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Relation between Paramylum Content and the Length of the Lag Period of Chlorophyll Synthesis during Greening of Dark-grown *Euglena gracilis*[1]

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ABSTRACT

***Euglena* cells, strains Z and *bacillaris*, were grown in the dark under various nutritional deficiencies. After 3 days of nondivision, cells were transferred to the light, and the following parameters were measured: the paramylum content at the time of illumination (zero time), the rate of paramylum consumption during the first 10 hours of greening, and the length of the lag phase of chlorophyll synthesis. Similar results were obtained with both strains and can be summarized as follows. *(a)* The use of various nutritional deficiencies allows the control, to a certain extent, of the amount of paramylum present at zero time. *(b)* The rate of paramylum consumption is proportional to the cellular paramylum content for values in excess of 50 picograms/cell. *(c)* The length of the lag phase increases rapidly when the cellular content of paramylum decreases below 50 picograms. This period can be greatly diminished by the addition of an exogenous organic carbon source. *(d)* The amount of paramylum (rate of paramylum consumption × length of lag phase) consumed during the lag phase is around 5 to 10 picograms/cell for cells which contain less than 50 picograms of paramylum/cell. It increases when the cellular paramylum content increases, this increment being more rapid for *bacillaris* than for Z cells.**

Paramylum, a β-1,3-glucan, is the characteristic carbohydrate reserve of the Euglenophyta (1). It accumulates when *Euglena* is grown on an organotrophic medium in the dark and is consumed either in the dark when cells receive, at the end of the exponential phase of growth, nitrogen and/or phosphorus (10), or when cells are transferred to the light (7, 8, 10, 23). In the latter case, it provides carbon materials and energy requirements during the early times of plastid formation before photosynthetic activity is able to satisfy cell demands (8, 22, 23).

When *Euglena* cells are transferred to the light, the active synthesis of Chl occurs after a certain lag time. Depending on culture conditions and/or the strain used, the duration of this lag changes from 3 hr (7, 10, 17) to 12 hr (15, 17, 26).

In previous experiments (10), we have demonstrated that there is a linear relation between the amount of paramylum utilized and Chl synthesized during the 1st day of greening. This relation exists only when paramylum consumption is in the range of 30 to 600 $pg \cdot cell^{-1} \cdot day^{-1}$. Thus one could suppose that another phenomenon appears in cells which consume less than 30 $pg \cdot cell^{-1} \cdot day^{-1}$.

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It seems worthwhile to study cultures containing small amounts of paramylum. Results shown here indicate that when the cellular content is below 50 pg the lag phase of Chl synthesis increases. Results obtained with the Z strain are compared with those obtained with *bacillaris*.

MATERIALS AND METHODS

Two strains of *Euglena gracilis*, Klebs Z Pringsheim (No. 1224-5/25) and *bacillaris* Pringsheim, have been used for these experiments.

Several growth media with known composition in carbon, phosphorus, and nitrogen have been employed (Table I). Media are identified by two letters and one number: in this manner, a PC_1 medium induces a growth deficiency first by phosphorus [P] then by carbon [C]; sodium butyrate is the carbon source [1]. Media which contain butyrate (class 1) have been prepared according to a technique already described (10). Media containing glutamate-malate (class 2) have been prepared either from the Difco powder (Difco Laboratories, Detroit) which gives the CN_2 medium (Table I) or by mixing the mineral fraction used for class 1 medium (10) and the organic mixture described in Table I. No difference has been detected between the two types of preparations. Deficiencies for class 2 media have been estimated by comparison with medium E (Table Ic).

Euglena cells were grown in the dark at 25 C and transferred, at the end of the exponential phase of growth, or early in the stationary phase, either into a 30 mM KCl solution, a mixture of 10 mM KCl, 20 mM citric acid (pH 4.5), or into the resting medium described by Cohen (3) following a technique previously described (11). After 3 to 5 days of resting, cells are illuminated with a sodium vapor lamp which gives 4700 lux at the level of cultures.

Chl and paramylum have been measured as described previously (10).

RESULTS AND DISCUSSION

INFLUENCE OF GROWTH CONDITIONS IN DARK ON LEVEL OF PARAMYLUM

Paramylum Content when Cells Are Transferred to Light (Zero Time). A study of the behavior of cells grown on different media led us to make the following observations. *(a)* The various nutritional deficiencies used result in cells with different amounts of paramylum. The cellular content ranges from 10 pg for CN_1 cultures to 450 pg for NC_1 cultures (Fig. 1). In the following experiments, we primarily utilized cells containing less than 200 pg of paramylum. *(b)* Cells often have a higher paramylum content when the resting phase is performed in Cohen's medium

Table I. *Composition of Different Media Used for Growth of Euglena*

Concentrations of vitamins B_1 and B_{12} are, respectively, 2 and 0.002 mg/l for class 1 media and 3 and 0.003 mg/l for class 2 media. The organic fraction thus constituted is mixed with the mineral fraction (Meb) described in a previous report (10), and each medium gives 10^9 cells/l. Final pH of the media is 7.4 for class 1 and 3.5 for class 2. Media are identified by two letters and one number. For instance, a CN_2 medium induces a growth deficiency first by carbon [C] then by nitrogen [N]; glutamate and malate are the carbon sources [2]. Medium E corresponds to balanced medium where nutrients are given in amounts proportional to the requirements of cells (10).

CLASS 1	E		PC_1			CN_1			CP_1			NC_1		
	a[1]	b[2]	a	b	c[3]	a	b	c	a	b	c	a	b	c
K_2HPO_4	15		15			48			17			45		
Phosphorus		2.6		2.6	1		8.3	3.2		2.9	1.1		7.8	3
NH_4Cl	120		262			210			210			120		
Nitrogen		32		70	2.2		56	1.8		56	1.8		32	1
Sodium butyrate	900		1130			900			900			1300		
Carbon		393		493	1.3		393	1		393	1		568	1.4

CLASS 2	PC_2			CN_2			CP_2			NC_2		
	a	b	c	a	b	c	a	b	c	a	b	c
K_2HPO_4	8.3			80			20			80		
Phosphorus		2.9	1.1		23.7	8.8		4.8	1.8		19	7.3
$(NH_4)_2HPO_4$	5			40			5			20		
Nitrogen		96.2	3		103.6	3.2		96.2	3		51.3	1.6
Glutamic acid L	1000			1000			1000			500		
Carbon		551	1.4		551	1.4		551	1.4		1063	2.7
Malic acid DL	400			400			400			2400		

[1]Amount of compounds added (mg/l).
[2]Resulting amount of elements (carbon, nitrogen or phosphorus) in the medium (mg/l).
[3]Ratio between the amount of an element added into a precise medium and that of a medium E.

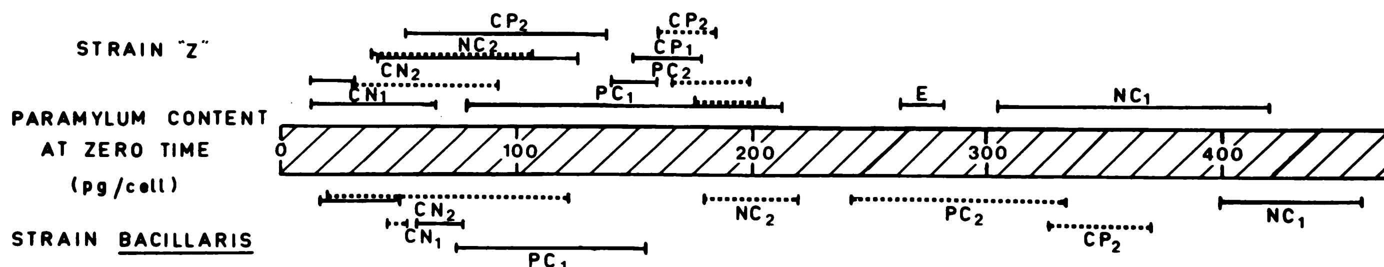

FIG. 1. Influence of the composition of the growth medium on the paramylum content at zero time of greening. Resting phase in 30 mM KCl (——); resting phase in Cohen's medium (3) (----). Composition of growth media is given in Table I. Paramylum content was measured when cells were transferred to the light (zero time of greening).

(Fig. 1). *(c)* Using the same culture conditions, *bacillaris* contains more paramylum than Z. *(d)* With *bacillaris*, extreme values of paramylum content are difficult to obtain. In media which result in a high paramylum content, most of the cells become bloated during growth. On the contrary, when cells have a low paramylum content, they die during resting or during the 1st day of greening. *(e)* Cells grown in class 2 media and rested in a KCl solution stop greening after the 1st day of illumination, whereas they continue when the resting period is on Cohen's medium. Such a phenomenon is not found with cells cultivated in class 1 media.

Rate of Paramylum Consumption during First 10 Hr of Greening. Paramylum consumption starts without a lag when cells are transferred to the light and continues linearly during 12 to 36 hr, depending on culture conditions and/or the strain used (8, 10, 23). For each culture condition, we measured paramylum consumption during first 10 hr of greening and compared it with the paramylum content at zero time (Fig. 2): a linear relation is obtained with values higher than 50 pg of paramylum/cell. Below that, the straight line bends and tends toward the origin. This seems confirmed by the linear regression calculated with values below 50 pg/cell.

Estimation of Carbohydrate Reserves. The results presented above furnish new information concerning the carbohydrate reserves in greening cells. Two types of observations can be made. First, at least three factors have an influence on the paramylum content: the proportions of carbon, nitrogen, and phosphorus during growth in the dark, as well as the origin of the carbon source (butyrate or glutamate-malate); the composition of resting medium; and the origin of the inoculum, a period of adaptation to a new growth medium is always required. Second, the rate of paramylum consumption during greening seems to be regulated by the paramylum content available. This balance, however, can be changed when cells receive, at the moment of transfer to light, nitrogen (10), or carbon (8).

INFLUENCE OF CARBOHYDRATE RESERVES ON LENGTH OF LAG PHASE OF CHLOROPHYLL SYNTHESIS

To determine the length of the lag phase of Chl synthesis, we first calculated the linear regression with values of Chl/cell ob-

tained during the linear phase of its synthesis and then estimated the length of the lag phase by the intercept of this straight line with the abscissa (Fig. 3). The lag is short (2.4 hr) for PC_1 cultures which contain a large amount of paramylum, 150 pg/cell, and long (11.6 hr) for CN_1 cultures which have a low paramylum content, 20 pg/cell. This lengthening of the lag is not necessarily accompanied by a difference in the quantity of Chl formed during greening.

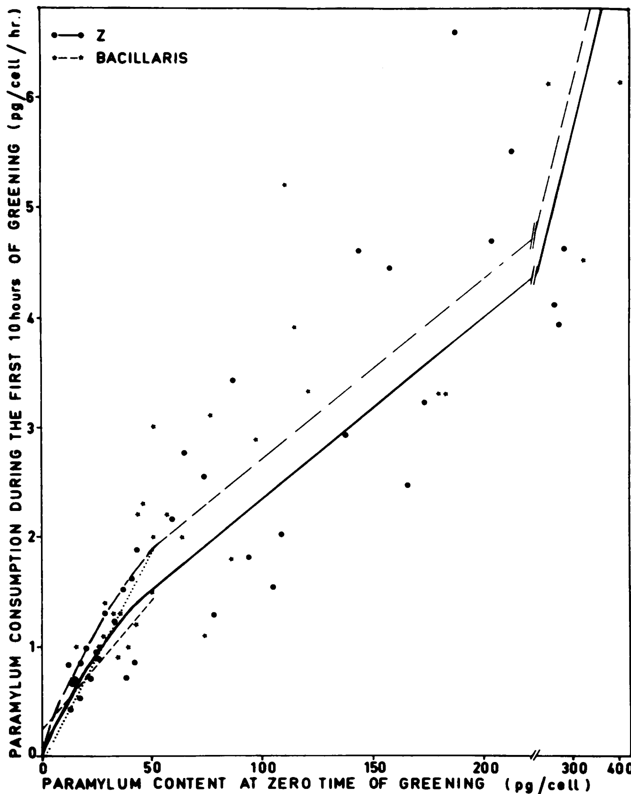

FIG. 2. Relation between paramylum content at zero time of greening and paramylum consumption during the first 10 hr of greening. For both strains, two regression lines are calculated, one with all the values of paramylum/cell (——, Z; — —, *bacillaris*) and one with values below 50 pg of paramylum/cell (- - -, Z; · · · · ·, *bacillaris*). The first regression lines were bent toward the origin; this is confirmed by the second regression lines.

Figure 4 confirms the existence of a relationship between the lag period and paramylum content. The lag phase, close to 3 to 5 hr for cells with more than 100 pg of paramylum, increases rapidly when the paramylum content decreases below 50 pg/cell. Because of the culture problems described above, only few experimental results were obtained with *bacillaris* containing below 20 pg/cell and above 150 pg/cell. The behavior of this strain seems similar to that of the Z strain.

A linear relation is obtained when we plot the inverse of the paramylum content *versus* the length of the lag phase (Fig. 5). The intercept of the regression line thus obtained with the ordinate gives an estimation of the theoretical minimum lag. This value is 3.2 hr for Z strain and 4.5 hr for *bacillaris;* they both represent the horizontal asymptotes drawn on Figure 4. Figure 5 shows values of paramylum content higher than 30 pg/cell. The equations of the regression lines of Figure 5 were used to calculate the curves in Figure 4; we see that the experimental points and the calculated curves fit well. Different results are obtained from calculations including values below 30 pg of paramylum/cell: the hyperbolic curves do not fit the experimental points, the theoretical minimum lag being close to 3 hr for both strains. This discrepancy may be due to the fact that we did not have greening cells with less than 12 pg of paramylum/cell, such cells die during resting or during the 1st day of greening. For these reasons, we cannot be sure that the two strains have a different minimum lag even though the two values for minimum lag calculated with cells having more than 30 pg of paramylum/cell were found significantly different by a *t* test.

AMOUNT OF PARAMYLUM CONSUMED DURING LAG PHASE

An estimation of the amount of paramylum consumed during the lag phase is obtained by multiplying the rate of paramylum consumption by the length of the lag phase. Omitting cultures which present a lag phase longer than 12 hr (because of the error inherent with the estimate of the rate of paramylum consump-

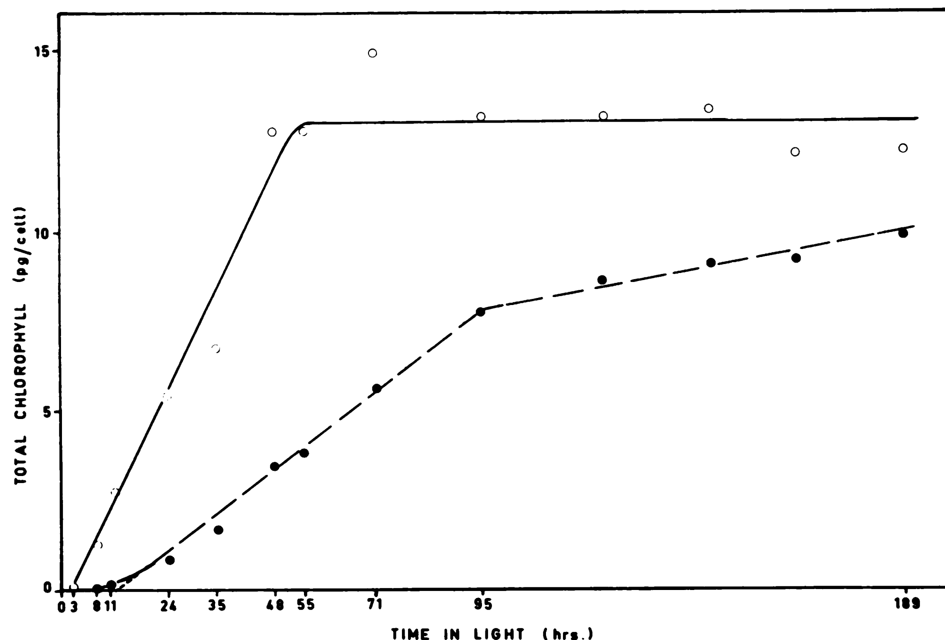

FIG. 3. Influence of the growth medium used in the dark on Chl synthesis during greening of *Euglena* Z cells. Growth in the dark on PC_1 medium (○——○); growth in the dark on CN_1 medium (●— —●). Resting phase in 30 mM KCl. Dashed line represents the linear regression calculated with values of Chl/cell during the active period of synthesis of the pigment. Its interception with the abscissa gives an estimate of the length of the lag phase of Chl synthesis.

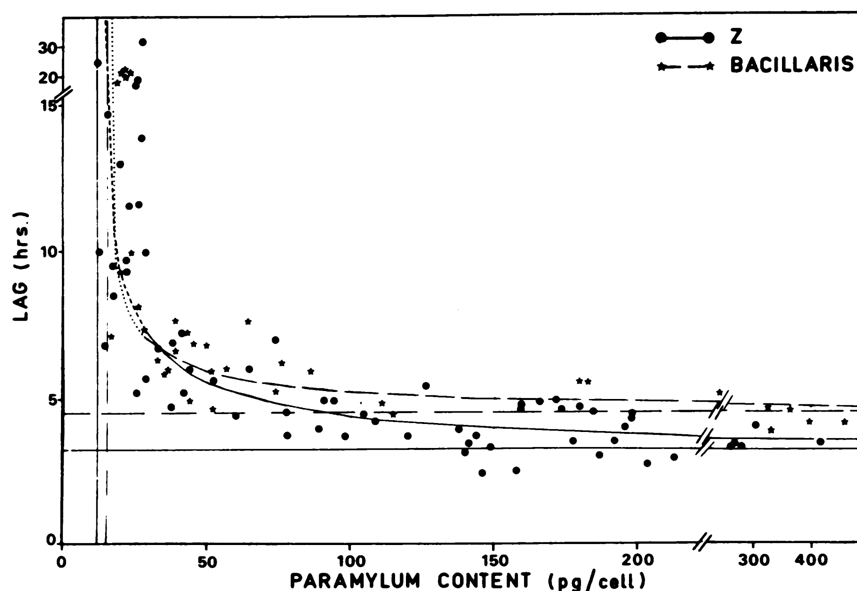

FIG. 4. Relation between paramylum content at zero time of greening and length of the lag phase of Chl synthesis. Horizontal asymptotes correspond to the minimum length of the lag phase of Chl synthesis calculated from Fig. 5 (——, Z; – –, *bacillaris*). Vertical asymptotes correspond to the estimate of the amount of paramylum/cell consumed during the lag phase of Chl synthesis, see Fig. 6 (——, Z; – –, *bacillaris*). The equations of the curves were calculated from the regression lines in Fig. 5. The equations are: y = 117.94/x + 3.17 for Z and y = 73.09/x + 4.48 for *bacillaris*. Curves for values below 30 pg/cell (· · · · ·); the equations, and hence the solid line curves, were calculated with values higher than 30 pg/cell.

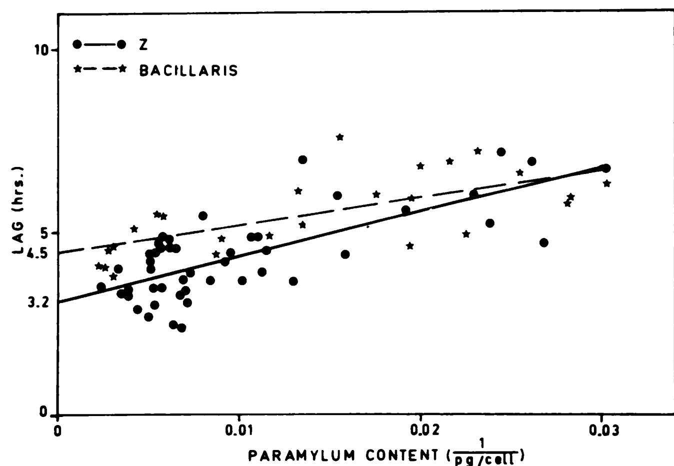

FIG. 5. Relation between the inverse of paramylum content at zero time of greening and length of the lag period of Chl synthesis. Regressions are: y = 3.17 + 117.94 x for Z and y = 4.48 + 73.09 x for *bacillaris*. Interception of these regression lines with the ordinate gives an estimate of the minimum length of the lag phase of Chl synthesis. These values are 3.2 hr for Z and 4.5 hr for *bacillaris*.

tion), we plotted the values calculated as described above *versus* the paramylum content at zero time (Fig. 6). The following points are worth noting.

1. For *bacillaris*, the curve obtained (Fig. 6) can be divided into two parts. The first part drawn with values of cellular paramylum content below 70 pg/cell shows a rapid increase in the paramylum consumption during lag phase. This increment becomes slower in the second part of the curve when the cellular paramylum content is higher than 100 pg/cell. Although we do not have values corresponding to small amounts of paramylum consumption, it seems that the curve intercepts the origin.

2. For Z cells, paramylum consumption during the lag phase of Chl synthesis is almost the same whatever the cellular paramylum content. However, by analogy with *bacillaris* we can suppose that the curve bends toward the origin for values below 30 pg/cell.

3. An average estimate is calculated with values of paramylum consumption measured with cells which contain less than 50 pg/cell. The two values obtained, one for Z and one for *bacillaris,* correspond to the vertical asymptotes drawn in Figure 4.

4. Differences noticed here are probably due to a strain difference.

It seems, from these results, that cells require the consumption of only a small amount of paramylum (5–10 pg/cell) to escape from the lag phase. This value represents a slight excess when compared with the endogenous metabolism which has been evaluated as 7 to 30 $pg \cdot cell^{-1} \cdot day^{-1}$ depending on culture conditions (10).

EFFECTS OF ADDITION OF ORGANIC CARBON TO *EUGLENA* CULTURES AT BEGINNING OF GREENING

Sodium butyrate, sodium acetate, or a mixture of sodium L-glutamate and sodium DL-malate were added to cultures when they were transferred to the light. Two cases have to be considered.

1. The lag is not modified when the organic carbon is added to cells nondeficient in paramylum: PC_1 or NC_1 cultures, for instance. Sometimes under these conditions, cells synthesize larger amounts of Chl during greening.

2. The lag is reduced when such additions are done with paramylum deficient cells, CN_1 or CN_2 cultures. Similar results are obtained with the three carbon sources (Fig. 7; Table II). This addition does not necessarily modify the total amount of Chl formed during greening. For an unknown reason, this effect is important when sodium butyrate is added to cells grown in the dark on glutamate-malate medium (CN_2). In all cases, cellular division occurs after the 1st day of greening, such division is not seen in control cultures which do not receive exogenous carbon.

These observations are consistent with those of Sisler and Klein (25). They found a decrease in the lag from 6 to 2 hr when glucose and light are given to 12-day-old bean plants. This lag is abolished even when plants received in addition δ-aminolevulinic acid. This has not been done with *Euglena* since under our conditions δ-aminolevulinic acid does not penetrate the cells.

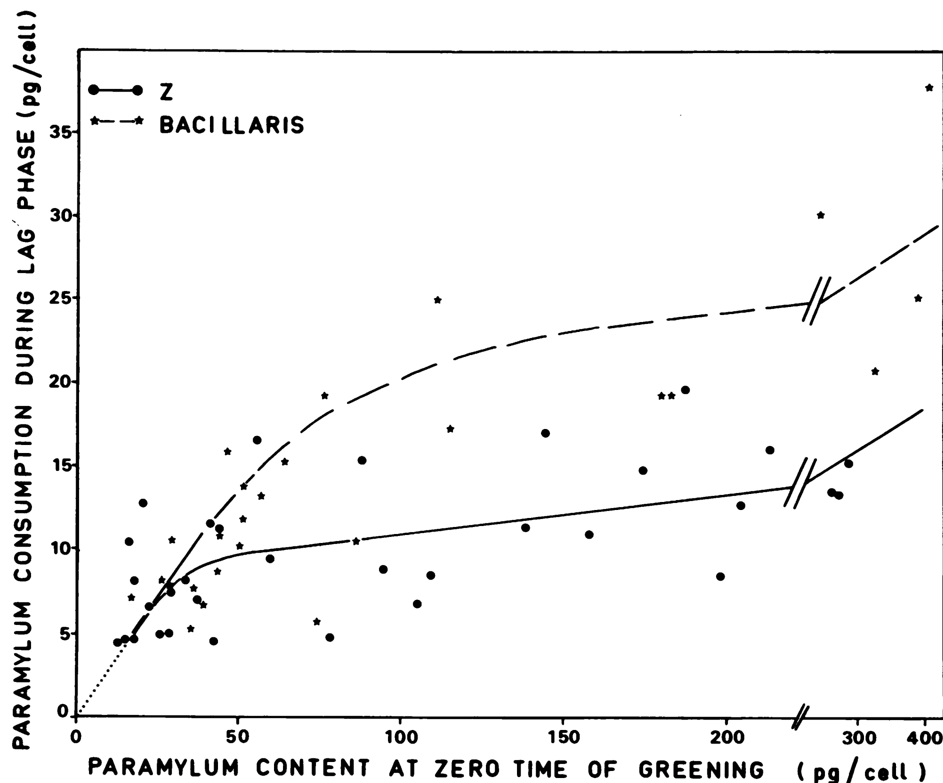

FIG. 6. Relation between paramylum content at zero time of greening and the amount of paramylum consumed during the lag phase of Chl synthesis. The amount of paramylum consumed during lag phase was estimated by the product: rate of paramylum consumption × length of lag phase.

Table II. *Changes in Length of Lag Phase of Chl Synthesis in Presence of Added Carbon Source*

The carbon source is added when cells were transferred to the light.

Strain	Growth medium[1]	Resting medium[2]	Compound added	Length of lag phase (hr)
Z	CN_1	KCl	none	6.0
			Na butyrate 27 mM	4.5
			Na acetate 12 mM	4.3
	CN_2	KCl	none	10.3
			Na butyrate 27 mM	5.6
			Na acetate 12 mM	3.1
Bac	CN_2	Cohen	none	8.5
			Na L-glutamate 30 mM Na DL-malate 13 mM	7.1

[1]Composition of growth media is given in Table I.

[2]Composition of resting media is given in Material and Methods.

CONCLUSIONS

The results reported here, probably valid only for cells subjected to a resting phase (24), furnish data concerning the influence of paramylum content on Chl synthesis during light-induced chloroplast development in *Euglena*. They lead us to make three types of remarks.

First, different parameters, for instance cellular division, increment in RNA content, or length of the lag phase of Chl synthesis, are strongly modified by the level of paramylum content (10). We observe that the critical level of paramylum/cell is different depending on the parameter studied. Actually, we can distinguish two discontinuities. The first one is situated at about 150 to 200 pg of paramylum/cell. Cellular division and the increment in RNA content observed during the greening of cultures, which contain more than 150 to 200 pg of paramylum/cell, are not measurable when the cellular content is below this threshold (10). The second one is located at about 30 to 50 pg of paramylum/cell. The most important modifications described here appear when cultures are below this level (see Figs. 2, 4, and 6). The meaning of these thresholds is at present unknown. One possibility, however, is that when illumination starts, cells give priority to plastid development. When the cells have excess of carbohydrate reserves, they might put in place new mechanisms which would allow either to accelerate plastid development or even to start cell division.

Second, the two strains studied respond in a similar manner to experimental changes. Only differences in the amount of paramylum consumed during the lag phase (Fig. 6) and, perhaps, in the minimum lag phase necessary (Fig. 4) have been detected. We must conclude that these parameters might be under genetic control. The limiting mechanisms which take place when paramylum is in excess (more than 50 pg/cell) might be, for the two strains, either of different kinds or with different functional rates.

Third, Ophir *et al.* (17) recently found that "old" cells have a lag phase of Chl synthesis longer than that of "young" cells. This lengthening of the lag of Chl synthesis is accompanied by a similar lengthening of the lag of O_2 evolution. An explanation of their results can be furnished by the present paper. The difference observed by the authors might be due to difference in the amount of paramylum available at the beginning of illumination, *i.e.* that young cells contain a larger amount of paramylum than old cells. We already know that the cellular paramylum content decreases at the end of the exponential phase of growth and during the stationary phase of growth (10).

One can ask if the variations of the length of the lag phase of chlorophyll synthesis similar to those of O_2 evolution (17) can be extended to other processes also occurring during chloroplast development. Although a systematic study has not been carried

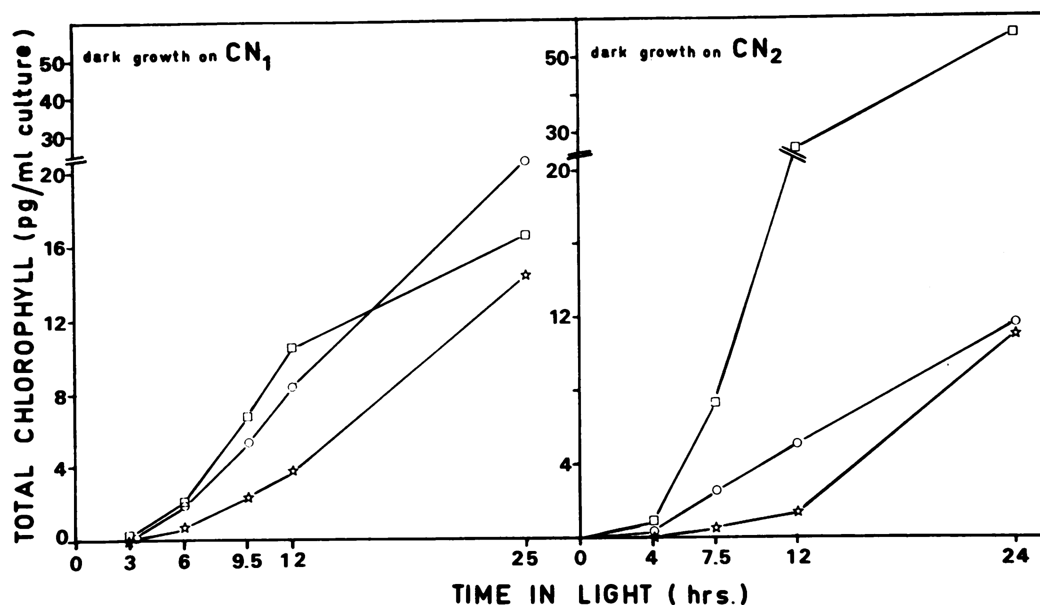

FIG. 7. Effect of added carbon source of Chl synthesis. Z cells were grown in the dark on CN medium and rested for 3 days in a 30 mM KCl solution. Carbon source is added when cells are transferred to the light. Control, no addition (☆——☆); Na butyrate, 27 mM (□——□); Na acetate, 12 mM (○——○).

Table III. *Classification of Few Mechanisms Which Occur during Chloroplast Development in Terms of Absence or Presence of Lag Period*

A. MECHANISMS DISPLAYING A LAG PERIOD

Mechanisms	lag period[1] Hr.	Ref.
Chlorophyll synthesis	3	7
	3	10
	4 or 12	17
	12	26
Oxygen evolution	3	6
	5 or 12	17
	12	26
Carotenoid synthesis	12	26
Plastid membrane formation	12	16
	3	21
Formation of cytochrome C_{552}	12	2
	3	24
Synthesis or activation of enzymes, for instance :		
δ-aminolevulinic acid dehydratase	3	19
ribulose-1,5-diphosphate carboxylase	12	2

B. MECHANISMS WITHOUT A LAG PERIOD

Mechanisms	Ref.
Polysome formation	14
Paramylum consumption	7,10,23
Synthesis or activation of enzymes, for instance :	
photoreactivating enzyme	5
glucose-6-phosphate dehydrogenase	7,24
NADP-linked triose phosphate dehydrogenase	2,24
ribonuclease	24
ribulose-5-phosphate kinase	20
plastid-associated deoxyribonuclease	9
several species of aminoacyl-tRNA synthetases	12,18

[1]Lag period represents the time between the onset of illumination and the phase of active synthesis of the mechanism studied. The length of lag period indicated for one mechanism is close to the one measured for chlorophyll synthesis under the same experimental conditions.

out, an indication of the answer is obtained when we compare results already published (Table III). We then notice that for one process which displays a lag (Table III, part A), this lag is close to the one measured for Chl under the same conditions. This might mean either that the process studied is under similar regulation or that it is dependent upon the presence and/or the activity of Chl. From the published results, it appears that these processes would be controlled directly or indirectly by the activity of the 68S chloroplast ribosomes. Only the synthesis of chloroplast and cytoplasmic ribosomal RNA might obey a different rule (3, 4, 13). Processes which do not have a lag (Table III, part B) do not seem to be influenced by changing culture conditions. In opposition to the other processes, these events would be controlled directly or indirectly by the activity of the 89S cytoplasmic ribosomes. No information is available concerning the control of polysome formation.

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LITERATURE CITED

1. BARRAS, D. R. AND B. A. STONE. 1968. Carbohydrate composition and metabolism in *Euglena*. *In:* D. E. Buetow, ed., The Biology of *Euglena*, Vol. II. Academic Press, New York. pp. 149-191.
2. BOVARNICK, J. G., J. A. SCHIFF, Z. FREEDMAN, AND J. M. EGAN. 1974. Events surrounding the early development of *Euglena* chloroplasts: cellular origins of chloroplast enzymes in *Euglena*. J. Gen. Microbiol. 83: 63-71.
3. COHEN, D. 1973. Photoregulation of formation and turnover of chloroplast-rRNA and cytoplasmic rRNA during chloroplast development in *Euglena gracilis* Klebs var. *bacillaris*

Pringsheim. Ph.D. thesis. Brandeis University, Waltham, Mass.
4. Cohen, D. and J. A. Schiff. 1973. Photoregulation of formation and turnover of chloroplast-rRNA (Chl rRNA) and cytoplasmic rRNA (Cyt rRNA) during chloroplast development in *Euglena gracilis* Klebs var. *bacillaris* Pringsheim. Biophys. Soc. Abst. 13: 111a.
5. Diamond, J., J. A. Schiff, and A. Kelner. 1975. Photoreactivating enzyme from *Euglena* and the control of its intracellular level. Arch. Biochem. Biophys. 167: 603-614.
6. Dupuy, J. 1969. Contribution à l'étude de la différenciation d'*Euglena gracilis* étiolées sous l'action de la lumière. Thèse de spécialité, No. 407, Faculté des Sciences de Lyon, France.
7. Dwyer, M. R. and R. M. Smillie. 1970. A light-induced β-1,3-glucan breakdown associated with the differentiation of chloroplasts in *Euglena gracilis*. Biochim. Biophys. Acta 216: 392-401.
8. Dwyer, M. R. and R. M. Smillie. 1971. β-1,3-Glucan: a source of carbon and energy for chloroplast development in *Euglena gracilis*. Aust. J. Biol. Sci. 24: 15-22.
9. Egan, J. M. and E. F. Carell. 1972. Studies on chloroplast development and replication in *Euglena*. III. A study of the site of synthesis of alkaline deoxyribonuclease induced during chloroplast development in *Euglena gracilis*. Plant Physiol. 50: 391-395.
10. Freyssinet, G., P. Heizmann, G. Verdier, G. Trabuchet, and V. Nigon. 1972. Influence des conditions nutritionnelles sur la réponse à l'éclairement chez les Euglènes étiolées. Physiol. Veg. 10: 421-442.
11. Freyssinet, G. and J. A. Schiff. 1974. The chloroplast and cytoplasmic ribosomes of *Euglena*. II. Characterization of ribosomal proteins. Plant Physiol. 53: 543-554.
12. Hecker, L. I., J. Egan, R. J. Reynolds, C. E. Nix, J. A. Schiff, and W. E. Barnett. 1974. Sites of transcription and translation for *Euglena* chloroplastic aminoacyl-tRNA synthetases. Proc. Nat. Acad. Sci. U.S.A. 71: 1910-1914.
13. Heizmann, P. 1974. La synthèse des RNA ribosomiques au cours de l'éclairement d'Euglènes étiolées. Biochim. Biophys. Acta 353: 301-312.
14. Heizmann, P., G. Trabuchet, G. Verdier, G. Freyssinet, and V. Nigon. 1972. Influence de l'éclairement sur l'évolution des polysomes dans des cultures d'*Euglena gracilis* étiolées. Biochim. Biophys. Acta 277: 149-160.
15. Holowinsky, A. W. and J. A. Schiff. 1970. Events surrounding the early development of *Euglena* chloroplasts. I. Induction by preillumination. Plant Physiol. 45: 339-347.
16. Klein, S., J. A. Schiff, and A. W. Holowinsky. 1972. Events surrounding the early development of *Euglena* chloroplasts. II. Normal development of fine structure and the consequences of preillumination. Dev. Biol. 28: 253-273.
17. Ophir, I., A. Talmon, S. Polak-Charcon, and Y. Ben-Shaul. 1975. Aspects of structure and photosynthetic competence of *Euglena* plastids under conditions of greening and degreening. Protoplasma 84: 283-295.
18. Parthier, B. and R. Krauspe. 1974. Chloroplast and cytoplasmic transfer RNA of *Euglena gracilis*. Transfer RNA^{Leu} of blue-green algae as a substitute for chloroplast $tRNA^{Leu}$. Biochem. Physiol. Pflanzen. 165: 1-17.
19. Richard, F. and V. Nigon. 1973. La synthèse de l'acide δ-amino-lévulinique et de la chlorophylle lors de l'éclairement d'*Euglena gracilis*. Biochim. Biophys. Acta 313: 130-149.
20. Salvador, G. 1971. Etudes de certaines modifications biochimiques et morphologiques provoqées par l'éclairement d'*Euglena gracilis* étiolée et définitivement blanchie. Thèse d'Ingénieur, No. 7, Université de Lyon I, France.
21. Salvador, G. F. 1973. Evolution du système plastidien d'*Euglena gracilis* étiolées à l'obscurité après stimulation lumineuse. Exp. Cell Res. 79: 479-484.
22. Schiff, J. A., M. H. Zeldin, and J. Rubman. 1967. Chlorophyll formation and photosynthetic competence in *Euglena* during light-induced chloroplast development in the presence of 3,(3,4-dichlorophenyl) 1,1-dimethyl urea (DCMU). Plant Physiol. 42: 1716-1725.
23. Schwartzbach, S. D., J. A. Schiff, and N. H. Goldstein. 1975. Events surrounding the early development of *Euglena* chloroplasts. V. Control of paramylum degradation. Plant Physiol. 56: 313-317.
24. Smillie, R. M., W. R. Evans, and H. Lyman. 1963. Metabolic events during the formation of a photosynthetic from a nonphotosynthetic cell. Brookhaven Symp. Biol. 16: 89-108.
25. Sisler, E. C. and W. H. Klein. 1963. The effect of age and various chemicals on the lag phase of chlorophyll synthesis in dark-grown bean seedlings. Physiol. Plant. 16: 315-322.
26. Stern, A. I., J. A. Schiff, and H. T. Epstein. 1964. Studies of chloroplast development in *Euglena*. V. Pigment biosynthesis, photosynthetic O_2 evolution and CO_2 fixation during chloroplast development. Plant Physiol. 39: 220-226.