Cell-Cell Interactions in the Testis*

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I. Introduction

A. Concept and categorization of cell-cell interactions

THE ability of cells to interact and communicate is a biological phenomenon that developed with the evolution of multicellular organisms. The cell-cell interactions that have evolved are essential for the survival of both simple organisms such as dicyostelium and complex organisms such as mammals. These cellular associations allow an organism to develop capacities that are greater than the simple sum of their individual parts. As early as 1878, Claude Bernard proposed that the "milieu interieur" (i.e. internally produced fluid environment) and a cybernetic-like control system (i.e. cell-cell interactions and communication) in multicellular organisms are needed to adaptively regulate the growth, development, and maintenance of normal tissue function (1, 2). The experimental analysis of cellular interactions was initiated with the investigation of cell aggregation in simple organisms such as sponges (3, 4) and progressed into the areas of embryology (5) and cell biology (6, 7). Analysis of cell biology on a molecular level has revealed the importance of cell-cell interactions during embryogenesis, organogenesis, and determination of cell lineage (reviews in Refs. 8–12). Alterations in these cell-cell interactions, therefore, may cause abnormal tissue physiology associated with disease states. Disorganization of normal cell-cell and cell-basement membrane interactions is associated with the process of metastasis and malignancy (13–16). Alterations in cellular differentiation associated with cell-cell interactions will also result in abnormal tissue functions (17, 18). Further examination of the cellular and molecular biology of abnormal tissue physiology is speculated to reveal that many cellular oncogenes (19) and tumor suppressor genes (20) may be associated with cell-cell interactions (21).

The increasing awareness of the importance of cell-cell interactions has become critical in disciplines such as endocrinology, cell biology, and pharmacology. This is illustrated in the more than 700 literature citations during the past year on the topic of cell-cell interactions. The cellular interactions identified have also increased dramatically in diversity. A categorization of cell-cell interactions is proposed to help classify the different types of interactions as well as understand the interrelationships between the different interactions in the control of tissue function (Table 1 and Ref. 22). Cellular interactions are classified into three general categories of environmental, nutritional, and regulatory interactions. The categorization of cell-cell interactions presented provides a vocabulary with which to distinguish the various types of interactions as well as classify the effects of specific interactions on cellular physiology. The classification of cell-cell interactions (22) shown in Table 1 will be used to more thoroughly discuss the specific cell-cell interactions in the testis.

B. Testis cell biology and function

The observation that early man domesticated animals through castration implies that the testis is likely one of the first organs investigated on a physiological level. This is supported by reference to the testis in ancient Greek, Roman, Egyptian, and Assyrian writings (23–26). Scientists engaged in the developing discipline of anatomy retained this interest in reproduction and

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investigated the details of testis and sperm cell structure, which played a role in the development of the cell theory (27-30). The advent of the light microscope facilitated anatomical analysis of the testis with often surprising detail (31-33). More recently, investigators (34-39) have explored the ultrastructure of the testis by means of the electron microscope. Comparative anatomy of the testis of a number of species indicates that although differences can be observed, the process of spermatogenesis and cellular associations are similar in many animals (40, 41).

In most mammals the process of spermatogenesis occurs within seminiferous tubules that release spermatzoa into a rete testis which is connected to the epididymis (Fig. 1). The seminiferous tubules are formed by the Sertoli cells that provide structural support for the developing germinal cells. Peritubular myoid cells surround the tubule and are in contact with the basal surface of the Sertoli cells. In the interstitium of the testis between tubules are the Leydig cells responsible for the production of androgen. The majority of research on the cell biology of the testis has focused on Sertoli, peritubular myoid, Leydig and/or developing germinal cells. Cell-cell interactions between all three of the somatic cells are possible; however, Sertoli cells appear to be the primary somatic cell to directly interact with the developing germinal cells. The cellular associations and interactions illustrated in Fig. 2 will be reviewed.

In addition to investigation of the anatomy of the testis, the observation of Smith in 1930 that removal of the pituitary inhibited the process of spermatogenesis (42, 43) initiated an intense investigation of the endocrine regulation of testis function that continues today (44, 45). Initial speculation was that hormones acted directly on germinal cells (46-49). Subsequent research and consideration of testis physiology, however, demonstrated that the actions of hormones are primarily confined to the somatic cells (review in Refs. 50 and 51). The appreciation that the endocrine hormones, particularly androgens, acted directly on the somatic cells of the testis, and not the germinal cells, led to initial speculations reviewed by Fritz in 1978 (50) regarding the potential importance of cell-cell interactions in the endocrine regulation of testis function.

Recent studies have identified numerous secretory products of specific testicular cell types. These products range from steroids to specific secreted proteins. Inter-

### Table 1. Categorization of cell-cell interactions

<table>
<thead>
<tr>
<th>Classification</th>
<th>Definition</th>
<th>Examples/mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental</td>
<td>Interactions that influence the extracellular environment of the cell to affect cell contacts and cytoarchitecture</td>
<td>Extracellular matrix; cell adhesion molecules</td>
</tr>
<tr>
<td>Nutritional</td>
<td>Interactions involved in the delivery of essential nutrients between cells</td>
<td>Transfer of energy metabolites, metals, or vitamins</td>
</tr>
<tr>
<td>Regulatory</td>
<td>Agents provided by a cell that through a signal transduction event regulates another cell's function on a molecular level</td>
<td>Paracrine/autocrine factors: growth factors; differentiation factors; cytokines</td>
</tr>
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![Fig. 1. Cell biology of the testis.](image-url)
estingly, most of the secretory products identified appear to be involved directly or indirectly in cell-cell interactions. For this reason, the major secretory products of Sertoli cells, Leydig cells, and peritubular cells will be categorized and tabulated for subsequent reference.

The secretory products of Sertoli cells and their proposed functions have previously been reviewed (37, 50, 52–59). The major Sertoli cell products (60–102) are shown in Table 2 and include transport/binding proteins, proteases, extracellular matrix components, growth factors, and cellular metabolites. These Sertoli cell secretory products will subsequently be discussed in reference to specific cell-cell interactions.

The critical secretory role of the Leydig cell was established with the early observations that Leydig cells are the primary site of androgen production (103, 104) which is under endocrine control (reviews in Ref. 105–107) and essential for the maintenance of spermatogenesis and the endocrine status of the male. Nonsteroidal Leydig cell secretory products have not been thoroughly investigated. Although few secretory products have been identified and/or characterized, the major secretory products known (Table 3) appear to be involved in cell-cell interactions.

The peritubular myoid cell has also recently been shown to secrete components that may be important for the maintenance and control of testicular function. As found with Sertoli cells and Leydig cells, the secretory products of peritubular cells identified (Table 3) also appear to be involved in cell-cell interactions.

C. Analysis of cell-cell interactions

A number of different experimental approaches can be taken to investigate cellular interactions and identify potential autocrine and paracrine agents (Table 4). A thorough examination requires a number of criteria to be considered including localization, production, secretion, characterization, action, and physiology. Few testicular cell interactions examined have considered all the criteria listed in Table 4. Therefore, examination of cell-cell interactions in the testis requires a critical consideration of the data available to determine which cellular interactions are simply speculated to exist vs. those more thoroughly established as important physiologically.

As demonstrated with many organs and tissues (121–125), the advent of cell culture procedures has provided

![Cell-cell interactions in the testis.](image-url)
TABLE 3. Major Leydig cell and peritubular cell secretory products

<table>
<thead>
<tr>
<th>Secretory product</th>
<th>Function and/or characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen (103, 104)</td>
<td>Steroid/endocrine/paracrine agent</td>
</tr>
<tr>
<td>POMC peptides (108-112)</td>
<td>Opioids/POMC regulatory agents</td>
</tr>
<tr>
<td>Inhibin (113)</td>
<td>Endocrine/paracrine regulatory agents</td>
</tr>
<tr>
<td>IGF-I (114)</td>
<td>Maintenance growth/differentiation</td>
</tr>
<tr>
<td>PMoS (117-119)</td>
<td>Paracrine regulatory agent</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor (120)</td>
<td>Inhibition of plasminogen activator activity</td>
</tr>
<tr>
<td>Fibronectin (115-116)</td>
<td>Extracellular matrix component</td>
</tr>
<tr>
<td>Collagen I (79)</td>
<td>Extracellular matrix component</td>
</tr>
<tr>
<td>Proteoglycans (80)</td>
<td>Extracellular matrix component</td>
</tr>
<tr>
<td>TGFs (81)</td>
<td>Growth stimulation/EGF-like</td>
</tr>
<tr>
<td>TGFβ (82)</td>
<td>Growth inhibition</td>
</tr>
<tr>
<td>IGF-I (114)</td>
<td>Maintenance growth/differentiation</td>
</tr>
</tbody>
</table>

a significant advance in an understanding of the molecular and cellular aspects of testis function. Initially organ culture of the testis was utilized (124-126); however, individual cell types were subsequently isolated and cultured to provide more definitive information regarding their functions and hormone responses. Methods have been developed to isolate purified preparations of Leydig cells (127-134), germinal cell populations at various stages of development (135-146), Sertoli cell populations (147-160), and peritubular myoid cells (161-165). Histochemical and immunocytochemical procedures have been developed to assess the purity of cell preparations (161, 162, 165, 166). Several cell lines have been developed for Sertoli cells and Leydig cells (167-169) which are useful for investigation of parameters such as signal-transduction events or when a product of interest is produced by the cells. The majority of experiments regarding cell-cell interactions in the testis have used primary cell cultures.

When an in vitro experimental approach is utilized it is inevitable and appropriate that questions regarding physiological relevance be raised (170). Eagle (171) provided insight early in the establishment of in vitro cell culture procedures when he stated "the study of discrete cells will not suffice to explain the interaction of many different cell types associated into organs which in turn exercise a mutual control." Therefore, a combination of an in vitro analysis to investigate molecular parameters and an in vivo analysis to investigate physiological parameters will be most useful to elucidate cell-cell interactions.

II. Specific Cell-Cell Interactions

Consideration of cell-cell interactions in the testis began with the identification of multiple testicular cell types which initiated studies that have continued for the past 100 yr. The increased amount of research in the area of cellular interactions in the testis over the past 20 yr can be illustrated by the number of literature citations obtained from a National Library of Medicine literature search on the general topic of cell-cell interaction in the testis (Fig. 3). A significant and continuous rise in the number of citations per year is apparent. For this reason there have been a number of informative reviews (22, 50, 51, 172-191) that address selected cellular interactions, individual agents involved in specific cellular interactions, or the research associated with a given laboratory.

A. Sertoli cell-germinal cell interactions

The early microscopic examination of spermatogenesis and the development of the spermatozoa within the seminiferous tubules were active areas of research between 1830 and 1860 (192). Although the concept of a support cell for the developing germinal cells was discussed, this idea was not seriously considered by figures such as Koelliker who suggested the presence of polygonal cells as a paved epithelium. In 1865, however, Enrico Sertoli (193) described the existence of special branched cells in the seminiferous tubules of the human testis. Sertoli postulated "the function of the branched cells is linked to the formation of spermatozoa." Further analysis of the Sertoli cell and its association with the developing germinal cells led to the concept reviewed by Peter in 1899 (31) that this "nurse cell" is essential for the germinal cell and the process of spermatogenesis. The research that has progressed since that time has refined these observations and provided insight into the molec-
Environmental. The cytoarchitectural arrangements between Sertoli cells and the developing germinal cells provide one of the most complex examples of an environmental cell-cell interaction. The columnar and convoluted structure of the Sertoli cells, which extends from the basal to the apical surface of the seminiferous tubule (review in Ref. 37), provides the physical support for the spermatogonia undergoing mitosis, the spermatocytes undergoing meiosis, and the spermatids undergoing the process of spermiogenesis to become spermatozoa (reviews in Refs. 194–196). The Sertoli cell is also required to maintain the germ cell syncitium that connects all the cells derived from an initial clone of cells. One of the initial environmental interactions postulated to be important was identified between Sertoli cells near the base of the epithelium as tight and gap junctions referred to as junctional specializations (197–199). These tight junctions were found to exclude the passage of macromolecules from the interstitial space to the lumen of the seminiferous tubules (200–202). These observations provided support for the existence of a blood-testis barrier initially postulated from an examination of the ionic, amino acid, and protein content of testicular fluids from the tubule, lymph, and plasma (203–206). The environmental interactions between Sertoli cells mediated by this tight junction, therefore, are essential in the creation of the blood-testis barrier and the maintenance of the unique microenvironment within the seminiferous tubule required for germinal cell development (207).

Specific types of environmental interaction identified between Sertoli cells and germinal cells include the ectoplasmic specializations (208–210). These are flattened cisternae of the Sertoli cell endoplasmic reticulum that appear in Sertoli cell membranes facing spermatocytes and the acrosomes of the spermatids (197–199, 211–214). These junctional specializations have generally been considered attachment devices (198, 199, 208, 215). Another specific environmental interaction identified between Sertoli cells and germinal cells is referred to as the tubulobulbar complex (209, 216, 217). This is a narrow tubular projection of plasma membrane from the heads of maturation-phase spermatids that invaginate the adjacent plasma membrane of Sertoli cells (218, 219). The functional significance of these structures remains to be determined (220).

In an elegant set of experiments designed to investigate the complexity of the structural interactions between Sertoli cells and germinal cells, a three-dimensional structure of an individual Sertoli cell was determined (212, 221, 222). These studies of Russell and colleagues demonstrated that an individual Sertoli cell can be in contact with 5 adjacent Sertoli cells at the basal surface of the cell and 47 adjacent germinal cells at various stages of development. The morphology of the Sertoli cell, therefore, is exceedingly complex, and the ability of an individual cell to be in contact with greater than 50 adjacent cells indicates the importance of environmental interactions between the cells.

The presence of various generations of spermatocytes and spermatids derived from individual spermatogonia (223) initiates waves of spermatogenesis that are not random but instead occur in a specific cyclic manner referred to as stages (224). Progression of these stages of germinal cells is referred to as the cycle of the seminiferous epithelium and suggests potential alterations in cellular function during this cycle (187, 224–228). Changes that occur in Sertoli cell size (229, 230) and lipid content (225, 231, 232) during the cycle have been examined. Transillumination procedures to microdissect different stages have been developed (233, 234) and used to investigate biochemical aspects of the cycle (review in Ref. 187). Comparison of stages in rodents vs. humans (235) implies that the dynamics of the cycle can vary between species. Experiments recently designed to manipulate the cycle of the seminiferous tubule were based on the dependence of spermatogenesis on retinoids (236–239). Vitamin A deficiency causes an arrest at the preleptotene stage of spermatogenesis (238, 240). Morales and Griswold (241) found that replacement of vitamin A to deficient animals restores spermatogenesis in a synchronized stage-specific manner. All the seminiferous tubules in a synchronized testis appear to be at the same stage of the cycle, which provides a technical advance to study the cycle of the seminiferous epithelium as well as
environmental interactions between the Sertoli cells and germinal cells (241–243).

The continuous nature of germinal cell development and the presence of the cycle of the seminiferous epithelium indicate that environmental interactions between Sertoli cells and germ cells are dynamic and will require a rapid rate of tissue remodeling. The initial aspect of tubule remodeling considered was the need to translocate early stage spermatocytes from the basal compartment to the apical compartment through the tight junctional complex (244–248). The production of plasminogen activator and other protease activities by Sertoli cells (72, 77, 249) may be needed in several aspects of tissue remodeling associated with translocation of spermatocytes, degradation of junction complexes, and release of mature spermatozoa into the lumen of the tubule. The function and control of the proteolytic activities in the tubule requires further investigation.

The presence of junctional contacts between Sertoli cells and germinal cells observed microscopically suggested that these environmental interactions may be needed for cell attachment and association (250, 251). Germinai cell shape and junctional interactions can be maintained in vitro (252), and the viability of spermatogenic cells is facilitated by coculture with Sertoli cells (253). The binding of germ cells to Sertoli cells is specific and temperature dependent (254). Proteins on the surface of spermatogenic cells potentially involved in the environmental interaction with Sertoli cells have been identified (255, 256), and specific Sertoli cell-secreted proteins have also been speculated to be involved in this attachment process (257). Sertoli cells also must adhere firmly to the membrane of the residual bodies of released spermatozoa to prevent their loss from the surface of the seminiferous epithelium (215). These residual bodies must be freed from the released spermatozoa, remain attached to the Sertoli cells (209, 258), and subsequently be phagocytized and metabolized by the Sertoli cells (227, 259).

**Nutritional.** The transport of essential nutritional components between Sertoli cells and germinal cells is critical for germ cell survival and metabolism. The earliest reference to the Sertoli cell was with regard to its probable “nurse cell” function for the developing spermatogenic cells (31, 193). A number of Sertoli cell functions have been identified on a molecular level that are directly or indirectly involved in the nutritional support of the germinal cells. These nutritional interactions between Sertoli cells and germinal cells are required due to the presence of the blood-testis barrier (207, 260, 261). One of the major functions of the Sertoli cells that has evolved is the ability to transport essential nutritional components to the spermatogenic cells sequestered in a serum-free unique microenvironment.

Early during fetal development, junctional interactions between the germ cells and immature Sertoli cells may facilitate transfer of nutritional agents between the cells (262–265). In the adult testis a complex interaction, referred to as a desmosome-gap junction, forms between Sertoli cells and germ cells (198, 212, 263, 266, 267). These junctions appear to form primarily between Sertoli cells and pachytene spermatocytes and are less frequent with spermatids (212). These junctional complexes are permeable to agents with mol wt less than 600–700 (263) and appear to transfer metabolic substances such as choline between Sertoli cells and germ cells (268). Due to the existence of the germ cell syncitium and cytoplasmic bridges between germinal cells, junctional contacts may not be necessary between each spermatogenic cell and Sertoli cell. Further examination of the functional importance and the components transported by gap junctions between the Sertoli cells and germinal cells is required.

Developing spermatogenic cells sequestered within the blood-testes barrier are not exposed to the high glucose levels in the interstitial fluid. Localization of metabolic enzymes such as ATPase in the tubule (269) and demonstration that Sertoli cells produce glucose metabolites such as inositol (270) suggested that Sertoli cells may provide energy metabolites to germinal cells. Sertoli cells have been shown to metabolize glucose to lactate and pyruvate resulting in high levels of extracellular metabolites (89–91, 271) that increase in response to FSH (90, 91, 272–274). Both pyruvate and lactate were found to support germinal cell metabolism and appear to provide an efficient energy source for the cells (90, 91, 272, 275–277). Analysis of metabolic enzymes, such as lactate and malate dehydrogenases (278), and the actions of FSH on glucose transport by Sertoli cells (279) also suggest that Sertoli cells may provide energy metabolites to spermatogenic cells. Whether the lactate and pyruvate can be transported by gap junctions between the cells or by active transport across the plasma membranes remains to be determined. Although the physiological significance of pyruvate and lactate production by Sertoli cells remains to be elucidated, the sequestration of the developing germinal cells within the blood-testis barrier suggests that the Sertoli cells will likely be required to provide an energy source for spermatogenic cells. Other cellular metabolites, such as coenzymes, nucleotides, and amino acids, may also require a nutritional interaction between these cells to support the process of spermatogenesis.

Although many nutritional components are small soluble molecules that can be transported by junctional interactions or secreted, a large number of substances
require binding proteins to facilitate transport due to low solubility or reactive chemical properties. Functional identification of Sertoli cell-secreted proteins has revealed the production of a number of different transport proteins (reviews in Refs. 53–56). The Sertoli cells appear to acquire essential nutritional components at the basal surface of the cell from specific binding proteins present in the interstitial fluid. The serum binding proteins can enter the cell and transfer the nutritional component to a newly synthesized binding protein. The testicular binding protein can then be secreted by the Sertoli cell and transport the nutritional component to developing germinal cells sequestered within the blood-testis barrier (Fig. 4). The first Sertoli cell transport protein identified was androgen binding protein (ABP) (Refs. 60–62; review Ref. 53). Although ABP is a useful marker of Sertoli cell function (280), the transport of androgen to germinal cells is not required (50). The postulated function for ABP is to localize the transport androgens throughout the male reproductive tract; therefore, ABP does not appear to mediate a nutritional interaction between Sertoli cells and germinal cells. An abundant Sertoli cell secretory product functionally identified is testicular transferrin (63). Transferrin is an iron-binding protein, previously thought to be primarily produced by the liver, that binds and transports iron in the circulatory system. Iron is bound by transferrin with an extremely high affinity due to the low solubility of iron. All cells require iron for cytochrome activity and respiration (281). The transferrin produced by Sertoli cells is the same gene product as serum transferrin produced by the liver with differences in glycosylation of the two proteins (282, 283). Transferrin delivers iron to the cell through a receptor-mediated process. Transferrin and the receptor complex are endocytosed to an acidified vesicle where iron is released, and the transferrin and receptor complex is recycled to the cell surface (284, 285). Transferrin receptors are present on all cells, and high concentrations of transferrin receptors have been localized on pachytene spermatocytes (286, 287). The observations from a number of laboratories support this proposed transferrin-mediated nutritional interaction between Sertoli cells and germinal cells (63, 287–292). Another metal binding and transport protein found to be synthesized and secreted by Sertoli cells is ceruloplasmin (64). Ceruloplasmin is a copper transport protein, also thought to be primarily produced by the liver, that binds and transports copper to cells that require copper as a coenzyme for proteins and feroxidase (293, 294). A nutritional interaction is proposed for ceruloplasmin that is similar to transferrin with the exception that the ceruloplasmin receptor complex appears to be degraded upon internalization. The nutritional interaction between Sertoli cells and germinal cells involving ceruloplasmin is not as thoroughly characterized as the nutritional interaction mediated by transferrin.

Lipids are another nutritional component that generally require a binding protein for transport. Several unique fatty acids have been identified in Sertoli cells and germinal cells, and the potential transport of these fatty acids from Sertoli cells to germinal cells has been postulated (295, 296). Although the production of general lipid transport proteins by Sertoli cells has not been thoroughly investigated, one specific lipid binding protein has been identified. A major Sertoli cell-secreted protein referred to as sulfated glycoprotein-1 (SGP-1) (66, 67) has been identified as a unique form of a sphingolipid-binding protein involved in sphingolipid solubilization (67, 68). The precursor form of sphingolipid activator protein that is involved in sphingolipid binding and metabolism (68) has been shown to have a high degree of homology with SGP-1 (67, 68). The lipid specificity of SGP-1 is unclear, and the possibility that SGP-1 may be involved in more general lipid binding remains to be investigated. The rapid expansion of the germ cell population during spermatogenesis is anticipated to require the transport of lipid precursors and specific fatty acids between Sertoli cells and germinal cells (295). In addition, the metabolism and degradation of the residual body by Sertoli cells are also anticipated to require lipid-binding proteins. Further investigation of lipid transport and the binding proteins involved is required.
Sertoli cells will also likely have developed a mechanism with which to transport vitamins to spermatogenic cells. Many vitamins utilize binding proteins in the circulatory system to facilitate their transport and localization. Preliminary evidence exists for the production of vitamin-binding proteins by Sertoli cells, such as folate-binding protein and biotin-binding protein, that may transport these vitamins to germinal cells (296). Further characterization and investigation of potential Sertoli cell-derived vitamin-binding proteins are required. As discussed previously, one vitamin that has been shown to be essential for testis function and spermatogenesis is vitamin A, retinoids (236-239, 298). Cellular retinoid-binding proteins have been localized in Sertoli cells (299-301) and germinal cells (300, 302). Sertoli cells respond directly to retinol and retinoic acid with an increase in several functional parameters (303-307), including transferrin production (Fig. 5). Vitamin A deficiency also directly influences Sertoli cell functions (308-311). Retinoids appear to act on Sertoli cells through the nuclear retinoid receptor to directly regulate Sertoli cell functions. Information regarding the actions of vitamin A on spermatogenesis, however, imply that retinoids also directly influence germ cell development (236-243, 312).

Due to the low solubility of retinoids, binding proteins in the serum, retinol-binding protein, and the cell, cellular retinol and retinoic acid binding proteins exist to facilitate retinoid transport. The majority of retinol accumulation by Sertoli cells is esterified with lipids and apparently stored as compounds such as retinol-palmi-tate esters (313, 314). Retinol-binding protein facilitates the uptake of retinol by Sertoli cells (315) and whether retinol, retinoic acid and/or retinol-esters are transported to germinal cells remains to be investigated. Although several studies have implied that Sertoli cells may be capable of producing a retinoid binding protein (316, 317), the detection and characterization of a Sertoli cell-derived retinoid binding protein that may facilitate retinoid transport to germinal cells remain to be elucidated.

**Regulatory.** Regulatory interactions between Sertoli cells and germinal cells were proposed as the concepts of paracrine factors and local trophic agents developed. Initial morphological observations regarding alterations of Sertoli cell-germinal cell associations led to several laboratories to postulate the potential presence of such interactions. Disruption of testis cell associations and morphology by heat (318), irradiation (319-321), cytotoxic agents (322), or disease states (323) suggested that regulatory interactions may exist between Sertoli cells and germinal cells. Analysis of the expression of specific genes in germ cells and Sertoli cells at different stages of the seminiferous cycle (324) and the effects of removal of germ cells on Sertoli cell products in vivo also suggested that germ cells may regulate Sertoli cell function (325, 326). In addition, the ability of hormones to indirectly influence germinal cell development through actions on the Sertoli cells supports the presence of interactions between the cells (50, 327, 328). Although these experiments and observations imply that interactions appear to occur between Sertoli cells and germinal cells, the specific type of interaction involved can not be distinguished. As previously discussed, alterations in critical environmental or nutritional interactions may have profound effects on spermatogenic cells and Sertoli cells. Further investigation of potential regulatory interactions between Sertoli cells and germinal cells requires the elucidation of the paracrine factors involved.

Initial experiments to investigate the effects of Sertoli cells on germinal cells utilized coculture of the cells. The presence of Sertoli cells in culture with spermatogenic cells has been shown to stimulate germ cell RNA and DNA synthesis (329), induce the appearance of germ cell surface antigens (330), and maintain spermatogenic cell glutathione synthesis (331). Although these observations do not demonstrate the presence of a regulatory interaction, these experimental systems may be used in the future to detect the potential presence of Sertoli cell-derived paracrine factor(s). Several Sertoli cell secretory products have been identified that may mediate regulatory interactions between Sertoli cells and germinal cells. Insulin growth factor-I (IGF-I) has been shown to be produced by Sertoli cells (83-86) and may act on meiotic spermatogenic cells at IGF-I receptors (85, 332). IGF-I has been shown to act on most cell types to maintain a number of cellular functions and regulate DNA synthesis. Since IGF-I is a ubiquitous paracrine factor, actions on germinal cells will likely be similar to those on somatic.
CELL-CELL INTERACTIONS IN THE TESTIS

February, 1991

53

cells. Further investigation of IGF-I and IGF-I receptor
gene expression and production by cells of the seminif-
erous tubule is required. Examination of direct actions
of IGF-I on germinal cells is also needed to further
elucidate IGF-I mediated regulatory interactions be-
tween Sertoli cells and spermatogenic cells. Two other
Sertoli cell-derived growth factors that have not been
fully characterized, but are speculated to act on germ
cells, are the seminiferous growth factor (SGF) (333–
335) and Sertoli cell-secreted growth factor (SCSGF)
(336). The mitogenic properties of SGF and SCSGF,
however, have been demonstrated on somatic cells, but
not spermatogenic cells. Further investigation is required
to determine whether SGF or SCSGF may mediate reg-
ulatory interactions between these cells and promote
growth of developing germinal cells. Transforming
growth factors-α (TGFα) and β (TGFβ) have also re-
cently been shown to be produced by Sertoli cells (81,
82) and potentially could influence germinal cells. Initial
studies imply that developing germinal cells do not con-
tain the epidermal growth factor (EGF) receptor (81,
337). The actions of TGFα on spermatogenic cells, there-
fore, appear unlikely but remain to be thoroughly inves-
tigated. TGFβ is similar to IGF-I in that it is a ubiquitous
paracrine factor that acts on many cell types. TGFβ may
mediate a regulatory interaction between Sertoli cells
and germinal cells; however, the actions of TGFβ on
spermatogenic cells remain to be elucidated. Other reg-
ulatory agents postulated to be produced by Sertoli cells
and act on germinal cells, such as interleukin-1 (IL-1)
that may stimulate germ cell growth (88, 190, 338) and
insulin growth factor-II (IGF-II) that may influence germ

cell metabolism (339), also require further examination
of both the sites of production and action of these poten-
tial paracrine factors.

Initial experiments to investigate the effects of ger-

minal cells on Sertoli cells also utilized coculture of the
cells. The presence of germinal cells in culture with
Sertoli cells has been shown to influence Sertoli cell
protein glycosylation (340), increase basal and FSH-
stimulated ABP production by Sertoli cells (341–343),
inhibit estradiol production by Sertoli cells (343), and
influence the vectorial secretion of ABP by Sertoli cells
(344). Pachytene spermatocytes generally were found to
have more dramatic effects than late stage spermatids,
and the effects of germ cells on Sertoli cells may be age
dependent (343, 345). These observations have been ex-
tended through analysis of the effects of conditioned
medium obtained from spermatogenic cell cultures on
Sertoli cells. Germ cell-conditioned medium was found
to stimulate the phosphorylation of specific proteins in
Sertoli cells (346); stimulate γ-glutamyl transpeptidase
activity in Sertoli cells (347); increase ABP production
and decrease estradiol synthesis by Sertoli cells (348,
349); decrease RNA synthesis in Sertoli cells (350); in-
crease transferrin production (351, 352) and transferrin
gene expression (353); and influence vectorial secretion
of proteins by Sertoli cells (352). Conditioned medium
from cultures of pachytene spermatocytes and early stage
round spermatids generally had the most dramatic ef-
facts. Initial characterization of the active component(s)
in the conditioned medium indicate the activity is heat
and trypsin sensitive (348, 349). Partial purification dem-
strated that the activity was present in a fraction
containing three polypeptides between 10 and 30 kilo-
daltons (353). Further purification and characterization
of the factors in germ cell conditioned medium are re-
duced to determine the number of potential factors
required for the biological activities and potential
identification of the paracrine factor. Due to the rela-
tively poor viability of germ cells in culture, an important
consideration that will require investigation is whether
the paracrine factor(s) is actively secreted by germ cells
or simply present in germinal cell cytosol and derived
from cell lysis. In addition to the above analysis of
germinal cell-derived paracrine factors, nerve growth
factor (NGF) has also been postulated to be a paracrine
factor in the seminiferous tubule (354). β-NGF gene
expression and NGF immunoreactivity have been iden-
tified in spermatocytes and early spermatids (355, 356).
NGF receptor gene expression has recently been identi-
cified in Sertoli cells under androgen regulation (354). The
observations that germ cells produce NGF and Sertoli
cells contain the NGF receptor have led these investi-
gators to postulate that NGF may mediate regulatory
interactions between germinal cells and Sertoli cells.
Demonstration that these and other potential factors can
be secreted by germinal cells and subsequently act on
Sertoli cells remains to be elucidated.

The physiological significance of the regulatory inter-
actions between Sertoli cells and germinal cells requires
further consideration. General factors required for the
maintenance of cellular function, such as IGF-I and
possibly TGFβ, are likely needed by all cell types. There-
fore, developing germinal cells may also require these
same factors. Whether unique paracrine factors mediate
Sertoli-germinal cell interactions is unclear at present.
Spermatogenesis and meiosis are among the most evo-

cutionarily conserved biological processes to assure prop-
agation of the species. For this reason, the process of
germinal cell development and cellular associations has
been shown to be similar in a large number of different
animals (41). To maintain a high degree of stability and
conservation, the majority of regulatory elements re-
quired for spermatogenesis will likely be associated with
the genome of the germinal cell and require minimal
external regulatory interactions with other cell types.
Therefore, spermatogenesis may be a passive process,
and the primary Sertoli cell-germinal cell interactions will be environmental and nutritional. Alternatively, spermatogenesis may be an active process and require a complex network of regulatory interactions to instruct germinal cells to proceed from one stage of development to the next. Further elucidation of Sertoli cell-germinal cell interactions will elucidate the degree to which germinal cell development is an active or passive process.

**B. Peritubular cell-Sertoli cell interactions**

The stromal cells that surround the seminiferous tubule and are in contact with the basal surface of the Sertoli cells are referred to as the peritubular myoid cells (357–366). The widespread occurrence of peritubular myoid cells among various species suggests that they are an integral functional component of the mammalian testis (357, 361–363, 365, 367). Differences in the thickness of the peritubular cell layers that surround the tubule in different species, however, have been observed (357, 362). The peritubular cells are thought to provide structural integrity for the tubule and also appear to be involved in contraction of the tubule (357, 362, 368, 369). A peritubular cell sheath is present early in development (370); however, myoid cells do not differentiate until the early stages of puberty (359, 363, 371, 372) in response in part to the actions of androgens (366, 373). Alterations in the peritubular cell layers have been shown to occur in pathological conditions of the testis generally associated with an increased thickness of the tissue (374–380).

Peritubular cell-Sertoli cell interactions are postulated to play an integral role in the maintenance of testis function (reviews in Refs. 22 and 178).

**Environmental.** One of the primary environmental interactions between peritubular cells and Sertoli cells is mediated by the complex extracellular matrix (*i.e.* basement membrane) between the two cell types. This extracellular matrix provides structural integrity for the tubule and assists in the maintenance of an efficient blood-testis barrier (200). The junctional interactions between peritubular cells and the extracellular matrix provide a permeability barrier or prefilter while the tight junctional interactions between Sertoli cells create a functional blood-testis barrier. The extracellular matrix between the peritubular cells and Sertoli cells appears to be produced cooperatively by the two cell types (79, 381, 392). Sertoli cells in culture produce laminin and collagen types I and IV (79) and unique proteoglycans (80). Peritubular cells in culture produce collagen type I (79), unique proteoglycans (80), and fibronectin (79, 115). Therefore, peritubular cells and Sertoli cells appear to produce individual components of the basement membrane, and the presence of both cell types appears necessary to generate an organized deposited extracellular matrix (79). The ability of peritubular cells and Sertoli cells to cooperate in the production of this extracellular matrix is postulated to be an essential environmental interaction between the cells to form the basement membrane of the seminiferous tubule (79). Coculture of the two cell types increased the attachment and viability of Sertoli cells (383, 384) and altered the pattern and rate of Sertoli cell migration (385, 386). Similar results have been observed when Sertoli cells are cultured on a cell line of peritubular cells (387). Since the primary environmental interactions between peritubular cells and Sertoli cells are mediated by the extracellular matrix, a number of laboratories have examined the effects of an extracellular matrix on Sertoli cells (388–392). The presence of an extracellular matrix in culture was found to increase cell attachment and viability, as well as promote a histotype that appears similar to that found *in vivo* with a columnar shape cell, nucleus near the basal surface of the cell, and tight junctions between Sertoli cells. Although the presence of an extracellular matrix can promote the structural differentiation of the Sertoli cell (388, 389, 392), the extracellular matrix may not influence Sertoli cell functions on a molecular level (392); that will require a regulatory type interaction. The presence of an extracellular matrix has also been shown to promote peritubular myoid cells in culture to express a morphology that is similar to that observed in the seminiferous tubule (393). Therefore, peritubular cells and Sertoli cells cooperate in the production of the basement membrane of the seminiferous tubule that, in turn, promotes structural differentiation of the peritubular cells and Sertoli cells.

The observations that the presence of an extracellular matrix promoted a columnar shaped cell *in vitro* with tight junctions between Sertoli cells led several laboratories to develop dual-chamber culture systems to investigate polarized secretion by Sertoli cells (394–397). A monolayer of Sertoli cells is placed on a permeable membrane to create an apical and basal chamber. The presence of an extracellular matrix was found to facilitate the polarization of the cell and create a permeable barrier (394). Sertoli cells secrete ABP, transferrin, plasminogen activator, and inhibit in a polarized manner dependent on the functional marker examined. Peritubular cells were found to increase the efficiency of the permeability barrier and altered the polarized secretion of Sertoli cell products (398–402). Therefore, an additional environmental interaction between peritubular cells and Sertoli cells is to promote and maintain the polarity of the Sertoli cell and vectorial secretion of cellular products.

Proteases and antiproteases are additional cellular products that can influence the environmental interactions between peritubular cells and Sertoli cells. Sertoli cells produce plasminogen activator (72), which could be
involved in the degradation and turnover of the basal lamina of the tubule and influence the junctional interaction between Sertoli cells. Peritubular cells have been shown to produce a protease inhibitor that inhibits plasminogen activator activity (120). The production of the antiprotease by peritubular cells, therefore, might regulate the level of protease activity present to control degradation of the extracellular matrix between Sertoli cells and peritubular cells, as well as maintain the blood-testis barrier (120). This hypothesis is supported by an investigation of the effects of proteases and antiproteases on the permeability barrier of Sertoli cells in a dual-chamber culture system (400).

Nutritional. Both Sertoli cells and peritubular cells are in contact with interstitial fluid such that essential components from the circulatory system can readily be obtained by both cell types. Junctional interactions between Sertoli cells and gap junctions between peritubular cells (200) can facilitate nutritional interaction between the individual cell types. Sertoli cells in vitro have been shown to metabolically cooperate with peritubular cells (403) and fibroblasts (404) through junctional contacts and transport metabolites such as nucleotide precursors. The presence of the basal lamina of the seminiferous tubule, which separates peritubular cells and Sertoli cells, however, prevents junctional contacts to form between the cells (200). Therefore, the metabolic cooperation observed in vitro between the cells is likely not present in vivo. Nutritional interactions between peritubular cells and Sertoli cells may not be important due to the availability of serum components to both cell types and lack of junctional interactions between the cells in vivo.

Regulatory. Regulatory interactions between peritubular cells and Sertoli cells were first postulated when it was observed that androgens alone could not promote peritubular cell differentiation, but gonadotropin action on Sertoli cells was also required to indirectly effect peritubular cell differentiation (366). Coculture of Sertoli cells and peritubular cells indicates that the presence of peritubular cells increases the production of ABP by Sertoli cells (383, 405); stimulates transferrin production by human Sertoli cells (406); alters the enzyme histochemistry of Sertoli cells (407); and influences the vectorial secretion of proteins by Sertoli cells (398, 401, 408). These observations imply that peritubular cells may influence Sertoli cell functions through a regulatory type interaction. Peritubular cells were subsequently shown to produce a nonmitogenic paracrine factor that modulates Sertoli cell function and has been termed PModS (117, 409). Serum-free peritubular cell-conditioned medium was found to stimulate the production of a number of proteins by Sertoli cells including ABP and transferrin (117, 409, 410). PModS has been purified and characterized and found to have a more dramatic effect on Sertoli cell functions in vitro than any individual regulatory agent previously identified, including FSH (118). An illustration of the effect of PModS on Sertoli cell function in comparison with other hormones is shown in Fig. 5. PModS is shown to stimulate transferrin production by Sertoli cells in culture to the same extent as a mixture of FSH, insulin, and retinol. A combination of PModS and these hormones results in an additive response with an 8- to 10-fold stimulation of Sertoli cell function. The actions of PModS on transferrin and ABP production correlate with an increase in steady state levels of messenger RNA, and the dramatic effects of PModS appear to be due to the signal transduction system utilized (119). Although PModS stimulates most functions associated with the differentiation of the cell, cellular functions apparently independent of Sertoli cell differentiation, such as aromatase activity, may be suppressed by PModS (411). The hypothesis has developed that PModS may have an important role in the induction and maintenance of Sertoli cell differentiation (22, 118, 119, 188). The in vivo actions of PModS, however, remain to be investigated.

Additional paracrine factors that have been identified to potentially mediate regulatory interactions between peritubular cells and Sertoli cells are growth factors. The EGF-like substance, TGFα has been shown to be synthesized and secreted by Sertoli cells and peritubular cells (81). The EGF-like growth factor previously shown to be produced by Sertoli cells (87) is thought to be TGFα (81). Functional EGF/TGFα receptors are expressed on peritubular cells but not Sertoli cells or germinal cells (81). Immunolocalization of the EGF receptor, however, indicated that the EGF receptor may be on Sertoli cells (337). TGFα/EGF stimulates peritubular cell growth but not Sertoli cell growth (81). EGF has been shown to influence Sertoli cell functions (412, 413); however, several Sertoli cell functions were not found to be influenced by TGFα/EGF in highly purified preparations of Sertoli cells (81). Further examination of the cellular localization of EGF receptor expression and the actions of EGF/TGFα in the seminiferous tubule is required to elucidate the role TGFα may have in regulatory interactions between peritubular cells and Sertoli cells.

TGFβ is a growth inhibitor that has also been shown to be produced by Sertoli cells and peritubular cells (82). TGFβ can inhibit TGFα/EGF-stimulated peritubular cell growth and promote peritubular cell migration and colony formation (82). TGFβ appears to promote the differentiation of peritubular cells and can increase the production of specific proteins (82) such as the plasminogen activator inhibitor antiprotease (414). TGFβ may also promote peritubular cell chemotaxis to assist in the morphogenesis of the seminiferous tubule (82). TGFβ
Nutritional. Both Sertoli cells and Leydig cells have

does not appear to have general effects on major Sertoli
cell functions (82); however, TGFβ may increase lactate
production by Sertoli cells (415). Further examination of
the production and action of TGFβ in the tubule is
required to determine the importance TGFβ may have
in mediating peritubular cell-Sertoli cell interactions.

Another growth factor produced by peritubular cells
and Sertoli cells is IGF-I, which is a somewhat ubiqui-
tously expressed growth factor that maintains cellular
metabolism and acts as a progression factor in the growth
of most cell types. IGF-I has been shown to directly
stimulate a number of Sertoli cell functions and facilitate
the growth of peritubular cells and prepubertal Sertoli
cells. Examination of the cellular localization of IGF-I
and IGF-I receptor gene expression in the seminiferous
tubule (83-86, 416-419) and the actions of IGF-I is
required to elucidate whether locally produced IGF-I may
mediate peritubular cell-Sertoli cell interactions. The
high concentration of IGF-I in the interstitial fluid
derived from the circulatory system is readily available to
both cell types; therefore, the physiological need for
actions of a locally produced IGF to mediate peritubular
cell-Sertoli cell interactions needs to be questioned.

C. Sertoli cell-Leydig cell interactions

The specialized cells within the interstitial tissue of
the testis referred to as Leydig cells (420) were distin-
guished morphologically by a number of early investiga-
tors (31–33). The functional importance of this cell was
realized with the demonstration that Leydig cells are the
site of androgen production (421-426) and that LH
through the actions of cAMP directly stimulates andro-
gen production (427-431). The local action of androgen
on testis function was initially demonstrated when testo-
sterone alone in the absence of gonadotropins was
shown to support spermatogenesis (432) at significantly
reduced androgen levels as observed in vivo (433, 434).
Androgens, therefore, were the first paracrine agents
involved in cell-cell interactions in the testis to be inves-
tigated. These observations initiated a relatively intense
analysis of Sertoli cell-Leydig cell interactions.

Environmental. The location of Leydig cells in the in-
terstitium and the presence of peritubular myoid cells and
the basement membrane of the seminiferous tubule do
not allow for physical contact between Sertoli cells and
Leydig cells. The inability of these cells to physically
interact indicates that direct environmental interactions
between Sertoli cells and Leydig cells are not possible in
vivo.

Nutritional. Both Sertoli cells and Leydig cells have
ready access to essential nutritional components in the
interstitial fluid derived from the circulatory system. For
this reason, and because the cells are unable to form
junctional contacts, nutritional interactions between
Sertoli cells and Leydig cells appear minimal. Leydig
cells, however, have been shown to have junctional in-
teractions between themselves (435-439) that can me-
diate nutritional interactions. Cooperativity between
Leydig cells mediated by steroids (440-442) and nonste-
roidal substances (443, 444) has been observed.

Regulatory. The identification of androgen production by
Leydig cells (421–431) and the ability of androgens to
maintain the process of spermatogenesis (432–434) led a
number of laboratories to investigate the actions of an-
drogens on Sertoli cells. Sertoli cells have been shown to
contain and express the androgen receptor (445–451),
which is influenced by hormones, sexual maturation, and
the cycle of the seminiferous epithelium (452–457). The
actions of androgens on Sertoli cell function have been
investigated, and the effects observed in vitro are gener-
ally negligible or less than the actions of FSH (308, 458–
462) as shown in Fig. 5. Although the androgen receptor
is present in Sertoli cells, a complete understanding of
the role of androgen actions on Sertoli cells requires
further investigation. The ability of peritubular cells to
enhance the actions of androgen on Sertoli cells, how-
ever, suggests that androgens may indirectly regulate
Sertoli cell function and points out the importance of
using a purified preparation of Sertoli cells to investigate
the actions of androgens (463). Current data imply that
an important regulatory interaction between Leydig cells
and Sertoli cells is mediated through the local production
and action of androgens, but the physiological signifi-
cance of this regulatory interaction remains to be fully
elucidated.

In addition to androgens, Leydig cells can potentially
influence Sertoli cells through nonsteroidal factors. A
number of peptides and proteins have been shown to be
produced or localized in Leydig cells that can potentially
have a regulatory action on Sertoli cells and the seminif-
erous tubule including renin (464–467), prodymorphin
(468), and oxytocin (469–471). The functional and phys-
iological roles of these peptides, however, remain to be
investigated. Another peptide produced by Leydig cells
that may regulate Sertoli cell and seminiferous tubule
function is POMC and related peptides (108–112, 472–
474). Several peptides that are derived from the POMC
protein and appear to be produced by Leydig cells include
β-endorphin, αMSH, and ACTH (108, 475). These pep-
tides have been postulated to be involved in the regula-
tion of Sertoli cell function (476-478). The actions of β-
endorphin, αMSH, and ACTH on Sertoli cell function
have been examined (479–485). The stimulating effects
of αMSH and ACTH on Sertoli cell function are small
and appear to involve in vitro alterations in cAMP levels.
The β-endorphin receptor has been shown to be present on Sertoli cells (479). Although β-endorphin alone was found to have little effect, β-endorphin can partially decrease the ability of FSH to stimulate Sertoli cell functions. The concentrations of these POMC peptides required to obtain effects on Sertoli cells in vitro were relatively high, and these peptides have not been shown to have dramatic effects on Sertoli cell function in comparison with factors such as FSH. Perfusion of the testis with these peptides revealed that ACTH, but not α-MSH or β-endorphin, can influence androgen production by Leydig cells (486, 487). Further work is required to determine the physiological significance of POMC-derived peptides and their importance in mediating regulatory interactions between Leydig cells and Sertoli cells.

The ability of Sertoli cells and the seminiferous tubules to affect Leydig cell function was initially hypothesized from observations that Leydig cell morphology was altered by tubules with abnormal function and spermatogenesis (488, 489). Damage of seminiferous tubule function with cytotoxic agents, vitamin A deficiency, or fetal irradiation was found to cause abnormal cytological features and function in adjacent Leydig cells (490). Analysis of testis morphology under pathological conditions (491–493) and in aged man (494) supports the interrelationship of the seminiferous tubules and Leydig cells. Cryptorchidism has been extensively utilized to inhibit seminiferous tubule and Sertoli cell function to examine subsequent effects on Leydig cell function and morphology (495–502). In addition to studies with damaged or abnormal testis, examination of normal testis has revealed that Leydig cell morphology changes with the seminiferous cycle being most dramatic at stages VII and VIII (503–505). These morphological studies of both abnormal and normal testis implied that a regulatory interaction may exist between Sertoli cells and Leydig cells. To extend these observations, several laboratories cocultured Leydig cells with Sertoli cells or seminiferous tubules. Sertoli cells were found to generally increase basal and hormone-stimulated Leydig cell functions, and FSH was found to indirectly, through the Sertoli cell, stimulate Leydig cell steroidogenesis (506–512). Seminiferous tubules in coculture, particularly at stages VI and VIII, were also found to influence Leydig cell function (513, 514).

Further examination of this potential regulatory interaction between Sertoli cells or seminiferous tubules utilized conditioned culture medium. A number of laboratories have identified a stimulatory activity in Sertoli cell-conditioned medium that increases both basal and hormone-stimulated Leydig cell function (515–527). Both FSH-dependent and -independent activities have been identified. Inhibitory activities in the conditioned medium that can decrease basal and hormone-stimulated Leydig cell steroidogenesis have also been identified (528–535). An interesting study with the use of spent media from seminiferous tubules isolated from normal and cryptorchid testis revealed that under normal conditions predominantly inhibitory activity was present whereas cryptorchidism induced the appearance of stimulatory activity (536). Although the contributions of germinal cells and peritubular myoid cells to the activities in conditioned medium from seminiferous tubules remain to be elucidated, observations imply that Sertoli cells may produce multiple factors that can influence Leydig cell function and size. Purification and characterization of these Sertoli cell products are required to determine the novelty of the substances investigated and to address the physiological significance of this proposed regulatory interaction between Sertoli cells and Leydig cells. Additional indirect support of the presence of local factor(s) in the testis that can regulate Leydig cell function is provided with the observations that testicular interstitial fluid has the ability to stimulate Leydig cell steroidogenesis (537–546). The stimulatory activity present in interstitial fluid is speculated to be derived from Sertoli cells; however, further analysis of the sites of action, sites of production, and biochemical properties of the active components is required.

Several Sertoli cell-secretory products have been identified that can potentially mediate regulatory interactions between Sertoli cells and Leydig cells. One of the first paracrine factors considered was estrogen. Sertoli cells can produce estrogen from androgens (92, 547), and estrogen generally has inhibitory effects on Leydig cell androgen production (548, 549). The proposal has been made that Sertoli cell-derived estrogen may mediate inhibitory actions on Leydig cell testosterone production; however, Leydig cells can also produce relatively high levels of estrogen (550), which could act as an autocrine factor for the cell. The physiological significance of estrogen-mediated regulatory interactions between Sertoli cells and Leydig cells, therefore, remains to be elucidated. Another factor postulated to be produced by Sertoli cells that can act on Leydig cells is a LHRH-like substance (551–557). Leydig cells from some species have been shown to contain receptors for LHRH (551, 558–561), and LHRH generally has long term inhibitory effects on Leydig cell steroidogenesis (562, 563). The production of an LHRH-like substance by Sertoli cells, however, has been questioned (564) and appears to be somewhat species specific in both the production and action of an LHRH-like substance. Although altered molecular forms of an LHRH molecule may be present in the testis (565–567), further analysis of species specificity, sites of production, sites of action, and biochemical characterization of such substances will be required.

Sertoli cells also produce a number of growth factors
that potentially mediate Sertoli cell-Leydig cell interactions. IGF-I is produced by Sertoli cells (83–86, 568–570) and can act on Leydig cells to regulate steroidogenesis (571–576). The ability of Sertoli cells to stimulate Leydig cell steroidogenesis in coculture of the two cell types can be partially inhibited with immunoneutralization with an IGF-I antibody (577). Leydig cells, however, can produce IGF-I (114) that can act as an autocrine factor for the cell. In addition, the concentration of liver-derived IGF-I in the circulatory system and interstitial fluid is approximately 2 orders of magnitude higher than the concentrations required to influence Leydig cell steroidogenesis. Therefore, the physiological relevance of an IGF-I mediated regulatory interaction between Sertoli cells and Leydig cells is questioned. Two other growth factors produced by Sertoli cells are TGFα and TGFβ (81, 82). Leydig cells contain the EGF/TGFα receptor and can respond to EGF or TGFα to influence steroidogenesis and cell growth (578–581). The potential production of TGFα/EGF by Leydig cells (582) or interstitial cells would question the importance of a paracrine interaction between Sertoli cells and Leydig cells. Leydig cell steroidogenesis can also be influenced by the actions of the growth inhibitor TGFβ (583–586). The TGFβ produced by Sertoli cells (82, 587) could act as a paracrine factor to regulate Leydig cell function and growth, but further investigation of additional sites of TGFβ production and action is required to elucidate the physiological relevance of this potential cell-cell interaction.

Inhibin (588) is a peptide hormone produced by Sertoli cells (reviews in Refs. 93, 589, and 590) under the control of FSH or agents that alter cAMP levels (591–596). Although a major function for inhibin is to act on the pituitary to regulate FSH production, potential local actions of inhibin have been postulated. Inhibin and its related protein activin both can influence Leydig cell steroidogenesis (597, 598). Therefore, inhibin may potentially mediate a regulatory interaction between Sertoli cells and Leydig cells. Leydig cells have been postulated to be involved in the regulation of Sertoli cell inhibin production (599, 600); however, Leydig cells have also been shown to produce both inhibin and activin (113, 601–603). The ability of both Sertoli cells and Leydig cells to produce inhibin and related peptides questions the relevance of a paracrine interaction between the cells mediated by inhibin. The observation that other cell types, such as germinal cells (604), may provide additional sites of action for inhibin or activin suggests that an understanding of the role inhibin/activin has in testicular cell-cell interactions will require further investigation of the sites of action and sites of production of inhibin/activin. Potential alternate or new biological activities of forms of inhibin such as the pro-a-c region of the inhibin subunits also needs to be examined. The role inhibin and its related peptides may have in the local regulation of testis function remains to be elucidated.

A number of potential paracrine factors may mediate regulatory interactions between Sertoli cells and Leydig cells (Table 5). Additional regulatory agents that can influence Leydig cell function, such as IL-1 (605, 606) and vasopressin-like peptides (607), may also be involved in this interaction but require further investigation. The biological activities present in conditioned medium will require biochemical characterization in order that the potential role these agents may have in cell-cell interactions may be understood. Many of the stimulatory and inhibitory effects observed with materials such as Sertoli cell-conditioned medium may potentially be attributed to the known regulatory agents investigated, such as IGF-I, IL-1, TGFα, or TGFβ. Although a list of potential paracrine factors is developing, further analysis is necessary to elucidate their involvement in cell-cell interactions and physiological relevance.

Although the production of androgen by Leydig cells and subsequent action on the seminiferous tubule is an essential regulatory interaction to maintain testis function, the physiological requirement for Sertoli cell-Leydig cell regulatory interactions requires further considera-

<table>
<thead>
<tr>
<th>Potential paracrine factor</th>
<th>Site production</th>
<th>Site action</th>
<th>Actions/proposed function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen</td>
<td>Leydig</td>
<td>Sertoli</td>
<td>Regulate/maintain function and differentiation</td>
</tr>
<tr>
<td>POMC peptides</td>
<td>Sertoli</td>
<td>Leydig</td>
<td>Decrease FSH actions</td>
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<tr>
<td>β-endorphin</td>
<td></td>
<td></td>
<td>Increase FSH actions/cAMP</td>
</tr>
<tr>
<td>MSH, ACTH</td>
<td></td>
<td></td>
<td>Increase steroidogenesis</td>
</tr>
<tr>
<td>Stimulatory factor (?)(FSH dependent or independent)</td>
<td>Sertoli</td>
<td>Leydig</td>
<td>Increase steroidogenesis</td>
</tr>
<tr>
<td>Inhibitory factor (?)</td>
<td>Sertoli</td>
<td>Leydig</td>
<td>Decrease steroidogenesis</td>
</tr>
<tr>
<td>LHRH-like factor</td>
<td>Sertoli</td>
<td>Leydig</td>
<td>Decrease steroidogenesis</td>
</tr>
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<td>Leydig</td>
<td>Decrease steroidogenesis</td>
</tr>
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<td>IGF-I</td>
<td>Sertoli</td>
<td>Leydig</td>
<td>Increase steroidogenesis</td>
</tr>
<tr>
<td>TGFα</td>
<td>Sertoli</td>
<td>Leydig</td>
<td>Decrease steroidogenesis/increase growth</td>
</tr>
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<td>Leydig</td>
<td>Increase steroidogenesis</td>
</tr>
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<td>Leydig</td>
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</tr>
<tr>
<td>Inhibin</td>
<td>Sertoli</td>
<td>Leydig</td>
<td>Increase steroidogenesis</td>
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</table>
tion. The concentration of androgen present in the adult testis is significantly higher than the concentrations needed to maintain germinal cell development (608–611). Local transient alterations in androgen production by 80–90% may not have major effects on Sertoli cell or germinal cell development. A stimulation in androgen production would not appear to be important for adult testis function. Although an active regulation of Leydig cell androgen production may be needed during embryonic, postnatal, and pubertal development, in the adult testis regulatory interactions between Sertoli cells and Leydig cells to influence Leydig cell steroidogenesis may not be physiologically important. Additional functions of the Leydig cells, however, may need to be activity regulated.

**D. Leydig cell-peritubular cell interactions**

The Leydig cell and peritubular myoid cell are both thought to be derived from the pluripotential urogenital mesenchyme. Although peritubular myoid cell differentiation occurs postnatally, Leydig cells appear to develop from precursor mesenchymal cells located in the peritubular region during fetal development (612, 613), and this development is thought to be similar to the pattern of Leydig cell development during postnatal life (438, 614). The origins of the Leydig cells and peritubular myoid cells, therefore, are closely associated. These cell types are not terminally differentiated, and both peritubular cells and Leydig cells proliferate in the adult and have a defined turnover time (615). The development and proliferation of these cell types require a continuous association between Leydig cells and peritubular myoid cells.

**Environmental.** In the adult tissue Leydig cells in the interstitium do not generally form a physical contact with differentiated peritubular myoid cells surrounding the tubule and in contact with the basal surface of the Sertoli cell. Outer layers of less differentiated peritubular myoid cells and undifferentiated fibroblasts or stromal cells and lymphatic endothelium generally separate Leydig cells from myoid cells. The inability of these cells to physically interact indicates that direct environmental interactions between Leydig cells and peritubular myoid cells are generally not present in the adult testis. During development, however, less mature or differentiated Leydig cells and peritubular cells may potentially form environmental interactions.

**Nutritional.** Both Leydig cells and peritubular myoid cells have ready access to essential nutritional components in the interstitial fluid derived from the circulatory system. The lack of apparent junctional interactions between these cell types and ready access to nutritional components implies that minimal nutritonal interactions between Leydig cells and peritubular myoid cells are needed. Although Leydig cells appear to interact among themselves (435–444), no apparent nutritional interactions between Leydig cells and peritubular myoid cells have been observed.

**Regulatory.** The primary regulatory interaction identified between Leydig cells and peritubular myoid cells is mediated by androgens. Peritubular myoid cell development is postulated to be dependent on androgens (366, 373). Inhibition of the androgen production by Leydig cells or the presence of antiandrogens will interfere with peritubular myoid cell development. The peritubular cells accumulate androgen (46) and contain the androgen receptor (456, 616, 617). The level of androgen receptor present in peritubular cells is approximately the same as present in Sertoli cells (617). Peritubular cells in organ culture (373) and isolated peritubular cells in culture respond to androgens (117, 161, 411, 463). The production of the testicular paracrine factor PModS by peritubular cells appears to be under androgen control (117, 463). Therefore, a cascade of cellular interactions is speculated to occur in response to LH and androgen. LH acts on Leydig cells to stimulate the production of androgen that subsequently acts on peritubular myoid cells to stimulate PModS production that modulates Sertoli cell functions and nutritional interactions essential for germinal cell development (117–119). This androgen-mediated regulatory interaction between Leydig cells and peritubular cells involving PModS is postulated to be important for the maintenance of testis function and the process of spermatogenesis (22, 118, 119, 188). Further analysis of the in vivo actions of PModS is required to determine the physiological importance of this cell-cell interaction.

Additional Leydig cell products that influence peritubular myoid cells have not been identified. Further analysis of biochemical markers for peritubular cell function, such as PModS, will be needed to elucidate potential nonsteroid-mediated regulatory interactions between Leydig cells and peritubular cells. Peritubular cell products that may act as paracrine factors and influence Leydig cell function include IGF-I (114), the EGF-like substance TGFα (81), and TGFβ (81). IGF-I and TGFβ may potentially stimulate Leydig cell steroidogenesis (571–576, 583–586) whereas TGFα may regulate Leydig cell growth and steroidogenesis (578–581). Although these growth factors are produced by peritubular cells and can act on Leydig cells, the role of these growth factors in mediating cell-cell interactions requires further investigation. The high concentration of serum-derived IGF-I in the interstitial fluid makes questionable the role of IGF-I as a mediator of regulatory interactions between
the cells. Further analysis of TGFα and TGFβ production and action in the interstitial tissue is also needed before speculations regarding their involvement in cellular interactions between Leydig cells and peritubular myoid cells can be postulated.

The androgen-mediated regulatory interactions between Leydig cells and peritubular myoid cells provide a potentially important indirect mode of androgen action in the testis. Therefore, the peritubular cells may play a critical role in the endocrine regulation of the process of spermatogenesis. The dramatic effects of PModS on Sertoli cell function and inability of purified Sertoli cells to respond to androgens in vitro support these speculations of the importance of this indirect mode of androgen action. Peritubular cell-Sertoli cell interactions provide an example of mesenchymal-epithelial cell interactions present in a variety of tissues. Steroid actions on tissues have been postulated to, in part, be mediated through mesenchymal-epithelial cell interactions (review in Ref. 11). The prostate stromal/mesenchymal cells, in response to androgens, appear to produce an inducer substance that regulates the functions and differentiation of the adjacent prostatic-epithelial cells (618–620). This proposed cell-cell interaction is similar to that proposed between Leydig cell cells, peritubular myoid cells, and Sertoli cells. The possibility that PModS may be a general mediator of androgen actions in other organs in addition to the testis has previously been postulated (22, 118), and preliminary evidence was provided with prostate cell culture-conditioned medium (621, 622). Further characterization of PModS and its potential expression and action in androgen-responsive tissues will be required to determine its tissue specificity.

E. Additional cell-cell interactions

Although the Leydig cells, peritubular myoid cells, Sertoli cells, and germinal cells are major functional cell types in the testis, several additional existing cell populations include the vasculature and lymphatic endothelium, lymphocytes, macrophages, and the stromal fibroblast cell population. Several studies have been initiated to investigate the physiology of these cell populations and their potential involvement in cell-cell interactions.

The stromal fibroblast population of cells in the interstitium and surrounding the outer layers of the seminiferous tubules is thought to contribute to the structural integrity of the tissue as well as contain a stromal/mesenchymal cell population from which Leydig cells and peritubular myoid cells have been speculated to be derived. Because Leydig cells and peritubular myoid cells proliferate and have a limited turn over time, a less differentiated population of precursor cells or a stem cell population of stromal/mesenchymal cells appears necessary to repopulate the cell types. Whether the Leydig cells and peritubular myoid cells are derived from the same precursor population of cells or separate precursor cell types is unclear. Studies to investigate the potential involvement of these cells in cellular interactions have focused on Leydig cells. Treatment of rats with the cytotoxic agent ethane dimethanesulfonate (EDS) has been found to destroy the mature Leydig cell population (623–625). Although EDS does influence Sertoli cell and peritubular myoid cell functions (626, 627), EDS has been utilized to examine Leydig cell regeneration and interactions with other cell types (628–632). Repopulation of Leydig cells after removal of EDS suggests that the Leydig cells are derived from the stromal fibroblast population of cells often associated with the outer layer of cells of the seminiferous tubule (628, 630–632). The regeneration of the Leydig cells from this stromal fibroblast population is speculated to be influenced by the seminiferous tubules and Sertoli cells for growth and possibly differentiation (629, 631, 632). Analysis of cell proliferation and pulse-chase radiolabeling experiments of the stromal/mesenchymal cell population, in comparison to other testicular cell types, support the hypothesis that Leydig cells may be derived from this cell population (633). These stromal/mesenchymal precursor cells can be isolated and cultured and appear to be responsive to the combined actions of LH and androgen (634). Further analysis of cellular interaction with this Leydig cell precursor population of stromal fibroblast cells requires characterization of the cell population and identification of functional parameters of the cells.

Another cell population in the testis is derived from the immune system. Macrophages have been shown to reside in the interstitial compartment of the testis (635–637). These testicular macrophages have been isolated and characterized (638). Testicular macrophages often physically associate with Leydig cells, and speculations of potential cellular interactions between the cells have been made (639). Interestingly, the conditioned medium from testicular macrophage cultures has been shown to stimulate Leydig cell steroidogenesis (640). Although testicular macrophages will likely have traditional bactericidal and phagocytic activities in the testis (641–647), potential environmental and regulatory interactions between testicular macrophages and other testis cell types, particularly Leydig cells, may also exist. Lymphocytes are also present in the testis and participate in the normal immunology of the animal. The testis, however, has been shown to be an immunologically protected tissue (648–651). Potential cellular interactions between testis cell types and these lymphocytes remain to be thoroughly investigated. Several testis cell products may interact with the lymphocyte population present. Sertoli cells appear to produce protein(s) that can inhibit lym-
phocyte proliferation (98, 652). The apparent Sertoli cell product IL-1 (88) may also potentially act as a paracrine factor to influence lymphocytes. Products of testis cell types may be needed to regulate local lymphocyte functions important for immunological protection of the tissue (653–655). In contrast, lymphocyte products, such as interferon and tumor necrosis factor, may be needed to influence testis cell functions (656–658). Further investigation of the reproductive immunology of the testis (652) is required to determine specific regulatory interactions between immune system cells, such as macrophages and lymphocytes, with other cell types in the testis.

Vascular and lymphatic endothelial cells are also relatively abundant cell populations in the testis. The arteries, veins, and capillaries that vascularize the testis at puberty are generally associated with the tubules (659–662). The permeability of this vasculature is relatively high such that substances introduced into the circulation equilibrate with testicular lymph (207, 260, 663). Potential interactions between this vasculature and other testicular cells, particularly the seminiferous tubule, may be mediated by angiogenic factors. Fibroblast growth factor (FGF) is one of the most potent and well characterized angiogenic factors that promotes the vascularization of tissues (664). FGF has been shown to be present in testis extracts (665, 666); however, the site of synthesis of testicular FGF remains to be elucidated. The ability of testis cells to produce FGF as an angiogenic factor to promote and maintain vascularization of the testis constitutes a potentially important regulatory cell-cell interaction. Other paracrine factors that may mediate regulatory interactions between the vasculature and other testis cell types, such as the renin-angiotensin or atrial natriuretic peptides, remain to be investigated. In addition to the vasculature, lymphatic endothelium often associates with the outer peritubular wall and envelops the Leydig cells (667–669). Although the association of lymphatic endothelium with tubules is variable among species, this cell population is thought to be important in the formation of the interstitium compartment and support of testicular lymph.

### III. Summary

#### A. Testicular cell-cell interactions

A general summary of the major categories of cell-cell interactions among the primary testicular cell types considered are illustrated in Table 6. The major environmental interactions occur between Sertoli cells and spermatogenic cells and between peritubular cells and Sertoli cells. The lack of physical contact between Leydig cells and Sertoli cells or peritubular myoid cells indicates that environmental interactions between these cells will not likely be an essential cell-cell interaction. The primary nutritional interaction in the testis is between Sertoli cells and germinal cells. Ready access to serum-derived nutritional components in interstitial fluid by the other cell types indicates that major nutritional interactions between Leydig, peritubular, and Sertoli cells will not be required. Regulatory interactions occur among all the cell types considered. Although further investigation of regulatory interactions between Sertoli cells and germinal cells is needed, initial studies suggest the presence of such interactions. Observations imply that the cellular anatomy of the testis and factors such as the blood-testis barrier play a major role in the types of cell-cell interactions that have evolved and are needed to maintain testis function. The cellular associations and physiology of the testis, however, differ depending on the stage of development. Therefore, the cell-cell interactions required and present in the prenatal testis, prepubertal testis, pubertal testis, and adult testis will be altered depending on the specific physiological requirements. As specific cellular interactions are more thoroughly characterized in the more mature stages of development, their role in the developing testis can be examined.

Several aspects of testis cell biology that are integrally associated with local cellular interactions involve growth and differentiation. All testicular cell types prepubertally require cell proliferation, which terminates for Sertoli cells at the early stages of puberty. All other testicular cell types, except Sertoli cells, require varying degrees of cell proliferation in the adult testis. Therefore, the local production and action of growth factors are required to regulate testis cell growth (review in Ref. 670). These

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Environmental</th>
<th>Nutritional</th>
<th>Regulatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertoli-germinal</td>
<td>Yes</td>
<td>Yes (e.g. transport proteins)</td>
<td>Yes (e.g. IGF-I)</td>
</tr>
<tr>
<td></td>
<td>(e.g. cytoarchitecture)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peritubular-Sertoli</td>
<td>Yes</td>
<td>No</td>
<td>Yes (e.g. PModS)</td>
</tr>
<tr>
<td></td>
<td>(e.g. basement membrane)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sertoli-Leydig</td>
<td>No</td>
<td>No</td>
<td>Yes (e.g. POMC peptides)</td>
</tr>
<tr>
<td>Leydig-peritubular</td>
<td>No</td>
<td>No</td>
<td>(e.g. androgen)</td>
</tr>
</tbody>
</table>

TABLE 6. Classification of testicular cell-cell interactions
growth factors can act as autocrine and paracrine factors to mediate regulatory interactions among various testis cell types. The majority of proposed potential growth factor-mediated cell-cell interactions are outlined in Fig. 6. All of these proposed interactions require further investigation to elucidate their physiological relevance. IGF-I can potentially mediate interactions between all the cell types; however, due to the high concentration of serum-derived IGF-I present in the interstitium, only Sertoli cell-germinal cell IGF-I-mediated interaction may be important. TGFα and TGFβ have antagonist growth effects and may also mediate interactions between all the cell types, but further analysis of the sites of production and action of TGFα and TGFβ is required. IL-1 has been postulated to be produced by Sertoli cells and potentially interact with Leydig cells and germinal cells. Interactions of IL-1 with other testicular cell types such as macrophages and lymphocytes may be of equal or greater physiological importance for testis function. FGF has been shown to be present in the testis; however, the site of synthesis of testicular FGF remains to be determined. FGF has the ability to influence Leydig cell (671–674) and Sertoli cell function (675); however, potential actions on lymphatic and vascular endothelial cells may be of equal or greater physiological relevance. Other potential testicular growth factors that require further characterization such as SGF (333, 334) and SCGSGF (336) may also mediate cell-cell interactions. Clearly, locally produced growth factors in the testis will be important mediators of regulatory interactions between various cell types; however, further investigation is needed to elucidate the specific interactions and growth factors that will be physiologically relevant in the regulation of testis cell growth.

Classical growth factors such as FGF or EGF/TGFα act to promote a cell into the growth cycle, and subsequently a progression factor such as IGF-I will facilitate DNA synthesis followed by the proliferation of the cell. The function and pharmacology of these growth factors are directed toward putting the cell into the cell cycle to allow cell proliferation. Differentiation of a cell is generally independent of cell growth and may require a separate class of regulatory agents to promote and maintain the differentiated state of the cell. Optimal differentiation of the cell often requires the cell to be at a specific stage of the cell growth cycle. Alteration of the cell cycle by growth factors, therefore, may indirectly affect cellular differentiation. As with most organs, the testis requires a set of differentiation type regulatory agents to promote development, control function, and maintain cellular differentiation. Although identification and analysis of differentiation type factors is generally more difficult than analysis of growth factors, a summary of the regulatory agents involved in cell-cell interactions is shown in Fig. 7. The endocrine hormones LH and FSH play an integral role in promoting and maintaining Leydig cell and Sertoli cell function, respectively. Androgen production by Leydig cells promotes and maintains the differentiation and function of peritubular myoid cells and Sertoli cells. PModS derived from peritubular cells appears to promote and control Sertoli cell function and differentiation. TGFβ is generally a growth inhibitor that stops cell growth and puts the cell into a stage in the cell cycle more responsive for optimal differentiation. Therefore, TGFβ can indirectly act as a differentiation factor by inhibiting growth. TGFβ has been shown to stimulate Leydig cell and peritubular cell function, and its potential role on germinal cells remains to be investigated. Inhibin/activin are also regulatory agents with hormone-like differentiation properties that are likely independent of growth similar to TGFβ. Inhibin has been postulated to be involved in Sertoli cell-Leydig cell interactions; however, further analysis of the sites of production and action of inhibin/actin is needed to fully elucidate involvement in cell-cell interactions. Further investigation of these and additional differentiation fac-

**Fig. 6.** Potential growth factor-mediated cell-cell interactions in the testis.

**Fig. 7.** Potential cell-cell interactions that regulate cellular differentiation.
tors is needed to fully understand the specific regulatory interactions involved in the control and maintenance of cellular differentiation in the testis.

B. Endocrine regulation of testis function

Analysis of cell-cell interactions in the testis has revealed that the actions of the endocrine system are often indirectly mediated through local cellular interactions. Leydig cells respond to the gonadotropin LH to increase androgen production. The response to LH, however, is the initiation of a cascade of events in which androgens can act on peritubular cells to promote PModS production that acts on Sertoli cells to influence functions essential for germ cell development (Fig. 7). This androgen-mediated mesenchymal (stromal)-epithelial cell interaction may be similar in other androgen-dependent organs during fetal development and in the adult (11, 22, 676). It is speculated that differentiation type factors such as PModS may mediate steroid- or hormone-induced interactions in a wide variety of organs throughout development. In addition to LH-induced cell-cell interactions, FSH actions on Sertoli cells also promote indirect effects on other testicular cell types. FSH will indirectly affect germinal cell development by altering Sertoli cell-germinal cell interactions. Sertoli cells also appear to produce FSH-dependent factors that can influence Leydig cell function. Therefore, the two major endocrine agents known to influence testis physiology act, in large part, indirectly through alterations in local cellular interactions. Further investigation of the effects of endocrine agents on cell-cell interactions in the testis will be required to elucidate male reproductive endocrinology. The evolution of local cellular interactions that influence tissue function under the control of the endocrine system provides an efficient mechanism to regulate tissue physiology. The next era for the field of endocrinology will be to elucidate how endocrine agents affect tissue function on a molecular level through local cell-cell interactions.

C. Future directions

Information is needed on the paracrine and autocrine factors that mediate testicular cell-cell interactions. Factors such as the peritubular cell-produced PModS, growth factors such as SGF and SCSGF, and the Sertoli cell-produced factors that influence Leydig cells need to be more thoroughly purified and characterized on the protein and molecular level. This is needed to identify the unique or common properties of the factors as well as provide reagents to further investigate the specific cellular interactions. Two initial parameters to be considered in the analysis of cell-cell interactions are demonstration of the sites of production and action of regulatory agents. The expression of a specific gene and the capacity of the cell to secrete the factor is one prerequisite for an agent to be involved in a local cellular interaction. Of equal importance is the demonstration of the site of action of paracrine or autocrine factors. Regulatory factors must have defined and significant actions on target cells to effect cellular growth, function, or differentiation. The majority of proposed cell-cell interactions in the testis require further analysis on the cellular and molecular level.

Elucidation of the biochemical properties of a potential paracrine/autocrine agent and analysis of its site(s) of production and actions in vitro, however, do not directly address the physiological relevance of the proposed cellular interaction. The only testicular cell-cell interaction that is known to be essential for testis function is the need for the actions of androgen. Although androgens are needed, the mode(s) of androgen actions remain to be investigated. Few of the proposed cell-cell interactions can be investigated thoroughly on a physiological level at this time. As specific cell-cell interactions are elucidated it will be critical to determine the physiological relevance and importance of these cellular interactions.

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