Comparison of the Proteins Present in HeLa Cell Interphase Nucleoskeletons and Metaphase Chromosome Scaffolds*

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The nucleoskeleton, a macromolecular complex whose major structural elements are DNA and protein, is purported to be at least in part responsible for the gross morphology of the nucleus. This structure can be isolated from the nuclei of cells in all stages of the cell cycle with the exception of mitosis when no nuclei are present. We had previously proposed that during mitosis, the nucleoskeletal elements are reorganized and become part of mitotic chromosomes (Keller, J. M., and Riley, D. E. (1976) Science 193, 399–401). In order to test this possibility, we have compared the proteins of the nucleoskeleton to chromosomal proteins of similar solubility by one- and two-dimensional polyacrylamide gel electrophoresis and by peptide mapping. We have found that the major proteins of the nucleoskeleton are also major proteins of the chromosome scaffold. In addition, our data indicate that at least two of the nucleoskeletal proteins may be modified during the transition of the cell into mitosis. These data suggest that the nuclear dissolution associated with open mitosis is accompanied by a gross rearrangement of the nucleoskeletal elements to form components of metaphase chromosomes. This reorganization may be triggered by modification of several of the nucleoskeletal proteins. These observations appear to distinguish the nucleoskeletal proteins from the major nuclear lamina proteins which have been shown by others to be dispersed throughout the cell at mitosis.

Riley et al. (1) have reported the isolation of a macromolecular complex purported to be responsible at least in part for the gross morphology of the nucleus. The important structural elements of this nuclear structure, referred to as the nucleoskeleton, consist of DNA and protein, although RNA and lipid are present. The protein component consists of only a small subset of the total proteins found within the nucleus. The nucleoskeleton DNA also appears to constitute only a fraction of the DNA found in the nucleus.

At the electron microscope level, the nucleoskeleton is composed of presumed remnants of nuclear pores, rods (250 × 60 nm) and thinner connecting strands (2–4). The pore-like structures and 250 nm rods are predominately proteinaceous as judged by their sensitivity to protease digestion. The thin connecting strands, on the other hand, appear to be composed of double-stranded DNA since they are susceptible to digestion with either DNase I or DNase II but not to nuclease S1.

Since digestion of the thin connecting strands results in the destruction of the gross morphology of the nucleoskeleton and release of the thick rods and pore-like structures, we concluded that this DNA is required to maintain the structural integrity of the nucleoskeleton.

Riley and Keller (5) were able to isolate nucleoskeletons from cells in all stages of the cell cycle except mitosis. At this stage, the nucleus is absent, thus rendering the original nucleoskeleton isolation procedure ineffective. Attempts to isolate nucleoskeleton-like structures from individual chromosomes have been unsuccessful. Therefore, to determine the fate of the nucleoskeletal proteins during mitosis, we have compared them by one- and two-dimensional polyacrylamide gel electrophoresis to metaphase chromosomal proteins of similar solubility. We will show that (a) the major nucleoskeletal proteins are present as major components of the chromosome scaffold; and (b) that at least two of the nucleoskeletal proteins appear to be modified during the transition into mitosis. A summary of this research has been presented previously (6).

Materials and Methods

Cells—HeLa cells were grown as described previously (1). Isolation of Nucleoskeletons—Unless otherwise stated, all steps were performed at 0–4 °C. HeLa S3 cells were washed once in PBS, swollen in 1/3 X RSB buffer (1 X RSB = 10 mM Tris, pH 7.4, 10 mM NaCl, 4.5 mM MgCl₂) for 10 min and lysed with a tight fitting Dounce homogenizer. The nuclei were washed three times with 1 X RSB at 800 × g for 5 min and suspended at no more than 6 × 10⁸ nuclei/ml in 1.7 M sucrose/TKM (TKM = 50 mM Tris, pH 7.4, 25 mM KCl, 5 mM MgCl₂). After addition of 0.1 volume of 10% (v/v) Tween 40 and 0.05 volume 10% (w/v) deoxycholate, the nuclei were centrifuged through a 10-ml layer of 2.2 M sucrose/TKM onto a 3.5-ml pad of 2.8 M sucrose/TKM at 53,000 × g for 45 min in a Beckman SW 27 rotor.

The material above the nuclei was removed by aspiration and sufficient TKM was added to the nuclei to reduce the sucrose concentration to 2 M. Thirty minutes after the addition of 1 volume of 2 M sucrose/TKM, 1 M MgCl₂, the partially swollen nuclei were homogenized for 10 s with a Polytron homogenizer at a speed sufficient to agitate the suspension without shearing the partially swollen nucleoskeletons. The partially swollen nucleoskeletons were mixed at 1 × 10⁹/tube into the top half of a discontinuous gradient consisting of 2.0 M sucrose/TKM, 0.5 M MgCl₂ and a 3-ml pad of 2.8 M sucrose/TKM. After centrifugation for 1 h at 82,000 × g in a SW 27 rotor, the partially swollen nucleoskeletons were located on top of the dense sucrose pad.

The partially swollen nucleoskeletons were swept off of the 2.8 M sucrose/TKM pad with TKM, 0.5 M MgCl₂ and allowed to fully swell by the addition of TKM, 0.5 M MgCl₂ until the sucrose concentration was reduced to 0.6 M. After the nucleoskeletons had swelled completely, they were concentrated by precipitation with cold 1 M sucrose/TKM and centrifuged through a 1 M sucrose/TKM cushion for 90 min at 27,000 × g.

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† The abbreviations used are: PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; Tris, 2-amino-2-(hydroxymethyl)-1-propanediol; EDTA, ethylenediaminetetraacetic acid; Tween 40, polyoxyethylene sorbitan monopalmitate; BNPS-skatole, 2-(2-nitrophenyl-sulfenyl)-3-methyl-3'-bromindolenine skatole.
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Completely, 1 volume of 1.25 M sucrose/TKM, 0.5 M MgCl₂ was added. The nucleoskeletons were then mixed into the top half of the upper layer of a discontinuous gradient consisting of 1.0 M sucrose/TKM, 0.5 M MgCl₂ and a 3-ml pad of 2.8 M sucrose/TKM. After centrifugation for 1 h at 82,000 × g in a SW 27 rotor, the nucleoskeletons were located on the 2.8 M sucrose/TKM pad. The material above the nucleoskeletons was aspirated and the nucleoskeletons were swept off of the 2.8 M sucrose/TKM pad with TKM, 0.5 M MgCl₂ and centrifuged at 9,000 × g for 30 min in a Sorvall HB-4 rotor.

Isolation of Chromosome Scaffolds—HeLa S3 cells were blocked in mitosis with 0.01 µg/ml of Vinblastine (Sigma) for 24 h. Chromosomes were prepared as described by Wray and Stubblefield (7). Nuclei and clumps of chromosomes were removed by repeated centrifugation at 400 × g for 2 min whereas chromosomes were pelleted at 1600 × g for 20 min in an IEC refrigerated centrifuge. Chromosome scaffolds were prepared as described by Adolph et al. (8) except that the DNA was digested for 30 min with 80 µg/ml of micrococcal nuclease (Sigma). The non-scaffold proteins were extracted by the addition of 1 volume of buffer containing 4 mM NaCl, 20 mM Tris (pH 9.0), 20 mM EDTA, 2 mM PMSF, and 0.2% (v/v) Ammonyx LO (Onyx Chemical Co., Jersey City, NJ). The chromosome scaffolds were purified by centrifugation at 9,000 × g for 1 h through a pad of 5% (w/v) sucrose, 2 M NaCl, 10 mM Tris (pH 9.0), 10 mM EDTA, 0.1% (v/v) Ammonyx LO, and 1 mM PMSF.

Polyacrylamide Gel Electrophoresis—To prepare the nucleoskeletal proteins for electrophoresis, the DNA within this sample was digested after an overnight dialysis against distilled water. The samples were then dried under vacuum and dissolved in 1% (w/v) SDS, 5% (v/v) 2-mercaptoethanol. One-dimensional polyacrylamide gel electrophoresis was performed as described by Laemmli (9). Two-dimensional polyacrylamide gel electrophoresis was performed as described by O’Farrell (10). Bio-Rad ampholytes (equal volumes of pH 4/6 and pH 6/8) were used in the isoelectric focusing. The pH gradient of the isoelectric focusing gels was estimated by slicing one gel into 5-mm sections which were placed in vials containing 1 ml of water. After 30 min, the pH was determined with a pH meter.

After electrophoresis, the gels were fixed and stained with 0.2% (w/v) Coomassie blue in methanol/acetic acid/water (5:1.5. v/v/v) and destained in 7.5% (v/v) acetic acid.

BNPS-skatole Digestion—Two hundred micrograms of nucleoskeletal or scaffold proteins were electrophoresed in an 8% polyacrylamide gel, stained, and destained as described in the preceding section. The portion of the gel containing the protein of interest was excised, destained completely in 50% (v/v) methanol, 50 mM Tris, pH 8.8, equilibrated for 20 min with 100 volumes of methanol/acetic acid/water (5:1.5, v/v/v) and lyophilized. After the gel was rehydrated in 0.025% (w/v) BNPS-skatole, 0.017% (v/v) tyrosine in 50% (v/v) acetic acid, 8.3% (v/v) ethanol for 15 min, the excess liquid was removed and the digestion permitted to continue for a further 48 h in the dark at room temperature (19). The gel was then incubated twice for 10 min with 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 62.5 mM Tris, pH 6.8, placed vertically in a well of a 15% polyacrylamide gel and immobilized with 1% agarose in the preceding buffer. Electrophoresis, staining, and destaining was performed as described in the preceding section.

Protein Determination—Protein concentrations were estimated by the method of Lowry et al. (11) with bovine serum albumin (Sigma) as the reference.

Photomicroscopy—The nuclear organelles were dialyzed overnight against several changes of 1 × RSB. The material was stained with 0.1 volume of acridine orange (100 µg/ml) and photographed immediately afterwards on Tri-X film with phase-contrast or epifluorescence optics. The Tri-X film was developed with Rodinal (Agfa-Cevaert, Inc., Teterboro, NJ) to the manufacturer’s specifications.

RESULTS

Morphological Examination of the Nucleoskeleton—In order to obtain a sufficient amount of protein with which to work, we found it necessary to alter the nucleoskeletal isolation procedure (described in detail under “Materials and Methods”). During the formulation of this procedure, we discovered a semistable intermediary stage that permitted us to examine the nucleoskeletal swelling process per se. If the nuclei are extracted with 0.5 M MgCl₂ in 2 M sucrose/TKM, they swell to only a fraction of the size they would attain in 0.5 M MgCl₂/TKM in the absence of sucrose. In this high

![FIG. 1. Phase and fluorescent photomicrographs of nuclei, partially swollen nucleoskeletons, and fully swollen nucleoskeletons. The nuclear organelles were isolated as described under "Materials and Methods," and dialyzed overnight against several changes of 10 mM Tris, 10 mM NaCl, 4.5 mM MgCl₂ (pH 7.4). The material was stained with 0.1 volume of acridine orange (100 µg/ml) and photographed immediately afterward with phase contrast (A, C, E) or epifluorescent illumination (B, D, F). Nuclei (A, B); partially swollen nucleoskeletons (C, D); fully swollen nucleoskeletons (E, F). The bar represents 20 µm.](http://www.jbc.org/)
sucrose-magnesium environment, the nuclei swell to approximately 1.5 to 2 times their original size and, except for the nucleoli, appear vacuous as compared to nonextracted nuclei which become very refractile to light after the discontinuous sucrose gradient (Fig. 1). Unlike the fully swollen nucleoskeletons, the partially swollen nucleoskeletons are readily visible by phase microscopy and possess sharply delineated boundaries. The DNA of these partially swollen structures, however, is not completely contained within these boundaries. Fluorescence microscopy of these structures in the presence of acridine orange, a fluorescent DNA-binding molecule, reveals that a halo exists around these structures suggesting that a fraction of the DNA protrudes from the partially swollen nucleoskeleton (Fig. 1).

Although the data of Cook et al. suggest that the halo around the partially swollen nucleoskeleton is generated by the fluorescent dyes (12), we believe that the fluorescence reveals the actual structure of these organelles. In agreement with their data, we find the halos immediately after staining with acridine orange but only after prolonged illumination when stained with ethidium bromide. However, if we first stain the partially swollen nucleoskeletons with acridine orange and then introduce ethidium bromide under the cover slip by capillary action we find that the halo disappears only a few minutes later. We suggest that ethidium bromide induces a temporary collapse of the DNA which is reversible with illumination and time. This hypothesis also explains why fully swollen nucleoskeletons appear smaller immediately after being stained with ethidium bromide than when stained with acridine orange or in the absence of either of the dyes whatsoever.

The partially swollen state of the nucleoskeleton is stable for at least 2 weeks at 4°C in the presence of the extracted proteins as long as the sucrose and magnesium are maintained at a concentration of 2 M and 0.5 M, respectively. Reducing the sucrose concentration to less than 0.6 M results in further swelling, whereas reducing both the sucrose and magnesium concentration to less than 0.1 M causes a decrease in nucleoskeletal size. An analysis of the proteins in these shrunken nucleoskeletons shows that the histones are present in relatively great abundance, suggesting that the solubilized proteins reassociate with the nucleoskeletons at lower magnesium levels and induce the condensation. The partially swollen nucleoskeleton is stable at low magnesium concentrations in the presence of low concentrations of magnesium if these structures are separated from the extracted proteins by centrifugation.

Complete swelling of the nucleoskeleton occurs in the presence of 0.5 M MgCl₂ when the sucrose concentration is reduced to 0.6 M or less. The nucleoskeleton swells to 4 to 7 times the size of the nucleus and becomes difficult to detect under phase optics (Fig. 1). Nucleoskeletons larger than 50 μm are almost invisible under these conditions except for a few internal, condensed bodies. Stained with acridine orange, however, the nucleoskeleton fluoresces lightly and is readily detected regardless of size (Fig. 1). We have followed the swelling of nuclei microscopically by introducing 0.5 M MgCl₂ under a cover slip by capillary action and have determined that the prominent fluorescent spots in the nucleoskeleton are the remnants of the nucleoli.

Characterization of the Nucleoskeletal Proteins during the Swelling Process—Riley et al. (1, 13) had previously shown that the nucleoskeletal proteins are a subset of the total proteins present in the nucleus. Since we altered the original isolation procedure and have isolation an intermediary stage, we reinvestigated the proteins associated with these structures. Fig. 2 shows that proteins with molecular sizes similar to those of the major nucleoskeletal proteins can be seen in the nucleus and are present in increasingly greater relative abundance during the isolation of the nucleoskeleton. Fig. 2 also shows that the partially swollen nucleoskeleton is depleted of many of the proteins found within the nucleus, but still contains proteins not apparent in the fully swollen nucleoskeleton. The bulk of the histones and many of the non-histone nuclear proteins have been extracted from the partially swollen nucleoskeleton. The remainder of the non-nucleoskeletal proteins are released when the nucleoskeletons are fully swollen.

Prominent in the partially swollen nucleoskeleton but usually not in the fully swollen nucleoskeleton (hereafter referred to solely as the nucleoskeleton) are several peptides with molecular weights of 65,000 to 75,000. A comparison of the nucleoskeletal proteins in Figs. 2 and 3, for example, demonstrates that the relative abundance of these proteins is quite variable in our preparations. We have observed that the
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FIG. 3. SDS-polyacrylamide gel of nucleoskeletal and chromosome scaffold proteins. Approximately 15 μg of protein were electrophoresed in each well in a 12% polyacrylamide gel. Lanes 1 and 5, protein standards (see Fig. 2). Lane 2, nucleoskeletal proteins. Lane 3, equal amounts of nucleoskeletal and chromosome scaffold proteins. Lane 4, chromosome scaffold proteins.

Relative abundance of these proteins in the nucleoskeletal fraction appears to be inversely related to the size distribution of the nucleoskeletons; the amount of these proteins relative to the other major nucleoskeletal proteins decreases as the average nucleoskeletal size increases. On the basis of molecular weight, these proteins appear to be analogous to the lamina proteins of rat liver which are located adjacent to the nucleoplasmic surface of the inner nuclear membrane (14–16). We have found that the lamina proteins of HeLa cells have similar solubility properties as those of the major nucleoskeletal proteins and suggest that their disappearance from the nucleoskeleton is caused by their arrangement in a nonexpandable macromolecular complex around the periphery of the nucleoskeleton. During the expansion of the nucleoskeleton, this shell breaks and is lost.

The proteins which remain in the nucleoskeleton comprise a discrete subset of the total nuclear proteins. Present in a relatively great abundance in each nucleoskeleton preparation examined are found major proteins with molecular weights of 54,000, 51,000, 45,000, and 43,000. In addition to these prominent proteins, a number of minor proteins are also present. The size distribution of nucleoskeletal proteins reported in this communication agrees with the estimate published previously (13) and indicates that similar structures have been isolated even though the purification procedure has been altered.

Comparison of the Nucleoskeletal and Chromosome Scaffold Proteins by One-dimensional Polyacrylamide Gel Electrophoresis—To determine the fate of the nucleoskeletal proteins during mitosis, we compared the electrophoretic pattern of the major nucleoskeletal proteins to various subfractions of chromosomal proteins of similar solubility. A direct 0.5 M MgCl₂ or 2 M NaCl extraction of chromosomes left a large spectrum of proteins which made analysis difficult (data not shown). However, isolation of the chromosome scaffold as described by Adolph et al. (8) left a fraction of the total chromosomal proteins which possessed solubility properties similar to the nucleoskeletal proteins and which was easier to analyze.

A one-dimensional polyacrylamide gel revealed that the nucleoskeleton and chromosome scaffold have many proteins in common with respect to molecular size (Fig. 3). The major nucleoskeletal proteins migrate with the same electrophoretic mobility as several of the major scaffold proteins. Many of the minor nucleoskeletal proteins also migrate with the same mobility as the minor proteins of the scaffold. Present in the scaffold, however, are two prominent proteins with molecular weights of 57,000 and 49,000 which are not found in the nucleoskeleton. The prominent nucleoskeletal protein of 67,000 daltons is not routinely seen. The middle lane of Fig. 3, an equal mixture of nucleoskeletal and chromosome scaffold proteins, demonstrates that the proteins of apparently identical molecular weight cannot be resolved.

The nucleoskeletal proteins present in the scaffold are indigenous to the scaffold and are not present because of the nuclear contamination of the chromosome preparations. We routinely are unable to detect by fluorescence microscopy any nuclei in our chromosome preparations immediately prior to the isolation of the scaffolds. We estimate that if nuclei are present, the peptide map of cognate nucleoskeletal and chromosome scaffold proteins. The peptide bond at tryptophan was cleaved with BNPS-skatole as described under "Materials and Methods." Lane 1, nucleoskeletal 54,000-dalton protein; lane 2, scaffold 54,000-dalton protein; lane 3, nucleoskeletal 51,000-dalton protein; lane 4, scaffold 51,000-dalton protein; lane 5, nucleoskeletal 45,000-dalton protein; lane 6, scaffold 45,000-dalton protein; lane 7, nucleoskeletal 43,000-dalton protein; lane 8, scaffold 43,000-dalton protein.
FIG. 5. Two-dimensional polyacrylamide gel of nucleoskeletal and chromosome scaffold proteins. Approximately 120 µg of protein was electrofocused for 16 h in the presence of Bio-Rad ampholytes (pH 4 to 8). Electrophoresis in the second dimension was in a 12% polyacrylamide gel. A, nucleoskeletal protein; B, chromosome scaffold protein.
present, they are present at a concentration less than $6 \times 10^7$/ml when he chromosomes are suspended at 10 $A_{260}$ units/ml as measured in 0.1 N NaOH (approximately equal to $3 \times 10^7$ nuclei/ml).

**Comparison of the Nucleoskeletal and Chromosome Scaffold Proteins by Peptide Mapping**—To determine whether the similarity of the molecular weight of cognate nucleoskeletal and scaffold proteins is fortuitous, we also compared the major nucleoskeletal and scaffold proteins via the cleavage products after BNPS-skatole digestion of peptide bonds adjacent to tryptophan. Fig. 4 shows that peptides of similar molecular weight are found in cognate nucleoskeletal and scaffold proteins and that the number of peptides observed for cognate proteins is nearly identical. Minor differences between cognate proteins are visible and may be caused by differences in conformation and perhaps accessibility around a tryptophan residue or by the presence of a minor contaminant which is not present in equal amounts in the nucleoskeleton and chromosome scaffold. One-dimensional polyacrylamide gel electrophoresis of nondigested material, however, reveals only one band for each protein in the stained gel (data not shown), whereas two-dimensional polyacrylamide gel electrophoresis indicates that there may be charge heterogeneity within these proteins (described in detail in following section).

**Comparison of the Nucleoskeletal and Chromosome Scaffold Proteins by Two-dimensional Polyacrylamide Electrophoresis**—We also compared the nucleoskeletal and chromosome scaffold proteins by two-dimensional polyacrylamide gel electrophoresis to assess for possible modifications during the transition from interphase into mitosis. Fig. 5 shows that the major nucleoskeletal and scaffold proteins are clustered within a small pH range. Fig. 6 shows more clearly that the major component of the nucleoskeleton and scaffold 54,000-dalton proteins possess identical isoelectric points. The nucleoskeleton 45,000- and 43,000-dalton proteins also possess isoelectric points indistinguishable from their scaffold counterparts. The major 51,000-dalton protein of the scaffold, on the other hand, appears to possess a different isoelectric point. Although this might indicate a difference in the primary structure, we suggest that a series of modifications have been imposed on this protein in the transition from interphase to mitosis. In the two-dimensional gel a series of discrete spots can be seen of proteins with molecular weights of 51,000 but which differ in isoelectric points, ranging from that of the major component of the nucleoskeletal 51,000-dalton protein to the major component of the scaffold 51,000-dalton protein, a difference of about 0.6 pH unit. The relatively simple peptide map after BNPS digestion of the scaffold 51,000-dalton protein also supports the hypothesis that these multiple 51,000-dalton scaffold proteins are related (Fig. 4).

The two-dimensional gel also indicates that a minor component of the nucleoskeleton 54,000-dalton protein may also be modified during the transition into mitosis. The nucleoskeleton contains two proteins with a molecular weight of 54,000: a major component with a PI of 5.35 and a minor component with a PI of 5.45. The scaffold, however, contains three components: a major component with a PI of 5.35, a minor component with a PI of 5.25, and a third component with a PI of 5.45 which is faintly visible.

The putative protein modifications of the scaffold proteins as revealed in these two-dimensional gels does not arise from the presence of the protease inhibitor, phenylmethylsulfonyl fluoride, during the isolation of these organelles. PMSF is a serine-active protease inhibitor but may also react via the serine of other proteins and alter their isoelectric points. To assess if the protein heterogeneity of the scaffold 51,000-dalton protein is due to the PMSF, we compared the proteins from scaffolds prepared in the presence and absence of PMSF and found that all components present in the scaffold isolated in the presence of PMSF were also present in the scaffolds isolated in the absence of PMSF (data not shown). Since none of the other components used during the isolation of either the nucleoskeleton or chromosome scaffolds are known to modify proteins, we believe that this protein heterogeneity is not an artifact arising during the isolation of the organelles.

**Discussion**

We have found that the major proteins of the nucleoskeleton are also present as major constituents of the chromosome scaffold fraction of metaphase chromosomes. Interestingly, even though many of the proteins are common to these structures, they appear to be arranged differently. The nucleoskeleton is an extended object, 4- to 7-fold larger in size than a nucleus. In addition, the proteinaceous components of the nucleoskeleton are found throughout this structure and do not appear to be localized exclusively interior to the nucleoskeletal DNA (2, 3). The chromosome scaffold proteins, however, are not found in an extended structure under conditions required to swell the nucleoskeleton. In this environment the chromosome scaffold is at most equal in size to the chromosome (8). Furthermore, the scaffold proteins appear to

![Fig. 6. Comparison of the isoelectric points of the major nucleoskeletal and chromosome scaffold proteins. The areas containing the major proteins of the gels in Fig. 4 were photographically enlarged to facilitate comparison. A, nucleoskeletal proteins; B, equal amounts of nucleoskeletal and chromosome scaffold protein; C, chromosome scaffold proteins.](http://www.jbc.org/)

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be located interior to the chromosomal DNA in high ionic strength buffers. We suggest that either the forces responsible for condensing the chromatin into chromosomes inhibit the swelling of the proteinaceous components or perhaps some rearrangement of the nucleoskeletal proteins has occurred during the transition into mitosis.

Our evidence indicates that alterations to the high salt residual proteins occur during the cycling of the cell into mitosis. At least two of the major nucleoskeletal proteins appear to have been modified during this transition. The isoelectric point of the 51,000-dalton protein has decreased about 0.6 pH unit and the evidence suggests that multiple modifications have occurred. A fraction of the 54,000-dalton peptide also appears to have been modified. Experiments which are currently in progress should reveal the nature of these modifications and if these modifications result in a rearrangement of the high salt residual proteins.

This study also demonstrates that the putative nuclear structural proteins behave differently during mitosis. The lamina proteins, a group of nuclear structural proteins located on the periphery of the nucleus, have been found to be dispersed throughout the cell during mitosis (14-15). The nucleoskeletal proteins, on the other hand, are repackaged with the genetic material during this stage of the cell cycle.

As we suggested earlier, the interphase nuclear structural proteins which are retained by the chromosomes may have dual functions which could include a contribution to the gross morphology of the chromosome in addition to a possible role in the maintenance of the structure of the interphase nucleus. We searched for these nuclear proteins in the chromosome via the chromosome scaffold solely as a means of analyzing chromosomal proteins with solubility properties similar to those of the nucleoskeletal proteins. Whether the chromosome scaffold represents a true subchromosomal structure or be the result of the solubility properties of these proteins remains to be determined (17, 18). Nevertheless, Hadlaczky et al. (18) have also suggested that proteins do possess a role in the gross morphology of the chromosome even though the scaffold per se may be an in vitro artifact.

Alternatively, the nucleoskeletal proteins may not be involved in metaphase chromosome structure. The nucleoskeletal proteins may be repackaged with the genetic material during mitosis in order to re-establish at least part of the nuclear framework as soon as possible after chromosome movement has been completed.

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