Dual Effects of Endogenous DNases on Transcriptionally Active and Inactive Chromatin

N. P. Borisova, G. V. Kostyuk, N. A. Shevchenko, P. Ya. Boikov, E. V. Rudakova, R. I. Papina, and A. A. Terent'ev

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Experiments on resting hepatocytes with inactive *c-fos* gene and active albumin gene. We revealed that DNA of the transcribed gene is less susceptible to the influence of endogenous Ca^{2+}/Mg^{2+}-dependent DNases in matrix-associated and highly soluble chromatin fractions. In the fraction of low soluble chromatin active gene was more accessible for DNases. Our results indicate that activity of endogenous DNases can change in the transcribed gene locus.

Key Words: *endogenous DNases; transcriptionally active chromatin*

Chromatin is involved in the regulation of gene activity. The regulatory role of chromatin is revealed in comparing euchromatic and heterochromatic regions that differ in structural and biochemical characteristics (*e.g.*, protein composition and DNA methylation) [2, 5]. Inactive genes are localized in region of heterochromatin with high degree of DNA packaging. Euchromatin has a less compact structure and includes active or activable genes [3]. Histones and non-histone structural proteins of chromatin play a role in the regulation of transcription. Histone acetylases and deacetylases and chromatin-remodeling complexes responsible for local structural reorganization of chromatin during activation or repression of transcription are the main elements of the transcription complex [4,7].

DNases are an important tool in studies of structural characteristics of chromatin. Endogenous Ca^{2+}/Mg^{2+}-dependent DNases (CMD) are the enzymes activated in isolated nuclei in the presence of Ca^{2+} and Mg^{2+} and inducing internucleosomal chromatin fragmentation. Endogenous CMD and exogenous DNases predominantly cleave transcriptionally active chromatin [8]. Previous comparative studies were performed with genes present in euchromatin and heterochromatin and, therefore, differing in DNA hydrolysis in various gene loci.

Here we compared chromatin fragmentation in loci of the inactive *c-fos* gene and active albumin (*Alb*) gene in resting hepatocytes. It is important that differences in activity and state of chromatin in loci of these genes are not associated with heterochromatinization. Published data show that local activity of endogenous CMD can vary during transcription and fragmentation of chromatin in the gene locus.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 140-160 g. Hepatocyte nuclei were isolated by tissue homogenization in 0.25 M sucrose in buffer A (20 mM NH_4Cl, 5 mM $CaCl_2$, and 50 mM Tris-HCl; pH 9.0), 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.

Chromatin fractions were obtained after activation of intranuclear CMD by incubation of nuclei in solution H containing 0.25 M sucrose, 10 mM $MgCl_2$, 1 mM $CaCl_2$, and 50 mM Tris-HCl (pH 8.0) at 30°C for 15 min. Highly soluble chromatin was extracted with buffer TM containing 0.2 mM $MgCl_2$ and 50 mM Tris-HCl (pH 7.5) at 4°C for 5 min (fraction P1) and then at 30°C for 20 min (fraction P2). Low soluble

Laboratory of Molecular Biology, Institute for Problem of Chemical Physics, Russian Academy of Sciences, Chernogolovka, Moscow region. ***Address for correspondence:*** alexei@icp.ac.ru. Terent'ev A. A.

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chromatin (fraction T) was extracted with 2 M NaCl in TM. The nuclear matrix (fraction NM) was obtained after washing with 1% Triton X-100.

For DNA purification from chromatin fractions the material was dissolved in 4 M guanidine thiocyanate (GTC), 20 mM sodium acetate, 1% sodium lauryl sarcoside, 1% β-mercaptoethanol, and 40 mM Tris-HCl (pH 8.0), incubated with 1% sodium dodecyl sulfate at 65°C for 2-3 min, deproteinized with phenol: chloroform:isoamyl alcohol (25:24:1) mixture, and treated with RNase A. Electrophoresis of DNA in 2% agarose gel (10 μg DNA per row) and Southern hybridization with labeled probes (constructs containing *v-fos*, viral analogue of *c-fos* gene, and *Alb* gene cDNA, for *c-fos* and *Alb* genes, respectively) were carried out.

Hepatocyte RNA was purified by tissue homogenization in GTC solution and centrifugation in a CsCl density gradient. After RNA electrophoresis under denaturing conditions (20 μg RNA per row) expression of *c-fos* and *Alb* genes was studied by Northern hybridization with the corresponding labeled probes.

RESULTS

For comparative analysis of fragmentation of transcribed and nontranscribed chromatin by endogenous CMD we used *c-fos* and *Alb* genes characterized by different expression in hepatocytes. *c-fos* gene is inactive in resting hepatocytes, while *Alb* gene was intensively expressed (Fig. 1).

For evaluation of chromatin nucleolysis in loci of various genes in hepatocyte nuclei endogenous CMD were activated by incubation in buffer H. Highly soluble chromatin (fractions P1 and P2) is most susceptible to DNases (Fig. 2, *a*, *b*). DNA degradation was less intensive in the low soluble chromatin fraction compared to highly soluble fractions (Fig. 2, *c*). The minimum fragmentation was noted in nuclear matrix (NM) DNA (Fig. 2, *d*).

Differences in fragmentation and solubility of chromatin from these fractions are associated with various protein environments of DNA in chromatin extracted under various conditions. The qualitative composition of chromatin proteins and strength of their interaction with DNA determine nucleolysis and chromatin hydrolysis by exogenous DNases. It should be emphasized that activity of endogenous DNases and other cell proteins is regulated by various factors. As differentiated from nucleolysis by exogenous enzymes, the process catalyzed by endogenous DNases has two constituents. The structural component depends on the composition of chromatin proteins. The functional component is determined by DNase activity in a certain locus of the nucleus. A comparative analysis of the effects produced by endogenous CMD on chromatin of *c-fos* and *Alb* gene loci showed that the functional component of nucleolysis plays an important role in DNA fragmentation.

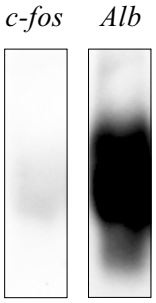

Fig. 1. Expression of *c-fos* and *Alb* genes in hepatocytes.

Since *c-fos* and *Alb* genes have different activity, it can be expected that DNases produce various effects in these gene loci. Our experiments showed that differences in DNA fragmentation in these gene loci are insignificant in highly and low soluble chromatin fractions (Fig. 2, *a*, *c*), but pronounced in the fraction of NM (Fig. 2, *d*). It should be emphasized that chromatin in active *Alb* gene was less fragmented than in the inactive *c-fos* gene.

Differential profiles of DNA served as the additional criterion of DNase activity for a detailed analysis of differences in chromatin fragmentation in soluble fractions. Each autoradiograph was obtained using DNA with a certain profile of the molecular-weight distribution. Subtraction of the curve for baseline DNA profiles from the densitogram allows evaluating the peculiar characteristics of genes. Differential profiles determined after this procedure are unsuitable for correct mathematical description of specific features, but reflect qualitative characteristics of chromatin fragmentation in a certain gene locus. If chromatin of the gene locus is fragmented similarly to nuclear chromatin, the differential profile appears like a straight line parallel to abscissa. Differences between the autoradiograph and electrophoretogram produce curvatures of the differential profile.

Experiments with highly soluble fractions P1 and P2 showed that chromatin in the *Alb* gene is less fragmented than in the *c-fos* gene (Fig. 3). In the locus of inactive *c-fos* gene chromatin was fragmented to di-, tri-, and tetranucleosomes. Larger chromatin fragments were present in the *Alb* gene locus. Therefore, highly soluble chromatin of the *Alb* gene locus was less susceptible to the influence of endogenous CMD (similarly to the fraction of NM). The fraction of low soluble chromatin had other characteristics. Fragmentation of inactive *c-fos* gene proceeded slower than that of active *Alb* gene (Fig. 3, *c*).

Published data show that transcribed chromatin is more susceptible to exogenous DNases [6]. Our results

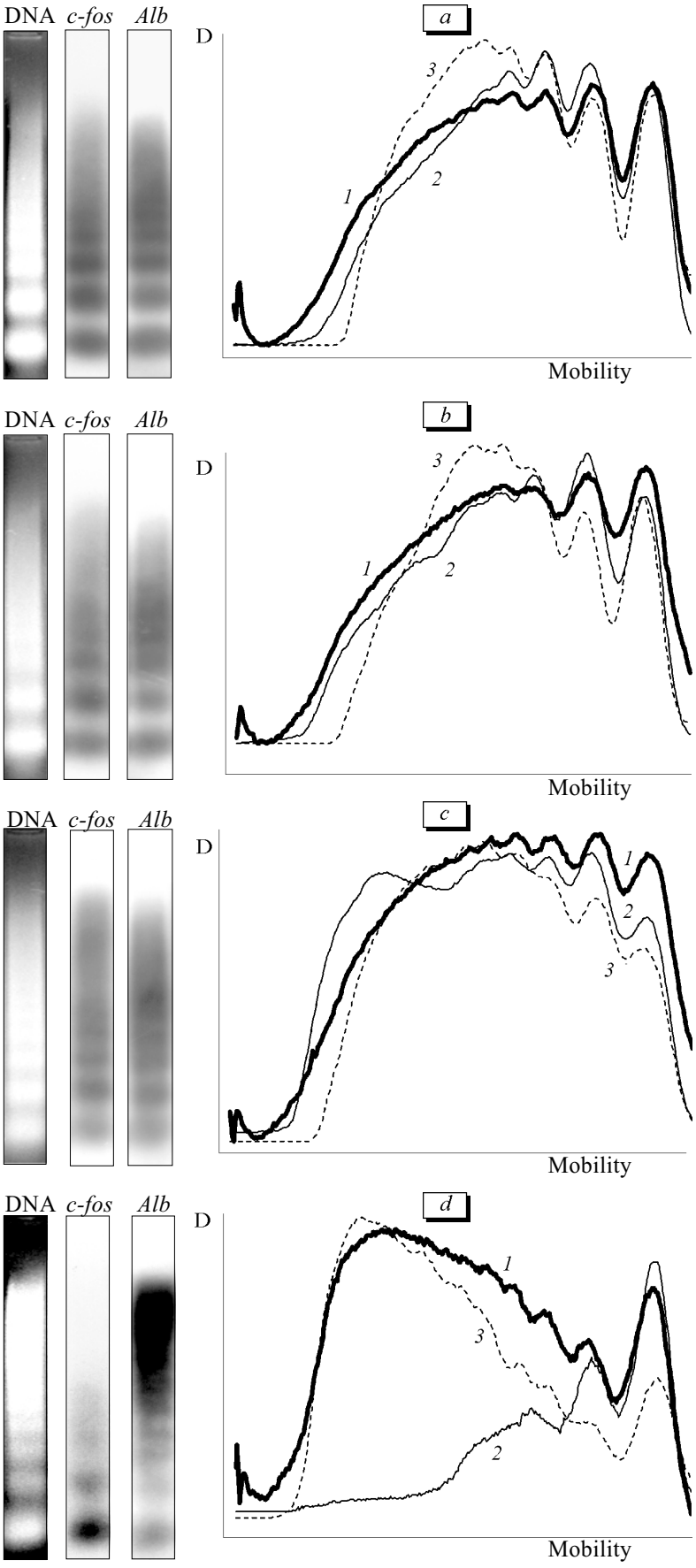

Fig. 2. Total and local chromatin fragmentation by endogenous Ca^{2+}/Mg^{2+}-dependent DNases. Electrophoretograms of DNA purified from fractions P1 (*a*) and P2 (*b*), low soluble chromatin fraction (*c*), and nuclear matrix (*d*) after activation of endogenous DNases. Autoradiographs obtained after hybridization of DNA from various fractions (*1*) with probes for *c-fos* (*2*) and *Alb* genes (*3*). *D*: optical density.

show that as differentiated from exogenous DNases, endogenous enzymes produce various effects. Chromatin is low fragmented in the locus of active *Alb* gene. Probably, in this gene locus DNA interacts with a greater number of transcriptional proteins. It can be suggested that inhibition of DNases is not related to their physical displacement from the transcribed locus by transcriptional proteins. In this instance, exogenous DNases would fragment transcribed chromatin to a lesser degree. We believe that transcriptional factors can inactivate local DNases. Probably, transcriptional factors and DNases are functionally interrelated. Therefore, inactivation of DNases can result from the influence of factors involved in transcription. These data indicate that the functional component of nucleolysis determines DNA profiles in highly soluble fractions of chromatin and NM.

The low soluble fraction of chromatin extracted in the presence of high salt concentration contains DNA strongly bound with structural proteins [1]. In this fraction the structural component plays an important role in nucleolysis. Therefore, the degree of chromatin fragmentation depends on the intensity of transcription. Transcribed chromatin of the *Alb* gene is more susceptible to DNases.

Interestingly, inactive and active genes were revealed in various fractions of chromatin. Since probes for *v-fos* and *Alb* genes include the transcriptional part of gene loci, it can be suggested that chromatin structure varies within the same gene locus. It cannot be excluded that these differences are related to heterogeneity of hepatocytes, and autoradiographs reflect various activity and structural organization of gene chromatin in individual cells. The distribution of intranuclear DNA in various gene loci requires further investigations.

Our results show that endogenous CMD produce various effects. In highly soluble fractions and NM intensively transcribed chromatin is low susceptible to nucleolysis by endogenous DNases. However, in the low soluble fraction inactive chromatin is fragmented to lesser degree. The functional and structural components of the effect of endogenous DNases were differently expressed in chromatin fractions obtained by us.

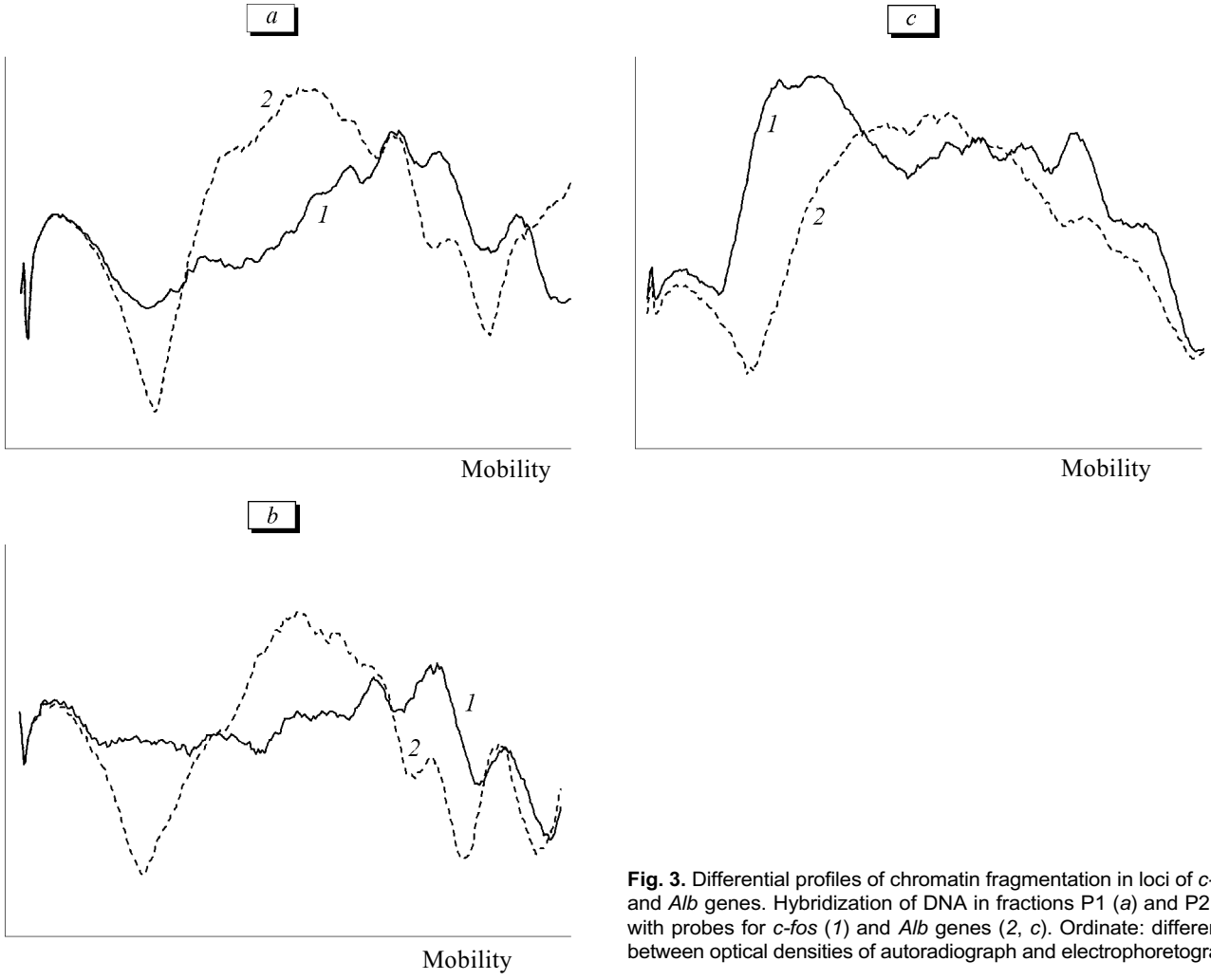

Fig. 3. Differential profiles of chromatin fragmentation in loci of *c-fos* and *Alb* genes. Hybridization of DNA in fractions P1 (*a*) and P2 (*b*) with probes for *c-fos* (*1*) and *Alb* genes (*2*, *c*). Ordinate: difference between optical densities of autoradiograph and electrophoretogram.

These data suggest that functional activity of endogenous DNases is regulated by transcriptional factors. Endogenous DNases can be used for selective fractionation of chromatin by structural and functional criteria.

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