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Mesenchymal (Stromal)–Epithelial Cell Interactions in the Testis and Ovary which Regulate Gonadal Function

Michael K. Skinner

Department of Pharmacology, Vanderbilt University, School of Medicine, Nashville, TN. 37232, USA.

Introduction

The ability of different cells to communicate and interact became essential with the evolution of multicellular organisms. Cell–cell interactions have become important for the maintenance and control of essentially every tissue's function, growth and differentiation. In addition, the ability of physiological systems such as the endocrine system to modulate local cell–cell interactions plays an important role in the regulation of tissue function. Numerous types of cell–cell interactions are possible and have previously been categorized into environmental, nutritional and regulatory cellular interactions (Skinner 1987). Both the testis and the ovary provide convenient model tissues to investigate more thoroughly cell–cell interactions on a molecular level. Observations made with these gonadal tissues provide insight into general cell–cell interactions that occur in many different tissues, as well as develop a better understanding of the regulation of testis and ovary function. This paper provides a brief overview of cell–cell interactions in the testis and ovary with emphasis on mesenchymal (stromal)–epithelial cell interactions. Mesenchymal–epithelial cell interactions will be stressed in view of the postulated importance of this cell–cell interaction for the development and functions of most tissues (Cunha *et al.* 1983).

Cell–Cell Interactions in the Testis

Interactions between Leydig cells, peritubular myoid cells, Sertoli cells and germinal cells are required for the maintenance of testis function and the process of spermatogenesis. Sertoli cells form the seminiferous tubule and provide the cytoarchitectural support for the developing germinal cells. Peritubular myoid cells surround and form the exterior wall of the tubule. Leydig cells are in the interstitium and provide the source of androgens. The interactions between peritubular cells and Sertoli cells provide an example of a mesenchymal (stromal)–epithelial cell interaction. The effect of this cellular interaction on germinal cell development and the influence of Leydig cells on this interaction will be discussed.

The different types of interactions will be classified as environmental, nutritional, or regulatory, as previously described (Skinner 1987). Environmental interactions are mediated by components such as extracellular matrix and cell adhesion molecules. Nutritional interactions are mediated through the transport of essential metabolites between different cell types. Regulatory interactions are mediated by a paracrine factor via a receptor-mediated signal transduction on a molecular level.

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Environmental interactions in the testis primarily occur between Sertoli cells and germ cells or between Sertoli cells and peritubular cells. The interaction between Sertoli cells and developing germinal cells is one of the most complex and elegant environmental cell–cell interactions known. The cytoarchitecture created by the Sertoli cell is essential for the process of spermatogenesis and an important environmental cell–cell interaction for testis function. The environmental interaction between peritubular cells and Sertoli cells is mediated by a complex extracellular matrix (basement membrane) between these cells. Both peritubular cells and Sertoli cells cooperate in the production of individual components of the extracellular matrix (Skinner *et al.* 1985). This extracellular matrix between peritubular cells and Sertoli cells provides structural integrity for the seminiferous tubule as well as promoting the structural differentiation of the Sertoli cell (Hadley *et al.* 1985). This environmental interaction, however, does not appear to regulate Sertoli cell function on a molecular level (Anthony and Skinner 1989). Although these environmental interactions may not actively regulate cellular function, they are critical for the maintenace of testis function and the process of spermatogenesis.

Nutritional interactions occur primarily between Sertoli cells and germinal cells. The creation of the blood–testis barrier by Sertoli cells requires that essential components (such as energy metabolites, vitamins and metals) be transported through or synthesized by the Sertoli cells and delivered to the developing germinal cells sequestered within the tubule (Skinner 1987). Other cell types within the testis are exposed to the circulatory system, and nutritional interactions are not as critical for cell function.

Regulatory interactions can occur between all the different cell types in the testis. Peritubular cell–Sertoli cell regulatory interactions have been identified through the identification of several paracrine factors. Peritubular cells produce a non-mitogenic paracrine factor, PModS, which modulates Sertoli cell function (Skinner and Fritz 1985). PModS has been purified and characterized (Skinner *et al.* 1988) and recently shown to act in part through a unique signal transduction system for Sertoli cells by increasing cGMP levels (Norton and Skinner 1989). PModS has more dramatic effects on Sertoli cell function than any individual regulatory agent previously identified, including FSH (Skinner *et al.* 1988; Norton and Skinner 1989). Therefore, it has been postulated that the regulatory interactions between peritubular cells and Sertoli cells mediated via PModS will be essential for the maintenance and control of testis function. Additional regulatory interactions between peritubular cells and Sertoli cells have been suggested through the recent identification of potential paracrine factors, transforming growth factor-α (TGFα) (Skinner *et al.* 1989*a*) and transforming growth factor-β (TGFβ) (Skinner and Moses 1989). Both Steroli cells and peritubular cells produced TGFα and TGFβ. The epidermal growth factor (EGF)-like peptide TGFα acts at the EGF receptor and mimics the actions of EGF. The EGF receptor in the seminiferous tubule appears to be primarily localized to the peritubular cell (Skinner *et al.* 1989*a*). Therefore, TGFα production by Sertoli cells can act as a paracrine factor to regulate peritubular cell growth while TGFα production by peritubular cells may act as an autocrine factor. TGFβ is a growth inhibitor that has a dramatic effect in promoting peritubular cell differentiation and migration (Skinner and Moses 1989). TGFβ, therefore, may also act as a paracrine and autocrine factor in the seminiferous tubule. The ability of peritubular cells and Sertoli cells to interact through regulatory interactions will indirectly influence the process of spermatogenesis through an influence on Sertoli cell–germ cell interactions. The environmental and nutritional interactions between Sertoli cells clearly will be important for germ cell development; however, whether regulatory interactions occur between these cells remains to be thoroughly investigated. A number of laboratories have indicated that germ cells can influence Sertoli cell function *in vitro*. The mechanisms and agents involved have not been identified; therefore, the physiological significance is unclear at present. An interesting area of research yet to be elucidated is whether the Sertoli cells simply

produce a passive microenvironment for the genome of the germ cells to control germ cell development or whether there is a more active process involving a complex network of regulatory interactions between Sertoli cells and germinal cells.

Leydig cells have an important regulatory interaction with the seminiferous tubules through the production and action of androgens. Androgens are required for the process of spermatogenesis and have been shown to act on the Sertoli cells that contain the androgen receptor. However, the direct actions of androgens on Sertoli cell function *in vitro* are limited. The specific actions and physiological significance of androgen regulation of Sertoli cell function requires further investigation. Peritubular cells also contain androgen receptors and provide a site for a regulatory interaction with Leydig cells. PModS production by peritubular cells is under androgen regulation (Skinner and Fritz 1985) and so provides a potential mode of action for androgens to regulate Sertoli cell functions indirectly (Skinner 1987). Because of the dramatic effects of PModS on Sertoli cell function, the ability of androgens to influence peritubular cell–Sertoli cell interactions via PModS is postulated to be a critical mode of androgen action in the control of testis function. For example, PModS has recently been shown to stimulate inhibin production by Sertoli cells (Skinner *et al.* 1989*b*). Androgens have no effect alone on inhibin production by Sertoli cells *in vitro*; however, the action of androgens in stimulating PModS production by peritubular cells provides a mechanism for androgens to indirectly regulate inhibin levels *in vivo* (Skinner *et al.* 1989*b*).

Sertoli cell–Leydig cell interactions have been postulated following observations of the effects that material derived from seminiferous tubules has on Leydig cell steroidogenesis. Since the characteristics of these active agents remain to be identified, the physiological significance of such regulatory interactions is unclear. Inhibin is an agent derived from Sertoli cells that has the potential to influence hormone action and steroidogenesis of Leydig cells (Hsueh *et al.* 1987); however, the physiological significance of such an interaction remains to be investigated. Since androgen production by Leydig cells is nearly an order of magnitude higher than required to maintain testis function, regulation of androgen production in the adult male appears to have negligible effect on testis function. The identification of additional important functions of the Leydig cells, other than androgen production, may help resolve this apparent paradox. For example, although the functions of proopiomelanocortin (POMC) peptides produced by Leydig cells is unclear, the regulation of POMC peptides by Leydig cells via local cell–cell interaction may have physiological significance that is yet to be identified.

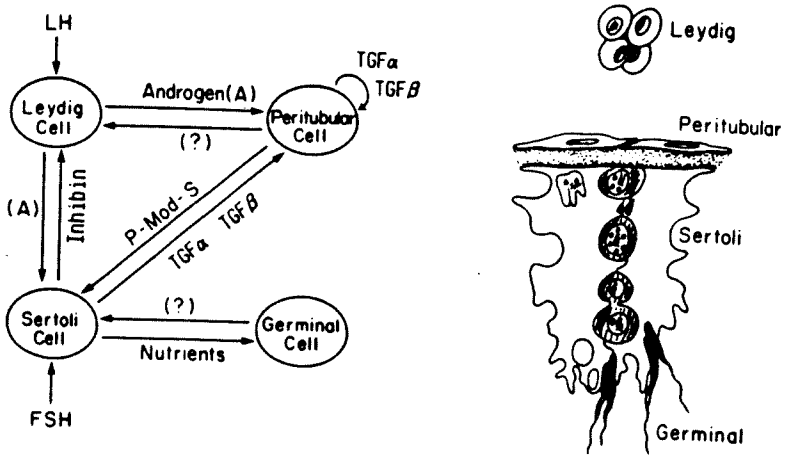

Fig. 1. Major cellular interactions in the testis.

The major cellular interactions discussed are summarized in Fig. 1. The identification of unique cellular interactions such as those mediated by PModS provide an insight into testis physiology as well as the potential future design of agents to modulate testis function. However, the cell-cell interactions presented are likely to be the first of many complex cell-cell interactions yet to be identified.

Cell-Cell Interactions in the Ovary

Interactions between theca cells, granulosa cells and the oocyte are required for the maintenance of ovarian function and the process of oogenesis. Granulosa cells provide the cytoarchitectural support for the developing oocyte and also help to form the follicle and antrum. Theca cells surround and form the exterior wall of the follicle. The interactions between theca cells and granulosa cells provide an example of a mesenchymal (stromal)-epithelial cell interaction. The effects of this cellular interaction on oocyte development and the influence of the endocrine system on this cellular interaction will be discussed.

Environmental interactions in the ovarian follicle occur between granulosa cells and either the oocyte or theca cells. Granulosa cell-oocyte interactions occur with the layer of cumulus granulosa cell surrounding the oocyte. These granulosa cells provide the physical support for the oocyte and have complex junctional interactions between the cells. Although this environmental cell-cell interaction is not as complex as Sertoli cell-germ cell environmental interactions, the granulosa cell-germ cell interaction is essential for oocyte development. Theca cells surround the follicle and form a complex extracellular matrix with the outer layer of mural granulosa cells. This environmental interaction between theca cells and granulosa cells is important to maintain the structural integrity of the follicle.

Nutritional interactions can occur between granulosa cells, theca cells and/or the oocyte. Granulosa cells have a complex network of gap junctions which allow the transport of essential components between cells. Granulosa cells and the oocyte also have junctions that allow the transport of nutritional substances between the cells. This complex reticulum aids the granulosa cells and oocyte to form a functional unit which is critical for the maintenance of ovarian function. The production of androgens by theca cells and subsequent utilization by granulosa cells as a substrate for the enzyme aromatase to produce oestrogen is a classic example of nutritional interaction between mesenchymal (stromal) cells and epithelial cells. This steroid-mediated theca cell-granulosa cell nutritional interaction is essential for the maintenance of ovarian function and the endocrine status of the animal.

Regulatory interactions occur between the three major cell types of the follicle to maintain and control ovary function, growth and differentiation. Granulosa cells and the oocyte are postulated to interact through the junctional network and the transport of cyclic nucleotides. The ability of regulatory agents such as cAMP to pass through the junctions constitutes a potentially important regulatory interaction between granulosa cells and the oocyte. The interactions between these cells, mediated by paracrine factors, are not known but may involve oocyte maturation inhibitor. Granulosa cells interact through autocrine mechanisms via the ability of granulosa products such as oestrogen, progestin and insulin-like growth factor (IGF) to modulate granulosa cell steroidogenesis. A number of potential regulatory agents such as renin, relaxin, angiotensin and prostaglandins are thought to participate also in regulating ovarian function. Although the physiological significance of these agents requires further investigation, local autocrine regulation of ovarian function is likely to be an important regulatory interaction needed to maintain ovarian function.

Regulatory interactions between theca cells and granulosa cells can be mediated via steroid and peptide factors. Progestin production by granulosa cells can influence theca cell function (Fortune 19[illegible] however, oestrogen may be a more important regulator of theca cell differentiation. [illegible]ese steroid-mediated interactions are essential for the main-

tenance of follicle development, and they influence the endocrine status of the female. There is a recent awareness of peptide-mediated interactions between theca cells and granulosa cells. Theca cells, but not granulosa cells, have been found to produce an EGF-like substance identified as TGFα (Skinner and Coffey 1988). TGFα can act to stimulate granulosa cell and theca cell growth so is assumed to be important for the stimulation of cell growth in the follicle (Fortune 1986). This theca cell–granulosa cell interaction provides physiological significance for the large number of reports regarding the actions of EGF on granulosa cell differentiation and growth (Hsueh *et al.* 1984). In addition to the production of the growth stimulator TGFα, theca cells were also found to produce the growth inhibitor TGFβ (Skinner *et al.* 1987). TGFβ can inhibit the ability of TGFα to stimulate either granulosa cell or theca cell growth. The inverse actions of TGFα and TGFβ are thought to provide an efficient mechanism to control the growth of the ovarian follicle. TGFβ has also been shown to stimulate the steroidogenic capacity and hormonal responsiveness of granulosa cells, probably through the ability of TGFβ to inhibit the growth phase of the cell. Therefore, TGFβ and possibly TGFα may also have a role in regulating the differentiation of follicular cells. Other peptide factors mediating granulosa cell–theca cell interactions, such as inhibin and IGF, require further investigation to determine the physiological significance of such interactions.

The major cellular interactions discussed are summarized in Fig. 2. The cell–cell interactions presented are probably only a few of many complex cell–cell interactions yet to be identified.

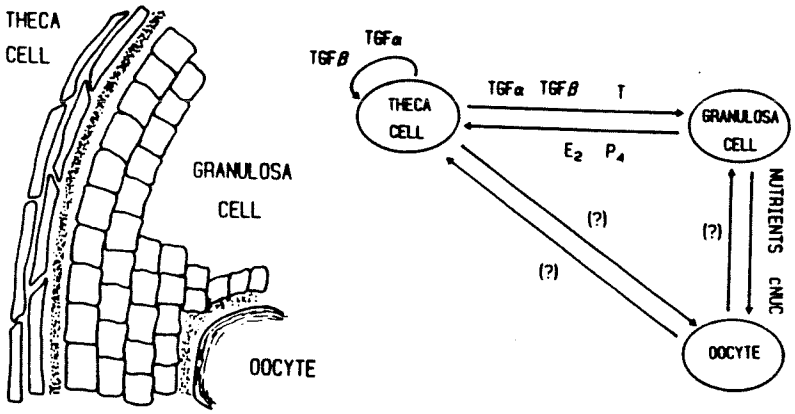

Fig. 2. Major cellular interactions in the ovary.

Summary

Clearly the interactions between different cell types within the ovary and testis are essential for gonadal function, growth and differentiation. The environmental interactions are critical for maintaining the structural integrity and differentiation of the tissues as well as being required for the process of gametogenesis. Nutritional interactions, particularly within the blood–testis barrier, are also essential for the maintenance of cell viability and for the process of germ-cell development. The regulatory interactions provide efficient local control mechanisms to modulate gonadal function on a molecular level. The ability of the endocrine system to influence local cell–cell interactions will initiate a cascade of events which can indirectly regulate the functions of many different cell types. Therefore, further investigation of cell–cell interaction in the testis and ovary is required to develop a complete understanding of reproductive endocrinology and the mechanisms regulating gonadal function.

Comparison of the cell–cell interactions in the testis and ovary reveals the general differences in the physiologies of the tissues. The somatic cells of the ovary require a rapid rate of growth during the process of folliculogenesis as well as a mechanism to inhibit growth for differentiated stages such as atresia. Hence, many of the cell–cell interactions identified in the ovary deal with growth regulation. The stomatic cells of the testis deal with the regulation of differentiation rather than of growth. It is evident that the cell–cell interactions that evolve for a tissue accommodate the physiological needs of the tissue.

Mesenchymal–epithelial cell interactions are claimed to be critical for the development and differentiation of tissues. Investigation of mesenchymal (stromal)–epithelial cell interactions in the testis (peritubular–Sertoli cell interactions) and the ovary (theca–granulosa cell interactions), reveals that this interaction is essential for gonadal function. Observations made in the testis provide biochemical support for the hypothesis that mesenchymal cells produce inducer substances that can regulate the cellular differentiation of the adjacent epithelial cell. Observations made in the ovary reveal the importance of mesenchymal–epithelial interaction in the regulation of tissue growth. Analysis of cell–cell interaction in these reproductive tissues, therefore, has provided insight into cell–cell interactions that occur in many tissues. It is suggested that mesenchymal (stromal) cells and epithelial cells of all organs will form a functional unit that is required for the maintenance and control of tissue function. Few cells will be autonomous in the maintenance of their function and this includes the individual cell types of the testis and ovary.

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