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TRANSCRIPTIONAL REGULATION OF CELL ADHESION AT THE BLOOD-TESTIS BARRIER AND SPERMATOGENESIS IN THE TESTIS

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Abstract

Spermatogenesis involves precise co-ordination of multiple cellular events that take place in the seminiferous epithelium composed of Sertoli cells and developing germ cells during the seminiferous epithelial cycle. Given the cyclic and co-ordinated nature of spermatogenesis, temporal and spatial expression of certain genes pertinent to a specific cellular event are essential. As such, transcriptional regulation is one of the major regulatory machineries in controlling the cell type- and stage-specific gene expression, some of which are under the influence of gonadotropins (e.g., FSH and LH) and sex steroids (e.g., testosterone and estradiol-17 β). Recent findings regarding transcriptional control of spermatogenesis, most notably target genes at the Sertoli-Sertoli and Sertoli-spermatid interface at the site of the blood-testis barrier (BTB) and apical ectoplasmic specialization (apical ES), respectively, involving in cell adhesion are reviewed and discussed herein. This is a much neglected area of research and a concerted effort by investigators is needed to understand transcriptional regulation of cell adhesion function in the testis particularly at the BTB during spermatogenesis.

INTRODUCTION

In mammalian testes, such as in rats, spermatogenesis is a highly co-ordinated event in which spermatogonia (2n) undergo a series of mitotic divisions and Type A spermatogonia differentiate into primary preleptotene spermatocytes. The primary preleptotene spermatocytes (2n) traverse the blood-testis barrier (BTB) which is created by adjacent Sertoli cells near the basement membrane and anatomically divides the seminiferous epithelium into the basal and apical compartment (Fig. 1), differentiating into leptotene and zygotene spermatocytes, so that diplotene spermatocytes undergo two meiotic divisions and form haploid spermatids (1n) in the apical (adluminal) compartment of the seminiferous epithelium behind the BTB. Thereafter, spermatids undergo a series of extensive morphological changes known as spermiogenesis (steps 1 to 19 in the rat testis) and spermatozoa are released from the seminiferous epithelium at spermiation. The synchronous nature of spermatogenesis that involves mitosis, BTB restructuring, cell cycle progression,

meiosis, spermiogenesis and spermiation, resulting in a specific pattern of cellular association at a given segment of the seminiferous tubule. Based on the unique cellular association pattern, the seminiferous epithelium can be classified into 12 stages and 14 stages in mouse and rat, respectively.^{1,2} Throughout these stages, developing germ cells remain attached to the Sertoli cells via specialized cell junctions for structural and nourishment support, many of these junctions are uniquely found in the testis, such as ectoplasmic specialization (ES) and desmosome-like junction.³ For instance, a specialized anchoring junction known as apical ES is restricted to the interface between Sertoli cells and spermatids (steps 8-19). Once apical ES appears at the Sertoli cell-step 8 spermatid interface, this is the *only* anchoring device to anchor developing spermatids until spermiation when apical ES begins to be engulfed by the Sertoli cell, analogous to "giant" endocytic vesicles undergoing internalization or endocytosis, forming an ultrastructure known as the tubulobulbar complex.³ As noted above, the BTB also undergoes extensive restructuring at the Sertoli-Sertoli cell interface at Stage VIII of the epithelial cycle in the rat testis to accommodate the transit of preleptotene spermatocytes at the site. Thus, it is conceivable that different cell-cell interacting events occur at the Sertoli-Sertoli (i.e., BTB) and the Sertoli-germ (i.e., apical ES, desmosome-like junction and gap junction) cell interface at respective stages of the epithelial cycle. As such, precise temporal and spatial regulation of gene expression in Sertoli and germ cells occur stage-specifically in the seminiferous epithelium. In fact, recent studies using microarray analysis have revealed at least 80 stage-regulated gene probe sets whose expression is 3-fold higher in mature Sertoli cells than germ cells and certain stage-regulated pathways in Sertoli cells pertinent to cell migration during the seminiferous epithelial cycle have been also identified.⁴ Therefore, transcriptional regulation of the cell-specific and stage-specific genes is essential to maintain the timely expression of specific genes during spermatogenesis.

TRANSCRIPTION FACTORS IN SERTOLI AND GERM CELLS CRUCIAL TO SPERMATOGENESIS

Some groups of transcription factors are ubiquitously expressed in Sertoli and germ cells throughout all stages of spermatogenic cycle that affect a relatively board spectrum of genes important for germ cell development. However, there are also differential expressions of selected transcription factors in Sertoli and germ cells crucial to exert stage-specific and cell type-specific gene regulation during spermatogenesis. Representative transcription factor families are summarized (Fig. 1) and briefly discussed in this chapter to illustrate how gene expression is co-ordinated in stage-specific and cell-specific manners in the seminiferous epithelium. However, emphasis is placed on transcriptional regulation of genes pertinent to maintain cell adhesion function at the BTB and the apical ES during spermatogenesis. However, it is noted that there are very few reports in the literature that examine the transcriptional regulation of adhesion protein complexes at the BTB and the apical ES. The goal of this chapter is to highlight and discuss some of these findings. But more importantly, this chapter attempts to provide some helpful guides in this much neglected area of research that deserves attention in future studies.

Reproductive Homeobox X-Linked (RhoX) Homeodomain Proteins

Reproductive homeobox X-linked (RhoX) genes clustered on the mouse X chromosome encode transcription factors that are selectively expressed in reproductive tissues.⁵ The twelve related homeobox genes (RhoX1-12) are selectively expressed in male reproductive tissue.⁵ However, most X-linked genes are inactivated in germ cells during spermatogenesis,⁶ but all RhoX genes are expressed in somatic cells in the testis.⁵ In particular, the expressions of RhoX1, 4 and 11 are high in Leydig cells; whereas all RhoX1-12 are expressed in Sertoli cells. The temporal and spatial expressions of different RhoX genes in the testis suggest that some RhoX genes might perform distinct cell type-specific functions during specific phases of spermatogenesis.^{5,7}

RhoX5 is the best studied RhoX gene. RhoX5^{-/-} males were sub-fertile with reduced number of mature spermatids in the seminiferous epithelium. This decline in mature spermatid number was due to an increase in apoptosis among spermatogonia and spermatocytes.⁵ Knockout of RhoX5 altered the expression of other genes in the testis as well,^{5,8} such as an increase in Unc5c expression. Unc5c is known to promote germ cell apoptosis, since Unc5c^{-/-} mice are having significantly fewer seminiferous tubules with apoptotic germ cells.^{9,10} These results thus suggest that RhoX5 negatively regulates Unc5c expression in Sertoli cells. These findings also implicate the likely transcriptional involvement of RhoX5 and Unc5c in germ cell apoptosis, possibly at the specialized junctions at the Sertoli-germ cell interface, such as desmosome-like junctions, gap junctions and apical ES since gap junctions may provide the necessary signaling information between Sertoli and germ cells to mediate the events of cell apoptosis, which should be explored in future studies.

Similar to RhoX2, 3, 10 and 11, RhoX5 is responsive to testosterone, thus it is a candidate to mediate testosterone-dependent events of spermatogenesis.^{5,7,11} Other studies have shown that testosterone is crucial to maintain the integrity of junctional complexes in the testis,¹² in particular BTB integrity,¹³ such as the re-assembly of occludin-based "new" TJ-fibrils behind preleptotene spermatocytes in transit at the BTB at Stage VIII of the seminiferous epithelial cycle via recycling transcytosis^{14,15} and/or de novo synthesis.^{16,17} It is important to determine if RhoX5 is involved in the transcriptional regulation of integral membrane proteins at the BTB, such as occludin, claudins, JAMs, N-cadherin, nectins, in future studies. On the other hand, RhoX1 is dominantly expressed in Sertoli cells in neonatal rats when they are still actively dividing, but its expression diminishes considerably when Sertoli cells enter terminal differentiation phase at day 10-15 postpartum in mice⁵ when BTB is established by day ~15. Such expression pattern suggests that RhoX1 might promote Sertoli cell proliferation and its reduced expression might be critical for the assembly of the BTB.

Androgen Receptor and Cell Adhesion Regulation at the Blood-Testis Barrier and Apical ES

Androgens are crucial in the maintenance of spermatogenesis.¹⁸ Testosterone and its metabolite, 5 α -dihydrotestosterone (DHT) mediate their effects via binding to intracellular androgen receptor (AR).¹⁹ AR belongs to nuclear receptor superfamily which is found in Sertoli, germ, Leydig and peritubular myoid cells in the testis, and it acts as a ligand-dependent transcription factor that mediates androgen-dependent gene regulation through

binding to the androgen response element (ARE) of the promoter region.²⁰ Apart from the classical testosterone-intracellular AR signaling pathway, recent studies have shown that testosterone also mediates its effects via nonclassical signaling cascades such as c-Src and MAPK in different cell types including testicular cells.^{21,22} Instead of binding to intracellular AR, testosterone has been shown to bind to membrane-associated AR, triggering a cascade of signaling events, resulting in the stimulation of calcium influx and the activation of MAP kinase pathways.²³⁻²⁵ Nonetheless, the activation of nonclassical testosterone action still requires nuclear AR.

The role of AR in the testis has been evaluated using various male total and conditional AR knockout mice models. The male total AR knockout mice (T-AR^{-y}) displayed female phenotype and had undescended testes with severe interruption in germ cell development.^{26,27} While AR specific knockout in germ cells (G-AR^{-y}) illustrated no apparent influence in fertility with normal spermatogenesis in the testis; however, AR knockout in peritubular cells (PM-AR^{-y}) led to reduced sperm count and drastic reduction in testis weight and these mice were infertile.^{28,29} Additionally, Sertoli cell function was impaired in PM-AR^{-y} mice with reduced seminiferous tubule fluid production and a decline in androgen-dependent gene expression.²⁹ Thus, AR apparently is not the crucial transcription factor in germ cells to maintain male fertility but to fine-tune spermatogenesis, yet it is critically important in peritubular myoid cell function and its loss would impede spermatogenesis. Two lines of cell type-specific AR knockout mice (AR^{-y}), namely Sertoli (S-AR^{-y}) and Leydig cell (L-AR^{-y}) specific knockouts, displayed testicular dysfunction with meiosis arrest, leading to infertility.³⁰⁻³³ For instance, diplotene spermatocytes fail to develop in S-AR^{-y} mice, while round spermatids fail to enter spermiogenesis in L-AR^{-y} mice.³⁰⁻³³ These results suggest that AR in Sertoli cells play a crucial role in the event of meiosis I and AR in Leydig cells are important to maintain spermiogenesis possibly through its effects on steroidogenic function.

Using microarray gene profiling, an array of genes was detected to be up- or down-regulated in prepubertal S-AR^{-y} mice compared to the wild-type.³⁴ For instance, serine protease inhibitor (Eppin), dopamine receptor 4 (Drd4) and glycerol-3-phosphate dehydrogenase 1 (Gpd1) are down-regulated in prepubertal S-AR^{-y} mice (postnatal day 10) *versus* normal mice.³⁴ Genes encoding two cell junction proteins including claudin-11 and laminin α 5 are also down-regulated in S-AR^{-y} mice at postnatal day 10.5.³⁵ However, it remains to be determined whether AR exerts a direct effect on the gene transcription of these two junction protein genes.

TRANSCRIPTION FACTORS INVOLVED IN THE MAINTENANCE OF SPERMATOGENIAL STEM CELLS

A_{single} spermatogonia were originally conceived to be the possible spermatogonial stem cells (SSC) in the testis.³⁶ However, two recent studies have unequivocally demonstrated that not all the spermatogonia A_{single} are 'true' SSC in rodent testes and it was estimated that there are only about 2,000 to 3,000 SSC among the 35,000 A_{single} spermatogonia per testis.^{37,38} Thus, it is apparent that only ~10% of the the spermatogonia are 'true' SSC in the testis. Nonetheless, understanding the mechanism regarding the self-renewal process of

spermatogonia (and/or SSC) during spermatogenesis is crucial and might provide new insights for treatment of male infertility. It is also important to determine if ‘true’ SSC express unique sets of genes (i.e., specific SSC markers) *versus* other spermatogonia including A_{single} spermatogonia.

Promyelocytic leukemia zinc-finger (Plzf) and B-cell lymphoma 6 member B (Bcl6b) are members of the bric-à-brac/track broad complex/pox viruses and zinc fingers (BTB/POZ) domain transcriptional repressors that are expressed in spermatogonia.^{39,40} Plzf and bcl6b regulate spermatogonial stem cell renewal.^{41,42} Transplantation studies have shown that Plzf^{-/-} spermatogonia are unable to repopulate the testis via mitosis in germ cell-depleted recipient testis,⁴² suggesting that Plzf is crucial to maintain spermatogonial stem cells. An array of genes involved in cell metabolism, cell cycle and cell differentiation was found to be significantly reduced in purified Plzf^{-/-} spermatogonia. Microarray studies have shown that genes such as testis specific X-linked Knockout of plzf showed a more pronounced effect on spermatogenesis since bcl6b null mice were shown to have offsprings of smaller litter size.⁴⁰ Male mice lacking Plzf showed a progressive loss of spermatogonia upon aging, leading to infertility eventually.⁴² Genes such as Tss and cyp11a1 and transcription factor including doublesex and mab-3 related transcription factor 2 (Dmrt2) are also down-regulated significantly *versus* normal mice.⁴² Some proteins, such as cyclinD2 and Ches1, however, are upregulated in Plzf^{-/-} mice. CyclinD2 is normally expressed in differentiated spermatogonia, spermatocytes and spermatids, but not in spermatogonial stem cells and it plays a role in spermatogonial differentiation.^{43,44} However, questions such as whether Plzf acts directly to alter the transcription machineries on these genes remain enigmatic. Except one, kit gene encoding the transmembrane receptor of stem cell factor, is the direct Plzf target gene identified so far.⁴⁵ Kit is the hallmark of differentiating spermatogonia, Plzf-mediated kit repression is believed to be crucial to maintain the population of spermatogonial stem cells. In fact, Plzf represses kit gene transcription via the binding to the Plzf binding site located upstream of the exon 1 of the kit promoter in spermatogonia.⁴⁵

It is no doubt that transcription factors expressed by germ cells are crucial for SSC renewal, recent studies have also showed that transcription factors expressed exclusively by Sertoli cells are also important regulators for this process.⁴⁶ Male mice with targeted disruption of Ets related molecule (ERM) displayed testicular atrophy with tubules devoid of germ cells, but having morphologically normal Sertoli cells at the age of 10 weeks postpartum.⁴⁶ Surprisingly, the lack of germ cells in the ERM^{-/-} mice was not due to the interruption of spermatogenic differentiation process since the first wave of spermatogenesis progresses normally and spermatogonia finally give rise to spermatids. In fact, the exhaustion of SSC after the completion of the first wave of spermatogenesis was the cause of germ cell depletion in the Erm knockout, illustrating Erm is crucial for SSC renewal.⁴⁶

Although Erm is a transcription factor exclusively expressed in Sertoli cells, a loss of Erm would impede changes in the expression of multiple genes, some of which are not *restricted* to Sertoli cells. In fact, a plethora of genes expressed in spermatogonial germ cells was altered in Erm knockout mice. A list of genes in Sertoli cells was found to be down-regulated (9- to 25-fold reduction) in Erm^{-/-} mice, illustrating a disruption of Erm in Sertoli

cells would impede gene expression in germ cells by microarray analyses including stromal cell-derived factor (SDF-1), chemokine ligand 5 (CXCL5), chemokine ligand 7 (CCL7) and matrix metalloproteinase 12 (MMP-12).⁴⁶ A recent study has confirmed that Erm directly binds to Ets binding site (EBS) of SDF-1 promoter region and is responsible for fibroblast growth factor 2-mediated SDF-1 gene activation in Sertoli cells and TM4 cells.⁴⁷ Based on studies in other systems, it is known that chemokines and MMP are crucial signaling molecules to maintain the stem cell niche. For instance, they play an important role in the recruitment of hematopoietic stem cells to the bone marrow.⁴⁸ Sertoli cells, the somatic supporting cells within seminiferous epithelium, might utilize these chemokines as niche signaling molecules to support spermatogonial stem cell renewal. In fact, alteration of Erm expression in Sertoli cells was shown to affect the expression of spermatogonia-specific genes such as *Plzf* (promyelocytic leukemia zinc finger) and *Stra8* (stimulated by retinoic acid gene 8).⁴⁹ Apparently, Sertoli cell-specific transcription factors are as important as spermatogonia-specific counterparts in the maintenance of spermatogonial stem cell niche. Further investigations are warranted to elucidate how Erm mediates transcription of other genes and how spermatogonial stem cells and Sertoli cells cross-talk with each other via the actions of Erm-regulated genes. Current studies have shown that some of the above-mentioned transcription factors such as Erm are under the control of glial cell line-derived neurotrophic factor (GDNF).⁵⁰

While SSC is restricted to the SSC niche in the testis, which is adjacent to the Sertoli cells, basement membrane and the interstitium, there is virtually no report in the literature that examines the cell-cell or cell-matrix junction which is essential to maintain the homeostasis of the SSC niche. A recent report using microarray pathway analyses have identified the most affected pathways during SSC differentiation are those involved in adherens junction, gap junction and actin cytoskeleton, illustrating there are functional cell junctions at the SSC niche, possibly at the SSC-SSC and the Sertoli cell-SSC interface.⁵¹ Junction proteins at these sites, analogous to BTB and apical ES, must also be transcriptionally regulated, which should be carefully examined in future studies.

TRANSCRIPTION FACTORS INVOLVED IN GERM CELL DIFFERENTIATION AND SPERMOGENESIS

DNA replication takes place in preleptotene spermatocyte and condensation of chromosomes commences in leptotene spermatocyte. Pairing of homologous chromosomes occurs in pachytene spermatocyte which allows the exchange of genetic material between homologous chromosomes. Pachytene spermatocyte further differentiates into diplotene spermatocytes (tetraploid, 4n) and each of which gives rise to two diploid secondary spermatocytes. Each secondary spermatocyte then enters the second meiotic division immediately and produces two haploid round spermatids (1n). Round spermatids undergo spermiogenesis with extensive morphological changes such as acrosome formation and tail elongation. Those events are tightly regulated by the expression of unique regulatory proteins that are expressed temporally and spatially in Sertoli and germ cells. Some transcription factors are of particular importance in germ cell differentiation and spermiogenesis. CREM, *a-myb*, *Cnot7* and *Rxrb* are representative examples. There are

recent reviews^{52,53} on the role of CREM in spermatogenesis, we therefore encourage readers to read those reviews for a more comprehensive view of the topic.

A-myb that expresses predominantly in male germ cells regulates the expression of genes involved in meiotic phase of spermatogenesis. Male germ cells that entered meiotic prophase were found to be arrested at pachytene stage when A-myb gene was disrupted in mice,⁵⁴ indicating A-myb is crucial to meiosis I. An array of genes expressed in primary spermatocytes such as phosphoglycerate kinase 2 (PGK2) and heat shock protein 70-2 (Hsp70-2) was also down-regulated significantly.⁵⁴ Based on the sequence analyses, it is known that myb-binding sites are present in the promoters of PGK2 and Hsp70-2 genes.

Knockout of Cnot7/CAF1, a CCR4-associated transcription cofactor, caused infertility in male mice.^{55,56} Unsynchronized development of germ cells was observed in the null mice. Although Cnot7 is expressed in both Sertoli and germ cells, spermatogenesis could be restored in *Kit* mutant mice transplanted with spermatogonial stem cells from Cnot7^{-/-} mice. These results indicate that Cnot7 in Sertoli cells, but not in germ cells, is responsible for germ cell development. Studies have also shown that Cnot7 binds the AF-1 domain of retinoid X receptor beta (Rxb, a nuclear receptor) and their association is important in Rxb-mediated gene transcription in spermatogenesis. Mice with target inactivation of Rxb in whole organism (Rxb^{-/-}) or in Sertoli cells (Rxb^{Sert/-}) were sterile, indicating that Rxb is crucial to maintain the functions of Sertoli cells essential for germ cell development.^{57,58} In fact, an array of genes involved in cholesterol metabolism (e.g., ABCA1 and SCARB1), cytoskeleton organization (e.g., Rai14 and Mtap7) and sex hormone signaling (e.g., FSHR and AR) have been found to be altered in their expression levels in the Rxb^{Sert/-} mice.⁵⁸ Apparently, Cnot7 and Rxb are two transcription factors that are inter-dependent and indispensable for spermatogenesis. Obviously, much research is needed to define the transcriptional and post-transcriptional regulation of genes in meiosis, such as the temporal and spatial expression of genes pertinent to meiotic regulation.

TRANSCRIPTION FACTORS INVOLVED IN ANCHORING JUNCTION DYNAMICS IN THE SEMINIFEROUS EPITHELIUM

During spermatogenesis, extensive cell junction restructuring take place between adjacent Sertoli cells at the BTB as well as between Sertoli cells and developing spermatids during spermiogenesis. For instance, the timely restructuring of BTB that occur at Stage VIII of the epithelial cycle is needed to allow the transit of preleptotene spermatocytes so that post-meiotic germ cell development can take place in a specialized microenvironment known as the apical compartment behind the immunological barrier conferred by the BTB.³ On the other hand, spermiogenesis is accompanied by progressive transit of developing spermatids across the epithelium, so that fully developed spermatids that are found at the adluminal edge can be released and enter the tubule lumen at spermiation.³ It is well-established that several biomolecules in the seminiferous epithelium including cytokines (e.g., TGF- β 3, TNF α , IL-1 α) and hormones (e.g., testosterone) are crucial regulators of junction dynamics and their concerted efforts maintain the BTB integrity while facilitating the transit of preleptotene spermatocytes at the BTB [for reviews, see refs. 59,60]. More recent studies have shown that the differential expression and bioavailability of junction proteins at the

cell-cell interface via different regulatory mechanisms, such as transcriptional, post-transcriptional and post-translational regulation, are important to regulate junction restructuring events at the BTB and the apical ES.^{59,61-63} Thus, the required junction proteins at the BTB and/or Sertoli-germ cell interface can be temporally and spatially expressed in such a way that the restructuring events of cell junctions in the seminiferous epithelium could be highly co-ordinated to facilitate the timely movement of developing germ cells.

It is well-documented in various epithelial cells that the expressions of junction proteins along the epithelium are tightly regulated by transcriptional modification of junction protein genes. A spectrum of transcription factors identified to regulate the expression of junction proteins in other epithelial cells are listed in Table 1. In the testis, some transcription factors are recently shown to play important roles in regulating the expression of junction proteins such as claudin-11 and junctional adhesion molecules (Fig. 2). For instance, WT1 is a zinc-finger transcription factor that regulates the apical ectoplasmic specialization (apical ES, a specialized adherens junction formed between Sertoli cells and elongating/elongated spermatids). Using microRNA targeting WT1 in Sertoli cells, disruption of apical ES was reported, indicating that WT1 might be involved in modulating the expression of the junction proteins that constitute the apical ES.⁶⁴ Although the detailed mechanism(s) by which WT1 regulates apical ES protein expression is unknown, it is clear that apical ES dynamics during spermiogenesis could be contributed by altering gene expression at this stage.

Apart from WT1, Sp/KLF transcription factor family has shown to regulate the expression of several junction molecules in Sertoli cells. For instance, we have shown that Sp1 and Sp3 upregulate the transcription of nectin-2 and junctional adhesion molecule-B (JAM-B) in Sertoli cells; whereas KLF4 is involved in regulating coxsackie and adenovirus receptor-like protein (CLMP).⁶⁵⁻⁶⁷ CREB and GATA proteins have also been demonstrated to be the transcription factors that up-regulate the basal transcription of claudin-11 and nectin-2 genes in Sertoli cells, both of which are critical integral membrane components of the BTB.^{65,68} Apart from controlling the basal expression of junction proteins in the seminiferous epithelium, several transcription factors were shown to interact with the promoters of junction protein genes upon cytokine stimulation so as to exert possible stage-specific regulation. For instance, TGF- β 2 is known to activate Smad3 and Smad4 proteins and promotes the binding of Smad proteins onto the TGIF motif of the JAM-B promoter, resulting in JAM-B gene repression.⁶⁷ Since JAM-B is one of the integral membrane proteins at the BTB to confer the barrier function, this TGF- β 2-induced JAM-B repression thus leads to a loss of TJ-associated integral membrane proteins (including JAM-B and perhaps other proteins such as ZO-1, an adaptor protein of JAM-B) at the Sertoli cell BTB, leading to its transient disruption of the Sertoli cell TJ-permeability barrier as earlier reported when the Sertoli cell epithelium was exposed to TGF- β 3.^{69,70} The findings regarding the role of Smad proteins on JAM-B repression are highly significant since previous immunostaining analyses have shown that there is a shift in the localization of Smad proteins from the cytosol to the nucleus in the Sertoli cell epithelium during specific stages of the seminiferous epithelial cycle.⁷¹ In short, by interacting stage-specific transcription factors, such as Smad3, with a respective promoter, stage-specific expression

or repression of junction proteins during the seminiferous epithelial cycle can be achieved. Such cyclic shuffling of specific transcription factors from the cytosol to the nucleus provides a precise but efficient mechanism to control stage-specific expression or repression of junction proteins in the seminiferous epithelium at the Sertoli-Sertoli and Sertoli-germ cell interface. The identification of testis- and stage-specific transcription factors might open a new window for the design of novel contraceptives that target specific germ cell differentiation process. For instance, contraceptives that can interfere with the cyclic shuffling of a particular stage-specific transcription factor to the nuclei to exert its function in regulating gene expression pertinent to a particular event of spermatogenesis would become novel compounds to disrupt spermatogenesis without perturbing the hypothalamic-pituitary-testicular axis.

CONCLUSION

Transcription factors reviewed and discussed herein play indispensable roles in spermatogenesis as their functions have been identified by both in vitro RNA knockdown and promoter studies and knockout animal studies. With recent advances in genomic and proteomic research, an array of genes and proteins, such as transcription factors, has been identified to be altered in knockout animals with the phenotypes displaying disrupted spermatogenesis and/or infertility. However, in many cases, it is not known whether those altered genes are the direct targets of the respective transcription factor(s). Besides, numerous scattered reports indicate that there are several transcription factors that can exert their effects in mediating spermatogenesis, in particular their role in regulating cell adhesion function at the BTB. However, the detailed mechanisms by which these transcription factors regulate spermatogenesis have not been studied. Perhaps a systematic approach is needed to investigate the role of different transcription factors in regulating blood-testis barrier dynamics and spermatogenesis, and how transcription factors regulate different sets of genes in an orderly manner in the seminiferous epithelium, coinciding with the stages of the seminiferous epithelial cycle of spermatogenesis.

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Figure 1.

A schematic representation of developing germ cells engulfed by two adjacent Sertoli cells in the seminiferous tubule. Spermatogonia undergo mitotic divisions for either self-renewal (for the maintenance of the stem cell pool at the niche*) or proliferation that gives rise to primary spermatocyte. Primary spermatocytes then undergo meiotic divisions behind the BTB, leading to the production of haploid round spermatids. These cells undergo the process of spermiogenesis to become elongated spermatids and are released to the seminiferous tubule lumen at spermiation. During spermatogenesis, numerous transcription factors expressed in Sertoli and/or germ cells, which are crucial for the development of mature spermatids. Tissue- or cell type-specific knockouts of these transcription factors in mice have shown that their loss would lead to male infertility. Alteration of gene profiles were observed in the knockout models and some representatives from a plethora of genes in the profiling studies are selected and illustrated herein.^{5,7,10,31,34,42,46,49,50,54,55,57,58} Upward arrow means an increase in its expression level; downward arrow means a decrease in its expression level; TF, transcription factor.

Figure 2.

A schematic representation of different cell junction-associated proteins and their transcriptional regulation in the seminiferous epithelium in rodent testes. Different types of cell junctions can be found between testicular cells at the Sertoli-Sertoli and Sertoli-germ cell interface. The blood-testis barrier (BTB) formed at the basal compartment is constituted by tight junction and the basal ectoplasmic specialization (basal ES, a testis-specific atypical anchoring junction-type). Apical ES is restricted to the interface at Sertoli cells and elongating spermatids. Once apical ES appears in step 8 spermatids, it is the *only* anchoring device at the Sertoli-spermatid interface which persists until step 19 spermatids in rats and step 16 spermatids in mice, when it is undergoing endocytosis to be recycled for the formation of new apical ES and to prepare the elongated spermatids for spermiation and the ‘degenerating’ apical ES was called apical tubulobulbar complex (apical TBC) (for a review, see ref. 3). ES (both apical and basal) is typified by the presence of actin filament bundles found near the plasma membrane of the Sertoli cell. Gap junction and anchoring junction can also be found elsewhere between Sertoli and germ cells. Different types of junctions is made up of different junction proteins and transcription factors listed in the figure are involved in regulating particular junction proteins in the testis [64, 65, 66, 68, and Lui et al (unpublished data)]. Upward arrow, transcription activation; downward arrow, transcription inhibition. TF, transcription factor.

Table 1

Transcription factors control the transcription of genes encoding cell junction proteins in other epithelial cells*

Transcription Factors	Junction Proteins	References
Slug	Occludin, claudin-1, E-cadherin, integrins (e.g., $\alpha 3$, $\beta 1$)	72-75
Snail	Occludin, claudins (e.g., claudin-1, -3, -4, -7), E-cadherin	72-74
Sp/KLF family	Claudins (e.g., claudin-1, -4), P-, E-cadherin, integrins (e.g., $\alpha 2b$, $\alpha 3$, $\alpha 5$), connexins (Cx40, Cx43)	74,76-81
Smad family	Claudin-1, E-cadherin	82,83
Nkx2.5	Connexins (Cx40, Cx43)	81,84
HNF	E-cadherin, connexin32	85,86
SIP1	Claudin-4, P-, E-cadherins, connexins (Cx26, Cx31)	74,87
GATA	N-cadherin, connexin40	84,88

* This table is prepared based on earlier reports and reviews, illustrating the transcriptional control of junction proteins in other epithelia. It is not intended to be exhaustive due to page limits, however, it highlights the development of this rapidly evolving field of research.