Plant Physiol. (1983) 73, 906–911
0032-0889/83/73/0906/06/$00.50/0

Ribulose Bisphosphate Carboxylase Synthesis in Barley Leaves

A DEVELOPMENTAL APPROACH TO THE QUESTION OF COORDINATED SUBUNIT SYNTHESIS[1]

Received for publication April 22, 1983 and in revised form August 2, 1983

HELEN T. NIVISON[2] AND C. RALPH STOCKING
Department of Botany, University of California, Davis, California 95616

ABSTRACT

The coordination of the synthesis of the large and small subunits of ribulose 1,5-bisphosphate carboxylase (RuBPCase) was studied in young light-grown barley (*Hordeum vulgare* L. var. UC566) leaves. Since a barley leaf is a continuum of different aged cells with the youngest cells at the base and the oldest at the tip, developmental changes could be investigated by comparing different leaf regions. The rate of total cytoplasmic protein synthesis increased to a maximum before the rate of total organelle protein synthesis. The different positions of the maxima suggested that the synthesis of the small RuBPCase subunit on cytoplasmic ribosomes and the large RuBPCase subunit on chloroplast ribosomes might not be coupled during barley leaf development. However, measurements of the amounts and rates of synthesis of the subunits showed that they were coupled. Although the amounts of the RuBPCase subunits increased from the younger to the older leaf regions, the subunits were present in an equimolar ratio. While the rates of synthesis of both subunits increased to a maximum in a midleaf region and then declined, the ratio of the rates remained constant. That the subunit amounts remained equimolar and the synthetic rates proportional while total RuBPCase synthesis was changing indicated that the synthesis of the subunits was closely coordinated during leaf development. A close coordination was also supported by the kinetics of the inhibition of subunit synthesis in the presence of cycloheximide.

Ribulose 1,5-bisphosphate carboxylase is composed of large and small subunits which are synthesized in the chloroplast and cytoplasm, respectively, then brought together and assembled into a holoenzyme within the chloroplast (6, 23). The two types of subunits occur in the holoenzyme in equal numbers (13), and free subunits are either absent or present in relatively small amounts (15, 21). These observations suggest that the synthesis of the large and small RuBPCase[3] subunits in the two different cellular compartments may be coordinated events.

Previous studies have both supported this suggestion and provided counter evidence. Studies with *Chlamydomonas* indicated coupling between the synthesis of each subunit and the complementary translation system (12). In contrast, results with higher plants such as barley (6), pea (5), and rye (10) seedlings, spinach protoplasts (18), and soybean leaf cells (2) suggested either coupling (12, 18), partial coupling (6), or no coupling (2, 10). These varied results may be due in part to the reliance of these studies on protein synthesis inhibitors, whether chemical (2, 5, 6, 12, 18) or environmental (10). Uncertainties concerning the specificity of the inhibitors and the extent of the deleterious consequences can present difficulties in interpretation.

As an alternative approach, in the present study we have made use of the endogenous changes in protein synthesis that occur during the growth and development of barley leaves. Barley leaves are composed of a developmental sequence of cells which extends from the base to the tip of the leaf (4). Cells are formed at the basal meristem, elongate in the lower leaf regions, and are then pushed upward by the elongation of later formed cells. Thus, the youngest cells are at the base of the leaf and the oldest at the tip. Since RuBPCase synthesis increases and then declines during the course of leaf development (8, 11), one would expect the pattern of RuBPCase synthesis to vary along the length of a barley leaf. As RuBPCase synthesis changes, the synthesis of the two subunits should change together if the processes are coordinated, or independently, if they are not.

MATERIALS AND METHODS

Sources of Chemicals. L-[U-^{14}C]leucine was obtained from New England Nuclear. Acrylamide, bisacrylamide, glycine, SDS, TEMED, EtSH, urea, and Coomassie Brilliant Blue R-250 were from Bio-Rad. Ampholines were from LKB. NCS Solubilizer was from Amersham. Lincomycin-HCl was a gift from the Upjohn Co.

Plant Growth. Barley seeds, *Hordeum vulgare* L. var UC566, obtained from Dr. Charles Schaller of the Agronomy Department, University of California, Davis, were grown in a soil mixture (3 parts sandy loam:2 parts peat:1 part sand) kept moist with half-strength Hoagland solution under a 16-h light (250–310 μE/m^2·s, 23.5°C) and 8-h dark (13.5°C) cycle. The temperatures were carefully maintained as leaf growth rates were found to be very temperature sensitive.

Preparation of Leaf Sections and Disks. On the 7th d after planting, disks were prepared from the first leaves of the seedlings. Leaves with lengths of 12.3 to 12.8 cm measured from the coleoptile tip were cut into six sections 2 cm long, or seven if the tissue covered by the coleoptile was being used. Sections were labeled with reference to the distance from the tip of the coleoptile (Fig. 1). Three disks, 4 mm in diameter, were punched from each section. Sections from the 0–2 region had to be unrolled and held flat for disk punching; to punch disks from the youngest region, −2–0, the coleoptile had to be peeled away and the leaf unrolled and held flat. Section cutting and disk punching were done under water.

Protein Synthesis Incubation. Leaf disk samples were vacuum infiltrated with 40 mM KH_2PO_4 brought to pH 6.8 with NH_4OH ('phosphate buffer'), then transferred to the incubation solution consisting of 0 to 5.0 mM [^{12}C]leucine and 0.4 to 0.5 μCi/ml

[1] Supported in part by Grant PCM 77-12456 A01 from the National Science Foundation.

[2] Present address: Section of Plant Biology, Plant Science Building, Cornell University, Ithaca, NY 14853. To whom correspondence should be addressed.

[3] Abbreviations: RuBPCase, ribulose 1,5-bisphosphate carboxylase; TEMED, *N,N,N',N'*-tetramethylenediamine; EtSH, 2-mercaptoethanol.

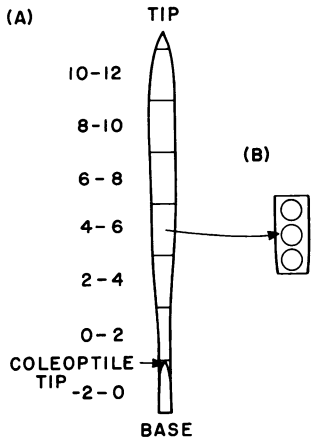

FIG. 1. Drawing illustrating the preparation of leaf sections and disks. A, Barley leaves were divided into 2-cm sections which were labeled with reference to the distance from the tip of the coleoptile; B, three disks were removed from each section.

[^{14}C]leucine (295 to 355 mCi/mmol) in phosphate buffer. During the incubation the temperature was 24°C, the light intensity was 160 to 170 $\mu E/m^2 \cdot s$, and gentle shaking kept the solutions mixed. The length of the incubation period was 1 to 4 h depending on the experiment. When protein synthesis inhibitors were used, they were included in the vacuum infiltration solution and in the incubation solution. At the end of the incubation period, leaf disks were rinsed with ice water, blotted, and frozen.

Determination of TCA-Insoluble Radioactivity. Frozen leaf disks were homogenized with 10 mM Tris-HCl, pH 7.4, in ground glass tissue grinders. The homogenate was made 10% (w/v) in TCA and kept overnight on ice. The precipitate was collected by centrifugation, dissolved in 0.05 N NaOH containing 200 μg/ml [^{12}C]leucine, and the protein again precipitated with TCA. The precipitate was collected as before, washed twice with 10% (w/v) TCA, and resuspended in 10% (w/v) TCA by sonication. Two aliquots from each sample were collected on glass fiber papers by filtering. Filters were rinsed with ethanol and ethyl ether, bleached with drops of 30% (v/v) H_2O_2 and light, dried, and the radioactivity measured in toluene scintillation fluid.

One-Dimensional Gel Electrophoresis. Leaf disk samples were homogenized with 15 mM Tris-HCl, pH 8, 5 mM EDTA, 5 mM DTT, and 3.3 mg/ml insoluble PVP. The homogenate was made 1% (w/v) in SDS and 0.14 M in EtSH, boiled for 3 min, and clarified by centrifugation. The protein in an aliquot of the supernatant was precipitated with acetone; the precipitate was dissolved in three parts solution A (0.20 M Tris-HCl, pH 6.7, 0.47 M EtSH, 2% [w/v] SDS), boiled for 3 min, and then mixed with one part solution B (1.4 M EtSH, 35% [v/v] glycerol, 0.07% [w/v] bromophenol blue). The specific volumes of solutions A and B were varied so that the final protein concentration was about 0.6 mg/ml; 10 μl of this solution were loaded into wells on the slab gel.

Slab gels had a 12.5% (w/v) acrylamide running gel in 0.375 M Tris-HCl, pH 8.9, 0.1% (w/v) SDS, and a 5% (w/v) acrylamide stacking gel in 0.15 M Tris-HCl, pH 6.7, 0.025% (w/v) SDS. The running buffer contained 0.025 M Tris, 0.19 M glycine, and 0.1% (w/v) SDS. Electrophoresis was at 10 mamp for 1 h, then 20 mamp for about 2 more h. Gels were fixed in 25% (v/v) isopropyl alcohol containing 10% (v/v) acetic acid, then stained and destained according to the method of Fairbanks *et al.* (9). Gels were then cut into strips and scanned at 580 nm.

Two-Dimensional Gel Electrophoresis. Leaf disk samples were homogenized in 15 mM Tris-HCl, pH 8, 5 mM EDTA, 5 mM $MgCl_2$, 1.33% (v/v) EtSH, and 3.3 mg/ml insoluble PVP. A portion of the resulting crude homogenate was made 2% (w/v) in SDS, boiled for 5 min, and clarified by centrifugation. An aliquot of the supernatant was lyophilized and the dry sample stored at −22°C. Just before use, the sample was dissolved in a solution of 8.5 M urea, 2% (w/v) Ampholines, pH 3.5 to 10, 5% (v/v) EtSH, and 8% (w/v) Nonidet P-40.

Isoelectric focusing gels were prepared essentially according to the method of O'Farrell (20). The upper electrolyte solution was 0.02 N $Ca(OH)_2$ with 0.04 N NaOH; the lower electrolyte solution was 0.06 N H_2SO_4. Electrophoresis was at 300 v for 19 h followed by 400 v for 1 to 1.5 h. Gels were then extruded into screw top culture tubes, frozen in a bed of pulverized dry ice, and stored at −22°C.

Electrophoresis in the second dimension was on SDS-polyacrylamide slab gels with the gel concentrations described above and the Laemmli (16) buffer system. The focusing gel was thawed and the gel segment containing RuBPCase protein was cut out. With no prior equilibration, the gel segments were sealed on the second dimension stacking gel between a lower layer of O'Farrell's solution 0 (20), made 1% (w/v) in agarose, and an upper layer of 1% (w/v) agarose in water. Electrophoresis was at 10 mamp for the first 30 min, 15 mamp for the next 30 min, and then 20 mamp until the tracking dye was near the lower edge of the slab gel. Gels were fixed, stained, and destained as described above, and then dried on Whatman 3MM chromatography paper.

Determination of Radioactivity in Second Dimension Gels. The stained RuBPCase subunit spots were cut from the dried gels, cut into thin strips, transferred to scintillation vials, and moistened with 50 μl of water. After 10 min, 10 ml of NCS scintillation fluid (1) were added and the capped vials were incubated at 43°C for 12 h. When the vials had cooled, the radioactivity was measured.

RESULTS AND DISCUSSION

Total, Cytoplasmic, and Organelle Protein Synthesis. The rate of total protein synthesis (Fig. 2A) increased to a maximum in region 6–8 and then declined. When protein synthesis inhibitors were included in the incubation solutions, the contributions of cytoplasmic and organelle protein synthesis to the total were determined. Figure 2B shows that cytoplasmic protein synthesis was maximal in region 4–6, while the rate of organelle protein synthesis continued to increase until region 6–8. Most of the organelle protein synthesis was probably due to the activity of chloroplast ribosomes, as 25% to 40% of the ribosomes in green leaves are chloroplast ribosomes while less than 1% are mitochondrial (17).

Since the small subunit of RuBPCase is a major product of cytoplasmic protein synthesis in green leaves, and the large subunit is the major product of chloroplast protein synthesis (3, 14), these results suggested that in region 4–6 small subunit synthesis might exceed large subunit synthesis and that more large than small subunit synthesis might occur in the adjacent older region. In other words, the synthesis of the subunits might not be synchronized during leaf development: small subunit synthesis might lead large subunit synthesis. This possibility was investigated through measurements of the amounts and rates of synthesis of the subunits.

Amounts of RuBPCase Subunits. The amounts of the subunits increased from the base to the tip of the leaf (Fig. 3A). Along most of the leaf the amounts of both subunits appeared to change in parallel. The relationship between the amounts could be seen more clearly when the data were expressed as the ratio of small to large subunits. Figure 3B shows that the ratio increased in the youngest leaf regions while it remained constant at about 1.3 along most of the length of the leaf.

This determination of subunit quantities was based on resolving the subunits by one-dimensional SDS gel electrophoresis,

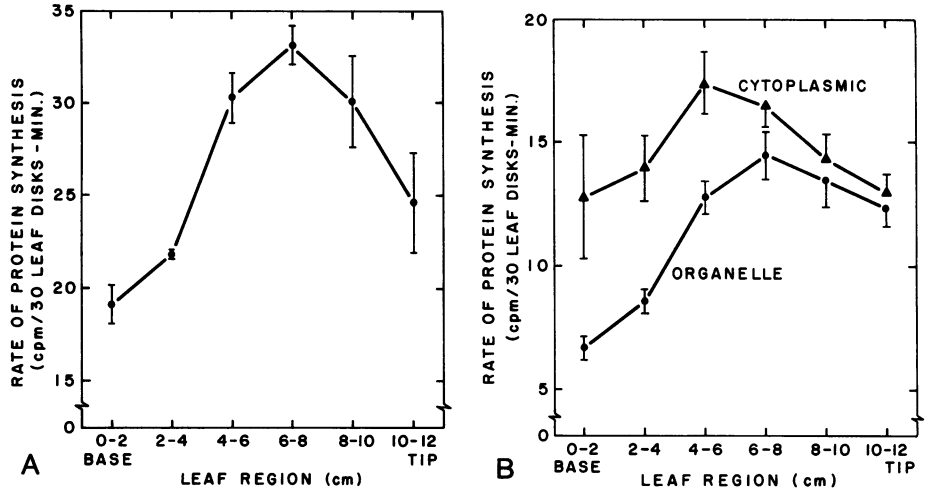

FIG. 2. Rates of protein synthesis in six leaf regions. Samples of 30 leaf disks were incubated under conditions for protein synthesis as described in "Materials and Methods" with 5.0 mM [^{12}C]leucine. The rates of incorporation of radioactivity into TCA-insoluble material are shown. A, Rates of incorporation of radioactivity into TCA-insoluble material in the absence of inhibitors ('total' protein synthesis); B, rates of incorporation of radioactivity into TCA-insoluble material in the presence of 200 μg/ml lincomycin ('cytoplasmic' protein synthesis) or 5 or 10 μg/ml cycloheximide ('organelle' protein synthesis). The mean incubation times were 100, 109, and 115 min for the treatments with no inhibitors, lincomycin, or cycloheximide, respectively. Values are means ± SE for three experiments (total protein synthesis), eight experiments (cytoplasmic protein synthesis), or six experiments (organelle protein synthesis).

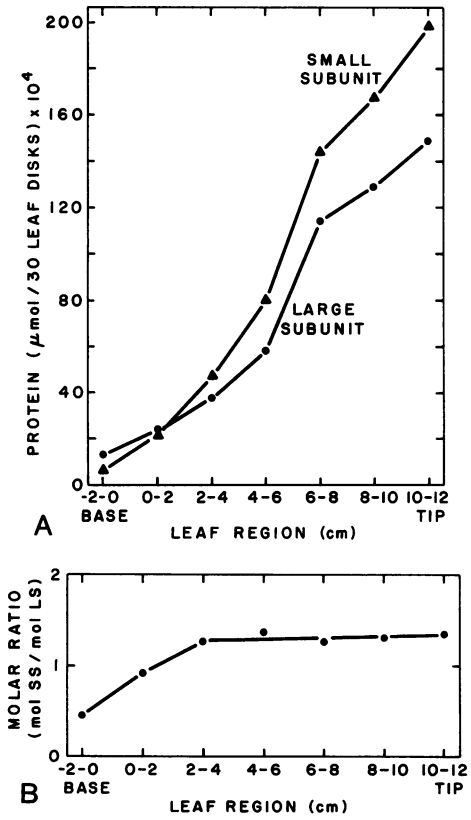

FIG. 3. Amounts of large and small RuBPCase subunits in each leaf region. Values for each region were determined using two samples which were prepared from two sets of 30 leaf disks, except that for region −2–0 only one set of 30 disks was used. Samples were electrophoresed on one-dimensional gels. Optical density data were converted to μg protein with a BSA standard curve; μmol were calculated using the molecular wt of 55,000 and 13,000 D for the large and small subunits, respectively. A, The amounts of the subunits; B, the ratios of moles of small subunit (SS) to moles of large subunit (LS).

then staining and scanning. However, comparisons between one- and two-dimensional gels suggested that the increase in the ratios in the young regions (Fig. 3B) reflected changes in amounts of proteins comigrating with the large subunit rather than changes in the relative amounts of the RuBPCase subunits (data not shown). Furthermore, differences in the Coomassie staining properties of the subunits contributed to the subunit ratio of 1.3. This conclusion is based upon an analysis of stained one-dimensional gels after electrophoresis of the purified RuBPCase holoenzyme. Scans of these gels also showed a subunit ratio of 1.3. Since the complete enzyme consists of equal amounts of the two kinds of subunits, this result meant that the small subunit stained preferentially and indicated that the subunits were present in equimolar amounts in those leaf samples. Thus, the data were consistent with the presence of equimolar amounts of both subunits.

Rates of Synthesis of RuBPCase Subunits. The rates of synthesis of the subunits (measured as the rates of [^{14}C]leucine incorporation) rose to a maximum in the middle of the leaf and then declined (Fig. 4A). The ratio of the rates for the two subunits was the same in all regions (Fig. 4B).

The ratio of the incorporation rates was about 8 and the ratio of the amounts of leucine in the subunits of barley leaves was calculated to be about 5 (19) using the data of Strobaek and Gibbons (24). The close agreement of these values suggests that the rates of large and small subunit synthesis were similar.

A precise quantitative comparison of the rates was not possible because the specific radioactivities of the leucine pools feeding large and small subunit synthesis were not known. Evidence that the specific radioactivities of the two pools were not identical was provided by an experiment in which leaf disks were incubated with or without [^{12}C]leucine before exposure to [^{14}C] leucine (data not shown). The ratio of radioactivity in large compared to small subunits was found to be lower for the samples that had been preincubated with [^{12}C]leucine, suggesting that the [^{12}C]leucine had diluted the [^{14}C]leucine in the chloroplasts more than in the cytoplasm. Apparently, the exogenous leucine did not distribute uniformly between cytoplasm and chloroplasts.

For information about coordinated synthesis, the relative rates of large and small subunit synthesis, as indicated by the ratios of

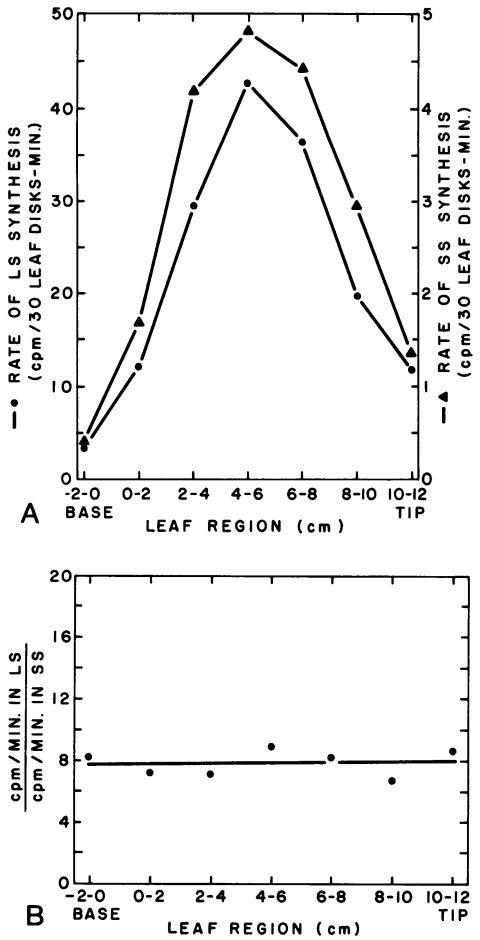

FIG. 4. Rates of synthesis of RuBPCase subunits in each leaf region. Samples of 30 leaf disks were incubated for 157 to 165 min in 0.48 mM leucine (1.04 μCi [^{14}C]leucine/μmol). The subunits were resolved by two-dimensional gel electrophoresis. A, Rates of large (—●) and small (—▲) subunit synthesis; B, ratio of the rate of large subunit synthesis to the rate of small subunit synthesis.

Table I. *Synthesis of the RuBPCase Subunits in the Presence or Absence of Inhibitors*

Sections from each of the three regions were cut from 30 leaves and combined. Thirty leaf disks from 10 randomly selected sections were prepared for each sample. Samples were incubated under conditions for protein synthesis as previously described, except that [^{12}C]leucine was omitted and either no inhibitor, 10 μg/ml cycloheximide, or 200 μg/ml lincomycin were included. Subunits were resolved by two-dimensional gel electrophoresis.

Leaf Region	Inhibitor	Incubation Time	Rate of Synthesis		Rate inhibition	
			LS	SS	LS	SS
		min	*cpm/30 leaf disks·min*		*%*	
0–2	No inhibitor	156	38.0	4.4	0	0
4–6		157	147.0	15.0	0	0
8–10		159	116.5	11.2	0	0
0–2	Cycloheximide	163	25.2	0.2	34	96
4–6		165	77.7	0.3	47	98
8–10		168	87.4	0.1	25	99
0–2	Lincomycin	173	6.9	2.6	82	41
4–6		175	21.5	10.1	85	32
8–10		177	14.7	6.5	87	42

the rates, are of less importance than the constant nature of these ratios (Fig. 4B). The ratios were the same along the length of the leaf, even though the rates of synthesis of the subunits changed. This relationship means that as the rates of synthesis of the subunits changed, they changed by the same proportions. Such balanced synthesis suggests that exact controls coordinated the synthesis of the subunits in the two different cellular compartments during leaf development.

Synthesis of the Subunits in the Presence of Inhibitors. To examine further the apparent coordination of large and small subunit synthesis, protein synthesis inhibitors were used in an attempt to perturb the coordination. When leaf disks were treated with cycloheximide or lincomycin, the synthesis of the subunits was inhibited differentially (Table I). Treatment with cycloheximide resulted in nearly complete inhibition of small subunit synthesis (96–99%) but only partial inhibition (25–47%) of large subunit synthesis. Likewise, treatment with lincomycin inhibited large subunit synthesis more than small subunit synthesis (82–87% as opposed to 29–46%). The response to inhibitors was similar in disks from young (0–2), intermediate (4–6), and older (8–10) leaf regions. This differential inhibition suggested that although the synthesis of the subunits appeared to be coordinated during development, the coordination could be disrupted by inhibiting either one of the translational systems.

A time course with cycloheximide provided additional information about this disrupted coordination. Figure 5 describes the synthesis of the subunits in region 4–6 during 4 h in the presence or absence of cycloheximide. In the absence of the inhibitor (Fig. 5A), [^{14}C]leucine incorporation was linear for at least 3 h into the small subunit and for at least 2 h into the large subunit. When cycloheximide was included, incorporation into the small subunit stopped after the first hour (Fig. 5B), while the rate of incorporation into the large subunit declined throughout the 4 h (Fig. 5, B and C). Other experiments (data not shown) indicated that the rate of synthesis of total chloroplast protein did not undergo this decline.

Using the data from Figure 5, A and B, the rates of [^{14}C] leucine incorporation (Fig. 5C) and the per cent inhibition of the rates due to cycloheximide were calculated. The latter values were plotted with a time scale which extended back to the time when the leaf disks first came in contact with cycloheximide, during the vacuum infiltration treatment 34 min before [^{14}C] leucine was added (Fig. 5D). It is interesting that the curve of large subunit inhibition extrapolates smoothly to 0% inhibition at the time when the inhibitor was first added. This means that the inhibition of large subunit synthesis may have started without a lag, or if there was a lag it cannot have lasted very long. That the large subunit began to be inhibited as soon, or almost as soon, as the small subunit again suggests a coordination between the synthesis of the subunits.

Our results contrast with those of Barraclough and Ellis (2), who used isolated soybean leaf cells. They found that in the absence of inhibitors, [^{35}S]methionine incorporation into the small RuBPCase subunit declined after the 1st h while incorporation into the large subunit continued at the original rate for at least 5 h. When the cells were incubated with cycloheximide, incorporation into the large subunit continued at almost the original rate for at least 4 h. Their conclusion was that the synthesis of the subunits was not tightly coupled over short time intervals. In contrast, our findings of equimolar subunit quantities, proportional rates of synthesis, and the response to cycloheximide support a coordination of protein synthesis between the cytoplasm and chloroplasts in the synthesis of RuBPCase. Possibly different controls operate in isolated soybean leaf cells and barley leaf disks, or one of the systems may have been altered during preparation.

Since this study was completed, Dean and Leech (7) have

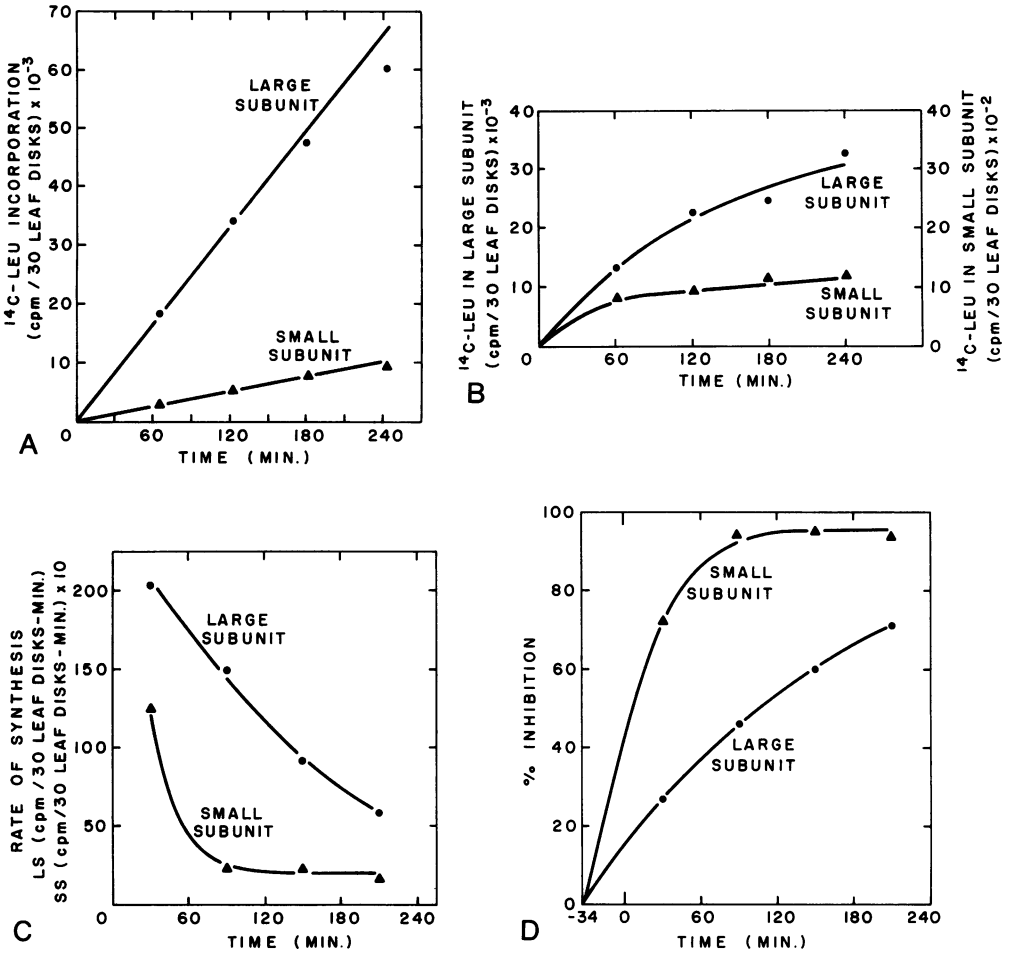

FIG. 5. Synthesis of the RuBPCase subunits in region 4–6 during a 4 h time course in the presence or absence of cycloheximide. Four samples of 30 leaf disks from region 4–6 were incubated without [^{12}C]leucine in the presence or absence of 10 μg/ml cycloheximide for the times shown. The RuBPCase subunits were resolved by two-dimensional gel electrophoresis. A, [^{14}C]leucine incorporation in the absence of cycloheximide; B, [^{14}C]leucine incorporation in the presence of cycloheximide; C, rate of [^{14}C]leucine incorporation in the presence of cycloheximide, calculated from the data in (B); D, per cent inhibition of the rate of [^{14}C]leucine incorporation in the presence of cycloheximide, calculated from the data in (A) and (B).

reported a developmental study of RuBPCase subunit synthesis in wheat leaves. Using different sampling methods and experimental techniques, they reached conclusions similar to those presented here.

Although measurements of subunit degradation were beyond the scope of the present study, recent results in another laboratory (22) suggest that in isolated pea chloroplasts large subunit degradation accompanies the assembly of the holoenzyme. Such degradation has not yet been demonstrated *in vivo* or in a whole cell system. Thus, to confirm and extend our observations it will be important for future developmental studies of RuBPCase synthesis to measure rates of subunit degradation.

Acknowledgment—We would like to thank Dr. Terence M. Murphy for his suggestions and for critical reading of the manuscript.

LITERATURE CITED

1. AMES GF-L 1974 Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs. Membranes, soluble, and periplasmic fractions. J Biol Chem 249: 634–644
2. BARRACLOUGH R, RJ ELLIS 1979 The biosynthesis of ribulose bisphosphate carboxylase. Uncoupling of the synthesis of the large and small subunits in isolated soybean leaf cells. Eur J Biochem 94: 165–177
3. 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 319: 223–234
4. BOFFEY SA, G SELLDEN, RM LEECH 1980 Influence of cell age on chlorophyll formation in light-grown and etiolated wheat seedlings. Plant Physiol 65: 680–684
5. CASHMORE AR 1976 Protein synthesis in plant leaf tissue. The sites of synthesis of the major proteins. J Biol Chem 251: 2848–2853
6. CRIDDLE RS, B DAU, GE KLEINKOPF, RC HUFFAKER 1970 Differential synthesis of ribulose diphosphate carboxylase subunits. Biochem Biophys Res Commun 41: 621–627
7. DEAN C, RM LEECH 1982 The co-ordinated synthesis of the large and small subunits of ribulose bisphosphate carboxylase during early cellular development within a seven day wheat leaf. FEBS Lett 140: 113–116
8. ELLIS RJ 1978 Chloroplast proteins and their synthesis. *In* G Norton, ed, Plant Proteins, Butterworths, London, pp 25–40
9. FAIRBANKS G, TL STECK, DFH WALLACH 1971 Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10: 2606–2617
10. FEIERABEND J, G WILDNER 1978 Formation of the small subunit in the absence of the large subunit of ribulose 1,5-bisphosphate carboxylase in 70S ribosome deficient rye leaves. Arch Biochem Biophys 186: 283–291
11. FRIEDRICH JW, RC HUFFAKER 1980 Photosynthesis, leaf resistances, and ribulose-1,5-bisphosphate carboxylase degradation in senescing barley leaves. Plant Physiol 65: 1103–1107
12. GIVAN AL, RS CRIDDLE 1972 Ribulosediphosphate carboxylase from *Chlamydomonas reinhardi*: purification, properties and its mode of synthesis in the cell. Arch Biochem Biophys 149: 153–163
13. GRAY JC, EA HOOPER, RN PERHAM 1980 Subunit stoichiometry of tobacco ribulose 1,5-bisphosphate carboxylase. FEBS Lett 114: 237–239
14. HIGHFIELD PE, RJ ELLIS 1978 Synthesis and transport of the small subunit of chloroplast ribulose bisphosphate carboxylase. Nature (Lond) 271: 420–424
15. IWANIJ V, N-H CHUA, P SIEKEVITZ 1975 Synthesis and turnover of ribulose

bisphosphate carboxylase and its subunits during the cell cycle of *Chlamydomonas reinhardtii*. J Cell Biol 64: 572–585

16. LAEMMLI UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond) 227: 680–685
17. LEAVER CJ 1979 Ribosomal RNA of plants. *In* TC Hall, JW Davies, eds, Nucleic Acids in Plants, Vol 1. CRC Press, Inc., Boca Raton, FL, pp 193–215
18. NISHIMURA M, T AKAZAWA 1978 Biosynthesis of ribulose-1,5-bisphosphate carboxylase in spinach leaf protoplasts. Plant Physiol 62: 97–100
19. NIVISON HT 1982 The synthesis of ribulose bisphosphate carboxylase in barley leaves—a developmental approach. Ph D thesis. University of California, Davis
20. O'FARREL PH 1975 High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250: 4007–4021
21. ROY H, H ADARI, KA COSTA 1979 Characterization of free subunits of ribulose-1,5-bisphosphate carboxylase. Plant Sci Lett 16: 305–311
22. ROY H, M BLOOM, P MILOS, M MONROE 1982 Studies on the assembly of large subunits of ribulose bisphosphate carboxylase in isolated pea chloroplasts. J Cell Biol 94: 20–27
23. SMITH SM, RJ ELLIS 1979 Processing of small subunit precursor of ribulose bisphosphate carboxylase and its assembly into whole enzyme are stromal events. Nature (Lond) 278: 662–664
24. STROBAEK S, GC GIBBONS 1976 Ribulose-1,5-diphosphate carboxylase from barley (*Hordeum vulgare*). Carlsberg Res Commun 41: 57–72