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The Blood-Testis Barrier and Its Implications for Male Contraception

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Abstract——**The blood-testis barrier (BTB) is one of the tightest blood-tissue barriers in the mammalian body. It divides the seminiferous epithelium into the basal and the apical (adluminal) compartments. Meiosis I and II, spermiogenesis, and spermiation all take place in a specialized microenvironment behind the BTB in the apical compartment, but spermatogonial renewal and differentiation and cell cycle progression up to the preleptotene spermatocyte stage take place outside of the BTB in the basal compartment of the epithelium. However, the BTB is not a static ultrastructure. Instead, it undergoes extensive restructuring during the seminiferous epithelial cycle of spermatogenesis at stage VIII to allow the transit of preleptotene spermatocytes at the BTB. Yet the immunological barrier conferred by the BTB cannot be compromised, even transiently, during the epithelial cycle to avoid the production of antibodies against meiotic and postmeiotic germ cells. Studies have demonstrated that some unlikely partners, namely adhesion protein complexes (e.g., occludin-ZO-1, N-cadherin-β-catenin, claudin-5-ZO-1), steroids (e.g., testosterone, estradiol-17β), nonreceptor protein kinases (e.g., focal adhesion kinase, c-Src, c-Yes), polarity proteins (e.g., PAR6, Cdc42, 14-3-3), endocytic vesicle proteins (e.g., clathrin, caveolin, dynamin 2), and actin regulatory proteins (e.g., Eps8, Arp2/3 complex), are working together, apparently under the overall influence of cytokines (e.g., transforming growth factor-β3, tumor necrosis factor-α, interleukin-1α). In short, a "new" BTB is created behind spermatocytes in transit while the "old" BTB above transiting cells undergoes timely degeneration, so that the immunological barrier can be maintained while spermatocytes are traversing the BTB. We also discuss recent findings regarding the molecular mechanisms by which environmental toxicants (e.g., cadmium, bisphenol A) induce testicular injury via their initial actions at the BTB to elicit subsequent damage to germ-cell adhesion, thereby leading to germ-cell loss, reduced sperm count, and male infertility or subfertility. Moreover, we also critically evaluate findings in the field regarding studies on drug transporters in the testis and discuss how these influx and efflux pumps regulate the entry of potential nonhormonal male contraceptives to the apical compartment to exert their effects. Collectively, these findings illustrate multiple potential targets are present at the BTB for innovative contraceptive development and for better delivery of drugs to alleviate toxicant-induced reproductive dysfunction in men.**

I. Introduction: Background and the Concept of the Blood-Testis Barrier

The blood-tissue barrier is a concept originally based on observations reported in the early twentieth century. When dyes were administered to laboratory animals, they failed to stain the testis and the brain (Ribbert, 1904; Bouffard, 1906; Goldmann, 1909). These findings thus led to the concept of the blood-testis barrier (BTB[1]) and the blood-brain barrier (BBB) (Fawcett et al., 1970; Setchell and Waites, 1975; Setchell, 2008; Easton, 2011). The term blood-testis barrier, also known as the Sertoli cell seminiferous epithelium barrier, however, was first used by Chiquoine (1964) in a study that examined the effects of cadmium toxicity as it related to testicular necrosis. However, the function of the BTB was not fully appreciated until the late 1960s, when it was reported that dyes that were capable of penetrating seminiferous tubules of prepubertal rats were excluded from tubules in adult rats (Kormano, 1967a,b, 1968). These earlier findings were followed by eminent investigations by Setchell and Waites (1975) and Setchell (2008), who collected fluids from different compartments in the testis (such as the rete testis, seminiferous tubule versus blood plasma and testicular lymph in rats and sheep) and demonstrated that there were significant differences in their fluid compositions, such as small hydrophilic organic compounds (e.g., inositol) and proteins, illustrating the presence of "restricted" communication between various fluid compartments in the testis (Setchell and Waites, 1975; Setchell, 2008). Subsequent eminent studies in the 1970s by Fawcett and Russell and their colleagues using electron microscopy further defined the ultrastructure of the BTB in the mammalian testis (Dym and Fawcett, 1970; Fawcett et al., 1970; Dym and Cavicchia, 1977; Russell and Peterson, 1985).

Most mammalian blood-tissue barriers, such as the BBB in the brain and the blood-retina barrier (BRB) in the eye, are constituted almost exclusively by the tight junction (TJ)-permeability barrier between endothelial cells of the small capillaries in the brain and supported in part by pericytes or perivascular macrophages (Hawkins and Davis, 2005; Easton, 2011; Paolinelli et al., 2011). Likewise, the BRB in the eye is constituted almost exclusively by the TJ barrier of retinal capillary endothelial cells (inner BRB) and supported by pericytes and retinal pigment epithelial cells (outer BRB) (Cunha-Vaz, 2004; Hosoya and Tachikawa, 2011). However, the BTB in mammalian testes, unlike other blood-tissue barriers, is constituted almost exclusively by specialized junctions between adjacent Sertoli cells near the basement membrane [a modified form of extracellular matrix (Dym, 1994; Siu and Cheng, 2004a, 2008)] in the seminiferous epithelium of the seminiferous tubule (Figs. 1, 2, and 3); and the seminiferous tubule is not penetrated by blood vessels, lymph vessels, or nerves. Instead, these are found in the interstitium between tubules (Setchell and Waites, 1975; Cheng and Mruk, 2010a) (Fig. 1). In rodents, the myoid cell layer in the tunica propria (Figs. 1 and 3) contributes significantly to the barrier function of the BTB because the passage of electron-opaque markers (e.g., lanthanum, thorium, colloidal carbon) was blocked by the myoid cell layer in ~85% of seminiferous tubules (Dym and Fawcett, 1970; Fawcett et al., 1970). Subsequent studies have shown that the myoid cell layer is less effective in restricting the penetration of electron-dense substances across tubules in the testes of primates (Dym, 1973) and therefore perhaps humans. It is noteworthy that the endothelial TJ barrier in microvessels found in the interstitium, however, contributes relatively little to BTB function in both rodents and primates (Dym and Fawcett, 1970; Dym and Cavicchia, 1977; Setchell, 2008; Cheng and Mruk, 2010a).

II. Spermatogenesis and the Blood-Testis Barrier

Spermatogenesis that takes place in the seminiferous epithelium (Fig. 2) is composed of a series of cellular events and can be divided as follows (Fig. 4): 1) spermatogonial renewal and proliferation via mitosis and differentiation; 2) cell cycle progression from type B spermatogonia to preleptotene spermatocytes, which takes place in the basal compartment, lying outside of the BTB; 3) cell cycle progression from zygotene and pachytene to diplotene spermatocytes, which is followed by meiosis I and II; 4) development of round spermatids to elongated spermatids, and then spermatozoa via sper-

[1]Abbreviations: ABC, ATP-binding cassette; AJ, adherens junction; AR, androgen receptor; Arp, actin-related protein; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; BPA, bisphenol A; BRB, blood-retina barrier; BTB, blood-testis barrier; CAR, coxsackievirus and adenovirus receptor; CDB-4022, [4*aRS*,5*RS*,9*bRS*]2-ethyl-2,3,4,4*a*,5,9*b*-hexahydro-8-iodo-7-methyl-5-[4-carbomethoxyphenyl]-1*H*-indeno-[1,2-*c*]-pyridine-hydrochloride; Cdc42, cell division cycle 42; c-Src, sarcoma-inducing gene of *Rous* sarcoma virus; Cx, connexin; c-Yes, a member of the Src kinase family, an oncogene identified in avian sarcoma, encoding a protein tyrosine kinase; DES, diethylstilbestrol; dpc, days postcoitus; Eps8, epidermal growth factor receptor pathway substrate 8; ES, ectoplasmic specialization; FAK, focal adhesion kinase; FITC, fluorescein isothiocyanate; FSH, follicle-stimulating hormone; GJ, gap junction; JAM, junctional adhesion molecule; JNK, c-Jun N-terminal kinase; KO, knockout; LH, luteinizing hormone; MAPK, mitogen-activated protein kinase; MDCK, Madin-Darby canine kidney; MDR, multidrug resistance protein; MMP-9, matrix metallopoteinase-9; MRP, multidrug resistance-related protein; MTM-R2, myotubularin-related protein 2; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cell; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OCTN, organic cation/carnitine transporter; P450arom, cytochrome P450 aromatase; PAR, partitioning-defective protein; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PTU, 6-propyl-2-thiouracil; RB, retinoblastoma protein; RNAi, RNA interference; SB202190, 4-[4-(4-fluorophenyl)-5-(4-pyridinyl)-1*H*-imidazol-2-yl]phenol; SH, Src homology; siRNA, small interfering RNA; SLC, solute carrier; SSC, spermatogonial stem cell; SU6656, 2,3-dihydro-*N*,*N*-dimethyl-2-oxo-3-[(4,5,6,7-tetrahydro-1*H*-indol-2-yl)methylene]-1*H*-indole-5-sulfonamide; TβR, transforming growth factor β receptor; TGF-β, transforming growth factor β; TJ, tight junction; TNF, tumor necrosis factor; TNFR, tumor necrosis factor α receptor; ZO-1, zonula occludens-1.

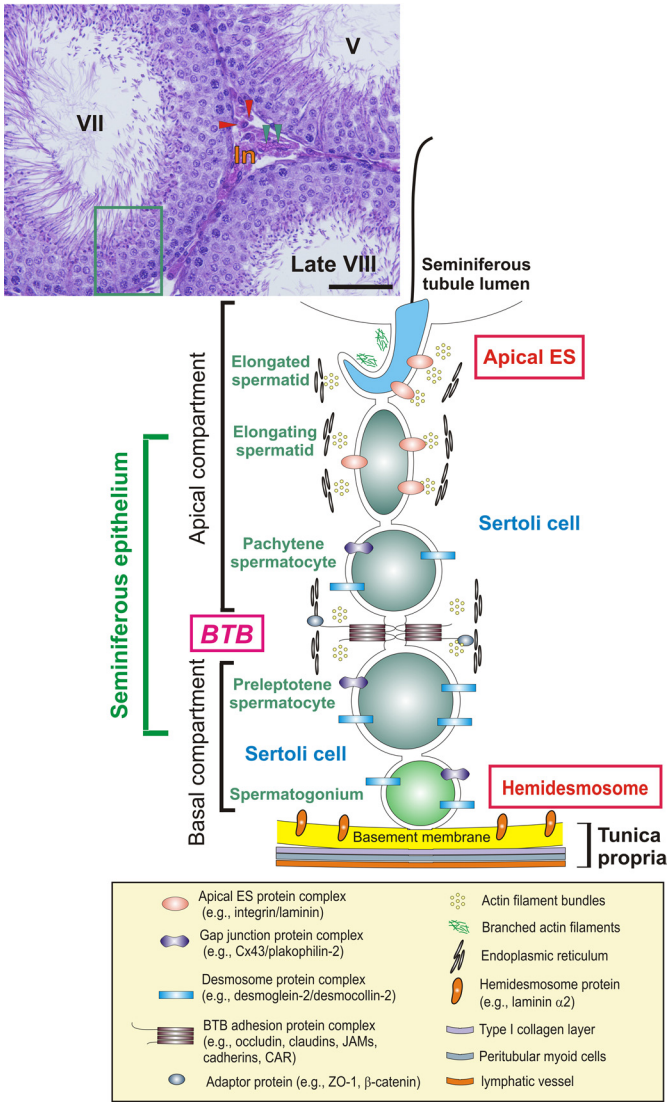

FIG. 1. The location of the BTB in the seminiferous epithelium of adult mammalian testes and its physiological relationship with developing germ cells during spermatogenesis. The micrograph is the cross-section of an adult rat testis showing three seminiferous tubules at stages V, VII, and late VIII of the seminiferous epithelial cycle (see Fig. 2 for detailed description of the epithelial cycle). The green boxed area is a section of the seminiferous epithelium that is magnified and represented by the schematic drawing shown below it, illustrating the intimate relationship between Sertoli and germ cells and the relative location of the BTB. Leydig cells (red arrowheads) that produce testosterone (via steroidogenesis) and a microvessel (e.g., erythrocytes inside the microvessel are denoted by two green arrowheads) found in the interstitial space (In) are noted. The BTB is located near the basement membrane in the tunica propria, which also segregates the epithelium into the basal and the apical (or adluminal) compartment. The BTB is constituted by coexisting TJ, basal ES, desmosome, and gap junction, and the ultrastructural features of the BTB as typified by the actin filament bundles sandwiched between the cisternae of endoplasmic reticulum and the plasma membranes of two apposing Sertoli cells (i.e., the basal ES) shown herein can be seen in the electron micrographs shown in Fig. 3. The apical ES also shares similar ultrastructural features with the basal ES, except that the actin filament bundles that are sandwiched between the cisternae of endoplasmic reticulum and the plasma membranes of the apposing Sertoli cell and elongating spermatids (step 8–19) are restricted *only* to the Sertoli cell (see also micrographs in Figs. 2 and 3). As discussed in the text, there is cross-talk between the apical ES, the BTB, and the hemidesmosome. These three ultrastructures also form a functional axis that regulates and coordinates junction-restructuring events during spermatogenesis. Scale bar, 60 μm.

miogenesis; and 5) spermiation, which all take place in the apical compartment behind the BTB. Spermatogenesis begins with differentiation of spermatogonial stem cells (SSC) residing within the spermatogonial stem cell niche, which is located in the basal compartment where several tubules converge and borders the interstitial tissue (de Rooij, 2009) to form A_{single} spermatogonia (Fig. 4). A_{single} spermatogonia were originally conceived to be the SSC in the testis (de Rooij and Russell, 2000; de Rooij, 2009). Subsequent studies, however, have shown that not all A_{single} spermatogonia are "true" SSC in rodent testes, and it was estimated that there are only ~2000 to 3000 SSC among the 35,000 A_{single} spermatogonia in each testis based on transplantation experiments (Nagano, 2003; Nakagawa et al., 2007) and morphological (Grisanti et al., 2009) analyses. In addition, A_{paired} spermatogonia were shown to be capable of switching back to become "true" SSC via dedifferentiation (Brawley and Matunis, 2004; Nakagawa et al., 2007). In short, an A_{single} spermatogonium will undergo 4 mitotic divisions to form a 16-cell chain of $A_{aligned}$ spermatogonia (Fig. 4), which will differentiate into A1 spermatogonia to be followed by 6 mitotic divisions to form 1024 preleptotene spermatocytes (Fig. 4). Preleptotene spermatocytes are the germ cells that are in transit at the BTB as clones connected by intercellular bridges (Fawcett, 1961; Miething, 2010), which will differentiate into zygotene and diplotene spermatocytes, to be followed by two meiotic divisions (meiosis I and II) to form 4096 haploid spermatids in mice and rats, theoretically (Fig. 4). However, because many of these germ cells undergo apoptosis, fewer than 25% will become spermatozoa (Billig et al., 1995; Shaha et al., 2010) via spermiogenesis to be released into the tubule lumen at spermiation (O'Donnell et al., 2011). Thus, the BTB provides a physical barrier to segregate the events of spermatogenesis, namely items 3 to 5, and allows these cellular events to take place in an immune-privileged site in the epithelium.

III. Structure and Functions of the Blood-Testis Barrier

A. Background and Unique Structural Features of the Blood-Testis Barrier

The BTB in mammalian testes, as noted above, is constituted by specialized junctions between adjacent Sertoli cells in the seminiferous epithelium near the basement membrane, away from the TJ barrier of the endothelial cells in the microvessels found in the interstitium (see Figs. 1 and 3). In the rat, the BTB begins to assemble by postnatal day ~15 to 16, and it is completed by postnatal day ~18 to 21 (Vitale et al., 1973; Bergmann and Dierichs, 1983; Russell et al., 1989; Toyama et al., 2001), coinciding with the time that Sertoli cells cease to divide (Orth, 1982). Instead of using TJ ultrastructures, which are the hallmark of the other blood-

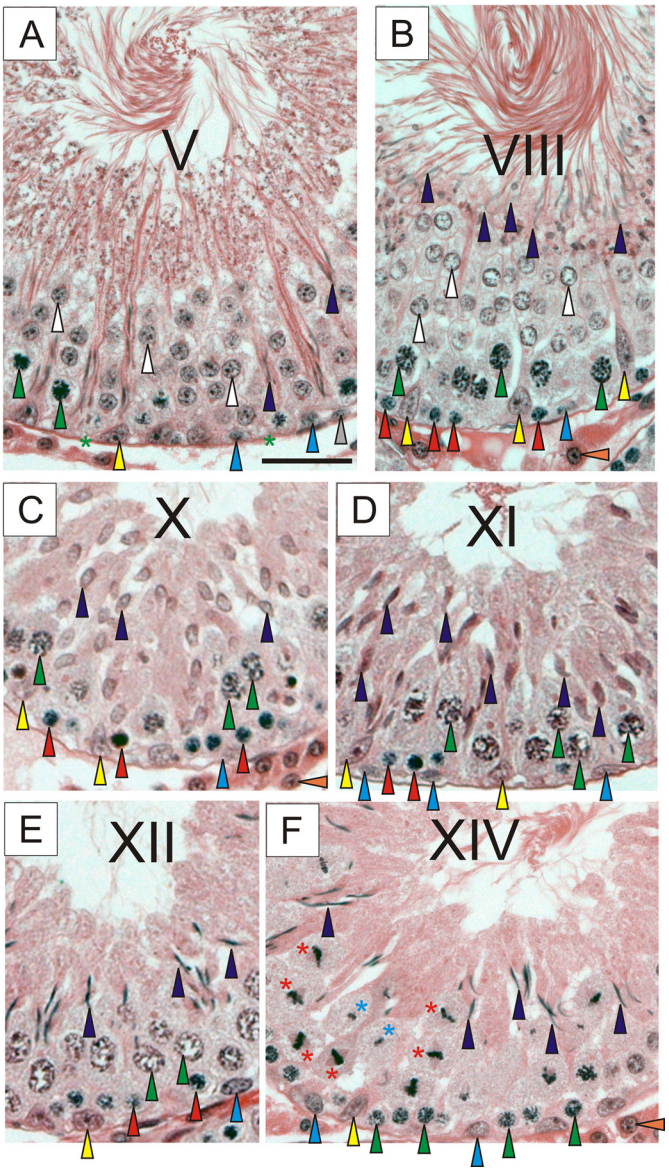

FIG. 2. Cross-sections of testes from adult rats illustrating some selected stages of the seminiferous epithelial cycle of spermatogenesis. The stages of the seminiferous epithelial cycle shown herein are tubules at stage V (A), VIII (B), X (C), XI (D), XII (E), and XIV (F). The sections herein are paraffin sections stained with hematoxylin and eosin. During spermatogenesis, the seminiferous epithelium (composed of Sertoli and germ cells) undergoes cyclic changes as the result of germ cell development, which is manifested by notable changes on the morphology and relative location of the spermatids (purple arrowheads, such as acrosome formation, elongation of the tail), round spermatids (white arrowheads) and spermatocytes (red and green arrowheads), and can be divided into 6, 12, and 14 stages in human, mouse, and rat, respectively (Leblond and Clermont, 1952; Parvinen, 1982; de Kretser and Kerr, 1988; Hess, 1990; Hess and de Franca, 2008). The duration of a complete seminiferous epithelial cycle takes ~8.6 days (Oakberg, 1956) and ~12.8 days (Clermont et al., 1959) to complete in mouse and rat, respectively, when a specific section of a tubule is monitored by stereomicroscopy to define changes from stages I to XII and I to XIV in these species. But because certain phases of the spermatogenesis, such as type A spermatogonial renewal and their subsequent differentiation, take longer time to complete, spermatogenesis in mouse, rat, and human was estimated to take 34 to 35, 48 to 53, and 64 days, respectively, to complete in studies using [^{3}H]thymidine or ^{32}P-thymidine incorporation (de Kretser and Kerr, 1988) (Table 2). In stage V tubule (A), the heads of elongating spermatids almost make contact with the Sertoli cell nucleus (yellow arrowheads) located near the basement membrane of the tunica propria (green asterisk). Yellow arrowhead, Sertoli cell; blue arrowhead, type B spermatogonium; gray arrowhead, A1 spermatogonium; green arrowhead, pachytene spermatocyte; white arrowhead, step 5 spermatid; purple arrowhead, step 17 spermatid. In stage VIII tubule (B), elongated spermatids line up at the luminal edge of the epithelium to prepare for sperm release at spermiation. Yellow arrowhead, Sertoli cell; blue arrowhead, A1, spermatogonium; red arrowhead, preleptotene spermatocyte; green arrowhead, pachytene spermatocyte; white arrowhead; step 8 spermatid; purple arrowhead, step 19 spermatid; orange arrowhead, Leydig cell in the interstitium. In stage X tubule (C), yellow arrowhead, Sertoli cell; blue arrowhead, A2 spermatogonium; red arrowhead, leptotene spermatocyte; green arrowhead, pachytene spermatocyte; purple arrowhead, step 10 spermatid; orange arrowhead, Leydig cell. In stage XI tubule (D), yellow arrowhead, Sertoli cell; blue arrowhead, A2 spermatogonium; red arrowhead, leptotene spermatocyte; green arrowhead, pachytene spermatocyte; purple arrowhead, step 11 spermatid. In stage XII tubule (E), yellow arrowhead, Sertoli cell; blue arrowhead, type A2 spermatogonium; red arrowhead, zygotene spermatocyte; green arrowhead, pachytene spermatocyte; purple arrowhead, step 12 spermatid. At stage XIV (F), meiosis I and II occur, and the two secondary spermatocytes that arise from meiosis I can be seen (red asterisks); also two step 1 spermatids that arise from telophase of meiosis II are also seen (blue asterisks). Yellow arrowhead, Sertoli cell; blue arrowhead, A3 spermatogonium; green arrowhead, early pachytene spermatocyte; purple arrowhead, step 14 spermatid; orange arrowhead, Leydig cell. Scale bar, 25 μm (A–F).

tissue barriers, such as the BBB and the BRB, to constitute the barrier *exclusively*, the BTB in the mammalian testis is constituted by *coexisting* TJ, basal ectoplasmic specialization [basal ES, a testis-specific *atypical* adherens junction (AJ) type], gap junction, and desmosome (Figs. 1 and 3; Table 1) (Gilula et al., 1976; Russell and Peterson, 1985; Pelletier and Byers, 1992; Pelletier, 2001; Cheng and Mruk, 2002, 2009a, 2010a; Mruk and Cheng, 2004b, 2011; Mruk et al., 2008; Vogl et al., 2008; Wong et al., 2008a; Mital et al., 2011). Some of the most distinctive and prominent ultrastructural features of the BTB are tightly packed actin-filament bundles that lie perpendicular to the plasma membrane. These are sandwiched between cisternae of the endoplasmic reticulum and the plasma membranes of apposing Sertoli cells known as the basal ES, and these ultrastructural features are found on both sides of adjacent Sertoli cells (Russell, 1993; Vogl et al., 2008; Cheng and Mruk, 2010a; Lie et al., 2010c) (Figs. 1 and 3). TJs, on the other hand, appearing as "kisses" between apposing Sertoli cell plasma membranes, are found between the basal ES, near the basement membrane (Fig. 3). It is noted that the ES was called "junctional specialization" when it was first identified to be a crucial structural component of the BTB (Flickinger and Fawcett, 1967) but renamed "ectoplasmic specialization" 10 years thereafter (Russell, 1977c). It is also noted that even though the BTB is one of the tightest blood-tissue barriers (Dym and Fawcett, 1970; Setchell and Waites, 1975; Cheng and Mruk, 2010a; Cheng et al., 2011a; Franca et al., 2011; Mital et al., 2011), it undergoes restructuring to facilitate the transit of preleptotene spermatocytes connected by intercellular bridges as "clones" (Fawcett, 1961; Miething, 2010) at stage VIII of the seminiferous epithelial cycle of spermatogenesis (Russell, 1977b).

Recent studies from multiple epithelia using cultured intestinal and renal epithelial cells and the small intes-

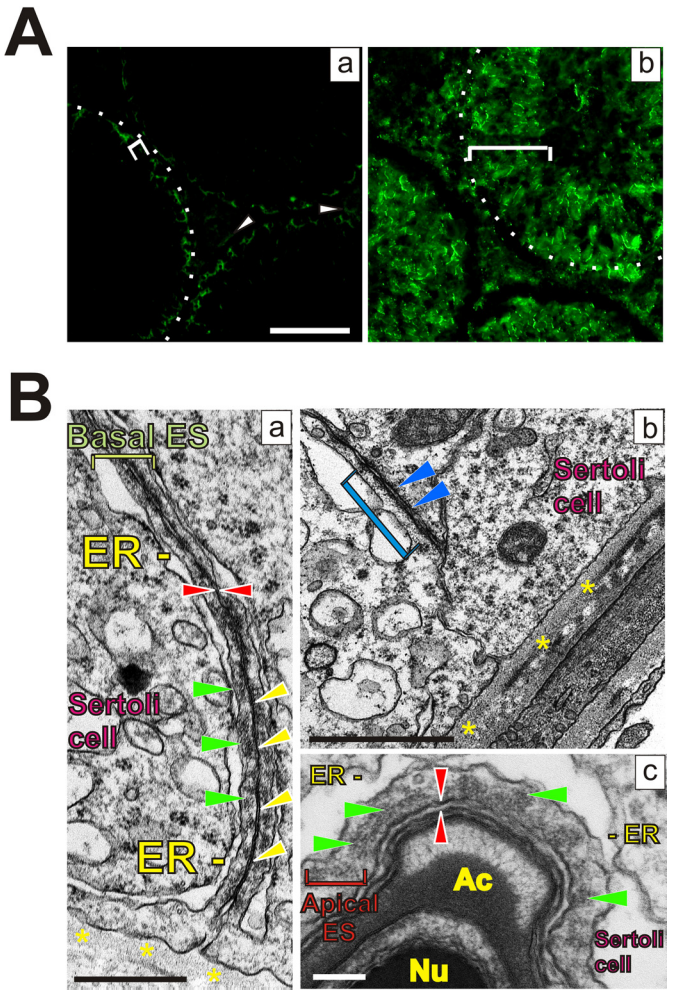

FIG. 3. Cellular, functional and ultrastructural features of the blood-testis barrier (BTB) in mammalian testes during the seminiferous epithelial cycle of spermatogenesis. A, an intact and functional BTB is found in each of the three adjacent seminiferous tubules as shown in a, in which FITC (M_r 389.39) administered to an adult rat (~300 g b.wt.) via the jugular vein was unable to pass through the BTB located near the basement membrane of the tunica propria (see broken white-line) to enter the apical compartment in each of these tubules (see the white bracket), even though FITC traversed the TJ barrier in the microvessels in the interstitial space (see green fluorescence in the interstitium annotated by the white arrowheads) (a). In the right panel, this rat was treated with $CdCl_2$ (3 mg/kg b.wt. i.p.) for 3 days (b), which is known to disrupt the BTB integrity, before administration of FITC at the jugular vein. The BTB was found to be disrupted in all three tubules in this cross-section of testis because FITC entered the apical compartment "freely," reaching the lumen of the seminiferous tubule (see the white bracket). Scale bars, 50 μm (a and b). B, the anatomy of the BTB. In a, this is an electron micrograph shown the typical ultrastructural features of the BTB created by two adjacent Sertoli cells lying on the basement membrane (yellow asterisks). Shown here are the basal ES, typified by the actin filament bundles (green arrowheads) sandwiched between cisternae of the endoplasmic reticulum (ER) and apposing plasma membranes of two Sertoli cells (apposing red arrowheads), TJ (yellow arrowheads) coexisting with the basal ES. In b, the desmosome (denoted by the blue arrowheads), which is also part of the components of the BTB, typified by the presence of electron dense substances along the two adjacent Sertoli cell and the absence of the distinctive actin filament bundles since desmosome is an intermediate-based cell-cell anchoring junction type. In c, the electron micrograph illustrates the apical ES surrounding the head of this elongating spermatid, in which the actin filament bundles (green arrowheads) are sandwiched between cisternae of the ER and the apposing plasma membranes of the Sertoli cell and the elongating spermatid (apposing red arrowheads), similar to the basal ES at the BTB shown in a, except that this typical features of the ES restricted *only* to the Sertoli cell. Scale bars, 0.5 μm (a), 1 μm (b), and 0.1 μm (c). Ac, acrosome; Nu, nucleus.

tinal epithelium ex vivo have provided a semiquantitative model of the TJ barrier (Watson et al., 2005; Van Itallie et al., 2008; Anderson and Van Itallie, 2009; Marchiando et al., 2010b), and these findings also illustrate that, similar to the BTB, the TJ barrier/blood-tissue barrier in these epithelia/endothelia is not static but is a dynamic ultrastructure (Cardoso et al., 2010; Steed et al., 2010). In short, the TJ barrier in these epithelia has two distinct but coexisting paracellular pathways to allow transport of biomolecules across the TJ: 1) the "pore" pathway, which is permeable to small solutes with a molecular radius <4 Å, and 2) the "nonpore" pathway in which temporary and transient breaks occur at cell-cell contacts, which are permeable to >4-Å molecules (Anderson and Van Itallie, 2009; Ivanov, 2011; Shen et al., 2011). Thus, it is currently not known whether these two pathways found at the TJ barrier in other epithelia are applicable to the BTB because of the presence of the basal ES, which apparently is being used to reinforce the "tightness" of the BTB, plus other coexisting junction types (e.g., desmosome, gap junction) at the site (see Figs. 1 and 3).

It is noteworthy that besides being found at the BTB, the ES is also found at the Sertoli cell-spermatid interface from steps 8 to 19 in rat testes during spermiogenesis, and it is known as the apical ES (Russell, 1977c). Once it appears, it is the *only* anchoring device, replacing desmosomes and gap junctions that are found between the Sertoli cell and step 1-to-7 spermatids. The ultrastructural features of the apical ES are identical to the basal ES except that the actin filament bundles and the cisternae of the endoplasmic reticulum unique to the ES are visible only on the Sertoli cell side (Russell, 1977c; Vogl et al., 2000; Toyama et al., 2003; Cheng and Mruk, 2010a) (Figs. 1 and 3). Perhaps it is because of these unique ultrastructural features [e.g., the unique actin filament bundles at the basal ES that confer an unusual adhesive force to the *coexisting* TJ, and other *coexisting* junction types at the BTB, most notably the desmosome (Fig. 3)] that the BTB is one of the tightest blood-tissue barriers in the mammalian body. The physiological significance of actin filament bundles at the basal ES that confer BTB function was first reported in the late 1980s. In a study using cytochalasin D to selectively disrupt actin filament bundles in the basal ES at the BTB in rats, BTB function was found to be disrupted when its integrity was assessed by the diffusion of 1) an electron-dense lanthanum salt across the BTB under electron microscopy and 2) radiolabeled inulin from the interstitial fluid and into the seminiferous tubule lumen by using micropuncture techniques to collect and compare radioactivity from fluids in these compartments (Weber et al., 1988). These data thus illustrate the significance of intact actin filament bundles to maintain BTB integrity. Subsequent studies that assessed the adhesive force conferred by the ES, such as the apical ES at the Sertoli-elongate spermatid interface, which shares ul-

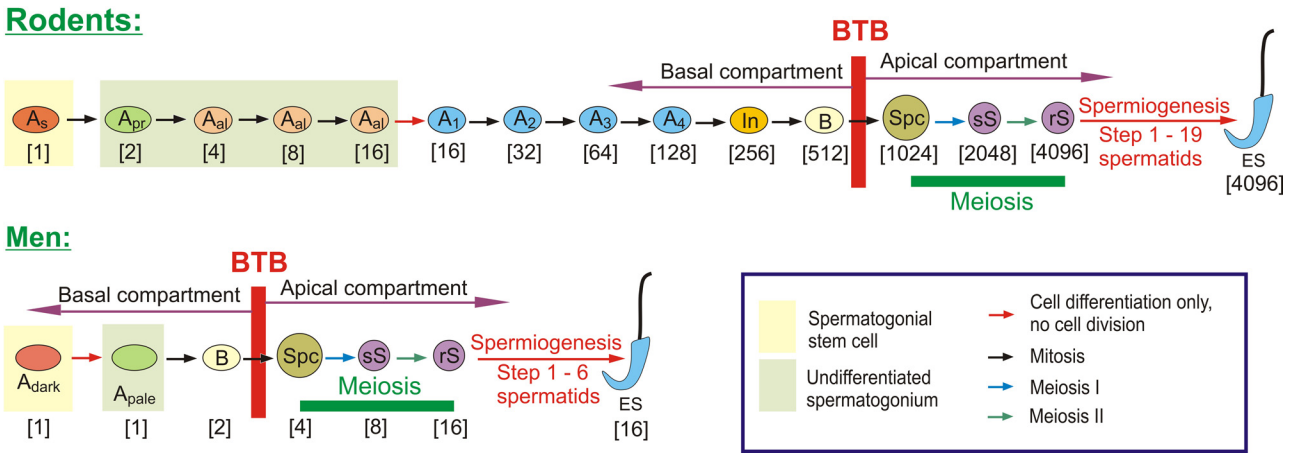

FIG. 4. A schematic illustration of spermatogenesis in rodents and humans involving cell division (mitosis, meiosis) and cell differentiation shows the significance of the BTB that segregates germ cells in the basal or the apical (adluminal) compartment of the seminiferous epithelium. The bracketed number below each germ cell type represents the number of daughter cells derived from an earlier progenitor cell via either mitosis (black arrow), meiosis [green bar, which includes meiosis I (blue arrow) and meiosis II (green arrow)], or transformation/differentiation without involving cell division (red arrow). In theory, 4096 elongated spermatids (ES) and thus spermatozoa are formed from a single A_{single} spermatogonium in rodents, but >75% of germ cells (e.g., spermatogonia, spermatocytes, and spermatids) undergo apoptosis and degeneration, so that the number of spermatozoa derived from a single spermatogonium is considerably less. A, type A spermatogonium; In, intermediate spermatogonium; B, type B spermatogonium; Spc, spermatocyte; sS, secondary spermatocyte; rS, round spermatid, which undergoes spermiogenesis involving step 1-to-19 and 1-to-6 spermatids in rats and humans, respectively, to form elongated spermatids; ES, elongated spermatid, which transforms and differentiates to spermatozoan before spermiation; A_s, A_{single} spermatogonium; A_{pr}, A_{paired} spermatogonium; A_{al}, $A_{aligned}$ spermatogonium; A_1-A_4 are differentiated spermatogonia. The BTB physically divides the seminiferous epithelium into the basal and apical (adluminal) compartment. All the events of spermatogonial mitotic division and differentiation take place in the basal compartment, when type B spermatogonia differentiate into preleptotene spermatocytes, which are the germ cells in transit at the BTB so that meiosis I/II and spermiogenesis take place behind the BTB in the apical compartment until spermiation (i.e., the release of sperm into the tubule lumen).

trastructural features similar to those of the basal ES at the Sertoli-Sertoli cell interface, have demonstrated that this is the "strongest" adhesion junction in the testis versus the desmosome and the gap junction (Wolski et al., 2005).

B. Functions of the Blood-Testis Barrier

1. "Fence" and "Gate-Keeper" Functions of the Blood-Testis Barrier.

a. Restricts paracellular flow of biomolecules. Anatomically speaking, the BTB divides the seminiferous epithelium into the basal and the apical (adluminal) compartments (Figs. 1–3). The primary function of the BTB is to restrict the paracellular "flow" of substances (e.g., water, electrolytes, ions, nutrients, hormones, paracrine factors, and biological molecules) across the Sertoli cell epithelium into the apical compartment (Fig. 3). Because the seminiferous epithelium is not penetrated by blood vessels or capillaries, lymphatic vessels, or even nerves, which are all located in the interstitium between seminiferous tubules (Fig. 1), the BTB thus regulates the entry of substances, both nutritional (e.g., sugars, amino acids) and vital molecules (e.g., hormones, electrolytes) and harmful toxicants (e.g., environmental toxicants, drugs, chemicals), into the apical compartment in which postmeiotic germ cell development (i.e., spermiogenesis and spermiation) takes place. This "selectivity" function of the BTB thus creates a unique microenvironment for postmeiotic spermatid development in the apical compartment of the seminiferous epithelium in mammalian testes (Fig. 1) during the seminiferous epithelial cycle of spermatogenesis (Fig. 2; Table 2).

b. Segregates cellular events during the epithelial cycle of spermatogenesis. The "fence" function of the BTB also segregates different cellular events of spermatogenesis as illustrated in Fig. 4. This morphological segregation imposed by the BTB is perhaps important for spermatogonial renewal, mitotic proliferation, and differentiation so that they can have unrestricted access to nutrients, hormones, and biomolecules released from microvessels and into the interstitial space (see Fig. 1), whereas further development of highly specialized and metabolically inactive developing spermatids are "shielded" behind the BTB in the immune-privileged apical compartment.

2. The Blood-Testis Barrier Creates an Immunological Barrier.

The "fence" and "gate-keeper" functions of the BTB also contribute, at least in part, to the immune-privileged status of the testis, such that the immunological response to autoantigens residing within germ cells undergoing meiosis and developing spermatids during spermiogenesis, many of which are expressed transiently, can be suppressed (Fijak et al., 2011; Meinhardt and Hedger, 2011). This is necessary to avoid the production of anti-sperm antibodies and autoimmune disease, which leads to male infertility (Francavilla et al., 2007). Thus, the BTB also creates an immunological barrier to sequester many germ cell-specific antigens, some of which are proto-oncogenes and oncogenes that arise or appear transiently during meiosis and spermiogenesis. In this context, it is noteworthy that autoantigens also reside within germ cells outside the BTB in

TABLE 1

Types of cell junctions and their constituent and peripheral proteins found at the blood-testis barrier (BTB) in rodent testes

Prepared based on reviews (Cheng and Mruk, 2002, 2009b, 2010a, 2011; Mruk and Cheng, 2004b, 2010, 2011; Lie et al., 2008, 2011a; Mruk et al., 2008, 2011; Siu and Cheng, 2008; Yan et al., 2008a; Li et al., 2009c; Wong and Cheng, 2009; Kopera et al., 2010; Morrow et al., 2010; Su et al., 2011a) and reports (Li et al., 2011b), many of which are cited in the text.

Junction Type	Component Proteins
Occluding junction	
Tight junction	
Integral membrane proteins	Occludin, claudin-1, -3, -4, -5, -7, -8, -11, JAM-A, JAM-B, CLMP, CAR
Adaptors	ZO-1, ZO-2, vinculin
Regulatory or signaling proteins	FAK, c-Src, c-Yes, p38 MAPK, ERK1/2, JNK, PKB (Akt), PAK, PI3K
Polarity proteins	PAR6, 14–3-3, PATJ, PALS1, Scribble, Crumbs, Cdc42
Protease	MMP9
Protease inhibitors	TIMP-1, α_2-macroglobulin
Trafficking proteins	Dynamin 2, Rab8B, clathrin, caveolin-1, EEA-1, Rab9, MTMR2, annexin II
Cytokines	TGF-β2, TGF-β3, TNFα, IL-1α
Drug transporters	P-glycoprotein (efflux pump), Oatp3 (influx pump)
Scaffolding proteins	Actin, collagen α3(IV)
Anchoring junction	
Cell-cell actin-based	
Basal ES	
Integral membrane proteins	N-cadherin, E-cadherin, nectin-2, CAR
Adaptors	α-catenin, β-catenin, γ-catenin, afadin, vinculin
Regulatory or signaling proteins	c-Src, c-Yes, CK2, Csk
Trafficking proteins	Dynamin 2, clathrin, caveolin-1, MTMR2, annexin II
Drug transporters	Oatp3
Scaffolding proteins	Actin
Actin regulatory proteins	Eps8, Arp2/3 complex, N-WASP, drebrin E
Cell-cell intermediate filament-based	
Desmosome	
Integral membrane proteins	Desmoglein 2, desmocollin 2, desmocollin 3
Adaptors	Plakoglobin (γ-catenin), plakophilin-1,- 2,- 4, desmoplakin
Regulatory or signaling proteins	c-Src, CK2, Csk
Scaffolding proteins	Vimentin
Communicating junction	
Gap junction	
Integral membrane proteins	Connexin-43, -33, -26, -32, -36, -37, -40, -43, -45, -46, -50, -57
Adaptors	Plakophilin 2, β-catenin, drebrin E
Regulatory or signaling proteins	c-Src
Scaffolding proteins	Actin

CK2, casein kinase 2; CLMP, CXADR-like membrane protein; Csk, carboxyl-terminal Src kinase; drebrin E, developmentally regulated brain protein E; EEA-1, early endosome antigen-1; N-WASP, neuronal Wiskott-Aldrich syndrome protein; PAK, p21-activated kinase; PALS1, protein associated with Lin seven-1; PATJ, Pals1-associated tight junction protein; TIMP-1, tissue inhibitor of metalloproteases-1, a metalloprotease inhibitor.

the basal compartment, such as preleptotene spermatocytes, type B spermatogonia, undifferentiated and differentiated type A spermatogonia, and SSCs (see Fig. 1), which are equally potent *and* capable of eliciting autoimmune responses (Yule et al., 1988, 1990), as well as cancer/testis antigens (Kalejs and Erenpreisa, 2005; Simpson et al., 2005; Wong et al., 2008a; Caballero and Chen, 2009), which are oncogenes transiently expressed in these more primitive germ cells. It is noteworthy that neither male rodents nor male humans develop antibodies against those autoantigens residing within germ cells outside the BTB except in pathological conditions.

TABLE 2

Duration of spermatogenesis and the seminiferous epithelial cycle in rodents and men

The duration of spermatogenesis (in days) is the time it takes for one type A spermatogonium to form 4096 mature spermatozoa (in theory; see Fig. 4) with half of the original number of chromosomes (see Fig. 4) in rodents, so that sperm will be released from the seminiferous epithelium into the tubule lumen at spermiation. This can be estimated by using [^{3}H]thymidine incorporation. Because of germ cell apoptosis and/or degeneration as a result of the limited number of Sertoli cells in the rat testis ($\sim 5 \times 10^6$ Sertoli cells/mouse testis and $\sim 30–40 \times 10^6$ Sertoli cells/rat testis)(Orth, 1982; Berndtson and Thompson, 1990; França et al., 1998), only ~25% of the germ cells become spermatozoa (Shaha, 2008; Shaha et al., 2010) and are released to the tubule lumen at spermiation (O'Donnell et al., 2011). The duration of the seminiferous epithelial cycle (or one cycle of the seminiferous epithelium) is the time it takes to complete the series of morphological changes between two appearances of the same developmental stage, such as stage VIII, in a given area of the seminiferous epithelium in a tubule under stereomicroscopy (Clermont, 1972; Parvinen, 1982; França et al., 1998; Hess and de França, 2008). Thus, the duration of spermatogenesis takes ~4.2 (~68 days) to ~4.5 cycles (~58 days) in humans (Amann, 2008) and rodents (Amann and Schanbacher, 1983), respectively, to complete to allow type A spermatogonia to differentiate to sperm. The stages of spermatogenesis are divided according to the specific cellular associations using cross-sections of the seminiferous tubules originally based on the use of Periodic acid-Schiff reaction (PAS) of the acrosome in developing spermatids (Clermont, 1972; Hess and de FrançaZ, 2008), which can easily be defined with the use of hematoxylin and eosin staining using paraffin sections of testes (see Fig. 2).

Species	Duration of Spermatogenesis		Duration of Seminiferous Epithelial Cycle		Stages of the Epithelial Cycle	
	days		*days*			
Rat	48–58	Clermont et al., 1959; Hilscher, 1964; França et al., 1998	12.8–12.9	Clermont and Harvey, 1965; Russell and Brinster, 1996; Aslam et al., 1999	I–XIV	Leblond and Clermont, 1952; Hess, 1990
Mouse	34–35	Oakberg, 1956; Clermont and Trott, 1969	8.6	Clermont and Trott, 1969; Russell et al., 1996	I–XII	França et al., 1998; Hess and de França, 2008
Human	64–68	Heller and Clermont, 1964; Amann, 2008	16	Amann, 2008	I–VI	Clermont, 1963

Studies have suggested that local immunoregulatory mechanisms operate to prevent testicular autoimmune disease, such as nonspecific immunosuppression, antigen presentation, lymphocyte trafficking, and/or suppressor T cells, as well as androgens. Perhaps selected populations of leukocytes may play a role to confer immune privilege to the basal compartment of the seminiferous epithelium outside the BTB (Mahi-Brown et al., 1988; Mital et al., 2010; Fijak et al., 2011).

In addition to the BTB, studies have shown that Sertoli cells per se may play a critical role in maintaining the testis as an immune-privileged organ by secreting immunosuppressive molecules to block immune response to transiently expressed autoantigens in developing germ cells during spermatogenesis, as demonstrated in cotransplantation experiments (Selawry and Cameron, 1993; Dufour et al., 2004; Shamekh et al., 2006; Fallarino et al., 2009; Yin et al., 2009; Mital et al., 2010). However, the identities of the immunosuppressive biomolecules as noted in these allogeneic and xenogeneic transplantation studies remain unknown; it is conceivable that they are composed of an array of molecules including cytokines (e.g., interleukins, interferons) and prostaglandins (Hein and O'Banion, 2009; Eyerich et al., 2010; Yang, 2010) because Sertoli cells are capable of producing cytokines and prostaglandins (Samy et al., 2000; Mruk and Cheng, 2004b; Meinhardt and Hedger, 2011). In this context, it is noteworthy that Sertoli cell lines (e.g., MSC-1) lack the immunoprotective properties associated with primary Sertoli cells (Dufour et al., 2008), illustrating that studies using Sertoli cell lines may not reflect the physiological function of the Sertoli cell in vivo. However, it remains to be determined whether other Sertoli cell lines also lack the immunoprotective properties of primary Sertoli cells cultured in vivo. In short, these findings illustrate that the BTB creates an immunological barrier impermeable to antigens residing in postmeiotic germ cells during spermiogenesis and spermiation; however, it also works in concert with other mechanism(s) to confer immune privilege to the testis. As noted above, because blood and lymphatic vessels reside outside of the seminiferous tubules in the interstitium (Setchell and Waites, 1975), and macrophages and other antigen presenting cells are found in the seminiferous epithelium only in pathological conditions (Mahi-Brown et al., 1988; Yule et al., 1990), there is a necessity for Sertoli cells to contribute, at least in part, to immune defense mechanism(s). Indeed, it has been shown that Sertoli and/or germ cells produce antiviral and antibacterial molecules, such as defensins (e.g., α-, β-defensin) (Com et al., 2003; Jin et al., 2010) and interferons (Dejucq et al., 1997; Dejucq et al., 1998a,b), and the production of interferons was shown to be mediated by toll-like receptors in Sertoli cells (Starace et al., 2008). It is noteworthy that *Escherichia coli* administered to seminiferous tubules in live mice were shown to rapidly reproduce to reach a maximal level within a day, but the bacteria level began to decline by day 5 and was completely eradicated in ~2 months, concomitant with infiltration of neutrophils and enhanced production of chemokines and inflammatory cytokines [e.g., tumor necrosis factor-α (TNFα)], resulting in infertility with an irreversible disruption of spermatogenesis (Nagaosa et al., 2009). Collectively, these findings illustrate that the seminiferous epithelium has its own immunosuppressive, antibacterial, and antiviral defense mechanism(s) to confer immune privilege to the testis instead of a total reliance on the BTB.

3. Confers Cell Polarity in the Seminiferous Epithelium. The BTB confers Sertoli cell polarity in the seminiferous epithelium. One of the most obvious features of cell polarity in the seminiferous epithelium is manifested by the localization of Sertoli cell nuclei, which are restricted to the basal compartment, lying adjacent to the tunica propria; and cytoplasmic organelles (e.g., Golgi apparatus, lysosomes) in the Sertoli cell are not uniformly distributed within its cytosol (Fig. 2). Furthermore, all apical ES ultrastructures at the Sertoli-spermatid interface are located in the apical compartment (see Figs. 1 and 3). Similar to the TJ barrier in other epithelia, which is crucial to maintain cell polarity, cell polarity in the seminiferous epithelium of adult mammalian testes is conferred, at least in part, by the BTB via the three polarity complexes or modules: the Crumbs (Crumbs/PatJ/Pals1), PAR (PAR3/PAR6/atypical PKC/Cdc42), and Scribble (Scribble/Dlg/Lgl) modules (Assémat et al., 2008; Iden and Collard, 2008; Wong and Cheng, 2009; Cheng and Mruk, 2010a; Harris and Tepass, 2010). Many of the components of these three polarity protein complexes have been identified in the testis, and their physiological significance in spermatogenesis has recently been reported (Wong et al., 2008c, 2009, 2010a). Besides the BTB, the apical ES (for reviews, see Wong et al., 2008a; Wong and Cheng, 2009) also confers spermatid polarity during spermiogenesis using similar polarity modules, so that developing spermatids can be properly oriented to occupy minimal space in the epithelium with their heads pointing toward the basement membrane and their tails toward the seminiferous tubule lumen (see Figs. 1 and 2). Based on earlier studies in *Drosophila melanogaster* and subsequent studies in mammalian epithelial cells, it is known that the localization of Crumbs and PAR modules at the TJ near the apical region and the Scribble module at the basolateral region of an epithelium are mutually exclusive (for reviews, see Assémat et al., 2008; Iden and Collard, 2008; Wong and Cheng, 2009). As such, each module recruits its unique binding partners, including adaptors, kinases, phosphatases, and different organelles, to create the cell polarity necessary for morphogenesis, development, and pathogenesis (Médina et al., 2002; Assémat et al., 2008; Huang and Muthuswamy, 2010; Marx et al., 2010). The most obvious cell polarity phenotype in the seminiferous epithelium conferred by

the BTB, besides the unique localization of Sertoli cell nuclei near the basement membrane, is that spermatogonial stem cells, undifferentiated and differentiated spermatogonia, and preleptotene spermatocytes are all sequestered outside the BTB in the basal compartment, whereas late spermatocytes (e.g., zygotene, pachytene, and diplotene spermatocytes), secondary spermatocytes, spermatids, and spermatozoa are restricted to the apical compartment in the seminiferous epithelium (Figs. 1 and 2). Furthermore, many organelles and ultrastructures, including the cytoskeleton, are not uniformly distributed within the Sertoli cell cytosol. For instance, actin filament bundles, and cisternae of endoplasmic reticulum are very abundant at the BTB at the site of the basal ES, and also at the apical ES, associating with developing spermatids during spermiogenesis (Yan et al., 2007; Vogl et al., 2008; Cheng and Mruk, 2009a, 2010a; Mruk and Cheng, 2010b) (Fig. 5). Recent studies have shown that polarity proteins, such as PAR3 and PAR6, also regulate protein distribution at the BTB and TJ permeability barrier function (Wong et al., 2008c) via an unexpected mechanism (Wong et al., 2009). For instance, it was shown that a knockdown of PAR3 or PAR6 by RNAi using corresponding specific siRNA duplexes in Sertoli cells cultured in vitro in which the TJ barrier mimicked the BTB in vivo (Fig. 6) led to a mislocalization of integral membrane proteins and/or peripheral adaptors at the BTB, such as JAM-A and α-catenin, perhaps via an increase in protein endocytosis, thereby destabilizing the TJ barrier (Wong et al., 2008c). Indeed, such effects were subsequently shown to be mediated by 14-3-3 (also known as PAR5), which regulated the events of protein endocytosis at the BTB (Wong et al., 2009). More important, Cdc42 in the PAR module is critical for cytokine-mediated [e.g., transforming growth factor (TGF) β3] protein endocytosis events at the BTB (Wong et al., 2010a), because earlier studies have shown that cytokines (e.g., TGF-β2, TGF-β3, and TNFα) regulate BTB function via an increase in the kinetics of endocytosis of integral membrane proteins at the BTB, facilitating the disruption of TJ fibrils above preleptotene spermatocytes in transit at stage VIII of the epithelial cycle (Yan et al., 2008b; Xia et al., 2009). These findings thus support the notion that polarity proteins (e.g., 14-3-3, Cdc42, PAR3, PAR6) are crucial regulators of endocytic vesicle-mediated protein trafficking events at the BTB (Wong et al., 2008c, 2009, 2010a). Indeed, recent studies in other epithelia have also supported the emerging concept that polarity proteins and endocytic pathways work together to create distinctive cellular domains in the polarized cell epithelium (Shivas et al., 2010).

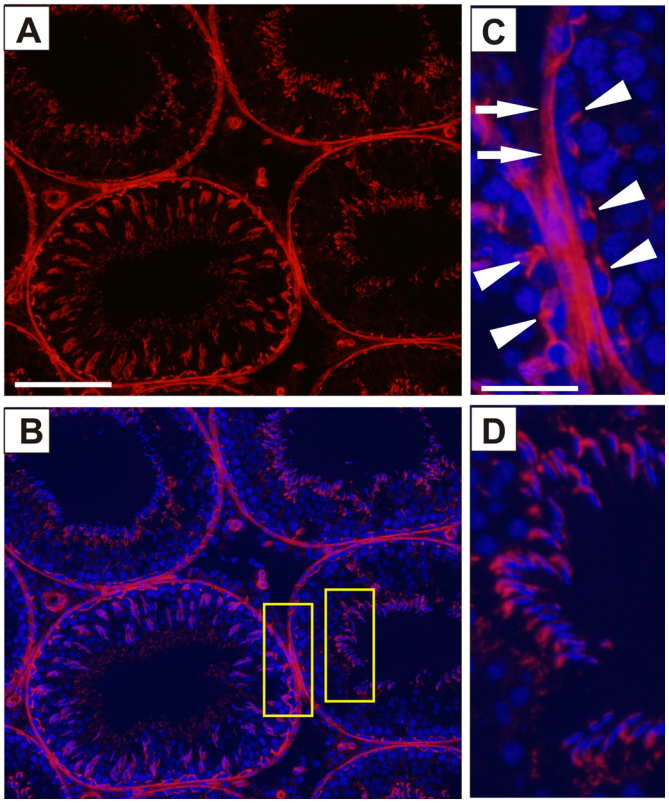

FIG. 5. Distribution of the actin-based cytoskeleton in the seminiferous tubules of adult rat testes. A, cross-section of an adult rat testis in which F-actin was visualized by staining with rhodamine-conjugated phalloidin (Invitrogen, Carlsbad, CA) (red fluorescence), and cell nuclei were stained with 4′,6-diamidino-2-phenylindole and shown in the merged image (B). The two boxed areas in B are magnified and shown in C and D illustrating the relative location of BTB in C as denoted by white arrowheads, and F-actin is also predominant in the tunica propria (see white arrows) associated with peritubular myoid cells. Extensive F-actin network is also detected at the apical ES, surrounding the heads of the elongating spermatids, as shown in D. Scale bars, 80 μm (A and B) and 20 μm (C and D).

C. An Intact and Functional Blood-Testis Barrier Is Necessary for Spermatogonial Stem Cell/Spermatogonial Differentiation during Spermatogenesis: an Emerging Concept

The emerging concept that the BTB is a physiologically significant ultrastructure in relation to the onset of spermatogonial differentiation, such as from type B spermatogonia to preleptotene spermatocytes during spermatogenesis, was based on earlier studies that examined the onset of premeiotic cell differentiation and meiosis and the timing of BTB assembly in the developing testis. For instance, preleptotene and leptotene spermatocytes are found in the seminiferous epithelium in rats by postnatal days 9 to 12 before the establishment of the BTB (Clermont and Perry, 1957). However, the differentiation of preleptotene spermatocytes, which is the only germ cell type that is in transit at the BTB, into zygotene spermatocytes to be followed by pachytene spermatocytes that occurs by postnatal days ~15 to 18 (Clermont and Perry, 1957) coincides with the establishment of the BTB (Vitale et al., 1973; Bergmann and Dierichs, 1983; Russell et al., 1989). It is also noted that Sertoli cells cease to divide and are fully differentiated by approximately postnatal day 15, with ~30 to 40 $\times$ 10^6 Sertoli cells per testis in rats (Orth, 1982; Berndtson and

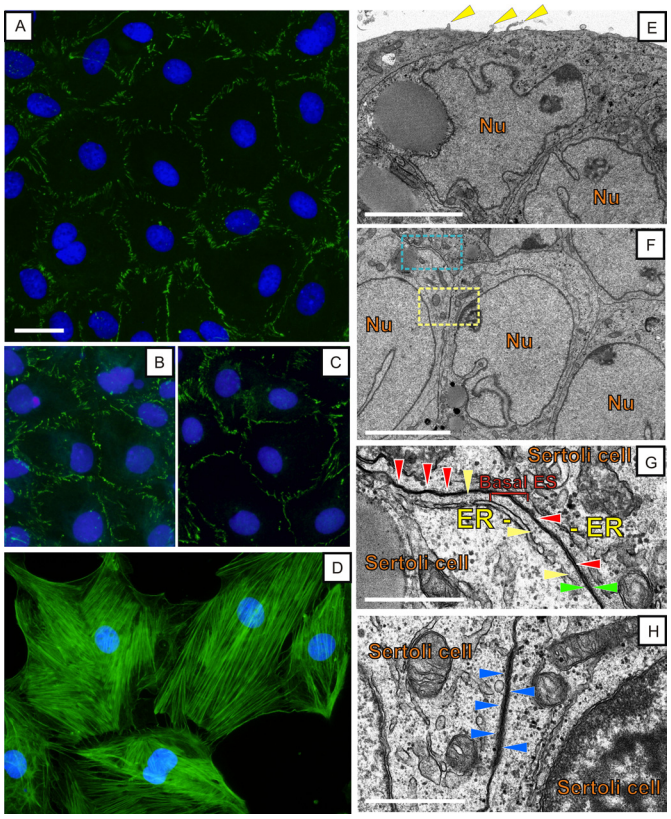

FIG. 6. Morphology and ultrastructural features of the Sertoli cell BTB in vitro. Sertoli cells were cultured at $\sim 0.02 \times 10^6$ cells/cm^2 (A–C) or at 0.0125×10^6 cells/cm^2 (D) on Matrigel-coated glass coverslips for 4 days. Thereafter, cells were fixed in 4% paraformaldehyde (w/v) in phosphate-buffered saline (10 mM sodium phosphate and 0.15 M NaCl, pH 7.4 at 22°C), permeabilized in 0.1% Triton X-100 (v/v) in phosphate-buffered saline, and stained with a monospecific antibody against ZO-1 (A, an TJ adaptor protein), occludin (B, a TJ-integral membrane protein), N-cadherin (C, a basal ES integral membrane protein), or stained for F-actin using FITC-conjugated phalloidin (Sigma-Aldrich, St. Louis, MO) (D). Cells were mounted in ProLong Gold antifade reagent with 4,6-diamidino-2-phenylindole (Invitrogen) to visualize cell nuclei. Both TJ and basal ES were established in these Sertoli cells cultured in vitro, confirming earlier findings that a functional TJ permeability barrier is established in Sertoli cells cultured in vitro, by measuring transepithelial electrical resistance across the Sertoli cell epithelium (Grima et al., 1998; Lui et al., 2001). The distinctive actin filament network is also found in these Sertoli cells as shown in D. E to H, electron micrographs of Sertoli cells (0.5×10^6 cells/cm^2) cultured in vitro on Matrigel-coated dishes for 4 days, and the ultrastructural features of the BTB detected in vivo are also found in this Sertoli cell epithelium (F–H), illustrating that a functional BTB has been established. E, two adjacent Sertoli cells with the distinctive cell nuclei (Nu) are noted, and microvilli are also found (yellow arrowheads), which are the typical features of Sertoli cells cultured in vitro. F, three adjacent Sertoli cells are noted (Nu) and the functional BTB encircled by the blue and yellow brackets are magnified and shown in G and H, respectively. G, basal ES is typified by the presence of actin filament bundles (yellow arrowheads), which are sandwiched between cisternae of the endoplasmic reticulum (ER) and the apposing Sertoli cell plasma membranes (see apposing green arrowheads), and TJ (red arrowheads) is found to coexist with basal ES. H, functional desmosome (blue arrowheads), which is typified by the presence of electron-dense substances on both sides of the two adjacent Sertoli cells, is also noted. Desmosome shown in H and the basal ES and TJ shown in G are the magnified images from the corresponding areas in F, illustrating the ultrastructures of the Sertoli cell BTB in vitro. In short, these observations illustrate that a functional BTB was established in these Sertoli cells cultured in vitro, mimicking the BTB in vivo by having the ultrastructures of the BTB found in vivo. Scale bars, 20 μm (A–D), 5 μm (E and F), and 1 μm (G and H).

Thompson, 1990) [and ~ 2–5×10^6 Sertoli cells per testis in mice (Vergouwen et al., 1993; França et al., 1998; Auharek and de França, 2010)], which persists through adulthood. In short, these findings suggest that Sertoli cells that cease to divide by postnatal day 15 are necessary for the establishment of a functional BTB, which in turn, allows the transit of preleptotene spermatocytes across the barrier and their differentiation into zygotene and pachytene spermatocytes. It is noteworthy that neonatal rats treated with diethylstilbestrol (DES), which delayed the establishment of the BTB by 4 weeks, was also shown to delay the first wave of spermiation by ~4 weeks, wherein spermatocytes failed to enter meiosis I/II and were found to undergo degeneration (Toyama et al., 2001) [note that spermiation occurs by postnatal day 45 in normal rats (Clermont and Perry, 1957)], illustrating meiotic arrest in the absence of a functional BTB.

A recent study using adjudin [1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carbohydrazide, formerly known as AF-2364, a potential male contraceptive (Cheng et al., 2001, 2005; Cheng and Mruk, 2010b)] to knock out virtually all germ cells from the seminiferous epithelium except SSCs and spermatogonia by perturbing germ cell adhesion to Sertoli cells has yielded some surprising results (Mok et al., 2011a). First, it was shown that in adult rats (~270–300 g b.wt.) treated with adjudin at either 50 mg/kg (low dose) or 250 mg/kg (high dose) b.wt. (one dose, via gavage), all the seminiferous tubules were devoid of germ cells by ~2 weeks except for SSCs/spermatogonia (Mok et al., 2011a). However, in the low-dose group, spermatogenesis reinitiated, beginning at ~20 weeks after treatment, and >70% of the tubules displayed normal spermatogenesis by 30 weeks; however, spermatogenesis failed to resume in the high-dose group (Mok et al., 2011a). We speculated that this might have been due to the depletion of SSCs/spermatogonia by adjudin, which is somewhat analogous to the effect of a "toxicant" on spermatogenesis. Surprisingly, the population of SSCs/spermatogonia remained relatively unaltered in *both* low- and high-dose groups (Mok et al., 2011a), illustrating that the inability to reinitiate spermatogenesis in the high-dose group is not the result of SSCs/spermatogonia loss. Instead, it was found that the BTB was disrupted in both treatment groups by 6 weeks after treatment (Mok et al., 2011a) when barrier integrity was assessed by a functional in vivo assay that monitored the ability of an intact BTB to block the diffusion of a small fluorescence tag (e.g., FITC or FITC-inulin) across the BTB (Li et al., 2006; Xia et al., 2009). It is noteworthy that the BTB was "resealed" in the low-dose group by 20 weeks but not in the high-dose group (Mok et al., 2011a), illustrating that a functional BTB is necessary for reinitiation of spermatogenesis after its disruption by toxicants. These findings are also supported by earlier studies in which rats were treated with 2,5-hexanedione (Boekelheide and Hall, 1991), dibromochloropropane (Meistrich et al., 2003), and procar-

bazine (Meistrich et al., 1999) or subjected to irradiation (Kangasniemi et al., 1996), which depleted most germ cells from the seminiferous epithelium, except spermatogonia and/or SSCs. Nonetheless, spermatogenesis failed to reinitiate in these animals because spermatogonia failed to differentiate beyond type A; however, it should be noted that BTB integrity was not examined in these animals. Nonetheless, these findings are in agreement with observations in the developing testis, which showed that the assembly of the BTB correlates with the onset of differentiation of spermatocytes and meiosis.

Studies using other genetic models also support the significance of the BTB in the initiation and maintenance of spermatogenesis. For instance, claudin-11(−/−) (Gow et al., 1999; Mazaud-Guittot et al., 2010) and occludin(−/−) (Saitou et al., 2000; Takehashi et al., 2007) mice failed to assemble TJ fibrils and to establish a functional BTB, and these mice were infertile, because occludins are known to oligomerize to form TJ strands via homologous and heterologous interactions of occludins localized on adjacent epithelial cells (e.g., Sertoli cells) (Blasig et al., 2011). In claudin-11(−/−) mice, spermatogenesis failed to proceed beyond meiosis (Mazaud-Guittot et al., 2010), whereas meiotic arrest was detected in occludin(−/−) mice by postnatal weeks ~36 to 60, even though occludin(−/−) mice remained fertile by postnatal week ~10 (Takehashi et al., 2007); perhaps other TJ proteins (e.g., claudins, JAMs) were capable of temporarily superseding the function of occludin, but not continuously into adulthood.

Collectively, these findings thus support the concept that the BTB is important to maintain and reinitiate spermatogenesis after toxicant-induced aspermatogenesis, perhaps at the stages of spermatogonial differentiation (such as from undifferentiated to differentiated type A spermatogonia and/or the transformation from type A to type B), meiosis, and the onset of spermiogenesis as demonstrated in toxicant and genetic models. However, it is noted that these events are marked with a complex and intriguing regulation at both cellular and molecular levels, as illustrated in gene profiling studies (O'Donnell et al., 2009; Carlomagno et al., 2010). Thus, it is not unexpected that the underlying mechanism(s) by which the BTB exerts its regulatory effect(s) remains unknown. Studies have shown, however, that spermatogonial stem cells (SSCs) reside in the stem cell niche, which is found in the area where several seminiferous tubules converge that also borders the interstitial tissue, near the BTB and microvessels in the interstitium (de Rooij, 2009; Phillips et al., 2010; Yoshida, 2010), perhaps making it easier for SSCs to have access to nutrients from the systemic circulation. It is possible, therefore, that SSCs acquire signals from polarized Sertoli cells near the basement membrane via gap junction (GJ), so that SSCs can undergo proper differentiation during the seminiferous epithelial cycle of spermatogenesis. This hypothesis is supported by studies showing that in connexin-43 knockout [Cx43(−/−)] mice, tubules were devoid of all germ cells except for spermatogonia (Brehm et al., 2007). More important was the fact that the remaining spermatogonia failed to differentiate beyond type A spermatogonia (Brehm et al., 2007), and Sertoli cells remained undifferentiated and proliferative well into adulthood. Thus, clusters of Sertoli cells were continuously found in the tubule lumen in these Cx43(−/−) mice (Sridharan et al., 2007), perhaps to "shed" the unwanted Sertoli cells as a result of unchecked cell proliferation. Although the ultrastructural features of the BTB in these Cx43(−/−) mice seemed to be unaffected when examined by electron microscopy (Carette et al., 2010b), an increase in the levels of occludin, N-cadherin, and β-catenin concomitant with a significant loss of ZO-1 at the BTB, as well as mislocalization of some of these BTB proteins, was detected when examined by dual-labeled immunofluorescence analysis (Carette et al., 2010b), illustrating a BTB malfunction at the physiological level. In short, it is likely that an intact *and* functional BTB provides and maintains a proper microenvironment via GJ signaling to allow spermatogonial differentiation within the stem cell niche during spermatogenesis. In fact, this hypothesis is supported by a recent gene profiling study in which SSCs induced to undergo differentiation by BMP4 (bone morphogenetic protein 4) were found to associate with a surge in cell adhesion proteins, including GJ-proteins (Carlomagno et al., 2010).

IV. Regulation of the Blood-Testis Barrier

A. Cross-Talk of Different Junction Types at the Blood-Testis Barrier to Confer Its Dynamic Nature during Spermatogenesis

1. Introduction. The BTB physically divides the seminiferous epithelium into the basal and apical compartments (see Fig. 1). It thus segregates the events of postmeiotic germ cell development (i.e., spermiogenesis) and spermiation (the release of sperm from the epithelium at stage VIII of the epithelial cycle in rats) from the systemic circulation, which take place in the apical compartment of the epithelium. As noted in section III.A, the BTB is constituted by *coexisting* TJ, basal ES, desmosome, and gap junction (Setchell, 2008; Vogl et al., 2008; Cheng and Mruk, 2009a, 2010a) (Figs. 3, 6, and 7; Table 1). It is possible that these multiple junction types, particularly the unusual ultrastructural features of the basal ES (e.g., the tightly packed actin filament bundles along the Sertoli-Sertoli cell interface, reinforcing its adhesive function) that coexist with the TJ (Figs. 3 and 7), make the BTB one of the tightest blood-tissue barriers in the mammalian body. However, the physiological significance of multiple junction types that constitute the BTB has remained unknown until recently. Emerging evidence illustrates that the gap junction (Li et al., 2009b, 2010) and desmosome (Lie et al., 2010b) at the

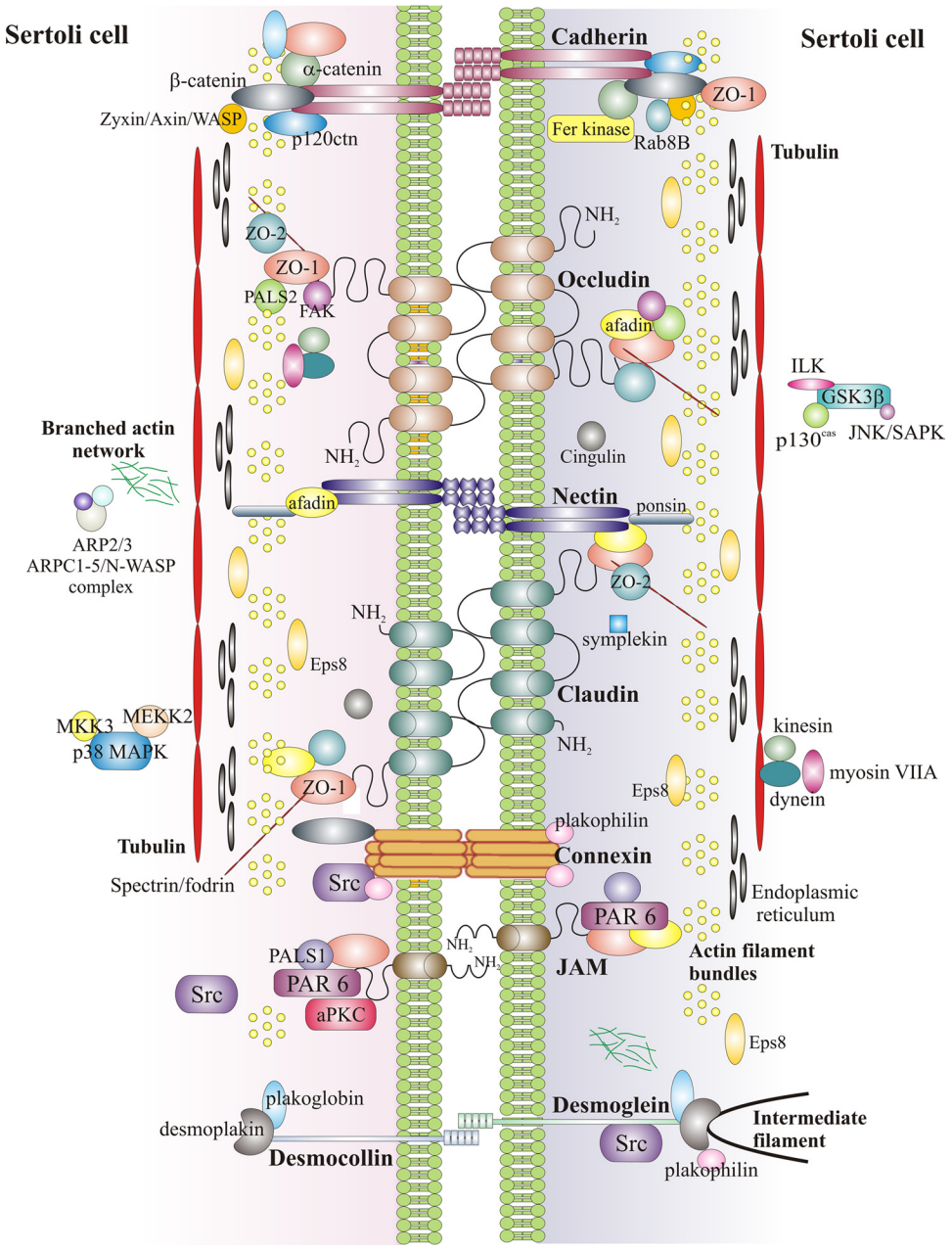

FIG. 7. A schematic drawing illustrating the molecular architecture of the constituent proteins at the BTB. The BTB is typified by the presence of actin filament bundles sandwiched between cisternae of the endoplasmic reticulum and the apposing plasma membranes of two adjacent Sertoli cells. Cell adhesion at the BTB is conferred by the presence of several integral membrane proteins and their adaptors, such as TJ protein complexes (occludin-ZO-1, claudin-ZO-1, JAM-ZO-1), basal ES protein complexes (cadherin-catenin, nectin-afadin), gap junction protein complex (connexin-43-plakophilin-2), and desmosome protein complex (desmoglein-2/desmocollin-2-plakophilin/plakoglobin). Adaptors in these protein complexes (e.g., ZO-1, β-catenin, afadin, plakophilin, plakoglobin) also recruit additional adaptors [e.g., zyxin, axin, Wiskott-Aldrich syndrome protein (WASP), ponsin] and regulatory proteins to the site, including nonreceptor protein kinases [such as Src family kinases (e.g., c-Src, c-Yes), Fer kinase, FAK], polarity proteins [e.g., PAR6, protein associated with Lin seven-1 (PALS1), PALS1-associated tight junction protein (PATJ), atypical PKC (aPKC)], GTPases (e.g., Rab8B, Cdc42), and MAPKs [e.g., p38 MAPK and JNK/stress-activated protein kinase (SAPK)]. The actin network is also maintained by the actin capping and bundling protein Esp8 and the actin nucleation Arp2/3 protein complex. Also present is the intermediate filament near the desmoglein-desmocollin complex, and the tubulin network with the motor proteins (e.g., myosin VIIA, dynein, kinesin) near the actin filament to facilitate preleptotene spermatocyte transit at the site.

BTB coordinate protein distribution at the basal ES and TJ, which is assisted in part by nonreceptor protein kinases such as c-Src, c-Yes, and FAK that associate with these junctions (Fig. 8). The net result regulates the temporal and spatial "opening" and "closing" of the barrier, which occurs at stage VIII of the epithelial cycle, to facilitate the transit of preleptotene spermatocytes, which move from the basal to the apical compartment while differentiating into leptotene and zygotene spermatocytes (Russell, 1977b; de Kretser and Kerr, 1988; Kerr et al., 2006) (Fig. 9). Furthermore, the basal ES and the TJ, while they are structurally "engaged" because they coexist at the BTB, can also become "disengaged" (Yan and Cheng, 2005), so that the integrity of the "old"

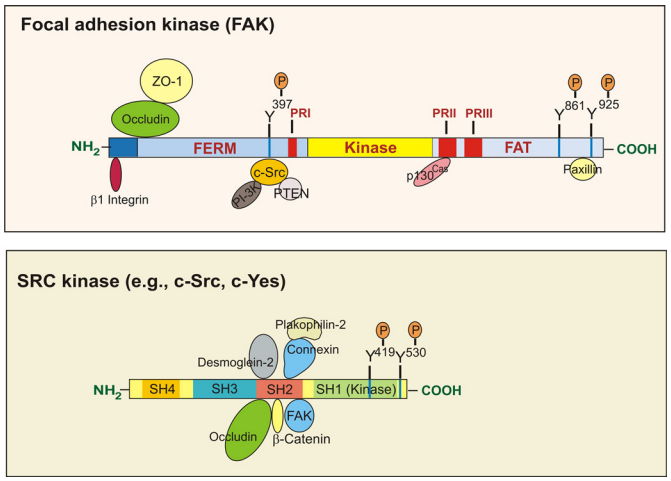

FIG. 8. A schematic drawing illustrating functional domains in non-receptor protein kinases FAK and Src. Both FAK and c-Src (the transforming, sarcoma-inducing gene of *Rous* sarcoma virus) are mediators of integrin-based signaling, most notably at the focal adhesion complex (also known as focal contact, which is an actin-based cell-matrix anchoring junction. Focal contact is not found in the testis. Instead, FAK and c-Src are components of the TJ and basal ES at the BTB, as well as the apical ES in the apical compartment). There are at least nine members in the Src kinase family: Src, Yes, Hck, Fyn, Fgr, Lyn, Lck, Blk, and Yrk. c-Src and c-Yes are found at the BTB and are structurally associated with the occludin-ZO-1 and the N-cadherin-β-catenin adhesion complexes. FAK consists of an N-terminal domain that binds β1-integrin, followed by a FERM (band 4.1, ezrin, radixin, moesin homology) domain, a catalytic kinase domain, and a FAT (focal adhesion targeting) domain near its C terminus. Also present are the three Pro-rich regions PRI, PRII, and PRIII, which also serve as the sites for the attachment of a number of adaptors and/or regulatory proteins (such as c-Src, PI-3K, PTEN, $p130^{Cas}$) after activation of FAK via one or several of its putative phosphorylation sites (e.g., Tyr-397, -407, -576, -577, -861, and -925). Members of the Src kinase family, such as c-Src and c-Yes, consist of four Src homology (SH) domains, SH1 to SH4 (Xu et al., 1997; Chong et al., 2005). The SH4 domain near the N terminus of Src kinase contains the myristoylation and membrane-localization site and a unique domain of 50 to 70 amino acid residues that has no similarity among members of the Src kinase family, thus making each Src kinase member a unique protein. SH2 and SH3 domains are involved in the interaction with phosphorylated Tyr residues of other proteins and Pro-rich regions, respectively. For instance, FAK interacts with c-Src/c-Yes at its SH2 domain. The SH1 domain is the catalytic kinase site. c-Src interacts with several BTB proteins: occludin, N-cadherin, CAR, desmoglein-2, connexin-43, plakophilin-2, β-catenin (Lee and Cheng, 2005; Wang et al., 2007; Li et al., 2009b; Lie et al., 2010b), and myotubularin-related protein 2 (Zhang et al., 2005), conferring proper phosphorylation status in many of the integral membrane proteins at the BTB to regulate cell adhesion. Src has two important phosphorylation sites at Tyr-530 and Tyr-419 near its C terminus. Upon phosphorylation of Tyr-530, Src assumes an inactive locked conformation via interaction between the SH3 and the SH1 (kinase) domain; however, dephosphorylation of Tyr-530 and autophosphorylation of Tyr-419 within the catalytic kinase domain induce Src to assume an active open conformation, making its catalytic domain active to induce Tyr phosphorylation of its substrates. Src and FAK form a functional dual kinase complex to affect multiple cellular functions (Brunton and Frame, 2008; Aleshin and Finn, 2010; Bolós et al., 2010; Cabodi et al., 2010a,b), including the testis (Yan and Cheng, 2006).

BTB above preleptotene spermatocytes in transit can be partially maintained when "old" BTB integral membrane proteins are transcytosed and recycled to assemble the "new" BTB behind transiting spermatocytes (Fig. 9). In this section, these recent findings are critically discussed, and we also propose a molecular/biochemical model in Fig. 9.

2. Desmosome. In the testis, the desmosome is a cell-cell intermediate filament-based anchoring junction, a strong and flexible adhesive junction (Getsios et al., 2004; Green and Simpson, 2007; Bass-Zubek et al., 2009; Green et al., 2010; Thomason et al., 2010; Lie et al., 2011a; Mruk and Cheng, 2011) (Table 3). In the testis, it is found most predominantly at the Sertoli-spermatid (from step 1 to 7 spermatids) interface and at the BTB between adjacent Sertoli cells (Figs. 3, 6, and 7). Proteins that constitute the desmosome can be classified into three groups with five major components as follows: 1) the desmosomal cadherins: desmogleins and desmocollins; 2) the plakin family cytolinker desmoplakin, and 3) the arm (armadillo) proteins: plakoglobin and plakophilin (Table 3; Figs. 3 and 7). A recent study has shown that many of these components are found in the testis, associating with either Sertoli or germ cells, or with both cell types, in the seminiferous epithelium (Lie et al., 2010b). On the basis of electron microscopy studies, it was reported that desmosomes found in the testis possessed the ultrastructural features of both the desmosome and gap junction and were designated desmosome-like junctions (Russell, 1977a). However, it is noted that putative gap junctions are also found in the testis (Enders, 1993; Vogl et al., 2008; Li et al., 2011a). However, because desmosomal cadherins, desmogleins, and desmocollins are incapable of forming connexons similar to connexins (e.g., connexin-43, connexin-33) to create functional hemichannels and/or gap junction channels so that the terminology of desmosome should be kept as it is in other organs, such as the skin and heart. Surprisingly, there are very few reports in the literature investigating the functions of desmosomes in the testis until recently (Lie et al., 2011a; Mruk and Cheng, 2011), possibly because of the absence of specific antibodies against many desmosomal component proteins. There are different functional domains within desmosomal cadherin desmogleins (Lie et al., 2011a). Using this information, we prepared a specific antibody against the intracellular proline-rich linker and repeat unit domain of rat desmoglein-2, which was used to investigate the function of desmosomes at the BTB (Lie et al., 2010b). Desmoglein-2 was found to partially colocalize with the basal ES protein N-cadherin and the TJ protein ZO-1 at the Sertoli cell BTB; it is noteworthy that desmoglein-2 was also found to interact structurally with c-Src, in addition to plakoglobin by coimmunoprecipitation (Lie et al., 2010b), illustrating that it may be a substrate of c-Src. It is possible that its phosphorylation status can be altered by c-Src to affect adhesion at the BTB. Although the knockdown of desmoglein-2 alone by RNAi using specific siRNA duplexes failed to compromise Sertoli cell TJ permeability barrier function, the silencing of desmoglein-2 induced mislocalization of ZO-1, but not occludin or N-cadherin, at the Sertoli-Sertoli cell interface, moving these proteins away from the plasma membrane and into the cell cytosol (Lie et al., 2010b). It is noteworthy that the simultaneous knockdown of desmoglein-2 and desmocollin-2 in

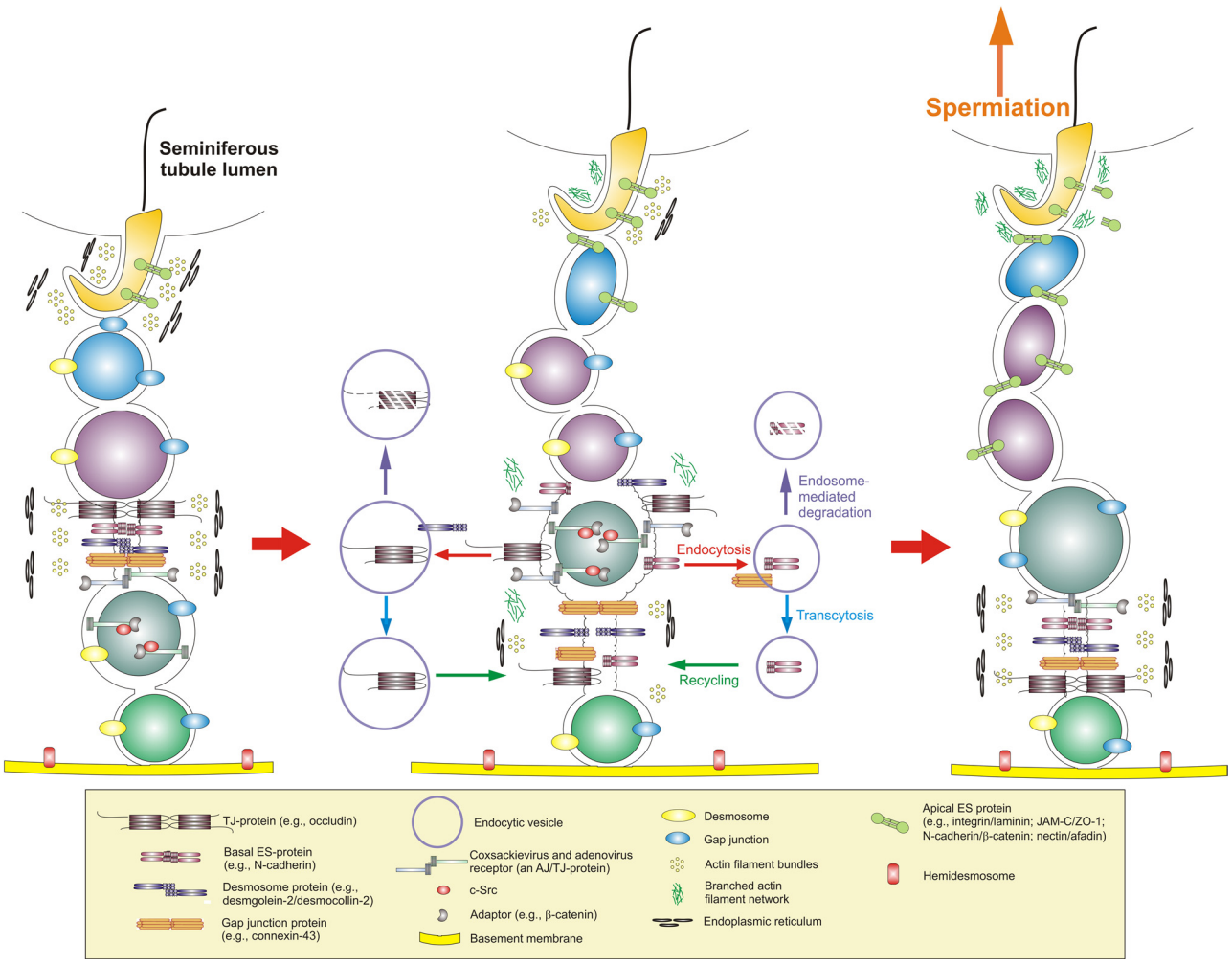

FIG. 9. A current model illustrating the maintenance of the immunological barrier integrity during the transit of preleptotene spermatocytes at the BTB during spermatogenesis. This model was prepared based on recent findings in the field as discussed in section IV. Left, schematic drawing of a tubule at stage VII of the epithelial cycle with an intact BTB above a preleptotene spermatocyte differentiated from a type B spermatogonium, showing several adhesion protein complexes of TJ, basal ES, desmosome, and gap junction. The typical actin filament bundles sandwiched in between cisternae of endoplasmic reticulum and the apposing Sertoli cell plasma membranes (or apposing Sertoli cell-elongating spermatid) are also shown at the BTB or at the apical ES. At late stage VII to early VIII of the epithelial cycle (center), cytokines (e.g., TGF-β3, TNFα) and testosterone induce endocytosis of integral membrane proteins (and/or their adaptors) (possibly also mediated by changes in their phosphorylation status induced by FAK and/or Src), so that these proteins are internalized, destabilizing the old BTB site to open up the TJ barrier for the transit of preleptotene spermatocytes. This endocytic vesicle-mediated protein trafficking event is facilitated by the concomitant action of polarity proteins (e.g., PAR6, 14-3-3, PAR3) and the combined action of Arp2/3 protein complex and Eps8. Some of the endocytosed proteins will be targeted for degradation, but others will be transcytosed and recycled to the new BTB site behind the transiting preleptotene spermatocyte. This establishment of a new BTB is also facilitated by de novo synthesis of BTB proteins mediated by testosterone. Also, the transit of spermatocytes across the BTB is facilitated because some of the integral membrane proteins at the BTB are also found in these germ cells (e.g., CAR), so that these proteins can form homotypic interactions between the transiting spermatocytes and Sertoli cells at the BTB to disallow an opened BTB (center). Thus, as shown on the right, these BTB restructuring events that occur at stage VIII of the epithelial cycle concomitant with spermiation will not compromise the integrity of the immunological barrier conferred by the BTB. This model also demonstrates the presence of multiple targets for male contraceptive development. For instance, a disruption of the polarity proteins that are involved in the endocytic vesicle-mediated protein trafficking events would disable the transit of preleptotene spermatocytes at the BTB, halting spermatogenesis. Such action is likely to generate minimal side effects because the site of action is localized at the BTB microenvironment and not systemic.

the Sertoli cell epithelium in vitro (see Fig. 6) was shown to reversibly disrupt the Sertoli cell TJ permeability barrier (Lie et al., 2010b), which also induced mislocalization of c-Src, ZO-1, occludin, and CAR [coxsackievirus and adenovirus receptor, an integrated component of the BTB and the apical ES (Mirza et al., 2006; Mirza et al., 2007; Wang et al., 2007)], moving from cell-cell interface to cell cytosol (Lie et al., 2010b), thereby destabilizing the Sertoli cell TJ barrier. More important, the use of a biochemical endocytosis assay indeed confirmed that the knockdown of both desmoglein-2 and desmocollin-2 could accelerate the internalization of CAR (Lie et al., 2010b). These findings are significant, because they demonstrate for the first time that desmosomes at the BTB confer more than an intermediate filament-based cell adhesion function; they indeed coordinate the function of other junctions at the BTB, such as the CAR-, occludin-, and/or N-cadherin-based adhesion protein complexes at the TJ and the basal ES, possibly via the association of desmosomal proteins (e.g., desmoglein-2)

TABLE 3
Desmosomal proteins found in the rat testis

Three classes of desmosomal proteins, desmosomal cadherins (A), plakins (B), and armadillo proteins (C) are found in the desmosome, which consist of five components: desmoglein (1), desmocollin (2), desmoplakin (3), plakoglobin (4) (also known as γ-catenin), and plakophilin (5). The presence of many of these desmosomal component proteins in Sertoli and/or germ cells was identified by reverse transcription-polymerase chain reaction and/or immunoblotting (Lie et al., 2010b). Sertoli cells were isolated from 20-day-old rat testes and germ cells were from adult rat testes; these cells had negligible contamination of other cells and did not come into contact with other cell types, including Leydig and peritubular myoid cells when assessed by immunoblotting using corresponding cell markers (Lie et al., 2010b).

Desmosomal Proteins	Sertoli Cells	Germ Cells
A. Desmosomal cadherins		
1. Desmoglein		
Desmoglein-1	−	+
Desmoglein-2	+	+
Desmoglein-3	−	−
Desmoglein-4	−	+
2. Desmocollin		
Desmocollin-1	−	+
Desmocollin-2	+	+
Desmocollin-3	+	−
B. Plakins		
3. Desmoplakin	+	+
C. Armadillo proteins		
4. Plakoglobin	+	+
5. Plakophilin		
Plakophilin-1	+	+
Plakophilin-2	+	+
Plakophilin-3	−	−
Plakophilin-4	+	+

+, presence; −, absence.

with c-Src, serving as a platform for signal transduction at the BTB (Lie et al., 2011a; Mruk and Cheng, 2011). However, much work is needed to define the role of c-Src (Figs. 8 and 9) in desmosome function at the BTB, and the precise mechanism(s) by which the desmoglein-2-c-Src protein complex regulates endocytic vesicle-mediated protein trafficking events at the BTB also needs to be better defined.

2. Gap Junction. The presence of gap junction at the BTB has been known for decades (Vogl et al., 2008), and its significance in testicular function (Enders, 1993; Pelletier, 1995; Pointis et al., 2005; Brehm et al., 2007; Gilleron et al., 2009a,b; Carette et al., 2010b; Pelletier et al., 2011), particularly in spermatogenesis (Pointis et al., 2010; Li et al., 2011a), has been extensively reviewed. However, the role of gap junction at the BTB as a signaling platform to confer cross-talk between different junction types to coordinate preleptotene spermatocyte migration at stage VIII of the epithelial cycle has only become known recently (Li et al., 2009b; Li et al., 2011a). Gap junction is an actin-based cell-cell communication junction found mostly at the BTB coexisting with basal ES but also at the apical ES between Sertoli cells and pre-step 8 spermatids (Figs. 1 and 10, A–C) (Cheng and Mruk, 2002; Vogl et al., 2008; Pointis et al., 2010; Li et al., 2011a). In this context, it is noteworthy that gap junction proteins, such as Cx43 and Cx26, are also integrated components of the apical ES (Cheng and Mruk, 2010a; Lie et al., 2011a). The gap junction is composed of aggregates of connexons between adjacent epithelial cells (Nakagawa et al., 2010; Li et al., 2011a; Maeda and Tsukihara, 2011) (Fig. 10C). Each connexon (Fig. 10B) is composed of a hexamer of gap junction proteins [known as connexins (Cx) (Fig. 10A), such as Cx43, Cx33, and Cx26, which are integral membrane proteins found in the testis (Li et al., 2011a); each connexin consists of four transmembrane domains, two extracellular domains, and three intracellular domains (Fig. 10A)] either of the same (monomeric) or different (heteromeric) type(s) (Fig. 10B). A connexon found in a cell by itself is a hemichannel, which can be either homomeric or heteromeric (Fig. 10B), whereas gap junction channel refers to connexons coupled between apposing cells, which can be homo- or heterotypic (Fig. 10C) (Alberts et al., 2002; Meşe et al., 2007; Li et al., 2011a). Small molecules and/or chemicals (e.g., inorganic ions, ATP, cyclic nucleotides, siRNA duplexes, glucose, polypeptides) can pass through these channels to coordinate different cellular events in an epithelium in response to changes in the environment (e.g., toxicants, temperature, pH, growth factors) (Loewenstein, 1981; Goldberg et al., 1999; Valiunas et al., 2005; Harris, 2007; Meşe et al., 2007).

Cx43, plakophilin-2 (a peripheral adaptor of desmosomal proteins and also connexins in the testis) (Table 3), and c-Src were recently shown to form a regulatory protein complex at the BTB in adult rat testes (Li et al., 2009b) (Fig. 7). This finding is consistent with an earlier report that Cx43-ZO-1-Src form a functional complex at the gap junction plaques in the cell epithelium of the 42GPA9 Sertoli cell line (Gilleron et al., 2008). Knockdown of Cx43 alone in Sertoli cells by RNAi did not interfere with Sertoli cell TJ permeability barrier function (Li et al., 2009b), consistent with a recent study reporting that the BTB remains intact in Sertoli cell-specific Cx43 knockout (KO) mice when examined by electron microscopy (Carette et al., 2010b), even though these mice were infertile. This was because spermatogonia failed to differentiate beyond type A to initiate spermatogenesis (Brehm et al., 2007), and Sertoli cells also failed to mature but continued to proliferate well into adulthood so that clusters of Sertoli cells were found in the tubule lumen in Cx43(−/−) mice (Sridharan et al., 2007). However, simultaneous knockdown of Cx43 *and* plakophilin-2 by RNAi was shown to perturb the Sertoli cell TJ barrier, which was mediated, at least in part, by changes in protein localization at the TJ (e.g., occludin and ZO-1) and basal ES (e.g., N-cadherin, CAR), which caused these proteins to move away from the cell surface and into the cytosol, probably resulting from an increase in protein endocytosis, thereby destabilizing the BTB (Li et al., 2009b). Indeed, recent studies have demonstrated that endocytic vesicle-mediated protein trafficking events are actively involved in the molecular remodeling of the gap junction plaque in the 42GPA9 Sertoli cell line via protein endocytosis (Gilleron et al., 2008, 2009b; Carette et al., 2009), possibly via a phosphorylation-independent mechanism (Carette et al., 2010a). However, some of the findings derived from studies using the

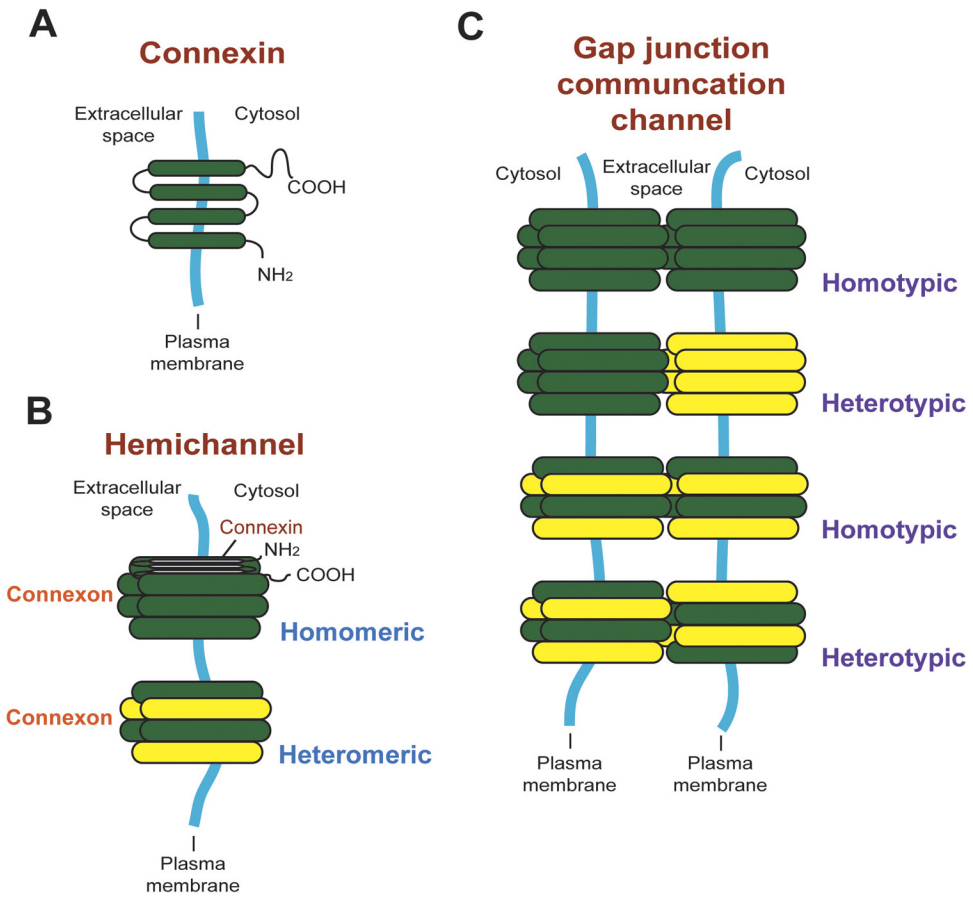

FIG. 10. Schematic illustration on the molecular architecture of connexin, connexon, hemichannel, and gap junction communication channel in gap junction. A, a typical connexin (e.g., Cx43, Cx26, Cx33) is composed of four transmembrane domains, two extracellular loops, one intracellular loop, and the intracellular N- and C-terminal tails. The C-terminal region confers most of the distinctiveness among connexins, which also contains phosphorylation sites for activation and inactivation and for interactions with binding partners (e.g., ZO-1, c-Src). B, an uncoupled functional connexon (also known as hemichannel) is composed of six connexins, which can be of the same (homomeric) or different (heteromeric) types. C, two coupled and compatible connexons create a gap junction communication channel between two adjacent Sertoli or Sertoli-germ cells, which can be homotypic or heterotypic.

42GPA9 Sertoli cell line will need to be verified using primary Sertoli cell cultures, because Sertoli cell lines may be regulated differently from differentiated and nonproliferating primary Sertoli cells (Fig. 6). For instance, Sertoli cell lines (e.g., MSC-1 Sertoli cell line) lack the immunosuppressive properties of primary Sertoli cells (Dufour et al., 2008). Nonetheless, studies that use the 42GPA9 Sertoli cell line are still very significant because they illustrate that gap junction dynamics are regulated by endocytic vesicle-mediated trafficking events. In short, the effects of Cx43 knockdown in primary Sertoli cells that impede protein distribution at the cell-cell interface can possibly be mediated by c-Src, which alters the phosphorylation status of these proteins (e.g., Cx43, plakophilin-2) at the site, causing the "closing" of the gap junction communicating channel (Li et al., 2009b). In fact, treatment of Sertoli cells having a functional TJ barrier with bisphenol A (BPA) was shown to perturb gap junction communication when assessed by a dye-transfer assay (Li et al., 2010). Likewise, gap junction communication was also shown to be perturbed when the 42GPA9 Sertoli cell epithelium was treated with 17α-ethynylestradiol (Tramoni et al., 2009). Studies have shown that Cx43 *alone* is crucial for the *reassembly* of a disrupted Sertoli cell BTB using the Ca^{2+}-switch model and the bisphenol A model, suggesting that the Cx43-based gap junction may be mediating intercellular communication during junction restructuring in the epithelium during spermatogenesis (Li et al., 2010). In short, these findings show that the gap junction plays a critical role to maintain the integrity of other junction types at the BTB, such as the TJ and/or the basal ES, so that their adhesive protein complexes can be properly localized to maintain BTB integrity. However, the mechanism(s) underlying this critical function of the gap junction is not known. For instance, is c-Src the cornerstone to coordinate signals downstream of the gap junction protein complex, thereby eliciting proper cross-talk among the TJ, basal ES, and desmosome? What are the identities of the chemical signals that pass through gap junction communication channels to mediate these effects at the BTB? These questions should be carefully addressed in future studies.

4. Tight Junction (Zonula Occludens) and Basal Ectoplasmic Specialization. At the BTB, the TJ coexists with the basal ES in the seminiferous epithelium near the basement membrane (Figs. 2, 3, and 7), so these two ultrastructures are discussed together. As shown in Fig. 3, the ultrastructural features of the basal ES (i.e., tightly packed actin filament bundles sandwiched between cisternae of the endoplasmic reticulum and the apposing plasma membranes of two Sertoli cells) is almost identical to the apical ES except that these features are found within both Sertoli cells, whereas they are limited only to the Sertoli cell side at the apical ES (Vogl et al., 2008; Cheng and Mruk, 2010a; O'Donnell et al., 2011). Because the basal ES coexists with the TJ, it remains difficult to identify proteins that are unique to the basal ES because putative TJ proteins (e.g., occludins, claudins, JAM-A, JAM-B) colocalize to the same site as basal ES proteins (e.g., N-cadherin, β-catenin) (Yan and Cheng, 2005). Nonetheless, it is very likely that proteins that are components of the apical ES are also found at the basal ES (Table 1). However, several important adhesion proteins (e.g., β1-integrin, laminin-α3, laminin-γ3, JAM-C) are restricted to the apical ES, whereas others are limited to the basal ES (e.g., JAM-A). In addition, many proteins are common to both the apical and basal ES (e.g., N-cadherin, E-cadherin, β-catenin, α-catenin, nectins, afadins, JAM-B, CAR) (Table 1). For example, N-cadherin (a basal ES protein) and occludin (a TJ protein) localize to the same site at the BTB, but they do not have direct protein-protein interaction, as confirmed by coimmunoprecipitation; instead, they are structurally linked via their corresponding peripheral adaptors α-catenin and ZO-1, respectively, possibly at stages I to VII and IX to XIV, but not at stage VIII of the epithelial cycle, which creates an "engaged" state (Yan and Cheng, 2005). Thus, ultrastructures, such as actin filament bundles, at the basal ES can be used to "reinforce" the cell-adhesive function of the TJ at the BTB. However, when testes are exposed to a toxicant (e.g., adjudin) or when the seminiferous epithelium is at stage VIII of the epithelial cycle when the BTB undergoes restructuring to accommodate the transit of preleptotene spermatocytes, α-catenin and ZO-1 become "disengaged" and do not physically interact with each other, such that a disruption of protein-protein interactions within the occludin-ZO-1 complex *or* the N-cadherin-α-catenin complex at the TJ or basal ES will not elicit an "immediate" disruption of the other and vice versa. This "engagement" and "disengagement" mechanism thus provides a novel mechanism to segregate the events of TJ and basal ES disruption (Yan and Cheng, 2005; Yan et al., 2008c) via a logical transition from an "assembled," "disassembling," and then "disassembled" state at the "old" BTB situated above preleptotene spermatocytes. This provides enough time for "new" TJ fibrils to be assembled behind transiting spermatocytes to establish the "new" BTB (see sections below for details) to avoid disruption of the immunological barrier, even transiently, during the seminiferous epithelial cycle of spermatogenesis (Fig. 9). The detailed biochemical and molecular events involving cytokines (e.g., TGF-β3, TNFα), sex steroids (e.g., testosterone, estradiol-17β), and endocytic vesicle-mediated intracellular trafficking (e.g., endocytosis, transcytosis, recycling) that regulate BTB dynamics during spermatogenesis will be summarized in sections IV.B, IV.C, and IV.D. On this note, it is important to examine in future studies the precise physiological role of the basal ES in BTB permeability function in addition to its structural role. For instance, can a selective knockdown of basal ES function impede the Sertoli cell TJ permeability barrier? Can knockdown of a basal ES adhesive protein (e.g., N-cadherin, nectin) impede the distribution or arrangement of actin filament bundles at the site?

B. Steroids

1. Testosterone. Testosterone is produced by Leydig cells located in the interstitium under the influence of LH, and it is one of the most important regulators of spermatogenesis in the hypothalamic-pituitary-testicular axis (Sharpe, 1994; Zirkin, 1998; McLachlan et al., 2002; Walker, 2010) in parallel to estradiol-17β produced by Leydig, Sertoli, and germ cells (O'Donnell et al., 2001; Carreau et al., 2009, 2010; Carreau and Hess, 2010). It is noted that the intratesticular testosterone level, such as in the seminiferous tubule fluid, is maintained at ~100 times the level found in the systemic circulation in both humans and rodents (10^{-7} versus 10^{-9} M) (Turner et al., 1984; Jarow and Zirkin, 2005) to sustain spermatogenesis. Earlier findings coupled with studies using transgenic mouse models have demonstrated that androgens are crucial in the regulation of almost every aspect of spermatogenesis, including spermatogonial proliferation and differentiation, germ cell cycle progression through meiosis, spermiogenesis, spermiation, and cell adhesion at the Sertoli-Sertoli and Sertoli-germ cell interface in the seminiferous epithelium (Wang et al., 2009; Ruwanpura et al., 2010; Verhoeven et al., 2010; O'Donnell et al., 2011) and to confer immune privilege in the testis (Meng et al., 2011). These effects are mediated via androgen receptor (AR) using genomic and/or nongenomic pathways (Walker, 2009, 2010; Lamont and Tindall, 2010; Shupe et al., 2011). For instance, it was shown that Sertoli cell-selective KO of AR in mice led to infertility manifested by meiotic arrest (Chang et al., 2004; De Gendt et al., 2004). Furthermore, Sertoli cell-specific AR-KO mice displayed a defective BTB, which was associated with a reduced expression of claudin-11, ZO-1, occludin, and gelsolin but with a significantly enhanced expression of vimentin (Wang et al., 2006; Willems et al., 2010a; Willems et al., 2010b). In addition, it was noted that Sertoli cell maturation and Sertoli cell polarization were also perturbed in these Sertoli cell-specific AR-KO mice, and that JAM-C (an

apical ES marker that is also restricted to the apical ES) was shown to be significantly reduced (Willems et al., 2010a), illustrating that androgens are crucial to both apical and basal ES function. It is noteworthy that in $AR^{flox(ex1\text{-}neo)/Y}$ mice that had a partial defect in androgen sensitivity by carrying this floxed allele, a marked reduction in AR protein levels in different tissues including the testis was observed, as well as a defect in spermiogenesis, and these mice were infertile (Holdcraft and Braun, 2004). Moreover the BTB in $AR^{flox(ex1\text{-}neo)/Y}$/*AMH-Cre* mice was also disrupted, possibly the result of a reduced expression of claudin-3 (Meng et al., 2005). It is noteworthy that peritubular myoid cell-selective AR-KO mice were also azoospermic and infertile with an ~86% reduction in germ cell number in particular elongating/elongated spermatids, and the testes of these myoid cell-specific AR-KO mice were marked by reduced seminiferous tubule fluid production, as well as reduced expression of androgen regulated genes by Sertoli cells (Welsh et al., 2009). However, proliferation of germ cells in normal fetal mice, known as gonocytes, at 15.5 days postcoitus (dpc) was found to be inhibited by androgens (Merlet et al., 2007). Furthermore, testicular feminized (*Tfm*) mice (mice with a testicular feminization mutation caused by a frame-shift mutation in the AR mRNA, lacking functional AR) were found to possess significantly more gonocytes per testis, and gonocytes in these *Tfm* mice had significantly higher mitotic capacity versus wild-type mice when assessed by 5-bromo-2′-deoxyuridine incorporation; yet the proliferation of gonocytes from both *Tfm* and normal mice was significantly reduced by dihydrotestosterone (10^{-6} M) at 15.5 dpc but not at 16.5 dpc (Merlet et al., 2007). Thus, these findings illustrate that although testosterone has a promoting effect on germ cell maturation in adult mice, there is a narrow window in the testis when it comes to fetal development in which testosterone can inhibit gonocyte proliferation at ~15 dpc.

A recent study in which adult rats treated with acyline [a gonadotropin-releasing hormone antagonist and a potent suppressor of circulating gonadotropins, such as FSH and LH; testosterone in the systemic circulation, and of intratesticular testosterone in both rodents and humans (Rivier et al., 1995; Herbst et al., 2002; Zhou et al., 2010)] was shown to disrupt BTB integrity, which could be rescued in part by FSH replacement but more significantly by human chorionic gonadotropin (McCabe et al., 2010), illustrating the significance of androgens in the maintenance of BTB integrity in adult rats. In addition, testosterone and FSH were also shown to be crucial to maintain the proper distribution of claudin-11 and JAM-A at the BTB in rodent testes (McCabe et al., 2010).

Other studies using Sertoli cells cultured in vitro (Byers et al., 1986; Janecki and Steinberger, 1986; Kaitu'u-Lino et al., 2007; Yan et al., 2008b) have also demonstrated the role of androgens in conferring BTB integrity, because the presence of androgens was shown to promote the assembly of the Sertoli cell TJ-permeability barrier and to lessen the cadmium-induced disruptive effects on the Sertoli cell TJ barrier function (Janecki et al., 1992; Chung and Cheng, 2001). Testosterone was also shown to stimulate the steady-state levels of occludin (Kaitu'u-Lino et al., 2007; Yan et al., 2008b) and claudin-11 (Kaitu'u-Lino et al., 2007) in the Sertoli cell epithelium *and* the proper localization of these proteins at the Sertoli-Sertoli cell interface. In addition, the use of 2,3-dihydro-*N*,*N*-dimethyl-2-oxo-3-[(4,5,6,7-tetrahydro-1*H*-indol-2-yl)methylene]-1*H*-indole-5-sulfonamide (SU6656), a selective inhibitor of c-Yes (a nonreceptor protein tyrosine kinase and a member of the Src kinase family) that blocked the function of c-Yes at the Sertoli cell BTB that led to a disruption of the TJ barrier could also be rescued by testosterone (Xiao et al., 2011), demonstrating the protective function of androgens at the BTB. Collectively, these findings illustrate unequivocally the promoting effects of androgens on the Sertoli cell BTB assembly, maintenance, and integrity both in vitro and in vivo, mediated, at least in part, via their actions on the maintenance of the steady-state levels of integral membrane proteins (e.g., occludin, claudin-11) and adaptors (e.g., ZO-1) at the site, as well as by enforcing the integrity of actin filament bundles at the basal ES (Xiao et al., 2011). Furthermore, recent studies have shown that testosterone also regulates BTB integrity via its effects on endocytic vesicle-mediated protein endocytosis, transcytosis, and recycling (Yan et al., 2008b; Su et al., 2010b), such as by "relocating" integral membrane proteins at the "old" BTB site above preleptotene spermatocytes in transit to the "new" BTB site behind these cells via transcytosis so that the immunological barrier can be maintained at stage VIII of the epithelial cycle (Cheng and Mruk, 2010a; Su et al., 2010b).

2. Estradiol-17β. In adult mammalian testes, testosterone (a C19 steroid) is irreversibly converted to estradiol-17β (a C18 steroid) by the aromatase complex in Leydig cells (but mostly in Sertoli cells in immature rats). This complex is composed of 1) an ubiquitous NADPH-cytochrome P450 reductase and 2) a specific cytochrome P450 aromatase (P450arom), which contains a heme and a steroid binding pocket (O'Donnell et al., 2001; Hess, 2003; Carreau and Hess, 2010). It was first reported that P450arom was present in immature germ cells in the mouse, including round spermatids, elongating spermatids, and elongated spermatids (Nitta et al., 1993). Since then, P450arom was demonstrated in both immature germ cells and ejaculated spermatozoa from rodents, bear, primates, and humans (Carreau et al., 2010). In short, Leydig cells are an important source of estrogens in adult testes; however, germ cells also account for ~60% of the aromatase activity in the testis, contributing significantly to the pool of estrogens, which is needed to maintain testicular

function (Carreau and Hess, 2010; Carreau et al., 2010). Estrogen exerts its biological functions via its interaction with the estrogen receptor (ER) (ERα and ERβ receptors are both found in the testis). This ligand/receptor interaction leads to conformational changes, altering ER interaction with 1) kinases and scaffolding adaptors in the cytoplasm to regulate cell signaling cascades (extranuclear nongenomic action) and/or 2) DNA to control a repertoire of transcription factors, coregulators, and other auxiliary proteins associating with ER to alter the expression of various genes (genomic action) (Cheskis et al., 2007; Watson et al., 2007). In adult mammalian testes, ERα is mostly restricted to Leydig and peritubular myoid cells and is weakly expressed in Sertoli cells, whereas ERβ is mostly restricted to Sertoli cells and weakly expressed in Leydig cells but abundantly expressed in spermatocytes, even weaker in round and elongating spermatids, but almost none in elongated spermatids (Carreau and Hess, 2010). The combined nongenomic and genomic actions of estrogens thus confers cell-specific function in the testis. Recent studies have also identified a third estrogen receptor: GPR30 (G-protein-coupled receptor, a transmembrane intracellular estrogen receptor) in rat pachytene spermatocytes and round spermatids that regulates germ cell apoptosis and differentiation (Chimento et al., 2010, 2011). GPR30 and ERα were also shown to be strongly expressed by Sertoli cells cultured in vitro and activated by estrogen treatment in immature rats (Lucas et al., 2008).

Estrogen is crucial to male reproductive function, including the male reproductive tract (e.g., efferent ductules, epididymis), testis, spermatogenesis, and the BTB (Li et al., 2009d; Carreau and Hess, 2010; Carreau et al., 2010; Joseph et al., 2010a,b, 2011; Cheng et al., 2011a; Hess et al., 2011). A role for estrogen as a locally acting male hormone in the testis was first demonstrated in adult mice deficient in ERα (Hess et al., 1997). These ERα-KO mice were infertile largely because of a defect in fluid reabsorption by efferent ductules of the epididymis, thereby disrupting spermatogenesis in the seminiferous epithelium (Hess et al., 1997). However, it is noteworthy that despite the loss of 1) ERα alone (Eddy et al., 1996; Hess et al., 1997) or ERα alone in somatic cells (Mahato et al., 2000, 2001), 2) ERβ alone (Krege et al., 1998; Antal et al., 2008), or 3) both ERα and ERβ (Dupont et al., 2000) in mice, or in rats treated with ERα agonist (16α-lactone-estradiol 2) or ERβ agonist (8β-vinyl-estradiol 2) (Allan et al., 2010), spermatogenesis developed normally. Even though ERα(−/−) adult mice were infertile [but not ERβ-null mice, because ERβ(−/−) mice exhibited no compromised fertility (Couse et al., 2001)]. But this was the result of defects in the reabsorption of luminal fluid in efferent ductules that led to pressure build-up in tubules, thereby adversely affecting the seminiferous epithelium instead of a direct effect on spermatogenesis (Hess et al., 1997). It is noteworthy that P450arom(−/−) mice [by deleting the *Cyp19* gene encoding P450arom (i.e., lacking estrogen synthesis entirely) without any loss of gonadotropins or androgens in the systemic circulation and/or the testis] were unable to synthesize endogenous estrogens, but these mice remained fertile until 18 weeks of age when postmeiotic defects began to show up, and these KO mice also had a reduced number of round and elongated spermatids as a result of spermiogenesis arrest by 1 year of age postpartum (Robertson et al., 1999). Still, spermatogenesis continued to function normally, at least in some tubules (Robertson et al., 1999). Rodents that have normal spermatogenesis in only 10% of their tubules are known to still be fertile (Robaire, 2003); thus, a loss of estrogen-synthesizing capability in these null mice failed to induce complete infertility. Thus, other factors, perhaps phytoestrogens and isoflavones [or even pseudoestrogens, such as diethylstilbestrol (DES), found in drinking water] or standard rodent diet, may have contributed to the replacement of the lost estrogens in these P450arom-null mice, maintaining spermatogenesis in some tubules. This possibility was indeed supported by a subsequent study in which P450arom-null mice fed on a soy-free diet indeed became infertile after 1 year with postmeiotic defects, incapable of completing spermiogenesis (Robertson et al., 2002). In addition, mice with mutations in the kisspeptin signaling pathway [e.g., Kiss1(−/−) or Gpr54(−/−) mice; kisspeptins are a family of peptide hormones that, together with the kisspeptin receptor (previously known as G-protein-coupled receptor 54), play a critical role in the regulation of the hypothalamic-pituitary-testicular axis, such as in the release of 1) gonadotropin-releasing hormone from the hypothalamus and 2) LH and FSH from the pituitary gland (d'Anglemont de Tassigny and Colledge, 2010; Hameed et al., 2011)] were found to have smaller testes and impaired spermatogenesis versus wild-type animals, lacking spermatids in the epithelium as the result of postmeiotic defects. On the other hand, diets supplemented with phytoestrogens (e.g., genistein and daidzein from soy/soybeans) were shown to have a significant improvement in spermatogenesis over a 7-month period with regard to testis weight and the number of elongated spermatids and spermatozoa (Mei et al., 2011). In summary, these findings illustrate that estrogens support somatic cells in the testis and the epididymis, as well as regulate germ cell apoptosis and spermiogenesis, in particular the transition of spermatocytes into round spermatids (Carreau and Hess, 2010; Carreau et al., 2010).

The first evidence that estrogens play a role in regulating BTB function came from an earlier study in the 1970s in which neonatal rats were treated with either β-estradiol-3-benzyoate or clomiphene citrate (a selective estrogen receptor modulator). In this study, BTB assembly was found to be delayed by up to 1 week (Vitale et al., 1973). In normal rats, the BTB is established by postnatal day ~18 to 21 (i.e., ~3 weeks) (Vitale et al.,

1973; Bergmann and Dierichs, 1983; Russell et al., 1989; Toyama et al., 2001). However, when neonatal rats were treated with DES, a synthetic nonsteroidal estrogen (five doses of DES at 10 μg per male pup, on postnatal days 1, 3, 5, 7, 9 and 11), the BTB failed to be assembled by postnatal week ~3 (Toyama et al., 2001). Instead BTB assembly was delayed by ~4 to 5 weeks (i.e., until postnatal weeks ~7–8) because the basal ES was not detected by electron microscopy until postnatal day 56 (Toyama et al., 2001). In these DES-treated rats, meiosis also failed to occur; pachytene spermatocytes were the only germ cells found in the seminiferous epithelium and many of these spermatocytes underwent apoptosis. When the BTB was finally assembled by postnatal day 56, step-1 to -4 spermatids were also found in the seminiferous epithelium (Toyama et al., 2001). This delay in BTB assembly is probably caused by the inability of the Sertoli cell to cease to proliferate and to differentiate after DES treatment as reported in a similar study (Sharpe et al., 1998). Collectively, these findings thus illustrate that estrogens can impede Sertoli cell BTB function. In addition, the onset of meiosis is tightly associated with the establishment of a functional and intact BTB. This postulate is further supported by recent observations using rats treated with a high dose of adjudin (125 or 250 mg/kg b.wt., by gavage) in which adjudin-induced BTB disruption in rats was shown to impede spermatogonial differentiation; spermatogenesis failed to reinitiate in these rats even though there was no significant loss of spermatogonial stem cells and spermatogonia (Mok et al., 2011a). It is noteworthy that when adult rats were treated with adjudin at 50 mg/kg b.wt. (a low-dose group in the same study), although BTB integrity in these rats remained intact by ~2 to 3 weeks after treatment (Mruk and Cheng, 2004b; Su et al., 2010a; Mok et al., 2011a), testes in these rats later displayed a transient and *reversible* disruption of the BTB. It is noteworthy that the disrupted BTB detected by 4 to 6 weeks was "resealed" by 20 to 30 weeks. Once the BTB became functional, a reinitiation of spermatogenesis was observed, germ cells began to repopulate the once "voided" seminiferous epithelium in this low-dose group, and fertility rebounded (Mok et al., 2011a).

The role of estrogens in BTB function is best illustrated in a study in which adult rats treated with short-term doses of bisphenol A (an estrogenic environmental toxicant used primarily to make polycarbonate plastic and epoxy resins) (five doses at 10 or 50 mg/kg b.wt.) was found to have no effects on BTB integrity. However, BPA or estradiol-17β administered to neonatal rats disrupted the BTB (Li et al., 2009d) in vivo. The disruptive effects of estrogens on the Sertoli cell TJ permeability barrier were also confirmed using Sertoli cells cultured in vitro that had an established barrier that mimicked the BTB in vivo and with the ultrastructures of TJ, basal ES, and desmosome when examined by electron microscopy (Siu et al., 2005) (see Fig. 6). More important, it was found that the estrogen-induced disruptive effects on the Sertoli TJ barrier were mediated by changes in the distribution of integral membrane proteins at the BTB, such as occludin, N-cadherin, and connexin 43, which moved from the cell-cell interface and into the cell cytosol via an increase in protein endocytosis, thereby destabilizing the TJ barrier function (Li et al., 2009d). In addition, BPA was shown to disrupt gap junction function when cell-cell communication was assessed by a gap junction dye-transfer assay (Li et al., 2010). These findings are also in agreement with an earlier study demonstrating that estrogens (e.g., 17α-ethynylestradiol) and progestins (e.g., medroxyprogesterone acetate, levonorgestrel) are capable of blocking gap junction communication using the 42GPA9 Sertoli cell line (Tramoni et al., 2009). These findings thus suggest that at stage VIII of the epithelial cycle, when preleptotene spermatocytes are in transit at the BTB, aromatase highly expressed by spermatocytes (Carreau et al., 2010) might produce an elevated level of estradiol-17β in the microenvironment, which might induce BTB restructuring via an increase in protein endocytosis to destabilize barrier function at the "old" BTB site above preleptotene spermatocytes in transit, to facilitate the entry of spermatocytes into the apical compartment. In contrast, androgens released into the microenvironment, by either Sertoli cells and/or Leydig cells, may assist in the assembly of the "new" BTB below spermatocytes in transit. As such, the tightly regulated but coordinated effects of estrogens and androgens, together with cytokines [e.g., TNFα, TGF-β2/-β3, interleukin-1α (IL-1α)] and nonreceptor protein tyrosine kinases (e.g., FAK, c-Src, and c-Yes) (see Fig. 9) thus provide an efficient system to maintain BTB integrity during the seminiferous epithelial cycle of spermatogenesis.

C. Nonreceptor Protein Kinases: Focal Adhesion Kinase, c-Src, and c-Yes

1. Introduction. Studies in other epithelia and blood-tissue barriers have demonstrated that the phosphorylation status of integral membrane proteins, peripheral adaptors/kinases/phosphatases, and/or scaffold proteins at the AJ (e.g., N-catenin, β-catenin) (Gumbiner, 2000; Dejana et al., 2008), TJ (e.g., occludins, claudins) (Cheng and Mruk, 2002; González-Mariscal et al., 2008; Dörfel et al., 2009; Findley and Koval, 2009; Raleigh et al., 2011), gap junction (e.g., Cx43, keratins) (Magin et al., 2007; Hesketh et al., 2009; Solan and Lampe, 2009; Maeda and Tsukihara, 2011), and desmosome (e.g., desmocollins, desmogleins, plakoglobin) (Aoyama et al., 2009; Thomason et al., 2010; Lie et al., 2011a) play a critical role in determining adhesive function at the cell-cell interface. The phosphorylation status of these proteins and their peripheral adaptors/regulators is regulated by nonreceptor protein kinases and/or lipid kinases in response to changes in the environment, growth and development, growth factors, cytokines, inflammation, infection, and oxidative stress (Cheng and Mruk,

2002; Hawkins and Davis, 2005; Xia et al., 2005a; Suzuki and Hara, 2011). This, in turn, regulates their distribution in cell epithelia and endothelia via changes in protein endocytosis, recycling and transcytosis (Mruk and Cheng, 2010a; Stuible and Tremblay, 2010; Funakoshi et al., 2011). For instance, Tyr-398 and Tyr-402, in a highly conserved sequence of occludin in humans, YETDYTT (residues 398–404 from the N terminus), near its C terminus, are known to be the putative phosphorylation sites of c-Src. Deletion of this YETDYTT domain (a substrate of c-Src), or the site-directed mutation of Tyr-398 and Tyr-402 in this domain, also abolished c-Src-mediated phosphorylation (Elias et al., 2009). It was also shown that phosphorylation of Tyr-398 and Tyr-402 prevents the interaction of occludin and ZO-1, dissociating the occludin-ZO-1 complex, thereby destabilizing the TJ barrier in colon carcinoma cell line (Caco-2), fibroblast cell line (Rat-1) or kidney cell lines (MDCK) (Elias et al., 2009). These findings are thus in agreement with other studies illustrating the significance of phosphorylation on the assembly of TJ proteins (e.g., occludin, claudin-5, ZO-1) into TJ fibrils at the TJ barrier in *X. laevis* oocytes (Cordenonsi et al., 1997, 1999), small intestine (Goldblum et al., 2011), or brain endothelial cells (Yamamoto et al., 2008).

The role of protein phosphorylation conferred by kinases and phosphatases and its significance in junction integrity at the BTB, however, was not known until the early 2000s when it was first demonstrated that inhibitors and/or activators of protein kinases and phosphatases could "manipulate" Sertoli cell TJ permeability barrier function (Li et al., 2001). Subsequent studies have also illustrated that the phosphorylation status of proteins at the apical ES (e.g., β-catenin), likely regulated by the c-Src/MTMR2 (myotubularin-related protein 2) protein complex, indeed regulated the binding between N-cadherin and β-catenin (Xia and Cheng, 2005; Zhang et al., 2005). Likewise, the phosphorylation status of occludin conferred by FAK at the Sertoli cell BTB also determines the integrity of the occludin-ZO-1 complex by regulating adhesion via changes in protein endocytosis (Siu et al., 2009b). The lack of studies in the literature on the role of kinases in BTB function is largely due to the lack of information on the constituent components on different junction types at the BTB until recently (see Table 1). In sections IV.C.2. and IV.C.3, we provide a summary and critical discussion on the role of several protein kinases on Sertoli cell BTB function and how these kinases regulate BTB restructuring during the seminiferous epithelial cycle in concert with cytokines, actin regulators, and steroids. However, it must be noted that several other nonreceptor protein kinases [e.g., CK1 (casein kinase 1) and CK2, which are also found at the BTB associating with the N-cadherin/β-catenin complex but not with nectin/afadin (Lee and Cheng, 2005)] (Cordenonsi et al., 1997, 1999; Smales et al., 2003; Dörfel et al., 2009) and GTPases (e.g., Rho kinase) (Yamamoto et al., 2008), can also regulate the phosphorylation status of occludin, tricellulin, or claudin-5 and adhesion in other epithelia and blood-tissue barriers (e.g., the blood-brain barrier).

2. Focal Adhesion Kinase. Focal adhesion kinase (FAK), as its name implies, is a nonreceptor protein tyrosine kinase found predominantly at the focal adhesion complex (also known as focal contact, an actin-based cell-matrix anchoring junction type), known to be involved in cell adhesion and migration during development, growth, inflammation, and tumorigenesis (Brunton and Frame, 2008; Chatzizacharias et al., 2010; Frame et al., 2010; Golubovskaya, 2010; Ning et al., 2010; Schaller, 2010) (Fig. 8). Subsequent studies have shown, however, that FAK is ubiquitously expressed in mammalian cells and tissues (Boutros et al., 2008), and other studies have also identified FAK at the BTB, at the apical ES and at the actin-based cell-cell TJ and anchoring junctions (Cheng and Mruk, 2009b). In contrast to members of the Src family kinases, FAK does not possess a myristoylation site to anchor itself to the plasma membrane, and it also lacks putative Src homology 2 (SH2) and SH3 domains required for protein-protein interactions, even though FAK has multiple binding partners in epithelia, including the seminiferous epithelium (Cheng and Mruk, 2009b; Frame et al., 2010; Schaller, 2010) (Fig. 8). FAK, similar to Src kinases, is most notably known for its involvement in integrin-based signaling; in fact, β1-integrin binds to the N-terminal region of FAK (Boutros et al., 2008; Cheng and Mruk, 2009b) (Fig. 8). Thus, it is not surprising that FAK forms a bona fide complex with β1-integrin at the apical ES (Siu et al., 2003b; Beardsley et al., 2006), because $\alpha6\beta1$-integrin-laminin-$\alpha3\beta3\gamma3$ is a predominant cell adhesion complex at the apical ES (Palombi et al., 1992; Salanova et al., 1995; Siu and Cheng, 2004b; Yan and Cheng, 2006), and it is critically involved in the release of sperm from the seminiferous epithelium at spermiation (O'Donnell et al., 2011). A recent study has demonstrated that the β1-integrin-FAK protein complex at the apical ES activates the p130Cas (p130 Crk-associated substrate)-DOCK180 (Dedicator of cytokinesis 180)-RhoA-vinculin signaling pathway downstream to disrupt elongated spermatid adhesion at spermiation (Siu et al., 2011). FAK contains at least six putative phosphorylation sites at Tyr-397, -407, -576, -577, -861, and -925 for its activation; of these, Tyr-397 is the only autophosphorylated site (Ilić et al., 1997). p-FAK-Tyr-397 and p-FAK-Tyr-576 are predominantly localized to the apical ES, displaying a stage-specific expression, and are the highest at stage VIII just before spermiation (Siu et al., 2003b). At the BTB, FAK is predominantly expressed at stages III to VI of the epithelial cycle but it is considerably diminished at stages VIII to IX at the time of preleptotene spermatocyte transit at the site. More importantly, p-FAK-Tyr-397 was shown to be significantly induced during the assembly of the Sertoli cell

TJ-permeability barrier (Siu et al., 2009c), illustrating the involvement of FAK in the assembly and maintenance of the BTB. Both FAK and p-FAK-Tyr-397 are localized to the Sertoli-Sertoli cell interface (Siu et al., 2009c). FAK also colocalized with both occludin and ZO-1, forming a functional occludin-ZO-1-FAK complex. A surge in interaction between FAK and occludin was also detected during cadmium-induced BTB disruption even though a declining FAK steady-state level was noted (Siu et al., 2009b), which apparently was being used to induce aberrant phosphorylation of occludin to elicit mislocalization of occludin and ZO-1, moving these proteins away from the cell-cell interface and into the cytosol, as the result of enhanced protein endocytosis (Siu et al., 2009c).

The physiological significance of FAK in BTB function was best demonstrated in a study by RNAi to knockdown FAK in Sertoli cell epithelium with a functional TJ permeability barrier (Siu et al., 2009b). Although the knockdown of FAK using specific siRNA versus nontargeting siRNA duplexes was capable of silencing >50% of FAK expression in these Sertoli cells with no effects on the levels of occludin and ZO-1 in the same experiment, the Sertoli cell TJ permeability barrier was disrupted. This also associated with mislocalization of occludin and JAM-A, but not ZO-1, when these two integral membrane proteins moved away from the cell surface and into the cell cytosol, thereby destabilizing the TJ barrier (Siu et al., 2009b). More importantly, it was observed that knockdown of FAK also rendered these cells insensitive to cadmium, because cadmium failed to perturb Sertoli cell barrier function in FAK-silenced cells (Siu et al., 2009b), illustrating that FAK is also one of the cellular targets of cadmium toxicity in the testis. Much research is needed in future studies to assess whether a FAK inhibitor delivered locally to the testis can be used to protect against cadmium-induced testicular injury.

3. c-Src and c-Yes. The Src (sarcoma-inducing gene of *Rous* sarcoma virus) family of nonreceptor protein tyrosine kinases is composed of at least nine members in rodents: Src, Yes, Hck, Fyn, Fgr, Lyn, Lck, Blk, and Yrk. Of these, c-Src is the best studied Src kinase family member, and since being discovered y by Francis Peyton Rous in 1911, it has been implicated in oncogenesis (Martin, 2001, 2009; Yeatman, 2004; Aleshin and Finn, 2010) (Fig. 8). Src kinases are also involved in embryonic development, cell growth, cell movement, and actin-cytoskeletal dynamics and, similar to FAK, are central to integrin-based signaling, most notably, at the focal adhesion complex at the cell-matrix interface (Huveneers and Danen, 2009; Guarino, 2010; Tegtmeyer and Backert, 2011). Members of the Src kinase family all have characteristic SH domains, such as SH1, SH2, SH3 (a proline-rich domain), and SH4 used for protein-protein interactions (see Fig. 8), so that it can recruit multiple partners to a specific cellular site to affect multiple cellular functions (Engen et al., 2008; Aleshin and Finn, 2010). Each Src kinase member, including c-Src and c-Yes, consists of four distinctive domains: an N-terminal SH4 domain, followed by a SH3 domain, a central SH2 domain and a SH1 tyrosine kinase domain (Aleshin and Finn, 2010) (Fig. 8). In the testis, both c-Src (Lee and Cheng, 2005; Goupil et al., 2011) and c-Yes (Xiao et al., 2011) are localized to the BTB and the apical ES in the seminiferous epithelium of adult rat testes. Although c-Yes and c-Src are two closely related members of the Src kinase family and they share redundancy in their signaling functions, they have different cellular functions in multiple epithelia (Summy et al., 2003; Sato et al., 2009). For instance, c-Yes is known to regulate endocytic vesicle-mediated protein trafficking events, most notably transcytosis, because it is monopalmitoylated at its SH4 domain. Thus, it can take part in the transport of the Golgi pool of caveolin [a marker of transcytosis (Hansen and Nichols, 2010)] to the plasma membrane, whereas c-Src is nonpalmitoylated, and it can shuffle between the plasma membrane and the late endosome/lysosome to regulate protein endocytosis (Sato et al., 2009), such as endosome-mediated protein degradation. In addition, overexpression of c-Yes in human colorectal carcinoma cells was shown to promote cancer spread and metastasis but not tumor growth (Barraclough et al., 2007). Likewise, c-Src overexpression also did not promote tumor growth, but it enhanced cell detachment, causing a delay in the G_2 phase of the cell cycle, and inducing nonapoptotic cell death in these cells, thereby preventing cancer metastasis (Welman et al., 2006). These findings thus clearly illustrate differences in c-Src and c-Yes signaling functions. Other Src kinases, such as Hck (and its truncated form) and Lyn, are also detected in the testis based on immunohistochemistry experiments, and truncated Hck was found to localize predominantly to the apical ES (Bordeleau and Leclerc, 2008; Goupil et al., 2011); however, their role at the BTB remains unexplored.

a. c-Src and blood-testis barrier function. c-Src is most notably detected in the seminiferous epithelium at the apical ES at stages VII to VIII of the epithelial cycle (Lee and Cheng, 2005), but p-c-Src-Tyr-416 is the predominantly activated c-Src at the apical ES, associating with β1-integrin (Wong et al., 2005b; Zhang et al., 2005) and laminin α3-, β3-, and γ3-chains (Yan and Cheng, 2006). However, c-Src was also detected at the BTB, associating with N-cadherin at the basal ES (Lee and Cheng, 2005). It also structurally associated with FAK as demonstrated by coimmunoprecipitation (Yan and Cheng, 2006). Furthermore, c-Src structurally interacts with desmoglein-2 at the Sertoli cell BTB (Lie et al., 2010b). In addition, c-Src also forms a functional protein complex with MTMR2 [a member of the lipid phosphatase MTM family known to be involved in endocytic vesicle-mediated trafficking events (Mruk and Cheng, 2010a)], which also localized predominantly at the BTB

(Zhang et al., 2005). Collectively, these findings suggest that c-Src is an integrated component of occludin-, N-cadherin-, and desmoglein-2-based protein complexes at the BTB, possibly regulating cell adhesion via its ability to maintain and/or alter the phosphorylation status of either occludin, N-cadherin, and/or desmoglein-2, thereby regulating their kinetics of endocytic vesicle-mediated protein endocytosis, recycling, and/or transcytosis during spermatogenesis. This hypothesis should be further evaluated in future studies.

b. c-Yes and blood-testis barrier function. In contrast to c-Src, c-Yes is predominantly localized to the BTB in the seminiferous epithelium in a stage-specific pattern, but the most intense expression is at stages VIII to IX at the time of preleptotene spermatocyte transit, colocalizing with occludin and N-cadherin (Xiao et al., 2011). More important, c-Yes also forms a structural complex with FAK, occludin, and N-cadherin, but not with JAM-A or CAR at the BTB (Xiao et al., 2011), consistent with an earlier study reporting that c-Yes structurally interacts with occludin at the TJ in kidney MDCK cells (Chen et al., 2002). These findings illustrate that c-Yes can potentially regulate the phosphorylation status of N-cadherin- and occludin-based adhesion complexes (but not the JAM-A- or CAR-based adhesion protein complex) to confer cell adhesion. This possibility is supported by findings in other studies using MDCK and human intestinal T84 cells in which c-Yes forms a complex with occludin and dissociation of c-Yes from the occludin complex leads to dephosphorylation of occludin, disrupting the TJ permeability barrier (Nusrat et al., 2000; Chen et al., 2002). In fact, a blockade of c-Yes function with the use of a selective inhibitor, SU6656, was shown to perturb Sertoli cell TJ barrier function. This was also associated with changes in the distribution of actin filaments in these cells in which actin filament bundles were found to move away from the cell-cell interface (Xiao et al., 2011), such as at the basal ES, into the cell cytosol, thereby destabilizing TJ fibrils near the cell surface. Likewise, occludin became mislocalized, moving away from the cell-cell interface and into cell cytosol, and it associated more extensively with the endocytic vesicle protein clathrin (Xiao et al., 2011). It is noteworthy that the disruptive effects of SU6656 were blocked in the presence of testosterone (Xiao et al., 2011), illustrating that c-Yes must be working in concert with AR in the microenvironment of the BTB to fine tune the timely restructuring of multiple junctions at the site to allow the transit of preleptotene spermatocytes without compromising the immunological barrier. The working model proposed in Fig. 11 forms a framework for the design of different functional studies in the years to come.

D. Cytokines

1. Introduction. Studies in multiple epithelia and/or blood-tissue barriers in various organs (e.g., kidney, small intestine, brain, eyes), including the BTB in adult mammalian testes, have demonstrated the role of cytokines, such as TNFα, interferon-γ, TGF-β2/-β3, IL-1α, and IL-12, in regulating TJ permeability barrier function under normal and pathological conditions (e.g., inflammation, tumorigenesis) (Walsh et al., 2000; Xia et al., 2005a; Lui and Cheng, 2007; Li et al., 2008, 2009a,c; Capaldo and Nusrat, 2009; Turner, 2009; Marchiando et al., 2010a; Roberson and Bowcock, 2010; John et al., 2011). It is noted that endocytic vesicle-mediated protein trafficking events (e.g., endocytosis, transcytosis, recycling, or intracellular protein degradation mediated by endosome- and/or ubiquitin-dependent pathways) that determine the levels of integral membrane proteins at the TJ barrier play a critical role in modulating the adhesive status of cell adhesion protein complexes (e.g., cadherins, occludins, JAMs, claudins) at the barrier, and protein endocytosis can be mediated by either clathrin- or caveolae-dependent pathways or macropinocytosis (Tuma and Hubbard, 2003; Maxfield and McGraw, 2004; Mehta and Malik, 2006; Clague and Urbé, 2010; Golachowska et al., 2010; Hsu and Prekeris, 2010). Recent studies have shown that these events are highly complicated. For instance, endocytosis of occludin at the TJ barrier in epithelia can be regulated via macropinocytosis (Bruewer et al., 2005), clathrin-mediated (Ivanov et al., 2004), or caveolae-mediated (Shen and Turner, 2005; Schwarz et al., 2007; Stamatovic et al., 2009; Marchiando et al., 2010b) pathways, depending on the tissue being investigated.

As discussed in section III.B.3, polarity protein complexes, namely the Crumbs (CRB), the PAR (partitioning-defective), and the Scribble/Dlg (Discs large)/Lgl (Lethal giant larvae), are crucial to confer Sertoli cell polarity at the BTB. Besides their role in conferring Sertoli cell polarity at the BTB *and* maintaining proper orientation of developing spermatids during spermiogenesis, recent studies have shown that polarity proteins, most notably PAR6 and 14-3-3θ (also known as PAR5), also confer cell adhesion at the Sertoli-Sertoli and Sertoli-spermatid interface at the BTB and apical ES, respectively (Wong et al., 2008c, 2009). This is achieved, at least in part, by the ability of PAR-based proteins (e.g., 14-3-3) to regulate cell adhesion at the Sertoli-Sertoli interface via changes in the kinetics of protein endocytosis. For instance, it was shown that the knockdown of 14-3-3θ by RNAi using specific 14-3-3θ siRNA duplexes in Sertoli cells cultured in vitro with an established functional TJ-permeability barrier led to an increase in endocytosis of JAM-A and N-cadherin, thereby destabilizing the Sertoli cell TJ barrier (Wong et al., 2009). In addition, a knockdown of either PAR3, PAR6 or 14-3-3θ by RNAi in Sertoli cells resulted in the redistribution of proteins (e.g., N-cadherin) at the Sertoli-Sertoli cell interface (Wong et al., 2008c; Wong et al., 2009), leading to a loss of BTB integrity. More important, it was recently demonstrated that the cytokine-

TABLE 4
Cytokines found in the rodent testes that regulate BTB function

	TGF-β2	TGF-β3	TNFα	IL-1α
Molecular mass	25-kDa Homodimer with two 12.5-kDa monomers	25-kDa Homodimer with two 12.5-kDa monomers	51-kDa Homotrimer with three 17-kDa monomers	17-kDa Monomer; 31-kDa precursor is also biologically active
Cellular association in adult testes	Sertoli cells, round spermatids	Sertoli cells, spermatocytes, round spermatids	Sertoli cells, spermatocytes, round spermatids, elongating and elongated spermatids	Sertoli cells, spermatocytes, round spermatids
Stage-specific expression/localization				
I-IV	−	+	+	−
V-VII	+	+	+	−
VIII-IX	−	+	−	+
X-XIV	−	−	+	+
References	Teerds and Dorrington, 1993	Mullaney and Skinner, 1993; Lui et al., 2003b	De et al., 1993; Siu et al., 2003a	Haugen et al., 1994; Syed et al., 1995

+, presence; −, absence.

mediated increase in protein endocytosis at the Sertoli cell BTB required the presence of Cdc42 [an integrated component of the PAR-based polarity protein complex (Iden and Collard, 2008; Wong and Cheng, 2009)], because overexpression of a dominant-negative Cdc42 mutant in Sertoli cells by site-directed mutagenesis blocked the TGF-β3-induced acceleration in protein endocytosis, as well as TGF-β3-induced TJ permeability barrier disruption (Wong et al., 2010a). These findings suggest that cytokines (e.g., TGF-β2, TGF-β3, TNFα, IL-1α) play a "commanding" role in regulating BTB dynamics and that they do not just regulate protein adhesion at the Sertoli-Sertoli cell interface via changes in the kinetics of endocytosis, recycling, and endosome- or ubiquitin-mediated protein degradation (Yan et al., 2008b; Xia et al., 2009; Su et al., 2010b; Lie et al., 2011b) or protein-protein interactions (Xiao et al., 2011). Instead, cytokines also work with polarity proteins, which are critical to regulate endocytic vesicle-mediated protein-trafficking events (Wong et al., 2010a), as well as actin dynamics (Sarkar et al., 2008; Lie et al., 2011b) and steroid-mediated actions at the BTB (Delfino et al., 2003; Xiao et al., 2011). Herein, we will critically evaluate the central "commanding" role of cytokines on BTB regulation based on recent findings in the literature.

2. Transforming Growth Factor β, Tumor Necrosis Factor α, and Interleukin-1α. Sertoli and/or germ cells are known to secrete an array of cytokines during the epithelial cycle, many stage-specifically, to regulate spermatogenesis (Skinner, 1993; Mruk and Cheng, 2004b; O'Bryan and Hedger, 2008; Guazzone et al., 2009) and the BTB (Li et al., 2009c; Cheng and Mruk, 2010a; Cheng et al., 2010). However, the best studied cytokines that regulate BTB dynamics are TGF-β2, TGF-β3, TNFα, and IL-1α (Lui et al., 2003a; Siu and Cheng, 2004a; Li et al., 2008; Lie et al., 2011b) (Table 4). These four cytokines in the seminiferous epithelium are the products of Sertoli and germ cells (in particular spermatocytes and early spermatids but not elongated spermatids except for TNFα, which is produced by elongated spermatids, and their receptors are mostly found in the Sertoli cell (see Table 4), such that their production at specific stages of the epithelial cycle can exert their effects on the BTB created by Sertoli cells, representing a unique but efficient system to regulate BTB restructuring during spermatogenesis. In addition, although the phenotypes induced by all four cytokines are similar, each cytokine mediates its effects via different mechanisms and/or signaling pathways. As such, transient restructuring events that are "localized" to the BTB (such as behind transiting preleptotene spermatocytes) induced by TGF-β3 can be regulated independently of TNFα- or IL-1α-mediated disruption above transiting spermatocytes, so that the immunological barrier can remain "sealed" during the passage of spermatocytes across the BTB at stage VIII of the epithelial cycle (see Fig. 9).

a. Transforming growth factors β2 and β3. TGF-β2 and TGF-β3 are both 25-kDa homodimeric glycoproteins, with each monomer being ~12.5 kDa (see Table 4). TGF-β exerts its biological effects by first binding to the TGF-β type II receptor (TβRII), which then recruits the type I receptor (TβRI), forming a TGF-β/TβRII/TβRI complex, except that TGF-β2 binds to the two receptors almost at the same time with the assistance of the type III receptor, β-glycan (Massagué, 2000; Massagué and Gomis, 2006). This protein complex, in turn, recruits other adaptors, mediating different signaling pathways downstream, involving different GTPases and mitogen-activated protein kinases (MAPKs), and regulating an array of cellular events and functions under normal and pathological conditions, including spermatogenesis (Lui et al., 2003a; Xia et al., 2005a; Loveland et al., 2007; Massagué, 2008; Worthington et al., 2011). For instance, TGF-β signaling downstream involving either p38 MAPK (Lui et al., 2003c; Wong et al., 2004) or ERK1/2 (Xia and Cheng, 2005) can disrupt either BTB and germ cell adhesion or BTB function only, respectively.

Lui et al. (2001) reported that the assembly of a functional TJ permeability barrier by Sertoli cells in vitro was associated with a significant decline in the expression of TGF-β2 and -β3, suggesting that the presence of these cytokines in the microenvironment might perturb TJ barrier function. Indeed, recombinant TGF-β3 added

to Sertoli cultures was shown to block the assembly (Lui et al., 2001) or the maintenance (Lui et al., 2003a) of the TJ barrier dose dependently, which is mediated by the p38 MAPK signaling pathway (Lui et al., 2003c). More important, these findings in vitro were subsequently confirmed and expanded in studies in vivo in which administration of TGF-β3 to the testis via intratesticular administration indeed induced BTB damage when examined by electron microscopy, and required the coupling of the adaptors TGF-β activated kinase 1 binding protein 1 and CD2-associated protein with the TGF-β3/TβRII/TβRI protein complex upstream (Xia et al., 2006). Perhaps the most important of all, the use of TGF-β3 at doses that were within the range of the levels of TGF-βs in the testis, rather than pharmacological doses, was found to induce BTB disruption when assessed by a functional in vivo BTB integrity assay, and similar to the in vitro findings, this TGF-β3-induced BTB disruption was reversible (Xia et al., 2009). Another line of research that illustrates the significance of TGF-βs on BTB function derives from the use of the cadmium-induced BTB disruption animal model, in which adult rats treated with $CdCl_2$ (at 3 mg/kg b.wt. i.p.) was shown to associate with a surge in TGF-β3 expression (Wong et al., 2004), and the use of a specific p38 MAPK inhibitor could block, at least in part, the cadmium-induced BTB disruption and the loss of occludin from the BTB site (Lui et al., 2003c; Wong et al., 2004). More recent studies have shown that TGF-β2 or -β3 also enhances clathrin-mediated protein endocytosis at the Sertoli cell BTB (Yan et al., 2008b; Xia et al., 2009), but instead of recycling the endocytosed proteins back to the cell surface, TGF-β-induced endocytosed proteins are targeted for endosome- and/or ubiquitin-mediated intracellular degradation (Yan et al., 2008b; Su et al., 2010b). In addition, a recent study has demonstrated that the TGF-β3-induced acceleration in protein endocytosis at the Sertoli cell BTB requires the activation and involvement of Cdc42 in the PAR-based polarity protein complex (Wong et al., 2010a). These findings thus illustrate that TGF-β2 and/or -β3 produced by Sertoli cells, spermatocytes, and round spermatids locally at or near the BTB microenvironment, such as at stage VIII of the epithelial cycle, can "open" the BTB via the p38 MAPK signaling pathway, which in turn accelerates protein endocytosis at the site and targets internalized proteins for endosome- or ubiquitin-mediated intracellular degradation. This redistribution of integral membrane proteins at the BTB therefore "destabilizes" the TJ barrier, leading to its transient disruption.

b. Tumor necrosis factor α. TNFα, also known as cachexin or cachectin, is a proinflammatory cytokine. As its name implies, TNFα is involved in inflammation; it also inhibits tumorigenesis and viral replication by inducing apoptosis (Kruglov et al., 2008; O'Bryan and Hedger, 2008; Guazzone et al., 2009). TNF is primarily produced by activated monocytes and macrophages in response to inflammation or infection (Fiers, 1991), and also by Sertoli cells, spermatocytes, and round, elongating, and elongated spermatids in the testis (De et al., 1993; Siu et al., 2003a) (Table 4). Interstitial cells, such as mast cells and macrophages, are also a major source of TNFα in the mammalian testis (Guazzone et al., 2009). TNF is produced initially as a type II integral membrane homotrimeric protein of ~78 kDa with each monomer of ~26 kDa (212-amino acid polypeptide) (Kriegler et al., 1988; Tang et al., 1996), which is subsequently cleaved by metalloprotease TNFα-converting enzyme (also known as ADAM17) (Black et al., 1997) to generate the 51-kDa soluble homotrimeric form (each monomer is ~17 kDa with 185 amino acids) of TNFα. Both the membrane bound and soluble forms of trimeric TNFα are biologically active. Transmembrane and soluble TNFα exert their biological effects by binding onto either one of two membrane-bound TNFα receptors (TNFR): TNFR1 (55–60 kDa) or TNFR2 (75–80 kDa) (Tartaglia and Goeddel, 1992). TNFR1 is found in most tissues and can interact with either a cytoplasmic TNFR1-associated death domain protein (a 34-kDa adaptor protein that is also a TNFR1-associated signal transducer) or the Fas-associated death domain protein (a 23-kDa adaptor protein), which can lead to TNFα-induced death signaling through a caspase-dependent apoptotic pathway (Hsu et al., 1996), such as to regulate Sertoli/germ cell survival or apoptosis in the testis. Thus, TNFα plays a significant role in determining the size of the germ cell population in the seminiferous epithelium via its effects on germ cell apoptosis, because more than 75% of germ cells that arise during spermatogenesis undergo spontaneous degeneration (Clermont, 1963; Huckins and Oakberg, 1978; Bartke, 1995; Billig et al., 1995; Shaha, 2008). In this context, it noteworthy that estrogen (e.g., estradiol-17β) also regulates germ cell (e.g., pachytene spermatocytes) apoptosis via its effects on cyclins A1 and B1 (Chimento et al., 2010, 2011), reactive oxygen species, as well as the Fas-FasL system (Lee et al., 1999; Shaha, 2008; Shaha et al., 2010; Aitken et al., 2011). It remains to be determined whether TNFα works independently or in concert with estrogen to maintain the optimal number of germ cells in the epithelium during spermatogenesis. Recent studies have also shown that TNFR1 signaling can also be mediated by the protein ubiquitin-mediated pathway (Wertz and Dixit, 2008) and the NF-κB pathway [nuclear factor κ-light-chain-enhancer of activated B cells, a transcription factor (Baker et al., 2011)] (Kim et al., 2011). TNFR2, in contrast, lacks the ability to bind to death domain adaptor proteins; instead, it primarily activates the NF-κB pathway (Rothe et al., 1995) or the JNK [c-Jun N-terminal kinase, also known as stress-activated protein kinase, a member of the MAPK family (Haeusgen et al., 2011)] signaling pathway (Dempsey et al., 2003). The TNFR2 pathway is also a drug target for autoimmune diseases (Faustman and Davis, 2010), but

its function in spermatogenesis remains somewhat obscure. In short, TNFα is a multifunctional cytokine in the testis. For instance, it stimulates Sertoli cell androgen receptor expression, iron transport to germ cells, and lactate supply to postmeiotic germ cells; it regulates spermiation (Delfino et al., 2003; Siu et al., 2003a; Lysiak, 2004); and TNFα is also a potent inhibitor of Leydig cell steroidogenesis (Hong et al., 2004). In the testis, TFNR1 is mostly expressed in Leydig cells, lymphocytes, macrophages, Sertoli cells, and germ cells, whereas TNFR2 is detected in peritubular myoid and Sertoli cells (Pentikäinen et al., 2001; Suescun et al., 2003; Schell et al., 2008). In short, the biological actions of TNFα depend on 1) the receptor subtype engaged (once TNFα binds to its receptor, TNFR also forms a trimeric protein complex to serve as a platform to recruit other proteins to the site), 2) the expression and/or recruitment of specific adaptors (e.g., TNFR1-associated death domain protein, Fas-associated death domain protein, receptor interacting protein, and TNF receptor associated factor-2) or other binding partners to the TNFα/TNFR complex, and 3) cross-talk between TNFR1 and TNFR2, which in turn, select the appropriate signaling pathways downstream (Xia et al., 2005a; Smith and Humphries, 2009; Naudé et al., 2011).

TNFα was first shown to perturb Sertoli cell TJ barrier function when its recombinant protein was added to Sertoli cell cultures in vitro (Siu et al., 2003a). This finding is in agreement with the role of TNFα in inducing an up-regulation of Fas expression in mouse Sertoli cells via the NF-κB pathway that triggers apoptosis, leading to a disruption of the BTB, underlying the pathogenesis of autoimmune orchitis (Yule and Tung, 1993; Starace et al., 2005; Guazzone et al., 2009; Pelletier et al., 2009). Similar to TGF-β3, administration of recombinant TNFα in vivo to rats via intratesticular injection at doses comparable with the highest level during the epithelial cycle, TNFα was also found to reversibly disrupt the BTB when examined ultrastructurally by electron microscopy and functionally when assessed by an in vivo BTB integrity assay (Li et al., 2006). Although the disruptive effects of both TGF-β3 and TNFα on Sertoli cell BTB function, both in vitro and in vivo, seem similar, TNFα-induced BTB disruption is *not* mediated via the p38 MAPK signaling pathway. Instead, TNFα exerts its effects via an entirely different pathway. Besides inhibiting the de novo synthesis of occludin by Sertoli cells, TNFα was shown to induce MMP-9 synthesis by Sertoli cells and the activation of pro-MMP-9 to its enzymatically activated form, which was probably used to cleave the existing collagen network in the basement membrane, perturbing the scaffolding function imposed by collagen and leading to BTB disruption (Siu et al., 2003a). These changes, in turn, created a negative feedback that caused TNFα to induce collagen synthesis and the production of tissue inhibitor of metalloproteinase 1, so that the former was needed to replenish the cleaved collagen network and the latter was needed to limit unwanted proteolysis in the seminiferous epithelium (Siu et al., 2003a). It is likely that, because of this unique mechanism, TNFα and other cytokines (e.g., TGF-β3) can regulate TJ barrier function differentially during the transit of preleptotene spermatocytes at the BTB to allow the maintenance of the immunological barrier.

c. Interleukin-1α. IL-1α is mostly a product of spermatocytes and round spermatocytes in rat testes (Haugen et al., 1994; Lie et al., 2011c), and its production by Sertoli cells is largely dependent on the presence of germ cells (Cudicini et al., 1997; Jonsson et al., 1999; Lie et al., 2011c). Its expression and production in the testis is stage-specific, being highest at stages VIII to IX of the epithelial cycle (Syed et al., 1995; Wahab-Wahlgren et al., 2000) (Table 4). Administration of IL-1α to the testis locally was shown to perturb BTB integrity; however, this disruptive effect was not mediated by reducing the steady-state level of integral membrane proteins at the BTB (e.g., occludin, JAM-A, N-cadherin), unlike TGF-β3 or TNFα; instead, IL-1α perturbed the orderly arrangement of actin filament bundles at the BTB, causing disintegration of actin filaments. This in turn led to a disruption of the BTB (Sarkar et al., 2008). Subsequent studies using Sertoli cells cultured in vitro having an established functional TJ barrier showed that although the levels of BTB integral membrane proteins (e.g., occludin) were not altered by IL-1α treatment, an increase in the kinetics of endocytosis of occludin *and* a decrease in its rate of degradation were detected, which in turn destabilized Sertoli-Sertoli cell adhesion, leading to a disruption of the TJ barrier (Lie et al., 2011b). These changes were mediated by a disruption of the actin network at the BTB via a redistribution of Eps8 (epidermal growth factor receptor pathway substrate 8) and a surge in the expression of Arp3 (actin-related protein 3) [Eps8 is an actin barbed-end capping and bundling protein recently shown to regulate actin bundling in the testis (Lie et al., 2009b), and Arp3 is part of the Arp2/3 protein complex, which is known to confer actin nucleation/branching in cell epithelia (Ahmed et al., 2010; Rottner et al., 2010; Cheng and Mruk, 2011; Firat-Karalar and Welch, 2011), including the seminiferous epithelium (Lie et al., 2010a)] at the Sertoli cell BTB (Lie et al., 2011b). Thus, the transient loss of Eps8 at the Sertoli-Sertoli cell interface via its redistribution renders the failure of actin filament bundles to remain intact at the basal ES, and the surge in Arp3 induces actin branching, increasing the plasticity and fluidity of the actin network, perturbing normal endocytic vesicle-mediated protein trafficking events. This, in turn, destabilizes BTB integrity (Lie et al., 2011c).

3. Cytokines and Steroids: Their Differential Effects on Blood-Testis Barrier Dynamics and the Regulatory Role of Cytokines on Steroid-Mediated Action on the Blood-Testis Barrier. As noted above, cytokines and estro-

gens (section IV.D.2) are known to have a disruptive effect on Sertoli cell BTB integrity. However, testosterone promotes BTB assembly and its maintenance (Meng et al., 2005; Xia et al., 2005b; Wang et al., 2006; Yan et al., 2008b) via its action on 1) production of proteins, such as caveolin-1, Rab11 (Su et al., 2010b), claudin-11 (Kaitu'u-Lino et al., 2007), occludin (Chung and Cheng, 2001), and their proper localization at the Sertoli-Sertoli cell interface (Kaitu'u-Lino et al., 2007; Su et al., 2010b); 2) proper protein-protein interactions (e.g., N-cadherin-β-catenin, c-Yes-occludin; c-Yes-N-cadherin; occludin-clathrin; N-cadherin-clathrin) (Zhang et al., 2005; Su et al., 2010b; Xiao et al., 2011); 3) proper recycling of endocytosed proteins back to the cell surface to maintain cell adhesion (Yan et al., 2008b; Su et al., 2010b); and 4) maintenance of actin filament bundles within the Sertoli cell (Xiao et al., 2011), which are necessary to maintain BTB integrity and function during the seminiferous epithelial cycle of spermatogenesis. Studies have shown that the "opposing" effects of cytokines/estrogens and androgens seem to be under the central "command" of cytokines. This possibility is not just novel, but perhaps essential physiologically, given the diverse cellular and molecular events that are occurring at the microenvironment of the BTB (Lie et al., 2009a). In addition, the number of differentiated Sertoli cells (Orth, 1982) that can support developing germ cells at a Sertoli/germ cell ratio of ~1:30 to 1:50 (Weber et al., 1983) in adult rat testes during the epithelial cycle is limited, with ~25 to 40 $\times$ 10^6 Sertoli cells per testis. For instance, TNFα is known to perturb Sertoli cell TJ-permeability barrier function by inhibiting the production of occludin, but not ZO-1, and by maintaining the proper level of occludin at the BTB, which is mediated by MMP-9 and collagens (Siu et al., 2003a). It can also stimulate Sertoli cell intercellular adhesion molecule-1 (De Cesaris et al., 1999) *and* AR (Delfino et al., 2003) expression, which is mediated by an activation of the JNK signaling pathway and the binding of NK-κB (a transcription factor) to the AR promoter, respectively. These findings thus illustrate that TNFα can either perturb BTB function via its direct effects on adhesion protein complexes at the BTB, perhaps at the "old" BTB site above preleptotene spermatocytes in transit, or promote BTB assembly at the "new" BTB site via androgen action beneath transiting spermatocytes, which occur at stage VIII of the epithelial cycle. The role of cytokines in mediating the effects of steroids on BTB function was also supported by recent studies in other blood-tissue barriers. For instance, using the human brain microvascular endothelial cell line hCMEC/D3 as an in vitro model of the blood-brain barrier, hydrocortisone was shown to stimulate the steady-state levels of occludin and claudin-5, but not claudin-1 and vascular endothelial cadherin, as well as to promote the TJ permeability barrier in hCMEC/D3 cells, making the barrier almost 4-fold "tighter" than controls; yet TNFα perturbed TJ barrier function and significantly blocked hydrocortisone-stimulated blood-brain barrier-protein expression (Förster et al., 2008), illustrating that TNFα can mediate the effects of steroids on TJ barrier function. In the section that follows, we provide a hypothetical model illustrating the central "commanding" role of cytokines on BTB regulation based on recent findings in the literature.

4. Cytokines, Steroids, Polarity Proteins, Nonreceptor Protein Kinases, and Actin Regulatory Proteins: Their Concerted Efforts to Regulate Blood-Testis Barrier Dynamics during Spermatogenesis. As depicted in Figs. 7 and 9, occludin-, claudin-, N-cadherin-, JAM-A-, JAM-B-, nectin-, and CAR-based adhesion complexes that confer BTB integrity during the seminiferous epithelial cycle of spermatogenesis are localized at the Sertoli-Sertoli cell interface. This is achieved by proper phosphorylation of integral membrane proteins, their corresponding adaptors, and associated polarity proteins via the action of nonreceptor protein kinases. Intact actin filament bundles at the basal ES that "reinforce" and support the tight junctions are maintained by the proper ratio of Eps8 to Arp2/3 protein complex. However, at stage VIII of the epithelial cycle, when preleptotene spermatocytes are in transit at the BTB (Parvinen, 1982; Hess and de Franca, 2008), several events occur as follows. First, androgen-induced de novo synthesis of integral membrane proteins (e.g., occludin, claudin-11), *and* their corresponding adaptors begin to assemble "new" TJ fibrils (Chung and Cheng, 2001; Kaitu'u-Lino et al., 2007) behind migrating spermatocytes to establish a "new" BTB. The establishment of a "new" BTB is also assisted by the relocation of TJ (e.g., occludin, claudins) and basal ES proteins (e.g., cadherins, nectins, CAR) from 1) the basolateral storage site, most likely mediated by GTPases and/or endosomes, and 2) the "old" BTB site above transiting spermatocytes via internalization and transcytosis of "used but still functional" integral membrane proteins, and to recycle these endocytosed proteins to assemble "new" TJ-fibrils. This endocytic vesicle-mediated event is facilitated by polarity proteins (e.g., 14-3-3, PAR3, PAR6, and Cdc42) (Wong et al., 2008c, 2009, 2010a) and androgen-induced expression of caveolin-1 and Rab11 (both are markers of transcytosis and recycling) (Su et al., 2010b). This action is mediated, at least in part, by TNFα, which is known to induce Sertoli cell androgen receptor expression (Delfino et al., 2003). Second, BTB disruption in vitro and in vivo induced by TNFα (Siu et al., 2003a; Li et al., 2006), TGF-β3 (Lui et al., 2003b; Xia et al., 2006, 2009), and IL-1α (Sarkar et al., 2008; Lie et al., 2011b) is known to be mediated by changes in endocytic vesicle-mediated trafficking events. Endocytosis of integral membrane proteins and their associated adaptors from the "old" BTB site is made possible by their phosphorylation, which is mediated by protein kinases (e.g., FAK, c-Src, c-Yes) (Lee and Cheng, 2005; Siu et al., 2009b,c; Lie et al., 2010b; Xiao et al., 2011) or dephosphorylation by

phosphatases (Li et al., 2001), altering their phosphorylation status. Some of these endocytosed proteins are used to establish the "new" BTB behind transiting spermatocytes under the influence of testosterone (Yan et al., 2008b; Su et al., 2010b), but some are targeted for intracellular degradation via endosome- or ubiquitin-dependent pathways that are regulated by cytokines (e.g., TGF-β2, TGF-β3, TNFα, IL-1α) (Yan et al., 2008b; Xia et al., 2009; Su et al., 2010b; Lie et al., 2011b), thereby destabilizing the "old" BTB site to prepare for its dissolution. Third, endocytic vesicle-mediated events are also facilitated by changes in the ratio of Esp8 to the Arp2/3 complex to increase the fluidity and plasticity of the BTB to assist in intracellular trafficking of proteins (e.g., endocytosis, transcytosis, recycling), which is also regulated by cytokines (e.g., IL-1α) (Lie et al., 2011b). Fourth, TJ and basal ES proteins expressed by transiting spermatocytes [e.g., CAR, JAMs, cadherins, nectins (Lee et al., 2003, 2004; Mirza et al., 2007; Wang and Cheng, 2007; Wang et al., 2007; Shao et al., 2008)] can form "transient" interlocking complexes with corresponding proteins found in Sertoli cells to avoid "unwanted" paracellular leakage of biomolecules and substances at the BTB (Wang and Cheng, 2007). In short, the concerted efforts of different molecules and mechanisms thus maintain the function of the immunological barrier when preleptotene spermatocytes are in transit across the BTB. It is envisioned that additional "players" and "regulators" will be added to this model depicted in Fig. 9 in the years to come. Nonetheless, this model serves as the framework upon which functional experiments can be designed in future studies.

V. Toxicants and Blood-Testis Barrier Function

Environmental toxicants, such as heavy metals (e.g., cadmium), that caused testicular injury (Parizek and Zahor, 1956; Parizek, 1960; Chiquoine, 1964) and BTB disruption (Setchell and Waites, 1970) were first reported more than 5 decades ago. In addition, bisphenol A was also shown to perturb the assembly of the BTB in immature rats (Li et al., 2009d), impeding male fertility via its effects on male germline stem cells and the expression of Sertoli cell junctional proteins (Salian et al., 2009a,b) after neonatal or perinatal exposure, consistent with findings in vitro in which bisphenol A was found to disrupt the expression of Sertoli cell junctional proteins (Fiorini et al., 2004; Li et al., 2009d). These findings are consistent with mounting evidence that prenatal and neonatal exposure of rodents to bisphenol A, even at the current "safe" dose level ($<$50 μg/kg b.wt./day) accepted by the U.S. Food and Drug Administration and the U.S. Environmental Protection Agency, is linked to changes in sexual differentiation, defects in male and female reproductive tracts, meiotic abnormalities in fetal oocytes, complications during pregnancy, and increases in malignancies in adult animals (Richter et al., 2007; Hunt et al., 2009; Vandenberg et al., 2009). A recent study has conclusively demonstrated a trend of reducing semen quality and increasing testicular cancer among Finnish men caused by exposure to environmental toxicants (Jørgensen et al., 2011), consistent with earlier studies that associated reduced sperm count and reproductive dysfunction in men exposed to environmental toxicants (Benoff et al., 2000; Phillips and Tanphaichitr, 2008; Lucas et al., 2009; Siu et al., 2009a; Bonde, 2010; Sakkas and Alvarez, 2010; Cheng et al., 2011b; Wong and Cheng, 2011). It is noteworthy that only in recent years, toxicant-induced testis damage was used by investigators as a model to study the regulation of the BTB at the cellular and molecular levels both in vitro and in vivo (Janecki et al., 1992; Chung and Cheng, 2001; Fiorini et al., 2004; Wong et al., 2004, 2010b; Siu et al., 2009a; Elkin et al., 2010; Li et al., 2010; Cheng et al., 2011b; Gualtieri et al., 2011; Wong and Cheng, 2011). However, it is of interest to note that not all toxicants that impede spermatogenesis in males can disrupt BTB integrity. For instance, in the same study in which $CdCl_2$ was found to disrupt BTB integrity, methoxyacetic acid and 1,3-dinitrobenzene, which affected pachytene spermatocyte and Sertoli cell function, respectively, were shown to have no effects on BTB integrity or function (Elkin et al., 2010). These findings thus illustrate that the BTB is vulnerable to some but not all toxicants (Li and Heindel, 1998; Yu et al., 2009; Wong et al., 2010b; Cheng et al., 2011b).

A. Sertoli Cell Differentiation Status and Blood-Testis Barrier Integrity and Their Impact on Spermatogenesis

In this context, it is of interest to note that exposure of neonatal rats to goitrogens, such as 6-propyl-2-thiouracil (PTU), to induce neonatal hypothyroidism leads to an increase in testis weight and size, as well as germ cell production (Cooke and Meisami, 1991; Cooke et al., 1991, 1992; Santos-Ahmed et al., 2011), possibly the result of reduced apoptosis, which is mediated via the Akt1 [Akt, also known as protein kinase B (PKB), is a Ser/Thr protein kinase family composed of Akt1, Akt2, and Akt3, the product of the normal gene homolog of *v-akt*, the transforming oncogene of AKT8 virus] signaling pathway (Santos-Ahmed et al., 2011). It is noted that rats exposed to PTU from postnatal days 1 through 25 displayed a delay in the differentiation of Sertoli cells, so that these cells continued to proliferate, and the BTB was not established by postnatal day 25 (De França et al., 1995). This increase in Sertoli cell number in the testis of PTU-treated rats thus enhanced the capacity of these cells to support more germ cells (and perhaps associated with an increase in Akt1 signaling), which, in turn, led to an increase in germ cell numbers and testis weight. At present, it is not known whether the BTB was established in these rats by the time they reached adulthood, but because these adult rats were fertile, it can be assumed that the BTB was functional. Nonetheless,

these findings illustrate that the assembly of the BTB is tightly linked to the maturation status of Sertoli cells and that the BTB can be developed only with differentiated Sertoli cells that have ceased to divide. The concept that proper terminal differentiation of Sertoli cells plus their mitotic quiescence are crucial to the establishment and/or maintenance of a functional BTB to support spermatogenesis is further supported by a recent study using Sertoli cell-specific conditional knockout mice of retinoblastoma protein (RB). Other studies have shown that the RB pathway is crucial in cell cycle control and cell differentiation by serving as a master regulator to coordinate cell cycle exit with differentiation (Lipinski and Jacks, 1999; Nguyen and McCance, 2005; Chinnam and Goodrich, 2011; McDuff and Turner, 2011). It was noted that RB(−/−) mice appeared normal by postnatal week 6 (that is, they were fertile with normal testis weights and spermatogenesis indistinguishable from wild-type mice); however, by postnatal weeks 10 to 14, these Rb(−/−) mice were infertile with severe Sertoli cell dysfunction, and Sertoli cells displayed defective regulation of multiple androgen-regulated genes and most notably a "leaky" BTB (Nalam et al., 2009). These findings thus illustrate that improper terminal differentiation of Sertoli cells seems *not* to affect the initial establishment of the BTB to support spermatogenesis in rats, but it fails to "maintain" BTB integrity, which in turn perturbs spermatogenesis. In addition, RB is known to impair cell cycle G_1- to S-phase progression by acting as a repressor of the E2F family of transcriptional activators, and recent studies have shown that E2F1 is a transcription factor possessing both proapoptotic and prosurvival properties (Udayakumar et al., 2010); thus, future studies should examine whether a disruption of the Sertoli cell BTB would also impede the events of apoptosis in the seminiferous epithelium.

B. The Cadmium Model

Although it has been known since the 1950s that exposure of adult rats to cadmium salts (e.g., cadmium chloride) induces severe testicular injury (Parizek and Zahor, 1956), its disruptive effects on the BTB were not known until Setchell and Waites (1970) illustrated that the Sertoli cell barrier is highly sensitive to $CdCl_2$. However, only in the 1990s did it become clear that cadmium (Hew et al., 1993b) and other toxicants [e.g., glycerol (Wiebe et al., 2000)] that induce *irreversible* male infertility are mediated, at least in part, via BTB damage by disrupting TJ-associated actin microfilaments and TJ fibrils, together with an inhibition on occludin expression) and/or microtubules in Sertoli cells. Herein, we provide a brief but critical and updated discussion on how this cadmium model has helped us and other investigators in the field to unravel the underlying signaling mechanisms that regulate BTB dynamics during spermatogenesis. These findings also illustrate some new avenues of research that can be tackled to develop novel male contraceptives and/or deliver drugs across the BTB, and perhaps other blood-tissue barriers.

1. Introduction. Cadmium is a major environmental toxicant that is released into the atmosphere as cadmium oxide, cadmium chloride, or cadmium sulfide via industrial activities, such as manufacturing of batteries and pigments, metal smelting and refining, and municipal waste incineration. It enters the food chain via contaminated water and food (World Health Organization, 2000; Agency for Toxic Substances and Disease Registry, 2008). Once ingested, cadmium enters eukaryotic cells via the ZIP (Zrt-, Irt-like Protein, representing a family of zinc transporters; Zrt1 and Zrt2 were initially found in yeast *Saccharomyces cerevisiae*, and Irt1, an iron and zinc transporter, was found in roots of *Arabidopsis thaliana*) transporters SLC39A8 and SLC39A14, which are also found in the testis (Dalton et al., 2005; Su et al., 2011a). However, Cd^{2+} also enters cells using the Fe^{2+}/H^{+} cotransporter divalent metal transporter 1 (Thévenod, 2010) and perhaps other transporters, such as influx pumps (Su et al., 2011a). Although the average person's intake of cadmium is only ~1 to 30 μg/person/day via food and water, with an additional 2 to 30 μg of cadmium per person per day among cigarette smokers (a single cigarette carries ~1–2 μg of cadmium) (Andersson et al., 1986; Agency for Toxic Substances and Disease Registry, 2008; Pan et al., 2010), it has an exceedingly long half-life, approximately 20 to 40 years in humans (Kjellström and Nordberg, 1978; World Health Organization, 2000; Wong et al., 2010b) and >200 days in rodents (Webb, 1975). Cadmium accumulates mostly in the liver and kidney but also in the testes (Waalkes et al., 1992; Järup and Akesson, 2009), largely because of high concentrations of metallothioneins [cysteine-rich low molecular mass metal-binding proteins localized to the membrane of the Golgi apparatus that protect cells from cytotoxicity of heavy metals (such as copper, selenium, and zinc) and xenobiotics (such as cadmium, mercury, silver, and arsenic) by binding to these metals through the thiol group of its cysteine residues] in these organs (Dalton et al., 1996; Siu et al., 2009a; Chiaverini and De Ley, 2010; Vesey, 2010; Wong et al., 2010b). As such, significant and harmful amounts of cadmium can indeed build up in a person over a period of time, overwhelming the capacity of metallothioneins.

2. Cadmium and Carcinogenesis—Molecular Targets and Mechanisms of Action. Cadmium is associated with increased risks in carcinogenesis in multiple organs, most notably kidney, prostate, liver, pancreas, lung, stomach, and testis in humans and rodents, and it was pronounced a potent carcinogen in rodents in the early 1960s (Waalkes et al., 1992; Waalkes, 2003; Goyer et al., 2004; Huff et al., 2007; Thévenod and Chakraborty, 2010). For instance, rats that received long-term exposure to cadmium aerosols, such as at 50 $\mu g/m^3$ $CdCl_2$, had a 70% greater incidence of lung carcinoma than control rats (Takenaka et al., 1983). Besides the organs listed above,

recent studies also link cadmium to different forms of cancer in multiple organs in humans, such as mouth, lung, head and neck, urinary bladder, and breast, based on data derived from workers occupationally exposed to cadmium and from heavy smokers (Waalkes et al., 1992; Huff et al., 2007; Barrett, 2009; Beveridge et al., 2010; Kazi et al., 2010; Khlifi and Hamza-Chaffai, 2010; Pan et al., 2010). The mechanisms of cadmium-induced carcinogenicity seem to be multifactorial, such as inducing DNA-protein cross-linking, inhibiting DNA damage repair, disrupting gene expression, inhibiting cell apoptosis (Waalkes et al., 1992; Waalkes, 2003; Huff et al., 2007), disrupting Wnt/β-catenin signaling function (Thévenod and Chakraborty, 2010), and modulating transcriptional regulation of stress-response genes (e.g., metallothioneins, heme oxygenase, heat shock proteins, and apoptosis-related genes) (Luparello et al., 2011). Cadmium also acts as a mitogen by promoting breast cancer cell proliferation (Siewit et al., 2010; Yu et al., 2010). It also induces tissue damage, such as in the kidney, where it induces apoptosis (0.1–10 μM) or necrosis (>50 μM) (Templeton and Liu, 2010). However, all the available evidence seemingly suggests that cadmium-induced oxidative stress plays a central role in cadmium carcinogenesis, because superoxide dismutase, catalase, NADPH oxidase, and/or glutathione peroxidase were shown to be altered significantly during cadmium-induced genotoxicity, tissue damage and aberrant gene expression (Waalkes, 2003; Joseph, 2009; Liu et al., 2009). It is noteworthy that cadmium-induced reproductive dysfunction is also likely to be mediated by an increase in oxidative stress, which, in turn, impedes cell junctions in the testis, including the BTB (Wong and Cheng, 2011).

3. Cadmium and Male Reproductive Dysfunction. Exposure of rodents to short-term doses of cadmium [e.g., $CdCl_2$ administered at 1–5 mg/kg b.wt. (i.e., at ~0.3–1.5 mg/rat, assuming the body weight of adult rats is ~300 g) at a single dose via intraperitoneal or subcutaneous injection] was known to induce serious testicular injury (e.g., sterilization, necrosis, germ cell depletion, interstitial tissue damage, and BTB disruption) more than 5 decades ago (Parizek and Zahor, 1956; Parizek, 1957, 1960; Setchell and Waites, 1970; Hew et al., 1993b; Siu et al., 2009a; Wong et al., 2010b). However, the disruptive effects of cadmium on male reproductive function in rodents and humans, particularly after low-dose exposure, such as at 5 to 50 μg/rat/day (i.e., at approximately 30- to 60-fold less than the short-term doses), were not known until recently (Telisman et al., 2007; Wirth and Mijal, 2010). Indeed, recent correlation studies have linked elevated and statistically different cadmium levels in blood and seminal plasma in infertile men with reduced semen quality (i.e., low sperm count and abnormal sperm motility) (Wu et al., 2008; Benoff et al., 2009; Rouiller-Fabre et al., 2009; Wirth and Mijal, 2010). For instance, it was shown that the mean seminal plasma cadmium level in infertile patients was 0.282 μg/l (n = 132 men; range, 0.241–0.348 μg/l) versus 0.091 μg/l (n = 14 men; range, 0.073–0.102 μg/l) in artificial insemination donors and 0.092 μg/l (n = 35 men; range, 0.080–0.111 μg/l) in general population volunteers (Benoff et al., 2009), illustrating a 3-fold increase in the seminal plasma cadmium level in infertile men versus unaffected subjects. Furthermore, the seminal plasma cadmium level in heavy smokers is also positively associated with male infertility compared with healthy subjects (Wu et al., 2008). In addition, administration of an environmentally relevant and low dose of cadmium to rats was also shown to induce asthenozoospermia (reduced sperm motility and quality), which is associated with infertility (Benoff et al., 2008). These findings, as summarized herein, are significant because they support the notion that the current trend of declining semen quality, such as sperm count and sperm motility among men in industrialized nations, is caused, at least in part, by low-level exposure to environmental toxicants such as cadmium.

4. Cellular Targets of Cadmium in the Testis. Setchell and Waites (1970) first reported that the BTB is the primary ultrastructural target of cadmium toxicity in the testis, with BTB disruption occurring before hemorrhage of microvessels in the interstitial tissue (but not in the brain or the epididymis in these rats) after the administration of ~7 mg of $CdCl_2$/kg b.wt. s.c. to adult rats (234–373 g b.wt.). In a subsequent study that used rats at ~300 g b.wt. treated with $CdCl_2$ at 3 mg/kg b.wt. i.p. to assess BTB integrity by a combination of techniques, including electron microscopy, histological and dual-labeled immunofluorescence analysis found the BTB to be disrupted by ~10 h after treatment. However, microvessels in the interstitium were not disrupted until ~20 h after treatment (Wong et al., 2004, 2005a). Most notably, actin filament bundles at the basal ES adjacent to the TJ were found to be disrupted (Wong et al., 2005a); these findings are consistent with those of an earlier report in which rats were exposed to cadmium at 1 mg/kg b.wt., where actin filaments at the BTB were also found to be disrupted (but *not* actin filaments in peritubular myoid cells) (Hew et al., 1993b). Collectively, these findings (Setchell and Waites, 1970; Hew et al., 1993b; Wong et al., 2004, 2005a) thus illustrate that although the BTB is one of the tightest blood-tissue barriers, it is more susceptible to cadmium-induced damage than the TJ barrier in microvessels located in the interstitium, the blood-brain barrier, or the blood-epididymal barrier, and cadmium does not disrupt actin filaments in other tissues indiscriminately, but only at the ES in the seminiferous epithelium.

Hew et al. (1993a) reported that cadmium at 1 mg/kg b.wt. i.p. induced failure of spermiation in rats with disrupted spermatid transport because elongated sper-

matids remained embedded within the seminiferous epithelium in late-stage VIII and stages IX and X, implying that cadmium is disrupting the apical ES at the Sertoli-spermatid interface, which is known to be crucial to spermatid transport across the epithelium during the epithelial cycle (Mruk and Cheng, 2004b). The possibility that the apical and basal ES are cellular targets of cadmium in the testis was not pursued until almost 3 decades later, when we first reported that Sertoli cell BTB dynamics were regulated by TGF-β3 (Lui et al., 2001), which was mediated by p38 MAPK downstream. The use of a specific p38 MAPK inhibitor, such as 4-[4-(4-fluorophenyl)-5-(4-pyridinyl)-1*H*-imidazol-2-yl]phenol (SB202190), was shown to block the TGF-β3-induced TJ permeability barrier disruption (Lui et al., 2003b). Subsequent in vivo studies using the cadmium model, which is known to disrupt the Sertoli cell TJ permeability barrier in vitro (Janecki et al., 1992; Chung and Cheng, 2001) have shown that the cadmium-induced BTB disruption is associated with an activation of TGF-β3 and also an activation of p38-MAPK (Lui et al., 2003c; Wong et al., 2004). More important, the cadmium-induced BTB disruption can either be partially blocked and delayed with the use of a p38-MAPK inhibitor, SB202190 (Lui et al., 2003c), or it can be worsened with the use of the JNK inhibitor dimethylaminopurine (which blocks the production of α_2-macroglobulin, a nonspecific protease inhibitor, in the seminiferous epithelium) (Wong et al., 2005a). In addition, cadmium was shown to induce disruption of actin filament bundles at the basal ES by electron microscopy (Wong et al., 2005a). Taken collectively, these findings illustrate that the basal ES (a testis-specific AJ type) at the BTB is one of the cellular targets of cadmium-induced toxicity in the testis. These findings are also in agreement with studies in epithelia other than the testis, such as the small intestine, kidney, liver, and skin, which imply that cell junctions are the target of different classes of reproductive toxicants, including cadmium (Fiorini et al., 2004; Zefferino et al., 2008; Pinton et al., 2009; Choi et al., 2010; Li et al., 2010). In addition, there is mounting evidence that both AJ (and/or TJ) (Prozialeck and Lamar, 1999; Prozialeck, 2000; Prozialeck et al., 2003; Jacquillet et al., 2007; Thompson et al., 2008; Siu et al., 2009b,c; Calabro et al., 2011) and gap junction (Fukumoto et al., 2001; Jeon et al., 2001; Guan et al., 2007; Tang et al., 2009; Vinken et al., 2010) are targets of cadmium toxicity in multiple epithelia in different organs, such as the kidney, heart, liver, ovary, and testis.

It is noted that many cell adhesion molecules at cell junctions are Ca^{2+}-dependent proteins, such as E-cadherin and occludin, which are also integral membrane proteins at the BTB (Cheng and Mruk, 2002). Using the techniques of equilibrium microdialysis and circular dichroism spectroscopy to study the interactions of Cd^{2+} with E-cadherin, it was shown that Cd^{2+} bound to a 13-amino acid residue peptide corresponding to a putative Ca^{2+}-binding motif of E-cadherin (Prozialeck and Lamar, 1999), illustrating that Cd^{2+} interacts with the Ca^{2+}-binding sites in Ca^{2+}-dependent E-cadherin, thereby substituting Ca^{2+} in these cell adhesion molecules. This, in turn, disrupts E-cadherin-based AJ in epithelia and endothelia, consistent with the hypothesis that E-cadherin may be a direct molecular target for cadmium toxicity (Prozialeck, 2000). This finding also explains earlier observations that short-term exposure of rodents to cadmium led to hemorrhagic injury (Parizek and Zahor, 1956; Wong et al., 2005a) because Cd^{2+} blocks the function of Ca^{2+}-dependent adhesion molecules (e.g., cadherins, occludins) at the TJ permeability barrier in microvessels, causing tissue hemorrhage in the interstitial tissue. In fact, based on mounting evidence in the field, the vascular system has recently been declared to be a target of metal toxicity, including cadmium (Prozialeck et al., 2008). The notion that Ca^{2+}-dependent cell adhesion molecules (e.g., cadherins, occludins) are the target of cadmium toxicity is also supported by the unusual vulnerability of the BTB to cadmium toxicity with BTB disruption preceding microvessel damage in the interstitium by as much as 10 to 14 h (Wong et al., 2005a). Unlike the endothelial TJ that confers barrier function in microvessels and capillaries in which cadherin-based AJs are shielded behind the TJ fibrils, which are constituted of Ca^{2+}-independent cell adhesion molecules (e.g., claudins; see Table 1) (Wong and Cheng, 2005), the BTB is composed of *coexisting* TJ and basal ES. Thus, cadherins [e.g., N-cadherin and E-cadherin, which are localized at the BTB (Lee et al., 2003, 2004)] are readily accessible by incoming cadmium. Yet cadmium must "work" its way through the endothelial TJ barrier, perhaps by initially mediating its disruptive effects via Ca^{2+}-dependent TJ proteins (e.g., occludins) before it can "reach" cadherins in the AJ lying behind the TJ to cause extensive damage, leading to hemorrhage. This explains the time difference between the disruption of the BTB and of the microvessels located in the interstitium of the testis after cadmium exposure (Setchell and Waites, 1970; Wong et al., 2005a).

Recent studies have also demonstrated that the FAK/occludin/ZO-1 protein complex at the BTB (Siu et al., 2009c) is a putative target of cadmium toxicity (Siu et al., 2009b). It is known that cadmium can perturb the Sertoli cell TJ permeability barrier in vitro (Janecki et al., 1992; Chung and Cheng, 2001). However, a knockdown of FAK by RNAi in the Sertoli cell epithelium with a functional TJ permeability barrier that mimics the BTB in vivo can desensitize these cells to $CdCl_2$ exposure (Siu et al., 2009b). This effect is probably mediated by the inability of the Sertoli cell to maintain the proper phosphorylation status of the occludin/ZO-1 complex as a result of FAK knockdown, altering the kinetics of protein endocytosis and recycling because integral membrane proteins (e.g., occludin) and adaptors (e.g., ZO-1)

at the site became mislocalized, moving away from the cell-cell interface and into the cell cytosol (Siu et al., 2009b). These findings are also consistent with the concept that cell adhesion molecules (e.g., E-cadherin) (Prozialeck and Lamar, 1999; Prozialeck, 2000; Prozialeck et al., 2002, 2003) and intercellular junction proteins (Fukumoto et al., 2001; Jeon et al., 2001; Fiorini et al., 2004; Thompson et al., 2008) are the target of cadmium toxicity in multiple epithelia and endothelia including the BTB.

5. Molecular Mechanisms of Cadmium-Induced Testicular Injury. Mounting evidence illustrates the importance of environmental toxicant-induced oxidative stress (e.g., induced by exposure to cadmium, BPA) in mediating disruption of cell junctions, such as the TJ, basal ES, desmosome and gap junctions, at the BTB that leads to reproductive dysfunction (Wong and Cheng, 2011). Besides directly acting on enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase) that modulate oxidative stress, toxicants (e.g., cadmium) can also induce oxidative stress via changes in cytokines, as well as mitogen-activated protein kinases (MAPK) (see Fig. 9). For instance, cadmium is known to activate TGF-β2 and -β3 and p-p38 MAPK in the testis before extensive cellular damage can be detected in the seminiferous epithelium (Wong et al., 2004). In fact, an increase in oxidative stress was seen in ~80% of clinically proven infertile men who had long-term exposure to environmental toxicants (e.g., cadmium, smoking), which are emerging as a major contributing factor in male infertility (Kiziler et al., 2007; Tremellen, 2008; Venkatesh et al., 2011). In addition, recent studies have shown that flavonoids (antioxidants, such as genistein, myricetin, and quercetin) can protect the intestinal TJ barrier function from oxidative stress-induced barrier disruption caused by inflammatory cytokines, enteric bacteria, and chemicals (e.g., acetaldehyde) (Suzuki and Hara, 2011), illustrating the damaging effects of oxidative stress on TJ barrier function. In this context, it is of interest to note that estrogens (or phytoestrogens) act as scavengers of free radicals, protecting the testis and the liver from oxidative stress-induced injury (Hamden et al., 2008, 2009). Thus, the protective role of estrogens in environmental toxicant-induced oxidative stress that leads to testicular injury must be carefully evaluated.

Studies in the past decade have shown that an activation of phosphatidylinositol 3-kinase (PI3K), a lipid kinase that activates PKB in the PI3K/PKB/mTOR (mechanistic target of rapamycin, a Ser/Thr kinase) signaling pathway is crucial to regulate cell growth, proliferation, differentiation, apoptosis, and intracellular protein trafficking in multiple epithelia (Kwiatkowska, 2010; Cockcroft and Garner, 2011). In adult rat testes, PI3K is localized intensely at the apical ES, as well as at the basal ES, and it is activated during adjudin-induced spermatid loss that mimics spermiation (Siu et al., 2005). Emerging evidence in the field based on multiple studies has shown that aberrant activation of PI-3K is the hallmark of the oxidative stress-induced junction disruption (Wong and Cheng, 2011) (Fig. 11). The activation of PI3K, such as during cadmium-induced oxidative stress in the testis, can modulate the activity of c-Src and/or FAK downstream (Fig. 11). Earlier studies have illustrated that both of these nonreceptor protein tyrosine kinase are localized to the BTB (Lee and Cheng, 2005; Siu et al., 2009c), FAK being a component of the occludin-ZO-1 adhesion protein complex (Siu et al., 2009b,c) and c-Src structurally interacts with desmoglein-2/desmocollin-2 adhesion (Lie et al., 2010b) and connexin-43/plakophilin-2 complexes (Li et al., 2009b) at the BTB. Thus, it is likely that both c-Src and FAK are being used to maintain the proper phosphorylation status of integral membrane proteins, such as occludin, at the site (Cheng and Mruk, 2009b; Siu et al., 2009b). It is known that occludins that are assembled into TJ fibrils must be properly phosphorylated at Ser, Thr, and Tyr residues (Sakakibara et al., 1997; Tsukamoto and Nigam, 1999), and changes in their phosphorylation status would move occludins away from TJ fibrils and toward the basolateral region of epithelial cells (Cordenonsi et al., 1997; Sakakibara et al., 1997), destabilizing cell adhesion at the BTB and leading to its disruption. In vitro studies using Sertoli cells that mimic the BTB in vivo have shown that treatment of these cells with either BPA or cadmium indeed inactivated c-Src (Li et al., 2009d) or FAK (Siu et al., 2009c), respectively, and these changes enhanced protein endocytosis at the Sertoli-Sertoli cell interface, moving integral membrane proteins (e.g., occludin, N-cadherin) away from the cell surface and into the cell cytosol, destabilizing the TJ barrier and resulting in its disruption (Li et al., 2009d; Siu et al., 2009c).

C. Regulation of Blood-Testis Barrier Dynamics during Spermatogenesis: a Model Based on Studies Using Cadmium

On the basis of recent studies in the field that were discussed above, cadmium-induced testicular injury is initially mediated via its direct effects on the activation of cytokines [e.g., TGF-β3] and/or the induction of oxidative stress in Sertoli cells at the BTB. As discussed in section IV.D, cytokines play a high-level commanding role in regulating BTB function, and this can also modulate cellular oxidative stress (see Fig. 11). This, in turn, may activate either FAK- or the PI3K-c-Src-PAR-based signaling pathway (Fig. 11). At the molecular level, activated FAK or c-Src will alter the phosphorylation status of adhesion protein complexes at the BTB, which can accelerate endocytic vesicle-mediated intracellular trafficking events aided by polarity proteins (e.g., 14-3-3, PAR6, Cdc42). The net result of these changes destabilizes cell adhesion at the BTB, leading to its disruption, and more cadmium can make its way into the apical compartment to induce additional activation of FAK and

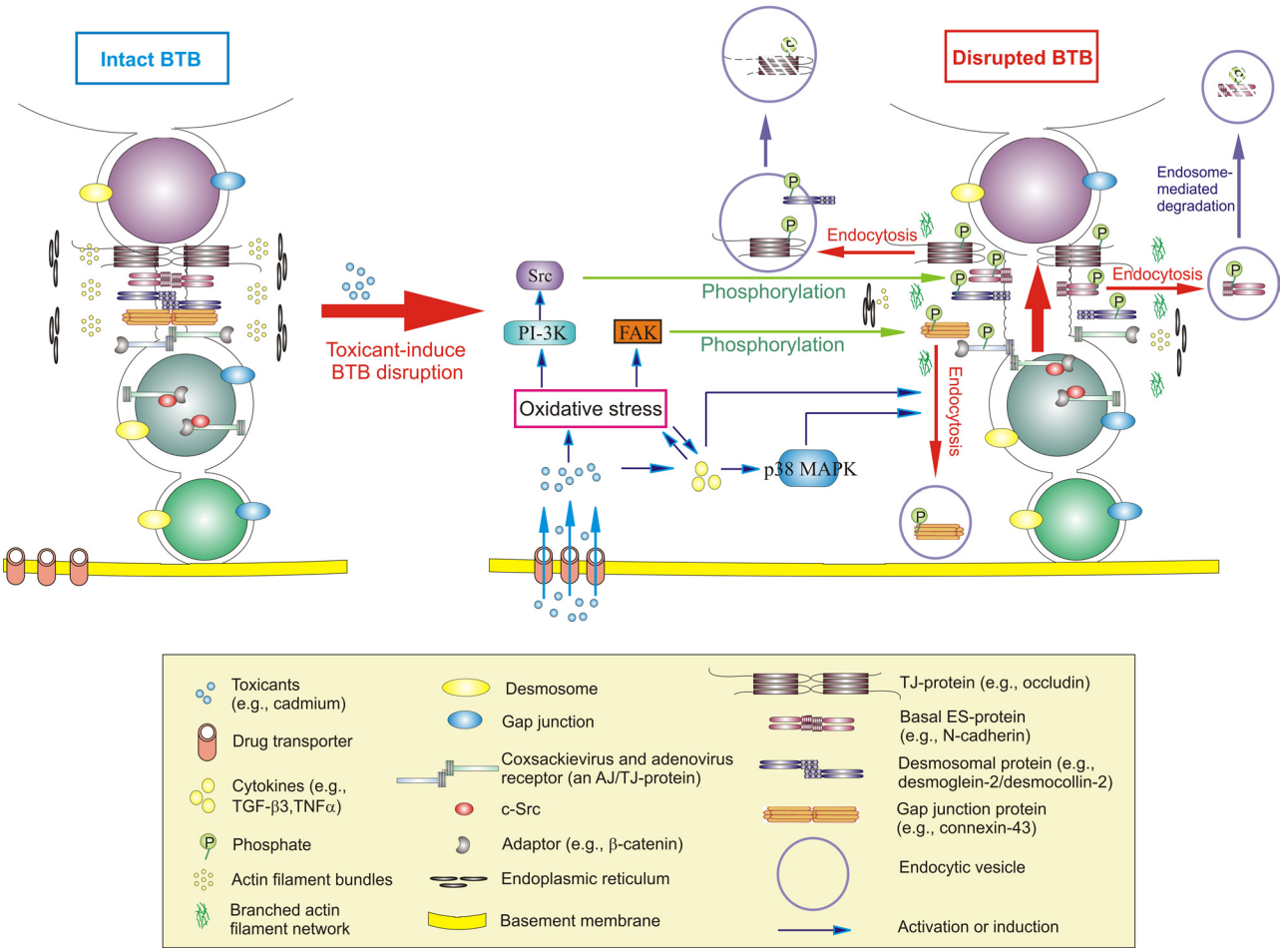

FIG. 11. A schematic drawing illustrating the molecular mechanism underlying BTB disruption induced by a toxicant (e.g., cadmium, BPA). Left, seminiferous epithelium of a normal tubule with an intact BTB. However, toxicants enter the Sertoli cells not at the BTB but instead at the plasma membrane via junction-associated "pores" and/or drug transporters. These toxicants can induce oxidative stress, which, in turn, mediates their effects on kinases (e.g., FAK, c-Src) causing unwanted protein endocytosis, which can destabilize the BTB. Alternatively, toxicants can also activate MAPK (e.g., p38 MAPK, ERK), which can also induce unwanted protein endocytosis. These effects can also be mediated via changes in the homeostasis of the Eps8 and Arp2/3 protein complex, compromising the optimal endocytic vesicle-mediated protein trafficking events, destabilizing cell adhesion at the BTB. The net result leads to a disruption of the BTB, which in turn affects germ cell adhesion in the basal and apical compartment of the epithelium, causing exfoliation of germ cells. These findings also illustrate potential targets that can be tackled to therapeutically manage toxicant-induced BTB disruption and the subsequent male reproductive dysfunction (e.g., reduced sperm count). For instance, the unwanted acceleration of protein endocytosis induced by cadmium or BPA can be prevented by modifying the action of polarity proteins (e.g., 14-3-3, PAR3, PAR6) and/or Arp3/3 complex to stabilize the BTB integrity. Functional studies can now be designed based on this hypothetical model to block or to therapeutically manage toxicant-induced BTB disruption.

c-Src at the apical ES, leading to germ cell loss from the epithelium. This thus induces male reproductive dysfunction as a result of premature germ cell loss, leading to reduced sperm count, poor semen quality, and eventually male infertility. In short, the model depicted in Fig. 11 provides a detailed roadmap for the intervention of cadmium toxicity in the testis by targeting different potential candidate molecules, such as FAK, c-Src, and polarity proteins.

VI. Drug Transporters, Blood-Testis Barrier Function, and Male Contraception

A. Introduction

As noted above, the BTB is a unique ultrastructure in the seminiferous epithelium, critical to spermatogenesis, particularly for the differentiation of spermatogonia into spermatocytes to initiate cell cycle progression that leads to meiosis (Mruk et al., 2008; Lie et al., 2009a), the reinitiation of spermatogenesis after toxicant-induced aspermatogenesis and infertility (Mok et al., 2011a) and spermiogenesis (Cheng and Mruk, 2010a; O'Donnell et al., 2011). In addition, studies from different transgenic mouse models, such as AR-KO mice or Sertoli cell-selective AR KO mice have demonstrated that loss of AR in Sertoli cells that leads to infertility is manifested by meiotic arrest, which is accompanied by a disruption of the BTB (Meng et al., 2005; Verhoeven et al., 2008, 2010; Willems et al., 2010a). Collectively, these findings illustrate the significance of the BTB in meiosis and spermiogenesis. However, the unusual features of the BTB, which make it one of the tightest blood-tissue barriers in the mammalian body, also pose a major obstacle in de-

livering male contraceptives, in particular drugs that exert their effects on postmeiotic spermatids that are sequestered behind the BTB. The BTB also restricts the delivery of therapeutic drugs to the seminiferous epithelium, such as for treatment of germ cell tumors, even though there are some advances using adjuvant chemotherapy in recent years (Mruk and Cheng, 2008; Wong et al., 2008b; Diamantopoulos and Kortsaris, 2010; Mok et al., 2011b; Powles, 2011). For instance, adjudin was shown to be a highly potent male contraceptive that disrupts germ cell adhesion, most notably elongating/elongated spermatids, to be followed by round spermatocytes and spermatocytes, causing transient infertility (Cheng et al., 2001, 2005; Mruk and Cheng, 2004a; Mruk, 2008; Cheng and Mruk, 2010b). However, less than 1% of the drug administered to adult rats by gavage could reach the testis (Cheng et al., 2005), thereby narrowing the margin between efficacy and toxicity. Moreover, an earlier study in rats to assess the permeability of the BTB to different test chemicals and drugs (e.g., salicylic acid, barbiturates, sulfonamides) have shown that the passage of nonelectrolytes and acidic drugs across the barrier is dependent on their molecular sizes and partition coefficients, respectively (Okumura et al., 1975). Thus, a thorough understanding of the biology and regulation of drug transport across the BTB is crucial to develop safe, reversible, and dependable male contraceptives.

B. Background and Classification of Drug Transporters, Their Function, and Cellular Distribution in the Testis

Drug transporters are required for a therapeutic drug (e.g., male contraceptive) and/or an environmental toxicant (e.g., cadmium, bisphenol A) to pass through a blood-tissue barrier (e.g., the BTB) (Dallas et al., 2006; Miller et al., 2008; Hartz and Bauer, 2010; Kis et al., 2010; Burger et al., 2011; Mruk et al., 2011; Niemi et al., 2011; Su et al., 2011a), unless its entry is mediated through a ligand-receptor mechanism (Mruk and Cheng, 2008; Wong et al., 2008b). Drug transporters are best studied in cancer cells, because many tumors display resistance to different classes of chemotherapeutic drugs. This is because drug transporters in cancer cells can *prevent* the entry of therapeutic drugs that are "harmful" to these cancer cells and/or pump "harmful" chemotherapeutic drugs out of cells (i.e., eliminating therapeutic drugs from a growing tumor) (Leslie et al., 2005; Löscher and Potschka, 2005; Miller et al., 2008; Poguntke et al., 2010; Robey et al., 2010; Shukla et al., 2011). Subsequent studies have shown that normal cells and tissues, such as the brain, liver, and testes (including Sertoli cells) and different germ cells (including spermatogonia, spermatocytes, developing spermatids) express high levels of different drug transporters (Leslie et al., 2005; Löscher and Potschka, 2005; Koshiba et al., 2008; Setchell, 2008; He et al., 2009; Mruk et al., 2011; Su et al., 2011a). Besides drugs (e.g., male contraceptives), cations, anions, electrolytes, steroids (e.g., corticosteroids), small biomolecules, and even xenobiotics, toxicants and certain sex hormones (e.g., estrogens, androgens) can enter or be eliminated from a cell (e.g., Sertoli cell) via drug transporters (Löscher and Potschka, 2005; Cérec et al., 2007; Koshiba et al., 2008; Setchell, 2008; Su et al., 2011a), illustrating their significance in conferring a unique microenvironment in the apical compartment behind the BTB for meiosis and postmeiotic spermatid development during spermiogenesis. Approximately 60% of the ~800 drug transporters known to exist to date in mammalian cells and tissues are integral membrane proteins (Rochat, 2009; Hosoya and Tachikawa, 2011; Mruk et al., 2011). Drug transporters can be categorized into 1) ATP-binding cassette (ABC) transporters (i.e., ATP-dependent drug transporters) and 2) solute carrier (SLC) transporters, the transport function of which does not require the consumption of ATP (Löscher and Potschka, 2005; Su et al., 2011a). Drug transporters can also be broadly classified as either efflux or influx pumps that transport drugs out of a cell (or prevent drugs from entering altogether) or into a cell, respectively.

1. ATP-Binding Cassette Drug Transporters in the Testis. All ABC drug transporters found in the testis are efflux pumps. These include 1) multidrug resistance proteins (MDRs; e.g., P-glycoprotein, also known as MDR1), 2) multidrug resistance-related proteins (MRP; e.g., MRP1), and 3) breast cancer resistance protein (BCRP, also known as ABCG2).

a. Multidrug resistance proteins. P-Glycoprotein (also known as MDR1 or ABCB1) is the best studied MDR in the testis. It is the product of the *MDR1* gene in humans, and of *mdr1a* (or *Abcb1a*) and *mdr1b* (or *Abcb1b*) genes in mice and rats (Setchell, 2008). P-Glycoprotein is an ABC transporter. This efflux pump displays drug resistance to a wide variety of drugs and compounds in cancer and normal cells, including Sertoli cells at the BTB (Miller et al., 2008; Aller et al., 2009; Kis et al., 2010; Mruk et al., 2011). In mammalian testes, P-glycoprotein is highly expressed by Sertoli cells, Leydig cells, macrophages, peritubular myoid cells, spermatogonia, and late spermatids, but it is not expressed by spermatocytes and round or early spermatids (Trezise et al., 1992; Melaine et al., 2002; Bart et al., 2004; Su et al., 2009). In studies using the *mdr1a*(−/−) and *mdr1b*(−/−) double-KO mice versus wild-type mice, P-glycoprotein was shown to actively pump the anti-Parkinson drug budipine out of the brain, but there was a 3.1-fold higher retention of budipine in the cerebrum of KO mice versus wild-type (Uhr et al., 2005). It is noteworthy that in these *mdr1a*(−/−) and *mdr1b*(−/−) double-KO mice, the penetration of steroids (e.g., aldosterone, cortisol, corticosterone, and progesterone) was also significantly enhanced in the testis versus wild-type animals (Uhr et al., 2002). However, the entry of cortisol (Karssen et al.,

2001) or prednisolone (Karssen et al., 2002) into the testis was unaffected in *mdr1a*(−/−) single-KO mice, illustrating that *mdr1b* alone can supersede the lost function of the *mdr1a* gene. These findings also illustrate that the BTB constituted by Sertoli cells determines the steroidal microenvironment, such as how much steroid is available in the apical compartment for meiosis and spermiogenesis. Recent studies by immunohistochemistry and dual-labeled immunofluorescence analysis have shown that P-glycoprotein localized most abundantly to the BTB in the seminiferous epithelium, as well as to the apical ES, in all stages of the epithelial cycle in the rat testis (Su et al., 2009). P-Glycoprotein also colocalizes with TJ (e.g., occludin, claudin-11, JAM-A, ZO-1) and basal ES (e.g., N-cadherin, β-catenin) proteins in the seminiferous epithelium in adult rat testes in vivo and also at the Sertoli-Sertoli cell interface in vitro in cells that had a functional TJ barrier (Su et al., 2009). After treatment of adult rats with adjudin to induce germ cell loss via a disruption of the apical ES (Cheng et al., 2005; Mruk et al., 2008; Cheng and Mruk, 2010b; Mok et al., 2011b), a *transient* surge in the expression of P-glycoprotein was detected by immunoblot analysis (Su et al., 2009). Such an increase in P-glycoprotein expression is probably needed to pump adjudin (or its metabolite) out of the testis to avoid damage to germ cell adhesion in the epithelium. In addition, it was shown that P-glycoprotein interacted with occludin, claudin-11, and JAM-A at the BTB by coimmunoprecipitation. More importantly, this protein-protein interaction was induced significantly after treatment of rats with adjudin at the time of germ cell loss, most notably elongating/elongated spermatids, from the epithelium in the apical compartment (Su et al., 2009). The molecular mechanism underlying this increase in association between P-glycoprotein and occludin-, claudin-11-, and JAM-A-based adhesion protein complexes at the BTB is not known, but it may be used to "close up" physiological "pores" at the BTB to disallow further entry of adjudin into the apical compartment to disrupt apical ES function.

b. Multidrug resistance-related proteins. MRP is another efflux transporter subfamily of the ABC transporter, consisting of MRP1 (ABCC1), MRP2 (ABCC2), and MRP3 (ABCC3), found in mammalian cells, as well as in plants and bacteria (Rappa et al., 1999; Pérez-Tomás, 2006; Paumi et al., 2009; Gu and Manautou, 2010; Wanke and Kolukisaoglu, 2010). MRP1 was found to be expressed at relatively high levels in the mouse testis versus other organs. It is detected in Sertoli cells, spermatogonia, round and elongating/elongated spermatids, and even spermatozoa (Stride et al., 1996), but mostly in Sertoli cells and Leydig cells of humans and mice (Flens et al., 1996). MRP1 is the best studied MRP in Sertoli cells, and it is known to transport a wide range of hydrophobic xenobiotics, hydrophilic organic anion conjugates, natural compounds (e.g., glutathione, glucuronide), and steroids (Setchell, 2008) in particular sulfated estrogens (e.g., estrone 3-sulate) in the presence of glutathione (Qian et al., 2001), illustrating that this efflux pump at the BTB plays a role in determining the levels of estrogens in the apical compartment necessary for spermiogenesis. Indeed, MRP1(−/−) mice displayed a significant disruption of the seminiferous epithelium after failing to pump the anticancer drug etoposide phosphate out of the epithelium, with tubules becoming devoid of almost all germ cells except spermatogonia and early spermatocytes by day 7 after administration (Wijnholds et al., 1998). In addition, MRP1 transports heavy metals (such as the xenobiotics sodium arsenate and antimony potassium tartrate) out of epithelial cells, as shown in MRP1-deficient mice (Lorico et al., 2002), illustrating it can protect the testis against infiltration of toxicants to disrupt spermatogenesis. In short, these studies illustrate the protective role of MRPs at the BTB to safeguard meiosis and spermiogenesis that occurs in the apical compartment of the seminiferous epithelium behind the BTB.

c. Breast cancer resistance protein. As its name implies, BCRP, a multidrug resistance ABC transporter, was initially found in breast cancer cells (Robey et al., 2009, 2010; Poguntke et al., 2010). However, it is also a crucial drug efflux pump that limits the penetration of different classes of drugs (Kodaira et al., 2010) or xenobiotics (Enokizono et al., 2008) into the testis. Recent studies have shown that BCRP also mediates renal urate secretion (i.e., pumping uric acid out of the epithelial cells into urine), and it is a target to manage gout disease (VanItallie, 2010). In the testis, BCRP was found in Sertoli cells, consistent with its localization at the BTB, as well as in round and elongating spermatids, peritubular myoid cells, and endothelial cells of microvessels in the interstitium (Bart et al., 2004). It is noteworthy that BCRP also limits the access of phytoestrogens into the testis in rodents, because higher concentrations of daidzein and genistein, but not coumestrol, were found to penetrate the testis in BCRP(−/−) mice versus wild-type animals (Enokizono et al., 2007). These findings thus illustrate that BCRP may play a significant role in determining the amount of phytoestrogens and/or estrogens that can enter the apical compartment behind the BTB to modulate meiosis and spermiogenesis, because estrogens are crucial to these events during spermatogenesis (see section IV.B.2). In addition to ABCG2, ABCG4 (an ABC transporter in human brain) is also found in the mouse brain (Koshiba et al., 2007), and it is highly expressed in the testis and implicated in the transport of sex steroids across the BTB (Koshiba et al., 2008), perhaps maintaining the proper testosterone to estrogen ratio at the BTB microenvironment to regulate junction restructuring (Fig. 9). This possibility should be vigorously investigated in future studies.

2. Solute Carrier Transporters. The SLC transporter superfamily consists of at least 47 SLC subfamilies (e.g.,

zinc efflux, metal ion transporter, foliate transporter) (Hediger et al., 2004), many of which are efflux pumps. However, the 1) SLC21/SLCO) [i.e., organic anion transporting polypeptide (OATP)] and 2) SLC22 [i.e., organic anion transporter (OAT)/organic cation transporter (OCT)/organic cation/carnitine transporter (OCTN)] subfamilies are two of the best studied transporters (both are influx pumps) to date, which includes studies in the testis (Kalliokoski and Niemi, 2009; Kis et al., 2010; Klaassen and Aleksunes, 2010; Su et al., 2011a), so that a brief discussion is provided herein. Transport of drugs across the cell membrane using SLC transporters does not require ATP, because the energy required for transport is derived from a gradient created by a primary active transport system, such as the electrochemical potential difference created by pumping ions out of a cell, or a glutathione electrochemical gradient, such as in oocytes) (Li et al., 2000), as well as via "pores" found in SLC transporters (e.g., OATs, OATPs) (Meier-Abt et al., 2005; Carl et al., 2010; Mruk et al., 2011).

a. SLC21/SLC (organic anion transporting polypeptide) transporters. The OATP subfamily has at least 10 and 15 isoforms found in humans and rodents, respectively, and most of them are influx pumps responsible for drug entry into cells (Hediger et al., 2004; Kalliokoski and Niemi, 2009; Fahrmayr et al., 2010). However, Oatp2 can mediate bidirectional transport of organic anions (Li et al., 2000). Oatp6b1 (Slco6b1, Tst1) and Oatp6c1 (Slco6c1, Tst2) are two testis-specific influx pumps expressed only by Sertoli cells, spermatogonia, and Leydig cells (Suzuki et al., 2003). Oatp3 (Slc21a7) is highly expressed by Sertoli cells in the testis, most notably at the BTB (Augustine et al., 2005; Su et al., 2011b); subsequent studies have shown that its expression at the BTB in the seminiferous epithelium is stage-specific, being highest at stages VII to X and colocalizing with putative BTB proteins (e.g., claudin-11, JAM-A, ZO-1, N-cadherin, β-catenin) (Su et al., 2011b). However, Oatp3 is also found at the apical ES and is intensely associated with developing elongating/elongated spermatids at stages VII to VIII of the epithelial cycle, colocalizing with apical ES proteins, laminin-α3, -β3, and -γ3 chains (Su et al., 2011b). In addition, Oatp3 structurally interacts with ZO-1, N-cadherin, and β-catenin at the BTB, and this association is significantly induced after exposure of adult rats to adjudin, when germ cell adhesion is being disrupted (Su et al., 2009). These findings thus illustrate that this drug transporter is involved in regulating how much drug can enter the microenvironment in the apical compartment behind the BTB. Thus, even if a toxicant or other drug is present in this microenvironment, developing germ cells can determine their "fate" by pumping unwanted drugs "in" or "out" to avoid adverse effects.

b. SLC22 transporters (organic anion transporter/organic cation transporter/organic cation/carnitine transporter). This subfamily is composed of three classes of transporters of OATs, OCTs, and OCTNs. OCT1 (Slc22a1), OCT3 (Slc22a3), OCTN1 (Slc22a4), OCTN2 (Slc22a5), and OCTN3 (Slc22a6) are expressed by Sertoli cells in the testis of either rodents or humans (Koepsell et al., 2007; Klaassen and Aleksunes, 2010), illustrating their likely involvement in BTB function to regulate drug entry into the testis. OCTN2 and OCTN3, both L-carnitine transporters, are also highly expressed in spermatozoa, particularly in the distal and proximal portion of the sperm tail, possibly involved in L-carnitine transport, which is critical for sperm maturation and metabolism during the epididymal transit (Kobayashi et al., 2007). A recent study has identified Oat6 (Slc22a20) to be an influx drug transporter specifically expressed by Sertoli cells (and not Leydig cells or spermatids), and it was shown to mediate the transport of estrone sulfate and dehydroepiandrosterone sulfate across a Chinese hamster ovary cell line stably expressing Oat6 (Schnabolk et al., 2010), illustrating its possible involvement in regulating the amount of steroids in the microenvironment of the apical ES behind the BTB.

C. Are Drug Transporters the "Obstacles" of Male Contraceptive Development?

1. Introduction. Unlike hormonal contraception (e.g., testosterone), which exerts its effects to disrupt the hypothalamic-pituitary-testicular axis (Page et al., 2008; Huhtaniemi, 2010; Wang and Swerdloff, 2010), or approaches that target the epididymis (O'Rand et al., 2007; Blithe, 2008; Kopf, 2008; Mruk, 2008; Sipilä et al., 2009), the development of nonhormonal male contraceptives, such as adjudin (Cheng et al., 2005; Cheng and Mruk, 2010b), bisdichloroacetyldiamines (Hogarth and Griswold, 2010; Amory et al., 2011; Hogarth et al., 2011), gamendazole (Tash et al., 2008a,b), indenopyridine CDB-4022 ([4*aRS*,5S*R*,9*bRS*]2-ethyl-2,3,4,4*a*,5,9*b*-hexahydro-8-iodo-7-methyl-5-[4-carbomethoxyphenyl]-1*H*-indeno-[1,2-*c*]-pyridine-hydrochloride, also known as RTI-4587-073) (Hild et al., 2004, 2007a,b; Koduri et al., 2008), and immunological approaches that target sperm-specific postmeiotic germ cell antigens (Suri, 2005; Mruk, 2008; McLaughlin and Aitken, 2011), requires a better understanding of the BTB because these compounds exert their effects, at least in part, behind the BTB in the apical compartment of the seminiferous epithelium (Mruk et al., 2008; Cheng and Mruk, 2010b; Mok et al., 2011b). As described above, the BTB largely dictates how much drug can enter the apical compartment of the seminiferous epithelium to exert its effects behind the immunological barrier. As briefly reviewed herein, drug transporters found in the testis pose a major hurdle in our efforts to develop a safe, effective, and reversible male contraceptive. It seems that "nature" has also installed an almost "bullet-proof" system in the testis to protect spermatogenesis from being disrupted by unwanted toxicants and/or compounds in which drug transporters are not limited to Sertoli cells that create the BTB; they are also found at relatively high levels in germ cells outside of the BTB (e.g., spermatogonia, early spermatocytes), as well as behind the

BTB (e.g., pachytene spermatocytes, spermatids, spermatozoa). Thus, even if drugs can somehow get access to the developing germ cells in the apical compartment by passing through the BTB, they can still be actively "pumped out" to protect spermatogenesis. Fortunately, several of the compounds that are being actively investigated in the field and that can possibly serve as potential nonhormonal contraceptives (e.g., adjudin, CDB-4022, gamendazole, bisdichloroacetyldiamines) are very potent molecules. For instance, submicrogram quantities of these compounds are needed to disrupt spermatogenesis, such as by damaging germ cell adhesion in the epithelium (e.g., adjudin, CDB-4022) and germ cell metabolism. Nonetheless, if these compounds can penetrate the Sertoli cell BTB efficiently to exert their effects in the apical compartment locally to perturb spermatogenesis, this, at least in principle, should be a relatively safe contraceptive approach, because the hypothalamus-pituitary-testicular axis is not affected and changes in secondary sexual characteristics (e.g., skeletal muscle mass/tone, sex drive), bone density, and blood pressure should be minimal, if any.

2. Entry of Male Contraceptives into the Apical Compartment behind the Blood-Testis Barrier. As discussed above, drug transporters play a very critical role in protecting the testes from xenobiotics, environmental toxicants, drugs, and preventing potential male contraceptives from damaging spermatogenesis, including 1) spermatogonial self-renewal and differentiation and germ cell cycle progression, these events occurring outside the BTB because spermatogonia and early spermatocytes are equipped with different efflux and influx pumps; 2) meiosis; 3) spermiogenesis; and 4) spermiation. Events 2 through 4 take place behind the BTB in the apical compartment of the epithelium. However, it was not known until recently whether any of the potential male contraceptives under development indeed penetrate the BTB using one or several of the dozens of drug transporters that are found in Sertoli cells (Mruk and Cheng, 2008; Mruk et al., 2011; Su et al., 2011a). It was investigated whether Oatp3 alone (an influx pump) or in combination with other SLC transporters (influx pumps) would regulate the entry of adjudin from the basal to the apical compartment across the Sertoli cell BTB using an in vitro system that mimics the BTB in vivo (Grima et al., 1992; Janecki et al., 1992; Okanlawon and Dym, 1996). These SLC transporters included the following:

1. *Slc22a5* (also known as OCTN2, involved in the transport of carnitine and organic cations),
2. *Slco6b1* (also known as testis-specific transporter-1 or gonad-specific transporter-1, implicated in Schwann cell development and involved in the transport of dehydroepiandrosterone sulfate, sex steroids and thyroid hormones), and
3. *Slco6c1* (also known as testis-specific transporter-2 or gonad-specific transporter-2, involved in the transport of thyroxine, taurocholic acid, dehydroepiandrosterone, which is highly expressed by Sertoli cells in the testis (Collarini et al., 1992; Mizuno et al., 2003; Suzuki et al., 2003; Augustine et al., 2005; Ueno et al., 2010) all of which are known to be involved in drug transport in epithelia under normal and pathological conditions (Rochat, 2009; Kis et al., 2010; Klaassen and Aleksunes, 2010)]

It was shown that when [^{3}H]adjudin was placed in the basal compartment of the bicameral unit, it was capable of traversing the Sertoli cell epithelium (an intact TJ-permeability barrier was established in these cultures when barrier function was assessed by quantifying transepithelial electrical resistance across the epithelium) (see Fig. 6) and reaching the apical compartment (Su et al., 2011b). More importantly, a 70% knockdown of Oatp3 by RNAi, on the basis of immunoblot analysis, could significantly reduce, by ~30%, the amount of [^{3}H]adjudin reaching the apical compartment versus controls in which Sertoli cells were transfected with nontargeting control siRNA duplexes. When all four influx pumps, namely Oatp3, *Slc22a5*, *Slco6b1*, and *Slco6c1*, were knocked down by RNAi, the amount of [^{3}H]adjudin that reached the apical compartment was reduced by >70% (Su et al., 2011b). These findings are significant, because they have demonstrated that influx transporters mediate the entry of a male contraceptive (e.g., adjudin) beyond the BTB into the apical compartment. Thus, much research is needed to understand the relationship between influx/efflux pumps and BTB restructuring and whether drug transporters regulate drug entry into the apical compartment via the TJ permeability barrier, as well as by other mechanism(s).

D. Drug Transporters and Blood-Testis Barrier Dynamics: Recent Advances

As discussed in sections IV.B and IV.C, estrogens, androgens, and/or their analogs (e.g., phytoestrogens) are substrates of several drug transporters, and they can be transported "into" and "out" of the apical compartment , illustrating that the combined action of influx and efflux pumps can determine, at least in part, the relative concentrations of estrogens and androgens in the microenvironment near the BTB, such as in the apical and basal compartment. This concept is critical in light of earlier observations that androgens promote (Janecki et al., 1992; Chung and Cheng, 2001; Meng et al., 2005; Wang et al., 2006; Siu et al., 2009b; McCabe et al., 2010; Xiao et al., 2011) and estrogens perturb (Cavicchia et al., 1996; Li et al., 2009d, 2010) Sertoli cell BTB integrity in in vitro and in vivo studies. However, as briefly summarized and discussed herein, studies investigating the transport of steroids using drug transporters are limited to sulfated estrogens, DHEA, and others; further investigation must assess whether estradiol-17β, testosterone, and/or dihydrotestosterone can penetrate the BTB equally well using drug pumps. It is noted that in rats, the transit of preleptotene spermatocytes across the

BTB that occurs at stage VIII of the epithelial cycle is critical for cell cycle progression and meiosis (Parvinen, 1982; Hess and de Franca, 2008; Lie et al., 2009a). Thus, primary spermatocytes can prepare themselves (e.g., diakinesis) for meiosis I and II to take place at stage XIV of the epithelial cycle, to be followed by postmeiotic spermatid development, and all these events occur in an immune-privileged site (i.e., the apical compartment) behind the BTB, which requires the contribution of both estrogens and androgens, among others, such as cytokines and polarity proteins (Wong and Cheng, 2009; Cheng et al., 2011a), to regulate these events (de Kretser and Kerr, 1988; Simpson et al., 2000; O'Donnell et al., 2001, 2011; McLachlan et al., 2002; Carreau and Hess, 2010; Carreau et al., 2010). The recent findings that local estrogen and androgen levels can be efficiently managed by drug transporters and also via the action of aromatase thus illustrate that androgen (or a proper ratio of androgen/estrogen regulated by drug transporters at different stages of the epithelial cycle such as at a ratio of androgen/estrogen >1) in the microenvironment above the preleptotene spermatocytes in transit across the BTB could assist in the assembly of TJ-fibrils to establish a "new" BTB, whereas estrogen (at a ratio of androgen/estrogen <1) can exert its effects to compromise the "old" BTB at the site beneath spermatocytes in transit, combined with the actions of cytokines and polarity proteins (e.g., 14-3-3, PAR6) to facilitate endocytosis and transcytosis of integral membrane proteins at the site, disassembling the TJ fibrils (see Fig. 9). As such, the immunological barrier can be maintained during the transit of spermatocytes at the BTB even though these cells are connected in clones by intercellular bridges (Fawcett et al., 1959; Fawcett, 1961; Weber and Russell, 1987; Tres et al., 1996; Kierszenbaum, 2002; Hamer et al., 2003; Greenbaum et al., 2006; Chang et al., 2010; Hermo et al., 2010).

VII. Conclusion, Outstanding Questions, and Future Perspectives

Herein, we presented a comprehensive overview on the structure, function, and regulation of the BTB and its involvement in different cellular events during spermatogenesis, such as spermatogonial differentiation, initiation of meiosis, or reinitiation of spermatogenesis after environmental toxicant-induced aspermatogenesis. We have also highlighted in different sections regarding the specific areas of research that deserve attention by investigators in future studies. We also present two important hypothetical models; one in Fig. 9 that details the current concept and molecular events of BTB restructuring to accommodate the transit of preleptotene spermatocytes at stage VIII of the epithelial cycle, where the integrity of the immunological barrier can be maintained. This model also reveals multiple new targets for male contraceptive development. For instance, if endocytic vesicle-mediated protein trafficking events are perturbed, preleptotene spermatocytes will fail to enter the apical compartment for the occurrence of meiosis I and II, shutting down spermatogenesis without any interference to the hypothalamic-pituitary-testicular axis, minimizing the risk of side effects, such as boss loss, muscular atrophy, hypertension, and secondary sexual characteristics in men. Of course, this approach will require a better understanding of drug transport mechanisms at the BTB, so that a drug (e.g., a small-molecule inhibitor of clathrin, caveolin-1, or a critical endosome component) can be targeted to the testis via a selected influx pump. The other model shown in Fig. 11 summarizes the current findings in the field underlying the molecular mechanisms of cadmium-induced testicular injury or BPA-induced BTB disruption in immature mammals. This model not only provides a new approach to manage cadmium- or toxicant-induced reproductive dysfunction in men, it also illustrates new candidate molecules to induce male infertility for contraceptive development. For instance, small-molecule inhibitors can be used to block the function of FAK, lessening (or even blocking) the toxicity of cadmium to the BTB, preventing the entry of cadmium to the apical compartment to disrupt germ cell adhesion. This approach can also prevent the entry of preleptotene spermatocytes into the apical compartment for further development, thereby disrupting spermatogenesis that leads to infertility. However, more resources are required to advance our current state of research in this rapidly developing field.

As a final note, there are major advances in the field regarding the delivery of drugs behind other blood-tissue barriers, in particular the blood-brain barrier, such as antiviral drugs or chemotherapeutic drugs, using nanotechnology for treatment of HIV/AIDS and cancer, respectively (Destache, 2009; Hartz and Bauer, 2010; Wong et al., 2010c; Invernici et al., 2011; Malam et al., 2011; Roger et al., 2011). Information obtained from these studies can possibly be applied to the delivery of male contraceptives behind the BTB to exert their effects in the immune-privileged adluminal compartment, which should be carefully evaluated in future studies.

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Wrote or contributed to the writing of the manuscript: Cheng and Mruk.

References

Ahmed S, Goh WI, and Bu W (2010) I-BAR domains, IRSp53 and filopodium formation. *Semin Cell Dev Biol* **21:**350–356.

Agency for Toxic Substances and Disease Registry (2008) Cadmium toxicity: ATSDR Case Studies in Environmental Medicine. Agency for Toxic Substances and Disease Registry, Atlanta, GA. Available at: http://www.atsdr.cdc.gov/csem/cadmium/docs/cadmium.pdf.

Aitken RJ, Findlay JK, Hutt KJ, and Kerr JB (2011) Apoptosis in the germ line. *Reproduction* **141:**139–150.

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, and Walter P (2002) *Molecular Biology of the Cell*, 4th ed, Garland Science, New York.

Aleshin A and Finn RS (2010) SRC: A century of science brought to the clinic. *Neoplasia* **12:**599–607.

Allan CM, Couse JF, Simanainen U, Spaliviero J, Jimenez M, Rodriguez K, Korach KS, and Handelsman DJ (2010) Estradiol induction of spermatogenesis is mediated via an estrogen receptor-α mechanism involving neuroendocrine activation of follicle-stimulating hormone secretion. *Endocrinology* **151:**2800–2810.

Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R, Harrell PM, Trinh YT, Zhang Q, Urbatsch IL, et al. (2009) Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* **323:**1718–1722.

Amann RP and Schanbacher BD (1983) Physiology of male reproduction. *J Anim Sci* **57 (Suppl 2):**380–403.

Amann RP (2008) The cycle of the seminiferous epithelium in humans: a need to revisit? *J Androl* **29:**469–487.

Amory JK, Muller CH, Shimshoni JA, Isoherranen N, Paik J, Moreb JS, Amory DW Sr, Evanoff R, Goldstein AS, and Griswold MD (2011) Suppression of spermatogenesis by bisdichloroacetyldiamines is mediated by inhibition of testicular retinoic acid biosynthesis. *J Androl* **32:**111–119.

Anderson JM and Van Itallie CM (2009) Physiology and function of the tight junction. *Cold Spring Harb Perspect Biol* **1:**a002584.

Andersson A, Page AL, and Stoeppler M (1986) Cadmium: uses, occurrence, and intake, in *Cadmium and Health: A Toxicological and Epidemiological Appraisal. Vol. 1. Exposure, Dose, and Metabolism* (Friberg L, Elinder CG, Kjellstrom T, and Nordberg GF eds) pp 23–63, CRC Press, Boca Raton, FL.

Antal MC, Krust A, Chambon P, and Mark M (2008) Sterility and absence of histopathological defects in nonreproductive organs of a mouse ERβ-null mutant. *Proc Natl Acad Sci USA* **105:**2433–2438.

Aoyama Y, Yamamoto Y, Yamaguchi F, and Kitajima Y (2009) Low to high Ca^{2+}-switch causes phosphorylation and association of desmocollin 3 with plakoglobin and desmoglein 3 in cultured keratinocytes. *Exp Dermatol* **18:**404–408.

Aslam H, Rosiepen G, Krishnamurthy H, Arslan M, Clemen G, Nieschlag E, and Weinbauer GF (1999) The cycle duration of the seminiferous epithelium remains unaltered during GnRH antagonist-induced testicular involution in rats and monkeys. *J Endocrinol* **161:**281–288.

Assémat E, Bazellières E, Pallesi-Pocachard E, Le Bivic A, and Massey-Harroche D (2008) Polarity complex proteins. *Biochim Biophys Acta* **1778:**614–630.

Augustine LM, Markelewicz RJ Jr, Boekelheide K, and Cherrington NJ (2005) Xenobiotic and endobiotic transporter mRNA expression in the blood-testis barrier. *Drug Metab Dispos* **33:**182–189.

Auharek SA and de França LR (2010) Postnatal testis development, Sertoli cell proliferation and number of different spermatogonial types in C57BL/6J mice made transiently hypo- and hyperthyroidic during the neonatal period. *J Anat* **216:**577–588.

Baker RG, Hayden MS, and Ghosh S (2011) NF-κB, inflammation, and metabolic disease. *Cell Metab* **13:**11–22.

Barraclough J, Hodgkinson C, Hogg A, Dive C, and Welman A (2007) Increases in c-Yes expression level and activity promote motility but not proliferation of human colorectal carcinoma cells. *Neoplasia* **9:**745–754.

Barrett JR (2009) Cadmium and breast cancer: exposure associated with basal-like phenotype. *Environ Health Perspect* **117:**A552.

Bart J, Hollema H, Groen HJ, de Vries EG, Hendrikse NH, Sleijfer DT, Wegman TD, Vaalburg W, and van der Graaf WT (2004) The distribution of drug-efflux pumps, P-gp, BCRP, MRP1 and MRP2, in the normal blood-testis barrier and in primary testicular tumours. *Eur J Cancer* **40:**2064–2070.

Bartke A (1995) Apoptosis of male germ cells, a generalized or cell type-specific phenomenon? *Endocrinology* **136:**3–4.

Bass-Zubek AE, Godsel LM, Delmar M, and Green KJ (2009) Plakophilins: multifunctional scaffolds for adhesion and signaling. *Curr Opin Cell Biol* **21:**708–716.

Beardsley A, Robertson DM, and O'Donnell L (2006) A complex containing α6β1-integrin and phosphorylated focal adhesion kinase between Sertoli cells and elongated spermatids during spermatid release from the seminiferous epithelium. *J Endocrinol* **190:**759–770.

Benoff S, Auborn K, Marmar JL, and Hurley IR (2008) Link between low-dose environmentally relevant cadmium exposures and asthenozoospermia in a rat model. *Fertil Steril* **89 (2 Suppl):**e73–e79.

Benoff S, Hauser R, Marmar JL, Hurley IR, Napolitano B, and Centola GM (2009) Cadmium concentrations in blood and seminal plasma: correlations with sperm number and motility in three male populations (infertility patients, artificial insemination donors, and unselected volunteers). *Mol Med* **15:**248–262.

Benoff S, Jacob A, and Hurley IR (2000) Male infertility and environmental exposure to lead and cadmium. *Hum Reprod Update* **6:**107–121.

Bergmann M and Dierichs R (1983) Postnatal formation of the blood-testis barrier in the rat with special reference to the initiation of meiosis. *Anat Embryol* **168:**269–275.

Berndtson WE and Thompson TL (1990) Changing relationships between testis size, Sertoli cell number and spermatogenesis in Sprague-Dawley rats. *J Androl* **11:**429–435.

Beveridge R, Pintos J, Parent ME, Asselin J, and Siemiatycki J (2010) Lung cancer risk associated with occupational exposure to nickel, chromium VI, and cadmium in two population-based case-control studies in Montreal. *Am J Ind Med* **53:**476–485.

Billig H, Furuta I, Rivier C, Tapanainen J, Parvinen M, and Hsueh AJ (1995) Apoptosis in testis germ cells: developmental changes in gonadotropin dependence and localization to selective tubule stages. *Endocrinology* **136:**5–12.

Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, et al. (1997) A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. *Nature* **385:**729–733.

Blasig IE, Bellmann C, Cording J, Vecchio GD, Zwanziger D, Huber O, and Haseloff RF (2011) Occludin protein family: oxidative stress and reducing conditions. *Antioxid Redox Signal* **15:**1195–1219.

Blithe D (2008) Male contraception: what is on the horizon? *Contraception* **78 (Suppl 4):**S23–S27.

Boekelheide K and Hall SJ (1991) 2,5-Hexanedione exposure in the rat results in long-term testicular atrophy despite the presence of residual spermatogonia. *J Androl* **12:**18–26.

Bolós V, Gasent JM, López-Tarruella S, and Grande E (2010) The dual kinase complex FAK-Src as a promising therapeutic target in cancer. *Onco Targets Ther* **3:**83–97.

Bonde JP (2010) Male reproductive organs are at risk from environmental hazards. *Asian J Androl* **12:**152–156.

Bordeleau LJ and Leclerc P (2008) Expression of hck-tr, a truncated form of the src-related tyrosine kinase hck, in bovine spermatozoa and testis. *Mol Reprod Dev* **75:**828–837.

Bouffard G (1906) Injection des couleurs de benzidine aux animaux normaux. *Ann Inst Pasteur* **20:**539–546.

Boutros T, Chevet E, and Metrakos P (2008) Mitogen-activated protein (MAP) kinase/MAP kinase phosphatase regulation: roles in cell growth, death, and cancer. *Pharmacol Rev* **60:**261–310.

Brawley C and Matunis E (2004) Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. *Science* **304:**1331–1334.

Brehm R, Zeiler M, Rüttinger C, Herde K, Kibschull M, Winterhager E, Willecke K, Guillou F, Lécureuil C, Steger K, et al. (2007) A Sertoli cell-specific knockout of connexin43 prevents initiation of spermatogenesis. *Am J Pathol* **171:**19–31.

Bruewer M, Utech M, Ivanov AI, Hopkins AM, Parkos CA, and Nusrat A, Ivanov AI, Hopkins AM, Parkos CA, and Nusrat A (2005) Interferon-γ induces internalization of epithelial tight junction proteins via a macropinocytosis-like process. *FASEB J* **19:**923–933.

Brunton VG and Frame MC (2008) Src and focal adhesion kinase as therapeutic targets in cancer. *Curr Opin Pharmacol* **8:**427–432.

Burger H, Loos WJ, Eechoute K, Verweij J, Mathijssen RH, and Wiemer EA (2011) Drug transporters of platinum-based anticancer agents and their clinical significance. *Drug Resist Updat* **14:**22–34.

Byers SW, Hadley MA, Djakiew D, and Dym M (1986) Growth and characterization of polarized monolayers of epididymal epithelial cells and Sertoli cells in dual environment culture chambers. *J Androl* **7:**59–68.

Caballero OL and Chen YT (2009) Cancer/testis (CT) antigens: potential targets for immunotherapy. *Cancer Sci* **100:**2014–2021.

Cabodi S, del Pilar Camacho-Leal M, Di Stefano P, and Defilippi P (2010a) Integrin signalling adaptor: not only figurants in the cancer story. *Nat Rev Cancer* **10:**858–870.

Cabodi S, Di Stefano P, Leal Mdel P, Tinnirello A, Bisaro B, Morello V, Damiano L, Aramu S, Repetto D, Tornillo G, et al. (2010b) Integrins and signal transduction. *Adv Exp Med Biol* **674:**43–54.

Calabro AR, Gazarian DI, and Barile FA (2011) Effect of metals on β-actin and total protein synthesis in cultured human intestinal epithelial cells. *J Pharmacol Toxicol Methods* **63:**47–58.

Capaldo CT and Nusrat A (2009) Cytokine regulation of tight junctions. *Biochem Biophys Acta* **1788:**864–871.

Cardoso FL, Brites D, and Brito MA (2010) Looking at the blood-brain barrier: molecular anatomy and possible investigation approach. *Brain Res Rev* **64:**328–363.

Carette D, Gilleron J, Decrouy X, Fiorini C, Diry M, Segretain D, and Pointis G (2009) Connexin 33 impairs gap junction functionality by accelerating connexin 43 gap junction plaque endocytosis. *Traffic* **10:**1272–1285.

Carette D, Gilleron J, Segretain D, and Pointis G (2010a) Heteromeric connexin 43/connexin 33 complex endocytosis: a connexin phosphorylation independent mechanism. *Biochimie* **92:**555–559.

Carette D, Weider K, Gilleron J, Giese S, Dompierre J, Bergmann M, Brehm R, Denizot JP, Segretain D, and Pointis G (2010b) Major involvement of connexin 43 in seminiferous epithelial junction dynamics and male fertility. *Dev Biol* **346:**54–67.

Carl SM, Lindley DJ, Couraud PO, Weksler BB, Romero I, Mowery SA, and Knipp GT (2010) ABC and SLC transporter expression and pot substrate characterization across the human CMEC/D3 blood-brain barrier cell line. *Mol Pharm* **7:**1057–1068.

Carlomagno G, van Bragt MP, Korver CM, Repping S, de Rooij DG, and van Pelt AM (2010) BMP4-induced differentiation of a rat spermatogonial stem cell line causes changes in its cell adhesion properties. *Biol Reprod* **83:**742–749.

Carreau S and Hess RA (2010) Oestrogens and spermatogenesis. *Philos Trans R Soc Lond B Biol Sci* **365:**1517–1535.

Carreau S, Delalande C, and Galeraud-Denis I (2009) Mammalian sperm quality and aromatase expression. *Microsc Res Tech* **72:**552–557.

Carreau S, Wolczynski S, and Galeraud-Denis I (2010) Aromatase, oestrogens and human male reproduction. *Philos Trans R Soc Lond B Biol Sci* **365:**1571–1579.
Cavicchia JC, Sacerdote FL, and Ortiz L (1996) The human blood-testis barrier in impaired spermatogenesis. *Ultrastruct Pathol* **20:**211–218.
Cérec V, Piquet-Pellorce C, Aly HA, Touzalin AM, Jégou B, and Bauché F (2007) Multiple pathways for cationic amino acid transport in rat seminiferous tubule cells. *Biol Reprod* **76:**241–249.
Chang C, Chen YT, Yeh SD, Xu Q, Wang RS, Guillou F, Lardy H, and Yeh S (2004) Infertility with defective spermatogenesis and hypotestosteronemia in male mice lacking the androgen receptor in Sertoli cells. *Proc Natl Acad Sci USA* **101:**6876–6881.
Chang YC, Chen YJ, Wu CH, Wu YC, Yen TC, and Ouyang P (2010) Characterization of centrosomal proteins Cep55 and pericentrin in intercellular bridges of mouse testes. *J Cell Biochem* **109:**1274–1285.
Chatzizacharias NA, Kouraklis GP, and Theocharis SE (2010) The role of focal adhesion kinase in early development. *Histol Histopathol* **25:**1039–1055.
Chen YH, Lu Q, Goodenough DA, and Jeansonne B (2002) Nonreceptor tyrosine kinase c-Yes interacts with occludin during tight junction formation in canine kidney epithelial cells. *Mol Biol Cell* **13:**1227–1237.
Cheng CY and Mruk DD (2002) Cell junction dynamics in the testis: Sertoli-germ cell interactions and male contraceptive development. *Physiol Rev* **82:**825–874.
Cheng CY and Mruk DD (2009a) An intracellular trafficking pathway in the seminiferous epithelium regulating spermatogenesis: a biochemical and molecular perspective. *Crit Rev Biochem Mol Biol* **44:**245–263.
Cheng CY and Mruk DD (2009b) Regulation of blood-testis barrier dynamics by focal adhesion kinase (FAK): an unexpected turn of events. *Cell Cycle* **8:**3493–3499.
Cheng CY and Mruk DD (2010a) A local autocrine axis in the testes that regulates spermatogenesis. *Nat Rev Endocrinol* **6:**380–395.
Cheng CY and Mruk DD (2010b) New frontiers in nonhormonal male contraception. *Contraception* **82:**476–482.
Cheng CY and Mruk DD (2011) Regulation of spermiogenesis, spermiation and blood-testis barrier dynamics: novel insights from studies on Eps8 and Arp3. *Biochem J* **435:**553–562.
Cheng CY, Mruk D, Silvestrini B, Bonanomi M, Wong CH, Siu MK, Lee NP, Lui WY, and Mo MY (2005) AF-2364 [1-(2,4-dichlorobenzyl)-*1H*-indazole-3-carbohydrazide] is a potential male contraceptive: a review of recent data. *Contraception* **72:**251–261.
Cheng CY, Silvestrini B, Grima J, Mo MY, Zhu LJ, Johansson E, Saso L, Leone MG, Palmery M, and Mruk D (2001) Two new male contraceptives exert their effects by depleting germ cells prematurely from the testis. *Biol Reprod* **65:**449–461.
Cheng CY, Wong EW, Lie PP, Li MW, Mruk DD, Yan HH, Mok KW, Mannu J, Mathur PP, Lui WY, et al. (2011a) Regulation of blood-testis barrier dynamics by desmosome, gap junction, hemidesmosome and polarity proteins: an unexpected turn of events. *Spermatogenesis* **1:**105–115.
Cheng CY, Wong EW, Lie PP, Li MW, Su L, Siu ER, Yan HH, Mannu J, Mathur PP, Bonanomi M, et al. (2011b) Environmental toxicants and male reproductive function. *Spermatogenesis* **1:**2–13.
Cheng CY, Wong EW, Yan HH, and Mruk DD (2010) Regulation of spermatogenesis in the microenvironment of the seminiferous epithelium: new insights and advances. *Mol Cell Endocrinol* **315:**49–56.
Cheskis BJ, Greger JG, Nagpal S, and Freedman LP (2007) Signaling by estrogens. *J Cell Physiol* **213:**610–617.
Chiaverini N and De Ley M (2010) Protective effect of metallothionein on oxidative stress-induced DNA damage. *Free Radic Res* **44:**605–613.
Chimento A, Sirianni R, Delalande C, Silandre D, Bois C, Andò S, Maggiolini M, Carreau S, and Pezzi V (2010) 17β-Estradiol activates rapid signaling pathways involved in rat pachytene spermatocytes apoptosis through GPR30 and ER α. *Mol Cell Endocrinol* **320:**136–144.
Chimento A, Sirianni R, Zolea F, Bois C, Delalande C, Andò S, Maggiolini M, Aquila S, Carreau S, and Pezzi V (2011) Gper and ESRs are expressed in rat round spermatids and mediate oestrogen-dependent4 rapid pathways modulating expression of cyclin B1 and Bax. *Int J Androl* http://dx.doi.org/0.1111/j.1365-2605.2010.01100.x.
Chinnam M and Goodrich DW (2011) RB1, development, and cancer. *Curr Top Dev Biol* **94:**129–169.
Chiquoine AD (1964) Observations on the early events of cadmium necrosis of the testis. *Anat Rec* **149:**23–35.
Choi YJ, Seelbach MJ, Pu H, Eum SY, Chen L, Zhang B, Hennig B, and Toborek M (2010) Polychlorinated biphenyls disrupt intestinal integrity via NADPH oxidase-induced alterations of tight junction protein expression. *Environ Health Perspect* **118:**976–981.
Chong YP, Ia KK, Mulhern TD, and Cheng HC (2005) Endogenous and synthetic inhibitors of the Src-family protein tyrosine kinases. *Biochem Biophys Acta* **1754:**210–220.
Chung NP and Cheng CY (2001) Is cadmium chloride-induced inter-Sertoli tight junction permeability barrier disruption a suitable in vitro model to study the events of junction disassembly during spermatogenesis in the rat testis? *Endocrinology* **142:**1878–1888.
Clague MJ and Urbé S (2010) Ubiquitin: same molecule, different degradation pathways. *Cell* **143:**682–685.
Clermont Y and Harvey SC (1965) Duration of the cycle of the seminiferous epithelium of normal, hypophysectomized and hypophysectomized-hormone treated albino rats. *Endocrinology* **76:**80–89.
Clermont Y and Perry B (1957) Quantitative study of the cell population of the seminiferous tubules in immature rats. *Am J Anat* **100:**241–267.
Clermont Y and Trott M (1969) Duration of the cycle of the seminiferous epithelium in the mouse and hamster determined by means of ^{3}H-thymidine and radioautography. *Fertil Steril* **20:**805–817.
Clermont Y (1963) The cycle of the seminiferous epithelium in man. *Am J Anat* **112:**35–51.
Clermont Y (1972) Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. *Physiol Rev* **52:**198–236.
Clermont Y, Leblond CP, and Messier B (1959) [Duration of the cycle of the seminal epithelium of the rat]. *Arch Anat Microsc Morphol Exp* **48:**37–55.
Cockcroft S and Garner K (2011) Function of the phosphatidylinositol transfer protein gene family: is phosphatidylinositol transfer the mechanism of action? *Crit Rev Biochem Mol Biol* **46:**89–117.
Collarini EJ, Kuhn R, Marshall CJ, Monuki ES, Lemke G, and Richardson WD (1992) Down-regulation of the POU transcription factor SCIP is an early event in oligodendrocyte differentiation in vitro. *Development* **116:**193–200.
Com E, Bourgeon F, Evrard B, Ganz T, Colleu D, Jégou B, and Pineau C (2003) Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. *Biol Reprod* **68:**95–104.
Cooke PS and Meisami E (1991) Early hypothyroidism in rats causes increased adult testis and reproductive organ size but does not change testosterone levels. *Endocrinology* **129:**237–243.
Cooke PS, Hess RA, Porcelli J, and Meisami E (1991) Increased sperm production in adult rats after transient neonatal hypothyroidism. *Endocrinology* **129:**244–248.
Cooke PS, Porcelli J, and Hess RA (1992) Induction of increased testis growth and sperm production in adult rats by neonatal administration of the goitrogen propylthiouracil (PTU): the critical period. *Biol Reprod* **46:**146–154.
Cordenonsi M, Mazzon E, De Rigo L, Baraldo S, Meggio F, and Citi S (1997) Occludin dephosphorylation in early development of *Xenopus laevis*. *J Cell Sci* **110:**3131–3139.
Cordenonsi M, Turco F, D'atri F, Hammar E, Martinucci G, Meggio F, and Citi S (1999) *Xenopus laevis* occludin. Identification of in vitro phosphorylation sites by protein kinase CK2 and association with cingulin. *Eur J Biochem* **264:**374–384.
Couse JE, Mahato D, Eddy EM, and Korach KS (2001) Molecular mechanism of estrogen action in the male: insights from the estrogen receptor null mice. *Reprod Fertil Dev* **13:**211–219.
Cudicini C, Kercret H, Touzalin AM, Ballet F, and Jégou B (1997) Vectorial production of interleukin 1 and interleukin 6 by rat Sertoli cells cultured in a dual culture compartment system. *Endocrinology* **138:**2863–2870.
Cunha-Vaz JG (2004) The blood-retinal barriers system. Basic concepts and clinical evaluation. *Exp Eye Res* **78:**715–721.
d'Anglemont de Tassigny X, and Colledge WH (2010) The role of kisspeptin signaling in reproduction. *Physiology* **25:**207–217.
Dallas S, Miller DS, and Bendayan R (2006) Multidrug resistance-associated proteins: expression and function in the central nervous system. *Pharmacol Rev* **58:**140–161.
Dalton T, Fu K, Enders GC, Palmiter RD, and Andrews GK (1996) Analysis of the effects of overexpression of metallothionein-1 in transgenic mice on the reproductive toxicology of cadmium. *Environ Health Perspect* **104:**68–76.
Dalton TP, He L, Wang B, Miller ML, Jin L, Stringer KF, Chang X, Baxter CS, and Nebert DW (2005) Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. *Proc Natl Acad Sci USA* **102:**3401–3406.
De Cesaris P, Starace D, Starace G, Filippini A, Stefanini M, and Ziparo E (1999) Activation of Jun N-terminal kinase/stress-activated protein kinase pathway by tumor necrosis factor α leads to intercellular adhesion molecule-1 expression. *J Biol Chem* **274:**28978–28982.
De França LR, Hess RA, Cooke PS, and Russell LD (1995) Neonatal hypothyroidism causes delayed Sertoli cell maturation in rats treated with propylthiouracil: evidence that the Sertoli cell controls testis growth. *Anat Rec* **242:**57–69.
De Gendt K, Swinnen JV, Saunders PT, Schoonjans L, Dewerchin M, Devos A, Tan K, Atanassova N, Claessens F, Lécureuil C, et al. (2004) A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. *Proc Natl Acad Sci USA* **101:**1327–1332.
de Kretser D and Kerr J (1988) The cytology of the testis, in *The Physiology of Reproduction* (Knobil E, Neill J, Ewing L, Greenwald G, Markert C, and Pfaff D eds) vol 1, pp 837–932, Raven Press, New York.
de Rooij DG and Russell LD (2000) All you wanted to know about spermatogonia but were afraid to ask. *J Androl* **21:**776–798.
de Rooij DG (2009) The spermatogonial stem cell niche. *Microsc Res Tech* **72:**580–585.
De SK, Chen HL, Pace JL, Hunt JS, Terranova PF, and Enders GC (1993) Expression of tumor necrosis factor-α in mouse spermatogenic cells. *Endocrinology* **133:**389–396.
Dejana E, Orsenigo F, and Lampugnani MG (2008) The role of adherens junctions and VE-cadherin in the control of vascular permeability. *J Cell Sci* **121:**2115–2122.
Dejucq N, Chousterman S, and Jégou B (1997) The testicular antiviral defense system: localization, expression, and regulation of 2′5′ oligoadenylate synthetase, double-stranded RNA-activated protein kinase, and Mx proteins in the rat seminiferous tubule. *J Cell Biol* **139:**865–873.
Dejucq N, Liénard MO, and Jégou B (1998b) Interferons and interferon-induced antiviral proteins in the testis. *J Reprod Immunol* **41:**291–300.
Dejucq N, Lienard MO, Guillaume E, Dorval I, and Jégou B (1998a) Expression of interferons-α and -γ in testicular interstitial tissue and spermatogonia of the rat. *Endocrinology* **139:**3081–3087.
Delfino FJ, Boustead JN, Fix C, and Walker WH (2003) NF-κB and TNF-α stimulate androgen receptor expression in Sertoli cells. *Mol Cell Endocrinol* **201:**1–12.
Dempsey PW, Doyle SE, He JQ, and Cheng G (2003) The signaling adaptors and pathways activated by TNF superfamily. *Cytokine Growth Factor Rev* **14:**193–209.
Destache CJ (2009) Chapter 12—Brain as an HIV sequestered site: use of nanoparticles as a therapeutic option. *Prog Brain Res* **180:**225–233.
Diamantopoulos N and Kortsaris A (2010) Testicular germ cell tumors. *J BUON* **15:**421–434.
Dörfel MJ, Westphal JK, and Huber O (2009) Differential phosphorylation of occludin and tricellulin by CK2 and CK1. *Ann N Y Acad Sci* **1165:**69–73.
Dufour JM, Dass B, Halley KR, Korbutt GS, Dixon DE, and Rajotte RV (2008) Sertoli

cell line lacks the immunoprotective properties associated with primary Sertoli cells. *Cell Transplant* **17:**525–534.
Dufour JM, Gores P, Hemendinger R, Emerich DF, and Halberstadt CR (2004) Transgenic Sertoli cells as a vehicle for gene therapy. *Cell Transplant* **13:**1–6.
Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, and Mark M (2000) Effect of single and compound knockouts of estrogen receptors α (ERα) and β (ERβ) on mouse reproductive phenotypes. *Development* **127:**4277–4291.
Dym M and Cavicchia JC (1977) Further observations on the blood-testis barrier in monkeys. *Biol Reprod* **17:**390–403.
Dym M and Fawcett DW (1970) The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol Reprod* **3:**308–326.
Dym M (1973) The fine structure of the monkey (*Macaca*) Sertoli cell and its role in maintaining the blood-testis barrier. *Anat Rec* **175:**639–656.
Dym M (1994) Basement membrane regulation of Sertoli cells. *Endocr Rev* **15:**102–115.
Easton AS (2011) Regulation of permeability across the blood-brain barrier, in *Biology and Regulation of Blood-Tissue Barriers* (Cheng CY ed) pp in press, Landes Bioscience, Austin, TX.
Eddy EM, Washburn TF, Bunch DO, Goulding EH, Gladen BC, Lubahn DB, and Korach KS (1996) Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. *Endocrinology* **137:**4796–4805.
Elias BC, Suzuki T, Seth A, Giorgianni F, Kale G, Shen L, Turner JR, Naren A, Desiderio DM, and Rao R (2009) Phosphorylation of Tyr-398 and Tyr-402 in occludin prevents its interaction with ZO-1 and destabilizes its assembly at the tight junctions. *J Biol Chem* **284:**1559–1569.
Elkin ND, Piner JA, and Sharpe RM (2010) Toxicant-induced leakage of germ cell-specific proteins from seminiferous tubules in the rat: relationship to blood-testis barrier integrity and prospects for biomonitoring. *Toxicol Sci* **117:**439–448.
Enders G (1993) Sertoli-Sertoli and Sertoli-germ cell communications, in *The Sertoli Cell* (Russell L and Griswold M eds) pp 447–460, Cache River Press, Clearwater, FL.
Engen JR, Wales TE, Hochrein JM, Meyn MA 3rd, Banu Ozkan S, Bahar I, and Smithgall TE (2008) Structure and dynamic regulation of Src-family kinases. *Cell Mol Life Sci* **65:**3058–3073.
Enokizono J, Kusuhara H, and Sugiyama Y (2007) Effect of breast cancer resistance protein (Bcrp/Abcg2) on the disposition of phytoestrogens. *Mol Pharmacol* **72:**967–975.
Enokizono J, Kusuhara H, Ose A, Schinkel AH, and Sugiyama Y (2008) Quantitative investigation of the role of breast cancer resistance protein (Bcrp/Abcg2) in limiting brain and testis penetration of xenobiotic compounds. *Drug Metab Dispos* **36:**995–1002.
Eyerich S, Eyerich K, Cavani A, and Schmidt-Weber C (2010) IL-17 and IL-22: siblings, not twins. *Trends Immunol* **31:**354–361.
Fahrmayr C, Fromm MF, and König J (2010) Hepatic OATP and OCT uptake transporters: their role for drug-drug interactions and pharmacogenetic aspects. *Drug Metab Rev* **42:**380–401.
Fallarino F, Luca G, Calvitti M, Mancuso F, Nastruzzi C, Fioretti MC, Grohmann U, Becchetti E, Burgevin A, Kratzer R, et al. (2009) Therapy of experimental type 1 diabetes by isolated Sertoli cell xenografts alone. *J Exp Med* **206:**2511–2526.
Faustman D and Davis M (2010) TNF receptor 2 pathway: drug target for autoimmune diseases. *Nat Rev Drug Discov* **9:**482–493.
Fawcett DW (1961) Intercellular bridges. *Exp Cell Res* **8:**174–187.
Fawcett DW, Ito S, and Slautterback D (1959) The occurrence of intercellular bridges in groups of cells exhibiting synchronous differentiation. *J Biophys Cytol* **5:**453–460.
Fawcett DW, Leak LV, and Heidger PM Jr (1970) Electron microscopic observations on the structural components of the blood-testis barrier. *J Reprod Fertil Suppl* **10:**105–122.
Fiers W (1991) Tumor necrosis factor. Characterization at the molecular, cellular and in vivo level. *FEBS Lett* **285:**199–212.
Fijak M, Bhushan S, and Meinhardt A (2011) Immunoprivileged sites: the testis. *Methods Mol Biol* **677:**459–470.
Findley MK and Koval M (2009) Regulation and roles for claudin-family tight junction proteins. *IUBMB Life* **61:**431–437.
Fiorini C, Tilloy-Ellul A, Chevalier S, Charuel C, and Pointis G (2004) Sertoli cell junctional proteins as early targets for different classes of reproductive toxicants. *Reprod Toxicol* **18:**413–421.
Firat-Karalar EN and Welch MD (2011) New mechanisms and functions of actin nucleation. *Curr Opin Cell Biol* **23:**4–13.
Flens MJ, Zaman GJ, van der Valk P, Izquierdo MA, Schroeijers AB, Scheffer GL, van der Groep P, de Haas M, Meijer CJ, and Scheper RJ (1996) Tissue distribution of the multidrug resistance protein. *Am J Pathol* **148:**1237–1247.
Flickinger C and Fawcett DW (1967) The junctional specializations of Sertoli cells in the seminiferous epithelium. *Anat Rec* **158:**207–221.
Förster C, Burek M, Romero IA, Weksler B, Couraud PO, and Drenckhahn D (2008) Differential effects of hydrocortisone and TNFα on tight junction proteins in an in vitro model of the human blood-brain barrier. *J Physiol* **586:**1937–1949.
Frame MC, Patel H, Serrels B, Lietha D, and Eck MJ (2010) The FERM domain: organizing the structure and function of FAK. *Nat Rev Mol Cell Biol* **11:**802–814.
Franca LR, Auharek SA, Hess RA, Dufour JM, and Hinton BT (2011) Morphofunctional and immunological aspects of the blood-testis and blood-epididymal barriers, in *Biology and Regulation of Blood-Tissue Barriers* (Cheng CY ed) pp in press, Landes Bioscience, Austin, TX.
França LR, Ogawa T, Avarbock MR, Brinster RL, and Russell LD (1998) Germ cell genotype controls cell cycle during spermatogenesis in the rat. *Biol Reprod* **59:**1371–1377.
Francavilla F, Santucci R, Barbonetti A, and Francavilla S (2007) Naturally-occurring antisperm antibodies in men: interference with fertility and clinical implications. An update. *Front Biosci* **12:**2890–2911.
Fukumoto M, Kujiraoka T, Hara M, Shibasaki T, Hosoya T, and Yoshida M (2001) Effect of cadmium on gap junctional intercellular communication in primary cultures of rat renal proximal tubular cells. *Life Sci* **69:**247–254.
Funakoshi Y, Hasegawa H, and Kanaho Y (2011) Regulation of PIP5K activity by Arf6 and its physiological significance. *J Cell Physiol* **226:**888–895.
Getsios S, Huen AC, and Green KJ (2004) Working out the strength and flexibility of desmosomes. *Nat Rev Mol Cell Biol* **5:**271–281.
Gilleron J, Carette D, Durand P, Pointis G, and Segretain D (2009a) Connexin 43 is a potential regulator of cell proliferation and apoptosis within the seminiferous epithelium. *Int J Biochem Cell Biol* **41:**1381–1390.
Gilleron J, Carette D, Fiorini C, Benkdane M, Segretain D, and Pointis G (2009b) Connexin 43 gap junction plaque endocytosis implies molecular remodelling of ZO-1 and c-Src partners. *Commun Integr Biol* **2:**104–106.
Gilleron J, Fiorini C, Carette D, Avondet C, Falk MM, Segretain D, and Pointis G (2008) Molecular reorganization of Cx43, ZO-1 and Src complexes during the endocytosis of gap junction plaques in response to a non-genomic carcinogen. *J Cell Sci* **121:**4069–4078.
Gilula NB, Fawcett DW, and Aoki A (1976) The Sertoli cell occluding junctions and gap junctions in mature and developing mammalian testis. *Dev Biol* **50:**142–168.
Golachowska MR, Hoekstra D, and van IJzendoorn SC (2010) Recycling endosomes in apical plasma membrane domain formation and epithelial cell polarity. *Trends Cell Biol* **20:**618–626.
Goldberg GS, Lampe PD, and Nicholson BJ (1999) Selective transfer of endogenous metabolites through gap junctions composed of different connexins. *Nat Cell Biol* **1:**457–459.
Goldblum SE, Rai U, Tripathi A, Thakar M, De Leo L, Di Toro N, Not T, Ramachandran R, Puche AC, Hollenberg MD, et al. (2011) The active Zot domain (aa 288–293) increases ZO-1 and myosin 1C serine/threonine phosphorylation, alters interaction between ZO-1 and its binding partners, and induces tight junction disassembly through proteinase activated receptor 2 activation. *FASEB J* **25:**144–158.
Goldmann EE (1909) [Die äußere und innere Sekretion des gesunden und kranken Organismus im Lichte der "vitalen Farbung"]. *Beitr Klin Chir* **64:**192–265.
Golubovskaya VM (2010) Focal adhesion kinase as a cancer therapy target. *Anticancer Agents Med Chem* **10:**735–741.
González-Mariscal L, Tapia R, and Chamorro D (2008) Crosstalk of tight junction components with signaling pathways. *Biochem Biophys Acta* **1778:**729–756.
Goupil S, La Salle S, Trasler JM, Bordeleau LJ, and Leclerc P (2011) Developmental expression of SRC-related tyrosine kinases in the mouse testis. *J Androl* **32:**95–110.
Gow A, Southwood CM, Li JS, Pariali M, Riordan GP, Brodie SE, Danias J, Bronstein JM, Kachar B, and Lazzarini RA (1999) CNS myelin and Sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. *Cell* **99:**649–659.
Goyer RA, Liu J, and Waalkes MP (2004) Cadmium and cancer of prostate and testis. *Biometals* **17:**555–558.
Green KJ and Simpson CL (2007) Desmosomes: new perspectives on a classic. *J Invest Dermatol* **127:**2499–2515.
Green KJ, Getsios S, Troyanovsky S, and Godsel LM (2010) Intercellular junction assembly, dynamics, and homeostasis. *Cold Spring Harb Perspect Biol* **2:**a000125.
Greenbaum MP, Yan W, Wu MH, Lin YN, Agno JE, Sharma M, Braun RE, Rajkovic A, and Matzuk MM (2006) TEX14 is essential for intercellular bridges and fertility in male mice. *Proc Natl Acad Sci USA* **103:**4982–4987.
Grima J, Pineau C, Bardin CW, and Cheng CY (1992) Rat Sertoli cell clusterin, α_2-macroglobulin, and testins: biosynthesis and differential regulation by germ cells. *Mol Cell Endocrinol* **89:**127–140.
Grima J, Wong CC, Zhu LJ, Zong SD, and Cheng CY (1998) Testin secreted by Sertoli cells is associated with the cell surface, and its expression correlates with the disruption of Sertoli-germ cell junctions but not the inter-Sertoli tight junction. *J Biol Chem* **273:**21040–21053.
Grisanti L, Falciatori I, Grasso M, Dovere L, Fera S, Muciaccia B, Fuso A, Berno V, Boitani C, Stefanini M, et al. (2009) Identification of spermatogonial stem cell subsets by morphological analysis and prospective isolation. *Stem Cells* **27:**3043–3052.
Gu X and Manautou JE (2010) Regulation of hepatic ABCC transporters by xenobiotics and in disease states. *Drug Metab Rev* **42:**482–538.
Gualtieri AF, Iwachow MA, Venara M, Rey RA, and Schteingart HF (2011) Bisphenol A effect on glutathione synthesis and recycling in testicular Sertoli cells. *J Endocrinol Invest* **34:**e102–e109
Guan K, Wagner S, Unsöld B, Maier LS, Kaiser D, Hemmerlein B, Nayernia K, Engel W, and Hasenfuss G (2007) Generation of functional cardiomyocytes from adult mouse spermatogonial stem cells. *Circ Res* **100:**1615–1625.
Guarino M (2010) Src signaling in cancer invasion. *J Cell Physiol* **223:**14–26.
Guazzone VA, Jacobo P, Theas MS, and Lustig L (2009) Cytokines and chemokines in testicular inflammation: A brief review. *Microsc Res Tech* **72:**620–628.
Gumbiner BM (2000) Regulation of cadherin adhesive activity. *J Cell Biol* **148:**399–404.
Haeusgen W, Herdegen T, and Waetzig V (2011) The bottleneck of JNK signaling: molecular and functional characteristics of MKK4 and MKK7. *Eur J Cell Biol* **90:**536–544.
Hamden K, Carreau S, Ayadi F, Masmoudi H, and El Feki A (2009) Inhibitory effect of estrogens, phytoestrogens, and caloric restriction on oxidative stress and hepato-toxicity in aged rats. *Biomed Environ Sci* **22:**381–387.
Hamden K, Silandre D, Delalande C, Elfeki A, and Carreau S (2008) Protective effects of estrogens and caloric restriction during aging on various rat testis parameters. *Asian J Androl* **10:**837–845.
Hameed S, Jayasena CN, and Dhillo WS (2011) Kisspeptin and fertility. *J Endocrinol* **208:**97–105.
Hamer G, Roepers-Gajadien HL, Gademan IS, Kal HB, and De Rooij DG (2003) Intercellular bridges and apoptosis in clones of male germ cells. *Int J Androl* **26:**348–353.
Hansen CG and Nichols BJ (2010) Exploring the caves: cavins, caveolins and caveolae. *Trends Cell Biol* **20:**177–186.

Harris AL (2007) Connexin channel permeability to cytoplasmic molecules. *Prog Biophys Mol Biol* **94:**120–143.
Harris KP and Tepass U (2010) Cdc42 and vesicle trafficking in polarized cells. *Traffic* **11:**1272–1279.
Hartz AM and Bauer B (2010) Regulation of ABC transporters at the blood-brain barrier: new targets for CNS therapy. *Mol Interv* **10:**293–304.
Haugen TB, Landmark BF, Josefsen GM, Hansson V, and Högset A (1994) The mature form of interleukin-1α is constitutively expressed in immature male germ cells from rat. *Mol Cell Endocrinol* **105:**R19–23.
Hawkins BT and Davis TP (2005) The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* **57:**173–185.
He L, Wang B, Hay EB, and Nebert DW (2009) Discovery of ZIP transporters that participate in cadmium damage to testis and kidney. *Toxicol Appl Pharmacol* **238:**250–257.
Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, and Bruford EA (2004) The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins: Introduction. *Pflugers Arch* **447:**465–468.
Hein AM and O'Banion MK (2009) Neuroinflammation and memory: the role of prostaglandins. *Mol Neurobiol* **40:**15–32.
Heller CH and Clermont Y (1964) Kinetics of the germinal epithelium in man. *Recent Prog Horm Res* **20:**545–575.
Herbst KL, Anawalt BD, Amory JK, and Bremner WJ (2002) Acyline: the first study in humans of a potent, new gonadotropin-releasing hormone antagonist. *J Clin Endocrinol Metab* **87:**3215–3220.
Hermo L, Pelletier RM, Cyr DG, and Smith CE (2010) Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 4: intercellular bridges, mitochondria, nuclear envelope, apoptosis, ubiquitination, membrane/voltage-gated channels, methylation/acetylation, and transcription factors. *Microsc Res Tech* **73:**364–408.
Hesketh GG, Van Eyk JE, and Tomaselli GF (2009) Mechanisms of gap junction traffic in health and disease. *J Cardiovasc Pharmacol* **54:**263–272.
Hess RA (1990) Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: Light microscopic observations of perfusion-fixed and plastic-embedded testes. *Biol Reprod* **43:**525–542.
Hess RA (2003) Estrogen in the adult male reproductive tract: a review. *Reprod Biol Endocrinol* **1:**52.
Hess RA, Bunick D, Lee KH, Bahr J, Taylor JA, Korach KS, and Lubahn DB (1997) A role for oestrogens in the male reproductive system. *Nature* **390:**509–512.
Hess RA and de Franca LR (2008) Spermatogenesis and cycle of the seminiferous epithelium, in *Molecular Mechanisms in Spermatogenesis* (Cheng CY ed) pp 1–15, Landes Bioscience/Springer Science+Business Media, Austin, TX.
Hess RA, Fernandes SAF, Gomes GR, Oliveira CA, Lazari MF, and Porto CS (2011) Estrogen and its receptors in efferent ductules and epididymis. *J Androl* http://dx.doi.org/10.2164/jandrol.110.012872.
Hew KW, Ericson WA, and Welsh MJ (1993a) A single low cadmium dose causes failure of spermiation in the rat. *Toxicol Appl Pharmacol* **121:**15–21.
Hew KW, Heath GL, Jiwa AH, and Welsh MJ (1993b) Cadmium in vivo causes disruption of tight junction-associated microfilaments in rat Sertoli cells. *Biol Reprod* **49:**840–849.
Hild SA, Attardi BJ, and Reel JR (2004) The ability of a gonadotropin-releasing hormone antagonist, acyline, to prevent irreversible infertility induced by the indenopyridine, CDB-4022, in adult male rats: the role of testosterone. *Biol Reprod* **71:**348–358.
Hild SA, Marshall GR, Attardi BJ, Hess RA, Schlatt S, Simorangkir DR, Ramaswamy S, Koduri S, Reel JR, and Plant TM (2007a) Development of L-CDB-4022 as a nonsteroidal male oral contraceptive: induction and recovery from severe oligospermia in the adult male cynomolgus monkey (*Macaca fascicularis*). *Endocrinology* **148:**1784–1796.
Hild SA, Reel JR, Dykstra MJ, Mann PC, and Marshall GR (2007b) Acute adverse effects of the indenopyridine CDB-4022 on the ultrastructure of Sertoli cells, spermatocytes, and spermatids in rat testes: comparison to the known Sertoli cell toxicant di-*n*-pentylphthalate (DPP). *J Androl* **28:**621–629.
Hilscher W (1964) [Contributions to the orthology and pathology of "spermatogoniogenesis" in rats]. *Beitr Pathol Anat* **130:**69–132.
Hogarth CA and Griswold MD (2010) The key role of vitamin A in spermatogenesis. *J Clin Invest* **120:**956–962.
Hogarth CA, Amory JK, and Griswold MD (2011) Inhibiting vitamin A metabolism as an approach to male contraception. *Trends Endocrinol Metab* **22:**136–144
Holdcraft RW and Braun RE (2004) Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. *Development* **131:**459–467.
Hong CY, Park JH, Ahn RS, Im SY, Choi HS, Soh J, Mellon SH, and Lee K (2004) Molecular mechanism of suppression of testicular steroidogenesis by proinflammatory cytokine tumor necrosis factor α. *Mol Cell Biol* **24:**2593–2604.
Hosoya KI and Tachikawa M (2011) The inner blood-retinal barrier: molecular structure and transport biology, in *Biology and Regulation of Blood-Tissue Barriers* (Cheng CY ed) pp in press, Landes Bioscience, Austin, TX.
Hsu H, Shu HB, Pan MG, and Goeddel DV (1996) TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* **84:**299–308.
Hsu VW and Prekeris R (2010) Transport at the recycling endosome. *Curr Opin Cell Biol* **22:**528–534.
Huang L and Muthuswamy SK (2010) Polarity protein alterations in carcinoma: a focus on emerging roles for polarity regulators. *Curr Opin Genet Dev* **20:**41–50.
Huckins C and Oakberg EF (1978) Morphological and quantitative analysis of spermatogonia in mouse testes using whole mounted seminiferous tubules. II. The irradiated testes. *Anat Rec* **192:**529–542.
Huff J, Lunn RM, Waalkes MP, Tomatis L, and Infante PF (2007) Cadmium-induced cancers in animals and in humans. *Int J Occup Environ Health* **13:**202–212.
Huhtaniemi I (2010) A hormonal contraceptive for men: how close are we? *Prog Brain Res* **181:**273–288.
Hunt PA, Susiarjo M, Rubio C, and Hassold TJ (2009) The bisphenol A experience: a primer for the analysis of environmental effects on mammalian reproduction. *Biol Reprod* **81:**807–813.
Huveneers S and Danen EH (2009) Adhesion signaling—crosstalk between integrins, Src and Rho. *J Cell Sci* **122:**1059–1069.
Iden S and Collard JG (2008) Crosstalk between small GTPases and polarity proteins in cell polarization. *Nat Rev Mol Cell Biol* **9:**846–859.
Ilić D, Damsky CH, and Yamamoto T (1997) Focal adhesion kinase: at the crossroads of signal transduction. *J Cell Sci* **110:**401–407.
Invernici G, Cristini S, Alessandri G, Navone SE, Canzi L, Tavian D, Redaelli C, Acerbi F, and Parati EA (2011) Nanotechnology advances in brain tumors: the state of the art. *Recent Pat Anticancer Drug Discov* **6:**58–69.
Ivanov AI (2011) Structure and regulation of intestinal epithelial tight junctions, in *Biology and Regulation of Blood-Tissue Barriers* (Cheng CY ed) pp in press, Landes Bioscience, Austin, TX.
Ivanov AI, Nusrat A, and Parkos CA (2004) Endocytosis of epithelial apical junction proteins by a clathrin-mediated pathway into a unique storage compartment. *Mol Biol Cell* **15:**176–188.
Jacquillet G, Barbier O, Rubera I, Tauc M, Borderie A, Namorado MC, Martin D, Sierra G, Reyes JL, Poujeol P, et al. (2007) Cadmium causes delayed effects on renal function in the offspring of cadmium-contaminated pregnant female rats. *Am J Physiol Renal Physiol* **293:**F1450–F1460.
Janecki A and Steinberger A (1986) Polarized Sertoli cell functions in a new two-compartment culture system. *J Androl* **7:**69–71.
Janecki A, Jakubowiak A, and Steinberger A (1992) Effect of cadmium chloride on transepithelial electrical resistance of Sertoli cell monolayers in two-compartment cultures–a new model for toxicological investigations of the "blood-testis" barrier in vitro. *Toxicol Appl Pharmacol* **112:**51–57.
Jarow JP and Zirkin BR (2005) The androgen microenvironment of the human testis and hormonal control of spermatogenesis. *Ann N Y Acad Sci* **1061:**208–220.
Järup L and Akesson A (2009) Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol* **238:**201–208.
Jeon SH, Cho MH, and Cho JH (2001) Effects of cadmium on gap junctional intercellular communication in WB-F344 rat liver epithelial cells. *Hum Exp Toxicol* **20:**577–583.
Jin JY, Zhou L, Wang Y, Li Z, Zhao JG, Zhang QY, and Gui JF (2010) Antibacterial and antiviral roles of a fish β-defensin expressed both in pituitary and testis. *PLoS One* **5:**e12883.
John LJ, Fromm M, and Schulzke JD (2011) Epithelial barriers in intestinal inflammation. *Antioxid Redox Signal* **15:**1255–1270.
Jonsson CK, Zetterström RH, Holst M, Parvinen M, and Söder O (1999) Constitutive expression of interleukin-1α messenger ribonucleic acid in rat Sertoli cells is dependent upon interaction with germ cells. *Endocrinology* **140:**3755–3761.
Jørgensen N, Vierula M, Jacobsen R, Pukkala E, Perheentupa A, Virtanen HE, Skakkebaek NE, and Toppari J (2011) Recent adverse trends in semen quality and testis cancer incidence among Finnish men. *Int J Androl* **34:**e37–e48
Joseph A, Hess RA, Schaeffer DJ, Ko C, Hudgin-Spivey S, Chambon P, and Shur BD (2010a) Absence of estrogen receptor α leads to physiological alterations in the mouse epididymis and consequent defects in sperm function. *Biol Reprod* **82:**948–957.
Joseph A, Shur BD, and Hess RA (2011) Estrogen, efferent ductules, and the epididymis. *Biol Reprod* **84:**207–217.
Joseph A, Shur BD, Ko C, Chambon P, and Hess RA (2010b) Epididymal hypoosmolality induces abnormal sperm morphology and function in the estrogen receptor α knockout mouse. *Biol Reprod* **82:**958–967.
Joseph P (2009) Mechanisms of cadmium carcinogenesis. *Toxicol Appl Pharmacol* **238:**272–279.
Kaitu'u-Lino TJ, Sluka P, Foo CF, and Stanton PG (2007) Claudin-11 expression and localisation is regulated by androgens in rat Sertoli cells in vitro. *Reproduction* 133:1169–1179.
Kalejs M and Erenpreisa J (2005) Cancer/testis antigens and gametogenesis: a review and "brain-storming" session. *Cancer Cell Int* **5:**4.
Kalliokoski A and Niemi M (2009) Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* **158:**693–705.
Kangasniemi M, Huhtaniemi I, and Meistrich ML (1996) Failure of spermatogenesis to recover despite the presence of a spermatogonia in the irradiated LBNF1 rat. *Biol Reprod* **54:**1200–1208.
Karssen AM, Meijer OC, van der Sandt IC, De Boer AG, De Lange EC, and De Kloet ER (2002) The role of the efflux transporter P-glycoprotein in brain penetration of prednisolone. *J Endocrinol* **175:**251–260.
Karssen AM, Meijer OC, van der Sandt IC, Lucassen PJ, de Lange EC, de Boer AG, and de Kloet ER (2001) Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. *Endocrinology* **142:**2686–2694.
Kazi TG, Wadhwa SK, Afridi HI, Kazi N, Kandhro GA, Baig JA, Shah AQ, Kolachi NF, and Khan S (2010) Evaluation of cadmium and zinc in biological samples of tobacco and alcohol user male mouth cancer patients. *Hum Exp Toxicol* **29:**221–230.
Kerr JB, Loveland KL, O'Bryan MK, and De Kretser DM (2006) Cytology of the testis and intrinsic control mechanisms, in *Knobil and Neill's Physiology of Reproduction*, 3rd ed (Neill JD ed) pp 827–947, Elsevier, New York.
Khlifi R and Hamza-Chaffai A (2010) Head and neck cancer due to heavy metal exposure via tobacco smoking and professional exposure: a review. *Toxicol Appl Pharmacol* **248:**71–88.
Kierszenbaum AL (2002) Keratins: unraveling the coordinated construction of scaffolds in spermatogenic cells. *Mol Reprod Dev* **61:**1–2.
Kim JY, Morgan M, Kim DG, Lee JY, Bai L, Lin Y, Liu ZG, and Kim YS (2011) TNFα-induced noncanonical NF-κB activation is attenuated by RIP1 through stabilization of TRAF2. *J Cell Sci* **124:**647–656.
Kis O, Robillard K, Chan GN, and Bendayan R (2010) The complexities of antiret-

roviral drug-drug interactions: role of ABC and SLC transporters. *Trends Pharmacol Sci* **31:**22–35.
Kiziler AR, Aydemir B, Onaran I, Alici B, Ozkara H, Gulyasar T, and Akyolcu MC (2007) High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects. *Biol Trace Elem Res* **120:**82–91.
Kjellström T and Nordberg GF (1978) A kinetic model of cadmium metabolism in the human being. *Environ Res* **16:**248–269.
Klaassen CD and Aleksunes LM (2010) Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev* **62:**1–96.
Kobayashi D, Tamai I, Sai Y, Yoshida K, Wakayama T, Kido Y, Nezu J, Iseki S, and Tsuji A (2007) Transport of carnitine and acetylcarnitine by carnitine/organic cation transporter (OCTN) 2 and OCTN3 into epididymal spermatozoa. *Reproduction* **134:**651–658.
Kodaira H, Kusuhara H, Ushiki J, Fuse E, and Sugiyama Y (2010) Kinetic analysis of the cooperation of P-glycoprotein (P-gp/Abcb1) and breast cancer resistance protein (Bcrp/Abcg2) in limiting the brain and testis penetration of erlotinib, flavopiridol, and mitoxantrone. *J Pharmacol Exp Ther* **333:**788–796.
Koduri S, Hild SA, Pessaint L, Reel JR, and Attardi BJ (2008) Mechanism of action of *l*-CDB-4022, a potential nonhormonal male contraceptive, in the seminiferous epithelium of the rat testis. *Endocrinology* **149:**1850–1860.
Koepsell H, Lips K, and Volk C (2007) Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm Res* **24:**1227–1251.
Kopera IA, Bilinska B, Cheng CY, and Mruk DD (2010) Sertoli-germ cell junctions in the testis: a review of recent data. *Philos Trans R Soc Lond B Biol Sci* **365:**1593–1605.
Kopf GS (2008) Approaches to the identification of new nonhormonal targets for male contraception. *Contraception* **78 (Suppl 4):**S18–S22.
Kormano M (1967a) Distribution of injected L-3,4-dihyrodxyphenylalanine (L-dopa) in the adult rat testis and epididymis. *Acta Physiol Scand* **71:**125–126.
Kormano M (1967b) Dye permeability and alkaline phosphatase activity of testicular capillaries in the postnatal rat. *Histochemie* **9:**327–338.
Kormano M (1968) Penetration of intravenous trypan blue into the rat testis and epididymis. *Acta Histochem* **30:**133–136.
Koshiba S, An R, Saito H, Wakabayashi K, Tamura A, and Ishikawa T (2008) Human ABC tarnsporters ABCG2 (BCRP) and ABCG4. *Xenobiotica* **38:**863–888.
Koshiba S, Ito T, Shiota A, Wakabayashi K, Ueda M, Ichinose H, and Ishikawa T (2007) Development of polyclonal antibodies specific to ATP-binding cassette transporters human ABCG4 and Abcg4: site-specific expression of mouse Abcg4 in brain. *J Exp Ther Oncol* **6:**321–333.
Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JA, and Smithies O (1998) Generation and reproductive phenotypes of mice lacking estrogen receptor β. *Proc Natl Acad Sci USA* **95:**15677–15682.
Kriegler M, Perez C, DeFay K, Albert I, and Lu SD (1988) A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell* **53:**45–53.
Kruglov AA, Kuchmiy A, Grivennikov SI, Tumanov AV, Kuprash DV, and Nedospasov SA (2008) Physiological functions of tumor necrosis factor and the consequences of its pathologic overexpression or blockade: mouse models. *Cytokine Growth Factor Rev* **19:**231–244.
Kwiatkowska K (2010) One lipid, multiple functions: how various pools of PI(4,5)P(2) are created in the plasma membrane. *Cell Mol Life Sci* **67:**3927–3946.
Lamont KR and Tindall DJ (2010) Androgen regulation of gene expression. *Adv Cancer Res* **107:**137–162.
Leblond CP and Clermont Y (1952) Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann N Y Acad Sci* **55:**548–573.
Lee J, Richburg JH, Shipp EB, Meistrich ML, and Boekelheide K (1999) The Fas system, a regulator of testicular germ cell apoptosis, is differentially up-regulated in Sertoli cell versus germ cell injury of the testis. *Endocrinology* **140:**852–858.
Lee NP and Cheng CY (2005) Protein kinases and adherens junction dynamics in the seminiferous epithelium of the rat testis. *J Cell Physiol* **202:**344–360.
Lee NP, Mruk D, Lee WM, and Cheng CY (2003) Is the cadherin/catenin complex a functional unit of cell-cell actin-based adherens junctions in the rat testis? *Biol Reprod* **68:**489–508.
Lee NP, Mruk DD, Conway AM, and Cheng CY (2004) Zyxin, axin, and Wiskott-Aldrich syndrome protein are adaptors that link the cadherin/catenin protein complex to the cytoskeleton at adherens junctions in the seminiferous epithelium of the rat testis. *J Androl* **25:**200–215.
Leslie EM, Deeley RG, and Cole SP (2005) Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BRCP (ABCG2) in tissue defense. *Toxicol Appl Pharmacol* **204:**216–237.
Li JC, Mruk D, and Cheng CY (2001) The inter-Sertoli tight junction permeability barrier is regulated by the interplay of protein phosphatases and kinases: an in vitro study. *J Androl* **22:**847–856.
Li L and Heindel J (1998) Sertoli cell toxicants, in *Reproductive and Developmental Toxicology* (Korach K ed) pp 655–694, Marcel Dekker, New York.
Li L, Meier PJ, and Ballatori N (2000) Oatp2 mediates bidirectional organic solute transport: a role for intracellular glutathione. *Mol Pharmacol* **58:**335–340.
Li MW, Mruk DD, and Cheng CY (2008) "Unlocking" the blood-testis barrier and the ectoplasmic specialization by cytokines during spermatogenesis: emerging targets for male contraception. *Immunol Endocr Metab Agents Med Chem* **8:**20–27.
Li MW, Mruk DD, and Cheng CY (2009a) Mitogen-activated protein kinases in male reproductive function. *Trends Mol Med* **15:**159–168.
Li MW, Mruk DD, and Cheng CY (2011a) Gap junctions and blood-tissue barriers, in *Biology and Regulation of Blood-Tissue Barriers* (Cheng CY ed) pp in press, Landes Bioscience, Austin, TX.
Li MW, Mruk DD, Lee WM, and Cheng CY (2009b) Connexin 43 and plakophilin-2 as a protein complex that regulates blood-testis barrier dynamics. *Proc Natl Acad Sci USA* **106:**10213–10218.
Li MW, Mruk DD, Lee WM, and Cheng CY (2009c) Cytokines and junction restructuring events during spermatogenesis in the testis: an emerging concept of regulation. *Cytokine Growth Factor Rev* **20:**329–338.
Li MW, Mruk DD, Lee WM, and Cheng CY (2009d) Disruption of the blood-testis barrier integrity by bisphenol A in vitro: is this a suitable model for studying blood-testis barrier dynamics? *Int J Biochem Cell Biol* **41:**2302–2314.
Li MW, Mruk DD, Lee WM, and Cheng CY (2010) Connexin 43 is critical to maintain the homeostasis of the blood-testis barrier via its effects on tight junction reassembly. *Proc Natl Acad Sci USA* **107:**17998–18003.
Li MW, Xia W, Mruk DD, Wang CQ, Yan HH, Siu MK, Lui WY, Lee WM, and Cheng CY (2006) Tumor necrosis factor α reversibly disrupts the blood-testis barrier and impairs Sertoli-germ cell adhesion in the seminiferous epithelium of adult rat testes. *J Endocrinol* **190:**313–329.
Li MW, Xiao X, Mruk DD, Lam YL, Lee WM, Lui WY, Bonanomi M, Silvestrini B, and Cheng CY (2011b) Actin binding protein drebrin E is involved in junction dynamics during spermatogenesis. *Spermatogenesis* **1:**123–136.
Lie PP, Chan AY, Mruk DD, Lee WM, and Cheng CY (2010a) Restricted Arp3 expression in the testis prevents blood-testis barrier disruption during junction restructuring at spermatogenesis. *Proc Natl Acad Sci USA* **107:**11411–11416.
Lie PP, Cheng CY, and Mruk DD (2011c) The biology of interleukin-1: emerging concepts in the regulation of the actin cytoskeleton and cell junction dynamics. *Cell Mol Life Sci* http://dx.doi.org/10.1007/s00018-011-0760-0.
Lie PP, Cheng CY, and Mruk DD (2009a) Coordinating cellular events during spermatogenesis: a biochemical model. *Trends Biochem Sci* **34:**366–373.
Lie PP, Cheng CY, and Mruk DD (2010b) Crosstalk between desmoglein-2/desmocollin-2/Src kinase and coxsackie and adenovirus receptor/ZO-1 protein complexes, regulates blood-testis barrier dynamics. *Int J Biochem Cell Biol* **42:**975–986.
Lie PP, Cheng CY, and Mruk DD (2011a) The biology of the desmosome-like junction: A versatile anchoring junction and signal transducer in the seminiferous epithelium. *Int Rev Cell Mol Biol* **286:**223–269.
Lie PP, Cheng CY, and Mruk DD (2011b) Interleukin-1α is a regulator of the blood-testis barrier. *FASEB J* **25:**1244–1253.
Lie PP, Mruk DD, and Cheng CY (2008) Dynamins, spermatogenesis and contraceptive development. *Immunol Endocr Metab Agents Med Chem* **8:**51–58.
Lie PP, Mruk DD, Lee WM, and Cheng CY (2009b) Epidermal growth factor receptor pathway substrate 8 (Eps8) is a novel regulator of cell adhesion and the blood-testis barrier integrity in the seminiferous epithelium. *FASEB J* **23:**2555–2567.
Lie PP, Mruk DD, Lee WM, and Cheng CY (2010c) Cytoskeletal dynamics and spermatogenesis. *Philos Trans R Soc Lond B Biol Sci* **365:**1581–1592.
Lipinski MM and Jacks T (1999) The retinoblastoma gene family in differentiation and development. *Oncogene* **18:**7873–7882.
Liu J, Qu W, and Kadiiska MB (2009) Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicol Appl Pharmacol* **238:**209–214.
Loewenstein WR (1981) Junctional intercellular communication: the cell-to-cell membrane channel. *Physiol Rev* **61:**829–913.
Lorico A, Bertola A, Baum C, Fodstad O, and Rappa G (2002) Role of the multidrug resistance protein 1 in protection from heavy metal oxyanions: investigations in vitro and in MRP1-deficient mice. *Biochem Biophys Res Commun* **291:**617–622.
Löscher W and Potschka H (2005) Drug resistance in brain diseases and the role of drug efflux transporters. *Nat Rev Neurosci* **6:**591–602.
Loveland KL, Dias V, Meachem S, and Rajpert-De Meyts E (2007) The transforming growth factor-β superfamily in early spermatogenesis: potential relevance to testicular dysgenesis. *Int J Androl* **30:**377–384.
Lucas B, Fields C, and Hofmann MC (2009) Signaling pathways in spermatogonial stem cells and their disruption by toxicants. *Birth Defects Res C Embryo Today* **87:**35–42.
Lucas TF, Siu ER, Esteves CA, Monteiro HP, Oliveira CA, Porto CS, and Lazari MF (2008) 17β-Estradiol induces the translocation of the estrogen receptors ESR1 and ESR2 to the cell membrane, MAPK3/1 phosphorylation and proliferation of cultured immature rat Sertoli cells. *Biol Reprod* **78:**101–114.
Lui WY and Cheng CY (2007) Regulation of cell junction dynamics by cytokines in the testis: a molecular and biochemical perspective. *Cytokine Growth Factor Rev* **18:**299–311.
Lui WY, Lee WM, and Cheng CY (2001) Transforming growth factor-β3 perturbs the inter-Sertoli tight junction permeability barrier in vitro possibly mediated via its effects on occludin, zonula occludens-1, and claudin-11. *Endocrinology* **142:**1865–1877.
Lui WY, Lee WM, and Cheng CY (2003a) TGF-βs: their role in testicular function and Sertoli cell tight junction dynamics. *Int J Androl* **26:**147–160.
Lui WY, Lee WM, and Cheng CY (2003b) Transforming growth factor-β3 regulates the dynamics of Sertoli cell tight junctions via the p38 mitogen-activated protein kinase pathway. *Biol Reprod* **68:**1597–1612.
Lui WY, Wong CH, Mruk DD, and Cheng CY (2003c) TGF-β3 regulates the blood-testis barrier dynamics via the p38 mitogen activated protein (MAP) kinase pathway: an in vivo study. *Endocrinology* **144:**1139–1142.
Luparello C, Sirchia R, and Longo A (2011) Cadmium as a transcriptional modulator in human cells. *Crit Rev Toxicol* **41:**75–82.
Lysiak JJ (2004) The role of tumor necrosis factor-α and interleukin-1 in the mammalian testis and their involvement in testicular torsion and autoimmune orchitis. *Reprod Biol Endocrinol* **2:**9.
Maeda S and Tsukihara T (2011) Structure of the gap junction channel and its implications for its biological functions. *Cell Mol Life Sci* **68:**1115–1129.
Magin TM, Vijayaraj P, and Leube RE (2007) Structural and regulatory functions of keratins. *Exp Cell Res* **313:**2021–2032.
Mahato D, Goulding EH, Korach KS, and Eddy EM (2000) Spermatogenic cells do not require estrogen receptor-α for development or function. *Endocrinology* **141:**1273–1276.
Mahato D, Goulding EH, Korach KS, and Eddy EM (2001) Estrogen receptor-α is required by the supporting somatic cells for spermatogenesis. *Mol Cell Endocrinol* **178:**57–63.

Mahi-Brown CA, Yule TD, and Tung KS (1988) Evidence for active immunological regulation in prevention of testicular autoimmune disease independent of the blood-testis barrier. *Am J Reprod Immunol Microbiol* **16:**165–170.
Malam Y, Lim EJ, and Seifalian AM (2011) Current trends in the application of nanoparticles in drug delivery. *Curr Med Chem* **18:**1067–1078.
Marchiando AM, Graham WV, and Turner JR (2010a) Epithelial barriers in homeostasis and disease. *Annu Rev Pathol* **5:**119–144.
Marchiando AM, Shen L, Graham WV, Weber CR, Schwarz BT, Austin JR 2nd, Raleigh DR, Guan Y, Watson AJ, Montrose MH, et al. (2010b) Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. *J Cell Biol* **189:**111–126.
Martin GS (2001) The hunting of the Src. *Nat Rev Mol Cell Biol* **2:**467–475.
Martin GS (2009) SRC substrate surprise. *Cancer Cell* **16:**176–178.
Marx A, Nugoor C, Panneerselvam S, and Mandelkow E (2010) Structure and function of polarity-inducing kinase family MARK/Par-1 within the branch of AMPK/Snf1-related kinases. *FASEB J* **24:**1637–1648.
Massagué J and Gomis RR (2006) The logic of TGFβ signaling. *FEBS Lett* **580:**2811–2820.
Massagué J (2000) How cells read TGF-β signals. *Nat Rev Mol Cell Biol* **1:**169–178.
Massagué J (2008) TGFβ in cancer. *Cell* **134:**215–230.
Maxfield FR and McGraw TE (2004) Endocytic recycling. *Nat Rev Mol Cell Biol* **5:**121–132.
Mazaud-Guittot S, Meugnier E, Pesenti S, Wu X, Vidal H, Gow A, and Le Maguer-esse-Battistoni B (2010) Claudin 11 deficiency in mice results in loss of the Sertoli cell epithelial phenotype in the testis. *Biol Reprod* **82:**202–213.
McCabe MJ, Tarulli GA, Meachem SJ, Robertson DM, Smooker PM, and Stanton PG (2010) Gonadotropins regulate rat testicular tight junctions in vivo. *Endocrinology* **151:**2911–2922.
McDuff FK and Turner SD (2011) Jailbreak: oncogene-induced senescence and its evasion. *Cell Signal* **23:**6–13.
McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, de Kretser DM, Pratis K, and Robertson DM (2002) Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. *Recent Prog Horm Res* **57:**149–179.
McLaughlin EA and Aitken RJ (2011) Is there a role for immunocontraception? *Mol Cell Endocrinol* 335:78–88.
Médina E, Lemmers C, Lane-Guermonprez L, and Le Bivic A (2002) Role of the Crumbs complex in the regulation of junction formation in Drosophila and mammalian epithelial cells. *Biol Cell* **94:**305–313.
Mehta D and Malik AB (2006) Signaling mechanisms regulating endothelial permeability. *Physiol Rev* **86:**279–367.
Mei H, Walters C, Carter R, and Colledge WH (2011) Gpr54−/− mice show more pronounced defects in spermatogenesis than Kiss1−/− mice and improved spermatogenesis with age when exposed to dietary phytoestrogens. *Reproduction* **141:**357–366.
Meier-Abt F, Mokrab Y, and Mizuguchi K (2005) Organic anion transporting polypeptides of the OATP/SLCO superfamily: identification of new members in non-mammalian species, comparative modeling and a potential transport mode. *J Membr Biol* **208:**213–227.
Meinhardt A and Hedger MP (2011) Immunological, paracrine and endocrine aspects of testicular immune privilege. *Mol Cell Endocrinol* **335:**60–68.
Meistrich ML, Wilson G, and Huhtaniemi I (1999) Hormonal treatment after cytotoxic therapy stimulates recovery of spermatogenesis. *Cancer Res* **59:**3557–3560.
Meistrich ML, Wilson G, Porter KL, Huhtaniemi I, Shetty G, and Shuttlesworth GA (2003) Restoration of spermatogenesis in dibromochloropropane (DBCP)-treated rats by hormone suppression. *Toxicol Sci* **76:**418–426.
Melaine N, Liénard MO, Dorval I, Le Goascogne C, Lejeune H, and Jégou B (2002) Multidrug resistance genes and P-glycoprotein in the testis of the rat, mouse, Guinea pig, and human. *Biol Reprod* **67:**1699–1707.
Meng J, Greenlee AR, Taub CJ, and Braun RE (2011) Sertoli cell-specific deletion of the androgen receptor compromises testicular immune privilege in mice. *Biol Reprod* **85:**254–260.
Meng J, Holdcraft RW, Shima JE, Griswold MD, and Braun RE (2005) Androgens regulate the permeability of the blood-testis barrier. *Proc Natl Acad Sci USA* **102:**16696–16700.
Merlet J, Racine C, Moreau E, Moreno SG, and Habert R (2007) Male fetal germ cells are targets for androgens that physiologically inhibit their proliferation. *Proc Natl Acad Sci USA* **104:**3615–3620.
Meşe G, Richard G, and White TW (2007) Gap junctions: basic structure and function. *J Invest Dermatol* **127:**2516–2524.
Miething A (2010) Local desynchronization of cellular development within mammalian male germ cell clones. *Ann Anat* **192:**247–250.
Miller DS, Bauer B, and Hartz AM (2008) Modulation of P-glycoprotein at the blood-brain barrier: opportunities to improve central nervous system pharmacotherapy. *Pharmacol Rev* **60:**196–209.
Mirza M, Hreinsson J, Strand ML, Hovatta O, Söder O, Philipson L, Pettersson RF, and Sollerbrant K (2006) Coxsackievirus and adenovirus receptor (CAR) is expressed in male germ cells and forms a complex with the differentiation factor JAM-C in mouse testis. *Exp Cell Res* **312:**817–830.
Mirza M, Petersen C, Nordqvist K, and Sollerbrant K (2007) Coxsackievirus and adenovirus receptor is up-regulated in migratory germ cells during passage of the blood-testis barrier. *Endocrinology* **148:**5459–5469.
Mital P, Hinton BT, and Dufour JM (2011) The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biol Reprod* **84:**851–858.
Mital P, Kaur G, and Dufour JM (2010) Immunoprotective Sertoli cells: making allogeneic and xenogeneic transplantation feasible. *Reproduction* **139:**495–504.
Mizuno N, Niwa T, Yotsumoto Y, and Sugiyama Y (2003) Impact of drug transporter studies on drug discovery and development. *Pharmacol Rev* **55:**425–461.
Mok KW, Mruk DD, Lee WM, and Cheng CY (2011a) Spermatogonial stem cells alone are not sufficient to re-initiate spermatogenesis in the rat testis following adjudin-induced infertility. *Int J Androl* http://dx.doi.org/10.1111/j.1365-2605.2011.01183.x.
Mok KW, Mruk DD, Lie PP, Lui WY, and Cheng CY (2011b) Adjudin, a potential male contraceptive, exerts its effects locally in the seminiferous epithelium of mammalian testes. *Reproduction* **141:**571–580.
Morrow CM, Mruk D, Cheng CY, and Hess RA (2010) Claudin and occludin expression and function in the seminiferous epithelium. *Philos Trans R Soc Lond B Biol Sci* **365:**1679–1696.
Mruk DD and Cheng CY (2004a) Cell-cell interactions at the ectoplasmic specialization in the testis. *Trends Endocrinol Metab* **15:**439–447.
Mruk DD and Cheng CY (2004b) Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocr Rev* **25:**747–806.
Mruk DD and Cheng CY (2008) Delivering non-hormonal contraceptives to men: advances and obstacles. *Trends Biotechnol* **26:**90–99.
Mruk DD and Cheng CY (2010a) The myotubularin family of lipid phosphatases in disease and in spermatogenesis. *Biochem J* **433:**253–262.
Mruk DD and Cheng CY (2010b) Tight junctions in the testis: new perspectives. *Philos Trans R Soc Lond B Biol Sci* **365:**1621–1635.
Mruk DD and Cheng CY (2011) Desmosomes in the testis. Moving into an uncharted territory. *Spermatogenesis* **1:**47–51.
Mruk DD (2008) New perspectives in non-hormonal male contraception. *Trends Endocrinol Metab* **19:**57–64.
Mruk DD, Silvestrini B, and Cheng CY (2008) Anchoring junctions as drug targets: role in contraceptive development. *Pharmacol Rev* **60:**146–180.
Mruk DD, Su L, and Cheng CY (2011) Emerging role for drug transporters at the blood-testis barrier. *Trends Pharmacol Sci* **32:**99–106.
Mullaney BP and Skinner MK (1993) Transforming growth factor-β (β1, β2, and β3) gene expression and action during pubertal development of the seminiferous tubule: potential role at the onset of spermatogenesis. *Mol Endocrinol* **7:**67–76.
Nagano MC (2003) Homing efficiency and proliferation kinetics of male germ line stem cells following transplantation in mice. *Biol Reprod* **69:**701–707.
Nagaosa K, Nakashima C, Kishimoto A, and Nakanishi Y (2009) Immune response to bacteria in seminiferous epithelium. *Reproduction* **137:**879–888.
Nakagawa S, Maeda S, and Tsukihara T (2010) Structural and functional studies of gap junction channels. *Curr Opin Struct Biol* **20:**423–430.
Nakagawa T, Nabeshima Y, and Yoshida S (2007) Functional identification of the actual and potential stem cell compartments in mouse spermatogenesis. *Dev Cell* **12:**195–206.
Nalam RL, Andreu-Vieyra C, Braun RE, Akiyama H, and Matzuk MM (2009) Retinoblastoma protein plays multiple essential roles in the terminal differentiation of Sertoli cells. *Mol Endocrinol* **23:**1900–1913.
Naudé PJ, den Boer JA, Luiten PG, and Eisel UL (2011) Tumor necrosis factor receptor cross-talk. *FEBS J* **278:**888–898.
Nguyen DX and McCance DJ (2005) Role of the retinoblastoma tumor suppressor protein in cellular differentiation. *J Cell Biochem* **94:**870–879.
Niemi M, Pasanen MK, and Neuvonen PJ (2011) Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacol Rev* **63:**157–181.
Ning L, Chen H, and Kunnimalaiyaan M (2010) Focal adhesion kinase, a downstream mediator of Raf-1 signaling, suppresses cellular adhesion, migration, and neuroendocrine markers in BON carcinoid cells. *Mol Cancer Res* **8:**775–782.
Nitta H, Bunick D, Hess RA, Janulis L, Newton SC, Millette CF, Osawa Y, Shizuta Y, Toda K, and Bahr JM (1993) Germ cells of the mouse testis express P450 aromatase. *Endocrinology* **132:**1396–1401.
Nusrat A, Chen JA, Foley CS, Liang TW, Tom J, Cromwell M, Quan C, and Mrsny RJ (2000) The coiled-coil domain of occludin can act to organize structural and functional elements of the epithelial tight junction. *J Biol Chem* **275:**29816–29822.
O'Bryan MK and Hedger MP (2008) Inflammatory networks in the control of spermatogenesis: chronic inflammation in an immunologically privileged tissue? *Adv Exp Med Biol* **636:**92–114.
O'Donnell L, Nicholls PK, O'Bryan MK, McLachlan RI, and Stanton PG (2011) Spermiation: the process of sperm release. *Spermatogenesis* **1:**14–35.
O'Donnell L, Pratis K, Wagenfeld A, Gottwald U, Müller J, Leder G, McLachlan RI, and Stanton PG (2009) Transcriptional profiling of the hormone-responsive stages of spermatogenesis reveals cell-, stage-, and hormone-specific events. *Endocrinology* **150:**5074–5084.
O'Donnell L, Robertson KM, Jones ME, and Simpson ER (2001) Estrogen and spermatogenesis. *Endocr Rev* **22:**289–318.
O'Rand MG, Widgren EE, Wang Z, and Richardson RT (2007) Eppin: an epididymal protease inhibitor and a target for male contraception. *Soc Reprod Fertil Suppl* **63:**445–453.
Oakberg EF (1956) Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. *Am J Anat* **99:**507–516.
Okanlawon A and Dym M (1996) Effect of chloroquine on the formation of tight junctions in cultured immature rat Sertoli cells. *J Androl* **17:**249–255.
Okumura K, Lee IP, and Dixon RL (1975) Permeability of selected drugs and chemicals across the blood-testis barrier of the rat. *J Pharmacol Exp Ther* **194:**89–95.
Orth JM (1982) Proliferation of Sertoli cells in fetal and postnatal rats: a quantitative autoradiographic study. *Anat Rec* **203:**485–492.
Page ST, Amory JK, and Bremner WJ (2008) Advances in male contraception. *Endocr Rev* **29:**465–493.
Palombi F, Salanova M, Tarone G, Farini D, and Stefanini M (1992) Distribution of β1 integrin subunit in rat seminiferous epithelium. *Biol Reprod* **47:**1173–1182.
Pan J, Plant JA, Voulvoulis N, Oates CJ, and Ihlenfeld C (2010) Cadmium levels in Europe: implications for human health. *Environ Geochem Health* **32:**1–12.
Paolinelli R, Corada M, Orsenigo F, and Dejana E (2011) The molecular basis of the blood brain barrier differentiation and maintenance. Is it still a mystery? *Pharmacol Res* **63:**165–171.
Parizek J and Zahor Z (1956) Effect of cadmium salts on testicular tissue. *Nature* **177:**1036–1037.

Parizek J (1957) The destructive effect of cadmium ion on testicular tissue and its prevention by zinc. *J Endocrinol* **15:**56–63.
Parizek J (1960) Sterilization of the male by cadmium salts. *J Reprod Fertil* **1:**294–309.
Parvinen M (1982) Regulation of the seminiferous epithelium. *Endocr Rev* **3:**404–417.
Paumi CM, Chuk M, Snider J, Stagljar I, and Michaelis S (2009) ABC transporters in *Saccharomyces cerevisiae* and their interactors: new technology advances the biology of the ABCC (MRP) subfamily. *Microbiol Mol Biol Rev* **73:**577–593.
Pelletier R (2001) The tight junctions in the testis, epididymis, and vas deferens, in *Tight Junctions* (Cereijido M and Anderson J eds) pp 599–628, CRC Press, New York.
Pelletier RM and Byers SW (1992) The blood-testis barrier and Sertoli cell junctions: structural considerations. *Microsc Res Tech* **20:**3–33.
Pelletier RM (1995) The distribution of connexin 43 is associated with the germ cell differentiation and with the modulation of the Sertoli cell junction barrier in continual (guinea pig) and seasonal breeders' (mink) testes. *J Androl* **16:**400–409.
Pelletier RM, Akpovi CD, Chen L, Day R, and Vitale ML (2011) Cx43 expression, phosphorylation, and distribution in the normal and autoimmune orchitic testis with a look at gap junctions joining germ cell to germ cell. *Am J Physiol Regul Integr Comp Physiol* **300:**R121–139.
Pelletier RM, Yoon SR, Akpovi CD, Silvas E, and Vitale ML (2009) Defects in the regulatory clearance mechanisms favor the breakdown of self-tolerance during spontaneous autoimmune orchitis. *Am J Physiol Regul Integr Comp Physiol* **296:**R743–R762.
Pentikäinen V, Erkkilä K, Suomalainen L, Otala M, Pentikäinen MO, Parvinen M, and Dunkel L (2001) TNF-α down-regulated the Fas ligand and inhibits germ cell apoptosis in the human testis. *J Clin Endocrinol Metab* **86:**4480–4488.
Pérez-Tomás R (2006) Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. *Curr Med Chem* **13:**1859–1876.
Phillips BT, Gassei K, and Orwig KE (2010) Spermatogonial stem cell regulation and spermatogenesis. *Philos Trans R Soc Lond B Biol Sci* **365:**1663–1678.
Phillips KP and Tanphaichitr N (2008) Human exposure to endocrine disrupters and semen quality. *J Toxicol Environ Health B Crit Rev* **11:**188–220.
Pinton P, Nougayrède JP, Del Rio JC, Moreno C, Marin DE, Ferrier L, Bracarense AP, Kolf-Clauw M, and Oswald IP (2009) The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. *Toxicol Appl Pharmacol* **237:**41–48.
Poguntke M, Hazai E, Fromm MF, and Zolk O, Fromm MF, and Zolk O (2010) Drug transport by breast cancer resistance protein. *Expert Opin Drug Metab Toxicol* **6:**1363–1384.
Pointis G, Fiorini C, Defamie N, and Segretain D (2005) Gap junctional communication in the male reproductive system. *Biochim Biophys Acta* **1719:**102–116.
Pointis G, Gilleron J, Carette D, and Segretain D (2010) Physiological and physiopathological aspects of connexins and communicating gap junctions in spermatogenesis. *Philos Trans R Soc Lond B Biol Sci* **365:**1607–1620.
Powles T (2011) Stage I nonseminomatous germ cell tumor of the testis: more questions than answers? *Hematol Oncol Clin North Am* **25:**517–527.
Prozialeck WC and Lamar PC (1999) Interaction of cadmium (Cd^{2+}) with a 13-residue polypeptide analog of a putative calcium-binding motif of E-cadherin. *Biochem Biophys Acta* **1451:**93–100.
Prozialeck WC (2000) Evidence that E-cadherin may be a target for cadmium toxicity in epithelial cells. *Toxicol Appl Pharmacol* **164:**231–249.
Prozialeck WC, Edwards JR, Nebert DW, Woods JM, Barchowsky A, and Atchison WD (2008) The vascular system as a target of metal toxicity. *Toxicol Sci* **102:**207–218.
Prozialeck WC, Grunwald GB, Dey PM, Reuhl KR, and Parrish AR (2002) Cadherins and NCAM as potential targets in metal toxicity. *Toxicol Appl Pharmacol* **182:**255–265.
Prozialeck WC, Lamar PC, and Lynch SM (2003) Cadmium alters the localization of N-cadherin, E-cadherin, and β-catenin in the proximal tubule epithelium. *Toxicol Appl Pharmacol* **189:**180–195.
Qian YM, Song WC, Cui H, Cole SP, and Deeley RG (2001) Gultathione stimulates sulfated estrogen transport by multidrug resistance protein 1. *J Biol Chem* **276:**6404–6411.
Raleigh DR, Boe DM, Yu D, Weber CR, Marchiando AM, Bradford EM, Wang Y, Wu L, Schneeberger EE, Shen L, et al. (2011) Occludin S408 phosphorylation regulates tight junction protein interactions and barrier function. *J Cell Biol* **193:**565–582.
Rappa G, Finch RA, Sartorelli AC, and Lorico A (1999) New insights into the biology and pharmacology of the multidrug resistance protein (MRP) from gene knockout models. *Biochem Pharmacol* **58:**557–562.
Ribbert H (1904) Die Abscheidung intravenos injizierten gelosten Karmins in den Geweben. *Z Allgem Physiol* **4:**201–214.
Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, and vom Saal FS (2007) In vivo effects of bisphenol A in laboratory rodent studies. *Reprod Toxicol* **24:**199–224.
Rivier JE, Jiang G, Porter J, Hoeger CA, Craig AG, Corrigan A, Vale W, and Rivier CL.A, Craig AG, Corrigan A, Vale W, and Rivier CL (1995) Gonadotropin-releasing hormone antagonists: novel members of the azaline B family. *J Med Chem* **38:**2649–2662.
Robaire B (2003) Advancing towards a male contraceptive: a novel approach from an unexpected direction. *Trends Pharmacol Sci* **24:**326–328.
Roberson ED and Bowcock AM (2010) Psoriasis genetics: breaking the barrier. *Trends Genet* **26:**415–423.
Robertson KM, O'Donnell L, Jones ME, Meachem SJ, Boon WC, Fisher CR, Graves KH, McLachlan RI, and Simpson ER (1999) Impairment of spermatogenesis in mice lacking a functional aromatase (cyp19) gene. *Proc Natl Acad Sci USA* **96:**7986–7991.
Robertson KM, O'Donnell L, Simpson ER, and Jones ME (2002) The phenotype of the aromatase knockout mouse reveals dietary phytoestrogens impact significantly on testis function. *Endocrinology* **143:**2913–2921.
Robey RW, Massey PR, Amiri-Kordestani L, and Bates SE (2010) ABC transporters: unvalidated therapeutic targets in cancer and the CNS. *Anticancer Agents Med Chem* **10:**625–633.
Robey RW, To KK, Polgar O, Dohse M, Fetsch P, Dean M, and Bates SE (2009) ABCG2: a perspective. *Adv Drug Deliv Rev* **61:**3–13.
Rochat B (2009) Importance of influx and efflux systems and xenobiotic metabolizing enzymes in intratumoral disposition of anticancer agents. *Curr Cancer Drug Targets* **9:**652–674.
Roger M, Clavreul A, Venier-Julienne MC, Passirani C, Montero-Menei C, and Menei P (2011) The potential of combinations of drug-loaded nanoparticle systems and adult stem cells for glioma therapy. *Biomaterials* **32:**2106–2116.
Rothe M, Sarma V, Dixit VM, and Goeddel DV (1995) TRAF2-mediated activation of NF-κB by TNF receptor 2 and CD40. *Science* **269:**1424–1427.
Rottner K, Hänisch J, and Campellone KG (2010) WASH, WHAMM, and JMY: regulation of Arp2/3 complex and beyond. *Trends Cell Biol* **20:**650–661.
Rouiller-Fabre V, Muczynski V, Lambrot R, Lécureuil C, Coffigny H, Pairault C, Moison D, Angenard G, Bakalska M, Courtot AM, et al. (2009) Ontogenesis of testicular function in humans. *Folia Histochem Cytobiol* **47:**S19–S24.
Russell L (1977a) Desmosome-like junctions between Sertoli and germ cells in the rat testis. *Am J Anat* **148:**301–312.
Russell L (1977b) Movement of spermatocytes from the basal to the adluminal compartment of the rat testis. *Am J Anat* **148:**313–328.
Russell L (1977c) Observations on rat Sertoli ectoplasmic ('junctional') specializations in their association with germ cells of the rat testis. *Tissue Cell* **9:**475–498.
Russell L (1993) Form, dimensions, and cytology of mammalian Sertoli cells, in *The Sertoli Cell* (Russell L and Griswold M eds) pp 1–37, Cache River Press, Clearwater, FL.
Russell LD and Brinster RL (1996) Ultrastructural observations of spermatogenesis following transplanation of rat testis cells into mouse seminiferous tubules. *J Androl* **17:**615–627.
Russell LD and Peterson RN (1985) Sertoli cell junctions: morphological and functional correlates. *Int Rev Cytol* **94:**177–211.
Russell LD, Bartke A, and Goh JC (1989) Postnatal development of the Sertoli cell barrier, tubular lumen, and cytoskeleton of Sertoli and myoid cells in the rat, and their relationship to tubular fluid secretion and flow. *Am J Anat* **184:**179–189.
Russell LD, França LR, and Brinster RL (1996) Ultrastructural observations of spermatogenesis in mice resulting from transplantation of mouse spermatogonia. *J Androl* **17:**603–614.
Ruwanpura SM, McLachlan RI, and Meachem SJ (2010) Hormonal regulation of male germ cell development. *J Endocrinol* **205:**117–131.
Saitou M, Furuse M, Sasaki H, Schulzke JD, Fromm M, Takano H, Noda T, and Tsukita S (2000) Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol Biol Cell* **11:**4131–4142.
Sakakibara A, Furuse M, Saitou M, Ando-Akatsuka Y, and Tsukita S (1997) Possible involvement of phosphorylation of occludin in tight junction formation. *J Cell Biol* **137:**1393–1401.
Sakkas D and Alvarez JG (2010) Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil Steril* **93:**1027–1036.
Salanova M, Stefanini M, De Curtis I, and Palombi F (1995) Integrin receptor $\alpha6\beta1$ is localized at specific sites of cell-to-cell contact in rat seminiferous epithelium. *Biol Reprod* **52:**79–87.
Salian S, Doshi T, and Vanage G (2009a) Neonatal exposure of male rats to bisphenol A impairs fertility and expression of Sertoli cell junctional proteins in the testis. *Toxicology* **265:**56–67.
Salian S, Doshi T, and Vanage G (2009b) Perinatal exposure of rats to bisphenol A affects the fertility of male offspring. *Life Sci* **85:**742–752.
Samy ET, Li JC, Grima J, Lee WM, Silvestrini B, and Cheng CY (2000) Sertoli cell prostaglandin D2 synthetase is a multifunctional molecule: its expression ann regulation. *Endocrinology* 141:710–721.
Santos-Ahmed J, Brown C, Smith SD, Weston P, Rasoulpour T, Gilbert ME, and Hixon ML (2011) Akt1 protects against germ cell apoptosis in the postnatal mouse testis following factational exposure to 6-*N*-propylthiouracil. *Reprod Toxicol* **31:**17–25.
Sarkar O, Mathur PP, Cheng CY, and Mruk DD (2008) Interleukin 1 alpha (IL1A) is a novel regulator of the blood-testis barrier in the rat. *Biol Reprod* **78:**445–454.
Sato I, Obata Y, Kasahara K, Nakayama Y, Fukumoto Y, Yamasaki T, Yokoyama KK, Saito T, and Yamaguchi N (2009) Differential trafficking of Src, Lyn, Yes and Fyn is specified by the state of palmitoylation in the SH4 domain. *J Cell Sci* **122:**965–975.
Schaller MD (2010) Cellular functions of FAK kinases: insight into molecular mechanisms and novel functions. *J Cell Sci* **123:**1007–1013.
Schell C, Albrecht M, Mayer C, Schwarzer JU, Frungieri MB, and Mayerhofer A (2008) Exploring human testicular peritubular cells: identification of secretory products and regulation by TNFα. *Endocrinology* **149:**1678–1686.
Schnabolk GW, Gupta B, Mulgaonkar A, Kulkarni M, and Sweet DH (2010) Organic anion transporter 6 (Slc22a20) specificity and Sertoli cell-specific expression provide new insight on potential endogenous roles. *J Pharmacol Exp Ther* **334:**927–935.
Schwarz BT, Wang F, Shen L, Clayburgh DR, Su L, Wang Y, Fu YX, and Turner JR (2007) LIGHT signals directly to intestinal epithelia to cause barrier dysfunction via cytoskeletal and endocytic mechanisms. *Gastroenterology* **132:**2383–2394.
Selawry HP and Cameron DF (1993) Sertoli cell-enriched fractions in successful islet cell transplantation. *Cell Transplant* **2:**123–129.
Setchell BP (2008) Blood-testis barrier, junctional and transport proteins and spermatogenesis, in *Molecular Mechanisms in Spermatogenesis* (Cheng CY ed) pp 212–233, Landes Bioscience/Springer Science+Business Media, LLC, Austin, TX.
Setchell BP and Waites GM (1970) Changes in the permeability of the testicular capillaries and of the 'blood-testis barrier' after injection of cadmium chloride in the rat. *J Endocrinol* **47:**81–86.

Setchell BP and Waites GM (1975) The blood-testis barrier, in *The Handbook of Physiology, Section 7 Endocrinology, Volume V, Male Reproductive System* (Hamilton DW and Greep RO eds) pp 143–172, American Physiological Society, Washington, DC.

Shaha C (2008) Estrogens and spermatogenesis, in *Molecular Mechanisms in Spermatogenesis* (Cheng CY ed) pp 42–64, Landes Bioscience/Springer Science+Business Media, LLC, Austin, TX.

Shaha C, Tripathi R, and Mishra DP (2010) Male germ cell apoptosis: regulation and biology. *Philos Trans R Soc Lond B Biol Sci* **365:**1501–1515.

Shamekh R, El-Badri NS, Saporta S, Pascual C, Sanberg PR, and Cameron DF (2006) Sertoli cells induce systemic donor-specific tolerance in xenogenic transplantation model. *Cell Transplant* **15:**45–53.

Shao M, Ghosh A, Cooke VG, Naik UP, and Martin-DeLeon PA (2008) JAM-A is present in mammalian spermatozoa where it is essential for normal motility. *Dev Biol* **313:**246–255.

Sharpe RM (1994) Regulation of spermatogenesis, in *The Physiology of Reproduction* (Knobil E, Neill J, Ewing L, Greenwald G, Markert C, and Pfaff D eds) vol 1, pp 1363–1434, Raven Press, New York.

Sharpe RM, Atanassova N, McKinnell C, Parte P, Turner KJ, Fisher JS, Kerr JB, Groome NP, Macpherson S, Millar MR, et al. (1998) Abnormalities in functional development of the Sertoli cells in rats treated neonatally with diethylstilbestrol: a possible role for estrogens in Sertoli cell development. *Biol Reprod* **59:**1084–1094.

Shen L and Turner JR (2005) Actin depolymerization disrupts tight junctions via caveolae-mediated endocytosis. *Mol Biol Cell* **16:**3919–3936.

Shen L, Weber CR, Raleigh DR, Yu D, and Turner JR (2011) Tight junction pore and leak pathways: a dynamic duo. *Annu Rev Physiol* **73:**283–309.

Shivas JM, Morrison HA, Bilder D, and Skop AR (2010) Polarity and endocytosis: reciprocal regulation. *Trends Cell Biol* **20:**445–452.

Shukla S, Ohnuma S, and Ambudkar SV (2011) Improving cancer chemotherapy with modulators of ABC drug transporters. *Curr Drug Targets* **12:**621–630.

Shupe J, Cheng J, Puri P, Kostereva N, and Walker WH (2011) Regulation of Sertoli-germ cell adhesion and sperm release by FSH and nonclassical testosterone signaling. *Mol Endocrinol* **25:**238–252.

Siewit CL, Gengler B, Vegas E, Puckett R, and Louie MC (2010) Cadmium promotes breast cancer cell proliferation by potentiating the interaction between ERα and c-Jun. *Mol Endocrinol* **24:**981–992.

Simpson AJ, Caballero OL, Jungbluth A, Chen YT, and Old LJ (2005) Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer* **5:**615–625.

Simpson E, Rubin G, Clyne C, Robertson K, O'Donnell L, Jones M, and Davis S (2000) The role of local estrogen biosynthesis in males and females. *Trends Endocrinol Metab* **11:**184–188.

Sipilä P, Jalkanen J, Huhtaniemi IT, and Poutanen M (2009) Novel epididymal proteins as targets for the development of post-testicular male contraception. *Reproduction* **137:**379–389.

Siu ER, Mruk DD, Porto CS, and Cheng CY (2009a) Cadmium-induced testicular injury. *Toxicol Appl Pharmacol* **238:**240–249.

Siu ER, Wong EW, Mruk DD, Porto CS, and Cheng CY (2009b) Focal adhesion kinase is a blood-testis barrier regulator. *Proc Natl Acad Sci USA* **106:**9298–9303.

Siu ER, Wong EW, Mruk DD, Sze KL, Porto CS, and Cheng CY (2009c) An occludin-focal adhesion kinase protein complex at the blood-testis barrier: a study using the cadmium model. *Endocrinology* **150:**3336–3344.

Siu MK and Cheng CY (2004a) Dynamic cross-talk between cells and the extracellular matrix in the testis. *BioEssays* **26:**978–992.

Siu MK and Cheng CY (2004b) Interactions of proteases, protease inhibitors, and the β1 integrin/laminin γ3 protein complex in the regulation of ectoplasmic specialization dynamics in the rat testis. *Biol Reprod* **70:**945–964.

Siu MK and Cheng CY (2008) Extracellular matrix and its role in spermatogenesis. *Adv Exp Med Biol* **636:**74–91.

Siu MK, Lee WM, and Cheng CY (2003a) The interplay of collagen IV, tumor necrosis factor-α, gelatinase B (matrix metalloprotease-9), and tissue inhibitor of metalloproteases-1 in the basal lamina regulates Sertoli cell-tight junction dynamics in the rat testis. *Endocrinology* **144:**371–387.

Siu MK, Mruk DD, Lee WM, and Cheng CY (2003b) Adhering junction dynamics in the testis are regulated by an interplay of β1-integrin and focal adhesion complex (FAC)-associated proteins. *Endocrinology* **144:**2141–2163.

Siu MK, Wong CH, Lee WM, and Cheng CY (2005) Sertoli-germ cell anchoring junction dynamics in the testis are regulated by an interplay of lipid and protein kinases. *J Biol Chem* **280:**25029–25047.

Siu MK, Wong CH, Xia W, Mruk DD, Lee WM, and Cheng CY (2011) The β1-integrin-p-FAK-p130Cas-DOCK180-RhoA-vinculin is a novel regulatory protein complex at the apical ectoplasmic specialization in adult rat testes. *Spermatogenesis* **1:**73–86.

Skinner M (1993) Secretion of growth factors and other regulatory factors, in *The Sertoli Cell* (Russell L and Griswold M eds) pp 237–247, Cache River Press, Clearwater, FL.

Smales C, Ellis M, Baumber R, Hussain N, Desmond H, and Staddon JM (2003) Occludin phosphorylation: identification of an occludin kinase in brain and cell extracts as CK2. *FEBS Lett* **545:**161–166.

Smith AJ and Humphries SE (2009) Cytokine and cytokine receptor gene polymorphisms and their functionality. *Cytokine Growth Factor Rev* **20:**43–59.

Solan JL and Lampe PD (2009) Connexin43 phosphorylation: structural changes and biological effects. *Biochem J* **419:**261–272.

Sridharan S, Simon L, Meling DD, Cyr DG, Gutstein DE, Fishman GI, Guillou F, and Cooke PS (2007) Proliferation of adult Sertoli cells following conditional knockout of the gap junctional protein GJA1 (connexin 43) in mice. *Biol Reprod* **76:**804–812.

Stamatovic SM, Keep RF, Wang MM, Jankovic I, and Andjelkovic AV (2009) Caveolae-mediated internalization of occludin and claudin-5 during CCL2-induced tight junction remodeling in brain endothelial cells. *J Biol Chem* **284:**19053–19066.

Starace D, Galli R, Paone A, De Cesaris P, Filippini A, Ziparo E, and Riccioli A, Ziparo E, and Riccioli A (2008) Toll-like receptor 3 activation induces antiviral immune responses in mouse Sertoli cells. *Biol Reprod* **79:**766–775.

Starace D, Riccioli A, D'Alessio A, Giampietri C, Petrungaro S, Galli R, Filippini A, Ziparo E, and De Cesaris P (2005) Characterization of signaling pathways leading to Fas expression induced by TNF-α: pivotal role of NF-κB. *FASEB J* **19:**473–475.

Steed E, Balda MS, and Matter K (2010) Dynamics and functions of tight junctions. *Trends Cell Biol* **20:**142–149.

Stride BD, Valdimarsson G, Gerlach JH, Wilson GM, Cole SP, and Deeley RG (1996) Structure and expression of the messenger RNA encoding the murine multidrug resistance protein, an ATP-binding cassette transporter. *Mol Pharmacol* **49:**962–971.

Stuible M and Tremblay ML (2010) In control at the ER: PTP1B and the down-regulation of RTKs by dephosphorylation and endocytosis. *Trends Cell Biol* **20:**672–679.

Su L, Cheng CY, and Mruk DD (2009) Drug transporter, P-glycoprotein (MDR1), is an integrated component of the mammalian blood-testis barrier. *Int J Biochem Cell Biol* **41:**2578–2587.

Su L, Cheng CY, and Mruk DD (2010a) Adjudin-mediated Sertoli-germ cell junction disassembly affects Sertoli cell barrier function in vitro and in vivo. *Int J Biochem Cell Biol* **42:**1864–1875.

Su L, Mruk DD, and Cheng CY (2011a) Drug transporters, the blood-testis barrier, and spermatogenesis. *J Endocrinol* **208:**207–223.

Su L, Mruk DD, Lee WM, and Cheng CY (2010b) Differential effects of testosterone and TGF-β3 on endocytic vesicle-mediated protein trafficking events at the blood-testis barrier. *Exp Cell Res* **316:**2945–2960.

Su L, Mruk DD, Lee WM, and Cheng CY (2011b) Drug transporters and blood-testis barrier function. *J Endocrinol* **209:**337–351.

Suescun MO, Rival C, Theas MS, Calandra RS, and Lustig L (2003) Involvement of tumor necrosis factor-α in the pathogenesis of autoimmune orchitis in rats. *Biol Reprod* **68:**2114–2121.

Summy JM, Sudol M, Eck MJ, Monteiro AN, Gatesman A, and Flynn DC (2003) Specificity in signaling by c-Yes. *Front Biosci* **8:**s185–s205.

Suri A (2005) Contraceptive vaccines targeting sperm. *Expert Opin Biol Ther* **5:**381–392.

Suzuki T and Hara H (2011) Role of flavonoids in intestinal tight junction regulation. *J Nutr Biochem* **22:**401–408.

Suzuki T, Onogawa T, Asano N, Mizutamari H, Mikkaichi T, Tanemoto M, Abe M, Satoh F, Unno M, Nunoki K, et al. (2003) Identification and characterization of novel rat and human gonad-specific organic anion transporters. *Mol Endocrinol* **17:**1203–1215.

Syed V, Stéphan JP, Gérard N, Legrand A, Parvinen M, Bardin CW, and Jégou B (1995) Residual bodies activate Sertoli cell interleukin-1α (IL-1α) release, which triggers IL-6 production by an autocrine mechanism, through the lipoxygenase pathway. *Endocrinology* **136:**3070–3078.

Takehashi M, Kanatsu-Shinohara M, Miki H, Lee J, Kazuki Y, Inoue K, Ogonuki N, Toyokuni S, Oshimura M, Ogura A, et al. (2007) Production of knockout mice by gene targeting in multipotent germline stem cells. *Dev Biol* **312:**344–352.

Takenaka S, Oldiges H, König H, Hochrainer D, and Oberdörster G (1983) Carcinogenicity of cadmium chloride aerosols in W rats. *J Natl Cancer Inst* **70:**367–373.

Tang P, Hung MC, and Klostergaard J (1996) Human pro-tumor necrosis factor is a homotrimer. *Biochemistry* **35:**8216–8225.

Tang Q, Dowd TL, Verselis VK, and Bargiello TA (2009) Conformational changes in a pore-forming region underlie voltage-dependent "loop gating" of an unapposed connexin hemichannel. *J Gen Physiol* **133:**555–570.

Tartaglia LA and Goeddel DV (1992) Two TNF receptors. *Immunol Today* **13:**151–153.

Tash JS, Attardi B, Hild SA, Chakrasali R, Jakkaraj SR, and Georg GI (2008a) A novel potent indazole carboxylic acid derivative blocks spermatogenesis and is contraceptive in rats after a single oral dose. *Biol Reprod* **78:**1127–1138.

Tash JS, Chakrasali R, Jakkaraj SR, Hughes J, Smith SK, Hornbaker K, Heckert LL, Ozturk SB, Hadden MK, Kinzy TG, et al. (2008b) Gamendazole, an orally active indazole carboxylic acid male contraceptive agent, targets HSP90AB1 (HSP90BHETA) and EEFA1 (eEF1A), and stimulates Il1a transcription in rat Sertoli cells. *Biol Reprod* **78:**1139–1152.

Teerds KJ and Dorrington JH (1993) Localization of transforming growth factor β1 and β2 during testicular development in the rat. *Biol Reprod* **48:**40–45.

Tegtmeyer N and Backert S (2011) Role of Abl and Src family kinases in actin-cytoskeletal rearrangements induced by the *Helicobacter pylori* CagA protein. *Eur J Cell Biol* http://dx.doi.org/10.1016/j.ejcb.2010.11.006.

Telisman S, Colak B, Pizent A, Jurasović J, and Cvitković P (2007) Reproductive toxicity of low-level lead exposure in men. *Environ Res* **105:**256–266.

Templeton DM and Liu Y (2010) Multiple roles of cadmium in cell death and survival. *Chem Biol Interact* **188:**267–275.

Thévenod F and Chakraborty PK (2010) The role of Wnt/β-catenin signaling in renal carcinogenesis: lessons from cadmium toxicity studies. *Curr Mol Med* **10:**387–404.

Thévenod F (2010) Catch me if you can! Novel aspects of cadmium transport in mammalian cells. *Biometals* **23:**857–875.

Thomason HA, Scothern A, McHarg S, and Garrod DR (2010) Desmosomes: adhesive strength and signalling in health and disease. *Biochem J* **429:**419–433.

Thompson J, Wong L, Lau PS, and Bannigan J (2008) Adherens junction breakdown in the periderm following cadmium administration in the chick embryo: distribution of cadherins and associated molecules. *Reprod Toxicol* **25:**39–46.

Toyama Y, Maekawa M, and Yuasa S (2003) Ectoplasmic specializations in the Sertoli cells: new vistas based on genetic defects and testicular toxicology. *Anat Sci Int* **78:**1–16.

Toyama Y, Ohkawa M, Oku R, Maekawa M, and Yuasa S (2001) Neonatally administered diethylstilbestrol retards the development of the blood-testis barrier in the rat. *J Androl* **22:**413–423.

Tramoni M, Gilleron J, Tahiri K, Carette D, Corvol MT, Segretain D, Pointis G, and Savouret JF (2009) Contraceptive steroids from pharmaceutical waste perturbate junctional communication in Sertoli cells. *Biochimie* **91:**1366–1375.

Tremellen K (2008) Oxidative stress and male infertility—a clinical perspective. *Hum Reprod Update* **14:**243–258.
Tres LL, Rivkin E, and Kierszenbaum AL (1996) Sak 57, an intermediate filament keratin present in intercellular bridges of rat primary spermatocytes. *Mol Reprod Dev* **45:**93–105.
Trezise AE, Romano PR, Gill DR, Hyde SC, Sepúlveda FV, Buchwald M, and Higgins CF (1992) The multidrug resistance and cystic fibrosis genes have complementary patterns of epithelial expression. *EMBO J* **11:**4291–4303.
Tsukamoto T and Nigam SK (1999) Role of tyrosine phosphorylation in the reassembly of occludin and other tight junction proteins. *Am J Physiol* **276:**F737–F750.
Tuma PL and Hubbard AL (2003) Transcytosis: crossing cellular barriers. *Physiol Rev* **83:**871–932.
Turner JR (2009) Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* **9:**799–809.
Turner TT, Jones CE, Howards SS, Ewing LL, Zegeye B, and Gunsalus GL (1984) On the androgen microenvironment of maturing spermatozoa. *Endocrinology* **115:** 1925–1932.
Udayakumar T, Shareef MM, Diaz DA, Ahmed MM, and Pollack A (2010) The E2F1/Rb and p53/MDM2 pathways in DNA repair and apoptosis: understanding the crosstalk to develop novel strategies for prostate cancer radiotherapy. *Semin Radiat Oncol* **20:**258–266.
Ueno M, Nakagawa T, Wu B, Onodera M, Huang CL, Kusaka T, Araki N, and Sakamoto H.L, Kusaka T, Araki N, and Sakamoto H (2010) Transporters in the brain endothelial barrier. *Curr Med Chem* **17:**1125–1138.
Uhr M, Ebinger M, Rosenhagen MC, and Grauer MT (2005) The anti-Parkinson drug budipine is exported actively out of the brain by P-glycoprotein in mice. *Neurosci Lett* **383:**73–76.
Uhr M, Holsboer F, and Müller MB (2002) Penetration of endogenous steroid hormones corticosterone, cortisol, aldosterone and progesterone into the brain is enhanced in mice deficient for both mdr1a and mdr1b P-glycoproteins. *J Neuroendocrinol* **14:**753–759.
Valiunas V, Polosina YY, Miller H, Potapova IA, Valiuniene L, Doronin S, Mathias RT, Robinson RB, Rosen MR, Cohen IS, et al. (2005) Connexin-specific cell-to-cell transfer of short interfering RNA by gap junctions. *J Physiol* **568:**459–468.
Van Itallie CM, Holmes J, Bridges A, Gookin JL, Coccaro MR, Proctor W, Colegio OR, and Anderson JM (2008) The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. *J Cell Sci* **121:**298–305.
Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, and Soto AM (2009) Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. *Endocr Rev* **30:**75–95.
VanItallie TB (2010) Gout: epitome of painful arthritis. *Metabolism* **59 (Suppl 1):**S32–S36.
Venkatesh S, Shamsi MB, Dudeja S, Kumar R, and Dada R (2011) Reactive oxygen species measurement in neat and washed semen: comparative analysis and its significance in male infertility asssessment. *Arch Gynecol Obstet* **283:**121–126.
Vergouwen RP, Huiskamp R, Bas RJ, Roepers-Gajadien HL, Davids JA, and de Rooij DG (1993) Postnatal development of testicular cell populations in mice. *J Reprod Fertil* **99:**479–485.
Verhoeven G, Denolet E, Swinnen JV, Willems A, Claessens F, Saunders PTK, Sharpe RM, and De Gendt K (2008) Contribution of recent transgenic models and trasncriptional profiling studies to our understanding of the mechanisms by which androgens control spermatogenesis. *Immunol Endocr Metab Agents Med Chem* **8:**2–13.
Verhoeven G, Willems A, Denolet E, Swinnen JV, and De Gendt K (2010) Androgens and spermatogenesis: lessons from transgenic mouse models. *Philos Trans R Soc Lond B Biol Sci* **365:**1537–1556.
Vesey DA (2010) Transport pathways for cadmium in the intestine and kidney proximal tubule: focus on the interaction with essential metals. *Toxicol Lett* **198:**13–19.
Vinken M, Ceelen L, Vanhaecke T, and Rogiers V (2010) Inhibition of gap junctional communication by toxic metals. *Chem Res Toxicol* **23:**1862–1867.
Vitale R, Fawcett DW, and Dym M (1973) The normal development of the blood-testis barrier and the effects of clomiphene and estrogen treatment. *Anat Rec* **176:**331–344.
Vogl A, Vaid K, and Guttman J (2008) The Sertoli cell cytoskeleton, in *Molecular Mechanisms in Spermatogenesis* (Cheng CY ed) pp 186–211, Landes Bioscience/ Springer Science+Business Media, Austin, TX.
Vogl AW, Pfeiffer DC, Mulholland D, Kimel G, and Guttman J (2000) Unique and multifunctional adhesion junctions in the testis: ectoplasmic specializations. *Arch Histol Cytol* **63:**1–15.
Waalkes MP (2003) Cadmium carcinogenesis. *Mutation Res* **533:**107–120.
Waalkes MP, Coogan TP, and Barter RA (1992) Toxicological principles of metal carcinogenesis with special emphasis on cadmium. *Crit Rev Toxicol* **22:**175–201.
Wahab-Wahlgren A, Holst M, Ayele D, Sultana T, Parvinen M, Gustafsson K, Granholm T, and Söder O (2000) Constitutive production of interleukin-1α mRNA and protein in the developing rat testis. *Int J Androl* **23:**360–365.
Walker WH (2009) Molecular mechanisms of testosterone action in spermatogenesis. *Steroids* **74:**602–607.
Walker WH (2010) Non-classical actions of testosterone and spermatogenesis. *Philos Trans R Soc Lond B Biol Sci* **365:**1557–1569.
Walsh SV, Hopkins AM, and Nusrat A (2000) Modulation of tight junction structure and function by cytokines. *Adv Drug Deliv Rev* **41:**303–313.
Wang C and Swerdloff RS (2010) Hormonal approaches to male contraception. *Curr Opin Urol* **20:**520–524.
Wang CQ and Cheng CY (2007) A seamless trespass: germ cell migration across the seminiferous epithelium during spermatogenesis. *J Cell Biol* **178:**549–556.
Wang CQ, Mruk DD, Lee WM, and Cheng CY (2007) Coxsackie and adenovirus receptor (CAR) is a product of Sertoli and germ cells in rat testes which is localized at the Sertoli-Sertoli and Sertoli-germ cell interface. *Exp Cell Res* **313:**1373–1392.
Wang RS, Yeh S, Chen LM, Lin HY, Zhang C, Ni J, Wu CC, di Sant'Agnese PA, deMesy-Bentley KL, Tzeng CR, et al. (2006) Androgen receptor in Sertoli cell is essential for germ cell nursery and junction complex formation in mouse testes. *Endocrinology* **147:**5624–5633.
Wang RS, Yeh S, Tzeng CR, and Chang C (2009) Androgen receptor roles in spermatogenesis and fertility: lessons from testicular cell-specific androgen receptor knockout mice. *Endocr Rev* **30:**119–132.
Wanke D and Kolukisaoglu HU (2010) An update on the ABCC transporter family in plants: many genes, many proteins, but how many functions? *Plant Biol* (*Stuttg*) **12 (Suppl 1):**15–25.
Watson CJ, Hoare CJ, Garrod DR, Carlson GL, and Warhurst G (2005) Interferon-gamma selectively increases epithelial permeability to large molecules by activating different populations of paracellular pores. *J Cell Sci* **118:**5221–5230.
Watson CS, Alyea RA, Jeng YJ, and Kochukov MY (2007) Nongenomic actions of low concentration oestrogens and xenoestrogens on multiple tissues. *Mol Cell Endocrinol* **274:**1–7.
Webb M (1975) Cadmium. *Br Med Bull* **31:**246–250.
Weber JE and Russell LD (1987) A study of intercellular bridges during spermatogenesis in the rat. *Am J Anat* **180:**1–24.
Weber JE, Russell LD, Wong V, and Peterson RN (1983) Three dimensional reconstruction of a rat stage V Sertoli cell: II. Morphometry of Sertoli-Sertoli and Sertoli-germ cell relationships. *Am J Anat* **167:**163–179.
Weber JE, Turner TT, Tung KS, and Russell LD (1988) Effects of cytochalasin D on the integrity of the Sertoli cell (blood-testis) barrier. *Am J Anat* **182:**130–147.
Welman A, Cawthorne C, Ponce-Perez L, Barraclough J, Danson S, Murray S, Cummings J, Allen TD, and Dive C (2006) Increases in c-Src expression level and activity do not promote the growth of human colorectal carcinoma cells in vitro and in vivo. *Neoplasia* **8:**905–916.
Welsh M, Saunders PT, Atanassova N, Sharpe RM, and Smith LB (2009) Androgen action via testicular peritubular myoid cells is essential for male fertility. *FASEB J* **23:**4218–4230.
Wertz IE and Dixit VM (2008) Ubiquitin-mediated regulation of TNFR1 signaling. *Cytokine Growth Factor Rev* **19:**313–324.
Wiebe JP, Kowalik A, Gallardi RL, Egeler O, and Clubb BH (2000) Glycerol disrupts tight junction-associated actin microfilaments, occludin, and microtubules in Sertoli cells. *J Androl* **21:**625–635.
Wijnholds J, Scheffer GL, van der Valk M, van der Valk P, Beijnen JH, Scheper RJ, and Borst P (1998) Multidrug resistance protein 1 protects the oropharyngeal mucosal layer and the tesicular tubules against drug-induced damage. *J Exp Med* **188:**797–808.
Willems A, Batlouni SR, Esnal A, Swinnen JV, Saunders PT, Sharpe RM, França LR, De Gendt K, and Verhoeven G (2010a) Selective ablation of the androgen receptor in mouse Sertoli cells affects Sertoli cell maturation, barrier formation and cytoskeletal development. *PLoS One* **5:**e14168.
Willems A, De Gendt K, Allemeersch J, Smith LB, Welsh M, Swinnen JV, and Verhoeven G (2010b) Early effects of Sertoli cell-selective androgen receptor ablation on testicular gene expression. *Int J Androl* **33:**507–517.
Wirth JJ and Mijal RS (2010) Adverse effects of low level heavy metal exposure on male reproductive function. *Syst Biol Reprod Med* **56:**147–167.
Wolski KM, Perrault C, Tran-Son-Tay R, and Cameron DF (2005) Strength measurement of the Sertoli-spermatid junctional complex. *J Androl* **26:**354–359.
Wong CH and Cheng CY (2005) The blood-testis barrier: its biology, regulation, and physiological role in spermatogenesis. *Curr Top Dev Biol* **71:**263–296.
Wong CH, Mruk DD, Lui WY, and Cheng CY (2004) Regulation of blood-testis barrier dynamics: an in vivo study. *J Cell Sci* **117:**783–798.
Wong CH, Mruk DD, Siu MK, and Cheng CY (2005a) Blood-testis barrier dynamics are regulated by α_2-macroglobulin via the c-Jun N-terminal protein kinase pathway. *Endocrinology* **146:**1893–1908.
Wong CH, Xia W, Lee NP, Mruk DD, Lee WM, and Cheng CY (2005b) Regulation of ectoplasmic specialization dynamics in the seminiferous epithelium by focal adhesion-associated proteins in testosterone-suppressed rat testes. *Endocrinology* **146:** 1192–1204.
Wong EW and Cheng CY (2009) Polarity proteins and cell-cell interactions in the testis. *Int Rev Cell Mol Biol* **278:**309–353.
Wong EW and Cheng CY (2011) Impacts of environmental toxicants on male reproductive dysfunction. *Trends Pharmacol Sci* **32:**290–299.
Wong EW, Mruk DD, and Cheng CY (2008a) Biology and regulation of ectoplasmic specialization, an atypical adherens junction type, in the testis. *Biochem Biophys Acta* **1778:**692–708.
Wong EW, Mruk DD, and Cheng CY (2008b) Delivery of contraceptives to men: lesson from other therapeutic drugs. *Immunol Endocr Metab Agents Med Chem* **8:**91–94.
Wong EW, Mruk DD, Lee WM, and Cheng CY (2008c) Par3/Par6 polarity complex coordinates apical ectoplasmic specialization and blood-testis barrier restructuring during spermatogenesis. *Proc Natl Acad Sci USA* **105:**9657–9662.
Wong EW, Mruk DD, Lee WM, and Cheng CY (2010a) Regulation of blood-testis barrier dynamics by TGF-β3 is a Cdc42-dependent protein trafficking event. *Proc Natl Acad Sci USA* **107:**11399–11404.
Wong EW, Sun S, Li MW, Lee WM, and Cheng CY (2009) 14-3-3 protein regulates cell adhesion in the seminiferous epithelium of rat testes. *Endocrinology* **150:** 4713–4723.
Wong EW, Yan HH, Li MW, Lie PP, Mruk DD, and Cheng CY (2010b) Cell junctions in the testis as targets for toxicants, in *Reproductive and Endocrine Toxicology* (Hoyer PB and Richburg JH ed) pp 167–188, Academic Press/Elsevier, Oxford.
Wong HL, Chattopadhyay N, Wu XY, and Bendayan R (2010c) Nanotechnology applications for improved delivery of antiretroviral drugs to the brain. *Adv Drug Deliv Rev* **62:**503–517.
World Health Organization (2000) Cadmium, in *Air Quality Guidelines*, pp 136–138, World Health Organization, Regional Office for Europe, Copenhagen, Denmark. Available at: www.euro.who.int/__data/assets/pdf_file/0005/74732/E71922.pdf
Worthington JJ, Klementowicz JE, and Travis MA (2011) TGFβ: a sleeping giant awoken by integrins. *Trends Biochem Sci* **36:**47–54.
Wu HM, Lin-Tan DT, Wang ML, Huang HY, Wang HS, Soong YK, and Lin JL (2008)

Cadmium level in seminal plasma may affect the pregnancy rate for patients undergoing infertility evaluation and treatment. *Reprod Toxicol* **25:**481–484.

Xia W and Cheng CY (2005) TGF-β3 regulates anchoring junction dynamics in the seminiferous epithelium of the rat testis via the Ras/ERK signaling pathway: an in vivo study. *Dev Biol* **280:**321–343.

Xia W, Mruk DD, Lee WM, and Cheng CY (2005a) Cytokines and junction restructuring during spermatogenesis—a lesson to learn from the testis. *Cytokine Growth Factor Rev* **16:**469–493.

Xia W, Mruk DD, Lee WM, and Cheng CY (2006) Differential interactions between transforming growth factor-β3/TβR1, TAB1, and CD2AP disrupt blood-testis barrier and Sertoli-germ cell adhesion. *J Biol Chem* **281:**16799–16813.

Xia W, Wong CH, Lee NP, Lee WM, and Cheng CY (2005b) Disruption of Sertoli-germ cell adhesion function in the seminiferous epithelium of the rat testis can be limited to adherens junctions without affecting the blood-testis barrier integrity: an in vivo study using an androgen suppression model. *J Cell Physiol* **205:**141–157.

Xia W, Wong EW, Mruk DD, and Cheng CY (2009) TGF-β3 and TNFα perturb blood-testis barrier (BTB) dynamics by accelerating the clathrin-mediated endocytosis of integral membrane proteins: a new concept of BTB regulation during spermatogenesis. *Dev Biol* **327:**48–61.

Xiao X, Mruk DD, Lee WM, and Cheng CY (2011) c-Yes regulates cell adhesion at the blood-testis barrier and the apical ectoplasmic specialization in the seminiferous epithelium of rat testes. *Int J Biochem Cell Biol* **43:**651–665.

Xu W, Harrison SC, and Eck MJ (1997) Three-dimensional structure of the tyrosine kinase c-Src. *Nature* **385:**595–602.

Yamamoto M, Ramirez SH, Sato S, Kiyota T, Cerny RL, Kaibuchi K, Persidsky Y, and Ikezu T (2008) Phosphorylation of claudin-5 and occludin by rho kinase in brain endothelial cells. *Am J Pathol* **172:**521–533.

Yan HH and Cheng CY (2005) Blood-testis barrier dynamics are regulated by an engagement/disengagement mechanism between tight and adherens junctions via peripheral adaptors. *Proc Natl Acad Sci USA* **102:**11722–11727.

Yan HH and Cheng CY (2006) Laminin α3 forms a complex with β3 and γ3 chains that serves as the ligand for α6β1-integrin at the apical ectoplasmic specialization in adult rat testes. *J Biol Chem* **281:**17286–17303.

Yan HH, Mruk DD, and Cheng CY (2008a) Junction restructuring and spermatogenesis: the biology, regulation, and implication in male contraceptive development. *Curr Top Dev Biol* **80:**57–92.

Yan HH, Mruk DD, Lee WM, and Cheng CY (2007) Ectoplasmic specialization: a friend or a foe of spermatogenesis? *BioEssays* **29:**36–48.

Yan HH, Mruk DD, Lee WM, and Cheng CY (2008b) Blood-testis barrier dynamics are regulated by testosterone and cytokines via their differential effects on the kinetics of protein endocytosis and recycling in Sertoli cells. *FASEB J* **22:**1945–1959.

Yan HH, Mruk DD, Lee WM, and Cheng CY (2008c) Cross-talk between tight and anchoring junctions - lesson from the testis, in *Molecular Mechanisms in Spermatogenesis* (Cheng CY ed) pp 234–254, Landes Bioscience/Springer Science+Business Media, Austin, TX.

Yang L (2010) TGFβ, a potent regulator of tumor microenvironment and host immune response, implication for therapy. *Curr Mol Med* **10:**374–380.

Yeatman TJ (2004) A renaissance for SRC. *Nat Rev Cancer* **4:**470–480.

Yin Z, Chen D, Hu F, Ruan Y, Li J, Wang L, Xiang Y, Xie L, Wang X, Ichim TE, et al. (2009) Cotransplantation with xenogenetic neonatal porcine Sertoli cells significantly prolongs islet allograft survival in nonimmunosuppressive rats. *Transplantation* **88:**339–345.

Yoshida S (2010) Stem cells in mammalian spermatogenesis. *Dev Growth Differ* **52:**311–317.

Yu X, Filardo EJ, and Shaikh ZA (2010) The membrane estrogen receptor GPR30 mediates cadmium-induced proliferation of breast cancer cells. *Toxicol Appl Pharmacol* **245:**83–90.

Yu X, Hong S, Moreira EG, and Faustman EM (2009) Improving in vitro Sertoli cell/gonocyte co-culture model for assessing male reproductive toxicity: Lessons learned from comparisons of cytotoxicity versus genomic responses to phthalates. *Toxicol Appl Pharmacol* **239:**325–336.

Yule TD and Tung KS (1993) Experimental autoimmune orchitis induced by testis and sperm antigen-specific T cell clones: an important pathogenic cytokine is tumor necrosis factor. *Endocrinology* **133:**1098–1107.

Yule TD, Mahi-Brown CA, and Tung KS (1990) Role of testicular autoantigens and influence of lymphokines in testicular autoimmune disease. *J Reprod Immunol* **18:**89–103.

Yule TD, Montoya GD, Russell LD, Williams TM, and Tung KS (1988) Autoantigenic germ cells exist outside the blood testis barrier. *J Immunol* **141:**1161–1167.

Zefferino R, Leone A, Piccaluga S, Cincione R, and Ambrosi L (2008) Mercury modulates interplay between IL-1β, TNF-α, and gap junctional intercellular communication in keratinocytes: mitigation by lycopene. *J Immunotoxicol* **5:**353–360.

Zhang J, Wong CH, Xia W, Mruk DD, Lee NP, Lee WM, and Cheng CY (2005) Regulation of Sertoli-germ cell adherens junction dynamics via changes in protein-protein interactions of the *N*-cadherin-β-catenin protein complex which are possibly mediated by c-Src and myotubularin-related protein 2: an in vivo study using an androgen suppression model. *Endocrinology* **146:**1268–1284.

Zhou W, Bolden-Tiller OU, Shetty G, Shao SH, Weng CC, Pakarinen P, Liu Z, Stivers DN, and Meistrich ML, Stivers DN, et al. (2010) Changes in gene expression in somatic cells of rat testes resulting from hormonal modulation and radiation-induced germ cells depletion. *Biol Reprod* **82:**54–65.

Zirkin BR (1998) Spermatogenesis: its regulation by testosterone and FSH. *Semin Cell Dev Biol* **9:**417–421.