CHANGES IN TEMPLATE PROPERTIES OF CHROMATIN DURING AMINOAZO-CARCINOGENESIS

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Male rats were kept on a diet containing the carcinogen 3'-methyl-4-dimethylaminoazobenzene. No difference from the control was found either in the electrophoretic spectrum of the total protein of deoxyribonucleoproteins (DNP) or nonhistone proteins, or in the character of the melting curves of DNA and DNP, or in the template ability of DNA isolated from rat liver. DNP of tumor tissue functioned more actively as template for RNA synthesis *in vitro* in a system with animal or bacterial RNA-polymerase, with low concentrations of substrates (of the order of $2 \cdot 10^{-5}$ M), but RNA synthesis did not differ from the control in the presence of high concentrations of each of the substrates ($2 \cdot 10^{-3}$ M). During lysis of the nuclei in a solution with alkaline pH the percentage of activity of RNA-polymerase that could be extracted from the complex with chromatin decreased considerably if the rats were fed with the carcinogen.
KEY WORDS: *chromatin; RNA-polymerase; chemical carcinogenesis.*

The chemical carcinogen 3'-methyl-4-dimethylaminoazobenzene (3'-MDAB), if fed to rats, can alter the level of RNA synthesis in nuclei *in vitro;* although above the normal level during the first few days of feeding, it then falls to 60% of the control [2, 3, 7]. Abnormalities in the RNA-synthesizing power of the nuclei include changes in the template for RNA synthesis and also changes in the enzymes catalyzing synthesis [4].

The ability of deoxyribonucleoproteins (DNP) and purified DNA to act as templates for RNA synthesis was studied.

EXPERIMENTAL METHOD

The isolation of DNP and DNA from rat liver nuclei and the analytical procedures were similar to those described earlier [3]. RNA-polymerase was isolated from liver nuclei by the method of Cunningham et al. [5], followed by reprecipitation with ammonium sulfate [6]. RNA synthesis by solubilized enzyme was carried out in systems whose composition was suggested by Gorbacheva et al. [6]. To the system for analysis of form A of RNA-polymerase 25 μg native rat liver DNA was added, and the same amount of denatured DNA was added to the system for the study of form B. Template activity of DNP with RNA-polymerase from rat liver was measured in a volume of 0.25 ml containing $5 \cdot 10^{-2}$ M Tris–HCl, pH 7.8, $8 \cdot 10^{-3}$ M $MgCl_2$, $2.5 \cdot 10^{-3}$ M $MnCl_2$, $8 \cdot 10^{-3}$ M KCl, $1.6 \cdot 10^{-2}$ M $(NH_4)_2SO_4$, $5 \cdot 10^{-4}$ M β-mercaptoethanol, and $4 \cdot 10^{-4}$ each of ATP, GTP, CTP and UTP; the last of these compounds was labeled with H^3 (0.5 Ci/mmole). The reaction was carried out for 10 min at 37°C. The significance of differences was estimated by Student's method [1].

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TABLE 1. Template Ability of DNP with RNA-Polymerase from *Escherichia coli* (in nmoles UMP; M±m)

Experimental conditions	Concentration of each substrate, in M ($\times 10^{-4}$)					
	0,2	0,4	2	4	16	24
Control	2,5±0,1	3,7±0,1	30±1	90±12	290±15	321±32
3'-MDAB, 130 days	5,1±0,2	10,5±0,5	77±3	176±2	300±31	374±47
P	<0,01	<0,01	<0,01	<0,02	>0,5	>0,5

Legend. Each sample contained 25 µg DNA in the DNP form and 5 units of RNA-polymerase. Reaction carried out for 10 min at 37°C.

EXPERIMENTAL RESULTS AND DISCUSSION

Metabolites of the carcinogen could induce disturbances of the DNA structure or change the interaction between protein and DNA. To analyze the possible changes in the secondary structure of the templates as a result of the action of metabolites of the dyes *in vivo*, melting curves were recorded by the optical density test for samples of DNA and DNP isolated on the 50th-130th day of feeding the rats with the carcinogen 3'-MDAB. The melting temperature of DNA of both the control and the experimental samples was 62-63°C and its hyperchromicity 39-40%. The melting temperature of DNP was 77-78°C and its hyperchromicity 37-38%. Within the limits of sensitivity of the method used, the secondary structure of the template thus remained unchanged. The electrophoretic spectra of nonhistone proteins or of total protein of DNP isolated from the liver of animals receiving 3'-MDAB also coincided with the control. No significant differences likewise were found in the content of nonhistone proteins, which were 23-25%.

The template activity of the purified DNA likewise was unchanged. For instance, on the 35th day of feeding with 3'-MDAB, after addition of a mixture of 6.4 µg purified DNA and 1.6 unit of RNA-polymerase from *Escherichia coli* to the incubation mixture, the incorporation of UMP-H^3 into the acid-insoluble fraction was 59±6 nmoles for the control and 58±7 nmoles for the experimental samples, with concentrations of $0.8 \cdot 10^{-4}$ M each of ATP, GTP, CTP, and UTP in the incubation medium. With an increase in the concentration of each of the ribonucleoside-triphosphates to $4 \cdot 10^{-4}$ M, the incorporation in the control and experimental samples was 264±31 and 269±66 nmoles UMP respectively.

The ratio between the template activity of DNA from normal liver and from the liver of animals receiving the carcinogen also was unchanged if the quantities of DNA and RNA-polymerase from *E. coli* in the incubation mixture were varied.

However, structural changes in the supramolecular organization of the chromatin could have changed its functional activity without causing any significant abnormality in the composition of the proteins and properties of the DNA. The results of investigation of the template activity of DNP with the aid of RNA-polymerase from *E. coli* are shown in Table 1. DNP isolated from the liver of rats receiving the carcinogen proved to be a better template for low concentrations of precursors of RNA synthesis. If these were present in high concentrations the template activities of the DNP were equal in the experimental and control samples.

Similar relations between the experimental and control samples of DNP also were observed if solubilized RNA-polyerase from normal liver was used. If 270 µg of enzyme protein and DNP isolated on the 60th day of feeding with the carcinogen, containing 25 µg DNA, were added to the incubation medium and if each of the triphosphates was present in a concentration of 10^{-5} M, the incorporation of UMP-H^3 was 1.3±0.1 and 1.9±0.1 nmole for the control and experimental samples ($P < 0.05$), but if the concentration of each triphosphate was $4 \cdot 10^{-4}$ M, incorporation of UMP was 39±3 and 32±1 nmoles respectively ($P < 0.5$).

The ability of RNA-polymerase to dissociate from the complex with chromatin also was studied in this investigation, and for that purpose the ratio between the RNA-polymerase activity of the residue and of the supernatant obtained after centrifugation (105,000g)

during purification of the RNA-polymerase was measured. Control animals were compared with animals receiving noncarcinogenic 2'-methyl-4-diemethylaminoazobenzene (2'-MDAB) or the carcinogen 3'-MDAB. RNA-polymerase activity of the residue, expressed as a fraction of activity in the supernatant for the control animals and for animals receiving 2'-MDAB or 3'-MDAB respectively, was 34, 24, and 44% in the system for testing form A and 38, 38, and 66% in the system for testing form B.

The quantity of synthesized RNA is an indicator both of the ability of DNP to form an initiating complex with RNA-polymerase and of the ability of the enzyme to dissociate from DNP after growth of the RNA chain has stopped. The writers showed previously that liver chromatin of rats receiving the carcinogen 3'-MDAB, at all stages of aminoazo-carcinogenesis, can bind more RNA-polymerase of *E. coli* added to the incubation mixture than normal liver chromatin [2]. In the presence of low concentrations of ribonucleoside triphosphates the quantity of RNA synthesized will evidently be determined mainly by the number of initiated RNA strands, and an increase in the ability of DNP to associate with RNA-polymerase will lead to an increase in the incorporation of UMP-H^3. If, however, virtually all the RNA-polymerase is bound with DNP (as can be observed in high concentrations of DNP and ribonucleoside triphosphates, or in isolated nuclei), a reduction in the ability of the DNP—RNA-polymerase complex to dissociate may lead to less likelihood of liberation of RNA-polymerase from the template and reinitiation of RNA synthesis and, consequently, to the observed [2] decrease in RNA-synthesizing power of the nuclei.

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