Developmental Changes in the Basement Membrane of the Normal and Hypothyroid Postnatal Rat Testis: Segmental Localization of Fibulin-2 and Fibronectin

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ABSTRACT

To characterize developmental changes in the extracellular matrix of the postnatal rat testis in relationship to the timing of germ cell maturation, we immunolocalized fibulin-1, fibulin-2, and other matrix components in the testses of normal and propylthiouracil (PTU)-induced hypothyroid animals. Unlike laminin, nidogen, and perlecan, which were present in the seminiferous tubule basement membrane (BM) throughout postnatal development, fibulins were found to disappear from the postnatal tubule BM. Fibulin-1 was no longer detected after Day 5 where-as fibulin-2 became localized in a segmental manner within the BM of each seminiferous tubule on Days 10 and 15 and disappeared by Day 20. Fibronectin showed a segmental pattern in the level of immunostaining of the tubule BM on Days 10 and 15, with a more uniform staining seen at earlier and later ages. Collagen VI was initially confined to the interstitial matrix in the Day 5 testis and became progressively more closely associated with the seminiferous tubule BM at later stages. The disappearance of fibulin-2 and the BM-association of collagen VI were both delayed in the PTU-treated testes. The developmental changes in the staining patterns for fibulin-2 and fibronectin coincide with the adhesion and alignment of peritubular cells on the inner seminiferous tubule BM. The delay in maturation of the seminiferous tubule BM in the testes of PTU-treated rats demonstrates a correlation between changes in the composition of the tubule BM and cellular development of the testis.

INTRODUCTION

The testis undergoes significant morphological and synthetic changes during the postnatal period in preparation for spermatogenesis at the time of puberty. These changes occur rapidly in the newborn rat by a series of highly coordinated processes involving cell interactions between the somatic and germ cell components of the testis. After a period of mitotic quiescence during embryogenesis, cell division of germ cells begins within 3 days after birth [1]. Around the same time, undifferentiated spermatogonia start to move from the center of the spermatogenic cords to reside on the basement membrane (BM) that was deposited around the cords during the embryonic period [1]. These cells become the differentiating type A spermatagonia, which enter mitosis starting around 12 days after birth and appear as haploid germ cells first around Day 25 (reviewed in [2, 3]). Proliferation and differentiation of the somatic cell types also occur during the early postnatal period to provide a structural and hormonal environment for the initiation and maintenance of spermatogenesis. Sertoli cell proliferation, begun during fetal development, ceases between Days 15 and 20 [4], with the coordinated appearance of biochemical markers of Sertoli cell maturation and establishment of the blood-testis barrier (reviewed in [5, 6]). Within the interstitium, division and differentiation of peritubular myoid cells is completed by Day 25 [7]. The proliferation of fetal Leydig cells present at birth appears to remain stable in number throughout life [8], while cells with the morphological characteristics of the adult type Leydig cells differentiate from a separate precursor population and are first observed on Day 10 [8], reviewed in [9]).

A number of studies have suggested that the BM around seminiferous tubules has an important role in supporting testis differentiation, influencing in particular the differentiation of peritubular cells [10] and the proliferation and differentiation of Sertoli cells and their interaction with germ cells [11-13]. A cooperative interaction between Sertoli and peritubular cells is necessary for the synthesis and deposition of the seminiferous tubule BM [14, 15], which is influenced by locally produced factors such as platelet-derived growth factor [16]. Abnormal thickening of the seminiferous tubule BM is frequently associated with reduced spermatogenic function in humans, and it has been suggested that deregulated production of BM components directly influences the phenotype of Sertoli and peritubular myoid cells [17].

Several studies suggest that a structural reorganization of BMs accompanies normal testicular development. Collagen VI is initially restricted to the interstitial matrix between seminiferous cords of the fetal and early postnatal rat testis, and subsequently it is observed to be associated with the BM around Sertoli cells and peritubular cells by Day 14, when these cell types are beginning to differentiate [18]. In the mouse, the appearance of the α3(IV) isoform chain of collagen IV in the BMs around seminiferous tubules coincides with the period when spermatogonia begin to proliferate and then migrate to the BM, suggesting that collagen α3(IV) chains may have a role in these processes [19]. It is now clear that a large degree of molecular heterogeneity exists in BMs of different tissues and at different stages of development [20-22], and novel BM components continue to be identified and characterized [22]. To better understand the precise extracellular matrix ligands that mediate different cell interactions in the postnatal testis, it is important to analyze in detail the molecular changes in BMs that are associated with key cellular changes in the developing testes.

In this study, we have explored the potential role of fi-
fibulins in testis development. Fibulins are a new class of matrix glycoproteins characterized by the presence of 9–11 epidermal growth factor-like repeats that include consensus sequences for Ca$^{2+}$-binding [23–25]. Two members have been identified so far, fibulin-1 and fibulin-2. Fibulin-1 is a 90-kDa monomer protein, and it has four alternate mRNA splice variants [23, 24]. Fibulin-2 has a larger N-terminal domain, and it is a 450-kDa dimer of two disulfide-bonded 195-kDa monomers [26]. The tissue expression patterns of fibulin-1 and fibulin-2 are broad in interstitial matrices and BMs of embryonic and adult tissues, but they are not ubiquitously expressed [24, 27–30]. Analyses of interactions with other extracellular matrix components have shown that both fibulin-1 and fibulin-2 bind strongly to nidogen and fibronectin and more weakly to some other components, by Ca$^{2+}$-dependent mechanisms in most cases [31, 32]. These studies suggest a potential role for fibulins in matrix assembly.

To determine whether fibulins may be involved in the structural reorganization of BMs during testis development, we used immunofluorescent staining to localize both fibulin-1 and fibulin-2 at different postnatal stages of the rat testis. We compared this to the localization of other components (laminin, nidogen, perlecan, collagen VI, and fibronectin). We also looked at propyl-thiouracil (PTU)-treated hypothyroid rats, in which testis development is significantly delayed [33–35], to correlate structural changes in the seminiferous tubule BM with other developmental changes.

**MATERIALS AND METHODS**

*Tissue Preparation for Immunolocalization and Histological Studies*

Outbred Sprague Dawley rats were obtained from Monash University Central Animal Services. Experimental procedures were approved by the Monash University Standing Committee on Ethics in Animal Experimentation and conformed to the National Health and Medical Research Council of Australia/CSIRO/ARC Code of Practice for the Care and Use of Animals for Experimental Purposes. Testes were removed from immature (5–40 days after birth) and adult (approximately 3 mo old) rats that had been killed by CO$_2$ anesthesia. To render rat pups hypothyroid, pregnant rats were monitored twice daily for time of delivery, and upon delivery lactating rats were provided with drinking water containing 0.1% (w:v) 6-propyl-2-thiouracil (PTU; Sigma Chemical Co., St. Louis, MO) sweetened with 0.0018% aspartame (Equal; Searle, Crows Nest, NSW, Australia) as previously described [36]. Rat pups were weaned at 22 days after birth, when the drinking water was changed to tap water.

Testes were dissected from control and treated animals on Days 5, 10, 15, 20, 30, and 40 days after birth, and from control adult animals. Body weights and testis weights were recorded. For immunolocalization studies, the testes were weighed and then immediately frozen in Tissue-Tek O.C.T. embedding compound (Miles Labs, Naperville, IL) by immersion in isopentane cooled on dry ice. For histological processing, testes were either fixed in Bouin's fixative for 5 h by immersion (on Days 5, 10, and 15) or by vascular perfusion followed by immersion (Day 20 and older). After transfer and washing in 70% ethanol, the samples were processed and embedded in paraffin using a standard protocol [37]. Five-micrometer sections were stained with Mayer's hematoxylin (Sigma) and mounted under Depex (BDH Chemicals Aust. Ltd., Kilsyth, Victoria, Australia).

**Antibodies**

Antibodies used in this study were polyclonal rabbit antisera raised against mouse EHS tumor laminin-1 [38],...
mouse nidogen [39], mouse perlecan [40], human collagen VI [41], recombinant mouse fibulin-1 [31], recombinant mouse fibulin-2 [24], and human plasma fibronectin (Life Technologies, Grand Island, NY). Antibodies against laminin-1 recognize the α1, β1, and γ1 chains, the latter two being common to most other laminin isoforms.

Immunofluorescence

Frozen sections were cut at 6 µm, air-dried onto gelatin-coated slides, and left unfixed. Slides were then rinsed in Tris-buffered saline, pH 7.2 (TBS), and antisera were applied at a 1:50 to 1:400 dilution in TBS, depending on the titer of the particular antiserum. Slides were incubated at room temperature for 1 h and washed in TBS before application of either fluorescein isothiocyanate (FITC)-conjugated sheep anti-rabbit IgG antiserum (Silenus, Melbourne, Victoria, Australia) diluted 1:100 in TBS or CY3-conjugated goat anti-rabbit IgG antibody (Jackson Immunoresearch, West Grove, PA) diluted 1:1000 in TBS. Both secondary antibodies yielded qualitatively identical results, while the latter reagent provided the more intense signal. After 1 h incubation in the secondary antibody, slides were washed in TBS and mounted in Fluorsave Reagent (Calbiochem-Behring Corp., San Diego, CA). Dried slides were viewed and photographed using a Zeiss Axioplan fluorescence microscope (Carl Zeiss, Inc., Thornwood, NJ).

Statistical Analysis

Tests and body weight data was analyzed by two-way analysis of variance (general linear model) followed by a multiple comparison procedure (Student-Newman-Keuls method). The level of significance was taken as p < 0.05.

RESULTS

Postnatal Changes in the Testis of Control Rats

Laminin, nidogen, and perlecan. Laminin, nidogen, and perlecan were shown by immunofluorescence to be localized to the BMs around seminiferous tubules in the adult rat testis and at all stages of postnatal development analyzed. The pattern of immunolocalization was very similar for all of these three BM components; it is shown in Figure 1 (left panels) for perlecan. In the Day 5 testis, a single continuous BM was seen around the tubules, with more diffuse, discontinuous immunoreactivity beneath this BM, and low levels of staining within the interstitial tissue. The BMs around small and large blood vessels in the testis were highly stained by all three antibodies (Fig. 1A). An increase in tubule diameter was evident by Day 10 (Fig. 1C) with an apparent reduction in the relative proportion of interstitial tissue. A second outer BM around the seminiferous tubules was now evident by immunostaining for perlecan (Fig. 1, C and E) and laminin and nidogen (not shown). These inner and outer BMs appeared to be closely associated when visualized by immunofluorescence microscopy. The diameter of the tubules continued to enlarge from Day 15 to Day 20, when the BMs of adjacent seminiferous tubules became closely apposed (Fig. 1G). The apparent volume of interstitial tissue relative to that of the seminiferous tubules was clearly diminished by this stage as well as in the adult (Fig. 1, G and I).

Histological analyses of postnatal testes showed changes in the arrangement of interstitial cells and germ cells from Day 5 to Day 10 (Fig. 2). On Day 5, cells in the interstitial connective tissue had no distinct orientation around the basal surface of seminiferous tubules (Fig. 2A), but by Day 10 a flattened single layer of cells was closely apposed to the basal surface of tubules (Fig. 2B). This layer of peritubular cells persisted through adulthood (data not shown). Germ cells were evident as gonocytes in the center of the seminiferous tubules on Day 5 (Fig. 2A), but by Day 10 few gonocytes remained and the germ cells had assumed a peripheral position at the basal surface of the tubules (Fig. 2B).

Collagen VI. Collagen VI immunoreactivity was confined to dense fibrillar bundles of extracellular matrix in the interstitial tissue of the Day 5 testis and the BM around blood vessels (Fig. 1B). No staining above background was seen in the single BM around seminiferous tubules, but strands of collagen VI-positive material were detected close to the BM. By Days 10 and 15, increased staining for collagen VI was seen associated with the BM (Fig. 1, D and F). Immunoreactivity was evident in both the inner and outer BMs in some regions, but collagen VI was not uniformly distributed throughout these BMs until Day 20 (Fig. 1H). In the adult testis, immunostaining for collagen VI around blood vessels and the seminiferous tubule BM (Fig. 1J) was indistinguishable from immunostaining for perlecan (Fig. 1I). Fibrillar strands of collagen VI continued to be abundant in the interstitial matrix of the adult testis (Fig. 1J).

Fibulin-1 and fibulin-2. Quite distinct patterns of immunolocalization were observed with antibodies to fibulin-1 and fibulin-2. Strong fibulin-1 immunoreactivity was seen in the tunica albuginea around the testis and in large blood vessels at all stages of development (not shown). Fibulin-1 was seen at very low levels in the BM around seminiferous tubules on Day 5 but was not detected at later stages (not shown). No reactivity for fibulin-1 was seen around small blood vessels in the interstitium. Fibulin-2 immunostaining was seen in the BM around seminiferous tubules.
on Day 5; this was continuous but not uniform in intensity (Fig. 3A). High levels of immunoreactivity were evident around large blood vessels and the tunica albuginea, but fibulin-2 was not detected in the interstitium surrounding the seminiferous tubules nor around small blood vessels (Fig. 3A). This pattern was quite different from the localization of other BM components such as perlecan (Fig. 1A).

By Day 10, fibulin-2 immunoreactivity was confined to discrete segments along the seminiferous tubule BM (Fig. 3C). This pattern was consistently seen in all BMs and was not restricted to a sub-population of tubules. This segmental pattern was also evident in the Day 15 testis, but at this stage larger unstained regions of BM separated the fibulin-2-positive segments (Fig. 3E). By Day 20, fibulin-2 was no longer localized to the tubular BMs and fibulin-2 immunoreactivity in the testis from Day 20 onwards was confined to the large blood vessels and the tunica albuginea (Fig. 3, G and I).

**Fibronectin.** The pattern of fibronectin staining in the Day 5 testis (Fig. 3B) was similar to that of perlecan (Fig. 1A) but with higher amounts present within the interstitial matrix. Uniform staining was seen along the seminiferous tubule BM. This pattern was unchanged in the Day 10 testis except for a distinct segmental distribution in the seminiferous BMs (Fig. 3D). This pattern was similar to that of fibulin-2 except that BM segments that were highly positive for fibronectin were separated by regions of lower immunoreactivity rather than no immunoreactivity. This segmen-
tal pattern was also observed in sections of the Day 15 testis (Fig. 3F) but was again continuous by Day 20 (Fig. 3H). Fibronectin continued to be associated with the seminiferous tubule BMs and interstitial matrix of the adult testis (Fig. 3J). In addition, fibrillar bundles of fibronectin-positive material in the interstitium of the adult testis appeared similar to collagen VI immunolocalization.

**Development of the PTU-Treated Testis**

Male newborn rats that had been treated with PTU from birth to the time of weaning at Day 22 were killed on postnatal Days 5, 10, 15, 20, 30, and 40. Testis weights of PTU-treated animals were significantly lower than those of controls on Days 20–40 (Fig. 4), as previously documented by Simorangkir et al. [35] for Days 20 and 30, and body weights of PTU-treated rats were significantly lower than those of untreated animals on Days 30 and 40 (Fig. 4). Frozen sections of the testes were analyzed by immunofluorescence with the panel of antibodies described above. Testes from 2–3 animals were analyzed at each time point, and similar patterns of immunoreactivity were seen with each antibody in all PTU-treated testes of the same age. PTU treatment resulted in a notable delay in the development of seminiferous tubules compared to those of untreated controls, as seen in testis sections immunostained for nidogen on Days 10, 20, and 30 (Fig. 5). Day 10 PTU-treated testes appeared similar in developmental stage to control Day 5 testis (compare Fig. 1A with Fig. 5J) with respect to the presence of a discontinuous outer, second BM around the seminiferous tubules. The diameter of PTU-treated seminiferous tubules on Day 30 was clearly smaller than that of control tubules (compare Fig. 5L with Fig. 5O). This inhibition of tubule growth and delay in the formation of a second outer BM was accompanied by a delay in the developmental changes seen in the control testis for the distribution of fibulin-2 (Fig. 5, A–C), fibronectin (Fig. 5, D–F), and collagen VI (Fig. 5, G–I). Fibulin-2 and fibronectin were uniformly distributed in the BM of seminiferous tubules on Day 10 but had a segmented distribution in the Day 20 and Day 30 PTU-treated testes (Fig. 5). Regions of immunoreactivity for fibulin-2 were still seen in the BM of some seminiferous tubules on Day 40 (not shown), but the majority of BMs were not immunostained with fibulin-2 antibodies on Day 40. This pattern contrasts with the complete disappearance of fibulin-2 from the tubule BM in the control testis by Day 20 (Fig. 3G).
collagen VI with the seminiferous tubule BM was also delayed in the PTU-treated testis, being found predominantly in the interstitial tissue on Days 10 and 20 and becoming more closely associated with the tubule BM by Day 30 (Fig. 5, G–I). High levels of collagen VI remained in the interstitial matrix in the PTU-treated testes on Day 30 and Day 40.

**DISCUSSION**

Fibulin-1 and fibulin-2 are two structurally related Ca\(^{2+}\)-binding extracellular matrix proteins that have previously been shown to be localized to interstitial matrices and BMs of adult and embryonic tissues [24, 27, 28, 30]. Interactions between fibulins and fibronectin, nidogen, and other matrix components has suggested a role for both fibulins in matrix assembly [31, 32], but their actual biological functions have not yet been elucidated. High levels of fibulin expression at sites of epithelial-mesenchymal interactions in embryos has led to the proposal that fibulins might mediate alterations in cell behavior, such as cell movement, during developmental processes [27, 29, 30]. However, fibulin-1 and fibulin-2 are not always coordinately expressed in tissues and are likely to have different structural and biological functions [24, 28, 42].

In this study, we show that fibulin-1 and fibulin-2 are present in the BM around developing seminiferous tubules in the newborn rat testis but disappear after Day 5. No fibulin-1 was detected around the seminiferous tubules from Day 5 onwards, but it remained at high levels in the tunica albuginea and around large blood vessels. Fibulin-2 also disappeared from the seminiferous tubule BM after Day 5 but showed a progressive disappearance in a localized, segmented pattern. Discrete, evenly spaced segments of the BM around each tubule showed fibulin-2 immunoreactivity in the normal Day 10 testis; these segments were more widely separated on Day 15 and had disappeared entirely by Day 20 in all regions of the testes. This unique distribution pattern is the first demonstration that there are localized changes in the composition of the BM around each seminiferous tubule during postnatal testicular development.

Our findings on the localization of laminin, nidogen, and perlecan are in agreement with previous studies showing BM components to be uniformly distributed in the postnatal seminiferous tubule BM [43, 44] and also present in the interstitium, where they are localized around blood vessels and around clusters of Leydig cells [45, 46]. We have shown that, in contrast, the distribution of fibronectin in the seminiferous tubule BM more closely correlates with the pattern of fibulin-2 localization. Distinct segments of the BM showed a higher concentration of fibronectin staining on Days 10 and 15, whereas lower levels of staining were evident between these segments. By Day 20, when fibulin-2 had disappeared from the BM, fibronectin immunoreactivity was more uniform throughout the BM of individual tubules, and this pattern remained in all BMs until adulthood. A distinct interaction between purified fibulin-2 and fibronectin has been demonstrated in vitro [32], and these two matrix components are co-distributed in extracellular microfibrils produced by fibroblasts in culture [47]. The segmented distribution pattern of fibulin-2 and fibronectin in the BMs around the developing seminiferous tubules suggests that these two matrix components interact in vivo such that fibronectin becomes more concentrated at sites where fibulin-2 is present. However, these data also show that the retention of fibronectin in the BM is not dependent on the presence of fibulin-2.

The localized segmental disappearance of fibulin-2 from the seminiferous tubule BM coincides with the alignment of interstitial cells on the BM between Days 5 and 10 of postnatal development and the start of their differentiation into contractile myoid cells [7]. After Day 10, a continuous second BM surrounds these peritubular cells, which become enclosed within the double BM around the developing seminiferous tubules. Both peritubular myoid cells and Sertoli cells synthesize laminin, perlecan, collagen IV, and nidogen [14, 44, 48, 49]; but in vitro coculture studies have shown that the assembly of a BM matrix requires an interaction between both cell types [14, 15]. Synthesis of fibronectin and interstitial collagens is restricted to peritubular cells [14, 15, 50]. It is not yet known which cells in the testis synthesize fibulin-1 and fibulin-2, but the absence of both fibulins in the interstitial matrix of the newborn testis suggests that they are likely to be products of Sertoli cells. The disappearance of both fibulins from the postnatal testis could be due to either the down-regulation of their expression or to proteolytic degradation. A segmental disappearance of fibulin-2 could be achieved through expansion of the BM in the absence of continued fibulin-2 synthesis and deposition into the matrix, with the distance increasing between the segments as the tubules enlarge. This segmented pattern could also be explained by degradation of fibulin-2 at initial sites of contact of the interstitial cells with the seminiferous tubules, with more extensive degradation as these cells envelop the entire BM. The periodicity of the fibulin-2-positive and -negative segments at different stages of testis development correlates well with the distribution of peritubular cells around the seminiferous tubules. Fibulin-2 is highly sensitive to degradation by a number of different proteases, including metalloproteinases [51]. Isolated peritubular myoid cells and Sertoli cells synthesize proteases including metalloproteinases [52, 53] that could be responsible for remodeling of the seminiferous tubule BM. Although coculture of Sertoli cells with peritubular myoid cells in vitro results in decreased rather than increased activity of metalloproteinases [53], it will be of interest to determine whether differentiation of these cells in the newborn testis is associated with an increased production of proteolytic enzymes that may contribute to the specific disappearance of fibulins from the BMs around seminiferous tubules while other BM components are retained.

The disappearance of fibulin-1 and fibulin-2 from the seminiferous tubule BM after Day 5 occurs at a time when the \(\alpha_3(IV)\) isoform chain of collagen IV is synthesized and deposited within the BM [19]. These changes demonstrate that the seminiferous tubule BM is undergoing subtle changes in its composition during the postnatal period before the onset of germ cell meiotic divisions. Previous studies have shown a change in the distribution of collagen VI within the postnatal rat testis, from an exclusive interstitial localization in the fetal and neonatal testis to a close association with the seminiferous tubule BM by Day 14 [18]. Our observations confirm these studies and demonstrate that the association of collagen VI with BMs occurs progressively from Day 10 to Day 20. Collagen VI is synthesized predominantly by mesenchymal cells in developing tissues [54] and is most likely produced by peritubular cells that also synthesize collagen I and fibronectin [14]. The alteration in the localization of collagen VI correlates with the alignment of interstitial cells against the developing...
mote their differentiation and maturation. Continued production of collagen VI by these cells would result in the deposition of collagen VI microfibrils between these two BMs. Collagen VI is not an integral component of BMs, but microfibrils are closely associated with BMs in a number of tissues and are predicted to link the BM with other components of extracellular matrix and thus stabilize tissue structure [55, 56]. It is thus possible that the association of collagen VI with seminiferous tubule BMs after Day 20 stabilizes them against further remodeling, when the full complement of differentiated somatic cells and differentiating germ cells is established. However, while the molecular structure of the BM is stabilized during this period, the tubule diameter continues to enlarge into adulthood, indicating continued synthesis and expansion of the tubular BMs.

Neonatal hypothyroidism causes a prolonged persistence of gonocytes within seminiferous tubules, a marked delay in onset of germ cell maturation, degeneration of primary spermatocytes, and prolonged proliferation and functional immaturity of Sertoli cells [33–35, 57]. Our analysis of tests from PTU-treated hypothyroid rats demonstrates a direct correlation between developmental changes in BM composition and the delay in germ cell development and Sertoli cell maturation that have been previously reported. Our results show that inhibited growth of the PTU-treated testsis and the delay in cellular maturation within the seminiferous tubules is accompanied by delays in the disappearance of fibulin-2 and in the association of collagen VI with the tubule BM. Neonatal hypothyroidism has also been shown to result in increased numbers of Leydig cells in the adult rat [58]. Our results suggest that PTU treatment affects the maturation of cell types that coordinately synthesize and assemble the BM and are responsible for developmentally regulated BM remodeling. Thus a delay in the maturation of peritubular myoid cells and/or Sertoli cells is implicated by our study.

Our observations taken together with other recent findings demonstrate that specific changes in the molecular composition of the seminiferous tubule BM coincide with important changes in cell interactions within the developing testis, both during normal development and in an experimentally induced delay in testicular development. A developmental cascade can now be predicted in which specific cell interactions are directly responsible for regulating the molecular structure of the BM, resulting in the establishment of new cell-matrix interactions and induction of somatic and germ cell differentiation pathways. In particular, we propose that a change in the composition of the seminiferous tubule BM, including loss of fibulins and appearance of collagen α3(IV) chains [19] allows adhesion of gonocytes to the seminiferous tubule BM, which is important for the initiation of germ cell differentiation [2]. The loss of fibulins from the BM may expose attachment sites on laminin, fibronectin, or other components important for germ cell attachment. It will be of interest to determine whether gonocytes preferentially adhere to regions of the seminiferous tubule BM that lack fibulin-2. It is likely that changes in the composition of the seminiferous tubule BM both influence and reflect the cessation of Sertoli cell and peritubular cell proliferation and that these changes promote their differentiation and maturation. The specific roles of fibulins, fibronectin, collagen IV isoforms, and collagen VI in these processes can now be examined experimentally.

REFERENCES


