Abstract. The nuclear matrix is concealed by a much larger mass of chromatin, which can be removed selectively by digesting nuclei with DNase I followed by elution of chromatin with 0.25 M ammonium sulfate. This mild procedure removes chromatin almost completely and preserves nuclear matrix morphology. The complete nuclear matrix consists of a nuclear lamina with an interior matrix composed of thick, polymorphic fibers and large masses that resemble remnant nucleoli. Further extraction of the nuclear matrices of HeLa or MCF-7 cells with 2 M sodium chloride uncovered a network of core filaments. A few dark masses remained enmeshed in the filament network and may be remnants of the nuclear matrix thick fibers and nucleoli. The highly branched core filaments had diameters of 9 and 13 nm measured relative to the intermediate filaments. They may serve as the core structure around which the matrix is constructed. The core filaments retained 70% of nuclear RNA. This RNA consisted both of ribosomal RNA precursors and of very high molecular weight hnRNA with a modal size of 20 kb. Treatment with RNase A removed the core filaments. When 2 M sodium chloride was used directly to remove chromatin after DNase I digestion without a preceding 0.25 M ammonium sulfate extraction, the core filaments were not revealed. Instead, the nuclear interior was filled with amorphous masses that may cover the filaments. This reflected a requirement for a stepwise increase in ionic strength because gradual addition of sodium chloride to a final concentration of 2 M without an 0.25 M ammonium sulfate extraction uncovered core filaments.
we found conditions that effectively remove chromatin with the least disruption of the non-chromatin nuclear structure. The procedure finally adopted (Capco et al., 1982; Fey et al., 1986) omits the initial isolation of nuclei, extracting instead the whole cell. After extraction with nonionic detergent, chromatin is digested with DNase I and removed with 0.25 M ammonium sulfate. This comparatively low ionic strength produces a more structurally complete nuclear matrix, freed of contaminating chromatin components (Fey et al., 1982). Resinless section EM shows this matrix to be a unique structure consisting of thick, lumpy fibers surrounded by the nuclear lamina. The fibers are sensitive to RNase A which causes them to collapse and aggregate into a few amorphous masses (Fey and Penman, 1988; Nickerson et al., 1989). The proteins of the matrix isolated by this mild procedure are distinct in different cell types. The cell type specificity of nuclear matrix proteins may reflect a nuclear matrix role in the selective activation of those genes which express differentiated function. This may be corollary to the matrix function as the underlying scaffolding for chromatin (Nickerson et al., 1989), selecting which chromatin regions are active in the cell.

The electron microscope shows only the surface morphology of the thick nuclear matrix fibers and not their interior structure. In particular, although RNA is essential to fiber organization, there has been no elucidation of its structural role. We, therefore, began to explore the inner structure of the nuclear matrix fibers using high ionic strength salt solutions to strip away the outer components. In this report we show that, underlying the thick matrix fibers, there is a network of thinner filaments composed of protein and RNA. The ionic strength of elution must be increased incrementally, first with 0.25 M ammonium sulfate and then 2 M NaCl, to reveal these filaments. Most nuclear RNA remained with these core filaments and intact RNA was required for filament integrity. These fibers may be a core around which other proteins are assembled in the complete nuclear matrix. We refer to them here as the core filaments of the nuclear matrix.

Materials and Methods

Cells
HeLa cells (S3) were grown in suspension culture at 4.0 x 10^5 cells/ml in MEM containing 7% (vol/vol) horse serum. HeLa cells (CCL2) and MCF-7 cells were grown in DME supplemented with 10% (vol/vol) FBS.

Cell Extractions
After a wash in PBS, cells were extracted in cytoskeleton buffer: 10 mM Pipes, pH 6.8, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl_2, 1 mM EGTA, 4 mM vanadyl ribosome complex, 1.2 mM PMSF, and 0.5% (vol/vol) Triton X-100. After 3 min at 4°C the cytoskeletal frameworks were separated from soluble proteins by centrifugation at 600 g for 3 min. In some experiments the cytoskeletal framework pellet was extracted in RSB- magik (10 mM Tris HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl_2, 4 mM vanadyl ribosome complex, 1.2 mM PMSF, 1% (vol/vol) Tween 40, and 0.5% (vol/vol) sodium deoxycholate) for 5 min at 4°C and pelleted as before. This step strips away the cytoskeletal leaving in the pellet nuclei with their attached intermediate filaments. In some experiments this step was eliminated, without affecting the morphology of the nuclear core filaments. Chromatin was removed with 50-200 µg/ml RNase-A free DNase I (Boehringer-Mannheim Biochemicals, Indianapolis, IN) at room temperature for 30 min in digestion buffer (10 mM Pipes, pH 6.8, 50 mM NaCl, 300 mM sucrose, 3 mM MgCl_2, 1 mM EGTA, 4 mM vanadyl ribosome complex, 1.2 mM PMSF, and 0.5% [vol/vol] Triton X-100). Ammonium sulfate was added from a 1 M stock solution to a final concentration of 0.25 M and the sample was again pelleted. This removed chromatin leaving a nuclear matrix-intermediate filament structure containing nuclear ribonucleoprotein complexes. This is the RNA-containing nuclear matrix. Cells can be extracted on a monolayer by gently adding and removing extraction solutions or can be extracted in suspension with gentle centrifugation steps between. Except for the DNase I digestion and 0.25 M ammonium sulfate elution, all steps were done at 4°C.

To reveal the core filaments of the nuclear matrix, the RNA-containing matrix, prepared as described above, was further extracted with 2 M NaCl. The structure was resuspended in digestion buffer and NaCl was added to a final concentration of 2 M from a stock solution of 4 M NaCl in digestion buffer before centrifugation as before. The core filaments of the matrix were no longer present after digestion with 10-100 µg/ml RNase A in digestion buffer. The RNase A had been boiled for 10 min to destroy any DNase or protease contaminants.

RNA Isolation and Analysis
RNA was isolated from each nuclear fraction by the guanidinium thiocyanate method followed by centrifugation through a cesium chloride cushion (Davis et al., 1986). [3H]RNAs were analyzed by electrophoresis in 1% agarose gels containing 2.2 M formaldehyde (Lehrach et al., 1977). After electrophoresis, gels were soaked in two changes of ethanol and then in 5% (vol/vol) PPO in ethanol for 1 h each. After precipitation of 2,5-diphenyloxazole by soaking in water for 1 h, the gel dried at 40°C under vacuum for fluorography.

Protein Analysis
Two-dimensional gel electrophoresis of 35S-labeled proteins was performed by the method of O'Farrell (1975) in 10% acrylamide gels. After electrophoresis, gels were processed with Resolution (EMCorp, Chestnut Hill, MA) for fluorography.

Electron Microscopy
After fractionation cells were fixed in 2.5% (vol/vol) glutaraldehyde in cytoskeleton buffer for 30 min at 4°C, washed first with digestion buffer and then three times in 0.1 M sodium cacodylate, pH 7.2, post fixed in 1% (vol/vol) OsO4 in the same cacodylate buffer for 5 min at 4°C, dehydrated in ethanol, and transferred to n-butanol before embedding in diethylene glycol distearate. Thin sections were cut and the embedding medium was removed with n-butanol. Sections were transferred to ethanol and dried through the CO2 critical point.

Results

Morphology of the Core Filaments of the Nuclear Matrix
The core filaments of the nuclear matrix were revealed by stripping the outer proteins of the thick nuclear matrix filaments. The first step was to prepare the nuclear matrix using 0.25 M ammonium sulfate. Cells were extracted with Triton X-100 in the nearly physiological ionic strength cytoskeleton buffer to remove membranes and soluble proteins (Capco et al., 1982, 1984; Fey et al., 1986). Most of the cytoskeletal proteins were then removed using the mixed detergents Tween 40 and sodium deoxycholate in a low ionic strength buffer, leaving a nucleus with no nuclear envelope but with intermediate filaments still attached to the nuclear lamina. The nucleus was then digested with DNase I, which cuts chromatin DNA between nucleosomes and the cleaved chromatin eluted with 0.25 M ammonium sulfate. The RNase inhibitor, vanadyl ribosome complex, was present during all steps.

Digestion with DNase I and elution with 0.25 M ammo-
Figure 1. The core filaments of the HeLa nuclear matrix. (a) Nuclear matrix. Cells grown in suspension were detergent extracted, digested with DNase I, and treated with 0.25 M ammonium sulfate to reveal the nuclear matrix. The nuclear interior contained thick nuclear fibers (F) together with dark granular nucleoli (N), bounded by the nuclear lamina (L), which also anchored the intermediate filaments extending from the lamina into the cytoplasmic space. This structure is the matrix-intermediate filament complex. (b) Core filaments of the nuclear matrix. The nuclear matrix-intermediate filament complex was further extracted with 2 M NaCl. This treatment removed most of the nuclear matrix proteins and revealed an underlying network of slender filaments. These HeLa core filaments were much thinner and more uniform than the original matrix fibers. (c) High-magnification view of HeLa core filaments. In this view core filaments could be clearly seen. The junctions, marked by arrowheads, were very smooth, without evidence for a separate junction structure.
nium sulfate removed >97% of nuclear DNA and most of the histones but left the interior nuclear matrix fibers. These interior nuclear fibers were bounded by the nuclear lamina, which also anchored the intermediate filaments extending from the lamina into the cytoplasmic space. This structure is the nuclear matrix-intermediate filament complex (Fig. 1a). The nuclear interior contained the polymorphic, knobbed fibers (F) of the matrix together with large, dark, granular nucleoli (N). The nuclear lamina (L) marked the boundary between the internal matrix fibers and the exterior intermediate filaments (IF).

Applying 2 M NaCl to the nuclear matrix-intermediate filament complex removed most of the nuclear matrix proteins and revealed an underlying network of slender filaments (Fig. 1b). These HeLa core filaments were much thinner and more uniform than the original matrix fibers. There were two filament types distinguished by their diameters: 9 and 13 nm. These diameters were measured relative to intermediate filaments since the absolute diameters were thickened somewhat by the carbon coating used to stabilize the specimen in the electron beam. The core filaments filled the nuclear space more densely and uniformly than the thick matrix fibers due, in part, to the salt-induced decrease in nuclear volume. The spatial distribution of the core filaments will be considered further below. Many nuclei had large, dark masses (M) with a granular morphology consistent with their being remnant nucleoli. The large dark mass (M) in the nucleus shown in Fig. 1b was typical in morphology though larger than average. These presumptive nucleolar remnants were less electron dense than those seen before the 2-M NaCl extraction (Fig. 1a). They were composed, in part, of granules ordered as though they aligned with interior filaments. Core filaments of larger than average diameter emanated from these presumptive nucleoli and might consist of the protein and RNA precursors to cytoplasmic ribosomes. The smaller dark masses are very likely unextracted fragments of the nuclear matrix.

The HeLa cells shown in Fig. 1 were grown in suspension. Cells growing in monolayer had a slightly different, more dispersed filament morphology. This may be due to the intermediate filaments which remain attached to the substrate, restricting nuclear shrinkage in 2 M NaCl. Figs. 2, A and B are medium and high magnification of the nuclear core filaments of an MCF-7 human breast carcinoma cell. The filament morphology was heterogeneous. There were two principal size classes of filaments with mean diameters of 9 and 13 nm. Some filaments showed repeating striations similar to, but not as clear as, those in the filaments reported by Jack-

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**Figure 2.** The core filaments of the MCF-7 nuclear matrix. (a) Core filaments. The nuclear lamina (L) connected the network of nuclear core filaments (below) to the cytoplasmic intermediate filament network (above). A residual mass (M) remained enmeshed in the network of filaments. (b) Higher magnification view of core filaments. In this higher magnification view, the tripartite junctions between core filaments could be seen more clearly. The network of core filaments connected to the lamina (L).
Figure 3. Nuclear matrix made by applying 2 M NaCl directly. Direct application of 2 M NaCl following DNase I digestion resulted in the aggregation of core filaments and other components as shown in this view of the nuclear interior.

Figure 4. Core filaments are removed by RNase A digestion. Treatment of the core filaments with 25 μg/ml RNase A for 10 min caused the removal of core filaments. Several empty nuclear shells (N) could be seen in this section, separated by regions of residual cytoskeleton (Cy) that consisted primarily of intermediate filaments.

He et al. Core Filaments of the Nuclear Matrix

The step wise application of salt was necessary to reveal core filaments. Applying 2 M NaCl directly to the nucleus after digestion with DNase I yielded the different and, apparently, less well preserved structure shown in Fig. 3. With this protocol, the nuclear material aggregated into amorphous masses. The two step procedure was far more effective in removing matrix proteins while leaving the core filaments distinct and intact. This difference was due to raising the ionic strength incrementally. Extraction with NaCl alone revealed similar core filaments provided the NaCl concentration was increased in graded steps (data not shown).

Even with a gentle, stepwise increase in salt, not every nucleus in a section was filled with core filaments. Some nuclei appeared partially or completely empty. This may be due to incomplete preservation of structure. Other cells appeared incompletely extracted with the core filaments hidden beneath the insufficiently removed covering proteins. In more recent experiments we have replaced the DNase A with a combination of Hae III and Pst I (data not shown). This combination of restriction enzymes removes 93% of DNA after 2 M NaCl extraction and reveals core filaments in every section. The inadequate preservation of structure in some cells and incomplete extraction in others is caused in some way by DNase I preparations and does not reflect a real and different morphology of core filaments in subsets of cells. It is possible that DNase I is disrupting the core filament structure, not as a nuclease, but as a high-affinity actin-binding protein (Pollard and Cooper, 1986). The large amount of actin in the core filament fraction (Fig. 6 c) and the localization of actin in the core filaments by immunogold staining with anti-actin mAbs (data not shown) lend some support to this interpretation.
Figure 5. Stereoscopic view of the nuclear core filament network. (a) MCF-7 core filament network. The network of core filaments (below) is bounded by the nuclear lamina (L). A dense mass of residual material (M) remained enmeshed in the network. (b) HeLa core filament network. In this higher magnification stereoscopic view, the core filaments can be seen connecting to filaments of the nuclear lamina (L) throughout the section. The filaments can be clearly seen entering the large dense mass of residual material (M).
The core filaments of the nuclear matrix were removed by mild digestion with RNase A (Fig. 4). Treatment of the core filament preparation with as little as 10 μg/ml of RNase A for 10 min resulted in their nearly complete disappearance leaving an empty nuclear shell. The appearance of this shell or lamina was unchanged by RNase A digestion. The intermediate filaments filling the cytoplasmic space could still be seen to connect to the empty nuclear lamina. The filament network clearly required intact RNA for its overall integrity but the exact location and structural role of this RNA is not yet known.

**Stereoscopic Images of the Nuclear Matrix Core Filament Network**

The three-dimensional organization of the nuclear matrix core filaments and their attachments to the nuclear lamina were best seen in stereo images (Fig. 5). While the conventional embedded section images metal stains on its surface, the resinless section displays its entire contents. The third dimension of the image can be presented by tilted stage stereoscopic micrographs. Fig. 5a presents a low-magnification stereoscopic view of the core filament network from a monolayer MCF-7 cell. Fig. 5b shows the core filaments of a suspension grown HeLa cell in stereo at higher magnification. The three-dimensional network of core filaments filled the section and appeared to be connected directly to the lamina fibers.

A number of dark, granular presumptive remnants of the nuclear matrix were enmeshed in the network. The stereoscopic image showed them enclosing bundles of core filaments. If these were actually matrix remnants, the images suggest that the core filaments may be extensively bundled into the thick matrix fibers. This is consistent with the very different spatial distributions of thick fibers and filaments (see Discussion).

**The Core Filament Proteins**

The relative content of protein and DNA in nuclear subfractions is shown in Table I. The nuclear matrix prepared using 0.25 M ammonium sulfate had <3% of the cellular DNA. Most of this was removed by the subsequent elution with 2 M NaCl and the core filaments retained <0.1% of the cellular DNA. The 2 M NaCl also removed 35% of the protein remaining after chromatin removal, though some of these proteins may derive from the remaining cytoskeleton.

The proteins of the nuclear matrix, prepared with DNase I and 0.25 M ammonium sulfate, were a moderately complex set, many of which vary with cell type (Fey and Penman, 1988). Extracting this nuclear matrix-intermediate filament complex with 2 M NaCl removed many proteins, as shown in Fig. 6b, leaving the core filaments. Some of these proteins may have been removed from the remaining cytoskeleton, which mostly consists of intermediate filaments; the proteins removed by 2 M NaCl from the nuclear matrix were a subset of those shown in Fig. 6b. The proteins of the core filaments were then released by treatment with RNase A (Fig. 6c), a treatment that removed core filaments but left the nuclear lamina and exterior intermediate filaments intact (Fig. 4). The protein fraction contained all the core filament proteins but may also have contained proteins released from outside the nucleus. Therefore, the protein composition of the core filaments shown in Fig. 6c is tentative. The predominant protein in this RNase A released core filament fraction had the mobility and isoelectric point of actin. Immunostaining of resinless sections with anti-actin antibodies and colloidal gold-coupled second antibodies showed that actin was present in the core filaments but not as actin filaments (data not shown). At least some of the actin released in RNase A was, therefore, released from the nucleus. The proteins remaining after RNase A digestion are shown in Fig. 6d. The predominant proteins were similar to proteins previously identified as keratins, vimentin, and lamins (Fey et al., 1986; Fey and Penman, 1988). It is clear that there is considerable overlap in the proteins released in 2 M NaCl (Fig. 6b) and those subsequently released by RNase A (Fig. 6c). This may reflect the incomplete extraction of some cells as discussed above.

**Table I. The Distribution of Protein and DNA in HeLa Nuclear Fractions**

| Fraction          | Nuclear protein* | Nuclear DNA* |
|-------------------|------------------|--------------|
|                   | %                |              |
| Chromatin         | 87.4             | 99.6         |
| 2 M NaCl released | 4.4              | 0.32         |
| RNase A released  | 3.3              | 0.006        |
| Remaining material| 4.9              | 0.005        |

HeLa cells (CCL2) were grown in monolayers and labeled with either [3H]methionine (25 μCi/ml) for 2 h or with [methyl-3H]thymidine (10 μCi/ml) for 24 h. Cells were sequentially fractionated as described in Materials and Methods and aliquots of the fractions were counted. Chromatin was removed by digestion with DNase I and elution with 0.25 M ammonium sulfate. The remaining nuclear structures were extracted with 2 M NaCl and then digested with RNase A. Material not released (Remaining material) was dispersed in 8 M urea for scintillation counting.

* (Counts per minute in fraction)/(total nuclear counts per minute) × 100.

† The chromatin fraction is the material released by DNase I digestion and 0.25 M ammonium sulfate extraction.

**RNA of the Nuclear Matrix Core Fibers**

Nuclear RNA consists of approximately equal amounts of ribosomal precursor RNA and heterogenous nuclear RNA (hnRNA). Ribosomal precursor RNA is largely, although not entirely, located in the nucleolus (Perry, 1962; Penman et al., 1968, 1969). The location of the hnRNA has been less certain, although it is known to be extranucleolar, in the nucleoplasm (Penman et al., 1968). Here we show that 70% of total nuclear RNA remained after extraction of the nuclear matrix with 2 M NaCl. Thus, most hnRNA was associated with the core filaments. This hnRNA is of very high molecular weight (Fig. 7).

HeLa cells were labeled for 2 h with [3H]uridine, a sufficient time for nuclear RNA to be completely labeled. A low level of actinomycin (0.04 μg/ml) was added to a second sample to selectively suppress the synthesis of ribosomal precursor RNA. After labeling, the cells were fractionated as before and the amount of radioactive RNA in the several nuclear subfractions determined (Table II). The RNA in each subfraction was purified and analyzed by gel electrophoresis (Fig. 7).

About 28% of nuclear RNA was released with the chromatin by DNase I digestion and 0.25 M ammonium sulfate extraction (Table I). An additional 4% was removed by a further 2 M NaCl extraction to reveal core filaments. The final 67% of nuclear RNA was retained with the core filaments. Treatment of cells with 0.04 μg/ml actinomycin D during...
Two-dimensional protein gel electrophoresis of HeLa nuclear fractions. HeLa cells, grown in monolayers, were labeled for 20 h with L\(^{35S}\)methionine (25 \(\mu\)Ci/ml) before extraction. Two-dimensional gel electrophoresis of \(35S\) labeled proteins was performed by the method of O'Farrell (1975) in 10\% acrylamide gels with \(7 \times 10^6\) cpm loaded per gel. Molecular weight markers are shown with units of kilodaltons and isoelectric point markers are shown above in units of pH. (a) Chromatin proteins removed from the nucleus by DNase I digestion and 0.25 M ammonium sulfate extraction. (b) Nuclear matrix proteins removed by 2 M NaCl. (c) Core filament proteins removed by RNase A digestion. (d) Proteins remaining after RNase A digestion. These were the proteins remaining in the pellet after RNase A digestion and centrifugation at 1,000 g for 5 min.

Labeling selectively inhibits ribosomal RNA synthesis and decreased the labeling in each fraction by about half.

The result of formaldehyde-agarose gel electrophoresis of these labeled RNA fractions is shown in Fig. 7. The mature 28S ribosomal RNA was present in both the chromatin (lane 1) and 2 M NaCl released fractions (lane 2), along with a smaller amount of 18S ribosomal RNA. The 45S ribosomal RNA precursor and the 41S, 36S, and 32S processing intermediates with some 28S RNA remained with the core filaments (lane 3), perhaps in remnant nucleoli. The selective suppression of ribosomal RNA synthesis before labeling showed the hnRNA distribution in the nuclear subfractions. The RNA released by 0.25 M ammonium sulfate after DNase I digestion was heterogeneous but much smaller, on average, than the hnRNA of the core filaments. The modal size of this RNA was \(\sim 2.5\) kb and it may consist of message sized molecules in transit to the cytoplasm. About 70% of nuclear hnRNA remained with the core filaments (Table II) and was highly enriched in very high molecular weight hnRNA (Fig. 7, lane 6) with a modal size of \(\sim 20\) kb. Thus the core fibers contained most hnRNA and almost all the very high molecular weight hnRNA.

Discussion

This report examines the filament network revealed by removing part of the nuclear matrix with high concentrations of salt. These filaments may serve as the core structure of the nuclear matrix. The core filaments were composed of a different set of proteins than the chromatin fraction and contained most of the nuclear RNA. Essentially all high molecular weight hnRNA and ribosomal RNA precursors were associated with this network and its structural integrity was destroyed by RNase A. This can explain the earlier finding that...
RNA was labeled and extracted from HeLa nuclear fractions as described in Table II and electrophoresed in a 1% (wt/vol) agarose gel as described in Materials and Methods with equal cpm of RNA loaded per lane. (Lanes 1-3) RNA from control cells. Lane 1 was the chromatin associated RNA released by DNase I digestion and 0.25 M ammonium sulfate extraction. Lane 2 was RNA released by 2 M NaCl from the complete nuclear matrix. Lane 3 was the RNA that remained after extraction with 2 M NaCl. The ribosomal precursor RNAs were not removed by 2 M NaCl (lane 3), but remained with the core structure. This included the 45S (13 kb), 41S (9.1 kb), and 32S (6.1 kb) rRNA species. The core structure retained some of the fully spliced 28S rRNA, but very little 18S rRNA. (Lanes 4-6) RNA from cells treated with 0.04 μg/ml actinomycin D. In these fractions, there was little labeled rRNA and the hnRNA could be better seen. In the chromatin associated hnRNA released by DNase I digestion and 0.25 M ammonium sulfate extraction (lane 4) there was a heterogeneous population of RNA of between 2 and 5 kb. Very little high molecular weight hnRNA (modal size, 20 kb) was removed with the chromatin or by 2 M NaCl (lane 5). This hnRNA was retained with the core structure (lane 6).

Although the core filaments were much more uniform in diameter and morphology than the highly polymorphic, thicker fibers of the complete matrix, they may still be heterogeneous. The average diameter of the core filaments was ~8-10 nm but there were filaments which were larger. Some of these thicker filaments emanated from what might have been residual nucleoli and may have been extended ribosomal subunits in transit to the cytoplasm.

The core filament network was highly branched with many filament junctions (Fig. 2, a and b). At these magnifications, no coupling structure could be seen and the filaments appeared to blend smoothly into one another. One possible means of joining filaments could be through their RNA.

### Table II. The Distribution of RNA in HeLa Nuclei

| Fraction       | % of Nuclear RNA | % of Control |
|----------------|------------------|--------------|
| Chromatin      | 16.9             | 28.7         |
| 2 M NaCl       | 2.5              | 4.2          |
| Core filaments | 39.4             | 67.1         |
| Total nuclear  | 58.8             | 100          |

|                | 0.04 μg/ml Actinomycin D |
|----------------|--------------------------|
| Chromatin      | 8.0                      | 27.4  | 47.4 |
| 2 M NaCl       | 1.2                      | 4.1   | 47.7 |
| Core filaments | 20.0                     | 68.6  | 50.8 |
| Total nuclear  | 29.2                     | 100   | 49.7 |

HeLa CCL2.2 cells (2.75 × 10^5 cells per incubation) were grown in [3H]uridine (50 μCi/ml) for 2 h at 37°C. The cells were grown with or without 0.04 μg/ml actinomycin D to inhibit ribosomal RNA synthesis and were fractionated as described in Materials and Methods.
decrease in nuclear size. Second, it separates into two steps the removal of chromatin and the major nuclear shrinkage.

2 M NaCl accomplishes two things. First, it increases the ionic strength in two steps and should cause a more gradual volume decrease in 0.25 M ammonium sulfate. When the nuclear radius decreased by as much as 20% corresponding to a volume decrease of 50%. There was a much smaller decrease in 0.25 M ammonium sulfate. The protein composition of these thick fibers is complex and specific to cell type (Fey and Penman, 1988). Further study of this phenomenon is clearly required.

The discovery of RNA-containing core filaments in the nuclear interior has given a new perspective to the work of Lothstein et al. (1985) who studied a 43S RNase A resistant nuclear particle generated by nuclease treatment of hnRNPs which had been released by sonication. This RNA-containing particle polymerized into 18–20-nm filaments under conditions of low ionic strength. The filaments had a visible helical repeat of 60 nm. These filaments, polymerized in vitro, have a different appearance from the core filaments of the nuclear matrix which have a smaller diameter and lack a helical repeat. Nevertheless, it is interesting to speculate that both nuclear core filaments and the in vitro polymerized filaments of Lothstein et al. (1985) may be assembled by related interactions between RNA and the hnRNP proteins A2, B1, and B2. The low ionic strength in our core filament extraction procedure could be omitted without changing the distribution of nuclear core filaments. Thus, we are satisfied that the core filaments were not generated during the extraction by a low ionic strength polymerization of hnRNP fragments. It may be, however, that the in vitro generated filaments may hold a clue to core filament composition.

While it is impossible to completely eliminate the possibility that the core filaments are generated during the extraction procedure, our confidence in their existence is strengthened by the observation of similar filaments in cells which have not been exposed to nuclease digestions, low ionic strength incubations, or high salt extractions. Previous reports from this laboratory have discussed nuclear filaments and the relationship of these filaments to the much more extensive network of nuclear matrix chromosomes. These nuclear filaments have a morphology similar to the core filaments described here. The filaments first become visible as the chromatin condenses in early prophase (Capco and Penman, 1983). The uncovered filaments remain attached to the chromosomes throughout mitosis (Capco and Penman, 1983; Wagner et al., 1986).

We have also seen nuclear filaments in adenovirus-infected HeLa cells (Zhai et al., 1987) as chromatin retracts, leaving filaments uncovered. At later stages of adenovirus infection these filaments are decorated with adenovirus nucleocapsids. The mitotic and adenovirus infected cells provide only occasional glimpses of nuclear filaments and the relationship of those to the much more extensive network of nuclear matrix core filaments discussed here is unclear.

Nuclear filaments have also been reported by Jackson and Cook (1988) who use a very different procedure to reveal.

| Figure 8: Model of nuclear matrix structure. The nuclear lamina with its nuclear pores forms the outer boundary of the nuclear matrix. Intermediate filaments (IF) radiate out from the lamina into the cytoplasmic space. The interior nuclear matrix is composed of thick, polymorphic fibers. These are the fibers revealed when chromatin is removed by digestion with DNase I and elution with 0.25 M ammonium sulfate. The protein composition of these thick fibers is complex and specific to cell type (Fey and Penman, 1988). Further extraction of this complete nuclear matrix with 2 M NaCl removes many of these matrix proteins and reveals the much thinner core filaments. Highly branched networks of hnRNA molecules held together by regions of stable duplex between complementary or nearly complementary sequences have been found in HeLa nuclei (Federoff et al., 1977). Interstrand bonding of hnRNA might occur at core filament junctions, a hypothesis we will examine further. There are several questions as yet unanswered about the preparation of the core filament network. It is not clear why the stepwise application of salt was necessary. Applying 2 M NaCl directly to a DNase I-treated nucleus produced a very different morphology, in which the core filaments are either obscured or not preserved. Further experiments, not reported here, show that a gradual increase in ionic strength appears to be important. NaCl alone could be used for eluting both chromatin and the outer nuclear matrix proteins provided it was applied step wise beginning at ~0.3 and increasing to 2 M. This procedure revealed core filaments similar to those seen after 0.25 M ammonium sulfate-2 M NaCl. One observation may be relevant. The 2 M NaCl caused a significant shrinkage of the nuclear structure. The nuclear radius decreased by as much as 20% corresponding to a volume decrease of ~50%. There was a much smaller volume decrease in 0.25 M ammonium sulfate. When the ionic strength is increased rapidly after DNase I digestion, the nuclear matrix may shrink rapidly while digested chromatin is being released. The matrix may be mechanically damaged by this procedure or chromatin remnants may be trapped. Further study of this phenomenon is clearly required.

The protocol using 0.25 M ammonium sulfate followed by 2 M NaCl accomplishes two things. First, it increases the ionic strength in two steps and should cause a more gradual decrease in nuclear size. Second, it separates into two steps the removal of chromatin and the major nuclear shrinkage.
them. Agarose-encapsulated cells are extracted with detergents and digested with the restriction enzyme Hae III. Chromatin is then removed, not by salt elution, but by electrophoresis, and the matrix is visualized by resinless section electron microscopy. This procedure removes chromatin incompletely; ≈25% of the DNA is retained, compared with <1% in our preparation. The remaining chromatin appears to cover the core filaments which are seen only in scattered uncovered regions. Filaments are not seen in their procedure if DNase I is substituted for the restriction enzyme. The unusual nature of the Jackson and Cook procedure makes it difficult to compare their results directly with those reported here. The filaments revealed have a similar appearance, but some of the properties may be different. The filaments seen with our procedure are not completely removed by DNase I and they have less pronounced and regular striations. The procedure that we report here for uncovering filaments is simple, rapid, and is easily employed in any laboratory.

In a previous paper (Nickerson et al., 1989), we showed directly that digesting nuclei with RNase A led to a collapse both of chromatin and of the underlying nuclear matrix. The results reported here suggest a mechanism for this phenomenon. The core filaments, which depend on intact RNA for their integrity, may be a basic component of the nuclear matrix. Disruption of nuclear RNA, either by digesting nuclei in vitro or by drug treatments in vivo, destroys the core filaments. This causes the collapse of the nuclear matrix, which may, in turn, affect the architecture of chromatin organized on the matrix. The RNA-dependent nuclear matrix may be the scaffolding used for the higher order folding of chromatin into the nucleus.

Some previous studies (Berezney and Coffey, 1974; Capco et al., 1982) have used an RNase A digestion step in the matrix isolation procedure. The resulting nuclear matrix retains many of its biochemical properties but its morphology is severely altered by the RNase A digestion (Fey et al., 1986; Nickerson et al., 1989). This was not apparent in previous studies that used Epon-embedded sections or unembedded whole mounts for EM.

The existence of hnRNA containing core filaments supporting the nuclear matrix may also be related to a long-standing conundrum of nuclear RNA metabolism. Both the mass and genetic complexity of hnRNA greatly exceed what would be expected if it consisted simply of mRNA precursors (Scheller et al., 1978; Salditt-Georgieff et al., 1981; Harpold et al., 1981). Rough estimates of hnRNA complexity and kinetic measurements of RNA metabolism show that the mRNA emerging from the nucleus is only a few percent of the hnRNA labeled in the nucleus. This is a much smaller conversion of nuclear RNA than can be explained by the loss of RNA during splicing. Either the processing of message precursor molecules is not conservative or, as experiments suggest, there are many hnRNA molecules that are not mRNA precursors. The most compelling demonstration that a major portion of hnRNA transcripts do not contain a message sequence are the experiments of Darnell and co-workers (Salditt-Georgieff et al., 1981; Harpold et al., 1981). These measurements show that 75% of hnRNA has no poly A and will not hybridize to cloned mRNA sequences. Also, the number of 5′-RNA caps is much larger than the number of caps entering polyribosomes. The inescapable conclusion is there are more hnRNA transcripts than there are mRNA precursors. Much of the hnRNA is unaccounted for in current theories of RNA metabolism and function.

The results presented here show that hnRNA is a component of the core nuclear matrix filaments. As such, it may play a role in nuclear architecture and in the organization of chromatin. Such a structural role may be only one of its multiple functions but it suggests a more complex role for nuclear RNA than has often been assumed. It is not surprising to find RNA playing a structural role in the cell. Half of cellular RNA is ribosomal and this ribosomal RNA is clearly essential for the assembly and maintenance of ribosome structure. In a similar way, hnRNA may serve as part of the basic internal structure of the nucleus. An important difference between ribosomes and core filaments is that while ribosomes consist of a few RNA species and a complex set of proteins, the core filaments have a complex set of RNAs.

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