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RNA-Dependent Nuclear Matrix Contains a 33 kb Globin Full Domain Transcript as Well as Prosomes but no 26S Proteasomes

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Abstract Previously, we have shown that in murine myoblasts prosomes are constituents of the nuclear matrix; a major part of the latter was found to be RNase sensitive. Here, we further define the RNA-dependent matrix in avian erythroblastosis virus (AEV) transformed erythroid cells in relation to its structure, presence of specific RNA, prosomes and/or proteasomes. These cells transcribe but do not express globin genes prior to induction. Electron micrographs show little difference in matrices treated with DNase alone or with both, DNase and RNase. In situ hybridization with alpha globin riboprobes shows that this matrix includes globin transcripts. Of particular interest is that, apparently, a nearly 35 kb long globin full domain transcript (FDT), including genes, intergenic regions and a large upstream domain is a part of the RNA-dependent nuclear matrix. The 23K-type of prosomes, previously shown to be co-localized with globin transcripts in the nuclear RNA processing centers, were found all over the nuclear matrix. Other types of prosomes show different distributions in the intact cell but similar distribution patterns on the matrix. Globin transcripts and at least 80% of prosomes disappear from matrices upon RNase treatment. Interestingly, the 19S proteasome modulator complex is insensitive to RNase treatment. Only 20S prosomes but not 26S proteasomes are thus part of the RNA-dependent nuclear matrix. We suggest that giant pre-mRNA and FDTs in processing, aligning prosomes and other RNA-binding proteins are involved in the organization of the dynamic nuclear matrix. It is proposed that the putative function of RNA within the nuclear matrix and, thus, the nuclear dynamic architecture, might explain the giant size and complex organization of primary transcripts and their introns. J. Cell. Biochem. 94: 529–539, 2005.

Key words: gene expression; globin; nuclear matrix; RNA processing; transcription; full domain transcripts; prosomes; proteasomes

For many years, the term "nuclear matrix" has remained to be an experimental definition for poorly defined remains of the cellular nucleus after extensive extraction with non-ionic detergents, high salt solutions, and treatment with nucleases [Berezney and Coffey, 1977; for review see Razin, 1997]. On the other hand, early work had already shown that up to 300 different proteins are constituents of this nuclear backbone, and that protein composition of the nuclear matrix changed in relation with species and cell differentiation [Capco et al., 1982]. In the last 10 years, electron micrographs from several laboratories have demonstrated more precisely filamentous networks of the nuclear matrix which were found to contain RNP particles as well as actin and lamins

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[Gounon and Karsenti, 1981; Hozak et al., 1995; Padros et al., 1997; Rando et al., 2000; Okorokov et al., 2002; Andrin and Hendzel, 2004].

The very notion of a nuclear matrix has gained new interest due to the increasingly convincing demonstration that in the nucleus genes are placed in a specific topological context and transcripts are localized in specific nuclear areas (for a review see [Razin et al., 2003]). A particularly striking example is the specific accumulation of globin transcripts around the nucleoli in uninduced avian erythroblastosis virus (AEV) transformed cells [Iarovaia et al., 2001], whereas after induction of globin gene expression these RNAs move to two nuclear spots, nuclear globin RNA processing centers (PCs) [De Conto et al., 1999; Iarovaia et al., 2001]. Furthermore, globin mRNA was found to appear in the cytoplasm in concise spots before entering translation [De Conto et al., 1999].

The notion that transcripts are specifically recognized in the nucleus and transported to specific areas of the cytoplasm is particularly evident in muscle cells. Indeed, mRNAs induced in myogenesis are inserted right into the parallel stripes of the sarcomeres in formation [Fulton and L'Ecuyer, 1993]; the same was found for specific types of prosomes [Foucrier et al., 1999]. This signifies that there must be a system of transcript recognition and selective transport, implying the existence of a well defined dynamic nuclear architecture.

Quite a few matrix proteins are well known and some have been characterized extensively (lamins [Hozak et al., 1995; Barboro et al., 2002, 2003], NuMA [Barboro et al., 2002, 2003], proteins of RNP particles [Mattern et al., 1996, 1999; Nickerson, 2001], MAR-binding proteins [von Kries et al., 1991, 1994a,b; Tsutsui et al., 1993; Weitzel et al., 1997; Lobov et al., 2000; Bode et al., 2003]. We could show recently that another major constituent of the nuclear matrix are prosomes [De Conto et al., 2000]. Prosomes constitute the core of 26S proteasomes but, long before this became known, we had revealed the existence of 20S prosomal particles associated with mRNPs [Schmid et al., 1984; Martins de Sa et al., 1986] as well as with the cytoskeleton [Arcangeletti et al., 2000]. Interestingly, these 20S prosomes, as free particles, display RNase activities [Jarrousse et al., 1999; Jorgensen and Hendil, 1999], whereas proteolysis necessitates first the assembly of 26S proteasomes by the addition of 19S modulator (or regulator) complexes; the latter recognize proteins and direct them into the proteolytic core.

Previously, we have extensively studied the distribution of prosomes on sub-networks of the cytoskeleton [Arcangeletti et al., 2000], where these protein complexes, made up of 2×14 subunits in variable composition, occupy various sub-networks according to their kind. Recently, we have started to investigate their presence in the nucleus. Working with myoblasts in division and during myogenic differentiation we could show that prosomes are not only genuine constituents of the nuclear matrix but, furthermore, that they form quite well defined sub-networks. Indeed, some types of prosomes accumulate around the nucleoli, which are linked by a quite well-defined prosome network extending to the nuclear membrane [De Conto et al., 1999]. Most interestingly, a major part of such matrix sub-networks, occupied by specific types of prosomes, is RNase sensitive. Reports on RNA being part of the nuclear matrix have been published previously [Capco et al., 1982; Nickerson, 2001; Barboro et al., 2002] and, recently, we have found that globin transcripts form part of the RNA-dependant nuclear matrix [Razin et al., 2004].

Having observed previously that specific types of prosomes co-localize with the globin full domain transcripts (FDTs) in the RNA PCs of the nuclei in AEV cells [Iarovaia et al., 2001], we decided to define better the RNA-dependent nuclear matrix in these transformed cells, where the globin genes are transcribed but not expressed. As the first step, we investigated by electron microscopy the matrix network before and after RNase treatment and found few differences. As in myoblasts, in erythroblasts prosomes constitute an integral part of the nuclear matrix of AEV cells in patterns that spare out the nucleoli. Globin transcripts, analyzed over a 35 kb long domain by genic, intergenic and upstream located riboprobes, occupy the nuclear matrix in slightly different patterns which are possibly related to differential processing. Most interestingly, prosomes but not the 19S modulator complex of 26S proteasomes are part of the RNase sensitive nuclear matrix, excluding the proteolytic function at that level. In contrast to intact AEV cells, where specific types of prosomes show different distribution, on nuclear matrices all prosomes are found in quite similar patterns.

MATERIALS AND METHODS

Cells

AEV cells of the line HD3 (clone A6 of the line LSCC [Beug et al., 1979] were grown in suspension in Dulbecce's modified Eagle's medium supplemented with 8% fetal bovine serum and 2% chicken serum.

Isolation of Nuclear Matrices

Nuclear matrices were isolated using a modification of the previously published protocol [Rzeszowska-Wolny et al., 1988]. Briefly, the cells were resuspended in a TM buffer (50 mM Tris-HCl (pH) 7.5, 3 mM $MgCl_2$) supplemented with 0.2 mM $CuCl_2$. All subsequent procedures were carried out at 0°C. Ten percent solution of NP40 was added to the suspension and the cells were destroyed by 10 strokes in a Dounce tissue homogenizer with a tight pestle. The nuclei were precipitated and washed twice with the TM buffer. Then they were treated with either DNase I (50 μg/ml) or with both DNase I (50 μg/ml) and RNase A (25 μg/ml). In both cases the incubation was for 20 min at room temperature. The suspension was then diluted with an equal volume of TM buffer supplemented with 4M NaCl. After incubation for 20 min on ice, the nuclear matrices were precipitated and washed sequentially two times with TM buffer supplemented with 2M NaCl and two times with TM buffer. The term "RNA-dependent nuclear matrix" is used in this article to indicate components of the nuclear matrix present in the matrices treated with DNase I alone but absent in the matrices treated with RNase A and DNase I. It is assumed that this part of the nuclear matrix is solubilized in a result of RNA removal.

For immunofluorescence analysis, the cells and nuclear matrices were spread on silane-coated microscopic slides using a "Cytospin" centrifuge. All samples were fixed with paraformaldehyde as described.

Electron Microscopy and Immunofluorescence Analysis

For immunofluorescence analysis, the cells and nuclear matrices were spread on silane-coated microscopic slides using a "Cytospin" centrifuge. All samples were fixed with paraformaldehyde as described [Arcangeletti et al., 1997]. Monoclonal antibodies directed against prosomal proteins p23K, p27K, and p30K were mouse ascitic fluids (ICN Biomedicals, Orsay Cedex, France). Antibodies against the 19S regulatory complex were purchased from AFFINITI BioReagents, Inc. (Golden, CO). The fixed cells and nuclear matrices were preincubated for 15 min with 1% BSA in PBS to reduce background staining and then incubated with primary p-mAbs (diluted 1:20) in PBS containing 0.2% BSA (incubation buffer) for 90 min at 37°C in a humid chamber. The cells were washed three times for 5 min with PBS and the reacting antibodies were revealed by anti-mouse IgG conjugated to Alexa 488 or Alexa 568 fluorochrome ("molecular probes"). The cells were washed with PBS and mounted in Mowiol (Calbiochem AG, Lucerne, Switzerland).

Electron microscopy of the nuclear matrices embedded in Epon and, after making ultrathin sections, stained with uranyl acetate was carried out essentially as described [Rzeszowska-Wolny et al., 1988].

RT-PCR Analysis

Total nuclear or nuclear matrix RNA (1 μg) treated with DNase I (PCR grade) (Invitrogen Corp., Carlsbad, CA) and then was reverse transcribed into cDNA with the aid of the 1st strand cDNA synthesis kit for RT-PCR (AMV). Each RT reaction was started from one of the "rev" PCR primers (see Table I). The synthesized cDNAs were treated with a mixture of RNase H and RNase A and amplified with Taq DNA polymerase (Roche, Basel, Switzerland). The products of PCR reactions were analyzed by agarose gel electrophoresis. In all RT-PCR experiments control amplifications on the templates incubated in RT mixture without reverse transcriptase were carried out. No amplification products were obtained in these experiments.

RESULTS

Isolation of RNA-Containing and RNA-Lacking Nuclear Matrices From Chicken Erythroleukemia Cells

The protocol for isolation of nuclear matrices from cultured chicken erythroleukemia cells (line HD3) was described previously [Rzeszowska-Wolny et al., 1988]. The essential differences of this protocol from the classical step-wise extraction procedure of Berezney and Coffey [1977] are: (1) stabilization of nuclear matrices with 0.2 mM Cu^{++} ions and (2) omission of the low-

TABLE I. PCR Primers and the Length of the Test Fragments

Region to be amplified	PCR primers	Expected length of the product (bp)
Test region "C"	C_dir TGCTGTCAAATTAGCCGAGT C_rev TGTGGTACACTGTGCTGTTG	302
Test region "D"	D_dir TGAAGAATTCAGAACATCAC D_rev CTAGTTTCCAGAATGTTCTG	306
Test region "E"	E_dir CAGAGCTCAATTCCATAGG E_rev TTATCTGGGGTACCTGCAT	265
Test region "F"	F_dir GCTCTTCTGGCTCATTTGT F_rev TCATCTCCCTTTCAGTCCC	218
Chloramphenicol acetylransferase (CAT) mRNA	CAT_dir TTCACATTCTTGCCCGCCTG CAT_rev ATCAGCACCTTGTCGCCTTG	376

salt extraction step. The rationales for these modifications were discussed previously [Razin et al., 1985]. In our earlier experiments, the micrococcal nuclease was used to digest both DNA and RNA in the course of nuclear matrix preparation [Rzeszowska-Wolny et al., 1988; Farache et al., 1990]. In order to obtain RNA-containing and RNA-lacking nuclear matrices, we have now used treatments with either DNase I alone or DNase I in combination with RNase A. Analysis of the distribution of pre-labeled RNA in the course of nuclear matrix isolation demonstrated that more than 85% of nuclear RNA remained in the nuclear matrices prior to RNase A treatment (not shown). When the nuclei were treated with RNase A, less than 2% of the initial amount of RNA was recovered in the matrix preparation (not shown). As far as DNA is concerned, both types of nuclear matrix preparations contained less than 1% of the initial amount of DNA. EM-pictures of RNA-containing and RNA-lacking nuclear matrices are shown in Figure 1. One can see that the removal of RNA does not affect drastically the morphology of matrices, although some minor changes can easily be detected (e.g., more prominent vacuolization of nucleoli in RNA-lacking matrices).

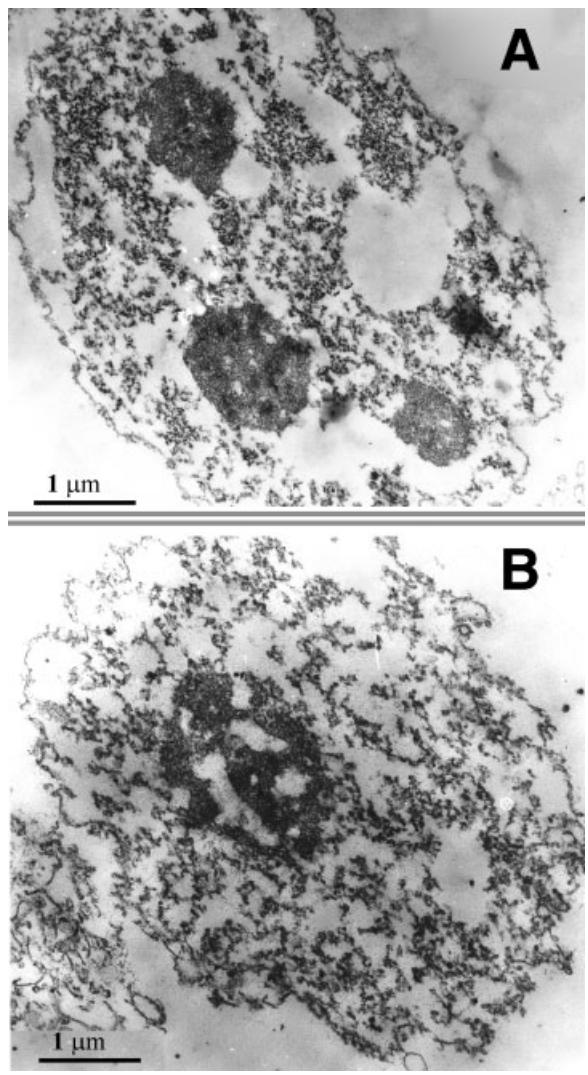

Fig. 1. Electron microscopic pictures of nuclear matrices prepared using treatment with DNase I alone (**A**) or with combination of DNase I and RNase A (**B**).

FDT of the Alpha-Globin Gene Domain Is Associated With the Nuclear Matrix After DNA Removal

For better characterization of RNA-containing matrices, the distribution of transcripts of the α-globin gene domain in these matrices was studied using in situ hybridization. A representative example of the results obtained is shown in Figure 2. It is evident that the transcripts of both genes (π, α^A; see scheme in Fig. 3) and intergenic regions (F- and C-probes) of the

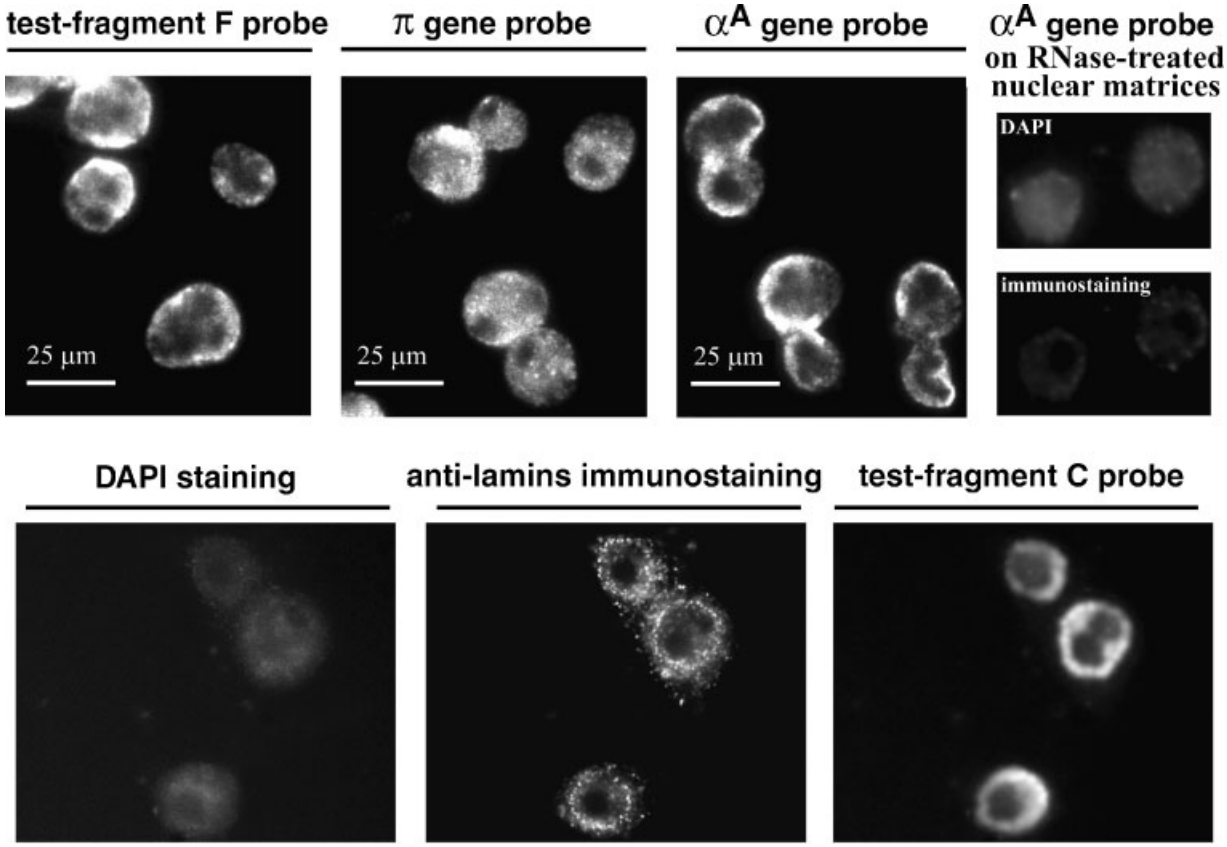

Fig. 2. Immunostaining of RNA-containing nuclear matrices from HD3 cells with single strand-specific ribo-probes recognizing the globin-direction transcript of the upstream non-coding area of the α-globin gene domain and coding sequences of globin genes. **First row**: Hybridization in situ with probes recognizing the globin-direction transcript of the test-fragment F (3.2 kb upstream to the π gene) and transcripts of the π and α^A genes). The **right panels** show hybridization of the α^A gene-specific probe with RNA-lacking nuclear matrices. **Second row**: Staining of the same group of nuclear matrices with DAPI, antibodies against lamins A and B, and in situ hybridization with a ribo-probe recognizing the test-fragment C (19.1 kb upstream to the π gene).

domain are present in nuclear matrices. The abundant transcripts (e.g., transcript of the π gene) are distributed almost randomly within the nuclear matrix with an obvious exclusion from the nucleoli. As it is not always easy to identify borders of nuclear matrices, which are poorly stained with DAPI, all samples were additionally stained with antibodies against lamins (Fig. 2, second row). It is important that in control experiment, when α^A gene-specific probe was hybridized to RNase-treated nuclear matrices, virtually no staining was observed (Fig. 2). This excludes the possibility that all signals observed in other experiments represent a result of non-specific sorption of the probe on nuclear matrix proteins or residual DNA.

In a previous study we showed that, in chicken erythroid AEV cells, a long upstream area of the alpha-globin gene domain is transcribed in the globin direction and that transcripts of the globin genes are parts of the nuclear matrix [Razin et al., 2004]. The full domain transcription unit starts near a putative locus control region (LCR) of the domain [Jarman et al., 1991], about 20.5 kb upstream to the π gene, and extends over the whole cluster of alpha-globin genes. In order to check if the alpha globin domain FDT also constitutes a part of the nuclear matrix, in addition to the gene transcripts, the RNA isolated from nuclear matrices was used as a template for RT-PCR reactions aimed to amplify four test regions (C, D, E, F) located respectively 19.1, 16.3, 13.3, and 3.2 kb upstream to the π gene. The PCR primers and the length of the test fragments are presented in Table I (see "Materials and Methods"). It should be mentioned that the upstream area of the chicken alpha-globin gene domain is transcribed in both directions [Sjakste et al., 2000], as part of the

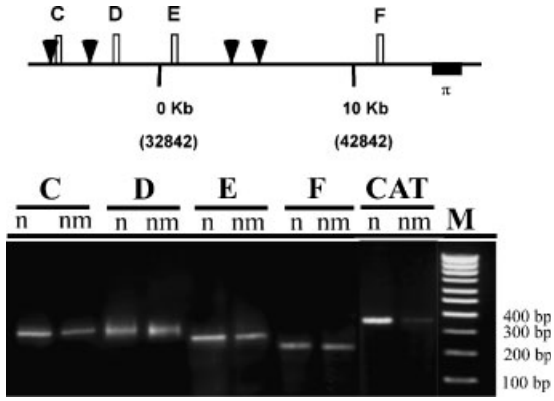

Fig. 3. Comparison of the representation of different test fragments in total nuclear RNA and nuclear matrix RNA. The products of RT-PCR reactions were separated by electrophoresis in agarose gels and photographed after staining with ethidium bromide. The slots are designated in the following manner: n, products of RT-PCR reaction on nuclear RNA; nm, products of RT-PCR reaction on nuclear matrix RNA. M, 100 bp size marker. The letters **C**–**F** above the slots indicate the test fragments studied in each case. The scheme in the **upper part** of the figure shows the positions of the test-fragments (C–F) relative to the π gene. The 0 point of the scale corresponds to an arbitrary chosen *Kpn*1 site colocalizing with the permanent site of hypersensitivity to DNase I, 13.34 kb upstream to the start of the π gene CDS. The figures in the parentheses indicate distances in the DNA sequence, deposited under the number AY016020 in the gene bank.

globin FDT, and also as part of the *ggPRX* gene (chicken analogue of the human gene "-14"), located on the opposite strand in all species tested. In order to escape confusion, RT reactions were hence started from strand-specific primers (the same as "rev" primers for PCR amplifications). These primers recognize the globin FDT exclusively.

The results of PCR amplification are presented in Figure 3. To have a positive control, we performed RT-PCR reactions using on total nuclear RNA as a template. The products of RT-PCR reactions carried out on total nuclear RNA ("n" in Fig. 3) and on nuclear matrix RNA ("nm" in Fig. 3) were run in parallel slots. It is evident that the RNA sequences transcribed in the globin direction from all test fragments are present in the nuclear matrix RNA roughly in the same quantities as in nuclear RNA. Thus, it may be concluded that the alpha-globin domain FDT, including genes as well as intergenic and upstream regions, is a component of the nuclear matrix. In control experiment, the distribution of chloramphenicol acetylransferase (CAT) mRNA transiently expressed in HD3 cells transfected by pCAT3-control vector (Promega, Madison, WI) was studied. As shown in Figure 3 (panel CAT) most of this RNA was extracted in the course of the nuclear matrix preparation. Hence, presence of virtually all globin FDT in the nuclear matrix can hardly represent a result of non-specific precipitation of RNA on the nuclear matrix proteins in the course of high salt extraction.

Prosomes and Proteasomes in the Nuclear Matrix

As demonstrated in our previous work, prosomes are genuine components of the nuclear matrix in myoblasts [De Conto et al., 1999]. We have now analyzed the distribution of prosomal antigens in chicken AEV cells and in nuclear matrices isolated from these cells. Three different monoclonal antibodies recognizing prosomal subunits p23, p27, and p30 were used in the present study. The results of immunostaining experiments are shown in Figure 4. One can see that the cellular distribution of p23-type prosomes differs significantly from that of p27 and p30 prosomes. The p23-type prosomes are excluded from the greater part of the nuclear space, except spherical internal structures representing the PCs, the RNA PCs [De Conto et al., 1997; Iarovaia et al., 2001]. The p27- and p30-type prosomes are distributed almost randomly both in nuclei and in the cytoplasm. Such obvious differences in the nuclear distribution of the three types of prosomal particles studied were not, however, seen at the nuclear matrix level (Fig. 4). All three prosomal antigens were distributed in a similar pattern in the nuclear matrices, with an obvious exclusion from nucleoli, which accumulate, however, to variable extent along their periphery. In this respect, their distribution resembles the distribution of matrix-bound transcripts of the alpha-globin gene domain (Fig. 2). It is evident that specific nuclear structures containing p23-type prosomes are not resistant to the treatments used for the nuclear matrix preparation.

In our previous study of prosomes present on the nuclear matrix of myoblasts [De Conto et al., 1999], the distribution of proteins belonging to the 19S regulatory subunit of proteasomes was not analyzed. In order to check if 26S proteasomes are genuine components of the nuclear matrix, we have now studied in parallel the presence in nuclear matrices of proteins participating in the formation of prosomes (20S proteasomes) and the 19S proteasomal regulatory complex. The results of IIF visualization of

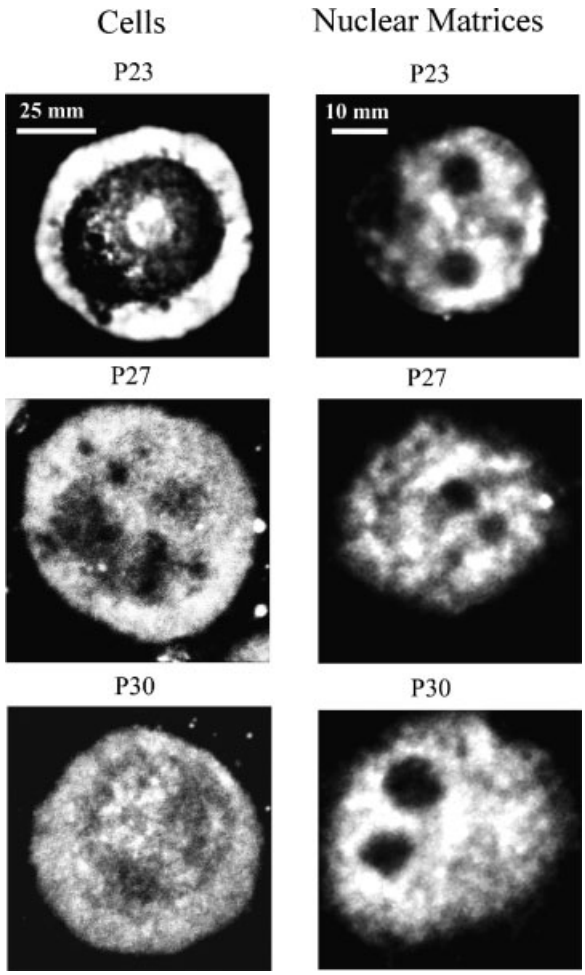

Fig. 4. Immunostaining of HD3 cells and RNA-containing nuclear matrices with the antibodies against prosomal antigens p23, p27, and p30, as outlined in the ''Materials and Methods.''

such proteins in RNA-containing and RNA-lacking nuclear matrices prepared from AEV cells are shown in Figure 5. It is visible that both, prosomal antigens and antigens of the 19S modulator complex of proteasomes, are present in RNA-containing nuclear matrices. The intensity of the prosomal signal decreases drastically upon RNA removal. In contrast, the 19S regulatory complexes seem to be equally represented in RNA-containing and RNA-lacking nuclear matrices. The presence of proteolytically active proteasomes on the RNA-dependent nuclear matrix can thus be excluded.

DISCUSSION

In this article, we describe three important findings. (1) The presence of specific pre-mRNA and FDTs as structural components in the nuclear matrix. (2) The presence of prosomes but not proteasomes on the RNA-dependent nuclear matrix. (3) The possible inter-dependence of RNA transcripts (in particular the globin FDT) and of prosomes on the matrix, pointing to the existence of a network on which gene-specific RNA processing and transport might occur. These data allow a novel interpretation for the very existence of giant RNA and their complex intron/exon organization.

One of the major actors on the nuclear matrix but also in the very process of transcription is nuclear actin; it was shown recently that the polymerase II transcription complex carries along actin monomers [Percipalle et al., 2003]. On the other hand, it is known for some time that polyribosomes are attached to the actin-based microfilaments [Singer, 1992; Singer and Green, 1997]. Actin is thus present not only on the nuclear matrix, and on the "holo-matrix" of cells devoid of nuclei during mitosis, but also as a carrier of the translation machinery in the cytoplasm. The present demonstration that globin pre-mRNAs and FDTs in processing are structural components of the matrix, may lead to the interpretation that, from transcription to translation, various actin-containing filaments may form the constitutive skeleton on which the mechanisms of gene expression operate.

On the other hand, in nucleus and cytoplasm, the prosomes seem to be associated to the transcripts, as well as to mRNA prior to translation [Martins de Sa et al., 1986; De Conto et al., 1999]. Based on their variability, due to the variation of the subunit composition of the individual particles, many types of prosomes exist, which seem to be related to specific patters of gene expression and cellular constituents. As outlined in the introduction, specific types of prosomes were found to be associated with specific gene transcripts; therefore the proposition was made, that the prosome system might be instrumental in selective mRNA transport [Scherrer and Bey, 1994]. In the cytoplasm, prosomes [Arcangeletti et al., 1997, 2000] as well as mRNA [Singer, 1992; Bassell et al., 1994] are present not only on the actin-based microfilaments, but also on sub-networks of the intermediate filaments. The latter appear, somehow, intercalated in between the nuclear and cytoplasmic types of actin-dependent networks carrying mRNA. Much further work will be necessary to get to the level of conclusive facts, all along this pathway. But a conceptual

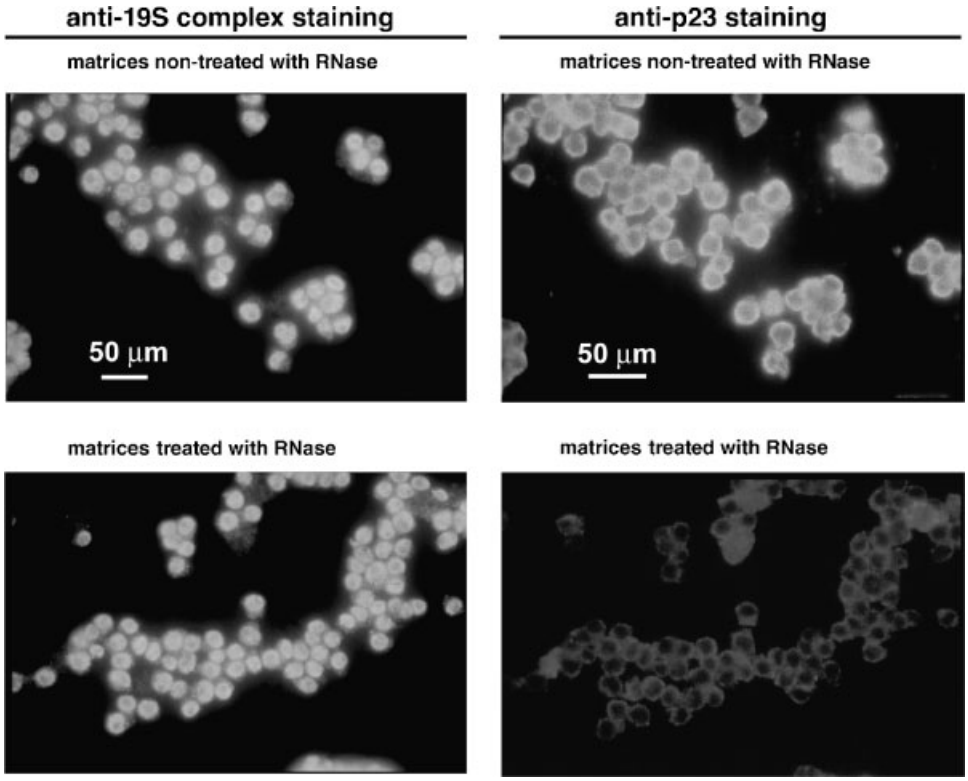

Fig. 5. Immunostaining of RNA-containing and RNA-depleted nuclear matrices with antibodies against prosomal antigen p23, and antibodies against the 19S regulatory complex of the 26S proteasomes.

scheme is in view, liable to explain selective RNA transport from nuclear sites of transcription to specific sites of translation, which involve as main actors the transcripts themselves, associated to prosomes as well as known components of the nuclear matrix and the cytoskeletal systems.

That RNA is a component of the nuclear matrix is not new [Nickerson, 2001]. But it is for the first time that we are able to show here that genuine and specific pre-mRNA and FDTs are, on the structural level, the material basis of the RNA-dependent matrix and, thus, the organizational principle of what may be called the dynamic nuclear architecture. Within the globin FDTs, not only genes and intergenic DNA are transcribed, but also a huge upstream area covering most of the domain. It was thus of importance to show that, apparently, most of this giant RNA is present in the nuclear matrix.

A function in RNA transport was suggested for prosomes [Scherrer and Bey, 1994], and the data presented here seem to support that notion. One of the important findings of the present study is that the proteolytic function of the prosomes as parts of proteasomes is not involved at this level. Indeed, we found that prosomes only, and not integral proteasomes, are part of the RNA-dependent matrix; at this level, a proteolytic function is, thus, excluded. It is interesting that, in contrast to prosomes, the 19S modulator complex of proteasomes resist the RNase treatment. Hence, it may constitute a part of the DNA–matrix complex. Proteasomes may be proteolytically effective in the modulation of the chromatin structure. However, it is also known that the 19S modulator complex of proteasomes is present in the nucleus as a free particle, like prosomes, being involved, beyond proteolysis, in other not yet defined functions.

Conceptually, the structural involvement of the globin FDT within the nuclear matrix may be of basic importance. Since the discovery of "giant" RNA [Scherrer and Darnell, 1962; Scherrer et al., 1963; Scherrer, 2003] and the conception of the pre-mRNA scheme [Scherrer et al., 1970], as well as the observation of the fragmentation of genes at the level of DNA and RNA splicing [Berget et al., 1977; Aloni et al., 1978; Bratosin et al., 1978], never a fully

satisfactory explanation of the function of this "surplus" RNA has been given. Based on our new observations we may propose a realistic and, apparently, more logical explanation of all this "extra" RNA and the corresponding "junk" DNA. The finding that FDTs and pre-mRNA are the structural carriers of the nuclear matrix, forming RNPs through their interaction with all kind of RNA-binding proteins, among them prosomes, suggests that such "extra" RNA might represent the blueprint of the organization of the dynamic nuclear architecture. Furthermore, assuming such a function, it becomes evident that the one-dimensional RNA may constitute the basis of a 3D-network, on which selective transcript processing and transport can occur in a gene-specific manner. The functional constraints, and the resulting complexity of such a network, may give significance to a major part of extragenic RNA transcribed along with the genes. Indeed, quite obviously, a precisely organized nuclear space has to be structured in a dynamic manner. In other terms, we suggest that introns and extragenic DNA initially sprung up from this genuine architectural function of transcripts and that, later on, differential splicing and other derived mechanisms took advantage of the fragmentation of genes in the organization of primary transcripts. Another type of genetic information, beyond the genetic code (c.f. Scherrer, "The Unified Matrix Hypothesis" [Scherrer, 1989]), may be involved in such a process, which secures selective transport and post-transcriptional regulation of gene expression within the "Cascade of Regulation" [Scherrer, 1980], from DNA to polyribosomes. This genomic information of a new kind may be based on the specific alignment of proteins along DNA and RNA, and their capacity to interact, among themselves and with the cellular networks, and hence guide selective processing and transport of transcripts on the gene expression pathway.

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