

# “Unlocking” the Blood-Testis Barrier and the Ectoplasmic Specialization by Cytokines During Spermatogenesis: Emerging Targets for Male Contraception

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**Abstract:** Cytokines are known to regulate an array of physiological functions in the testis, including cell differentiation, apoptosis, steroidogenesis, and cell division. Recent studies have illustrated that cytokines also take a crucial role in the regulation of junction dynamics. These include the regulation of cell-cell adhesion and tight junction permeability barriers in multiple epithelia and endothelia, such as those found in the small intestine, kidney, skin, and testis. In this review, we summarize recent findings in this field with an emphasis on the role of cytokines in junction restructuring events during spermatogenesis in the seminiferous epithelium of testes. This review also identifies several areas of research that functional studies can be designed to unravel the physiological significance of cytokines in junction restructuring at the Sertoli-Sertoli or Sertoli-germ cell interface in the seminiferous epithelium. It is expected that multiple cytokines, such as TGF- $\beta$ 3 and TNF $\alpha$ , are working in concert with other yet-to-be identified molecules to coordinate the intriguing events of junction restructuring during different stages of the seminiferous epithelial cycle in adult testes in mammals.

**Key Words:** Cytokines, transforming growth factors, tumor necrosis factor, testis, spermatogenesis, Sertoli cells, blood-testis barrier, tight junction, adherens junction, ectoplasmic specialization.

## INTRODUCTION

Cytokines are peptides that have diverse physiological functions in mammals. They are ubiquitously expressed in most cell types. These pleiotropic peptides are secreted for autocrine, paracrine and/or endocrine regulation of different physiological processes and some of them have functional redundancy [1-3].

Among their numerous physiological roles, cytokines have been reported to serve as important regulators of junction integrity in a number of epithelia and endothelia [4,5] including the seminiferous epithelium in the testis (for reviews, see [6,7]). Recent studies have shown that cytokines can affect two discrete junctional sites in the seminiferous epithelium, namely the blood-testis barrier (BTB) and the apical ectoplasmic specialization (ES). The BTB is composed of tight junctions (TJ), adherens junctions (AJ), desmosome-like junctions, and gap junctions formed between adjacent Sertoli cells near the basement membrane in mammalian testes. This barrier physically segregates the seminiferous epithelium into the apical (or adluminal) and basal compartment, conferring cell polarity (for reviews, see [8-10]). On the other hand, the ES, a testis-specific AJ, is restricted to the interface of Sertoli cells and elongating/elongated spermatids (step 8 spermatids and beyond in the rat) and of inter-Sertoli cells at the BTB. These two ES sites are named the apical and basal ES, respectively, according to their cellular localization (for reviews, see [9,11,12]).

At stage VIII of the seminiferous epithelial cycle, preleptotene or leptotene spermatocytes traverse the BTB [13], entering the adluminal compartment to continue their development. Spermiation takes place concurrently at the luminal edge to release spermatozoa into the seminiferous tubular lumen. Thus, it is conceivable that extensive restructuring at the Sertoli-Sertoli and Sertoli-germ cell interface occurs during spermatogenesis in addition to the molecular and cellular events associated with meiosis and spermiogenesis.

Different cytokines have been reported to disrupt TJ-barrier integrity in the testis, intestine or brain (Table 1). It has been postulated that developing germ cells cooperate with Sertoli cells to determine the levels of cytokines in the BTB microenvironment to facilitate junction restructuring (for reviews, see [6,7]). In this short review, we focus our discussion on TGF- $\beta$ 3 and TNF $\alpha$  since much

of the work reported in recent years is related to these two cytokines. However, this is not to rule out the participation of other cytokines, such as interferon- $\gamma$  (IFN $\gamma$ ) and interleukin-1 $\alpha$  in junction restructuring events as there is emerging evidence which suggests the involvement of these two cytokines in the regulation of junction permeability (see Table 1). Our goal in this short review is to evaluate critically current findings on the role of cytokines on the BTB and ES dynamics in the testis. It has become apparent in recent years that these two ultrastructures can serve as targets for the development of novel non-hormonal contraceptives for men.

## ROLES OF CYTOKINES IN JUNCTION RESTRUCTURING

In the seminiferous tubule, germ cells are major contributors of pro-inflammatory cytokines. The levels of pro-inflammatory cytokines produced and secreted by Sertoli and Leydig cells are relatively low [6,14]. Yet receptors for these cytokines, such as TGF- $\beta$ s and TNF $\alpha$ , are predominantly expressed in Sertoli cells. This is not entirely unexpected since germ cells are not equipped with an intrinsic mechanism for cell movement, unlike fibroblasts or macrophages. The translocation of germ cells across the BTB and their progressive migration towards the lumen of the seminiferous tubule relies largely on Sertoli cells, which is known to provide the necessary propulsive force. The effects of cytokines on the Sertoli cell cytoskeleton possibly facilitates the “uplifting” of developing germ cells from the basal to the adluminal compartment.

The translocation of germ cells across the seminiferous epithelium including the BTB in the adult testis during different stages of the seminiferous epithelial cycle was originally conceived as an unregulated cellular phenomenon by some investigators in the field. However, recent findings have implicated the role of cytokines in junction restructuring during spermatogenesis in the seminiferous epithelium. Earlier *in vitro* studies have shown that the endogenous mRNA levels of TGF- $\beta$ 3 [15] and TNF $\alpha$  [16] in Sertoli cells were lower during the assembly of the inter-Sertoli cell TJ barrier. This seems to suggest that the presence of these cytokines could perturb the Sertoli cell TJ-barrier formation. The inclusion of recombinant TGF- $\beta$ 3 or TNF $\alpha$  in the primary Sertoli cell culture was shown to perturb the TJ-barrier dose-dependently and reversibly [15,16]. Subsequent *in vitro* and *in vivo* studies using specific inhibitors of signal transducers downstream of TGF have shown that TGF- $\beta$ 3 mediates its effects on the inter-Sertoli cell TJ barrier *via* the p38 MAPK signal transduction pathway [17-19]. These studies thus demonstrated for the first time that the BTB integrity could be regulated biochemically by cytokines.

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**Table 1.** Effects of Cytokines on Different Blood-Tissue Barriers in Mammalian Tissues and Organs\*

Blood-tissue barrier	Cytokine	Biological effect(s)	Action mediator(s)	References
Blood-brain barrier	TNF $\alpha$ , IL-1 $\beta$ CCL2	Increase in barrier permeability Redistribution and decline of ZO-1 and occludin Redistribution of ZO-2 and claudin-5	Nitric oxide ERK signal transduction pathway Loss of caveolin-1	[5,67-71]
Blood-retina barrier	TGF $\beta$ VEGF	Increase in BRB permeability Increase in MMP-9 Decline in occludin level	n.k.	[72,73]
TJ-barrier in small intestine	TNF $\alpha$ , IFN $\gamma$	Increase in TJ barrier permeability Internalization of junctional integral membrane proteins	Myosin light chain kinase NF- $\kappa$ B; independent of apoptosis	[4,74-78]
	TGF $\beta$	Redistribution of JAM4 <i>via</i> endocytosis	Ligand-of-Numb protein X1	[79]
TJ-barrier in lung	TNF $\alpha$	Disassembly and redistribution of JAM-A from intercellular junctions	n.k.	[80,81]
TJ-barrier in heart	TGF $\beta$ s	Decline in paracellular adhesion Redistribution of $\beta$ -catenin and E-cadherin	Rho GTPase	[82]
TJ-barrier in skin	TNF $\alpha$ , IL-1 $\beta$ TNF $\alpha$	Increase in MMP-9 Reduction of vascular permeability Redistribution of VE-cadherin	Tyrosine phosphorylation	[41,83,84]

\*This Table is not intended to be exhaustive, the examples listed here represent selected studies illustrating the pivotal role of cytokines on tight junction barrier function in different mammalian organs and tissues. Many important references are not listed due to space limitations. n.k., not known.

In the earlier *in vivo* study, results were obtained using an cadmium chloride animal model which compromised germ cell adhesion, thereby leading to massive loss of germ cells from the epithelium [19,20]. We thus sought to investigate if TGF- $\beta$ 3 would also perturb Sertoli-germ cell adhesion using the Adjudin animal model. In this animal model, germ cells become depleted from the epithelium due to the disruption of Sertoli-germ cell anchoring junctions whilst the integrity of the BTB remains unaffected, at least during the time of anchoring junction restructuring [9]. Using this model, it was shown that TGF- $\beta$ 3 indeed is involved in AJ dynamics *via* the ERK signal transduction pathway [21].

Subsequent studies have shown that TGF- $\beta$ 3 can selectively activate p38 MAPK and ERK signal transduction pathways through its differential interaction with two upstream signal transducers, TAB1 and CD2AP, respectively. It was known that when TGF- $\beta$ 3 associated with its receptor T $\beta$ R1, this protein complex would become activated to elicit downstream signaling. If this activated TGF- $\beta$ 3/T $\beta$ R1 protein complex associated with both TAB1 and CD2AP, then p38 and ERK MAP kinases were activated, leading to a disruption of the BTB and Sertoli-germ cell AJ [22]. However, when the activated TGF- $\beta$ 3/T $\beta$ R1 protein complex associated with CD2AP selectively, only Sertoli-germ cell AJ were compromised without perturbing the BTB integrity [22].

Obviously, one can argue that the amount of cytokine administered to adult rat testes locally to elicit changes in BTB and Sertoli-germ cell adhesion [22,23] represents a physiological level that can be reached in the BTB microenvironment during the seminiferous epithelial cycle. Using a solid-phase immunoblot-based assay, the endogenous level of TNF $\alpha$  in the adult rat testis (~1.6 gm/testis) was shown to be  $\sim 0.5 \pm 0.15 \mu\text{g}$  [23]. This illustrates that the amount of TNF $\alpha$  administered (2  $\mu\text{g}$  per testis) to induce reversible BTB disruption [23] was likely attainable at the BTB microenvironment when preleptotene spermatocytes traverse the barrier at stage VIII of the seminiferous epithelial cycle. Furthermore, we have shown unequivocally that TNF $\alpha$  can increase BTB permeability transiently and reversibly using a functional test of the BTB integrity, which assesses the diffusion of fluorescein isothiocyanate (FITC, Mr 389), a small fluorescent molecular probe. When administered to adult rats *via* the jugular vein in normal adult rat testes, FITC was restricted from the adluminal compartment by the BTB. A surge in the TNF $\alpha$  level *via* local administration led to the diffusion of FITC across the BTB into the adluminal compartment, indicating a loss of BTB integrity. FITC became restricted to the basal

compartment again when the BTB was resealed after the metabolic clearance of the cytokine [23]. This indicates that cytokines can induce a transient loss of BTB integrity. Hence, any changes in the function of junctions resulted from cytokine injection were unlikely the result of cytotoxicity.

Collectively, these findings suggest that throughout spermatogenesis, except at stage VIII of the seminiferous epithelial cycle, the T $\beta$ R1 receptor, when activated by TGF $\beta$ 3, recruits only CD2AP to selectively disrupt AJ to facilitate germ cell migration *via* the ERK signal transduction pathway. On the other hand, the elevated TGF $\beta$ 3 level at stage VIII triggers the T $\beta$ R1 receptor to recruit both TAB1 and CD2AP, which in turn activate the p38 and ERK signal transduction pathways to increase the BTB permeability and perturb the Sertoli-germ cell adhesion. However, it remains to be determined how T $\beta$ R1 can recruit and activate signal transducers in a stage-specific manner since T $\beta$ R1 is expressed constitutively on Sertoli cells.

#### COORDINATION OF SIGNALING EVENTS BETWEEN THE BTB AND APICAL ES DURING CYTOKINE-INDUCED JUNCTION RESTRUCTURING

Due to their pleiotropic nature, cytokines are capable of activating different signaling pathways to elicit different physiological responses. However, studies on the signal transduction pathway involved in junction restructuring were limited to few selected cytokines. During the reversible BTB damage induced by TNF $\alpha$  [23] and TGF $\beta$ 3 [22] in the seminiferous epithelium, the ERK and p38 MAPK signal transduction pathways are activated. These two signaling pathways are responsible for the loss of junction integrity at the apical ES and the BTB, respectively [22]. However, it is likely that other signal transduction pathways are involved in the cytokine-induced junction restructuring. For instance, T $\beta$ R2 has been demonstrated to mediate the loss of tight junction integrity *via* Par6, a protein involved in the formation and maintenance of cell polarity (for a review, see [24]). A comprehensive investigation of signal transduction pathways involved in cytokine-mediated junction restructuring is urgently needed to identify all players in this cellular event.

As discussed above, cytokines can mediate disruption of junction integrity at the apical ES, as well as at the BTB, which are located at the opposite ends of the columnar Sertoli cells in the seminiferous epithelium *in vivo*. Thus, it is hypothesized that there is crosstalk between the apical ES and the BTB. The receptors for

TNF reside basally on Sertoli cells [16]. It is noteworthy that intratesticular administration of TNF $\alpha$  induces not only the disruption of the BTB integrity reversibly, but also of the apical ES, leading to a transient germ cell loss from the epithelium [23]. This indicates the presence of crosstalk between the apical ES and the BTB to coordinate cytokine-induced junction restructuring.

While the precise signaling pathway(s) responsible for the cross-talk between the apical ES and the BTB is unclear, this plausibly involves the integrin-laminin protein complex. Using the adjuvant model, it was reported that the toxicant-induced increase in the TGF- $\beta$ 3 level that led to the disruption of germ cell adhesion was associated with a surge in integrin- $\beta$ 1 [21], a major integral membrane component of the apical ES (for reviews, see [25-27]). Integrin is tightly linked to downstream signaling molecules, such as integrin-linked kinase (ILK) and phosphoinositide 3-kinase (PI 3-K), which are crucial for regulating junction restructuring in the testis [28,29]. It is thus likely that activation of the integrin-laminin protein complex at the apical ES can transduce signals to the BTB or *vice versa* so as to coordinate the simultaneous occurrence of spermatiation and BTB restructuring at stage VIII of the seminiferous epithelial cycle.

### CELLULAR MECHANISM

Even though it has been established that cytokines can down-regulate the steady-state levels of integral membrane proteins to affect junction dynamics [7,22,23], the cellular mechanisms which mediate these effects remain to be elucidated. It is possible that cytokines act at multiple levels, including the assembly of a functional protein complex and the maintenance of junction integrity. The latter can be affected by a number of different factors which lead to changes in protein-protein interactions [9,23,30]. In the following sections, the regulation of junction integrity by cytokines is introduced. These hypotheses are made based on the effects of cytokines in other endothelia or epithelia and are likely to be modified following future studies.

### ENZYME HOMEOSTASIS OF UBIQUITIN LIGASES, PROTEASES AND PROTEASE INHIBITORS, AND PROTEIN KINASES AND PHOSPHATASES

Different groups of enzymes, namely proteases, protein kinases and E3 ubiquitin ligases, have been reported to participate in junction restructuring [6,31,32]. Cytokines have been postulated to regulate the homeostasis of these enzymes to mediate junction restructuring at the BTB and apical ES.

The basement membrane is a modified form of the extracellular matrix (ECM) in the seminiferous tubule, and proteolysis of the ECM serves as an important mechanism for cell migration. It has been demonstrated previously that BTB integrity can be regulated in part by an interplay of proteases and their corresponding inhibitors (for reviews, see [6,33]). For instance, cleavage of the collagen network in the ECM promoted by TNF $\alpha$  is mediated through matrix metalloprotease-9 (MMP-9), and this in turn leads to junction disassembly in the BTB. Interestingly, there is a concomitant surge in the level of tissue inhibitor of metalloproteases-1, the inhibitor of MMP-9 [16]. This may serve as a negative feedback to compensate for the loss of collagen in the ECM. This negative feedback mechanism induced by TNF $\alpha$  strengthens the postulate that cytokines play an important role in junction restructuring.

Furthermore, phosphorylation of junctional proteins is one of the post-translational modifications responsible for regulating cell adhesion (for reviews, see [31,34-36]). For instance, adherens proteins such as cadherin are destabilized upon tyrosine phosphorylation at specific site(s). Thus, any change in the kinetics of protein tyrosine kinases (PTK) and protein tyrosine phosphatases would affect cell-cell adhesion ([29]; for reviews, see [31,36]). Cytokines are generally able to activate a number of PTKs [37-40]. For instance, Src kinase family, which belongs to the PTK families, can

be activated by TNF $\alpha$  to induce a surge in transendothelial permeability. On the other hands, tyrosine phosphorylation and subsequent endocytosis of vascular endothelial cadherin (VE cadherin) [41] have been shown to be mediated by Fyn, a non-receptor member of the Src family. This leads to a decline in the VE cadherin level at the cell-cell interface and an increase in permeability [42]. Therefore, cytokines may regulate cell adhesion by affecting the phosphorylation state of junctional proteins.

Furthermore, ubiquitination has been suggested to play a significant role in junction restructuring [32]. Ubiquitination of proteins can either lead to proteasomal degradation, lysosomal degradation or protein trafficking. The ubiquitin tag on a protein can be removed by the deubiquitinating enzyme to rescue the protein from degradation or to recycle it to its original subcellular location [43,44]. Cytokines can regulate junction integrity *via* the ubiquitination of signal transducers involved in junction restructuring. The ubiquitination of RhoA induced by TGF- $\beta$ 3 was shown to mediate the disruption of occludin [24]. As such, it is possible that cytokines can induce the ubiquitination of junctional proteins to affect their availability at the cell-cell interface and hence junction integrity. For instance, it was recently shown that Itch is responsible for the proteasomal degradation of occludin [45].

### TRANSCRIPTIONAL CONTROL

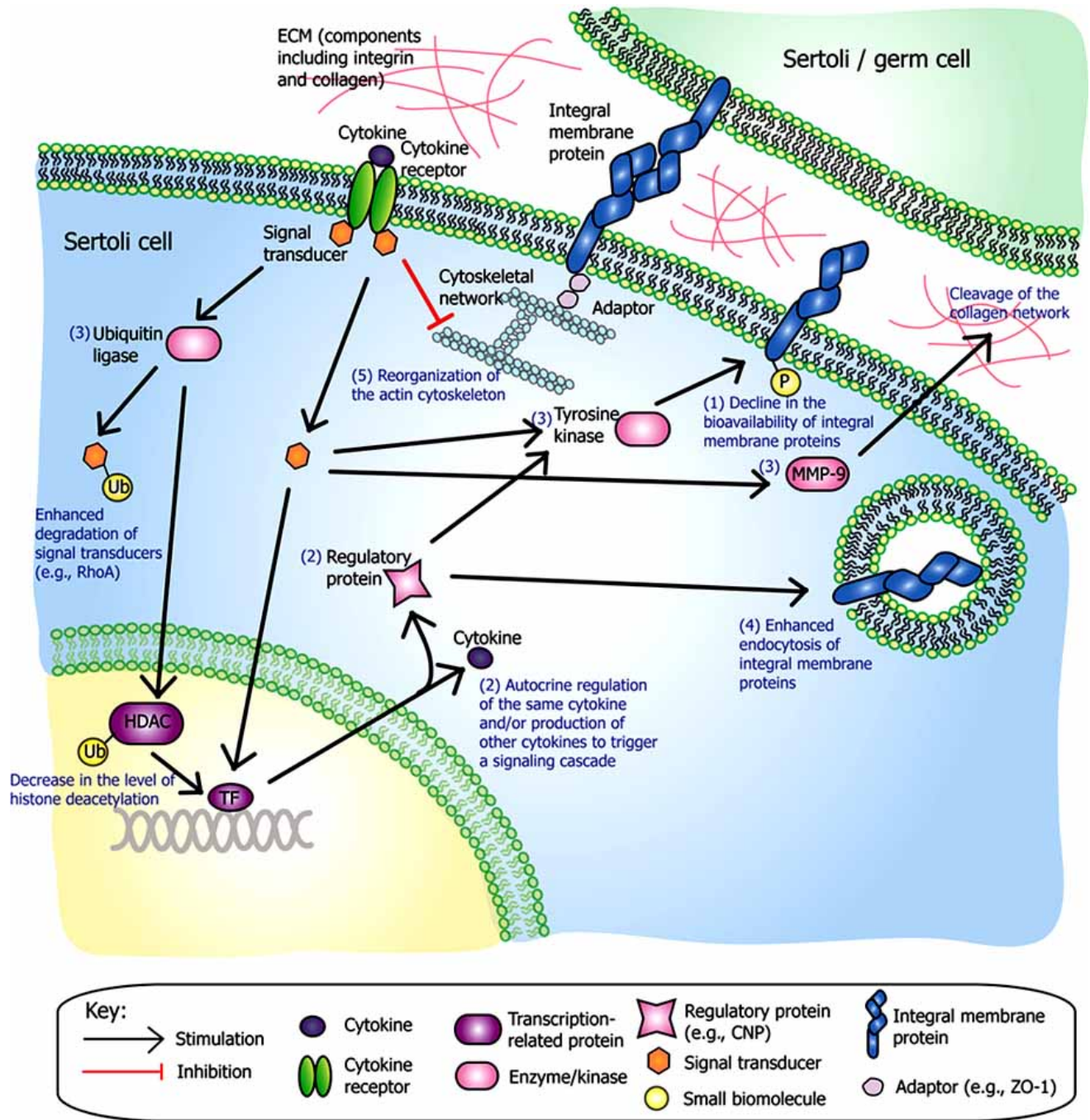
Cytokines regulate junction dynamics *via* their effects on the de novo synthesis of integral membrane proteins, peripheral adaptors and signaling proteins (e.g., kinases). For instance, the expression of occludin, an integral membrane TJ protein, is down-regulated by TNF $\alpha$  and IFN $\gamma$  [46]. As the turnover rate of junctional proteins is high, a decrease in the synthesis of nascent occludin would cause a drastic decline in its level at the cell-cell interface, possibly leading to the disruption of TJ integrity.

It should be noted that the Smad pathway is not activated during TGF- $\beta$ 3-induced perturbation of the BTB and apical ES [21]. However, it is possible that TGF- $\beta$ 3 may exert its effect on the transcriptional level independent of the Smad pathway. Through mediation of TGF $\beta$  stimulated factor 1 in pituitary derived GH3 cells, TGF- $\beta$ 3 can stimulate the transcription of C-type natriuretic peptide (CNP) [47], a peptide recently shown to affect BTB dynamics in adult rat testes [48].

Besides disrupting junction integrity, transcription regulation is important in junction assembly. The nuclear expression of NF- $\kappa$ B, an important signal transducer of TNF $\alpha$ , is higher during stages I to VII of the seminiferous epithelial cycle, which coincides with the stage specificity of TNF $\alpha$ . This leads to the postulate that NF- $\kappa$ B may mediate the transcriptional control of TNF $\alpha$  in testis [49]. Indeed, TNF $\alpha$ , with the help of NF- $\kappa$ B, induces expression of the androgen receptor in Sertoli cell [50], whereas testosterone has been shown to rescue the BTB damage induced by cadmium chloride [51]. Moreover, NF- $\kappa$ B was shown to mediate the proteasomal degradation of histone deacetylase 1 by various cytokines [49], suggesting that transcriptional control occurs through histone acetylation and deacetylation. Taken collectively, these observations suggest that cytokines regulate both junction disassembly and assembly *via* transcriptional control.

### PROTEIN TRAFFICKING AND INTERNALIZATION

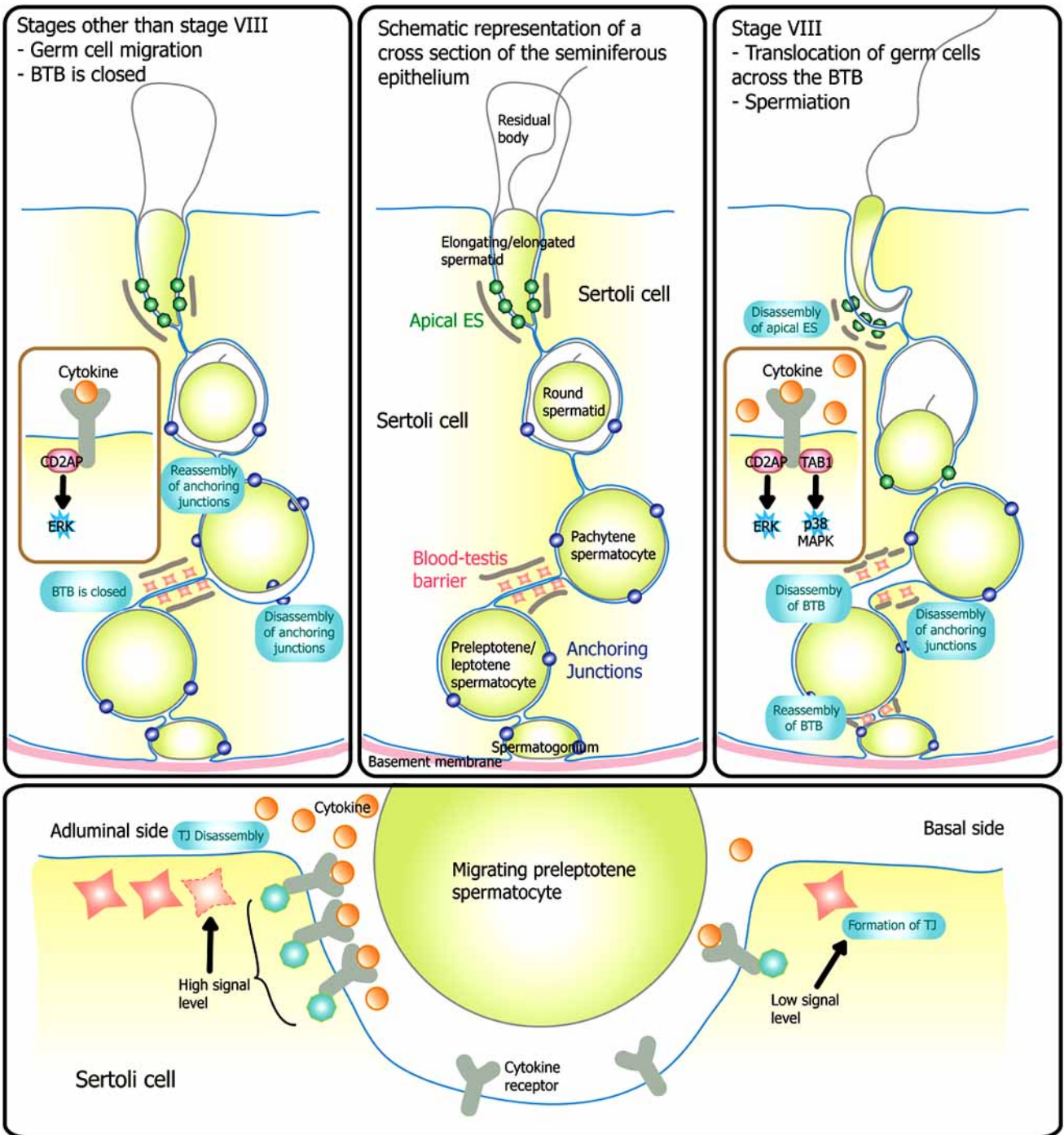
Integral membrane proteins at the Sertoli-Sertoli and/or Sertoli-germ cell interface are not static. Instead, there is constant movement of junction proteins to and from the plasma membrane [32,52,53]. Hence, the trafficking of proteins would change the bioavailability of junctional proteins at the cell-cell interface. Recent studies have demonstrated that junction disassembly at the BTB can be mediated by endocytosis of junctional proteins [48]. However, it is still unclear if cytokines can affect protein trafficking to regulate the junction integrity.



**Fig. (1).** A schematic diagram illustrating different cellular events regulated by cytokines for junction restructuring in the seminiferous epithelium during spermatogenesis. Upon coupling with cytokines such as TGFβ3 and TNFα, the corresponding receptors become activated. Various signal transducers are then recruited to the ligand-receptor complex to be followed by their activation. A number of cellular events have been shown to become up-regulated by signal transducers in junction restructuring during spermatogenesis. These include (1) a disruption of junction complexes at the cell-cell interface via changes in the phosphorylation of junctional proteins, (2) transcriptional regulation on the production of cytokines and other regulatory proteins; which in turn (3) regulates the homeostasis of kinases, phosphatases, proteases and/or protease inhibitors; (4) besides, an enhanced endocytosis of integral membrane proteins at the cell-cell interface can also occur; all these contribute to (5) a reorganization of the cytoskeleton.

Cytokines may affect the assembly of functional junction complexes at the cell-cell interface to facilitate passage of germ cells. The significance of cell adhesion molecules in junction assembly has been reported previously [54-56]. For instance, E-cadherin has been suggested to recruit occludin and other junctional proteins along the cytoskeleton to the cell-cell interface for junction assembly [55]. When junction disassembly was induced by TNFα and

TGF-β3 in the seminiferous epithelium, a significant decline in the level of N-cadherin [22,23]. This decline in N-cadherin may contribute to a decrease and a delay in the assembly of the junctional protein complex in germ cells. This decrease may facilitate the migration of germ cells towards the lumen of the seminiferous epithelium.



**Fig. (2).** A schematic diagram showing the dynamic changes occurring at the cell-cell interface during spermatogenesis in the seminiferous epithelium of adult rat testes. The middle panel in the upper part of the figure shows a general view of the cross-section of the seminiferous epithelium. The panel on the left represents junction restructuring during the migration of developing germ cells throughout spermatogenesis, except for stage VIII of the seminiferous epithelial cycle. The low level of cytokines present at the BTB microenvironment activates the T $\beta$ RI complex to recruit CD2AP only, which then activates the ERK signal transduction pathway. The BTB remains intact during these stages while there are constant engagement and disengagement of adherens junctions to mediate the movement of germ cells across the seminiferous epithelium. The panel on the right represents junction restructuring that occurs during stage VIII of the seminiferous epithelial cycle. The high level of cytokines present at the BTB microenvironment activates the T $\beta$ RI complex to recruit both CD2AP and TAB1, which activates the ERK and p38 MAPK signal transduction pathways. Adherens junctions are disrupted to mediate the migration of germ cells. Functional junction complexes at the BTB above preleptotene/leptotene spermatocytes become disengaged to mediate the translocation of these spermatocytes. At the same time, junctions are assembled beneath these spermatocytes in order to maintain the integrity of the BTB. This figure was prepared based on a recent study from our laboratory [22]. However, it should be noted that the mechanism(s) responsible for the differential association of the signal transducers, such as CD2AP and TAB1 with the activated TGF- $\beta$ 3/T $\beta$ R1 protein complex, is entirely unknown. The lower part of the figure shows a magnified view of the region near the migrating preleptotene spermatocyte at stage VIII of the seminiferous epithelial cycle. Above the migrating spermatocyte (the left hand side of this figure), TJ disengage to allow the migration of germ cells, whereas TJs are formed simultaneously between Sertoli cells beneath the migrating spermatocyte for the maintenance of the BTB integrity. It is speculated that the localized production of cytokines and/or their receptors, as well as the level of activated signal transducers, are needed for junction restructuring.

Interestingly, some cytokines have been found to mediate the translocation of cells without the loss of tight junction integrity. During the movement of germ cells across the BTB, maintenance of the integrity of the barrier is important to maintain other functions of the BTB. At low concentration, IFN $\gamma$  can induce the translocation of *E. coli* through caveolin and lipid-raft mediated endocytosis without loss of tight junction integrity [57]. Selected cytokines may also facilitate the passage of germ cells across the BTB in a similar manner.

### CYTOSKELETAL NETWORK

Integral membrane proteins of TJ and ES are linked to the cytoskeletal network *via* adaptor proteins. The cytoskeletal network contributes to the maintenance of junction integrity, as well as to direct junctional proteins-containing intracellular vesicles to the plasma membrane [9]. Transient disruption of the cytoskeleton or detachment of peripheral membrane proteins from the network would result in junction disassembly [9,25]. For instance, TNF $\alpha$ - or TGF $\beta$ 3-induced junction disruption is accompanied by a disruption of actin filament bundles at the apical ES and BTB [22,23]. However, it remains to be determined if damage to actin filament bundles induced by cytokine treatment mediates junction disassembly or is the result of this.

Actin is the major constituent of the actin-based cytoskeleton. The dynamics between filamentous and globular actin are regulated by polymerization and depolymerization. Enhanced depolymerization of actin filaments would result in disruption of the cytoskeletal network. It has been reported that TNF $\alpha$  can elicit reorganization of the actin cytoskeleton [58-60]. For instance, in pulmonary artery endothelial cells, TNF $\alpha$ -induced barrier disruption is accompanied by the depolymerization of F-actin and a stimulation of actin synthesis [61]. The PTK Fyn may also play a role in the actin reorganization in the testis during spermatogenesis since abnormal vesicular structures were observed in Fyn-deficient adult mice [62]. As discussed above, Fyn can mediate the TNF $\alpha$ -induced increase in transendothelial permeability. Hence, TNF $\alpha$  may disrupt junction integrity partly through disruption of the cytoskeletal network.

### HOW DOES CYTOKINE-INDUCED RESTRUCTURING IN THE SEMINIFEROUS EPITHELIUM FIT INTO THE CURRENT CONCEPT OF GERM CELL MOVEMENT DURING SPERMATOGENESIS? AN EMERGING NEW CONCEPT

A number of theories were proposed to address the mechanism of germ cell movement across the BTB during spermatogenesis (for a review, see [9]). A widely accepted one is known as the zipper theory (for reviews, see [9,10,63]). It states that at the BTB, existing tight junctions above migrating preleptotene or leptotene spermatocytes break down while new tight junctions form beneath them. This postulate accounts for the maintenance of the BTB integrity throughout spermatogenesis and was supported by observations with electron microscopy, in which preleptotene or leptotene spermatocytes were seen to be trapped in-between two tight junctions [64,65].

The junction restructuring theory [9] was then proposed by our research group to illustrate the junction restructuring based on recent biochemical and molecular findings in the field. In this theory, it was postulated that junctions are intricately regulated by different junction-associated proteins (e.g. proteases/protease inhibitors and kinases/phosphatases) and junction assembly and disassembly is coordinated *via* different signal transduction pathways. Based on available data as briefly reviewed above, we hypothesize that cytokines elicit a localized signal for determining the state (either open or closed) of the BTB. These signals are likely determined by the relative ratio of available cytokines in the intercellular space and their corresponding receptors on Sertoli cells (Fig. 2).

It is hypothesized that localized secretion of cytokines (e.g., TGF- $\beta$ 3 and TNF $\alpha$ ) and perhaps CNP [22,23,48] by Sertoli and/or

germ cells (most likely preleptotene spermatocytes) into the intercellular space above migrating preleptotene spermatocytes may trigger transient disruption of the TJ-barrier. On the other hand, secretion of a relatively lower level of these molecules into the intercellular space beneath migrating preleptotene spermatocytes may stimulate the formation of TJ fibrils. Alternatively, this may be achieved by redistributing cytokine receptors and/or CNP receptors on Sertoli cells near migrating preleptotene spermatocytes. Collectively, localized levels of cytokines and their receptors may lead to a cascade of signals, which would either disassemble or reassemble the TJ.

In addition, the cytoskeleton may mediate junction restructuring induced by cytokines since it has been proposed to facilitate junction restructuring *via* various pathways, which includes the directional transport of internalized junctional proteins [66]. Because of their ability to reorganize the cytoskeleton [58-60], cytokines could possibly utilize the cytoskeleton for junction restructuring. Perhaps, testosterone, follicle stimulating hormone (FSH), as well as other yet-to-be identified molecule(s) that promote formation of the TJ-barrier also participate in the assembly and maintenance of the TJ.

According to the zipper theory and the junction restructuring theory, precise regulation of junctional complexes must exist to coordinate the concurrent disassembly and assembly of Sertoli cell junctions at opposite ends of migrating preleptotene and leptotene spermatocytes. The hypothesis proposed herein attempts to explain the role of cytokines in these events. Obviously, additional studies by investigators in the field should be conducted in the years to come to validate this emerging concept.

### CONCLUDING REMARKS

The recent findings reviewed herein have opened up unprecedented opportunities for male contraceptive development since a number of molecules could be potentially targeted to perturb spermatogenesis without affecting the hypothalamic-pituitary-testicular axis. Cytokines themselves could not serve as targets for male contraception as these peptides have multiple physiological functions in different organs and tissues. Thus, any disruption in the production of cytokines by Sertoli and/or germ cells in adult testes would lead to undesirable side effects. These include affecting the function of Leydig cells, which can in turn result in a compromise of the hypothalamic-pituitary-testicular axis.

Alternatively, effectors of activated cytokine/receptor protein complexes, including the adaptors TAB1 and CD2AP, and associated protein kinases and phosphatases, can be potential targets for disrupting the migration and adhesion of male germ cells. Affecting these molecules may result in premature release of germ cells into the lumen of the seminiferous tubule or stop the passage of migrating preleptotene and leptotene spermatocytes across the BTB. In this way, spermatogenesis would be perturbed without affecting the androgen microenvironment in the testis and the hypothalamic-pituitary-testicular axis.

Finally additional research should be directed at understanding how cytokines regulate the function of the BTB and apical ES. These include determining the functional interaction of cytokines with each other, as well as with other regulatory molecules such as CNP, but more importantly the existence of crosstalk between the apical ES and BTB. This information will be instrumental in identifying suitable molecule(s) for arresting spermatogenesis, which can then be expanded to develop new male contraceptives.

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## ABBREVIATIONS

AJ	= Adherens junction
BTB	= Blood-testis barrier
BRB	= Blood-retina barrier
CCL2	= Chemokine (C-C motif) ligand 2
CD2AP	= Cluster of differentiation 2 associated protein
CNP	= C-type natriuretic peptide
ECM	= Extracellular matrix
ERK	= Extracellular signal-regulated kinase
ES	= Ectoplasmic specialization
FITC	= Fluorescein isothiocyanate
FSH	= Follicle stimulating hormone
IFN	= Interferon
IL	= Interleukin
ILK	= Integrin-linked kinase
JAM	= Junctional adhesion molecule
MMP-9	= Matrix metalloprotease-9
Mr	= Molecular weight
NF-κB	= Nuclear factor-κB
p38 MAPK	= p38 mitogen-activated protein kinase
PI 3-K	= Phosphoinositide 3-kinase
PTK	= Protein tyrosine kinase
TAB1	= Transforming growth factor-β activated kinase (TAK) 1 binding protein 1
TβR	= Transforming growth factor β receptor
TGF-β3	= Transforming growth factor-β3
TJ	= Tight junction
TNFα	= Tumor necrosis factor α
VE-cadherin	= Vascular endothelial-cadherin
VEGF	= Vascular endothelial growth factor
ZO	= Zonula occludens

## REFERENCES

- Evans, C. H. *J. Cell. Biochem.*, **1993**, *53*, 277.
- Oppenheim, J. J. *Int. J. Hematol.*, **2001**, *74*, 3.
- Cohen, M. C.; Cohen, S. *Am. J. Pathol.*, **1996**, *105*, 589.
- Turner, J. R. *Am. J. Pathol.*, **2006**, *169*, 1901.
- Stamatovic, S. M.; Dimitrijevic, O. B.; Keep, R. F.; Andjelkovic, A. V. *Acta Neurochir. Suppl. (Wien)*, **2006**, *96*, 444.
- Siu, M. K. Y.; Cheng, C. Y. *BioEssays*, **2004**, *26*, 978.
- Xia, W.; Mruk, D. D.; Lee, W. M.; Cheng, C. Y. *Cytokine Growth Factor Rev.*, **2005**.
- Dym, M.; Fawcett, D. W. *Biol. Reprod.*, **1970**, *3*, 308.
- Mruk, D. D.; Cheng, C. Y. *Endocr. Rev.*, **2004**, *25*, 747.
- Pelletier, R. M.; Byers, S. W. *Microsc. Res. Tech.*, **1992**, *20*, 3.
- Russell, L. D. *Tissue Cell*, **1977**, *9*, 475.
- Vogl, A. W.; Pfeiffer, D. C.; Mulholland, D. J.; Kimel, G.; Guttman, J. *Arch. Histol. Cytol.*, **2000**, *63*, 1.
- Russell, L. D. *Am. J. Anat.*, **1977**, *148*, 313.
- Fijak, M.; Meinhardt, A. *Immunol. Rev.*, **2006**, *213*, 66.
- Lui, W. Y.; Lee, W. M.; Cheng, C. Y. *Endocrinology*, **2001**, *142*, 1865.
- Siu, M. K. Y.; Lee, W. M.; Cheng, C. Y. *Endocrinology*, **2003**, *144*, 371.
- Lui, W. Y.; Lee, W. M.; Cheng, C. Y. *Biol. Reprod.*, **2003**, *68*, 1597.
- Lui, W. Y.; Wong, C. H.; Mruk, D. D.; Cheng, C. Y. *Endocrinology*, **2003**, *144*, 1139.
- Wong, C. H.; Mruk, D. D.; Lui, W. Y.; Cheng, C. Y. *J. Cell Sci.*, **2004**, *117*, 783.
- Hew, K.; Heath, G. L.; Jiwa, A. H.; Welsh, M. J. *Biol. Reprod.*, **1993**, *49*, 840.
- Xia, W.; Cheng, C. Y. *Dev. Biol.*, **2005**, *280*, 321.
- Xia, W.; Mruk, D. D.; Lee, W. M.; Cheng, C. Y. *J. Biol. Chem.*, **2006**, *281*, 16799.
- Li, M. W. M.; Xia, W.; Mruk, D. D.; Wang, C. Q. F.; Yan, H. H. N.; Siu, M. K. Y.; Lui, W. Y.; Lee, W. M.; Cheng, C. Y. *J. Endocrinol.*, **2006**, *190*, 313.
- Bose, R.; Wrana, J. L. *Curr. Opin. Cell Biol.*, **2006**, *18*, 206.
- Yan, H. H. N.; Mruk, D. D.; Lee, W. M.; Cheng, C. Y. *BioEssays*, **2006**, *29*, 36.
- Hervy, M.; Hoffman, L.; Beckerle, M. C. *Curr. Opin. Cell Biol.*, **2006**, *18*, 524.
- Chen, X.; Gumbiner, B. M. *Curr. Opin. Cell Biol.*, **2006**, *18*, 572.
- Siu, M. K. Y.; Cheng, C. Y. *Biol. Reprod.*, **2004**, *70*, 945.
- Siu, M. K. Y.; Wong, C. H.; Lee, W. M.; Cheng, C. Y. *J. Biol. Chem.*, **2005**, *280*, 25029.
- Chung, N. P. Y.; Mruk, D. D.; Mo, M. Y.; Lee, W. M.; Cheng, C. Y. *Biol. Reprod.*, **2001**, *65*, 1340.
- Sallee, J. L.; Wittchen, E. S.; Burrige, K. *J. Biol. Chem.*, **2006**, *281*, 16189.
- Lui, W. Y.; Lee, W. M. *Mol. Cell. Endocrinol.*, **2006**, *250*, 25.
- Wong, C. H.; Cheng, C. Y. *Curr. Top. Dev. Biol.*, **2005**, *71*, 263.
- Lee, N. P. Y.; Cheng, C. Y. *J. Cell. Physiol.*, **2005**, *202*, 344.
- Alema, S.; Salvatore, A. M. *Biochim. Biophys. Acta*, **2007**, *1773*, 47.
- Zhang, J.; Mruk, D. D.; Cheng, C. Y. *J. Cell. Physiol.*, **2005**, *204*, 470.
- Mon, N. N.; Hasegawa, H.; Thant, A. A.; Huang, P.; Tanimura, Y.; Senga, T.; Hamauchi, M. *Cancer Res.*, **2006**, *66*, 6778.
- Yagi, R.; Waguri, S.; Sumikawa, Y.; Nada, S.; Oneyama, C.; Itami, S.; Schmedt, C.; Uchiyama, Y.; Okada, M. *EMBO J.*, **2007**, *26*, 1234.
- Lee, C. W.; Lin, C. C.; Lin, W. N.; Liang, K. C.; Luo, S. F.; Wu, C. B.; Wang, S. W.; Yang, C. M. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2007**, *292*, L799.
- Ulanova, M.; Marcet-Palacios, M.; Munoz, S.; Asfaha, S.; Kim, M. K.; Schreiber, A. D.; Befus, A. D. *Biochem. Biophys. Res. Commun.*, **2006**, *351*, 431.
- Menon, C.; Ghartey, A.; Canter, R.; Feldman, M.; Fraker, D. L. *Ann. Surg.*, **2006**, *244*, 781.
- Angelini, D. J.; Hyun, S.-W.; Grigoryev, D. N.; Garg, P.; Gong, P.; Singh, I. S.; Passaniti, A.; Hasday, J. D.; Goldblum, S. E. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2006**, *291*, L1232.
- Mukhopadhyay, D.; Riezman, H. *Science*, **2007**, *315*, 201.
- Ciechanover, A.; Iwai, K. *IUBMB Life*, **2004**, *56*, 193.
- Traweger, A.; Fang, D.; Liu, Y.-C.; Stelzhammer, W.; Krizbai, I. A.; Fresser, F.; Bauer, H.-C.; Bauer, H. *J. Biol. Chem.*, **2002**, *277*, 10201.
- Mankertz, J.; Tavalali, S.; Schmitz, H.; Mankertz, A.; Riecken, E. O.; Fromm, M.; Schulzke, J. D. *J. Cell Sci.*, **2000**, *113*, 2085.
- Ohta, S.; Takeuchi, M.; Deguchi, M.; Tsuji, T.; Gahara, Y.; Nagata, K. *Biochem. J.*, **2000**, *350*, 395.
- Xia, W.; Mruk, D. D.; Cheng, C. Y. *Proc. Natl. Acad. Sci. U. S. A.*, **2007**, *104*, 3841.
- Vashisht Gopal, Y. N.; Van Dyke, M. W. *Cell Cycle*, **2006**, *5*, 2738.
- Delfino, F. J.; Boustead, J. N.; Fix, C.; Walker, W. H. *Mol. Cell. Endocrinol.*, **2003**, *201*, 1.
- Chung, N. P. Y.; Cheng, C. Y. *Endocrinology*, **2001**, *142*, 1878.
- Tepass, U.; Harris, K. P. *Trends Cell Biol.*, **2007**, *17*, 26.
- Chiba, H.; Kojima, T.; Osanai, M.; Sawada, N. *Sci. STKE*, **2006**, *316*, pe1.
- Meyer, R. A.; Laird, D. W.; Revel, J.-P.; Johnson, R. G. *J. Cell Biol.*, **1992**, *119*, 179.
- Capaldo, C. T.; Macara, I. G. *Mol. Biol. Cell*, **2007**, *18*, 189.
- Rajasekaran, A. K.; Hojo, M.; Huima, T.; Rodriguez-Boulan, E. *J. Cell Biol.*, **1996**, *132*, 451.
- Clark, E.; Hoare, C.; Tanianis-Hughes, J.; Carlson, G. L.; Warhurst, G. *Gastroenterology*, **2005**, *128*, 1258.
- Wojciak-Stothard, B.; Entwistle, A.; Garg, R.; Ridley, A. J. *J. Cell. Physiol.*, **1998**, *176*, 150.
- Koukouritaki, S. B.; Vardaki, E. A.; Papakonstanti, E. A.; Lianos, E.; Stournaras, C.; Emmanouel, D. S. *Mol. Med.*, **1999**, *5*, 382.
- Papakonstanti, E. A.; Stournaras, C. *Mol. Biol. Cell*, **2004**, *15*, 1273.
- Goldblum, S. E.; Ding, X.; Campbell-Washington, J. *Am. J. Physiol.*, **1993**, *264*, C894.
- Maekawa, M.; Toyama, Y.; Yasuda, M.; Yagi, T.; Yuasa, S. *Biol. Reprod.*, **2002**, *66*, 211.
- Russell, L. D.; Peterson, R. N. *Int. J. Cytol.*, **1985**, *94*, 177.
- Mruk, D. D.; Cheng, C. Y. Unpublished observation.
- Russell, L. D. Form, dimensions, and cytology of mammalian Sertoli cells. *The Sertoli cell*; Cache River Press: Clearwater, FL, 1993; pp 1-37.
- Vogl, A. W.; Vaid, K. S.; Guttman, J. A. The Sertoli cell cytoskeleton. *Molecular Mechanism in Spermatogenesis*; Landes Bioscience: Austin, TX, 2008 (in press).
- Boveri, M.; Kinsner, A.; Berezowski, V.; Lenfant, A.-M.; Draing, C.; Cecchelli, R.; Dehouck, M.-P.; Hartung, T.; Prieto, P.; Bal-Price, A. *Neuroscience*, **2006**, *137*, 1193.
- Miller, F.; Fenart, L.; Landry, V.; Coisne, C.; Cecchelli, R.; Dehouck, M.-P.; Buee-Scherrer, V. *Eur. J. Neurosci.*, **2005**, *22*, 835.
- Song, L.; Ge, S.; Pachter, J. S. *Blood*, **2007**, *109*, 1515.
- Stamatovic, S. M.; Dimitrijevic, O. B.; Keep, R. F.; Andjelkovic, A. V. *J. Biol. Chem.*, **2006**, *281*, 8379.
- Dimitrijevic, O. B.; Stamatovic, S. M.; Keep, R. F.; Andjelkovic, A. V. *J. Cereb. Blood Flow Metab.*, **2006**, *26*, 797.
- Behzadian, M. A.; Wang, X. L.; Windsor, L. J.; Ghaly, N.; Caldwell, R. B. *Invest. Ophthalmol. Vis. Sci.*, **2001**, *42*, 853.
- Antonetti, D. A.; Barber, A. J.; Khin, S.; Lieth, E.; Tarbell, J. M.; Gardner, T. W. *Diabetes*, **1998**, *47*, 1953.

- [74] Bruewer, M.; Luegering, A.; Kucharzik, T.; Parkos, C. A.; Madara, J. L.; Hopkins, A. M.; Nusrat, A. *J. Immunol.*, **2003**, *171*, 6164.
- [75] Ma, T. Y.; Boivin, M. A.; Ye, D.; Pedram, A.; Said, H. M. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **2005**, *288*, G422.
- [76] Ma, T. Y.; Iwamoto, G. K.; Hoa, N. T.; Akotia, V.; Pedram, A.; Boivin, M. A.; Said, H. M. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **2004**, *286*, G367.
- [77] Sanders, D. S. A. *J. Clin. Pathol.*, **2005**, *58*, 568.
- [78] Wang, F.; Graham, W. V.; Wang, Y.; Witkowski, E. D.; Schwarz, B. T.; Turner, J. R. *Am. J. Pathol.*, **2005**, *166*, 409.
- [79] Kansaku, A.; Hirabayashi, S.; Mori, H.; N, F.; Kawata, A.; Ikeda, M.; Rokukawa, C.; Kurihara, H.; Hata, Y. *Oncogene*, **2006**, *25*, 5071.
- [80] Martinez-Estrada, O. M.; Manzi, L.; Tonetti, P.; Dejana, E.-B.; Bazzoni, G. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2005**, *288*, L1081.
- [81] Gon, Y.; Wood, M. R.; Kiosses, W. B.; Jo, E.; Sanna, M. G.; Chun, J.; Rosen, H. *Proc. Natl. Acad. Sci. U. S. A.*, **2005**, *102*, 9270.
- [82] Dokic, D.; Dettman, R. W. *Dev. Biol.*, **2006**, *299*, 489.
- [83] Shellman, Y. G.; Makela, M.; Norris, D. A. *Melanoma Res.*, **2007**, *16*, 207.
- [84] Kimber, I.; Cumberbatch, M.; Dearman, R. J.; Bhushan, M.; Griffiths, C. E. *Br. J. Dermatol.*, **2000**, *142*, 401.

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