Germ Cell Transport Across the Seminiferous Epithelium During Spermatogenesis

Transport of germ cells across the seminiferous epithelium is crucial to spermatogenesis. Its disruption causes infertility. Signaling molecules, such as focal adhesion kinase, c-Yes, c-Src, and intercellular adhesion molecules 1 and 2, are involved in these events by regulating actin-based cytoskeleton via their action on actin-regulating proteins, endocytic vesicle-mediated protein trafficking, and adhesion protein complexes. We critically evaluate these findings and provide a hypothetical framework that regulates these events.

Spermatogenesis is a physiological process in which spermatogonia (diploid, 2n) transform into spermatooza (haploid, 1n) via mitosis, meiosis, and spermiogenesis, so that a sperm can fertilize an egg to generate an offspring, which is essential to the perpetuation of a species (FIGURE 1) (25, 69, 85). Spermatogenesis takes place in the epithelium of the seminiferous tubule, the functional unit of the testis that produces upward of 300 million sperms/day from each man after puberty, and sperm production continues well into the adulthood until death (2, 14, 17). The seminiferous epithelium is physically divided into two compartments, the basal and the adluminal (apical) compartments, by the blood-testis barrier (BTB), also known as the Sertoli cell epithelial barrier, which is created by specialized coexisting actin-based tight junction ( TJ), basal ectoplasmic specialization (ES), and gap junction (GJ), as well as intermediate filament-based desmosome (14, 29, 75). Although the gross morphological events (20, 47, 74) and the hormonal regulation of spermatogenesis, such as by testosterone released from Leydig cells in the interstitium (69, 85, 91) and also by estradiol-17β derived from Sertoli and germ cells in the seminiferous epithelium (10, 11, 71), are known, the molecular and cellular events underlying spermatogenesis remain poorly understood. FIGURE 1 is a schematic drawing of the cross section of a seminiferous tubule at stage VII of the epithelial cycle of spermatogenesis in the rat testis, illustrating that the seminiferous epithelium is comprised of only Sertoli and germ cells at different stages of their development (FIGURE 2). The Sertoli cell provides structural and nourishment supports to developing germ cells at a germ-to-Sertoli cell ratio of ~50:1 throughout the epithelial cycle (108), illustrating its pivotal role and intimate relationship with germ cells in spermatogenesis. In fact, there are testis-specific F-actin-rich junctions at the Sertoli-spermatid and Sertoli-Sertoli cell interface known as the apical and the basal ectoplasmic specialization (ES), respectively (FIGURE 1). In rat testes, Sertoli cells cease to divide by ~17 days postpartum (dpp) (72), and the number of Sertoli cells remains relatively constant thereafter throughout adulthood in both rodents and humans (86). For instance, the number of Sertoli cells per testis in adult mice (5), rats (8), and men (24) are 4, 30, and 1,850 million, respectively.

Cellular events that take place during spermatogenesis are cyclic in nature. The concept of the seminiferous epithelial cycle of spermatogenesis is based on studies using periodic acid-Schiff reaction (PAS) to visualize developing spermatids in seminiferous tubule (21, 22, 47) in which germ cells at different stages of their development are associated with Sertoli cells in the seminiferous epithelium cyclically, displaying a specific pattern of association. A typical seminiferous epithelial cycle of stages I–XIV in the rat testis is shown in FIGURE 2A, illustrating at each stage of the epithelial cycle that only unique types of germ cells are present and specific cellular events occur. For instance, preleptotene spermatocytes are only found at stages VII and VIII of the cycle, spermiation occurs at stage VIII, and meiosis only takes place at stage XIV of the epithelial cycle (FIGURE 2, A AND B). Also, the appearance of these stages are continuous along the seminiferous tubule and can be divided into I–XIV, I–XII, and I–VI in rats, mice, and humans, respectively, where each stage has a defined duration (1, 37, 64). For instance, stages VII and VIII have a duration of 56 and 29.1 h, respectively, and the entire cycle of I–XIV takes ~12.8 days to complete in the rat (3, 21). Earlier studies using [3H]thymidine incorporation have shown that it takes ~58 days for A1 spermatogonia to differentiate into step 19 spermatids, and each...
FIGURE 1. The biology of spermatogenesis

Left: a schematic drawing that illustrates the cross section of the seminiferous tubule, the functional unit in the testis that produces ~300–400 million spermatozoa per day in a man after puberty at ~12 yr of age, which persists throughout his entire life. Spermatogenesis takes place in the seminiferous epithelium, which is composed of only Sertoli and germ cells, located above the tunica propria. Spermatogonia that reside near the basement membrane derived from spermatogonial stem cells (SSC) enter spermatogenesis and differentiate into As (Asingle), to be followed by A1–A4 and intermediate (In) spermatogonia, until type B spermatogonia transform to preleptotene spermatocytes, which are the germ cells that must be transported across the blood-testis barrier (BTB) at stage VIII of the epithelial cycle. The BTB also divides the seminiferous epithelium into the adluminal and the basal compartment. Meiosis I and II, and all the cellular events of postmeiotic development known as spermiogenesis and spermiation, all take place in the adluminal compartment. During these processes, millions of spermatozoa are formed efficiently, and developing germ cells are also being transported progressively from the basal to the adluminal compartment, and finally to the edge of the seminiferous tubule lumen, so that mature spermatozoa can be released at spermiation to enter the seminiferous tubule lumen for their eventual maturation in the epididymis. ES, ectoplasmic specialization.
cycle takes 12.8 days to complete, such that A1 spermatogonia have to go through the epithelial cycle 4.5 times to become step 19 spermatids, as depicted in FIGURE 2B, in which spermatogonia progress through A1–A4 to be followed by intermediate and type B spermatogonia, meiosis, and spermiogenesis.

Spermatogonial stem cells, type A and type B spermatogonia, reside at the basal compartment of the seminiferous epithelium (FIGURE 1). Once type B spermatogonia differentiate to preleptotene spermatocytes at stage VII of the epithelial cycle, they are being transported across the BTB while transforming to leptotene spermatocytes, so that meiosis I/II and spermiogenesis all take place in the adluminal compartment, behind the BTB. Furthermore, spermatids are also transported across the epithelium during spermiogenesis (FIGURE 2A). In short, germ cells, including spermatocytes and spermatids, are being transported across the epithelium during the epithelial cycle, so that step 19 spermatids line up at the edge of the tubule lumen at stage VIII to prepare for their release at spermiation (17, 70). It is conceivable that there is extensive remodeling of junctions at the Sertoli cell-cell and Sertoli-germ cell interface during spermatogenesis, given the large number of spermatozoa that are produced each day in an

![Image of spermatogenesis stages](image-url)

FIGURE 2. The seminiferous epithelial cycle of spermatogenesis
A unique feature of spermatogenesis is the cyclic association of germ cells with the Sertoli cell (annotated by “red” arrowhead) in the seminiferous epithelium, such as in the rat testis as illustrated in A, in which stages I–XIV can be defined (note: only I–XII and I–VI stages are found in the mouse and human testis, respectively). Each stage of the cycle shown in A illustrates unique association of specific germ cells and the Sertoli cell in the seminiferous epithelium, and the different germ cell types that are found in each stage are shown in B. For instance, in stage VII of the epithelial cycle, which lasts for ~29.1 h in the rat, stage 19 spermatids line up near the tubule lumen to prepare for spermation. It is also noted that preleptotene spermatocytes that derive from type B spermatogonia that first appear in stage VII are being transported across the BTB in stages VII–VIII. Thus, in stage VIII, only type A1 spermatogonia, preleptotene spermatocytes, pachytene spermatocytes, and steps 8 and 19 spermatids are found. Also, meiosis only takes place in stage XIV in the rat testis. In short, the entire epithelial cycle from I to XIV takes ~12.9 days to complete. However, it takes a type A1 spermatogonium in stage II to become a step 19 spermatid in stage VIII through the epithelial cycle ~4.5 times, which lasts for ~58 days in the rat. A1, type A1 spermatogonium; A1m, type A1 spermatogonium undergoes mitosis; In, intermediate spermatogonium; Inm, intermediate spermatogonium undergoes mitosis; B, type B spermatogonium; Bm, type B spermatogonium undergoes mitosis; P, preleptotene spermatocyte; L, leptotene spermatocyte; Z, zygotene spermatocyte; P, pachytene spermatocyte; 1–19, step 1 to step 19 spermatids that are formed during spermiogenesis; ES, ectoplasmic specialization.
Germ Cell Transport During Spermatogenesis

Although germ cells are noted to move progressively from the base of the seminiferous epithelium toward the luminal edge during the epithelial cycle of spermatogenesis (FIGURE 2A), in contrast to fibroblasts, macrophages, neutrophils, and some other mammalian cells, they are not motile cells per se, lacking the ultrastructures of lamellipodia and filopodia to engage in motility, even though both Sertoli and germ cells express Cdc42, Rho, and Rac GTPases, as well the Arp2/3 protein complex and other cytoskeleton regulatory proteins necessary to elicit cell locomotion, as found in other mammalian cells (13, 18, 33, 97, 111). For instance, step 17 spermatids that are transported to the site near the luminal edge of the apical compartment in stage IV are being transported back to the base of the epithelium, near the basal compartment in stage V, before they are transported again back to the adluminal compartment and differentiate into step 18 spermatids in stage VI, until step 19 spermatids begin to line up near the tubule lumen at stage VII to prepare for their release at spermiation at stage VIII, when they are transformed to spermatozoa (FIGURE 2A). Furthermore, germ cells, in particular postmeiotic spermatids, are metabolically quiescent cells (with few mRNA and ribosomes but plenty of miRNAs), except that they undergo extensive morphological transformation including 1) condensation and packaging of the genetic materials in the spermatid head, 2) development of the acrosome over the head region, and 3) packaging of the mitochondria into the mid-piece and elongation of the tail. Thus the translocation of germ cells across the epithelium during spermatogenesis is not the result of germ cell movement per se; instead, these cells are being “transported” (FIGURE 3). On the other hand, although Sertoli cells are motile cells when cultured in vitro, capable of traversing pores in transwell inserts such as bicameral units (66), analogous to fibroblasts, macrophages, and metastatic cancer cells, Sertoli cells are not motile cells in the seminiferous epithelium in vivo. Instead, Sertoli cell nuclei are localized near the basement membrane, and specialized junctions between adjacent Sertoli cells create the BTB to provide an immunological barrier to sequester postmeiotic spermatid development from the systemic circulation, and this blood-tissue barrier remains functional through the epithelial cycle, and it is one of the tightest blood-tissue barriers in the mammalian body (14, 29, 75) (FIGURES 1 and 2). However, Sertoli cells extend their cytoplasmic processes well in the adluminal compartment so that multiple germ cells at different stages of their development are in close contact with the Sertoli cell to obtain structural, nutritional, paracrine, and hormonal supports (FIGURE 1). Thus it has been postulated that Sertoli cells are using the cytoskeletal elements wherein microtubules serve as the “tracks” to transport the “cargos,” i.e., germ cells, to their destinations during spermatogenesis (48, 82, 83, 97, 102). Although the precise mechanism(s) underlying this event remains to be fully elucidated, recent studies have identified multiple actin regulatory proteins and signaling molecules that are involved in germ cell transport. These data have shed insightful information in this process, which are critically evaluated herein.

Transport of Preleptotene Spermatocytes at the BTB

Preleptotene spermatocytes differentiated from type B spermatogonia first appear at stage VII and persist only through stage VIII of the cycle (see FIGURE 2B). Preleptotene spermatocytes connected in clones via intercellular bridges (28) (also called tunneling nanotubes) are the only germ cells that are being transported across the BTB at stages VII–VIII of the cycle (82). Studies based on morphological analysis have suggested that to maintain BTB integrity at stages VII–VIII, “new” BTB is being assembled behind preleptotene spermatocytes that are in transit at the BTB before “old” BTB above these cells is completely degenerated (FIGURE 4) (16, 17, 63, 81, 95). This hypothesis has recently been confirmed using confocal microscopy in which TJ-protein claudin-3 is used as a marker to track the transit of preleptotene spermatocytes across the BTB in the mouse testis (90). Furthermore, this concept is also supported by earlier studies illustrating that androgens (e.g., testosterone) promote BTB assembly (19, 39, 61, 107), whereas cytokines (e.g., TGF-β3, TNF-α) promote BTB disruption, suggesting the synergistic interactions of these two classes of molecules that have antagonistic effects on the BTB function can elicit the establishment of a “new” BTB behind the transiting preleptotene spermatocytes (induced by testosterone) before the “old” BTB is being disassembled (facilitated by cytokines). Indeed, this concept is supported by subsequent biochemical studies in which testosterone induces protein endocytosis, so that TJ proteins at the “old” BTB site can be transcytosed and recycled to the “new” BTB behind the preleptotene spermatocytes, whereas cytokines (e.g., TGF-β2) also facilitate endocytosis,
but the endocytosed TJ proteins are targeted to endosome-mediated degradation pathway (122). These findings are also supported by dual-labeled immunofluorescence analysis using specific markers of endocytosis, transcytosis, recycling, and endosome-mediated degradation (92). Furthermore, cytokine-mediated enhancement in the kinetics of protein endocytosis at the Sertoli cell BTB is a Cdc42-dependent cellular event, since overexpression of a dominant-negative mutant of Cdc42 was found to abolish the TGF-β3-induced acceleration in protein endocytosis (114). Since Cdc42 is an integrated component of the Par (partitioning-defective)-based polarity protein complex, namely

---

FIGURE 3. A schematic drawing that illustrates the concept of junction restructuring in the seminiferous epithelium in relation to spermatid transport during spermiogenesis and spermiation

For the transport of spermatids across the seminiferous epithelium during the epithelial cycle of spermatogenesis, apical ectoplasmic specialization (ES) is the anchoring junction responsible for this cellular event. Left: this represents the state of an intact apical ES at the Sertoli-spermatid interface, such as in a stage VII tubule. Apical ES first appears in step 8 spermatids; once it forms, it replaces desmosome and gap junction as the only anchoring device to confer spermatid adhesion and polarity, and it persists until step 19 spermatids in stage VII to early stage VIII until spermiation takes place in late stage VIII of the epithelial cycle. Spermatids anchor to the Sertoli cell at the apical ES using integral membrane proteins, such as ICAM-2, nectins (nectin 3 is spermatid-specific, but nectin 2 is found in both Sertoli cells and spermatids), and most notably αβ1-integrin/laminin-333 (integrin is Sertoli cell-specific, whereas laminin is spermatid-specific). Apical ES is maintained via a network of actin filament bundles that are sandwiched between cisternae of endoplasmic reticulum and the Sertoli cell plasma membrane. The actin filament bundles, in turn, are maintained by actin bundling proteins, such as Eps8 and palladin, and also signaling molecules p-FAK-Tyr407, p-FAK-Tyr397, c-Yes, and c-Src. In early stage VIII, the expression of actin bundling proteins (e.g., Eps8 and palladin) is downregulated, and the expression of actin branching protein, the Arp2/3 complex, is induced. This combined effect changes the F-actin from its “bundled” to “branched/un-bundled” configuration efficiently. Such reorganization of F-actin network thus destabilizes the adhesion protein complexes, and the nonreceptor protein tyrosine kinases (e.g., c-Yes and c-Src) also facilitate endocytic vesicle-mediated protein trafficking, such as endocytosis, transcytosis, and/or recycling. The recycling and/or endosome-mediated degradation of integral membrane proteins at the apical ES further destabilizes spermatid adhesion, facilitating the release of sperm at spermiation. Furthermore, the transcytosed and recycled apical ES proteins facilitate the assembly of “new” apical ES when step 8 spermatids appear in stage VIII tubules (see FIGURE 2). In short, this effectively re-organizes actin filament bundles at the apical ES via the intricate spatiotemporal expression of actin bundling proteins (e.g., Eps8, palladin) and actin branching/un-bundling proteins (e.g., the Arp2/3 complex) mediated by p-FAK-Tyr407 and p-FAK-Tyr397, thus rapidly converting actin filaments from “bundled” and “branched/un-bundled” configuration and vice versa. These changes coupled with endocytic vesicle-mediated trafficking events mediated by c-Yes and c-Src provide the means to facilitate spermatid transport across the seminiferous epithelium during spermatogenesis.
the Par6/3-Pals1/Patj/Cdc42 complex, at the BTB (111, 113), polarity proteins are likely involved in the transport of preleptotene spermatocytes at the BTB by maintaining the immunological barrier integrity. This possibility is further strengthened by the findings that a knockdown of 14-3-3 (also known as Par5) impedes Sertoli cell TJ permeability barrier function by accelerating the endocytosis of TJ-integral membrane proteins JAM-A and N-cadherin, thereby destabilizing the TJ function (115). It is obvious that cytoskeletons are also involved in these events since the transport of preleptotene spermatocytes requires the coordinated action of F-actin- and microtubule-based cytoskeletons. Although the precise mechanism(s) regarding the involvement of cytoskeletons in spermatocyte transport remains unknown, it has been speculated that the presence of actin-based motor proteins myosin VIIa (101), myosin Va (41), and MyRIP [motor recruiter myosin Va, VIIa-Rab27a/b interacting protein (35)], as well as microtubule-based motor proteins dynein (30) and kinesin [e.g., the testis-specific kinesin-2 family members 3A (KIF3A) and 3B (KIF3B) (38)] in the testes are crucial to this event (30, 42, 48, 98). For instance, it is likely that actin-based motor proteins facilitate the transport of preleptotene spermatocytes across the BTB by using the microtubule as the track, analogous to the transport of intracellular cargoes in other epithelia (6, 43, 45, 60).

The BTB is a unique blood-tissue barrier since it is composed of an extensive network of actin filament bundles that line perpendicular to the apposing plasma membranes of the adjacent Sertoli cells and they are found on both sides of these cells (FIGURE 1). This network of actin filament bundles

---

**FIGURE 4.** A schematic drawing that illustrates the concept of junction restructuring at the blood-testis barrier (BTB) in relation to preleptotene spermatocyte transport across the immunological barrier during spermatogenesis

Left: a preleptotene spermatocyte at stage VII of the epithelial cycle just arising from type B spermatogonium is located behind the intact BTB in a stage VII tubule. The BTB integrity is conferred by two arrays of actin filament bundles, which are also maintained by actin bundling proteins, such as Eps8 and palladin. BTB integrity is also conferred by adhesion protein complexes, such as occludin-ZO-1, N-cadherin-β-catenin, ICAM-1-ZO-1, as well as signaling proteins p-FAK-Tyr407, c-Yes, and c-Src. Similar to the apical ES, actin filament bundles at the basal ES are located between the cisternae of endoplasmic reticulum and the Sertoli cell plasma membrane. Middle: as described in the text, laminin fragments that are generated at the apical ES during spermiation via the action of MMP-2 on laminin chains possess biological activity to induce BTB restructuring, which is mediated by changes in the spatiotemporal expression of actin bundling proteins (e.g., Eps8, palladin), branched actin polymerization protein (e.g., the Arp2/3 complex which is activated by N-WASP to induce barbed end actin polymerization), and signaling proteins p-FAK-Tyr407, c-Yes, and c-Src. Also, soluble ICAM-1 (sICAM-1) is generated from ICAM-1 via the action of MMP-9, which promotes BTB restructuring. The net result of these changes induces reorganization of the F-actin network, causing actin filament bundles altered from a “bundled” to a “branched/unbundled” configuration, thereby destabilizing adhesive function of the TJ, basal ES, and gap junction, and also facilitating endocytic vesicle-mediated protein trafficking. Thus the “old” BTB above the preleptotene spermatocyte is disrupted. Right: at the same time the actin filament bundles are altered from a “bundled” to a “branched/unbundled” configuration, transcytosis and recycling have facilitated the assembly of a “new” BTB located at the basal region of the preleptotene spermatocyte. The preleptotene spermatocyte will be transformed into leptotene spermatocyte at stages IX–XI to be followed by zygotene spermatocyte at stages XII–XIII (FIGURE 2). Using such a mechanism, the immunological barrier can be maintained during the transport of preleptotene spermatocytes across the BTB at stage VII of the epithelial cycle. ES, ectoplasmic specialization.
thus confers the unusual adhesive strength to the Sertoli cells that constitute the BTB (14, 29, 102). Studies have shown that these actin filament bundles are actively maintained by actin barbed-end capping and bundling protein Eps8 (epidermal growth factor receptor pathway substrate 8) (55), and actin cross-linking and bundling protein palladin (80). However, the actin filament bundles at the BTB must be rapidly “de bundled” and also “rebundled” during the epithelial cycle to confer their plasticity to facilitate the transport of preleptotene spermatocytes. To make this rapid reorganization of actin filament network possible, it is now known that the actin barbed-end nucleation protein Arp2/3 complex that induces branched actin polymerization (54) and actin-branching and cross-linking protein filamin A (96) are also found at the BTB in the rat testis. It is likely that the stage-specific and spatiotemporal expression of these proteins at the BTB during the epithelial cycle facilitates the timely reorganization of the actin filament bundles from their “bundled” to “de bundled” configuration and vice versa, so that adhesion microdomain(s) between Sertoli cells can be rapidly reorganized, such as at the “new” and the “old” BTB sites during the transit of preleptotene spermatocytes. For instance, Eps8 that confers actin filaments and their bundled configuration is highly expressed at the BTB in stages VI–VII of the epithelial cycle but diminished to an almost non-detectable level by stage VIII (55), whereas Arp3 that induces branched actin polymerization to facilitate the conversion of actin filaments from the bundled to de bundled configuration is virtually undetectable at stages VI–VII of the cycle but prominently expressed at stage VIII (54). This thus provides a unique mechanism to elicit timely reorganization of the network of actin filament bundles at the BTB, synchronizing with the cellular events of preleptotene spermatocyte transport at the BTB (12).

Transport of Spermatids Across the Seminiferous Epithelium

Although the transport of spermatids across the seminiferous epithelium depicted in FIGURE 3 does not require the coordinated assembly and disassembly of “new” and “old” BTB, respectively, as during the transit of preleptotene spermatocytes across the BTB, this process is somewhat more complicated, and therefore less is known (93, 103, 119). In the mammalian testis, morphological studies in rodents, such as rats, have illustrated that postmeiotic spermatids are transported from the base of the ad lumenal compartment toward the luminal edge at stages I–IV (FIGURE 2). However, spermatid transport is not unidirectional during the epithelial cycle, since elongating spermatids return to the base at stage V before they are transported back to the apical region of the ad lumenal compartment again at stages VI–VII, until spermatids line up at the edge of the tubule lumen to prepare for spermiation at late stage VIII (FIGURE 2). Furthermore, step 8–19 spermatids are oriented such that their heads are pointing to the basement membrane with their tails toward the tubule lumen, displaying strict cell polarity. Interestingly, the only anchoring device that anchors these spermatids to the Sertoli cell in the epithelium that also confers polarity is the apical ES. Although apical ES shares virtually identical ultrastructural features as basal ES in which bundles of actin filaments are sandwiched between the cisternae of endoplasmic reticulum and the apposing Sertoli-spermatid plasma membranes, these actin bundles are limited only to the Sertoli cell side of the junction (FIGURE 1). The apical and basal ES share some common integral membrane proteins, adaptors, and regulatory protein kinases, yet they each have their unique component proteins (15, 17). These findings thus illustrate that spermatid transport likely requires the involvement of different signaling and regulatory molecules, as well as different signaling pathways vs. the transport of preleptotene spermatocytes at the BTB.

Nonetheless, the defining events of spermatid transport mediated by the apical ES remain to be the effective reorganization of actin microfilaments from their bundled to de-bundled/branched configuration and vice versa, so that spermatids can undergo rapid “de-adhesion” and “re-adhesion” as they are transported along the epithelium, as depicted in FIGURE 3. These changes are mediated via the intricate action of actin bundling proteins (e.g., Eps8, palladin) and branching/debundling-inducing proteins (e.g., the Arp2/3 complex, filamin A), as shown in FIGURE 3. Recent findings have shown that actin barbed-end capping and bundling protein Eps8 is highly expressed at the apical ES from stage V to early stage VIII (55), and another actin bundling protein palladin also displays a similar stage-specific pattern of expression at the apical ES at these stages (80). Specifically, the expression of Eps8 and palladin at the apical ES initially covers the entire head of spermatids; however, by mid to late stage VII and early stage VIII, they become predominantly expressed at the concave side of spermatid heads (55, 80). Due to their spatiotemporal expression at these stages, these two actin bundling proteins are used to confer actin filament bundles surrounding the heads of the spermatids at the apical ES to facilitate spermatid transport. However, the expression of both Eps8 and palladin reduce considerably at the mid to
late stage VIII, and they become virtually nondetectable at the time of spermiation (55, 80), which is necessary to allow apical ES degeneration, in which actin filament bundles are replaced with truncated and de-fragmented microfilaments likely mediated by metalloproteases (e.g., MMP2) (88, 123) to prepare for spermiation. On the other hand, although Arp3 is highly expressed at the apical ES at stages VI–VII, but it is restricted to the concave side of spermatid heads (54), where endocytic vesicle-mediated protein trafficking takes place (103). It is likely that the branched actin polymerization induced by Arp3 at the site facilitates the generation of branched/debundled actin network, which in turn assists protein endocytosis as noted in other epithelia (26, 34). Thus “old” apical ES proteins (e.g., N-cadherin, nectins, and integrins) at the degeneration site can be transcytosed and recycled to assemble “new” apical ES for the newly arisen step 8 spermatids at stage VIII of the epithelial cycle (FIGURE 2). Although both actin bundling proteins Eps8 (55) and palladin (80), as well as branched actin-inducing protein Arp3 (54), are overlappingly expressed at the concave side of spermatid heads in mid to late stage VII, they can be differentially activated, such as via phosphorylation by p-FAK-Tyr407 (56) and c-Yes (121) at this site. As such, their concerted efforts facilitate spermatid transport across the seminiferous epithelium during the epithelial cycle. Recent studies have shown that these events are also facilitated by the timely and spatiotemporal expression of other actin-binding proteins at the apical ES. For instance, drebrin E, an actin-binding protein that displays high affinity for Arp3, is found to express and colocalize with Arp3 at the apical ES (52), illustrating its likely involvement in spermatid transport. Furthermore, Rai14, an actin-binding protein that colocalizes and structurally interacts with F-actin and palladin also displays similar spatiotemporal expression as of palladin near the tip of the spermatid heads at stage VII (79), illustrating the tightly regulated transformation process of the F-actin network at the apical ES, alternating between their bundled and de-bundled/branched configuration, is mediated by two groups of proteins. At present, the precise mechanism(s) by which the microtubules that serve as the tracks so that the cargoes (i.e., spermatids) carried by the vehicles (i.e., actin microfilaments) can be transported across the epithelium remains unknown. It is likely that motor proteins associated with the microtubules (e.g., dynnein, kinesin) and actin filaments (e.g., myosin Va, myosin VIIa) are involved (FIGURE 3). In this context, it is noted that there are no reports in the literature that shed light on understanding the roles of these proteins in regulating spermatid transport, except that a recent study has reported that MARK4 (microtubule affinity-regulating kinase 4), a microtubule stabilizing protein, is structurally associated with β-tubulin, but not β-catenin, Eps8, or occludin in the rat testis, and MARK4 as well as MARK1–3 are highly expressed at the apical ES in virtually all stages of the epithelial cycle except at stage VIII, when spermiation takes place (98), illustrating a functional microtubule network is necessary for spermatid transport during spermiogenesis. However, the mechanism(s) by which the microtubule network coordinates with the actin microfilaments to elicit the transport of spermatids across the epithelium remains unknown. Thus much research is needed to understand the role of motor proteins, and plus- and minus-end proteins in both cytoskeletons in this event.

Role of Regulatory Proteins and Phagocytosis in Germ Cell Transport

Intercellular Adhesion Molecules

Intercellular adhesion molecules (ICAMs; ICAM-1, -2, -3, -4, and -5) are a subgroup of the immunoglobulin superfamily cell adhesion molecule (IgSF CAM) family, which are expressed in epithelial and endothelial cells, fibroblasts, neurons, and leukocytes, including Sertoli and/or germ cells in the testis (40, 65, 119). ICAMs, in particular ICAM-1 and -2, are best known for their involvement in the transmigration of leukocytes across the endothelium during infection and inflammation so that the TJ-barrier remains “sealed” during the transit of leukocytes (31, 68, 99), which is somewhat analogous to the transport of preleptotene spermatocytes across the BTB at stage VIII of the epithelial cycle (106). Recent studies have shown that ICAM-1 and -2 expressed by Sertoli and germ cells in the rat testis are involved in germ cell transport in the seminiferous epithelium during spermatogenesis (116, 117). ICAM-1 and its truncated form, namely soluble ICAM-1 (sICAM-1), which consisted only the five Ig-like extracellular domains lacking the transmembrane and cytoplasmic domains, are shown to have antagonistic effects on the Sertoli cell TJ-permeability barrier function (117). sICAM-1 is derived from ICAM-1 via proteolytic cleavage mediated by MMP-9 at the BTB microenvironment (59), similar to its generation in other epithelia and endothelia (46, 65, 109). Furthermore, the production of sICAM-1 is regulated by TNF-α (59), and earlier studies have shown that TNF-α is a crucial regulator of BTB dynamics in the testis (14, 51). Overexpression of ICAM-1 in Sertoli cells promotes, whereas sICAM-1 disrupts, the Sertoli cell TJ-barrier function (117), indicating that their spatiotemporal expression
at the microenvironment of the BTB can promote either assembly or disassembly of TJ fibrils at the corresponding basal or apical region of the preleptotene spermatocyte in transit at the BTB (FIGURE 4). This thus maintains the immunological barrier during the transport of preleptotene spermatocytes at the BTB, which are connected in “clones” via intercellular bridges. Additionally, the antagonistic effects of ICAM-1 and sICAM-1 are mediated by the proline-rich tyrosine kinase 2 (Pyk2)/p-Pyk2-Tyr402 signaling molecule (117), which is a nonreceptor protein tyrosine kinase structurally related to FAK (32, 44, 57), suggesting that Pyk2 and p-Pyk2-Tyr402 likely act as molecular “switches” that can turn “on” and “off” BTB to accommodate the transit of preleptotene spermatocytes at the site. On the other hand, ICAM-2, which is restrictively expressed at the apical ES, is involved in spermatid transport across the seminiferous epithelium during spermiogenesis (116). It is likely that ICAM-1 and -2 are working in concert with adhesion (e.g., occludin, N-cadherin, JAM-A) and regulatory proteins (e.g., Pyk2, p-Pyk2-Tyr402, c-Src) at the apical and basal ES to confer rapid adhesion and de-adhesion at the Sertoli-Sertoli and Sertoli-germ interface to facilitate germ cell transport during spermatogenesis (FIGURES 3 AND 4).

Src Protein Kinases: the Role of c-Yes and c-Src

c-Yes and c-Src are members of the Src nonreceptor protein tyrosine kinase family, which are downstream signaling mediators of integrin-based receptors at focal adhesion complex (FAC), an actin-based anchoring junction at the cell-extracellular matrix (ECM) interface also known as focal contact (9, 120). In short, c-Yes and c-Src mediate signals downstream following activation of integrin receptor by its ligands, usually fragments of laminins or collagens, regulating cell adhesion, migration, differentiation, and proliferation (100, 120). In the testis, basement membrane (BM) is a modified form of ECM (27, 87). However, unlike other epithelia, no ultrastructure analogous to FAC are found at the Sertoli cell-basement membrane (BM) in the seminiferous epithelium (63, 87). Instead, proteins that are usually restricted to FAC, such as c-Yes, c-Src, FAK, vinculin, integrins, and laminins, are detected at the apical ES and/or basal ES at the Sertoli-spermatid and/or Sertoli-Sertoli cell-cell interface, respectively (15, 17, 63). Studies in other epithelia have shown that c-Src shuttles between plasma membrane and late endosomes or lysosome, whereas c-Yes is associated with the Golgi pool of caveolin, illustrating c-Src and c-Yes are involved in endocytic vesicle-mediated protein degradation and transcytosis/recycling, respectively (58, 84, 94). In the testis, c-Yes and c-Src are expressed by Sertoli and germ cells (49, 121). A loss of c-Yes function by using a selective inhibitor, such as SU6656, disrupts the organization of F-actin in Sertoli cells, inducing truncation of microfilaments (121). This, in turn, causes mislocalization of TJ (e.g., occludin) and basal ES (e.g., N-cadherin) proteins at the Sertoli cell BTB, in which occludin and N-cadherin are relocated from near the cell surface to cell cytosol and associated with clathrin, thereby destabilizing the TJ-permeability barrier (121). These studies in vitro using a selective inhibitor of c-Yes have recently been confirmed both in vitro and in vivo when c-Yes is knocked down by RNAi (118). More important, a significant increase in actin polymerization is noted following c-Yes knockdown by RNAi (118). As such, the knockdown of c-Yes in vivo induces changes in F-actin organization in Sertoli cells in which microfilaments assume an unbounded and branched configuration, thereby perturbing the localization of occludin and N-cadherin at the BTB and also nectin-3 at the apical ES, destabilizing apical and basal ES function (118). On the other hand, c-Src is localized at the BTB in virtually all stages of the epithelial cycle but also prominently at the apical ES in stage VI and VII tubules (120); however, in stage VIII tubules, c-Src is mostly localized to the residual body, implicating its likely involvement in Sertoli cell phagocytic activity (120). Nonetheless, p-Src-Tyr416 remains robustly expressed at the apical ES in stage VIII tubules until spermiation takes place (110). Collectively, these findings illustrate that c-Yes and c-Src are crucial regulators of cell adhesion in the seminiferous epithelium at the Sertoli-spermatid and Sertoli-Sertoli interface, inducing rapid adhesion and de-adhesion via changes in the underlying F-actin organization, the attachment site for occludin- or N-cadherin-based adhesion protein complexes.

Focal Adhesion Kinase

Focal adhesion kinase (FAK), also known as protein tyrosine kinase 2 (PTK2), is a nonreceptor protein kinase serving as the downstream signaling transducer of integrin-based receptor, regulating cell movement mediated through Rho GTPases in multiple mammalian cells, such as fibroblasts, macrophages, keratinocytes, cancer cells, and others (4, 36, 76). FAK, as its name implies, is an integrated component of FAC. In the mammalian testis, however, there is no ultrastructure similar to FAC at the Sertoli cell-basement membrane interface. Instead, FAK is restricted mostly to the basal compartment in the seminiferous epithelium of the rat testis, consistent with its localization at the BTB (89). However, p-FAK-Tyr977 and p-FAK-Tyr576...
are restrictively and spatiotemporally expressed at the apical ES (56, 89), and their expression persists from stages V to VIII but most robust at stages VII–VIII until shortly before spermiogenesis (7, 89), illustrating its likely involvement in spermatid transport and the release of sperms at spermiogenesis (53, 70). On the other hand, p-FAK-Tyr407 is found at both the apical and the basal ES in virtually all stages of the epithelial cycle except that its expression at the apical ES is diminished considerably to a level virtually nondetectable at stage VIII, but it is still highly expressed at the basal ES/BTB at this stage (56), illustrating the two forms of FAK, namely p-FAK-Tyr397 and p-FAK-Tyr407, may play different roles in regulating apical and basal ES dynamics.

Different mutants of p-FAKs, such as FAK Y397E and FAK Y397F vs. FAK Y407E and FAK Y407F, as well as double mutant of FAK Y397E-Y407E in which Tyr (Y) is replaced with either Glu (E) or Phe (F) via site-directed mutagenesis, making it either phosphomimetic or nonphosphorylatable, respectively, have been prepared for their overexpression in Sertoli cell epithelium in vitro (56). It is noted that this Sertoli cell in vitro system mimics the Sertoli cell BTB in vivo since both functional TJ-permeability barrier is detected, and ultrastructures of TJ, basal ES, gap junction, and desmosome permeability barrier is detected, and ultrastructure of spermatids display these mutants have shown that overexpression of an analogous to those found in vivo are also found in these cultures (14, 50, 63, 112). Using this well characterized Sertoli cell BTB system, studies using these mutants have shown that overexpression of p-FAK-Tyr407 and -Tyr397 in Sertoli cells display antagonistic effects on the TJ-permeability barrier, in which the former促进ates and the latter disrupts Sertoli cell TJ barrier (56), illustrating that the p-FAK-Tyr407 and -Tyr397 serve as molecular "switches" that turn the BTB on and off during the epithelial cycle. This BTB promoting effect of p-FAK-Tyr407 is also noted in studies wherein overexpression of FAK Y407E phosphomimetic mutant is shown to protect the Sertoli cell TJ-permeability barrier from the disruptive effects induced by PFOS (perfluorooctanesulfonate) (105). However, the use of a FAK miRNA miR-135 that specifically knocks down FAK is shown to potentiate the disruptive effects of PFOS on the Sertoli cell TJ-barrier function (105). Collectively, these findings illustrate the promoting effects of p-FAK-Tyr407 on the Sertoli cell BTB function.

Studies in vivo by overexpressing p-FAK-Tyr397 in the rat testis via the use of FAK Y397E phosphomimetic mutant has confirmed its promoting effects on the apical ES, since its overexpression induces defects in spermiation in which elongated spermatids fail to be transported to the adluminal edge of the apical compartment to prepare for spermiogenesis due to the persisting presence of apical ES at stage VIII when it should have been degenerated (104). For instance, F-actin remains robust at the apical ES in late stage VIII when the actin filament bundles at the site should have been replaced by debundled/branched actin filaments that are rapidly undergoing de-polymerization to facilitate spermatid transport and their release into the tubule lumen (104). This persistence of F-actin at the apical ES is mediated by the continual expression of actin bundling proteins Eps8 and palladin at the site, and the presence of F-actin also retains cell adhesion proteins nectin-2 and -3 to mediate spermatid adhesion, causing failure in spermatid transport to their targeted site and to undergo spermiogenesis (104). These findings thus demonstrate unequivocally that p-FAK-Tyr397 is a crucial signaling molecule that is involved in mediating the events of spermatid transport by regulating the logical sequence of spatiotemporal expression of actin bundling proteins Eps8 and palladin, and also cell adhesion proteins nectin-2 and -3, via its effects on the organization and configuration of actin filament bundles. FIGURE 4 thus summarizes the physiological role of FAK, in particular its p-FAK-Tyr407 and -Tyr397, on the transport of germ cells at the BTB and also across the seminiferous epithelium during the epithelial cycle of spermatogenesis.

**Role of Phagocytosis**

Phagocytosis is a cellular event best studied in macrophages (67, 78). This event is crucial to spermatid transport during spermiogenesis since cytoplasmic debris derived from spermatids must be removed from the epithelium by Sertoli cells before spermiogenesis (23). Phagosomes are F-actin-coated vesicles of >0.2 μm in diameter (67), and phagocytosis is an actin-dependent cellular event (73). Studies using F-actin-depolymerizing drugs, such as cytochalasin D and latrunculins (77), are known to block phagocytosis such as in macrophages and Sertoli cells (62, 83). During spermiogenesis, cytosolic components, including organelles not necessary for the formation of haploid elongated spermatids, are being sequestered to an ultrastructure known as the residual body (RB) (FIGURE 2). RB is then phagocytosed by the Sertoli cell, becoming phagosomes, which are transported to Sertoli cell lysosomes at the base of the seminiferous tubule for their eventual degradation at stages VIII–IX of the cycle (25, 70, 74). Although the formation of RB is F-actin dependent (83), the transport of RB as phagosomes to the base of the Sertoli cell for degradation is a microtubule-dependent event (83). Thus the transport of spermatids during spermiogenesis is tightly coordinated with the formation and the eventual degradation of RB via phagocytosis, and if this process is broken down,
spermatogenesis may be disrupted. However, the biology of phagocytosis in the testis remains poorly understood. A recent report has demonstrated high expression of p-FAK-Tyr407 in structures analogous to RB and phagosomes in the seminiferous epithelium of the rat testis in virtually all stages of the epithelial cycle except at stages VIII–IX when its expression at the RB/phagosomes is considerably diminished (56), because phagosomes are transported to the base of the seminiferous epithelium for degradation (23). In light of the fact that p-FAK-Tyr407 (56, 93) and p-FAK-Tyr397 (89, 104) are regulators of BTB and apical ES remodeling, respectively, and these events are pertinent to germ cell transport during spermatogenesis, the likely involvement of p-FAK-Tyr407 in spermatid transport and phagocytosis is plausible. This possibility must be carefully investigated in future studies.

Concluding Remarks and Future Perspectives

As briefly but critically discussed herein, the complex molecular events underlying the transport of preleptotene spermatocytes at the BTB and also postmeiotic spermatid transport across the adluminal compartment during spermatogenesis are unfolding. It is noted that the actin-based cytoskeleton network plays a critical role in these events, involving the participation of actin bundling, barbed-end capping, cross-linking, nucleation, branch-inducing, and de-polymerization proteins, as well as cell adhesion proteins. More importantly, ICAMs, Src kinases, and FAK are also involved in these events by serving as the molecular switches that turn on and off some necessary events, such as branched actin polymerization induced by p-FAK-Tyr407. However, it remains to be carefully examined regarding the involvement of tubulin-based microtubule network in these events, since many of the studies in the literature on microtubulin are limited on the identification and expression of tubulins and tubulin-binding and regulatory proteins in the testis. Nonetheless, the hypothetical models depicted in FIGURES 3 AND 4 provide the detailed information regarding germ cell transport during spermatogenesis. These two figures also highlight areas that deserve careful investigation in the years to come.

Current address of X. Xiao: Department of Reproductive Physiology, Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang 310013, China (e-mail: xiao_xiang2@163.com).

This work was supported by grants from the National Institute of Child Health and Human Development (U54 HD-029990 Project 5 to C.Y. Cheng; R01 HD-D56034), and The Hong Kong General Research Fund (HKBU 261812 to C.K.C.W.).

No conflicts of interest, financial or otherwise, are declared by the author(s).


References


75. Pelletier RM. The blood-testis barrier: the junctional permeability, the proteins and the lipids. Prog Histochem Cytochem 46: 49–127, 2011.


