Cell-Cell Interactions in the Testis

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INTRODUCTION

Cell-cell interactions play an integral role in the cell biology of an individual cell as well as in the physiology of a whole organ or tissue. Interactions between cells result in the maintenance or alteration of several cellular parameters, including differentiation, function, and growth (Fig. 1). A particular differentiated state of a cell is associated with a set of specific cellular functions and products. A specific function may be present in two distinct differentiated states of the cell, although the overall physiological functions of the cell may be quite different. The control of a specific cellular function may not relate to a change in the differentiated state of the cell, for it is but one of a host of functions needed to define a given differentiated state. Cellular differentiation and function then are two related but distinct cell parameters. Growth can be associated with either the stimulation or inhibition of cell proliferation. Following cell division, a specific differentiated state of the cell along with the control of specific cellular functions must be established. Cell-cell interactions between two different cell types have an important role in maintaining and regulating the differentiation, function, and growth of a cell. Factors maintaining these cellular parameters are as important as those involved in altering or regulating them. It is unlikely that any individual cellular interaction could maintain and regulate all cellular processes. Therefore, a number of different types of cell-cell interactions are required.

The types of specific cellular interactions possible are numerous. An attempt will be made to divide into three categories the majority of cell-cell interactions that can occur (Fig. 1). The first category deals with the extracellular environment of the cell. Interactions mediated by extracellular matrix and specific proteins such as cell adhesion molecules make up the environmental category. This type of interaction has an important role in providing the proper structural support and surroundings for the cell to maintain a normal morphology and differentiated state. This interaction is also very important during development and morphogenesis. The second category, called nutritional, deals with a cell obtaining essential components needed for survival. The maintenance of normal cellular functions requires many externally derived components such as energy metabolites. When an essential component such as a sugar metabolite, vitamin, or metal is derived from one cell type and delivered to a different cell type, a nutritional type of cell-cell interaction has occurred. The third category of cell-cell interaction deals with a component that is produced by one cell type that acts on a second cell type to cause a signal transduction that induces a cellular response on the molecular level associated with the differentiation, function, or growth of the cell. This type of interaction is called regulatory. Interactions of this type are
important in the control and maintenance of many cellular parameters. Regulatory agents involved in this type of interaction are paracrine factors. This interaction generally requires a receptor-mediated event to induce a second messenger that alters cellular parameters at the molecular level.

All three of these categories of cell-cell interactions are equally important and required for normal cellular function. There are some distinct differences among these types of interactions, however, in their overall effects on the cell. A regulatory interaction by definition requires a signal transduction to maintain or regulate some cellular response. Generally, neither an environmental nor a nutritional interaction is linked to a signal transduction that controls cellular responses on the molecular level. An environmental interaction provides the proper structural support and surroundings that do not involve a nutritional or regulatory interaction. The supply of essential substrates and metabolites through a nutritional type interaction is also not generally performed by either an environmental or regulatory interaction. Therefore, it is a combination of these different types of cellular interactions that provides the proper control and maintenance of the unique physiology associated with a given cell type.

This introduction provides the general concepts and vocabulary required to further discuss the interactions that occur between different testicular cell types that influence cellular differentiation, function, and growth. The cell types that will be considered are shown in Figure 2. Other cell types in the testis will not be considered due to their low abundance or lack of characterization. The Leydig cells are present in the interstitial tissue and produce androgens. The peritubular myoid cells surround the seminiferous tubule and, in cooperation with Sertoli cells, form a basal lamina. Sertoli cells form the seminiferous tubule and provide the cytoarchitectural support for the developing germinal cells. The interactions that occur among the Leydig cells, peritubular cells, and Sertoli cells will be discussed. The germinal cells undergoing spermatogenesis at different stages of development are supported between adjacent Sertoli cells. The interactions that occur between germinal cells and Sertoli cells will also be discussed. Although interactions may be possible between germinal cells and other somatic cell types, these interactions have not been directly demonstrated, and therefore, will not be considered. Clearly, the relationship between Sertoli cells and germinal cells will have profound effects on the process of spermatogenesis. Prior to a discussion of

FIGURE 1. Schematic of the effects of cell-cell interactions between different cell types, resulting in the maintenance and control of cellular differentiation, function, and growth. Classification of the major types of cell-cell interactions are defined as environmental, nutritional, and regulatory.
FIGURE 2. Schematic of the major cell types in the testis and the specific cells involved in cell-cell interactions.

the specific cellular interactions, the methodological considerations in an analysis of cell-cell interactions will be presented.

ANALYSIS

Biochemical analysis of cellular interactions regarding site of synthesis, site of action, characterization of components involved, and influence on target cell function generally requires an *in vitro* cell culture system. Although an *in vitro* system is useful, the actual definition of the different aspects of a cellular interaction generally is done *in vitro*. Once a specific cellular interaction is defined *in vitro*, however, an *in vivo* analysis is required to determine the physiological significance of the interaction. Because the majority of the cellular interactions identified in the testis are in the process of being defined, *in vitro* systems are generally used at present.

Several aspects of cell culture need to be considered in both establishing and using an *in vitro* system, including cell viability, cell type purity, and cellular functions. The purity of a cell population of interest is often critical to any data interpretations. The influence of a regulatory agent on a mixed culture system is difficult to directly interpret due to the number of possible cellular interactions that may alter the overall actions observed. For this and other reasons, investigators have made attempts to obtain pure testicular cell populations. Germinal cell isolation procedures have been reported and improved by a number of methods. Viability is often the limiting aspect of isolated germinal cells, but a number of areas of research use these isolated populations. Sertoli cell isolation procedures have also been reported and recently improved. Germinal cells and peritubular cells are the primary contaminants of Sertoli cell preparations. Hypotonic treatment of Sertoli cell cultures has been used to reduce germinal cell contamination. Peritubular cell contamination can be reduced by additional enzymatic treatments during Sertoli cell preparation. Fibronectin is a protein produced by peritubular cells in high amounts, but not produced in detectable amounts by Sertoli cells. This protein provides a convenient marker for peritubu-
lar cell contamination. Recently, it was also found that peritubular cells contain desmin as a cytoskeletal component, whereas Sertoli cells do not contain this substance (data not shown). Desmin has provided a convenient marker to assess purity of the Sertoli cell preparations cytochemically. Leydig cell populations have also been isolated for use in vitro. Contaminants of these cell populations have not been well-characterized and generally are present in low amounts depending on the isolation procedures used. Peritubular cell cultures can be obtained, but characterization of contaminating cell populations has not been reported. Viability of the isolated cell populations varies with the cell type and isolation procedure, with the order of viability and length of culture generally being peritubular cell > Sertoli cell > Leydig cell > germinal cell. The use of testicular cell lines to study aspects of cell function, regulation, or interactions must be interpreted with caution. Due to the changes that occur during transformation, any observations made will need to be repeated and confirmed with primary cell culture.

Investigation of cellular interactions requires a method to assess the maintenance or alteration of cell differentiation, function, or growth. Morphological analysis can be used to analyze the effects of specific types of interactions on the histology of the cell. This procedure has been used extensively to assess environmental interactions mediated by extracellular matrix and cell adhesion molecules. More quantitative methods are generally used to analyze alterations in cellular function and differentiation. Investigation of regulatory interactions generally requires a quantitative analysis of some aspect of cell differentiation or function. The methods used to quantitate the production of specific cellular components. These may be cellular substances such as cyclic nucleotides, phosphorylated proteins, metabolic substrates, or enzymatic activities. Secreted substances are also used to assess an alteration in cellular function and differentiation.

Analysis of secreted proteins has provided markers to investigate cellular interactions as well as to provide insight into the functions of the cells. Radiolabeled secreted proteins from peritubular cells and Sertoli cells are shown in Figure 3. Prior to concluding that a secreted protein is derived from a specific cell type, either radiolabeling of the protein or the presence at its mRNA must be demonstrated. This is due to the many sources of contamination possible. One peritubular cell-secreted protein that has been functionally identified is fibronectin at 200 kDa, and as shown in Figure 3, Sertoli cells do not produce detectable amounts of this protein. Several major products of Sertoli cells have been functionally identified. Transferrin is an iron-binding protein produced by Sertoli cells, which appears to be the same gene product as serum transferrin produced by the liver. Production of testicular transferrin is hormonally regulated and provides a convenient marker for the regulation of Sertoli cell function. Ceruloplasmin is a copper transport protein of 130 kDa that is also a major product of Sertoli cells. Several other major secreted proteins have been purified and characterized; these proteins in general, however, have not been functionally identified. Minor secretory products, that make up less than one percent of the total protein, which have been functionally identified, include androgen-binding protein (ABP) and plasminogen activator. Quantitative assays are available for transferrin, ABP, and plasminogen activator. Therefore, these proteins can be used as markers to investigate cellular interactions directed at Sertoli cells. Androgen production by Leydig cells is generally the marker used to analyze regulation of cellular function and differentiation. Unique and useful markers for germinal cells are not currently available.

Investigation of cellular interactions between somatic cells in the testis is
Peritubular (Myoid) Cell

Fibronectin

200-

Ceruloplasmin

130-

Transferrin

75-

Others:

Androgen Binding Protein (ABP)

Plasminogen Activator (PA)

30-

30-

Radiolabeled Secreted Proteins ($^{35}$S-met, $^{3}$H-gly)

**FIGURE 3.** Fluorogram of radiolabeled secreted proteins from peritubular cells and Sertoli cells. Major secretory products that have been functionally identified are designated and aligned with the appropriate bands, whereas minor Sertoli cell secretory products that have been functionally identified are listed as others.

Currently in progress in a number of laboratories. In part, this is due to the availability of cell culture systems and unique markers of cellular function. In addition, it is clear that the somatic cells play an important role in the maintenance and control of testicular function. Detailed analysis of cellular interactions directed at germinal cells, however, will require technical advances both in improving cell culture systems and developing unique and convenient markers of cellular function and differentiation.

**SPECIFIC CELL-CELL INTERACTIONS**

A brief review will be given of the major cell-cell interactions in the testis that have been identified and investigated. The different categories of interactions,
environmental, nutritional, and regulatory, will be considered when appropriate. The identification of a possible cellular interaction through the demonstration that a crude material contains a relevant biological activity will be mentioned when confirmed by several laboratories. Detail, however, will be presented for the cellular interactions in which the components involved have been characterized.

Sertoli Cell-Germin al Cell Interaction

The cytoarchitectural arrangements between Sertoli cells and germinal cells provides one of the most complex and interesting examples of an environmental cellular interaction. A number of laboratories have provided insight into this interaction through several types of morphological analysis (review reference 26). An appreciation for the complexity of Sertoli-germin al cell interactions was provided by a three-dimensional reconstruction of a Sertoli cell.27,28 Clearly, this environmental interaction plays an integral role in the maintenance of the germin al cell syncytium, the integrity of the seminiferous tubule, and the overall process of spermatogenesis. Extracellular components that may be involved in this interaction have not been identified. The lack of a complex extracellular matrix (ECM) may reflect the dynamic nature of the process of spermatogenesis, which may be inhibited by the presence of a complex ECM. The inability to maintain mammalian spermatogenesis in vitro may reflect, in part, the inability to maintain the complex cytoarchitecture between Sertoli cells and germinal cells. This is supported by the observation that in species with a less complex environmental interaction, in vitro spermatogenesis is possible.29

Nutritional interactions between Sertoli and germinal cells have been postulated since the first description of the cell by Sertoli, when he described it as a nurse cell.30 A number of investigators have confirmed this concept through functional identification of Sertoli cell secretory products. With the presence of an efficient blood-testis barrier, most of the components generally available to cells from the circulatory system must be produced or transported by the Sertoli cells for delivery to the developing germinal cells. Sertoli cells have been shown to produce the energy metabolites, pyruvate, and lactate,31 that subsequently can be used by germinal cells.32 Through this nutritional interaction, it has been proposed that Sertoli cells may provide the energy metabolites required for germinal cell development.31-33 Proteins involved in the transport of essential components have also been shown to be produced by Sertoli cells. One such protein synthesized and secreted by Sertoli cells is testicular transferrin,34 which is postulated to be involved in the transport of iron to developing germinal cells.35 Serum transferrin has been shown to deliver iron to the basal surface of the Sertoli cell, which internalizes the transferrin and iron.36 The serum transferrin is then recycled while the iron is transported to newly synthesized testicular transferrin. The testicular transferrin iron complex is then secreted and binds to specific receptors on germinal cells.35,36 Upon internalization of the transferrin by germinal cells, the iron is then used in critical cellular processes such as respiration. This pathway may be generalized to many components that must be transported across Sertoli cells to germinal cells. Another such protein is testicular ceruloplasmin, which is synthesized and secreted by Sertoli cells and postulated to be involved in copper transport to developing germinal cells.37 From these examples it is apparent that nutritional interactions between Sertoli cells and germinal cells play an integral role in the maintenance of germinal cell development, as was originally proposed in 1865.38
Sertoli-germinal cell interactions involved in a signal transduction and resulting in a regulatory type of interaction have not been well-characterized. Several laboratories have demonstrated that the presence of germinal or germinal cell fractions can influence Sertoli cell function as measured by different endpoints. Therefore, it has been postulated that regulatory interactions may occur between Sertoli cells and germinal cells. The possibility, however, that this interaction may be an environmental type of interaction needs to be considered. Characterization of the components involved and their mode of action will be required to determine if regulatory interactions are present between Sertoli cells and germinal cells.

**Sertoli Cell-Leydig Cell Interactions**

Sertoli cells and Leydig cells do not have environmental interactions between themselves because the cells are not in contact with each other. Due to the fact that both cell types are accessible to the circulatory system, nutritional interactions would also not appear to be required. Regulatory interactions, however, between Sertoli cells and Leydig cells have been identified. The primary components involved in this regulatory interaction are steroids. The early observation that an inhibition of androgen produced by Leydig cells would interfere with spermatogenesis leads to the proposal that testosterone acted on the seminiferous tubule to maintain spermatogenesis. It was then demonstrated that this interaction was indirectly mediated through the somatic cells (review reference 24). Sertoli cells have been shown to contain androgen receptors and respond to testosterone using several parameters. Many of the actions of testosterone on Sertoli cells are small, however. Therefore, it is not presently known whether the cells simply do not respond well in vitro or whether some additional aspect of androgen action is not understood. Another steroid implicated in Sertoli-Leydig cell interactions is estrogen. Sertoli cells isolated from prepubertal animals are capable of producing estrogen. The adult Sertoli cells lose the ability to produce estrogen, but during development, the estrogen produced by Sertoli cells is postulated to act on Leydig cells to inhibit androgen production. The actions of estrogens on Leydig cell steroidogenesis are not completely understood at present and are being investigated by several laboratories.

Regulatory interactions between Sertoli cells and Leydig cells mediated by nonsteroidal substances have also been postulated. Leydig cells produce pro-opiomelanocortin-derived peptides, which may regulate Sertoli cell function. The responses obtained in vitro with these peptides are small; therefore, the significance of this interaction is not clear at present. Factors produced by Sertoli cells that regulate Leydig cell function have also been investigated. Although a number of laboratories have demonstrated the presence of biologically active substances that appear to be produced by Sertoli cells or seminiferous tubules that can modulate Leydig cell steroidogenesis, the characterization and mode of action of the components involved remains to be delineated. From the information available, it appears that regulatory interactions between Sertoli cells and Leydig cells are important for the development and maintenance of testicular function. Further characterization of the components involved in these cellular interactions will provide more insight into their role in the control of spermatogenesis.
SKINNER: CELL-CELL INTERACTIONS

Sertoli Cell-Peritubular (Myoid) Cell Interactions

The complex extracellular matrix, basement membrane, formed between Sertoli cells and peritubular cells provides a classic example of an environmental interaction. This matrix helps to maintain the structural integrity of the seminiferous tubule and provides the proper surroundings for the peritubular cells and the basal surface of the Sertoli cells. Both Sertoli cells and peritubular cells aid in the production and formation of this basement membrane. Sertoli cells produce laminin, collagen type IV, and collagen type I, whereas peritubular cells produce fibronectin and collagen type I. Both cells produce unique secreted and cell-surface-associated proteoglycans. The deposition of a complex matrix in vitro requires the presence of both cell types. The presence of a complex extracellular matrix in vitro has dramatic effects on Sertoli cell morphology and may aid in maintaining the differentiated state of the cell. This environmental interaction clearly has an important influence in the differentiation and function of both Sertoli cells and peritubular cells in addition to providing a structural support for the tubule.

Because both Sertoli cells and peritubular cells are in contact with the circulatory system, nutritional interactions would not appear to be required, nor have any been identified. Regulatory interactions between the cells have been postulated and identified. Initial investigations used the coculture of Sertoli cells and peritubular cells. It was demonstrated that coculture of the cells stimulated ABP production by Sertoli cells. This resulted in the proposal that peritubular cells may secrete a paracrine factor that could influence Sertoli cell function. Serum-free peritubular cell-conditioned medium was found to stimulate transferrin production by Sertoli cells in a dose-dependent manner. Results implied that peritubular cells produced a factor that could modulate Sertoli cell function and was termed P-Mod-S. Size exclusion chromatography demonstrated that P-Mod-S has a molecular weight between 50,000 and 60,000 as shown in Figure 4. Analysis

![Graph](image)

**FIGURE 4.** Size-exclusion high-pressure liquid chromatography profile of peritubular cell-secreted proteins. The ability of individual fractions to stimulate transferrin production by Sertoli cells was assessed and represented in the bar graph as ng transferrin/µg Sertoli cell DNA. Elution of total protein was monitored at 280 nm and represented in the line graph. Standardization of the column for molecular weight determination is listed with the inset molecular masses in kDa.
TABLE 1. Effects of Regulatory Agents on Testicular Transferrin Production by Sertoli Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>ng Transferrin/μg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>FSH</td>
<td>100 ng/ml</td>
<td>58 ± 6</td>
</tr>
<tr>
<td>Insulin</td>
<td>5 μg/ml</td>
<td>56 ± 5</td>
</tr>
<tr>
<td>Retinol</td>
<td>0.35 μM</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.1 μM</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>FIRT</td>
<td>Same</td>
<td>109 ± 8</td>
</tr>
<tr>
<td>P-Mod-S</td>
<td>20 μg/ml</td>
<td>115 ± 11</td>
</tr>
</tbody>
</table>

*Sertoli cells from 20-day-old rats were cultured and treated at the time of plating. On day 2 of culture, medium was replenished and the cells were retreated. A 72 hr medium collection on day 5 of culture was used for analysis. FIRT represents a combination of FSH, insulin, retinol, and testosterone. Transferrin levels in the medium were determined with a radioimmunoassay,18 and data was normalized for μg Sertoli cell DNA at the time of the final medium collection. Data are presented as the mean ± SEM for three experiments done in triplicate; n = 9.

of the biological activity of P-Mod-S demonstrated that it could stimulate both transferrin and ABP production by Sertoli cells to a greater extent than any hormone previously known to influence Sertoli cell function,37 including follicle-stimulating hormone (FSH) (TABLE 1). Previous observations have indicated that a combination of FSH, insulin, retinol, and testosterone (FIRT) is required to obtain maximum stimulation of Sertoli cell function.18,38 P-Mod-S has the ability to stimulate to the same extent as this combination of regulatory agents (TABLE 1). These results indicate that peritubular cells produce a paracrine factor, termed P-Mod-S, that may have a significant role in regulating Sertoli cell function and differentiation. The relationship of P-Mod-S to growth factors was analyzed using mitogenic assays on a number of different cell types and cell lines. It was found that P-Mod-S was nonmitogenic on all the cell types tested.37 Therefore, P-Mod-S appears to be a nonmitogenic paracrine factor. P-Mod-S has subsequently been purified using reverse phase chromatography (data not shown). Two peaks of activity were detected and designated P-Mod-S (A) and P-Mod-S (B). P-Mod-S (A) is a 55 kDa acid-sensitive glycoprotein, whereas P-Mod-S (B) is a 59 kDa protein. The further characterization and relationship of these two proteins is currently under investigation.

The production of P-Mod-S by peritubular cells may play an integral role in the regulation of Sertoli cell function and, indirectly, in the maintenance and control of the process of spermatogenesis. P-Mod-S appears to be a potent nonmitogenic paracrine factor. The nonmitogenic character of a paracrine factor may provide a specificity for both the site of action and mode of action that a growth factor would not necessarily have, due to its more generalized effects. The possibility that a class of nonmitogenic paracrine factors may be involved in specific cell-cell interactions associated with a given cell type or tissue will be a potentially important area of research. A number of developmental biologists have demonstrated that the mesenchymal cells associated with a tissue appear to direct the differentiation and development of the adjacent epithelial cell (review reference 59). The presence of mesenchymal inducer substances, paracrine factors, involved in this mesenchymal-epithelial cell interaction have been postulated. Peritubular-Sertoli cell interactions provide a classic example of mesenchymal-epithelial cell interactions. P-Mod-S may be a good candidate for a mesenchymal-inducer substance in
the seminiferous tubule. Quantitative probes, however, will need to be developed to analyze the participation of P-Mod-S in testis development and the specificity of the interaction.

**Leydig Cell-Peritubular Cell Interactions**

The lack of direct contact between peritubular cells and Leydig cell indicates that environmental interactions would not appear to be required. In addition, both cell types are in contact with the circulatory system, indicating that nutritional interactions also may not be necessary. Regulatory interactions, however, between the two cell types have been postulated. Quantitation of androgen receptors in the seminiferous tubule demonstrates that peritubular cells contain a high percentage of the total androgen receptors. This correlates with the observation that peritubular cell development may be under androgen control. With these results and the fact that peritubular cells produce a paracrine factor that can influence Sertoli cell function, the hypothesis was tested that androgens may act on peritubular cells to indirectly influence Sertoli cell function. Primary cultures of peritubular cells were found to respond to androgen by increasing the amount of P-Mod-S activity present in conditioned medium. Another experiment was done using the coculture of Sertoli cells and peritubular cells. Testosterone treatment of monocultures of Sertoli cells resulted in a very small stimulation in ABP production. However, with the presence of peritubular cells in coculture, testosterone stimulated ABP production to the same extent as a combination of hormones known to maximally stimulate ABP synthesis. Therefore, the presence of peritubular cells significantly augmented the actions of androgen on Sertoli cells, which may be indirectly mediated through the production of P-Mod-S by peritubular cells. These observations imply that androgens produced by Leydig cells may act on peritubular cells to stimulate the production of the paracrine factor, P-Mod-S, that influences Sertoli cell functions involved in the process of spermatogenesis. More direct quantitation of the effects of androgens on P-Mod-S production will be required to confirm this hypothesis.

With these observations, the direct versus indirect actions of androgens on Sertoli cell function will need to be considered. Clearly, the indirect mode of androgen action mediated by way of the peritubular cells may be significant due to the potent regulatory activity of P-Mod-S on Sertoli cell function. The inability of Sertoli cells in culture to respond well to androgens supports the concept that indirect interactions may be involved. The relationship and overall effects of androgen action mediated directly on Sertoli cells and mediated indirectly by way of peritubular cells will need to be elucidated prior to understanding the mode of androgen action in the testis.

**DISCUSSION**

Characterization of a specific cell-cell interaction requires consideration of the following: identification of the cell types involved (i.e., site of synthesis and site of action); characterization of the component involved; analysis of the mode of action; analysis of its influence on cell differentiation, function, and growth; and an investigation of the physiological significance of the specific cellular interaction. Classification of the type of interaction is also needed. In the testis, few cell-cell interactions have been completely characterized, but all the different types of
FIGURE 5. Schematic of the major cell types in the testis and some of the specific interactions between these cells. Interactions represented as a (?) indicate a cellular interaction that has not been identified and/or in which the components involved have not been characterized.

interactions appear to be required. Environmental interactions in the testis are primarily confined to the seminiferous tubules. These include the basement membrane between peritubular cells and Sertoli cells and the complex cytoarchitecture between Sertoli cells and germinal cells. Nutritional interactions primarily appear between Sertoli cells and germinal cells due to the physiology of the blood-testis barrier. Several of the specific regulatory interactions discussed are represented in Figure 5. Androgen action on the seminiferous tubule is mediated by Sertoli cells and peritubular cells. Peritubular cell-Sertoli cell interactions by way of P-Mod-S are also shown. The possible interactions listed as question marks represent an interaction that has not been identified or rigorously characterized. Further elucidation of the majority of these cell-cell interactions is needed to understand the cell biology of the testis. Information available indicates that many of these cellular interactions may have a significant role in the maintenance and control of testicular function.

Analysis of specific cell-cell interactions must eventually lead to the question of the physiological significance of the interactions in vivo. This question is often very difficult to answer due to many unknown cellular parameters. Demonstration of the presence of a cell-cell interaction may provide insight into the cell biology of a given cell type that was previously not known. On the other hand, demonstration of the lack of a cellular interaction may also be very important. With this in mind, an important cell-cell interaction to consider in the testis is one between Sertoli cells and germinal cells. Clearly, both environmental and nutritional interactions occur between these cells. The possible presence or absence of regulatory interactions needs to be considered, however. Spermatogenesis is one of the most evolutionarily conserved biological processes. For this reason, it appears plausible that the information required for germinal cell development is present in the genome of the germinal cell. In support of this idea, several species have been shown to have very limited somatic cell-germinal cell association. For these reasons the need to invoke a complex network of regulatory interactions between Sertoli cells and germinal cells to control different stages of development needs to be evaluated. An alternate type of cell-cell interaction could be a more passive
process in which regulatory (i.e. signal transduction type) interactions would not be needed. The Sertoli cell may simply provide the germinal cell with the proper environment, metabolites, and nutrients to allow the process of spermatogenesis to proceed. Therefore, the primary Sertoli cell-germinal cell interactions that would occur are environmental and nutritional. This passive process relies on the assumption that germinal cell development does not require externally derived regulatory agents or signals. The only requirement would be the delivery of essential nutritional components. Therefore, two possibilities exist for Sertoli cell-germinal cell interactions involving either a passive or active process. At present, the information available does not permit the determination of which process occurs. Therefore, whether Sertoli cell-germinal cell interactions involve a passive or active process to support spermatogenesis remains to be elucidated. The existence of either process will need to be considered in the characterization of this cellular interaction.

A final point deals with the concept of regulation versus maintenance. The process of spermatogenesis is a constant that has minimal alteration in rate or output in a nonseasonal breeder. Data indicate that germinal cell development occurs at a near optimal rate, with one of the major limiting factors being the space available in the seminiferous tubule. Therefore, the process of spermatogenesis does not appear to be regulated, but simply maintained. With this in mind, and the fact that Sertoli cells have an important role in the process of spermatogenesis, the question is raised as to whether Sertoli cell functions are simply maintained or actively regulated in the adult. Clearly, Sertoli cell function and differentiation are regulated during development. Maintenance of optimal Sertoli cell function and differentiation, however, in the adult would appear to be needed to allow for optimal germinal cell development. The concept of whether Sertoli cell function in the adult is maintained or regulated has not been rigorously investigated. In support of the idea of optimal maintenance of function is the observation that both androgen and FSH levels in vivo appear to be in excess of those required to stimulate the Sertoli cell. Demonstration of whether Sertoli cell function in the adult is simply optimally maintained or regulated (i.e. changing) remains to be elucidated. The distinction between maintenance and regulation does not reduce the importance of the endocrinology or regulatory agents involved, but simply broadens our understanding of testicular physiology and the cell biology of the testis.

SUMMARY

In conclusion, the information available indicates that the interactions between different cell types in the testis play an important role in the control and maintenance of testicular functions. Further characterization of these interactions will clearly provide insight into the cell biology of the testis and into the regulation of cellular differentiation, function, and growth. It is apparent that no testicular cell type is autonomous, but that there is communication and cooperation between all cell types in the testis.

REFERENCES

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