Interrelationships between Germ Cell Differentiation and Transformation of Basolateral Profile of Sertoli Cells during Rat Spermatogonial Cycle*

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Summary. After treatment with either trypsin, 8N HCl or 5N KOH, or with mechanical dissociation, normally hidden aspects of the seminiferous epithelium were exposed to observation by scanning electron microscopy (SEM). These included the basal surface of seminiferous epithelial cells, the basolateral processes of the Sertoli cell, junctions of the processes, and the basal or adluminal recesses.

With the progressing stages of the spermatogonial cycle, three kinds of spermatogonia show different profiles and topographic relations. The basolateral processes of the Sertoli cells can be categorized into four types: conical, wedge-shaped, sheet-like and cup-shaped processes. The first two of the basolateral processes are joined together by close contact and/or overlapping junctions to form the floor of the basal recesses, and they encircle small-sized spermatogonia. The sheet-like processes mutually join by seam line junctions to form the ceiling of the basal recesses. During the spermatogonial cycle, the basal recesses first appear as separated lacunae, then form continuous labyrinth-like trenches, and finally make complicated honeycomb-like lacunae. The cup-shaped processes also are joined by close contact and/or overlapping junctions and are tightly attached by the primary spermatocytes with doughnut-like or linear bodies. The cordal arrangement and adluminal shift of the diverse spermatogonia will be discussed along with the cyclic transformations of Sertoli cell processes and their junctions.

The seminiferous epithelium of adult rats consists of two distinct cell populations: Sertoli and germ cells. The Sertoli cells with highly irregular contours represent the non-dividing population of cells, and are characterized by crypts and recesses in which the germ cells and the residual cytoplasmic bodies of the spermatids are lodged (GRAVIS, 1978). Several investigators have discerned a continuous belt-like layer of Sertoli-Sertoli junctions running close to the base of the Sertoli cells and dividing the interior of the seminiferous tubules into two compartments: basal and adluminal (RUSSELL et al., 1983; PLOEN and RITZEN, 1984). Basally located germ cells consist of A- and B-spermatogonia and preleptotene spermatocytes which are located in the basal recesses. RUSSELL (1976) has concluded that the Sertoli cells play an important role

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in the transfer of spermatocytes from the basal compartment to the adluminal one. Furthermore, it has been determined that the Sertoli cells and the basally located germ cells have different appearances during the spermatogenic cycle (PERET et al., 1961; SCHULZE, 1974). The basolateral processes of the Sertoli cell join together to form the basal and adluminal recesses. Though the two-dimensional relationships between Sertoli cell processes and the germ cells have been examined by light microscopy and transmission electron microscopy (TEM) (ELFTMAN, 1950; WEGER et al., 1983; WONG and RUSELL, 1983), there has been no previous detailed study by scanning electron microscopy (SEM). SEM combined with chemical-enzymatic digestion and/or tape-stripping techniques allows us to observe the hidden basal surfaces of the seminiferous epithelium and of the basal or adluminal recesses situated both below and above the continuous belt-like layer of Sertoli-Sertoli junctions. The purpose of this SEM study is to clarify the hidden basal profiles of the seminiferous epithelium, the basal or adluminal recesses, the basolateral processes of the Sertoli cells and junctional patterns of the processes which are altered in each progressive stage during one spermatogonial cycle.

MATERIALS AND METHODS

Wistar strain male rats more than 40 days old were used. The animals were anesthetized by intramuscular injection of sodium pentobarbital. Under deep anesthesia, the thorax was opened and a cannula was introduced into the left ventricle or the thoracic aorta. The rats were perfused with physiological saline through the cannula, and the right testis was removed. The left testis was further perfused with a fixative (2.5% glutaraldehyde-2% formaldehyde in 0.1% cacodylate buffer, pH 7.4).

SEM: In order to observe the hidden basal surface, and the basal and adluminal recesses below and above the continuous Sertoli-Sertoli junction layer, fresh or fixed testicular blocks were treated enzymatically or chemically to remove the following unnecessary cellular and fibrous components:

1) Enzymatic digestion: To expose the basal surfaces of the epithelial cells, fresh blocks from the right testis were digested in 0.1M phosphate buffer or Eagle buffer containing collagenase (150-170 I.U./ml, Type II, Sigma Co.) and trypsin (40 I. U./ml, Merk) for 60 or 90 min at 37°C, and then fixed for 2 hr in the 2.5% glutaraldehyde fixative. The technical procedure for preparing such biological specimens has been described in detail in our previous reports (HAMASAKI et al., 1983, 1985).

2) Chemical maceration: In order to expose the basal recesses, fresh blocks of the right testis were immersed first in Hartman's solution containing 30 mM KCl for 1 hr, and then in the 2.5% glutaraldehyde fixative for more than 3 hr. Next, these blocks were hydrolyzed with 8N HCl for 60 or 90 min at 60°C or with 5N KOH for 10 min at 60°C. These specimens were postfixed with buffered 2% OsO₄ solution, dehydrated in a series of acetone and critical-point-dried with liquid CO₂.

3) Mechanical dissection: For exposing the adluminal recesses just above the epithelial continuous layer, critical-point-dried specimens were stripped using adhesive tape.

All specimens thus treated were examined and photographed with a Hitachi HFS-2 field emission SEM.
RESULTS

1. Basal surfaces of Sertoli cells and three kinds of spermatogonia

A continuous Sertoli-Sertoli cell junctional area divides the seminiferous epithelium into two compartments: basal and adluminal. All of the spermatogonia and some of the spermatocytes were located in the basal portion, while most of the spermatocytes and the more developed spermatids were lodged in the adluminal portion.

When fresh (unfixed) testicular blocks were enzymatically digested, the basal portions of the seminiferous epithelia were successfully exposed, and were found to be composed of Sertoli cells and many spermatogonia. The basal surfaces of major Sertoli cells showed large (270–430 \( \mu \text{m}^2 \)) hexagonal contours, whereas the basal surfaces of some cells were small (180–340 \( \mu \text{m}^2 \)) and pentagonal to square in contour (Fig. 1).

From their differences in size, contour and position, the basally located sper-
matogonia were subdivided into three kinds: small-sized (S-), medium-sized (M-) and large-sized (L-) cells. The S-cells were spherical or conical in contour. They appeared to circumscribe the basal portion of a Sertoli cell and were connected by intercellular bridges, forming partially opened polygons but not closed ones. These polygons were integrated into one row in most cases, though occasionally into two rows of S-cells. The latter arrangement was often found in epithelial segments* where there was a small basal area of the Sertoli cells. The M-cells were spindle-shaped or discoid and interconnected with the bridges. They were arranged in a simple or complicated cord (Fig. 1, 2). L-cells exhibited a flat discoid or polygonal contour and were arranged in a similar manner as the M-cells (Fig. 2, 3). Of these three kinds of spermatogonia, the S-cells lacked a cytoplasmic process, while M- and L-cells extended lengthy ones. Compared with the M-cells, L-cells showed extremely elongated and ramified processes, ultimately resembling twigs (Fig. 3). In most M- and L-cells the processes and cell bodies were elongated along the junctional lines between adjoining Sertoli cells, but in some cells they crossed the basal trunk of a Sertoli cell (Fig. 1, 3).

*Each stage of the spermatogonial cycle is represented by the features of an epithelial segment of the given length as described by PEREY et al. (1961).
2. Basal recesses, basal processes of Sertoli cells, and junctions of the processes

The L- and M-cells were mounted in shallow lacunae, called the basal recesses, which were formed by depressions between adjoining Sertoli cells. In contrast, the S-cells were located in deep basal recesses. Therefore, the latter cells had a smaller basal area facing the basement membrane of the seminiferous epithelium than did the former two types of cells (Fig. 1-3). Furthermore, the S-cells were gradually surrounded by the basal recesses of adjoining Sertoli cells (Fig. 1, 4) until they were completely embedded in the basal recesses (Fig. 2). When the basal processes and trunks of adjoining Sertoli cells were to a certain extent removed, embedded S-cells were disclosed (Fig. 2). Though the floor of the basal recesses was formed by the basal processes of adjoining Sertoli cells, these processes appeared first as thorny or conical in contour and then gradually modulated into a wedge-shaped contour. These processes joined together in overlapping and/or close contact junctions and then gradually embraced the S-cells (Fig. 4). Some of the basal recesses were often revealed in the collagenase-treated preparations (Fig. 1, 2, 4). In extremely rare cases, most of the basal recesses could be observed even after enzymatic digestion (Fig. 5a).

RUSSELL (1976) has reported that the shrinkage of basally located germ cells is caused by immersion in the hypertonic fixative, and that these cells are separated from
the surrounding Sertoli cells. A similar shrinkage of germ cells was also observed when rat testicular tubules were immersed in K⁺-rich Ringer solution. When K⁺-pretreated and fixed blocks were chemically digested with 8N HCl at 60°C, the excess cellular and matrix components in the tubular lamina propria and the shrunken germ cells were removed. The basal recesses of the seminiferous epithelium were readily revealed through this chemical digestion process (Fig. 5b-d). In the epithelial segment

Fig. 4. Sertoli cells show conical (C) and wedge-shaped (W) basal processes. The latter processes encircle the deeply embedded S-cells. The facing processes of two Sertoli cells join by overlapping (arrowheads) and/or close contact (arrow) junctions. Treated with collagenase and trypsin for 90 min at 37°C. ×6,600

Fig. 5. The pleomorphic basal recesses are widely exposed by the digestion technique. The fresh (unfixed) tubules are enzymatically digested (a), while the K⁺-treated and fixed tubules are chemically hydrolyzed (b-d). a. The separated basal recesses are arranged in a cord. One M-cell (asterisk) remains. ×460. Inset. High magnification of the box. These recesses are connected by narrow drains (arrowheads). ×860. b. The series of basal recesses becomes deeper as evidenced by the notches in the cytoplasmic processes of the proliferating L-cells (L). ×910. c. Most of the basal recesses are continuous with each other, forming labyrinth-like trenches. ×910. d. The basal recesses are the deepest of all the recesses and separate the basal trunks of the individual Sertoli cells. In the ceilings of the basal recesses, transitional chambers (arrowheads) are formed secondarily. ×900. Inset. High magnification of the transitional chamber. ×1,900
Fig. 5. Legend on the opposite page.
where M-cells are located, individual basal recesses showed shallow, separated lacunae, which were, strictly speaking, connected by narrow drains (Fig. 5a). These drains correspond to the furrows from which the intercellular bridges have possibly been pulled away. In the epithelial segment containing the L-cells, the basal recesses were deeper than the separated recesses and showed continuous lacunae because of the presence of wide drains (Fig. 5b-c). The wide drains are composed of depressions in which elongated cytoplasmic processes and bridges might be located previously. Most of the basal recesses formed labyrinthine trenches, while some of them remained as separated lacunae (Fig. 5b) or newly constructed transitional chambers (Fig. 5c). The S-cells (possibly B-spermatogonia to preleptotene spermatocytes) were located in the transitional chambers corresponding to the intermediate location between basal and adluminal recesses. In the epithelial segment where the S-cells were locating after differentiation from the M- or L-cells, the basal recesses were deepest, and their assemblages were then transformed from trenches into a honeycomb. This transformation caused the adjoining Sertoli cells to further separate (Fig. 5d). In the ceilings of the honeycomb-shaped recesses, transit chambers showing variable configurations were found more frequently.

Fig. 6. A K+-pretreated, fixed tubule macerated in 8N HCl solution. Cup-shaped processes (asterisk) which correspond to the precursor of the transitional chamber are revealed in the ceiling of the basal recesses. The sheet-like processes mutually join by the seam line junctions (arrowheads). ×4,600

Fig. 7. A fresh tubule enzymatically digested. As the S-cells (S) shift adluminally, they change from spherical to sphericoconical contour. SP spermatocytes. ×2,600
3. Basolateral processes of the Sertoli cell and their junctions

The floor of the basal recesses, the so-called basal processes, appeared first as elongated conical processes and then as flattened wedge-shaped processes of the Sertoli cells. In rare cases these two types of processes were fairly well preserved even after enzymatic digestion (Fig. 4). However, the majority of the basal processes were partially destroyed after hydrolysis (Fig. 5d). In contrast to the basal wedge-shaped processes, the configuration of the lateral sheet-like processes was well preserved even after HCl digestion. The ceiling of the basal recesses, the so-called lateral processes, was formed by the sheet-like processes of the adjoining Sertoli cells (Fig. 6). The sheet-like processes joined together along many seam lines, but not with overlapping and/or close contact junctional lines. Both of the sheet-like processes and the seam lines formed the basal septum of the Sertoli-Sertoli continuous layer. Some of the sheet-like processes occasionally changed to cup-shaped processes to form transitional

Fig. 8. A K+ untreated and fixed tubule macerated in 5N KOH for 10 min at 60°C and stripped by an adhesive tape. Most of the spermatocytes (SP) and the adluminal side of the Sertoli-Sertoli continuous layer are exposed. Some S-cells (arrowheads) lie under the septum of the Sertoli-Sertoli continuous layer. ×910

Fig. 9. Adluminal recesses of the fixed seminiferous epithelium successfully exposed by simple tape-stripping. The lower halves of the cup-shaped processes extend radially from the Sertoli cell trunk (SE) and mutually join by close contact (arrow) and/or overlapping (large arrowhead) junctions. Doughnut-like or linear structures (small arrowheads) are found on these processes near the Sertoli cell trunks. ×1,800. Inset. High magnification of the doughnut-like structure. ×3,900
chambers (Fig. 6). The transitional chambers were transformed from spherical to sphericoconical in contour (Fig. 7), fitting nicely to the S-cell configurations.

Primary spermatocytes were recognized in the adluminal recesses above the Sertoli-Sertoli continuous layer, while the S-cells were found below this layer (Fig. 8). When most spermatocytes and the upper halves of the cup-shaped processes were mechanically removed, the lower halves of the processes could be exposed. These processes resembled frills similar those seen on frilled neck lizards (Fig. 8, 9). Some spermatocytes were surrounded by cup-shaped processes extending radially from the trunk of a Sertoli cell (Fig. 9). The lower halves of these processes joined each other by overlapping and/or close contact junctions. In the adluminal recesses near the trunk of the Sertoli cell, doughnut-like structures were often found, with linear ones occasionally observed.

DISCUSSION

1. Relationship between the three kinds of spermatogonia and the spermatogonial cycle
Basally located spermatogonia were subdivided into three kinds: large-sized (L-), medium-sized (M-) and small-sized (S-) cells, according to their differences in size, contour and topographical feature. Proliferation and differentiation of these spermatogonia occurred during the first and second spermatogonial cycles of spermatogenesis, as previously reported for rats (Clermont and Bustos-Obregon, 1968; Huckins, 1971; Clermont and Hermo, 1975) and for the armadillo (Weaker, 1977). Undifferentiated spermatogonia proliferate during the first spermatogonial cycle, while differentiated spermatogonia are produced in the second cycle. Regarding the topology of the three kinds of spermatogonia, our SEM study has clarified that the S-cells are located in the deep basal recesses and that the M- and L-cells are in the shallow recesses. Judging from the cytological, topographical and morphological relationships between these three kinds of spermatogonia and basal recesses, the M- and L-cells (possibly undifferentiated spermatogonia) are found in the shallow recesses of one epithelial segment and show probable representative cells in the first spermatogonial cycle. The S-cells (possibly differentiated spermatogonia) are located in the deep recesses of the other epithelial segment, being conceivably representative cells in the second spermatogonial cycle. It remained unclear, however, as to what stage of the spermatogonial cycle the disclosed epithelial segment corresponds. To clarify this problem, it will be necessary to use a combination of SEM and TEM.

2. Cordal arrangement and adluminal shift of the basally located germ cells
Recently, fibronectin has been demonstrated as occurring in the basement membrane of the seminiferous epithelium (Sakasita, 1983; Tung et al., 1984). This is similar to that found in other epithelia (Seguchi, 1985; Kondo, 1985). It is known that fibronectin serves as a substratum for moving mesodermal cells (Nakatsuji and Johnson, 1984; Darribere et al., 1985). Furthermore, it has been observed in cordally arranged mesenchymal cells in vitro that their cytoplasmic processes, such as lamellipodia and filopodia, elongate reticularly while showing repeated fusions and separations (Kaneko and Dan, 1986). Reticularly connected processes of L-spermatogonia were also observed by SEM. Interposing mitotic activities of these
L-cells conceivably change the arrangement of these cells and bring about their cord-like arrangement.

In contrast, S-spermatogonia exhibiting small spherical contour without cytoplasmic processes were located in the deep basal recesses. The basal area of these S-cells facing the basement membrane of the seminiferous epithelium was extremely small. Fibronectin conceivably has less effect on the migration of the S-cells along the basement membrane. Furthermore, it is known that shifting cells of the gastrula in vitro change from a spherical to a flat polarized contour and that their nonshifting cells are spherical (KOMAZAKI, 1986). The S-cells shifted adluminally, but did not migrate to form a cord along the basement membrane of the epithelium. The adluminal shift of the S-cells is probably related to the succeeding transformations which occur in the basolateral processes of adjoining Sertoli cells, in the junctional pattern of these processes and in the basal recesses as follows.

Basal processes of the Sertoli cells changed from thorny or conical to wedge-shaped contours with adluminal shifting of the S-cells. Conical processes have already been demonstrated by TEM, in Stage V of rat Sertoli cells (Wong et al., 1983). The wedge-shaped processes which surrounded the S-cells were first demonstrated in our SEM study and seemed to be a flattening of the conical processes. When the M- or L-cells migrate cordally, the wedge-shaped processes change into sheet-like processes to form the ceiling of the basal recesses.

When K⁺-pretreated and fixed tubules were chemically digested, most of the basal recesses in the seminiferous epithelium were easily observed by SEM. The basal recesses were recognized first as shallow separated lacunae, which gradually changed to continuous labyrinth-like trenches and finally modulated into the deep honeycomb-shaped lacunae during the progression of the spermatogonial cycle. The basal recesses were pleomorphic; these configurations matched those of the diverse spermatogonia. Transitional chambers were then formed from the ceiling of the basal recesses. These chambers have also been observed by TEM (RUSSELL, 1976). The formation of these chambers is coupled with a modulation of the sheet-like processes into cup-shaped ones. The cup-shaped processes then form the septum of the adluminal recesses in which primary spermatocytes are located.

The primary spermatocytes are joined by doughnut-like or linear bodies. These bodies conceivably represent gap junctions. RUSSELL and PETERSON (1985) have reported that these junctions between Sertoli cells and germ cells appear to facilitate the upward movement of germ cells. There are also two kinds of junctions between adjoining Sertoli cells. Gap junctions between Sertoli cells and germ cells have been observed frequently by routine TEM or TEM using the freeze-fracture technique (McGINLEY et al., 1980; RUSSELL, 1980; SZÖLLŐSI and MARCAILLOU, 1980; RUSSELL and PETERSON, 1985). Our SEM study has also demonstrated the existence of variable types of junctions between adjoining Sertoli cells, as previously reported in TEM and freeze-fracture studies (NICANDER, 1976; RUSSELL, 1976; PELLETIER and FRIEND, 1983; NAGANO and SUZUKI, 1983; RUSSELL, 1984; SUN and GONDOS, 1986). Among the basolateral processes of adjoining Sertoli cells, the formation of overlapping and/or close contact junctions was first found in our SEM study. These junctions changed to form seam lines (possibly tight junctions) and then the seam lines were resolved and eventually reformed to the former two types of junctions. It has also been confirmed that the junctional structures of the basolateral processes are dynamic in their cyclic elimination or turnover during spermatogenesis (NICANDER, 1967; RUSSELL, 1976; NAGANO and SUZUKI, 1983; PELLETIER and FRIEND, 1983; RUSSELL, 1984). Our SEM
study has demonstrated that many epithelial components, such as the spermatogonia, the basolateral processes of Sertoli cells and their junctional patterns, as well as the basal recesses, can mutually transform according to the stage of the spermatogonial cycle. Their transformations may be related to either the migration of M- or L-cells along the basement membrane of the epithelium or to the adluminal shift of the S-cells.

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