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LIPID PEROXIDATION AND POLYMERASE ACTIVITIES OF LIVER CHROMATIN FRACTIONS OF AGING RATS

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The aging process is accompanied by definite changes in structure and function of the cell genome [8]. The writers previously demonstrated slowing of replication and transcription processes and changes in the distribution of DNA-polymerase activity in subcellular fractions, and also of certain structural properties of the liver chromatin of rats in [9, 10, 12].

Replication and transcription processes in the cell nucleus are located in the macromolecular protein—nucleic acid—lipid chromatin complex, which is the structural and functional form of organization of the nuclear genome. Endogenous DNA- and RNA-polymerase activities of chromatin determinable in vitro must evidently correlate with the intensity of replication and transcription processes in vivo.

Lipids, which are components of the nuclear genetic apparatus, can evidently perform regulatory functions [1]. In particular, the phospholipid sphingomyelin, which destabilizes the DNA double helix, can activate replication and transcription processes [2]. It has also been suggested that lipid peroxidation (LPO) of chromatin [1, 5] may be one factor regulating replication and transcription processes when disturbed during aging. The possibility therefore cannot be ruled out that disturbance of the regulatory effect of peroxidized chromatin phospholipids on its function may be one cause of changes observed in function of the nuclear genome during aging.

The aim of this investigation was to study relations between the intensity of NADPH- and ascorbate-dependent LPO and activities of DNA- and RNA-polymerases in fractions of actively transcribed and repressed liver chromatin of mature and old rats.

EXPERIMENTAL METHOD

Male Wistar rats aged 8 months (mature, 200-300 g) and 26 months (old, 300-400 g) were used. The animals were decapitated under superficial ether anesthesia during the morning hours, the liver was removed from them and preparations of actively transcribed and repressed chromatin were isolated from it [10]. DNA- and RNA-polymerase activities were determined by the methods in [6, 11]. Activities of DNA-polymerases α and β were separated on the basis of their differential sensitivity to N-ethylmaleimide, and activities of RNA-polymerases I and II on the basis of their differential sensitivity to α-amanitine. LPO of chromatin (NADPH- and ascorbate-dependent: NDP and ADP, respectively) was assessed on the basis of

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TABLE 1. Endogenous DNA- and RNA-Polymerase Activities of Liver Chromatin Fractions from Mature and Old Rats

Repressed chromatin		Actively transcribed chromatin	
8 months	26 months	8 months	26 months
Total DNA-polymerase activity			
169 754	160 030	165 997	82 271*
Activity of DNA-polymerase α			
110 831	85 786	140 325	75 023
Activity of DNA-polymerase β			
58 925	74 244	25 672	7 248*
Total RNA-polymerase activity			
116 725	86 158	176 564	281 722
Activity of RNA-polymerase I			
53 839	39 604	52 703	145 834*
Activity of RNA-polymerase II			
62 886	46 554	123 861	135 888

Legend. $^{*}p < 0.05$, $^{**}p < 0.01$ (compared with 8 months); enzyme activity expressed in cpm/mg DNA. Number of experiments: 6-20.

TABLE 2. Accumulation of MDA (in nmoles/mg protein) and Its Derivatives in Liver Chromatin Fractions from Mature and Old Rats

	8 months		26 months	
	repressed chromatin	actively transcribed chromatin	repressed chromatin	actively transcribed chromatin
NDP	1985	3624	1343	2060
NDP, Δ	233	817	172	207*
AZP	998	1750	800	1126

Legend. $^{*}p < 0.05$ (compared with 8 months); NDP) NADPH-dependent LPO; NDP, Δ) its enzymic component; AZP) ascorbate-dependent LPO. Number of experiments: n = 6.

accumulation of malonic dialdehyde (MDA) and its derivatives during incubation of the samples for 2 h at 37°C [4]; the enzymic component of NDP also was determined by subtraction of the value remaining after boiling the samples for 7 min from the total value. The experimental results were subjected to statistical analysis by nonparametric tests [3].

EXPERIMENTAL RESULTS

During aging a decrease was observed in total DNA-polymerase activity in the fraction of actively transcribed chromatin (Table 1). This was due mainly to a decrease in activity of reparative DNA-polymerase β, in agreement with the decrease in the intensity of DNA replication in the rat liver during aging which we observed previously [7].

The writers previously discovered a decrease in the intensity of incorporation of ^{3}H-orotate into RNA of liver chromatin fractions of rats with aging [10]. In connection with those data, some rather unexpected results were obtained when RNA-polymerase activities of the chromatin fractions were determined. As Table 1 shows, during aging activity of RNA-polymerase I increases in the actively transcribed chromatin fraction also. The increase in RNA-polymerase activity can probably regarded as an attempt at compensating the decrease in transcription through a nonspecific feedback mechanism. This increase may also be the result of strengthening of DNA—protein bonds during aging.

Determination of the intensity of LPO in liver chromatin fractions from mature and old rats showed that the aging process in accompanied by a decrease in activity of certain parameters, which are more marked in the actively transcribed chromatin fraction (Table 2). The observed decrease in the intensity of MDA accumulation could be linked with changes both in the content of natural antioxidants in old age [5] and in the fatty-acid composition of chromatin-bound phospholipids, and this will be a topic for future research.

On the other hand, the change in activities of DNA- and RNA-polymerases in the actively transcribed chromatin fraction may be due to a decrease in the intensity of LPO processes in this chromatin fraction.

The results thus confirm the concept of a possible role of changes in activity of LPO regulation in the modular mechanisms of aging.

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