Effect of Low-Dose Gamma Radiation on Nuclear RNA-Polymerase Activity and Protein Synthesis in Liver Cells of Different-Age Chicks

G. Khozhiakhmedov, T. Islamov, and D. Kh. Khamidov

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The effect of low-dose (0.05 Gy) gamma irradiation of chicks in the preincubation period on subsequent liver cell RNA-polymerase activity and protein synthesis is studied at different ages. It is shown that irradiated chicks demonstrate an increased level of RNA-polymerase and increased incorporation of labeled amino acids into cytoplasmic proteins. Apparently, low-dose ionizing radiation induces activation of the liver cell genome, this being one of the main mechanisms of the general stimulatory effect of low doses.

Key Words: *low-dose radiation; RNA-polymerase; protein synthesis*

Low-dose ionizing radiation stimulates a broad spectrum of biochemical processes in the cell, which ultimately result in accelerated growth and development of organisms. The known data regarding the effect of low-dose radiation are concerned mostly with the specific components of plasma membranes [1,8] and the activity of integral membrane enzymes [4,8]. At the same time, it has been shown that low doses induce changes in numerous other structures [5,10]. A few data on low dose-induced changes of the cell nuclear apparatus have been obtained on the model of adult tissues but provide no information about the effect of low doses on various organelles. In view of this, the goal of the present study was to investigate nuclear RNA-polymerase activity and protein synthesis in the liver cells of intact and preincubation-irradiated chicks of different age.

MATERIALS AND METHODS

Chicks 1, 10, 20, 30, 40, 50, and 60 days old were examined. Preincubation radiation of eggs was carried out on a Gaboi gamma-apparatus; the dose was 0.05 Gy and the dose rate 0.11 A/kg. Cytoplasmic protein synthesis was judged by the incorporation of a mixture of labeled amino acids into the proteins of intact and irradiated tissue [6]. The components of the protein-synthesizing apparatus (ribosomes, protein translation factors, tRNA, and aminoacyl-tRNA synthetases) were introduced in a cell-free system as a fraction obtained by centrifugation of cell homogenate at 30,000 *g* (S-30 fraction). The final concentration of S-30 fraction was 10-20 units according to absorption at wavelength 260 nm. The system contained 30 mM Tris-HCl (pH 7.6), 3.5 mM $MgCl_2$, 85 mM KCl, 7 mM 2-mercaptoethanol, 1.5 mM ATP, 0.1 mM GTP, 0.6 mM CTP, 10 mM creatine phosphate, 0.02 mg/ml creatine kinase, 40 μM of each unlabeled amino acid, and 3 μM of the mixture of ^{14}C-labeled amino acids with a specific radioactivity of 1.8 mCi/ml.

The hepatocyte nuclei were isolated routinely [11]: liver tissue was homogenized in 10 volumes of solution containing 0.25 M sucrose and 10 mM Tris-HCl (pH 7.6); the nuclei were sedimented and layered onto 2.2 M sucrose solution with subsequent centrifugation. Total nuclear polymerase

Institute of Biochemistry of the Uzbekistan Republic Academy of Sciences, Tashkent

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activity was estimated in 250 μl of solution containing 6 mM Tris-HCl (pH 7.6), 1 mM $MgCl_2$, 1 mM dithiothreitol, 200 mM $(NH_4)_2SO_4$, 0.4 mM GTP, 0.4 mM ATP, 0.4 mM CTP, and 0.03 mM ^{3}H-UTP (specific radioactivity 0.9 mCi/ml) [11]. The reaction was started by adding nuclei (25 μg DNA) to the reaction mixture. Incubation was carried out for 30 min at 37°C, and the reaction was terminated by the addition of precooled 10% trichloroacetic acid. Sediments were transferred to Millipore filters (Synpor); filters were washed with 5% trichloroacetic acid and 85% ethanol, and radioactivity was recorded in a Rack-beta 1217 liquid-scintillation counter.

RESULTS

In the first series of experiments we studied the dynamics of nuclear RNA-polymerase activity of liver cells from intact and preincubation-irradiated chicks in relation to age. As is shown in Table 1, intact chicks exhibit a rise of enzyme activity that is in direct proportion to age up to the 50th day. At this time the RNA-polymerase activity begins to decrease, and it continues to drop during the later period of observation. In experimental chicks an enhancement of enzyme activity was recorded at all times of testing. The degree of the rise was also a function of age. Thus, maximal activity was attained by the 40th day (206%), and starting from the 50th day the activity dropped gradually. However, it is worth noting that during the entire period of observation the RNA-polymerase activity of experimental birds exceeded that of intact chicks. These results are in agreement with the published data concerning low-dose radiation-induced elevated RNA synthesis in the liver of young rats [7].

The next series of experiments focused on liver cell cytoplasmic protein synthesis. Starting from the

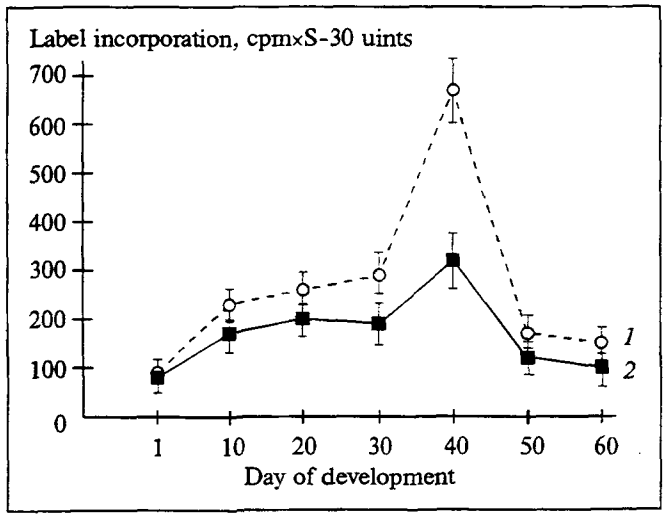

Fig. 1. Dynamics of liver protein synthesis in the course of development of preincubation–irradiated (*1*) and control (*2*) chick.

10th day (Fig. 1), protein synthesis rose, peaking at the 40th day. In later periods of postnatal development a drop in labeled amino acid incorporation into hepatocyte proteins was recorded.

In all tested periods of development the experimental chicks demonstrated an enhanced incorporation of labeled amino acids in hepatocyte proteins. The dynamics of this phenomenon conformed to that of RNA-polymerase activity. Even by the 50th and 60th day, when protein synthesis was significantly diminished in intact chicks; incorporation of the radioactive label into synthesized proteins remained elevated in the experimental group. Comparable results were obtained by other investigators [2,3] who studied the effect of radiation before and during incubation of eggs on the cells of endocrine organs. The elevated level of protein synthesis was accompanied by an increase of chromatin matrix activity and acceleration of specific ultrastructural differentiation. Accelerated cell differentiation under the influence of low-dose

TABLE 1. Effect of Preincubation Radiation on Nuclear RNA–Polymerase Activity in the liver of chicks of different age ($M \pm m$).

Day of development	^{14}C-UMP incorporation,cpm		% change
	control	experiment	
1	652±56	896±81	137
10	1736±227	2420±189	133
20	1943±465	2810±173	144
30	1914±98	2962±107	156
40	3356±189	6954±215	206
50	1265±115	1785±245	141
60	897±75	1512±97	168

Note. Each group consisted of 8 chicks.

radiation is considered to result from an increase of RNA synthesis which, in turn, boosts the rate of protein synthesis [7,9].

Thus, it seems that the earlier-proposed hypothesis [4] regarding an active participation of plasma membrane components in the realization of the effect of low-dose radiation does not fully reflect the mechanisms of the low dose-induced stimulating effect. Our finding that preincubation radiation affects RNA-polymerase activity and protein synthesis in hepatocytes points to participation of the genome in the stimulating effect of low-dose radiation on cell metabolism.

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