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Differences in chromatin domain organization in chicken erythrocytes and cultured transformed erythroblasts induced to differentiation, revealed by nucleoprotein celite chromatography

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Chromatin domain organization was studied in non-differentiated chicken erythroblasts transformed by a temperature-sensitive avian erythroblastosis virus (HD3 cells), the same cells induced to differentiate and chicken erythrocytes. Nucleoprotein celite chromatography was used as the methodical approach. The method enables to discriminate several types of DNA–protein interactions in the nucleus including two types of DNA complexes with nuclear matrix proteins; it is also very sensitive to DNA breakage. In non-differentiated erythroblasts most part of DNA was involved in the tight complexes with nuclear matrix proteins. Formerly we have shown that these were the replicative complex proteins; DNA in most of the cells was not interrupted by breaks.

Differentiation of HD3 cells was accompanied by massive apoptotic DNA fragmentation, degradation of the tight complexes between DNA and the nuclear matrix, complete detachment of the chromatin fraction from the nuclear matrix. In mature chicken erythrocytes, a very different chromatin domain structure was observed. DNA was high-molecular, most part of it was bound to the nuclear matrix, it was equally distributed between the "loose" and the "tight" complexes with nuclear matrix proteins. It is concluded that chromatin domain changes coupled to differentiation in HD3 cells do not correspond to those occurring during natural differentiation of erythrocytes.

Key words: chicken erythrocytes, differentiation, nuclear matrix, HD3 cells, nucleoprotein-celite-chromatography

INTRODUCTION

It is becoming increasingly clear that transcription control is carried out at several interconnected levels. Beside nucleosomal organization and interaction between transcription factors and gene promoters and other regulatory elements, the long-range organization of chromatin in loops or domains seems to play a role in transcriptional regulation. Differentiation of avian erythrocytes is coupled to numerous rearrangements of the chromatin domain structure at different levels of their organization (reviewed in [1]). Chromatin condensation in erythrocytes is provided by binding several specific proteins to multiple DNA sites [2, 3]. On the contrary, chromatin loops lose several anchoring sites to the nuclear matrix [3]; intranuclear matrix fibrils degrade to some extent [4]. During the preceding decennia, attention of the researchers was mainly focused on studies of interactions of regulatory proteins with individual sequences that were considered to be crucial in the mechanisms of the differentiation (reviewed in [5]. However, these data alone cannot explain the mechanism of the differentiation. Nowadays many researchers become again interested in the regulatory role of rearrangements of the chromatin domain structure [6, 7]. Application of different approaches to the study of chromatin domain rearrangements should accelerate progress in this field. For many years we used nucleoprotein-celite chromatography for this purpose [8]. The method enables to discriminate among several types of DNA–protein interactions in the nucleus, including two types of DNA complexes with nuclear matrix proteins that are not revealed by other approaches. In the present paper we report the data on differences in the nucleoprotein spectrum of avian erythroid cells of different degree of differentiation:

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chicken erythrocytes, non-differentiated transformed chicken erythroblasts and the same cells induced to differentiate.

MATERIALS AND METHODS

Cells

Chicken erythrocytes were obtained from chicken blood as described [13], and lysed for the nucleoprotein celite chromatography. AEV-transformed chicken erythroblasts (clone HD3 [9]) were cultivated in DMEM medium supplemented with 2% chicken serum and 8% calf serum at 37 °C. Cell differentiation was induced by addition of 1-(5 isoquinolylsulfonyl) 2-methylpiperazine dihydrochloride (Sigma) to a final concentration of 20 μM and incubation of the cell cultures at 42 °C. The percentage of differentiated cells was determined by the benzidine reaction, their viability was evaluated by the trypan blue exclusion test.

Nuclear matrix isolation

Cells were lysed in 1mM $CuSO_4$, 10 mM PIPES pH 7.8, 100 mM NaCl, 0.3 M sucrose, 3 mM $MgCl_2$, 0.5% Triton X100; the nuclei were pelleted by low-speed centrifugation. Presence of copper ions enabled to preserve the nuclear matrix structure of erythroid cells [10]. Nuclear matrices were isolated according to the lithium iod dodecylsulfate (LIS) procedure [11]. DNA in isolated nuclei was fragmented by treatment with restrictase Sau3AI (80 U/ml, 1 h, 37 °C) prior to the nuclear matrix extraction.

NPC-chromatography

Nucleoprotein celite chromatography (NPC-chromatography) was performed as described [11]. About 4×10^7 of cells, nuclei or nuclear matrices were lysed in 1 ml of 1 mM $CuSO_4$, 10 mM PIPES pH 7.8, 100 mM NaCl, 0.3 M sucrose, 3 mM $MgCl_2$, 0.5% Triton X100. The lysate was directly applied on a precooled (0 °C) water-coated column of Celite R-630 (Fluka). The column was rinsed with 50 ml of 5 mM $MgCl_2$, 10 mM Tris HCl, pH 7.4 (breakthrough fraction) and 80 ml of NaCl (0–3 M) at a linearly increasing concentration was pumped through the column; the eluate was collected in two fractions. Then a gradient of LiCl–urea (0–4 M; 8M) was applied in the same manner. Finally the column was gradually heated from 0 °C to 100 °C under a constant flow of 4 MLiCl, 8 M urea solution. The eluate obtained after each gradient was divided into two fractions: by volume in NaCl and LiCl–urea fradients, below 70 °C and between 70 °C and 100 °C for the temperature gradient. Nucleic acids from the fractions were concentrated by absorption on hydroxyapatite and elution with 1 ml of 0.24 M phosphate buffer. DNA was purified with a Wizard DNA Clean-Up System kit (Promega) and subjected to electrophoresis in 1% agarose in 1TBE buffer. The protocol is schematically presented in Fig. 1.

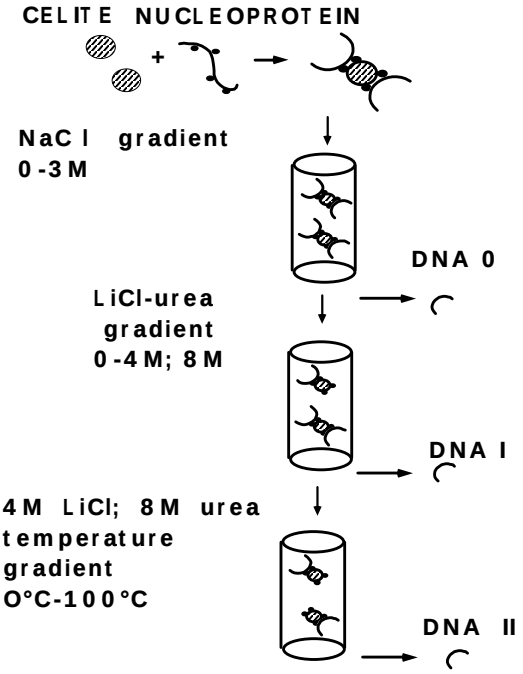

Fig. 1. Schematic presentation of the nucleoprotein celite chromatography method

RESULTS

NPC chromatography of the erythroid cell subnuclear fractions

Although formerly we have widely used NPC-chromatography for characterization of DNA–protein interactions in various cells, erythroid cells have never been studied. Taking into account the fragility of the nuclear matrix network in these cells [10] we have performed several model experiments in order to characterize the chromatographical distribution of different nucleoprotein complexes extracted from HD3 cells. It is generally accepted that DNA eluted in the NaCl gradient (DNA 0) is bound to histones, the LiCl–urea gradient destroys "weak" bonds between nuclear matrix proteins and DNA, the DNA I fraction is eluted, a high temperature is necessary to release DNA from the tight complex with the replication machinery enzymes [8]. Chromatin and the nuclear matrix were extracted from the non-induced HD3 cells. Figure, 2a presents the elec-

tropherograms of DNA precipitated from different chromatographic fractions of the soluble chromatin preparation extracted with LIS from the restrictase-treated nuclei. As expected, the oligonucleosomal DNA fragments are predominantly eluted with NaCl gradient. In the nuclear matrix preparation extracted with LIS from the restrictase-treated nuclei, DNA was distributed mostly between the first fraction of the LiCl–urea gradient and the second fraction of the temperature gradient (Fig. 2b). Thus, in HD3 cells NPC-chromatography also reveals tight and weak DNA-nuclear matrix complexes. Apparently the nuclear matrix structure was preserved during its isolation following a special protocol elaborated for erythroid cells [10, 11].

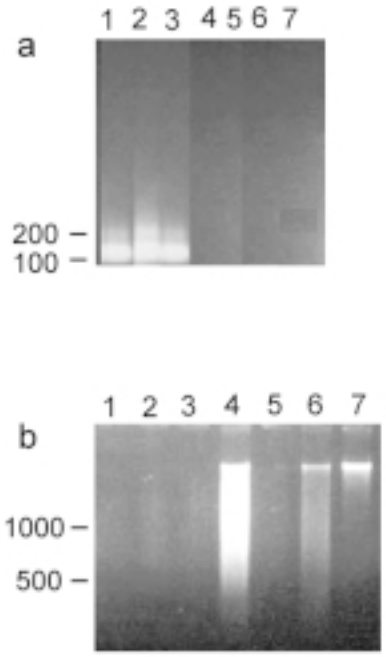

Fig. 2. Electrophoregrams of DNA precipitated from nucleoprotein celite chromatography fractions of subnuclear preparations of HD3 cells.
a – soluble chromatin; *b* – nuclear matrix isolated according the LIS procedure. *1* – breakthrough fraction; *2, 3* – Na Cl gradient fractions (DNA0); *4, 5* – LiCl–urea gradient fractions (DNA I); *6, 7* – temperature gradient fractions (DNA II). Arrows indicate positions of molecular weight markers (base pairs)

NPC chromatography of erythroblast nuclei and erythrocyte cell lysates

NPC-chromatography is extremely sensitive to rearrangements of chromatin domain structure as compared to conventional methods [8]. When intact nuclei are applied on a celite column, the integrity of the chromatin domains can be tested. If the domains are intact, DNA can be released from the column only after dissociation of an extremely tight complex of nuclear matrix proteins with DNA, and DNA is eluted as one fraction at the end of the temperature gradient. If the DNA–matrix complex is loosened, part of DNA is eluted by LiCl–urea. Detachment of the chromatin fraction of the nuclear matrix results in appearance of DNA eluted with NaCl fom DNA–histone complexes [8]. Figure 3a presents DNA electropherograms obtained after NPC chromatography of the HD3 cell nuclei directly applied on the column. Most part of the DNA is eluted in the high temperature fraction; DNA in this fraction is high-molecular. Fractions of DNA eluted with NaCl and LiCl–urea gradients are heterogeneous in size; part of them are fragmentized up to nucleosome ladders. The observed disconnection of the domains is due to presence of a subpopulation of apoptotic cells in the culture (about 5% according to the trypan blue exclusion test). Induction of HD3 to globin synthesis provoked dras-

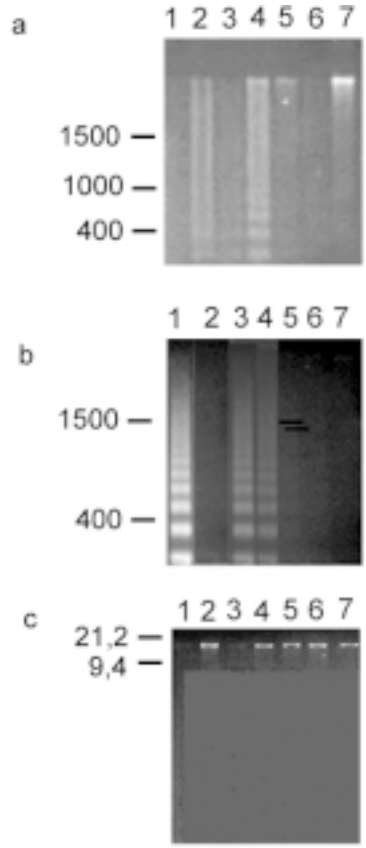

Fig. 3. Electrophoregrams of DNA precipitated from the nucleoprotein celite chromatography fractions of nuclei or cell lysates of erythroid cells.
a – nuclei of non-differentiated HD3 cells; *b* – nuclei of differentiated HD3 cells; *c* – lysate of chicken erythrocytes. *1* – breakthrough fraction; *2, 3* – NaCl gradient fractions (DNA0); *4, 5* – LiCl–urea gradient fractions (DNA I); *6, 7* – temperature gradient fractions (DNA II). Arrows indicate positions of molecular weight markers (base pairs for *a* and *b*, kilobases for *c*)

tic changes in the chromatin domain organization (Fig. 3b). The culture was incubated for 3 days at 42 °C in presence of the inducer. In general, it reflects massive apoptotic fragmentation of DNA (37% of the cells were stained with trypan blue), most part of DNA of oligonucleosome size is eluted in the breakthrough fraction, thus histone–DNA interactions appear to be altered in these apoptotic nucleosomes. No DNA fraction eluted by high temperature is observed any more. Thus, all the chromatin domains are disconnected from the replication complex. DNA still involved in nucleoproteins is distributed between the DNA I fraction weakly attached to the nuclear matrix and the chromatin fraction (DNA 0). Although 57% of these cells synthesized globin according to the benzidine test, changes in the organization of the chromatin domains coupled to erythroid differentiation were different from analogous rearrangements in erythrocytes. The NPC-chromatogram of lysate of chicken erythrocytes (Fig. 3c) drastically differs from that of HD3 cells. High molecular DNA appears in fractions of all gradients. Most part of DNA is bound to the nuclear matrix, as it is eluted as DNA I and DNA II fractions.

DISCUSSION

In the present study we have applied the nucleoprotein celite chromatography method to study overall changes of chromatin domain organization during induction of erythroid differentiation in AEV-transformed chicken erythroblasts (HD3 cells) and compared it to the domain organization in chicken erythrocytes. In non-differentiated erythroblasts most part of the DNA is involved in the tight complexes with nuclear matrix proteins. Formerly we have shown that these were the replicative complex proteins [8]. Apparently in most of the cells DNA is not interrupted by breaks, thus DNA cannot be eluted before the tight bond is destroyed. The small DNA fraction eluted by NaCl and LiCl–urea gradients is probably due to the population of apoptotic cells in the culture. The differentiation of HD3 cells is accompanied by massive apoptotic DNA fragmentation, degradation of the tight complexes between DNA and the nuclear matrix, complete detachment of the chromatin fraction from the nuclear matrix. In mature chicken erythrocytes, a very different chromatin domain structure is observed. DNA is high-molecular, most part of it is bound to the nuclear matrix, it is equally distributed between the "loose" and the "tight" complexes with nuclear matrix proteins. Presence of an important "loosely" bound DNA fraction indicates a probable disconnection of DNA domains; induction of a double strand break between the sites of the "strong" and the "loose" DNA bonds with the nuclear matrix proteins enables the release of the DNA I fraction during chromatography. Large-scale fragmentation of DNA has been detected in Xenopus erythrocytes [12]; apparently the same process occurs in chicken

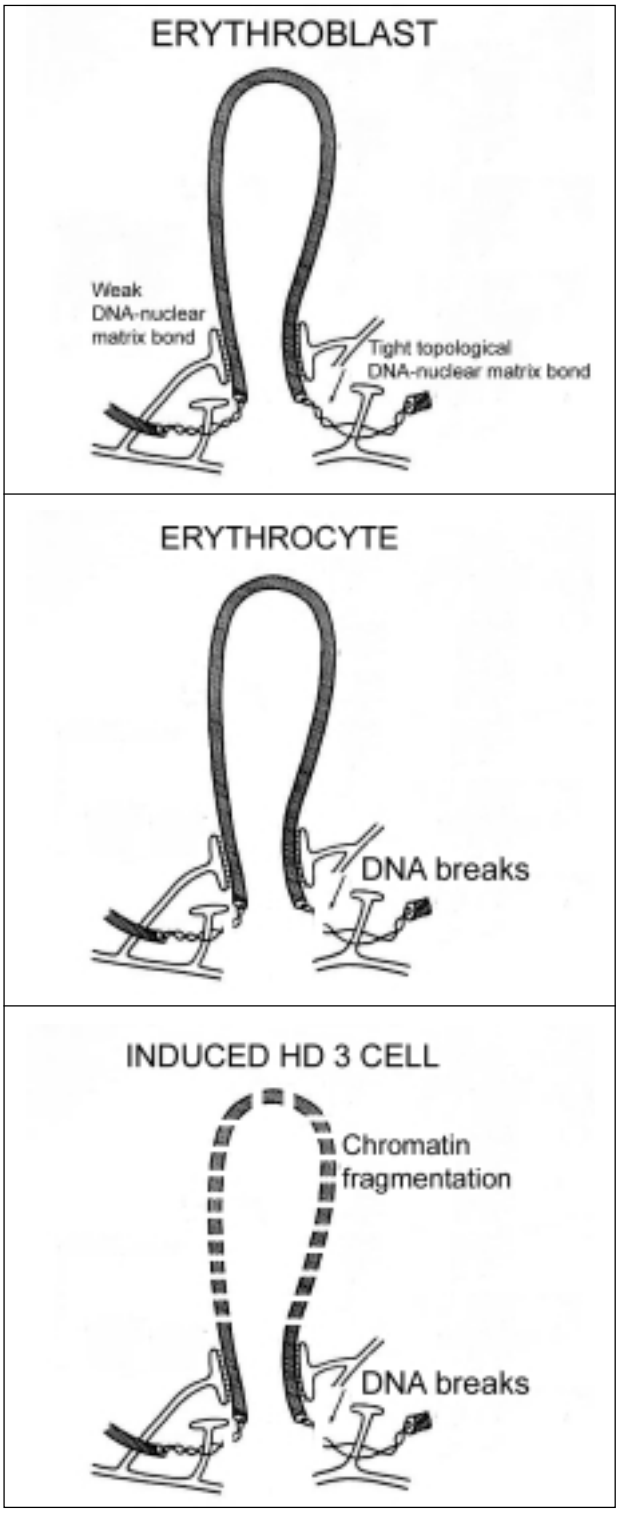

Fig. 4. Schematic presentation of changes in chromatin domain structure coupled to differentiation in HD3 cells and erythrocytes

erythrocytes. Degradation of part of nuclear matrix proteins or release of these proteins from DNA–protein complexes is another probable mechanism of the appearance of the DNAI fraction. Detachment of DNA from the nuclear matrix during avian erythrocyte maturation was reported by several researchers. In chicken alpha-globin, the number of attachment sites to the nuclear matrix decreases in erythrocytes as compared to erythroblasts [13]; in the same gene, the domain specific group of proteins tightly attached to DNA forms a link between DNA and the nuclear matrix in erythroblasts, but it becomes detached from the nuclear matrix in erythroblasts [14]. The process of differentiation in avian erythrocytes is followed by a decrease in the number of nuclear matrix proteins, histone deacetylase activity and in the number of transcription factors bound to the nuclear matrix [15, 16]. Despite the functional decline of the nuclear matrix enzyme machinery during erythroid differentiation, interactions of DNA with nuclear matrix proteins persist in avian erythrocytes, erythrocyte DNA can be cross-linked to the nuclear matrix proteins with *cis*-platinum [17]; moreover, specific nuclear matrix interacting sequences are involved in these interactions [18]. Differences in the chromatin domain changes during normal erythrocyte differentiation and differentiation of the transformed erythroblasts are shown in Fig. 4. HD3 cells are considered to correspond to hematopoetic cells of the red lineage arrested in early stages of differentiation [19]. Apparently the way these cells undergo differentiation is very different from the normal erythroid differentiation, at least at the level of chromatin domain reorganization, as the differentiation is followed by apoptotic fragmentation of the chromatin.

Taken together, our data illustrate the informative value of nucleoprotein celite chromatography. In rather simple experiments we have managed to reveal profound changes in the chromatin domain organization coupled to erythroid differentiation. We believe that when applied together with methods of molecular genetics our approach will become a potent tool for studying functional changes in the chromatin domains.

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