Note

Chloroplast Replication in *Euglena gracilis* Grown in Cadmium-Ion Containing Media

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As cadmium ion inhibited the normal process of cell division in *Euglena gracilis*, many types of abnormal-shaped cells were observed in cadmium-ion containing ([+Cd]) media.^{1,2)} The average chlorophyll content per cell increased in [+Cd] media. This result suggests that the average chloroplast number per cell may increase in these [+Cd] media. Under normal growth conditions, *E. gracilis* has a constant number of chloroplasts (about 10) as the rate of chloroplast replication is tightly coupled to the rate of cell division.^{3,4)} On the other hand, the replication of chloroplasts is independent of cell division in vitamin B₁₂-deficient ([−VB₁₂]) *Euglena* cells, in which both of the average chlorophyll content and chloroplast number per cell increase.⁴⁾ The relationship between the replication of chloroplasts and cell division in [+Cd] media has not been clarified. In the present study, the effects of cadmium ion on the average chlorophyll content and chloroplast number per cell were examined to provide evidence that the replication of chloroplasts is independent of cell division in [+Cd] media also.

E. gracilis, strain Z, was a generous gift from the Institute of Applied Microbiology, The University of Tokyo. Cells were grown in an 1-liter Erlenmeyer flask with 500 ml of modified Hutner's medium under white-cool light (2800 lux) at 25°C on a shaker.^{1,2)} Two types of growth media were prepared; control and [+Cd] ones. The [+Cd] medium was prepared by adding cadmium chloride (20 µg Cd/ml) into the control medium. Cell number was counted with a Coulter counter through a 100-µm diameter orifice. Chlorophyll content was determined by the method of Arnon.⁵⁾ Chloroplast number was counted under a fluorescence photomicroscope.⁶⁾ About 100 cells were examined for each sample. Dry weight of cells was determined as reported previously.¹⁾

Figure 1 shows the relationship between cell number and chlorophyll content during growth in both of control and [+Cd] media. Cell division was more strongly inhibited by cadmium ion than chlorophyll synthesis. This result implies that the average chlorophyll content per cell increased in the [+Cd] medium. Table I shows that the

FIG. 1. Changes of Cell Number (solid line) and Chlorophyll Content (dotted line) per ml during Growth in Control Medium (●) and [+Cd] Medium (▼).

TABLE I. AVERAGE DRY WEIGHT AND CHLOROPHYLL CONTENT PER CELL AT THE END OF LOGARITHMIC GROWTH STAGE (5th day)

Medium	Dry weight (ng/cell)	Chlorophyll content (pg/cell)
Control	0.65	7.1
[+Cd]	2.15	24.0

average dry weight and chlorophyll content in the [+Cd] medium were about three times higher than the respective values in the control medium. The average chlorophyll content per dry weight in the [+Cd] medium was similar to that in the control medium. Considering the result that the average cell volume in [+Cd] media was about three times higher than the normal volume,²⁾ the increase in the average chlorophyll content per cell in the [+Cd] medium may be attributed to cell enlargement, but not to the activation of chlorophyll synthesis. Figure 2 shows the average number of chloroplasts per cell during growth in the control medium as well as in the [+Cd] medium. In the control medium, the average number of chloroplasts per cell remained nearly constant over a period of 9 days. This result suggests that the chloroplasts divided at about the same rate as the cells in the control medium. In the [+Cd] medium, the average number of chloroplasts per cell increased with growth time. The average number of chloroplasts per cell at the end of growth in the [+Cd] medium was about twice the normal number. These results show that cadmium ion inhibited the cell division more strongly than the replication of chloroplasts, and that the replication of chloroplasts was not tightly coupled to the cell division in the [+Cd] medium. Therefore, the replication of chloroplasts in [+Cd] media is considered to

FIG. 2. Changes of the Average Number of Chloroplasts per Cell during Growth in Control Medium (●) and [+Cd] Medium (▼).

be independent of cell division. In $[-VB_{12}]$ cells, both of the average chlorophyll content and chloroplast number per cell are several times higher than those in control cells

as the result of cell enlargement.⁴⁾ The present study indicates that the effects of cadmium addition and those of the removal of vitamin B_{12} are very similar to each other.

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