Synaptonemal Complexes Are Integral Components of the Isolated Mouse Spermatocyte Nuclear Matrix

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ABSTRACT Synaptonemal complexes (SCs) have been isolated as integral components of the nuclear matrix from purified mouse pachytene spermatocytes. These nuclear synaptonemal complex-matrices are prepared by extracting Triton X-100-treated nuclei with low (0.2 M) and high (1.0 or 2.0 M) NaCl, DNase I, and RNase A to remove 85% of the nuclear proteins, 97% of the RNA, and 99% of the DNA. Studies with the light and electron microscopes indicate that these matrices, while lacking a distinct lamina, contain nuclear pores interconnected by a fiber network, residual nucleoli, and interchromatin fibers. In addition, the pachytene spermatocyte matrices contain residual XY heterochromatin and the principal components of the SCs, including two lateral elements, a central element, a presumptive centromere, and attachment plaques. These SCs are preserved within the matrix and retain their structural association with the pore-fiber complex, even when subjected to strong dissociating conditions.

Nuclear matrices from pachytene spermatocytes and spermatids (steps 1–8), when analyzed by SDS PAGE, contain an array of polypeptides distinct from those of mouse liver nuclear matrices. Proteins of spermatogenic matrices range in M_r from 8,000 to ~150,000. The prominent lamina proteins (M_r ~ 60,000–70,000) of somatic nuclear matrices are either absent or represent only a minor part of the spermatogenic matrix. The polypeptide composition of the pachytene spermatocyte and spermatid matrices are similar, although minor quantitative and qualitative differences are evident. These observations suggest that the SC constituents may consist of a heterogeneous group of proteins present in low proportion relative to total matrix proteins, or they may be retained, but in a different form, within the spermatid matrix.

Mammalian spermatogenesis is an intricate process of differentiation, involving germ cell proliferation and renewal, meiosis, and spermiogenesis. The product, haploid spermatozoa, are genetically diverse due to the meiotic events of gene recombination and chromosome reassortment. The exchange of gene sequences, in particular, is thought to be mediated by the synaptonemal complexes. Evidence suggests that these tripartite structures facilitate synapsis, recombination, and the eventual disjunction of the homologous parental chromosomes (for reviews see references 5 and 58).

Although the synaptonemal complexes (SCs) are considered to play a central role in genetic recombination, an understanding of their formation, structure, and function has been limited to morphological and histochemical studies (for reviews see references 37, 55, 57, and 76). The SC consists of two dense, rodlike lateral elements separated by a central region containing a medial central element and periodic transverse microfilaments. Lampbrush loops of chromatin radiate from the SC, each possibly being attached to a DNase-sensitive strand that extends axially through each lateral element (13, 49, 70). Each telomeric end of the SC is embedded firmly in the nuclear envelope by a broad attachment plaque (26, 77, 78). Cytochemical studies suggest that the SC is composed primarily of protein, and only trace amounts of DNA and RNA (59, 69, 70, 71). However, the polypeptide constituents of the SC and their molecular organization have yet to be defined.

The extensive integration of the SC with chromatin and other nuclear elements has precluded its isolation as a distinct entity. Efforts to assess the structural integrity of the SC suggest that its elements are associated closely with the pore-lamina complex (22, 56), an integral component of the somatic nuclear matrix (2, 9, 68). The proteinaceous SCs appear to be resistant to high salt, DNase, and RNase treatment (17, 18, 70, 71). Thus, a morphological analysis of nuclear matrices isolated from mouse testicular cells by salt extraction reveals the presence of "SC-like" structures (20). The structural resilience of

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the SCs is also evident from their persistence in pore-lamina preparations of Chinese hamster spermatocytes (75). These observations indicate that it may be feasible to prepare SCs as integral constituents of spermatocyte nuclear matrices. Since discrete populations of mammalian spermatocytes and other stages of germ cell differentiation can be isolated (3, 4, 48, 67), molecular studies on the constituents of the SCs are possible.

The procedure subsequently developed permits the progressive depletion of protein, RNA, and DNA from purified spermatocyte nuclei. It yields nuclear matrices containing the principal elements of the SC, complete with the central and two lateral elements, a presumptive centromere, and the terminal attachment plaques. Moreover, biochemical data show that nuclear matrices from pachytene spermatocytes and spermatids (steps 1–8) both contain protein constituents distinct from those present in somatic nuclear matrices.

MATERIALS AND METHODS

Materials: Male mice, CD-1 strain, were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Bovine serum albumin (BSA, fraction V), trypsin (bovine pancreas, type III), deoxyribonuclease (DNase I; DN-CL and DN-EP), ribonuclease (pancreatic RNase A), and soybean trypsin inhibitor (type I-S) were obtained from Sigma Chemical Co. (St. Louis, MO). The protease inhibitor Ep-475 was donated generously by Dr. A. L. Goldberg, Harvard Medical School, Boston, MA. Collagenase was a product of Worthington Biochemical Corp. (Freehold, NJ). TETKO Inc. (Elmsford, NY) supplied the Nitex nylon filter.

Isolation of Spermatogenic Cells: Spermatogenic cell suspensions were prepared from adult mouse testes (3, 4, 67). Decapsulated testes were incubated in 0.5 mg of collagenase/ml of sterile, enriched Krebs-Ringer bicarbonate buffer (EKRB) at 33°C for 15 min to dissociate the seminiferous tubules from interstitial tissue. The dispersed tubules were washed free of remaining interstitial cells and then incubated with 0.5 mg of trypsin/ml and 1 μg DNase (DN-CL)/ml EKRB for 15 min at 33°C to produce a monodisperse cell suspension. The cells were filtered through Nitex cloth (80-μm mesh), washed in 0.5% BSA (wt/vol), 100 μg soybean trypsin inhibitor/ml EKRB, and then filtered through Nitex cloth (35-μm mesh). When required, discrete populations of germ cells were isolated by sedimentation velocity at unit gravity by using a 2–4% BSA gradient in EKRB. The purity of the isolated pachytene spermatocytes (>89%) and spermatids (>88%) was monitored by Nomarski differential interference optics. These cells retain their structural integrity as assessed by trypan blue exclusion (>98%) and by several biochemical parameters (3, 4).

Isolation of Nuclei from Spermatogenic Cells: Seminiferous epithelial cells or purified spermatogenic cells were suspended in 17 mM $MgCl_2$, 40 mM KCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 20 mM Tris-HCl at pH 7.7 for 1 min at 4°C and centrifuged at 225 *g* for 5 min. The crude nuclear pellet was suspended in 1% Triton X-100, 0.32 M sucrose, in MKPT buffer (10 mM $MgCl_2$, 40 mM KCl, 1 mM PMSF, and 20 mM Tris-HCl at pH 7.7) for 10 min at 4°C. A 1-ml sample was underlayed with 1 ml of 1.1 M sucrose in MKPT and centrifuged at 5,000 *g* for 10 min at 4°C. The pure nuclear pellet was washed once with 0.32 M sucrose in MKPT, sedimented for 10 min at 135 *g*, and resuspended in the same medium. The nuclei were stored at 4°C until used within 12–16 h for preparing nuclear matrices.

Preparation of Spermatogenic Nuclear Matrices: Isolation of spermatogenic matrices involved the successive extraction of DNA, RNA, and chromosomal proteins by a series of nuclease digestions and salt extractions (Fig. 1). Two alternate procedures were used.

PEOCEDURE I: Nuclei were treated with 0.2 M NaCl, 10 mM $MgCl_2$, 1 mM PMSF, and 20 mM Tris-HCl (pH 7.4) [low salt] at 4°C for 10 min and centrifuged at 135 *g* for 10 min. The partially extracted nuclei were incubated with 100 μg of DNase I (DN-CL)/ml and 100 μg RNase/ml of 100 mM $MgCl_2$, 1 mM PMSF, and 20 mM Tris-HCl (pH 7.4) [low DNase-RNase] for 20 min at 24°C, and then centrifuged for 10 min at 600 *g*. The nuclear preparation was resuspended in 2 M NaCl, 100 mM $MgCl_2$, 1 mM PMSF, and 20 mM Tris-HCl (pH 7.4) [high salt] for 20 min at 4°C and sedimented at 600 *g* for 20 min. Finally, the extracted nuclei were incubated in 250 μg of DNase I/ml and 250 μg RNase/ml of 100 mM $MgCl_2$, 1 mM PMSF, and 20 mM Tris-HCl (pH 7.4) [high DNase-RNase] for 30 min at 24°C, and centrifuged at 600 *g* for 10 min. The final spermatogenic nuclear matrices were washed once in 20 mM Tris-HCl (pH 7.4).

PROCEDURE II: An alternate procedure involved the extraction of chromosomal proteins with 1 M rather than 2 M NaCl, after digesting the nuclei with DNase and RNase. This reduced the harshness of the high salt extractions and increased the yield of matrix protein. Nuclei were incubated with 100 μg of DNase I/ml and 100 μg of RNase/ml of 10 mM $MgCl_2$, 1 mM PMSF, and 20 mM Tris-HCl (pH 7.4) [DNase-RNase] for 60 min at 24°C. The preparation was sedimented at 500 *g* for 2 min and resuspended in 1 M NaCl, 10 mM $MgCl_2$, 1 mM PMSF, and 20 mM Tris-HCl (pH 7.4) [1 M NaCl] for 30 min at 4°C. After centrifugation at 800 *g* for 10 min, the nuclear pellet was extracted successively with DNase-RNase (100 μg/ml), then with 1 M NaCl, and again with DNase-RNase (100 μg/ml). The final nuclear matrix preparation was recovered after a wash with 20 mM Tris-HCl (pH 7.4).

Preparation of Liver Nuclei and Matrices: Mouse liver nuclei were prepared according to Blobel and Potter (10), and were then extracted by using a procedure modified after that of Berezney and Coffey (9). The nuclei first were digested with exogenous DNase I (10 μg/ml) for 15 min at 24°C. The original procedure was then followed, except that each step was repeated twice and 1 mM PMSF was added to all solutions. This modified protocol reduced the harsh treatment of nuclei during the low magnesium and high salt extractions and thereby permitted a more accurate comparison of this procedure with the gentler protocol used for isolating spermatogenic matrices.

Liver nuclear matrices also were prepared by a modification of procedure I. After the initial low DNase-RNase treatment, the nuclear preparation was incubated in 1% Triton X-100, 100 mM $MgCl_2$, 1 mM PMSF, and 20 mM Tris-HCl (pH 7.4) for 10 min at 4°C and then washed in 100 mM MgC'_2, 1 mM PMSF, and 20 mM Tris-HCl (pH 7.4) at 4°C for 20 min. The spermatogenic matrix isolation procedure was then followed without further modification.

Morphological Procedures: SCs were identified in isolated nuclei by observing silver-stained whole mount preparations with the light microscope. Pachytene spermatocyte nuclei were treated hypotonically for 10 min at 4°C in EKRB/H_2O (1:3, vol/vol). A few drops of this suspension were placed on a glass slide, fixed with 4% paraformaldehyde (pH 8.2), and dried with 0.4% Photoflo (pH 8.2) (56). The preparations were stained by the Ag-I method of Bloom and Goodpasture (11) as modified by Lau et al. (46). The specimens were treated with borate buffer (pH 9.0) for 20 min, washed in deionized water, and incubated with 50% $AgNO_3$ for ~27 h in a humidified chamber at 50°C. After staining was complete, the slides were rinsed in deionized H_2O and air-dried.

Whole mount preparations of nuclear matrices were examined by using phase contrast and Nomarski differential interference optics. The matrices were swollen by a 2- to 3-min exposure to 3 M urea, 0.1% SDS, 20 mM Tris-HCl (pH 7.4), placed on a microscope slide, and overlayed with a coverslip. Whole mounts of urea/SDS-treated nuclear matrices were also prepared on carbon-formvar-coated grids for observation with the electron microscope. Samples were fixed by floating the grids on 4% paraformaldehyde in 0.1 M sucrose (pH 8.0), protected with a coating of 1% Ficoll-1% dimethylsulfoxide, treated with 0.4% Photoflo (pH 8.0), and air-dried (56, 60). Grids were stained with ethanolic phosphotungstic acid and observed with a Philips 200 electron microscope.

For examination of thin section preparations with the electron microscope, nuclei of purified pachytene spermatocytes and spermatids (steps 1–8) were fixed separately with 2% glutaraldehyde in 0.32 M sucrose, 1 mM $MgCl_2$, and 0.07 M cacodylate buffer (pH 7.4) at 4°C for 90 min. Nuclear matrices of the purified cell types were fixed for 90 min with 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C. All preparations were postfixed with cacodylate-buffered 1% OsO_4 for 60 min at 4°C, washed with deionized H_2O, dehydrated stepwise through graded solutions of ethanol and propylene oxide, and embedded in Spurr resin (72). Thin sections were cut on a Sorvall MT-2B ultramicrotome, double stained with 2% uranyl acetate and lead citrate (66), and examined by using a Zeiss EM 10 electron microscope.

Chemical Determinations: Chemical analyses were performed to determine the DNA, RNA, protein, phospholipid, and carbohydrate content of nuclei and nuclear matrices. These structures were isolated from spermatogenic cells and from purified pachytene spermatocytes and spermatids (steps 1–8). The isolated nuclei and nuclear matrices were extracted with 0.2 N perchloric acid (PCA) at 4°C for 10 min and then centrifuged at 2,000 *g* for 10 min. Carbohydrate was measured in the supernatant (25). Total phospholipid was extracted from the PCA precipitate with ethanol and ethanol/chloroform (3:1, vol/vol) (61) and determined by the procedure of Chen et al. (15). Alternatively, RNA was hydrolyzed from the PCA precipitate (61) and quantitated by spectrophotometric techniques (31). Total RNA, phenol-extracted from seminiferous tubules and purified by $CsSO_4$ gradient (42), and tubular protein (32) were used as standards. DNA content was determined by the method of Thomas and Farquhar (74).

Total protein was quantitated by an assay modified from that of Bradford (12). Nuclear protein samples were dissociated in 6 M guanidine hydrochloride (G·HCl), 75 mM dithiothreitol (DTT), 5 mM EDTA, and 0.5 M Tris-HCl (pH 8.6) for 60 min at 33°C in a total volume of 50 μl. Each sample was acidified by the addition of 15 μl of 0.5 N HCl and diluted with deionized H_2O to 800 μl. Matrix protein samples were dissociated in 50 mM DTT and 20 mM Tris-HCl (pH 7.4) at room temperature for 10 min in a total volume of 28 μl. The samples were acidified with 3 μl of 0.5 N HCl and diluted to 800 μl with deionized H_2O. Protein samples from both nuclei and matrices were then mixed thoroughly

before and after the addition of 100 μl of the Bio-Rad reagent (Bio-Rad Laboratories, Richmond, VA). Absorbance was quantitated at 595 nm in a Beckman Acta CIII spectrophotometer.

SDS PAGE

SAMPLE PREPARATION: Nuclei and 0.2 M NaCl-extracted nuclei were dissociated in 6 M G·HCl, 5 mM EDTA, 75 mM DTT, and 0.5 M Tris-HCl (pH 8.6) at 33°C for 60 min, and centrifuged at 216,000 *g* for 14 h to pellet the DNA. Other protein samples to be aminoethylated were dissociated under similar conditions, and alkylated by adding ethyleneimine to a final concentration of 0.25 M, aerating with N_2, and incubating in the dark for 60 min at 24°C. All protein samples were dialyzed, lyophilized, and then solubilized in 2% SDS, 10% glycerol, 100 mM DTT, and 6.25 mM Tris-HCl (pH 6.8).

ELECTROPHORESIS AND STAINING: Proteins were resolved by SDS PAGE (45) in 14 cm × 13 cm × 0.75 mm slab gels consisting of a 7–18% linear gradient of polyacrylamide. The gels were stained overnight in 0.1% Coomassie Blue, 45% methanol, 10% acetic acid, and then destained. Alternatively, the gels were stained with silver by a modification of the technique of Morrissey (53). After electrophoresis, the gel was fixed overnight in 50% methanol and 0.037% formaldehyde. The gel was then soaked in 32 μM DTT for 30 min, incubated in 0.1% $AgNO_3$ (wt/vol) for 30 min, and rinsed rapidly in distilled H_2O. Development of the silver stain was accomplished by soaking the gel in 0.02% formaldehyde and 3% sodium carbonate (wt/vol). Further development of the stain was prevented by the addition of 0.1 vol of 2.3 M citric acid for 10 min. The gel was washed with several changes of H_2O for 30 min and stored in 0.03% sodium carbonate.

RESULTS

Matrix Isolation and Morphology

The SCs can be visualized in spermatocytes by using phase-contrast optics after silver staining (23, 33, 63). Silver primarily binds to basic proteins, and preferentially stains the lateral elements of the SC (24). In this study, the protocol (Ag-I) is applied to monitor SCs in pachytene spermatocyte nuclei after their isolation by hypotonic treatment and sucrose gradient centrifugation (procedure I; Fig. 1). SCs are discernible in the isolated nuclei as dark, thick threads, each having an enlarged terminus that corresponds to the telomeric centromere (Fig. 2). Several nucleoli and the heterochromatic XY body are also observed.

Spermatogenic nuclei progressively contract to ~50% of their original diameter during the sequential treatments with low and high salt, DNase, and RNase. The resulting nuclear matrices appear as condensed, aggregated spheres when observed with the phase-contrast microscope. These nuclear matrices remain intact when subjected to the moderately strong dissociating conditions of 1 or 2 M NaCl. Even when exposed to 3.0 M urea/0.1% SDS, the matrix only expands to its original or a slightly greater diameter. The structure remains completely intact, appearing as a transparent, fibrous sphere with distinct threadlike SCs extending across the interior. These SCs are similar in appearance to those in Ag-I stained nuclei (cf. Figs. 2 and 3*a*). The other internal structures of these urea/SDS-treated matrices can be observed quite readily with the phase-contrast microscope. Also, when using Nomarski optics, the attachment plaques are still discernible as knoblike structures at the termini of the SCs (Fig. 3*b*). The SCs appear to be enmeshed in a network of finer matrix fibers. Although the nuclear synaptonemal complex-matrix is stable under these dissociating conditions, it dissolves readily in the presence of >75 mM DTT (see references 19, 70, 71).

The ultrastructure of nuclei and nuclear matrices from purified pachytene spermatocytes was evaluated by examining thin section and whole mount preparations (Figs. 4 and 6–12). Isolated nuclei contain intact SCs with distinct lateral elements, a central element, and terminal attachment plaques (Fig. 4*a*).

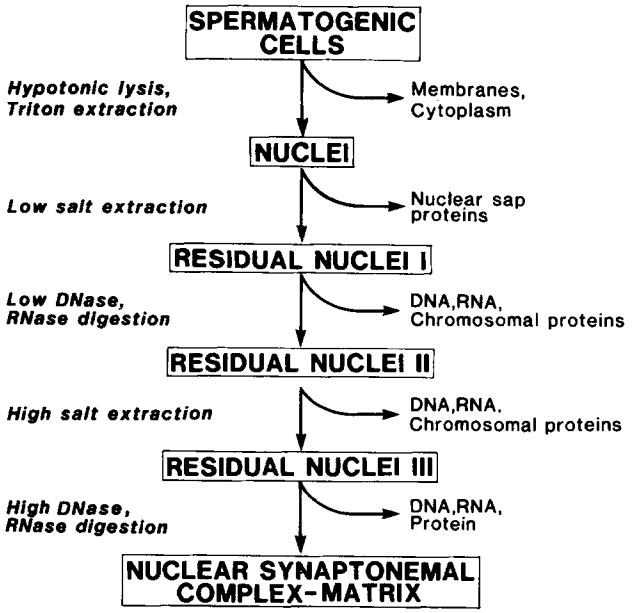


FIGURE 1 Flow diagram of procedure I used to isolate the nuclear synaptonemal complex-matrix from purified mouse pachytene spermatocytes, and the nuclear matrix from purified spermatids (steps 1–8). The germ cells were hypotonically lysed, extracted with 1% Triton X-100, and centrifuged through sucrose to separate the nuclei from contaminating cytoplasm and membranes. The purified nuclei were extracted with low salt (0.2 M NaCl) to remove nuclear sap proteins (see Materials and Methods). DNA, RNA, and chromosomal proteins were then extracted from the nuclei by successive treatment with low DNase-RNase (100 μg/ml), high salt (2 M NaCl), and high DNase-RNase (250 μg/ml). The preparation of nuclear synaptonemal complex-matrices or spermatid matrices was obtained after a final Tris-HCl wash.

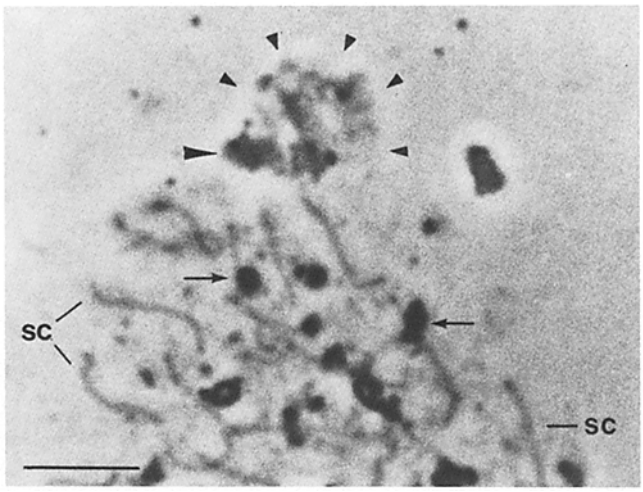


FIGURE 2 Phase-contrast micrograph of a hypotonically-treated pachytene spermatocyte nucleus stained by the Ag-I procedure. Synaptonemal complexes (*SC*) are evident as dark threads, each having a knoblike terminus corresponding with the telomeric centromere. The heterochromatic body (small arrowheads), containing the X and Y chromosomes in end-to-end association (large arrowhead), is located at the periphery of the nucleus. Several nucleoli (arrows) are distributed throughout the nucleus. Bar, 5 μm. × 3,100.

Sparse transverse microfilaments span the central region between the central and two lateral elements (Figs. 4*a* and 7). A well-developed lamina is not observed within intact sperma-

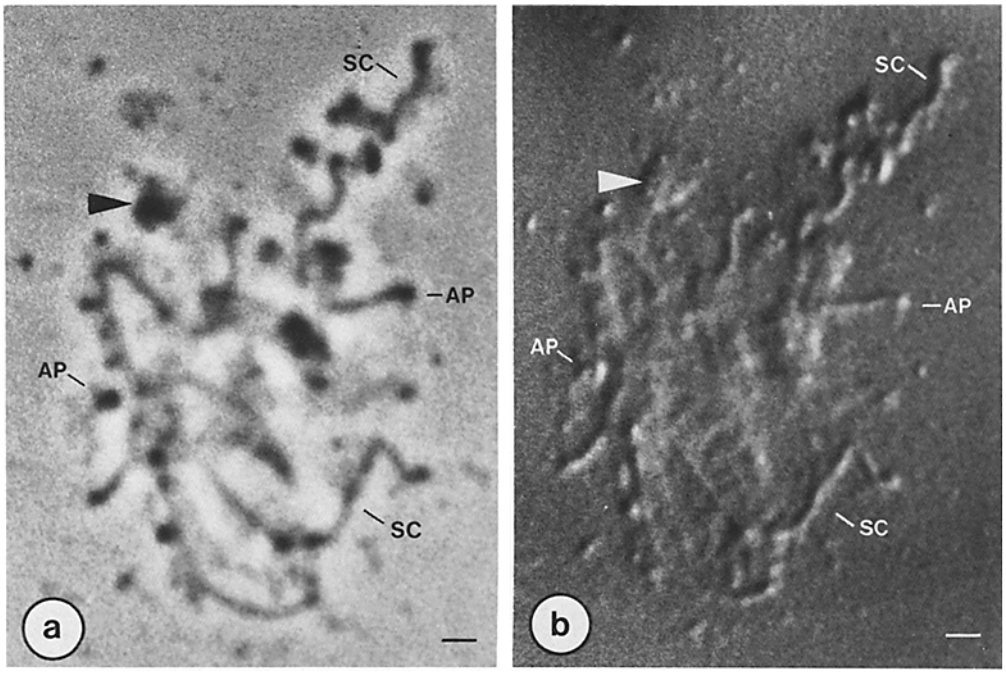


FIGURE 3 Whole mount preparation of a pachytene spermatocyte nuclear matrix observed by (*a*) phase-contrast and (*b*) Nomarski differential interference optics. Treatment with 3.0 M urea/0.1% SDS causes an expansion and subsequent spreading of the matrix, allowing visualization of the *SC* without prior fixation or staining. The *SCs* appear as thick, threadlike structures with bulblike attachment plaques (*AP*) at either end. Several nucleoli also are observed (arrowheads). Bar, 1 μm. × 4,400.

tocyte nuclei, in agreement with earlier morphological observations (28, 29, 73).

The pachytene spermatocyte nuclear matrix consists of a pore-fiber complex, residual nucleoli, and interchromatin fibers (Fig. 4*b*), comparable in most respects with the nuclear matrix of somatic cells. In addition, the spermatocyte matrix contains residual XY heterochromatin and the principal elements of the SCs. However, the fibrous lamina, a typical component of the somatic matrix, is not evident in these preparations. Instead, the nuclear pores are connected by thin interpore fibers (Fig. 8), comparable with the pore-fiber complex of Xenopus oocytes (34, 44). Interchromatin fibers form two distinct regions (Fig. 4*b*). The matrix interior is composed of dense, fibrillar material, while the peripheral areas contain small "vesicles" ranging from 0.25 to 0.43 μm in diameter (Fig. 6). The latter contain either flocculent material or large, dense granules. These stable vesicles usually appear in clusters at the nuclear periphery, and are surrounded by loosely arranged, interchromatin fibers. In a few preparations, the vesicles appear contiguous with the pore-fiber complex, but whether they are formed from invaginations of the nuclear envelope is not clear. Individual SCs are recognizable by their distinct morphology (Figs. 9 and 10; cf. references 27 and 54). The two dense, parallel, lateral elements, each ~45-nm thick, are separated by a central region 100-nm wide. Within this region is the narrower central element with a diameter of ~20 nm. Relatively few transverse microfilaments are evident in the central region. In favorable preparations, the thickened ends of the lateral elements are contiguous with the broader attachment plaques.

Ultrastructural analysis of the pachytene spermatocyte matrix was also performed on whole mounts prepared after spreading in 3.0 M urea/0.1% SDS. This procedure displays the matrix contents in a two-dimensional array. SCs with a central element, two lateral elements, and terminal attachment plaques are observed (Fig. 11). Their average length is 16 μm (range: 12–19 μm). Regions of fibrous material may adjoin the attachment plaques of several SCs, as observed previously in whole mount microspreads of meiocytes (56). The presumptive centromeric region of the SC is characterized by a spherical, dense body on both lateral elements close to the telomeric region. A tuft of fibrillar material radiates from each attachment plaque. Ultrastructural details of individual SCs reveal that the two lateral elements and flanking central element (Fig. 12) are narrower than those observed in thin section (Figs. 9 and 10). This possibly is due to some longitudinal stretching of the SC during whole mount preparation of urea/SDS-treated matrices (22, 56). In addition, altered staining characteristics or partial extraction of protein constituents of the SC may have occurred.

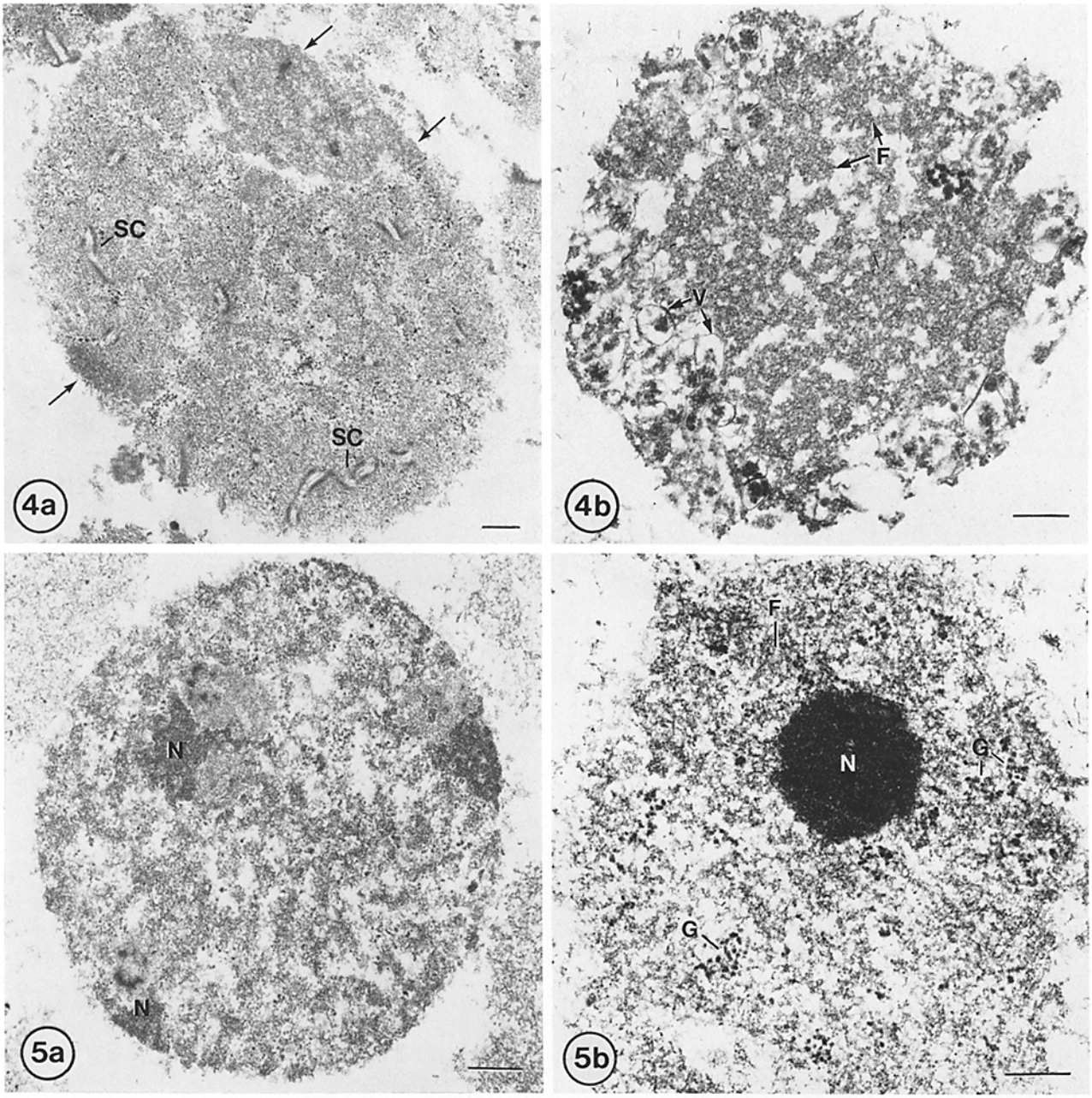
The spermatid (steps 1–8) nucleus typically contains prominent nucleolar-like structures and increased amounts of heterochromatin (Fig. 5*a*). Nuclear matrices from spermatids, like those of spermatocytes, exhibit an indistinct lamina, a pore-fiber complex, residual nucleoli, and an interchromatin fiber network that is interspersed with dense, interchromatin granules (Fig. 5*b*). In contrast with pachytene spermatocyte matrices, SCs, XY heterochromatin, and peripheral vesicles are not observed in spermatid matrices.

Gross Biochemical Composition

The molecular composition of the nuclei and nuclear matrices prepared from spermatogenic cells and purified populations of pachytene spermatocytes and spermatids has been analyzed (Table I). Extraction of these nuclei using the sequence of low and high concentrations of NaCl, DNase, and RNase removes 85% of the nuclear protein, 97% of the RNA, and 99% of the DNA. The resulting nuclear matrices consist primarily of proteins (90–99%) with residual amounts of DNA, RNA, phospholipid, and carbohydrate. The spermatid matrix is similar in composition to that of pachytene spermatocytes, except that proportionately less protein is removed from the spermatid nucleus during the isolation procedure (Table I).

Polypeptide Composition of Spermatogenic Nuclear Matrices

The matrix of spermatogenic cells, a mixture of spermatocytes and spermatids, consists of a complex array of polypep-



FIGURES 4 and 5 Thin section electron micrographs of nuclei and nuclear matrices from purified pachytene spermatocytes and spermatids (steps 1–8). Uranyl acetate and lead citrate stain. Fig 4: (*a*) Electron micrograph of pachytene spermatocyte nucleus. During fixation of the nuclei, the $MgCl_2$ concentration is decreased from 10 to 0.1 mM to disperse the highly condensed chromatin and to facilitate morphological examination of the internal nuclear structure. Intact SCs are observed coursing throughout the interior of the nucleus. Nuclear membranes have been removed by treatment with Triton X-100. A distinct lamina is not detectable (arrows). Bar, 0.5 μm. × 11,300. (*b*) Representative thin section electron micrograph of pachytene spermatocyte nuclear matrix. The interchromatin fiber network consists of a peripheral zone of small vesicles (*V*) surrounding an internal region of condensed fibrillar material (*F*). Bar, 0.5 μm. × 16,300. Fig. 5: (*a*) Electron micrograph of the nucleus of an early spermatid. The nucleus contains an increased amount of heterochromatin and several prominent nucleolarlike structures (*N*). Bar, 0.5 μm. × 16,100. (*b*) Electron micrograph of spermatid matrix. The matrix structure consists of a pore-fiber complex, a central nucleolarlike structure (*N*), and an internal network of interchromatin granules (*G*) and fibers (*F*). Bar, 0.5 μm. × 19,500.

tides ranging in M_r from 8,000 to ~150,000. These matrix constituents are distinct from total nuclear proteins, as evident from the recovery of different proteins in the successive nuclear and supernatant preparations (Fig. 13). The 0.2-M NaCl and 2-M NaCl treatments both remove nuclear sap and nonhistone chromosomal proteins of diverse M_rs, while most of the histones are extracted by 2 M NaCl. The nuclease digestions and final Tris-HCl wash primarily remove low-M_r proteins (M_r < 32,000). The successive nuclear pellets, from intact nuclei through to the final spermatogenic matrix, reveal an enrichment of high-M_r polypeptides (M_r > 44,000) and a concomitant decrease in histones and certain other polypeptides (Fig. 13).

Protein constituents of the spermatogenic matrix differ markedly from those found in somatic nuclear matrices (Fig. 14, *a* and *b*). By contrast with the liver matrix, which has the expected triplet polypeptides with M_rs of 61,000, 64,000, and

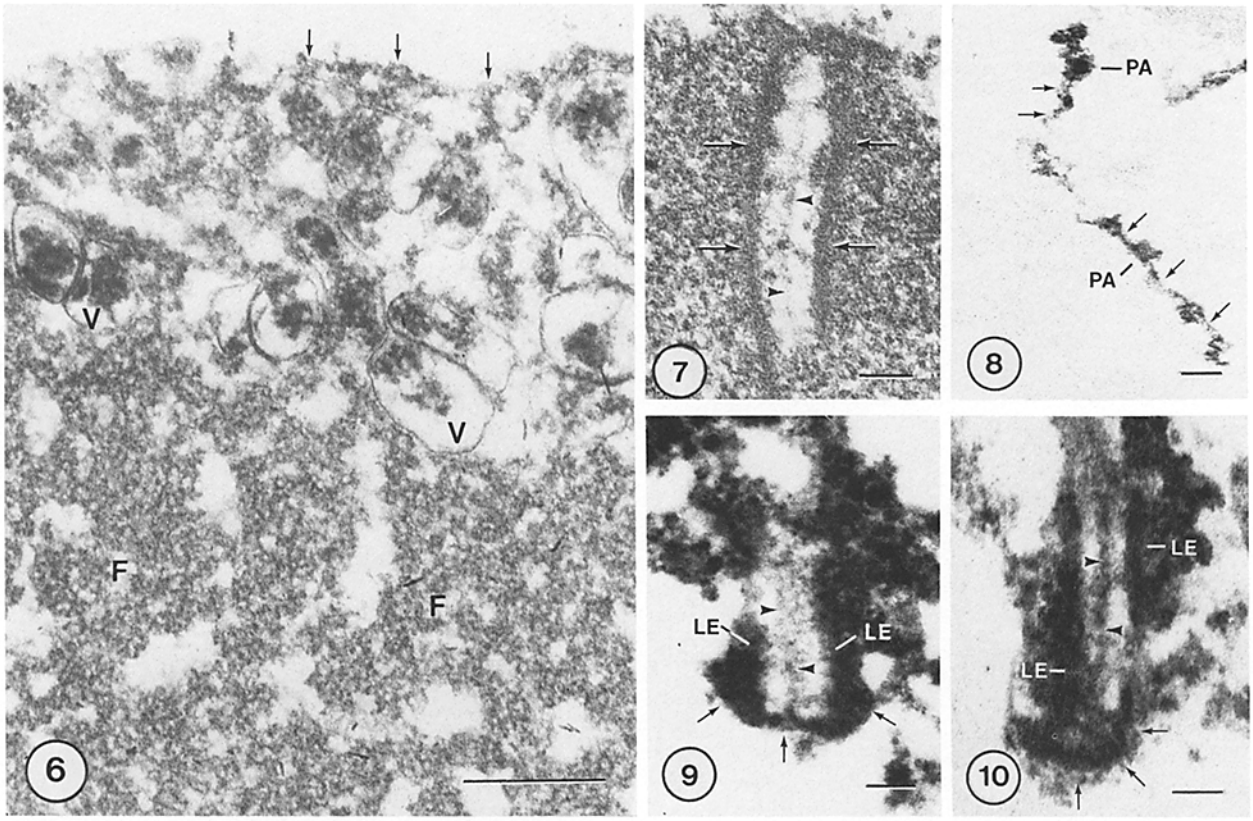


FIGURE 6–10 High magnification, thin section electron micrographs of pachytene spermatocyte nuclei and nuclear matrix components. Uranyl acetate and lead citrate stain. Fig. 6: The internal structure of the pachytene spermatocyte nuclear matrix. Dense interchromatin fibers (*F*) form the matrix interior. Vesicles (*V*) located in peripheral areas contain dense granules or flocculent material, and is surrounded by the pore-fiber complex (arrows). Bar, 0.5 μm. × 39,800. Fig. 7: Ultrastructure of the SC contained within the pachytene spermatocyte nucleus. The lateral elements (arrows) and central element (arrowheads) are similar to those of intact cells. The central region contains a few transverse microfilaments. Bar, 0.1 μm. × 75,000. Fig. 8: A segment of the pore-fiber complex. Residual pore annuli (*PA*) are interconnected by a network of thin fibers (arrows). Bar, 0.1 μm. × 56,000. Figs. 9 and 10: Ultrastructure of the SCs contained within the pachytene spermatocyte matrix. The two lateral elements (*LE*) and central element (arrowheads) are similar in morphology and dimension to those of isolated nuclei. The thickened ends of the lateral elements are associated closely with the attachment plaque (arrows). A few transverse microfilaments span the central region. Fig. 9: Bar, 0.1 μm. ×66,900. Fig. 10: Bar, 0.1 μm. × 70,000.

65,000, the spermatogenic matrix contains only one prominent protein (M_r ~ 63,000) and a minor component (M_r ~ 64,500) in this M_r range (Fig. 14*b*). Whether these two proteins are constituents of the pore-fiber complex remains to be determined. In addition, spermatogenic nuclear matrices contain prominent proteins having M_rs of 9,000, 20,500, 23,500, 27,000, 28,500, 30,000, 34,500, 36,000, 46,000, 52,500, 61,000, and 81,000. These results are not unique to procedure I, since this protocol, when used to isolate liver nuclear matrices, yields constituents identical to those obtained by the modified Berezney and Coffey protocol (Fig. 14, *a* and *b;* cf. reference 8: Fig. 5; and reference 9).

The unique polypeptide pattern of the spermatogenic nuclear matrix is highly reproducible, even when using different preparative methods and various protease inhibitors. The yield of spermatogenic matrix protein of 15% for procedure I is increased to 25% by using procedure II (1 M NaCl), which yields matrices with protein constituents identical to procedure I. The apparent complexity of the spermatogenic matrix is not due to limited proteolysis, since matrices isolated in the presence of protease inhibitors exhibit almost identical polypeptide profiles. Inhibitors used include: (*a*) no inhibitor; (*b*) 1 mM PMSF; (*c*) 1 mM PMSF and 100 μM Ep-475 (a thiol protease inhibitor, reference 38); (*d*) 1 mM PMSF and 100 μg soybean trypsin inhibitor (STI)/ml; and (*e*) 1 mM PMSF, 100 μM Ep-475, and 100 μg STI/ml. The inhibitors were added to all media used for the isolation of nuclei and nuclear matrices. Comparable polypeptide profiles were obtained after reduction and alkylation of matrix components with ethyleneimine, which blocks cysteinyl groups under reducing conditions.

The possible co-isolation of adventitiously bound proteins with the nuclear matrix was studied by incubating spermatogenic [^{35}S]methionine-labeled, cytoplasmic proteins (1,000 cpm/μg protein) with intact cells during hypotonic lysis. Comparing the specific activity of the nuclear matrix with the original cytoplasmic proteins shows that the latter comprises <9% of total matrix protein. This apparent binding of cytoplasmic proteins to the nuclear matrix may occur adventitiously. However, a major portion of this probably reflects a dynamic exchange of polypeptides between the cytoplasmic and nuclear compartments (64).

Polypeptide Composition of Pachytene Spermatocyte and Spermatid Nuclear Matrices

Components of the nuclear matrix may undergo marked transitions during meiotic prophase, the two meiotic reduction divisions, and spermiogenesis. In addition, proteins unique to

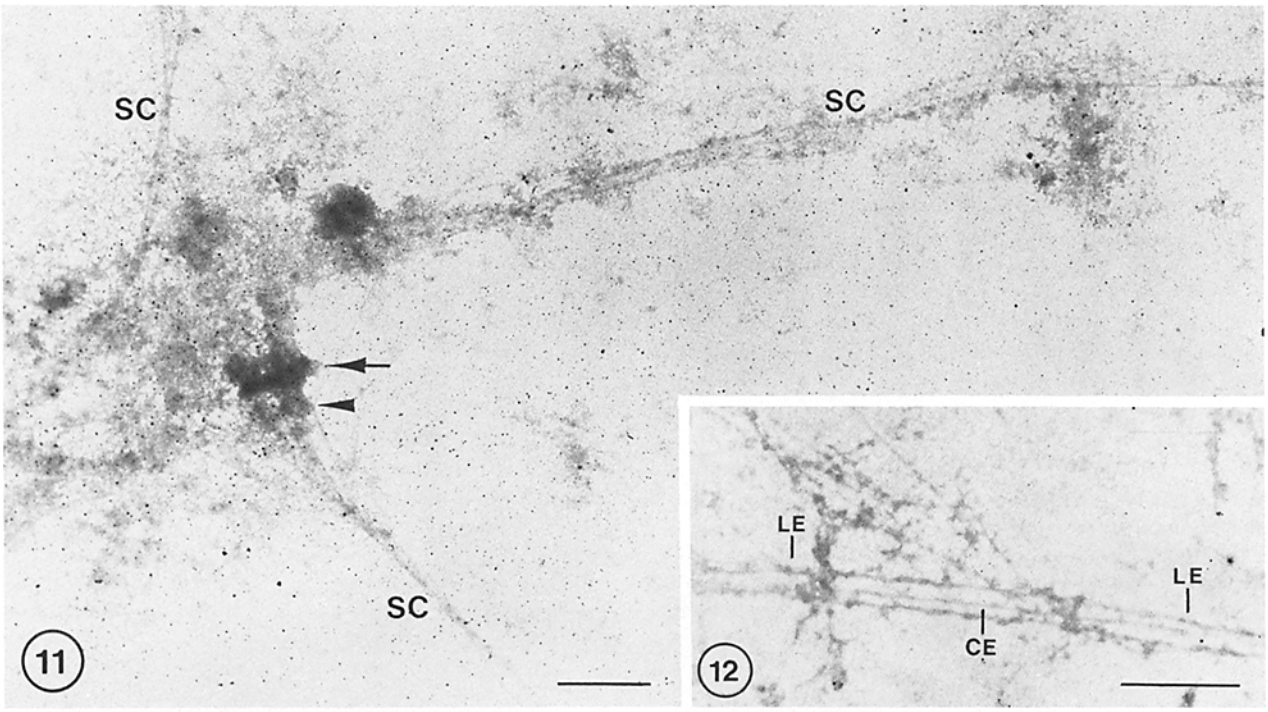


FIGURE 11 and 12 Whole mount electron micrograph of urea/SDS-treated nuclear matrices isolated from pachytene spermatocytes. Ethanolic phosphotungstic acid was used as the stain. Fig. 11: The telomeric region of three SCs remain associated at regions of fibrous material. The presumptive telomeric centromere region of the SC (arrowhead) appears as spherical differentiations near the termini of each lateral element. Arrows denote the broad attachment plaque. Bar, 1 μm. × 12,100. Fig. 12: Ultrastructure of individual SC components in the pachytene spermatocyte nuclear matrix. Two lateral elements (*LE*) flank a narrower central element (*CE*). In comparison with thin sectioned preparations, the lateral dimensions of SC components in urea/SDS-treated matrices are reduced by 65%. Bar, 0.5 μm. × 30,100.

TABLE I

Biochemical Composition of the Nucleus and Nuclear Matrix of Mouse Spermatogenic Cells *

	Nucleus	Nuclear matrix	Percent of nucleus§	Percent matrix composition
	pg			
Spermatogenic cells‡				
Protein	45.74 ± 2.55	8.58 ± 0.59	18.8	89.5
DNA	7.00 ± 0.94	0.44 ± 0.06	6.3	4.6
RNA	1.36 ± 0.09	0.10 ± 0.09	7.4	1.0
Carbohydrate	ND ± —	0.24 ± 0.03	—	2.5
Phospholipid	0.30 ± 0.03	0.23 ± 0.04	76.7	2.4
Pachytene Spermatocyte				
Protein	155.17 ± 17.67	22.87 ± 0.77	14.7	99.0
DNA	10.18 ± 0.55	0.12 ± 0.02	1.2	0.5
RNA	3.80 ± 0.21	0.11 ± 0.02	2.8	0.5
Spermatid (steps 1–8)				
Protein	46.37 ± 4.22	11.47 ± 0.66	24.7	98.9
DNA	4.15 ± 0.32	0.08 ± 0.01	1.9	0.7
RNA	1.00 ± 0.01	0.05 ± 0.01	5.0	0.4

* Data represent mean ± SE for three preparations, each performed in duplicate.
‡ Spermatogenic cells include pachytene spermatocytes, round spermatids (steps 1–8), and condensing spermatids (steps 9–16).
§ Percentage calculations assume total protein, DNA, and RNA (and for "Spermatogenic Cells", carbohydrate and phospholipid) to be equivalent to 100%. ND, not determined due to contaminating sucrose.

the SC may contribute to differences in the composition of the pachytene spermatocyte and spermatid (steps 1–8) matrices, particularly since SCs are absent from spermatids. However, although minor quantitative and qualitative differences exist, the polypeptide patterns observed for these two cell types are remarkably similar (Fig. 15). The spermatid matrix contains two distinct protein species with M_rs ~ 16,000 and 22,000. But, the majority of the pachynema matrix proteins are comparable with those present in early spermatids.

DISCUSSION

In the present study, procedures have been developed for preparing SCs as integral components of the nuclear matrix from purified mouse pachytene spermatocytes. Sequential extraction of pachynema nuclei yields SCs within residual proteinaceous structures resembling somatic nuclear matrices. These nuclear synaptonemal complex-matrices also contain a pore-fiber complex, residual nucleoli, and an interchromatin

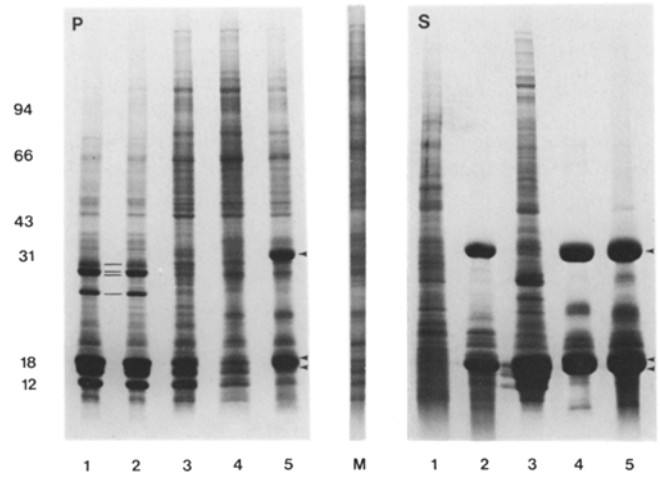


FIGURE 13 SDS PAGE of nonaminoethylated nuclear proteins recovered in the pellets and supernatants during the successive steps of procedure I for isolating spermatogenic matrices. The proteins from each pellet and supernatant fraction are prepared as described under Materials and Methods and resolved on a 7–18% SDS-polyacrylamide linear gradient gel, followed by staining with Coomassie Blue. Several prominent nuclear proteins (M_r ~26,500, 28,500, 29,000, and 30,000) are extracted during the matrix isolation procedure (bars). The position of DNase (DN-EP; M_r ~31,000) and RNase A (M_r ~12,600) are indicated by single and double arrowheads, respectively. The mobility of RNase is decreased anomalously during electrophoresis. Standard proteins used for molecular weight (M_r) calibration include: Phosphorylase a, 94,000; bovine serum albumin, 66,000; ovalbumin, 43,000; DNase I, 31,000; β-lactoglobulin, 18,300; and cytochrome c, 12,400 (left-hand numerals times 1,000). *P:* Nuclear proteins recovered at successive stages of matrix preparation. Total nuclear proteins (lane *1*) and nuclear proteins recovered after 0.2 M NaCl extraction (lane *2*), low DNase-RNase digestion (lane *3*), 2 M NaCl extraction (lane *4*), and high DNase-RNase digestion (lane *5*). *M:* Spermatogenic nuclear matrix proteins obtained using procedure I. *S:* Supernatant proteins recovered after treatment of nuclei with 0.2 M NaCl (lane *1*), low DNase-RNase (lane *2*), 2 M NaCl (lane *3*), high DNase-RNase (lane *4*), and Tris-HCl (lane *5*).

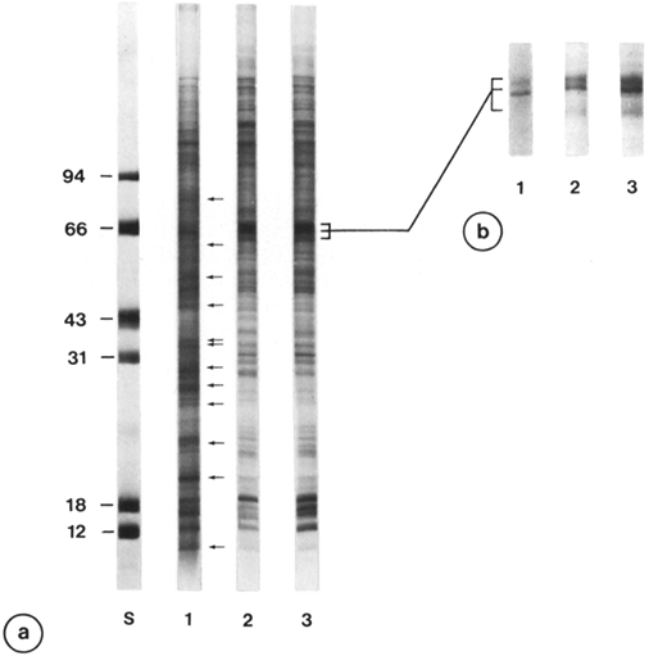


FIGURE 14 Comparison of nonaminoethylated polypeptides present in nuclear matrices of mouse liver cells and spermatogenic cells by SDS PAGE (for details, see legend to Fig. 13). (*a*) The spermatogenic matrix contains a complex array of polypeptides distinct from those of liver matrices (arrows). Prominent 60–70,000-M_r polypeptides (bracket; also *b*) of the liver matrices may be either absent or represent only a minor component of spermatogenic matrices. Lanes: (*S*) M_r standards (times 1,000); (*1*) spermatogenic nuclear matrices; (*2*) liver nuclear matrices prepared by procedure I for isolating spermatogenic matrices; (*3*) liver nuclear matrices prepared by a modification of the Berezney and Coffey procedure (9). (*b*) The 60–70,000-M_r components of spermatogenic and liver matrices are analyzed by high resolution PAGE. Matrix proteins are resolved as in *a*, except that electrophoresis is continued for an additional 1.5 h. The liver matrices contain three polypeptides with ~M_rs of 61,000, 64,000, and 65,000, comparable with other somatic matrices. The spermatogenic matrix contains one prominent protein in this molecular weight range (M_r ~ 63,000), and a minor component (M_r ~ 64,500). Lanes *1*, *2*, and *3* correspond with those in *a*.

fiber network. Significantly, the pachytene spermatocyte matrix includes residual XY heterochromatin and SCs still associated with the pore-fiber complex. The integral character of the SCs with the nuclear matrix is clearly evidenced by the structure remaining intact when exposed to strong dissociating conditions (3 M urea/0.1% SDS). Even in the absence of surrounding chromatin, the SCs remain continuous along their length and are comparable morphologically with SCs in surface-spread spermatocytes (22, 56). Examination of thin section preparations further demonstrates that the SCs, consisting of the central and two lateral elements, are preserved during the extraction protocol with minimal structural damage. However, the transverse microfilaments of the SCs are observed infrequently in SCs of pachynema nuclei and their matrices, confirming previous observations of the labile nature of these structures (56, 70). Further analysis of the molecular structure and composition of the pachytene spermatocyte matrix is necessary to understand the structural and functional relationship between the SCs and other matrix components.

The procedures used for isolating nuclear matrices from spermatogenic cells have been validated with biochemical analyses. First, the present technique yields spermatocyte and spermatid nuclear matrices in high purities (>88%) and in acceptable quantities (~25% yield from 10^8 cells). Second, comparable with somatic nuclear matrices, the germ cell matrix consists of only 15–20% of total nuclear protein, <5% of the RNA, and <2% of the DNA. Third, the complex polypeptide pattern of the spermatogenic matrices is highly reproducible, even when isolated using different ionic conditions and various protease inhibitors. Finally, the adventitious binding of cytoplasmic proteins to the nuclear matrix during isolation is minimal (<9%). These observations suggest that the unique morphology and biochemical composition of the pachytene spermatocyte and round spermatid matrices represent inherent features of nuclei in differentiating spermatogenic cells.

An understanding of the role of the nuclear matrix in mediating various nuclear functions has been based primarily on studies of somatic cells (2, 7, 68). The present study now provides definitive evidence for the presence of a nuclear matrix within differentiating male germ cells. However, these spermatogenic nuclear matrices do not appear to contain the three lamina proteins (M_r ~ 60–70,000) that usually comprise ~40% of the total matrix protein in most somatic cells (1, 6, 9, 20, 43), including those of mouse liver. The absence of a distinct lamina in spermatocytes corroborates the present morphological evidence with an earlier report by Fawcett (28). Moreover, in a recent immunofluorescent study, an antibody

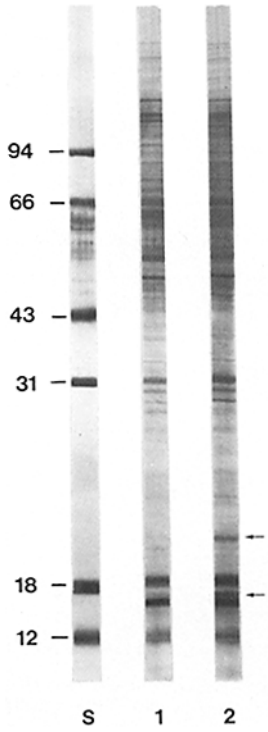


FIGURE 15 Comparison of the polypeptides comprising nuclear matrices isolated from purified populations of pachytene spermatocytes and spermatids (steps 1–8). Matrix samples prepared by procedure II are aminoethylated, resolved on a 7–18% linear gradient SDS polyacrylamide gel and then stained with silver, which increases protein sensitivity 10–15-fold. The pachytene and round spermatid matrices contain similar polypeptides. Those protein bands unique to the spermatid matrix are denoted by arrows. Lanes (*S*) M_r standards (times 1,000); (*1*) pachytene spermatocyte matrices; and (*2*) spermatid (steps 1–8) matrices.

directed against lamins of chick erythrocytes did not bind to nuclei of either chick or mouse spermatocytes (73). On this basis, it seems unlikely that the prominent 63,000-M_r and the minor 65,500-M_r polypeptides are constituents of any lamina structure. Similarly, the nuclear matrix of *Drosophila* embryos and the pore-fiber complex of *Xenopus* oocytes do not contain the prominent triplet polypeptides of M_rs ~ 60–70,000 (30, 43).

The peculiar composition of the germ cell nuclear matrix may be due to the transformation of mitotically dividing spermatogonia into spermatocytes undergoing meiosis. In somatic cells, the pore-lamina complex is disassembled during mitotic prophase, and the depolymerized lamina proteins are retained within the cytoplasm. After mitosis, these proteins are reassembled to form the pore lamina-complex of the two daughter cells (21, 35, 36, 39, 41). There is evidence to suggest that this lamina recycling process is altered when the germ cell enters meiosis (73). Dissolution of the lamina also must occur at some time during the 12.5-d long meiotic prophase, paralleling the comparable event in mitosis. The absence of a distinct lamina and prominent 60–70,000-M_r polypeptides in the pachynema matrix provides evidence for this gradual disassembly process, which may facilitate movement of the homologous chromosomes during synapsis and disjunction (29, 73). The continued absence of a typical lamina structure and its polypeptides in the spermatid matrix, however, suggests that a reassembly of the depolymerized lamina proteins does not occur after meiosis. The nuclear matrix of the postmeiotic germ cell does not contain a typical lamina, although nuclear pores are retained and progressively concentrated in the postacrosomal region of the spermatid nucleus (14, 29). Instead, the matrix structure may be stabilized by novel polypeptides specific to meiosis and spermiogenesis.

Morphological evidence for the occurrence of synaptonemal complexes in isolated spermatocyte nuclear matrices is compelling. Other structures present in the pachynema matrix include the residual XY heterochromatin, a prominent nucleolus, and peripheral vesicles. These components do not exist in the spermatid nuclear matrix. Yet, the protein constituents of these two germ cell nuclear matrices are remarkably similar. Only the spermatid matrix contains two major distinct polypeptides (M_rs ~16,000 and 22,000). There are several reasonable explanations for these observations. It is plausible that the morphological transformation occurring between meiosis and spermiogenesis may simply reflect a recycling process or a novel structural reorganization of existing nuclear matrix proteins (21, 35, 36, 39, 41). Alternatively, assembly of these organelles may be promoted by only a limited number of nuclear constituents, too few to be detected by the techniques used. It also should be noted that nuclear matrix proteins do not undergo major changes during differentiation of Friend erythroleukemia cells and sea urchin embryogenesis (47, 65).

Studies of germ cell nuclear matrices at other stages of spermatogenesis will permit a more detailed analysis of matrix transformation during this highly complex process of differentiation. It also will provide unique opportunities to study the temporal synthesis of the germ cell nuclear matrix during meiotic prophase. In addition to a structural transformation of the matrix, changes may occur in matrix-associated processes such as the initiation of DNA synthesis during mitotic proliferation, premeiotic replication, and pachynema DNA scission-repair, and the synthesis and processing of RNA during meiotic prophase (2, 16, 40, 50, 51, 52, 62). Finally, the application of immunological probes and further fractionation of the matrix will facilitate the identification and analysis of the SC constituents.

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