

PRIMARY EFFECT OF DIMETHYLAMINOAZOBENZENE ON TRANSCRIPTION OF THE UNIQUE RAT LIVER DNA

T. V. BARSKAYA, N. V. ZAKHAROVA and Ju. M. OLENOV

Institute of Cytology of the Academy of Sciences of the USSR, Leningrad, 190121, USSR

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1. Introduction

At present there is convincing evidence of alteration of the RNA population in tumors [1–6]. Study of the alterations in RNA population after carcinogen treatment before the appearance of the tumor is of great importance for understanding carcinogenesis mechanisms. There are many papers dealing with the effect of different carcinogens on the RNA synthesis, both inhibitory and stimulating [7]. At present DNA–RNA hybridization is the most sensitive technique available for determining the differences in genomic expression.

In cancer research this technique has been used to compare the nuclear and cytoplasmic RNA obtained from the normal tissue with that of the liver tissue undergoing carcinogenesis. The results of competitive hybridization studies obtained by Shearer and authors [8–10] show that there are some RNA species in the liver cell cytoplasm of rat with carcinogen diet which in the normal liver are found in the nuclei only. The authors explain this fact by alterations in RNA transport from nucleus to cytoplasm. This view is confirmed by competitive hybridization of nuclear and cytoplasmic RNA observed by Garrett and coauthors [11]. However their experiments were carried out with more than 6 days carcinogen diet. Also hybridization conditions employed (low nucleic acid concentration and short incubation times) provided evidence on the hybridization of RNA with the rapidly reassociating repeated DNA sequences only [12–15]. Nevertheless none of the previous experimentators which measured the RNA–DNA hybridization of higher organisms observed hybrids between RNA and nonrepeated DNA sequences in case of cancer research.

So our task was to study the effect of hepatotropic carcinogen on a population of RNAs in different stages after a short treatment. The present work was undertaken to investigate the effects of 4-dimethylaminoazobenzene on the transcription of rat liver unique DNA after one day treatment.

2. Materials and methods

130–150 g Male rats were used. 4-Dimethylaminoazobenzene (DAB) in oil (50 mg/200 g body wt) was injected intraperitoneally 24 h before the animals were sacrificed. 40 min before slaughter [^{14}C] orotic acid (100 $\mu\text{Ci}/\text{rat}$) was administered to both control and carcinogen-treated rats.

Nuclear RNA was extracted with hot phenol at the temperature interval of 65–85°C [16] and treated with DNAase (Worthington, RNAase-free) and pronase (Serva). This fraction of RNA mostly consists of the precursor of information RNA. Specific radioactivity of RNA preparations was 1500–2000 counts/min/ μg .

DNA was isolated by a modified phenol–detergent procedure [17] and treated with RNAase (Reanal) and pronase. DNA was sheared in 0.14 M NaCl solution by sonication to fragments of ~500 nucleotide pairs, sedimenting at 7 S. After denaturation (20 min in boiling water bath) and reassociation to $\text{Cot} = 100$ in 0.12 M phosphate buffer, pH 6.8 (PB) at 60°C DNA was separated into single- and double-stranded fragments on hydroxyapatite columns according to Britten's procedure [12]. Slowly reassociating DNA which had renatured at $\text{Cot} > 100$ was eluted with 0.12 M PB.

DNA-RNA hybridizations were run at 66°C in 0.24 M PB solution at various Cot values using excess of rat liver DNA [15]. Hybridization samples were treated with RNAase (20 $\mu\text{g}/\text{ml}$, 15 min). After precipitation with cold 5% trichloroacetic acid hybrids were collected on membrane filters (HUFS, Czechoslovakia).

3. Results and discussion

Figure 1 illustrates the hybridization of slowly reassociating unique DNA sequences with control RNA and RNA from DAB-treated livers, the latter hybridizing to markedly smaller extent. This difference in hybridization values has been reproducibly demonstrated using at least two different RNA preparations. The experimental points of one curve differ from those of the other curve at a confidence level of 0.99 ($p < 0.01$). The above data thus seem to suggest that DAB induces specific alterations in the nuclear RNA population. The observed changes might result from changing the intensity of transcription of DNA sites that are active in the control.

To investigate the peculiarities of changes in the population of RNA synthesized on unique DNA sequences, competitive hybridization studies were performed. The competitive efficiency of unlabeled liver nuclear RNA from control rats and those treated for one day with DAB has been determined. Results of competition studies are shown in fig.2. For these studies duplicate

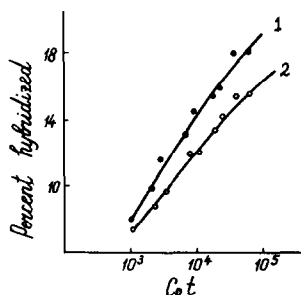


Fig.1. Hybridization capacity of nuclear [^{14}C] RNA isolated from livers of control (1) and DAB-treated rats (2) with the fraction of slowly renaturing DNA. 2–3 μg [^{14}C] RNA was incubated in 0.1–0.2 ml of 0.24 M PB, the ratio DNA/RNA = 150. Abscissa: Cot value. Ordinate: percent of hybridization.

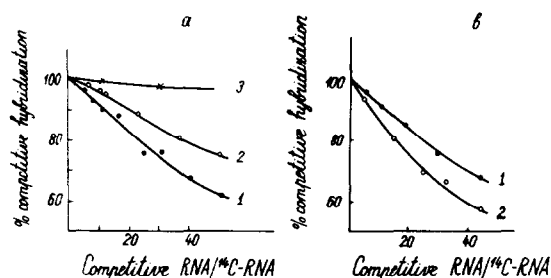


Fig.2. Competitive hybridization. Comparison of normal liver RNA (1) with that from liver nuclei of rats treated with DAB for 1 day. (2) [^{14}C] -Labelled RNA from normal (a) and DAB-treated liver (b), 5 μg , was incubated in 0.2 ml of 0.24 MPB, pH 6.8, with the fraction of unique DNA, 300 μg , $Cot = 5000$, in the presence of increasing amounts of unlabelled competing RNA. (3) Unspecific hybridization; competitor-cytoplasmic RNA from *E. coli*. Abscissa: the ratio of the amount of competitive RNA to [^{14}C] RNA in a hybridization sample. Ordinate: percent of competitive hybridization (hybridization in the absence of competitor is taken for 100%).

samples were run at each competition point. At the highest levels of competition the experimental points differ from those of the other curve with $p < 0.02$ and 0.05 in fig.2a and 2b correspondingly. Figure 2a shows that after one day of treatment nuclear RNA has less competition efficiency for control nuclear RNA than the control RNA. These results as the data shown in fig.1 suggest that carcinogen induces a decrease in the relative amount of RNAs transcribed from unique DNA sequences in liver cells, namely partial blocking of some unique DNA sites transcribed in the control is observed.

Results of analogous competition studies are shown in fig.2b. The hybridization curves differ significantly, this difference demonstrating that after one day of treatment nuclear RNA has more competition efficiency for the same RNA, than the control RNA. These data seem to suggest that carcinogen induces the appearance of some new RNA components which are not detectable in control, namely partial deblocking of some unique DNA sites is observed.

Thus in liver nuclei one day treatment of DAB induces blocking of some unique DNA sites. Along with these changes the partial deblocking of the other unique DNA sites seems to occur. The observed changes in

the RNA population under the action of DAB might be associated with the alterations of some tissue specific syntheses characteristic of the primary effect of carcinogens and of many tumors [7]. The activation of some unique DNA sequences might be associated for example with the appearing of proteins which are normally characteristic of embryo cells. Further investigations for clarifying the nature of alterations observed after short time of carcinogen treatment in the process of transcription are required.

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