NUCLEAR MATRIX OF HeLa S_3 CELLS

Polypeptide Composition during Adenovirus Infection and in Phases of the Cell Cycle

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ABSTRACT

A subnuclear fraction has been isolated from HeLa S_3 nuclei after treatment with high salt buffer, deoxyribonuclease, and dithiothreitol. This fraction retains the approximate size and shape of nuclei and resembles the nuclear matrix recently isolated from rat liver nuclei. Ultrastructural and biochemical analyses indicate that this structure consists of nonmembranous elements as well as some membranous elements. Its chemical composition is 87% protein, 12% phospholipid, 1% DNA, and 0.1% RNA by weight. The protein constituents are resolved in SDS-polyacrylamide slab gels into 30–35 distinguishable bands in the apparent molecular weight range of 14,000–200,000 with major peptides at 14,000–18,000 and 45,000–75,000. Analysis of newly synthesized polypeptides by cylindrical gel electrophoresis reveals another cluster in the 90,000–130,000 molecular weight range. Infection with adenovirus results in an altered polypeptide profile. Additional polypeptides with apparent molecular weights of 21,000, 23,000, and 92,000 become major components by 22 h after infection. Concomitantly, some peptides in the 45,000–75,000 mol wt range become less prominent. In synchronized cells the relative staining capacity of the six bands in the 45,000–75,000 mol wt range changes during the cell cycle. Synthesis of at least some matrix polypeptides occurs in all phases of the cell cycle, although there is decreased synthesis in late S/G_2. In the absence of protein synthesis after cell division, at least some polypeptides in the 45,000–75,000 mol wt range survive nuclear dispersal and subsequent reformation during mitosis. The possible significance of this subnuclear structure with regard to structure-function relationships within the nucleus during virus replication and during the life cycle of the cell is discussed.

The periphery of the mammalian cell nucleus is the site of several cellular and virus-specific processes. Usually, events localized to the nuclear periphery have been said to occur at or near the nuclear envelope, as this is by microscopy the most prominent structural element. Ultrastructural studies show that the components of this periphery also include numerous pore complexes

and an underlying dense lamella (see review, reference 19). Cytological techniques indicate that heterochromatin aggregates are associated with the lamella (see review, reference 19). Cellular events which appear to involve peripheral nuclear structures include nucleocytoplasmic exchange (see review, reference 19), dispersal and reformation of the nuclear envelope at mitosis (16), synthesis of late replicating DNA (25, 27, 43), and resumption of RNA synthesis in late mitosis (41). Virus-associated events which occur at this location include the assembly of *Herpes* virions (13) and the final steps of uncoating of adenovirus (9, 10). the periphery is also the site of expression of one group of tumor-specific antigens found in some virus-transformed cell lines (20, 28). In addition, escape of adenovirus progeny from the nucleus occurs only after nuclear integrity has been disrupted (17).

Recent morphological and biochemical evidence indicates that there is a nuclear matrix interior to the envelope which forms a proteinaceous network extending from the periphery to the interior and which may span the entire volume of the nucleus (2, 5). These observations focus attention on the possible involvement of this peripheral-internal complex as a whole in the functional-structural relationships that occur within the nucleus, and thus may necessitate a re-evaluation of the role of the nuclear envelope itself in cellular and viral processes.

We have isolated and characterized structures from purified, "detergent-cleaned" nuclei of HeLa S_3 cells which appear by phase-contrast microscopy to retain the approximate size and shape of nuclei. Ultrastructural and biochemical analyses indicate that these structures are nuclear matrices which retain some membranous elements. We have examined the polypeptide constituents of such subnuclear structures and find specific variations associated with adenovirus 2 infection and with different phases of the cell cycle. In addition, we report that some polypeptides of the matrix survive mitosis and are reutilized in the subsequent reformation of the nucleus after cell division.

MATERIALS AND METHODS

Cell Culture, Infection, and Synchronization

HeLa S_3 cells were maintained at 37°C in suspension culture in Eagle's minimal medium (15) supplemented with 7% calf serum and 2 mM glutamine. Cells were infected with adenovirus type 2 at a concentration of 2,000 viral particles per cell as previously described (22). Cells were synchronized by a double thymidine blockade (7), and by selective detachment of mitotic cells from monolayer cultures (38) as previously described (21). Cells were allowed to proceed into subsequent stages of the cell cycle by incubation at 37°C.

Cells were exposed for 2 h to radioactive amino acids (5 μCi/ml) at a concentration of 1×10^7 cells/ml in Eagle's medium prewarmed to 37°C containing 1% the normal concentration of amino acids. This was supplemented with 6% dialyzed calf serum, 1% calf serum, and 2 mM glutamine. With these conditions there was increasing incorporation of [^{3}H]amino acids (uniformly labeled, 1 mCi/ml, New England Nuclear, Boston, Mass.) into total cellular insoluble radioactivity which reached a maximum by 2 h. This was observed in medium containing either 1% or 10% amino acids.

Isolation of the Nuclear Matrix

Routinely, $8\text{–}10 \times 10^8$ cells were washed once in Eagle's spinner salts and suspended at a concentration of 10^7/ml in 0.01 M Tris-HCl, pH 7.2, containing 0.01 M NaCl and 1.5 mM $MgCl_2$ (RSB). Nonidet P-40 (NP40; Shell Chemical Co., New York) was added to a final concentration of 0.25% (8) for 30 min at 5°C. After centrifugation, cells were suspended in RSB at the same concentration, and disrupted in a Dounce homogenizer. This and subsequent steps in the procedure were monitored by phase-contrast microscopy. The number of strokes needed to release nuclei without breakage was carefully monitored, and this was particularly important with virus-infected cells because nuclei from these cells were more fragile. Nuclei were collected by centrifugation at 200 *g* for 3 min, washed several times, and suspended in RSB. Both NP40 detergent treatment and Dounce homogenization were essential to obtain clean nuclei as judged by electron microscopy. Treatment with NP40 did not result in visible damage to nuclei.

Detergent-cleaned nuclei were obtained essentially as described by Holtzman et al. (23). The nuclear suspension was treated with the detergent mixture Tween-desoxycholate (Tw-Doc) at a 1:40 dilution of a stock solution (2 vol 10% Tween-40 plus 1 vol 10% sodium desoxycholate) and agitated in a vortex mixer for 30 s. Nuclei were immediately collected by centrifugation at 200 *g* for 3 min, washed with RSB and suspended with vigorous pipetting in 0.01 M Tris-HCl, pH 7.4, containing 0.5 M NaCl and 0.05 M $MgCl_2$ (HSB) at a concentration of 2×10^7 nuclei/ml. The resulting nucleohistone gel was digested with 50 μg/ml deoxyribonuclease I (DNase; Sigma Chemical Co., St. Louis, Mo.) for 30–60 min at 37°C. Nuclear matrices were collected by centrifugation at 600 *g* for 10 min and incubated at 37°C for 30 min in 0.01 M Tris-HCl, pH 7.4, containing 0.01 M dithiothreitol, 0.01 M NaCl, and 0.01 M EDTA (NEB). After centrifugation, the subnuclear fraction was suspended in 4 ml of 0.01 M Tris-HCl, pH 7.4, contain-

ing 0.001 M EDTA (TE), and sedimented through a discontinuous sucrose gradient to a 60% sucrose cushion by centrifugation at 5°C in an SW27 rotor for 10 min at 10,000 rpm. The gradient consisted of a cushion of 60% (wt/wt) sucrose overlaid with 3 ml each of 1.20, 1.18, and 1.16 g/cm^3 sucrose prepared in TE buffer. No membranous fractions of lower density were observed. The subnuclear fraction was collected, washed in TE buffer, and used immediately or stored in 10% trichloracetic acid (TCA) at −20°C. Routinely, the final matrix preparation contained 0.5–1.0 mg of protein. The plasma membrane activity ouabain-sensitive ATPase (14), the microsomal activity glucose-6-phosphatase (44), as well as the lysosomal activity β-*N*-acetylglucosaminidase (4) were not demonstrable in these preparations. Mixing experiments did not indicate that freely dissociable inhibitors of enzymatic activities had been co-isolated with the matrix.

This procedure is derived from that previously used in our laboratory and thought to yield inner nuclear membrane. As discussed below, it is now clear that, although this fraction contains membranous elements, it is more appropriately termed a nuclear matrix.

Electron Microscopy

Detergent-cleaned nuclei and nuclear matrices were fixed at 5°C either in a 1:1 mixture of Karnovsky's fixative (26) and 2% osmium tetroxide in distilled water for 30 min, or in 2% glutaraldehyde in TE buffer for 1 h. Samples were washed several times in TE buffer and postfixed at 0°C for 1–3 h with 1% osmium tetroxide in 0.1 M sodium phosphate buffer, pH 6.9. Samples were then washed in buffer, dehydrated in a graded ethanol series, and embedded in Epon. Silver sections cut with a diamond knife were collected on Formvar-coated grids and were stained with aqueous uranyl acetate and lead citrate. Grids were examined by use of either a Hitachi-12 or a Philips 201S electron microscope.

Preparation of Other Cellular Fractions

A plasma membrane fraction was isolated by the method of Atkinson and Summers (3). Purification was monitored by phase-contrast microscopy and the final "ghost-fraction" resembled that previously reported.

Cytoplasm and nucleoplasm were prepared as the 100,000 *g* supernates isolated from crude fractions. Crude cytoplasm is the postnuclear supernate of RSB-NP40-treated cells. Crude nucleoplasm is the supernate remaining after matrices have been pelleted after HSB-DNase treatment.

Chemical Analyses and Extraction with Triton X-100

DNA was determined by the diphenylamine reaction and RNA by the orcinol procedure as described by Shatkin (40), and protein by a modification of the Lowry technique (33). To determine the phospholipid content, we first extracted phospholipids with 20 vol of chloroform-methanol (2:1, vol/vol) at 4°C for 30 min. Unsolubilized material was pelleted by centrifugation at 600 *g* for 10 min at 4°C and re-extracted with the same solvent at room temperature for 30 min according to the method of Ray et al. (35). The combined extracts were washed with 20 vol of glass-distilled water at 4°C for at least 48 h according to the procedure of Folch et al. (18). Quantitation of phosphorus was carried out by the method of Chen (11). The total amount of phospholipid present was estimated to be 25 times the amount of phosphorus present in the extract (36). ^{32}P-Labeled lipids were further characterized by silica gel chromatography according to the method of Skipski et al. (42). Lipids were located by autoradiography and were identified by comparison with the migration of authentic standards received from Dr. John Cronan (Yale University, New Haven, Conn.). Areas of ^{32}P-radioactivity were scraped from the plate and suspended in Aquasol (New England Nuclear, Boston, Mass.), and the relative amounts of radioactivity were determined.

When the subnuclear fraction was extracted with Triton X-100, the preparation was suspended in TE at a concentration not exceeding 5 mg protein/ml. A sufficient volume of 10% Triton X-100 was added to a final detergent concentration of 2% and extraction was carried out for 30 min at 4°C. Unsolubilized material was collected by centrifugation at 600 *g* for 10 min, the precipitate was washed with TE, and the remaining lipids were extracted with chloroform-methanol.

Determination of Acid-Insoluble Radioactivity

TCA-insoluble radioactivity was determined in cells and subcellular fractions as previously described (41).

Electrophoresis in SDS-Polyacrylamide Gels

After precipitation with cold 10% TCA, samples were prepared for electrophoresis by washing twice with 5% TCA followed by extraction of lipids with acetone for 20 min at 5°C and washed once in acetone. For electrophoresis in 18-cm cylindrical gels, the delipidated matrix was solubilized for 30–60 min at 37°C in 0.1 M sodium phosphate buffer, pH 7.2, containing 0.1% sodium dodecyl sulfate (SDS). Proteins were reduced with 2-mercaptoethanol and alkylated with iodoacetamide as previously described (21). After dialysis for 16 h against SDS-phosphate buffer, the polypeptides were analyzed by electrophoresis (29). For electrophoresis in 13 cm × 15 cm × 1.7-mm slab gels, the delipidated matrix was solubilized in 10% glycerol, 5% mercaptoethanol and 3% SDS in 0.0625 M Tris-HCl, pH 6.8 (30). Details are indicated in the appropriate legends. Generally, 100–200 μg of protein were used for cylindrical gel electrophoresis and 20–60 μg for slab gel electrophoresis.

Cylindrical gels were cut into 1- or 2-mm slices which were dissolved in 3 ml of scintillation fluid (toluene containing 0.5% PPO and 0.05% POPOP) containing 3% (vol/vol) Protosol (New England Nuclear) for 18 h at 37°C and radioactivity was determined. Alternatively, 2-mm slices were solubilized in 0.3 ml of a solution containing 9 parts Protosol, 10 parts toluene, and 1 part water at 60°C for 90 min and frozen for 1 h, and radioactivity was determined after the addition of 4 ml toluene scintillation fluid. Gradient slab gels were stained with 0.2% Coomassie blue in 50% methanol and 7% acetic acid. Molecular weight (mol wt) calibration of all gels was approximated according to the method of Shapiro et al. (39) utilizing thyroglobulin, (170,000), bovine serum albumin (68,000), immunoglobulin heavy chain (50,000), ovalbumin (49,000), pepsin (35,000), deoxyribonuclease 1 (31,500), immunoglobulin light chain (25,000), apoferritin (18,500), and bovine hemoglobin (14,500) as standards.

RESULTS

Isolation and Biochemical Characterization of Nuclear Matrices

At each stage of the isolation procedure, samples were examined by phase-contrast and electron microscopy to monitor structural integrity and background contamination. After combined treatment with NP40 and Tw-Doc, phase-contrast microscopy revealed that populations of nuclei were obtained which were apparently free of cytoplasmic remnants (Fig. 1, panel A). Examination by electron microscopy of random samples through several pellets of these detergent-cleaned nuclei confirmed these results and indicated the absence of recognizable subcellular elements in the preparations, as well as that such nuclei were bounded by an electron-dense structure different from the underlying chromatin, as previously reported (23). Recognizable nuclear envelopes and nuclear pores were observed only before the final detergent (Tw-Doc) wash. Treatment of these detergent-clean nuclei with high salt buffer (HSB) and with DNase resulted in shrunken structures that contained prominent nucleoli (Fig. 1, panel B). After dilution of the high salt buffer and treatment with dithiothreitol (DTT), these structures assumed a more or less spherical shape, were approximately two-thirds the size of the original nuclei (Fig. 1, panel C), and lacked visible intranuclear detail such as nucleoli (Fig. 1, panel C). Subsequent sedimentation through sucrose resulted in significant breakage of the subnuclear structures which was particularly apparent in elec-

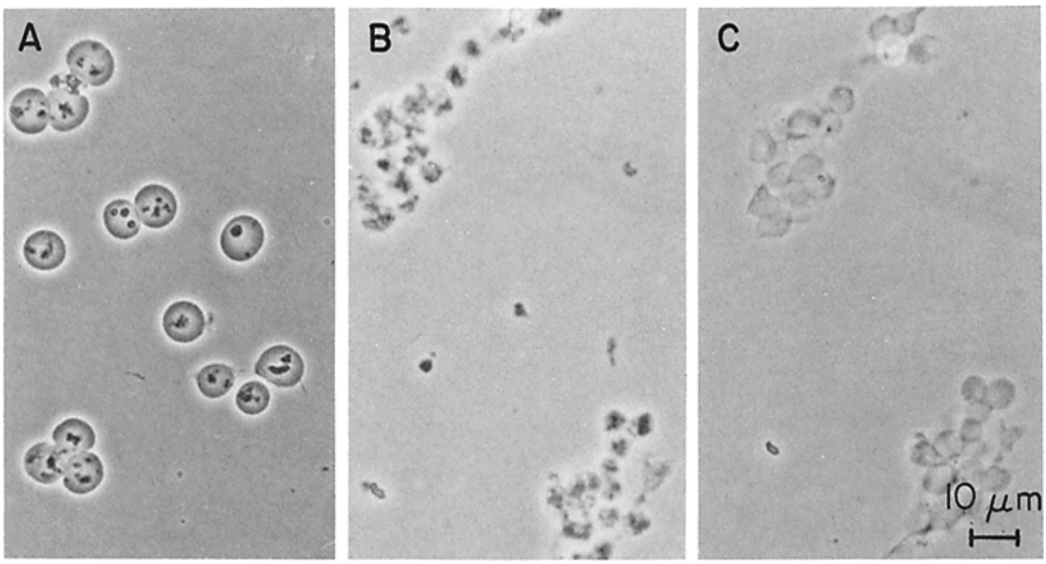

FIGURE 1 Phase-contrast microscopy of the nuclear fractions. Aliquots of nuclear preparations were observed as wet mounts by phase-contrast microscopy. After final detergent treatment (Tw-Doc) the nuclei contained obvious nucleoli and appeared free of cytoplasmic remnants (panel *A*). Subsequent treatment with HSB-DNase resulted in shrunken structures which contained nucleoli (panel *B*). This slide was then flooded with DTT-NEB buffer. The resulting structures had a spherical shape about 70% the diameter of the original nuclei in RSB, and nucleoli were not apparent (panel *C*). All micrographs × 725.

tron micrographs (Fig. 2 A, B). Unbroken structures contained a peripheral component which was somewhat granular in appearance and which was continuous with internal elements, as well as residual nucleolar elements (Fig. 2, panels A and C). The intact structures resemble the nuclear matrix isolated from rat liver (5, 6). Fragments of an 80-Å tripartite structure with the appearance of a typical unit membrane occurred at intervals around the periphery of some nuclear matrices (Fig. 2, panel D); the frequency of nuclear matrices having this structure varied between isolations but never exceeded 5%. The linear arrays of granular material with interspersed dense structures (Fig. 1, panels A and B) were interpreted to represent structures which had been disrupted in sedimentation.

This nuclear matrix fraction is by weight 87% protein and 12% phospholipid, with the remainder being nucleic acid (Table I). The relative amounts of protein and phospholipid have not varied more than 2–3% in several determinations. On the basis of the recovery of phospholipid, the final yield of matrix structures from detergent-cleaned nuclei ranged from 33% to 37%. On the basis of this observation, it can be estimated that an individual matrix contains an average of 12% of the protein of a detergent-cleaned nucleus. In the absence of dithiothreitol treatment, 25–30% of the RNA of detergent-cleaned nuclei was recovered and sedimentation analysis indicated that this was predominantly preribosomal RNA (unpublished observations). Because the amounts of DNA and RNA were barely at the level of biochemical detection, these components were also measured by the recovery of radioactivity in preparations from cells that had been prelabeled with [^{14}C]thymidine or [^{14}C]uridine. Residual amounts equal to no more than 0.2% of the original DNA and 1% of the original RNA were detected. The potential coisolation of nucleoplasmic and cytoplasmic proteins was also evaluated by reconstruction experiments using extracts labeled with [^{3}H]amino acids. In separate experiments a cytoplasmic extract containing 1.1×10^7 cpm and a nuclear extract containing 2.9×10^5 cpm were mixed with detergent-cleaned nuclei. The specific activity of these mixtures was compared to the specific activity of the final nuclear matrix fraction. As judged by this procedure, adventitious proteins from the cytoplasm and nucleoplasm should constitute no more than 2% and 9%, respectively, of the total protein of this subnuclear fraction.

Since the nuclear matrix fraction contains a significant amount of phospholipids (Table I), the identity and the proportional amounts of various phospholipids were determined in preparations obtained from cells exposed to [^{32}P]orthophosphate for 1, 6, and 18 h (Table II). After the extraction of lipids, phospholipids were separated by chromatography on silica gel plates. The matrix fraction contained the major phospholipids commonly found associated with mammalian membranes. In addition, the proportional amounts of two of these phospholipids varied with the time of exposure to radioactivity. Radioactivity was rapidly incorporated into phosphatidylethanolamine as has been observed in endoplasmic reticulum (12). In contrast, phosphatidylcholine has a longer synthesis or turnover time so that this fraction constituted nearly 50% of the total phospholipid at the longest labeling time. While a subnuclear fraction lacking phospholipids has been isolated from rat liver cells with the nonionic detergent Triton X-100 (1, 2), we found that only 45% of the total phospholipids of either detergent-cleaned nuclei or nuclear matrices of HeLa cells was extracted by 2% Triton X-100, even at a detergent-to-phospholipid ratio (wt/wt) fourfold greater than that previously reported. Although there was no exclusive extraction of a particular phospholipid, it appears that some phospholipids were solubilized to a greater extent. It can be calculated from the data (Tables I and II) that 67% of the phosphatidylserine and phosphatidylinositol were solubilized, compared to only 16% and 18% of the phosphatidylethanolamine and sphingomyelin, respectively. There was little or no protein released.

Polypeptide Constituents in Uninfected and Virus-Infected Cells

The polypeptide constituents of the nuclear matrix fraction were analyzed by electrophoresis in polyacrylamide gels. As expected, resolution in gradient slab gels was better than that obtained in cylindrical gels. After staining, the former system yielded 30–35 distinguishable bands in the apparent molecular weight range of 14,000–200,000, with major peptides in the 45,000–75,000 and 14,000–18,000 range (Fig. 3). This profile was unchanged even after extensive reduction and al-

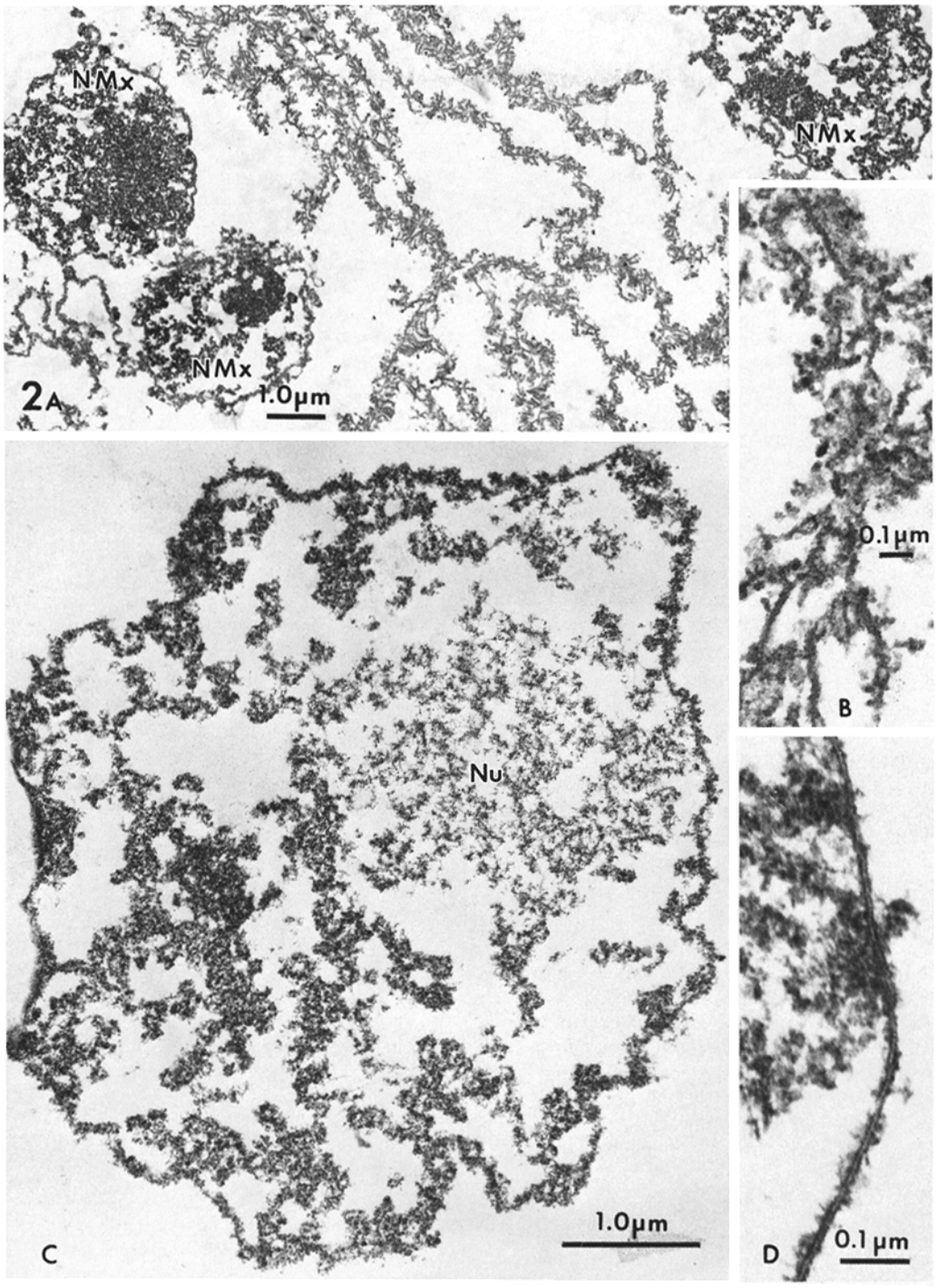

FIGURE 2 Ultrastructure of the nuclear matrix. Sections through nuclear matrices were examined by electron microscopy (see Materials and Methods). Intact matrices (*NMx*) were observed, as well as apparent disrupted matrices which appear as linear arrays of granular material (panel *A*) × 8,000. When examined at higher magnification, the linear arrays of granular material appear to be held together by an interspersed dense structure (panel *B*) × 45,000. Three regions can be recognized in an individual nuclear matrix: the nuclear lamella which forms the periphery of the matrix, the internal portion of the matrix which extends inwards from the lamella, and the residual protein component of the nucleolus (*Nu*; panel *C*) × 20,000. 80-Å tripartite structures were observed at infrequent intervals around the periphery of fewer than 5% of intact matrices (panel *D*) × 100,000.

TABLE I

Biochemical Composition of Nuclear Matrix

Biochemical component	Nuclear fraction*		
	Detergent-cleaned nuclei	Nuclear matrix	Nuclear matrix treated with Triton X-100
Protein	50.5	87.0	92.3
DNA	45.9	1.1	1.2
RNA	1.2	0.05	0.05
Phospholipid	2.3	11.8	6.9

* Percent composition by weight.

Isolation of the nuclear fractions, treatments with detergents, and biochemical analyses were done as described in Materials and Methods.

TABLE II

Relative Amounts of Phospholipids

Exposure time	Percent component*				
	APL	PE	PS + PI‡	PC	Sph
h					
1	13.4	34.2	18.2	30.9	3.4
6	16.4	23.1	17.0	40.0	3.2
18	12.1	12.8	12.4	48.2	14.7
1§	11.3	52.2	11.0	21.3	5.1

For exposure times up to 6 h, 1×10^9 cells, washed twice with phosphate-free medium (37°C) without serum, were resuspended in 200 ml of the same medium supplemented with 5% dialyzed calf serum and 2 mM glutamine. To deplete phosphate pools, cells were incubated for 30 min before the addition of 10 mCi [^{32}P]orthophosphate (New England Nuclear, Boston, Mass.). Cultures were incubated at 37°C and the pH of the medium was adjusted as needed with 5% $NaHCO_3$. For longer exposure times, 1×10^9 cells were suspended in 1,000 ml phosphate-free medium and incubated for 30 min at 37°C before the addition of 10 mCi of [^{32}P]orthophosphate. The nuclear matrix fraction was isolated and the relative amounts of the phospholipids were determined (see Materials and Methods).

* Abbreviations used: acidic phospholipids, APL; phosphatidylethanolamine and its lysoderivative, PE; phosphatidylserine, PS; phosphatidylinositol, PI; phosphatidylcholine and its lysoderivative, PC; sphingomyelin, Sph.

‡ PS and PI do not separate in this system.

§ Composition after extraction with 2% Triton X-100.

kylation for 4 h of SDS-solubilized samples, or by heating to 90°C for increasing lengths of time in the presence of 1% SDS and 0.01% mercaptoethanol followed by reduction and alkylation. Low molecular weight bands in the 14,000–18,000 range are presumptive histone polypeptides since they were preferentially extracted with 0.25 N HCl, 0.3 M $MgCl_2$, or 0.3 M KCl, and can be preferentially labeled in S-phase cells with basic amino acids. However, densitometer tracings have indicated that the proportion of histones was constant at 23–28%, regardless of the time of isolation in the cell cycle. By comparison, analysis of polypeptides in cylindrical gels after labeling with radioactive amino acids revealed two major clusters of polypeptides in the 90,000–130,000 and 50,000–75,000 regions (Fig. 4, panel A). To maximally display these two clusters, we found it necessary to carry out electrophoresis for a length of time that resulted in the histones migrating off the gel. When electrophoresis was carried out for shorter periods of time, routinely 22–26% of the radioactivity was present in the histone region. It is of interest that many of the peptides in the >50,000 range appeared to be glycopeptides. When cells were exposed to [^{3}H]glucosamine and the polypeptides of the isolated nuclear matrix

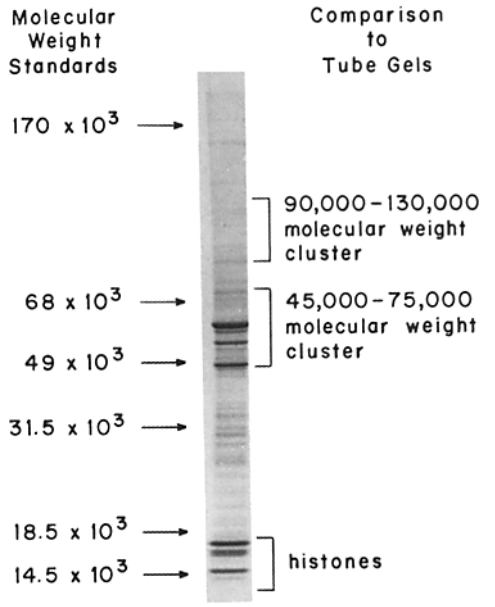

FIGURE 3 Electrophoretic profile of stained polypeptide components of the nuclear matrix fraction. The nuclear matrix fraction was isolated and solubilized as described in Materials and Methods. A 50-μg sample was analyzed by electrophoresis for 6.5 h at 150 V in a 6–20% SDS-polyacrylamide slab gel. Gels were stained with Coomassie blue and were calibrated for molecular weight with standard polypeptides (see Materials and Methods).

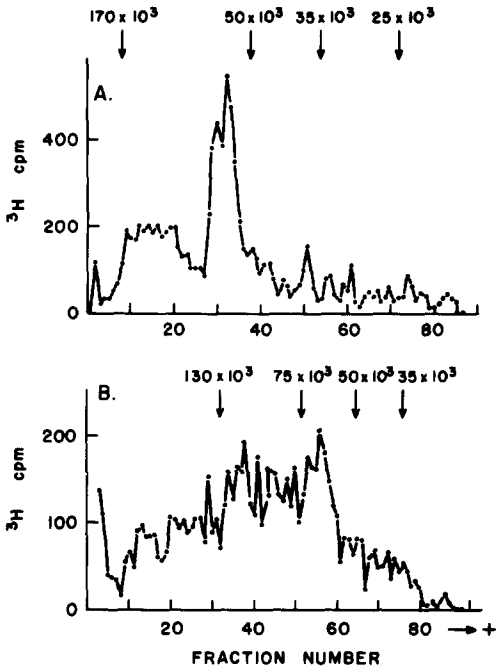

FIGURE 4 Electrophoretic profile of radioactive polypeptide components of the nuclear matrix fraction. (A) Cells at a concentration of 10^7/ml were exposed to 5 μCi/ml of [^{3}H]mixed amino acids for 2 h in medium containing 1% of the normal concentration of amino acids (see Materials and Methods). The nuclear matrix fraction was isolated, polypeptides were prepared, and a 100-μg sample was analyzed by electrophoresis for 18 h at 7 mA/gel in a 7.5% SDS-polyacrylamide cylindrical gel. Radioactivity was determined in 2-mm slices (see Materials and Methods). (B) Cells at a concentration of 10^7/ml were exposed to 5 μCi/ml of [^{3}H]glucosamine (1.2 Ci/mmol, New England Nuclear, Boston, Mass.) at 37°C for 6 h in complete medium. Nuclear matrix fraction was isolated, and a 100-μg sample of polypeptides was analyzed by electrophoresis for 18 h at 7 mA/gel in a 5% SDS-polyacrylamide cylindrical gel.

were analyzed by electrophoresis, approximately 85% of the radioactivity in the sample was associated with the polypeptides in this high molecular weight region (Fig. 4, panel B).

To determine whether or not the polypeptide profile of the nuclear matrix fraction was unique, we also analyzed other cellular fractions. This analysis was carried out with uninfected HeLa cells, as well as with HeLa cells infected by adenovirus 2. In the latter instance, viral infection provided a system whereby known marker polypeptides could be recognized in the nucleoplasmic and cytoplasmic fractions of the cells. It is clear that the total polypeptide profile of the nuclear matrix fraction from either infected or uninfected HeLa cells differs significantly from either the nucleoplasmic or cytoplasmic fractions (Fig. 5). This is particularly evident for virus-infected cells where the cytoplasmic fraction yielded structural viral polypeptides which migrated like hexon, penton, and fiber polypeptides. As expected, many of the same polypeptides were observed in the nucleoplasmic fraction. Differences between the polypeptide profiles of the nuclear matrix of infected and uninfected cells were also observed (Fig. 5, lanes *c* and *d*). Adenovirus-infected cells yielded three prominent additional polypeptides with apparent molecular weights of 21,000, 23,000, and 92,000. In comparison to the polypeptides prepared from virions, it is clearly seen that of these products associated with viral infection, only the 21,000 mol wt polypeptide corresponded to a virion component. Although their identity remains to be established, it is apparent that the 23,000 and 92,000 mol wt bands migrate as p-VII, the precursor to the core protein, and as the viral 100K protein, respectively. It is also clear that they were preferentially associated with the matrix fraction. In contrast, several of the polypeptides in the 45,000–75,000 mol wt cluster observed in uninfected nuclear matrix fractions are barely stained in preparations obtained 22 h after infection (compare Fig. 3 with Fig. 5, lanes *c* and *d*). This is especially true of three major polypeptides of approximately 49,000, 53,000, and 57,000 mol wt.

It was of interest to compare the profile of nuclear matrix polypeptides to that of a plasma membrane fraction which may contain similar cytoskeletal elements. This fraction was prepared from infected and uninfected cells according to the procedure of Atkinson and Summers (3). It is clear that the polypeptide profiles of the cytoplasmic membrane fraction are different from those of nuclear matrix fractions (Fig. 6). Again, the profile of polypeptides from infected cell plasma membranes is different from that of the corresponding fraction from uninfected cells.

Polypeptide Constituents during the Cell Cycle

To determine whether or not there were any cell cycle-related changes in the polypeptides of this nuclear matrix fraction, we have examined the polypeptide composition and synthesis of this frac-

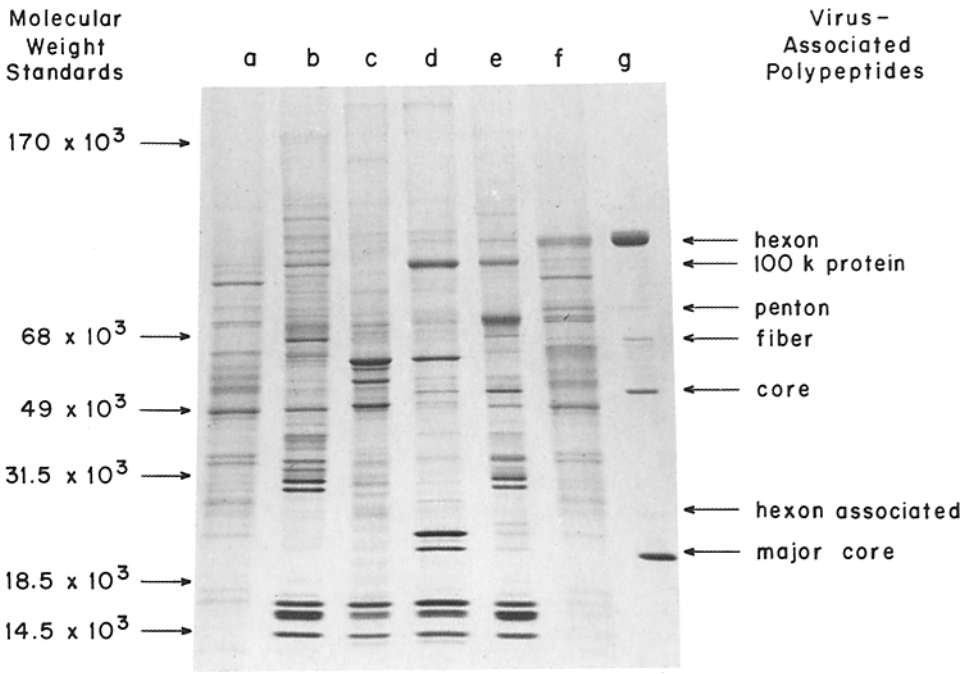

FIGURE 5 Electrophoretic profile of nuclear matrix fraction, nucleoplasm, and cytoplasm from uninfected and 22-h adenovirus 2-infected cells. Approximately 50-μg samples were analyzed from each cellular fraction. Electrophoresis in and staining of gradient slab gels were carried out as described in the legend to Fig. 3. Adenovirus 2 polypeptides were assigned as suggested by Maizel et al. (31). The sample order is: *a*, cytoplasm from uninfected cells; *b*, nucleoplasm from uninfected cells; *c*, nuclear matrix from uninfected cells; *d*, nuclear matrix from infected cells; *e*, nucleoplasm from infected cells; *f*, cytoplasm from infected cells; and *g*, purified adenovirus 2 polypeptides.

tion during the cell cycle, as well as the fate at mitosis of these polypeptides, utilizing populations of synchronized cells. Unlike the effects observed after virus infection, no major differences were seen in the overall pattern of the polypeptide profile from early G_1, mid S, and late S/G_2 cells as compared to random cells (Fig. 7). However, in each preparation the relative staining capacity of the major peptides in the 45,000–75,000 mol wt range was altered. Only the matrix fraction from mid S-phase cells yielded a profile similar to that of random cells in that the usual three major polypeptides at apparent molecular weights of 49,000, 53,000, and 57,000 were most pronounced. In contrast, there were six to seven nearly equally stained polypeptides in this range in preparations from early G_1 and late S/G_2 cells. The minor differences between the profiles, such as the polypeptide at 73,000 mol wt from S-phase cells, were consistent observations.

As an approach to the biosynthesis of nuclear matrix polypeptides, the incorporation of radioactivity into the high molecular weight polypeptide components was determined as a function of the cell cycle (Fig. 8). At the indicated times after synchronization, samples of the culture were exposed to tritiated amino acids, the nuclear matrix was isolated, and the polypeptides were separated by electrophoresis. Incorporation into the clusters of high molecular weight components (Fig. 4) compared to total nuclear matrix protein was essentially constant during G_1 and early S, but decreased as the cell population approached mid S to late S/G_2 (Fig. 8). This result was found with late S/G_2 populations whether they were obtained after release from thymidine blockade or by incubation of selectively detached mitotic cells. As expected, incorporation into the histone region of the gel occurred in S-phase cells. It is recognized that there is loss of synchrony with these procedures, but these results are consistent with a general lack of synthesis and/or assembly of high molecular weight components before mitosis.

To determine the fate of the matrix polypep-

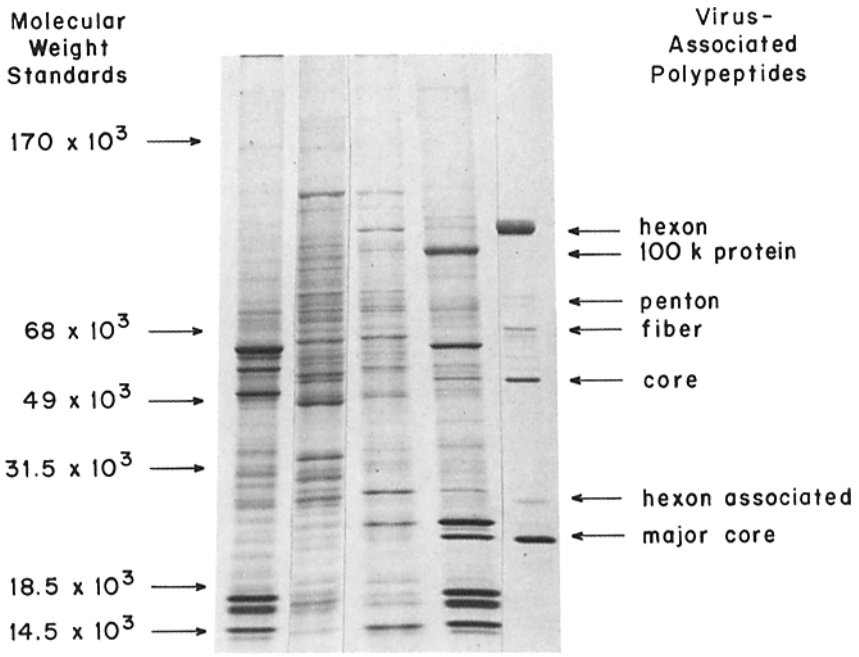

FIGURE 6 Electrophoretic profile of nuclear matrix and cytoplasmic membrane fraction from uninfected and 22-h adenovirus 2-infected cells. Electrophoretic conditions, molecular weight standards and viral polypeptides were as described in the legend of Fig. 5. For convenience, the figure is a composite of a single gel in which some lanes have been discarded. The sample order is: *a*, nuclear matrix from uninfected cells; *b*, plasma membrane fraction from uninfected cells; *c*, plasma membrane fraction from infected cells; *d*, nuclear matrix from infected cells; and *e*, purified adenovirus 2 polypeptides.

tides at the time of mitosis, we exposed monolayer cultures synchronous for S phase to [^{3}H]amino acids as they proceeded through S-phase and G_2 portions of the cell cycle (Fig. 9). Mitotic cells were then collected by selective detachment and allowed to enter G_1 in the presence of an inhibitor of protein synthesis. Under these conditions, there is nuclear reformation in the absence of protein synthesis (41). This should avoid possible complications of reutilization of radioactivity. When the nuclear matrix was isolated and analyzed for the presence of radioactive polypeptides, the acrylamide gel profile revealed that some polypeptides in the 50,000–75,000 mol wt range had survived. It is not clear at which times during S phase and G_2 such peptides were synthesized; nevertheless, this result indicates that there is survival of at least some polypeptides during the dissolution and reformation of the nucleus. Polypeptides larger than 90,000 mol wt were not labeled relative to the amount seen in short labeling conditions with unsynchronized populations (Fig. 4, panel A).

DISCUSSION

A subnuclear fraction has been isolated from nuclei of HeLa cells which resembles the nuclear matrix described in rat liver nuclei. These predominantly proteinaceous structures tend to maintain the shape and approximate size of nuclei and contain only minimal amounts of nucleic acids. Our procedure yields a subnuclear fraction with some membranous components which presumably represent portions of the inner nuclear membrane, unlike the matrix isolated from rat liver. However, ultrastructural evidence for a membrane was observed on only an occasional matrix in a given preparation. Riley et al. (37) have reported that a similar structure with a biochemical composition of 72% protein, 10% phospholipid, 14% DNA, and 4% RNA could be isolated from detergent-cleaned nuclei of HeLa cells, in the presence of high amounts of magnesium (0.5 M $MgCl_2$). Thus, two different isolation procedures yield subnuclear fractions with similar phospholipid content, and

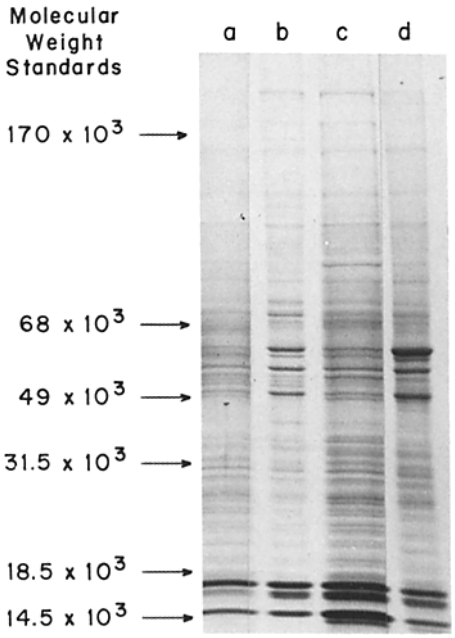

FIGURE 7 Electrophoretic profile of the polypeptides from synchronized cells. Profiles are from nuclear matrices isolated from cells at various times after synchronization. Electrophoresis in slab gel was carried out as described in the legend of Fig. 3. The sample order is: *a*, 50-μg sample from G_1 cells—selectively detached mitotic cells were allowed to proceed into G_1 for 2½ h; *b*, 60-μg sample from mid-S-phase cells—cells released from a double thymidine blockade were allowed to proceed into S phase for 3 h; *c*, 80-μg sample from late S/G_2 cells—cells released from a double thymidine blockade were allowed to proceed through S and into G_2 for 8 h; and *d*, 40-μg sample from unsynchronized log-phase cells.

these results suggest that the membrane component of HeLa nuclear matrices is more stable than that of rat liver matrices. In addition, Triton X-100 only partially removed the phospholipids of either HeLa nuclei or nuclear matrices, unlike the situation reported for rat liver nuclei. Furthermore, in our hands the procedures reported for isolating such subnuclear structures from rat liver nuclei have not proved successful with HeLa cells. Thus, nuclear structures and associations of macromolecules composing nuclei may not necessarily be similar from cell to cell. We also found that the relative composition of the various phospholipids varied in a consistent manner with the length of exposure to radioactive phosphate, and this suggests that as in other cellular membranes, phospholipid components of the nuclear envelope turn over at different rates.

By analogy to the investigations of the cytoplasmic membrane of red blood cells, the term nuclear ghost has been suggested for this fraction (37). This implies that such subnuclear structures are predominantly membranous in nature. On the contrary, our ultrastructural and biochemical data indicate that the structure is predominantly proteinaceous and composed of multiple components which include the dense lamella, internal matrix, and residual nucleolus.

The role of the nuclear matrix with regard to

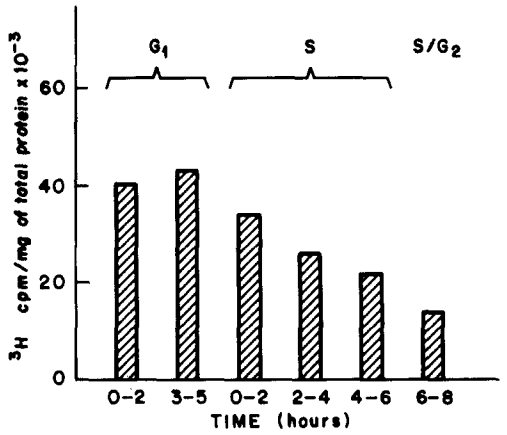

FIGURE 8 Incorporation of radioactive amino acids into high molecular weight polypeptides in synchronized populations. This figure is a composite of two separate experiments. In one experiment, mitotic cells were collected by selective detachment and permitted to enter G_1 by incubation at 37°C. After 1 h (0–2 h), when the cells had completed mitosis, one half of the culture was exposed to [³H]mixed amino acids at 5 μCi/ml and the remainder after 3 h (3–5 h). In the second experiment, cells in S phase obtained by release from a double thymidine blockade were maintained at 37°C and at subsequent (0–2)-h, (2–4)-h, (4–6)-h, and (6–8)-h intervals were exposed to [³H]mixed amino acids at 5 μCi/ml. Cells were maintained in complete medium after synchronization until exposed to radioactivity, at which time cells were suspended in medium containing 1% amino acids (see Materials and Methods). At the end of each 2-h pulse period, the nuclear matrix fraction was isolated, the amount of protein was determined, and the polypeptides were separated by electrophoresis in cylindrical gels. The radioactivity in matrix polypeptides excluding putative histones was summed. The data were calculated as total counts per minute in high molecular weight polypeptides per total milligrams of isolated nuclear matrix protein (counts per minute per milligram total protein).

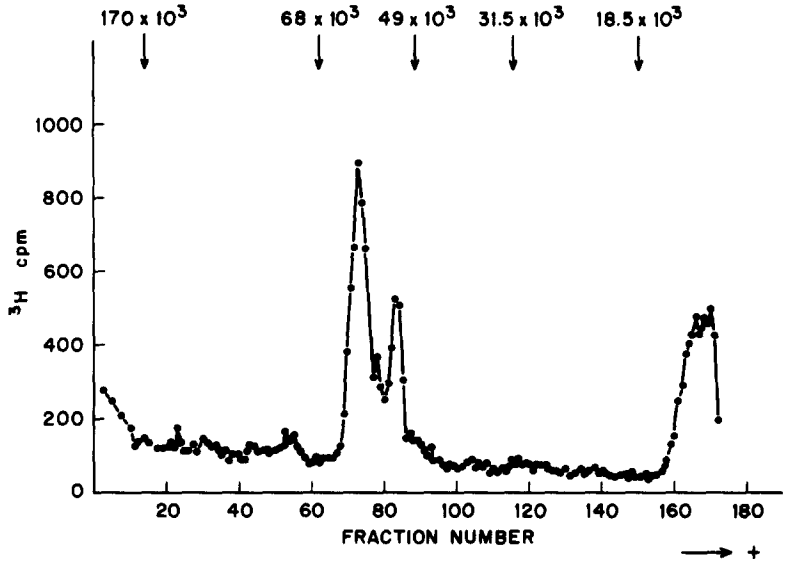

FIGURE 9 Conservation of polypeptide components through mitosis. S-phase cells, obtained by a double thymidine blockade, were used to establish monolayer cultures in complete monolayer medium containing [^{3}H]mixed amino acids at a concentration of 2 μCi/ml, and 8 h later mitotic cells were collected. The population at a concentration of 5 × 10^5 cells/ml with a metaphase index of 95% was permitted to enter G_1 by incubation at 37°C in complete medium in the presence of cycloheximide at a concentration of 100 μg/ml. The subnuclear fraction was isolated; and the constituent radioactive polypeptides were prepared and analyzed by electrophoresis for 18 h at 7 mA/gel in an 18-cm, 7.5% SDS-polyacrylamide cylindrical gel (see Materials and Methods).

nuclear function and intranuclear organization remains to be determined. For example, both rat liver and HeLa nuclei yield matrices with small amounts of residual DNA which is highly resistant to DNase attack. In general, nucleic acids do interact with specific proteins in such a manner that the complex is relatively resistant to digestion by nucleases. It is conceivable that such DNA sequences represent initiation sites for replication, especially since in rat liver the matrix DNA has been shown to be newly replicated (6). Alternatively, this DNA could represent a subset of sequences which interact with proteins to form fixing points for intranuclear organization. Previous speculations have involved the nuclear envelope in both processes. One way to probe for a functional significance of residual DNA is the infection of cells with adenovirus. In fact, we find that the nuclear matrix from infected cells yields pieces of newly synthesized presumptive viral DNA (F. Davis, unpublished observation). The use of specific fragments of adenovirus DNA generated by restriction endonucleases should permit us to determine whether or not the matrix-associated DNA represents unique viral sequences.

The nuclear matrix from rat liver yielded three major polypeptides in the 60,000–70,000 mol wt range, a small cluster in the 50,000 region, and no distinct peptides in molecular weight ranges corresponding to histones. By electrophoresis in 6–20% gradient acrylamide slab gels of HeLa nuclear matrix polypeptide components, we find at least 6 polypeptide bands with 3 being very prominent in the 45,000–75,000 mol wt range, as well as at least 24 other polypeptide bands. 3 of these 24 are presumably histones. Despite the relative loss of resolution in tube gel electrophoresis, the use of relatively short labeling times has allowed us to demonstrate a second high molecular weight polypeptide cluster at 90,000–130,000. It is conceivable that this group of polypeptides has a much shorter half-life and is therefore not a prominent feature of stained slab gels. Future experiments using [^{35}S]methionine combined with a more rigorous analysis of synthesis and turnover of individual polypeptides by autoradiography in slab gels should help to clarify this situation as well as the biogenesis in general of matrix components. In addition, many of the proteins in the >50,000 mol wt region appear to be glycoproteins since

they can be labeled with glucosamine. Thus, the approximate molecular weight estimates must be cautiously interpreted because the rate of migration of glycoproteins in sodium dodecyl sulfate polyacrylamide gel electrophoresis need not be an accurate measurement of size (32).

It is of interest that the polypeptide composition of the nuclear matrix appears to vary with the life cycle of the cell and after infection with adenovirus. In the latter instance, three major polypeptides in the 45,000–75,000 mol wt range tend to be reduced in amounts and three new polypeptides appear which migrate as the virus-coded proteins, major core protein VII at 21,000 mol wt, p-VII precursor to major core at 23,000 mol wt, and 100K at 92,000 mol wt. Neither nucleoplasm nor cytoplasm yields such a relative abundance of p-VII in comparison to VII. It is conceivable that this protein could be involved in a "fixing-point" for the initiation of viral assembly in association with the nuclear matrix. Unlike the effects seen as a result of virus infection, major additions in the polypeptide profiles of nuclear matrix preparations were not observed during the life cycle. However, the relative intensity of stain among the polypeptides in the 45,000–75,000 molecular weight range does change, although it is not clear whether this indicates the presence of different amounts of the polypeptides or changes in functional groups associated with them which can be stained. Assignment and/or identification of specific polypeptides as structural components of the matrix remain to be sorted out. It is conceivable that actin, myosin, and/or tropomyosin are components of this structure since nuclear extracts from many eukaryotic organisms contain these proteins (34). In fact, actin from several cellular sources has been estimated to have a molecular weight in the range of 43,000–48,000 (34). At least some of the polypeptides survive mitosis, and initial experiments (P. Mancini, unpublished observation) have indicated that they can be labeled when detergent-cleaned nuclei are iodinated *in situ* by the lactoperoxidase method (24). It is conceivable that limited proteolytic digestion and/or cross-linking of proteins would probe for topographical relationships, including any structural rearrangements at the time of cell division.

As with the isolation of any subcellular fraction, the final product is defined operationally and there is concern for its purity. In toto, our data indicate that a distinct microscopic element with a unique polypeptide profile has been isolated from the nucleus. Appropriate controls attest to the relative purity of our nuclear matrix fraction. In addition, our data indicate that it should provide another cellular element with which to explore structure-function relationships in synchronized and infected cultured mammalian cells.

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