EVIDENCE FOR CONTINUOUS POTENTIAL FOR GENE TRANSCRIPTION DURING THE CELL CYCLE OF A EUKARYOTE

by

Frank Samuel Baechtel

Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Biochemistry and Nutrition

APPROVED:

Dr. R. R. Schmidt, Chairman

Dr. C. J. Ackerman

Dr. N. R. Krieg

Dr. E. R. Stout

Dr. J. L. Hess

February, 1970

Blacksburg, Virginia

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Regulated growth and division are the fundamental processes which characterize the normal living cell. For an organism to adapt adequately to its environment, to maintain uniform growth, and to produce viable offspring, it must coordinate and control its innumerable metabolic functions. The information required for the synthesis of the proteins vital to the regulation of cellular processes resides in the organism's DNA. Although prior research has shown that regulation of cellular events is achieved through controls on the transcription of the information contained in the DNA, it has been only recently that the mechanisms underlying the timing of these controls have begun to be explained.

Efforts to reveal the controls exerted upon the timing of cellular events through a cell cycle, have focused upon the use of synchronous and synchronized cultures of microorganisms. Synchronous cultures allow the investigation of the metabolism of a single cell throughout its cell cycle while providing sufficient material to meet current analytical levels of measurement. The most widely utilized microorganisms for synchronous culture studies are bacteria, yeasts, and algae. It is from these classes of organisms that much of our current knowledge of the timing of cellular control mechanisms has been derived. An excellent review of the patterns of enzyme synthesis in both bacteria and yeasts has recently been published

by Mitchison (1).

Although most enzymes in prokaryotes are synthesized in a stepwise fashion (2-5), and a few are synthesized linearly (2,4), the potential for gene expression appears to be continuous through the cell cycles of these organisms (2-3,6). Evidence that gene expression can be continuous stems from observations that specific enzymes can be induced or derepressed at all times in their cell cycles (2-5,7-8).

Under conditions of full repression, the rate of enzyme synthesis is continuous and linear (2,7). In addition, the doubling in rate of linear enzyme synthesis appears to coincide with the time of structural gene replication and the time of doubling in potential for gene expression. Thus, linear enzyme synthesis lends indirect support to the proposal that the prokaryotic genome is continuously available for transcription.

Periodic enzyme synthesis apparently is not a result of full repression, but rather an effect of an oscillating endproduct repression. The rate of enzyme synthesis varies inversely with the level of endproduct, and the periodic bursts of enzyme synthesis need not occur during periods of gene replication. Thus, control of enzyme synthesis by oscillatory repression obscures the timing of changes in potential for gene expression. However, specific induction and derepression studies have shown that the potential for enzyme synthesis exists at all times in the cell cycle for periodically synthesized enzymes (7).

Differences are found among the eukaryotes in the manner in which gene transcription is controlled. The budding yeast appears to have restricted expression (9-11) for gene transcription during the cell cycle. The only time in the cell cycle when inducers or repressors affect the rate of enzyme synthesis is during the periods when an enzyme is normally synthesized.

In contrast, the fission yeast, Schizosaccharomyces pombe, has unrestricted potential (12) for the synthesis of certain derepressible enzymes (e.g., sucrase, alkaline phosphatase) throughout the cell cycle as in prokaryotes; however this organism differs from prokaryotes in that there is a lag between chemical replication of genes and their "functional" replication (i.e., potential to be transcribed).

For Chlorella, a highly compartmentalized eukaryote, the potential for transcription of a given gene (i.e., nitrite reductase) has been interpreted as being restricted to a certain period in the cell cycle (13). However, the induction data from Chlorella may have led to an incorrect conclusion concerning the potential for gene transcription in this organism.

A consideration of the enzymes previously selected for studies (13-14) of gene expression in Chlorella (i.e., acid and alkaline phosphatases, nitrite reductase) reveals that the choices may have been poor.

It has been reported (15) that multiple forms exist for

both acid and alkaline phosphatases in other organisms. Thus, the potentials observed for synthesis of these enzymes at any time in the cell cycle may well be a composite expression of the structural genes for two or more enzymes under control of the same corepressor. The principal objection to the study of acid and alkaline phosphatases, however, is that the manner of their derepression (i.e., by removal of P_i from the culture medium) may lead to a concommitant derepression of polyphosphatases (16) which yield P_i from endogenous phosphates; thus leading to repression of the synthesis of these enzymes. Studies on the derepression of acid and alkaline phosphatases during synchronized growth of C. pyrenoidosa, strain 211-8b, have shown that the intracellular orthophosphate level increases through the cell cycle when the cells are cultured on phosphate-free medium (14).

Another enzyme studied in Chlorella has been nitrite reductase. Knutsen (13) assumed that the rates of induction of nitrite reductase could be quantitatively measured by measuring the disappearance of nitrite from the medium. Because a constant number of cells was induced (in continuous light) at each interval in the cell cycle, the increase in cell volume during synchronous growth might have reduced the effective light intensity per cell to a level no longer sufficient to support maximal induction rates. This criticism is supported by the observation in our laboratory (17) that the rate of induction

of nitrate reductase changes with light intensity. Furthermore, the expression of Knutsen's (13) induction data on a per cell basis, rather than on a per ml basis as by other workers (8), also obscures the actual change in potential during the latter period of the cell cycle.

Since the question of timing of expression of gene potential in Chlorella has not been answered unequivocally, the present research was undertaken. Its objective has been to utilize Chlorella to measure the changes in potential for gene expression of an inducible enzyme which could be assayed directly and for which no multiple forms had been reported.

The enzyme selected for this study is the anaplerotic enzyme, isocitrate lyase (ICLase). This enzyme is found in many microorganisms (18), and catalyzes the cleavage of isocitrate to succinate and glyoxylate. Isocitrate lyase is the key enzyme of the glyoxylate cycle and the operation of this cycle depends greatly upon the activity of ICLase.

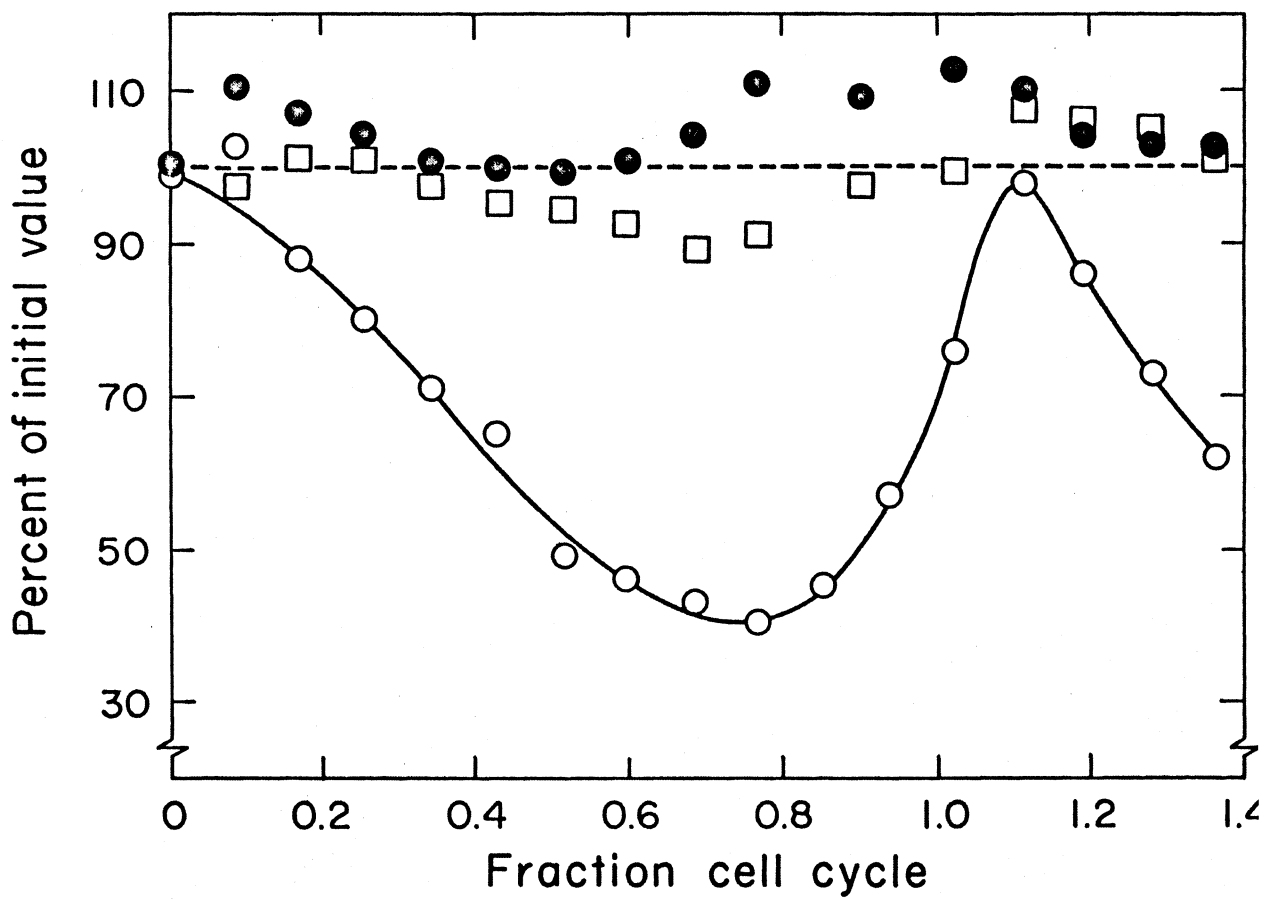
Studies using strain 211-8p of Chlorella pyrenoidosa have indicated that the synthesis of isocitrate lyase is induced by acetate and repressed by both glucose and light (19). Acrylamide gel electrophoresis has shown that only one form of ICLase exists in this organism.

METHODS AND MATERIALS

Organism and culture conditions. The high temperature strain 7-11-05 (20) of the unicellular green alga, Chlorella pyrenoidosa, was cultured under conditions which result in a division number of four. Synchronized cells were obtained by subjecting the cells to at least four alternating cycles of 10 h light and 8 h dark. The resultant synchronized cells were then cultured approximately 22 h in continuous light (at which time the cultures were composed of $\frac{1}{2}$ daughter cells), to attempt to abolish any metabolic disturbances that might be due to the alternate light-dark regime. From these cells, an extremely uniform population of daughter cells was selected by isopycnic centrifugation of the culture on Ficoll density gradients (21). The resultant synchronous cells were resuspended in pre-equilibrated medium and cultured during the cell cycle studies according to the mass culture technique of Hare and Schmidt (22) with the following modification: since changes in light intensity influence metabolic patterns (23), the culture was diluted with fresh, pre-equilibrated medium in a manner such that the turbidity and accumulation of protein of the growing culture remained within $\pm$ 11 percent of its initial value for the entire cycle (Fig. 1) thus insuring nearly constant light conditions during the cell cycle.

Induction of isocitrate lyase. Induction of isocitrate lyase (threo D_s-Isocitrate glyoxylate-lyase, EC 4.1.3.1) was

Fig. 1. Actual (uncorrected)patterns of dry weight and turbidity during synchronous growth of C. pyrenoidosa (strain 7-11-05). ● , dry weight per ml; □ , turbidity per ml; ○ , cell number per ml. The continuous dilution procedure of Hare and Schmidt (22) was used during the synchronous growth studies. The dilution rates were: 0-1 h, no dilution; 1-7 h, 13 percent dilution; 8 h, 10 percent dilution; 9 h, 5 percent dilution; 10 h, 10 percent dilution; 11-12 h, 13 percent dilution; 14-15 h, 15 percent dilution.


Percent of initial value
110
90
70
50
30
0
0.2
0.4
0.6
0.8
1.0
1.2
Fraction cell cycle

accomplished by placing actively growing cultures in the dark and adding the inducer, sodium acetate. For preliminary studies on the induction process, cells from continuously-lighted cultures were harvested and diluted with fresh medium to achieve a final cell concentration of 38×10^6 cells per ml. During the synchronous growth studies, cells were removed from the central culture, centrifuged 2 min at room temperature and resuspended in fresh medium to a concentration of 38×10^6 cells per ml. Immediately after dilution or resuspension, the cells were placed in the dark at 38.5° and 4% CO_2 in air bubbled through the culture. Zero time in the induction was considered to be when sodium acetate was added to the cultures to achieve a final concentration of 0.2% (w/v).

Preparation of cells for analysis. Aliquots of cells for enzymic assay were harvested at appropriate times and frozen at -20° with no further treatment until assayed for ICLase. Cells which had been frozen and thawed showed no diminution in ICLase activity when compared with the activity of fresh cells which had been sonicated in a Raytheon 10kc sonic oscillator for 12 min at maximum amperage.

During the synchronous growth studies, cells were harvested by centrifugation for DNA and protein analysis, washed 1x with deionized water, recentrifuged, and the pellets frozen at -20° for later analysis.

Assay of isocitrate lyase. Assay of ICLase was carried out with cells which had been frozen a minimum of 24 h. The

frozen cells were thawed rapidly, resuspended with a Dounce homogenizer and kept on ice until assayed. Enzymic activity was determined by measuring the amount of glyoxylate formed during a 20 min incubation at 38.5°. Contents of the reaction mixture are given in Table 1. The reaction was begun by addition of enzyme and terminated by adding 0.5 ml of a solution composed of 2 parts 20% TCA and 3 parts 0.1% 2,4-dinitrophenylhydrazine (made up in 2N HCL). The killed reactions were allowed to stand 30 min with occasional mixing. After 30 min, 2.5 ml of 4% (w/v) NaOH were added, the mixtures centrifuged and exactly 5 min after addition of base, the absorbance at 445nm due to formation of glyoxylate-dinitrophenylhydrazone (19), was determined in a Beckman DB spectrophotometer.

Quantitation of total cellular DNA and protein. The frozen whole cell preparations referred to above were used for analysis of DNA and protein.

DNA was extracted by the method of Schmidt and Thannhauser (24) as modified by Flora (25). After extraction, DNA was quantified colorimetrically by the Burton diphenylamine method (26) as modified by Flora (25). Total protein was quantified by the Lowry procedure (27). Bovine serum albumin was used as the standard protein.

Studies with cycloheximide. In studies using cycloheximide (Actidione), the compound was added in concentrated form to achieve a final concentration of 3 μg per ml of culture (28).

Sources of chemicals. Threo D_s (+) isocitric acid (lot

TABLE I

STANDARD REACTION MIXTURE FOR ASSAY OF ISOCITRATE LYASE ACTIVITY

Component	ml	μmoles
Glutathione (reduced)	0.05	10
Threo D_s (+) isocitrate	0.05	10
Tris-Cl buffer, pH 7.6	0.70	100
Thawed cell suspension	0.20	---
Total	1.00	

97B-7374) and reduced glutathione were obtained from Sigma Chemical Company, St. Louis, Mo. Grade A tris (hydroxymethyl) aminomethane was a product of Calbiochem, Los Angeles, Calif. Cycloheximide was kindly provided by Dr. G. S. Fonken of the Upjohn Pharmaceutical Company, Kalamazoo, Mich.

RESULTS AND DISCUSSION

Kinetics of protein and DNA accumulation during the cell cycle. The accumulation of total cellular protein and dry weight (Fig. 2) was essentially exponential during the cell cycle. A deviation from log-linearity occurred in the protein pattern at about one cell cycle. This deviation was observed in a number of cell cycle studies in this laboratory and could be an effect of a decrease in the accumulation of some major structural protein fraction. That the deviation from log-linearity is not the result of a decreased ability to synthesize all proteins during the mitotic period is supported by the observation that many enzymes (29-31) in Chlorella are synthesized continuously through this period of cellular development.

In contrast to the exponential accumulation of protein, DNA increased four-fold in a stepwise fashion (Fig. 3) during the cell cycle. The difference in patterns of DNA and protein accumulation precludes a tight coupling between gene replication and transcription and most proteins.

Assay of isocitrate lyase. Comparison of the absorption spectrum of the phenylhydrazone of the product formed during the induction period, to the absorption spectra (Fig. 4) of phenylhydrazones of standard glyoxylate, pyruvate and alpha-ketoglutarate indicated the major reaction product was glyoxylate.

Fig. 2. Accumulation of total cellular dry weight and protein during synchronous growth of C. pyrenoidosa (strain 7-11-05). ○ , protein per ml; ● , dry weight per ml; □ , cell number per ml. Data converted from that in Fig. 1 according to the procedure of Hare and Schmidt (22).

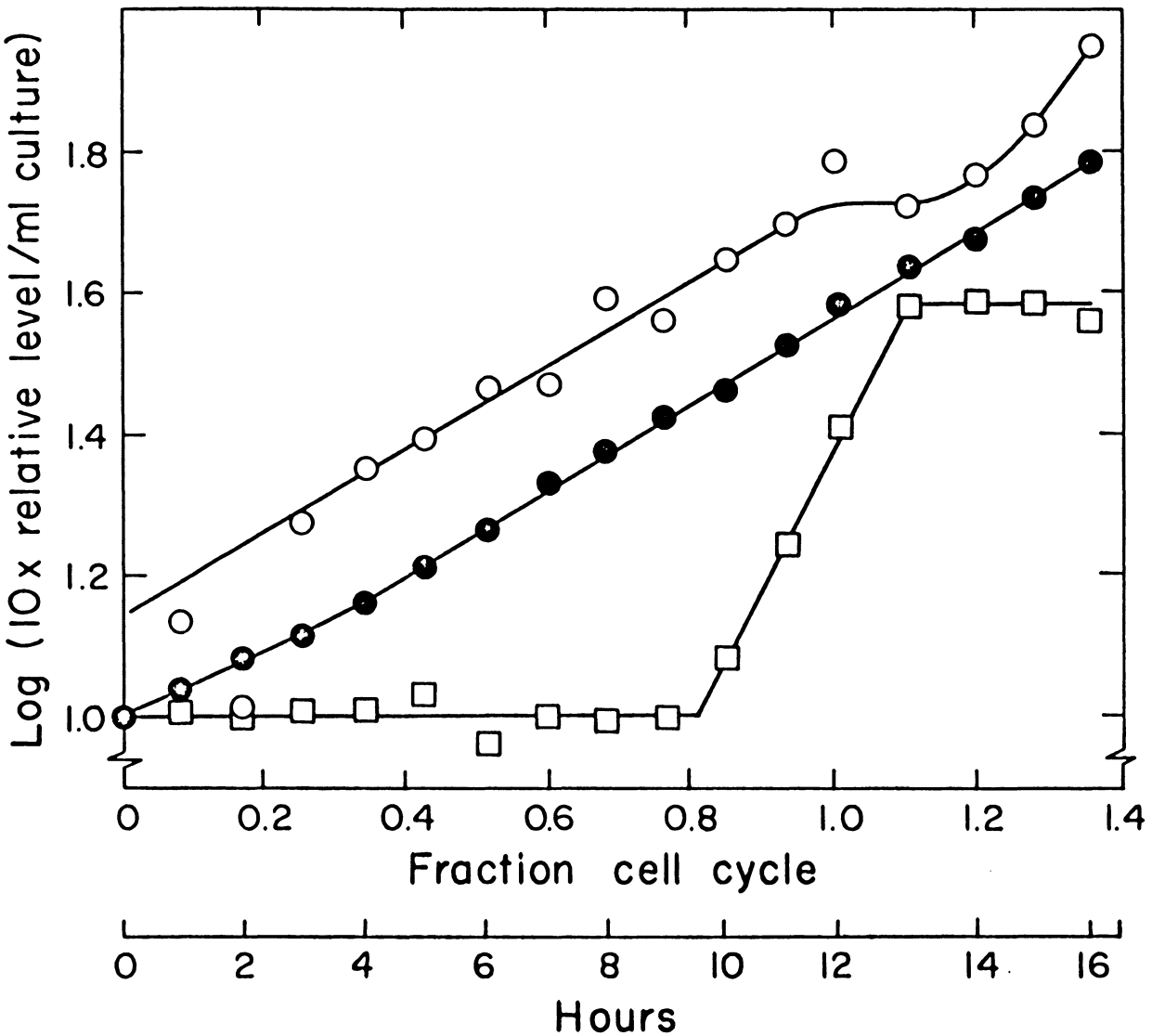

Log (10x relative level/ml culture)
1.0
1.2
1.4
1.6
1.8
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Fraction cell cycle
0
2
4
6
8
10
12
14
16
Hours

Fig. 3. Patterns of total cellular DNA and isocitrate lyase in continuous light during synchronous growth of C. pyrenoidosa (strain 7-11-05). O , DNA per ml; ● , isocitrate lyase activity per ml.

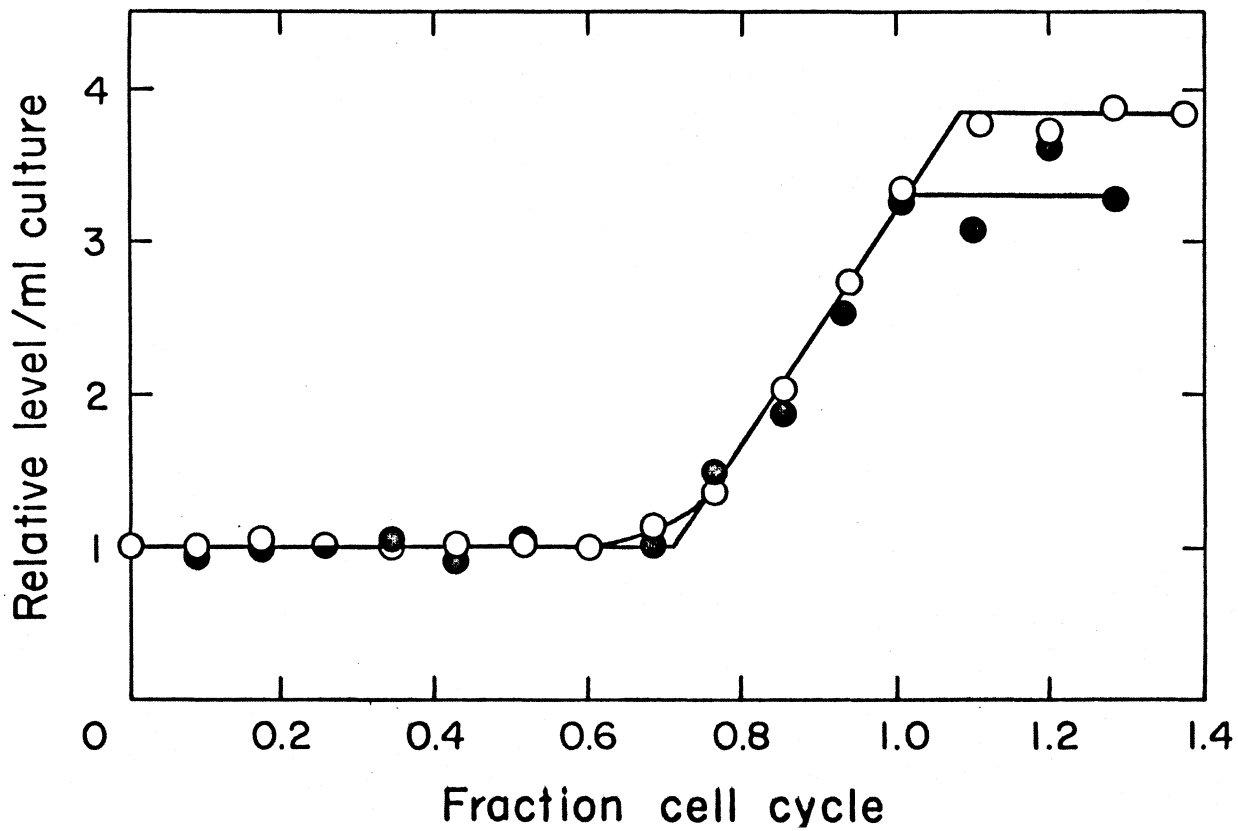
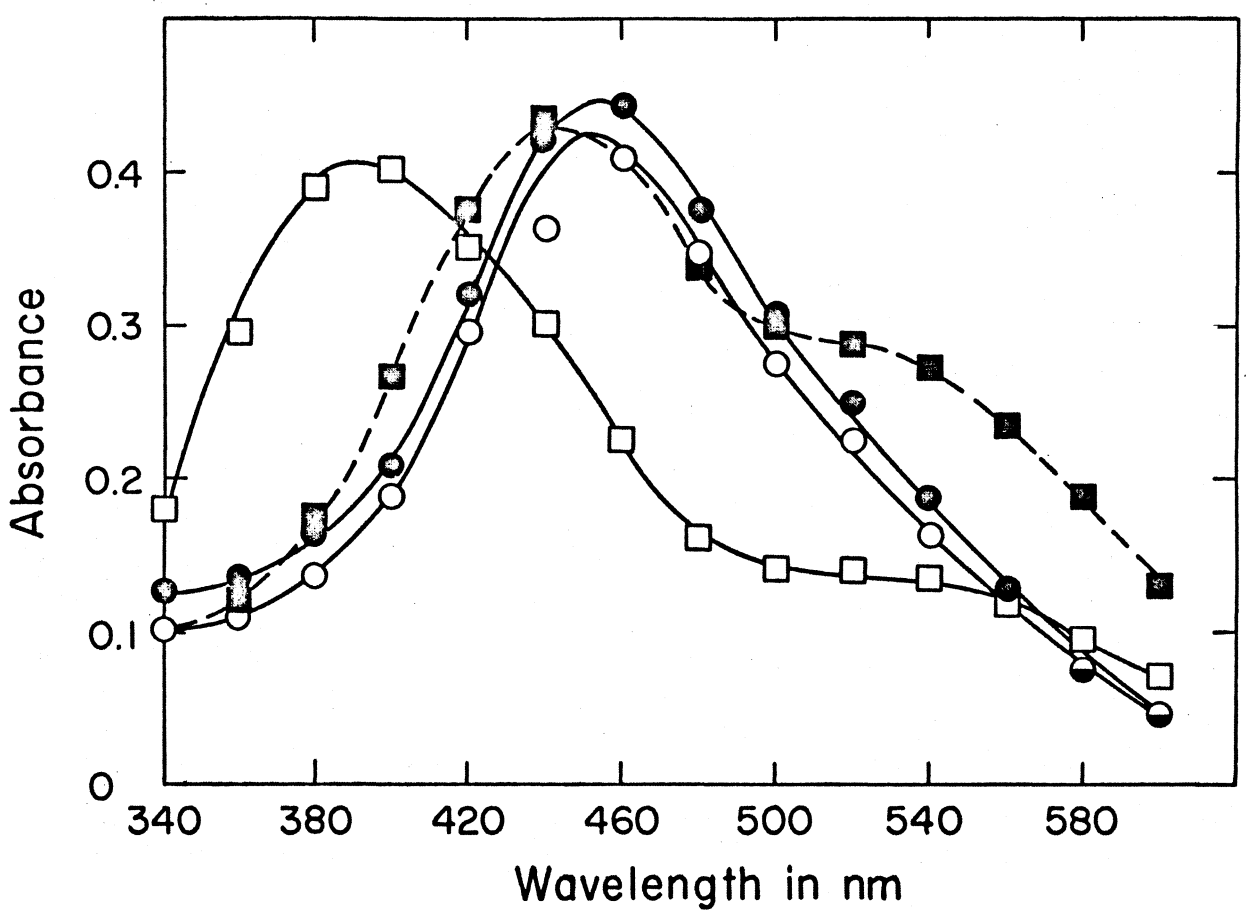

Relative level/ml culture
4
3
2
1
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Fraction cell cycle

Fig. 4. Absorption spectra of phenylhydrazones of standard alpha-ketoglutarate (□), glyoxylate (●), pyruvate (■), and product of enzymic reaction (○).


Absorbance
0
0.1
0.2
0.3
0.4
340
380
420
460
500
540
580
Wavelength in nm

The saturation of isocitrate lyase in 0.2 ml of cell sonicate (corresponding to 24×10^6 cells) was reached at approximately 10mM isocitrate (Fig. 5). Although 0.2 ml of sonicate, or equivalent frozen whole cells, was subsequently used with 10mM isocitrate in routine assays, sufficient substrate was present to support a linear production of glyoxylate with twice as much sonicate (Fig. 6).

Characteristics of isocitrate lyase induction. A quadratic relationship has been reported (32) to exist between the concentration of inducer and the rate of beta-galactosidase synthesis. The quadratic nature of the induction process also can be seen in Fig. 7 for isocitrate lyase. The quadratic induction curves at different concentrations of acetate are linear when expressed in their square root form. Identical maximal induction rates were observed over a wide concentration range (0.05-0.2%) of acetate. However concentrations over two percent completely inhibited induction. Although only darkness was required to initiate induction of the enzyme, acetate was required to sustain a maximal induction rate.

This strain of C. pyrenoidosa differs from strain 211-8b (19) in that induction in the latter organism would not occur in the absence of acetate. Another major difference is that glucose completely represses induction in the latter but not the former organism. Apparently the requirement for acetate is not rigid because in other species of algae the enzyme can be induced by compounds other than acetate (18, 33).

Fig. 5. Glyoxylate accumulation vs. isocitrate concentration for 0.2 ml of cell sonicate (corresponding to 24×10^6 cells) of C. pyrenoidosa (strain 7-11-05) during 20 min of incubation at 38.5°.

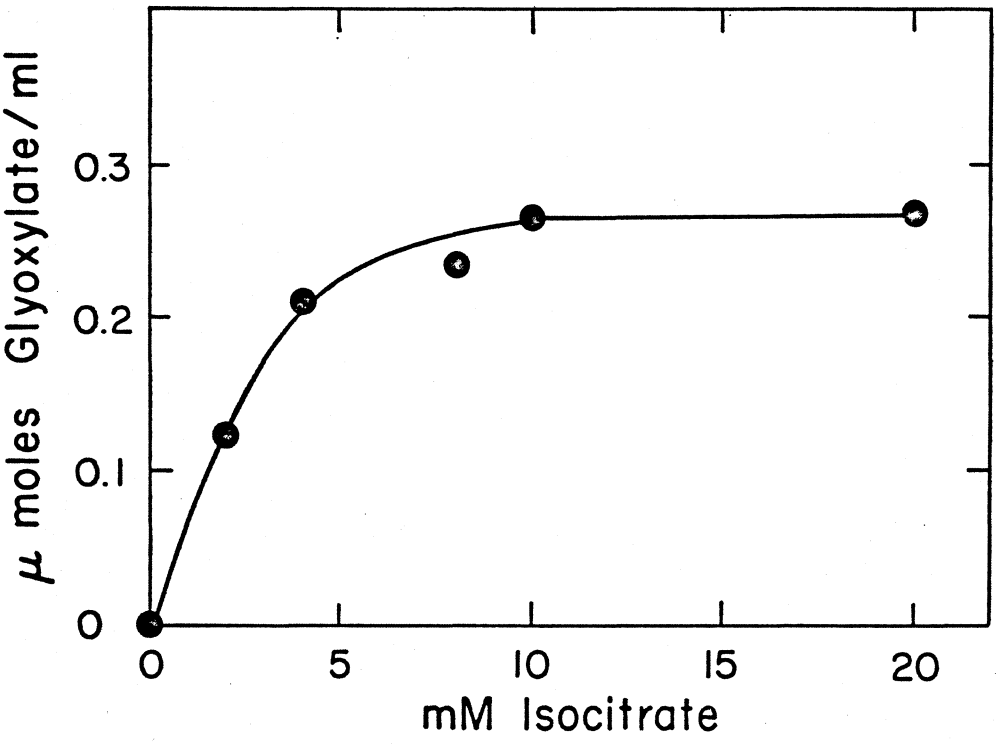

μ moles Glyoxylate/ml
0.3
0.2
0.1
0
0
5
10
15
20
mM Isocitrate

Fig 6. Glyoxylate accumulation vs. ml sonicate during a 20 min incubation at 38.5^{o}. $12x10^{6}$ cells equivalent to 0.1 ml sonicate. Assays carried out with 10mM isocitrate present.

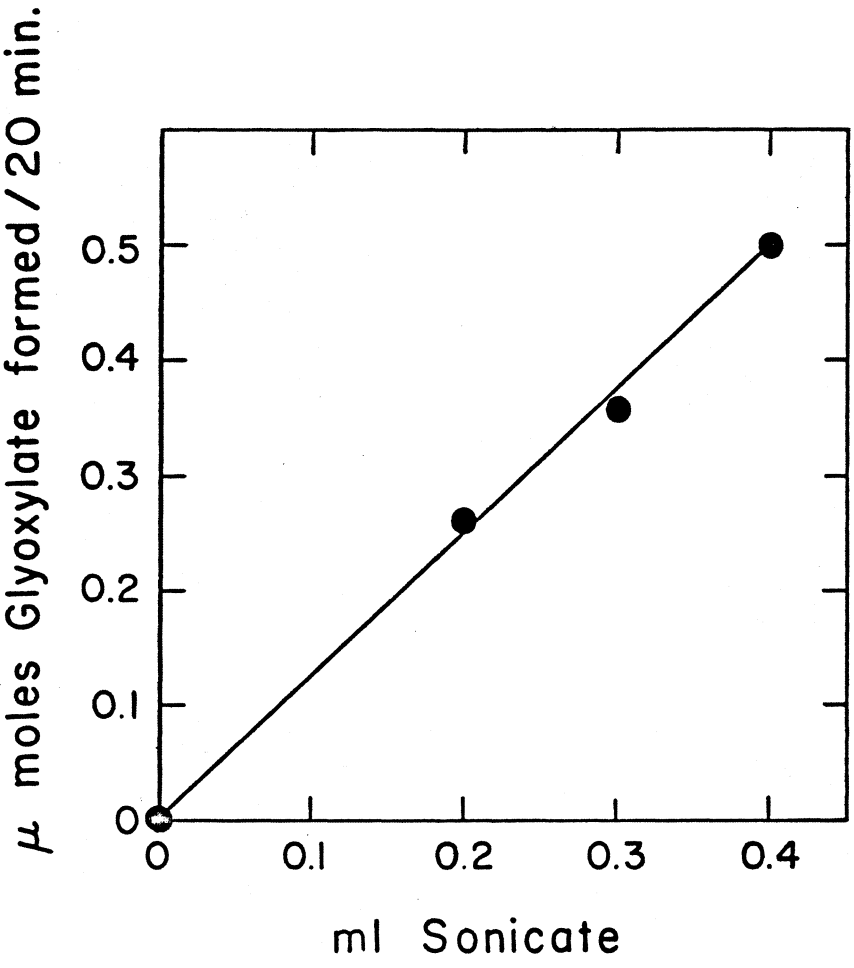
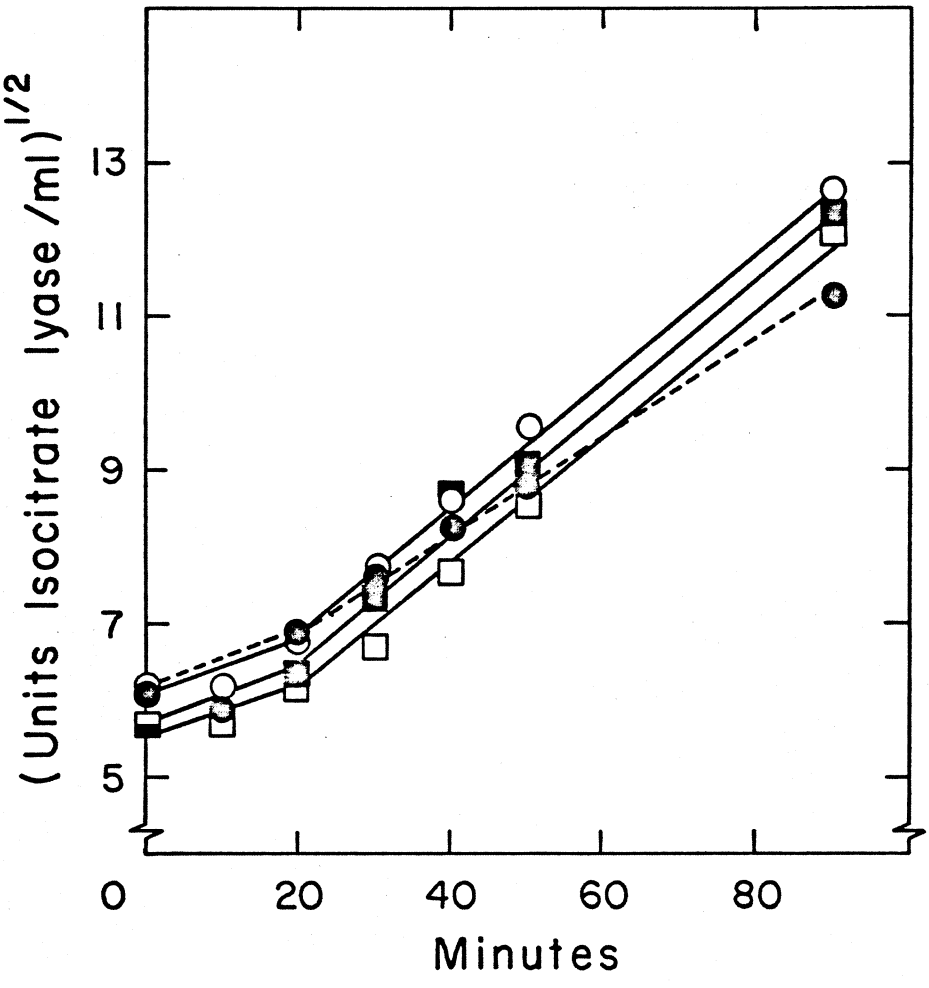

μ moles Glyoxylate formed / 20 min.
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
ml Sonicate

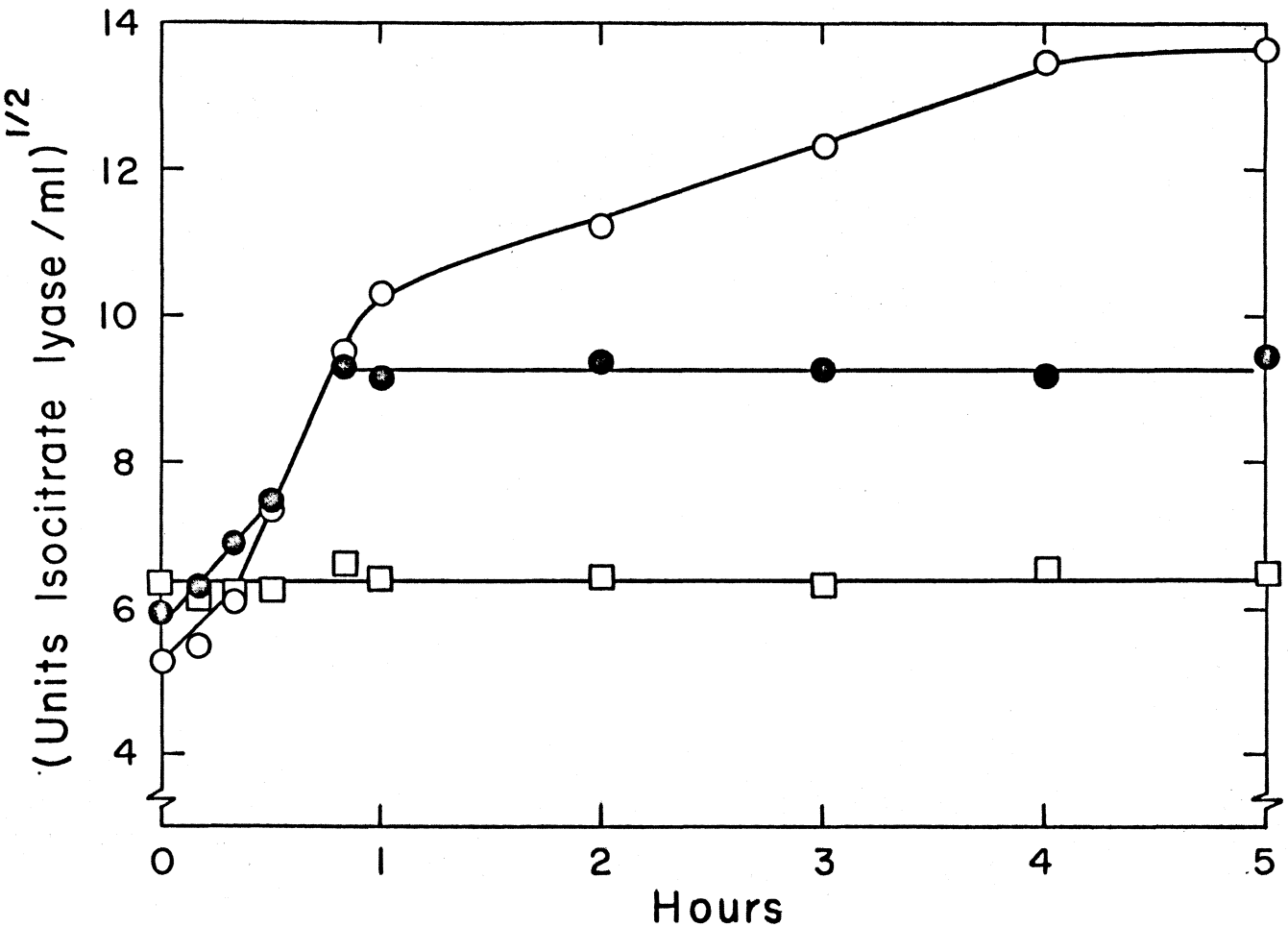
Fig. 7. Influence of different concentrations of acetate on the rate of induction of isocitrate lyase of C. pyrenoidosa (strain 7-11-05). ● , no acetate; ○ , 0.05 percent acetate; ■ , 0.1 percent acetate; □ , 0.2 percent acetate. All acetate percentages are final concentrations in the cultures. One unit of isocitrate lyase activity catalyzes the production of one μmole of glyoxylate during 20 min of incubation at 38.5°.


(Units Isocitrate lyase /ml)$^{1/2}$
13
11
9
7
5
0
20
40
60
80
Minutes

That the increase of isocitrate lyase activity actually represents de novo synthesis and not merely activation of pre-existing enzyme was shown by the sensitivity of the apparent induction to cycloheximide, a protein synthesis blocking agent. Synthesis of the enzyme could be blocked by addition of the inhibitor (Fig. 8) at the beginning or during the middle of the induction period. The enzyme also appears to be stable for at least 5 h after its synthesis is blocked.

Activity of isocitrate lyase during the cell cycle. The pattern of ICLase synthesis during the cell cycle (in continuous light) paralleled the stepwise increase of total cellular DNA, except the enzyme and DNA increased 3.3-and 4-fold, respectively (Fig. 3). Since the enzyme appears to be stable during the absence of its synthesis (Fig. 8), its constant activity during the first 0.8 of the cell cycle indicates detectable synthesis does not occur during this period of cellular development. The subsequent finding (see next section) that the synthesis of ICLase can be induced in the dark during this period, suggests that the structural gene for this enzyme is fully repressed and that its corepressor is likely produced through light-dependent reactions. The burst of synthesis of ICLase (in the absence of inducer) concurrent with that of DNA cannot be explained solely as a gene dosage effect under full repression. That is to say, if the activity of the original structural gene dosage is so highly quenched (repressed) so as to prevent any detectable

Fig. 8. Effect of cycloheximide on the induction of isocitrate lyase of C. pyrenoidosa (strain 7-11-05). ○ , acetate (0.2% w/v) added at zero time; □ , acetate (0.2% w/v) and 3μg/ml cycloheximide added at zero time; ● , acetate (0.2% w/v) added at zero time and 3 μg/ml cycloheximide added at 50 min.


14
12
10
8
6
4
(Units Isocitrate lyase/ml)^1/2
0
1
2
3
4
5
Hours

synthesis of ICLase to occur, then a four-fold increase in this dosage under full repression also would not result in measurable enzyme synthesis. Thus, the inference can be made that synthesis of ICLase must reflect a significant change in its endogenous corepressor or repressor level relative to its structual gene dosage. The reduction in photosynthetic rate (34) or the increased endogenous respiration rate (35) during this period of cellular development could be influencing corepressor levels. Alternatively, the rate of synthesis of aporepressor might be reduced during the period of gene replication.

Potential for isocitrate lyase gene expression during the cell cycle. ICLase synthesis could be induced throughout the cell cycle, indicating that the structural gene for this enzyme is continuously available for transcription in this eukaryote. The time courses of induction, in their square root forms, are shown in Fig. 9 for three different hours in the cell cycle. Each time course is an example of one of the three characteristic increases in rate of ICLase synthesis during different periods of the cell cycle. Furthermore, Table II lists for each hour in the cell cycle: (a) the slope of the line obtained when the induction data are plotted in their square root forms, (b) the acceleration in the rate of ICLase synthesis, and (c) the relative increases of the acceleration in rate of ICLase synthesis during the cell cycle.

The slope of the line on the square root plot of an induction time ccurse is related to the acceleration in rate of

Fig. 9. Induced rates of isocitrate lyase synthesis vs. time at different hours in the cell cycle of C. pyrenoidosa (strain 7-11-05). ○ , 5th h; ● , 11th h; □ , 12th h.

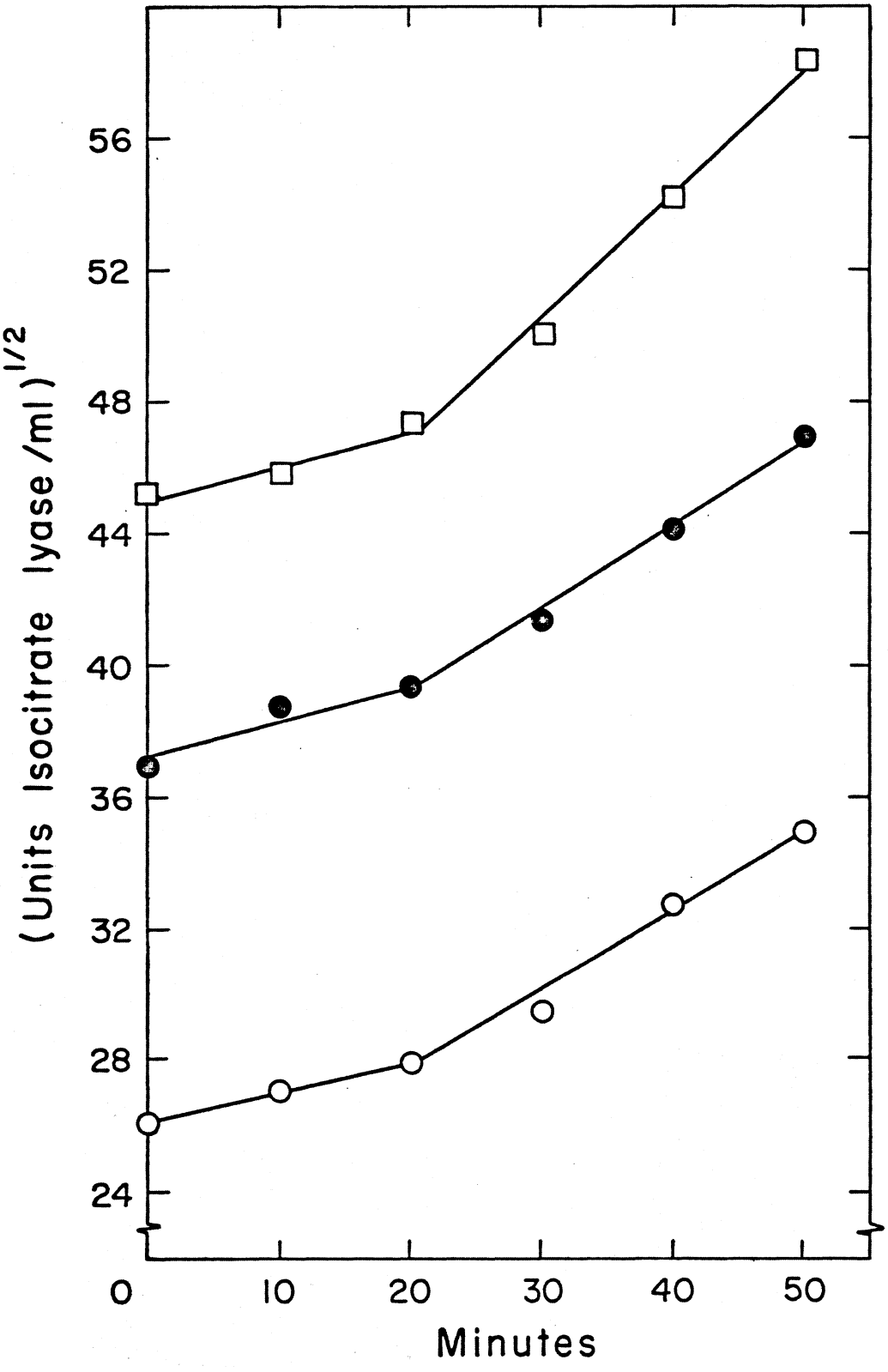

(Units Isocitrate lyase /ml)$^{1/2}$
56
52
48
44
40
36
32
28
24
0
10
20
30
40
50
Minutes

Table II

INDUCED RATES OF ISOCITRATE LYASE DURING THE CELL CYCLE OF *C. pyrenoidosa* (strain 7-11-05)

HOUR	FRACTION CELL CYCLE	SLOPE[a]	ACCELERATION[b]	RELATIVE INCREASE IN ACCELERATION
0	0.0000	2.08	8.66	0.914
1	0.085	2.18	9.50	1.000
2	0.170	2.02	8.16	0.860
3	0.255	2.26	10.22	1.080
4	0.341	2.22	9.84	1.040
5	0.426	2.28	10.38	1.100
6	0.511	----	----	-----
7	0.597	2.24	10.04	1.060
8	0.682	2.20	9.68	1.020
9	0.766	2.20	9.68	1.020
10	0.850	2.08	8.66	0.914
11	0.932	2.80	15.68	1.650
12	1.020	4.30	37.00	3.900
13	1.110	4.30	37.00	3.900
14	1.190	4.56	41.60	4.390
15	1.280	4.20	35.20	3.710

[a]/ 10 x slope of the line obtained when the induction data is plotted on a square root basis and expressed as (units isocitrate lyase induced per ml)$^{\frac{1}{2}}$ per min.

[b]/ Acceleration in the rate of isocitrate lyase induction; acceleration equals 2 x slope^2.

enzyme synthesis (i.e., 2 x slope^2 equals the acceleration) in the induced culture. Since the acceleration in rate of ICLase synthesis remained constant for most of the cell cycle, and dramatically increased four-fold during the time of DNA replication (Figs. 10,11), it appears that this parameter is a measure of the potential for gene expression throughout the cell cycle.

A considerable lag exists between the time when DNA replication begins and the first detectable rise in potential for ICLase gene expression (Fig. 10). Since the DNA has increased approximately 2.25-fold by the time of the initial rise in potential, and considering that Chlorella has been reported (36) to undergo a nuclear division after each round of replication, transcription of newly replicated genes appears to be delayed until the nuclear division after their replication. Mitchison (1) has reported even a longer time lag to exist between the replication of genes and their availability for transcription during the cell cycle of S. pombe. Although the lag is apparent at the beginning of the increase in ICLase potential, the variation among the experimental points (accelerations in rate of ICLase synthesis) at the end of the cell cycle prohibits an unequivocal determination of the time when the potential for gene expression reaches a four-fold increase.

Control of gene expression in eukaryotes. The recent work by Mitchison (12) has shown that S. pombe, a fission yeast,

Fig. 10. Patterns of total cellular DNA and acceleration in rate of induction of isocitrate lyase by acetate in the dark during synchronous growth of C. pyrenoidosa (strain 7-11-05). ● , DNA per ml; O , acceleration in rate of isocitrate lyase induction.

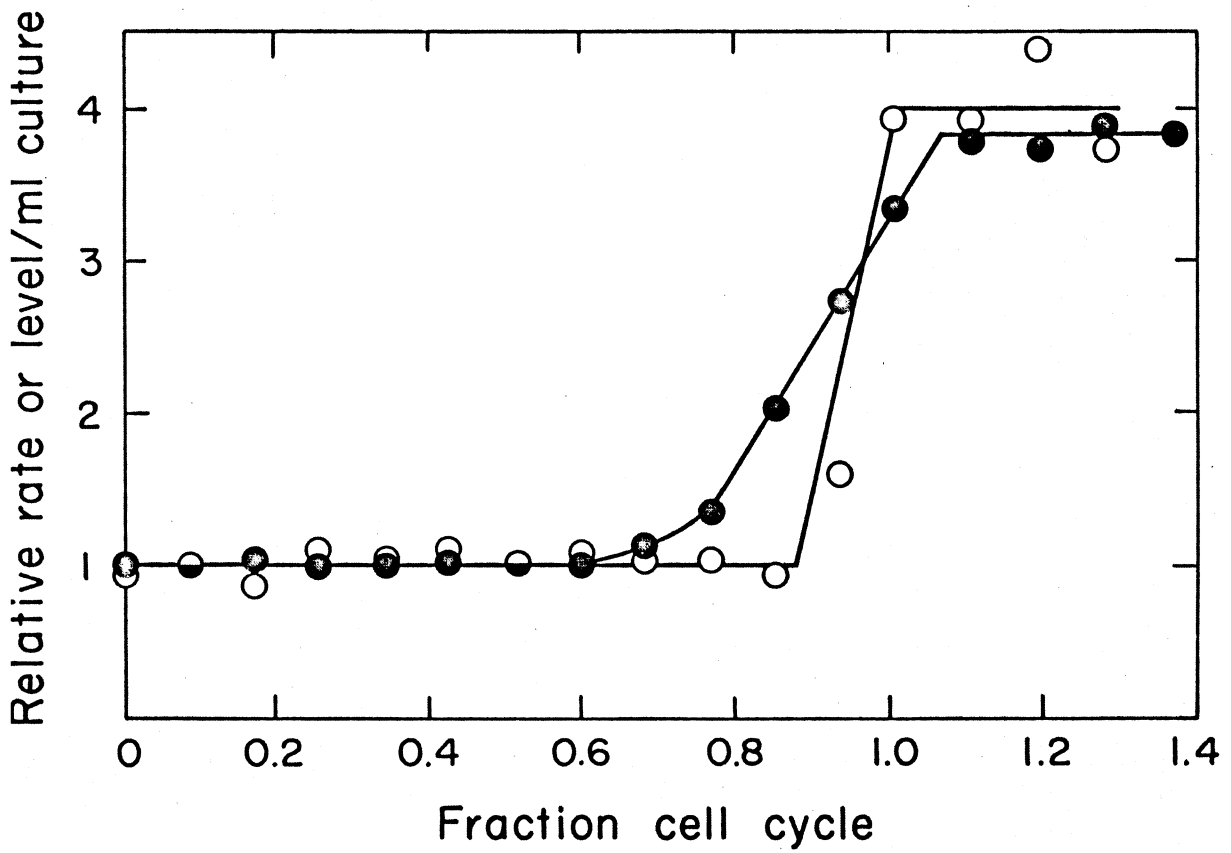
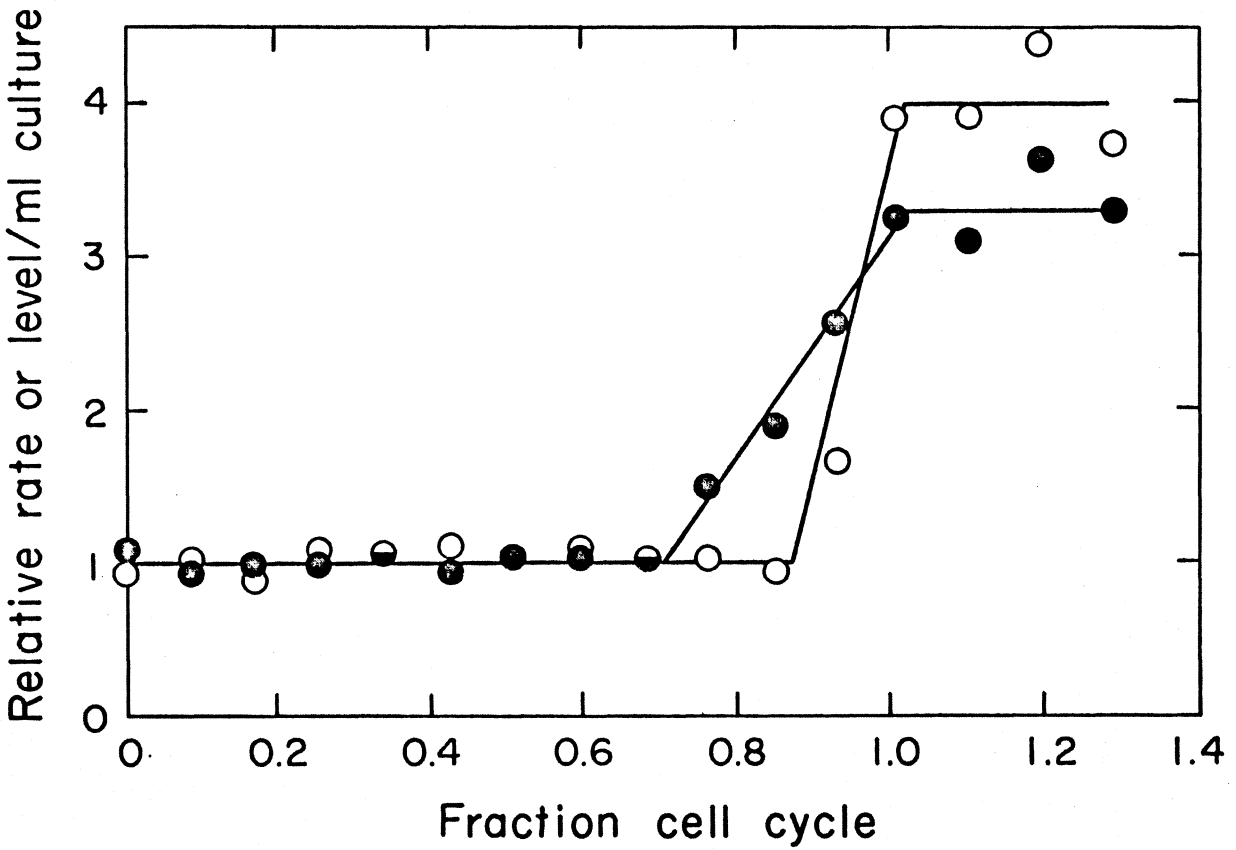

Relative rate or level/ml culture
4
3
2
1
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Fraction cell cycle

Fig. 11. Isocitrate lyase synthesis in continuous light and acceleration in rate of induction of isocitrate lyase by acetate in the dark during synchronous growth of C. pyrenoidosa (strain 7-11-05). ● , isocitrate lyase activity per ml; O , acceleration in rate of isocitrate lyase induction.


Relative rate or level/ml culture
0
1
2
3
4
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Fraction cell cycle

can regulate gene expression in a manner similar to that of prokaryotes. However, this organism differs from prokaryotes in that a delay exists between the time of replication and expression of structural genes. The present study has revealed that Chlorella is like S. pombe in the regulation of gene expression and timing during the cell cycle. The mechanism responsible for the delay in expression of newly replicated genes would appear to be a fruitful area for further study in eukaryotes.

SUMMARY

1. Synchronous cultures of *Chlorella pyrenoidosa* have been utilized to measure the potential for expression of the structural gene for isocitrate lyase during the cell cycle.
2. Synthesis of the enzyme could be induced by placing cultures in the dark on acetate, with the induction process occuring in a quadratic fashion. By addition of cycloheximide, the synthesis of ICLase was shown to be the result of *de novo* protein synthesis.
3. The pattern of uninduced ICLase synthesis in continuous light paralleled the stepwise increase in the total cellular DNA.
4. ICLase could be induced at all times in the cell cycle, indicating that the potential for gene expression is continuous in this organism.
5. A time lag was observed between the beginning of DNA replication and the initial rise in potential for ICLase gene expression.

ACKNOWLEDGEMENTS

The author is extremely indebted to his advisor, Dr. R. R. Schmidt, for the capable guidance and personal understanding extended him during these years of graduate education.

It has been the author's good fortune to be associated with the talented members of Dr. Schmidt's research group. Without their unselfish aid this research would have been impossible.

To the Department of Biochemistry and to the taxpayers of the United States, goes the author's gratitude for the financial support he received while at this institution.

To his wife, the author expresses his most sincere appreciation for her love, tolerance and understanding.

BIBLIOGRAPHY

1. J. M. Mitchison, Science, 165 (1969) 657.

2. P. L. Kuempel, M. Masters and A. B. Pardee, Biochem. Biophys. Res. Commun., 18 (1965) 858.

3. M. Masters and A. B. Pardee, Proc. Nat. Acad. Sci. U. S., 54 (1965) 64.

4. W. D. Donachie, Nature, 205 (1965) 1084.

5. M. Masters, P. L. Kuempel and A. B. Pardee, Biochem. Biophys. Res. Commun., 15 (1964) 38.

6. W. D. Donachie and M. Masters, Genet. Res., 8 (1966) 119.

7. M. Masters and W. D. Donachie, Nature, 209 (1966) 476.

8. W. D. Donachie and M. Masters, in G. M. Padilla, G. L. Whitson and I. L. Cameron, "The Cell Cycle: Gene-Enzyme Interactions", Academic Press, New York, 1969, p 37.

9. J. Gorman, P. Tauro, M. LaBerge and H. O. Halvorson, Biochem. Biophys. Res. Commun., 15 (1964) 43.

10. P. Tauro and H. O. Halvorson, J. Bacteriol., 92 (1966) 652.

11. H. O. Halvorson, R. M. Bock, P. Tauro, R. Epstein and M. LaBerge, in I. L. Cameron and G. M. Padilla, "Cell Synchrony-Studies in Biosynthetic Regulation", Academic Press, New York, 1966, p 102.

12. J. Mitchison and J. Creanor, J. Cell Sci., 5 (1969) 373.

13. G. Knutsen, Biochim. Biophys. Acta,103 (1965) 495.

14. G. Knutsen, Biochim. Biophys. Acta, 161 (1968) 205.

15. P. Tauro and H. O. Halvorson, J. Bacteriol., 92 (1966) 652.

16. A. L. Baker and R. R. Schmidt, Biochim. Biophys. Acta, 93 (1964) 180.

17. J. G. Weeks and J. B. Flora, unpublished data from this laboratory (1969).

18. W. G. Haigh and H. Beevers, Arch. Biochem. Biophys., 107 (1964) 147.

19. P. J. Syrett, J. Exptl. Bot., 17 (1966) 641.

20. C. Sorokin and J. Myers, Science, 117 (1953) 330.

21. T. O. Sitz, submitted to Science (1970).

22. T. A. Hare and R. R. Schmidt, Appl. Microbiol., 16 (1968) 496.

23. G. R. Molloy, unpublished data from this laboratory (1969).

24. G. Schmidt and S. J. Thannhauser, J. Biol. Chem., 161 (1945) 83.

25. J. B. Flora, Ph.D. thesis, Virginia Polytechnic Institute, Blacksburg, Virginia (1969).

26. K. Burton, Biochem. J., 62 (1956) 315.

27. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193 (1951) 265.

28. E. C. Herrmann, Ph.D. thesis, Virginia Polytechnic Institute, Blacksburg, Virginia (1969).

29. R. A. Johnson and R. R. Schmidt, Biochim. Biophys. Acta, 129 (1966) 140.

30. S. R. C. Shen and R. R. Schmidt, Arch. Biochem. Biophys., 115 (1966) 13.

31. F. E. Cole and R. R. Schmidt, Biochim. Biophys. Acta, 90 (1964) 616.

32. W. Gilbert and B. Muller-Hill, Proc. Nat. Acad. Sci. U. S., 56 (1966) 1891.

33. J. F. Hogg and H. L. Kornberg, Biochem. J., 86 (1963) 462.

34. C. Sorokin, Physiol. Plantarum, 10 (1957) 659.

35. S. G. Curnutt and R. R. Schmidt, Exptl. Cell Res., 36 (1964) 102.

36. F. Wanka and P. F. Mulders, Arch. Mikrobiol., 58 (1967) 257.

The vita has been removed
from the scanned document

EVIDENCE FOR CONTINUOUS POTENTIAL FOR GENE TRANSCRIPTION DURING THE CELL CYCLE OF A EUKARYOTE

ABSTRACT

by

Frank Samuel Baechtel

Synchronous cultures of Chlorella pyrenoidosa (strain 7-11-05) have been utilized to measure the potential expression of the structural gene for isocitrate lyase (threo D_s-Isocitrate glyoxylate-lyase, EC 4.1.3.1) during the cell cycle. Synthesis of the enzyme could be induced by placing cultures in the dark on acetate, with the induction process occurring in a quadratic fashion. By addition of cycloheximide during the course of induction, the increase in isocitrate lyase activity was shown to result from de novo protein synthesis. In the absence of protein synthesis the enzyme was stable for at least five hours.

The pattern of uninduced isocitrate lyase synthesis during the cell cycle in continuous light, paralleled the stepwise increase of total cellular DNA. The enzyme appeared to be fully repressed for most of the cell cycle, and was derepressed during the time of DNA replication.

Isocitrate lyase could be induced at all times in the cell cycle, indicating that the potential for gene expression is continuous in this eukaryote. A time lag was observed between the beginning of DNA replication and the initial rise in potential for isocitrate lyase gene expression. The control of gene expression in Chlorella appeared to be similar to that found in a fission yeast.