Biochimica et Biophysica Acta, 547 (1979) 531—543

BBA 47720

EVENTS SURROUNDING THE EARLY DEVELOPMENT OF *EUGLENA* CHLOROPLASTS

16. PLASTID THYLAKOID POLYPEPTIDES DURING GREENING

SCOTT BINGHAM * and JEROME A. SCHIFF **

Institute for Photobiology of Cells and Organelles, Brandeis University, Waltham, MA 01254 (U.S.A.)

(Received November 28th, 1978)

Key words: Chloroplast development; Thylakoid polypeptides; Coding; Plastid membranes; (Euglena)

Summary

Using sulfolipid to locate plastid thylakoid membranes in gradients from dark-grown resting cells it has been possible to study the plastid thylakoid membrane polypeptides of *Euglena gracilis* var. *bacillaris* undergoing light-induced chloroplast development. All plastid thylakoid bands seen in dark-growing wild-type cells and in mutant W_3BUL in which plastid DNA is undetectable, are observed to increase in amount during plastid development. Others, which are undetectable in dark-grown wild-type and W_3BUL increase greatly during plastid development and appear to be those associated with pigment-protein complexes. The data obtained from experiments where the polypeptides were labeled with ^{35}S during development, either continuously or in pulses, were consistent with these findings. Cycloheximide strongly inhibited the increases in amount in all bands and chloramphenicol or streptomycin produced a lower level of inhibition in all bands indicating tight control of the formation of each plastid membrane constituent by the others. The formation of a polypeptide band of 25 000 molecular weight, thought to be a part of a pigment-protein complex of the thylakoid, and chlorophyll synthesis were inhibited identically by these antibiotics.

* Present address: Department of Chemistry, University of Colorado, Boulder, CO 80309, U.S.A.
** To whom reprint requests should be directed.

Introduction

In the accompanying paper [1], we have described methods for locating the plastid thylakoid material in gradients from dark-grown and light-grown cells of wild-type *Euglena gracilis* var. *bacillaris* and from mutants using the thylakoid sulfolipid as a specific marker [1]. From these studies we concluded that all of the proplastid thylakoid membrane polypeptides of dark-grown wild-type cells are probably coded in nuclear DNA. It was evident, however, that light-grown wild-type cells contain a number of thylakoid polypeptides not found in dark-grown cells. It was of some interest, then, to investigate the changes in the polypeptide pattern during light-induced chloroplast development in non-dividing cells of *Euglena*, the standard developmental system used to study greening in this organism. This is the subject of the present paper. Brief accounts of this work have appeared previously [2—4].

Materials and Methods

Growth of organism and conditions for chloroplast development. E. gracillis Klebs var. *bacillaris* Cori was maintained in light or darkness on Hutner's pH 3.5 medium as previously described [5,6]. Non-dividing cells to be used for chloroplast development were obtained by employing a centrifugation step [7]; resuspension of the cells was in a pH 6.8 resting medium [8]. Dark grown non-dividing cells were placed in white light (150 ft-candles, measured with a Weston model 756 sunlight illumination meter) to allow chloroplast development to take place [8]; in experiments with chloramphenicol, light from red fluorescent lamps was used at an intensity (60 ft-candles) which allowed the same amount of chlorophyll formation during development as in white light.

Labeling of cells with $^{35}SO_4^{2-}$. In experiments where resting cells were to be labeled with $^{35}SO_4^{2-}$ during chloroplast development, wild-type *Euglena* were grown on pH 3.5 medium minus the $MgSO_4 \cdot 7\ H_2O$. The magnesium requirement was met by substituting $MgCl_2 \cdot 4\ H_2O$. The only sulfur source, then, was in the trace elements, about 200 μM. Cells grown in the low SO_4^{2-} medium had a generation time identical to the cells grown on normal medium and, after transfer to resting medium, the low SO_4^{2-} cells accumulated chlorophyll identically to control cells. 1 μCi/ml $^{35}SO_4^{2-}$ was added to the rested cultures at the onset of illumination when the label was to be present throughout chloroplast development; in pulse experiments it was added at the beginning of the 1 h pulse at 12, 24, 48, and 72 h of illumination.

$^{35}SO_4^{2-}$ uptake was estimated from the amount of label remaining in the medium as described previously [9]. Incorporation into lipid was determined by resuspending a cell pellet from 1—3 ml of cells in 1 ml of water. Lipids were extracted and the radioactivity present in the extract counted as described previously [1]. Measurement of incorporation into protein was accomplished as described previously from a 1 ml cell suspension [1]. Control experiments indicated that all residual radioactivity from the medium washed off the filters and that all [^{35}S]sulfolipid was removed in the ethanol wash. At cell numbers below $2.3 \cdot 10^6$ (used in the experiments to be described) protein placed on the filters resulted in counts proportional to the number of cells.

Inhibition by antibiotics. In experiments employing antibiotics as inhibitors of chloroplast development, cycloheximide was added at the appropriate time to the culture to a final concentration of 15 μg/ml from a Millipore filter-sterilized stock solution of 1 mg/ml in water. Chloramphenicol was administered to the cells at a final concentration of 2 mg/ml as described previously [10]. Since streptomycin sulfate would interfere with $^{35}SO_4^{2-}$ uptake, the streptomycin sulfate was converted to streptomycin chloride by addition of a stoichiometric amount of $BaCl_2$ and removal of $BaSO_4$. This stock streptomycin chloride solution, after filter sterilization, was then added to resting cultures at a final concentration of 0.05%. The streptomycin chloride inhibited chlorophyll synthesis normally and did not interfere with $^{35}SO_4^{2-}$ uptake.

Other methods. Cell counting, chlorophyll, carotenoid, and protein determinations were carried out as previously described [1]. Methods for determination of lipid soluble radioactivity on sucrose gradients, membrane isolation, solubilization of polypeptides, SDS-polyacrylamide gel electrophoresis, gel scanning, and calibration of gels using protein molecular weight standards were also described previously [1]. To measure protein accumulation in the polypeptide bands during chloroplast development, the area under each peak of a densitometer scan was estimated by tracing the peaks, cutting the peak regions from the tracing paper, and weighing the paper on a Mettler balance. The system was calibrated using known amounts of bovine serum albumin; for amounts of bovine serum albumin below 20 μg a linear relationship exists between the weight of the tracing paper and the amount of protein applied to the gel.

Radioactivity incorporated into polypeptides was determined as described previously [1]. To verify that the radioactivity was associated with the individual bands and was not merely smeared through the gel, the gels were dried and placed in contact with Kodak X-omat M X-ray film [11] which showed that the pattern of radioactive bands coincided with the stained bands in the gel.

Results and Discussion

Sulfolipid as a thylakoid marker during chloroplast development

Light-induced plastid development from the proplastid in dark-grown non-dividing cells of *Euglena* begins with a lag period of about 12 h under our conditions after which there is a period of rapid development until about 72—96 h when development ceases with the maturation of the chloroplast [6,12]. It was important to show that sulfolipid is a good marker for thylakoid membranes at various times of development, as we have previously shown for dark-grown and light-grown cells [1]. Fig. 1 shows that sulfolipid and chlorophyll concentrations follow each other closely across the gradients yielding the same three peaks (cf. Ref. 1) at all developmental times. The ratio of sulfolipid to chlorophyll is constant across the gradients at all developmental times. As might be expected there is an increase in both sulfolipid and chlorophyll as plastid development proceeds. Since our previous studies [1] have shown that the middle fraction represents fairly pure plastid thylakoid material and that the material

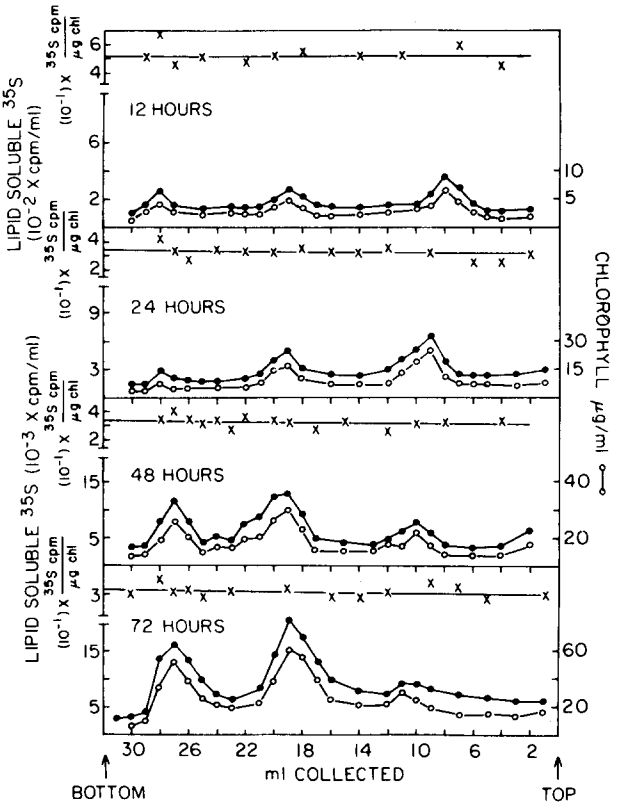

Fig. 1. Coincidence of sulfolipid and chlorophyll on sucrose step gradients prepared from equal numbers of dark-grown resting *Euglena* cells undergoing light-induced plastid development for various times. Also shown is the sulfolipid to chlorophyll ratio across the gradients.

found in this fraction is a constant proportion of the total thylakoid material recovered [1], the middle fractions from the developmental series were used to prepare gels dislplaying the plastid thylakoid polypeptides.

Thylakoid polypeptides during chloroplast development

Figs. 2 and 3 show the patterns found on SDS-polyacrylamide gels using solubilized middle fractions from the gradients. The gels were prepared in two ways. For qualitative comparison of various developmental stages, equal amounts of thylakoid protein were placed on the gels at various times. For quantitative comparison, thylakoid protein from equal numbers of cells were placed on the gels for separation. As may be seen, all bands increase in amount during chloroplast development but some increase more than others such as bands 16—19, 25, 29. There is reason to think that these bands are components of the pigment-protein complexes of the photosynthetic apparatus [13] (Kaufman, L., Bingham, S., Lyman, H. and Schiff, J.A., unpublished results). In the qualitative patterns (Fig. 2, see also Fig. 3 in the accompanying paper [1]) these bands predominate in the final mature chloroplast compared with

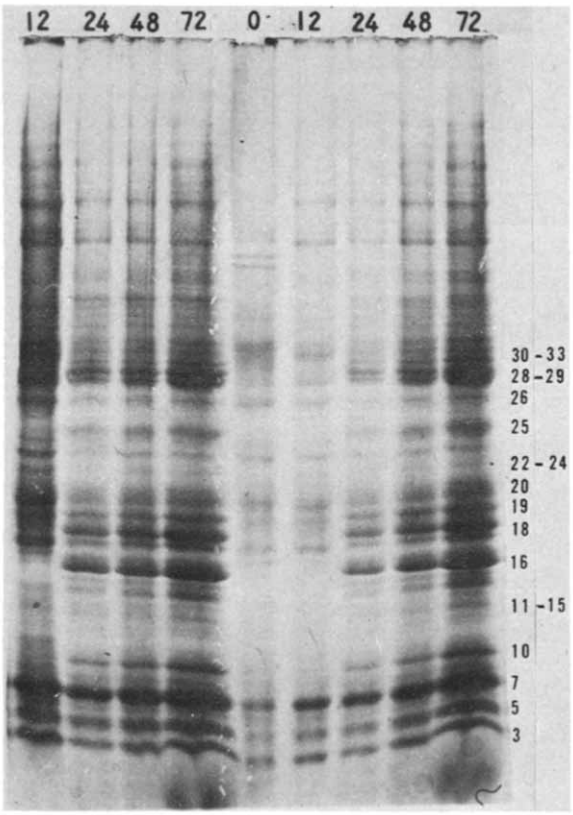

Fig. 2. SDS-polyacrylamide gels prepared from the plastid thylakoid (middle) fraction of the sucrose gradients isolated from dark-grown resting cells of *Euglena* undergoing light-induced chloroplast development. The numbers along the top indicate the number of hours the cells have been in the light; the numbers along the side are the numbers of the bands to be compared with the tracings in other figures. The patterns to the left of 0 h resulted from placing equal amounts of protein on the gels, 0 h and the patterns to the right resulted from placing the protein from equal numbers of cells on the gels.

the bands found in the proplastid patterns from dark-grown cells. Several of these bands appear to arise de novo during greening, but of course the apparent absence of a band from the dark-grown cells, may only mean it is below the limit of detection.

Labeling of plastid thylakoid polypeptides during chloroplast development

The incorporation of sulfur label from $^{35}SO_4^{2-}$ was used as a measure of the biosynthesis of various components. Control experiments (data not shown) indicated that carrier-free sulfate in the medium resulted in optimum uptake of label into the cells and into various components over the course of development and that uptake was essentially complete after 12 h of development when the label was added at zero time. Incorporation into cellular protein was also complete by 12 h of development but the labeling of sulfolipid showed a lag of about 12 h, like the lag in chlorophyll synthesis and rose to become constant by 48 h of development.

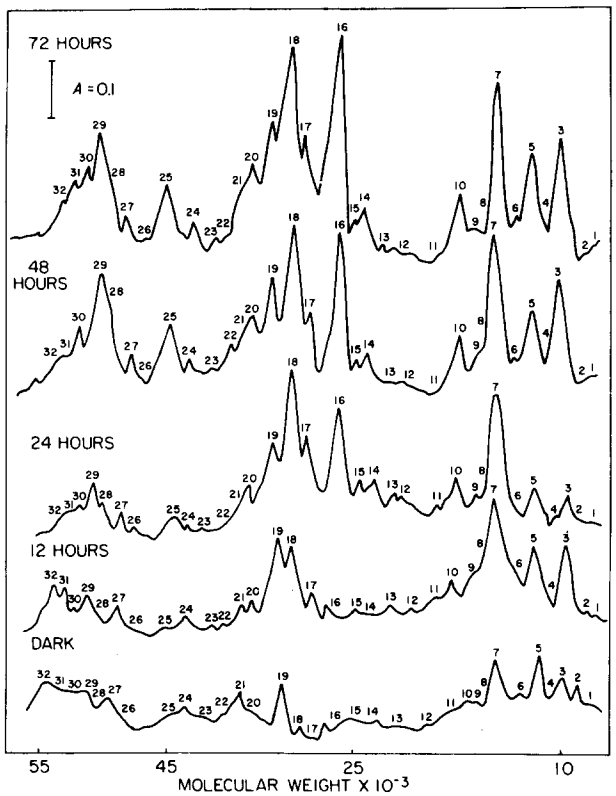

Fig. 3. Quantitative comparison of plastid thylakoid polypeptides during light-induced plastid development in dark-grown resting cells of *Euglena* (densitometer tracings of the gel patterns on the right hand side of Fig. 2).

Incorporation of the sulfur label into representative plastid thylakoid polypeptide bands is shown in Fig. 4. Band 16 is undetectable in preparations from dark-grown cells but is synthesized rapidly during plastid development. Protein and radioactivity in this band rise concomitantly leading to a constant specific activity during development as might be expected for synthesis de novo. Bands 3 and 7 are present as distinct bands in the preparations from dark-grown cells (7 being higher in protein than 3). During plastid development, protein in these bands rises at a lower rate than radioactivity leading to an increase in specific activity. Since unlabeled protein is present at the beginning, it is reasonable to suppose that the increase in specific activity is due to a combination of net synthesis and turnover. It is interesting to compare this with rRNA synthesis during chloroplast development in *Euglena* [7]. The cytoplasmic rRNAs are present at high levels at the start of plastid development and their labeling with $^{32}PO_4^{3-}$ represents considerable turnover during plastid development. Plastid rRNAs which increase in amount during development from very small amounts become labeled in a manner expected for net synthesis. The same situation seems to apply in the case of the polypeptides under discussion, those that rise from undetectable levels during development show net synthesis

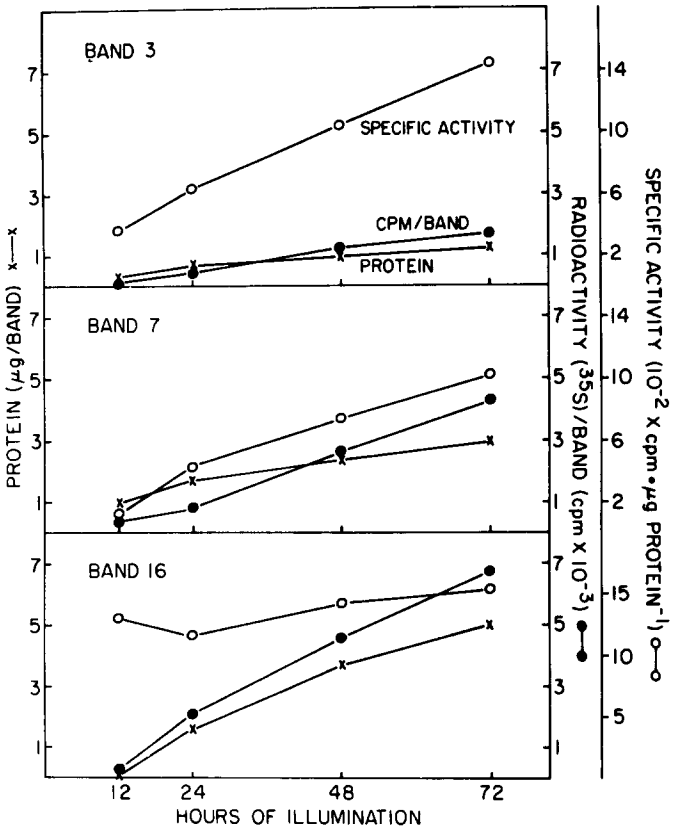

Fig. 4. Protein, radioactivity and specific radioactivity of representative plastid thylakoid polypeptide bands during light-induced chloroplast development in dark-grown resting cells of *Euglena*.

while those which rise from some preexisting concentration show turnover. Perhaps those bands, like number 16 which seem to be made de novo (and appear to be associated with pigment binding proteins in the thylakoids; [13], (Kaufman, L., Bingham, S., Lyman, H. and Schiff, J.A., unpublished results) are controlled or coded in the chloroplast, like plastid rRNA. Those that are present in the dark-grown cells and increase during development we know are not coded in plastid DNA [1] (they are probably coded in nuclear DNA) and show turnover like cytoplasmic rRNAs known to be nuclear coded.

Addition of inhibitors of protein synthesis at the beginning of greening gave the results of Fig. 5. Cycloheximide, a specific inhibitor of translation on the 87 S cytoplasmic ribosomes of *Euglena* [15], produces a large inhibition of synthesis of all polypeptide bands when equal cell numbers are compared. Streptomycin, a specific inhibitor of translation on the 70 S ribosomes of the chloroplast [16,17] inhibits somewhat less, but the formation of all bands is once again inhibited, within the precision of the data. As might be expected, those bands which show the greatest rise in concentration during greening show the greatest effect of the antibiotics. When compared qualitatively on the basis of equal cells numbers, bands 16 and 18 are among those whose synthesis is

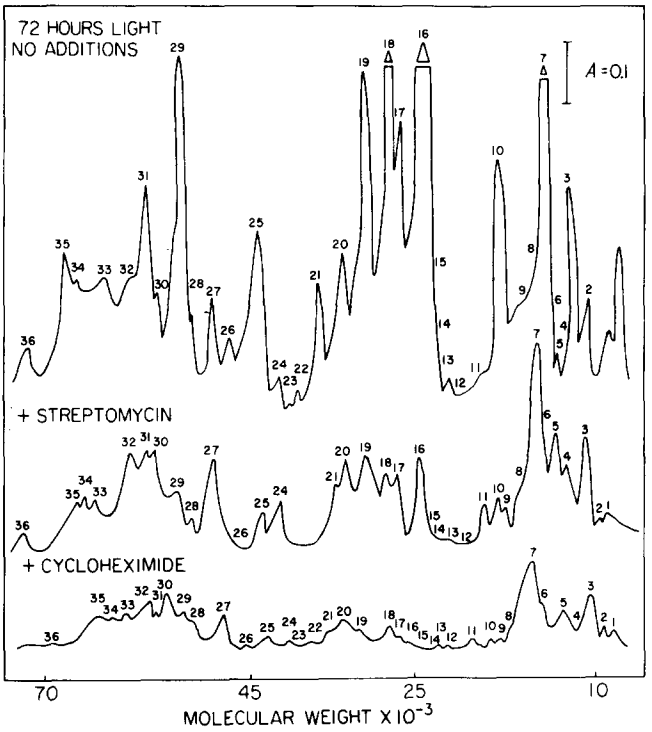

Fig. 5. Comparison of plastid thylakoid polypeptide patterns (middle fraction of sucrose gradient) at the end of plastid development in the absence or presence of inhibitors of protein synthesis. Protein from equal numbers of cells were placed on the gels.

inhibited more by cycloheximide than by streptomycin. Comparing the antibiotic-treated patterns with the untreated cells (Fig. 5), the bands which increase in concentration greatly during greening such as 10, 16, 18, 19, 25, and 29 show the greatest differences.

Fig. 6 shows the behavior of band 16 during development in the presence and absence of inhibitors of protein synthesis. Band 16 is thought to be a component of the light-harvesting pigment-protein complex ([13], and Kaufman, L., Bingham, S., Lyman, H. and Schiff, J.A., unpublished results) and its behavior closely parallels that of chlorophyll both in the kinetics of formation and in the extent of inhibition by cycloheximide and streptomycin.

To rule out indirect effects of the antibiotics on the formation of thylakoid polypeptides and to compare the actual rates of labeling, 1 h pulse experiments were conducted at various times of development. Cycloheximide was again used as a specific inhibitor of synthesis on cytoplasmic ribosomes, but chloramphenicol [15,18] was substituted for streptomycin which is known to require a longer time for permeation into cells and plastids [19]. This necessitated the use of red light for greening to avoid the detoxification of chloramphenicol known to occur in white light [10]. Control experiments (data not shown) indicated that uptake of label into the cells and into total cell protein was comparable at all times and in all treatments with and without antibiotics.

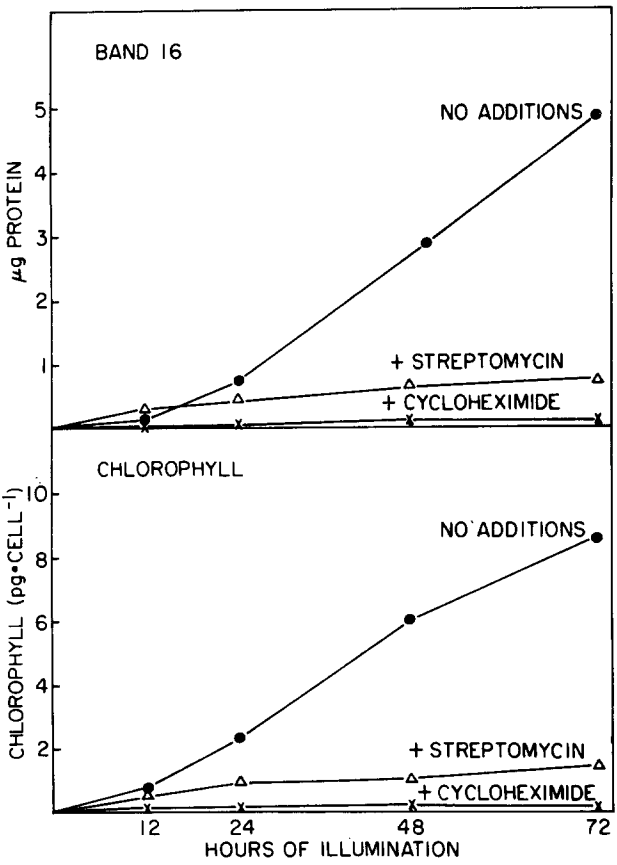

Fig. 6. Comparison of formation of band 16 and chlorophyll during light-induced chloroplast development in dark-grown resting cells of *Euglena* in the presence or absence of inhibitors of protein synthesis.

Fig. 7 shows that in white light, labeling of the thylakoid polypeptides is maximal at about 24 h of chloroplast development, a time of active chloroplast synthesis. The incorporation of label at all times is greatly inhibited by cycloheximide, corroborating the results of the experiments where label and the antibiotics were present from the beginning of development. Inhibition, therefore, is probably not due to side effects of cycloheximide resulting from prolonged treatment. In an intensity of red light, chosen to give the same amount of chlorophyll synthesis as in white light (see Materials and Methods), the maximum incorporation is somewhat delayed and is maximal at 48 h of development with substantial inhibition, again, by chloramphenicol. The values shown are averages over all the bands with the 95% confidence of the means shown as flags. It is astonishing how uniformly all of the polypeptide bands behave to the antibiotics which corroborates the data shown previously for continuous application of antibiotics and inhibitors during plastid development.

While certain bands may show more inhibition with one antibiotic or the other under conditions of continuous or pulse labeling, the incorporation of

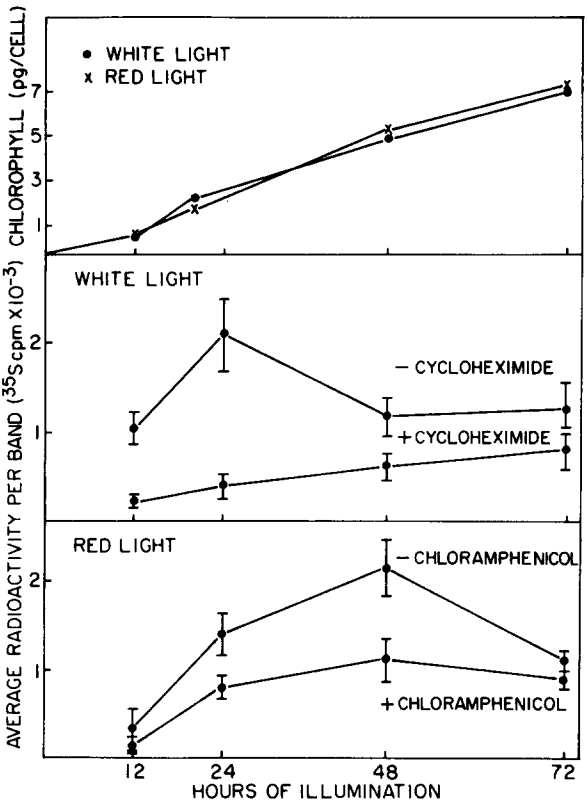

Fig. 7. Pulse labeling of thylakoid membrane polypeptides during greening. Dark-grown resting cells of *Euglena* were exposed to light to induce chloroplast development. Chlorophyll was measured at various times during development in cells exposed to white or red light. Cell aliquots were pulsed for 1 h with $^{35}SO_4^{2-}$ at 12, 24, 48, and 72 h. In white light some cell aliquots also received cycloheximide at the beginning of the pulse; in red light some cell aliquots received chloramphenicol 8 h before addition of $^{35}SO_4^{2-}$. At the end of the 1 h pulse the cell aliquots were processed to extract and purify the thylakoid membranes and to separate the polypeptides on SDS gels. The points shown are the mean incorporation into all bands on the SDS gels plus or minus the 95% confidence interval of the mean. The mean ($\bar{x}$) was computed from the sum of radioactivity in all bands divided by the number of bands. The standard deviation, s, of the measurements was computed in the usual way and the standard error of the mean $s_{\bar{x}}$ was computed from $S_{\bar{x}} = s/\sqrt{N}$, where N is the number of measurements. $S_{\bar{x}}$ was then multiplied by t^{95}_{N-1} to obtain the 95% confidence interval of the mean.

radioactivity varies enough with time of development to make it difficult to discern a pattern that can be relied upon. Rather, plastid membrane thylakoid polypeptides behave rather uniformly to the antibiotics suggesting that under normal conditions of development, one cannot distinguish conclusively in which compartment a given band is translated. As we have pointed out before, there is considerable evidence that the synthesis of plastid membranes is under tight control in *Euglena* and that the inhibition of any component can bring about a halt in the synthesis of others and, consequently, in membrane assembly. Other systems are not under such tight coordinate control [20—25] but under certain experimental conditions evidence of some control can be found [20—23,26]. The same explanation can be applied here to the formation of thylakoid membrane polypeptides: inhibition of any component including

chlorophyll, carotenoids or any of the membrane polypeptides brings about an inhibition of others and, therefore, in the normal greening system one cannot infer where translation of a given component is occurring. It appears that it may be possible to interfere with this regulatory system by using light-dark regimes which uncouple the synthesis of various components [13]. Thus it has been inferred from such experiments, that band 16 and others thought to be part of the pigment-protein complex of the light-harvesting system are translated in the cytoplasm of *Euglena* while another band in the 50 000—60 000 molecular weight region is thought to be translated in the plastid. Further work will be necessary to uncouple the tight regulation of components to find out where *Euglena* thylakoid membrane components are synthesized and how their synthesis is programmed during normal chloroplast development. Although the one-dimensional gradient gels used here provide a much improved separation of polypeptides compared to non-gradient gels, better separation may still be possible, perhaps through the use of two-dimensional gels. Perhaps better separation methods will show some of the present bands to be composed of more than one component.

Conclusions

The results presented here and in the accompanying paper [1] give some insight into the coding of components of *Euglena* plastid thylakoid membranes and how their synthesis is controlled. The fact that the dark-grown wild-type cells containing normal proplastids and cells of the mutant W_3BUL (in which plastid DNA is undetectable) yield identical plastid thylakoid polypeptide patterns indicates that all of the proplastid thylakoid polypeptides are coded outside plastid DNA, probably in nuclear DNA [1]. The fact that the same levels are present in both types of cells indicates that the regulation of their formation is also the same. Since protochlorophyll(ide) is present in wild type but absent from W_3BUL and since this pigment is thought to control the photoinduction of plastid processes during chloroplast development, it appears that the presence or absence of protochlorophyll(ide) does not control the formation of low levels of nuclear-coded plastid thylakoid polypeptides in dark-grown wild type and in W_3BUL. Other lipid components such as carotenoids do appear to exert control in darkness since dark-grown wild-type cells treated with SAN 9789 to inhibit carotenoid biosynthesis lose plastid thylakoid polypeptides progressively on being grown in the dark and a new low-steady-state level of sulfolipid is established on adding the inhibitor. Thus there appear to be regulatory systems common to dark-grown wild type and to W_3BUL which control the expression of nuclear-coded information for the formation of proplastid membranes.

On light induction of plastid development in dark-grown cells, a new developmental program is instituted. Only a very limited light induction is possible in W_3BUL under control of the non-plastid blue light receptor [7,12,14] but this induction does not include thylakoid polypeptides since the patterns in light and dark-grown cells of W_3BUL are identical. This is in agreement with the lack of light induction of those soluble enzymes of the chloroplast in W_3BUL thought to be nuclear coded [16]. In wild type however, where the blue-red

absorbing protochlorophyll(ide) system is present, light induces many new plastid proteins to form. Among these are approximately ten plastid thylakoid polypeptides which are undetectable in dark-grown cells but rise to become major membrane components by the end of chloroplast development. Many of these are plastid membrane polypeptides which are thought to be associated with pigments in the mature thylakoids. Remembering that membrane components regulate each other's formation in *Euglena*, it is not surprising that during light-induced plastid formation, the kinetics of formation of these bands and their inhibition by inhibitors of protein synthesis in cytoplasm and plastid closely resemble that of chlorophyll. A long biosynthetic pathway like that leading to chlorophyll probably has enzymes which are nuclear coded and cytoplasmically synthesized as well as enzymes which are plastid coded and synthesized on plastid ribosomes. Inhibition of chlorophyll synthesis would be expected to prevent the formation of the other components through its regulatory influence. Since these plastid membrane polypeptide components are undetectable in W_3BUL [1], they are candidates for coding in plastid DNA. This evidence, by itself, however, is not conclusive since the absence of plastid DNA also means the absence of plastid ribosomes and other plastid gene products which might have regulatory influences on structural genes in other compartments such as the nucleus. However, the evidence for plastid translation, and perhaps transcription, is somewaht strengthened by the finding that at least 5 of the bands which appear during plastid development are translated in isolated chloroplasts of *Euglena*.

The other plastid membrane polypeptides which are low in concentration in dark-grown wild-type cells and in W_3BUL and are presumably nuclear coded and cytoplasmically translated are found to increase on light induction of plastid development in *Euglena*. Thus as for nuclear-coded cytoplasmically synthesized soluble enzymes of the plastid, these components are regulated to be present at a low level in dark-grown cells but are induced to increase greatly during plastid development; they adhere to one developmental program in darkness but change to another on light induction of chloroplast development.

Thus plastid thylakoid polypeptides appear to be very similar to their soluble plastid counterparts in where they are coded (nucleus and plastid), where they are synthesized (cytoplasmic ribosomes and plastid ribosomes) and how they are regulated by light. The membrane system, however, is different from the soluble components, in that one membrane component can influence the formation of the others through regulation to insure that the total membrane is assembled in the proper sequence with the proper amounts of components at each point. Perhaps we should think of the regulation of plastid thylakoid membrane formation in *Euglena* as we would think of the synthesis of an enzyme with many subunits and coenzymes; in order to synthesize either one there must be coregulation of the formation of the components to ensure that the final structure is assembled correctly.

Acknowledgements

This work was supported by grants GM 14595 from the National Institutes of Health. This work, in part, was taken from a thesis submitted to the

Graduate Faculty of Brandeis University by S.B. in partial fulfillment of the requirements for the Ph.D. degree. J.A.S. is a Abraham and Etta Goodman Professor of Biology.

References

1 Bingham, S. and Schiff, J.A. (1979) Biochim. Biophys. Acta 547, 512—530
2 Bingham, S. and Schiff, J.A. (1976) Plant Physiol. 57, S-73
3 Bingham, S. and Schiff, J.A. (1976) in Genetics and Biogenesis of Chloroplasts and Mitochondria (Bücher, Th., Neupert, W., Sebald, W. and Werner, S., eds.), pp. 79—86, Elsevier, Amsterdam
4 Bingham, S. and Schiff, J.A. (1978) Plant Physiol. 61, S-83
5 Greenblatt, C.L. and Schiff, J.A. (1959) J. Protozool. 6, 23—28
6 Stern, A.I., Schiff, J.A. and Epstein, H.T. (1964) Plant Physiol. 39, 226—231
7 Cohen, D. and Schiff, J.A. (1976) Arch. Biochem. Biophys. 177, 201—216
8 Holowinsky, A.W. and Schiff, J.A. (1970) Plant Physiol. 45, 339—347
9 Hodson, R.C., Schiff, J.A. and Scarsella, A.J. (1968) Plant Physiol. 43, 570—577
10 Vaisberg, A.J., Schiff, J.A., Li, L. and Freedman, Z. (1976) Plant Physiol. 57, 594—601
11 Lai, E. (1978) Ph.D. Thesis, Brandeis University, Waltham, MA
12 Schiff, J.A. (1973) in Advances in Morphogenesis (Abercombie, M. and Bracket, J., eds.), Vol. 10, pp. 265—312, Academic Press, New York
13 Gurevitz, M., Kratz, H. and Ohad, I. (1977) Biochim. Biophys. Acta 461, 475—488
14 Schiff, J.A. (1978) in Chloroplast Development (Akoyunoglou, G., et al., eds.), pp. 747—767. Elsevier, Amsterdam
15 Avadani, N.G. and Buetow, D.E. (1972) Biochem. J. 128, 353—365
16 Bovarnick, J.G., Schiff, J.A., Freedman, Z. and Egan, J.M. (1974) J. Gen. Microbiol. 83, 63—71
17 Schwartzbach, S.D. and Schiff, J.A. (1974) J. Bacteriol. 120, 334—341
18 Smillie, R.M., Bishop, D.G., Gibbons, G.C., Graham, D., Grieve, A.M., Raison, J.K. and Reger, B.J. (1971) in Autonomy and Biogenesis of Chloroplasts and Mitochondria (Boardman, N.K., Linnane, A.W. and Smillie, R.M., eds.), pp. 422—433, North Holland, Amsterdam
19 Bovarnick, J.G., Chang, S., Schiff, J.A. and Schwartzbach, S.D. (1974) J. Gen. Microbiol. 83, 51—62
20 Hoober, J.K. (1970) J. Biol. Chem. 245, 4327—4334
21 Eytan, G. and Ohad, I. (1970) J. Biol. Chem. 245, 4297—4307
22 Apel, K. and Schweiger, H.G. (1973) Eur. J. Biochem. 38, 373—383
23 Machold, O. and Aurich, O. (1972) Biochim. Biophys. Acta 281, 103—112
24 Hachtel, W. (1976) in Genetics and Biogenesis of Chloroplasts and Mitochondria (Bücher, Th., Neupert, W., Sebald, W. and Werner, S., eds.), pp. 49—52, Elsevier, Amsterdam
25 Ellis, R.J. (1976) in The Intact Chloroplast (Barber, J., ed.), pp. 335—364, Elsevier, Amsterdam
26 Kaveh, D. and Harel, E. (1973) Plant Physiol. 51, 671—676