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PIOTR WIDŁAK, JACEK ROGOLIŃSKI and JOANNA RZESZOWSKA-WOLNY

INTERACTION OF DNA WITH NUCLEAR SKELETON IN RAT HEPATOCYTES

Institute of Oncology, Department of Tumour Biology,
Wybrzeże Armii Czerwonej 15; 44-100 Gliwice, Poland

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We have characterized fractions liberated from rat liver cell nuclei digested with DNase I and treated with buffers containing 0.1, 0.5 and 2 M NaCl. Analysis of DNA and proteins present in these fractions as well as in nuclear matrix, confirms the chromatin model according to which transcriptionally active loops interact differently with nuclear skeleton than inactive ones.

Recently proposed "domain model" for eukaryotic DNA functioning assumes that DNA is organized into topologically constrained loops which may exist in different conformations [1-3]. It was suggested that cell type specific control of gene expression depends on a combination of stable and dynamic DNA-nuclear skeleton interactions [3]. In this work we characterize four fractions containing DNA and proteins differing in type of their interaction with nuclear structures.

MATERIALS AND METHODS

Nuclear matrix was isolated according to Berezney & Coffey [4] with some modifications as described elsewhere [5, 6]. Proteins of different fractions were characterized by SDS polyacrylamide gel electrophoresis according to Laemmli [7]. DNA electrophoresis was performed on 1% agarose gel according to Maniatis *et al.* [8]. The enrichment of different fractions in particular sequences was assayed on the basis of DNA - DNA dot - blot hybridization using Gene Screen membrane, as previously described [5].

RESULTS AND DISCUSSION

Purified rat liver nuclei were suspended in 0.1 M NaCl and digested with DNase I for 30 - 120 min. Proteins and DNA liberated from the nucleus during this incubation were collected by centrifugation as fraction 1. Treatment of the pellet with 0.5 M NaCl liberated DNA and proteins of fraction 2. Further fractions contained the material liberated (fraction 3) and remaining in pellet (nuclear matrix) after treatment with 2 M NaCl. Figs. 1 and 2 present electrophoregrams of DNA and proteins appearing in these fractions.

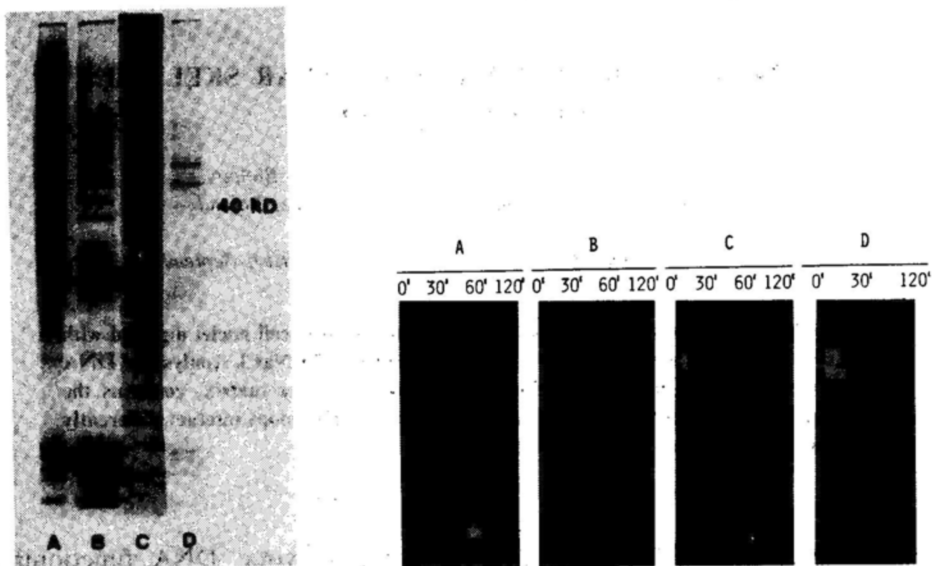

Fig. 1. Electrophoretical comparison of DNA present in the fractions liberated by different salt concentrations after the indicated time of DNase I digestion. A, fraction 1 (0.1 M NaCl); B, fraction 2 (0.5 M NaCl); C, fraction 3 (2 M NaCl); D, nuclear matrix fraction

Fig. 2. Electrophoretical comparison of proteins present in the fractions liberated by different salt concentrations (cf. Fig. 1). A, fraction 1; B, fraction 2; C, fraction 3; D, nuclear matrix fraction. DNase I digestion time 30 min

DNA. Even in the absence of exogenous nucleases rat liver DNA isolated from purified nuclei was always degraded to some extent because of high level of internal nucleases present in this tissue (lanes showing time 0 on Fig. 1).

The DNA of fraction 1 was more sensitive to the action of endogenous and exogenous nucleases than DNA from other fractions (lanes A on Fig. 1). Digestion with DNase I caused an increase in the amount of DNA in fraction 1, whereas the amount of DNA in fraction 3 and nuclear matrix decreased. During prolonged digestion the level of DNA in fraction 2 was rather stable and reached 60-70% of total DNA.

Table 1

DNA sequences enriched in fraction 2 and nuclear matrix

Fraction 2	Nuclear matrix
Repetitive sequences present in bands after EcoRI and MspI	Genes: KC JE DHFR TK
Oncogenes: erbA erbB fms	hsp 70-like myc fos

Fraction 2 and nuclear matrix of rat liver cells were assayed by dot-blot hybridization for the presence of different types of sequences (Table 1). Fraction 2 was enriched in repetitive sequences and sequences hybridizing to some oncogenes as erbA, erbB and v-fms. These genes are transcribed neither in normal nor in regenerating rat liver [9]. Nuclear matrix fraction showed the enrichment in sequences coding for genes actively transcribed in normal rat liver or induced for transcription during liver regeneration [9]. Also in fraction 1 the sequences hybridizing to KC gene were found to be enriched. During DNA degradation sequences hybridizing to KC increased in fraction 1 and decreased in the nuclear matrix fraction.

Proteins. Fraction 1 contained many different nonhistone proteins of high molecular weight and the complete set of core histones. Histone H1 was not present or present in very low concentration in comparison to other proteins.

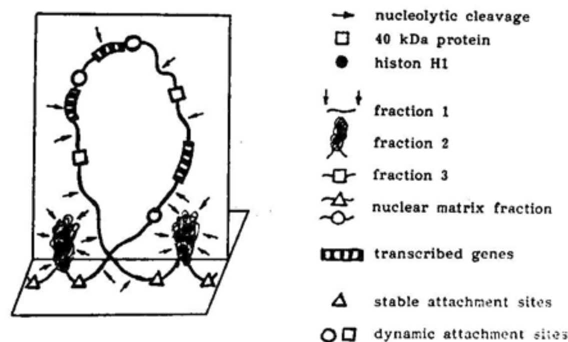

Fig. 3. Proposed model of interaction between nuclear matrix and different domains of DNA. Domains in the extended conformation contain active and potentially active genes (nuclear matrix fraction and probably fractions 1 and 3). Domains in the condensed conformation contain noncoding sequences and nonactive genes (fraction 2)

Histones were the most prominent proteins of fraction 2. In this fraction histone H1 was present in similar amounts as core histone proteins (Fig. 2B), suggesting that fraction 2 contains compact forms of chromatin. Among the proteins present in fraction 3 predominating was that of about 40 kDa (Fig. 2C). Proteins found in nuclear matrix were mainly lamins of about 40 kDa to 70 kDa (Fig. 2D).

Our results not only fit but also supplement the model proposed by Bodnar [3], in which DNA loops containing active or potentially active fragments of the genome are extended into the internal fibrillar network and are more closely attached to nuclear skeleton than inactive ones. Our suggestions are summarized in Fig. 3.

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