Published in final edited form as:

Biochem J. 2011 May 1; 435(3): 553–562. doi:10.1042/BJ20102121.

Regulation of spermiogenesis, spermiation and blood–testis barrier dynamics: novel insights from studies on Eps8 and Arp3

C. Yan Cheng¹ and Dolores D. Mruk

The Mary M. Wohlford Laboratory for Male Contraceptive Research, Center for Biomedical Research, Population Council, 1230 York Avenue, New York, NY 10065, U.S.A.

Abstract

Spermiogenesis in the mammalian testis is the most critical post-meiotic developmental event occurring during spermatogenesis in which haploid spermatids undergo extensive cellular, molecular and morphological changes to form spermatozoa. Spermatozoa are then released from the seminiferous epithelium at spermiation. At the same time, the BTB (blood–testis barrier) undergoes restructuring to facilitate the transit of preleptotene spermatocytes from the basal to the apical compartment. Thus meiotic divisions take place behind the BTB in the apical compartment to form spermatids. These germ cells enter spermiogenesis to transform into elongating spermatids and then into spermatozoa to replace those that were released in the previous cycle. However, the molecular regulators that control spermiogenesis, in particular the dynamic changes that occur at the Sertoli cell–spermatid interface and at the BTB, are not entirely known. This is largely due to the lack of suitable animal models which can be used to study these events. During the course of our investigation to develop adjuvin [1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carbohydrazide] as a potential male contraceptive, this drug was shown to ‘accelerate’ spermiation by inducing the release of premature spermatids from the epithelium. Using this model, we have identified several molecules that are crucial in regulating the actin filament network and the unique adhesion protein complex at the Sertoli cell–spermatid interface known as the apical ES (ectoplasmic specialization). In the present review, we critically evaluate these and other findings in the literature as they relate to the restricted temporal and spatial expression of two actin regulatory proteins, namely Eps8 (epidermal growth factor receptor pathway substrate 8) and Arp3 (actin-related protein 3), which regulate these events.

Keywords

adjuvin; blood–testis barrier; intermediate filament; seminiferous epithelial cycle; spermatogenesis; tubulobulbar complex

Introduction

Most changes in cell morphology, plasticity and movement resulting from cues received from the environment, growth and development, stress, cytokines, toxicants or during

pathogenesis are regulated by the actin-, intermediate filament- and/or tubulin-based cytoskeletons [1–6]. This applies to virtually all epithelial cells, including those in the seminiferous epithelium of the mammalian testis. Interestingly, the actin network in the seminiferous epithelium, which is composed of only Sertoli cells and germ cells which are at different stages of development (i.e. spermatogonial stem cells, spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa) (Figure 1), is notably different from actin networks found in other epithelia in several ways. First, actin filament bundles found in Sertoli cells at the ES {ectoplasmic specialization; a testis-specific atypical AJ (adherens junction) found at the Sertoli cell–spermatid and Sertoli–Sertoli cell interface known as the apical and basal ES respectively [7–9]} (Figures 1 and 2) are non-contractile [9,10], even though motor proteins (e.g. myosin VIIa) are present at the ES [10,11]. Secondly, actin filament bundles are tightly packed, arranged parallel to the Sertoli cell plasma membrane, and sandwiched in between apposing cell membranes of Sertoli cell–spermatid or Sertoli–Sertoli cell and cisternae of endoplasmic reticulum (Figures 1 and 2). Although other studies have shown that the ES is one of the strongest adhesive junctions [12,13], it still is subjected to extensive restructuring. This thus facilitates the transit of spermatids across the epithelium during spermiogenesis, as well as the transit of preleptotene spermatocytes across the BTB (blood–testis barrier) at stage VIII of the seminiferous epithelial cycle of spermatogenesis [14,15]. Thirdly, although Sertoli cells cultured *in vitro* are highly motile and capable of traversing the membranes of transwell (i.e. bicameral) units similar to metastatic cancer cells [16,17], Sertoli cells are in fact not motile *in vivo*. Instead, they are static, ‘nurse-like’ cells needed for germ cell development with each Sertoli cell ‘engulfing’ approx. 30–50 developing germ cells, but they do alter their cell shape to accommodate morphological changes of spermatids during spermiogenesis. Moreover, Sertoli cells are the only structural and ‘scaffolding’ cells in the seminiferous epithelium that confer BTB function via co-existing TJs (tight junctions), basal ES, desmosomes and gap junctions located near the basement membrane in the seminiferous epithelium, since microvessels in the interstitial space between tubules contribute relatively little to the BTB. On a final note, all of these junctions link to either the actin, the intermediate filament or the tubulin network, and they are interconnected structurally and functionally.

In the present review, we critically discuss results from recent studies relating to two actin regulatory proteins: Eps8 (epidermal growth factor receptor pathway substrate 8) (Figure 3) and Arp3 (actin-related protein 3) (Figure 4) that work together to modulate actin dynamics within the seminiferous epithelium, in particular at the apical ES and the BTB. Eps8 is a multifunctional actin regulatory protein [18–20]. Depending on its association with different binding partners; IRSp53 (insulin receptor tyrosine kinase substrate p53), Abi-1 (Abelson interacting protein-1), or Sos1 (son of sevenless 1) and Abi-1, the Eps8 protein complex can regulate actin bundling [21], capping of actin barbed-ends [22] or Rac (a GTPase) activation [23] (Figure 4), all of which regulate actin dynamics [20]. Arp3, on the other hand, is a component of the Arp 2/3 complex, which is one of the major powerhouses that creates a branched actin network in cells. Although the functional Arp2/3 nucleation complex is composed of seven subunits, Arp2, Arp3 and ARPCs (Arp2/3 complex subunit) 1–5 [24–26] (Figure 4), the Arp2/3 complex is not active. Instead it is activated by WASP (Wiskott–Aldrich syndrome protein) family proteins, namely N-WASP (neuronal-WASP), SCAR/

WAVE (suppressor of cAMP receptor/WASP family verprolin homologous) and cortactin to initiate actin nucleation/branching on a pre-existing actin filament [20,25,27,28] (Figure 4). It is worth noting that besides Eps8 and Arp3, many other proteins that work with Eps8 and the Arp2/3 complex have recently been identified in the testis [29,30]. In the present review, we will focus our discussion largely on the actin-based cytoskeleton and the significance of actin regulatory proteins in spermiogenesis and spermiation, as well as BTB dynamics since few studies are found in the literature relating to the roles of the intermediate filament- or tubulin-based cytoskeletons in spermatogenesis [20,31].

The Seminiferous Epithelial Cycle and Spermiogenesis

In the mammalian testis, spermiogenesis begins immediately after meiosis II, which takes place in a specialized microenvironment in the apical compartment of the seminiferous epithelium at stage XIV, XII or VI of the seminiferous epithelial cycle in the rat, mouse or human respectively (for reviews, see [32,33]). During spermiogenesis, round spermatids (whose precursor cells are the secondary spermatocytes) undergo extensive morphological, cellular and molecular transformations via a series of 19, 16 or 6 steps in the rat, mouse or human respectively. This is typified by the condensation of genetic material which forms the nucleus in the spermatid head, the development of the acro-some partly covering the nucleus and the elongation of the tail along with the formation of tightly packed mitochondria in the midpiece (for reviews, see [33–36]) (Figure 2). Interestingly, development of the acrosome which ‘caps’ the spermatid nucleus during spermiogenesis has allowed investigators to classify developing spermatids by the PAS (periodate–Schiff) reaction and to divide the seminiferous epithelium into discrete stages (I–XIV, I–XII or I–VI in the rat, mouse or human respectively). In essence, each stage is comprised of unique cellular associations between developing germ cells (in particular spermatids) and Sertoli cells, and these cellular associations can be clearly observed in cross-sections of seminiferous tubules [37,38] (Figure 2). Once fully developed elongated spermatids have formed and residual bodies have been phagocytosed by Sertoli cells which occurs at the end of spermiogenesis, spermatozoa are released from the seminiferous epithelium (i.e. spermiation) into the tubule lumen so that they can be transported to the epididymis for further maturation (for reviews, see [33,39]).

Besides the highly organized and intricate cellular events that occur during spermiogenesis (for reviews, see [11,33,36,40,41]), the transformation of spermatids is also marked by other important cellular events which are critical for the completion of spermatogenesis. First, CT (cancer/testis) antigens and germ cell-specific antigens (for reviews, see [7,42–44]), many of which are expressed only transiently during post-meiotic germ cell development throughout spermiogenesis, are sequestered away from the systemic circulation by the BTB. The BTB is formed by 15–17 days post-partum in the rat [45] and at puberty in humans by 12–13 years of age [46], which also plays a significant role in mammals to confer, at least in part, an immune-privilege status to the testis [47]. Thus the BTB prohibits the production of anti-sperm auto-antibodies, at least in post-meiotic spermatids during spermiogenesis and spermiation, which would otherwise induce male infertility. Secondly, there is extensive junction restructuring at the Sertoli cell–spermatid interface to allow the transit of developing spermatids across the epithelium until stages VII–VIII when elongated

spermatids properly 'line-up' near the luminal edge in preparation for spermiation (for reviews, see [15,20,48–50]). As such, the events of germ cell movement are synchronized precisely with the events of germ cell development. In the following sections, we highlight several important studies that have reported interactions among different actin regulatory proteins, as well as discuss their highly restricted spatial and temporal expression patterns in the seminiferous epithelium. We also discuss how actin regulatory proteins interact with polarized protein complexes at the Sertoli cell–spermatid interface (i.e. apical ES) to induce changes in cell plasticity and polarity, thereby facilitating rapid transformations in cell shape via protein endocytosis, recycling, transcytosis and endosome- or ubiquitin-mediated degradation.

The Actin Network at the Ectoplasmic Specialization

During spermiogenesis, a unique AJ type known as the apical ES emerges at the interface between Sertoli cells and elongating spermatids at step 8 and beyond in the rat (Figures 1 and 2) (for reviews, see [8,11,14]). Once it appears, it becomes the only adhesive device that anchors spermatids to Sertoli cells, replacing desmosome-like and gap junctions that are present between Sertoli cells and pre-step 8 spermatids or between Sertoli cells–spermatocytes. Besides being the only anchoring device used by developing spermatids, the apical ES is also known to maintain proper spermatid polarity and orientation within the epithelium so that the heads of all elongating/elongated spermatids point towards the basement membrane (for reviews, see [8,10,48]). This permits for tight arrangement of a maximal number of spermatids during spermatogenesis (Figure 2). Although this apical ES function has been largely a speculation for decades [51,52], it was only recently reported that this polarity function is made possible by the presence of several polarity protein complexes at the apical ES, namely the PAR3 (partitioning defective protein 3)/PAR6/Cdc42 (cell division cycle 42), the PALS1 (protein-associated with Lin-Seven 1)/PATJ (PALS1-associated TJ protein)/CRB (Crumbs) and the Scribble/LGL1/2 (lethal giant larvae 1/2) protein complexes (for reviews, see [14,48,53]). Although these polarity proteins were initially identified in *Caenorhabditis elegans*, a homologue for each of these proteins has been found in mammals and reported to be restricted to the TJ to confer cell polarity [48,54,55]. Interestingly, many of these proteins have been demonstrated to be components of the apical ES, and their roles in conferring spermatid polarity have been demonstrated [56,57].

The most obvious and unique ultrastructural feature of the apical ES is the precise arrangement of actin filament bundles that are sandwiched in between apposing plasma membranes of the Sertoli cell/elongating spermatid and the cisternae of endoplasmic reticulum (Figures 1 and 2). The apical ES is limited only to the Sertoli cell without any notable ultrastructural features visible in the spermatid (Figure 2) (for a review, see [14]). The ES is also found at the Sertoli–Sertoli cell interface (i.e. basal ES) and restricted to the BTB near the basement membrane, coexisting with TJs, desmosomes and gap junctions. However, unlike the apical ES, the ultrastructural features of the basal ES are found within both Sertoli cells (Figure 2) (for a review, see [14]). Thus the basal ES is believed to work with TJs at the BTB to confer adhesion and cell polarity to Sertoli cells in the testis so that Sertoli cell nuclei and some of their organelles (e.g. Golgi apparatus, endoplasmic

reticulum) can maintain their polarized localization. This is different from other epithelia in which TJs exclusively play a significant role to confer cell polarity (for reviews, see [48,54,55]).

Moreover, the apical ES at the Sertoli cell–spermatid (step 8 and beyond) interface was shown to be a significantly stronger testis-specific AJ compared with desmosome gap junctions at the Sertoli cell–spermatid (pre-step 8) or the Sertoli cell–spermatocyte interface when the adhesive force between these junctions was quantified by using a micropipette pressure transducing system [12]. Interestingly, although the adhesive force that was required to detach post-step 8 spermatids from Sertoli cells (i.e. disrupting the apical ES, 8.82×10^{-7} pN) was almost twice as much as that needed to detach pre-step 8 spermatids from Sertoli cells (i.e. desmosome gap junctions, 4.73×10^{-7} pN) [12], the apical ES undergoes extensive restructuring from step 8 to 19 spermatids to coincide with changes in shape and location which result from their morphological transformation and movement across the seminiferous epithelium during the epithelial cycle of spermatogenesis (Figures 1 and 2). Studies have shown that this rapid restructuring of the apical ES during spermatogenesis is mediated by the differential effects of the actin bundling/barbed end capping protein Esp8 [21,22,58] and the actin nucleation/branching Arp2/3–N-WASP–Cdc42 protein complex [26,59–62] via their highly regulated temporal and spatial expression patterns [29,30]. This is discussed in the next section, and a hypothetical model based on these findings is depicted in Figure 5.

An Emerging Concept of Actin Regulation which Mediates Apical ES Restructuring to Facilitate Spermatid Movement Across the Seminiferous Epithelium During Spermiogenesis

Recent studies have shown the expression of Eps8 and Arp3 to be stage-specific during the seminiferous epithelial cycle of spermatogenesis, and these proteins localized predominantly at the apical and basal ES [29,30]. For instance, it was shown that Eps8, an actin-bundling protein, localized largely to the apical ES at stages V–VII of the epithelial cycle, co-localizing with F-actin (filamentous actin) and coinciding with the time when actin filament bundles were needed to maintain the integrity of the apical ES [30]. However, at stage VIII, just prior to spermiation, the apical ES degenerates via internalization of adhesion proteins to give rise to an ultrastructure visible by fluorescence or electron microscopy as the tubulobulbar complex [39,63]. The tubulobulbar complex seems to resemble a giant clathrin-based endocytic vesicle [39,63] and is typified by invaginations of Sertoli cell membranous structures [63,64], and during this time the presence of Eps8 diminished to a virtually undetectable level when examined by immunohistochemistry or dual-labelled immunofluorescence analysis [30]. On the other hand, Arp3, an actin nucleation/branching protein, also localized predominantly at the apical ES at stage V–VII, co-localizing with F-actin as well, and likewise its level became virtually undetectable at stage VIII when tubulobulbar complexes were present at the Sertoli cell–elongated spermatid interface [29]. These findings coupled with studies using inhibitors or specific siRNA (small interfering RNA) duplexes which blocked the function of Arp3 [29] or Eps8 [30] respectively, have prompted us to hypothesize that their unique and highly restricted temporal and spatial

expression/localization patterns at the apical ES at stages V–VII of the epithelial cycle facilitate the rapid de-bundling, bundling and branching of actin filaments to allow the transit of elongating/elongated spermatids across the seminiferous epithelium during spermiogenesis (Figure 5). However, during spermiation, when there is virtually no apical ES restructuring, the expression of Eps8 and Arp3 is barely detectable (e.g. at early stage VIII just prior to spermiation). It is possible that during this time, components of the endocytosed adhesion protein complexes can be ‘recycled’ via transcytosis to the ‘newly’ developed elongating spermatids via spermiogenesis for the assembly of the ‘new’ apical ES (Figure 5). In short, this novel mechanism allows rapid restructuring of the apical ES and efficient utilization of apical ES proteins via ‘transcytosis’ and ‘recycling’ (Figure 5).

The model depicted in Figure 5 is further supported by studies using an *in vivo* model involving the administration of adjuvin [1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carbohydrazide]. When adult rats are treated with adjuvin, disruption of the apical ES was preferentially induced prior to the disruption of desmosome-like and gap junctions between Sertoli cells and pre-step 8 spermatids or spermatocytes [65]. Subsequent *in vitro* studies using the micropipette pressure transducing system indeed confirmed that the apical ES is more susceptible to adjuvin treatment compared with desmosome-like junctions at the Sertoli cell/germ cell interface since it took significantly less ‘force’ to pull post-step 8 spermatids apart from Sertoli cells compared with pre-step 8 spermatids following treatment of Sertoli/germ cell cocultures with adjuvin [13]. A significant loss of Eps8 at the apical ES was detected within 1 h in rats treated with adjuvin, and by 24 h virtually no immunoreactive Eps8 was found at the apical ES [30]. Although Arp3 did not diminish significantly at the apical ES by 24 h post-treatment, immunoreactive Arp3 at the apical ES became disorganized and mis-localized, moving from the concave to the convex side of the spermatid head, possibly to induce ‘premature’ actin branching before spermiation [29]. Collectively, these findings illustrate that adjuvin induces actin branching at the apical ES via changes in the localization of Arp3. This is concomitant with the de-bundling of actin filaments at the apical ES because a loss of Eps8 at this site induced spermatid ‘release’, in a sense mimicking the normal physiological ‘spermiation’ that takes place at late stage VIII of the epithelial cycle, except that it occurred prematurely in response to adjuvin treatment (Figure 5).

Nevertheless, additional research is needed to further confirm the hypothesis depicted in Figure 5. For instance, it remains to be determined biochemically whether protein endocytosis, recycling and transcytosis indeed occur at the apical ES, analogous to proteins at the basal ES and TJ at the BTB [66,67].

Actin Dynamics at the Btb

In virtually all cell epithelia, with the notable exception of the seminiferous epithelium, TJs (zonula occludens) reside at the apical region of cells, followed by AJs (zonula adherens) and desmosomes (macula adherens), which collectively create the junctional complex (for reviews, see [68,69]). Gap junctions usually lie behind the junctional complex, and then hemidesmosomes and focal contacts are found at the cell–matrix interface [68]. It is because of this morphological intimacy between TJs and AJs that a damage to the former, such as

that induced by xenobiotics or toxicants, can lead to a disruption of the latter, and vice versa, and an eventual dissolution of the junctional complex (for reviews, see [68,69]). However, in the testis, a well-defined junctional complex does not even exist since TJs lie closest to the basement membrane (a modified form of the extracellular matrix [70]), and they coexist with AJs and desmosomes, as well as with gap junctions to constitute the BTB, such that each junctional type (e.g. TJ) is not segregated from the others (e.g. basal ES) (for reviews, see [8,11,14]) (Figure 2). The BTB also physically divides the seminiferous epithelium into the basal and the apical compartment with post-meiotic germ cell development, namely spermiogenesis and spermiation, taking place in the apical compartment behind the BTB (Figure 1). BTB integrity, however, cannot be compromised, even transiently, during spermiogenesis to avoid the production of anti-sperm antibodies, since numerous specific autoantigens arise in spermatids during spermiogenesis and spermiation, so that the BTB confers immune privilege to the testis [47].

During spermiogenesis, extensive restructuring of the apical ES occurs as discussed above. Thus why doesn't restructuring of the apical ES during the transit of spermatids across the epithelium induce disruption to the BTB? Recent studies on the highly restricted temporal and spatial expression of Eps8 and Arp2 have shed light on a novel mechanism (Figure 5) [29,30]. For instance, the expression of Eps8 at the BTB is high at all stages of the epithelial cycle to maintain tightly packed actin filament bundles that constitute basal ES function (Figure 5), except at stage VIII when the BTB undergoes extensive restructuring to facilitate the transit of preleptotene spermatocytes [30]. This is also the stage when Eps8 expression at the BTB considerably diminishes [30] to induce de-bundling of actin filaments, which facilitates 'breakdown' of the basal ES and the BTB. Interestingly, the expression of Arp3 is also induced considerably at the BTB at stage VIII of the epithelial cycle. Thus this allows actin branching, increasing the 'fluidity' and 'plasticity' of the BTB. This concomitant decline in Eps8 [30] and surge in Arp3 at the BTB [29] at stage VIII of the epithelial cycle contributes to a state where the basal ES is disrupted by reducing the 'rigid' actin network at the BTB. This then facilitates the transit of preleptotene spermatocytes at the site (Figure 5). Due to the tightly regulated temporal and spatial expression of these two actin regulators, apical or basal ES restructuring that takes place in the apical compartment or BTB respectively, will not 'provoke' a disruption of the other junction, in a way segregating restructuring at opposite ends of the Sertoli cell epithelium during the seminiferous epithelial cycle of spermatogenesis (Figure 5).

Concluding Remarks and Future Perspectives

In the present review, we have proposed a novel mechanism that is based on the restricted temporal and spatial expression of two actin regulators, Eps 8 (an actin-bundling-inducing protein) and Arp3 (an actin nucleation/branching inducing protein) (Figure 5). Changes in the 'plasticity' of the actin filament network at the apical and basal ES as a result of actin filament bundling/debundling and actin branching allow timely disruption of the ES throughout the seminiferous epithelial cycle. This also allows the segregation of restructuring events that occur at the apical and basal ES so that spermatid movement during spermiogenesis does not compromise the integrity of the BTB. However, much more work is needed to fine-tune the hypothesis depicted in Figure 5. For instance, what are the

downstream and upstream signalling events involving Eps8 or Arp3? What are the roles of other actin regulatory proteins, such as nebulin [71], WASP [72], motor proteins (e.g. myosin) [1], GTPases (e.g. Cdc42) [1,48], formins [25,26,73,74], profilin [75–77], the cofilin–coronin–Aip1 protein complex [78] and phospholipids [79] in Eps8- and Arp3-mediated events or other pertinent events in the seminiferous epithelium [26,59]? How do actin dynamics contribute to the events of protein endocytosis, recycling and transcytosis that occur during the epithelial cycle in particular at the apical ES during spermiogenesis? Do they work similarly as found in other epithelia [80–83]? Some of these questions should be carefully tackled by functional experiments in the years to come.

Acknowledgments

Funding: Studies from this laboratory were supported by grants from the National Institutes of Health [grant numbers NICHD R01 HD056034 and R01 HD056034-02S1 (to C.Y.C.); U54 HD029990 Project 5 (to C.Y.C.); and R03 HD061401 (to D.D.M.)].

References

1. Parsons JT, Horwitz AR, Schwartz MA. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat Rev Mol Cell Biol.* 2010; 11:633–643. [PubMed: 20729930]
2. Oda T, Maeda Y. Multiple conformations of F-actin. *Structure.* 2010; 18:761–767. [PubMed: 20637412]
3. Harris TJC, Tepass U. Adherens junctions: from molecules to morphogenesis. *Nat Rev Mol Cell Biol.* 2010; 11:502–514. [PubMed: 20571587]
4. Green KJ, Getsios S, Troyanovsky S, Godsel LM. Intercellular junction assembly, dynamics, and homeostasis. *Cold Spring Harb Perspect Biol.* 2010; 2:a000125. [PubMed: 20182611]
5. Kueh HY, Mitchison TJ. Structural plasticity in actin and tubulin polymer dynamics. *Science.* 2009; 325:960–963. [PubMed: 19696342]
6. Etienne-Manneville S. From signaling pathways to microtubule dynamics: the key players. *Curr Opin Cell Biol.* 2010; 22:104–111. [PubMed: 20031384]
7. Wong EWP, Mruk DD, Cheng CY. Biology and regulation of ectoplasmic specialization, an atypical adherens junction type, in the testis. *Biochem Biophys Acta.* 2008; 1778:692–708. [PubMed: 18068662]
8. Yan HHN, Mruk DD, Lee WM, Cheng CY. Ectoplasmic specialization: a friend or a foe of spermatogenesis? *BioEssays.* 2007; 29:36–48. [PubMed: 17187371]
9. Mruk DD, Cheng CY. Cell-cell interactions at the ectoplasmic specialization in the testis. *Trends Endocrinol Metab.* 2004; 15:439–447. [PubMed: 15519891]
10. Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocr Rev.* 2004; 25:747–806. [PubMed: 15466940]
11. Vogl, A.; Vaid, K.; Guttman, J. The Sertoli cell cytoskeleton. In: Cheng, CY., editor. *Molecular Mechanisms in Spermatogenesis.* Landes Bioscience/Springer Science+Business Media, LLC; Austin, TX: 2008. p. 186–211.
12. Wolski KM, Perrault C, Tran-Son-Tay R, Cameron DF. Strength measurement of the Sertoli-spermatid junctional complex. *J Androl.* 2005; 26:354–359. [PubMed: 15867003]
13. Wolski KM, Mruk DD, Cameron DF. The Sertoli-spermatid junctional complex adhesion strength is affected *in vitro* by adjuvin. *J Androl.* 2006; 27:790–794. [PubMed: 16809272]
14. Cheng CY, Mruk DD. A local autocrine axis in the testes that regulates spermatogenesis. *Nat Rev Endocrinol.* 2010; 6:380–395. [PubMed: 20571538]
15. Cheng CY, Mruk DD. An intracellular trafficking pathway in the seminiferous epithelium regulating spermatogenesis: a biochemical and molecular perspective. *Crit Rev Biochem Mol Biol.* 2009; 44:245–263. [PubMed: 19622063]

16. Mruk DD, Zhu LJ, Silvestrini B, Lee WM, Cheng CY. Interactions of proteases and protease inhibitors in Sertoli-germ cell cocultures preceding the formation of specialized Sertoli-germ cell junctions *in vitro*. *J Androl*. 1997; 18:612–622. [PubMed: 9432134]
17. Mruk DD, Siu MKY, Conway AM, Lee NPY, Lau ASN, Cheng CY. Role of tissue inhibitor of metalloproteases-1 in junction dynamics in the testis. *J Androl*. 2003; 24:510–523. [PubMed: 12826691]
18. Di Fiore PP, Scita G. Eps8 in the midst of GTPases. *Int J Biochem Cell Biol*. 2002; 34:1178–1183. [PubMed: 12127568]
19. Ahmed S, Goh WI, Bu W. I-BAR domains, IRSp53 and filopodium formation. *Semin Cell Dev Biol*. 2010; 21:350–356. [PubMed: 19913105]
20. Lie PPY, Mruk DD, Lee WM, Cheng CY. Cytoskeletal dynamics and spermatogenesis. *Philos Trans R Soc Lond B Biol Sci*. 2010; 365:1581–1592. [PubMed: 20403871]
21. Disanza A, Mantoani S, Hertzog M, Gerboth S, Frittoli E, Steffen A, Herhoerster K, Kreienkamp H, Milanese F, Di Fiore PP, et al. Regulation of cell shape by Cdc42 is mediated by the synergic actin-bundling activity of Eps8-IRSp53 complex. *Nat Cell Biol*. 2006; 8:1337–1347. [PubMed: 17115031]
22. Disanza A, Carlier MF, Stradal TEB, Didry D, Frittoli E, Confalonieri S, Croce A, Wehland J, Di Fiore PP, Scita G. Eps8 controls actin-based motility by capping the barbed ends of actin filaments. *Nat Cell Biol*. 2004; 6:1180–1188. [PubMed: 15558031]
23. Scita G, Tenca P, Areces LB, Toccheti A, Frittoli E, Giardina G, Ponzanelli I, Sini P, Innocenti M, Di Fiore PP. An effector region in Eps8 is responsible for the activation of the Rac-specific GEF activity of Sos-1 and for the proper localization of the Rac-based actin-polymerizing machine. *J Cell Biol*. 2001; 154:1031–1044. [PubMed: 11524436]
24. Dos Remedios CG, Chhabra D, Kekic M, Dedova IV, Tsubakihara M, Berry DA, Nosworthy NJ. Actin binding proteins: regulation of cytoskeletal microfilaments. *Physiol Rev*. 2003; 83:433–473. [PubMed: 12663865]
25. Install RH, Machesky LM. Actin dynamics at the leading edge: from simple machinery to complex networks. *Dev Cell*. 2009; 17:310–322. [PubMed: 19758556]
26. Campellone KG, Welch MD. A nucleator arms race: cellular control of actin assembly. *Nat Rev Mol Cell Biol*. 2010; 11:237–251. [PubMed: 20237478]
27. Goley ED, Welch MD. The ARP2/3 complex: an actin nucleator comes of age. *Nat Rev Mol Cell Biol*. 2006; 7:713–726. [PubMed: 16990851]
28. Weaver AM, Heuser JE, Karginov AV, Lee W, Parsons JT, Cooper JA. Interaction of cortactin and N-WASP with Arp2/3 complex. *Curr Biol*. 2002; 12:1270–1278. [PubMed: 12176354]
29. Lie PPY, Chan AYN, Mruk DD, Lee WM, Cheng CY. Restricted Arp3 expression in the testis prevents blood-testis barrier disruption during junction restructuring at spermatogenesis. *Proc Natl Acad Sci USA*. 2010; 107:11411–11416. [PubMed: 20534520]
30. Lie PPY, Mruk DD, Lee WM, Cheng CY. 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*. 2009; 23:2555–2567. [PubMed: 19293393]
31. Lie PPY, Cheng CY, Mruk DD. The biology of the desmosome-like junction: a versatile anchoring junction and signal transducer in the seminiferous epithelium. *Int Rev Cell Mol Biol*. 2011; 286:223–269. [PubMed: 21199783]
32. Hess, RA.; de Franca, LR. Spermatogenesis and cycle of the seminiferous epithelium. In: Cheng, CY., editor. *Molecular Mechanisms in Spermatogenesis*. Landes Bioscience/Springer Science +Business Media, LLC; Austin, TX: 2008. p. 1-15.
33. de Kretser, D.; Kerr, J. The cytology of the testis. In: Knobil, E.; Neill, J.; Ewing, L.; Greenwald, G.; Markert, C.; Pfaff, D., editors. *The Physiology of Reproduction*. Vol. 1. Raven Press; New York: 1988. p. 837-932.
34. Ruwanpura SM, McLachlan RI, Meachem SJ. Hormonal regulation of male germ cell development. *J Endocrinol*. 2010; 205:117–131. [PubMed: 20144980]
35. Rajender S, Rahul P, Mahdi AA. Mitochondria, spermatogenesis and male infertility. *Mitochondrion*. 2010; 10:419–428. [PubMed: 20595008]

36. Toshimori K. Dynamics of the mammalian sperm head: modifications and maturation events from spermatogenesis to egg activation. *Adv Anat Embryol Cell Biol.* 2009; 204:5–94. [PubMed: 19552107]
37. LeBlond C, Clermont Y. Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann NY Acad Sci.* 1952; 55:548–573. [PubMed: 13139144]
38. Clermont Y. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. *Physiol Rev.* 1972; 52:198–235. [PubMed: 4621362]
39. O'Donnell L, Nicholls PK, O'Bryan MK, McLachlan RI, Stanton PG. Spermiation: the process of sperm release. *Spermatogenesis.* 2011;10.4161/spmg.1.1.14525
40. Kierszenbaum AL, Rivkin E, Tres LL. Molecular biology of sperm head shaping. *Soc Reprod Fertil Suppl.* 2007; 65:33–43. [PubMed: 17644953]
41. Kierszenbaum AL. Mammalian spermatogenesis *in vivo* and *in vitro*: a partnership of spermatogenic and somatic cell lineages. *Endocr Rev.* 1994; 15:116–134. [PubMed: 8156936]
42. Caballero OL, Chen YT. Cancer/testis (CT) antigens: potential targets for immunotherapy. *Cancer Sci.* 2010; 100:2014–2021. [PubMed: 19719775]
43. Simpson AJG, Caballero OL, Jungbluth A, Chen YT, Old LJ. Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer.* 2005; 5:615–625. [PubMed: 16034368]
44. Suri A. Family of sperm associated antigens: relevance in sperm-egg interaction and immunocontraception. *Soc Reprod Fertil Suppl.* 2007; 63:445–453. [PubMed: 17566290]
45. Bergmann M, Dierichs R. Postnatal formation of the blood-testis barrier in the rat with special reference to the initiation of meiosis. *Anat Embryol.* 1983; 168:269–275. [PubMed: 6660566]
46. Furuya S, Kumamoto Y, Sugiyama S. Fine structure and development of Sertoli junctions in human testis. *Arch Androl.* 1978; 1:211–219. [PubMed: 736673]
47. Meinhardt A, Hedger MP. Immunological, paracrine and endocrine aspects of testicular immune privilege. *Mol Cell Endocrinol.* 2011; 335:60–68. [PubMed: 20363290]
48. Wong EWP, Cheng CY. Polarity proteins and cell-cell interactions in the testis. *Int Rev Cell Mol Biol.* 2009; 278:309–353. [PubMed: 19815182]
49. Cheng CY, Mruk DD. The biology of spermatogenesis: the past, present and future. *Philos Trans R Soc Lond B Biol Sci.* 2010; 365:1459–1463. [PubMed: 20403863]
50. Cheng CY, Wong EWP, Yan HHN, Mruk DD. Regulation of spermatogenesis in the microenvironment of the seminiferous epithelium: new insights and advances. *Mol Cell Endocrinol.* 2010; 315:49–56. [PubMed: 19682538]
51. Russell LD, Peterson RN. Sertoli cell junctions: morphological and functional correlates. *Int Rev Cytol.* 1985; 94:177–211. [PubMed: 3894273]
52. Russell LD, Clermont Y. Anchoring device between Sertoli cells and late spermatids in rat seminiferous tubules. *Anat Rec.* 1976; 185:259–278. [PubMed: 937734]
53. Golachowska MR, Hoekstra D, van Ijzendoorn SC. Recycling endosomes in apical plasma membrane domain formation and epithelial cell polarity. *Trends Cell Biol.* 2010; 20:618–625. [PubMed: 20833047]
54. Assemat E, Bazellieres E, Pallesi-Pocachard E, Le Bivic A, Massey-Harroche D. Polarity complex proteins. *Biochim Biophys Acta.* 2008; 1778:614–630. [PubMed: 18005931]
55. Iden S, Collard JG. Crosstalk between small GTPases and polarity proteins in cell polarization. *Nat Rev Mol Cell Biol.* 2008; 9:846–859. [PubMed: 18946474]
56. Wong EWP, Mruk DD, Lee WM, Cheng CY. Par3/Par6 polarity complex coordinates apical ectoplasmic specialization and blood-testis barrier restructuring during spermatogenesis. *Proc Natl Acad Sci USA.* 2008; 105:9657–9662. [PubMed: 18621709]
57. Wong EWP, Sun S, Li MWM, Lee WM, Cheng CY. 14-3-3 protein regulates cell adhesion in the seminiferous epithelium of rat testes. *Endocrinology.* 2009; 150:4713–4723. [PubMed: 19608648]
58. Hertzog M, Milanese F, Hazelwood L, Disanza A, Liu H, Perlade E, Malabarba MG, Pasqualato S, Maiolica A, Confalonieri S, et al. Molecular basis for the dual function of Eps8 on actin dynamics: bundling and capping. *PLoS Biol.* 2010; 8:e1000387. [PubMed: 20532239]
59. Firat-Karalar EN, Welch MD. New mechanisms and functions of actin nucleation. *Curr Opin Cell Biol.* 2010; 23:1–10. [PubMed: 21190823]

60. Harris KP, Tepass U. Cdc42 and vesicle trafficking in polarized cells. *Traffic*. 2010; 11:1272–1279. [PubMed: 20633244]
61. Derivery E, Gautreau A. Generation of branched actin networks: assembly and regulation of the N-WASP and WAVE molecular machines. *BioEssays*. 2010; 32:119–131. [PubMed: 20091750]
62. Kurisu S, Takenawa T. The WASP and WAVE family proteins. *Genome Biol*. 2009; 10:226. [PubMed: 19589182]
63. Young JS, Guttman JA, Vaid KS, Vogl AW. Tubulobulbar complexes are intercellular podosome-like structures that internalize intact intercellular junctions during epithelial remodeling events in the rat testis. *Biol Reprod*. 2009; 80:162–174. [PubMed: 18799754]
64. Russell LD. Further observations on tubulobulbar complexes formed by late spermatids and Sertoli cells in the rat testis. *Anat Rec*. 1979; 194:213–232. [PubMed: 464323]
65. Chen YM, Lee NPY, Mruk DD, Lee WM, Cheng CY. Fer kinase/Fer T and adherens junction dynamics in the testis: an *in vitro* and *in vivo* study. *Biol Reprod*. 2003; 69:656–672. [PubMed: 12700184]
66. Yan HHN, Mruk DD, Lee WM, Cheng CY. 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*. 2008; 22:1945–1959. [PubMed: 18192323]
67. Xia W, Wong EWP, Mruk DD, Cheng CY. TGF- β and TNF α 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*. 2009; 327:48–61. [PubMed: 19103189]
68. Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*. Garland Science; New York: 2002.
69. Wong CH, Cheng CY. The blood-testis barrier: its biology, regulation and physiological role in spermatogenesis. *Curr Topics Dev Biol*. 2005; 71:263–296.
70. Dym M. Basement membrane regulation of Sertoli cells. *Endocr Rev*. 1994; 15:102–115. [PubMed: 8156935]
71. Pappas CT, Bliss KT, Zieseniss A, Gregorio CC. The nebulin family: an actin support group. *Trends Cell Biol*. 2011; 21:29–37. [PubMed: 20951588]
72. Padrick SB, Rosen MK. Physical mechanisms of signal integration by WASP family proteins. *Annu Rev Biochem*. 2010; 79:707–735. [PubMed: 20533885]
73. Schonichen A, Geyer M. Fifteen formins for an actin filament: a molecular view on the regulation of human formins. *Biochem Biophys Acta*. 2010; 1803:152–163. [PubMed: 20102729]
74. Paul AS, Pollard TD. Review of the mechanism of processive actin filament elongation by formins. *Cell Motil Cytoskeleton*. 2009; 66:606–617. [PubMed: 19459187]
75. Bugyi B, Carlier MF. Control of actin filament treadmilling in cell motility. *Annu Rev Biophys*. 2010; 39:449–470. [PubMed: 20192778]
76. Dominguez R. Structural insights into de novo actin polymerization. *Curr Opin Struct Biol*. 2010; 20:217–225. [PubMed: 20096561]
77. Yarmola EG, Bubb MR. How depolymerization can promote polymerization: the case of actin and profilin. *BioEssays*. 2009; 31:1150–1160. [PubMed: 19795407]
78. Lin MC, Galletta BJ, Sept D, Cooper JA. Overlapping and distinct functions for cofilin, coronin and Aip1 in actin dynamics *in vivo*. *J Cell Sci*. 2010; 123:1329–1342. [PubMed: 20332110]
79. Saarikangas J, Zhao H, Lappalainen P. Regulation of the actin cytoskeleton-plasma membrane interplay by phosphoinositides. *Physiol Rev*. 2010; 90:259–289. [PubMed: 20086078]
80. Romer W, Ontani LL, Sorre B, Rentero C, Berland L, Chambon V, Lamaze C, Bassereau P, Sykes C, Gaus K, Johannes L. Actin dynamics drive membrane reorganization and scission in clathrin-independent endocytosis. *Cell*. 2010; 140:540–553. [PubMed: 20178746]
81. Galletta BJ, Mooren OL, Cooper JA. Actin dynamics and endocytosis in yeast and mammals. *Curr Opin Biotechnol*. 2010; 21:604–610. [PubMed: 20637595]
82. Conibear E. Converging views of endocytosis in yeast and mammals. *Curr Opin Cell Biol*. 2010; 22:513–518. [PubMed: 20538447]

83. Lajoie P, Nabi IR. Lipid rafts, caveolae, and their endocytosis. *Int Rev Cell Mol Biol*. 2010; 282:135–163. [PubMed: 20630468]
84. Lee NPY, Cheng CY. Adaptors, junction dynamics, and spermatogenesis. *Biol Reprod*. 2004; 71:392–404. [PubMed: 15056568]
85. Mruk DD, Silvestrini B, Cheng CY. Anchoring junctions as drug targets: role in contraceptive development. *Pharmacol Rev*. 2008; 60:146–180. [PubMed: 18483144]
86. Stokes DL. Desmosomes from a structural perspective. *Curr Opin Cell Biol*. 2007; 19:565–571. [PubMed: 17945476]
87. Johnson K, Boekelheide K. Dynamic testicular adhesion junctions are immunologically unique II Localization of classic cadherins in rat testis. *Biol Reprod*. 2002; 66:992–1000. [PubMed: 11906918]
88. Li MWM, Mruk DD, Cheng CY. Mitogen-activated protein kinases in male reproductive function. *Trends Mol Med*. 2009; 15:159–168. [PubMed: 19303360]
89. Cheng CY, Wong EWP, Lie PPY, Li MWM, Su L, Siu ER, Yan HHN, Jayakanthan M, Mathur PP, Bonanomi M, et al. Environmental toxicants and male reproductive function. *Spermatogenesis*. 2011; 10.4161/spmg.1.1.13971
90. Wong EWP, Mruk DD, Lee WM, Cheng CY. Regulation of blood-testis barrier dynamics by TGF- β is a Cdc42-dependent protein trafficking event. *Proc Natl Acad Sci USA*. 2010; 107:11399–11404. [PubMed: 20534521]

Abbreviations

adjudin	1-(2,4-dichlorobenzyl)- <i>1H</i> -indazole-3-carbohydrazide
AJ	adherens junction
Arp	actin-related protein
ARPC	Arp2/3 complex subunit
BTB	blood-testis barrier
Cdc42	cell division cycle 42
Eps8	epidermal growth factor receptor pathway substrate 8
ES	ectoplasmic specialization
F-actin	filamentous actin
PAR	partitioning defective protein
N-WASP	neuronal WASP
SCAR/WAVE	suppressor of cAMP receptor/WASP family verprolin homologous
TJ	tight junction
WASP	Wiskott-Aldrich syndrome protein

Figure 1. A schematic drawing illustrating the relative location of apical ES, basal ES at the BTB, gap junction and desmosome-like junction in the seminiferous epithelium of adult mammalian testes

It is noted that the BTB is constituted by coexisting TJ, apical ES, desmosome-like junction and gap junction (see text). Apical ES and basal ES are an actin-based testis-specific AJ type restricted to the Sertoli cell–spermatid (step 8–19 spermatid) interface and Sertoli–Sertoli interface respectively, typified by the actin filament bundles sandwiched in between cisternae of ES and the apposing plasma membranes of Sertoli–spermatid or Sertoli–Sertoli cell (see Figure 2). Gap junction (actin-based cell–cell junction) and desmosome-like junction (intermediate filament-based cell–cell junction) are found at the Sertoli–Sertoli, Sertoli–spermatogonium, Sertoli–spermatocyte and Sertoli–pre-step 8 spermatid interface.

Integrated membrane proteins at the ES, gap junction, TJ and desmosome-like junction are linked to the actin network (for ES, gap junction and TJ) or intermediate filaments (for desmosome-like junction and hemidesmosome) via adaptors [31,84,85].

Figure 2. Anatomical features of the ES in the testis

(A–C) Corresponding cross-sections of seminiferous tubules at stages VI, VIII and XIV of the seminiferous epithelial cycle in the adult rat testis, illustrating the intimate relationship between germ cells at different stages of their development with Sertoli cells in the seminiferous epithelium. (A) Developing step 18 spermatids in a stage VI tubule are embedded deep within the seminiferous epithelium and anchored to Sertoli cells by the apical ES (see circled area) only. The BTB created by coexisting TJs, basal ES, desmosome-like and gap junctions of adjacent Sertoli cells near the basement membrane (see boxed area) divides the epithelium into the apical and the basal compartments, which remains ‘closed’ at this stage. However, step 19 spermatids aligned at the adluminal edge of the tubule lumen in preparation for spermiogenesis in a stage VIII tubule (B) when the BTB undergoes extensive restructuring (‘open’) to facilitate the transit of preleptotene spermatocytes from the basal to the apical compartment. Thereafter, all step 19 spermatids

transformed to sperm are released into the tubule lumen at late stage VIII and round spermatids transform to step 14 spermatids at stage XIV shown in (C), and late spermatocytes undergo meiosis at stage XIV of the epithelial cycle (C) to give rise to spermatids. Spermatocytes undergoing meiosis are clearly visible and marked with a red asterisk illustrating late anaphase. Es, elongating spermatid; Rs, round spermatid; Sg, spermatogonium; Pm, peritubular myoid cell; SC, Sertoli cell. (D) The apical ES shown in the circled region in (A–C) is magnified in this electron micrograph, illustrating the ultrastructural features of an apical ES, which is typified by the presence of actin filament bundles (white arrowheads) sandwiched in between cisternae of endoplasmic reticulum (ER) and the apposing plasma membrane of the Sertoli cell (green arrowhead) and the elongating spermatid (red arrowhead), and these typical ultrastructural features are restricted only to the Sertoli cell side at the apical ES. Chromatin is condensed and packed into the spermatid head as the nucleus. The developing acrosome (Ac) capping part of the nucleus is also visible. (E) The basal ES at the BTB as shown in the boxed region in (A–C) is magnified in this electron micrograph. The basal ES is ultrastructurally identical with the apical ES except that its typical feature, namely the actin filament bundles (black arrowheads) that are sandwiched in between cisternae of ER and the apposing plasma membranes of two adjacent Sertoli cells (apposing green arrowheads) are found within both Sertoli cells. The basal ES coexists with TJs as illustrated by yellow arrowheads illustrating the ‘kisses’ between apposing Sertoli cell plasma membranes. It is noted that the BTB is found near the basement membrane. Scale bars in (A–C), (D) and (E) are 20 μm , 0.2 μm and 0.5 μm respectively.

Figure 3. Different effects of Eps8 on the actin-based cytoskeleton network in the seminiferous epithelium of the rat testis

Depending on its various binding partners, Eps8 can modulate the actin network via its interaction with different proteins. Abi-1, Abelson interacting protein-1; IRSp53, insulin receptor tyrosine kinase substrate p53; Sos1, son of sevenless 1.

Figure 4. Differential effects of Eps8 and the Arp2/3 protein complex on the actin-based cytoskeleton network

(A) Eps8 functions to stabilize actin filament bundles found at the apical and basal ES in the seminiferous epithelium. (B) The Arp2/3 protein complex is composed of 7 subunit proteins, namely Arp2, Arp3 and ARPC1–5, which must be activated by N-WASP and SCAR/WAVE N-WASP. This protein complex can induce nucleation (branching) of an existing actin filament, thereby de-stabilizing the apical and basal ES, which is necessary for the movement of developing spermatids and the transit of preleptotene spermatocytes across the BTB respectively.

Figure 5. A hypothetical model based on the highly restricted temporal and spatial expression of Eps8 and Arp3 at the apical and basal ES in the seminiferous epithelium, which affects the status of actin filament bundles at these sites to facilitate germ cell transit

In (A), elongating spermatids are anchored to the Sertoli cell in the seminiferous epithelium via apical ES as shown in the left-hand panel. Integral membrane proteins at the apical ES are contributed mostly by the integrin–laminin (e.g. $\alpha 6 \beta 1$ -integrin–laminin $\alpha 3 \beta 3 \gamma 3$) complex, nectin–afadin and N-cadherin– β -catenin [8]. It is worth noting that these apical ES proteins are actin-based adhesion protein complexes. Intracellularly, however, the intracellular tail of cadherin can associate with the Armadillo family protein plakoglobin

(also known as γ -catenin) and plakophilins, which bind to the desmosomal cytoskeletal adaptor protein desmoplakin [20,86]. Thus N-cadherin can be seen to co-localize with intermediate filaments by fluorescence microscopy [87]. The integrity of the apical ES is maintained by Eps8 with the proper orientation of the actin filament bundles. This actin network becomes disorganized, which is mediated by Arp2/3 protein complex during spermiogenesis to facilitate the movement of elongating spermatid across the epithelium (middle panel), and at stage VIII of the epithelial cycle, actin filament bundles are disrupted entirely to facilitate the release of spermatozoa at spermiation (left-hand panel). These changes are also facilitated by endocytosis of integral membrane proteins at the apical ES so that the internalized endocytic vesicles can be transcytosed and recycled to assemble the 'new' apical ES in recently formed step 8 spermatids during spermiogenesis. The 'loss' of integral membrane proteins at the apical ES via endocytosis thus further 'destabilizes' the apical ES. In **(B)**, left-hand panel, an intact BTB is illustrated, which is maintained by the restricted expression of Eps8 needed for the integrity of actin filament bundles. At the time of BTB restructuring at stage VIII of the epithelial cycle, the expression of Eps8 diminishes. This is replaced by the Arp2/3 protein complex, which destabilizes actin filament bundles via the formation of a branched actin network, facilitating the internalization of integral membrane proteins [e.g. occludin, connexin 43 (Cx43)] (right-hand panel). This event of protein endocytosis is aided by an increase in the phosphorylation of integral membrane proteins induced by activated FAK (focal adhesion kinase), activated c-Src and/or p38 MAPK (mitogen-activated protein kinase) [88,89]. Previous studies have also illustrated the involvement of polarity proteins: PAR6, PAR3 and 14-3-3 (also known as PAR5) and Cdc42, in endocytic vesicle-mediated protein trafficking events (e.g. protein endocytosis and recycling) at the Sertoli cell BTB [56,57,90]. Endocytosed proteins can be targeted for endosome-mediated degradation or be recycled and transcytosed to another site, further destabilizing the BTB. ZO-1 (zonula occludens-1) and PKP-2 (plakophilin-2) are the corresponding adaptor proteins of the TJ protein occludin and the gap junction (GJ) protein Cx43.