There is no doubt that direct correlation does not exist between the ability of cells to repair DNA and the SCE level in all cases, for many factors can influence the course of these processes in cells under normal conditions and during changes in physiological status. In the writers' view, toxic products (endotoxins, free-radical products) produced in NSLD may cause injury to DNA that is manifested as an increase in the frequency of SCE. Antibiotic treatment may lead to an increase in bacteriolysis, with massive release of bacterial toxins, and may increase the degree of injury to the cell DNA. The sharply reduced ability of the patients' lymphocytes to repair DNA may be due to two causes: inhibition of activity of the DNA excision repair enzymes by toxic products, and deficiency of repair enzymes for the "healing" of both types (endogenous and UV-induced) DNA injuries.

Injury to the chromosomal material, manifested as an increase in the frequency of SCE, observed during an inflammatory process, and the simultaneous decrease in the ability of lymphocytes to repair DNA may thus be evidence of disturbance of the intracellular mechanisms aimed at maintaining the structural integrity of DNA.

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INCREASED NUMBER OF REPEATED NUCLEOTIDE SEQUENCES IN TRANSCRIPTIONALLY ACTIVE DNA AND POLY A^+-mRNA FROM RAT LIVER AND INDUCTION OF ITS INCREASED TRANSLATION ACTIVITY

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KEY WORDS: DNA, liver, induction

It was shown previously that the number of repeated sequences (RS) [4-8] in the composition of transcriptionally active DNA (taDNA) [4, 5] is increased in rat liver cells under the influence of various factors inducing gene expression (cortisol, phenobarbital, regeneration). These RS are actively transcribed and are found among giant nuclear RNA (gnRNA) [4]. No appreciable changes take place in the composition of the unique sequences of liver taDNA on induction of transcription by cortisol [4]. We know that cortisol induces enzymes of gluconeogenesis in the liver of animals [15], and that certain xenobiotics, including the aminoazo

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dye 3'-methyl-4-dimethylaminoazobenzene (3'MeDAB), which possesses potential hepatocarcinogenic activity, induce microsomal mono-oxygenases, that are responsible for eliminating such compounds [9].

The present investigation showed that during induction by cortisol and 3'-MeDAB the increase in the number of RS in TaDNA is accompanied by an increase in the number of RS in the transcribed polyA$^+$-mRNA during induction. This correlates with the increased translation efficiency of induced polyA$^+$-mRNA, in the absence of any qualitative differences in the spectrum of protein products synthesized in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 2-2.5 months. The adrenals were removed from some animals 3 days before the experiment to lower the endogenous glucocorticoid level. Cortisol was injected intraperitoneally into intact rats in a dose of 5 mg/100 g body weight 3-4 h before sacrifice. 3'-MeDAB was injected intraperitoneally into intact rats in a dose of 25 mg/100 g body weight in 1 ml of vegetable oil 24 h before sacrifice. Rats receiving the corresponding volume of solvent served as the control.

DNA was fractionated by a modified [3] phenol-salt method without detergent [11], by means of which, by using different conditions of deproteinization, three DNA fractions could be isolated: TaDNA, which is deproteinized by a mixture of (v/v) 1 M NaCl and 75% phenol (pH 6.4), and accounts for 15-20% of the total DNA; transcriptionally inactive DNA (tiDNA), which is deproteinized by a mixture (v/v) of 0.14 M NaCl and 66% phenol (pH 8.5) and accounts for about 70% of the total DNA; the third fraction is firmly bound with proteins and, under the conditions of the method, is extracted from the final interphase layer by treatment with pronase and subsequent purification [3]. The results obtained previously suggest that this fraction includes potentially active regions of the genome [5], and it will henceforth be called paDNA.

Polysomes were isolated by the method in [10] followed by affinity chromatogrpahy on polyG-sepharose to isolate polyA$^+$-mRNA. Synthesis of labeled ^{32}P-cDNA on polyA$^+$-mRNA was carried out by means of reverse transcriptase from avian myeloblastosis virus [10], using ^{32}P-lGTP and unlabeled dNTP as the precursors. The yield of cDNA, with specific activity of 10^6 cpm/μg, was 0.3-0.5 μg/μg of polyA$^+$-mRNA; the cDNA varied in size from 4S to 9S, with a maximum in the 7S region [1]. Translation products of polyA$^+$-mRNA in a rabbit reticulocyte system were analyzed electrophoretically, followed by radiofluorography [2]. The ^{32}P-cDNA, read from polyA$^+$-mRNA isolated from the liver of intact and induced animals, was hybridized with total liver DNA, fragmented by ultrasound, present in an excess of about 10^5 times [4]. The degree of hybridization and renaturation was determined from the ratio between the amount of ^{32}P-cDNA or unlabeled DNA (in the case of renaturation), resistant to S_1-nuclease (from Sigma, USA), and the total quantity of ^{32}P-cDNA (in the case of hybridization) or of unlabeled DNA (renaturation) in the sample, depending on the value of Cot. Cot stands for the produce of the DNA concentration and renaturation time, expressed in moles of nucleotides per liter per second, at 60°C in 0.12 or 0.35 M Na-phosphate buffer — 0.1% sodium dodecylsulfate (pH 6.8). On renaturation in 0.36 M Na-phosphate buffer values of Cot were normalized in accordance with the data in [12].

EXPERIMENTAL RESULTS

Comparison of the renaturation curves of taDNA from the liver of intact animals with those of taDNA from the liver of rats induced with cortisol and 3'-MeDAB shows that both inducers lead to an increase in the number of repeat sequences present in taDNA. However, repeats activated by different inducers differed in the frequency of their occurrence in taDNA. Under the influence of cortisol there was a significant increase in the number of moderately repeating nucleotide sequencies, denaturing in the region of Cot 10^{-1}-10^{-2}, in taDNA compared with the control (Fig. 1a). Conversely there was a decrease in the number of the corresponding repeats in the composition of paDNA (Fig. 1b). These results, obtained in the present investigation by S_1-nuclease hydrolysis, confirm the results of the writers' previous investigations, in which single- and double-stranded DNA was separated by chromatography on hydroxyapatite [4, 6].

Induction by 3'-MeDAB also caused an increase in the number of repeats in taDNA (Fig. 1a), but in this case the frequency of repetition of inducible sequences was higher than on induction by cortisol (Cot $\leq 10^{-2}$). 3'-MeDAB caused a decrease in the number of these repeats

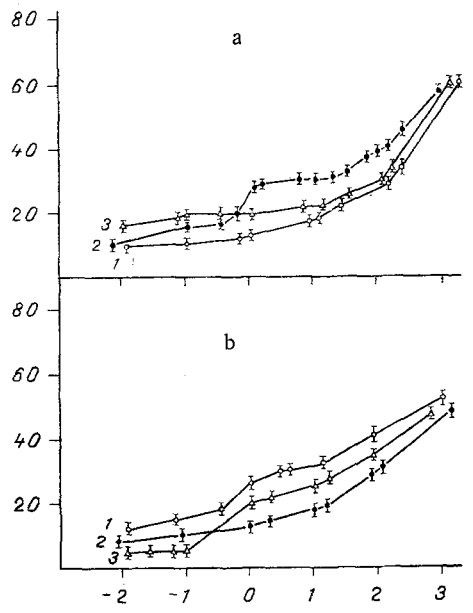

Fig. 1

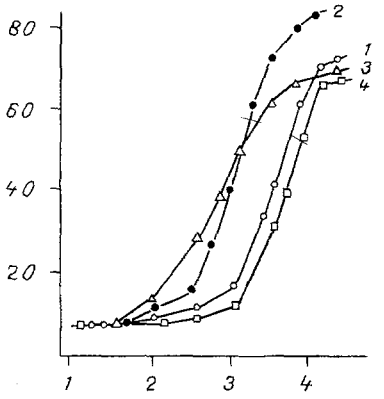

Fig. 2

Fig. 1. Effect of induction by cortisol and 3'-MeDAB on renaturation kinetics of rat liver DNA I (a) and DNA III (b). Abscissa (here and in Fig. 2), log Cot; ordinate, renaturation (in %). 1) Intact animals; 2) induction by cortisol; 3) induction by 3'-MeDAB. Each point on curves corresponds to statistical mean result of 5-7 determinations.

Fig. 2. Effect of induction by cortisol and 3'-MeDAB on hybridization of total liver DNA with ^{32}P-cDNA, read from rat liver polyA$^+$-mRNA. Ordinate, hybridization (in %). 1) Intact animals; 2) induction by cortisol; 3) induction by 3'-MeDAB; 4) after adrenalectomy. Typical results of 4-5 determinations given.

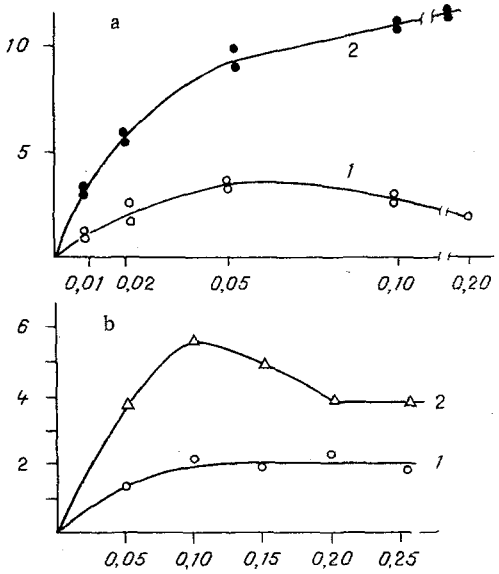

Fig. 3. Effect of induction by cortisol (a) and 3'-MeDAB (b) on translation activity of rat liver polyA$^+$-mRNA in a rabbit reticulocyte system. Abscissa, mRNA concentration (in μg/5 μl); ordinate, radioactivity (in cpm × 10^{-3}). 1) Intact animals; 2) inducer.

in the composition of paDNA (Fig. 1b). On induction there were no appreciable changes in the renaturation characteristics of tiDNA [6].

It was shown previously that RS arising in taDNA during induction are widely represented in cortisol-induced gnDNA [4]. To discover whether these sequences are preserved in polysomal polyA$^+$-mRNA, induced by different inducers, we investigated the hybridization kinetics of cDNA synthesized on the corresponding polyA$^+$-mRNA with total liver DNA. The hybridization curves show the appearance of RS in the composition of the induced polyA$^+$-mRNA compared with the control, consisting of polyA$^+$-mRNA of intact and adrenalectomized animals (Fig. 2). Judging by hybridization with cDNA, polyA$^+$-mRNA induced by 3'-MeDAB is characterized by the appearance of nucleotide sequences with a higher frequency of repetition than those in the composition of polyA$^+$-mRNA induced by cortisol (Fig. 2: 2, 3). For instance, for induction by cortisol (Fig. 2: 2) Cot 1/3 was about 600, compared with about 300 for induction by 3'-MeDAB.

It can be concluded from these results that during induction the number of repeating elements in transcribed polyA$^+$-mRNA increases, evidently due to an increase in the number of repeats in taDNA under the influence of inducers and their transcription. Do the induced polyA$^+$-mRNA differ in their functional characteristics from polyA$^+$-mRNA isolated from intact rat liver? To answer this question, we compared the transcription activity of polyA$^+$-mRNA from induced and intact animals in a cell-free protein-synthesizing system. Judging by the results, polysomal polyA$^+$-mRNA from the liver of induced rats translates several times more efficiently than polyA$^+$-mRNA from the liver of intact animals (Fig. 3). Electrophoretic analysis of the translation products of polyA$^+$-mRNA from liver polysomes of intact and induced animals in vitro did not reveal any new fractions in the composition of the protein products synthesized. An increase in translation activity of cortisol-induced polyA$^+$-mRNA in a protein-synthesizing system from wheat germ was described previously, and it was explained by possible unique structural features of the induced polyA$^+$-mRNA [1]. The results of the present investigation and data in the literature suggest that the increase in translation activity of polyA$^+$-mRNA takes place regularly under the influence of various inducers. It seems logical to suggest also that RS with an unstable conformation in chromatin may provide a system of rapid response of the genetic apparatus to an extremal situation, by acting as transcription intensifiers in the composition of taDNA (for example, due to the appearance of extra transcription initiation sites) [13], and as translation intensifiers in the composition of induced polyA$^+$-mRNA. The results of the present investigation are in agreement with existing views on the action of genetic inducers. We know that on administration of inducers to animals synthesis of certain enzymes or of other proteins responsible for adaptation of the animal to new environmental conditions increases: to stress, to a deficiency or excess of certain food factors, etc. [14]. Induction evidently leads to rapid adaptation of the organism to new metabolic and environmental conditions, not only through intensification of transcription of certain genes, but also through an increase in translation activity of the newly induced mRNA. Inducers evidently act as signals of extremal situations, which require activation of the universal reserve capacity of the genome, maintaining survival under extraordinary conditions. Under these circumstances strengthening of gene expression takes place at all stages of this multistaged process.

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