Functional Association of Immediate Early Gene *c-fos* with Nuclear Matrix

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 2, pp. 194-197, February, 2003
Original article submitted August 2, 2002

Accumulation of *c-fos* gene locus DNA in the nuclear matrix of hepatocyte nuclei was observed during induction of *c-fos* with cycloheximide. No enhanced association with the nuclear matrix was detected for inactive immunoglobulin gene locus. The use of endogenous DNases allows isolation of nuclear matrix preparations enriched with transcribing chromatin.

Key Words: *nuclear matrix; c-fos gene*

Cell nucleus has a complex skeletal structure — nuclear matrix (NM) with a wide spectrum of functions. Various pathologies caused by mutations in genes encoding NM proteins confirm the important physiological role of NM. Regulation of cell division includes binding to nuclear lamin, a protein encoded for by retinoblastoma susceptibility gene, which reflects a possible role of nuclear skeletal proteins in oncological processes [8].

DNA-binding sites on NM (MAR) can be divided into constitutive and functional (facultative) [5,14]. Constitutive MAR do not depend on gene status, they represent loop domains of the chromatin and are present in the same gene locus irrespective of the cell type or physiological status, for example, a constitutive MAR is situated in the immunoglobulin kappa gene locus [7]. Functional associations with NM are determined by activity of transcription in the gene locus [5,14]. Moreover, the interactions of many transcription factors with NM [9], frequently observed association of transcribed chromatin with NM [4,12], and other facts indicate that NM can regulate the transcription process [1].

There are many different methods for NM purification, and NM preparations obtained by different scientists have different characteristics [12]. We obtained NM preparations after activation of endogenous DNAses [3] and showed that association of the immediate early gene *c-fos* with NM increased after its induction in hepatocytes with translation inhibitor cycloheximide (CH). The specificity of the detected functional association of *c-fos* gene with NM was confirmed by the absence of this phenomenon in the immunoglobulin gene locus, which is inactive in hepatocytes.

Here we investigated association of chromatin with NM structures.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (140-160 g). Immediate early gene *c-fos* was activated by intraperitoneal injection of CH (3 mg/kg) in 0.9% NaCl. Liver samples for the analysis were collected 1 and 2 h after CH injection, which corresponded to the time of maximum expression of *c-fos* gene.

Hepatocyte nuclei were isolated after homogenization of tissue in 0.25 M sucrose solution in buffer A (20 mM NH_4Cl, 5 mM $CaCl_2$, 50 mM Tris-HCl, pH 9.0) with subsequent destruction of membranes in the same solution in the presence of 0.2% Triton X-100 and purification through a layer of 1 M sucrose in buffer A.

Purification of NM was carried out after activation of nuclear DNases by 15-min incubation of the

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0007-4888/03/1352-0167$25.00

nuclei in solution H (0.25 M sucrose, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 50 mM Tris-HCl, pH 8.0) at 30°C. The nuclear material was washed twice in TM solution (0.2 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5): 5 min at 4°C and then 20 min at 30°C (highly soluble chromatin). The final NM preparation was obtained after washing with 2 M NaCl in TM solution and 1% Triton X-100 in TM solution (poorly soluble chromatin).

DNA from NM preparations was isolated by dissolving the material in GTC solution (4 M guanidine thiocyanate, 20 mM Na acetate, 1% Na lauryl sarcosyl, 1% β-mercaptoethanol, 40 mM Tris-HCl, pH 8.0) with subsequent 2-3-min incubation at 65°C with 1% Na dodecylsulfate, deproteinization in phenol:chloroform:isoamine alcohol mixture (25:24:1), and RNAse A treatment. After DNA electrophoresis in 1.2% agarose (10 μg DNA per row) Southern hybridization with labeled probes (v-fos probe for *c-fos* gene and IgG light chain C_K constant fragment, $IgG(C_K)$, for immunoglobulin) was carried out.

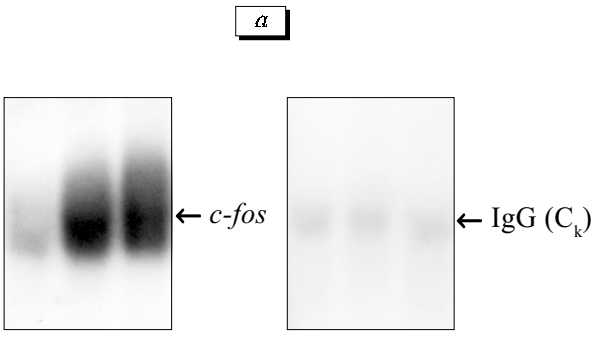
Hepatocyte RNA was isolated by tissue homogenization in GTC solution with subsequent ultracentrifugation in a CsCl gradient. After RNA electrophoresis under denaturing conditions (20 μg RNA per row) the expression of *c-fos* and $IgG(C_K)$ genes was evaluated by Northern hybridization with the corresponding probes.

RESULTS

Activation of early gene *c-fos* after various stress exposures, including inhibition of protein synthesis, was studied in detail [2,10]. Our results (Fig. 1, *a*) are in line with the data on *c-fos* activation during the first 2 h after treatment with CH (a potent inhibitor of translation). On the other hand, $IgG(C_K)$ is inactive in hepatocytes of both controls and CH-treated animals (Fig. 1, *a*). Pronounced induction of *c-fos* gene allows evaluation of functional local changes in chromatin structure associated with activation of transcription.

Inhibition of translation with CH reduced solubility of chromatin, *i.e.* decreased the volume of highly soluble material (Fig. 2, *a*) and reduced the degree of DNA degradation by nuclear DNases (Fig. 2, *b*). This probably reflects the process of chromatin compaction in hepatocytes. The content of chromatin in NM preparation after treatment with CH increases to 20% (*vs.* 5% in the control). On the one hand, accumulation of chromatin in NM can be explained by low degree of DNA hydrolysis, (Fig. 2, *b*). On the other hand, activity of RNA synthesis in hepatocytes increases 2 h after CH treatment [15], which attests to functional nature of these changes in NM. Further studies showed that activation of transcription at the level of individual loci is accompanied by gene-specific interactions of chromatin with NM.

In order to investigate the association of chromatin of *c-fos* and $IgG(C_K)$ gene loci with NM, DNA purified from NM was hybridized with the corresponding radioactive probes (Fig. 2, *b*). It was found that CH-induced activation of *c-fos* gene was associated with accumulation this gene DNA in NM. Interestingly, the 3-fold increase in *c-fos* gene expression (Fig. 1, *b*) was associated with approximately 6-fold increase in the content of this gene DNA in NM (Fig. 2, *c*). These data indicate a nonlinear correlation between gene activity and its functional association with NM. This can be due to the fact that interactions with NM are determined by many factors of different nature and it is difficult to evaluate the contribution of each factor into the observed changes. In contrast to *c-fos* gene, no changes in the association with NM were observed for the $IgG(C_K)$ gene locus repressed in hepatocytes both in the control and after CH treatment (Fig. 2). Association of $IgG(C_K)$ gene with NM both in the control and after CH treatment (Fig. 2, *b*) can be ex-



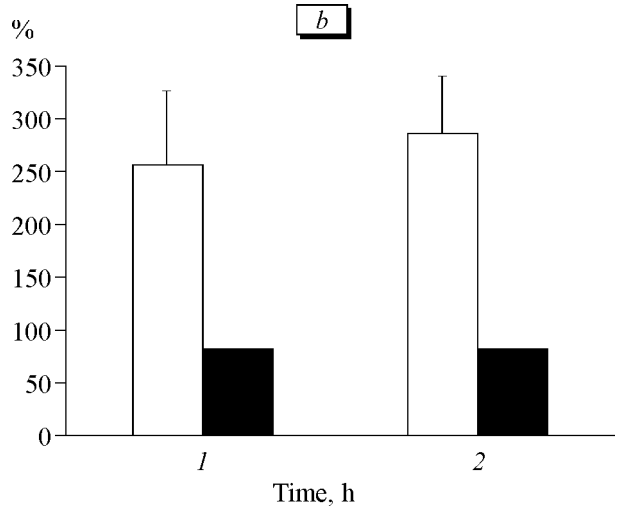


Fig. 1. Expression of *c-fos* and $IgG(C_K)$ genes in hepatocytes in the control and after cycloheximide treatment. *a*) northern hybridization of hepatocyte RNA with *v-fos* and $IgG(C_K)$ probes; *b*) changes in optical density of radioautographs obtained by Northern hybridization compared to the control (100%).

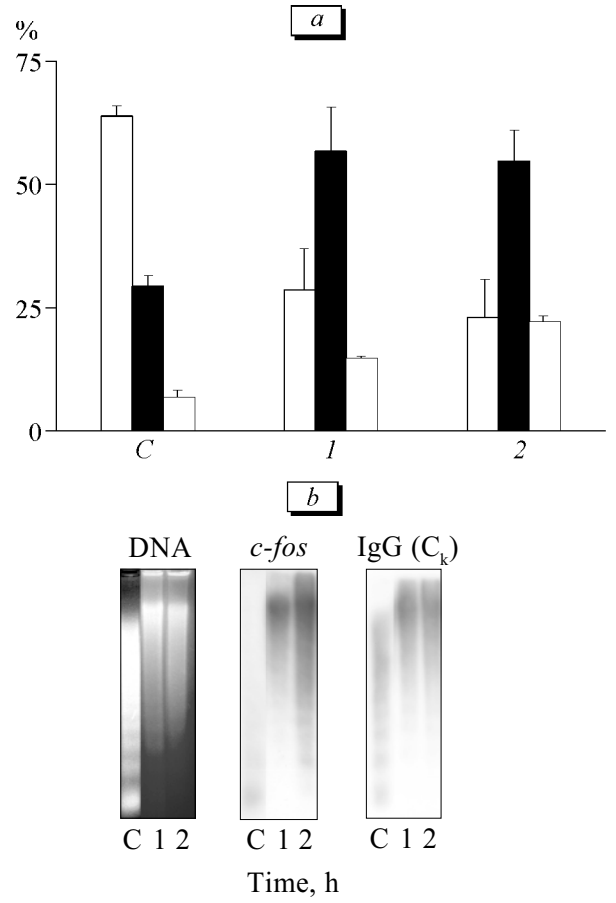


Fig. 2. Changes of total chromatin structure in hepatocytes in comparison with local reorganization of chromatin in *c-fos* gene locus. *a*) relative content of chromatin in soluble fractions and nuclear matrix fraction. Total chromatin content in hepatocytes is taken as 100%. Light bars: easily soluble chromatin; cross-hatched bars: poorly soluble chromatin; dark bars: nuclear matrix. *b*) electrophoregram of DNA purified from nuclear matrix fraction and radioautographs of Southern hybridization of this DNA with *v-fos* and *IgG*(C_κ) genes probes. *c*) changes in optical density of radioautographs obtained in Southern hybridization compared to the control (100%).

plained by the presence of constitutive MAR in this gene locus [7]. Hence, we detected functional association of *c-fos* gene with NM, caused by changes in its transcription activity.

The data on the association of transcribed chromatin with NM are contradictory and, as was shown, this association depends on the method of NM preparation. EDTA disturbs specific interactions between transcribed chromatin and NM [12], which can be due to destabilization of chromatin structure at low Mg^{2+} concentrations. In our experiments NM was isolated in TM buffer containing Mg^{2+}. It should be also noted that isolation of NM includes treatment with exogenous DNases, otherwise the greater part of chromatin is bound to NM and evaluation of specific features of interactions of transcribed chromatin with NM is impossible. We used nuclear (not exogenous) Ca^{2+}, Mg^{2+}-dependent DNases (incubation in buffer H) for destruction of DNA. We believe that this approach preserves the native chromatin structure, because activity of endogenous DNases is functionally regulated by intranuclear processes (replication, reparation, transcription) [6,13], which can also promote the detection of specific DNA interactions with NM structures.

The relationship between transcription and NM was discussed for a long time; it was hypothesized that NM is involved in transcription process. The mechanisms of such relationships are little known, but one of the most demonstrative facts is that many transcription factors are strongly bound with NM [1,11]. Many of these transcription factors participate in *c-fos* gene regulation [11], but it is unknown, which of these factors determined its induction in response to CH. It is also known that CH can induce stabilization of mRNA of some genes, *i.e.* accumulation of *c-fos* gene mRNA can be caused by both gene activation and stabilization of its mRNA [10].

Our data on accumulation of *c-fos* gene locus chromatin in NM suggest that CH activates *c-fos* gene. Similar data were previously obtained for *c-myc* gene [3]. Taking into account that many factors regulating activity of *c-fos* gene were detected in NM [1,2,11], we hypothesize that the association with NM observed during gene induction is mediated by binding of regulatory protein factors to its promoter.

Hence, our findings indicate that NM preparations adequately reflect the function of chromatin in various gene loci. A correlation between *c-fos* gene induction and its association with NM was revealed. The proposed method for NM purification helps to isolate NM preparations enriched with transcribed DNA and, evidently, with protein factors participating in the transcription.

The authors are grateful to Prof. F. L. Kiselev (Institute of Carcinogenesis, N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences) for plasmid containing *v-fos* gene DNA and to Dr. S. M. Deev, Doct. Med. Sci. (V. A. Engel'gardt Institute of Molecular Biology, Russian Academy of Sciences) for IgG(C_K) containing plasmid.

The study was supported by GNTP grant "Human Genome" and grant No. 211 from the 6th Expert Competition (1999) of Scientific Projects on Basic and Applied Research of Young Scientists of Russian Academy of Sciences.

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