Effect of Gamma-Irradiation on the RNA* Content of *Euglena* Cells

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The RNA content of the non-irradiated growing cells changes during the growth cycle. In most cases, it reaches a maximum in logarithmic phase. Thus, the cells of logarithmic phase generally contain more RNA than those of stationary phase.

When the cells in these two growth phases, logarithmic and stationary, were irradiated by the gamma rays of 1×10^5 *r*, the RNA content per cell sensitively decreases by about 30% during 6 hours after the irradiation in the cells of logarithmic phase. The rate of RNA decrease seems to be proportional to the RNA content of the cells before the irradiation. The RNA content of the irradiated cells remains unchanged for the period ranging from 12 hours to 11 days after the irradiation.

Contrary to the above case, no change in the RNA content per cell is recognized before and after the irradiation in the cells of stationary phase.

INTRODUCTION

When a living cell is irradiated by a ionizing radiation, the effects of the irradiation on the cell are variable according to the physiological conditions of the cell.[5] Though many investigators have reported that a rapidly growing cell is generally more radiosensitive than a slowly or non-growing cell,[1,6,11, etc.] only a little result has been reported on the radiosensitivity of *Euglena* cells. In the present work the effect of gamma-irradiation on the RNA content of the cells is compared between those of logarithmic and stationary phases to study the radiosensitivity of living cell under two different physiological conditions.

MATERIAL AND METHODS

A photosynthetic *Euglena* species, *Euglena gracilis var. bacillaris* was used in the present work.

The cells were cultured at 30°C in a peptone nutrient solution[12] and were gathered centrifugally from both cultures of logarithmic phase incubated for 5 days and those of stationary phase incubated for 20 days. They were washed twice centrifugally with distilled water. By these washings, dead cells were eliminated from the living ones. Only the latter cells were resuspended in 200 *ml* of distilled water.

A half volume of this cell suspension was exposed to gamma rays of 1×10^5 *r* for 30 minutes and the other half was used as the control. The irradiation was

* Abbreviation used in this paper: RNA; ribonucleic acid, RNA-P; ribonucleic acid-phosphorus.

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carried out with the Co-60 gamma-ray irradiation facility of the Institute for Chemical Research of Kyoto University. After the irradiation, the cell suspension was divided into 8 aliquots and each aliquot was transferred into 8 flashes which contain each 500 *ml* of fresh culture medium and was incubated under a continuous illumination at 30°C, until it was used for the measurment of RNA content. The non-irradiated control cell suspension was also cultured in the similar manner.

RNA was extracted mainly by a modified method of Schmidt and Thannhauser.[2,7,8] The method of Ogur and Rosen[10] was also tried. As these two methods of analysis gave similar results, only the results obtained by the former method were described in the present paper.

RESULTS

1) RNA Content per Cell of the Non-irradiated Cells

Although the present quantitative determinations of the RNA content of the *Euglena* cells include some errors, the results obtained show that the RNA content of the non-irradiated cells changes during the growth cycle. When the cells of stationary phase containing about 2 to 5 $\times 10^{-6}$ μg RNA-P per cell were transplanted into a fresh culture medium, the RNA content of these cells increases according to incubation time and reaches the maximum in the logarithmic phase of the fresh culture. After the logarithmic phase, the RNA content decreases according to the age of the culture (Fig. 1, A). In most cases, the RNA-P content of the cells of logarithmic phase was about 8 to 11 $\times 10^{-6}$ μg per cell and that of stationary phase, about 3 to 5 $\times 10^{-6}$ μg.

Contrary to the above mentioned case, when the cells of logarithmic phase containing about 10 to 11 $\times$ 10^{-6} μg RNA-P per cell were transplanted into a fresh culture medium, the RNA-P content of these cells decreases steadily from the beginning to the end of the culture period. (Fig. 1, B).

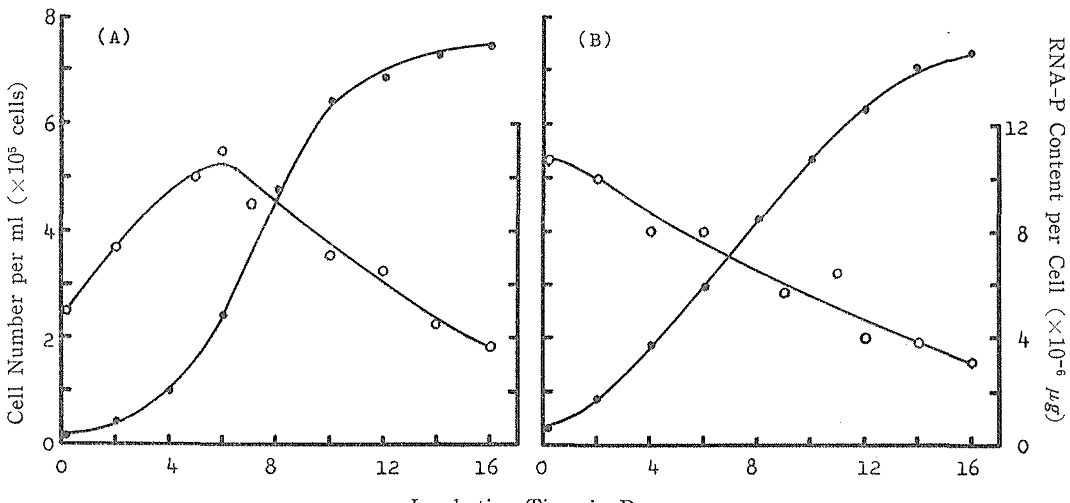
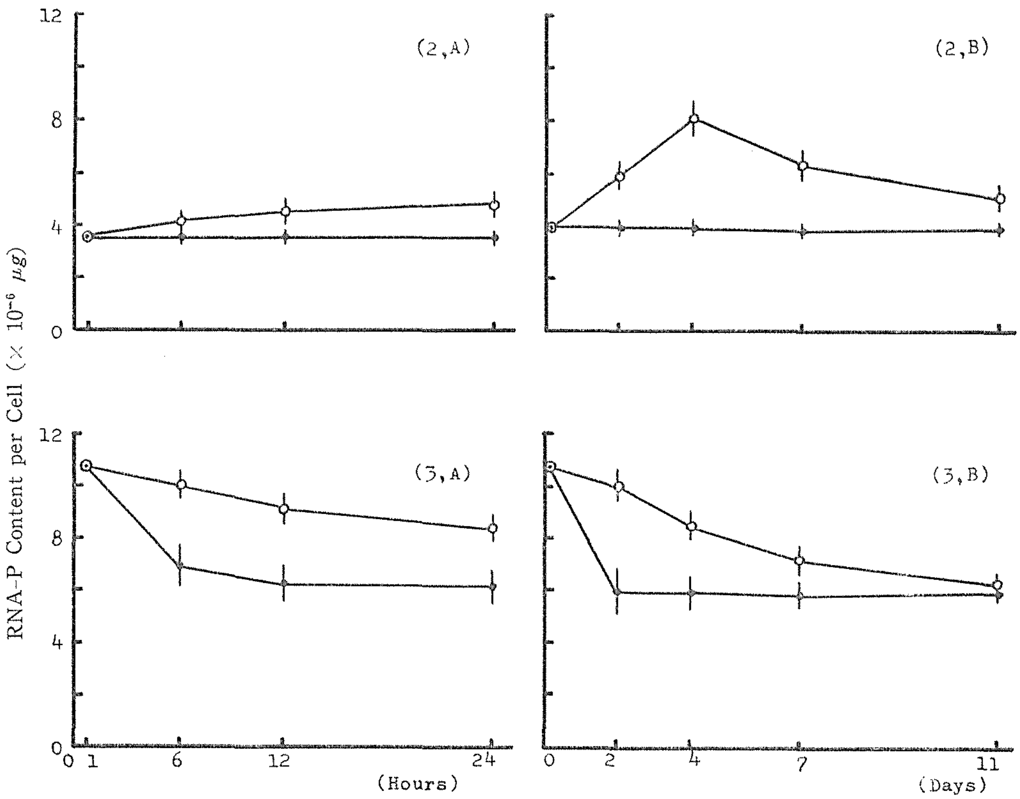


Fig. 1. Relation between cell growth and RNA-P content per cell.
Cell growth, (-●-); RNA-P, (-○-).
(A), Stationary phase cells and (B), Logarithmic phase cells.

2) RNA Content per Cell of the Irradiated Cells

When the cells of stationary phase, which contain about 3 to 5×10^{-6} μg RNA-P per cell were irradiated by the gamma rays of 1×10^{5} r for 30 minutes, the RNA-P content per cell of these cells does not change before and after the irradiation (Fig. 2, A and B).

Contrary to this case, when the cells of logarithmic phase, which contain about 10 to 11×10^{-6} μg RNA-P per cell, were irradiated by the above mentioned dosage of gamma rays, the RNA-P content per cell decreases by about 30 %, sometimes by 50 %, at 6 hours after the irradiation in comparison with that of the non-irradiated cells at the same incubation time (Fig. 3, A and B). The rate of RNA-P decrease of the irradiated cells seems to be proportional to their RNA-P content before the irradiation (Fig. 4). At about 12 hours after the irradiation, the RNA-P content reaches a constant level and remains unchanged for one week or more.



Incubation Time after Gamma-Irradiation

Figs. 2. and 3. RNA-P contents of irradiated and non-irradiated cells. (2) shows the case of stadionary phase cells and (3), that of logarithmic phase cells. (A), incubation time in hours and (B), that in days. Irradiated cells, (-●-) and non-irradiated cells, (-○-).

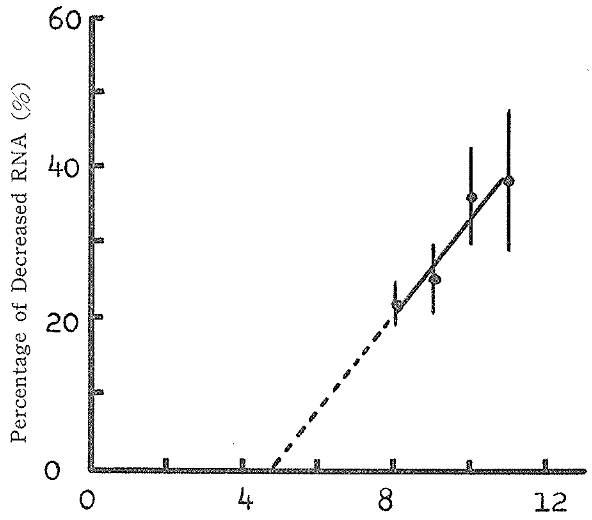


Fig. 4. RNA-P content per cell before irradiation and its decrease rate by gamma-irradiation.
The cells employed for RNA measurment were incubated for 6 hours after the irradiation of 1×10^5 r gamma rays for 30 minutes.

DISCUSSION AND CONCLUSION

The RNA content per cell of the logarithmic phase cells sensitively decreases by about 30%, sometimes by 50% by the gamma-irradiation of 1×10^5 r for 30 minutes. In other words, the RNA of the logarithmic phase cells is considerably unstable to gamma-irradiation. The degree of the RNA decrease by the irradiation appears to be in proportion to the RNA content per cell before the irradiation. This decrease of RNA content may suggest the following points: first, a part of the RNA is decomposed by the irradiation to some low molecular substances[3)] which are not estimated by the present method of RNA determination; secondly, some part of the RNA diffused out of the irradiated cells,[12)] that is, there is a possibility that they are transferred into the dispersion medium of the irradiated cell suspension as free RNA. It is hardly possible, however, to state whether the first or the second assumption is correct from the results obtained in the present experiment.

Contrary to the above mentioned case, no apparent change in RNA content per cell is recognized, when the stationary phase cells are exposed to the gamma-irradiation of the same dosage as above. That is, the RNA content per cell does not change before and after the irradiation.

From the present result, it is assumed that the RNA of the logarithmic phase cells may consist of at least two kinds of RNA; one stable to ionizing irradiation and the other unstable, while the RNA of the stationary phase cells mainly of the former RNA.

In most unicellular organisms, such as *Euglena* cells,[12)] Yeast cells[4)] etc., the cell growth is inhibited by the irradiation dosage used in the present study, and the irradiation dosage of this level frequently brings about an albino mutant in *Euglena* cells.[9,12] These effects of ionizing irradiation occur in the cells of both logarithmic and stationary phases. On the other hand, many investigations have shown that rapidly growing cells are more sensitive to ionizing irradiation than slowly or non-growing cells.[1,6,11] In the present work, too, a similar result is observed between the cells of logarithmic and those of stationary phases, at least as to the RNA decrease by gamma-irradiation.

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