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Cytoplasmic and chloroplast synthesis of phycobilisome polypeptides

(translation inhibitors/phycobiliproteins/anchor protein/linker protein)

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ABSTRACT *In vivo* labeling of eukaryotic phycobilisomes in the presence of inhibitors of translation on 70S and 80S ribosomes demonstrates that some of the polypeptides of this light-harvesting complex are synthesized in the cytoplasm while others are synthesized in the chloroplast. The major pigmented polypeptides, the α and β subunits of the biliproteins (molecular weights between 15,000 and 20,000) and the anchor protein (molecular weight about 90,000) are translated on 70S ribosomes. This suggests that these polypeptides are made within the algal chloroplast. Because the α and β subunits comprise a group of closely related polypeptides, the genes encoding these polypeptides may reside in the plastid genome as a multigene family. Other prominent phycobilisome polypeptides, including a nonpigmented polypeptide that may be involved in maintaining the structural integrity of the complex, are synthesized on cytoplasmic ribosomes. Because the synthesis of phycobilisomes appears to require the expression of genes in two subcellular compartments, this system may be an excellent model for: (*i*) examining interaction between nuclear and plastid genomes: (*ii*) elucidating the molecular processes involved in the evolution of plastid genes: (*iii*) clarifying the events in the synthesis and assembly of macromolecular complexes in the chloroplast.

Phycobilisomes are the major light-harvesting complexes in the prokaryotic blue-green and eukaryotic red algae (1–3) and may represent as much as 40–60% of the soluble cellular protein (1). These complexes contain both pigmented and nonpigmented polypeptides (4, 5). The phycobilisomes of some organisms consist of 10 distinct polypeptides (5), whereas in other organisms there are about 20 polypeptides (6). The pigmented polypeptides, phycoerythrin, phycocyanin, and allophycocyanin, serve to efficiently capture light energy and transfer it to the photosynthetic reaction centers (7, 8), whereas the nonpigmented polypeptides may be involved in assembly and stabilization of the complex (9). The major pigmented polypeptides are composed of α and β subunits. The α and β subunits of the different biliprotein classes are related and share considerable homology in their amino acid sequences (10). Associated with allophycocyanin, a third polypeptide, γ, has been detected (11). Several spectral forms of the individual biliproteins exist (11), but whether the α and β subunits of these different spectral forms are encoded by one gene or more than one gene is still unclear.

Elucidation of the arrangement of phycobilisome constituents has been achieved by partial dissociation of the complex in conjunction with spectral analysis and electron microscopic examination (12–16) and has been confirmed by fluorescence emission analysis with picosecond laser spectroscopy (17, 18).

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The use of mutants has been very effective in establishing a more detailed molecular architecture of the phycobilisome (19, 20). Little was known, however, about the sites of phycobilisome biosynthesis.

In this study, we labeled *in vivo* the phycobilisomes of the eukaryotic algae *Porphyridium aerugineum*, *Porphyridium cruentum*, and *Cyanidium caldarium*. Using drugs that block translation on either 80S or 70S ribosomes, we demonstrated that eukaryotic phycobilisomes contain polypeptides of both organelle and cytoplasmic origin. This suggests that some phycobilisome genes have been transferred from the plastid genome to the nuclear genome in the course of higher algal phycobilisome evolution. Comparative studies of the structure of phycobilisome genes and the biosynthesis of these complexes in the prokaryotic blue-green and eukaryotic red algae may help to test the endosymbiont hypothesis of plastid evolution. Furthermore, the finding that the biliprotein polypeptides are synthesized on plastid ribosomes provides the first example of a multigene family encoded by a plastid genome.

MATERIALS AND METHODS

All chemicals used, unless otherwise indicated, were reagent grade. The acrylamide and *N,N'*-methylenebisacrylamide were purchased from Bio-Rad; the sodium dodecyl sulfate, from Pierce (sequanal grade); and the $^{35}SO_4$, from Amersham (SJS.1).

Growth Conditions. *P. aerugineum* (UTEX 755) was grown at 22°C in Bristol's solution (21) modified according to J. Coleman (personal communication) [250 mg of $NaNO_3$, 70 mg of $MgSO_4 \cdot 7H_2O$, 15 mg of $CaCl_2 \cdot 2H_2O$, 25 mg of NaCl, 1 mg of iron ammonium citrate, 1 ml of Gaffron's trace elements (22), 40 mg of $NaSiO_3 \cdot 9H_2O$, and 80 mg of K_2HPO_4 per liter; 0.1 mg of each of vitamin B_{12}, biotin, and thiamine per liter; 4.0 mM Tris·HCl (pH 8.0); and 5% soil extract]. *P. cruentum* (UTEX 161) was grown in Jone's artificial sea water (23) at 22°C, while *C. caldarium* (Carolina Biological, Burlington, NC) was grown at 39°C in Allen's medium (24). All cultures were bubbled continuously with 5% CO_2/95% air, and illumination was from fluorescent tubes (100 $\mu E \cdot m^{-2} \cdot sec^{-1}$).

***In Vivo* Labeling.** For *in vivo* labeling of the phycobilisome polypeptides of the three algae examined, 100 ml of midlogarithmic-phase cells were washed twice with their respective growth medium minus sulfate (and minus soil extract if originally present) and resuspended in 50 ml of the wash medium. For inhibition of translation on 70S ribosomes, we used chloramphenicol (300 μg/ml); for inhibition of translation on 80S ribosomes, we used cycloheximide (1 μg/ml). The cultures were bubbled with air for 10 min in the light (approximately the same intensity as during growth), at 22°C for *P. aerugineum* and *P. cruentum* and at 39°C for *C. caldarium*, prior to the addition of $^{35}SO_4$. Generally, 0.5 mCi (1 Ci = 3.7×10^{10} Bq) of $^{35}SO_4$

was added to each culture after the preincubation. Labeling was for 1 hr at 22°C for the *Porphyridium* species and at 39°C for *C. caldarium*.

Phycobilisome Isolation. After *in vivo* labeling, the cells were washed in 0.65 M phosphate buffer (pH 7.0), and the phycobilisomes were isolated by the method of Williams *et al.* (20) except that protease inhibitors (1 mM phenylmethylsulfonyl fluoride/1 mM benzamidine·HCl/5 mM ε-amino-*n*-caproic acid) were added to all solutions. Briefly, the cells were lysed by passage through a French pressure cell (2,500 psi; 1 psi = 6.89 kPa), the lysate was made 1% in Triton X-100 and incubated for 30 min, and the remaining intact cells and membranous debris were pelleted by centrifugation at 30,000 × *g* for 30 min. For optimal breakage, *C. caldarium* was passed through the French pressure cell twice. The supernatant was layered over a sucrose step gradient of 3.0 ml each of 1.0 M, 0.75 M, 0.50 M, and 0.25 M sucrose (plus protease inhibitors) buffered according to Williams *et al.* (20) and was centrifuged for 18 hr at 18°C in a SW-27 rotor at 25,000 rpm. For *P. cruentum*, the 0.25 M sucrose layer was eliminated, and a bottom layer of 2.0 M sucrose was added.

Sample Preparation. The intact phycobilisome band from the sucrose gradient was diluted 4–5 times with 0.75 M phosphate buffer (pH 8.0) and centrifuged for 2 hr at 45,000 rpm (at 18°C) in a 50 Ti rotor. Pellets were solubilized in 0.1 M Na_2CO_3/0.1 M dithiothreitol to an A_{620} of 0.3/20 μl for *P. aerugineum* and *C. caldarium* and an A_{550} of 0.6/20 μl for *P. cruentum* and were treated with 0.5 vol of 5% sodium dodecyl sulfate/30% sucrose/0.1% bromphenol blue. Phycobilisomes of *P. aerugineum* and *C. caldarium* were electrophoresed on 12–18% polyacrylamide gradient gels containing 8 M urea. Phycobilisomes of *P. cruentum* were electrophoresed on 7.5–15% polyacrylamide gradient gels at 4°C. The phycobilisome samples of *P. cruentum* were boiled for 1 min prior to loading the gel to dissociate high molecular weight phycobilisome complexes (they appear as stable pigmented complexes near the top of the gel unless the sample is heated). Molecular weight standards were phosphorylase B (97,000), bovine serum albumin (66,000), ovalbumin (45,000), trypsinogen (24,000), β-lactoglobin (18,400), and lysozyme (14,300). After staining with Coomassie brilliant blue G-250, destaining, and fluorography (25) were carried out, and the gel was dried and exposed to XAR-5 film.

RESULTS

Using sodium dodecyl sulfate/polyacrylamide gel electrophoresis, we resolved several polypeptide constituents of phycobilisomes of *P. aerugineum*, *C. caldarium*, and *P. cruentum*. Generally, the banding patterns observed for the polypeptide constituents of isolated phycobilisomes from these eukaryotic algae were consistent with patterns observed by others (26, 27). There were differences in the proportion of nonpigmented bands to phycobiliprotein bands, but such differences may reflect differences in growth conditions or culture densities when harvested. *In vivo* experiments performed with these organisms were repeated three times with little variation in the results.

The profile of phycobilisome polypeptides of *P. aerugineum* (Fig. 1, lane 1) shows that the polypeptides ranged in molecular weight from 15,000 to 95,000. The high molecular weight protein (band 1) is thought to be the anchor protein (28), the polypeptide involved in the attachment of the phycobilisome to the thylakoid membranes, whereas the prominent stained bands near the bottom of the gel (bands 9–12) are the major pigmented components of the complex (α and β subunits of the phycobiliproteins). Because the molecular weights of the major pigmented polypeptides are similar, some of these broad bands

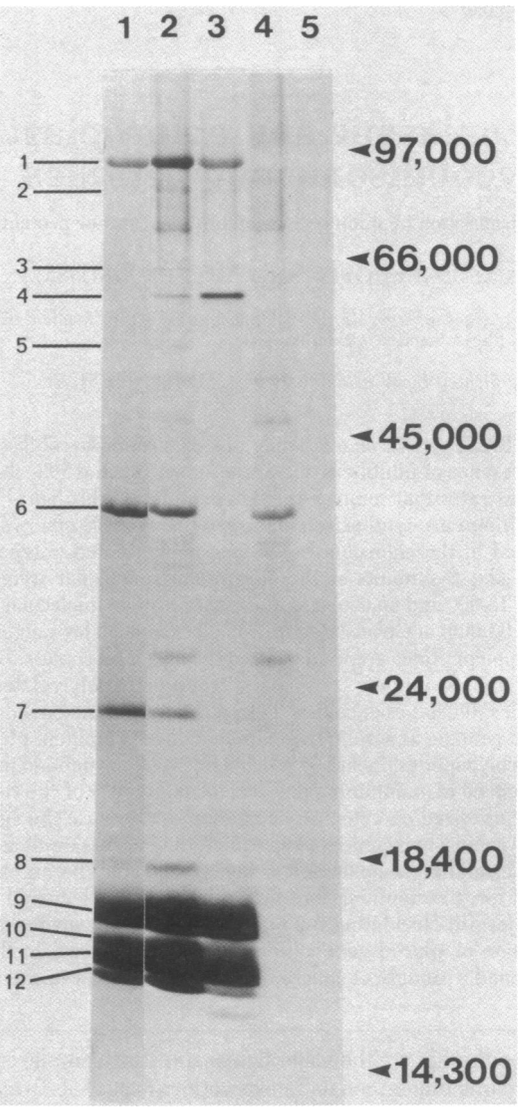

FIG. 1. Synthesis of phycobilisome polypeptides of *P. aerugineum*. Growth of *P. aerugineum*, *in vivo* labeling with and without inhibitors of translation, phycobilisome isolation, polyacrylamide gel electrophoresis, and fluorography were as described. Lanes: 1, stained profile of *P. aerugineum* phycobilisome polypeptides; 2, phycobilisome polypeptides labeled *in vivo*; 3, same as lane 2 but in the presence of cycloheximide; 4, same as lane 2 but in the presence of chloramphenicol; 5, same as lane 2 but in the presence of cycloheximide and chloramphenicol.

may represent more than one species. All of the stained polypeptides of *P. aerugineum* incorporated ^{35}S *in vivo* (Fig. 1, lane 2). Labeled bands that do not correspond to stained polypeptides can be seen, but they are generally less intense than the bands that do correspond to phycobilisome constituents. A heavily labeled band (molecular weight, 27,000), consistently observed, that does not correspond to a stained polypeptide is between band 6 and 7. It may represent a minor component rich in methionine or cysteine (or both), a poorly staining phycobilisome polypeptide, or contamination of the phycobilisome preparation with other cellular constituents. Minor stained and labeled bands also may result from a small amount of proteolysis of phycobilisome polypeptides (in spite of the fact that protease inhibitors were included in all steps of the isolation). One polypeptide that might have resulted from partial proteolysis is band 2. We observed various amounts of this stained band in different preparations, and, although we generally found

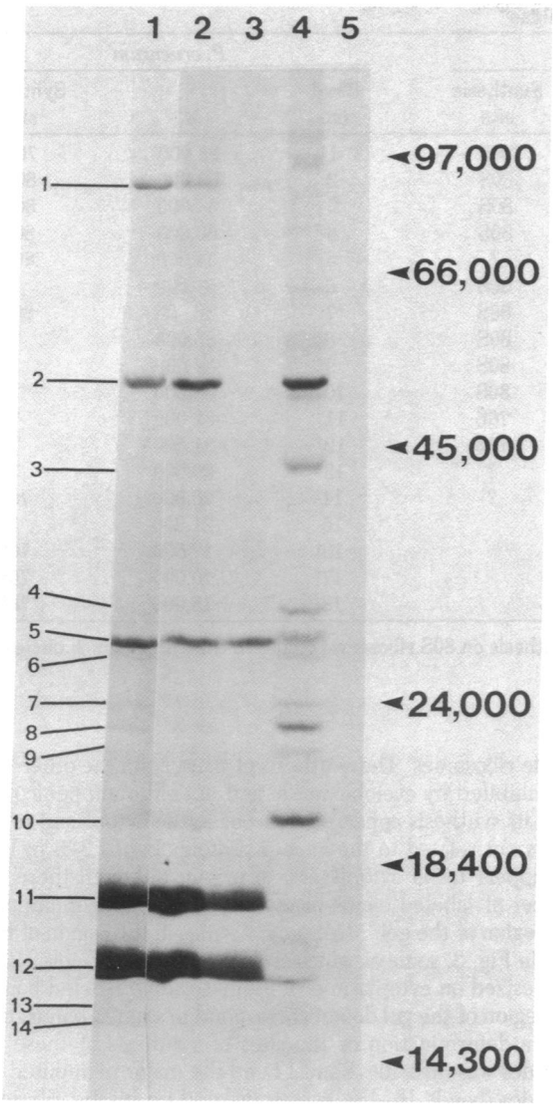

FIG. 2. Synthesis of phycobilisome polypeptides of *C. caldarium*. Procedures used are in the legend of Fig. 1. Lanes: 1, stained profile of *C. caldarium* phycobilisome polypeptides; 2, phycobilisome polypeptides labeled *in vivo*; 3, same as lane 2 but in the presence of cycloheximide; 4, same as lane 2 but in the presence of chloramphenicol; 5, same as lane 2 but in the presence of cycloheximide and chloramphenicol.

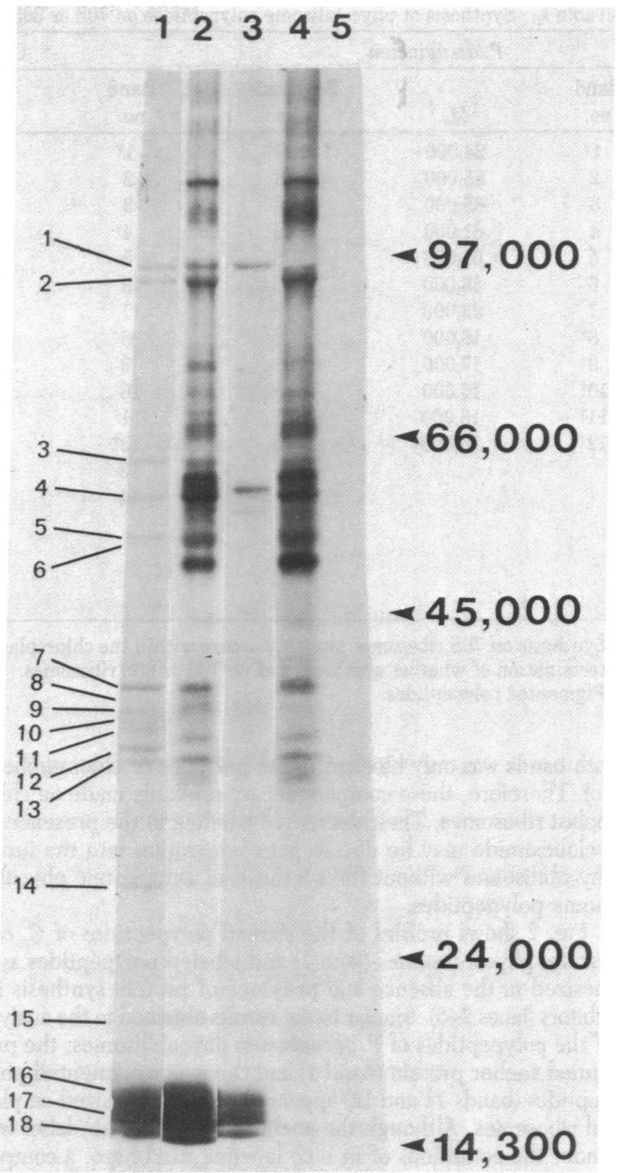

FIG. 3. Synthesis of phycobilisome polypeptides of *P. cruentum*. Procedures used are in the legend of Fig. 1. Lanes: 1, stained profile of *P. cruentum* phycobilisome polypeptides; 2, phycobilisome polypeptides labeled *in vivo*; 3, same as lane 2 but in the presence of cycloheximide; 4, same as lane 2 but in the presence of chloramphenicol; 5, same as lane 2 but in the presence of cycloheximide and chloramphenicol.

that band 1 predominated over band 2, in other published profiles the lower species (band 2) appears to predominate (27).

Bands 3, 4, and 5 in Fig. 1 are minor stained bands that were consistently observed but only clearly visible when the gels were overloaded. It is uncertain whether they are low-abundance phycobilisome constituents or contaminants. Lanes 3 and 4 show phycobilisome polypeptides that were labeled *in vivo* in the presence of cycloheximide and chloramphenicol, respectively, while lane 5 shows labeling in the presence of both inhibitors. Cycloheximide blocks translation on 80S cytoplasmic ribosomes, whereas chloramphenicol blocks translation on 70S plastid ribosomes (29). A reciprocal labeling pattern observed in the presence of these inhibitors (polypeptides synthesized in the presence of one inhibitor were not synthesized in the presence of the other inhibitor) was consistent with chloramphenicol inhibiting chloroplast protein synthesis and cycloheximide inhibiting cytoplasmic protein synthesis. Although chloramphenicol also would block translation on mitochondrial ribosomes, it does not seem likely that phycobilisome constituents are synthesized in the mitochondria. The inclusion of both inhibitors during *in vivo* protein synthesis completely blocked new synthesis of any phycobilisome constituent (Fig. 1, lane 5). A comparison of lanes 3 and 4 in Fig. 1 demonstrates that the anchor protein (band 1) and the major pigmented bands (bands 9–12) were synthesized in the presence of cycloheximide but not in the presence of chloramphenicol and, therefore, are probably chloroplast translation products.

Band 6 (Fig. 1), a prominent phycobilisome polypeptide, was synthesized on cytoplasmic ribosomes. Although the function of band 6 is uncertain, it may correspond to a linker polypeptide that has been localized to the periphery of the phycobilisome complex (27). The synthesis of polypeptides 7 and 8 (a pigmented polypeptide) was considerably lowered in the presence of either inhibitor. However, longer exposures of the fluorographed gel (not shown) indicated that the synthesis of

Table 1. Synthesis of phycobilisome polypeptides on 70S or 80S ribosomes in algae*

P. aerugineum			*C. caldarium*			*P. cruentum*		
Band no.	M_r	Synthesis site	Band no.	M_r	Synthesis site	Band no.	M_r	Synthesis site
1†	94,000	70S	1†	94,000	70S	1†	95,000	70S
2	85,000	70S	2	54,000	80S	2	93,000	80S
3	65,000	70S	3	44,000	80S	3	64,000	80S
4	61,000	70S	4	33,000	80S	4	60,000	80S
5	55,000	70S	5	30,000	?	5	54,000	80S
6	38,000	80S	6	28,000	80S	6	51,000	?
7	23,000	70S	7	24,500	80S	7	36,000	80S
8†	18,000	70S	8	23,500	80S	8	35,000	?
9†	17,000	70S	9	23,000	80S	9†	34,000	?
10†	16,500	70S	10	20,000	80S	10	33,000	?
11†	16,000	70S	11†	17,500	70S	11†	32,000	?
12†	15,500	70S	12†	16,500	70S	12†	31,000	?
			13	15,500	?	13†	30,000	?
			14	15,000	?	14	26,000	70S
						15	21,000	?
						16†	17,500	70S
						17†	16,000	70S
						18†	15,000	70S

*Synthesis on 70S ribosomes probably occurs within the chloroplast, whereas synthesis on 80S ribosomes occurs in the cytoplasm. ?, unclear determination of whether synthesis was on 70S or 80S ribosomes.
†Pigmented polypeptides.

both bands was only blocked in the presence of chloramphenicol. Therefore, these components are probably made on chloroplast ribosomes. Their decreased labeling in the presence of cycloheximide may be due to poor integration into the intact phycobilisomes without the synthesis of cytoplasmic phycobilisome polypeptides.

Fig. 2 shows profiles of the stained polypeptides of *C. caldarium* phycobilisomes (lane 1) and labeled polypeptides synthesized in the absence and presence of protein synthesis inhibitors (lanes 2–5). Similar to the results obtained in the analysis of the polypeptides of *P. aerugineum* phycobilisomes, the presumed anchor protein (band 1) and the major pigmented polypeptides (bands 11 and 12) appeared to be synthesized on plastid ribosomes. Although the anchor protein did not label well under the conditions of *in vivo* labeling used here, a comparison of lanes 3 and 4 in Fig. 2 and longer exposures of the fluorographed gel (not shown) indicated that this high molecular weight protein was made in the presence of cycloheximide but not in the presence of chloramphenicol. Other polypeptides of *C. caldarium* phycobilisomes were synthesized on 80S ribosomes. The major component, band 2 (molecular weight, 54,000), was translated on cytoplasmic ribosomes, as were less prominent polypeptides (Fig. 2, bands 3, 4, 6–10). Some of these less prominent polypeptides probably represent cytoplasmic contamination of the phycobilisome preparation. Generally, the cytoplasmically synthesized polypeptides incorporated more label in the presence of chloramphenicol than in its absence (compare lanes 2 and 4 of Fig. 2). It is uncertain why this occurred. Because of low incorporation of label, we could not determine the site of translation of bands 13 and 14. Band 5, on the other hand, was labeled in the presence of either inhibitor and may represent two comigrating components.

Analysis of phycobilisomes of *P. cruentum* supports the general observation of the previous analyses. *P. cruentum* phycobilisomes contained between 18 and 20 polypeptides as determined by polyacrylamide gel electrophoresis. A profile is shown in Fig. 3, lane 1. The anchor protein, band 1 (28), has a molecular weight of 95,000. A comparison of lanes 3 and 4 in Fig. 3 indicates that this polypeptide was synthesized on 70S, organelle ribosomes. The synthesis of band 2, on the other hand, was inhibited by cycloheximide and not chloramphenicol. Because its synthesis appeared to occur in the cytoplasm, it probably is not related to the anchor protein. Bands 3–6 in Fig. 3 also appear to be cytoplasmic in origin, although there are a number of labeled bands of cytoplasmic origin contaminating that region of the gel. Most polypeptides in the region of bands 7–13 in Fig. 3, some of which are pigmented (see Table 1), were synthesized on cytoplasmic ribosomes. Some labeled bands in this region of the gel do not correspond to stained bands; therefore, a determination of the sites of synthesis of these polypeptides was difficult. Band 14 and the major pigmented polypeptides (bands 16–18) were translated on plastid ribosomes. Band 15 was synthesized in the presence of either cycloheximide or chloramphenicol; therefore, the site of synthesis of this polypeptide could not be determined. (Band 15, like band 5 of *C. caldarium* phycobilisomes, may represent two species, with one component synthesized inside the organelle and the other in the cytoplasm.)

In Table 1 we list the molecular weights of the phycobilisome polypeptides from the three algae examined and the site at which these polypeptides appear to be translated. We indicate with an asterisk which of the polypeptides we visualize as being pigmented. Small differences in the molecular weights of the individual polypeptides among studies (compare our molecular weights with those in refs. 26 and 27) may be due to the use of different marker proteins and electrophoresis systems. Although it was difficult to determine on which set of ribosomes some of the phycobilisome polypeptides are synthesized (especially for *P. cruentum*), the site of synthesis of many were clearly resolved. The major colored polypeptides (molecular weights between 15,000 and 20,000) and the anchor protein of all three organisms examined were synthesized on 70S ribosomes and, therefore, most likely are translated and perhaps transcribed within the chloroplast. Other prominent components were cytoplasmic in origin. These polypeptides must pass through the chloroplast envelope to reach their site of assembly and function. The cytoplasmically synthesized phycobilisome constituents may represent linker polypeptides, involved in

maintaining the phycobilisome structure, as has been suggested for band 6 of *P. aerugineum* and band 2 of *C. caldarium* (27), or they may represent minor pigmented polypeptides.

DISCUSSION

Although more than one theory has been postulated to explain the origin of plastids, perhaps the leading theory suggests that plastids arose through serial endosymbiosis (30, 31). In the course of plastid evolution, many functions of the invading organism (perhaps a cyanobacterium) were transferred to the genome of the host organism. Evidence for gene transfer is accumulating (32). From this study, it seems likely that some phycobilisome genes have been transferred from the plastid genome to the nuclear genome and that at least one such gene is involved in linking the phycobilisome constituents together. A critical discussion of linker polypeptides has recently appeared (33). Studies to define which cytoplasmically synthesized phycobilisome polypeptides of the red algae have analogues in the phycobilisomes of cyanobacteria and the nature of the genes encoding these polypeptides (structural plus flanking regions) will be important in our understanding of gene evolution and the process of gene transfer (from endosymbiont to host genome).

This study also indicates that the pigmented polypeptides are synthesized on chloroplast ribosomes; therefore, the genes encoding these polypeptides probably are localized on the plastid DNA. Thus far, the genes for polypeptides translated within the plastid have been localized only to the plastid genome (34). The major pigmented polypeptides are composed of α and β subunits. As indicated by amino acid sequence analyses (35–39), the α subunits among the biliproteins are related as are the β subunits. The α and β subunits also are related to each other. Furthermore, each biliprotein class may contain more than one type of α and more than one type of β subunit (11). The data presented here in conjunction with data establishing the similarities among biliprotein polypeptides suggests that a multigene family encoding these polypeptides exists on the chloroplast DNA. The arrangement of these genes on the plastid genome, their nucleotide sequences, and the coordination of their synthesis with nuclear encoded components will help elucidate the evolution of these genes, their regulation, and the molecular events involved in the biosynthesis of phycobilisomes.

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