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Review

Regulation of spermatogenesis in the microenvironment of the seminiferous epithelium: New insights and advances☆

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ABSTRACT

Spermatogenesis is a complex biochemical event, involving the participation of the hypothalamus and the pituitary gland via secretion of the hypothalamus hormone GnRH, and two pituitary hormones FSH and LH. Thus, the hypothalamic–pituitary–testicular axis is a crucial regulatory axis for testicular function. Recent studies have shown that in the microenvironment of the seminiferous epithelium, wherein each Sertoli cell supports ~30–50 germ cells at different stages of development, locally produced autocrine and paracrine factors are also involved in spermatogenesis, in particular at the level of cell junctions. These cell junctions at the Sertoli–Sertoli and Sertoli–germ cell interface are crucial for coordinating different events of spermatogenesis by sending signals back-and-forth between Sertoli and germ cells, in order to precisely regulate spermatogonial cell renewal by mitosis, cell cycle progression, meiosis, spermiogenesis, germ cell movement across the epithelium, spermiation and germ cell apoptosis. In this minireview, we provide an update on these latest findings for an emerging new concept regarding the presence of a local "apical ectoplasmic specialization–blood–testis barrier–hemidesmosome/basement membrane" functional axis that regulates the events of spermiation and blood–testis barrier (BTB) restructuring via paracrine/autocrine factors and polarity proteins produced locally in the seminiferous epithelium. These findings provide a new window of research for investigators in the field to tackle the functional regulation of spermatogenesis.

Contents

1. Introduction

The seminiferous tubule is the functional unit in the testis that produces spermatozoa (haploid, 1*n*) from spermatogonia (diploid, 2*n*) during spermatogenesis. Spermatogenesis takes place in the seminiferous epithelium of the seminiferous tubule, which is com-

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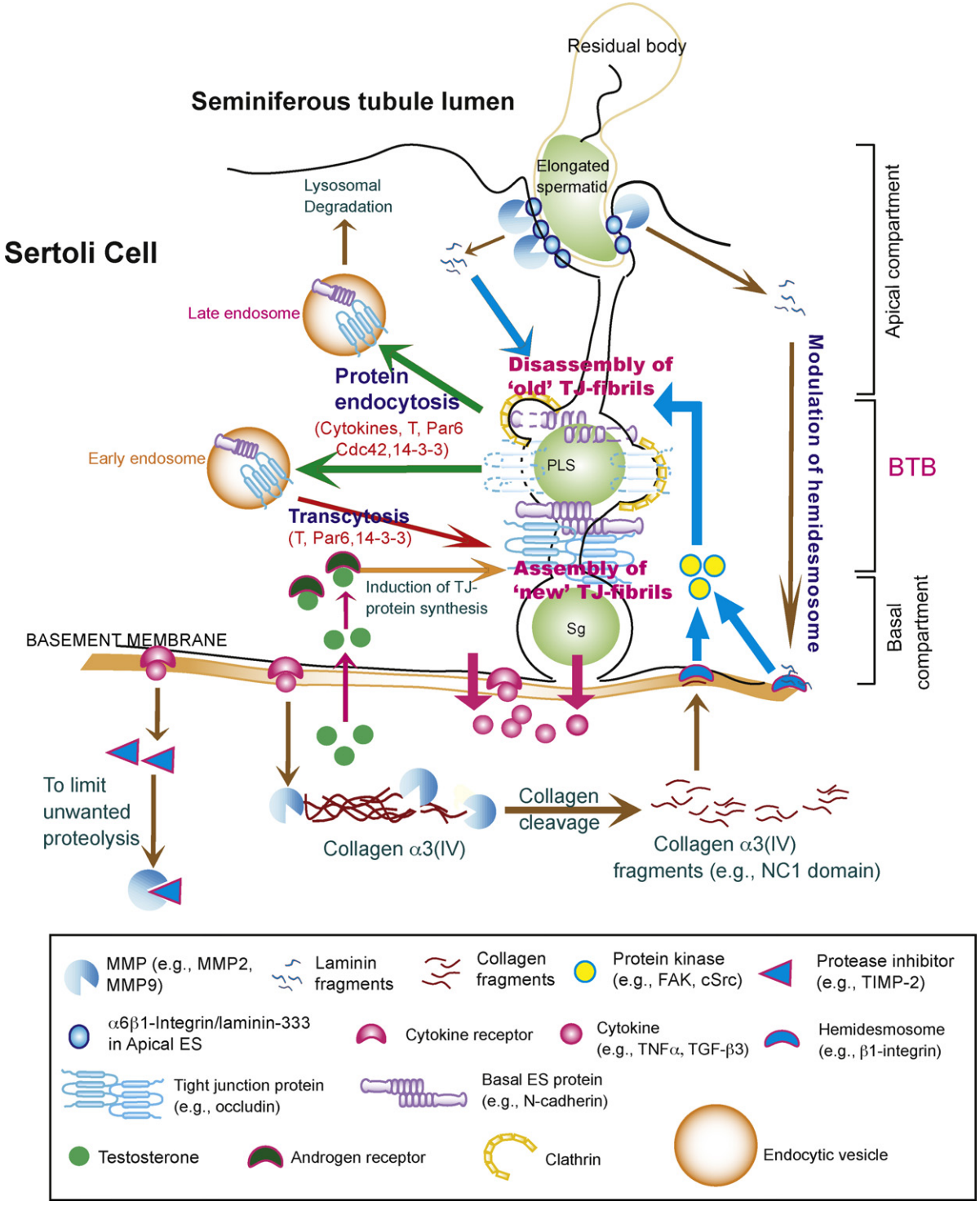

Fig. 1. A schematic drawing illustrating the local "apical ES–BTB–basement membrane/hemidesmosome" regulatory axis to coordinate the events of spermiation and BTB restructuring that occur simultaneously at stage VIII of the seminiferous epithelial cycle of spermatogenesis in the seminiferous epithelium of adult rat testes. At spermiation, the α6β1-integrin/laminin-333 adhesion complex at the apical ES is disrupted, likely via the action of MMP-2 at the site, with the generation of the biologically active laminin fragments. These fragments were shown to induce BTB disruption *directly*, such as by reducing the steady-state levels of integral membrane proteins at the BTB. Additionally, cytokines (e.g., TNFα, TGF-β2, and TGF-β3) and testosterone (T) were also shown to induce protein endocytosis at the BTB. The combination of these events 'de-stabilizes' the BTB, facilitating the transit of primary preleptotene spermatocytes at the BTB. Furthermore, these laminin fragments can also disrupt the BTB *indirectly* via effects on the hemidesmosome, such as by reducing the β1-integrin level in the hemidesmosome. This, in turn, further 'de-stabilizes' the BTB integrity. Additionally, biologically active collagen fragments, such as the NC1 domain, can also 'de-stabilizes' the BTB, working in concert with the laminin fragment, perhaps via FAK and c-Src at the site. Equally important, testosterone, which is known to promote BTB integrity, can enhance *de novo* synthesis of TJ-proteins (e.g., occludin), as well as promote transcytosis of endocytosed proteins from 'old' TJ-fibrils above the primary spermatocyte in transit at the BTB to 'new' TJ-fibrils beneath the spermatocyte. Furthermore, as discussed in the text, TNFα, was also shown to induce androgen receptor expression (see text for detail). Thus, TNFα can promote the assembly of new TJ-fibrils behind a primary spermatocyte in transit while it perturbs the 'old' TJ-fibrils above the migrating germ cell. In short, this novel functional axis in the seminiferous epithelium coordinates the events of spermiation and BTB restructuring which takes place concurrently at stage VIII of the epithelial cycle. It is anticipated that this model will be updated in the years to come as additional findings become available in the field. This model, however, serves as a reference for investigators in the field. Sg, spermatogonium; PLS, primary preleptotene spermatocyte.

posed of four distinctive phases: mitosis, meiosis, spermiogenesis, and spermiation that occur during the 14 stages of the seminiferous epithelial cycle of spermatogenesis in rats, 12 stages in mice, and 6 stages in men. The seminiferous epithelium is composed of germ cells and the Sertoli cell in which Sertoli cells lay adjacent to the tunica propria with physical contact with the basement membrane (see Fig. 1). Spermatogenesis is supported by the pituitary hormone follicle stimulating hormone (FSH) and the male sex hormone testosterone produced by Leydig cells in the interstitium with the FSH receptor and the androgen receptor being restricted to Sertoli cells in the seminiferous epithelium (for reviews, see de Kretser and Kerr, 1988; Sharpe, 1994; Mruk and Cheng, 2004; Walker, 2009). In short, the Sertoli cell in the seminiferous epithelium is the target for both FSH and testosterone, illustrating its crucial role in supporting germ cell maturation throughout spermatogenesis (Sharpe, 1994; Walker, 2009). Besides, estrogens also play an important role in different aspects of germ cell development, such as in apoptosis (Shaha, 2008). Recent studies have demonstrated that cytochrome P450 aromatase (P450arom), which irreversibly converts androgens into estrogens, and estrogen receptors (e.g., ERβ), which mediate the action of estrogens, are found in germ cells including spermatocytes, round spermatids, and elongated spermatids, as well as in Leydig and Sertoli cells (for a review, see Carreau, 2008). These findings thus illustrate that cells in the seminiferous epithelium are capable of producing estrogens from testosterone to regulate spermatogenesis (for reviews, see Shaha, 2008; Carreau, 2008; Hess, 2003).

The seminiferous epithelium in the mammalian testis is segregated into the basal and the apical (adluminal) compartment by the blood–testis barrier (BTB) which is created by adjacent Sertoli cells via co-existing tight junction (TJ), basal ectoplasmic specialization [a testis-specific atypical adherens junction (AJ) type], desmosome-like junction and gap junction (for a review, see Cheng and Mruk, 2002) (see Fig. 1). Mitosis occurs in type A spermatogonia residing in the basal compartment to produce additional germ cells, some of which differentiate into type B spermatogonia and primary preleptotene spermatocytes (de Kretser and Kerr, 1988; Sharpe, 1994). Preleptotene spermatocytes are the germ cells in transit at the BTB at stages VIII–IX of the seminiferous epithelial cycle in rats while differentiating into leptotene and zygotene spermatocytes. Once primary spermatocytes enter the apical compartment, they differentiate into diplotene spermatocytes and undergo diakinesis at stages X–XIII. These spermatocytes then enter metaphase I to undergo meiosis I, to be followed immediately by meiosis II which produces haploid spermatids at stage XIV of the epithelial cycle in rat testes (Parvinen, 1982; Hess and de Franca, 2008). Thus, there are extensive restructuring of cell junctions at the Sertoli–Sertoli cell and Sertoli–spermatocyte interface to facilitate the transit of primary spermatocytes at stages VIII–IX of the epithelial cycle. Once spermatids are formed, they undergo an extensive phase of developmental changes known as spermiogenesis, which is typified by the condensation of the genetic material to form the nucleus in the spermatid head, the formation of the acrosome above the spermatid head, elongation of the tail, and the packaging of the mitochondria into the mid-piece. Spermatids can be classified into one of 19 steps in rats depending on the phase of their development (de Kretser and Kerr, 1988; Parvinen, 1982). At stage VIII of the epithelial cycle, fully developed spermatids (i.e., spermatozoa) undergo spermiation, thus, spermatozoa detach from the seminiferous epithelium, entering the tubular lumen for further maturation in the epididymis. This thus involves disruption of the cell adhesion complex at the elongated spermatid–Sertoli cell interface.

While these morphological changes during spermatogenesis have been known for almost six decades (LeBlond and Clermont, 1952; Clermont, 1972; Clermont et al., 1993), the molecular and biochemical mechanism(s) that regulate and/or coordinate these junction restructuring events, namely BTB restructuring and spermiation taking place at opposite ends of the Sertoli cell epithelium, remain largely unexplored and unknown until recently. In this short review, we attempt to provide a critical discussion based on recent findings in the field regarding the regulation of these events in the microenvironment of the seminiferous epithelium. For instance, biologically active laminin peptides generated locally at the apical ectoplasmic specialization (apical ES) during spermiation can regulate BTB restructuring (Yan et al., 2008a), and coordinate these two seemingly unrelated events in the seminiferous epithelium occurring simultaneously at stage VIII of the epithelial cycle (Fig. 1). We also discuss recent findings that components of the polarity protein complexes (e.g., Par3, Par6, and 14-3-3 also known as Par5) at the BTB (Wong et al., 2008a, 2009) and proteins (e.g., β1-integrin) at the hemidesmosome (Yan et al., 2008a) also play a crucial role to maintain the homeostasis of the BTB during spermatogenesis (Fig. 1). Taken collectively, these data illustrate the presence of an apical ES–BTB–hemidesmosome functional axis to regulate local cellular events in the microenvironment of the seminiferous epithelium during spermatogenesis (Fig. 1). These findings also illustrate several potential targets that can be manipulated to induce infertility and to understand the mechanism(s) by which environmental toxicants induce reproductive dysfunction in males.

2. Ectoplasmic specialization (ES)

ES is an atypical adherens junction (AJ) type uniquely found in the testis, which appears at the interface between Sertoli cells and step 8 spermatids during spermiogenesis known as apical ES (for recent reviews, see Yan et al., 2007, 2008b; Wong et al., 2008b). Once apical ES appears, it is the *only* anchoring device present between these cells, and it persists through step 19 spermatids until spermiation in rat testes (for a review, see Yan et al., 2007). However, ES is also found between Sertoli cells at the BTB, *co-existing* with TJ, gap junction and desmosome-like junction to constitute the BTB (Yan et al., 2008b). Ultrastructurally, ES is typified by the presence of actin filament bundles sandwiched in between cisternae of endoplasmic reticulum and the plasma membrane of the Sertoli cell with this feature being limited only to the Sertoli cell side at the apical ES, but these structures are present on both sides of the two adjacent Sertoli cells at the basal ES at the BTB. Some of the known adhesion protein complexes and their peripheral adaptors at the apical ES and basal ES are cadherin/catenin, and nectin/afadin (Mruk and Cheng, 2008). The best studied cell adhesion complex at the apical ES, however, is the laminin 333-α6β1-integrin in the rat testes wherein laminin α3β3γ3 chains are restricted to the elongating/elongated spermatids (Yan and Cheng, 2006) with the α6β1-integrin residing on Sertoli cells (Palombi et al., 1992; Salanova et al., 1998). Studies by immunohistochemistry and dual-labeled immunofluorescence analysis have demonstrated that laminin α3 and γ3 chains co-localized to the same site at the apical ES, most predominantly expressed at stage VII–VIII tubules prior to spermiation (Yan and Cheng, 2006). This pattern of stage-specific localization of the laminin chains in the seminiferous epithelium of adult rat testes coincided with MMP-2 as earlier reported (Siu and Cheng, 2004a), implicating MMP-2 can proteolytically cleave the laminins at the apical ES at spermiation (Fig. 1).

3. The blood–testis barrier (BTB)

The BTB is an important ultrastructure in the seminiferous epithelium of mammalian testes. In rats, the BTB forms at ~age

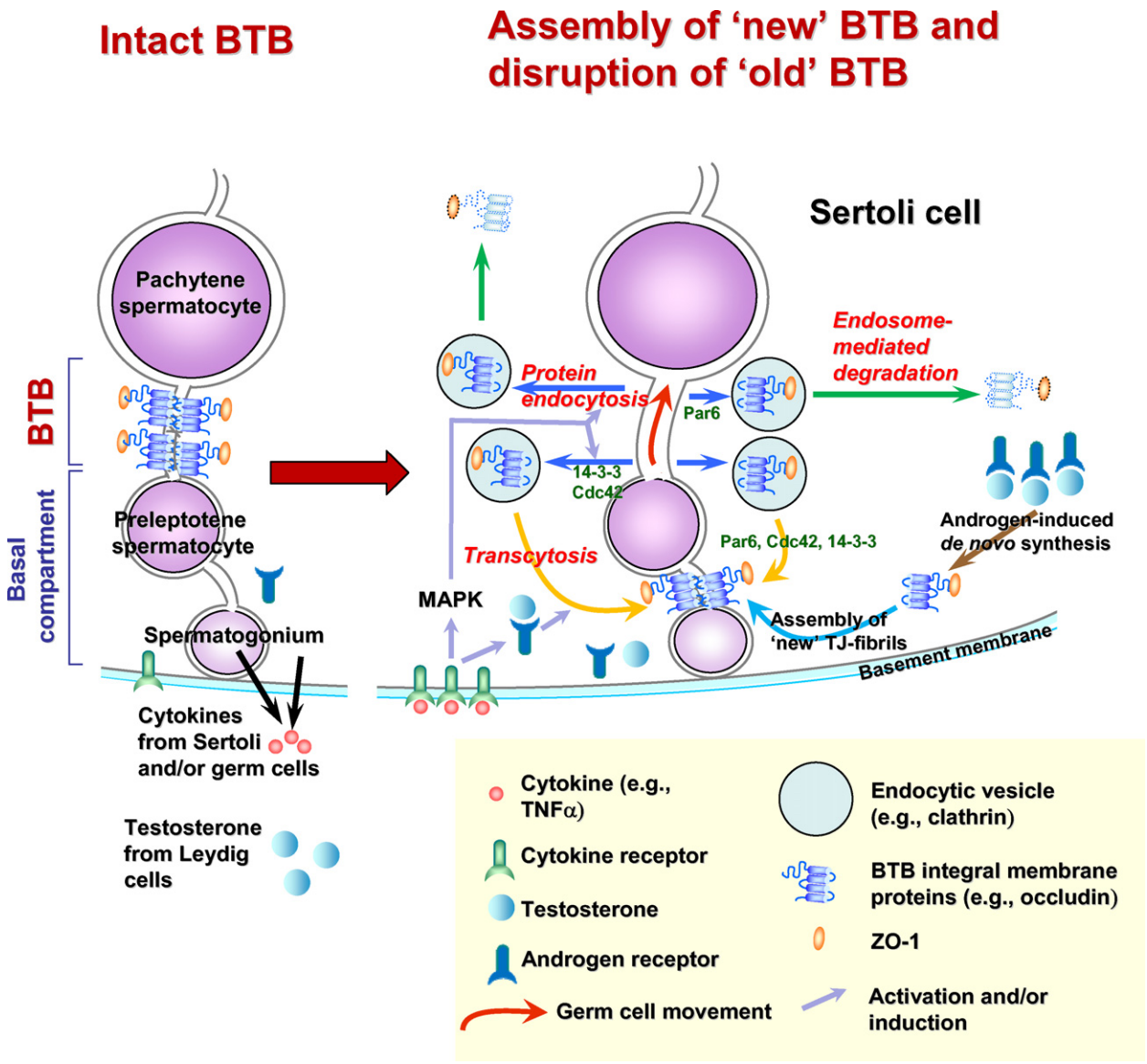

Fig. 2. A local regulatory mechanism to facilitate the transit of primary preleptotene spermatocytes at the BTB while maintaining its immunological barrier function. As discussed in detail in the text, this model illustrates the coordinated effects of cytokines (e.g., TNFα, TGF-β2, TGF-β3) and testosterone on junction dynamics in the testis, involving polarity proteins (e.g., Par3, Par6, 14-3-3, Cdc42). This model also illustrates the intricate interactions of cytokines, testosterone and polarity proteins on protein endocytosis, endosome-mediated degradation and transcytosis (recycling) and *de novo* synthesis to facilitate the transit of primary preleptotene spermatocytes at the BTB while maintaining the immunological barrier function.

15–18 days post-partum and is created by adjacent Sertoli cells near the basement membrane (Mruk and Cheng, 2008). By electron microscopy, the BTB is composed of TJ, basal ES, gap junction and desmosome-like junction, and it forms one of the tightest blood–tissue barriers in mammals (Setchell, 2008; Vogl et al., 2008; Yan et al., 2008c). Some of the known adhesion protein complexes that constitute the BTB are shown in Figs. 1 and 2, such as occldin-ZO-1, claudin-ZO-1, JAM-A-ZO-1, N-cadherin-ß-catenin, and nectin-afadin. Recent studies have shown that androgens (e.g., testosterone) are crucial to maintain the integrity of the BTB (Meng et al., 2005; Wang et al., 2006; Janecki et al., 1991; Chung and Cheng, 2001). For instance, testosterone was shown to facilitate the reassembly of the BTB following exposure of the Sertoli cell BTB to cadmium (Siu et al., 2009a), an environmental toxicant known to induce testicular injury via its rapid and disruptive effects on the BTB (Setchell and Waites, 1970) by causing disruption of the TJ-fibrils (Hew et al., 1993) (for a review, see Siu et al., 2009b). Recent studies have also demonstrated that the occludin/ZO-1/FAK is a crucial regulatory complex at the BTB (Siu et al., 2009c), and a loss of FAK function within this complex by RNAi using FAK-specific siRNA duplexes in Sertoli cells cultured *in vitro* with an established TJ-barrier can render the BTB insusceptible to cadmium-induced disruption (Siu et al., 2009a). Additionally, a disruption of the gap junction function by silencing connexin 43 *and* plakophilin-2 (a novel protein complex at the BTB) *simultaneously*, but not connexin 43 *alone*, by RNAi can also perturb protein distribution at the Sertoli–Sertoli cell interface, such as the occludin-ZO-1 protein complex, which moved away from the cell surface and into the cytosol, transiently disrupting the Sertoli cell TJ-permeability barrier when assessed by transepithelial electrical resistance (TER) across the Sertoli cell epithelium (Li et al., 2009). This recent finding illustrates not only the significance of the connexin 43/plakophilin-2 protein complex at the BTB, it also demonstrates the physiological significance of the *co-existing* junction types at the BTB. For instance, it is likely that the gap junction and desmosome-like junction provide the cross-talk to coordinate

the function of multiple junctions at the BTB to effectively safeguard the immunological barrier function during the transit of primary spermatocytes at the BTB during the seminiferous epithelial cycle, such as at stages VIII–IX.

4. Basement membrane

In mammalian testes, the basement membrane is a modified form of extracellular matrix, also called the basal lamina, that is known to regulate Sertoli cell function (for a review, see Dym, 1994). The basement membrane is composed of two major building blocks: type IV collagen and laminins (for a review, see Siu and Cheng, 2004b). It was shown that the inclusion of an anti-collagen antibody to Sertoli cells cultured *in vitro* with an established TJ-barrier could reversibly perturb transepithelial electrical resistance (TER) across the cell epithelium (Siu et al., 2003a), illustrating an inhibition of the collagen function (such as scaffold) can perturb the Sertoli cell BTB function. This finding also illustrates the presence of functional cross-talk between the basement membrane and the Sertoli cell in the seminiferous epithelium. While the basement membrane also maintains a pool of proteases, protease inhibitors, cytokines and growth factors, many of which are known regulators of Sertoli and germ cell functions, such as BTB integrity and cell cycle progression, very few studies are found in the literature to define the role(s) of basement membrane in spermatogenesis. In one of these few studies, TNFα was shown to induce Sertoli cell matrix metalloprotease-9 (MMP-9) production and promote the activation pro-MMP-9 (Siu et al., 2003a). As the collagen α3(IV) chain is a putative substrate of MMP-9, and fragments of the collagen chain (e.g., its non-collagenous domain 1, NC1 domain) are biologically active peptides that are known to regulate multiple biological events including cell movement at the cell–matrix focal adhesion complex (also known as focal contact) (for a review, see Siu and Cheng, 2004b). We speculated that TNFα in the basement membrane can regulate Sertoli cell BTB function via its effects on MMP-9, which can generate biologically active fragments (e.g., NC1 domain) from collagen α3 (IV) chain to regulate Sertoli cell TJ-permeability barrier (Fig. 1). This is an area of research that should be addressed in future functional studies.

5. Hemidesmosome

While the focal contact (a cell–matrix actin-based anchoring junction type, also known as focal adhesion complex) is not found in the testis, the hemidesmosome (a cell–matrix intermediate filament-based anchoring junction) is readily detected in the testis by electron microscopy (Mruk and Cheng, 2004; Siu and Cheng, 2004b). However, its biochemical composition in the testis remains unexplored. A recent study has shown that laminin α2 chain and β1-integrin are two putative components of the hemidesmosome in the rat testis (Yan et al., 2008a). Moreover, a disruption of the β1-integrin function by RNAi at the hemidesmosome in Sertoli cells cultured *in vitro* appears to perturb the Sertoli cell TJ-permeability barrier (Yan et al., 2008a), illustrating that a blockade of hemidesmosome function can perturb cross-talk between the BTB and hemidesmosome in the testis. In fact, studies in cancer biology have supported a functional axis between hemidesmosomes and blood–tissue barriers. For instance, during TJ disruption and a loss of cell polarity during oncogenesis, an increase in interactions between TJ-associated proteins (e.g., ErbB, an epidermal growth factor receptor variant) and α6β4-integrin in the hemidesmosome was detected. This 'newly' formed protein complex, in turn, served as a docking platform for signal transduction to promote cell proliferation (Carraway and KL, 2007). Much research is needed to explore the functional significance of hemidesmosome in spermatogenesis, in particular its cross-talk with the BTB and perhaps apical ES during the seminiferous epithelial cycle.

6. The apical ES–BTB–hemidesmosome functional axis

As discussed in Section 2, biologically active laminin fragments are likely generated at spermiation via the action of MMP-2 on laminin chains at the apical ES (Yan et al., 2008a; Siu and Cheng, 2004a) (Fig. 1). Indeed, recombinant protein fragments of both laminin β3 (e.g., domain I) and γ3 chains (e.g., domain IV) were capable of perturbing the Sertoli cell TJ-permeability barrier and/or the steady-state levels of BTB-associated proteins occludin and JAM-A, and hemidesmosome-associated protein β1-integrin (Yan et al., 2008a). Additionally, these findings were validated by overexpression of laminin γ3 domain IV or laminin β3 domain I in primary Sertoli cells cultured *in vitro* with an established TJ-barrier that mimicked the BTB *in vivo* (Yan et al., 2008a). These findings illustrate the presence of a functional axis between the apical ES and BTB via the production of an autocrine-like laminin peptide fragment at the apical ES at spermiation near the luminal edge of the seminiferous epithelium, which can *directly* modulate the BTB function near the basement membrane (Fig. 1). Since the expression of β1-integrin, a hemidesmosome-associated protein, in these primary Sertoli cell cultures with a functional TJ-barrier was shown to be inhibited by transient overexpression of laminin fragments or following the inclusion of recombinant proteins in the media, these findings suggest the likely presence of a functional loop between the apical ES/BTB and the hemidesmosome, and that the laminin peptide fragment can also *indirectly* modulate the BTB function. Indeed, a knock-down of β1-integrin by using specific siRNA duplexes versus non-targeting control siRNA duplexes has shown that a disruption of the hemidesmosome function leads to a transient disruption of the Sertoli cell barrier function (Yan et al., 2008a). We thus propose the presence of a functional apical ES–BTB–hemidesmosome axis in the seminiferous epithelium that can coordinate and regulate the two cellular events namely apical ES disruption at spermiation and BTB restructuring to facilitate the transit of primary preleptotene spermatocytes that occur at the opposite ends of the seminiferous epithelium at stage VIII of the epithelial cycle (Fig. 1).

7. Cytokines and testosterone in junction restructuring in the testis

Cytokines, such as TNFα, TGF-β2, and TGF-β3, and testosterone are important regulators of testicular functions including junction dynamics in the testis (for reviews, see Mruk and Cheng, 2004; Siu and Cheng, 2004b; Skinner, 1993; O'Bryan and Hedger, 2008). In this section, we provide brief background information on these molecules and summarize recent findings in the field pertinent to their role in regulating spermatogenesis in particular junction dynamics in the testis (see Fig. 2).

7.1. TNFα (tumor necrosis factor-α)

TNFα was initially identified as a cytokine produced by endotoxin-stimulated macrophages that induced necrosis of transplanted tumors (Carswell et al., 1975). TNFα (~50 kDa) is a homotrimer consisting of three identical subunits of ~17 kDa each. In the mammalian testis, such as in rodents, TNFα is a product of interstitial macrophages, round and elongating spermatids, pachytene spermatocytes (De et al., 1993) and Sertoli cells (Siu et al., 2003a). TNFα binds to either one of its two receptors: TNFR1 (p55, CD120a) and TNFR2 (p75, CD120b), which are mostly restricted to Sertoli and Leydig cells in the testis (but apoptotic germ cells

express TNRF1). TNFα exerts its biological effects by regulating inflammatory responses, such as stimulating IL-1 and IL-6 production (Cerami, 1992). It is noted that the expression of TNFα receptor by Sertoli cells is induced by FSH (Mauduit et al., 1996). TNFα is also known to induce cell death (e.g., germ cell apoptosis (Theas et al., 2008)) via its binding to TNFR1, and its subsequent interaction with the TNFR-associated death domain protein (TRADD) or the Fas-associated death domain protein (FADD), which is followed by an activation of the caspase-dependent apoptotic pathway (e.g., procaspase 8) (Hsu et al., 1996) or the mitochondrial pathway such as Bid and Bax (Pei et al., 2007). Indeed, TNFR1-positive germ cells are apoptotic, and TNFα can also trigger germ cell apoptosis by acting together with the local cell death regulatory systems, such as the Fas–Fas ligand system (Riccioli et al., 2000). The binding of TNFα to TNFR1 can induce a pro-inflammatory response (e.g., cell proliferation) by activating NF-κB and possibly p38 MAPK in multiple cell epithelia (O'Bryan and Hedger, 2008; Idriss and Naismith, 2000). TNFα is also an inhibitor of Leydig cell steroidogenesis via the NFκB signaling pathway (Hong et al., 2004) by blocking the expression and/or protein production of Leydig cell steroidogenic enzymes and/or proteins, such as P450scc, P450c17, 3β-hydroxysteroid dehydrogenase (Li et al., 1995; Xiong and Hales, 1993, 1994) and steroidogenic acute regulatory protein (StAR) (Mauduit et al., 1998). However, TNFα can also stimulate Sertoli cell androgen receptor expression by up-regulating NFκB (Delfino et al., 2003). Additionally, TNFα enhances the expression of plasminogen activator inhibitor-1 (PAI1) by peritubular myoid cells in the testis (Le Magueresse-Battistoni et al., 1997), illustrating its involvement in the homeostasis of proteolytic events (e.g., phagocytic reabsorption and proteolytic degradation of residual bodies by Sertoli cells at spermiation) in the seminiferous epithelium during spermatogenesis. Recent studies have also demonstrated that TNFα perturbs the blood–retinal barrier, blood–intestine barrier, and Sertoli cell BTB (for a review, see Siu and Cheng, 2004b). TNFα was shown to disrupt the Sertoli cell BTB integrity by first activating MMP-9 in the basement membrane, which, in turn, cleaved the collagen α3(V) chain (a major component of the basement membrane), perturbing the scaffolding function of the basal lamina, leading to BTB disruption (Siu et al., 2003a). Administration of TNFα to adult rat testes *in vivo* was shown to induce *reversible* BTB disruption (Li et al., 2006), confirming its disruptive effects on the Sertoli cell TJ-permeability barrier *in vitro* (Siu et al., 2003a). In short, TNFα has multiple biological effects on somatic cells (e.g., Sertoli cells) and/or germ cells in the testis, as well as other organs. Thus, its stimulatory, inhibitory, pro-apoptotic, pro-inflammatory, or destructive effects in cell epithelia depend on the receptor subtype engaged, and the expression and/or presence of specific adaptors or target proteins within each specific cell type.

7.2. Transforming growth factor-β2 and -β3 (TGF-β2 and -β3)

TGF-β1, -β2 and -β3 are homodimers of ~25 kDa consisting of two identical subunits of ~12 kDa. Their receptors (type I, TβRI, 55 kDa; and type II, TβRII, 85 kDa) are found in the testes and they are crucial to spermatogenesis (for reviews, see Lui et al., 2003a; Loveland et al., 2007; Guazzone et al., 2009). In adult testes, TGF-βs are expressed in early spermatids, pachytene spermatocytes, Sertoli cells, peritubular myoid cells and Leydig cells (Lui et al., 2003a; Guazzone et al., 2009). However, TGF-β2 and -β3, but not TGF-β1, were shown to have disruptive but reversible effects on the Sertoli cell TJ-permeability barrier function, apparently mediated via the p38 MAPK signaling pathway (Lui et al., 2001, 2003b). Subsequent *in vivo* studies have confirmed the earlier *in vitro* results illustrating the *reversible* but disruptive effects of TGF-β3 on the BTB integrity are mediated via the p38 MAPK signaling pathway (Lui et al., 2003c; Wong et al., 2004; Xia et al., 2009). Moreover, TGF-β2 or -β3 induced its disruptive effects on the BTB integrity by enhancing the kinetics of clathrin-mediated internalization of occludin, JAM-A and/or N-cadherin at the BTB using techniques of biotinylation and endocytosis assay (Xia et al., 2009; Yan et al., 2008d). More important, TGF-β3 targeted the endocytosed proteins to an endosome-mediated intracellular degradation pathway, such that the endocytosed proteins (e.g., occludin) associated more extensively with Rab 9, a late endosome marker, thereby reducing the steady-state level of integral membrane proteins at the BTB and compromising the TJ-barrier integrity (Yan et al., 2008d).

7.3. Testosterone

The role of testosterone in regulating and maintaining spermatogenesis has been known for decades (Sharpe, 1994; Zirkin, 1998). For instance, the intratesticular testosterone level found in rete testis fluid and seminiferous tubule fluid in both men and rodents is 50–100-fold higher than those found in the systemic circulation (Turner et al., 1984; Jarow and Zirkin, 2005), which apparently is needed to maintain germ cell development and normal Sertoli cell physiology in the seminiferous epithelium. Even though the androgen receptor is mostly restricted to Sertoli cells in males (Griswold et al., 1995) to mediate androgen action, germ cells associate intimately with Sertoli cells in the seminiferous epithelium via specialized cell junctions (e.g., gap junctions) and cytoplasmic bridges (Fawcett, 1961) through which chemical signals, biological factors, and/or biomolecules induced by androgen can be transported back-and-forth between these cells. Besides genomic action, recent studies have shown that androgens can also exert their effects via non-genomic action without involving the androgen receptor by activating c-Src/ERK1/2 in the EGFR (epidermal growth factor receptor) signaling pathway (for a recent review, see Walker, 2009). It is known that testosterone is crucial in the maintenance of elongating/elongated spermatid adhesion in the testis, in particular the apical ES in adult testes (Beardsley and O'Donnell, 2003; Zhang et al., 2005; Xia et al., 2005), and possibly involves phosphorylated (activated)-FAK (Beardsley et al., 2006; Siu et al., 2003b). Also, testosterone is important in the maintenance of the BTB integrity in rodents (Meng et al., 2005; Wang et al., 2006). Recent studies have shown that testosterone, similar to cytokines (e.g., TGF-β2, TGF-β3 and TNFα), also enhance the endocytosis of integral membrane proteins at the BTB (e.g., occludin, N-cadherin). However, the endocytosed proteins are rapidly recycled back to the Sertoli cell surface, perhaps to a different cellular location via transcytosis (Yan et al., 2008d). Yet, cytokine-induced endocytosed proteins are targeted to undergo endosome-mediated degradation as illustrated by their increased association with the late endosome marker Rab9 (Yan et al., 2008d).

7.4. Coordinated effects of cytokines and testosterone to facilitate the transit of primary preleptotene spermatocytes at the BTB while maintaining the immunological barrier function

As discussed above, cytokines and testosterone have opposing effects on the integrity of the junctional complexes in the testes with cytokines (e.g., TNFα, TGF-β3) promoting BTB disruption (Siu et al., 2003a; Li et al., 2006; Lui et al., 2003c; Xia et al., 2009, 2006) and testosterone facilitating BTB assembly and/or maintenance (Meng et al., 2005; Wang et al., 2006), respectively. The findings recently reported (Xia et al., 2009; Yan et al., 2008d) and summarized above regarding the differential effects of cytokines (e.g., TGF-β2, TGF-β3, TNFα) and testosterone on protein endocytosis, recycling and endosome-mediated degradation in Sertoli cell BTB are significant. Collectively, these findings illustrate that cytokines are likely working in concert with testosterone to assem-

ble "new" TJ-fibrils behind a primary preleptotene spermatocyte in transit at the BTB via the action of testosterone or TNFα-induced androgen receptor production to: (i) augment androgen action by inducing de novo synthesis of BTB integral membrane proteins (Chung and Cheng, 2001) and/or (ii) promote transcytosis (Yan et al., 2008d) by relocating integral membrane proteins from the "old" to the "new" BTB site; whereas cytokines (e.g., TGF-β2, TGF-β3, TNFα) induce the dissolution of the "old" TJ-fibrils via enhanced endocytosis and endosome-mediated degradation above the spermatocyte in transit (see Fig. 2). Using such an efficient mechanism, the immunological barrier can be maintained during the transit of preleptotene spermatocytes at the BTB at stage VIII of the epithelial cycle with the "new" BTB site being assembled *before* the "old" BTB site is broken down (Fig. 2). It is likely that polarity proteins, such as Par3, Par6, and 14-3-3 are involved in these events since these proteins were recently shown to be involved in protein endocytosis at the Sertoli cell BTB (Wong et al., 2008a, 2009) (Fig. 2). It is likely that testosterone- and/or cytokine-induced endocytosis and the subsequent recycling or endosome-mediated degradation is facilitated by these polarity proteins. For instance, it was shown that the transient knock-down of Par3 or Par6 in Sertoli cells cultured *in vitro* with an established TJ-permeability barrier that mimics the BTB *in vivo* caused a mislocalization of JAM-A and/or N-cadherin, the corresponding integral membrane protein at the TJ and basal ES at the BTB in rat testes, from the Sertoli cell surface and into the cytosol, inducing transient TJ-barrier disruption (Wong et al., 2008a). Subsequent studies have shown that this mislocalization is likely the result of an enhancement in protein endocytosis induced by 14-3-3 (Wong et al., 2009). These findings also illustrate that polarity proteins are working in concert with protein complexes at the BTB to modulate BTB restructuring during the seminiferous epithelial cycle of spermatogenesis.

8. Future perspectives

We have herein discussed the presence of a novel functional axis in the seminiferous epithelium of adult rat testes known as the apical ES–BTB–hemidesmosome axis or the apical ES–BTB–basement membrane axis (Fig. 1), which illustrates cross-talk between different cellular ultrastructures in the seminiferous epithelium to coordinate different cellular events during the epithelial cycle. Obviously, there are many open questions that remain to be addressed in future studies. For instance, what is the precise biochemical composition of the hemidesmosome in adult testes besides laminin α2 and β1-integrin? What are the downstream signaling events of β1-integrin at the hemidesmosome following its activation by the biologically active laminin fragments? Is there any cross-talk between β1-integrin found at the apical ES and at the hemidesmosome in the testis? What is the target signaling protein(s) at the BTB that is being activated by the biologically active laminin fragments; is it FAK in the recently identified occludin/ZO-1/FAK protein complex (Siu et al., 2009a,c) since FAK is a known mediator of integrin-based signaling events (Boutros et al., 2008)? What is the physiological and/or functional relationship between cytokines and testosterone that modulate BTB restructuring? Can cytokines modulate androgen receptor expression by Sertoli cells? Finally, what is the biological mechanism(s) that generates biologically active laminin and/or collagen fragments to regulate BTB function *directly* and/or *indirectly* via the hemidesmosome? The two models depicted herein (see Figs. 1 and 2) will serve as the basis for many future functional studies, and many of the above open questions can now be tackled. It is obvious that recent advances in cell and molecular biology and biochemistry are yielding unprecedented opportunities for investigators in the field for years to come.

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