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Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat

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Whether nucleoskeletons seen after extracting cells are preparative artefacts is controversial. Using an extraction method that preserves vital nuclear functions, we have visualized part of a nucleoskeleton by electron microscopy of thick resinless sections. Cells encapsulated in agarose microbeads are lysed using Triton in a physiological buffer; the agarose coat prevents aggregation and protects fragile cell contents. These extracted cells are accessible to small molecules and transcribe and replicate at rates close to those *in vivo*. After electroeluting most chromatin after treatment with *Hae*III, a skeleton is uncovered which ramifies throughout the nucleus. Individual filaments are ~10 nm wide with an axial repeat of 23 nm, characteristic of intermediate filaments.

Key words: chromatin/intermediate filaments/nucleoskeleton/nuclear matrix/nuclear structure

Introduction

Whether there is a skeletal structure that ramifies throughout the nucleus has been controversial since the beginning of the century (Wilson, 1928; Cook, 1988). The controversy centres on whether any sub-structure seen after fixation is produced artefactually. RNA, DNA and protein are so highly concentrated in the nucleus that they might be expected to aggregate if the ionic conditions were altered. Rather extreme conditions are used to extract cells because chromatin and nuclei aggregate into an unworkable mess at physiological salt concentrations (Fredericq, 1971). Extracted structures include matrices, scaffolds and nucleoids (Agutter and Richardson, 1980; MacGillivray and Birnie, 1986); some have been implicated as the sites of replication, transcription and repair (Pardoll *et al.*, 1980; Jackson *et al.*, 1984). Although these structures are generally similar, sceptics point to the differences. Thus, extracting liver cells in roughly similar ways can leave an internal matrix (Berezney and Coffey, 1977) or external envelope (Aaronson and Blobel, 1975); extracting nuclei with high salt concentrations gives matrices but low concentrations yield dispersed chromatin unassociated with any visible skeleton (Miller, 1984). Variability may stem from differences in the sequence of operations (Kaufmann *et al.*, 1981), oxidative cross-linking of protein (Berezney and Coffey, 1977), the assembly of ribonucleoprotein particles into filaments induced by low salt concentrations (Lothstein *et al.*, 1985), heat shock (Evan and Hancock, 1985; Welch and Suhan, 1985; Littlewood *et al.*, 1987; McConnell *et al.*, 1987) and copper binding by mercaptoethanol (Lebkowski and Laemmli, 1982).

The high chromatin concentration within unextracted nuclei obscures any sub-structure, but occasionally filamentous networks are seen after extraction with Triton and slightly hypo- or hypertonic buffers (see, e.g. Capco *et al.*, 1984; Fey *et al.*, 1986). The obvious networks of matrices, scaffolds and nucleoids—often associated with cytoplasmic intermediate filaments—become visible only after exposure to abnormally high salt concentrations.

Recently we devised a method for isolating chromatin using *physiological* salt concentrations (Cook, 1984; Jackson and Cook, 1985a; Jackson *et al.*, 1988). Cells are encapsulated in 0.5% agarose microbeads of ~50 μm in diameter. As agarose is freely permeable to small molecules, cells can be grown in beads or extracted and then manipulated without aggregation. We disrupt membranes using Triton X-100 in a 'physiological' buffer (pH 7.4) containing 22 mM Na^+, 130 mM K^+, 1 mM Mg^{2+}, <0.3 μM free Ca^{2+}, 132 mM Cl^-, 11 mM phosphate, 1 mM ATP and 1 mM dithiothreitol (Jackson *et al.*, 1988). This buffer preserves gross structure and maintains DNA integrity: function is also preserved since such encapsulated nuclei synthesize RNA and DNA authentically at >85% of the rate found *in vivo* (Jackson and Cook, 1985b, 1986b; Jackson *et al.*, 1988).

Figure 1 outlines our approach for visualizing a nucleoskeleton. Living cells are labelled for 24 h with [^{3}H]thymidine, encapsulated, re-grown for 1 h and lysed. The dense mass of chromatin that might obscure any skeleton can be removed electrophoretically after fragmentation with a nuclease. After fixation in 2.5% glutaraldehyde, residual material is visualized by electron microscopy using thick (150–300 nm) resinless sections. Loss of ^{3}H from beads reflects the amount of DNA (and chromatin) removed electrophoretically. All manipulations, from lysis to fixation, take place in the physiological buffer and nuclease digestions are conducted at 33°C to minimize any thermally-induced aggregation.

Results

The morphology of isotonically extracted cells

Electron micrographs of thin sections of extracted cells show that nuclear morphology is exceptionally well-preserved (Figure 2A and B); heterochromatin remains condensed and associated with the nuclear periphery. Thin sections held in resins are not particularly suitable for visualizing diffuse networks in three dimensions, so we prepared thicker (150–300 nm) resinless sections (Fey *et al.*, 1986; Wagner *et al.*, 1986). These confirm that overall nuclear morphology is preserved, despite some collapse of cytoskeleton away from the surrounding agarose (Figure 2C and D). Under high power (Figure 2E and F), few filaments can be differentiated from chromatin.

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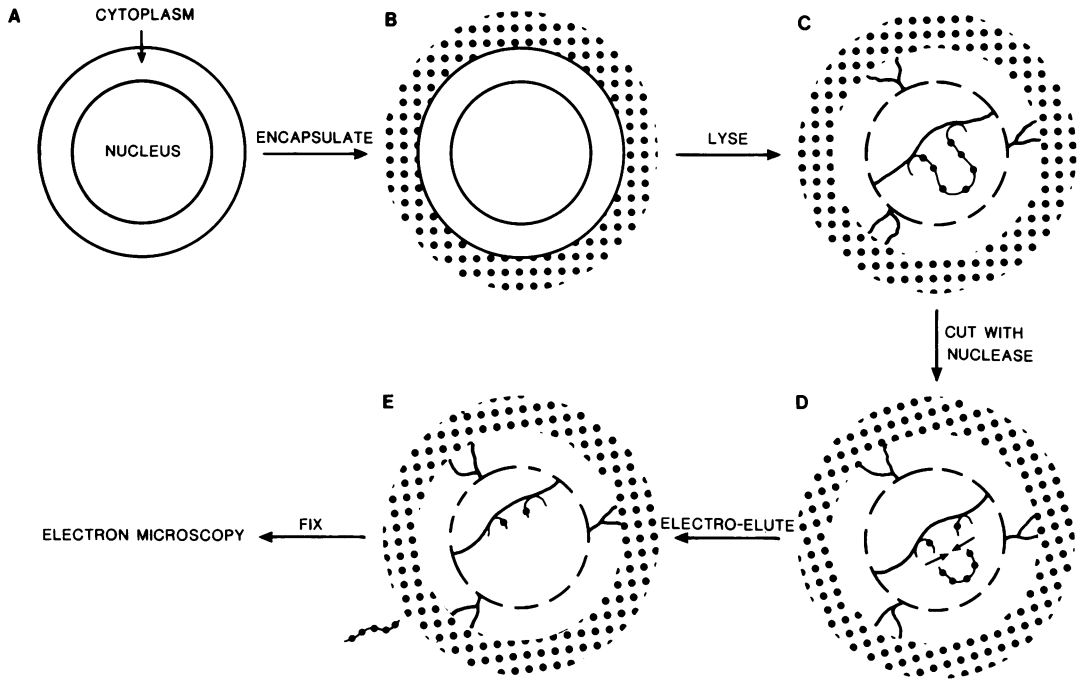

Fig. 1. Outline of experimental approach. Cells (**A**) are encapsulated (**B**) in agarose microbeads (stippled area), lysed (**C**) and washed in a physiological buffer. Structures too large to escape through agarose are left in beads and include the cytoskeleton, nuclear lamina (dashed circle) and chromatin (looped 'beads on a string') which obscures any underlying nucleoskeleton. Chromatin is fragmented (**D**) by addition of a nuclease (arrows) and small unattached pieces removed electrophoretically (**E**). Finally, samples are fixed and viewed in the electron microscope: any underlying nucleoskeleton can now be seen in the relatively empty nucleus.

Uncovering a nucleoskeleton

Extracted cells were treated with *Hae*III, subjected to electrophoresis to remove chromatin fragments, fixed and then thick sections prepared. Chromatin particles containing DNA fragments up to 150 kb pass through agarose; we use levels of nuclease that fragment DNA into pieces of 1–10 kb (Jackson and Cook, 1985b; unpublished data).

Subjecting undigested but extracted cells to electrophoresis has little effect on morphology; most nuclear material cannot be electroeluted (see later). After digestion with moderate concentrations of *Hae*III, sufficient chromatin is electroeluted (measured by loss of [^{3}H]DNA) that a nucleoskeleton is partially uncovered (Figure 3A). Filaments ramify throughout the nucleus from lamina to nucleolus with residual clumps of chromatin strung along them. Figure 3(B and C) shows higher magnifications of the same region of a different section. Most importantly, these stereo pictures show filaments and associated chromatin suspended in space without collapse onto any surface. One drawback of using a supporting resin that is removed after sectioning is that material unattached to the grid surface—directly or indirectly—is lost and densities are underestimated.

More nucleoskeleton can be uncovered by treatment with higher concentrations of nuclease but then the nuclear region often collapses, making it difficult to distinguish (unpublished results). This can be prevented partially by an additional pre-fixation with 0.25% glutaraldehyde prior to electroelution; then chromatin still electroelutes to leave an extensive network of filaments (Figure 4). Again it is important to view these stereoscopically to appreciate how diffuse the network really is.

Dimensions of the nucleoskeleton

Figure 5(A) illustrates the skeleton relative to circles with diameters roughly those of a nucleosome (10 nm), one turn of the solenoid (30 nm) and a chromatin loop of 500 closely-packed nucleosomes containing 100 kb DNA (Cook and Brazell, 1975; Paulson and Laemmli, 1977). Although skeletal segments vary in length, it is easy to imagine how each might associate with a chromatin loop.

Images of 10 different filaments are shown at high magnification in Figure 6(A). It is difficult to determine their real width as the depth of stain is unknown, but they have an apparent width of 25.3 nm (SD = 3.6; n = 150), similar to the main cytoskeleton filaments (width = 25.7 nm; SD = 4.3; n = 156) which are probably Triton-insoluble intermediate filaments (Steinert and Parry, 1985). Nucleosomes, discs with true diameters of 11 nm when viewed from above their flat face, appear as 37.2 nm discs (SD = 2.3; n = 167; only circular nucleosomes viewed from above were counted). If we assume that nucleosomes and filaments are coated with stain to equal depths, then the real widths of the nuclear and cytoplasmic filaments are 7.5 nm (SD = 1.1) and 7.6 nm (SD = 1.3) respectively. Different types of coating accentuate different details of filament morphology, for example their helix (Figure 6, A1–2 and B1–2), surface detail (A3–5 and B3–7) or repeat structure (A6–10 and B8–10).

Nuclear and cytoplasmic filaments have similar axial repeats (Figures 3–6), with repeat lengths (measured in >100 different filaments from various preparations) of 22.9 nm (SD = 2.8) and 23.6 nm (SD = 3.2) respectively. A repeat was only observed if moisture was excluded during

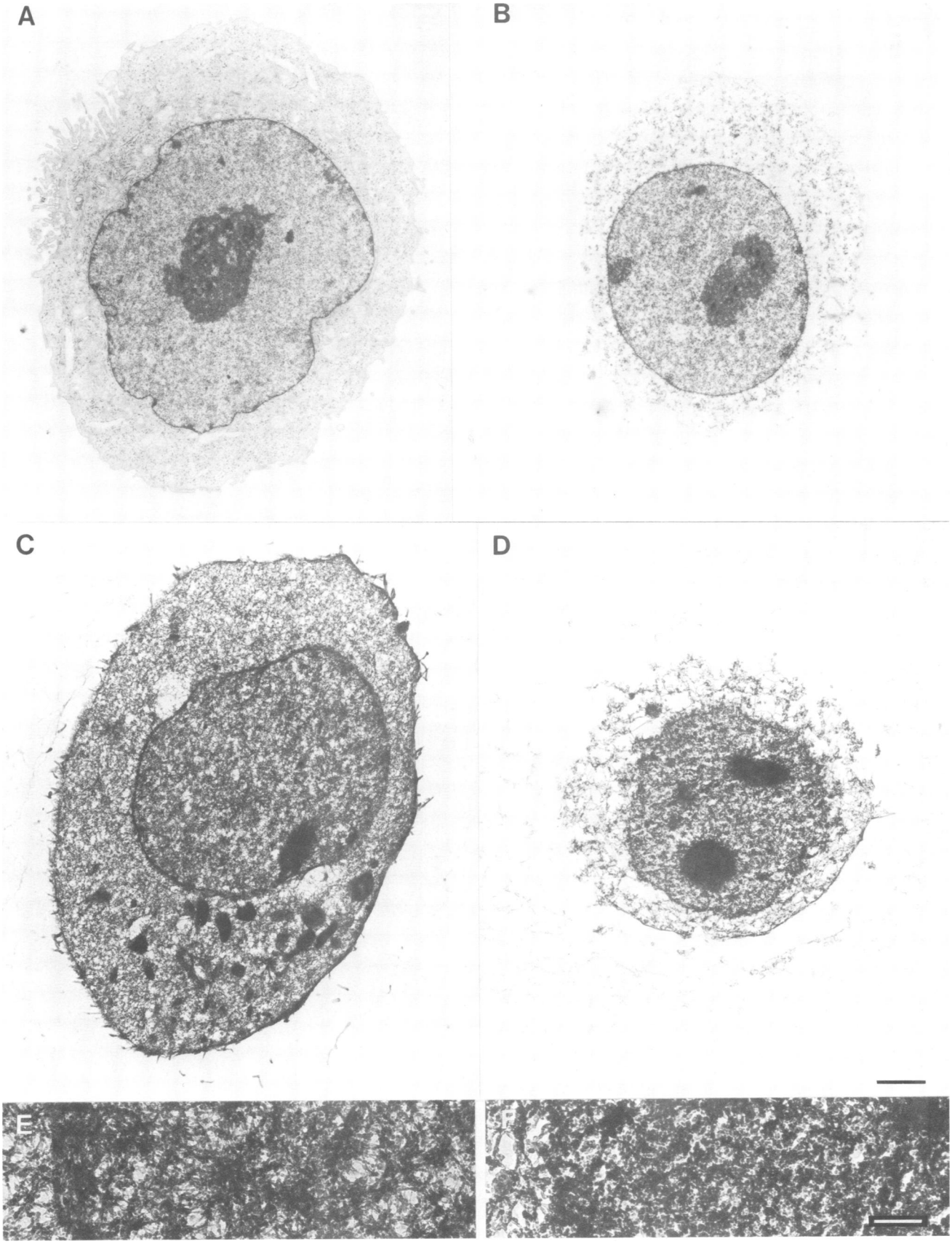

Fig. 2. Electron micrographs of sections of encapsulated cells, before and after extraction. (**A**) Thin (50 nm) section, unextracted cell. (**B**) Thin section, extracted cell. (**C**) and (**E**) Thick resinless section (150 nm), unextracted cell. (**D**) and (**F**) Thick resinless section (150 nm), extracted cell. (A)–(D) and (E) and (F) are at the same magnification; the bars are 2 and 0.5 μm respectively. In (E) and (F) the cytoplasm is to the far left.

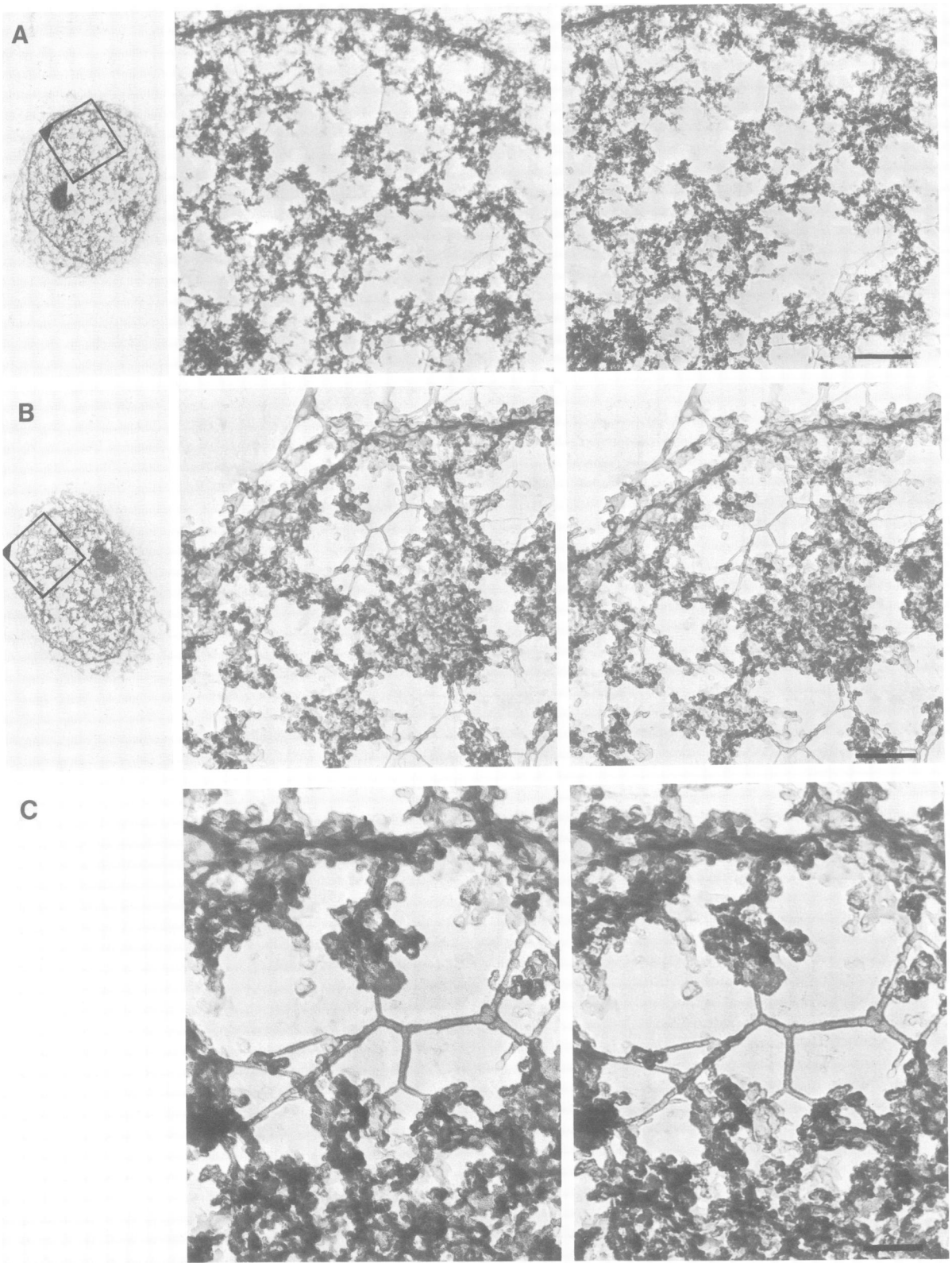

Fig. 3. Stereo pairs of electron micrographs of 250 nm thick sections of extracted cells. Either 85% (**A**) or 80% (**B** and **C**) of the chromatin has been removed. (C) is a higher magnification of (B). The rectangle in the left-hand panel shows the area viewed in the stereo pair to the right: the top left-hand corner is filled in for orientation. Bars represent 0.5 (A), 0.25 (B) and 0.1 μm (C).

critical point drying (Ris, 1985); failure to do so yielded filaments of variable width. [Note that no cytoplasmic microtrabecular network is visible in our carefully dried samples (Ris, 1985).]

These results show that nuclear and cytoplasmic filaments have similar dimensions. However, the nuclear network is more branched than the cytoskeleton (Figures 3–5) and in some regions nearly all the network is made up of double

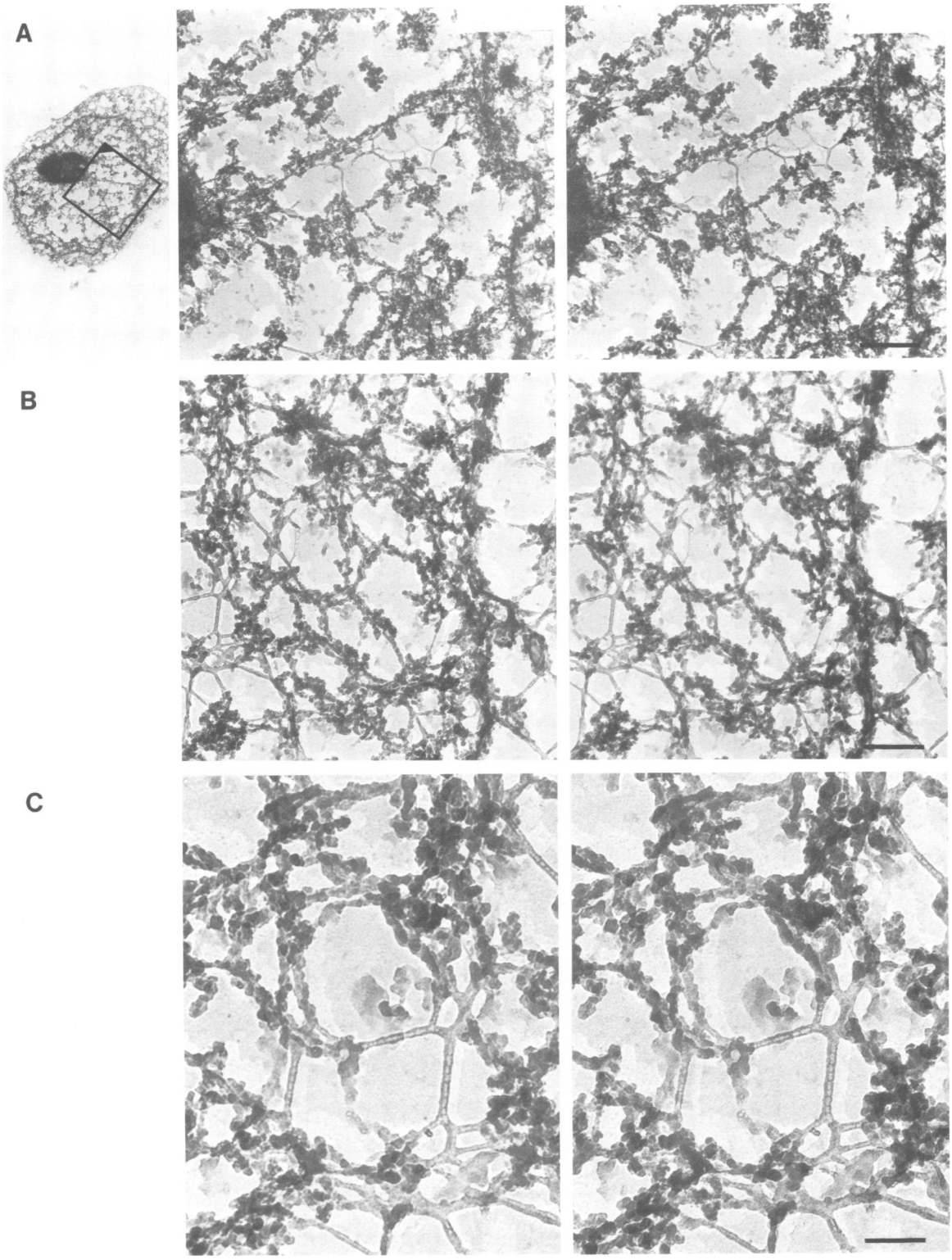

Fig. 4. Stereo pairs of electron micrographs of 250 nm thick section of extracted cells treated with 0.25% glutaraldehyde prior to electroelution. 87% of chromatin was removed. The rectangle in the left-hand panel in (**A**) shows the area viewed in the stereo pair to the right. In (**B**), the cytoplasmic region is to the right. (**C**) Shows a higher power of the left-hand side of (B). Bars represent 0.5 (A), 0.25 (B) and 0.1 μm (C).

filaments, as if the whole structure had been duplicated (Figure 5B).

The effects of RNase and DNase treatments

After treating encapsulated and lysed cells with DNase I rather than *Hae*III, all but 2% of chromatin may be electro-eluted leaving a range of structures. About 85% are collapsed (Figure 7A); a minority have a recognizable nuclear region (Figure 7B) but pre-fixation with glutaraldehyde prior to electroelution increases this proportion. Chromatin, cytoskeleton and nucleoskeleton appear denatured and the latter lacks a repeat and is concentrated at the nuclear periphery or around nucleoli (Figure 7C). As even lower concentrations of DNase have this destructive effect, we prefer to use *Hae*III.

There have been many suggestions that a nucleoskeleton

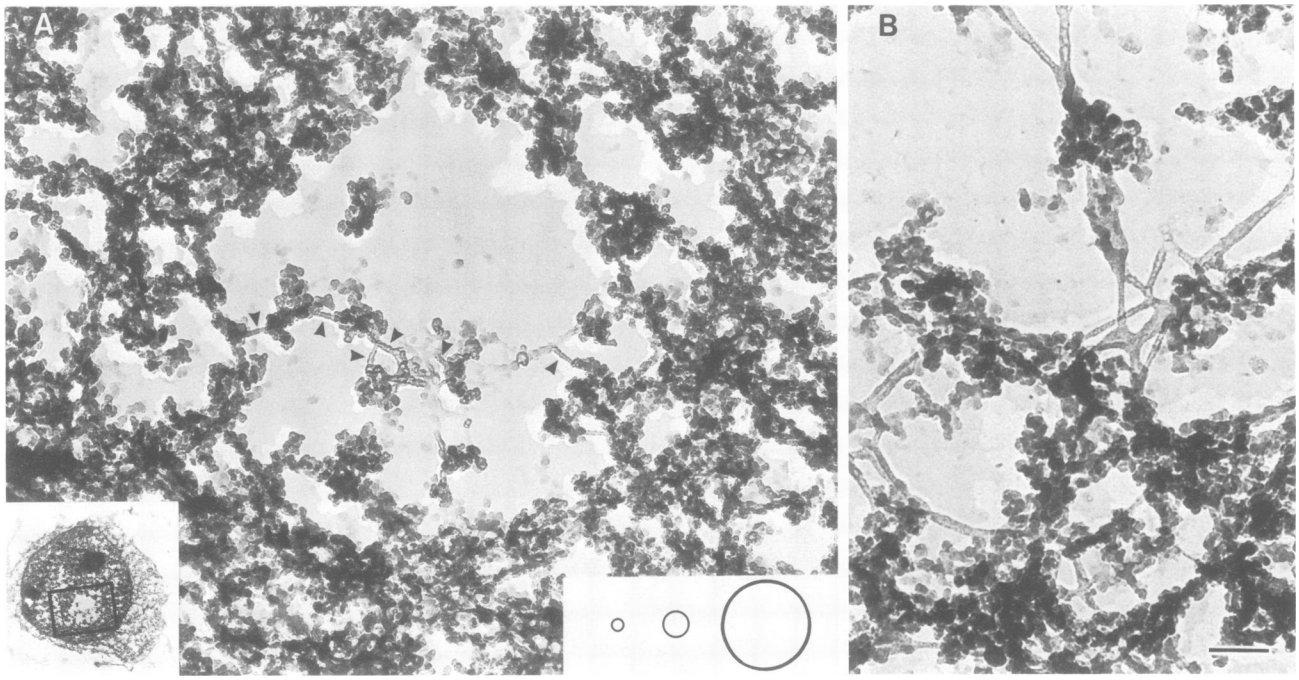

Fig. 5. Details of nuclear organization in thick sections. (**A**) The size of the nucleoskeleton relative to that of a nucleosome (small circle), solenoid (middle circle), chromatin loop (large circle). 25% of chromatin was removed. The insets show a low power view for orientation and circles with diameters representing 10, 30 and 90 nm. Arrowheads point to the path of the underlying skeleton. (**B**) A duplicated skeleton. The bar represents 0.1 μm.

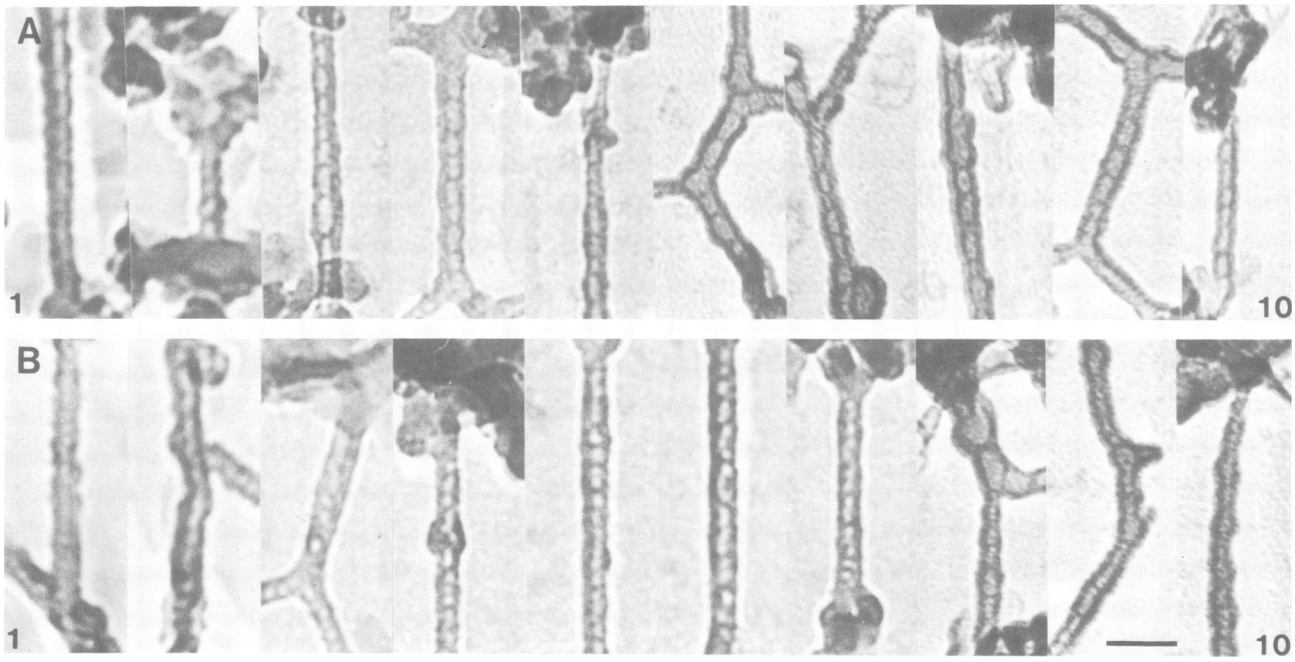

Fig. 6. Nuclear and cytoplasmic filaments have similar dimensions. Electron micrographs of 10 typical nuclear (**A**) and cytoplasmic (**B**) filaments. A1–2 and B1–2: low-angle (20°) carbon coating. A3–5 and B3–7: high angle (70°) rotary shadowing. A6–10 and B8–10: low angle rotary platinum/palladium shadowing followed by high angle rotary carbon shadowing. The bar represents 50 nm.

might contain RNA. Therefore we treated extracted cells with *Hae*III and sufficient RNase A to remove 95% of nascent RNA (Jackson and Cook, 1985b). This induced aggregation at the nuclear periphery giving various structures like those in Figure 7(D and E). Although chromatin clumps remain strung out throughout the nuclear region as though still associated with a skeleton, no filaments with a repeat were visible (Figure 7F). Therefore these results are equivocal: the persistence of a chromatin network implies that the skeleton resists RNase but no characteristic filaments are seen.

As both RNase and DNase are used at such high

concentrations and as the material becomes so aggregated and distorted, we are loath to draw any firm conclusions from these experiments.

Preservation of function

Ultimately the criticism that any skeleton seen *after* fixation is an artefact can never be completely answered. However, preservation of function *prior* to fixation implies that artefacts are unlikely to be generated *before* fixation. Therefore we compared replication and transcription rates *in vivo* with those found at the moment of fixation.

Elsewhere we have shown that lysed cells replicate their chromatin template at the sub-optimal pH and Mg^{2+} concentration of our buffer at 27% of the rate *in vivo* (Jackson *et al.*, 1988) and under optimal conditions this increases to 85% (Jackson and Cook, 1986b). Furthermore, this activity is authentic as it is only found in S-phase cells and it resists electroelution (Jackson and Cook, 1986a,b). Any assessment of how much activity is retained after nucleolytic treatment and electroelution is complicated by the fact that the template is now truncated: elongation

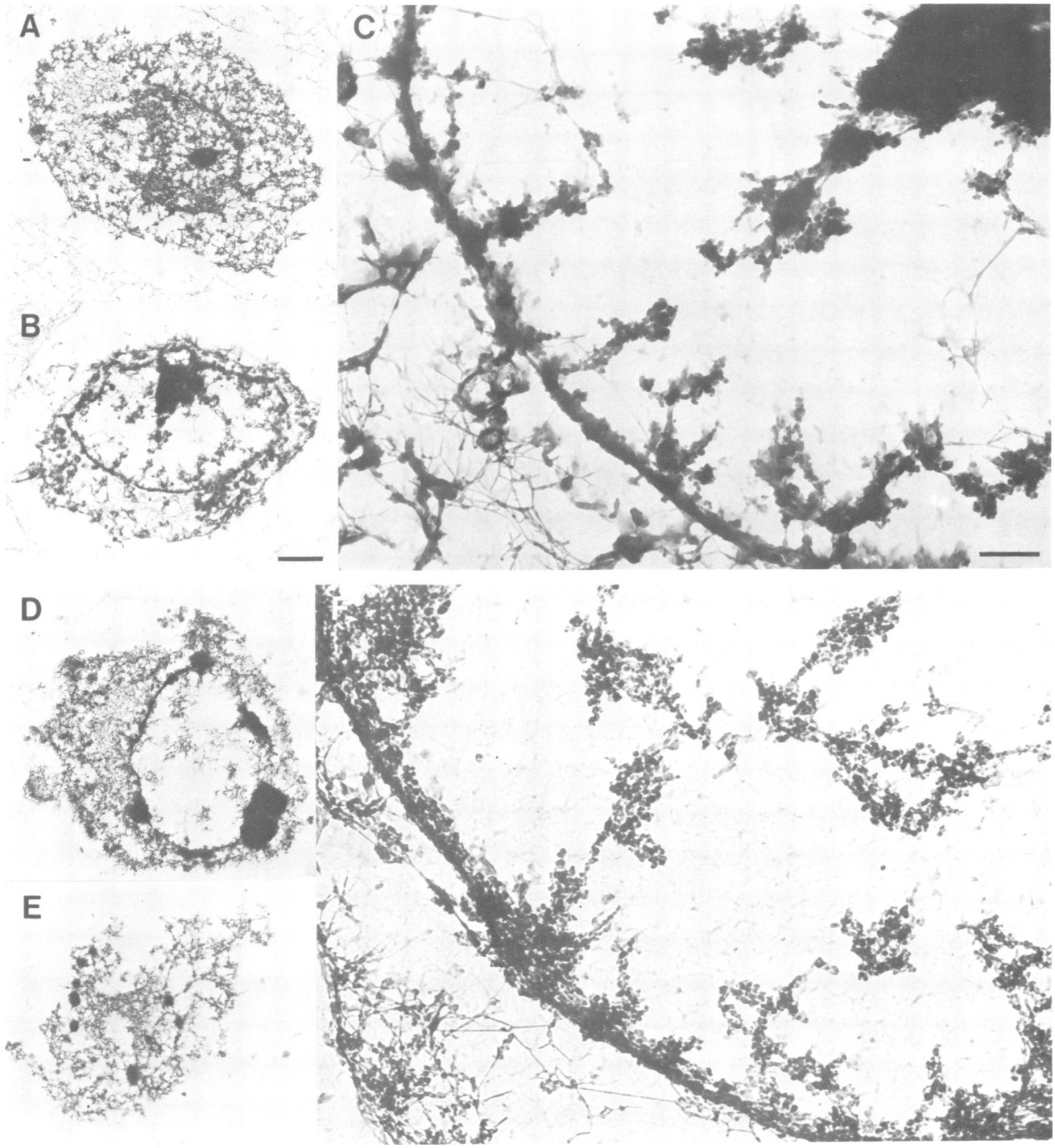

Fig. 7. The effects of treatment with DNase I or RNase A and *Hae*III on nuclear morphology. Encapsulated cells were treated with DNase (**A–C**) or RNase and *Hae*III (**D–F**), subjected to electrophoresis and thick sections photographed. 98% (A–C) and 88% (D–F) of chromatin was removed. (C) shows a high-power magnification of a region of (B). Bars represent 1 (A, B, D, E) and 0.25 μm (C, F).

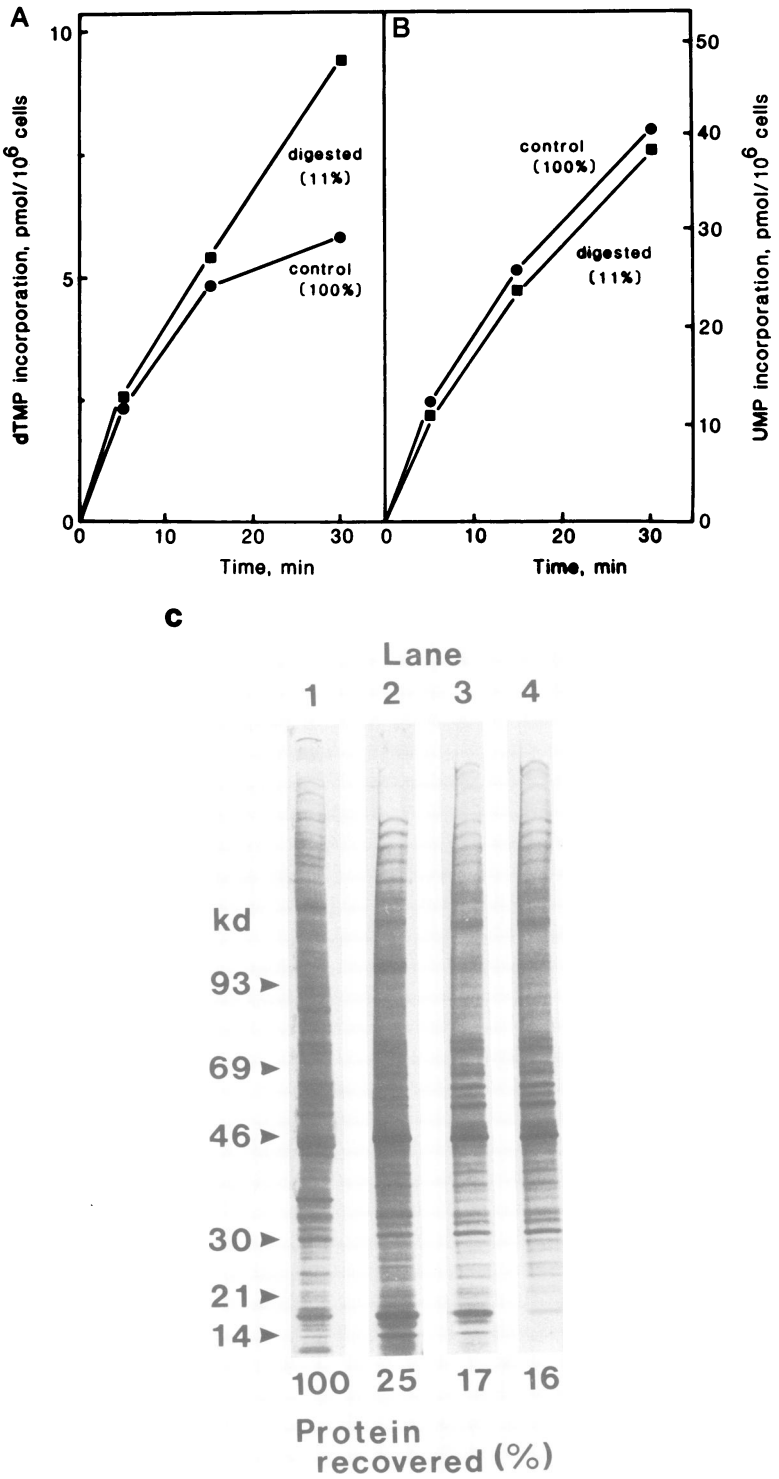

Fig. 8. Preservation of nuclear function and structure. (**A**) and (**B**) Cells grown in [^{3}H]thymidine were encapsulated, lysed, and incubated with (■) or without (○) *Hae*III; then 89% chromatin was removed electrophoretically and the rates of incorporation of dTTP (A) and UTP (B) into acid-insoluble material determined. During incubation with nuclease an aberrant soluble activity is generated which initiates at newly-cut ends in chromatin and which has altered kinetics: this activity slightly increases the rate in (A) (Jackson and Cook, 1986c). (**C**) Cells, pre-labelled with [^{35}S]methionine and [^{3}H]thymidine, were encapsulated, lysed and incubated with or without *Hae*III, 95% chromatin electroeluted and proteins remaining in beads visualized by autoradiography of a polyacrylamide gel. **Lane 1**: unextracted cells. **Lane 2**: extracted cells. **Lane 3**: extracted cells after electrophoresis. **Lane 4**: extracted cells, treated with *Hae*III, after electrophoresis. Each lane received the same number of cell equivalents, except lane 1 which received one-third the number. The position of size markers (kd) and percentage of ^{35}S remaining in beads are given.

proceeds so efficiently under optimal conditions that truncated templates are replicated in a few seconds. However, the initial rate can be slowed by reducing the dTTP concentration (Jackson and Cook, 1986b). Then, 89% of chromatin can be removed without any loss of activity (Figure 8A).

As we do not know the transcription rate *in vivo*, it is impossible to determine relative efficiencies *in vitro*. However, our encapsulated preparation—under optimal conditions—transcribes twice as efficiently as nuclei prepared conventionally and the activity again resists electroelution (Jackson and Cook, 1985a,b; Jackson *et al.*, 1988). Removal of 89% of chromatin only slightly reduces the transcription rate (Figure 8B). (Sub-optimal concentrations of triphosphates were used as before.)

This shows that our encapsulated preparation transcribes and replicates *in vitro* at rates close to those *in vivo*: furthermore, these high efficiencies are maintained up to fixation.

The proteins of treated nuclei

Figure 8(C) illustrates the proteins labelled with [^{35}S]-methionine that remain in beads at different stages in our procedure. Extraction with Triton removes 75% of total cell protein (lane 2) and electrophoresis removes a further 8% (lane 3). After treatment with *Hae*III, electrophoresis removes 85% of chromatin but only a further 1% of ^{35}S—this is almost entirely histone (lane 4); most proteins remain attached to elements too large to escape. Digestion and electrophoresis specifically remove chromatin without widescale protein loss.

Discussion

Potential artefacts

The ionic conditions present from cell lysis to fixation differ from those found in living cells in two major respects, the high concentration of Cl^- and the brief exposure to the Triton. To our knowledge, neither induces formation of filaments. A potential artefact is associated with the temperature of nucleolytic digestion. When cells are 'heat-shocked' at about 42°C, a characteristic set of proteins associate with karyoskeletal elements (Welch and Suhan, 1985). In nuclei isolated conventionally some aggregation even occurs at 37°C, but not at 33°C (Evan and Hancock, 1985; Littlewood *et al.*, 1987; see also McConnell *et al.*, 1987); our preparation is not so sensitized (Jackson *et al.*, 1988). Nevertheless we digested at 33°C, although we obtained similar results at 37°C (results not shown). Other artefacts might be associated with *Hae*III fragmentation and electroelution but we can imagine no gentler procedures for removing chromatin and again we know of no evidence that these induce aggregation. (The maximum concentration of *Hae*III used was 2 μg/ml.) We did not generally use DNase I since it depolymerizes actin filaments (Hitchcock *et al.*, 1976), its action depends on the precise ionic conditions (Jackson and Cook, 1986a) and it proved very destructive at the concentrations needed (Figure 7). A filamentous skeleton was also seen using an isotonic buffer (Jackson and Cook, 1985a) which contains EDTA and so lacks magnesium ions (unpublished data).

The best evidence that our preparation is free of artefact up to fixation is circumstantial: replication and transcription are maintained at *in vivo* rates even after lysing, washing, incubation with *Hae*III and electroelution (Figure 8). If networks do form artefactually, they cannot interfere with these functions, as does heat-shock.

It is more difficult to be confident that artefacts were not induced on, or after, fixation even though we use procedures that minimize their creation. Thus, we fix in a Ca^{2+}-free buffer at pH 7.4 with glutaraldehyde, conditions which best preserve nuclear morphology (Skaer and Whytock, 1977) and prevent ribonucleoprotein aggregating into filaments (Lothstein *et al.*, 1985). We also critical-point dry using conditions which both allow (Capco *et al.*, 1984; Wolosowick and Porter, 1979) and prevent (Ris, 1985) subsequent formation of the microtrabecular lattice: both yield filamentous nuclear structures, but detailed morphology was only seen in carefully-dried samples (Ris, 1985). It seems unlikely that the skeleton is a fixation artefact as such artefacts should depend on concentration, yet we see similar skeletons over a 10-fold range of chromatin concentration and in both nucleus and cytoplasm. Furthermore, lamina and nucleoli should nucleate artefactual aggregates, but the stereo pictures clearly show networks extending through three-dimensional space. Finally, conditions thought to be best by others (Ris, 1985) yield the best-preserved repeating structure. Nevertheless, in view of the long history of artefacts, we must interpret these images cautiously, however appealing their structure.

A nucleoskeleton

Bearing in mind these reservations, we find clear evidence for a skeleton that ramifies throughout the nucleus and which is associated with residual clumps of chromatin. Strong but circumstantial evidence suggests that these nucleofilaments are members of the intermediate filament family. These filaments, 7–15 nm wide with an axial repeat of 21–25 nm (Milam and Erickson, 1982; Steinert and Parry, 1985), ramify throughout the cytoplasm, connecting plasma and nuclear membranes (Georgatos and Blobel, 1987a,b). They are homologous to the nuclear lamins (Aebi *et al.*, 1986; McKeon *et al.*, 1986; Franke, 1987) and can bind to DNA (Ward *et al.*, 1984). They are not extracted by Triton and would be expected to be the main cytoplasmic filaments in our preparations; such cytoplasmic filaments are equal in width to the nucleofilaments. Both contain the axial 23 nm repeat characteristic of intermediate filaments. Our nucleofilaments differ from aggregated ribonucleoprotein particles which form filaments 18 nm wide with a 60 nm repeat (Lothstein *et al.*, 1985). Our results suggest that intermediate filaments provide the skeleton not only for the cytoplasm, but also for the nucleus.

Finding one type of filament does not exclude the possibility that the nucleoskeleton contains other types. We leave open whether RNA-containing filaments also form part of the skeleton as RNase induced aggregation (Figure 7D and E). [But note that RNase-sensitive filaments are seen after exposure to low salt concentrations known to aggregate ribonucleoprotein into thick filaments (Lothstein *et al.*, 1985).] Other cytoskeletal elements might also extend into nuclei; indeed, actin and tubulin cosediment with many different sub-nuclear structures (MacGillivray and Birnie, 1986).

The visualization of chromatin attached to a nucleoskeleton makes explicit a problem posed initially by the discovery of looped DNA (Cook and Brazell, 1975; Paulson and Laemmli, 1977): how might skeleton and attached DNA be replicated? If duplicated simultaneously, we might expect to see a double skeleton as in Figure 5(B). Then specific attachments of DNA to skeleton—and so specific patterns of expression—might be inherited through mitosis (Cook, 1973; Jackson *et al.*, 1984).

The active site of replication and transcription

As essentially all cellular replicative and transcriptional activities remain in beads when chromatin is removed and as the skeleton also remains (Figure 8A and B), it is attractive to suppose that functional polymerases are attached to this skeleton. If so, polypeptides of one family, the intermediate filaments, provide the structural base for replication and all stages of mRNA metabolism (Jackson *et al.*, 1981, 1982), from synthesis at the nucleoskeleton, transport through the nuclear pores and lamins (Blobel, 1985) to translation in the cytoplasm (Cervera *et al.*, 1981). This raises the intriguing possibility that messages remain attached to filaments which connect specific genes with specific cytoplasmic sites, so allowing targeting to specific destinations (Blobel, 1985; Lawrence and Singer, 1986). Information might also be channelled through intermediate filaments in the reverse direction, from membrane to genes. An association of active transcription complexes with intermediate filaments—which tend to be soluble in hypotonic conditions (Zackroff and Goldman, 1979)—might explain why a nucleoskeleton is never seen in 'Miller' spreads of nascent transcripts prepared by dropping nuclei on water (Miller, 1984).

Materials and methods

Cell encapsulation and lysis

Suspension cultures of HeLa cells were grown in minimal essential medium plus 5% newborn calf serum for 18–24 h in [methyl-^{3}H]thymidine (0.05 μCi/ml; ~60 Ci/mmol) to label uniformly their DNA, then encapsulated in 0.5% agarose (Cook, 1984) and any large beads that might block pipettes removed by filtration through 150 μm monofilament nylon mesh (Jackson *et al.*, 1988). Next, encapsulated cells were regrown for 1 h, washed in ice-cold pH 7.4 buffer, Triton X-100 added to 0.5% and after 15 min on ice washed 3 times in cold pH 7.4 buffer. pH 7.4 buffer (Jackson *et al.*, 1988) is made by adding 100 mM KH_2PO_4 to 130 mM KCl, 10 mM Na_2HPO_4, 1 mM $MgCl_2$, 1 mM Na_2ATP (Sigma Type II), 1 mM dithiothreitol to bring the pH to 7.4. As the acidity of the ATP varies from batch to batch, various amounts of KH_2PO_4 must be added, but they never exceed 1% (v/v) and generally increased K^+ to 130.8 mM and PO_4^{3-} to 11.6 mM.

Digestion with nucleases

Encapsulated cells (2.5×10^7/ml) were lysed, washed and incubated in an equal volume of buffer at 33°C for 0–80 min with 20–200 U/ml *Hae*III (100 U/μg), 20 μg/ml DNase I or 10 μg/ml RNase A. Chromatin was electroeluted from beads under isotonic conditions using a 0.8% agarose gel (4 h at 4 V/cm; buffer was recirculated to prevent pH drift). The buffering capacity of the pH 7.4 buffer was increased for this by increasing the phosphate ion concentration at the expense of the chloride ion so that it contained 78.6 mM KCl, 10 mM Na_2HPO_4, 20 mM K_2HPO_4, 9.4 mM KH_2PO_4, 1 mM $MgCl_2$, 1 mM dithiothreitol and 1 mM Na_2ATP. Beads were recovered, washed in pH 7.4 buffer, and the amount of chromatin (i.e. ^{3}H) remaining in beads determined by scintillation counting (Jackson *et al.*, 1988). The average size of electroeluted DNA was determined by inspecting the ethidium-stained gel.

Electron microscopy

After electrophoresis beads were recovered, washed in the pH 7.4 buffer and fixed by adding glutaraldehyde to 2.5%, post-fixed in 2% osmium tetroxide and stained in 0.5% uranyl acetate. Thin sections were prepared after embedding in Epon–Araldite and stained with lead citrate. Thick sections were prepared using the removable embedding compound diethylene glycol distearate (Capco *et al.*, 1984), using the procedure described in detail by Fey *et al.* (1986). The samples illustrated in Figure 4 were treated with 0.25% glutaraldehyde prior to electroelution and fixation in 2.5% glutaraldehyde. Samples were critical point dried taking care to ensure they were free of water (Ris, 1985) and stabilized by coating with carbon (unidirectionally or by rotary shadowing at angles of 20° or 70°). Occasionally samples were coated with platinum/palladium at 20° then carbon at 70° (Figure 3B and C; Figure 6A6–10 and B8–10). Catalase crystals were used as size markers (Wrigley, 1968).

Other procedures

Rates of replication and transcription were measured by addition of appropriate precursors to the pH 7.4 buffer (Jackson and Cook, 1985b, 1986a; Jackson *et al.*, 1988).

Protein recoveries were determined using cells labelled overnight in medium lacking methionine and supplemented with 2% normal medium, 5% dialysed FCS and L-[^{35}S]methionine (1 or 0.1 μCi/ml; 800 mCi/mmol) by measuring the amount of label before and after lysis, nuclease treatment and electroelution using 7–20% polyacrylamide gradient gels and autoradiography (Jackson *et al.*, 1988).

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