COMPLEMENTARY CHROMATIC ADAPTATION
IN A FILAMENTOUS BLUE-GREEN ALGA

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ABSTRACT
Fluorescent and red light environments generate greatly different patterns of pigmentation and morphology in *Fremyella diplosiphon*. Most strikingly, red-illuminated cultures contain no measurable C-phycoerythrin and have a mean filament length about 10 times shorter than fluorescent-illuminated cultures. C-phycoerythrin behaves as a photoinducible constituent of this alga. Spectrophotometric and immunochemical procedures were devised so that C-phycoerythrin metabolism could be studied quantitatively with [14C]-phenylalanine pulse-chased cultures. Transfer of red-illuminated cultures to fluorescent light initiates C-phycoerythrin production by essentially de novo synthesis. C-phycoerythrin is not degraded to any significant extent in cultures continuously illuminated with fluorescent light. Transfer of fluorescent-illuminated cultures to red light causes an abrupt cessation of C-phycoerythrin synthesis. The C-phycoerythrin content of cultures adapting to red light decreases and subsequently becomes constant. Loss of C-phycoerythrin is not brought about by metabolic degradation, but rather by a decrease in mean filament length which is effected by transcellular breakage. In this experimental system, light influences intracellular C-phycoerythrin levels by regulating the rate of synthesis of the chromoprotein.

INTRODUCTION
Environmental conditions exert a strong influence upon the pigment composition of many algae (Hallåld, 1970). Complementary chromatic adaptation is a spectacular response of some blue-green and red algae to alterations in the energy distribution in the visible light environment. As a consequence of this phenomenon, the pigment which absorbs the incident wavelengths of light most strongly becomes predominant. Gaidukov (1902) first described complementary chromatic adaptation in blue-green algae. He observed that *Oscillatoria sancta* assumed a red coloration after growth under green light and a blue-green tint after growth under orange light. Gaidukov (1902) ascribed these color variations to the synthesis of different kinds of pigments. Kylin (1912) and Boresch (1919, 1921), further, correctly perceived that such color changes in blue-green algae are primarily a specific consequence of alterations in the relative proportions of the red and blue phyco-biliprotein constituents. The demonstration that phyco-biliproteins function as accessory pigments (Engelmann, 1883; Emerson and Lewis, 1942; Haxo and Blinks, 1950; Duysens, 1951; French and Young, 1952) provided a rationale for complementary chromatic adaptation: alterations in the levels of these chromoproteins permitted maximal utilization of the available light energy for photosynthetic purposes.

The principal concern of the work reported in
this communication was to define, in broad metabolic terms, how light influences C-phycocerythrin (PE)\(^1\) metabolism during complementary chromatic adaptation in *Fremyella diplosiphon*. The net intracellular levels of individual phycobiliproteins may decrease during complementary chromatic adaptation in growing blue-green algae. Such light-induced decreases could be brought about either by acceleration in the rate of phycobiliprotein degradation or by deceleration in the rate of phycobiliprotein synthesis and dilution with growth. It is impossible a priori to distinguish between these alternatives. Experimentally, “synthesis-versus-degradation” problems have been most readily approached with inducible cellular constituents, such as β-galactosidase in *Escherichia coli* (see Hogness et al., 1955). The levels of inducible components are very low in the absence of appropriate environmental stimuli. Spectral response curves for complementary chromatic adaptation in *Tolypholytx tenuis* (Fujita and Hattori, 1962) indicated that quanta with wavelengths greater than about 585 nm do not stimulate PE synthesis. This suggested that it might be possible to utilize restricted conditions of illumination to give PE the status of an inducible cell constituent during complementary chromatic adaptation.

The filamentous blue-green alga *F. diplosiphon* was selected for these experiments because it shows striking complementary chromatic adaptation and because its trichomes, unlike those of *T. tenuis* and many other filamentous blue-green algae, do not clump together into large masses or adhere to the sides of the culture flasks. This latter feature greatly reduces the variability between replicate samples obtained from liquid cultures.

Numerous differences in pigmentation and morphology exist between *F. diplosiphon* adapted to fluorescent and to red light. The kinetics of the changes in pigmentation and morphology which occur in *Fremyella* during complementary chromatic adaptation were studied by transferring fluorescent-illuminated cultures to red light. These experiments made use of a microscale purification procedure to obtain PE for specific activity measurements from pulse-chased cultures. It is shown that fluorescent light may be utilized to “induce” PE production in red light-adapted cultures, and that red light may be employed to turn off PE synthesis in fluorescent light-adapted cells.

### EXPERIMENTAL SECTION

#### General Culture Conditions

*F. diplosiphon* (B. and F.) Drouet (strain 481) was obtained in axenic culture from the collection at Indiana University (Starr, 1964). Dr. F. Drouet (personal communication) now considers *F. diplosiphon* to be a growth form of *Calothrix parietina* (Näg.) Thur. The organism was grown at 32 ± 2°C in cotton-plugged, shaker-agitated, 2800-ml Fernbach flasks in modified Medium C (Kratz and Myers, 1955). Sodium ferric diethylenetriamine pentaacetate (Sequestrene) (Geigy Chemical Corp., Ardsley, N. Y.) was added to a final concentration of 40 mg/liter of medium as a substitute for Fe\(_2\)(SO\(_4\))\(_3\)-6H\(_2\)O, and the KNO\(_3\) concentration was increased to 2 g/liter. The pH of the medium was adjusted to 7.0 with 1 N NaOH before autoclaving. Fluorescent illumination (250 foot candles [ft-c]) was provided by a bank of Sylvania F 30 T12-W-RS lamps. Incandescent illumination (100 ft-c) was derived from a rack of 60-W, 120-V Sylvania F 30 T12-W lamps. Red illumination (26 ft-c) was obtained by interposing a 1/8-inch thick EvrKleer transparent amber cast acrylic filter (no. 2422, Rohm and Haas Co., Philadelphia, Pa.) between General Electric 60A21/R red lamps and the culture flasks.

#### Light Measurements

Absorption spectra of whole cells and crude extracts were determined with a Cary model 14 recording spectrophotometer equipped with a model 1462 scattered transmission accessory (Cary Instruments, Monrovia, Calif.). Light-source emission spectra were measured with an ISCO spectroradiometer (Instrumentation Specialties Co., Lincoln, Neb.). Incident light intensities at the shaker surfaces were determined either by manual integration of light-source emission spectra from 400 to 730 nm or with a Weston model 756 sunlight illumination meter (Weston Electrical Instrument Corp., Newark, N.J.).

#### Culture and Harvesting Procedures for Metabolism Experiments

Pulse-chased, fluorescent light-adapted cultures for metabolic experiments were initiated by adding 15...
µCi of L-[14C]phenylalanine and a small liquid inoculum containing 3–4 mg dry weight of healthy incandescent light-grown cells to 500 ml of medium. The cultures were allowed to proliferate under fluorescent illumination until the cells in 1 ml of medium contained about 5.1 µg of chlorophyll a (chl a). Fresh medium (500 ml) containing a 2,000-fold molar excess of unlabeled L-phenylalanine was then added to the cultures. Chasing under fluorescent illumination proceeded until the chl a level again reached about 5.1 µg/ml. The protocol for preparing red light-adapted, pulse-chased cultures was identical, except that (a) inocula were obtained from red light-adapted cultures, (b) the cultures were grown under red light, and (c) the chase with unlabeled L-phenylalanine was started when a chl a level of 3.3 µg/ml was attained. Both L-[14C]phenylalanine (uniformly labeled) (New England Nuclear [Boston, Mass.], NEC-284, sp act 472 mCi/mmol, stated radiochemical purity > 99.6%) and unlabeled L-phenylalanine (Nutritional Biochemicals Corp., Cleveland, Ohio) were dissolved in 10 ml of medium and added to the culture flasks via a sterile Swinnex-13 filter unit containing a 0.22 µm filter (Millipore Corp., Bedford, Mass.).

At each sampling point, duplicate aliquots were removed for estimation of phycobiliproteins (20 ml), for estimation of chl a and total counts incorporated (5 ml), and for estimation of growth (2 ml), and were transferred to graduated 15-ml conical glass centrifuge tubes. During sampling, sterile technique was used and red light-illuminated cultures were manipulated only under red light or in darkness. Samples could be harvested and washed quantitatively if the filaments were pelleted with an IEC model UV centrifuge (Damon/IEC Div., Needham Hts., Mass., no. 240 head, 2,000 rpm, 5 min, 23°C) and the supernatants were carefully removed by hand with a Pasteur pipette. Harvested cell pellets were washed rapidly either three times (phycochlorin, growth aliquots) or four times (chl a aliquots) with 10 ml of distilled water.

Chl a and Phycobiliprotein Quantitation

Blue-green algae contain chl a only (Bogorad, 1962). Chl a was quantitatively removed from washed cell pellets by extraction with two 3.0-ml aliquots of absolute methanol in dim light at about 23°C. The chl a content of the combined extracts was determined from the optical density of the solution at 665 nm. The extinction coefficient of chl a at 665 nm in absolute methanol is 74.5 ml/mg-cm (MacKinney, 1941). Absorption spectra of methanol extracts indicated that pheophytinization did not occur to any significant extent during chl a extraction.

To estimate phycobiliprotein contents, washed cell pellets were resuspended to a total volume of 6.0 ml with 0.01 M sodium phosphate, pH 7.0, 0.15 M NaCl (buffered saline). The cell suspensions were transferred to Beckman no. 302235 tubes (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), quick-frozen in liquid nitrogen, capped with Parafilm, and stored at −20°C in darkness before disruption. Thawed cell suspensions were immersed in a stirred ice bath and sonicated with the microtip of a Heat Systems model W185D sonifier (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) operated at power setting 6. Each aliquot of cells was exposed to 4 min of sonication, with alternate 1-min periods of sonication and cooling. Light microscope examinations demonstrated that this procedure quantitatively disrupts the cells. Over the cell concentration ranges used in these experiments, the amount of each water-soluble phycobiliprotein released into the buffered saline solution was directly proportional to the number of cells disrupted. Sonicates were transferred to Beckman polycarbonate tubes and centrifuged at 4°C and 81,000 g at average radius of centrifugation (Rv) for 45 min. This procedure pellets the membrane fragments of the cells, which contain chl a and most of the carotenoids. Phycobiliproteins could not be detected in membrane fragments which were resuspended in buffered saline. The supernatant (crude extract) contains the phycobiliproteins and other soluble proteins. Phycobiliproteins are responsible for essentially all of the visible light absorption above 500 nm in the crude extracts. The upper 5.5 ml of the supernatants were carefully withdrawn and their absorption spectra were determined from 730 to 500 nm. Insertion of the optical densities of the crude extracts at 562, 615, and 652 nm into equations derived from extinction coefficients of the purified phycobiliproteins permitted the PE, C-phycocyanin (PC), and allophycocyanin (APC) concentrations in the crude extracts to be ascertained.

The extinction coefficient of each of the three F. diplosiphon phycobiliproteins, purified by the procedure of Bennett and Bogorad (1971), was determined in buffered saline at 562, 615, and 652 nm. These wavelengths correspond to the absorption maxima of PE, PC, and APC, respectively, in buffered saline. This solvent was selected because it provides optimal conditions for precipitin reactions with rabbit antisera (Kabat and Mayer, 1967). The method of Lowry et al. (1951) was used to estimate phycobiliprotein concentrations for extinction coefficient calculations and also to estimate total soluble protein concentrations in crude algal extracts. Standard curves for the Lowry protein measurements were obtained from solutions prepared with cytochrome c which had been thoroughly desiccated over P2O5 in vacuo. Equations for calculating phycobiliprotein concentrations in crude extracts were derived by combining the extinction coefficients with three simultaneous equations having the form ODx = (PPE, XCPFL) + (PPE, XPC) + (PPE, XAPC).
cells were prepared. These samples were quantitatively transferred to preweighed glass tubes and dried to constant weight in a vacuum oven at 100°C. When determining pigment fractions of the total cellular dry weight, duplicate aliquots of washed cells were prepared. These samples were analyzed quantitatively with regard to their phycobiliprotein and chl a content, and their dry weights were determined directly. Turbidity measurements were made to estimate growth during metabolic experiments. Washed cell pellets were brought to volume with distilled water and the optical density of the cell suspensions was determined at 730 nm in 1-cm cuvets with a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Under these conditions, cellular dry weight in mg/ml ≈ 0.265 (OD730), between 0.075 and 0.275 OD736 units.

**Dry Weight and Growth Determinations**

To estimate dry weights, washed cell pellets were quantitatively transferred to preweighed glass tubes and dried to constant weight in a vacuum oven at 100°C. When determining pigment fractions of the total cellular dry weight, duplicate aliquots of washed cells were prepared. These samples were analyzed quantitatively with regard to their phycobiliprotein and chl a content, and their dry weights were determined directly. Turbidity measurements were made to estimate growth during metabolic experiments. Washed cell pellets were brought to volume with distilled water and the optical density of the cell suspensions was determined at 730 nm in 1-cm cuvets with a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Under these conditions, cellular dry weight in mg/ml ≈ 0.265 (OD730), between 0.075 and 0.275 OD736 units.

**Immunological Procedures**

Antibodies to each of the purified phycobiliproteins were prepared for use in metabolic experiments and in Ouchterlony double-diffusion tests. For primary immunization, female New Zealand White rabbits were each injected intracutaneously in the back with 5-10 mg of purified phycobiliprotein in a thick emulsion prepared from equal volumes of Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.) and buffered saline. After 6 wk, a secondary response was effected by the injection of 3-5 mg of purified phycobiliprotein in buffered saline into the marginal ear vein. Major bleedings were carried out 6-9 days after the secondary response was stimulated if the antibody titers were sufficiently high, judging from the three-tube test of Chase (1967). Quantitative precipitin curves indicated that specific antibody levels of 4-5 mg/ml of serum were usually elicited by this immunization protocol. Antisera from the first bleeding after the secondary response were used in all experiments.

Antibody purification was carried out at 4°C. Globulin and IgG fractions were prepared by chromatography of globulin fractions on DEAE-cellulose following the procedure of Koshland et al. (1962). Globulin and IgG fraction were dialyzed extensively against buffered saline, centrifuged at 10,000 g for 15 min, and stored in 5-ml aliquots at −20°C. Globulin and IgG fractions were used in Ouchterlony double-diffusion tests and precipitin reactions, respectively.

Quantitative precipitin curves were determined according to Kabat and Mayer (1967, pp. 73-76). The total protein content of immunoprecipitates was estimated by a micro-Folin procedure (Kabat and Mayer, 1967, pp. 537-558), using cytochrome c as standard. Precipitin reactions were carried out in buffered saline in conical 15-ml, graduated Kimble polycarbonate centrifuge tubes (Kimble Products Div., Owens-Illinois, Inc., Toledo, Ohio). Precipitin mixtures were capped with Parafilm and left standing in the dark in an ice bath for 24-36 h before the precipitates were harvested. Precipitates were washed twice with two 6-ml volumes of ice-cold buffered saline. Quantitative precipitin reaction supernatants were tested for residual soluble antigen (PE) with a Blak-Ray ultraviolet lamp (Ultra-Violet Products, Inc., San Gabriel, Calif.). Because of its intense orange fluorescence, PE may be unambiguously detected by this procedure at concentrations greater than 0.5 µg/ml.

To prepare Ouchterlony double-diffusion plates, 1 g Difco bacto agar and 20 mg Merthiolate were dissolved with stirring in 100 ml buffered saline, using a double boiler arrangement. Level, 0.5% agar-coated Kodak slide projector plates (3½ x 4 inches, Eastman Kodak Co., Rochester, N.Y.) were each overlaid with 20 ml of the liquefied agar solution. Antigen and antibody wells were cut into solidified

### Table I

<table>
<thead>
<tr>
<th>Phycobiliprotein</th>
<th>Extinction coefficients</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>λ = 562 nm</td>
</tr>
<tr>
<td>PC</td>
<td>2.41</td>
</tr>
<tr>
<td>APC</td>
<td>0.849</td>
</tr>
<tr>
<td>PE</td>
<td>9.62</td>
</tr>
</tbody>
</table>

Phycobiliprotein concentration equations

\[
[PC] = \frac{OD_{615} - 0.474(OD_{652})}{5.34}
\]

\[
[APC] = \frac{OD_{615} - 0.208(OD_{652})}{5.09}
\]

\[
[PE] = \frac{OD_{652} - 2.41(PC) - 0.849(APC)}{9.62}
\]
agar layers with a no. 5 cork borer, and a distance of 1.8-1.9 cm separated their centers. Plates were allowed to develop for 36 h at 23°C after the addition of 100 μl antibody and antigen aliquots, in buffered saline, to various wells. Results of double-diffusion tests were not qualitatively altered from those illustrated when antigen concentrations were either increased or decreased 10-fold.

**Preparation and Counting of Radioactive Materials**

Washed immunoprecipitates containing [14C]phenylalanine-labeled PE were carefully resuspended with several drops of 0.5 M NaOH and quantitatively transferred to numbered Whatman filter paper disks (2.4 cm diameter, no. 3 qualitative). After drying, the disks were processed by the method of Mans and Novelli (1961) to selectively extract coprecipitating nucleic acid and lipid components of the cell extracts. The 90°C, 10% TCA extraction was limited to 15 min. For total soluble protein radioactivity measurements, crude extracts were applied to filter paper disks and processed identically.

The magnitude of radioactive "pools" remaining at the end of chase periods was estimated by extracting washed cells twice with 3.0 ml of ice-cold 5% TCA and determining the fraction of the total counts which were soluble. Total incorporated radioactivity measurements during metabolic experiments were determined with cells which had been extracted with methanol for chl a determinations. It was necessary to utilize cells so extracted in order to eliminate carotenoid quenching. Methanol-extracted cells and aliquots of 5% TCA extracts were transferred to glass vials, air-dried for 24 h, and processed for counting by the method of Tishler and Epstein (1968). These samples, as well as the nucleic acid and lipid extracted immunoprecipitate and total soluble protein disks, were counted in a Picker 220 liquid scintillation counter (Picker Corp., Cleveland, Ohio) after 10 ml of toluene-Omnifluor (New England Nuclear) solution was added to the counting vials.

**Filament Length Measurements**

Small culture samples, obtained with a sterile inoculating loop, were gently transferred to slides. The filaments were allowed to settle for several minutes and then photographed, without the use of a cover slip, at 15X on 35-mm film. Prints were made on 8 X 10 inch paper. The lengths of at least 100 filaments from each sample were determined with a Keuffel and Esser no. 620305 map measure (Keuffel & Esser Co., Morristown, N.J.). A stage micrometer scale was magnified to the same extent as the filaments in order to calibrate the map measure.

**Immunoprecipitates Containing 14CO2-Labeled PE**

Several methods were used to check anti-PE specificity in precipitin reactions with crude extracts. The specific activity (ca. 500 cpm/μg) of PE from fluorescent-illuminated, [14C]phenylalanine-labeled cultures was not sufficiently high to permit anti-PE specificity to be evaluated by analysis of the distribution of radioactivity in dissociated antigen-antibody complexes on sodium dodecyl sulfate (SDS) gels. PE having a high enough specific activity for this purpose (ca. 10,000 cpm/μg) was obtained by allowing a small inoculum of F. diplosiphon to proliferate under fluorescent illumination in a closed system in the presence of 5% 14CO2, Ba14CO3 (New England Nuclear, NEC-009, sp act: 54.9 mCi/mmol) was mixed with sufficient nonradioactive BaCO3 to reduce the specific activity to 125 μCi/mmol, and 14CO2 was generated by acidification of the mixture with 2 M H3PO4.

Solutions containing 8 M urea, 0.72 M 2-mercaptoethanol, 0.002 M phenylmethylsulfonyl fluoride (PMSF) (see Schulze and Colowick, 1969), 4.8% ethanol, and 0.5 M Tris-HCl, pH 8.3, were used to dissociate and reduce the antigen-antibody complexes. This solution (300 μl) was added to washed immunoprecipitates containing 100 μg of labeled PE and the mixture was placed in a boiling water bath for 1 min to solubilize the precipitates. The solutions were then covered tightly with Parafilm and incubated at 37°C for 3 h to permit sulfhydryl reduction. The solutions were cooled to 23°C and mixed with 250 μl of a solution containing 5% SDS, 20% (wt/vol) iodoacetamide (Calbiochem, San Diego, Calif.) and 0.5 M Tris-HCl, pH 8.5. The tubes were then capped with Parafilm and left in darkness at room temperature for 1 h to permit alkylation of the dissociated, reduced complexes. Alkylation was stopped by the addition of 25 μl of 14.4 M 2-mercaptoethanol. The mixtures were then dialyzed against several 50-ml volumes of 2% SDS, 0.01 M sodium phosphate, pH 7.0. Dialysates were concentrated approximately twofold by placing the bags on small mounds of Sephadex G-200.

Concentrated dialysates were subjected to SDS gel electrophoresis and the gels were fixed, stained, and scanned at 620 nm following the procedures of Bennett and Bogorad (1971), with several modifications: (a) electrode and gel buffer strengths were halved, (b) electrophoresis was carried out for 6.5 h, (c) the methylene bisacrylamide concentration was doubled, (d) a 10 mg/ml ammonium persulfate solution was used, and (e) gel tubes were immersed in a stirred 23°C water bath during polymerization. With these modifications, the resolution in the 25,000-15,000 molecular weight region is greatly increased. Gels
were calibrated with $\beta$-galactosidase (130,000), glutamate dehydrogenase (53,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), and ribonuclease A (13,700). Sources and molecular weight references for these standard proteins are listed in Weber and Osborn (1969).

Stained scanned gels were cut into approximately 1.1-mm thick slices with a device formed from an alternating series of razor blades and washers. Slices were transferred to glass counting vials, air-dried, solubilized, and counted following the procedure that was described for methanol-extracted cells. Background was determined with identically processed slices of gels devoid of radioactivity. Channel ratio measurements indicated that the solubilized gel slices were counted with a uniform efficiency of about 86%.

RESULTS

Pigmentation and Morphological Characteristics of F. diplosiphon Adapted to Fluorescent and to Red Illumination

Fig. 1 illustrates the emission spectra of the fluorescent and red light sources used, together with absorption spectra of intact cells and crude extracts of F. diplosiphon grown under these conditions of illumination.

In vivo spectra were obtained with suspensions containing approximately 0.68 mg cellular dry weight per ml. Chl $\alpha$, PC, and PE are primarily responsible for the peaks occurring in vivo at 678–680, 624–629, and 569 nm, respectively. An APC peak is not observed in vivo. However, APC absorption is responsible for the prominent shoulder at 649–653 nm in crude extracts of F. diplosiphon grown under fluorescent light. Peaks in the in vivo absorption spectrum are also found at about 490 nm (carotenoids) and 430 nm (Soret band of chl $\alpha$) (see Emerson and Lewis, 1942). The fluorescent light emission peak is very close to the absorption maximum of PE in vivo. Absorption spectra of intact F. diplosiphon adapted to red light do not contain any suggestion of a PE peak.

Table II enumerates the quantitative differences in pigmentation between F. diplosiphon adapted to fluorescent and to red light. Phycobiliprotein contents were estimated from analyses of absorption spectra of crude algal extracts with the information contained in Table I. The values listed in Table II were determined with cultures having a cellular dry weight of 0.25–0.35 mg/ml when harvested. Per unit of cellular dry weight, cultures adapted to fluorescent illumination have at least 205 times more PE, approximately 3.1 times less PC, 1.7 times less APC, and about 1.5 times more chl $\alpha$ than cultures adapted to red illumination. The information in Table II also demonstrates that phycobiliproteins constitute the bulk of the soluble protein in F. diplosiphon under the culture conditions used in these experiments.

The absence of PE from red light-adapted cultures may be demonstrated with Ouchterlony double-diffusion tests. The results of such tests with purified phycobiliproteins and crude extracts of fluorescent- and red light-adapted cultures are illustrated in Fig. 2. Both of the PE precipitin lines were red and fluoresced orange, and the PE lines both were blue and fluoresced red. No cross-reaction was observed between any of the phycobiliproteins, which indicates that the protein components of PE, PC, and APC from F. diplosiphon are unrelated. The subunit molecular weights of these phycobiliproteins are also distinct (Bennett and Bogorad, 1971). Lines of identity were ob-
TABLE II
Intensity Differences between the Fluorescent and Red Light Sources and Differences in Pigmentation and Morphology between F. diplosiphon Adapted to these Conditions of Illumination

<table>
<thead>
<tr>
<th>Illumination*</th>
<th>Fluorescent</th>
<th>Red</th>
</tr>
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<tbody>
<tr>
<td>Incident light intensity</td>
<td>666 µW/cm², 290 ft-c$</td>
<td>282 µW/cm², 26 ft-c$</td>
</tr>
<tr>
<td>Percentage of total soluble protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE</td>
<td>21.0</td>
<td>&lt;0.11</td>
</tr>
<tr>
<td>PC</td>
<td>14.3</td>
<td>47.3</td>
</tr>
<tr>
<td>APC</td>
<td>9.5</td>
<td>15.9</td>
</tr>
<tr>
<td>Total</td>
<td>44.8</td>
<td>63.3</td>
</tr>
<tr>
<td>Percentage of total cellular dry weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE</td>
<td>6.17</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>PC</td>
<td>4.40</td>
<td>13.7</td>
</tr>
<tr>
<td>APC</td>
<td>3.17</td>
<td>5.37</td>
</tr>
<tr>
<td>Chl. a</td>
<td>2.42</td>
<td>1.58</td>
</tr>
<tr>
<td>Total</td>
<td>16.16</td>
<td>20.77</td>
</tr>
<tr>
<td>Mean filament length</td>
<td>460 µm</td>
<td>50 µm</td>
</tr>
</tbody>
</table>

* Emission spectra of the fluorescent and red light sources are illustrated in Fig. 1.
† Obtained by manual integration of emission spectra from 400 to 730 nm.
$ Measured with a Weston model 756 sunlight illumination meter.
|| Determined by serological methods.

Figure 2 Ouchterlony double-diffusion tests with crude extracts and purified phycobiliproteins from F. diplosiphon. Key to antibody and antigen wells: aPE, anti-C-phycoerythrin; aPC, anti-C-phycocyanin; aAP, anti-allophycocyanin; PE, purified C-phycoerythrin; PC, purified C-phycocyanin; APC, purified allophycocyanin; FCE, crude extract of cells adapted to fluorescent illumination; RCE, crude extract of cells adapted to red illumination.

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...tained between purified and impure phycobiliproteins, suggesting that the antigenic properties of these compounds are not altered in the course of purification. No precipitin lines ever formed between wells containing crude extracts of red light-adapted cultures and anti-PE. Double-diffusion tests are more sensitive than spectral measurements with crude extracts as a means of estimating the PE content of red light-adapted cultures. If the anti-PE preparation was adequately diluted, orange-fluorescing precipitin lines formed in double-diffusion tests with 100-µl PE aliquots having a concentration as low as 0.02 mg/ml. Orange-fluorescing lines failed to form in parallel double-diffusion tests in which 100 µl of a concentrated crude extract from a red light-adapted culture (total protein concentration, 17.9 mg/ml) was added to the antigen well. Serial dilutions of the concentrated crude extract also failed to give precipitin lines, demonstrating that antigen excess did not inhibit the precipitin reaction. On the basis of these results, it was concluded that PE is less...
than \((0.02/17.9)\times 100 = 0.11\%\) of the soluble protein in red light-adapted cultures.

There are striking morphological differences between \(F. diplosiphon\) adapted to fluorescent and to red illumination. Fluorescent-illuminated cultures have a mean filament length of about 460 \(\mu\text{m}\). The average filament contains approximately 45–60 cells. Filaments of red-illuminated cultures, on the other hand, have a mean length of about 50 \(\mu\text{m}\) and each of these filaments contains 10–15 cells. Cell size and shape are altered by the different conditions of illumination (see Fig. 3). Fluorescent-illuminated cells are larger, more cylindrically shaped, and have less pronounced constrictions at their cross-walls than red light-illuminated cells. After numerous liquid transfers under red illumination, conspicuous cyanophycin granules accumulate adjacent to the cross-walls between cells. The light-induced changes in pigmentation and morphology in \(F. diplosiphon\) which have been described are fully reversible with growth.

**Metabolic Experiments**

**GENERAL INTRODUCTION:** Investigations of PE metabolism in \(F. diplosiphon\) were carried out with the objective of answering three questions:

(a) Is PE a stable molecule in fluorescent light-adapted cultures? (b) When fluorescent light-adapted cultures are transferred to red light, is the preexisting PE actively degraded, or is it simply diluted with growth? and (c) Upon transfer of red light-adapted cultures to fluorescent illumination, is PE synthesized \textit{de novo} or from existing polypeptides? To answer these questions, pulse-chased fluorescent- or red-illuminated cultures were prepared. During the post-chase period, fluorescent-illuminated cultures were either left under fluorescent light or transferred to red light, and red-illuminated cultures were transferred to fluorescent light. In each experimental situation, aliquots were removed as the cultures proliferated. These samples were quantitatively disrupted and their PE contents were estimated spectrophotometrically. The addition of excess anti-PE to crude extracts permitted the chromoprotein to be isolated on a microscale for specific activity determinations. Measured specific activities were compared with those predicted simply on the basis of dilution (continuous fluorescent illumination), cessation of synthesis (transfer to red light), and \textit{de novo} synthesis (transfer to fluorescent light). Total incorporated radioactivity levels and morphological

**Figure 3** Morphological differences between filaments of \(F. diplosiphon\) adapted to fluorescent (upper) and red (lower) illumination. Bar = 10 \(\mu\text{m} \times 2,600\).
features of the cultures were also taken into consideration. It was then possible to determine if degradative metabolic forces play a significant role in the regulation of PE levels and if other cellular proteins are utilized to any appreciable extent in the synthesis of PE when its formation is "induced" by fluorescent illumination.

The cultures were labeled with [14C]phenylalanine because: (a) it was hoped that an amino acid would be more or less specifically incorporated into protein constituents, and (b) Kiyohara et al. (1960) observed that reasonable quantities of phenylalanine were taken up by T. tenuis during heterotrophic growth. These workers found that arginine supported even better dark growth in T. tenuis than phenylalanine, but arginine would probably be a poor choice for metabolic labeling. Simon (1971) has demonstrated that blue-green algal cyanophycin granules, considered to be inherently unstable protein storage bodies, are copolymers of arginine and aspartic acid.

At the beginning of the sampling periods in all metabolic experiments, less than 1% of the radioactivity in the cells was soluble in ice-cold 5% TCA. The chases were not 100% effective in any of these experiments, perhaps because of incorporated radiolytic or other contaminants in the [14C]phenylalanine preparation which were not diluted by the addition of a large excess of unlabeled phenylalanine. It was established that concentration of the cultures by medium evaporation did not occur to any significant extent (less than 1%) in the course of these investigations.

USE OF ANTIBODY TO PURIFY PE FROM CRUDE EXTRACTS

Anti-PE (IgG fraction) was employed for the quantitative and selective removal of PE from crude extracts for specific activity determinations. Specific activities were calculated by dividing the corrected counts per minute in the precipitates by the number of micrograms of PE in the precipitates. Several experiments were carried out in order to demonstrate that the precipitin reactions were both quantitative and selective.

Fig. 4a illustrates the results of quantitative precipitin tests with purified PE and anti-PE. In these experiments, 1-ml volumes of buffered saline, containing progressively increasing quantities of purified PE, were mixed with 100-µl volumes of purified antibody. Control tubes received 100 µl of an IgG preparation, equal in protein concentration to the anti-PE solution, from nonimmunized rabbits. Under the conditions of these tests, up to about 80 µg of purified PE could be quantitatively precipitated out of solution.

In order to determine the antigen concentrations required to yield antibody excess when precipitating PE from crude extracts, precipitin reactions were carried out with dilution series of crude extracts from [14C]phenylalanine-labeled, fluorescent-illuminated F. diplosiphon crude extract.

Figure 4 (a) Quantitative precipitin curve for purified PE and anti-PE. The supernatant test consisted of examining the solution with ultraviolet light and checking for the orange fluorescence of PE after the immunoprecipitates had been harvested by centrifugation. (b) Quantitative precipitation of PE from a [14C]phenylalanine-labeled, fluorescent-illuminated F. diplosiphon crude extract.
determined from absorption spectra of the crude extracts, using the information in Table I. The washed precipitates were extracted for lipids and nucleic acids and then counted, following procedures outlined in the Experimental Section. The results, illustrated in Fig. 4b, indicated that PE could be quantitatively precipitated from 1 ml of crude extract containing up to 65 µg of PE. The absence of PE in precipitin reaction supernatants was confirmed spectrophotometrically. Parallel IgG control series indicated that nonspecific precipitation of crude extract components during incubation periods was minimal. Before precipitin reactions in metabolic experiments, all crude extracts were diluted to give a PE concentration of 35–40 µg/ml to insure antibody excess.

Direct proportionality in the labeled crude extract precipitin reactions did not necessarily imply monospecific precipitation of PE, since this result would have been obtained if contaminating materials precipitated in constant proportion with PE. To be assured of anti-PE specificity, it was therefore necessary to determine the fraction of the total radioactivity in the precipitates which was associated with PE.

Fig. 5 illustrates the results of SDS gel electrophoresis of dissociated antigen-antibody complexes which were derived from crude extracts of fluorescent-illuminated cultures grown in the presence of MC0 2. Four major protein peaks were observed. Molecular weight calibration of the gels demonstrated that these peaks corresponded to the heavy and light IgG subunits and the heavy and light PE subunits. Approximately 80% of the total radioactivity in the gels was associated with the heavy and light PE subunits. The major radioactive contaminant was associated with a band having an apparent molecular weight of about 29,000. Before staining, this band demonstrated an orange fluorescence, characteristic of the heavy PE subunit, under ultraviolet illumination. This “contaminant” may therefore be a fraction of the heavy PE subunits which, for some unknown reason, displayed anomalous mobility during electrophoresis.

Cultures for metabolic experiments were labeled with [14C]phenylalanine, not with 14CO2. The immunoprecipitation specificity estimate obtained by SDS gel electrophoresis of 14CO2-labeled antigen-antibody complexes is probably low, because 14CO2 undoubtedly labels nucleic acids and lipids more heavily than [14C]phenylalanine, and, for technical reasons, the washed precipitates could not be extracted for these constituents before electrophoresis.

Estimates of anti-PE specificity with [14C]phenylalanine-labeled crude extracts were obtained by experiments with artificial mixtures. Crude extracts were prepared from red-illuminated cultures grown in the presence of [14C]phenylalanine. As discussed previously, these extracts do not contain detectable levels of PE. Unlabeled, purified PE was added to these crude extracts and then recovered by the addition of anti-PE in excess. If the immunoprecipitation were totally specific, no radioactivity should have been present in the precipitates. The results of artificial mixture experiments are summarized in Table III. Approximately 86–90% of the algal protein in precipitates formed from crude extracts having low PE/PC ratios was PE. Subtraction of counts obtained with IgG control tubes corrected for only about 20% of the nonspecific precipitation. The uncorrected contamination was probably due to coprecipitating proteins which could not be removed during precipitate washing.

In metabolic experiments with pulse-chased, red-light-illuminated cultures transferred to fluorescent illumination, PE specific activities were estimated when the PE/PC ratio became about 20% of that in cultures fully adapted to fluorescent illumination. The average soluble protein specific activities were determined, and counts in the precipitates

![Figure 5](https://example.com/figure5.png)

**Figure 5** SDS gel electrophoresis of a reduced alkylated immunoprecipitate prepared by adding anti-PE in excess to a 14CO2-labeled, fluorescent-illuminated *F. diplosiphon* crude extract. Key to abbreviations: H-Ab, heavy γ-globulin subunit; GPDH, glyceraldehyde-3-phosphate dehydrogenase marker; L-Ab, light γ-globulin subunit; H-PE, heavy C-phycoerythrin subunit; L-PE, light C-phycoerythrin subunit.
Crude extracts were prepared from [14C]phenylalanine-labeled, red-illuminated F. diplosiphon. Such extracts do not contain detectable levels of PE. Measured amounts of unlabeled, purified PE were added to the crude extracts and then quantitatively recovered from the mixtures by the addition of anti-PE in excess. From measurements of the radioactivity in the precipitates and the specific activity of the soluble protein in the labeled crude extracts, the extent of nonspecific coprecipitation may be ascertained.

* Contained 200 µg of PC; average specific activity of protein in crude extract was 270 cpm/µg. Total radioactivity was 112,640 cpm/ml.

† Averages of duplicates.

§ Added to give 10% of the PE/PC mass ratio in fully adapted fluorescent-illuminated cultures.

|| Added to give 20% of the PE/PC mass ratio in fully adapted fluorescent-illuminated cultures.

were corrected on the basis of 10% nonspecific precipitation. In metabolic experiments with pulse-chased, fluorescent-illuminated cultures, counts in the precipitates were corrected simply by subtracting the counts present in IgG control tubes. Under these conditions, about 5% of the algal protein in the precipitates was probably not PE. IgG controls did not correct for counts associated with these contaminants.

**CONTINUOUS FLUORESCENT ILLUMINATION:** The results of an experiment designed to determine the stability of PE in cells fully adapted to and growing under fluorescent illumination are illustrated in Fig. 6. Samples were removed from a pulse-chased, fluorescent-illuminated culture that was left under fluorescent light. The radioactivity content of the cells per unit volume of culture (■■■■■■) increased by 2% during the sampling period. The culture was healthy, judging from the absence of vacuoles in the cells, and continued to grow, as is demonstrated by the steady increase in dry weight per unit volume of culture (Δ—Δ—Δ). The PE/dry weight ratio increased by about 6-8% over the sampling period. As the culture proliferated, the PE content (○—○—○) increased and consequently the PE specific activity (not shown) decreased. The total radioactivity associated with PE in the cells per unit volume of culture (■■■■■■■■■■■■) was obtained at each sampling point by multiplying the PE concentration (micrograms per milliliter culture) by the PE specific activity (counts per minute per microgram). The least-squares line through these products had essentially no slope. Scatter among these products was due primarily to variability in estimates of PE concentration, not in PE specific activity.

The total radioactivity of the PE per unit volume of culture was essentially constant; this shows that PE is a stable molecule under these conditions. New PE was synthesized, but was unlabeled, owing to the effectiveness of the chase. On the other hand, the PE labeled during the pulse period was not degraded. The PE concentration times specific activity curve that would have been obtained if PE had been degraded, without recycling, at 15% of the rate of the new PE increase has been calculated and is plotted (+——+——+).
Although no measurable PE degradation could be observed in this experiment, destruction at a rate of up to about 5% of the net PE accumulation would probably have been undetectable.

**RED-TO-FLUORESCENT LIGHT TRANSFERS:** The results of a red-to-fluorescent illumination transfer experiment are shown in Fig. 7. The pulse-chased, red light-illuminated culture was left under red light for 20 h and then transferred to fluorescent illumination. For undetermined reasons, it was particularly difficult to obtain effective chases with red-illuminated cultures. The total radioactivity in the cells increased by about 10% during the overall sampling period in this experiment. Transfer to fluorescent illumination initiated PE synthesis (○—○—○). The rate of accumulation of chl a (X—X—X) was accelerated, while the level of PC (○—○—○) increased slightly and then became stationary. (In several other experiments, the PC level continued to increase at a slow rate over the entire sampling period.) These changes in rate of accumulation are in the direction of complementary chromatic adaptation. On a dry weight basis (see Table II), fluorescent light-adapted cultures have more PE and chl a and less PC than red light-adapted cultures. No net decreases in the phycobiliprotein or chl a levels of the cultures were ever observed in red-to-fluorescent light transfer experiments. The last sample taken in this experiment had a PE/PC ratio that was about 20% of that in fully-adapted fluorescent-illuminated cultures. In this sample, the PE specific activity was 5.2 cpm/µg, whereas that of the total soluble protein was 76 cpm/µg.

These data indicate that no more than about 8% of the PE synthesized in response to fluorescent light could have been derived from preexisting proteins. However, in view of the 10% increase in radioactivity of the cells during the sampling period, a large fraction of the counts in the PE can probably be attributed to the ineffectiveness of the chase.

**FLUORESCENT-TO-RED LIGHT TRANSFERS:** A fluorescent-to-red light transfer experiment is illustrated in Fig. 8. While under fluorescent illumination, the dry weight, phycobiliprotein, and chl a levels of the culture all increased, but the total incorporated radioactivity remained essentially constant. A decrease in the PE specific activity (□—□—□) occurred because of the synthesis of unlabeled PE. After transfer to red illumination, the PE specific activity became constant, indicative of a cessation of PE synthesis. The PC content of the culture (○—○—○) increased dramatically and, in this experiment, no decrease in the level of PC was observed at any time. However, net decreases in the levels of PE (○—○—○), chl a (X—X—X), dry weight
FIGURE 8 Complementary chromatic adaptation after transfer of fluorescent light-adapted cultures to red illumination.

(\(\triangle\) -- \(\triangle\) -- \(\triangle\)), and total radioactivity (■■■■) occurred in parallel shortly after transfer to red light. After these decreases, the PE, chl \(a\) and total radioactivity levels remained constant, but the dry weight of the culture increased. The changes in pigmentation which took place after transfer to red light were exactly opposite to those observed in red-to-fluorescent light transfer experiments (see Fig. 7). In this experiment, the PE level decreased by 16.2\% and the total incorporated radioactivity decreased by 15.8\%. Equivalent losses in PE and total incorporated radioactivity should be observed as a consequence of lysis if the chase has been effective, if PE synthesis has stopped, and if these two components are evenly distributed throughout the cell population.

MEAN FILAMENT LENGTH REDUCTION DURING ADAPTATION TO RED LIGHT:

There are morphological aspects to complementary chromatic adaptation. The mean filament length of fluorescent-illuminated cultures is much greater than that of red-illuminated cultures (see Table II). Fig. 9 demonstrates that the long filaments of fluorescent-illuminated cultures fragment very soon after transfer to red light. Furthermore, the decrease in mean filament length (+---+) occurs simultaneously with losses in PE and total incorporated radioactivity.

Fig. 10 a is a photomicrograph of \(F.\) diplosiphon filaments that were removed from a fluorescent-to-red light transfer immediately before the major period of mean filament length reduction. Large numbers of dark, biconcave cells, known as necridia or separation disks, are scattered throughout the filaments. These structures disappear from the cultures after filament fragmentation and have not been observed in cultures fully-adapted to red light. Fig. 10 b is a high power photomicrograph of an infrequently observed necridium from a fluorescent-illuminated culture.

Lamont (1969) has summarized evidence indicating that transcellular filament breakage almost invariably occurs across necridia, resulting in lysis of the cell at the locus of breakage. Transcellular filament breaks may also occur across vegetative cells (Lamont, 1968; F. Drouet, personal communication). If filament fragmentation during fluorescent-to-red light transfer experiments were achieved exclusively by transcellular breakage, a substantial fraction of the cell population would be lysed. Cells in filaments of fluorescent-illuminated \(F.\) diplosiphon are about 8–10 \(\mu\)m long. Transcellular breakage of filaments 450–600 \(\mu\)m long into filaments having a length of about 50 \(\mu\)m would result in the lysis of one out of every six or seven
cells (16.5% to 14.3%). Losses in PE and total radioactivity occur at about these levels in fluorescent-to-red light transfer experiments and are temporally preceded by morphological indicators of incipient lysis (necridia). During the period of mean filament length reduction, phycobiliproteins may also be observed in the culture medium. A loss in the morphological integrity of a fraction of the cell mass, rather than active metabolic degradation, is responsible for the observed diminution in the PE content of fluorescent-illuminated cultures adapting to red light.

**DISCUSSION**

PE is synthesized but not measurably degraded in *F. diplosiphon* adapted to and growing under fluorescent illumination. This fact is demonstrated by the constant total radioactivity associated with the PE per unit volume of proliferating pulse-chased cultures. The PE specific activity remains
constant after pulse-chased, fluorescent-illuminated *F. diplosiphon* cultures are transferred to red light because synthesis of unlabeled PE is shut off and no additional dilution of radioactive PE occurs. The PE content of *F. diplosiphon* cultures decreases, however, during complementary chromatic adaptation accompanying the shift from fluorescent to red illumination. The decrease in PE level which is observed after transfer to red light is due to cell lysis, not to measurable active metabolic degradation. These results lead to the conclusion that the PE is a stable molecule in *F. diplosiphon* and that light influences the intracellular levels of this chromoprotein solely by increasing or decreasing its rate of synthesis. The methods employed in these experiments must be regarded as providing minimum estimates for PE degradation rates, since the extent of metabolic recycling of degraded labeled constituents could not be measured. Destructive forces may play an important role in PE metabolism under different environmental circumstances and in stationary or necrotic cultures, but PE is not degraded to any significant extent in growing cultures under the conditions of these experiments.

Transfer of pulse-chased, red-illuminated, PE-less cultures to fluorescent light induced PE production. When the PE/PC ratio reached 20% of that observed in cells fully adapted to fluorescent light, the specific activity of PE was 8% of that observed in the average soluble protein. The majority of the PE synthesized was, therefore, not derived from other preexisting soluble proteins.

The mechanism by which light influences the rate of PE synthesis is not known. Light does not appear to be absolutely required for PE synthesis in all blue-green algae. *Nostoc muscorum* A, which does not adapt chromatically, continues to produce PE when grown heterotrophically in complete darkness (Lazaroff, 1966). Scheibe (1972) has recently provided limited evidence for the existence of a green-red photoreversible pigment in *T. tenuis*. Referring to action spectrum data obtained by Fujita and Hattori (1962) and by Lazaroff (1966), Scheibe has proposed a hybrid model in which light modifies the properties of the photoreversible pigment, which in turn "controls" phycobiliprotein synthesis and morphogenesis. According to this model, green light promotes PE synthesis and growth as short filaments, and red light promotes PE synthesis and long filaments. This relationship does not hold for *F. diplosiphon*, since green light stimulates the production of long filaments and red light generates cultures composed of short filaments.

Once PE synthesis is induced by light, further levels of control come into play. Since measurable pools of phycoerythrobilin and PE apoprotein have not been detected in the present work, the synthesis of these moieties is probably tightly coordinated. It is also probable that a mechanism exists for the stoichiometric synthesis of the heavy and light PE subunits. It is not known at present if the protein components of the two subunits are related.

When attempting to determine action spectra for phycobiliprotein synthesis in *T. tenuis*, Hattori and Fujita (1959) did not observe any effect of light upon the chl *a* content of the organism. Light has a strong influence upon both the chl *a* and the phycobiliprotein content of *F. diplosiphon*. Fluorescent illumination provides large quantities of light that the phycobiliproteins absorb well, but relatively little light in the wavelength regions of maximal chl *a* absorption. Red light, on the other hand, has large quantities of energy that may be absorbed by the long wavelength band of chl *a*, but comparatively little that is absorbed by phycobiliproteins. The ratio of total phycobiliprotein to chl *a* in *F. diplosiphon* cultures adapted to red illumination is 2.15 times greater than that in cultures adapted to fluorescent illumination. Thus, changes in chl *a* content are also a component of complementary chromatic adaptation in this system. Jones and Myers (1965) previously observed light quality to have a strong influence upon the chl *a* content of *Anacystis nidulans*.

Complementary chromatic adaptation has been regarded heretofore as a rather specific alteration in the phycobiliprotein complement in response to changes in the light environment's quality. However, quite marked morphological and probably many other less obvious enzymatic changes also occur in *F. diplosiphon*. These manifold changes may be direct or indirect consequences of light absorption by a "master switch" pigment or by several discrete photoreceptors.

The findings discussed here underscore difficulties that systematists face in their efforts to create a meaningful blue-green algal taxonomy. Alterations in either the availability of exogenous combined nitrogen (Bennett, 1972) or in the energy distribution in the visible light environ-
ment can convert *F. diplosiphon* into strikingly different morphological variants. If standard taxonomic criteria were used, these forms would be classified as totally different organisms. Furthermore, the experiments concerned with the adaptation of fluorescent-illuminated cultures to red light illustrate how incorrect physiological conclusions may be reached; cell lysis could easily have been interpreted to mean PE degradation in this particular situation if PE levels and specific activities had been the only parameters measured.

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