Chapter 17
Drebrin and Spermatogenesis

Haiqi Chen, Michelle W.M. Li, and C. Yan Cheng

Abstract Drebrin is a family of actin-binding proteins with two known members called drebrin A and E. Apart from the ability to stabilize F-actin microfilaments via their actin-binding domains near the N-terminus, drebrin also regulates multiple cellular functions due to its unique ability to recruit multiple binding partners to a specific cellular domain, such as the seminiferous epithelium during the epithelial cycle of spermatogenesis. Recent studies have illustrated the role of drebrin E in the testis during spermatogenesis in particular via its ability to recruit branched actin polymerization protein known as actin-related protein 3 (Arp3), illustrating its involvement in modifying the organization of actin microfilaments at the ectoplasmic specialization (ES) which includes the testis-specific anchoring junction at the Sertoli-spermatid (apical ES) interface and at the Sertoli cell-cell (basal ES) interface. These data are carefully evaluated in light of other recent findings herein regarding the role of drebrin in actin filament organization at the ES. We also provide the hypothetical model regarding its involvement in germ cell transport during the epithelial cycle in the seminiferous epithelium to support spermatogenesis.

Keywords Testis • Spermatogenesis • Drebrin • Blood-testis barrier • Arp2/3 complex • Branched actin polymerization • F-actin • Ectoplasmic specialization • Actin microfilament bundles • Seminiferous epithelial cycle

17.1   Introduction

In the mammalian testes, the functional unit that produces an upward of 200 million sperm per day in each man after puberty at ~12–13 years of age versus 40 million sperm per day in a male rat by 45-day postpartum (dpp) is the seminiferous tubule via

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spermatogenesis (Johnson et al. 1980; Amann and Howards 1980). Spermatogenesis takes place in the seminiferous epithelium that encircles the inner surface of seminiferous tubules which is composed of only Sertoli cells and germ cells. The Sertoli cell provides nutritional and structural support for germ cell maturation (Xiao et al. 2014; de Kretser and Kerr 1988; Bardin et al. 1988). In short, spermatogenesis is comprised of four distinctive phases of cellular events, which include (1) self-renewal of undifferentiated spermatogonia and proliferation of spermatogonia via mitosis, and their differentiation to type A and type B spermatogonia, (2) transformation of type B spermatogonia to preleptotene spermatocytes and progression of spermatocytes to undergo meiosis, (3) postmeiotic differentiation of round spermatids to elongated spermatids via spermiogenesis, and (4) the release of sperms at spermiation (Schlatt and Ehmcke 2014; O'Donnell et al. 2011; de Kretser and Kerr 1988; Ehmcke and Schlatt 2006). Since germ cells in particular spermatids are highly differentiated and metabolically quiescent cells, they rely almost exclusively on the Sertoli cell for the provision of nutrients, biomolecules, paracrine, and structural supports (Mruk and Cheng 2004b; Cheng and Mruk 2002). On the other hand, Sertoli cells by 15–17 dpp (day postpartum) in rodents and ~12–13 years of age in humans are differentiated and ceased to divide (Orth 1982; Sharpe et al. 2003) so that Sertoli cells support only a relatively small population of germ cells and most germ cells undergo apoptosis (Billig et al. 1995) in order to maintain a Sertoli/germ cell ratio of ~1:30–1:50 in the seminiferous epithelium in rodents (Weber et al. 1983).

In a cross section of the seminiferous tubule, germ cells at different developmental states are associated with Sertoli cells in the seminiferous epithelium, displaying a unique pattern of Sertoli-germ cell association particularly regarding changes of the spermatid head (e.g., acrosome biogenesis) and elongating of the tail which can be divided into 12, 14, and 6 stages in the mouse, rat, and man, respectively, known as the seminiferous epithelial cycle of spermatogenesis (Clermont and Leblond 1955; Amann 2008; Hess and de Franca 2008). For example, the release of fully developed elongated spermatids (i.e., spermatozoa) from the seminiferous epithelium into the tubule lumen at the luminal edge of the apical compartment and blood-testis barrier (BTB) remodeling near the basal compartment both take place concurrently at stage VIII of the epithelium cycle in the rat and mouse testis, whereas meiosis takes place at stages XIV and XII in the rat and mouse testis, respectively (Parvinen 1982; Hess and de Franca 2008; Leblond and Clermont 1952; Clermont 1972).

17.2 Actin Cytoskeleton, Ectoplasmic Specialization (ES), and Spermatogenesis

While the cellular events that take place in the seminiferous epithelium during the epithelial cycle are known for more than six decades (Parvinen 1982; Hess and de Franca 2008; Leblond and Clermont 1952; Clermont 1972), molecular mechanism(s) that regulate these events remain relatively unknown until recent years. Studies have shown that actin-based cytoskeleton is crucial for these events in particular by conferring structural support to Sertoli cells, maintaining cell polarity and junction integrity as well as by participating in endocytic vesicle-mediated protein trafficking

(O'Donnell 2014; Qian et al. 2014; Cheng and Mruk 2015; Wong and Cheng 2009). Studies in the past two decades have shown that the highly coordinated cellular events that take place in the seminiferous epithelium during the epithelial cycle are mediated at the Sertoli cell-cell and Sertoli-germ cell interface, relying on rapid restructuring of the unique cell junctions at these sites, such as gap junctions (Cheng and Mruk 2010, 2002; Hess and de Franca 2008; Hermo et al. 2010; Mruk and Cheng 2004a, b). Interestingly, the most extensive cell junction noted in the testis is the actin-rich cell-cell anchoring junction known as ectoplasmic specialization (ES), which is found at the Sertoli cell-cell or Sertoli-spermatid interface known as the basal and apical ES, respectively (Cheng and Mruk 2010; Russell and Peterson 1985; Wong et al. 2008a; Yan et al. 2007). The ES is typified by the presence of an array of actin microfilament bundles that lie perpendicular to the Sertoli cell plasma membrane, which are sandwiched between the cisternae of endoplasmic reticulum and the apposing Sertoli-spermatid plasma membranes at the apical ES or the apposing Sertoli cell-cell plasma membranes, thereby containing two arrays versus one array of actin microfilament bundles at the basal ES, respectively (Fig. 17.1). In the rat testis, the basal ES is found to coexist with tight junction (TJ) and gap junction (GJ), which together with the intermediate filament-based desmosome constitutes the BTB (Russell 1979, 1977a; Vogl et al. 2008; Mruk and Cheng 2004a; Wong et al. 2008b). Due to the presence of the actin microfilament bundles on both sides of the Sertoli cells at the basal ES, the BTB is one of the tightest blood-tissue barriers (Cheng and Mruk 2012; Pelletier 2011; Franca et al. 2012). The BTB also divides the seminiferous epithelium into the basal and the apical (adluminal) compartments (Fig. 17.1). Thus, postmeiotic spermatid development takes place in a unique microenvironment supported entirely by the Sertoli cells known as the adluminal (apical) compartment (Fig. 17.1), which is sequestered from the systemic circulation (Cheng and Mruk 2012; Pelletier 2011; Franca et al. 2012). On the other hand, apical ES that shares similar ultrastructural features with the basal ES is found restrictively at the Sertoli-spermatid (step 8–19 spermatids in the rat testis) interface during spermiogenesis (Russell 1977b; Yan et al. 2007; Vogl et al. 2008, 2000). Unlike basal ES which coexists with TJ and GJ, when apical ES appears in step 8 spermatids in the rat testis, it replaces GJ and desmosome at the Sertoli-spermatid interface, becoming the only anchoring device until the release of elongated spermatids at spermiation. Furthermore, there is only a single array of actin microfilament bundles restricted to the side of Sertoli cell at the apical ES. While the network of actin microfilament bundles at the ES is crucial to confer unusual adhesive strength to the BTB and developing spermatids, these microfilaments rapidly reorganize during the epithelial cycle to facilitate the transport of preleptotene spermatocytes across the BTB at stage VIII as well as the progressive transport of developing spermatids across the adluminal compartment so that fully developed spermatids (i.e., spermatozoa) can line up near the tubule lumen to prepare for their eventual release at spermiation. Studies have shown that the rapid reorganization of these actin microfilaments such as their conversion from a bundled to an unbundled/branched configuration and vice versa is mediated via the intriguing actions of three classes of proteins: (1) the actin-bundling proteins such as Eps8 (also an actin barbed end capping protein) (Lie et al. 2009), palladin (also an actin-cross-linking protein) (Qian
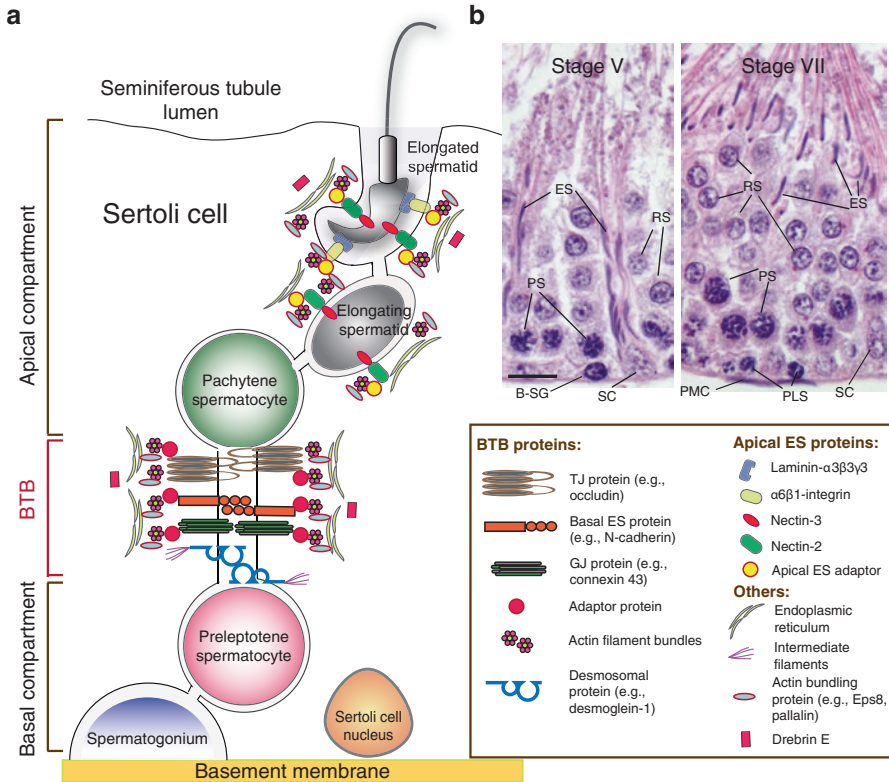
Fig. 17.1 Morphological features and relative location of the actin-rich ES in the seminiferous epithelium of adult rat testes. (**a**) A schematic drawing of the cross section of a seminiferous tubule, illustrating the blood-testis barrier (BTB) that physically divides the seminiferous epithelium into the basal and the adluminal (apical) compartments. Preleptotene spermatocytes derived from type B spermatogonia at stage VII must be transported across the immunological barrier at stage VIII of the epithelial cycle. The BTB integrity is maintained by different cell adhesion complexes of the TJ (e.g., occludin-ZO-1), basal ES (e.g., N-cadherin-β-catenin), and gap junction (e.g., connexin 43/plakofilin-2) which all use actin microfilaments for their attachment, as well as the desmosome (e.g., desmoglein-2/desmocollin-2) which uses vimentin-based intermediate filaments for attachment. Developing spermatids (steps 8–19) in the adluminal compartment remain attached to the Sertoli cell via apical ES by unique adhesion protein complexes such as nectin-afadin and laminin-integrin, of which nectin-3 and laminin-α3β3γ3 are specific to spermatids, whereas necin-2 can be found in both Sertoli cells and spermatids, and α6β1-integrin is Sertoli cell-specific. The typical feature of the ES is the actin microfilament bundles that lie perpendicular to the Sertoli cell plasma membrane, and they are sandwiched between cisternae of endoplasmic reticulum and the apposing plasma membrane of the adjacent Sertoli cells called basal ES *vs.* Sertoli-spermatid called apical ES. These actin microfilament bundles thus confer the ES its unusual adhesive strength to support Sertoli cell adhesion at the BTB and the spermatid adhesion at the ES during spermiogenesis. (**b**) These two micrographs illustrate the typical cell-cell interactions between different germ cells and Sertoli cells at stage V (*left panel*) and stage VII (*right panel*) of the epithelial cycle. For instance in stage V, elongating spermatids (step 17) are found deep inside the base of the adluminal compartment, almost touching the Sertoli cell nucleus (SC), whereas at stage VII, elongated spermatids (step 19) begin to line up near the tubule lumen to prepare for their release into the tubule lumen at stage VIII of the cycle. Scale bar, 60 μm. Abbreviations: *B-SG* type B spermatogonium, *ES* elongating/elongated spermatid, *PLS* preleptotene spermatocyte, *PMC* peritubular myoid cell, *PS* pachytene spermatocyte, *RS* round spermatid, *SC* Sertoli cell

et al. 2013), fascin 1 (Gungor-Ordueri et al. 2014a), plastin 3 (Li et al. 2015c), and ezrin (Gungor-Ordueri et al. 2014b) versus (2) branched actin polymerization (or nucleation) proteins such as the Arp2/3 complex (Lie et al. 2010) and filamin A (Su et al. 2012) and (3) actin nucleation proteins that generate long stretches of micro-filaments which can be assembled into actin bundles such as formin 1 (Li et al. 2015b). These proteins coordinate to regulate actin microfilament remodeling at the basal ES and apical ES via their stage-specific and spatiotemporal expression, which, in turn, facilitate the transport of preleptotene spermatocytes across the BTB and the transport of elongating spermatids across the adluminal compartment, respectively. The coordinated efforts of those actin regulatory proteins have recently been reviewed (Li et al. 2015a; Cheng and Mruk 2015; Su et al. 2013). Herein, we focus our discussion on drebrin, a family of proteins known to regulate the action of the Arp2/3 complex which is a barbed end nucleation protein that effectively induces branched actin polymerization, thereby converting actin microfilaments from a lin-ear to a branched configuration. Thus, actin microfilaments can no longer resume a bundled configuration, thereby destabilizing adhesion protein complexes at the Sertoli cell-cell and/or Sertoli-spermatid interface. These changes also facilitate endocytic vesicle-mediated protein trafficking events, such as endocytosis, trans-cytosis, and recycling, necessary to reassemble "new" basal ES/BTB and apical ES before or following degeneration of the "old" ES.

17.3   Drebrin in the Testis

Drebrin, also known as developmentally regulated brain protein, is an actin-binding protein first identified in chicken brain (Shirao and Obata 1985). Drebrin is a grow-ing protein family which now contains the embryonic type (E) and the adult type (A) in mammals, both of which originate from a single gene (Shirao and Obata 1986; Majoul et al. 2007). As a member of the actin-depolymerizing factor homol-ogy (ADF-H) domain-containing family of actin-binding proteins, drebrin binds to actin microfilaments. It alters the helical twist of actin filaments, stabilizes their structures, and strengthens their stiffness (Sharma et al. 2011; Mikati et al. 2013). The coiled-coil (CC) domain and the helical (hel) domain of drebrin, besides the N-terminal ADF-H domain, also contribute to its intrinsic actin-binding activity (Fig. 17.2) (see also Fig. 1.3 in Chap. 1) (Hayashi et al. 1999; Worth et al. 2013; Xu and Stamnes 2006). Multiple binding partners have been identified for drebrin to date (Table 17.1). The drebrin-based protein complex is known to stabilize F-actin, modifies microfilament structure, or moderates the interactions of other actin-binding proteins with actin microfilaments. For instance, drebrin binds to afadin to connect F-actin to nectin-based integral membrane proteins (Rehm et al. 2013). It competes with tropomyosin for F-actin binding and inhibits actin-binding and cross-linking activity of α-actinin (Ishikawa et al. 1994). Furthermore, drebrin is a negative regulator of HIV entry into epithelial cell, as well as HIV-mediated cell fusion by regulating F-actin rearrangement (Gordon-Alonso et al. 2013), and it is also a critical player in glioma cell invasiveness (Terakawa et al. 2013). In addition,
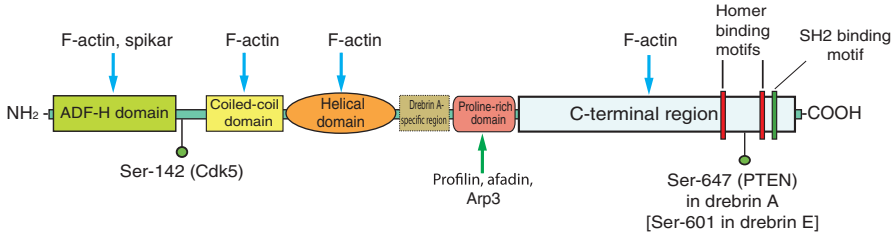
Fig. 17.2 Functional domains in drebrin. Different functional domains along the polypeptide sequence of drebrin, applicable to both drebrin A and drebrin E, are shown herein, except that in drebrin A, there is a drebrin A-specific region located behind the helical domain. Drebrin contains an actin-depolymerizing factor homology (ADF-H) domain, a coiled-coil domain (CC), a helical domain (Hel), as well as a large domain called the blue box which are all capable of interacting with F-actin via their corresponding actin-interaction domain, conferring drebrin its actin-binding protein ability (Worth et al. 2013; Majoul et al. 2007). In addition, spikar is shown to bind to the ADF-H domain (Yamazaki et al. 2014). Drebrin also contains a proline-rich (PP) domain known to bind profilin (Mammoto et al. 1998), afadin (Rehm et al. 2013), and possibly Arp3 (Li et al. 2011). There are also two homer-binding motifs near the C-terminus that interact with cupidin/homer2 (Shiraishi-Yamaguchi et al. 2009) and also an SH2-binding motif. There are two putative phosphorylation sites in drebrin: Ser-142 and Ser-647. Cdk5 is the kinase known to phosphorylate drebrin at Ser-142 (Worth et al. 2013). PTEN is known to dephosphorylate Ser-647 in drebrin A (Kreis et al. 2013), which corresponds to Ser-601 in drebrin E based on sequence homology alignment analysis

Fig. 17.3 A hypothetical model regarding the involvement of drebrin E in the remodeling of the apical ES to facilitate the release of sperms at spermiation. In stage VII tubules, such as the one shown on the *left panel*, the apical ES is supported by the actin microfilament bundles, which are likely maintained through an upregulation of actin-bundling proteins such as Eps8, ezrin, and palladin that confer the actin microfilaments their bundled configuration, which in turn support the functionality of adhesion protein complexes at the site. However, at late stage VII, at the concave (ventral) side of the spermatid head, an upregulation of drebrin E begins to occur (Li et al. 2011). This thus recruits more Arp3 to the site via the high affinity of drebrin E to Arp3 as shown in the testis (Li et al. 2011), so that the intrinsic branched actin polymerization activity of the Arp2/3 complex converts actin microfilaments from a bundled to an unbundled/branched configuration, promoting endocytic vesicle-mediated trafficking events, creating a transient ultrastructure known as apical TBC (tubulobulbar complex) (Vogl et al. 2013), referring to the aggregates of numerous endocytic vesicles readily visible by fluorescence microscopy. The events of endocytosis, transcytosis, and recycling are necessary to recycle apical ES proteins (e.g., integrins, nectins, laminins) from the "old" apical ES to assemble "new" apical ES when step 8 spermatids develop from step 7 arise at stage VIII of the epithelial cycle without requiring de novo synthesis of all the apical ES and pertinent proteins such as peripheral adaptors. This event of actin microfilament reorganization becomes more widespread at stage VIII when virtually all the actin microfilament bundles are replaced by unbundled/branched actin microfilaments to facilitate the release of sperm at spermiation. These changes are also assisted by a downregulation of actin-bundling proteins at the site such as Eps8, ezrin, and palladin, as well as an upregulation of cofilin-1 which cleaves actin microfilaments into shorter fragments to facilitate the breakdown of cell adhesion function conferred by adhesion protein complexes. Collectively, these changes lead to apical ES degeneration to coordinate spermiation

although drebrin knockout mice were viable, abnormalities were observed in cortical neurons, mainly associating with dendritic spines (Table 17.2). While drebrin A and E isoforms are both detected in the brain, only drebrin E is found in the rat testis, and it is predominantly expressed by Sertoli cells instead of germ cells (Li et al. 2011). In Sertoli cells cultured in vitro with an established functional TJ-permeability barrier, drebrin E is localized in cell cytoplasm as well as near the cell surface, co-localizing with F-actin (Li et al. 2011). In adult rat testes, drebrin E displays a restrictive, but stage-specific, localization at the ES during the epithelial cycle and co-localizes with F-actin both at the apical ES and the basal ES/BTB. At
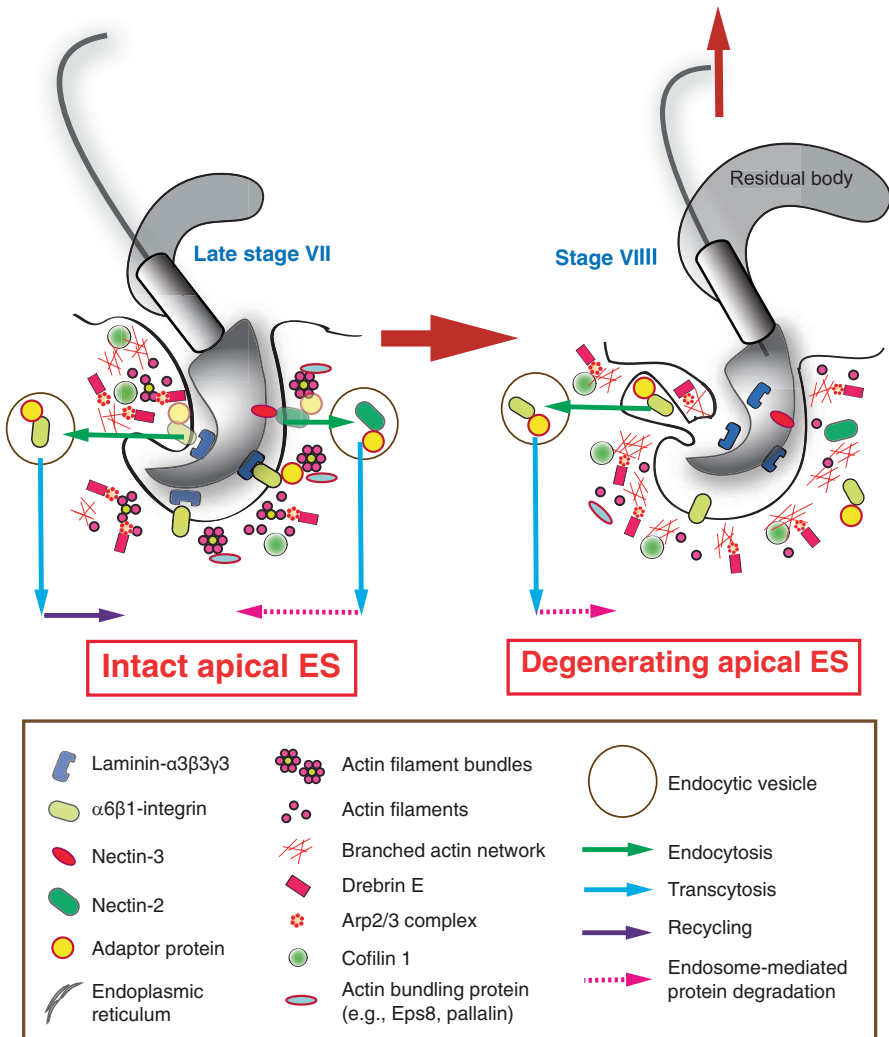
Table 17.1 Binding partners of drebrin

Arp3	Rat Sertoli cells	Recruitment of Arp3 by drebrin E at the Sertoli cell BTB that induces actin branching, converting actin microfilaments in Sertoli cells from a bundled to unbundled/branched configuration that facilitates endocytic vesicle-mediated protein trafficking	n.d. in neither Arp3 nor drebrin E	Li et al. (2011)
Afadin	HUVEC	Afadin serves as a linker between drebrin and nectins in adherens junction	PR domain in drebrin; PR1 and PR2 regions in afadin	Rehm et al. (2013)
Connexin 43	Vero cells; mouse astrocytes	Drebrin acts as a linker between connexin 43 and submembranous actin cytoskeleton	n.d. in drebrin; C-terminal region (amino acids 234–382) in Cx43	Butkevich et al. (2004)
EB3	Rat cortical neurons	Binding of drebrin to EB3 regulates interaction between F-actin and microtubules to support growth cone formation and neuritogenesis	n.d. in drebrin; 3 amino acid inserts in EB3 that are not found in EB1	Geraldo et al. (2008)
Spikar	Rat hippocampal neurons	Extranuclear spikar (a drebrin-binding protein in the brain) accumulation mediated by drebrin that increases dendritic spine density	N-terminal region (amino acids 1–134) in drebrin; N-terminal region (amino acids 88–376) in spikar	Yamazaki et al. (2014)
PTEN	Rat hippocampal or cortical neurons	PTEN negatively regulates S647-phosphorylation of drebrin via dephosphorylation	n.d. in drebrin; C2 domain (amino acids 182–354) in PTEN	Kreis et al. (2013)
Profilin	Rat brain	Drebrin likely regulates actin microfilament via its interaction with profilin	Likely PR domain in drebrin; n.d. in profilin	Mammoto et al. (1998)
Cupidin/ Homer2	Mouse cerebella; rat hippocampal neurons	Deletion of Cdc42-binding region of cupidin disturbs dendritic drebrin distribution in hippocampal neurons	Homer-binding motifs at the C-terminus of drebrin; N-terminal EVH-1 domain in cupidin/ homer2	Shiraishi-Yamaguchi et al. (2009)

[a]This Table is not intended to be exhaustive; the binding partners of drebrin that are selected herein represent those that are found in the testis, or their function is consistent with regulation of spermatogenesis based on our current understanding in the field. However, studies are needed to confirm if many of these proteins are indeed putative binding partners of drebrin in the testis since there are few studies in the literature that explore the role of drebrin in spermatogenesis. *n.d.* not determined, *HUVEC* human umbilical vein endothelial cells, *PR domain* proline-rich domain, *EB3* end-binding protein 3, *PTEN* phosphatase and tensin homolog

Table 17.2 Function of drebrin which is assessed based on studies using genetic models

Drebrin A	Global knockout	Drebrin A-specific KO (drebrin E remained functional in these KO mice) led to a loss of homeostatic synaptic plasticity (HSP) at excitatory synapses of adult cerebral cortex, illustrating drebrin A may be involved in Alzheimer's disease pathogenesis	Cre-Lox P recombination	Aoki et al. (2009)
Drebrins A and E	Global knockout	Total drebrin KO led to a decrease in the number of neurotransmitter receptors, impaired dendritic spine morphogenesis, and reduced memory-related synaptic plasticity in the hippocampus	Cre-Lox P recombination	Jung et al. (2015)
Drebrin A	Global knockout	An impairment of context-dependent fear learning in adult drebrin A KO mice, suggesting a role of drebrin A in cognitive function	Cre-Lox P recombination	Kojima et al. (2010)

KO knockout

the apical ES, drebrin E is first detected at stage V, surrounding the entire head of elongating spermatids; and by stage VII, its localization is shifted mostly to the concave (ventral) side of the spermatid head, co-localizing with two other actin regulatory proteins: actin barbed end capping and bundling protein Eps8 and branched actin nucleation protein Arp3 (Li et al. 2011). However, by stage VIII of the epithelium cycle, drebrin E is downregulated considerably (Li et al. 2011). Drebrin E appears to regulate actin organization in the testis via its association with Apr3 with high affinity, but not Eps8, playing a role in branched actin polymerization. Interestingly, when adult rats were treated with adjudin, a male contraceptive known to induce exfoliation of germ cells in particular elongating/elongated spermatids from the epithelium (Grima et al. 2001; Cheng et al. 2005; Mruk et al. 2006), the localization of drebrin E was grossly affected (Li et al. 2011), similar to Arp3 (Lie et al. 2010) and Eps8 (Lie et al. 2009) following adjudin treatment. This mislocalization and downregulation of drebrin E alongside with Arp3 and Eps8 thus perturb the proper organization of F-actin at the apical ES, thereby causing a disruption of spermatid adhesion that leads to premature spermatid release (Cheng et al. 2011). Drebrin may also be involved in the crosstalk between actin-based and microtubule-based cytoskeleton. For instance, drebrin A was shown to interact directly and specifically with microtubule (MT) end-binding protein 3 (EB3) in growth cone filopodia, and this interaction induces the formation of MT-containing filopodia (Geraldo et al. 2008). Moreover, drebrin A, which is highly concentrated in dendritic spines (Kojima and Shirao 2007; Ferhat 2012), facilitates the entry of MT into dendritic spines following its overexpression in neurons cultured in vitro, resembling the phenotype following an increase in the concentration of stabilized F-actin filaments in neurons (Merriam et al. 2013). Interestingly, a mis-localization of Arp3 and a considerable surge in Arp3/N-WASP interaction were detected following the knockdown of EB1, a plus-end-binding protein of MT, in Sertoli cells

with an established TJ-permeability barrier, thereby impeding MT organization as well as proper organization of actin microfilaments of ES at the cell-cell interface to maintain the barrier function (Tang et al. 2015). This finding illustrates that EB1, a close cousin of EB3, is involved in both MT and F-actin regulations. Taken together, these data suggest that drebrin E may be involved in regulating MT dynamics via its interaction with Arp3 and EB1 in Sertoli cells, which will need further verification in future investigation.

17.4   Drebrin E and Ectoplasmic Specialization (ES) Dynamics

Apical ES is a hybrid atypical adherens junction because this cell-cell anchoring junction is constituted by proteins that are found in AJ (e.g., nectins, afadins, N-cadherin), TJ (e.g., JAM-C, CAR), gap junction (e.g., Cx43), and focal adhesion complex (e.g., α6β1-integrin, laminin-α3, laminin-β3, laminin-γ3, FAK) in other epithelia, illustrating it is composed of proteins more than just components of adherens junctions (Wong et al. 2008a; Cheng and Mruk 2010). In the rat testis, the stage-specific and spatiotemporal expression of drebrin E at the apical ES resembles that of Arp3 (Li et al. 2011). Arp3 is a component of the Arp2/3 protein complex which when activated by N-WASP upstream triggers barbed end actin nucleation (Weaver et al. 2003), causing branched actin polymerization on preexisting actin filaments. This thus converts existing actin microfilaments from a linear to a branched configuration, destabilizing adhesion protein complexes at the site, facilitating endocytic vesicle-mediated protein trafficking events (Cheng and Mruk 2011). During the epithelial cycle, Arp3 expresses predominantly at the apical ES in stage VII tubules, mostly at the concave side of the head of step 19 spermatids (Lie et al. 2010), converting the site into an ultrastructure known as apical tubulobulbar complex (TBC) (Vogl et al. 2013), which is also the site where endocytic vesicle-mediated trafficking takes place extensively including protein endocytosis, transcytosis, and recycling so that "old" apical ES proteins can be recycled to assemble "new" apical ES when step 8 spermatids arise at stage VIII of the cycle. However, at stage VIII when spermiation takes place and the apical ES undergoes degeneration, Arp3 becomes downregulated considerably and diminished to an almost undetectable level (Lie et al. 2010). Drebrin E, besides expresses similarly to Arp3 spatiotemporally during the epithelial cycle, also has high affinity to bind to Arp3, illustrating that drebrin E may recruit Arp3 to the ES to induce F-actin reorganization and to alter apical ES dynamics to facilitate spermiation. It has been reported that AJ integrity in endothelial cells is stabilized by drebrin since the knockdown of drebrin by RNAi in human umbilical vein endothelial cell (HUVEC), an endothelial cell line, leads to impairment of endothelial cell-cell junctions by causing mis-localization of afadin, thereby impeding the localization of nectin-2 and nectin-3 at the cell-cell interface (Rehm et al. 2013). It is of interest to note that treatment of Sertoli cells with a functional TJ barrier in vitro with TNFα and TGFβ3 that downregulates drebrin E expression also

increases structural interaction between drebrin E and Arp3 (Li et al. 2011), illustrating an increase in the intrinsic branched actin polymerization activity. This observation is important since it suggests that cytokines may be playing a role in regulating F-actin organization. Studies have shown that TNFα and TGFβ3 as well as their specific receptors are expressed stage-specifically during the epithelial cycle as reviewed (Li et al. 2009), and they also work in concert with testosterone to regulate endocytic vesicle-mediated protein trafficking events at the basal ES (Yan et al. 2008; Xia et al. 2009). These findings seemingly suggest that drebrin E may also be involved in apical ES endocytic function. This notion is supported by findings of a recent report that drebrin E knockdown by RNAi in Coca2 cells caused mis-localization of apical proteins involved in directing recycling vesicles for lysosome-mediated protein degradation (Vacca et al. 2014), illustrating the involvement of drebrin E in degradation function of lysosomes via its effects on endocytic vesicle-mediated protein trafficking in intestine epithelial cells. In summary, drebrin E serves as a platform to recruit actin regulatory proteins such as Arp3 to the apical ES for actin remodeling. It also participates in the endocytic vesicle-mediated trafficking events to confer plasma membrane plasticity, accommodate changes in spermatid shape, promote germ cell transport, and induce junction restructuring.

17.5 Drebrin, Cofilin, and the Arp2/3 Complex and F-actin Organization

Actin depolymerization factor (ADF, also known as destrin)/cofilin is a family of actin-binding proteins composed of destrin, cofilin-1 (in non-muscle cells, also known as n-cofilin), and cofilin-2 (in muscle cells) (Bowman et al. 2000; Shirao and Gonzalez-Billault 2013). Cofilin has the intrinsic activity to sever and disassemble/depolymerize actin microfilaments, creating new filament fragments with both barbed (+) and pointed (−) ends, so that the Arp2/3 complex can induce branched actin polymerization at the barbed end, also the fast growing end of an actin filament (Ichetovkin et al. 2002), which is essential for rapid turnover of F-actin in mammalian cells for microfilament remodeling (De La Cruz and Gardel 2015; Ohashi 2015; Rust 2015; Bernstein and Bamburg 2010), including Sertoli cells in response to changes in cellular environment in the epithelium during the epithelial cycle. Cofilin also depolymerizes actin monomers from the pointed (−) end of an existing microfilament, which can be recycled to the barbed (+) end for actin nucleation by working in concert with the Arp2/3 complex (Condeelis 2001; DesMarais et al. 2004) to rapidly create a network of branched actin filaments. Studies in synaptic biology have also shown that ADF/cofilin is working in close contact with drebrin for the formation of dendritic spine from dendritic filopodia, involving both actin filaments and microtubules (Shirao and Gonzalez-Billault 2013). This is analogous to Sertoli cells establishing close contacts with developing spermatids during spermiogenesis, such as the establishment of apical ES to confer spermatid transport. In short, emerging evidence has suggested that drebrin is playing a crucial role

in mediating changes in the organization of F-actin in specific cellular structures, such as the ES, by working in concert with cofilin and the Arp2/3 complex to modify F-actin organization. For instance, drebrin has been shown to oppose the ability of cofilin to promote actin severing and depolymerization through competitive binding to F-actin and vice versa (Zhao et al. 2006; Grintsevich and Reisler 2014). Additionally, drebrin also contains an ADF homology domain (Lappalainen et al. 1998; Shirao 1995; Kojima et al. 1993; Shirao et al. 1988), making it structurally related to the cofilin. In fact, besides drebrin E (Li et al. 2011), cofilin-1 is a component of the apical ES in the rat testis, restrictively expressed at the concave side of the spermatid head at late stage VII–stage VIII of the epithelial cycle in an ultrastructure called apical tubulobulbar complex (TBC) (Guttman et al. 2004) which is also the site where extensive endocytic vesicle-mediated protein trafficking takes place due to the appearance of multiple giant endocytic vesicles (Vogl et al. 2013, 2014). Thus, it is conceivable that a disruption of cofilin-1 function at the apical ES/apical TBC would impede spermatogenesis. It is of interest to note that knockout of the cofilin-1 gene in mice leads to embryonic lethality (Gurniak et al. 2005). For instance, cofilin-1 mutant embryos had defects in neural crest-derived tissues, lacking actin microfilament bundles in neural crest cells, thereby impairing neural crest cell migration which thus impedes neural development, causing embryonic lethality (Gurniak et al. 2005). As such, the function of cofilin-1 in spermatogenesis is not known. Studies have shown that cofilin can be inactivated via phosphorylation on its Ser-3 residue by LIM kinases (LIMKs, Lin-11/Isl-1/Mec-3 kinases), namely, LIMK1 and LIMK2 (Takahashi et al. 2003), or reactivated via dephosphorylation at Ser-3 residue by phosphatases such as Slingshot (SSH encoded by the *Ssh* gene) which is a family of phosphatases known to dephosphorylate ADF/cofilin, thereby modulating cofilin function (Niwa et al. 2002). In this context, it is of interest to note that LIMK2 is known to be expressed predominantly by germ cells in the mouse (Takahashi et al. 2002), whereas LIMK1 is expressed in the rat testis (Lui et al. 2003). Furthermore, LIMK1 is working in concert with ROCK upstream and β2-integrin at the apical ES to regulate spermatid adhesion in the testis (Lui et al. 2003). However, the role of LIMK1 and LIMK2 in cofilin-1 activation in the testis remains unexplored. Interestingly, LIMK1-deficient mice developed normally and were fertile except displaying abnormalities in hippocampal dendritic spine structure (Meng et al. 2002). On the other hand, the testis of LIMK2-deficient mice when all three LIMK2 isoforms were disrupted was reduced by 20% at 2–4 months of age by weight, and >50% of the tubules were devoid of spermatids (including round, elongating, and elongated spermatids) with fewer spermatocytes and had signs of germ cell apoptosis and necrosis (Takahashi et al. 2002, 2003), illustrating a gross disruption of spermatogenesis. However, these mice remain fertile (Takahashi et al. 2002) since it is noted that rodents remain fertile even with a decline of 90% of spermatogenetic outputs (Robaire 2003). Interestingly, *Ssh-3* KO mice were fertile and healthy (Kousaka et al. 2008). However, it remains to be assessed if the triple KO of the three Slingshot phosphatases SSH1, SSH2, and SSH3 which are found in mammalian tissues (Ohta et al. 2003) would affect spermatogenesis in particular spermiogenesis and ES function. Since specific KO of cofilin-1 in podocytes (also known as glomerular visceral epithelial cells) in the kidney was recently found to

cause failure in selective filtration barrier function (Garg et al. 2010), thus, it is noteworthy to examine the phenotype of the Sertoli cell-specific KO of cofilin-1 *vs.* triple KO of *Ssh-1*, *Ssh-2*, and *Ssh-3* in mice.

17.6 Drebrin E, Cofilin-1, the Arp2/3 Complex, and Spermiation

Based on such intimate functional and structural relationship between drebrin, cofilin, and the Arp2/3 complex as briefly discussed herein, we propose a hypothetical model depicted in Fig. 17.3, illustrating their likely involvement in actin remodeling at the apical ES. In brief, at stage VII, the expression of drebrin E at the apical ES, mostly at the concave side of spermatid heads known as apical TBC, is robust and intense, which diminishes somewhat by stages late VII–early VIII and considerably weakened in stage VIII when spermiation begins to take place (Li et al. 2011). On the other hand, cofilin-1 is intensely expressed at the apical ES/apical TBC in late stage VII–stage VIII tubules at the same site (Guttman et al. 2004) to confer endocytic vesicle-mediated protein trafficking events including endocytosis, transcytosis, and recycling to facilitate recycling of the "old" apical ES proteins (e.g., β1-integrin, laminins, nectin-2, nectin-3, afadin) for the assembly of "new" apical ES when step 8 spermatids arise at stage VIII of the cycle. However, cofilin-1 remains considerably expressed at the apical ES (Guttman et al. 2004) when drebrin E (Li et al. 2011) and Arp3 (Lie et al. 2010) are virtually not expressed at stage VIII. This downregulation of drebrin E is necessary since both drebrin E and cofilin-1 competes to bind onto the actin microfilaments at the same site (Zhao et al. 2006; Grintsevich and Reisler 2014). Thus, cofilin-1 further severs and depolymerizes actin microfilaments at the apical ES, causing truncation of actin microfilaments, preparing the eventual release of fully developed spermatids (i.e., spermatozoa) at spermiation, possibly in conjunction with the action of MMP-2 (Siu and Cheng 2004). It is obvious that the model depicted in Fig. 17.3 will be updated as more data are available in future years.

17.7 Drebrin E and BTB Remodeling During the Epithelial Cycle

The BTB is one of the tightest blood-tissue barriers in the mammalian body. However, unlike other tissue barriers such as the blood-brain barrier and the blood-retina barrier (Cheng 2012), the BTB undergoes extensive remodeling during the epithelial cycle to accommodate the transport of germ cells of >40-μm in diameter across the barrier. For instance, preleptotene spermatocytes derived from type B spermatogonia that first appear in stage VII tubules and are connected in clones by intercellular bridges must be transported across the BTB at stage VIII, so that spermatocytes can be prepared for meiosis that takes place in stage XIV tubules in the

rat testis (Xiao et al. 2014; Parvinen 1982; Leblond and Clermont 1952). Thus, extensive junction restructuring including actin- and MT-based cytoskeleton reorganization is required to accommodate these events. Drebrin is known to be associated with cell junctions in a number of epithelial cells (e.g., MDBK cells (Madin and Darby bovine kidney epithelial cells) and A431 cells (human epithelial carcinoma cells) in which drebrin is localized to actin microfilament bundles at the cell-cell contact sites at AJ (Peitsch et al. 1999, 2001; Keon et al. 2000). Drebrin also binds to the C-terminal cytoplasmic domain of connexin 43, involving in the maintenance of gap junction function (Butkevich et al. 2004). In the rat testis, drebrin E is also found at the basal ES, a testis-specific AJ which also coexists with GJ, at the BTB based on studies by immunohistochemistry and immunofluorescence analysis (Li et al. 2011). Drebrin E is highly expressed at the BTB at stages IV–V of the epithelial cycle, but it diminishes somewhat at stage VII and considerably weakened by stage VIII (Li et al. 2011), coinciding with the restructuring of BTB. This apparent downregulation is necessary for the transport of preleptotene spermatocytes across the barrier. It is of interest to note that the expression of Arp3 at the basal ES/BTB is most intensely in stage VIII tubules (Lie et al. 2010). Since drebrin E binds to the same site as cofilin-1 in the actin microfilament and their binding is thus mutually exclusive (Zhao et al. 2006; Grintsevich and Reisler 2014), the transient downregulation of drebrin E at stage VIII may be necessary to facilitate cofilin-1 to exert its actin severing/depolymerization intrinsic activity on the actin microfilaments at the basal ES. These changes thus lead to the formation of more truncated actin microfilaments at the site, in conjunction with a robust expression of Arp3 at the BTB in stage VIIl tubules (Lie et al. 2010), thus enhancing branched actin polymerization, thereby converting the F-actin network from a bundled to a branched/unbundled configuration. These changes, in turn, facilitate endocytic vesicle-mediated protein trafficking events so that BTB proteins (e.g., occludin, ZO-1, N-cadherin, β-catenin) from the "old" BTB located at the apical region of the preleptotene spermatocytes can undergo endocytosis, transcytosis, and recycling for the assembly of the "new" BTB behind these germ cells under transport at the barrier (Fig. 17.4). Other biomolecules, such as cytokines (e.g., TGF-ßs, TNF-α) and testosterone and/or its receptor which are known to modulate endocytic vesicle-mediated protein trafficking events (Yan et al. 2008; Xia et al. 2009), are also involved in BTB remodeling as depicted in Fig. 17.4.

17.8   Future Perspectives and Concluding Remarks

Based on recent advances in the field, we conclude that drebrin plays an important role in regulating both apical ES and basal ES/BTB in the testis. Drebrin is also crucial for the transport of germ cells across the BTB and the seminiferous epithelium during the epithelial cycle as noted in both Figs. 17.3 and 17.4. However, many
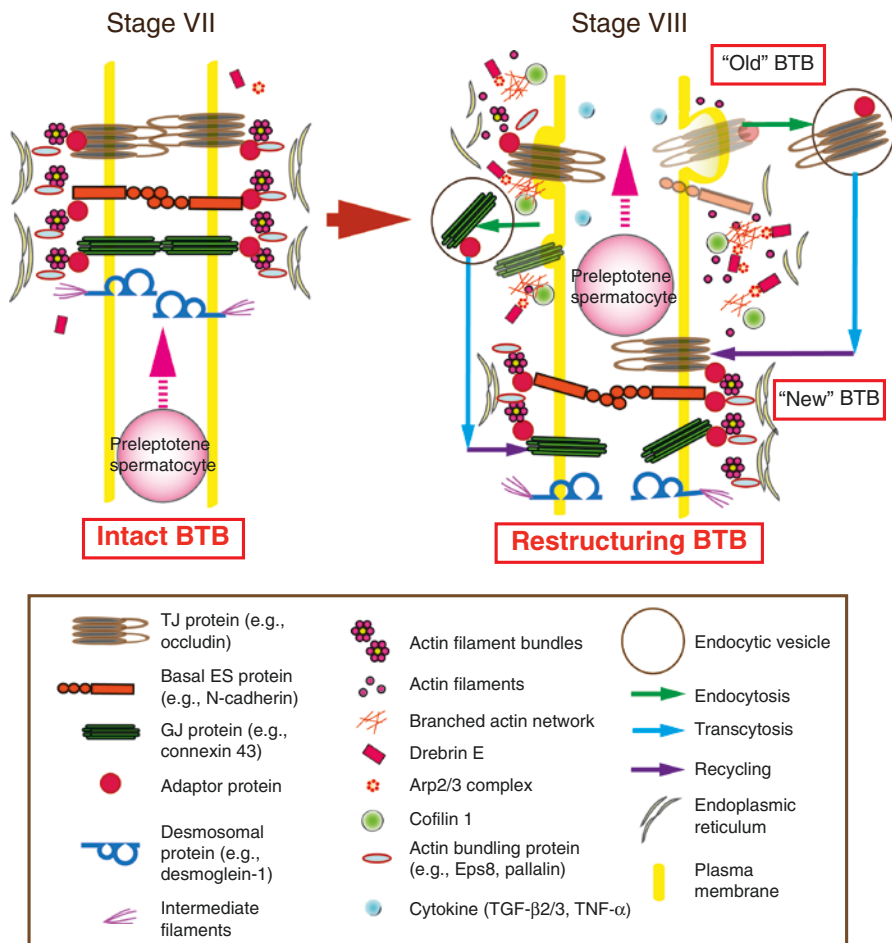
Fig. 17.4 A hypothetical model regarding the involvement of drebrin E in the remodeling of the BTB to facilitate the transport of preleptotene spermatocytes across immunological barrier. At stage VII, the BTB is intact which is supported by the actin microfilament bundles at the basal ES due to the upregulation of actin-bundling proteins such as Eps8, palladin, ezrin, plastin 3, and others (see *left panel*). However, preleptotene spermatocytes transformed from type B spermatogonia at stage VII begin to be transported across the immunological barrier at late stage VII. In late stage VII–early stage VIII, an upregulation of drebrin E recruits Arp3 to the site, so that the intrinsic branched actin polymerization activity of the Arp3 converts actin microfilaments from a bundled to an unbundled/branched configuration; this is also associated with a surge of cofilin-1 which further assists the breakdown of actin microfilaments coupled with a downregulation on the expression of actin-bundling proteins such as Eps8, palladin, ezrin, and plastin 3. These changes thus promote endocytic vesicle-mediated protein trafficking events including endocytosis, transcytosis, and recycling. Thus, "old" BTB above the preleptotene spermatocytes can be transcytosed and recycled to assemble "new" BTB below these spermatocytes to maintain the BTB integrity while preleptotene spermatocytes are being transported across the barrier

open questions remain unanswered. Since the intrinsic actin-binding capacity of drebrin is modulated by its phosphorylation and dephosphorylation, the kinases, phosphatases, and other signaling molecule(s) that are involved in drebrin activation at the ES remain to be identified. Studies in recent years have shown that cyclin-dependent kinase 5 (Cdk5)-p35, a Ser/Thr protein kinase, can phosphorylate drebrin to modulate neuronal migration (Tanabe et al. 2014). Phosphatase and tensin homolog (PTEN), a known binding partner of drebrin (Table 17.1), was also shown to dephosphorylate drebrin at Ser-647 in the brain to regulate neuronal activity (Kreis et al. 2013). It will be of interest to determine if these proteins or other testis-specific kinases and phosphatases would play a role in modulating the function of drebrin. Furthermore, fascin 1, an actin filament-bundling protein in the testis, is a component of apical ES (Gungor-Ordueri et al. 2014a). Interestingly, its stage-specific expression pattern in the seminiferous epithelium closely mimics drebrin E in which fascin 1 is predominantly expressed at the apical ES at stage VII but quickly diminishes by stage VIII (Gungor-Ordueri et al. 2014a). An earlier study has reported that the actin-bundling activity of fascin can be inhibited by drebrin in filopodia (Sasaki et al. 1996). Is it possible that drebrin E modulates or fine-tunes the actin-bundling activity of fascin 1 at the apical ES? Also, what are the phenotypes following an inactivation of drebrin E in the testis? Additionally, the physiological relationship of drebrin and cofilin-1 in the testis will need to be better defined. These questions need to be addressed in future studies.

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