The Nucleoskeleton: A Permanent Structure of Cell Nuclei Regardless of Their Transcriptional Activity

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Nuclear matrix or nucleoskeleton is thought to provide structural basis for intranuclear order. However, the nature of this structure is still uncertain because of numerous technical difficulties in its visualization. To reveal the “real” morphology of the nucleoskeleton, and to identify possible sources of structural artifacts, three methods of nucleoskeleton preparations were compared. The nucleoskeleton visualized by all these techniques consists of identical elements: nuclear lamina and an inner network comprising core filaments and the “diffuse” nucleoskeleton. We then tested if the nucleoskeleton is a stable structure or a transient transcription-dependent structure. Incubation with transcription inhibitors (α-amanitin, actinomycin D, and DRB) for various periods of time had no obvious effect on the morphology of the nucleoskeleton. A typical nucleoskeleton structure was observed also in a physiological model—in transcriptionally inactive mouse 2-cell embryos and in active 8- to 16-cell embryos. Our data suggest that the nucleoskeleton is a permanent structure of the cell nucleus regardless of the nuclear transcriptional state, and the principal architecture of the nucleoskeleton is identical throughout the interphase.

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INTRODUCTION

The nucleoskeleton, or nuclear matrix, is a complex nuclear structure that is thought to provide a structural basis for nuclear compartmentalization and function. Numerous nuclear activities have been shown to be associated with the nuclear matrix (for reviews see [1–4]). Newly replicated DNA [5, 6] and various components of replication machinery [7–10] are retained in nuclear matrix preparations. When the soluble nuclear proteins and most of the chromatin are removed from the nucleus under gentle conditions, the remaining nucleoskeleton retains the ability to replicate DNA at rates close to those in vivo [11]. A significant part of the replication process takes place in “replication factories” attached to the nucleoskeleton [12, 13]. Transcriptionally active DNA [14], nascent pre-mRNA [15], and RNA polymerases [16, 17] are also associated with the nuclear matrix, and most of the transcriptional activity is retained after gentle chromatin removal [18]. Transcription factors can be linked to the nuclear matrix; for instance, several members of the steroid receptor superfamily are bound to the nuclear matrix [19–21] in a hormone-dependent and tissue- and cell-type-specific manner ([22, 23], for review see also [24]). The components of the splicing machinery have been shown to associate with the nuclear matrix [25, 26]. Nuclear matrices contain preassembled spliceosomes, which can splice pre-mRNA efficiently when some soluble splicing factors are added [27].

Structurally, the visualized nucleoskeleton consists of lamina and an inner network of 9- to 13-nm-thick “core filaments” covered with material referred to as “diffuse nucleoskeleton.” Remnants of nucleoli, nuclear bodies, and clusters of interchromatin granules are also observed in such preparations. The molecular composition of this inner structure is very complex (see e.g. [1, 28]). The core filaments of the nucleoskeleton morphologically resemble cytoplasmic intermediate filaments [29, 30]. They show positive immunoreactivity with an antibody directed against an epitope shared by all intermediate filament proteins, and, in addition, the diffuse nucleoskeleton is labeled by anti-lamin A antibodies at nodes of the branching core filaments [31]. NuMA (nuclear-mitotic apparatus protein) is another interesting candidate for being a component of the core filaments. Zeng and colleagues [32] localized NuMA at the core filaments, but there was no further confirmation.

In summary, the nucleoskeleton, or the nuclear matrix, is structurally and functionally complex, and
many of its properties still remain elusive. Although the techniques used for the visualization of the nucleoskeleton have highly improved, it is still unclear, for example, to what extent the isolated nuclear matrix preparations correspond to the existing in vivo structure. Nevertheless, preparation of nucleoskeletons using gentle techniques definitely shifted the idea about intranuclear skeleton from suggestion to reality. Possibly the best argument is that the nucleoskeleton contains many structures we know from intact nuclei and, above all, it preserves the main physiological activities—the ability to replicate and transcribe DNA. Currently two terms are used in literature: nuclear matrix and nucleoskeleton. The first was originally introduced for the definition of residual structure remaining in the nucleus after removal of chromatin and soluble proteins. We prefer to use the term nucleoskeleton with the understanding that this term describes nuclear structure participating in DNA replication and gene expression, which can be visualized by gentle extraction methods.

In order to assess gross dynamic changes of the nucleoskeleton, we have examined the connection between the transcriptional state of the nucleus and the morphology of the nucleoskeleton using two models: (1) cells treated with transcription inhibitors and (2) cells of early mouse embryos with various states of transcriptional activity. We have tested the possibility that the visualized nuclear matrix/nucleoskeleton represents a transient complex that forms as a result of transcription and RNA-processing activities rather than a permanent structure of the cell nucleus actively participating in regulation of gene expression [33, 34]. In order to interpret the results correctly, we first compared three relatively gentle techniques of nucleoskeleton preparation. The results show that all these methods reveal essentially identical structures that are present in cell nuclei regardless of the level of their transcriptional activity and suggest that the nucleoskeleton is a permanent structure of cell nuclei.

**MATERIALS AND METHODS**

**Cell Culture**

Human cervical carcinoma (HeLa) cells were grown at 37°C in suspension culture in Eagle minimum essential medium (S-MEM; Sigma, Cat. No. M-4767) supplemented with 5% (v/v) fetal calf serum. Before the experimental procedures, cells were encapsulated in agarose beads as described previously [35]. Cells embedded in agarose beads were regrown for 4 h before they were used in experiments.

**Early Mouse Embryos**

Two-month-old C57/CBA females were first injected with pregnant mare serum gonadotropin (4 iu per animal), then after 48 h with human chorionic gonadotropin (30 iu per animal), and mice were mated. Early two-cell stage embryos were collected after 32 h and eight-cell stage embryos after 56 h. Embryos were washed in Sörensens phosphate buffer (SB; 70 mM Na2HPO4, 30 mM KH2PO4, pH 7.4) and their zona pellucida was removed using 0.5% pronase in SB. Embryos were then washed several times in SB, embedded individually into small blocks of 1.5% low-melting-point agarose, and subjected to the nucleoskeleton visualization procedure.

**Treatment with Transcription Inhibitors**

HeLa cells in agarose beads were spun down, the medium was replaced with culture medium containing one of the transcription inhibitors, and the cells were then incubated at 37°C. Transcription inhibitors used were 0.5 μg/ml actinomycin D (Sigma, Cat. No. A-1410) for 4 h, 50 μg/ml DRB (Calbiochem, Cat. No. 287891) for 6 h, 100 μg/ml α-amanitin (Boehringer Mannheim, Cat. No. 161 284) for either 3 or 6 h. After incubation with inhibitors, the cells in agarose beads were washed twice in PBS and subjected to the nucleoskeleton preparation procedure or used for immunofluorescence.

To investigate the effect of treatment with transcription inhibitors on the fate of the newly synthesized RNA, BrU (Aldrich Chemical Co., Cat. No. 85,018-7) was added to the culture medium from 100 mM stock solution to final concentration of 50 mM. The cells were pulse labeled for 15 min at 37°C, washed with PBS and with fresh culture medium, and grown for 1 or 4 h in the presence of the above-listed transcription inhibitors or in fresh culture medium for control. The cells were then washed twice in PBS and fixed for subsequent immunofluorescence visualization of nascent transcripts.

**Visualization of the Nucleoskeleton**

Cells in agarose beads or embryos embedded in agarose blocks were permeabilized with 0.2% Triton X-100 in "physiological buffer" (PB; 130 mM KCl, 10 mM Na2HPO4, 1 mM MgCl2, 1 mM MnCl2, 1 mM Na2ATP, 1 mM DTT, 2.5 U/ml human placental ribonuclease inhibitor (Amer- sham, Cat. No. E 23102), 0.1 mM PMSF, pH adjusted to 7.4 by adding 100 mM KH2PO4 solution) for 10 min on ice. After that, one of the following protocols was used.

Restriction enzymes and electroleuton (after [12], slightly modified). After a wash in four changes of ice-cold PB, DNA was cut with 1650 U/ml EcoRI and 340 U/ml HaeII in PB containing 25 U/ml human placental ribonuclease inhibitor for 20 min at 33°C. Agarose beads with cells were then placed in wells of 0.8% agarose gel, and unattached chromatin fragments were electroleutoned at 4 V/cm for 3 h at 4°C.

Cross-linking and DNase I treatment (modified from [36]). Cell structures were cross-linked with 2% formaldehyde in PB for 10 min at 4°C. After thorough washing in PB (four changes, 5 min each), DNA was digested with 400 U/ml RNase-free DNase I (Bohringer Mannheim, Cat. No. 776 785) in PB containing 25 U/ml human placental ribonuclease inhibitor for 50 min at 32°C. Samples were then washed in four changes of ice-cold PB to remove unattached chromatin fragments.

DNase I treatment (modified from [37]). After permeabilization, cells were washed in four changes of ice-cold PB, incubated with 400 U/ml DNase I in PB containing 25 U/ml human placental ribonuclease inhibitor for 30 min at room temperature, and washed in four changes of PB.

In all three cases, removal of the chromatin was followed by fixation in 2.5% glutaraldehyde in PB for 1 h at 4°C, unless the samples were used for evaluation of the DNA content. Samples were then dehydrated and embedded into diethylene glycol distearate (DGD; EMCorp, Cat. No. ET2M) as described earlier [12]. Sections 600 nm thick were cut using a diamond knife and placed onto Pioloform-coated grids (agar, grids preincubated with poly-L-lysine in water), DGD was dissolved in n-butanol, n-butanol was sequen-
and observed immediately under electron microscope (Philips CM100).

Analysis of the DNA Content

After chromatin removal described above, cells were fixed in 4% paraformaldehyde in PB for 30 min at 4°C, washed in PBS, and mounted in Mowiol (Hoechst, Cat. No. 4-88) containing 0.08 μg/ml DAPI (Boehringer Mannheim, Cat. No. 236276) for DNA counter-staining along with control cells. Digital images of 200 nuclei from each sample were taken using a Hamamatsu C5985 CCD camera on an Olympus Vanox-S fluorescence microscope using identical conditions. Total signal intensity in each nucleus was measured using Leica image processing software, and percentage of chromatin removal by each technique was calculated.

Immunofluorescence

Cells were fixed in a mixture of 2% formaldehyde and 0.1% Triton X-100 in PBS for 20 min at 4°C, washed in three changes of PBS for 10 min each, and incubated for 1 h at room temperature with anti-RNA polymerase II mouse monoclonal antibody directed against the CTD domain (a kind gift from Dr. Marc Vigneron) or with anti-bromodeoxyuridine mouse monoclonal antibody (Boehringer Mannheim, Cat. No. 1170 376), both diluted in PBS to final concentration 5 μg/ml. After three washes in PBS, cells were incubated with FITC-conjugated sheep anti-mouse IgG antibody (Sigma) diluted in PBS 1:100 for 45 min in dark, washed in three changes of PBS, and mounted in Mowiol containing 0.08 μg/ml DAPI. Cells were observed under an Olympus Vanox-S fluorescence microscope and digital images were taken using a Hamamatsu C5985 CCD camera.

RESULTS

Nucleoskeleton in Control Cells

The nucleus of a HeLa cell from which chromatin was removed by electroelution after treatment with restriction enzymes is shown in Fig. 1. The visualized nucleoskeleton consists of nuclear lamina and an inner network of "core filaments" mostly covered with granulofilibrillar material referred to as "diffuse nucleoskeleton." Nucleoli are also visible. Frequently, one can see that the inner nucleoskeleton network radiates from nucleoli to lamina and these radial branches are interconnected by additional nucleoskeletal structures.

The Comparison of the Various Methods of the Nucleoskeleton Visualization

We compared three different relatively gentle techniques of the nucleoskeleton visualization in order to choose the most suitable one for our following experiments. The results are shown in Fig. 2. The morphology of the nucleoskeleton is essentially identical in all three cases. Comparison of nucleoskeletons obtained after treatment with restriction enzymes followed by electroelution of chromatin fragments (Fig. 2A) or by DNase I treatment with (Fig. 2B) or without preceding cross-linking (Fig. 2C) revealed only somewhat different degrees of preservation of the inner network. The structures were less preserved after treatment with DNase I without cross-linking, while the two other techniques gave very close degrees of preservation. The efficiency of chromatin removal in the three described protocols was measured by calculating the mean intensity of DAPI fluorescence per nucleus. In comparison with control cells with no DNA removed, the electroelution removed 90.6 ± 8.3% of DNA, DNase I treatment with preceding cross-linking 86.3 ± 9.7% of DNA, and DNase I treatment without cross-linking 93.6 ± 10.1% of DNA. Thus, in the subsequent experiments, we used for nucleoskeleton visualization the DNase I treatment with preceding cross-linking technique, as it produced morphologically equally good results compared to the electroelution but was easier and faster to perform.

Nucleoskeleton in Cells Treated with Transcription Inhibitors

In order to establish the relationship between the transcriptional state of the nucleus and the nucleoskeleton structure, we compared the nucleoskeleton in control cells and in cells in which transcription was inhibited for various intervals by inhibitors with different mechanisms of action.

α-Amanitin is an inhibitor of RNA polymerases II and III, but not RNA polymerase I. It inhibits RNA polymerase II via disruption of its largest subunit [39]. We first verified the effect of treatment with α-amanitin using immunofluorescence as shown in Figs. 3A-
A decrease of the speckled staining was observed after 3 h of incubation with 100 μg/ml α-amanitin (Fig. 3B); after 6 h of inhibition, the fluorescent signal practically disappeared (Fig. 3C). An electron micrograph of the nucleoskeleton of a cell after 6 h of α-amanitin treatment (Fig. 4B) shows that it has the same basic structural features as in control cells (Figs. 1 and 4A). The network of core filaments covered with diffuse skeleton remains undisrupted.

Actinomycin D (AMD) intercalates into double-stranded DNA in GC-rich regions and inhibits elongation phase of transcription. At high concentrations it inhibits essentially all RNA synthesis in all cell types [40]. The concentration used in this paper inhibits most of the transcriptional activity, as verified using radioactively labeled RNA precursors (data not shown). After 4 h of treatment with AMD, the main structural components of the nucleoskeleton—a network of core filaments covered with diffuse material and attached to nuclear lamina and nucleolar remnants—are clearly visible, as shown in Fig. 4C (compare to control cells in Figs. 1 and 4A).

DRB inhibits transcription in a more general way by inactivation of various protein kinases [41]. After 6 h of inhibition (Fig. 4D), the morphology of the nucleoskeleton is essentially the same as in control cells (see Figs. 1 and 4A).

We have also compared the lifetime of newly synthesized RNA in control cells and in cells treated with transcription inhibitors. Following the pulse labeling of control cells, Br-labeled RNA was observed initially in 86% of cells. After 1 h of growth without labeled RNA precursors, around 35% of nuclei still retained the label, while after 4 h less than 3% of nuclei were labeled. The respective values in the presence of transcription inhibitors were 41 and 2.5% for α-amanitin, 38 and 4% for actinomycin D, and 34 and 3% for DRB.

Nucleoskeleton in Early Mouse Embryos

The nucleoskeleton in G1 cells of early two-cell embryos with switched-off transcription was compared to nucleoskeleton in eight-cell embryos, in which the transcription is completely activated. The electron micrographs of resinless sections of the nucleoskeletons in two-cell and in eight-cell mouse embryos are shown in Fig. 5. In general, the nucleoskeleton in mouse two-cell and eight-cell embryos, and blastocysts (not shown), is very similar and resembles the nucleoskel-
etton structure in somatic cells. The only obvious difference is that lamina in embryonic cells appears thinner than in somatic cells, around 60 nm versus 75 nm, respectively.

**DISCUSSION**

Various Methods Reveal the Same Morphology of the Nucleoskeleton

Many various techniques have been invented for isolation and visualization of the nuclear matrix. They have been criticized for using steps that may cause precipitation of proteins and other major artifacts, such as incubation in hypotonic media, ATP depletion of the nucleus after permeabilization with detergents, changing the intranuclear redox conditions, “stabilization” of nuclear structure at 37°C or in the presence of Cu²⁺ ions, and incubation in high-salt media (see e.g. [42], recently reviewed in [43]). A gentler technique using “physiological” conditions, which is practically free of the disadvantages listed above, was developed by Jackson et al. [11]. In this protocol, cells are first

![Image of nucleoskeleton in cells treated with transcription inhibitors.](image)
encapsulated in agarose beads to protect them from mechanical distortions and lysed with a moderate concentration of Triton X-100 under controlled conditions, and chromatin is then removed by treatment with restriction enzymes and by electroelution. The residual nuclear structures retain the ability to replicate and transcribe DNA at rates close to those in vivo [11, 18]. We have compared this probably most gentle method used so far with two other techniques that use DNase I. All three techniques were tested using the same buffer conditions. They provided similar degrees of chromatin removal and revealed three principal structural components of the nucleoskeleton—lamina, network of core filaments, and the diffuse nucleoskeleton—with essentially identical quality. Obviously, the two better methods allow a reliable and repetitive visualization of the nucleoskeleton.

Nucleoskeleton and the Functional State of the Nucleus

Nuclear matrix preparations usually retain the majority (about 70%) of the total nuclear RNA [30] and the structure is destroyed by RNase treatment [31, 37, 44]. Only a very minimal nuclear matrix structure was observed in the nuclei of transcriptionally inactive cells, such as bird erythrocytes [45]. Based on these observations, the nucleoskeleton is sometimes viewed as a transient structure resulting from aggregation of the products of RNA synthesis and processing [33, 34]. We addressed this question by an application of several transcription inhibitors with different mechanisms of action. The principal architecture of the nucleoskeleton was not disrupted after a prolonged inhibition of RNA synthesis by α-amanitin, actinomycin D, or DRB. There have been several reports that transcription inhibitors also reduce the mRNA export to cytoplasm (see e.g. [46]). To exclude the possibility that newly synthesized RNA was upon inhibition of transcription retained in the nucleus and contributed to the observed structure, we checked also the lifetime of the nascent transcripts by pulse-labeling with BrU and then growing the cells without labeled precursors for various periods of time. The results show practically complete depletion of labeled RNA from nuclei during the period of treatment both in control cells and in cells treated with transcription inhibitors. Thus, it is highly unlikely that the observed structure of the nucleoskeleton represents aggregation of the transcription products. Our results clearly demonstrate that the nucleoskeleton is a permanent nuclear structure and not a transient, transcription-dependent structure. The collapse of the nucleoskeleton after RNase digestion may be caused by disruption of more stable RNAs, essential for maintaining the architecture of the nucleoskeleton, such as snRNPs [47], hnRNPs [26, 28, 31], or the stable subpopulation of poly(A)⁺ RNA that does not serve as mRNA which will be transported to the cytoplasm for translation [48].

The inhibition of transcription was also shown to cause changes in overall nuclear morphology and in rearrangement of rDNA and disruption of nucleolar structure in cells treated with various doses of actinomycin D [49], redistribution of heterochromatin masses upon treatment with α-amanitin [50], and dis-
perspective of chromosome territories as an effect of α-amanitin and DRB treatment [51]. Nickerson and colleagues [52] studied the relationship between RNA synthesis and chromatin architecture. They have shown a parallel decay of chromatin and of nuclear matrix organization after treatment with actinomycin D or DRB and suggested that the cause for chromatin rearrangement was a disruption of underlying nuclear matrix in the absence of transcription. The results presented here are more consistent with a model in which chromatin rearrangement is caused by modulation of chromatin attachment to the nucleoskeleton. The contradiction with the earlier data may be due to the differences in the nucleoskeleton/nuclear matrix isolation procedure as most of the older papers used rather disruptive conditions.

Interestingly, when the largest subunit of RNA polymerase II, through which it is anchored to the nucleoskeleton [53, 54], is destroyed, no significant structural changes of the nucleoskeleton are observed. This suggests that RNA polymerase II is not a key structural component of the nucleoskeleton and the association can be of a dynamic nature.

The results obtained with the use of transcription inhibitors could be questioned, however, because of nonphysiological treatment; therefore we chose a natural physiological model of a prolonged inhibition of transcription, which is provided by early mammalian embryonic development. Transcription in the maturing oocyte is repressed upon nuclear envelope breakdown at the onset of the first meiotic division, and the embryo is transcriptionally inactive shortly after fertilization. In the mouse, the embryo enters the transcriptionally permissive state at the late one-cell stage (18–19 h postinsemination) [55–58]. The activation of the zygotic genome (ZGA) takes place in two phases. During the first phase, also termed “minor ZGA,” just a few genes are expressed and as a consequence a small set of proteins is synthesized at the early two-cell stage (G1/S): the 70-kDa heat-shock proteins [59], the TRC (transcription-requiring complex) [60], the U2afbp-rs splicing factor [61], and the translation initiation factor, eIF-4C [62]. The transcriptional activity during this phase is very low, which was confirmed by microinjection reporter genes into the pronuclei of a one-cell embryo [63–65], for review see also [66]). After the first cleavage, this transcription is repressed unless the microinjected genes possess appropriate enhancers [66], and a sharp burst of transcriptional activity characterizing the “major ZGA” is observed only in the late (G2) two-cell embryo, when the second round of DNA replication is completed [67, 68]. RNA synthesis is therefore repressed during at least 20 h from the beginning of nuclear maturation to the onset of minor ZGA, and only small amounts of RNA are synthesized before the completion of the second round of DNA replication. We compared the nucleoskeleton in G1 cells of early two-cell embryos before the onset of major transcriptional activation and that in nuclei of eight-cell embryos, in which the transcription is completely activated. Nucleoskeleton has the same typical structure at both stages assessed, which shows that the nucleoskeleton is formed also in nuclei with minimal transcriptional activity. There have been no electron microscopic data on the nucleoskeleton of early mammalian embryos. The present paper therefore gives for the first time a description of the nucleoskeleton in mouse embryonic nuclei on the ultrastructural level.

Prather and Shatten [69] studied the distribution of several nuclear matrix antigens in oocyte germinal vesicles, in zygotic pronuclei, and in two- and eight-cell mouse embryos by immunofluorescence. They have shown that the antigens P11 (a 68-kDa antigen associated with nuclear pores and colocalizing with snRNPs) and P12 (a triplet of proteins, M, 35,000, 70,000, and 140,000) are present in the germinal vesicles and then disappear in metaphase II oocytes, are absent from zygotic pronuclei, and demonstrate bright staining again in late two-cell and in eight-cell embryos. At the same time, the P1 antigen (a triplet of proteins that cover the chromosomal mass) and lamin B were present in the nuclei of all cells studied. Another physiological model—mitogenic lymphocyte activation—was used by Shaly et al. [70]. They have shown that peripherin and P12 antigens did not reorganize during blastogenesis, while labeling of P11 and snRNP increased markedly and redistributed. Our data are in agreement with these previous findings that the nuclear matrix/nucleuskeleton has some dynamic components and some permanent, transcription-independent components. The studies on avian erythrocytes [45] that show absence of the nuclear matrix in mature genomically inactive cells and its presence in embryonic erythrocytes may reflect the same fact; however, the basic transcription-independent structure may be more sensitive to isolation procedure and removed under the harsher conditions they used. The results presented here definitely do not exclude the dynamic nature of the nucleoskeleton that is to be expected; they show that the nucleoskeleton is not a direct—and only temporary—result of RNA synthesis; however, it is a permanent structure of an interphase nucleus.

The cell nucleus undergoes significant structural changes in the progress of the cell cycle. It disassembles at the onset of mitosis and reassembles again in telophase. Little is known about the detailed behavior of the nucleoskeleton in these transition periods. However, we can claim that the nucleoskeleton is present throughout the whole interphase. This statement is supported by following observations: (1) in unsynchronized culture of HeLa cells, in G1 (our observations, not shown), G1, G2 [31], and S cells [12, 13], the overall...
structure of the nucleoskeleton is indistinguishable and (2) G1 cells of early 2-cell embryos contain the same typical nucleoskeletal structure as the 8- to 16-cell embryos and blastocysts in various stages of the cell cycle.

CONCLUSIONS

The paper confirms that the current methods of nucleoskeleton visualization provide sufficient tools for studying the nucleoskeleton without major structural artifacts. The nucleoskeleton has essentially the same morphology in various cell types: it consists of lamina and a network of core filaments mostly covered with diffuse nucleoskeleton. The results obtained with the use of transcription inhibitors and in early mouse embryos indicate that the nucleoskeleton is a permanent structure of the cell nucleus present regardless of its transcriptional state and of the cell cycle stage of interphase.

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REFERENCES


