Planta (1983) 158:249–258

Planta

Photocontrol of the polypeptide composition of *Euglena*

Analysis by two-dimensional gel electrophoresis

Antonio F. Monroy and Steven D. Schwartzbach
Cell Biology and Genetics Sections, School of Life Sciences, University of Nebraska, Lincoln, NE 68588-0118, USA

Abstract. Two-dimensional gel electrophoresis resolved total cellular protein from *Euglena gracilis* Klebs var. *bacillaris* Cori into 650 polypeptides detectable by silver staining. Exposure of dark-grown resting *Euglena* to light for 72 h increased the relative amounts of 79 polypeptides and decreased the relative amounts of 72 polypeptides. Four polypeptides whose level increased upon light exposure were undetectable in the bleached mutant W_3BUL. Since W_3BUL has lost most if not all of its chloroplast genome, these polypeptides may be coded by the chloroplast genome. Although the majority of the polypeptides studied appear to be coded by the nuclear or mitochondrial genomes, seven polypeptides which were present in W_3BUL were not detectable in another chloroplast-DNA-deficient, bleached mutant, W_{10}BSmL. It appears that a number of nonchloroplast genes are no longer expressed in this mutant. Exposure of dark-grown resting cells of the bleached mutant, W_3BUL, to light increased the relative amounts of 12 polypeptides and decreased the relative amounts of 14 polypeptides. Since W_3BUL lacks detectable protochlorophyll(ide), the chloroplast photoreceptor, the levels of these polypeptides are regulated by light acting through a nonchloroplast photoreceptor. Exposure of cells to light had no detectable effect on the relative amounts of those polypeptides which were present in W_{10}BSmL, even though W_{10}BSmL has a nonchloroplast photoreceptor. Either *Euglena* contains multiple nonchloroplast photoreceptors, some of which are absent from W_{10}BSmL, or the nonchloroplast photoreceptor present in W_{10}BSmL is uncoupled from some of the events normally controlled by that photoreceptor.

Key words: *Euglena* – Light and polypeptide synthesis – Mutant (*Euglena*) – Polypeptide coding.

Introduction

Light acting through a chloroplast-localized photoreceptor, protochlorophyll(ide), and a blue-absorbing nonchloroplast photoreceptor regulates organelle biogenesis in *Euglena* (reviewed in Schiff and Schwartzbach 1982). Exposure of dark-grown resting *Euglena* to light induces the synthesis of chloroplast-localized enzymes such as NADP-glyceraldehyde-3-phosphate dehydrogenase (Horrum and Schwartzbach 1980a) and valyl-tRNA synthetase (McCarthy et al. 1982). The synthesis of mitochondrial enzymes such as fumarase and succinate dehydrogenase (Horrum and Schwartzbach 1980b) as well as microbody enzymes such as glycolate dehydrogenase (Horrum and Schwartzbach 1981) is also increased by exposure of the cells to light. The induction of organelle enzymes by light is, however, a selective process as evidenced by the failure of light exposure to alter the levels of hydroxypyruvate reductase, a microbody enzyme, and serine hydroxymethyltransferase, an enzyme found in the cytoplasm and mitochondria of *Euglena* (Horrum and Schwartzbach 1980c). Just as exposure of cells to light increases the specific activity of certain enzymes, the specific activity of other enzymes such as NAD-glyceraldehyde-3-phosphate dehydrogenase (Bovarnick et al. 1974) and pyruvate kinase (Dockerty and Merrett 1979) decreases after exposure of cells to light. Since chloroplast development in *Euglena* can occur in the absence of a net synthesis of protein (Bovarnick et al. 1974; Horrum and Schwartzbach 1980a), the degradation of preexisting proteins is thought to provide the amino acids required by the developing chloroplast. It is not known whether light induces the degradation of specific proteins or whether all cellular proteins are turning over in resting cells and light simply inhibits the resynthesis of those proteins which are gratituous for phototrophic growth.

Although light clearly regulates the polypeptide composition of *Euglena*, the extent and level, i.e. transcriptional, translational, posttranslational, at which light regulates the synthesis of cytoplasmic, chloroplast, mitochondrial and microbody enzymes is still unclear. Studies of changes in enzyme specific activity are time consuming, they only provide information regarding a single enzyme, changes which are found can be the consequence of enzyme activation or inactivation, and enzymes are normally selected for study because the investigator assumes that they will be induced or repressed by light. Two-dimensional gel electrophoresis is a technique which is free from the problems associated with studies of specific enzymes and which rapidly provides a global picture of changes in cellular polypeptide levels. Polypeptides are chosen for study based on their physical properties (charge and molecular weight) (O'Farrell 1975) and changes in staining intensity of a given polypeptide are directly proportional to changes in polypeptide levels (Merril et al. 1982). Since the investigator has not selected the polypeptides to be studied, the regulatory patterns observed are truly representative of the changes occurring in response to a specific environmental agent.

We have used two-dimensional gel electrophoresis to analyze the changes occurring when dark-grown resting cells of *Euglena* are exposed to light; the results are reported in this paper. Of 640 ± 45 polypeptides resolved from wild-type cells and detected by silver staining, the relative amounts of 151 specific polypeptides are photoregulated.[1] Studies with two bleached mutants of *Euglena*, W_3BUL and W_{10}BSmL, indicate that associated with the loss of the chloroplast genome is a loss of the ability to control the levels of specific polypeptides by the presence of light. Some photoregulated polypeptides which are present but not photoregulated in W_3BUL are not detectable in W_{10}BSmL. A brief report of this work was presented at the 1981 meeting of the American Society of Plant Physiologists (Monroy and Schwartzbach 1981).

Materials and methods

Strains; culture conditions. Euglena gracilis Klebs var. *bacillaris* Cori, maintained in our laboratory in the dark for many years, and the bleached mutants W_3BUL and W_{10}BSmL derived from this strain (Schiff and Schwartzbach 1982) were used throughout this work. Conditions for cell growth, preparation of resting cells and light induced chloroplast development were as described in Horrum and Schwartzbach (1980b).

Extraction of total cellular protein. Nucleic-acid-free total cellular protein samples were obtained by a modification of the procedure of Van Etten et al. (1979). Cells were harvested at 0–4° C by centrifugation for 5 min at 4000 *g*, washed two times with 10% methanol and stored at −70° C for subsequent protein extraction. The frozen cell pellets were rapidly thawed and resuspended in water-saturated phenol (13 ml per 200 ml culture). An equal volume of extraction buffer (0.7 M sucrose; 0.05 M 2-amino-2-(hydroxymethyl)-1,3-propanediol [Tris]; 0.03 M HCl; 0.05 M disodium ethylenediaminetetraacetate; 0.1 M KCl, 2%, v/v, β-mercaptoethanol) was rapidly added and the proteins were extracted into the phenol phase by vigorous shaking. After phase separation, the upper phenol phase was removed and reextracted with an equal volume of extraction buffer; after phase separation, proteins were precipitated from the phenol phase at −20° C by the addition of five volumes of 0.1 M ammonium acetate in methanol. The precipitated protein was washed three times with 0.1 M ammonium acetate in methanol, once with ethyl ether, dried at 45° C, and was resuspended with the aid of a tissue homogenizer in a minimal volume (2 ml/200 ml culture) of O'Farrell's lysis buffer (9.5 M urea; 2%, v/v, Nonidet P-40; 1.2%, v/v, Ampholine pH 5–7; 0.8% v/v, Ampholine, pH 3.5–10; 5%, v/v, β-mercaptoethanol) (O'Farrell 1975). The solubilized protein was clarified by centrifugation at room temperature for 10 min at 1000 *g* and either immediately subjected to electrophoresis or stored at −20° C. Two-dimensional gels of total cellular protein prepared by this procedure are virtually identical to gels prepared from protein extracted by resuspending cells directly in lysis buffer or by sonicating cells in lysis buffer.

Two-dimensional gel electrophoresis. Proteins were separated by two-dimensional gel electrophoresis essentially as described by O'Farrell (1975). Isoelectric focusing gels (4%, w/v, acrylamide; 1.2%, v/v, pH 5–7, Ampholine; 0.8%, v/v, pH 3.5–10, Ampholine; 9 M urea; 2%, v/v, Nonidet P-40) were prepared in glass tubes, 130 mm long, 3 mm inner diameter, to a length of 115 mm. The polymerized gels were overlayered with 0.03 ml of lysis buffer and pre-run for 15 min at 200 V, 30 min at 300 V and 30 min at 400 V using 20 mM NaOH in the upper reservoir (cathode) and 10 mM H_3PO_4 in the lower reservoir (anode). After the end of the pre-run, the sample (60 µg protein) contained in 0.04–0.06 ml of lysis buffer was loaded onto the gel, the sample was overlyered with 0.015 ml of a mixture of 9 M urea and 1% Ampholine (0.6% pH 5–7, 0.4% pH 3.5–10) and subjected to electrophoresis at 400 V for 13 h and then at 800 V for 1 h. Sample protein concentrations were determined by the Coomassie-Blue method (Spector 1978) using bovine serum albumin as a standard. The isoelectric focusing gel was equilibrated for 1 h in 5 ml of sodium dodecyl sulfate (SDS)-sample buffer (0.625 M Tris-HCl, pH 6.8; 10%, v/v, glycerol; 5%, v/v, β-mercaptoethanol; 2.3% SDS), immediately placed on top of a SDS-slab gel, and fused into place with a solution of 1% (w/v) agarose containing 0.0002% (w/v) bromophenol blue prepared in SDS-sample buffer. Molecular-weight standards (12,300–200,000) were placed in a slot formed on the top side of the staking gel and were used for the determination of apparent molecular weights. The second-dimension slab gel (120 mm long, 150 mm wide, 0.8 mm thick) consisted of a 8.25–11.5% (w/v) linear polyacrylamide gradient resolving gel containing 0.375 M Tris-HCl, pH 8.8; 0.1% (w/v) SDS; 0.05% (v/v) glycerol; and a 25-mm long, 4% (w/v) stacking gel containing 0.125 M Tris HCl, pH 6.8; 0.1% (w/v) SDS. Both buffer

1 For the sake of brevity, polypeptides whose relative levels are increased or decreased as a result of light exposure will be referred to as photoregulated polypeptides. The use of this term is not meant to imply that light acts at the transcriptional or translational level

chambers contained 0.025 M Tris, 0.19 M glycine, 0.1% SDS. Electrophoresis was at a constant voltage (110 V) for 6–7 h at which time the dye front was approx. 1 cm from the bottom of the gel. The gels were fixed overnight in 50% methanol, 10% acetic acid.

Silver-nitrate staining procedure. The fixed gels were stained by a modification of the procedure of Oakley et al. (1980); this modification maintains the high sensitivity of the original procedure while reducing the background and increasing the reproducibility from gel to gel. By carefully controlling the staining process, direct visual comparisons could be made between gels stained on the same day. The fixed gels were allowed to expand during three 10-min washes with 250 ml of 5% methanol, 7% acetic acid. The gels were then soaked individually for 30 min in 10% glutaraldehyde (200 ml/gel). The glutaraldehyde was removed over a 2-h period by six rinses with glass-distilled water (250 ml/gel). The gels were then transferred to a large partitioned plastic (plexiglass) container and soaked in 100 ml/gel of ammoniacal silver which was prepared immediately before use by mixing 4 ml of 20% (w/v) $AgNO_3$, 1.4 ml fresh NH_4OH, 21 ml 0.36% (w/v) NaOH and distilled water to a final volume of 100 ml. After 10 min soaking, the gels were rinsed with two changes of distilled water and stored in distilled water. The stained proteins were visualized by transferring a gel to a plastic box containing 250 ml of 0.0025% (w/v) citric acid, 0.012% (v/v) formaldehyde. After 1.5 min incubation with gentle shaking (the incubation time required for optimal resolution on stained gels containing 60 µg protein), the visualization reaction was stopped by addition of 250 ml of 0.75 mM NaOH. After 1 min, the gel was transferred for 2 min to a tray containing 250 ml of 0.75 mM NaOH and the stained gels were stored in distilled water. This staining procedure permits detection of a protein band containing approx. 2 ng of protein.

Analysis of polypeptide patterns. The absolute position of a polypeptide on a two-dimensional gel varies from one gel to the next. The relative position of a protein, i.e. position relative to neighboring proteins, however, is constant and portions of gels can be superimposed. For comparative analysis, a plastic transparency was placed over a photographic enlargement of the stained gel. The gel was then divided into vertical and horizontal sectors based on the positions of landmark polypeptides, i.e. polypeptides present in all of the gels to be examined. Within a sector, the polypeptide patterns were superimposable. For each pair of gels, differences in the relative amounts of each polypeptide to be compared were recorded directly upon the transparencies. The transparencies recording differences for each gel pair in five independent experiments were then superimposed sector by sector and those polypeptides whose relative amounts were altered in at least four out of five independent experiments were considered to be photoregulated. The figures illustrate representative gel pairs.

Chemicals. Ampholines were purchased from LKB Instruments, Rockville, Md., USA, Nonidet P-40, ultrapure urea, and molecular-weight standards from Bethesda Research Laboratories, Gaithersburg, Md., USA. Glutaraldehyde was supplied by Eastman Kodak, Rochester, N.Y., USA.

Results

Limitations to visual quantitation of relative protein levels in silver-stained two-dimensional gels. Staining polyacrylamide gels with silver provides a highly sensitive method for detecting proteins. The actual staining intensity obtained is, however, dependent upon which of several staining protocols is used (Ochs et al. 1981), the amino-acid composition of the polypeptide being stained (Merril et al. 1982), the amount of time the gel is exposed to developer, and the batch of reagents actually used to stain a specific gel (Merril et al. 1982). For a given polypeptide on a set of gels stained simultaneously, staining intensity is proportional to protein concentration over a 40-fold concentration range (Merril et al. 1982). When dealing with total cellular proteins, the relative concentrations of the most abundant and least abundant proteins could conceivably differ by more than 40-fold, thus exceeding the linear range of the stain. Furthermore, visual comparisons of staining intensity are not strictly quantitative. A number of preliminary experiments were therefore performed in order to determine the reproducibility from gel to gel of the staining procedure, the polypeptide pattern, and the relative increase in protein required to produce a visually detectable increase in staining intensity.

By standardizing the time a gel was exposed to developer (3 min) and staining all of the gels to be compared at the same time, two types of changes were observed when increasing amounts of total *Euglena* protein were resolved by two-dimensional gel electrophoresis. As the amount of protein applied to the gel was increased, the number of polypeptides detected was increased. For polypeptides which were detectable at the lowest protein load, an increase in the absolute amount of protein resulted in an increase in staining intensity. For proteins present in low amounts, a 1.5–2-fold increase in amount produced a visually detectable increase in staining intensity while for proteins present in high amounts (most intensely stained polypeptides), a threefold increase in amount produced a visually identifiable increase in staining intensity (data not shown). If equal amounts of protein were loaded onto gels, there were no detectable differences in staining intensity. In five independent experiments, 60 µg of total cellular protein from wild-type *Euglena* were resolved into 640 ± 45 polypeptides. It appears that the technique is highly reproducible and that two- to threefold differences in the amounts of specific polypeptides can be visually detected.

Light-dependent changes in the relative amounts of protein extracted from wild-type Euglena. Exposure of dark-grown resting cells to light for 72 h to complete chloroplast development increased the relative amounts of 79 specific polypeptides (squares,

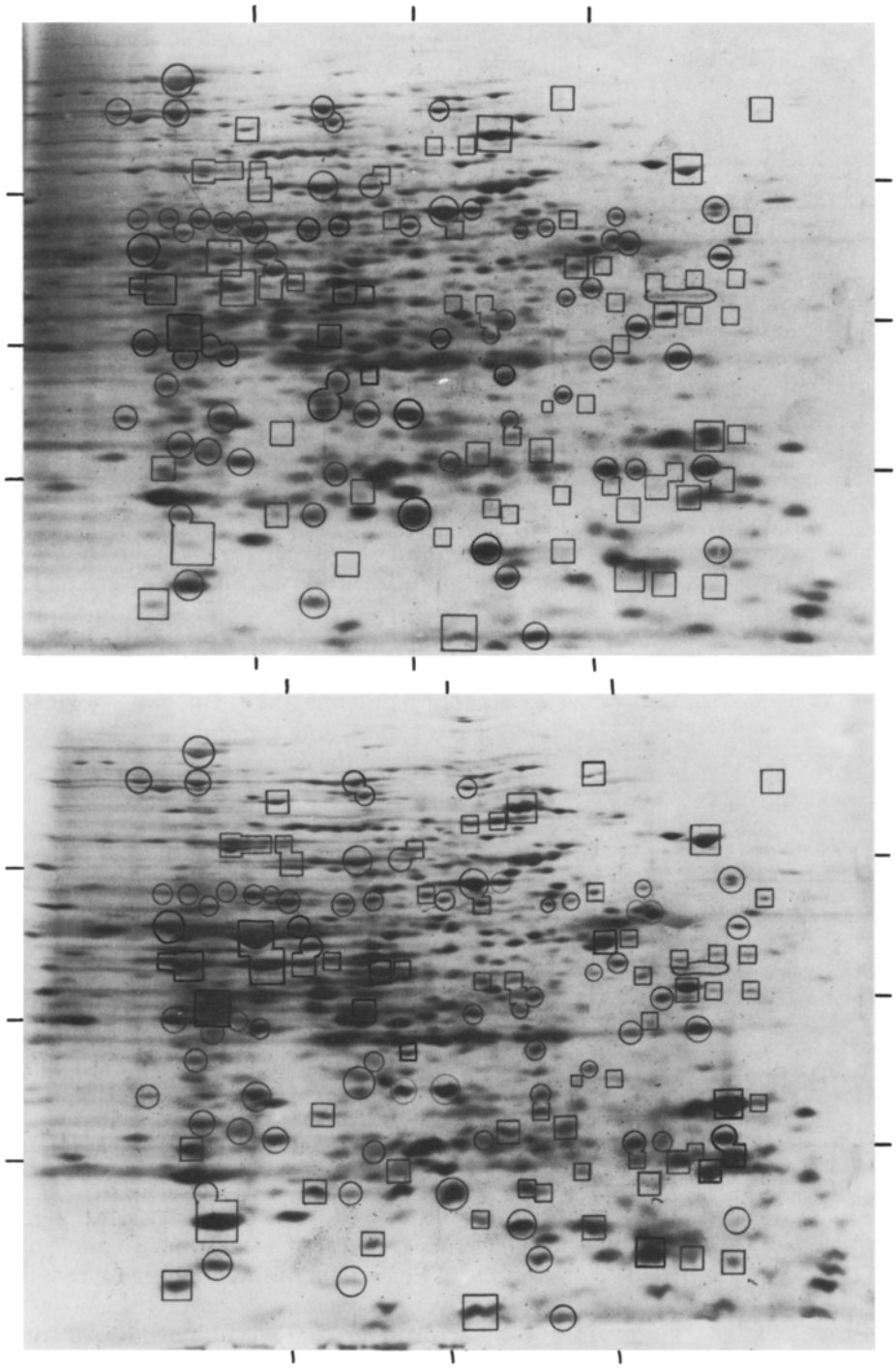

Fig. 1. Silver-stained two-dimensional gel of 60 μg total cellular protein extracted from dark-grown, resting *Euglena* maintained in the dark (*top*) or exposed to light for 72 h (*bottom*). Polypeptides whose relative amounts decreased or increased upon light exposure are enclosed in circles or squares, respectively. The lines on the edge of the electrophoretogram indicate the positions of the sectors used for polypeptide analysis

Fig. 1). Of these polypeptides, 64 increased in relative amount in each of five independent experiments while 15 polypeptides increased in relative amount in four out of five independent experiments. Exposure of the cells to light also decreased the relative amounts of 72 specific polypeptides (circles, Fig. 1). Of these polypeptides, 39 decreased in relative amount in each of five independent experiments while 33 polypeptides decreased in relative amount in four out of five independent experiments. Although additional alterations were detected, changes observed in less than four independent experiments are not reported. The relative amount of the majority of the polypeptides resolved appeared to be unaltered by light exposure, indicating that light selectively regulated the polypeptide composition of *Euglena*.

A composite map indicating the relative posi-

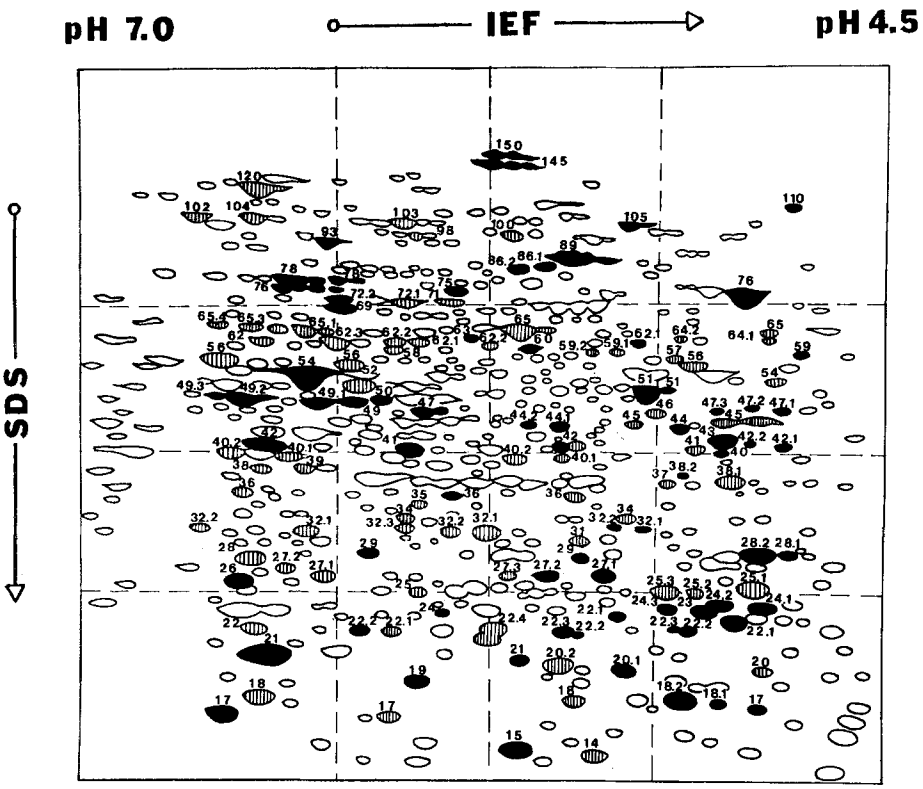

Fig. 2. Composite diagram indicating the relative positions of all polypeptides detected on silver-stained two-dimensional gels of 60 μg of total cellular protein of *Euglena*. Solid and striped spots indicate those polypeptides whose relative amount increased and decreased, respectively, upon light exposure. A number corresponding to the apparent MW (dalton $\times 10^{-3}$) of the polypeptide rounded to the nearest 1000 appears above each photoregulated polypeptide. In the case of polypeptides within a sector having the same apparent molecular weight, a decimal point is assigned starting with the most acidic polypeptide

tions and shapes of all of the polypeptides resolved from 60 μg of total cellular protein extracted from dark-grown resting cells maintained in the dark or exposed to light for 72 h is shown in Fig. 2. Landmark polypeptides which were present in all of the gels studied were used to divide the map into four sectors in both the isoelectric-focusing dimension and the sodium-dodecyl-sulfate dimension. As proposed by Pedersen et al. (1978), each polypeptide is identified by an alphanumeric system consisting of a letter (A–D; A being the most acidic) corresponding to the isoelectric-focusing sector in which the polypeptide is found and a number corresponding to the apparent molecular weight of the polypeptide rounded to the nearest 1000. For proteins of the same molecular weight, a decimal is added with the most acidic protein corresponding to 0.1.

Light-dependent changes in the polypeptide composition of the bleached mutants W_3BUL and $W_{10}BSmL$. A number of mutants have been used to determine the genome coding for specific proteins and the photoreceptor regulating the synthesis of specific polypeptides. W_3BUL is a bleached mutant which lacks photoconvertable protochlorophyll(ide) (reviewed in Schiff and Schwartzbach 1982). It contains nuclear-coded plastid-membrane polypeptides (Bingham and Schiff 1979) and a plastid remnant which undergoes limited morphological changes upon light exposure (Osafune and Schiff 1980). $W_{10}BSmL$ is another bleached mutant which differs from W_3BUL in that it lacks colored carotenoids (Fong and Schiff 1979) and a plastid remnant (Osafune and Schiff 1980). Chloroplast valyl-tRNA synthetase is detectable and light-induced in W_3BUL (McCarthy et al. 1982), but this enzyme, as well as alkaline DNase (Egan et al. 1975), is undetectable in $W_{10}BSml$ (McCarthy et al. 1982). Fragments of the chloroplast genome, predominantly rRNA genes, have been found in some bleached mutants and there is some evidence that these fragments, but not the entire genome, could be present at low copy numbers, but unexpressed in W_3BUL and $W_{10}BSmL$ (Heizmann et al. 1981, 1982; Schwarzbach et al. 1976). Thus, the nuclear genome is the probable coding site for the chloroplast-localized polypeptides found inW_3BUL and $W_{10}BSmL$. In both W_3BUL and $W_{10}BSmL$ light induces paramylum degradation (Horrum and Schwartzbach 1980b; Schwartzbach et al. 1975) and transiently increases and decreases the rate of amino-acid incorporation into protein (Schwartzbach and Schiff 1979), indicating that both mutants contain a non-chloroplast photoreceptor. Light induces the syn-

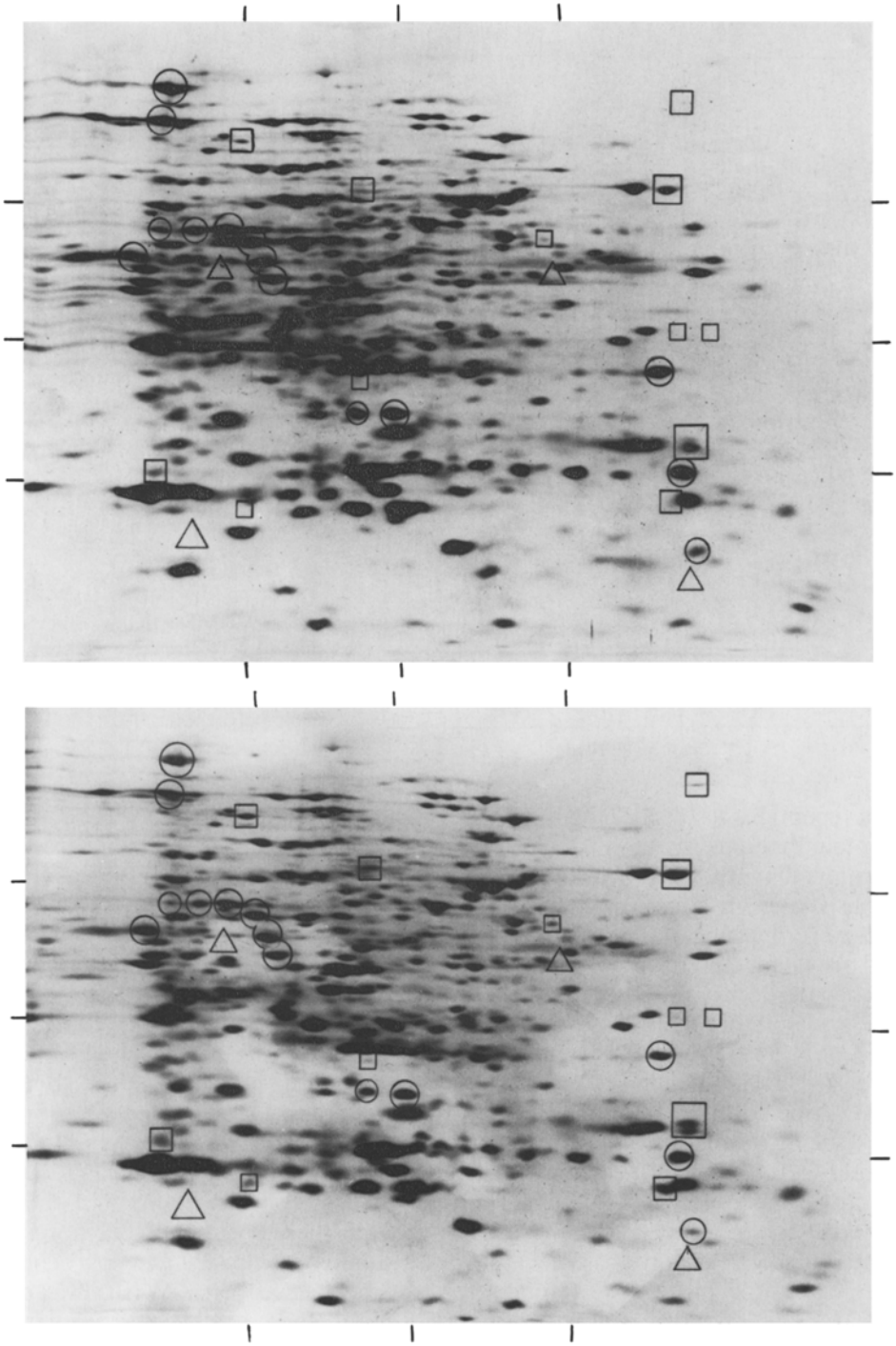

Fig. 3. Silver-stained two-dimensional gel of 60 μg total cellular protein extracted from dark-grown, resting cells of the bleached mutant W_3BUL maintained in the dark (*top*) or exposed to light for 72 h (*bottom*). Polypeptides enclosed in circles or in squares as in Fig. 1. *Triangles* indicate the positions of polypeptides which are detectable in wild-type cells but undetectable in W_3BUL. The lines on the edge of the electrophoretogram indicate the position of the sectors used for polypeptide analysis

thesis of cytoplasmic rRNA (Cohen and Schiff 1976), of fumarase (Horrum and Schwartzbach 1980b) and of phosphoglycerate phosphatase (James and Schwartzbach 1982) in W_3BUL but not in W_{10}BSmL, indicating either the presence of multiple photoreceptors only a few of which are present in W_{10}BSmL, or an uncoupling between the single photoreceptor and the processes controlled by that photoreceptor. In order to determine the presumptive coding site for each photoregulated polypeptide, the photoreceptor regulating polypeptide accumulation, and the differences between the polypeptide composition of the mutants W_3BUL and W_{10}BSmL, two-dimensional polypeptide maps of the bleached mutants have been compared to those of wild-type cells.

A comparison of the two-dimensional polypeptide maps of dark-grown resting wild-type cells

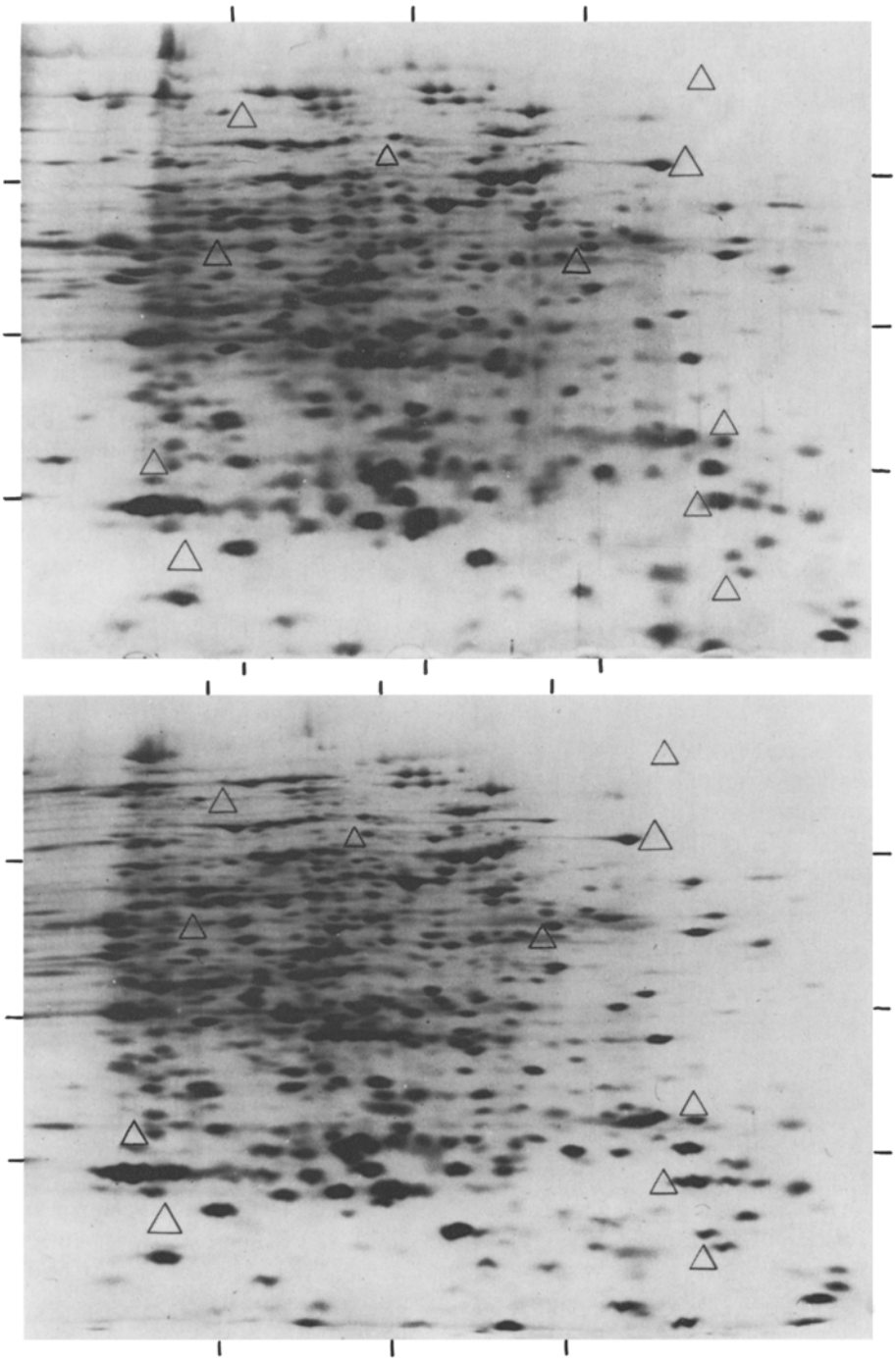

Fig. 4. Silver-stained two-dimensional gel of 60 μg total cellular protein extracted from dark-grown, resting cells of the bleached mutant W_{10}BSmL maintained in the dark (*top*) or exposed to light for 72 h (*bottom*). *Triangles* indicate the positions of polypeptides which are detectable in wild-type cells but which are undetectable in W_{10}BSmL. The lines on the edge of the electrophoretogram indicate the positions of the sectors used for polypeptide analysis

with those of dark-grown resting cells of the bleached mutant W_3BUL maintained in the dark or exposed to light for 72 h (Fig. 3) showed that four polypeptides, A17, B51, D54 and D21, whose relative amount increased in wild-type cells upon light exposure, were undetectable in W_3BUL. Thus, all of the polypeptides whose relative amounts decrease in wild-type cells upon light exposure, all but four of the polypeptides whose relative amounts increase in wild-type cells upon light exposure, and all of the polypeptides whose relative amounts are unaffected by light exposure appear to be coded by either the nuclear or mitochondrial genome. A comparison of the two-dimensional polypeptide maps of dark-grown, resting, wild-type cells with dark-grown resting cells of the bleached mutant W_{10}BSmL maintained in the dark or exposed to light for 72 h (Fig. 4) showed

that in addition to the four polypeptides undetectable in W_3BUL, there were seven polypeptides, A110, A76, A28.2, A22.1, D93, C75 and D26, which though present in W_3BUL were undetectable in W_{10}BSmL. As found for alkaline DNase (Egan et al. 1975) and chloroplast valyl-tRNA synthetase (McCarthy et al. 1982), although these polypeptides appear to be coded by the mitochondrial or nuclear genome, they are undetectable in the bleached mutant W_{10}BSmL.

Exposure of dark-grown resting W_3BUL to light for 72 h increased the relative amounts of 12 polypeptides, A110, A76, A42.1, A42.2, A28.2, A22.1, B62.1, C75, C36, C22.2, D93, and D26 (Fig. 3), and decreased the relative amounts of 14 polypeptides, A38.1, A25.1, A20, C62.3, C56, C52, C32.1, C32.2, D120, D104, D65.1, D65.2, D65.3 and D56 (Fig. 3). Although many of these photoregulated polypeptides are present in W_{10}BSmL, light exposure had no effect on polypeptide levels in this bleached mutant (Fig. 4).

Discussion

Light regulates the expression of the nuclear and chloroplast genomes of *Euglena*. The complexity of total cellular RNA derived from single-copy nuclear DNA increases during the first 12 h of light exposure of the cells and then declines (Curtis and Rawson 1979). Polyadenylated-RNA sequences present 24 h after light exposure differ from those present prior to light exposure (Verdier 1979), indicating that light induces the transcription of some nuclear genes while repressing the transcription of other genes. A large fraction of the chloroplast genome is transcribed in the dark (Chelm and Hallick 1976; Rawson and Boerma 1976) and light exposure induces the expression of additional sequences (Chelm and Hallick 1976; Rawson and Boerma 1979). Most sequences expressed in the dark continue to be expressed in the light (Chelm et al. 1979; Rawson and Boerma 1979) but the abundance of some of their transcripts increases five- to tenfold (Rawson et al. 1981). Some chloroplast genome transcripts present in dark-grown cells can not be detected 4 h after light exposure but they are detectable 72 h after light exposure (Chelm et al. 1979). Other transcripts are not detected in the dark but they are present in the light (Chelm et al. 1979).

Visual comparisons of two-dimensional gels indicate that changes in the relative levels of specific polypeptides parallel the changes in the levels of nuclear and chloroplast genome transcripts. The relative amounts of 79 specific polypeptides increased in response to light exposure. This increase is probably the result of a de-novo enzyme synthesis resulting from increased mRNA levels which are in turn the result of the light-dependent increase in the transcription of specific nuclear and chloroplast genes. Since light induces the synthesis of mitochondrial (Horrum and Schwartzbach 1980b) and microbody enzymes (Horrum and Schwartzbach 1981) as well as chloroplast enzymes (Horrum and Schwartzbach 1980a; McCarthy et al. 1982), it will be interesting to determine the intracellular localization of the photoregulated polypeptides.

In resting (carbon-starved) cells, the amino acids required by the developing chloroplast appear to be obtained through the degradation of preexisting proteins (Bovarnik et al. 1974; Schwartzbach and Schiff 1979). Since a decrease in the level of 72 specific polypeptides occurs in response to light exposure coincidently with a decrease in the complexity of total nuclear transcripts (Verdier 1979; Curtis and Rawson 1979), it is likely that light acts at the transcriptional level by selecting which proteins can be resynthesized rather than at the posttranslational level by selecting which proteins will be degraded. Although nonselective protein degradation followed by selective resynthesis is an energetically wasteful way in which to alter the polypeptide composition of a cell, it may be the only way in which a cell can maintain the selectivity required to adapt its polypeptide composition to diverse environmental conditions.

Bleached mutants of *Euglena* are readily obtained. The common characteristic of these mutants is that they have lost their chloroplast genomes (Schiff and Schwartzbach 1982). Many of these mutants have retained fragments of chloroplast DNA (usually rRNA cistrons) in low-copy numbers, but this DNA does not appear to be expressed (Schwartzbach et al. 1976; Heizmann et al. 1981, 1982). All but four of the polypeptides whose amounts increase and all of the polypeptides whose amount decreases after exposure of cells to light were present in the mutant W_3BUL at the same level as in dark-grown, resting, wild-type cells, indicating that most of the photoregulated polypeptides were coded by the nuclear and possibly mitochondrial genomes. Based on its isoelectric point, its molecular weight and its relative abundance (McFadden et al. 1975; Sagher et al. 1976), one of the four polypeptides undetectable in W_3BUL, polypeptide D54, is probably the large subunit of ribulose bisphosphate carboxylase, a polypeptide whose gene has been mapped on *Euglena* chloroplast DNA (Stiegler et al. 1982). At the

present time, the function and intracellular localization of the other polypeptides not found in W_3BUL is unknown.

It has been assumed that bleached mutants are capable of expressing nuclear genes for chloroplast-localized enzymes to the same extent as dark-grown wild-type cells. Thus, the presence of an enzyme in W_3BUL has been used as evidence that this enzyme is coded by nuclear DNA. Some enzymes present in W_3BUL, however, are undetectable in the mutant W_{10}BSmL (McCarthy et al. 1982; Egan et al. 1975).

In contrast to measurements of enzyme activity, visual inspection of two-dimensional gels provides information concerning the amount of a polypeptide; quantitation is not dependent upon the production of a functional polypeptide. Of the 72 identified polypeptides whose level decreased upon exposure of wild-type cells to light, all 72 are present in both W_3BUL and W_{10}BSmL; they are nuclear-coded polypeptides which are probably required for heterotrophic growth and at least partially gratuitous for phototrophic growth. Of the 75 identified polypeptides which are present in W_3BUL and whose level increased upon exposure of wild-type cells to light, seven are undetectable in W_{10}BSmL. Associated with the loss of the chloroplast during the bleaching process, W_{10}BSmL appears to have lost the ability to express or post-transcriptionally process and accumulate the product of some, but not all, of the nuclear genes coding for light-induced and possibly chloroplast-localized enzymes. Since bleaching selectively represses the accumulation of nuclear-gene products, polypeptide levels in bleached mutants can not be the only criteria used to determine the coding site for a specific polypeptide. Thus, those polypeptides other than D54 which are not found in W_3BUL are only tentatively identified as coded by the chloroplast genome.

In addition to lacking most if not all of their chloroplast genome, bleached mutants lack photo-transformable protochlorophyll(ide), the chloroplast-localized photoreceptor regulating organelle biogenesis (Schiff and Schwartzbach 1982). Photoresponses maintained by the bleached mutants must be mediated by a nonchloroplast photoreceptor. Exposure of W_3BUL to light increased the relative amounts of 12 polypeptides and decreased the amounts of 14 polypeptides. A nonchloroplast photoreceptor apparently regulates the levels of these polypeptides. Although W_{10}BSmL contains a nonchloroplast photoreceptor (Horrum and Schwartzbach 1980b), light exposure had no effect on polypeptide levels in this mutant. A number of polypeptides whose levels are regulated by the nonchloroplast photoreceptor are present in W_{10}BSmL; they are no longer photoregulated. Taken together, these results indicate that *Euglena* may contain multiple photoreceptors or that in W_{10}BSmL, the nonchloroplast photoreceptor has been uncoupled from some of the events regulated by that photoreceptor. As W_{10}BSmL lacks carotenoids (Fong and Schiff 1979), the possibility exists that carotenoids as well as the ubiquitous blue-absorbing system which has been termed cryptochrome (Senger 1980) regulate macromolecule synthesis in *Euglena*.

This work was supported by National Institutes of Health grant GM26994 and National Science Foundation grant PCM 8202472.

References

Bingham, S., Schiff, J.A. (1979) Events surrounding the early development of *Euglena* chloroplasts. 15 Origin of plastid thylakoid polypeptides in wild type and mutant cells. Biochim. Biophys. Acta **547**, 512–530

Bovarnick, J.G., Schiff, J.A., Freedman, Z. Egan Jr., J. (1974) Events surrounding the early development of *Euglena* chloroplasts. 4. Cellular origin of chloroplast enzymes in *Euglena*. J. Gen. Microbiol. **83**, 63–71

Chelm, B.K., Hallick, R.B. (1976) Changes in the expression of the chloroplast genome of *Euglena gracilis* during chloroplast development. Biochemistry **15**, 593–599

Chelm, B.K., Hallick, R.B., Gray, P.W. (1979) Transcription program of the genome of *Euglena gracilis* during chloroplast development. Proc. Natl. Acad. Sci. USA **76**, 2258–2262

Cohen, D., Schiff, J.A. (1976) Events surrounding the early development of *Euglena* chloroplasts. Photoregulation of the transcription of chloroplast and cytoplasmic ribosomal RNAs. Arch. Biochem. Biophys. **177**, 201–216

Curtis, S.E., Rawson, J.R.Y. (1979) Measurement of the transcription of nuclear single-copy deoxyribonucleic acid during chloroplast development in *Euglena gracilis*. Biochemistry **18**, 5299–5304

Dockerty, A., Merrett, M.J. (1979) Isolation and enzymic characterization of *Euglena* proplastids. Plant Physiol. **63**, 468–473

Egan, J.M., Dorsky, D., Schiff, J.A. (1975) Events surrounding the early development of *Euglena* chloroplasts. 6. Action spectra for the formation of chlorophyll, lag elimination in chlorophyll synthesis, and appearance of TPN-dependent triose phosphate dehydrogenase and alkaline DNase activities. Plant Physiol. **56**, 318–323

Fong, F., Schiff, J.A. (1979) Blue-light-induced absorbance changes associated with carotenoids in *Euglena*. Planta **146**, 119–127

Heizmann, P., Hussein, D.Y., Nicolas, P., Nigon, P., Bernardi, G. (1981) The chloroplast genome of bleached mutants of *Euglena gracilis*. Biochim. Biophys. Acta **653**, 412–415

Heizmann, P., Hussein, D.Y., Nicolas, P., Nigon, V. (1982) Modifications of chloroplast DNA during streptomycin induced mutagenesis in *Euglena gracilis*. Curr. Genet. **5**, 9–15

Horrum, M.A., Schwartzbach, S.D. (1980a) Nutritional regulation of organelle biogenesis in *Euglena*. Repression of chlo-

rophyll and NADP-glyceraldehyde-3-phosphate dehydrogenase synthesis. Plant Physiol. **65**, 383–386

Horrum, M.A., Schwartzbach, S.D. (1980b) Nutritional regulation of organelle biogenesis in *Euglena*: photo and metabolite induction of mitochondria. Planta **149**, 376–383

Horrum, M.A., Schwartzbach, S.D. (1980c) Absence of photo and nutritional regulation of two glycolate pathway enzymes in *Euglena*. Plant Sci. Lett. **20**, 133–139

Horrum, M.A., Schwartzbach, S.D. (1981) Nutritional regulation of organelle biogenesis in *Euglena*. Induction of microbodies. Plant Physiol. **68**, 430–434

James, L., Schwartzbach, S.D. (1982) Differential regulation of phosphoglycolate and phosphoglycerate phosphatase in *Euglena*. Plant Sci. Lett. **27**, 223–232

McCarthy, S.A., James, L., Schwartzbach, S.D. (1982) Photo and nutritional regulation of chloroplast valyl-tRNA synthetase in *Euglena*. Arch. Microbiol. **133**, 149–154

McFadden, B.A., Lord, J.M., Rowe, A., Dilks, S. (1975) Composition, quaternary structure, and catalytic properties of D-ribulose-1,5-biphosphate carboxylase from *Euglena gracilis*. Eur. J. Biochem. **54**, 195–206

Merril, C.R., D. Goldman, Van Keuren, M.L. (1982) Simplified silver protein detection and image enhancement methods in polyacrylamide gels. Electrophoresis **3**, 17–23

Monroy, A., Schwartzbach, S.D. (1981) Regulation of polypeptide synthesis in greening *Euglena*. (Abstr.) Plant Physiol. **67**, Suppl. 33

O'Farrell, P.H. (1975) High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**, 4007–4021

Oakley, B.R., D.R. Kirsch, Morris, N.R. (1980) a simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. Anal. Biochem. **105**, 361–363

Ochs, D.C., McConkey, E.H., Sammons, D.W. (1981) Silver stains for proteins in polyacrylamide gels: A comparison for six methods. Electrophoresis **2**, 304–307

Osafune, T., Schiff, J.A. (1980) Events surrounding the early development of *Euglena* chloroplasts 17. Light-induced changes in a proplastid remnant in mutant W_3BUL. J. Ultrastruct. Res. **73**, 64–76

Pedersen, S., Reeh, S., Friesen, J.D., Bloch, P.L., Neidhardt, F.C. (1978) A nomenclature for *E. coli* proteins. Cell **14**, 186–190

Rawson, J.R.Y., Boerma, C.L. (1976) A measurement of the fraction of chloroplast DNA transcribed during chloroplast development in *Euglena gracilis*. Biochemistry **15**, 588–592

Rawson, J.R.Y., Boerma, C.L. (1979) Hybridization of EcoRI chloroplast DNA fragments of *Euglena* to pulse labeled RNA from different stages of chloroplast development. Biochem. Biophys. Res. Commun. **89**, 743–749

Rawson, J.R.Y., Boerma, C.L., Andrews, W.H., Wilkerson, C.G. (1981) Complexity and abundance of ribonucleic acid transcribed from restriction endonuclease fragments of *Euglena* chloroplast deoxyribonucleic acid during chloroplast development. Biochemistry **20**, 2639–2644

Sagher, D., Grosfeld, H., Edelman, E. (1976) Large subunit ribulose bisphosphate carboxylase messenger RNA from *Euglena* chloroplasts. Proc. Natl. Acad. Sci. USA **73**, 722–726

Schiff, J.A., Schwartzbach, S.D. (1982) Photocontrol of chloroplast development in *Euglena*. In: Biology of *Euglena*, vol. III: Physiology, pp. 313–352, Buetow, D.W., ed. Academic Press, New York London

Schwartzbach, S.D., Hecker, L.I., Barnett, W.E. (1976) Transcriptional origin of *Euglena* chloroplast tRNAs. Proc. Natl. Acad. Sci. USA **73**, 1984–1988

Schwartzbach, S.D., Schiff, J.A. Goldstein, N.H. (1975) Events surrounding the early development of *Euglena* chloroplasts. V. Control of paramylum degradation. Plant Physiol. **56**, 313–317

Schwartzbach, S.D., Schiff, J.A. (1979) Events surrounding the early development of *Euglena* chloroplasts. 13. Photocontrol of protein synthesis. Plant Cell Physiol. **20**, 827–838

Senger, H. (ed) (1980) The blue light syndrome. Springer, Berlin Heidelberg New York

Spector, T. (1978) Refinement of the Coomassie Blue method of protein quantitation. Anal. Biochem. **86**, 142–146

Stiegler, G.L., Matthews, H.M., Bingham, S.E. Hallick, R.B. (1982) The gene for the large subunit of ribulose-1,5-bisphosphate carboxylase in *Euglena gracilis* chloroplast DNA: localization, polarity, cloning, and evidence for an intervening sequence. Nucleic Acid Res. **10**, 3427–3443

Van Etten, J.L., Freer, S.N., McCune, B.K. (1979) Presence of a major (storage?) protein in dormant spores of the fungus *Botryodiplodia theobromae*. J. Bacteriol. **138**, 650–652

Verdier, G. (1979) Poly (adenylic acid)-containing RNA of *Euglena gracilis* during chloroplast development. I. Analysis of their complexity by hybridization to complementary DNA. Eur. J. Biochem. **93**, 573–580

Received 24 December 1982; accepted 17 March 1983