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Complexes of nuclear matrix DNA with proteins tightly bound to DNA contain a specific small-size RNA of a novel type

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Analysis of DNA-protein structures composed of nuclear matrix attached DNA and the most tightly bound proteins was performed. Although the previously described non-histone proteins (1) were present the buoyant density of the complex was the same as that of pure DNA. RNA inaccessible to RNase in 0.4 M NaCl but digestible in low ionic strength buffer was detected. This RNA is not a nascent one. It turned out to be homogeneous and represent a novel type of small nuclear RNA. Partial sequence of this RNA is presented.

During the past decade much work was done to investigate the architecture and functional role of a skeletal structure in the eukaryotic nucleus. This structure was detected in 1960 (2) and thoroughly examined several years later in a series of experiments by Berezney and Coffey (for review see 3). Since then it has become evident that virtually all types of intranuclear activity in some way depend on this structure: compactization (4), replication (5, 6) and transcription (7, 8) of DNA, RNA processing (9) and splicing (10), ADP-ribosylation of nuclear proteins (11), etc. Nuclear matrix seems to be an obligatory component of functioning nucleus in living cell (12).

The major component of the nuclear matrix is protein (13). Since most functions of this structure involve nucleic acids, the interactions of these nuclear components became the hot spot for study. In our laboratory, we characterized a specific group of non-histone proteins located at, or in the closest vicinity of, the site of DNA loop attachment to the nuclear matrix (1). These proteins turned out to be extremely protease-resistant (14, 15), immunologically related to topoisomerase I (16) and in some way connected with cell differentiation (17).

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In this work, we planned to analyze the buoyant densities of various preparations of the complex in CsCl gradients in order to determine 1/ whether these complexes were as homogeneous as they appeared in electron microscope (15) and 2/ what was the DNA/protein ratio of the complex.

MATERIALS AND METHODS

Cell culture. Mouse plasmacytoma cell line P3O1 was used as in (15).
Nuclear matrix isolation: was done essentially as described earlier (15).
DNA-protein complex isolation was done as described (15) except that 0.5% sarkosyl and not urea was used for matrix lysis.
RNA isolation. DNA-containing fractions of the gradient were collected and dialyzed. After ethanol precipitation, the pellet was dissolved in 10 mM Tris-HCl, pH 7.8, 0.5 mM EDTA (TE buffer), and $MnCl_2$ was added up to 2 mM and Tris-HCl, pH 7.8, up to 50 mM. Then 50 ug/ml of DNase I (Worthington; free of RNase) was added and the mixture was incubated for 1 h at 37°C. Pronase and phenol treatment were done according to (18).
RNA dephosphorylation and labeling were done according to (18).
RNA sequencing was done as described by Donis-Keller (19).
RNase A digestion. DNA-protein complexes were dissolved in TE buffer and incubated with RNase A either without salt or after addition of NaCl to 0.4 M.
$H_3{}^{32}PO_4$ labeling of the cells. The cells were incubated for 48 h in a medium without phosphorus and then 0.5 mCi/ml of the labeled compound was added. The cells were harvested 20 h later.
Pulse labeling with [^{3}H]-uridine. The labeled compound (20 Ci/mmole; Izotop, USSR) was given for 5 or 30 min at a concentration of 1 mCi/ml; after the incubation the cells were chilled on ice and the nuclear matrix was isolated.
3′ nucleotide analysis. The ^{32}P-labeled material was analyzed after CsCl centrifugation and dialysis as described in (20).
Gel electrophoresis was done according to (18).

RESULTS

The buoyant density of the complex in CsCl was determined using purified mouse DNA as an internal marker. All nuclear matrix DNA fragments banded exactly at the same position as marker DNA, e.g. at 1.7 g/cm^3 (21) (Fig.1A), although they were complexed with the whole set of previously described proteins (1) (not shown). Moreover, the buoyant density of the complex did not depend upon DNA fragment length since all the preparations gave identical results (samples contained from 5 to 0.3% of total nuclear DNA).

In order to explain this phenomenon we presumed that the complex included another component with buoyant density in CsCl greater than that of DNA. The quantity of this component should exactly compensate for the expected shift to lower densities induced by proteins of the complex. RNase A treatment of isolated complexes in fact caused such a shift (Fig. 1B); as it could not be explained by artificial RNase A association with these com-

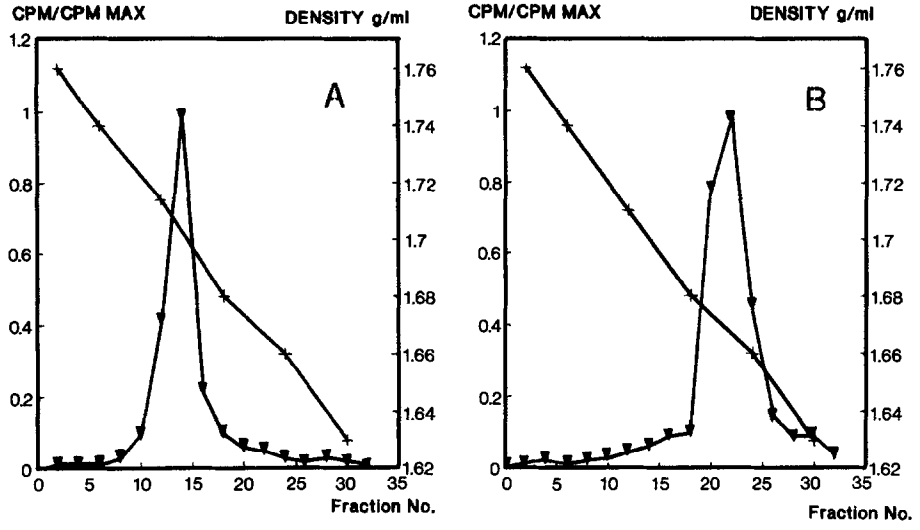

Fig. 1. CsCl gradient centrifugation of nuclear matrix attached DNA-protein complex before (A.) or after (B.) RNase A treatment. (▼) - DNA-protein complex; (+) - density curve.

plexes (no changes in protein profile) we assumed RNA presence within the complex as a working hypothesis.

The presence of ribonucleotides in the complex was checked next: cells were labeled with $H_3{}^{32}PO_4$ for 24 h and DNA-protein complexes were purified by two successive CsCl centrifugation. Over 99% of the labeled material after the second centrifugation banded in DNA containing fractions of the gradient, therefore the presence of free RNA in this fraction was highly improbable. Dialysed samples were treated with RNase A and T_2 (DNase free), and subjected to two-dimensional thin-layer chromatography (20); a distinct pattern of 3′-ribonucleotides can be seen (Fig.2). Thus there is RNA in DNA-protein complexes at sites of DNA loop attachment to nuclear matrix.

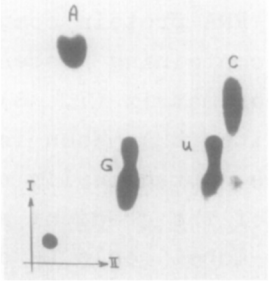

Fig. 2. Two-dimensional thin-layer chromatography of RNase digest of ^{32}P-labelled and CsCl-purified DNA-protein complex.

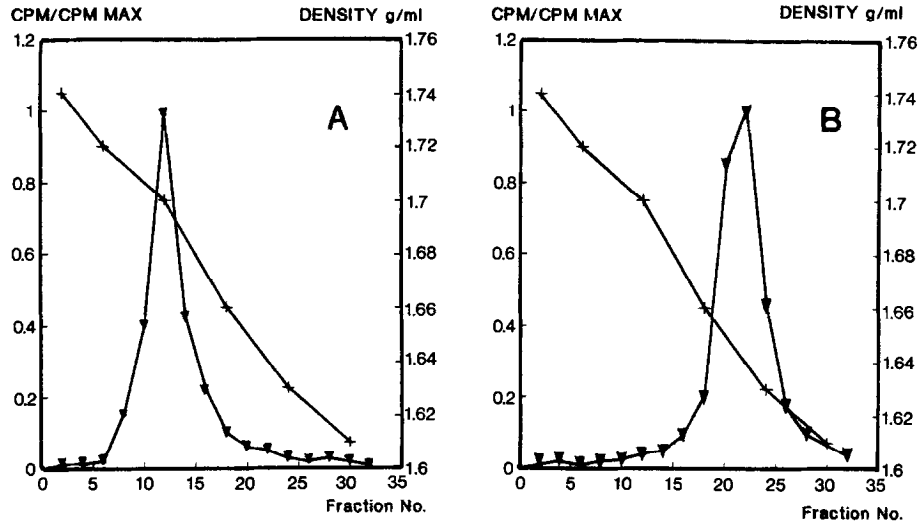

Fig. 3. CsCl gradient centrifugation of the complex, which was purified by centrifugation and digested by RNase A in 0.4 M NaCl (A) or in low ionic strength buffer (B). (▼) - DNA-protein complex; (+) - density curve.

To investigate the structure of RNA in the complex we treated isolated complex with RNase A in different salt solutions. The buoyant density was reduced only after RNase A treatment in low ionic strength buffer (Fig. 3). Hence, RNA of the complex either possessed a prominent secondary structure, or was base-paired with DNA. This result was further confirmed using direct visualization of RNA: RNase A digestion in a low ionic strength buffer eliminated virtually all RNA (Fig.4, lane B) while the same treatment in 0.4 M NaCl had less dramatic effect (Fig. 4, lane C) although some RNA degradation occurred even in this case (compare Fig.4, lane A & C). Thus we propose that the RNA component of the complex exists at least partially in a double-stranded form.

Among nuclear DNA-RNA-protein complexes one cannot neglect transcription complexes containing nascent RNA because a) they are located at the nuclear matrix (7, 8) and b) represent a kind of DNA loop attachment site (22). When in vivo pulse-chase labeling experiments were done, practically no labeled material was found at DNA position of the gradient after 30 min [^{3}H]-uridine pulse (Fig.5A). The [^{3}H]-label could be detected at DNA position only after 16 h incubation (Fig. 5B). Hence, this RNA cannot be a part of the transcription complex.

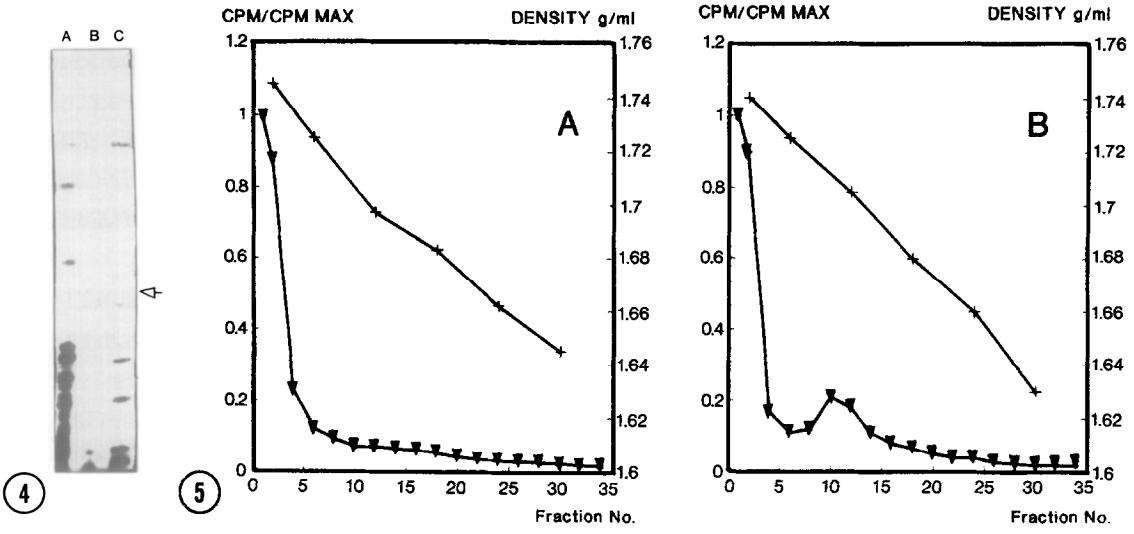

Fig. 4. Electrophoretic separation of RNA fragments of the complex after ^{32}P-labelling in kinase reaction. A: untreated sample; B: complex pretreated with RNase A in low ionic strength buffer; C: complex pretreated with RNase A in 0.4 M NaCl. Arrow correspond to the position of 28 bases fragment.

Fig. 5. CsCl gradient centrifugation of the complexes atfer in vivo pulse-label with ^{3}H-uridine. A: 30 min pulse; B: 16 hr label. (▼) - ^{3}H-labelled material; (+) - density curve.

Such a conclusion prompted us to analyze this RNA using the enzyme sequencing protocol (19). To obtain homogeneous preparations we separated 5′-end labeled RNA fragments in polyacrylamide gel, extracted the most prominent bands and analyzed them. RNA fragments were found to be homogeneous: first by the 5′ analysis of purified bands and later by the direct sequencing of such fragments. The data on one of them are presented (see Fig.6).

DISCUSSION

The main result of the work presented here is the identification of a specific RNA located at particular sites of the eukaryotic chromosome. Amazingly, the role of RNA in the genome organization has been under investigation for more than 15 years. One of the first results concerned with the role of RNA in

AGAAGACACC CUGAUUUAAC UUCUGGUAUC GGAAGAUGCA AGAGCCGAAC CAGA

Fig. 6. Partial sequence of RNA, found in the DNA-protein complex.

chromosome packing was obtained by Stonington & Pettijohn (23) who showed that the folded conformation of *E.coli* genome depended on RNA-DNA interactions. In eukaryotes alkali-labile sites regularly spaced with intact DNA were detected (24, 25) and RNA was identified as a linker providing for the integrity of chromosomal DNA (26). Several models for DNA loop organization, which involved RNA, were put forward (27).

Previously, Leibovitch et al. (28) reported an unusually high buoyant density of DNA fragments associated with tightly bound to DNA proteins in CsCl gradients. A high quantity of nuclease S1 sensitive regions is characteristic for these DNA fragments (28, 29); evidently, this will be the case if degradation of a complementary RNA strand occurs, or if there is an imperfect complementarity between DNA and RNA strands.

However, despite the data available on possible role of RNA in maintenance of genome structure, we could find no information about specific RNA fragments in DNA attachment complexes to nuclear matrix. Meanwhile, results presented here agree with the idea of a small specific RNA associated with DNA-protein complexes. It seems that RNA fragments associated with DNA are uniform at least for a given type of cells; therefore this RNA may play some specific role in chromatin organization and functioning.

As the isolation of RNA fragments involved several enzymatic treatments we could not expect to isolate intact RNA. The largest fragment observed up to date was no less than 150 bases.

The sequence obtained was analyzed as in (30) using the Microgenie software. The only strong homology found was with bacteriophage T7 genome, but we considered it to be accidental. Stop codons have been found in two possible reading frames of the sequenced fragment; taken together with the absence of pulse label in the analyzed fraction (Fig. 5) these data agreed with the idea of a special role played by this molecule.

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