Stage-dependent Changes in Localization of a Germ Cell-specific Lamin during Mammalian Spermatogenesis*

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We had earlier identified a 110/120-kDa protein specific to nuclear matrix of rat pachytene spermatocytes (Behal, A., Prakash, K., and Rao, M. R. S. (1987) J. Biol. Chem. 262, 10898–10902). This protein is now shown to be a disulfide-linked homodimer of a 60-kDa polypeptide. Indirect immunofluorescence and Western blot analyses using anti-120-kDa polyclonal antibodies have shown that this protein is a component of the pore-complex lamina structure of spermatogonia. As germ cells enter meiotic prophase and the lamina structure disassembles, this polypeptide is redistributed in the nucleus and can be isolated as a component of synaptonemal complexes. Following meiotic division, this 60-kDa protein is relocalized in the lamina, then representing the sole major component of the lamina structure of round spermatids. The identity of the 60-kDa protein in the pore-complex lamina fraction and synaptonemal complexes was further confirmed by two-dimensional analysis of iodinated tryptic peptides. Such an analysis has also shown that the germ cell-specific 60-kDa protein is related but not identical to somatic lamin B.

The nuclear lamina is a proteinaceous meshwork which lies subjacent to the inner nuclear membrane (1, 2). It not only serves as a skeletal framework for the nuclear envelope but also provides attachment sites to the interphase chromatin (3, 4). The nuclear lamina is generally comprised of three major polypeptides called lamins, which in higher vertebrates are termed lamins A, B, and C and L_I, L_{II}, and L_{III} in amphibians. This classification is based on the difference in structural and biochemical properties of the lamins. On comparison of lamins from various amphibian cells, it has been observed that there are cell type-specific differences in their expression (5, 6). The somatic cells of Xenopus laevis contain two lamin polypeptides L_I and L_{II}, whereas in oocytes only lamin L_{III} is expressed. Lamin L_{III} is also expressed in other highly differentiated cells such as neurons, myocytes, and Sertoli cells along with L_I and L_{II}. However, the expression of a unique lamin L_{IV} seems to be restricted to the later stages of spermatogenesis (7). Cell type and developmentally controlled variations in lamin expression are also observed in higher vertebrates (8, 9). Since the lamina structure provides

sites for anchoring the chromatin, it is possible that these variations may orient the organization of chromatin in a stage-specific manner during development.

The nuclear lamina was shown to be a dynamic structure in that it disassembles prior to the onset of mitotic (10, 11) and meiotic (12, 13) divisions. This is thought to facilitate the process of chromosome condensation which occurs in the subsequent stages of cell divisions. During oogenesis, the lamina structure is reformed at diplotene stage (13), whereas during spermatogenesis of amphibians, it does not reappear until the meiotic division is completed (12). Very little is known about lamin expression during mammalian gametogenesis.

One interesting feature during the meiotic cell division is the coincidence of depolymerization of the lamina structure with appearance of the synaptonemal complexes (SCs).[1] This has led to the hypothesis that a part of lamina, on disassembly, may comprise a component of SCs. Both the lamina and SCs are resistant to nuclease digestion and are insoluble in nonionic detergents and high salt. Although this similarity provides a logical support to the above concept, to date there is no biochemical evidence to this effect.

We had identified previously, a 120-kDa[2] protein specific to the pachytene nuclear matrix of rat testis and absent in many somatic tissues (14). Indirect immunofluorescence studies with polyclonal antibodies raised against this protein had shown predominant localization in the pachytene nucleus with very little fluorescence in spermatogonia and round spermatids. The polyclonal antibodies also recognized antigens in rat oocytes and pachytene nuclei of mouse and monkey testis. Based on these observations, we had speculated that this protein could play an important role in meiosis, particularly in chromosome pairing and genetic recombination.

We have now carried out a more detailed study on the localization of this antigen in premeiotic, meiotic, and postmeiotic germ cells and show that this protein is actually a homodimer of a 60-kDa polypeptide. We find this polypeptide to be a germ cell-specific lamin during the spermatogonial stage which can be isolated as a component of SCs of pachytene spermatocytes. Furthermore, we report the existence of a lamina structure in mammalian round spermatids having this 60-kDa polypeptide as the only major component.

MATERIALS AND METHODS

Animals—Male Wistar rats were used for all the studies. Testes from 35- to 40-day-old rats were used for the isolation and analysis of pachytene stage nuclei while adult rats (60-day old) were used to obtain a complete representation of all the stages of spermatogenic

[1] The abbreviations used are: SCs, synaptonemal complexes; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DTT, dithiothreitol.

[2] We had earlier estimated the molecular weight of this protein as 110,000 (14). A more accurate molecular mass is 120 kDa.

cells. Analysis of the spermatogonial nuclei was done from the testes of 10-day-old rats.

Isolation of Nuclei—Total testicular nuclei were isolated from decapsulated testes as described (15). Pachytene spermatocyte nuclei were purified from total nuclear preparation by employing the "STA-PUT" technique (15, 16). Round spermatids and pachytene spermatocytes were isolated from the testes of 40-day-old rats by the centrifugal elutriation technique (16) with minor modifications. Briefly, testes from three rats were teased gently in 30 ml of phosphate-buffered saline containing 0.1% glucose and 400 μg/ml of collagenase (Sigma, Type IV). After incubation at 34 °C for 30 min, the suspension was filtered through two layers of cheesecloth. The single cell suspension was centrifuged at 1,000 × g for 10 min. The cell pellet was washed once with and subsequently suspended in 25 ml of elutriation buffer (10 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.9% NaCl, 0.1% glucose, and 0.2% bovine serum albumin). Centrifugal elutriation was carried out using Beckman JE-6 rotor in the J2-21M centrifuge. Round spermatids and pachytene spermatocytes were collected and checked for purity in a Nikon Diaphot TMD inverted microscope. Nuclei from these cells were prepared by suspending them in 0.34 M sucrose, 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, and centrifugation through a cushion of 1 M sucrose in the same buffer at 15,000 × g for 10 min.

Preparation of Nuclear Matrix, Nuclear Lamina, and SCs—Nuclear matrices were prepared according to the method of Behal *et al.* (14). Nuclear lamina of spermatogonia (10-day-old rats) and round spermatid nuclei were prepared according to the procedure described by Dwyer and Blobel (17). SCs from pachytene spermatocytes were isolated by employing the procedure of Heyting *et al.* (18).

Indirect Immunofluorescence—Indirect immunofluorescence assays of nuclei with anti-120-kDa polyclonal antibodies were done as described (14).

One- and Two-dimensional PAGE Analysis, Western Blotting, and Peptide Mapping—The nuclear matrix preparation was solubilized in a buffer containing either 100 mM or 25 mM DTT and analyzed by SDS-PAGE as described (14, 19). Protein concentration of the samples prepared was determined by turbidometric method (20). Two-dimensional electrophoretic analysis (non-equilibrium pH gradient electrophoresis/SDS-PAGE) was done as described by O'Farrel (21). For Western blotting studies, the proteins were electrophoretically transferred onto nitrocellulose membranes (Schleicher & Schuell) and after reacting with anti-120-kDa antibodies, the filters were probed with goat anti-rabbit IgG peroxidase conjugate (22). The polypeptides of interest were cut out from the polyacrylamide gel and were radioiodinated with ^{125}I *in situ*, digested with L-1-tosyl-amido-2-phenylethyl chloromethyl ketone-treated trypsin (Worthington), and analyzed on cellulose-coated thin layer chromatography plates (E. Merck) according to the method of Elder *et al.* (23).

Electron Microscopy—The samples were fixed for 15 min at 0 °C with 4% paraformaldehyde, made fresh in 10 mM sodium cacodylate buffer, pH 7.3. A drop of the suspension was placed on Formvar-coated grids, drained and stained with ethanolic phosphotungstic acid, and examined under Philips EM 300 Å electron microscope at 60 KV. Isolated pachytene nuclei were processed for immunoelectron microscopy as described (3). Briefly, pachytene nuclei were treated with anti-120-kDa antibodies in SUCNM buffer (0.25 M sucrose, 0.05 M sodium cacodylate, pH 7.4, 0.1 M NaCl, and 0.005 M $MgCl_2$) for 30 min at room temperature, washed twice with SUCNM, and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG for another 30 min at room temperature. The washing was repeated, and the nuclei were then fixed for 15 min at 0 °C in 2.5% gluteraldehyde in SUCNM buffer and rinsed in 0.05 M Tris-HCl, pH 7.6. The diaminobenzidine reaction was carried out for 5 min at room temperature, and the nuclei were then washed, post-fixed in 1% OsO_4,
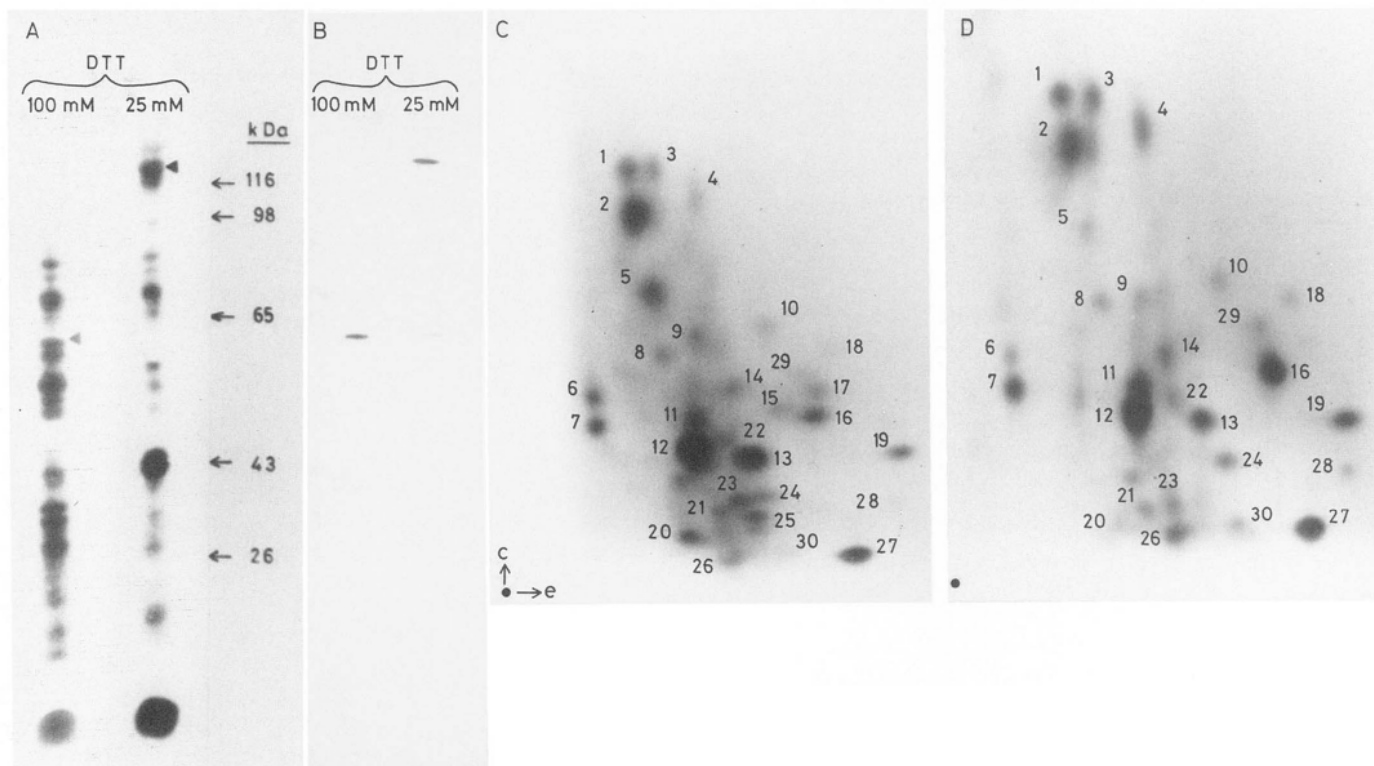
FIG. 1. **Pachytene nuclear matrix specific 120-kDa protein is a homodimer of a 60-kDa polypeptide.** Nuclear matrices were prepared from rat pachytene spermatocytes. They were solubilized in buffer containing either 25 or 100 mM DTT and were analyzed by SDS 5–15% gradient PAGE. Coomassie Blue-stained pattern (*A*) and Western blot analysis (*B*) with anti-120-kDa antibodies. The extent of dissociation of 120-kDa polypeptide into 60-kDa monomer varied with different preparations. The Coomassie Blue-stained and the Western blot patterns are from two independent matrix preparations. The two *arrowheads* indicate the bands corresponding to 120- and 60-kDa polypeptides reacting with antibodies. 70 μg (25 mM DTT) and 100 μg (100 mM DTT) of solubilized protein were loaded on the gel. *C* and *D* represent the two-dimensional analysis of iodinated tryptic peptides. The 120-kDa (*C*) and 60-kDa (*D*) bands were iodinated with ^{125}I *in situ*, digested wtih L-1-tosyl-amido-2-phenylethyl chloromethyl ketone-treated trypsin, and the released ^{125}I-labeled peptides were subjected to electrophoresis (*e*) in the first dimension and chromatography (*c*) in the second dimension (20).

FIG. 2. **60-kDa polypeptide is a component of lamina structure of spermatogonia.** Total testicular nuclei from 20-day-old rats were treated with anti-120-kDa antibodies and subsequently with fluorescein isothiocyanate-labeled anti-rabbit goat IgG as described earlier (14). *A* and *B*, phase contrast micrographs; *C* and *D*, immunofluorescence pattern. × 400. *P*, pachytene; *Sg*, spermatogonia. *E* and *F* represent SDS-PAGE and Western blot patterns, respectively, of proteins solubilized from the pore-complex lamina fractions isolated from liver (*L*) and testes of 10-day-old rats (enriched with spermatogonia). *Lane M* represents the molecular weight markers. *G* represents the two-dimensional analysis of radioiodinated tryptic peptides of the 60-kDa polypeptide band reacting with anti-120-kDa antibodies in the *Sg lane*.
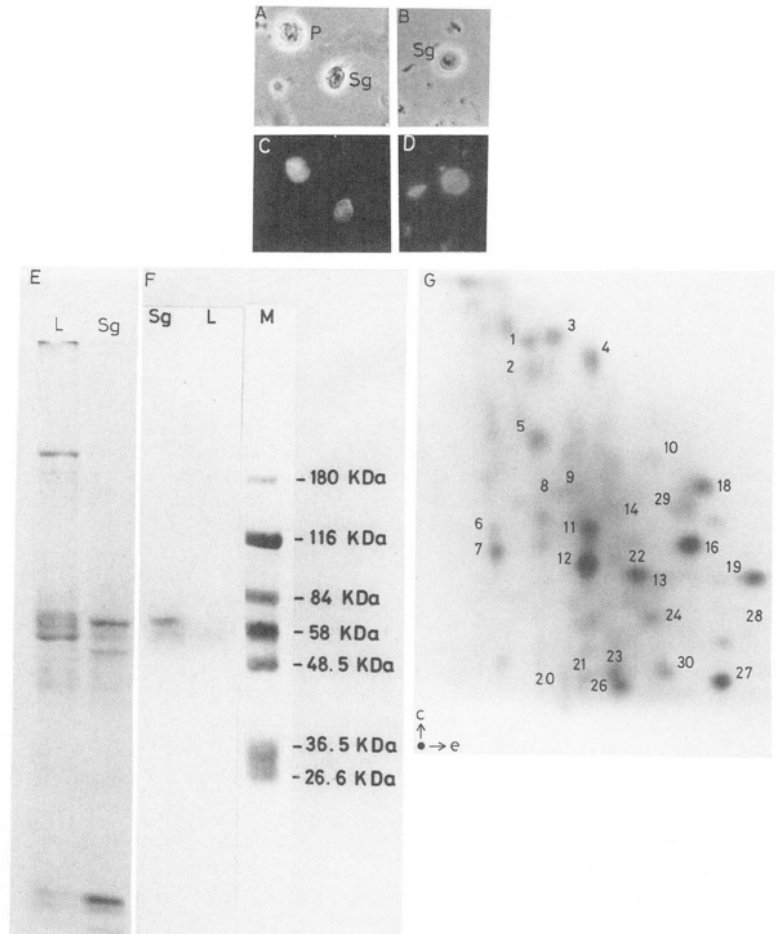
dehydrated in propylene oxide, embedded, and thin sections (silver) were taken for observation.

RESULTS

120-kDa Protein Is a Homodimer of 60-kDa Protein—We had shown earlier the presence of a polypeptide around 120 kDa, specific to the nuclear matrix of rat pachytene spermatocytes (14). We have now isolated and solubilized nuclear matrix from pachytene nuclei of rat testes, using higher (100 mM) and lower (25 mM) concentrations of DTT. Fig. 1A shows the SDS-PAGE pattern of such preparations. At 100 mM DTT, many of the higher molecular weight polypeptides disappeared with a concomitant increase in the number as well as the intensity of lower molecular weight polypeptides. It can be seen that the 120,000 molecular weight protein, which is quite prominent at lower (25 mM) DTT concentration, has almost disappeared at higher (100 mM) DTT concentration. At the same time, a protein around 60,000 molecular weight makes its appearance at 100 mM DTT concentration. The Western blot analysis (Fig. 1B) of the polypeptides, using anti-120-kDa antibodies, showed strong reactions at 120-kDa region (25 mM DTT) and at 60-kDa region (100 mM DTT). This suggests that the 120-kDa protein may be a homodimer of the 60-kDa polypeptide, arising out of disulfide linkages. Further evidence for the identity of these two proteins was obtained from two-dimensional tryptic peptide analysis of ^{125}I-labeled polypeptides (Fig. 1, *C* and *D*). It is clear from the figure that the majority of the tryptic peptides derived from the 60- and 120-kDa polypeptides are very similar. The variation in the intensities of some of the spots between the two polypeptides may be due to the differential

accessibility of the protein inside the gel to the reaction components. Theoretically one would also expect some additional spots in the map of 120-kDa polypeptide due to the presence of disulfide linkages in the dimer form, and the spots numbered *15*, *17*, and *25* might represent such disulfide-linked peptides.
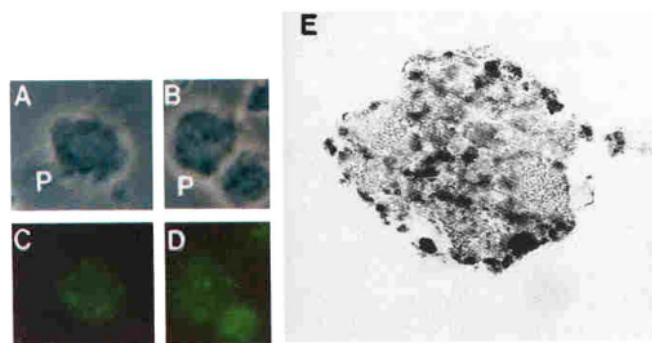
60-kDa Polypeptide Is a Component of Pore-complex Lamina Fraction of Spermatogonia—In our earlier studies, the immunofluorescence of spermatogonial and round spermatid nuclei was very minimal as compared with that of pachytene nuclei (14). A more careful examination showed that the fluorescence in the spermatogonial nuclei is localized around the periphery of the nucleus (Fig. 2, *A–D*). This kind of fluorescence is reminiscent of somatic nuclei stained with anti-lamin antibodies (8, 24, 25). To confirm the presence of the 60-kDa antigen in the lamina structure of spermatogonial nuclei, we isolated the pore-complex lamina fraction from testicular nuclei of 10-day-old rats (age at which majority of the spermatogenic cells are spermatogonia with a few preleptotene spermatocytes), according to the method of Dwyer and Blobel (17). The Coomassie Blue-stained pattern of the polypeptides separated by SDS-PAGE and the corresponding Western blot pattern are shown in Fig. 2, *E* and *F*. The Western blot pattern showed reaction only with a 60-kDa polypeptide of the spermatogonia. The tryptic pattern of this polypeptide shown in Fig. 2G reveals that majority of the spots are similar to those of the 60-kDa polypeptide present in the nuclear matrix of pachytene spermatocytes. The pattern also showed a few additional spots which were absent in that of the 60-kDa polypeptide of pachytene nuclear matrix.

It is very likely that the band corresponding to the 60-kDa polypeptide is cross-contaminated with somatic lamin(s) (see "Discussion") of the same mobility. These results, therefore, confirm the identity of the 60-kDa polypeptide and show that it is a part of the lamina structure of spermatogonial nucleus. The distribution of fluorescence pattern all over the pachytene nucleus suggests that when the spermatogonial cells enter meiotic prophase, following the disassembly of the lamina structure, the 60-kDa polypeptide is redistributed in the nucleus.
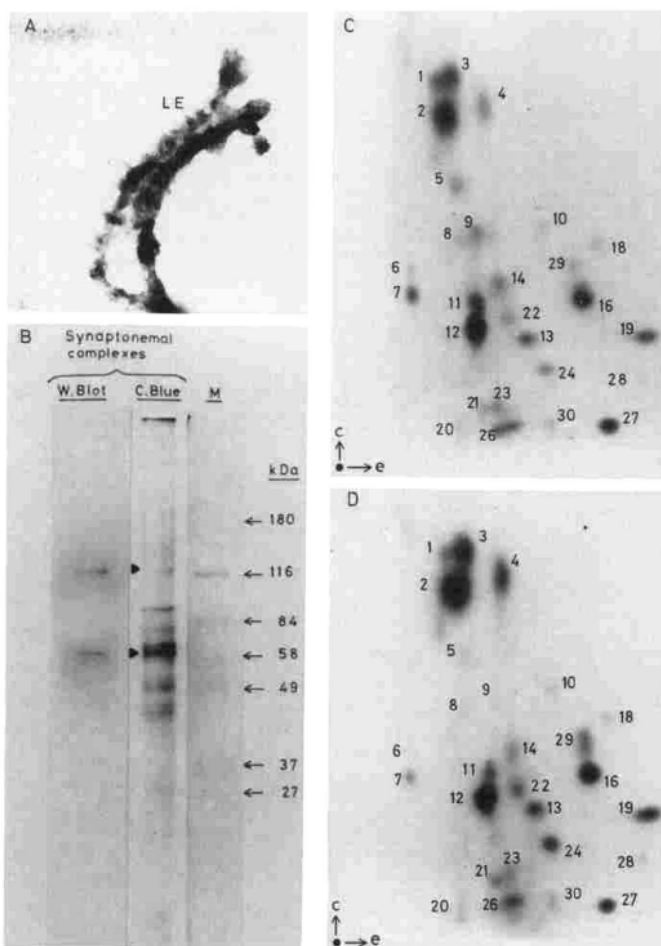
Germ Cell-specific 60-kDa Polypeptide Is a Component of Isolated SCs—The above data prompted us to ask whether the 60-kDa polypeptide, on redistribution, has any specific localization in the nuclei of primary spermatocytes. We have repeated our earlier immunofluorescence studies (14) with 100 µg of polyclonal antibodies (IgG) and the fluorescence patterns of pachytene nuclei are shown in Fig. 3 (*A–D*). With this reduced primary antibody concentration, it can be seen that there are some areas of relatively brighter fluorescence, which suggest that there may be specific sites of localization for this protein in the nuclei of primary spermatocytes. Our preliminary immunoelectron microscopic data shown in Fig. 3*E* also confirms such a distribution of the antigen in the pachytene nucleus.

SCs were originally isolated as part of the pachytene nuclear matrix (14, 26). We have employed here the method developed recently by Heyting *et al.* (18) to isolate the SCs free of other nuclear contaminants. Electron microscopic observation of this SC fraction confirmed the identity of the preparation (Fig. 4*A*). The SDS-PAGE pattern of the SC-associated polypeptides is shown in Fig. 4*B*. The polypeptide pattern we have obtained here is very similar to that reported by Heyting *et al.* (18), being enriched in proteins of 50,000–70,000 molecular weight range. The Western blot pattern of these polypeptides, using anti-120-kDa antibodies, showed reaction both at 60- and 120-kDa regions (Fig. 4*B*). When the corresponding polypeptides were subjected to tryptic peptide map analysis, the patterns obtained (Fig. 4, *C* and *D*) were similar to each other and were characteristic of the 60-kDa polypeptide pattern described above.

Germ Cell-specific 60-kDa Polypeptide Is a Major Component of Lamina of Round Spermatids—Since the SCs disappear following the meiotic division, we were curious to study the fate of the 60-kDa antigen after meiosis. The immunofluorescence pattern of nuclei isolated from adult rat testes,
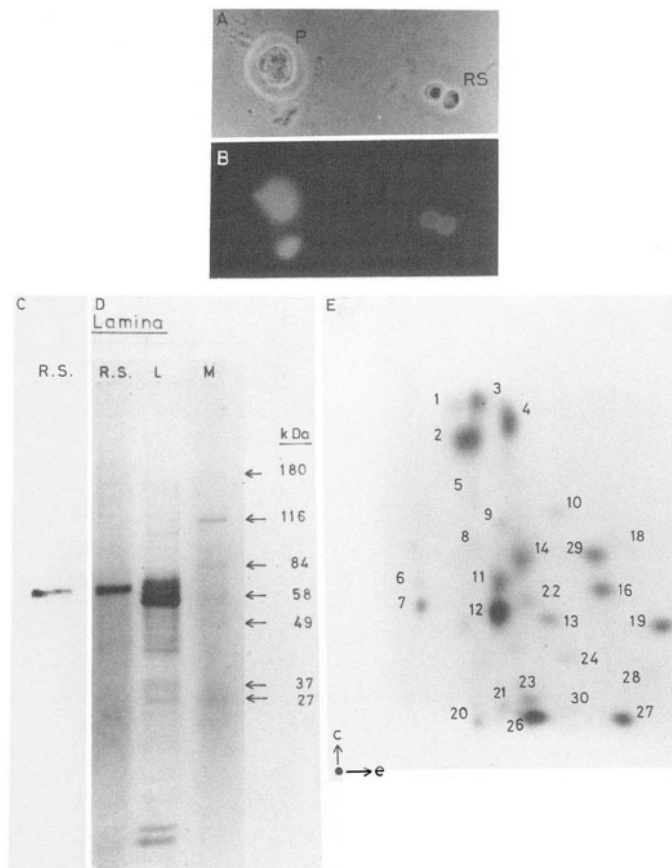

Fig. 4. **60-kDa polypeptide is a component of SCs isolated from rat pachytene spermatocytes.** SCs were isolated from purified rat pachytene spermatocytes as described (18). *A*, represents an electron micrograph of the preparation showing the characteristic features of the SCs. *LE*, lateral element. × 56,000. The proteins associated with the SCs were solubilized and analyzed on a polyacrylamide gel. *B*, the polypeptides were visualised by Coomassie Blue staining and analyzed by Western blot analysis with anti-120-kDa antibodies. The *arrowheads* indicate the polypeptides reacting with the antibodies. *C* and *D* represent the two-dimensional tryptic peptide analysis of the 60- and 120-kDa polypeptides, respectively, reacting with the antibodies in the Western blot.

Fig. 3. **Indirect immunofluorescence pattern of pachytene spermatocyte nuclei with anti-120-kDa antibodies.** × 400. *A* and *B* represent phase contrast micrographs; *C* and *D* represent the immunofluorescence pattern. *P*, pachytene. The electron micrograph in *E* represents a pachytene nucleus-treated sequentially with anti-120-kDa antibodies, horseradish peroxidase-conjugated goat anti-rabbit IgG, and subsequent reaction with diaminobenzidine and H_2O_2. × 1400.

using anti-120-kDa antibodies revealed that the round spermatids were decorated only on their periphery (Fig. 5, *A* and *B*). This suggested a relocalization of the 60-kDa polypeptide into the lamina structure of the haploid gametes following meiosis. To confirm this, the pore-complex lamina fraction was isolated from purified round spermatids, and associated polypeptides were analyzed by SDS-PAGE. Fig. 5*D* shows that only one major band of 60 kDa was present in the lamin region of the round spermatids, which reacted with the anti-120-kDa antibodies (Fig. 5*C*). This polypeptide also had a very similar tryptic peptide map (Fig. 5*E*) as that of the 60-kDa polypeptide from pachytene nuclear matrix. The round spermatids from chicken *Xenopus* and mouse have been shown previously to lack a lamina structure (12). On the contrary, the above results show that mammalian round spermatids do have a lamina structure, comprised solely of the germ cell-specific 60-kDa lamin.

Relationship between the Germ Cell-specific 60-kDa Polypeptide and the Somatic Lamins—One logical question that arises at this stage is the relationship of the 60-kDa polypeptide with somatic lamins. To answer this, we excised the
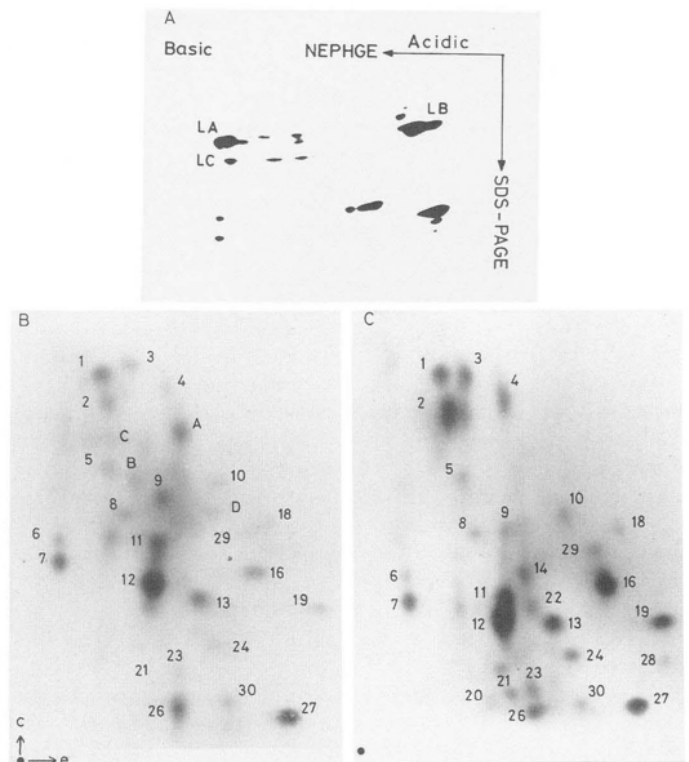
FIG. 5. **60-kDa polypeptide is a component of pore-complex lamina structure of round spermatids.** Total testicular nuclei from adult rats were processed for indirect immunofluorescence as described in Fig. 1. *A*, phase contrast micrograph; *B*, immunofluorescence pattern. × 400. *RS*, round spermatids; *P*, pachytene. *C* represents the Western blot pattern of the round spermatid lamina fraction, *R.S. D*, Coomassie Blue-stained patterns of the proteins associated with pore-complex lamina fraction, isolated from purified round spermatids and rat liver, separated by SDS-PAGE. *E* represents the two-dimensional tryptic peptide map analysis of the polypeptide of round spermatid lane reacting with the antibodies.

FIG. 6. *A*, two-dimensional gel-non-equilibrium pH gradient electrophoresis pattern (*NEPHGE/SDS-PAGE*) of liver lamins. *LA*, lamin A; *LB*, lamin B; *LC*, lamin C. *B* and *C* represent two-dimensional tryptic peptide analysis of somatic lamin B (*B*), and 60-kDa polypeptide associated with the pachytene nuclear matrix (*C*). The peptides *A–D* are specific to lamin B which are absent in the 60-kDa polypeptide.

Coomassie Blue-stained bands of liver lamins separated on a two-dimensional polyacrylamide gel (Fig. 6*A*) and subjected them to radioiodination and tryptic peptide map analysis. Among the somatic lamins, only lamin B yielded radioiodinated peptides similar to those of the germ cell-specific 60-kDa polypeptide (Fig. 6, *B* and *C*; peptide maps of lamins *A* and *C* are not shown). It can be seen from the figure that, although the germ cell-specific 60-kDa polypeptide shares many of the spots with lamin B, the latter has some additional major spots (*spots A–D*) which are absent in the map of 60-kDa polypeptide. Similarly, the germ cell-specific polypeptide has some spots (*spots 14, 20, 28*) that are absent in somatic lamin B. We have also carried out two-dimensional analysis of a mixture of lamin B and 60-kDa polypeptide-derived radioiodinated peptides and found that most of the common spots comigrate (data not shown). This homology strongly supports our contention that the 60-kDa polypeptide is a lamina protein and moreover, raises the possibility of it being a germ cell-specific variant of lamin B.

DISCUSSION

The present investigation was aimed at further characterization of the germ cell-specific nuclear matrix protein, identified by us earlier and shown to be conserved in mammals

(14). The results presented here show that the 120-kDa polypeptide is actually a homodimer of a 60-kDa germ cell-specific lamin. The strong tendency of this protein to remain as a dimer even at very high concentrations of DTT was observed only in the pachytene stage. This cannot be explained solely by intermolecular disulfide linkages. Probably some stage-specific modifications of the protein like phosphorylation might be responsible for such an interaction as in the case of somatic lamins, where such homotypic associations have been demonstrated (27). However, it cannot be ascertained at this stage whether the dimerization of this polypeptide is an artifact of isolation or represents a functional structure *in vivo*.

Our conclusion that this polypeptide is a germ cell-specific lamin is based on the following evidence. 1) The fluorescence is observed only at the nuclear cortex of spermatogonia and round spermatids. 2) The 60-kDa polypeptide is a component of the pore-complex lamina fraction isolated from spermatogonia-enriched cell population and round spermatids. 3) Western blot analysis with anti-120-kDa antibodies showed a strong reaction with spermatogonial and round spermatid lamina fraction, while they had very little affinity toward liver lamins. 4) The iodinated tryptic map of the 60-kDa polypeptide was similar to that of lamin B. In addition, this germ cell-specific protein has a pI of 5.6 (14), which is close to that of lamin B. The observation that, despite the similarity, the antibodies did not react with the somatic lamins in the Western blot analysis is not surprising, since the anti-somatic lamin antibodies also have been shown not to react with meiotic and post-meiotic germ cells of rat (12). We would like to emphasize that the method of tryptic peptide analysis employed detects only radioiodinated peptides and does not

represent all the tryptic peptides. Radioiodination occurs mostly at tyrosine, histidine, and phenylalanine residues (23), and hence some of the peptides would not be represented in the tryptic map. The fact that some of the lamin B-specific spots are also observed in the tryptic map of 60-kDa polypeptide from spermatogonia-enriched cell population (Fig. 2G) suggests that the 60-kDa polypeptide band of Fig. 2E might be cross-contaminated with somatic lamin B (contributed either by testicular somatic cells or lamin B present in spermatogonia). A separate and more detailed study is required to determine the extent of lamin B representation in spermatogonia and to map the antigenic determinants of the germ cell-specific lamin which distinguish it from its somatic counterpart.

The observation that the 60-kDa germ cell-specific lamin is the sole major component of the lamina structure in round spermatids has shown for the first time that the lamina structure is indeed reformed following the meiotic division of mammalian germ cells. Earlier, Stick and Schwarz (12) had shown that during chicken spermatogenesis, the lamina structure disappears in primary spermatocytes and is not reformed following the meiotic division. They had obtained similar results in *Xenopus* and rat. These studies, however, were carried out with antibodies against the somatic lamins and therefore could not detect the lamina structure in round spermatids. More recently, Benavente and Krohne (7) have shown that in *Xenopus*, there is a lamina structure in the spermatids, made up of a single germ cell-specific lamin L_{IV} which is expressed only during spermiogenesis. It remains to be seen whether the 60-kDa germ cell-specific lamin reported here has any relationship with the *Xenopus* lamin L_{IV}. However, a careful examination of the lamins reported in the literature has revealed striking similarities between the tryptic map of the lamin reported here and that of the oocyte-specific lamin L_{III} in *Xenopus* (6).

As mentioned earlier, the appearance of the SCs in the meiotic prophase coincides with the disappearance of the lamina structure (18). This correlation has been the basis of the hypothesis that lamina components may be the precursor(s) of SCs. Our earlier experiments on the identification of the 120-kDa protein as a germ cell-specific protein were done on isolated pachytene nuclear matrices, which also contain SCs as an integral part (26). Our previous fluorescence studies did not yield any conclusive evidence on the localization of this antigen in the pachytene nucleus. A clearer fluorescence pattern of the pachytene nuclei described in the present study has shown localization of fluorescence in patches, corresponding to the phase dense regions of the pachytene nuclei. The fluorescence pattern we have observed is quite different from that obtained by Heyting and co-workers (28). Using monoclonal antibodies against 30- and 33-kDa polypeptides, they have shown them to be localized to the lateral elements of the SCs. One likely possibility is that the bright fluorescent patches we have observed around the periphery of the nucleus might represent the attachment plaque regions, where the initiation of chromosome pairing occurs at their telomeric ends. However, we cannot ascertain at present whether the patches of fluorescence within the pachytene nucleus represent peripheral or internal structures. Our preliminary immunoelectron microscopic studies on isolated pachytene nuclei also show localization of this antigen around the periphery as patches (Fig. 3E). The SCs by definition not only include the lateral and central elements but also the attachment plaques. In the present study, we have also isolated SCs from rat pachytene spermatocytes using the method of Heyting *et al.* (18) and analyzed the polypeptide composition. The results
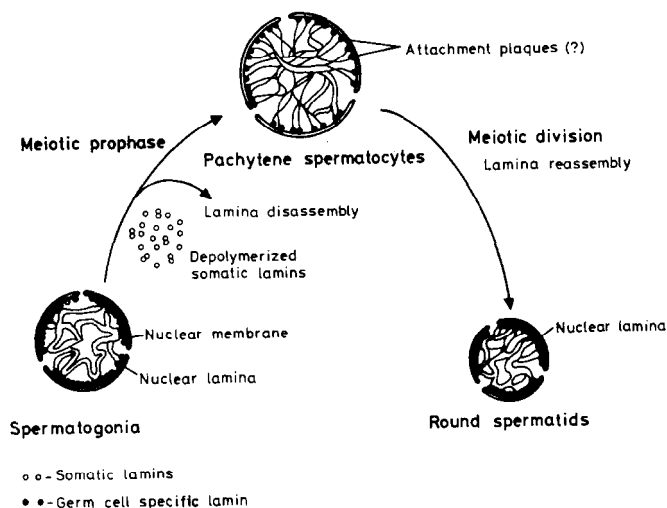

FIG. 7. **Schematic representation of the fate of nuclear lamina during spermatogenesis.**

we have obtained clearly show that the 60-kDa polypeptide is a component of the isolated SCs. It may be pertinent to point out here that Heyting *et al.* (18) had also noticed a 60-kDa polypeptide in the SDS-PAGE pattern of SCs and had suggested it to be a lamin-related polypeptide. We are aware of the possibility that since both the lamina structure and SCs have similar biochemical properties in terms of their resistance to high salt and non-ionic detergents, the germ cell-specific lamin may get coisolated along with the SCs. Immunoelectron microscopic studies on surface spread SCs are required for a final confirmation.

In our earlier studies, we had detected a cross-reacting antigen in the germ cells of other mammalian species, in addition to rat ovaries (14). More recently, we have detected a similar nuclear distribution of this antigen in the male germ cells of frog, grasshopper, rooster, and even plant meiocytes.[3] The high conservation of this protein through evolution goes in conjunction with the evolutionary stability of SCs and ascribes to it a crucial role in aiding the pairing and recombination of homologous chromosomes.

Thus, the results presented in this paper show that a germ cell-specific lamin is expressed in the spermatogonial cells of rat and following the disassembly of the lamina structure during meiotic prophase, it is redistributed in the meiotic cells. After meiotic division it is relocalized in the lamina and is the only lamin polypeptide organizing the lamina structure in round spermatids. A schematic representation of the fate of the nuclear lamina during spermatogenesis, as indicated by our results, is depicted in Fig. 7. The mechanism of selective retention of this protein at the time of meiotic prophase with a concomitant degradation of somatic lamins is open to speculation. Lamins are known to undergo post-translational modifications like phosphorylation (10, 29), isoprenylation (30, 31), and methylation (32), which in turn regulate their behavior during different stages of cell cycle. In this context, the 120-kDa polypeptide (homodimer of 60-kDa lamin) was found to be highly phosphorylatable in the pachytene nuclear matrix (14). In addition, the 60-kDa germ cell-specific lamin, having extensive homology to lamin B, may undergo other modifications as well, which protect it from degradation and facilitate its redistribution. Further biochemical studies on this germ cell specific lamin should yield answers to many of these questions.

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[3] L. Sudhakar, unpublished observations.

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