REVIEW

Molecular mechanisms by which hormones and cytokines regulate cell junction dynamics in the testis

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Abstract

Hormones and cytokines are known to regulate cellular functions in all tissues including testis. These two groups of biomolecules exert a broad spectrum of effects on various aspects of spermatogenesis. Among them, one of the regulatory effects on spermatogenesis is to modulate cell junction restructuring between Sertoli cells and between Sertoli and germ cells in the seminiferous epithelium. The restructuring of cell junctions is crucial to enable the migration of germ cells along the seminiferous epithelium from the basement membrane towards the tubular lumen, and at the same time for their attachment to Sertoli cells for support. This review will summarize the recent findings that focus on the role of hormones (FSH and testosterone) and cytokines (transforming growth factor- β s and tumor necrosis factor- α) on cell junction restructuring in the testis and the molecular mechanisms.

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Introduction

Spermatogenesis is a process by which spermatogonia (diploid) undergo a series of events, including mitotic and meiotic divisions and morphological differentiation, to become haploid spermatozoa. During spermatogenesis, intricate interactions between Sertoli cells and between Sertoli and germ cells are important for germ cell differentiation and these can be achieved via the precise organization of different types of cell junctions (for review, see [Cheng & Mruk 2002](#)). For instance, neighboring Sertoli cells closely associate with each other to form the blood–testis barrier (BTB) that is constituted by inter-Sertoli tight junctions (TJ) and basal ectoplasmic specializations (ES, an atypical adherens junction (AJ) type). The BTB is important for several reasons. First, it creates a microenvironment for germ cell development and confers apical–basal polarity. Secondly, it segregates meiotic germ cells apart from the systemic circulation, thereby protecting them from autoimmune recognition and potential cytotoxic substances. Thirdly, it must open and close at time intervals to allow the passage of preleptotene spermatocytes from basal to adluminal compartment at stages VIII–IX of the spermatogenic cycle. Fourthly, the delivery of drugs or contraceptives must take into account the constraints imposed by the BTB. Besides, germ cells intimately associate and interact with Sertoli

cells for structural support via the AJs and gap junctions, and eventually detach from the epithelium into the tubule lumen at spermiation. Therefore, the junctions at the BTB and the Sertoli–germ cell interface are undergoing extensive restructuring (disassembly and reassembly of junctions) to allow the movement of germ cells along the seminiferous epithelium. Worthy of note is the timely opening and closing of junctions at the BTB that are crucial to allow germ cell migration without affecting the integrity of the barrier.

Hormones and cytokines are known biomolecules that regulate a wide spectrum of cellular functions in different tissues such as testes. In this review, we shall highlight the molecular mechanisms by which testosterone, FSH, transforming growth factor- β s (TGF- β s), and tumor necrosis factor- α (TNF- α) exert their effects in regulating cell junction restructuring pertinent to spermatogenesis. The architecture of cell junctions in the testis has been extensively reviewed elsewhere ([Lui & Lee 2006](#), [Sofikitis *et al.* 2008](#), [Wong *et al.* 2008](#)). Readers are strongly encouraged to read those reviews for a more comprehensive view of the topic.

Testosterone

Testosterone is a male sex hormone secreted by Leydig cells under the control of luteinizing hormone (LH)

released from the anterior pituitary. It is essential for the development of male phenotype and sexual behavior among other tissues (Rahman & Christian 2007). For instance, the differentiation of round spermatids to elongated spermatids at stages VII–VIII requires the action of testosterone (Sun *et al.* 1990, McLachlan *et al.* 1994). While testosterone is produced in the interstitial compartment under the influence of LH, the expression of the androgen receptor (AR) in adult testes is stage-specific and is the highest at stages VII–VIII (Bremner *et al.* 1994, Shan *et al.* 1997).

Several *in vivo* models including hormone-suppression, hormone-restoration and hypophysectomy are available for the study of the hormonal regulation of spermatogenesis by testosterone (Huang *et al.* 1987, Sun *et al.* 1989, O'Donnell *et al.* 1994). Extensive studies have been performed in the past two decades using the suppression and restoration models to understand how testosterone affects spermatogenesis. Testosterone has been shown to exert its effect to control the fate of developing germ cells, in particular the round spermatids (Sun *et al.* 1990, McLachlan *et al.* 1994). For instance, the suppression of intratesticular testosterone by testosterone and estradiol implant could interrupt the conversion of round spermatids to elongated spermatids between stages VII and VIII, but

could be restored to normal upon high-dose testosterone treatment (O'Donnell *et al.* 1994). Subsequent studies confirmed that the absence of the elongated spermatids in testosterone-suppressed rats was due to the fact that round spermatids were prematurely detached from the epithelium (O'Donnell *et al.* 1996). These studies suggest that testosterone affects the adhesive function between round spermatids and Sertoli cells, leading to the sloughing of round spermatids from the epithelium.

The detailed mechanism of how testosterone alters the adhesive function has been recently unraveled. Two protein complexes linking between round spermatids and Sertoli cells at the apical ES, the cadherin/cadherin and $\alpha 6 \beta 1$ -integrin/laminin $\gamma 3$ interlocks, are the affected targets in the testosterone-suppression model (Wong *et al.* 2005, Zhang *et al.* 2005b). In normal rat testis, actin-linked N-cadherin/ β -catenin complexes in both Sertoli cell and round spermatid join together to form intact actin-based cadherin/cadherin AJ interlocks at the cell–cell surface, and such interaction is tightly controlled by kinases and phosphatases such as c-src and myotubularin-related protein 2 (Zhang *et al.* 2005a; Fig. 1). For the $\alpha 6 \beta 1$ -integrin/laminin $\gamma 3$ interlock, the interaction of peripheral proteins, focal adhesion kinase (FAK) and c-src, with $\beta 1$ -integrin on

Figure 1 A schematic drawing illustrates the regulation of apical ES by testosterone. In the presence of testosterone, intact apical ES are established and constituted by cadherin/cadherin (Zhang *et al.* 2005b) and integrin/laminin interlocks (Wong *et al.* 2005). Cadherin and integrin are closely associated with their cytoplasmic interacting partners to form stable ES (Wong *et al.* 2008). In the absence of testosterone, a significant increase in phosphorylation of β -catenin (Zhang *et al.* 2005b) and FAK (Wong *et al.* 2005) are observed. The change of phosphorylated state of the cytoplasmic partners destabilizes the protein interlocks, resulting in the disassembly of the apical ES. α -cat, α -catenin; β -cat, β -catenin; MTMR2, myotubularin-related protein 2; FAK, focal adhesion kinase; ES, ectoplasmic specializations.

the Sertoli cell side is important to maintain the integrity of the $\alpha 6\beta 1$ -integrin/laminin $\gamma 3$ interlock (Yan & Cheng 2006).

By the use of the testosterone-suppression model again, it has been found that in the absence of testosterone there are apparent changes in the degree of protein–protein interactions and the levels and phosphorylation states of several ES proteins at the apical ES, which in fact alters the architecture of the ES and leads to the detachment of round spermatids from the epithelium (Wong *et al.* 2005, Zhang *et al.* 2005b; Fig. 1). Significant increases in protein levels of c-src, $\beta 1$ -integrin as well as FAK and tyrosine phosphorylation of both FAK and β -catenin were detected in testosterone-depleted testes. Tyrosine phosphorylation of β -catenin indeed favors the dissociation of the N-cadherin/ β -catenin complex, thus destabilizing the cadherin/cadherin interlock found at the interface of Sertoli cells and round spermatids (Zhang *et al.* 2005b). In the absence of testosterone, the increase in the association of c-src with FAK (but not with $\beta 1$ -integrin) under the activation of extracellular signal-regulated kinase (ERK) also affects the integrity of the $\alpha 6\beta 1$ -integrin/laminin $\gamma 3$ interlock at the Sertoli cell/spermatid interface (Wong *et al.* 2005). Taken collectively, the two major junction interlocks at the apical ES are seriously disrupted in testosterone-suppressed testis and testosterone thus plays a major role to regulate the adhesive function between Sertoli cells and round spermatids via its action on the protein complexes at the apical ES.

Apart from its role to regulate the dynamics of the apical ES and spermiogenesis in the testis, recent studies have demonstrated that testosterone (2×10^{-7} M) also controls the bioavailability of several junction proteins at the BTB by accelerated endocytosis (Yan *et al.* 2008a). Although the concentration of testosterone used in this study was greater than the K_d of AR ($2\text{--}5 \times 10^{-10}$ M), the results obtained in the study are of physiological significance (Wilson & French 1976) since this is the testosterone concentration in the testis which is 100-fold that of the serum. The reason for the presence of a supraphysiological testosterone level (greater than K_d of the AR) in the testis is unknown; perhaps this is reflected by a different regulation of testosterone entry to the cell. In the rat testis, the androgen-binding protein that regulates the bioavailability of testosterone in extracellular fluid is produced by the Sertoli cells and found within the seminiferous tubular compartment. However, Yan *et al.* have found that the addition of testosterone into the Sertoli cell culture having well-established TJ and AJ can accelerate the internalization of junction proteins from the cell surface. Junction proteins including occludin, junctional adhesion molecule-A (JAM-A) and N-cadherin were the target proteins being internalized into the clathrin vesicles

and subsequently targeted to early endosomes for transcytosis. It was found that testosterone could enhance the recycling of the internalized junction proteins back to the cell surface (Yan *et al.* 2008a) and such cycling of junction proteins back and forth between cell surfaces is important in controlling the transient opening of the BTB to allow the migration of spermatocytes. It is postulated that the internalization of BTB junction proteins at the apical end of the migrating spermatocytes facilitates cell movement. Once the spermatocytes move along, testosterone enhances recycling of the internalized junction proteins back to the Sertoli cell surface at the basal region of the spermatocytes to reseal the BTB (Yan *et al.* 2008a).

Earlier studies by Chung & Cheng (2001) have revealed that testosterone can up-regulate other tight junction components including claudin-11, claudin-1, E-cadherin and β -catenin at the mRNA levels in the rat testis. Until recently, Kaitu'u-Lino *et al.* (2007) have demonstrated that testosterone regulates claudin-11 expression and promotes the localization of claudin-11 and occludin at Sertoli–Sertoli cell interfaces. Along this line, studies by Meng *et al.* (2005) have shown that testosterone positively regulates the expression of claudin-3 in mice, which is believed to be a transient component of newly-formed tight junctions at the BTB. Although knockout of claudin-11 causes male infertility in mice (Gow *et al.* 1999), there is still little definitive data available on the detailed regulatory mechanism of claudin members in the testis by testosterone. Taken collectively, testosterone is a master regulator in controlling the bio-availability of the tight junction proteins at the BTB via post-translational and transcriptional pathways.

Generation of the AR knockout (ARKO) and Sertoli cell-selective (SC) ARKO mice in fact has provided significant insights towards understanding the role of androgen in spermatogenesis (Yeh *et al.* 2002, Chang *et al.* 2004, De Gendt *et al.* 2004, Holdcraft & Braun 2004, Denolet *et al.* 2006). Mice from these knockout models are all infertile. In particular, it was found that spermatogenic arrest occurred at the pachytene stage in ARKO mice and resulted in severe testis atrophy (80% smaller than wild-type; Yeh *et al.* 2002); whereas SC ARKO testes (28% smaller than wild-type) displayed spermatogenic arrest at the late spermatocytes/spermatid stages (De Gendt *et al.* 2004, Holdcraft & Braun 2004). The numbers of round spermatids and elongated spermatids in SC ARKO mice were reduced to 0–3% respective to the wild-type (De Gendt *et al.* 2004). The fact that SC ARKO mice present a testicular phenotype similar to that of the testosterone-suppressed rodent model further strengthens the idea that testosterone and its receptor play specific roles in Sertoli–germ cell adhesion in the seminiferous

epithelium. With the advance of microarray technique, Denolet *et al.* have analyzed and compared the gene profiles of wild-type and SC ARKO mice. A spectrum of genes related to junction restructuring, including structural components (claudin and nectin-like molecule-1) and regulatory components (serine-type protease inhibitors), has been found to be differentially expressed in the SC ARKO mice respective to the wild-type (Denolet *et al.* 2006), which further suggests that testosterone is indeed a key player in modulating junction dynamics in the testis. It is apparent that testosterone is not only involved in round spermatid-Sertoli cell adhesion at the apical ES, but also functions as a positive regulator to maintain the integrity of the BTB.

FSH and estrogen

FSH is vital for normal spermatogenesis in the rodents (for reviews, see McLachlan *et al.* 2002, O'Donnell *et al.* 2005). For instance, male mice lacking a functional FSH receptor had impaired fertility and defected elongated spermatids and Sertoli cells (Krishnamurthy *et al.* 2000, Grover *et al.* 2004). The expression of FSH receptors on Sertoli cells in adult rats is also stage-specific, being the highest at stages IX and X (Kliesch *et al.* 1992).

Rat models having both intratesticular testosterone and FSH suppression have been used to study the effect of hormonal suppression on spermiation. Mature spermatids are attached to Sertoli cells via the apical ES. During spermiation, the apical ES are likely to be removed, which is subsequently followed by the formation of tubulobulbar complex (TBC) and the detachment of spermatids from the Sertoli cells. It was found that T+FSH-suppression severely interrupted spermiation in the rats, more significantly than T- or FSH-suppression alone (Saito *et al.* 2000, Beardsley & O'Donnell 2003). Immunohistochemistry analyses have shown that the β 1-integrin remained associated with the elongated spermatids in the T+FSH-suppressed testis after the removal of the apical ES. The association of the β 1-integrin in mature spermatids was the likely cause of spermiation failure (Beardsley & O'Donnell 2003, Beardsley *et al.* 2006). These data suggest that testosterone and FSH act synergistically to support spermiation possibly by mediating the dissociation of the β 1-integrin in mature spermatids at spermiation. Another *in vivo* study in hypophysectomized rats has shown that concurrent testosterone plus FSH treatment, but not testosterone alone, is able to restore normal spermiogenesis and that this restoration is associated with the reorganization of F-actin and vinculin at the ES (Muffly *et al.* 1994). Other than rat models, studies on Djungarian hamsters have also demonstrated that exogenous supply of FSH to

short-day photoperiod (8 h light:16 h darkness) hamsters whose gonadotropins are suppressed could restore the localization of junction proteins such as actin and epsin at the apical ES, and claudin-11 and zonula occludens-1 (ZO-1) at the basal region of the seminiferous epithelium (Tarulli *et al.* 2006). Using the same animal model, Tarulli *et al.* (2008) have recently found that similar to claudin-11, claudin-3, occludin and JAM-A can rapidly be reorganized at the BTB upon FSH replacement. These results strongly suggest that in addition to spermiation, FSH regulates the integrity and functionality of the BTB via reorganization and relocation of junction proteins.

An *in vitro* study has confirmed that FSH is important to stimulate the relocation of ES proteins such as epsin and in the presence of FSH, cultured Sertoli cells are capable of forming not only the classical AJs, but also the AJ belts and the testis-specific ES which contains actin and epsin (Sluka *et al.* 2006). Other than ES proteins, FSH also regulates the tight junction proteins such as coxsackievirus and adenovirus receptor (CAR) and claudin-11 in the testis (Mirza *et al.* 2007). It has been reported that FSH up-regulates the CAR mRNA in cultured immature rat Sertoli cells. However, in claudin-11 expression, rat and mouse Sertoli cells responded to FSH treatment oppositely. It was found that FSH could partially stimulate claudin-11 mRNA in cultured rat Sertoli cells, and it inhibited the expression of claudin-11 mRNA in mouse Sertoli cells (Hellani *et al.* 2000, Kaitu'u-Lino *et al.* 2007). Despite the fact that the importance of FSH in regulating junction restructuring has been known for a long time, the molecular mechanism explaining how FSH exerts its effect remains enigmatic. Further studies in this area are highly warranted.

Apart from FSH, the effects of estrogen on spermiation have been recently examined (D'Souza *et al.* 2008). Confocal microscopic studies have confirmed that administration of 17 β -estradiol to rats, which causes a rise in intratesticular 17 β -estradiol and suppression of circulating FSH and intratesticular testosterone could result in the disruption of the TBC in elongated spermatids and spermiation failure. It is believed that 17 β -estradiol affects the Sertoli cell cytoskeleton and Arp2/3 complex which are critical for *de novo* polymerization of actin during TBC formation (D'Souza *et al.* 2008).

Molecular mechanism of cytokines on regulating junction restructuring in the testis

TGF- β s and TNF- α are known to regulate multiple physiological functions including germ cell development, Leydig cell steroidogenesis and extracellular matrix (ECM) biosynthesis in the testis (for reviews, see

Lui *et al.* 2003a, Siu & Cheng 2004). In addition, there is accumulating evidence showing that TGF- β s (TGF- β 2 and TGF- β 3) and TNF- α are actively involved in junction restructuring in the seminiferous epithelium, thus facilitating the movement of developing germ cells. These cytokines are secreted by Sertoli and germ cells in a stage-specific manner. For instance, TGF- β 3 and TNF- α are expressed at their highest in stages VII–VIII tubules (Lui *et al.* 2003b, Siu *et al.* 2003), similar to the expression of the AR. Recent studies have unraveled the molecular mechanism by which these cytokines exert their effects to modulate junction dynamics. In fact, TGF- β s and TNF- α regulate the junction restructuring via various control mechanisms at the transcriptional, post-transcriptional and post-translational levels.

Transforming growth factor- β s

TGF- β s are a group of cytokines that have been extensively studied by both *in vitro* and *in vivo* models in regard to their roles in junction restructuring (Lui *et al.* 2003b,c, Xia & Cheng 2005, Xia *et al.* 2006). TGF- β 3 is a crucial cytokine that modulates the disassembly of the BTB, the apical ES and the AJ by down-regulating the expression of integral membrane proteins such as occludin and N-cadherin (Lui *et al.* 2001, 2003b, Xia & Cheng 2005, Xia *et al.* 2006). It is noted that TGF- β 3 can exert distinctive effects on junction restructuring by interacting with selective adaptors to evoke different signaling pathways. The interaction of the TGF- β 3/T β RI complex with both CD2AP and TAB1 adaptors triggers the activation of both p38 and ERK pathways, resulting in the disruption of the BTB and apical ES in the seminiferous epithelium (Lui *et al.* 2001, 2003b, Xia *et al.* 2006). However, if the TGF- β 3/T β RI complex interacts with CD2AP alone, only the ERK pathway will be activated. Such activation can effectively disrupt the AJs between Sertoli and germ cells, but not the BTB and basal ES (Xia & Cheng 2005, Lui & Cheng 2007).

While TGF- β 3 was found to differentially regulate apical ES and/or BTB junctions, TGF- β 2 was shown to reduce the junctional adhesion molecule-B (JAM-B) protein level via transcriptional repression in cultured Sertoli cells (Wang & Lui 2009). In the testis, JAM-B is expressed by Sertoli cells and localized at the apical ES to form interlocks with (JAM-C) for spermatid attachment (Gliki *et al.* 2004). It was found that TGF- β 2 exerted its negative regulatory effects on the JAM-B transcription via activation of Smad proteins. Activated Smad proteins effectively displaced Sp1 proteins from the TGIF motif of JAM-B promoter, resulting in JAM-B repression (Wang & Lui 2009).

It is apparent that the reduction of the junction protein level within cells, either by suppressing *de novo* protein synthesis or promoting its protein degradation,

is an effective approach to modulating junction restructuring. A recent study has shown that TGF- β 2 can alter the bioavailability of junction proteins at the cell–cell interface by accelerating protein degradation (Yan *et al.* 2008a). It has been found that both TGF- β 2 and testosterone can accelerate the kinetics of internalization of the BTB proteins from the cell surface. However, the fates of internalized proteins triggered by TGF- β 2 and testosterone are entirely different. TGF- β 2 accelerates the internalization of integral membrane proteins (such as JAM-A and occludin) via a clathrin-coated pit and targets the internalized proteins into late endosomes for degradation, leading to the disassembly of the BTB; whereas testosterone accelerates the internalization of integral membrane proteins and their transcytosis to form new junction fibrils beneath the migrating spermatocyte (Yan *et al.* 2008a).

Tumor necrosis factor- α

TNF- α is produced by Sertoli and germ cells in the testis and is a crucial cytokine that regulates a wide range of cellular processes in the testis (Mruk & Cheng 2004). It controls steroidogenesis in Leydig cells and survival of germ cells by interfering signaling transduction of the Fas-ligand system (Pentikainen *et al.* 2001, Hong *et al.* 2004). Analysis of adult rats having chronically systemic administration of TNF- α has uncovered the possible role of TNF- α on junction restructuring in the testis (Mealy *et al.* 1990). It was found that the loss of germ cells from the seminiferous epithelium in this animal model was not due to inflammatory responses. In fact, TNF- α exerted its effect on Sertoli–germ cell interface, resulting in premature germ cell loss from the epithelium (Mealy *et al.* 1990). To elucidate the mechanism on how TNF- α perturbs cell junctions between Sertoli and germ cells, Li *et al.* (2006) have established another *in vivo* model by local administration of recombinant TNF- α into rat testes. It was found that there was a significant increase in FITC diffusion across the BTB in the seminiferous tubules along with the reduction of occludin and ZO-1 protein levels in the testis lysate, which clearly illustrates the disruption of the BTB upon TNF- α treatment (Li *et al.* 2006). Apart from the BTB impairment, TNF- α is capable of causing disorganization of the actin bundles and cisternae of endoplasmic reticulum at the apical ES, leading to the release of premature spermatids (Li *et al.* 2006; Fig. 2).

Like occludin and ZO-1, coxsackie- and adenovirus receptor-like membrane protein (CLMP) is another TJ protein at the BTB which can be negatively regulated by TNF- α . In a recent study, it has been demonstrated that TNF- α can act on the CLMP mRNA transcript and destabilize the transcript by promoting the binding of

Figure 2 A schematic drawing illustrates the effect of $\text{TNF-}\alpha$ on junction restructuring in the seminiferous epithelium. Intact ES and BTB are maintained in the absence of $\text{TNF-}\alpha$. At stage VIII of the epithelial cycle, a surge of $\text{TNF-}\alpha$ triggers different MAPK pathways and regulates the opening of the BTB (Li *et al.* 2006, Sze *et al.* 2008) as well as the apical ES (Mealy *et al.* 1990) by controlling the junction protein levels and altering the transcription of junction proteins to allow the migration of preleptotene/leptotene spermatocytes across the BTB and the release of mature spermatids to the tubular lumen. It is also noted that at stage VIII, testosterone enhances transcytosis of occludin in Sertoli cells, possibly from the cell surface at the apical end of the migrating spermatocytes to cell surface at the basal end to reassemble the BTB (Yan *et al.* 2008a). $\text{TNF-}\alpha$, tumor necrosis factor- α ; TNFRI, tumor necrosis factor receptor I; ZO-1, zonula occludens-1, BTB, blood-testis barrier; ES, ectoplasmic specializations.

an RNA-binding protein, tristetraprolin, at the 3'UTR region under the activation of the c-Jun N-terminal kinase (JNK) pathway (Sze *et al.* 2008). This study illustrates that $\text{TNF-}\alpha$ disassembles TJ proteins at the BTB, possibly including regulation at post-transcriptional level by affecting the mRNA stability (Fig. 2).

Bioactive peptides released from proteolysis

Apart from the above-mentioned regulator, an emergence of significance in relation to the testis is the role of biological peptides generated by the breakdown of adhesion complexes and ECM proteins. The recently identified autocrine axis, coordinating the events of BTB restructuring and spermiogenesis which take place concurrently at the opposite ends of the seminiferous epithelium, is proposed to be mediated by fragments of laminin chains released from the dissociation of spermatid-Sertoli cell adhesion (Yan *et al.* 2008b).

The disruption of the BTB by $\text{TNF-}\alpha$ and other cytokines is also proposed to be mediated by collagen fragments resulting from proteolytic cleavages of the ECM proteins (Siu *et al.* 2003, Yan *et al.* 2008a). These laminin and collagen fragments that coordinate events within the testis are in fact functioning as paracrine factors. Sertoli cells produce a variety of matrix metalloproteases and tissue inhibitors of metalloproteases (Siu *et al.* 2003, Siu & Cheng 2004). Studies of the proteolysis and proteolytic fragments in the testis deserve greater attention, as the identification of unique coordination pathways in regulating junction restructuring has the potential for the development of a non-hormonal male contraceptive.

Concluding remarks

Undoubtedly, these biomolecules are important regulators in many aspects of spermatogenesis, especially junction restructuring, and in most of cases, detailed

mechanisms on how these molecules exert their functions have been examined. However, there are still ample scattered data in the literature showing that other biomolecules such as hepatocyte growth factor and nitric oxide play a role in junction restructuring *in vitro* (Lee & Cheng 2003, Catizone *et al.* 2008), yet their effects have not been fully examined in animal models. Needless to say, much of the research efforts should be allocated to uncovering the underlying mechanisms of each individual regulator. Even so, the challenge we have to confront is how to delineate the possible coordination between these biomolecules in modulating junction restructuring in the testis. The use of advanced cell biology techniques, such as over-expression and gene knock-down (RNAi) studies in primary cell cultures and the application of bioinformatics should, hopefully, be helpful in expanding this area of research, and even in assuring that a systematic overview of spermatogenesis under the control of various regulators can be simulated in the near future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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