STUDIES ON THE REGULATION OF RIBULOSE-1,5-DIPHOSPHATE CARBOXYLASE SYNTHESIS DURING THE CELL CYCLE OF THE EUCARYOTE CHLORELLA

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Summary: The patterns of synthesis of ribulose-1,5-diphosphate carboxylase were measured in synchronous and synchronized cultures of Chlorella under conditions in which the cells divided into either 4 or 8 daughter cells. The synthesis of the carboxylase occurred in the absence of DNA replication and could be changed from a periodic to continuous pattern during the cycle by increasing the growth rate and the division number of the cells. The evidence suggests that endproduct repression might play the major role in regulating the timing of synthesis of this enzyme during the cell cycle.

The manner in which procaryotes and eucaryotes regulate enzyme synthesis and gene expression during their cell cycles has been the target of many recent studies (1).

Many structural genes in procaryotes appear to be available for transcription throughout their cell cycles and the periodic patterns observed for enzyme synthesis are proposed to result from oscillatory endproduct repression (2).

Although gene transcription appears to be regulated in a similar manner during the cell cycle of most procaryotes, differences appear to exist among the eucaryotes in the way this process is regulated. In a fission yeast (3) and in Chlorella (4,5) the structural genes of certain derepressible enzymes appear to be available for transcription throughout the cell cycle except that in the former organism the transcription of newly replicated genes is restricted for a short period. Mammalian cells are similar to the fission yeast in that certain genes are available for transcription for most of the cell cycle (6). In contrast, transcription of specific genes in budding yeasts is restricted to discrete periods of the cell cycle (7).

The present study was initiated to gain insight into the mechanism(s) regulating the synthesis of the constitutive enzyme, ribulose-1,5-diphosphate

carboxylase during the cell cycle of the highly compartmentalized eucaryote, Chlorella.

MATERIALS AND METHODS

Organism and cultural conditions. The thermophilic strain 7-11-05 of the unicellular alga (8), Chlorella pyrenoidosa, was cultured in the apparatus described by Hare and Schmidt (9) under a light intensity of either 550 or 1,100 f.c. to produce cells with a division number of 4 or 8, respectively. At 550 and 1,100 f.c., the cells were synchronized by 4 alternating light-dark cycles of 10:8 and 8.5:7 hours, respectively. Synchronous daughter cells were selected from continuously-lighted cultures by a new equilibrium centrifugation procedure which employs Ficoll density gradients (10). The turbidity of growing synchronized and synchronous cultures was held essentially constant by continuous dilution (9).

Preparation of ribulose-1,5-diphosphate. This compound was enzymatically synthesized (11) and the barium salt was converted to its hydrogen form with cation-exchange resin (AG 50W-x12, 200-400 mesh, hydrogen form). The authenticity, purity, and concentration of the enzymatically-synthesized product was determined by procedures used by Paulsen and Lane (12).

Assay of ribulose-1,5-diphosphate carboxylase. Approximately 0.05 ml packed volume of cells was harvested by centrifugation, washed twice with 0.01 M Tris-HCl buffer (pH 8.1), resuspended in 15 ml of 0.1 M Tris-HCl buffer (pH 8.6) containing 0.002 M dithiothreitol, and then sonicated for 12 minutes in a Raytheon 10 kc sonic oscillator.

These sonicates were assayed for the carboxylase by a procedure similar to that of Paulsen and Lane (12). The volume of the standard reaction mixture was 0.04 ml and contained (in μmoles): $NaH^{14}CO_3$, 2.1 (0.3-0.6 μc/μmole); ribulose-1,5-diphosphate, 0.3; $MgCl_2$, 2.7; dithiothreitol, 0.04; Tris-HCl buffer (pH 8.6), 8; Chlorella sonicate, 0.02 ml. Enzyme incubations were performed at 38.5° for 15 minutes and terminated by addition of 0.1 ml of 3.2 N HCl. After centrifugation, 0.1 ml of supernatant was pipetted into a count-

ing vial, dried at 95° for 25 minutes, and its radioactivity determined after addition of 2 ml H_2O and 15 ml of a scintillation counting solution (13).

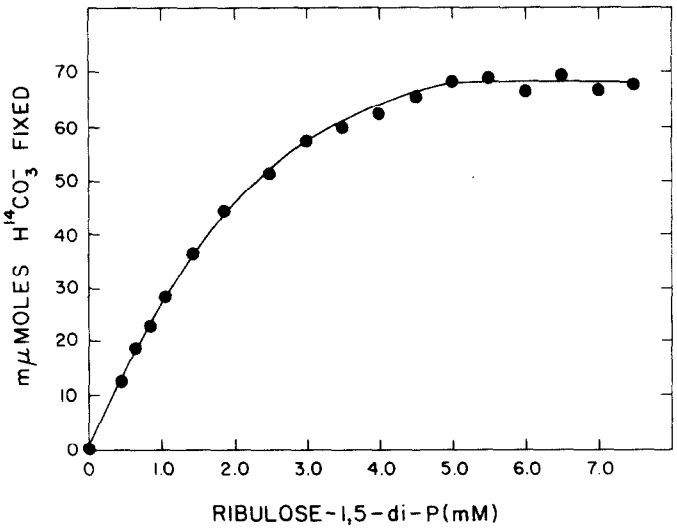

Fig. 1. Activity of ribulose-1,5-diphosphate carboxylase from Chlorella pyrenoidosa (strain 7-11-05) at different ribulose-1,5-diphosphate concentrations. The concentrations of other components same as in standard reaction mixture.

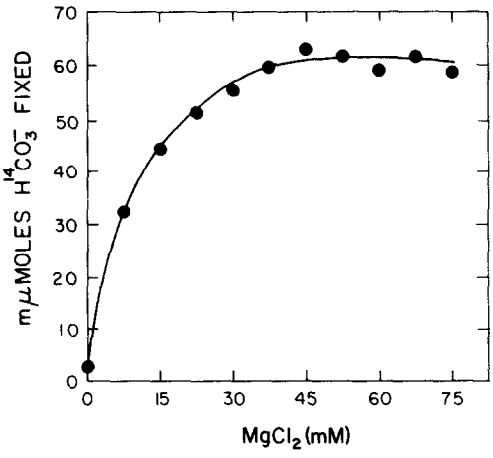

Fig. 2. Activity of ribulose-1,5-diphosphate carboxylase from Chlorella pyrenoidosa (strain 7-11-05) at different $MgCl_2$ concentrations. The concentrations of other components same as in standard reaction mixture.

RESULTS AND DISCUSSION

Substrate and cofactor studies. The enzymatic incorporation of $H^{14}CO_3^-$ into acid-stable molecules had an absolute requirement for ribulose-1,5-diphos-

phate. Concentrations of at least 5 mM ribulose-1,5-diphosphate (Fig. 1) and 45 mM $MgCl_2$ (Fig. 2) were required for maximal carboxylase activity. Kinetic studies showed the standard assay mixture to support linear incorporation of $H^{14}CO_3^-$ by the ribulose-1,5-diphosphate carboxylase for at least 27 minutes for the enzyme levels employed.

Ribulose-1,5-diphosphate carboxylase activity during the cell cycle. If gene transcription is regulated in Chlorella by a temporal mechanism similar to that in budding yeasts (7), the timing of specific enzyme synthesis within the cell cycle should not be altered significantly by changes in growth rate; whereas, it likely would be altered if endproduct repression plays the major role in controlling the timing of gene expression during the cell cycle as in

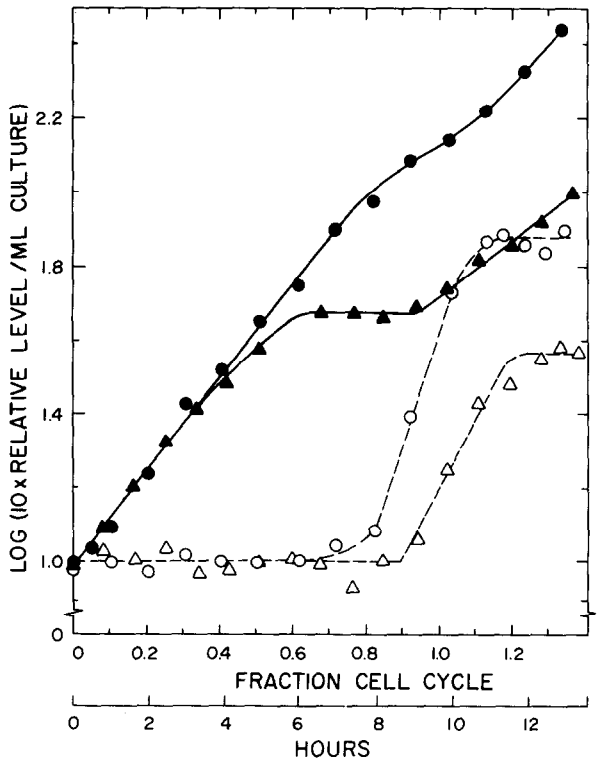

Fig. 3. Patterns of ribulose-1,5-diphosphate carboxylase activity in synchronized cells of Chlorella pyrenoidosa (strain 7-11-05) with a division number of 8 and 4. Carboxylase activity, ● and ▲, and cell number, ○ and △, in cultures with division number of 8 and 4, respectively. Initial cell number per ml averaged 140 x 10^6.

procaryotes (2). Since an increase in light intensity from 550 to 1,100 f.c. changed ribulose-1,5-diphosphate carboxylase accumulation from a periodic to continuous pattern (Fig. 3), when the growth rate (Fig. 4) and division number of synchronized cells approximately doubled, the timing of synthesis of this enzyme does not appear to be regulated as in the budding yeasts.

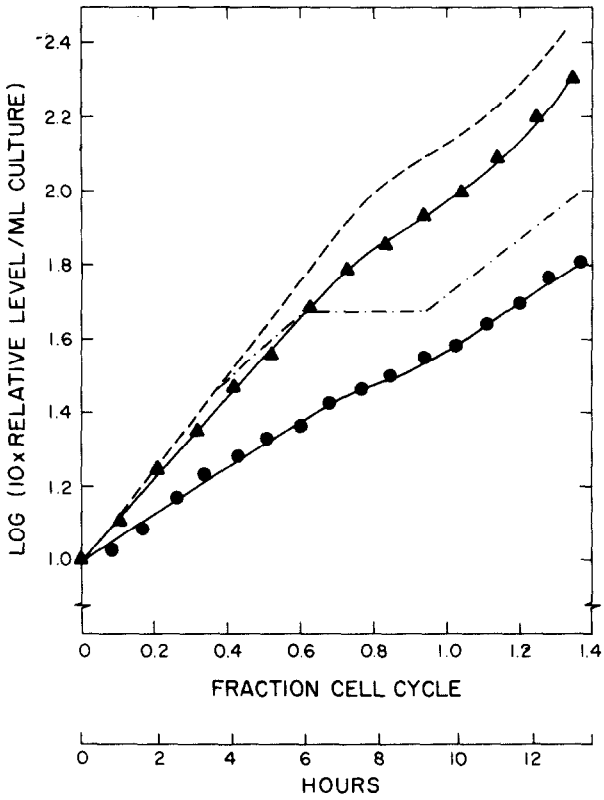

Fig. 4. Patterns of ribulose-1,5-diphosphate carboxylase activity and total cellular protein accumulation in synchronized cells of Chlorella pyrenoidosa (strain 7-11-05) with a division number of 8 and 4. Carboxylase activity, – – – and – • – , and protein ▲ and ● , in cells with a division number of 8 and 4, respectively. Protein data from Flora (19) and Hopkins (20).

The addition of cycloheximide (2.5 µg/ml) to synchronized cells, during the first 0.5 of the cell cycle, almost immediately inhibited further increase in carboxylase activity, suggesting that the increases in activity represented de novo synthesis of the carboxylase (Fig. 5). Because the synthesis of ribulose-1,5-diphosphate carboxylase was inhibited by chloramphenicol but not by

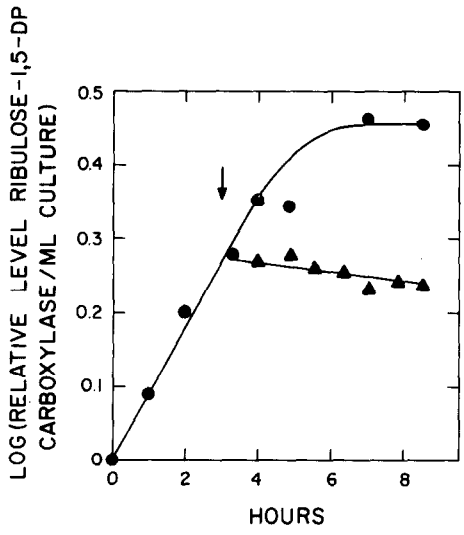

Fig. 5. Effect of cycloheximide (2.5 µg/ml) on ribulose-1,5-diphosphate carboxylase accumulation in synchronized cells of Chlorella pyrenoidosa (strain 7-11-05) with a division number of 4. ● , carboxylase activity in control culture, and ▲ , carboxylase activity in treated culture. Cells (70 x 10^6/ml) were cultured without dilution.

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cycloheximide (15 µg/ml) in Euglena gracilis, Smillie et al. (14) suggested that this enzyme is synthesized on chloroplast ribosomes. Cycloheximide inhibition of carboxylase synthesis in Chlorella might reflect a different mode of action of the inhibitor rather than a different intracellular site of synthesis of the enzyme, i.e., in Chlorella total RNA accumulation is blocked almost immediately while protein accumulation continues at a reduced rate for approximately 2 hours after cycloheximide addition (15).

Synchronous cells (gradient selected) with a division number of 4 showed essentially the same pattern (Fig. 6) of carboxylase synthesis as their light-dark synchronized counterpart (Fig. 3), indicating the pattern in synchronized cells is not a manifestation of the synchronization procedure. In these synchronous cells DNA accumulation was observed (10) to be restricted to the last 0.5 of the cell cycle; therefore, synthesis of the carboxylase cannot be solely attributed to an increase in number of carboxylase structural genes (Fig. 6).

The continuous DNA pattern reported (16) earlier for this organism re-

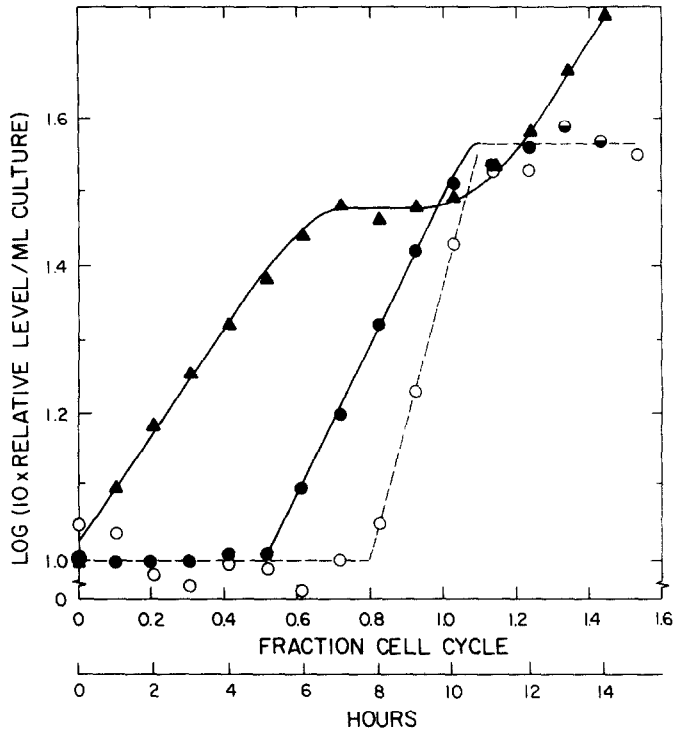

Fig. 6. Patterns of ribulose-1,5-diphosphate carboxylase activity (▲) and total cellular DNA accumulation (●) in synchronous cells (O) of Chlorella pyrenoidosa (strain 7-11-05) with a division number of 4. DNA data from Sitz et al. (10).

sulted from analytical difficulties which are discussed by Hopkins et al. (17). These workers also have observed total DNA to increase as a single step in the last 0.5 of the cycle in this organism with a division number of 8.

The different patterns of carboxylase synthesis are explainable by a model in which feedback repression, by one or more of the photosynthetic products, acting as a corepressor, regulates synthesis of the carboxylase during the cell cycle. The close similarity in the patterns of accumulation of total protein and the carboxylase at the high growth rate (Fig. 4) suggests that the concentration of photosynthetic product(s) (i.e., corepressor) does not reach repressive levels because their utilization for growth approaches the photosynthetic capacity of the cells. At the lower growth rate, the rate of synthesis of the carboxylase greatly exceeds that of protein accumulation during

the initial 0.6 of the cell cycle (Fig. 4). The cessation in enzyme accumulation with continued accumulation in protein and mass, suggests that enough enzyme had accumulated by approximately 0.5 of the cell cycle to catalyze sufficient CO_2 fixation to support exponential growth and to repress net synthesis of the carboxylase for almost the remainder of the cell cycle.

A reduction in the pools of photosynthetic products during cell division could result in the high photosynthetic rate observed (18) after this cellular event and in the complete derepression of carboxylase synthesis as indicated by the identical rates of accumulation of the enzyme during early development in cells growing at different rates (Fig. 4).

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