FLUCTUATIONS IN THE POLYSOME CONTENT OF GAMMA-IRRADIATED TETRAHYMENA PYRIFORMIS[1]

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Received June 19,1972

SUMMARY

Sublethal doses of gamma-radiation lead to a rapid, dose-dependent decrease in the polysome content of Tetrahymena pyriformis, followed by an increase to normal and supra-normal levels. The radiation-induced disaggregation of the polysomes is prevented by cycloheximide, but not by puromycin. The post-irradiation recovery is prevented by actinomycin D. These data suggest that radiation decreased the rate of initiation of protein synthesis relative to the rate of elongation and that the later increase in the polysome content may require new RNA synthesis.

INTRODUCTION

A variety of disturbances in protein synthesis can be directly measured as alterations in the polysome content of the cells. Both increases and decreases in polysome content of irradiated cells have been reported (1-7). Unfortunately, no single study has provided a comprehensive description of the immediate vs. long-term effects of both large and small doses of ionizing radiation.

The present investigation was initiated in order to analyze the time course of changes in the polysome content following different doses of radi-

1. Portions of these data were presented at the 4th International Cell Cycle Conference, San Antonio, Texas, April, 1971 and at the 25th Annual Meeting of the Society of General Physiologists, Woods Hole, Mass., September, 1971.
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ation. Our investigations show that the polysome content of γ-irradiated Tetrahymena first decreases and then increases to normal and supra-normal levels. Studies with radiation in combination with specific inhibitors of RNA and protein synthesis suggest that radiation interferes with an early step in protein synthesis.

MATERIALS AND METHODS

Preparation and irradiation of cultures: Cultures of the ciliated protozoan Tetrahymena pyriformis, strain GL-C (obtained from Dr. G. Whitson), were grown in a medium consisting of 1% (w/v) proteose-peptone (Difco), 0.1% liver extract (Nutritional Biochemicals Corp.) and 0.13% $Na_3PO_4 \cdot 12\ H_2O$, streptomycin sulphate (50 μg/ml), and penicillin G (100 units/ml). One hundred ml-portions of medium in 140 mm petri dishes were inoculated with 2 ml of a stock culture and incubated at 25°C. Log-phase cultures (20-21 hrs after inoculation; 4-5 generations) were pooled and aliquots of equivalent volume were taken. In some experiments the cells were treated with inhibitors: puromycin dihydrochloride (Nutritional Biochemicals Corp.) 430 μg/ml (8); cycloheximide (Mann Laboratories Inc.), 5 μg/ml (9); or actinomycin D (Merck, Sharp, and Dohme) 10 μg/ml (10). Gamma-radiation was obtained from a ^{60}Co source at dose rates of 6.9 to 7.6 kR/min.

Isolation of polysomes: Samples of cell cultures were chilled in an ice bath. The cells were collected by a low speed centrifugation (2000 g, 2 min) and lysed by the addition of 0.3 ml of a solution containing buffer [50 mM KCl, 10 mM tris(hydroxymethyl)aminomethane, pH 7.6, and 10 mM $MgCl_2$], saturated indole (11), and 0.5% Triton X-100. The entire lysate was layered over cold 18.2 to 41.7% linear sucrose gradients made with ribonuclease-free sucrose (Schwarz-Mann) in the buffer. Gradients were centrifuged in a SW 41 rotor in a Beckman L2-65B Preparative Ultracentrifuge (2-4° C, 80 min, 40,000 rpm). The gradients were analyzed from top to bottom by displacing the tube contents with 50% sucrose at a known flow rate with an ISCO Model D Fractionator. The absorbance at 260 nm of the effluent was recorded con-

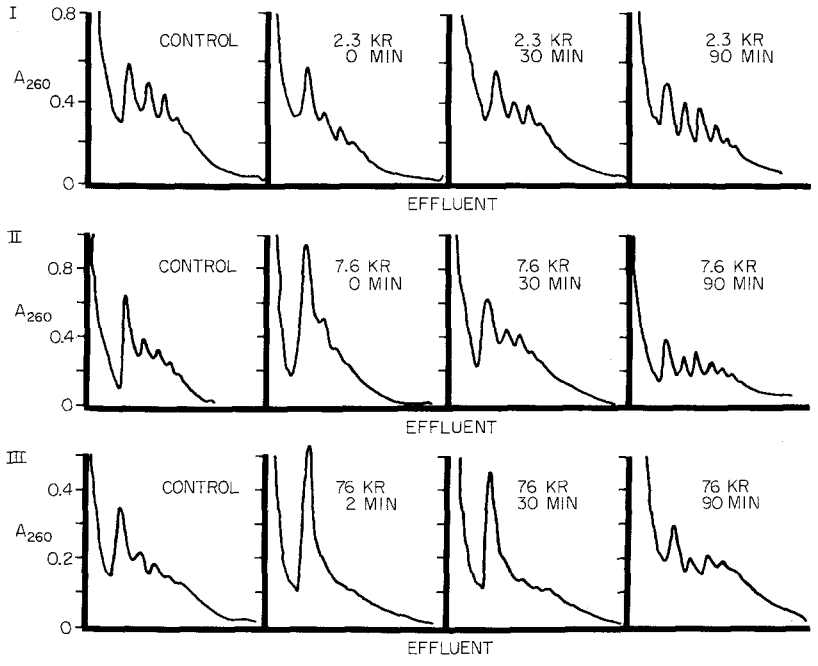

Figure 1. Fluctuations in the polysome content after γ-irradiation. In three separate experiments polysomes were isolated from unirradiated cells or from irradiated cells at the designated times after irradiation with I) 2.3 kR, II) 7.6 kR, or III) 76 kR.

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tinuously with a Gilford 2400 Spectrophotometer equipped with a modified Gilford flow cell having a 2 mm light path. The approximate ratio of polysomes to total ribosomes (monosomes plus polysomes) in experimental cells was calculated from planimeter measurements of the appropriate areas on the spectrophotometric record. The normalized polysome content (P) was obtained by dividing that value by the control value.

RESULTS

Gamma-irradiation of log-phase cultures of Tetrahymena with sublethal doses leads to a rapid dose-dependent decrease in the polysome content and a concomitant increase in the number of monosomes (Fig. 1). Although there were less polysomes of any size class following irradiation, the relative loss of heavy polysomes was greater than that of small oligomers. The initial decrease in polysome content of irradiated cells is followed by a

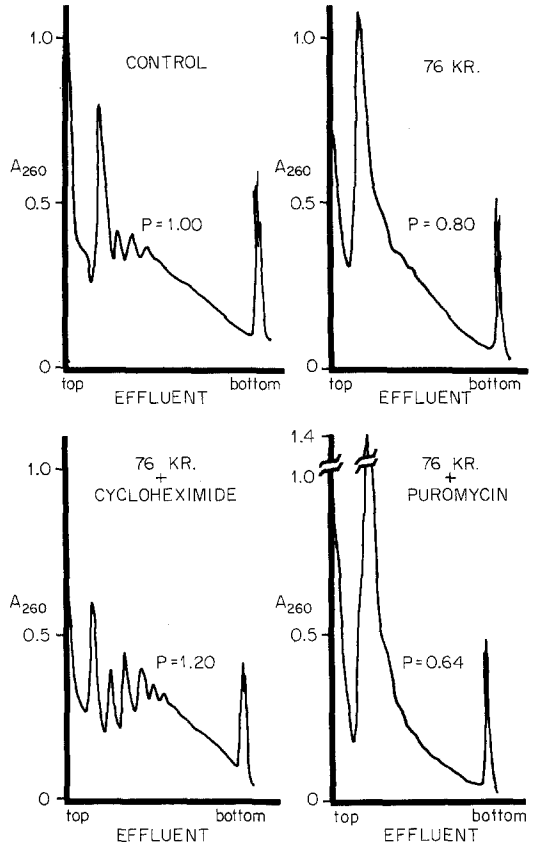

Figure 2. Effects of cycloheximide and puromycin on initial radiation response. Cultures were treated with either cycloheximide (5 μg/ml) or puromycin (430 μg/ml) beginning 2 min and 20 min, respectively, prior to irradiation with 76 kR. Polysomes were isolated immediately after irradiation. The normalized polysome ratio (P) obtained from unirradiated, cycloheximide treated cells was 1.11, whereas unirradiated, puromycin treated cells had a value equal to 0.62.

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recovery and then an "overshoot". After 2.3 kR of γ-radiation, the reformation of polysomes is detectable within 15 min, and the polysome content returns to normal in less than one hr and becomes significantly greater than normal within 1.5 hrs. Following progressively larger doses of γ-radiation (up to 76 kR) not only is the extent of the initial dissociation of polysomes greater but the times required for the recovery and for the "overshoot" are longer.

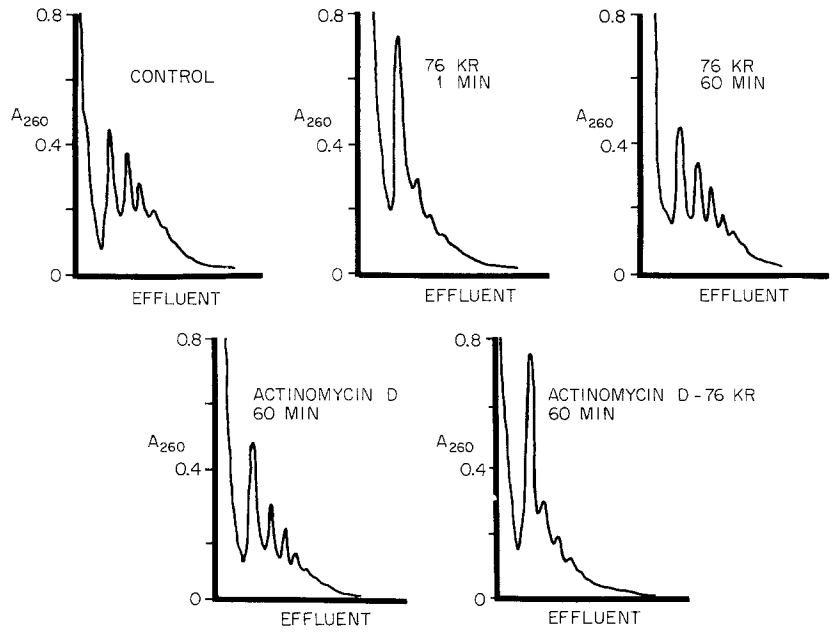

Figure 3. Effect of actinomycin D on the reformation of dissociated polysomes. Actinomycin D (10 μg/ml) was added to cultures 20 min before the end of irradiation with 76 kR, and the incubation was continued. Polysomes were isolated 1 min and 60 min after irradiation.

The effects of two inhibitors of protein synthesis, cycloheximide and puromycin, on normal cells and on cells receiving 76 kR of γ-radiation are reported in Fig. 2. In the presence of cycloheximide the polysome distribution patterns of both irradiated and unirradiated cells remain similar to that of the control cells. In contrast, puromycin reduces the amount of polysomes both with and without irradiation.

Actinomycin D prevents the reformation of polysomes previously dissociated by 76 kR of γ-radiation during a period in which the drug has little effect on the distribution of polysomes from unirradiated cells (Fig. 3).

DISCUSSION

Gamma-irradiation causes an immediate, dose-dependent decrease in the number of polysomes of Tetrahymena and an accompanying elevation of the monosome content (Fig. 1). Following irradiation the relative loss of heavy

polysomes was greater than that of small oligomers. The largest dose employed (76 kR) is less than 1/5 of the LD_{50} (400 kR) of this radioresistant organism (12). Cycloheximide prevents the radiation-induced disaggregation of polysomes (Fig. 2). Since this antibiotic is known to interfere with translocation and thereby "freeze" the ribosomes on the mRNA (13), it appears that the radiation response depends upon the continued movement of ribosomes along the mRNA. Puromycin, which itself causes polysome disaggregation by removing nascent peptides (14), neither prevents nor materially enhances the radiation-induced breakdown of polysomes. These results suggest that there may be a decrease in the rate of initiation of protein synthesis relative to the rate of translation as a result of irradiation. Alternatively, radiation might lead to a premature dissociation of ribosomes from messages after at least one translocation.

Following the dissociation of polysomes by radiation, the polysome content returns to normal and later exceeds the level found in the unirradiated control (Fig. 1). The recovery is more rapid at low doses of radiation, an effect which may be due to a dose-dependent damage to component(s) necessary for the re-initiation process.

We have demonstrated a pattern of decrease in polysome content followed by an increase to greater than normal levels. The extent of loss of polysomes is dose-dependent, as is the delay in the time required to reach and exceed the normal level. An analogous pattern of changes in rate of protein synthesis with longer recovery periods has been detected in plant cells following massive doses of ionizing radiation (15). If the patterns which we have detected are general, then the apparent discrepancies in the literature (1-7) may be the result of studying only a limited selection of doses and post-irradiation times.

The recovery may be dependent upon RNA synthesis, since it is blocked by actinomycin D (Fig. 3). The observations suggest that a species of RNA which is required for initiation of protein synthesis is labilized following

exposure to γ-radiation. We do not know the type of RNA which might be synthesized to allow the post-irradiation recovery of polysomes. In Tetrahymena both mRNA (9) and the large ribosomal RNA (16) are known to be labile to heat. Radiation is known to produce a transient inhibition of mRNA synthesis in rat liver (17) and peanut cotyledons (18) and rRNA synthesis in HeLa cells (19). Positive identification of the radiation-sensitive RNA species will be a part of our plan for further investigation into the mechanism of the immediate radiation response of polysomes identified in the present study.

Acknowledgements: This work was supported by Contract No. W-109-31-ENG-78 (Report Number COO-78-277) with the Division of Biology and Medicine, U.S. Atomic Energy Commission. One of us (G.S.K.) was the recipient of a National Science Foundation Undergraduate Summer Research Stipend during a portion of this research.

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