Increased Interaction of Proteins in Nuclear Extract from Mouse Liver and Lung Tumors with TATA-Containing Oligonucleotide

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Increased TATA-binding activity of proteins in nuclear extracts from murine hepatocarcinoma HA-1 and murine Lewis lung adenocarcinoma was demonstrated. The dependence of the amount of formed complexes on protein concentration, displacement of labeled ^{32}P-TATA-containing oligonucleotide by its unlabeled analog, and weak interaction with an oligonucleotide containing damaged TATA box confirm specificity of the formed complexes.

Key Words: *TATA-binding protein; RNA polymerase; transformation*

TATA-binding protein (TBP) interacts with RNA polymerases I, II, and III and is a key factor in gene expression. TBP is bound to proteins, TBP-associated factors, and thereby participates in the formation of three transcription complexes, SL1, TFIID and TFIIIB [7], determining its involvement into transcription of RNA polymerase I, II, and III, respectively. The effect of TBP on transcription of a certain gene depends on the composition and location of the regulatory elements of the promoter [3,9]. There are two main mechanisms for recruitment of TBP to the promoters. In TATA-containing promoters, TPB directly binds to this sequence, while in non-TATA-containing promoters (*e.g.* promoters of RNA polymerase I and II) TBP interacts with other proteins bound to the promoter.

In human colon carcinoma, expression of TBP gene and protein content are higher than in normal epithelium [6]. Expression of TBP gene is enhanced in human lung and breast carcinomas and lung carcinoma cell lines [12]. Increased concentrations of TBP were observed in cells treated with phorbol ester, potential activator of protein kinase C, or expressing Ras oncogene [5,13]. The decrease in TBP content also induces specific changes in gene expression. Disruption of one TBP gene in chicken B cell line leads to disturbances in the expression of cell cycle regulator protein cdc25B phosphatase [11]. Heterozygous disruption of TBP gene in these cells caused abnormalities in cell growth and size and delayed mitosis. The involvement of TBP into transcription executed by PNA polymerase II and its recruitment to TATA-containing promoters is essential for initiation of cell transformation [7]. However, it remains unknown whether TBP-induced changes in transcription effected by RNA polymerases I and III [7] are essential for transformation and whether disturbances in the systems of transcription of nuclear RNA polymerases are necessary and sufficient for tumor development [7,14].

The aim of our study was to evaluate TATA-binding activity of TBP, a protein component of nuclear extracts from liver and lung tumors of A/Sn and C57Bl/6 mice, respectively.

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MATERIALS AND METHODS

Hepatocarcinoma HA-1 [8] and Lewis lung adenocarcinoma obtained from Tumor Strain Bank of Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, were used in the experiments. The tumors were maintained on A/Sn and C57Bl/6 mice, respectively, in a solid form. To this end, tumor cells were implanted into thign muscles every 2 weeks.

For isolation of nuclear extracts, peripheral parts of tumor nodes from decapitated animals were used (n=2-4). The tissue samples were placed into cold buffer containing 140 mm KCl, 1 mM EDTA, and 10 mM HEPES (pH 7.6, 0°C). Samples of the liver and lungs from intact mice of the corresponding strains served as the control. Immediately after decapitation of animals (n=4-6) the organs were perfused with cold buffer through the portal vein (liver) or through the heart (lungs). Nuclear extracts from the analyzed tissues were prepared as described elsewhere [5] with some modifications [10]. All manipulations were carried out at 0°C. Protein concentration was measured by the method of Bradford [2].

Oligodeoxyribonucleotides identical to 26-b.p. TATA-containing site of AdML promoter 5'-tga agg ggg gcTATAAAAggg ggtgc-3' (TATA-OH) and the control oligonucleotide with damaged TATA box 5'-tga agg tgg gcTGCTAGCgct ggt gg-3' (sequences on nontranscribed strands are presented, TATA boxes are shown by capital letters) were synthesized on an ASM-102I automatic synthetizer (Biosset) by the H-phosphate method and purified by electrophoresis in 12% denaturating PAAG. One oligonucleotide strand was labeled using [^{32}P]-ATP (Biosan) and T4 polynucleotide kinase (SibEnzyme) at 37°C for 1 h [1]. Then, the ^{32}P-labeled oligonucleotide was annealed with equimolar amount of unlabeled complimentary oligonucleotide at 80°C and slowly (for 3 h) cooled at room temperature. The duplexes were separated from the label and single-stranded oligonucleotides in 8% non-denaturating PAAG (0.5x TBE buffer).

Binding of nuclear extract proteins with ^{32}P-TATA-OH was carried out in a buffer of the following composition: 25 mM HEPES-KOH (pH 7.6), 5 mM $MgCl_2$, 80 mM KCl, 1 mM dithiotreitol, 0.2 mM EDTA, 0.2 mM EGTA, 0.01% NP-40, and 10% glycerol. First, 12 μg nuclear extract protein was preincubated with sonicated salmon sperm DNA (1 μg per 7 μg protein) for 15 min at 0°C for preventing binding of the nucleotide with nuclear proteins characterized by high nonspecific affinity to DNA. Then, 5 nM ^{32}P-oligonucleotide was added to the reaction mixture and the incubation was carried out at room temperature for 15 min. The complexes were analyzed by electrophoresis in 5% PAAG under nondenaturating conditions at 4°C (1x TBE buffer). The gels were dried and exposed with X-ray film at -70°C.

For evaluation of the differences in the binding of labeled TATA-OH with proteins from tumors and normal tissues, fragments of the gels (after electrophoresis and exposure with X-ray film) corresponding to TBP-probe complexes were cut and their radioactivity was measured on a RackBeta 1209 counter (LKB-Wallac).

For evaluation of the effect of cold nucleotide on binding of nuclear extract proteins with ^{32}P-oligonucleotide, a constant amount of protein (12 μg) was preincubated with increasing concentrations of cold oligonucleotide for 15 min and then with ^{32}P-oligonucleotide in a constant concentration of 5 nM for 15 min at room temperature. The reaction mixture was analyzed by electrophoresis in 5% PAAG under nondenaturating conditions at 4°C (1x TBE buffer). The complexes were detected by radioautography.

RESULTS

In all cases, we observed the formation of only one type of complexes with ^{32}P-TATA-OH and only one band in the gel corresponding to these complexes; the amount of complexes formed with proteins from hepatocarcinoma and lung adenocarcinoma surpassed the corresponding value for normal tissue by 60 and 30%, respectively (Fig. 1). It should be noted that the amount of formed complexes depended on protein concentration (Fig. 2), which attested to specificity of these complexes. ^{32}P-TATA-

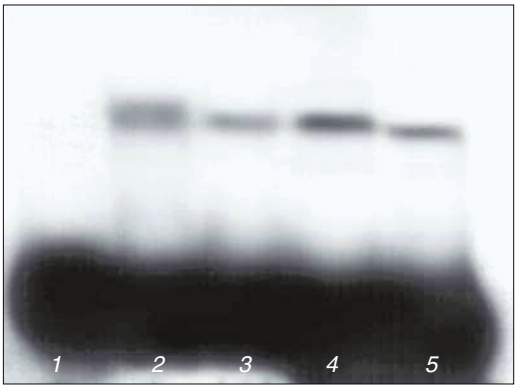

Fig. 1. Binding of nuclear extract proteins (12 μg) from mouse lung adenocarcinoma and hepatocarcinoma with ^{32}P-TATA-OH (5 nM). *1*) free ^{32}P-TATA-OH; *2*) lung adenocarcinoma; *3*) control, lungs of intact mice; *4*) hepatocarcinoma; *5*) control, liver from intact mice.

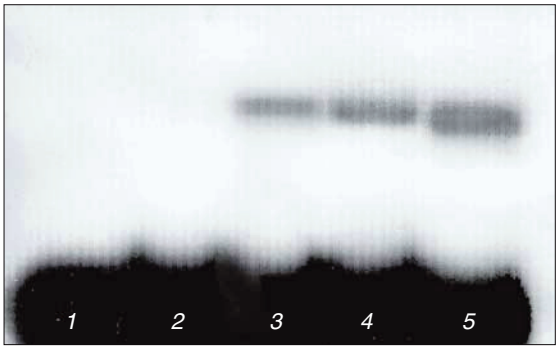

Fig. 2. Concentration dependence of binding of nuclear extract proteins from the lungs of intact mice with ^{32}P-TATA-OH (*2-5*). *1*) free ^{32}P-TATA-OH; *2*) 2 μg; *3*) 4 μg; *4*) 8 μg; *5*) 12 μg nuclear extract proteins.

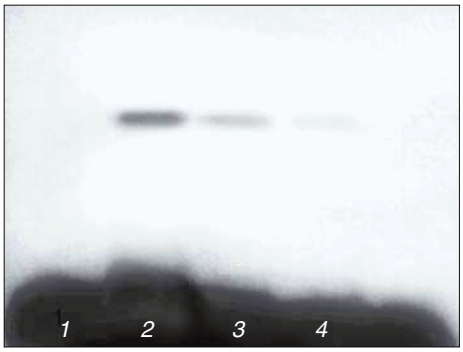

Fig. 3. Displacement of ^{32}P-TATA-OH (5 nM) from its complexes with TBP from lung adenocarcinoma with cold TATA-OH. *1*) control, free ^{32}P-TATA-OH; *2*) binding in the absence of cold TATA-OH; *3*) 25-fold molar excess; *4*) 100-fold molar excess of cold TATA-OH.

OH was almost completely replaced from the complexes even in the presence of a 25-fold excess of cold oligonucleotide (Fig. 3). This also confirmed specific nature of the formed complexes with ^{32}P-TATA-OH and the absence of the nonspecific component in the interaction. Thus, our findings suggest that ^{32}P-TATA-OH interacts only with TBP. An additional proof was low binding of nuclear extract protein with oligonucleotide containing damaged TATA box. It is known that TBP exhibits low affinity to non-TATA-containing DNA. Thus, our experiments demonstrated increased TATA-binding activity of TBP and changes in the transcription apparatus of murine HA-1 hepatocarcinoma and Lewis lung adenocarcinoma.

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