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DNA-PROTEIN COMPLEXES OF THE NUCLEAR MATRIX: VISUALIZATION AND PARTIAL CHARACTERIZATION OF THE PROTEIN COMPONENT

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Summary. Complexes composed of DNA attached to the nuclear matrix and of proteins most tightly bound to DNA are visualized as globular particles 25-35 nm in diameter. Their morphology depends greatly on the isolation conditions: a Cs salts/urea combination permits the isolation while CsCl/sarcosyl destroys the particles. The preparation is shown to have the same protein content regardless of the treatment employed. The proteins of the complex are resistant to SDS and pronase treatment and to phenol/chloroform extraction while being associated with DNA.

INTRODUCTION. During the past decade much attention was given to the investigation of the internal skeletal structure of the eukaryotic nucleus described in (1,2). This structure is involved not only in chromatin compactization (3) but serves as a structural milieu for quite a number of intranuclear activities, such as replication, and transcription (4-6) and primary transcript processing(7).

Such a diversity in the functional activities associated with the nuclear matrix makes it necessary to comprehend how the sites of DNA attachment to the nuclear matrix fibril are organized. It seems obvious that there must be at least several types of such sites: some of them are transitory and made up by an engaged replication fork or a transcription complex while others are permanent and involved in maintaining the compact structure of chromatin. These situations are discussed in a recent review (8).

Analysis of DNA fragments remaining attached to nuclear matrix fibrils led to the identification of a consensus sequence quite

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similar to that of a TOPO II recognition site (9-13). It seems that eukaryotic replication origins also reside in the nuclear matrix (14-17) and, moreover, it is possible that there is a consensus sequences among them (18).

Another approach to investigation of the whole complex of attachment and not only of DNA fragments associated with the nuclear matrix is to isolate DNA-protein complexes located within a nuclear matrix fibril. Such an approach allows one to isolate all types of DNA-protein complexes, provided one can find a way to stabilize a particular complex; an additional advantage concerns the possibility to identify and analyze proteins associated with a given type of DNA fragment. We have developed a procedure for isolation of a DNA-protein complex, which enabled us to identify seven proteins most tightly bound to matrix DNA (19). Functional analysis of such complexes was performed to show that they might play a role in defining what parts of the genome would be transcribed in a given type of differentiated cell (20). In our studies presented here we tried to visualize such complexes prepared under different conditions and to investigate some properties of their protein component.

METHODS

Cell culture. A murine plasmacytoma cell line P_3O_1 kindly provided by Dr.I.N.Trakht (All Union Research Center for Cardiology) was used throughout this study. The cells were cultured in roller bottles containing Eagle's medium supplemented with calf serum (10%), penicillin (100 u/ml) and streptomycin (100 ug/ml). [^{3}H]-thymidine (0,3 uCi/ml) was added 24 hours prior to harvest.

Nuclear matrix isolation. A procedure described in (19) was used with minor modifications. (1). $CuSO_4$ concentration in lysis buffer was reduced to 1 mM; (2). DNase I digestion was performed at a DNase concentration of 10 ug/ml and a $MnCl_2$ concentration of 1.2 mM.

Nuclear matrix lysis and DNA-protein complex isolation. The lysis was performed as described in (19) with several alterations. When CsCl or Cs_2SO_4/urea method was used, nuclear matrices were suspended in an appropriate salt solution (ρ 1.7 for CsCl or 1.2 for Cs_2SO_4) made up to 4 M with urea. After incubation for 1 hour at 60°C a CsCl/urea preparation was spun in a 50Ti rotor (Beckman) at 40 krpm and 16°C for 48 h. Cs_2SO_4/urea preparation was treated in the same way and layered on top of a Cs_2SO_4/urea gradient made up by layers of an increasing density: ρ 1.35; 1.5; 1.65; 1.8. This preparation was spun in a SW 50.1 (Beckman) at 32 krpm and 20°C for 24 h. A Cs_2SO_4/sarcosyl preparation was treated and

centrifuged as described in (19). Gradient fractions were obtained after piercing the tube bottom. Radioactivity was measured as described in (19). DNA containing fractions were pooled and, after dialysis against triethanolamine-EDTA buffer (5 mM-0.1 mM), processed for electron microscopy or protein analysis.

Protein analysis. Iodination of the protein component, DNA digestion sample preparation and electrophoresis were done as described in (19).

Deproteinization was carried out according to (21).

Electron microscopy. Dialysed samples were treated with glutaraldehyde (2.5%, 1 h, 20°C) and prepared for microscopy as described in (22). Ethidium bromide was used as an effective mediator for adsorption of nucleic acids molecules to carbon support (23). The sample droplet contained 100-200 ug/ml of ethidium bromide. Specimens were dehydrated by ethanol, rotary shadowed with platinum-palladium alloy and examined in a Philips-400 electron microscope. Micrographs were taken at 10000 - 15000x.

RESULTS

The general scheme for isolation of DNA-protein complexes was the same as elsewhere (19): nuclear matrices were subjected to lysis by different dissociating agents at a high ionic strength (Cs ions) and purified by isopycnic gradient centrifugation. Gradient fractions containing DNA were combined and analyzed by electron microscopy. It turned out that the results of such an experiment depended most strongly upon the combination of dissociating agents which were used to lyse nuclear matrix preparation. Whenever we used Cs_2SO_4/urea (see Methods) we got lots of clusters or aggregates associated with DNA fibers (Figure 1A). One can see that the structure of such clusters makes it difficult to identify individual globules although it is quite evident that the clusters are composed of them. In order to disperse these clusters, we substituted urea with sarcosyl in nuclear matrix lysis and subsequent Cs_2SO_4 centrifugation. Much to our surprise however, such a treatment destroyed not only the clusters but also the globules; only DNA-like fibrils could be seen in DNA-containing fractions of the gradient (Figure 1B).

Another attempt to disperse the clusters while preserving the structural integrity of the particles was based on the different dissociating properties of Cs_2SO_4 and CsCl (24).One can see (Figure 1C) that the combined treatment with CsCl/urea yielded well-dispersed globular particles associated with DNA.

It may be of interest that such a diversity in the appearance did nothing to modify protein content of these structures (Figure

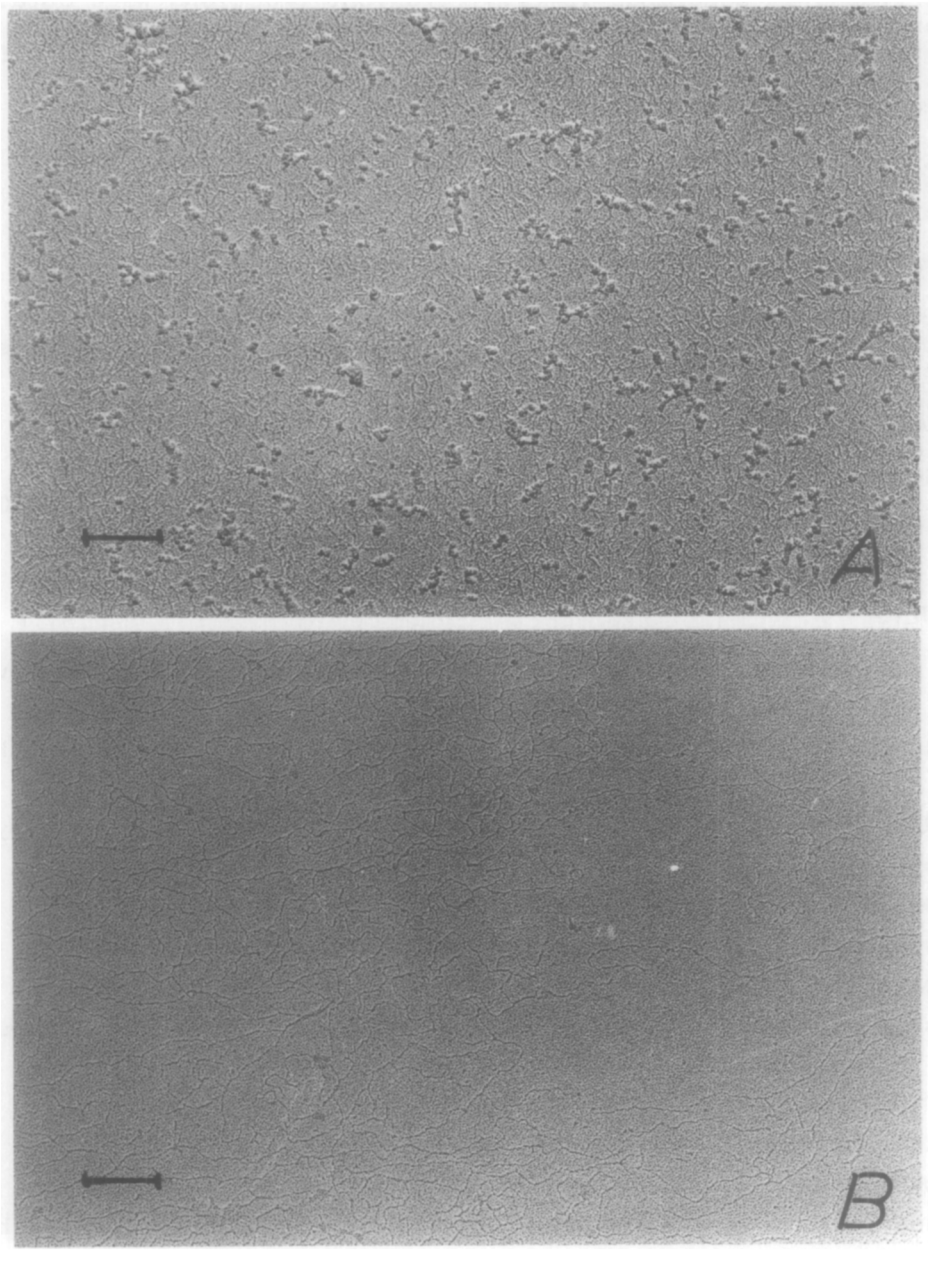

Figure 1. Electron microscopy of the complex composed of proteins most tightly bound to matrix attached DNA. The isolation procedures are described in **METHODS**. A. DNA-containing fractions of a Cs_2SO_4/urea gradient. B. The same of Cs_2SO_4/sarcosyl gradient. C. The same of a CsCl/urea gradient. Bars represent: A=360 nm, B=282 nm, C=220 nm.

2A). It seems that only proteins tightly bound to DNA survive the combined treatment with Cs salts and urea or sarcosyl. Based on these observations, we presume that detergent treatment affects

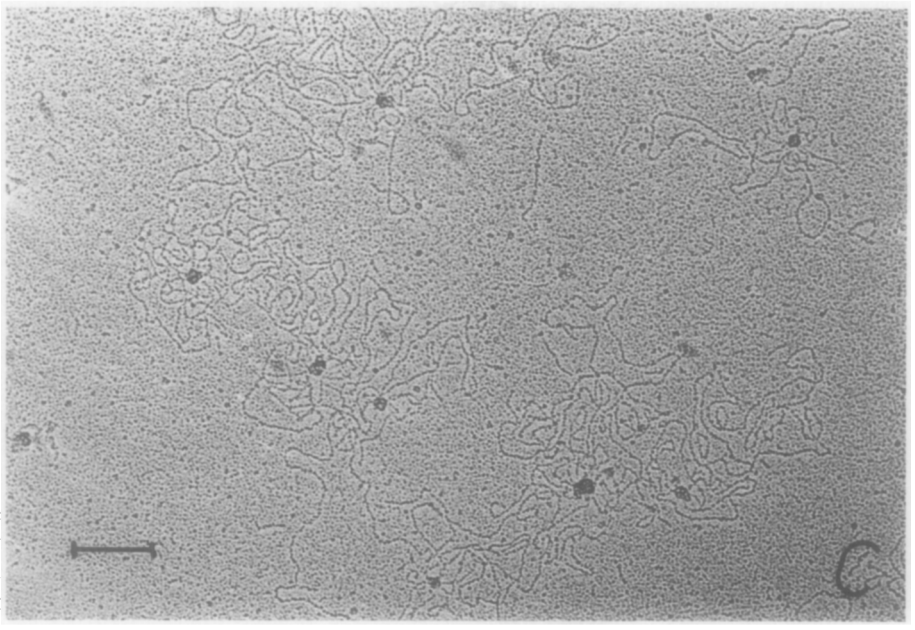

Fig. 1 - Continued

intermolecular contacts among the proteins of the particle but not protein-DNA bonds.

DNA-protein particles isolated from nuclear matrices by the CsCl/urea procedure vary in size from 25 to 35 nm although, in most cases, their diameter is somewhere about 30 nm. It seems that there are more than two DNA fibers associated with each particle Also it is worth noting that they are globular in shape without irregularities; thus it is highly unlikely that the particle originates as a result of adventitious protein aggregation.

As we have shown previously (19, 20) there are seven protein species composing the complex. The nature of the bonds involved in their attachment to a DNA molecule makes them unique among other nuclear proteins. To date the only other complex described initially for the total DNA preparation (21) can be compared to that obtained in our work. Since 1979 when Krauth and Werner identified two protease resistant DNA-linked proteins in total DNA after phenol deproteinization (21), there remained the possibility that these proteins represented a subset of the nuclear-matrix located complex identified in our work (19). In order to clarify this point we compared proteins obtained by our method with those prepared by Krauth and Werner's procedure (21). It turned out that these proteins were identical (compare Figures 2A and 2B). Using the deproteinization procedure (21) we obtained the same number of

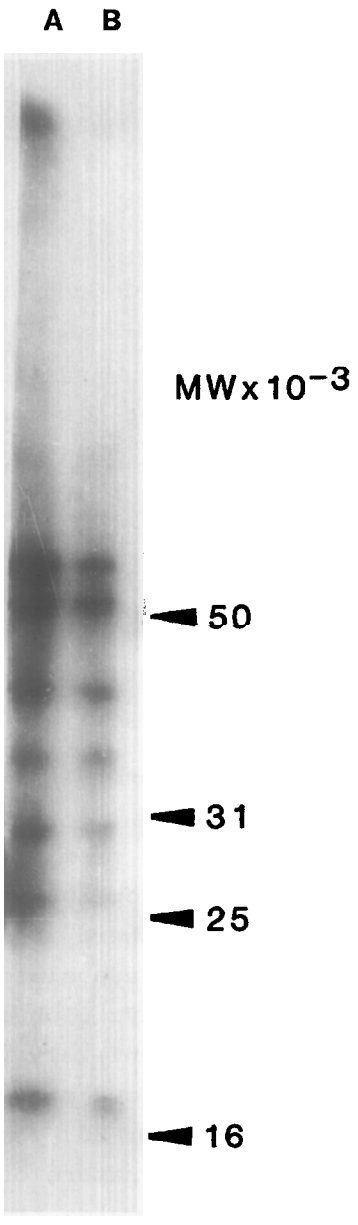

Figure 2. Proteins most tightly bound to nuclear matrix attached DNA. A: Proteins of the complex isolated by isopycnic centrifugation as described in METHODS. B: Proteins of the complex isolated by DNA-deproteinization procedure of Krauth and Werner (21).

protein bands whatever was as a starting material used: whole cells (as in 21), purified nuclei, nuclear matrices or DNA-protein complexes gave the same result (Figure 2B). The presence of additional proteins in our preparations was the only difference from the data of Werner's group (21, 25-26); possible reasons for this difference will be given below (see Discussion). Here, we

would like to stress that the proteins tightly bound to DNA remain attached to DNA bound to the nuclear matrix regardless of the procedure employed for their purification, including such drastic treatments as ionic detergent and high salt/urea treatment, protease digestion and phenol extraction (the latter methods were employed as in (21)).

DISCUSSION

Visualization of the complexes. One of the main results in our work presented here is visualization of the complexes made up by DNA bound to the nuclear matrix and proteins in Cs salt/urea preparations (Figures 1A and 1C). It is quite understandable that the clusters of globular particles seen in Cs_2SO_4/urea samples can efficiently be dispersed in the CsCl/urea procedure (24). The main question that can be only formulated here is whether those clusters are products of unspecific aggregation or they are indicative of insufficient dispersion in nuclear matrix lysis. In other words are those clusters artifacts or do they represent genuine parts of a nuclear matrix fibril? Unfortunately, so far we cannot prove either of the alternatives.

As there were no particles visible after detergent treatment, one is tempted to conclude that some detergent-sensitive bonds are responsible for maintaining the globular structure; it is also possible that some component of the complex is lost upon detergent treatment but we cannot detect such a loss on a protein gel. It would be relevant to note that, as such particles are formed by proteins associated with DNA, it means that their integrity may depend on the DNA moiety as well.

Dispersion of the particle due to detergent treatment was probably the cause for its "invisibility" in a previous work of Werner and collaborators (25); they reported that proteins could be seen in contact with DNA only after dinitrofluorobensene modification and subsequent reaction with appropriate antibodies (25).

Several authors have reported on DNA-protein particles identified as chromatin substructures (27-29) although no data on their location in the nuclear matrix are available. The size of particles reported previously is identical with that obtained in our work and, moreover, is almost the same as the length of the axial repeat in nuclear matrix fibrils (30). It is tempting to conclude that a nuclear matrix fibril is composed of identical

protein particles, some of which are associated with DNA. Such a chain of particles is then severed randomly in the case of Cs_2SO_4/urea treatment or at every particle in the case of CsCl/urea procedure. Evidently more profound changes take place in detergent treatment.

Proteins of the complex. The main point that we should like to stress here is the identity of proteins obtained previously (19) and in this work. It seems that only this set of proteins can survive harsh treatments with high salt/urea as well as with detergents. Evidently, even a less severe treatment is sufficient to remove all other proteins; but the most striking property of these proteins is their resistance to protease digestion and phenol/chloroform extraction. These latter characteristics as well as the molecular weight of two larger proteins in the complex are quite similar to those of proteins isolated by Werner and his colleagues (21, 25-26). The only difference in our results and those of Werner's group lies in the number of protein species: while these authors described only two, the same procedure in our hands gave seven proteins (Figure 2). We believe that the loss of lower molecular weight proteins may occur due to the method of sample preparation for electrophoresis described by Krauth and Werner (21) and employed by this group ever since (25-26): after DNase I digestion of the complex during dialysis the protein aggregates formed were sedimented (21). We believe that aggregation is sufficient to bring down only large proteins while the smaller ones remain in the supernatant. It is also possible that DNase digestion performed at low pH (pH 5.0 in /21/) cannot completely degrade DNA; therefore, two larger proteins are liberated later, during 2-mercaptoethanol treatment in Laemmli's sample buffer: as we have shown, these two proteins can be cleaved off by such a treatment (19).

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