Scanning Microscopy

Volume 1 | Number 3 Article 39

4-20-1987

# Germ Cell-Somatic Cell Relationships: A Comparative Study of Intercellular Junctions During Spermatogenesis in Selected Non-Mammalian Vertebrates

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Scanning Microscopy, Vol. 1, No. 3, 1987 (Pages 1249-1255) 0891-7035/87$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

# GERM CELL-SOMATIC CELL RELATIONSHIPS: A COMPARATIVE STUDY OF INTERCELLULAR JUNCTIONS DURING SPERMATOGENESIS IN SELECTED NON-MAMMALIAN VERTEBRATES

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(Received for publication January 16, 1987, and in revised form April 20, 1987)

## Abstract

Specialized germ cell-somatic cell relationships were surveyed in the testis of species representative of four classes of non-mammalian vertebrates. Desmosome-like junctions were present in all classes studied. In the teleost fish studied (bluegill; Lepomis macrochirus), small, infrequent desmosomes, seen between the spherical cyst cells and spermatocytes, were characterized by poorly represented subsurface densities. In the bullfrog (Rana catesbeiana), similar desmosome-like junctions were found between cyst cell processes and spermatocytes. Reptilian (turtle; Pseudameys scripta) desmosome-like junctions between Sertoli cells and germ cells were heterogeneous and more numerous than those junctions found in fish and amphibians. In general, the reptilian desmosome-like junctions were extensive structures displaying 10 nm filaments associated with the Sertoli cell component of the junctions. Regions within the desmosome where the two plasma membranes converged suggested that gap junctions were a component of the desmosome-like junctions. "Desmosome-gap" junctions persisted in turtle spermatids for sometime after nuclear elongation had commenced. In birds (chicken; Gallus domesticus), "desmosome-gap" junctions, similar to those seen in turtles were described between both spermatocytes and Sertoli cells, and spermatids and Sertoli cells. These junctions were frequently lined by saccules of endoplasmic reticulum. The presence of gap junctions suggest the evolution of mechanisms for somatic cell-germ cell communication although more species should be examined to confirm this hypothesis.

Key Words: Gap junctions, Desmosome-like junctions, Testis, Spermatids, Spermatogenesis, Non-mammalian vertebrates, Cell junctions, Cyst cells, Sertoli cells, Germ cells.

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## Introduction

The rat and mouse, as well as other mammals have served as the primary models for understanding testicular structure and somatic cell-germ cell relationships. Thus, considerable information has been obtained and the topic reviewed (Russell, 1980; Russell and Peterson, 1985). Non-mammalian species possess a great diversity of testicular organization (Roosen-Runge, 1962; Van Tienhoven, 1983) as well as great variations in sperm morphology (Retzius, 1904). Investigations, in non-mammalian species, have focused primarily on acrosomal, nuclear or flagellar changes occurring in germ cells during spermiogenesis (Dadone and Narbaitz, 1967; Gronberg and Telkka, 1968; Nicander, 1970). Studies generally have not been expanded beyond this scope although some exceptions are noted (Zlotnik, 1947; Clark, 1967; Gunwardana and Scott, 1977; Grier, 1981).

In particular, there are few studies of somatic cell-germ cell junctions in non-mammalian species. We note, however, that Osman et al. (1980), Cooksey and Rothwell (1973), and Xia et al. (1986) have reported some data on junctions for the chicken testis. We set forth to examine and to characterize adhering (desmosome-like junctions) and communicating (gap junctions) cell-to-cell relationships in species representing four classes of non-mammalian vertebrates.

## Materials and Methods

### Animals

The class and species of adult animals utilized were: Pisces (bluegill, Lepomis macrochirus), Amphibia (bullfrog, Rana catesbeiana), Reptilia (turtle, Pseudameys scripta) and Aves (chicken, Gallus domesticus). Bluegills were obtained locally (Carbondale, IL) and bullfrogs were obtained both commercially (Kons Sci., Germantown, WI) and locally from April-July. Turtles were obtained both commercially (Kons Sci., Germantown, WI) and locally June-August. Chickens were purchased from a local breeder in July.

### Tissue Preparation

All species were perfused through the vascular system using hydrostatic pressure (hanging bottles; 122 cm elevation). Bluegills,

anesthestized with Quinaldine®, were perfused through a 23-gauge needle inserted into the atrium. After severing the hepatic portal vein, a Ringer's solution (0.65% NaCl; Humason, 1972) was briefly perfused until the gills and other major vessels cleared of blood. Subsequently, the animal was perfused with cacodylate (0.05M; pH 7.4) buffered 5% glutaraldehyde fixative for one hour. Perfusion success was approximately 50%.

Bullfrogs were pithed and then perfused via the truncus arteriosus with a 23-gauge needle. After severing the ventricle, Ringer's solution was perfused until the liver cleared. The animals were subsequently perfused with fixative for 1 hour. Perfusion success was approximately 90%.

Turtles were anesthetized with Nembutal® (20 mg/kg body weight) and the plastron removed to expose the heart. The pericardial sac was opened and heparin (1.0 ml) was injected directly into the ventricle. Fifteen minutes later an 18-gauge needle was inserted into the arch of the right aorta and secured with suture. The ventricle was cut and turtle Ringer's (Humason, 1972) briefly perfused through the animal until the liver cleared of blood. The animals were subsequently perfused with fixative for 45 minutes. The perfusion success rate was 90-95%.

For chickens, heparin was injected i.p. 15 minutes prior to perfusion (Russell et al. 1986) and animals anesthetized with 1.0-1.5 ml Nembutal® (25 mg/kg body weight). Animals were perfused through an 18-gauge needle inserted into the ascending part of the arch of the aorta, with bird Ringer's (Humason, 1972) until the liver cleared, and then with fixative for 1 hour. The perfusion success rate was approximately 75%.

After perfusion with fixative the testes were removed, diced with a razor blade and immersed in fixative for 1 hour. Tissues were washed in their respective buffer solutions for 24 h, postfixed in a mixture of 1.0% $OsO_4$ and 1.25% $K_4Fe(CN)_6$ (Russell and Burquet, 1977), dehydrated in ascending concentrations of ethanol, infiltrated with propylene oxide and embedded in Araldite (CY 212). Thick sections were obtained and appropriate areas selected for thin sectioning. Animals not undergoing active spermatogenesis i.e. animals in which all phases of spermatogenesis were not present, were excluded from the study. Silver and silver-gold sections stained with uranyl acetate and lead citrate were examined on a Hitachi H500H electron microscope.

## Results

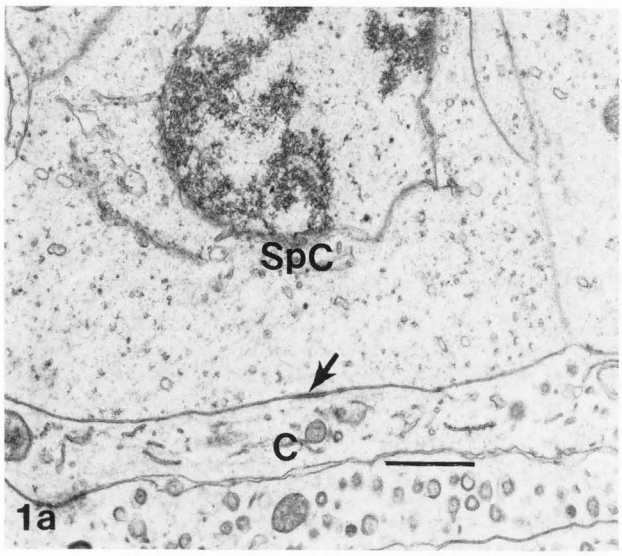
The general organization of the testis in non-mammalian vertebrates has previously been described (Rugh, 1939; Risley, 1938; Zlotnick, 1947; Aire et al., 1980; Grier, 1981; Van Tienhoven, 1983; Mendonca and Licht, 1986). Briefly, pisces demonstrate spermatogenesis within cysts whose walls are formed by somatic cells (often referred to as nurse cells, cyst cells or Sertoli cells). The organization of reptilian and avian testes closely resembles the mammalian testis in that they show seminiferous tubules containing somatic cells (Sertoli cells) and associated germ cells, many of which are adlumenal and thus not sequestered by junctions from the lumenal environment. Amphibian spermatogenesis begins in cysts but during mid-spermiogenesis cysts open to expose elongating spermatids to the tubular lumen.

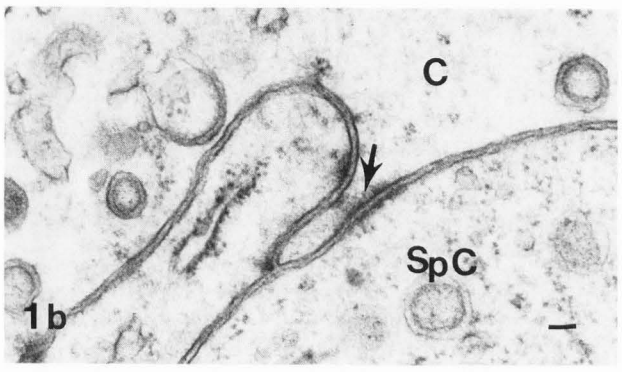
Classical appearing desmosomes (Farquhar and Palade, 1963) were not seen in any species; however, rudimentary junctions which we have termed as being "desmosome-like" (Russell, 1977) are present in all of the representative species studied. In the bluegill this junctional type was only seen joining spermatocytes to cyst cells. The spermatocyst was formed by a single somatic cell and contained germ cells in synchronous stages of development. No processes from the cyst cell extended inward among germ cells. Desmosome-like junctions were seen between germ cells and the cyst wall (Fig. 1a). Poorly represented subsurface densities of the desmosome-like junction (up to 35 nm in height) were the characteristic feature noted. The intercellular space was unmodified in width but generally contained a moderately dense material (Figs. 1b and 1c).

Unlike the bluegill, bullfrog cyst cells frequently exhibited processes extending between germinal cells within the cyst. It was in this position that small desmosome-like junctions were occasionally seen between spermatocytes and cyst cells (Figs. 2a and 2b). The subsurface densities of such junctions were more pronounced than those noted in the bluegill.

Reptiles and birds demonstrate seminiferous epithelia in which the somatic cells are termed Sertoli cells. In turtles, desmosome-like junctions were seen between germ cells (primary spermatocytes, Figs. 3a-c; spermatids, Figs. 3d-e) and Sertoli cells and these junctions could be described as morphologically heterogeneous. The simplest spermatocyte junctions were best described as areas of apposed plasma membranes separated by a 16-18 nm space. The junctions were flanked by sparse subsurface densities (Fig. 3a). Other junctional sites showed an abundance of 10 nm filaments associated with the subsurface density on the Sertoli aspect of the junction (Fig. 3b). More elaborate spermatocyte junctions displayed not only filaments but an additional area in which the plasma membranes converged focally to leave a 3-4 nm intercellular space (suggesting the presence of gap junctions; Figs. 3b-c). Gap junctions are usually indicated by the greatly reduced intercellular space such as that demonstrated herein, however, positive identification requires the verification of characteristic intramembranous particles using freeze-fracture techniques. Spermatid desmosome-like junctions seen in young round spermatids, remained associated with the spermatids until well after nuclear elongation and chromatin condensation had begun (Fig. 3d). Smooth endoplasmic reticulum was closely associated with both sides of the desmosome-like junction.

Chicken desmosome-like junctions were abundant, quite variable in appearance and were



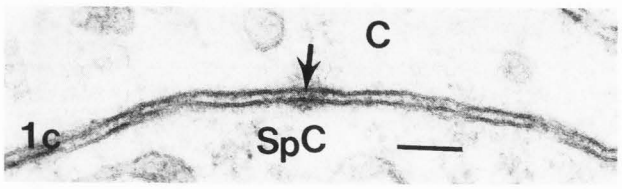
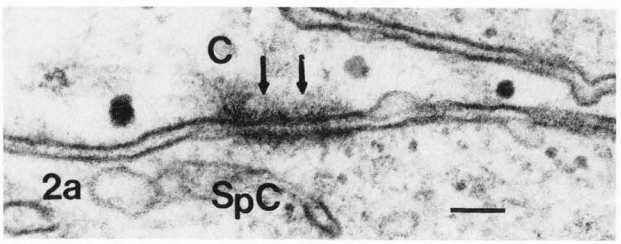




Fig. 1. Bluegill testis showing the relationship of somatic or cyst cells to germ cells. In Figure 1a, a portion of a spherical cyst cell (C) is related to a spermatocyte (SpC) on one of its surfaces. A small desmosome-like junction is indicated (arrow). Bar = 1.0 µm. At a higher magnification (1b and 1c; Bar = 0.1 µm) other junctions (arrow) characterized by dense material on both the cyst and germ cell counterparts are seen between a spermatocyte and cyst cell. Figures 1b-c show that the intercellular space is denser in the region of the junction.



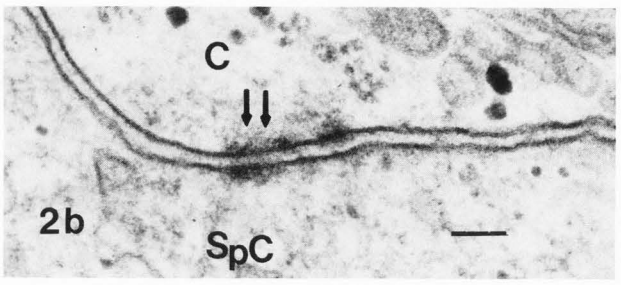
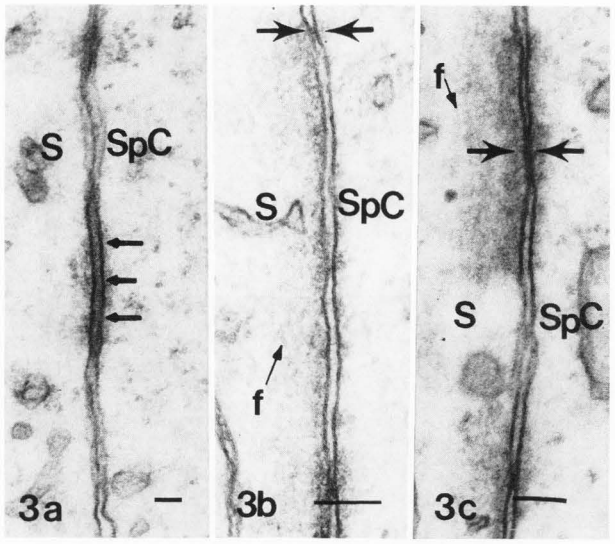




Fig. 2. Bullfrog desmosome-like junctions (arrows) between spermatocyte (SpC) and cyst (C) cell processes. A meager subsurface density is noted on both the cyst and germ cell aspect of the junctional site. Although the intercellular space is denser in the region of the junction, no intermediate dense line is present. Bar = 0.1 µm.

formed by Sertoli cells and germ cells (primary spermatocytes Figs. 4a-c; spermatids Figs. 4d-f). Desmosome-like contact between primary spermatocytes and Sertoli cells was generally extensive, however, small junctions were also noted (Fig. 4a). Endoplasmic reticulum was frequently seen associated with the Sertoli cell counterpart of the junction and to a lesser extent with the spermatocyte counterpart of the junction (Fig. 4c). The intercellular space (10-12 nm) was generally very uniform and occupied by dense material. Within the junctional domain some junctions showed apposed membranes 3-4 nm apart, suggesting the presence of focal gap junctions within the confines of the desmosome (Figs. 4b and 4d). In spermatids, junctions were characterized by focal accumulations of smooth endoplasmic reticulum within the Sertoli cell and to some degree within the spermatid (Figs. 4e-4g). The smooth endoplasmic reticulum was generally uniformly spaced at 35-45 nm from the Sertoli plasma membranes and either lined the junction or extended deeply within the cell. Junctions were also present in spermatids which had initiated nuclear elongation and remain during the early phases of chromatin condensation (Fig. 4g).

## Discussion

The mammalian testis demonstrates an adhesive junctional type which is spot-like or macular yet has only a poorly represented or



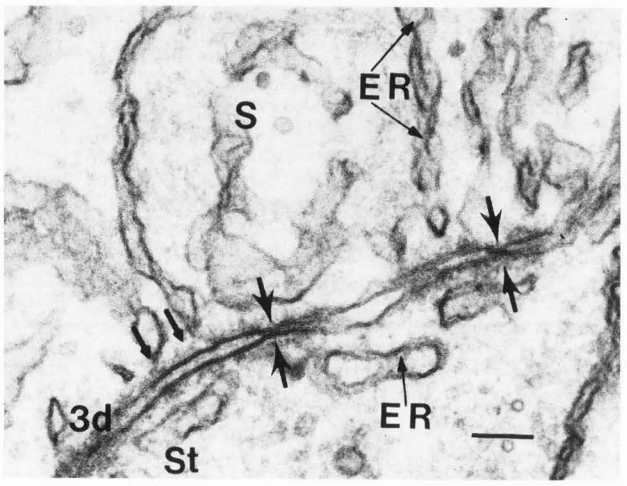




Fig. 3. Turtle desmosome-like (isolated arrows) and gap junctions (opposing arrows) between spermatocytes (SpC) and Sertoli cells (S) showing the heterogeneity of these junctions. In Figure 3a, the least elaborate junctional form displays evenly spaced plasma membranes, a dense intercellular space (~15-20 nm across) and sparse subsurface densities. In Figures 3b and 3c the junction is extensive showing prominent subsurface densities as well as 10 nm filaments(f) on the Sertoli counterpart of the junction. In some regions the membranes are closely opposed with an apparent 3-4 nm intercellular space and are presumed to be gap junctions. Figure 3d shows a turtle desmosome-like junctions (small arrows) and associated focal gap junctions (opposing arrows) between an elongating spermatid (St) and a nearby Sertoli cell (S). Subsurface densities are associated with the Sertoli cell and spermatid counterparts of the junction. In some regions the plasma membranes converge leaving a 3-4 nm intercellular space. These regions are assumed to be gap junctions. Smooth endoplasmic reticulum (ER) is associated with both the Sertoli cell and germ cell aspect of the junction. Bar = 0.1 μm.

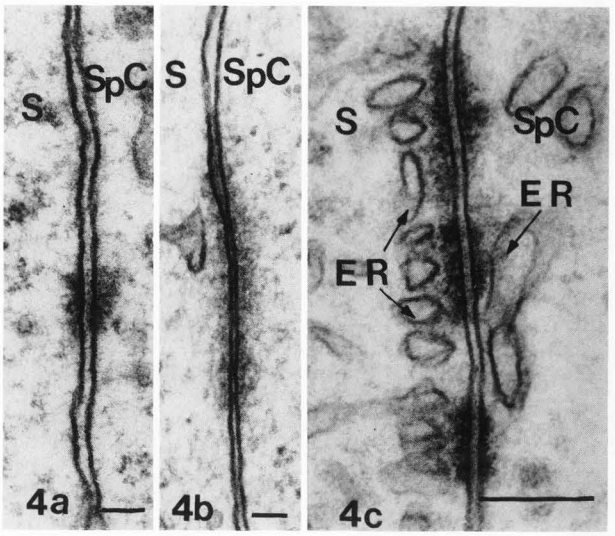
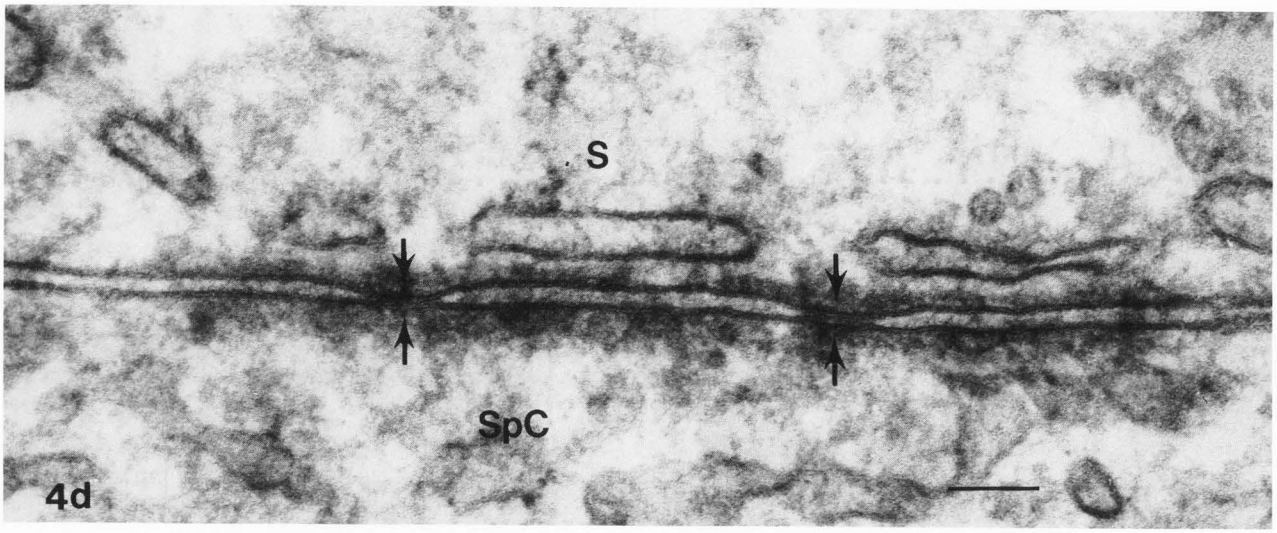


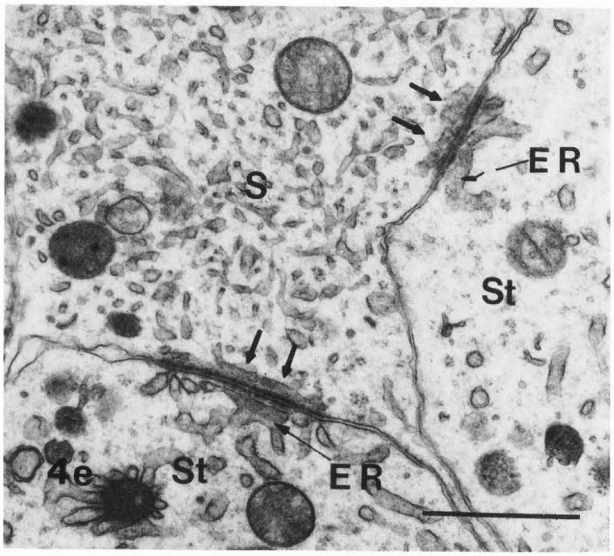

Fig. 4a-c. Rooster desmosome-like junctions found between spermatocytes (SpC) and Sertoli cells (S). Figures 4a and 4b represent the least elaborate junctional types seen. Subsurface densities are found associated with the Sertoli and germ cell counterparts of the junction and in some regions (Fig. 4b) the intercellular space is greatly reduced. Bar = 0.1 μm. Figure 4c represents a more elaborate spermatocyte-Sertoli cell desmosome-like junction which is characterized by a more prominent subsurface density associated with both the spermatocyte and Sertoli cell counterparts of the junction. Cisternae of smooth endoplasmic reticulum (ER) are associated with the surface specializations in both the Sertoli and germ cell counterparts of the junction. Bar = 1.0 μm. Figure 4d shows a junction at a high magnification in which the intercellular space is reduced focally to 3-4 nm in two regions (opposing arrows). Bar = 0.1 μm.

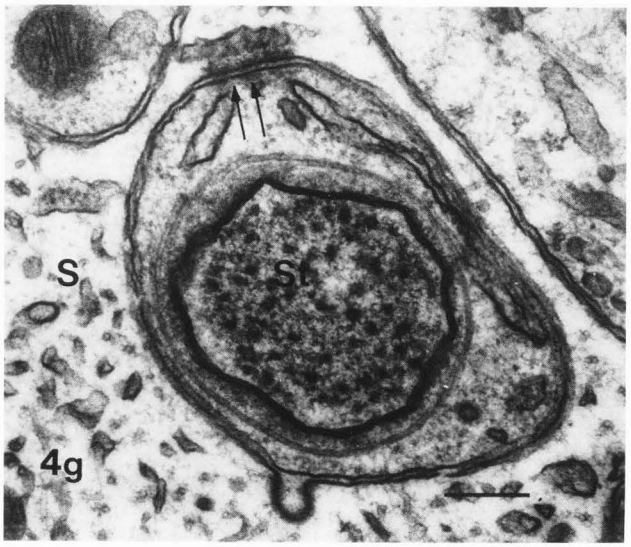
Fig. 4e. A survey micrograph showing the relationship of young round spermatids to the body of an adjacent Sertoli cell. Desmosome-like junctions (arrows) are seen joining these two cells in this region. Endoplasmic reticulum is preferentially associated with the subsurface density in both the spermatid and Sertoli aspect of the junction. Bar = 1.0 μm.

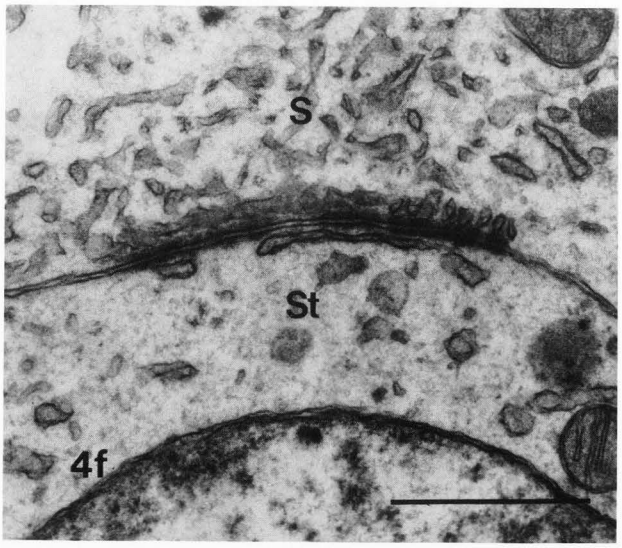
Fig. 4f. A high magnification micrograph showing the type of junction illustrated in Figure 4e. Endoplasmic reticulum is associated with the junction and dense material is present in the intercellular space in the region of the junction. Bar = 1.0 μm.

Fig. 4g. A survey micrograph showing an elongating spermatid undergoing nuclear condensation. Junctions similar to those shown in Figure 4c, but smaller, were generally noted (arrows). Bar = 1.0 μm.




non-existent intermediate dense line appearing, in section like that of a zonula adherens (Russell, 1977). Since this junctional type does not conform to any one of the categories of classical junctions (Farquhar and Palade, 1963; Staehelin and Hull, 1978), we referred to it as "desmosome-like" (Russell, 1977). It most closely resembles a macula adherens since it is both macular and frequently shows 10 nm filaments. The present study has shown that non-mammalian vertebrates also possess germ cell-somatic cell specializations resembling those of mammals which we also term as being "desmosome-like." The peculiar characteristics of testis desmosome-like junctions in non-mammalian vertebrates and mammals (lack of an intermediate dense line as well as an asymmetrical distribution of intermediate filaments) suggests that they are highly conserved during evolution.

In the fish (bluegill) and amphibian (bullfrog) studied, junctions were rarely found,

poorly developed and only noted between spermatocytes and cyst cells. The cysts cells, which completely surrounded the germinal elements, provided a closed environment which would sequester germinal cells and allow clones of like cells to develop simultaneously. As noted above, the elongate spermatids of the bullfrog are exposed to the tubular lumen by virtue of cyst opening. These spermatids appear to be held by another holdfast device--the ectoplasmic specialization (Sprando and Russell, 1987). Thus, there appears to be little necessity for specialized cell-to-cell adhesion properties in species with cystic spermatogenesis.

Representative reptilian (turtle) and avian (chicken) species displayed numerous desmosome-like junctions. In these species the majority of round germ cells were positioned on the lateral aspect of Sertoli cells (terminology of Wong and Russell, 1983). In this position the germinal cells would be vulnerable to a variety of tissue stresses which could easily displace them into the lumen of the seminiferous tubule. Thus it might be expected in such a situation that the desmosome-like junctions would be well-developed and numerous. Desmosome-like junctions were present in early turtle and chicken spermiogenesis until mid-spermiogenesis when another holdfast device developed (the ectoplasmic specialization; Sprando and Russell, 1987). The reptilian (turtle) junction most closely represented that seen in mammals (Russell, 1977). The chicken demonstrated junctions which were modified by an accumulation of smooth endoplasmic reticulum adjacent to the subsurface density of the junction. A very recent report notes the presence of this junction-associated smooth endoplasmic reticulum in chickens, but no function was suggested for this array of ER (Xia et al., 1986).

The turtle and chicken also showed a further modification of the desmosome-like junction--that being the presence of apparent focal gap junctions. Such junctions have been described for the rat where the plasma membranes of two cells converge to leave a narrow (2-4 nm) intercellular space and where freeze-fracture shows small aggregations of intramembranous particles characteristic of gap-junctions (Russell, 1977; McGinley et al., 1979; Pelletier and Friend, 1983). Very little is known about physiologic cell-to-cell communication via gap junctions in general, and even less is known about the smaller varieties of gap junctions such as those described in the present report and how they might play a role in communication between Sertoli cells and germ cells. Their presence in reptiles and in birds suggests that non-cystic spermatogenesis is modified sufficiently to require direct Sertoli-germ cell coupling, whereas, cystic spermatogenesis provides a closed environment controlled by the spherical nature of the cyst cells and junctions between them (Sertoli cell barrier). In such a closed environment direct somatic cell-germ cell communication may not be necessary. That amphibian germ cells have been shown to undergo substantial development in vitro without the presence of cyst cells argues for the relative unimportance of somatic-germ cell, cell-to-cell communication (Risley and Eckhardt, 1979).

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## Discussion with Reviewers

Y. Clermont: Is it not premature to call such junctions "desmosome-gap" junctions when the direct demonstration of gap-component, i.e., connexons, have not been demonstrated yet in such location by means of scanning electron microscopy on freeze-fractured material?
Authors: We agree that absolute proof would require freeze fracture; however, we believe we are safe in calling these junctions "desmosome-gap" based upon their striking similarity to those junctions in mammals where connexons have been demonstrated (McGinley et al., 1979).

Y. Clermont: Is it not dangerous to make generalizations about the evolutionary pathways of somatic cell-germ cell communication from the examination of only four animal species? If one considers the wide diversity of intercellular junctional structures, are the observations reported here considered a sufficient sample? Is it possible that what is observed here is the expression of the variability in structure of junctional structures, and that such a variation has no direct relation on the position of animals on the evolutionary scale?
Authors: Because we cannot examine every non-mammalian species, we only make suggestions about the evolutionary features of each class, but we certainly agree that more sampling is necesary to solidify our statements regarding evolutionary features of junctions.

Reviewer IV: Your micrographs, especially Figure 4c, show alignment of SER with desmosomal junctions. What, in your opinion, is the structural-functional significance of this?
Authors: We do not know their function but the micrographs and published reports (Russell, 1977) indicate that smooth endoplasmic reticulum is only seen in the reptiles and birds but is not present in amphibia and mammals. The general functional significance of junction-associated endoplasmic reticulum is thus in question.

Reviewer IV: Might the atypical structure of desmosomes which you describe be a reflection of the rapid turnover in the testis?
Authors: The dynamics of spermatogenesis might certainly be reflected in desmosome structure as is suggested for mammals (Russell and Peterson, 1985).

Reviewer IV: The desmosomes of the rooster between spermatocytes and Sertoli cells appear to have a dense central line...was this investigated at higher magnification (resolution)?
Authors: We searched our micrographs and screened those published and did not find a dense central line. These findings are similar to Cooksey and Rothwell (1973) and Osman et al. (1980).

M.R. Bakst: Speculate on the relationship, if any, between the type of cell junctions observed between germ-somatic and somatic-somatic cells in the four classes of animals examined and the establishment of a blood-testis barrier as described by Dym (1973) in the monkey and Osman et al. (1980) in the chicken.
Authors: In both mammals and non-mammalian vertebrates studied to date, the Sertoli cell barrier excludes materials from the circulatory and lymphatic systems. There may not be a relationship between the junctions forming the Sertoli cell barrier and those forming between somatic cells and germ cells.

M.R. Bakst: Clarify what constituted a successful perfusion.
Authors: The testis clears of blood, turns slightly yellow with time and hardens.

## Additional Reference

Dym M. (1973). The fine structure of the monkey (Macaca) Sertoli cell and its role in maintaining the blood-testis barrier. Anat. Rec. 175, 639-656.