the mRNA, translated by the wheat germ system, which codes for the 32,000 mol wt polypeptide. The appearance of this mRNA on day 4 appears to be light-dependent.

The initiation of the accumulation of the mRNAs coding for the LSu and pSSu of RuBPCase between days 2 and 3 appears to be light-independent and coincides with the appearance of both chloroplast ribosomal RNA (4) and RuBPCase protein in the cotyledon (Fig. 1). The accumulation of mRNA until day 5 is positively related to the accumulation of RuBPCase protein. In the dark-grown tissue the decline in the rate of accumulation of RuBPCase protein appears to be preceded by a decline in the levels of LSu and pSSu mRNA as assayed *in vitro*. It is also apparent from Figures 6 and 7 that after day 4 there is a general decline in the levels of mRNAs assayed in the translation systems. However, the decline not only in the levels of the mRNAs extracted from the light-grown cotyledons of the later stages of seedling development but also in the protein-synthesizing activity of isolated plastids takes place while the accumulation of RuBPCase protein is maintained. This observation remains to be explained. The results may be interpreted to suggest that a light-dependent factor increases the efficiency of RuBPCase mRNA translation *in vivo*. On the other hand, the rate of accumulation of RuBPCase may be sustained by pools of previously synthesized subunits. The latter proposal would be supported by the finding that the protein synthesizing activity of the plastids declines after day 5.

The data presented here indicate that in cucumber cotyledons chloroplast protein synthesis initially takes place in a burst lasting for approximately 48 h. After this period there appears to be a decline in chloroplast protein synthetic activity to a level presumably sufficient to replace protein turnover.

Acknowledgments—We would like to thank B. Forde, K. Dosser, and E. Weir for critical discussion and assistance; also R. J. Ellis and S. H. Howell for helpful comments on the manuscript. R. W. acknowledges receipt of an Science Research Council postgraduate studentship.

LITERATURE CITED

1. APEL K, K KLOPPSTECH 1978 The plastid membranes of barley (*Hordeum vulgare*). Light induced appearance of the mRNA coding for the apoprotein of light harvesting Chl *a/b* binding protein. Eur J Biochem 85: 581–588
2. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
3. AVIV H, P LEDER 1972 Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA 69: 1408–1412
4. BECKER WM, CJ LEAVER, EM WEIR, H RIEZMAN 1978 Regulation of glyoxysomal

enzymes during germination of cucumber. Plant Physiol 62: 542–549
5. BEDBROOK JR, G LINK, DM COEN, L BOGORAD, A RICH 1978 Maize plastid gene expressed during photoregulated development. Proc Natl Acad Sci USA 73: 3060–3064
6. BLAIR GE, RJ ELLIS 1973 Protein synthesis in chloroplasts. I. Light driven synthesis of the large subunit of fraction I protein by isolated pea chloroplasts. Biochim Biophys Acta 463: 185–215
7. BOTTOMLEY W, TJV HIGGINS, PR WHITFELD 1976 Differential recognition of chloroplast and cytoplasmic messenger RNA by 70S and 80S ribosomal systems. FEBS Lett 63: 120–124
8. BOTTOMLEY W, D SPENCER, PR WHITFELD 1974 Protein synthesis in isolated spinach chloroplasts. Comparison of light-driven and ATP-driven synthesis. Arch Biochem Biophys 164: 106–117
9. CHUA N-H, GW SCHMIDT 1978 Post translational transport into intact chloroplasts of a precursor to the small subunit of ribulose 1,5 bisphosphate carboxylase. Proc Natl Acad Sci USA 75: 6110–6114
10. DOBBERSTEIN R, G BLOBEL, N-H CHUA 1977 *In vitro* synthesis and processing of a putative precursor for the small subunit of ribulose 1,5 bisphosphate carboxylase of *Chlamydomonas reinhardii* Proc Natl Acad Sci USA 74: 1082–1085
11. DOCKERTY A, JM LORD, MJ MERRETT 1977 Development of ribulose-1,5-diphosphate carboxylase in castor bean cotyledons. Plant Physiol 59: 1125–1127
12. ELLIS RJ, 1977 Protein synthesis in isolated chloroplasts. Biochim Biophys Acta 463: 185–215
13. GREBANIER AE, KE STEINBACK, L BOGORAD 1979 Comparison of the molecular weights of proteins synthesized by isolated chloroplasts with those that appear during greening in *Zea mays*. Plant Physiol 63: 436–439
14. HARTLEY MR, A WHEELER, RJ ELLIS 1975 Translation of messenger RNA for the large subunit of fraction I protein in a heterologous cell-free system. J Mol Biol 91: 67–77.
15. HUISMAN JG, AFM MOORMAN, FN VERKLEY 1978 *In vitro* synthesis of chloroplast ferredoxin as a high molecular weight precursor in a cell-free protein synthesizing system from wheat germ. Biochem Biophys Res Commun 82: 1121–1131
16. KESSLER SW 1975 Rapid isolation of antigens from cells with a staphylococcal protein A-antibody absorbant. Parameters of the interaction of antibody-antigen complexes with protein A. J Immunol 115: 1617–1634
17. LAEMMLI UK 1970 Cleavage of structural proteins during the assembly of the head proteins of bacteriophage T4. Nature 227: 680–685
18. LEAVER CJ J INGLE 1971 The molecular integrity of chloroplast ribonucleic acid. Biochem J 123: 235–243
19. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275.
20. MANS RV, AD NOVELLI 1961 Measurement of the incorporation of radioactive amino acids into protein by filter paper disc method. Arch Biochem Biophys 94: 48–56
21. MARCU K, B DUDOCK 1974 Characterization of a highly efficient protein synthesizing system derived from commercial wheat germ. Nucle Acids Res 1: 1385–1397
22. MENDIOLA-MORGENTHALER LR, JJ MORGENTHALER, CA PRICE 1976 Synthesis of coupling factor CF_1 protein by isolated spinach chloroplasts. FEBS Letts 62: 96–100
23. MILLER J 1972 Experiments in Molecular Genetics. Cold Spring Harbor, New York
24. MODOLELL J 1971 An S-30 system from *E. coli*. *In* JA Last, AI Laskin, eds, Protein Biosynthesis in Bacterial Systems. Marcel Dekker, New York, pp 2–28
25. MOHR H 1977 Phytochrome and chloroplast development. Endeavour, New Series 1: 107–114
26. REISFELD A, J GRESSEL, KM JAKOB, M EDELMAN 1978 Characterization of the 32,000 dalton membrane protein. 1. Early synthesis during photoinduced plastid development of *Spirodella*. Photochem Photobiol 27: 161–165
27. RIEZMAN H, EM WEIR, CJ LEAVER, DE TITUS, W BECKER 1980 Regulation of glyoxysomal enzymes during germination of cucumber. *In vitro* translation and characterization of four glyoxysomal enzymes. Plant Physiol 65: 40–46
28. SCHMIDT GW, A DEVILLERS-THIERY, H DESRUISSEAUX, G BLOBEL, N-H CHUA 1979 NH_2 terminal amino acid sequences of precursor and mature forms of ribulose 1,5 bisphosphate carboxylase small subunit from *Chlamydomonas reinhardii*. J Cell Biol 83:615–622
29. SIDDELL SG, RJ ELLIS 1975 Protein synthesis in chloroplasts VII. Characteristics and products of protein synthesis *in vitro* in cytoplasts and developing chloroplasts from pea leaves. Biochem J 146: 675–685
30. SILVERTHORNE J, RJ ELLIS 1980 Protein synthesis in chloroplasts VIII. Differential synthesis of chloroplast proteins during spinach leaf development. Biochim Biophys Acta 607: 319–330
31. SMITH SM, RJ ELLIS 1979 Processing of small subunit precursor of ribulose bisphosphate carboxylase and its assembly into whole enzyme are stromal events. Nature 278: 662–664
32. WALDEN R 1979 Synthesis of chloroplast proteins during germination and early development of cucumber (*Cucumis sativus*). PhD thesis. Edinburgh University
33. WALDEN R, CJ LEAVER 1978 Regulation of chloroplast protein synthesis during germination and early development of cucumber. *In* G Akoyunoglou, JH Argyroudi-Akoyunoglou, eds, Chloroplast Development-Elsevier/North Holland, Amsterdam, pp 251–256
34. WHEELER AM, MR HARTLEY 1976 Major mRNA species from spinach chloroplasts do not contain poly(A). Nature 257: 66–67