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Review

Biology and regulation of ectoplasmic specialization, an atypical adherens junction type, in the testis

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Abstract

Anchoring junctions are cell adhesion apparatus present in all epithelia and endothelia. They are found at the cell–cell interface (adherens junction (AJ) and desmosome) and cell–matrix interface (focal contact and hemidesmosome). In this review, we focus our discussion on AJ in particular the dynamic changes and regulation of this junction type in normal epithelia using testis as a model. There are extensive restructuring of AJ (e.g., ectoplasmic specialization, ES, a testis-specific AJ) at the Sertoli–Sertoli cell interface (basal ES) and Sertoli-elongating spermatid interface (apical ES) during the seminiferous epithelial cycle of spermatogenesis to facilitate the migration of developing germ cells across the seminiferous epithelium. Furthermore, recent findings have shown that ES also confers cell orientation and polarity in the seminiferous epithelium, illustrating that some of the functions initially ascribed to tight junctions (TJ), such as conferring cell polarity, are also part of the inherent properties of the AJ (e.g., apical ES) in the testis. The biology and regulation based on recent studies in the testis are of interest to cell biologists in the field, in particular their regulation, which perhaps is applicable to tumorigenesis.

Keywords: Anchoring junction; Adherens junction; Testis; Spermatogenesis; Ectoplasmic specialization; Atypical adherens junction

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1. Introduction

Cell–cell adhesion between epithelial cells is mediated by tight junctions (TJs) and anchoring junctions, such as the cell–cell actin-based adherens junctions (AJs) and cell–cell intermediate filament-based desmosomes [1–3]. In mammalian epithelia, AJs are adhesive structures that link integral membrane proteins to actin microfilament network through various intracellular adaptor proteins which maintain tissue integrity [2]. Initially seen as static adhesive structures, AJs are now known to undergo constant restructuring during both normal and pathological conditions by internalization, recycling, and endosome-mediated degradation of junctional proteins [4–6]. In addition, recent findings have shown that AJs also function as a signaling platform. For instance, it is known that β-catenin acts as a transcriptional cofactor in Wnt signaling, regulating gene expressions which are important for cellular proliferation and differentiation [7–10]. α-Catenin and p120 catenin also involve in signaling processes by transmitting extracellular signals across the cells and affect subsequent cellular behaviors [9]. In this review, we focus much of our discussion on AJ dynamics in the testis. Advancements in understanding the biology and regulation of AJs based on studies in other epithelia or endothelia are also highlighted in order to provide a balanced treatment on this topic.

Throughout spermatogenesis, extensive junction restructuring, in particular AJs at the Sertoli–Sertoli and Sertoli–germ cell interfaces, takes place in the seminiferous epithelium [11–13]. This allows developing germ cells to migrate from the basal compartment of the seminiferous epithelium towards the adluminal compartment for further development [14] (Fig. 1). Thus extensive AJ turnovers occur at the cell–cell interfaces and this makes testis a unique organ to study AJ dynamics. In the past decade, studies have shown that germ cell development during spermatogenesis and cancer cells during tumorigenesis share some similarities as both cell types are rapidly dividing. For instance, a new class of proteins known as cancer/testis (CT) antigens is found to express in a range of human cancers where these proteins are normally restricted to germ cells and trophoblasts only (Table 1). The first CT antigen that was identified was MAGEA which is restrictively expressed in melanoma, breast carcinomas and testis [15]. The exact functional roles of CT antigens in tumorigenesis and spermatogenesis remain to be defined but several CT antigens are thought to have fundamental roles in tumorigenesis [15–17]. Additionally, a few CT antigens which are restricted to spermatocytes and spermatids were shown to be related to Sertoli–germ cell interactions (Table 1). Thus, a thorough understanding in the involvement of these CT antigens (e.g., TPX1, SPA17) (Table 1) in AJ dynamics during spermatogenesis may indeed provide new insights underlying cancer development which also involves cell movement. Furthermore, the migration of germ cells across the seminiferous epithelium may also share some links with cancer metastasis. In this context, it is of interest to note that epithelial–mesenchymal transition (EMT), an important mechanism in cancer progression and invasion where cancer cells lose cell–cell contacts by down-regulating junction proteins, such as E-cadherin, in order to acquire migratory ability, was shown to be regulated by transforming growth factor-β (TGF-β) [18–20]. TGF-β is a crucial cytokine recently shown to be involved in facilitating TJ and AJ dynamics in the testis via different downstream MAP kinase signaling molecules [21–23]. Apparently, TGF-β also disrupts cell–cell adhesion in the seminiferous epithelium to allow germ cells migration via down-regulation of TJ and AJ proteins such as occludin, ZO-1, N-cadherin [23].

The seminiferous epithelium is composed of two types of cells: Sertoli cells and germ cells at different stages of development, which together rest on the tunica propria. Tunica propria consists of an acellular zone: basement membrane and type I collagen fibril network, and a cellular zone: peritubular myoid cell layer and the lymphatic components. The major functions of Sertoli cells include: 1) provide structural supports and nourishment to developing germ cells, 2) confer cell polarity, and 3) create an immunological barrier to sequester post-meiotic germ cell antigens from the systemic circulation [13,24–28]. The later function is conferred by the blood–testis barrier (BTB), which is constituted by TJs, the basal ectoplasmic specialization (basal ES), the basal tubulobulbar complex (basal TBC) (both are testis-specific actin-based AJs), and the desmosome-like junctions between adjacent Sertoli cells [27–29]. In contrast to other epithelia where the AJ adhesion belt is distinctly situated underneath TJs near the apical portion of the cell epithelium, basal ES, basal TBC and desmosome-like junctions are present alongside with TJs at close proximity to the basement membrane (a modified form of extracellular matrix) [30] in the seminiferous epithelium (Fig. 1). The BTB also segregates the seminiferous epithelium into the basal and adluminal compartments (Fig. 1). Furthermore, another type of ES, the apical ES, is found between Sertoli cells and developing spermatids (from step 8 and beyond) where TJs are not present [12,13,31,32] (Fig. 1).

Since the biology of the testis may not be familiar to some readers, we first provide a succinct background on the physiology and cell biology of the seminiferous epithelium. In the second part, we discuss some of the recent findings involving the roles of (1) kinases and phosphatases, (2) proteases and protease inhibitors, (3) endocytosis and (4) cell polarity proteins in AJ dynamics in the testis. This information shall be helpful not only to reproductive biologists, but the fascinating features of AJ dynamics during spermatogenesis in the testis may elicit interests amongst cell biologists in other disciplines.

2. Biology of AJ in the seminiferous epithelium

In adult rat testis, germ cells at different stages of their development in the seminiferous epithelium display a unique pattern of association with Sertoli cells which can be classified into fourteen stages of I to XIV (Fig. 2) [33,34]. These stages in turn constitute one cycle of the seminiferous epithelium. The seminiferous epithelium in a given stage is composed of a different combination of germ cell types, such as spermatogonia, spermatocytes, round spermatids, elongating spermatids, and elongated spermatids, and their association with Sertoli cells. Furthermore, the development of round spermatids into elongated spermatids via spermiogenesis is also divided into 19 steps (step 1 through

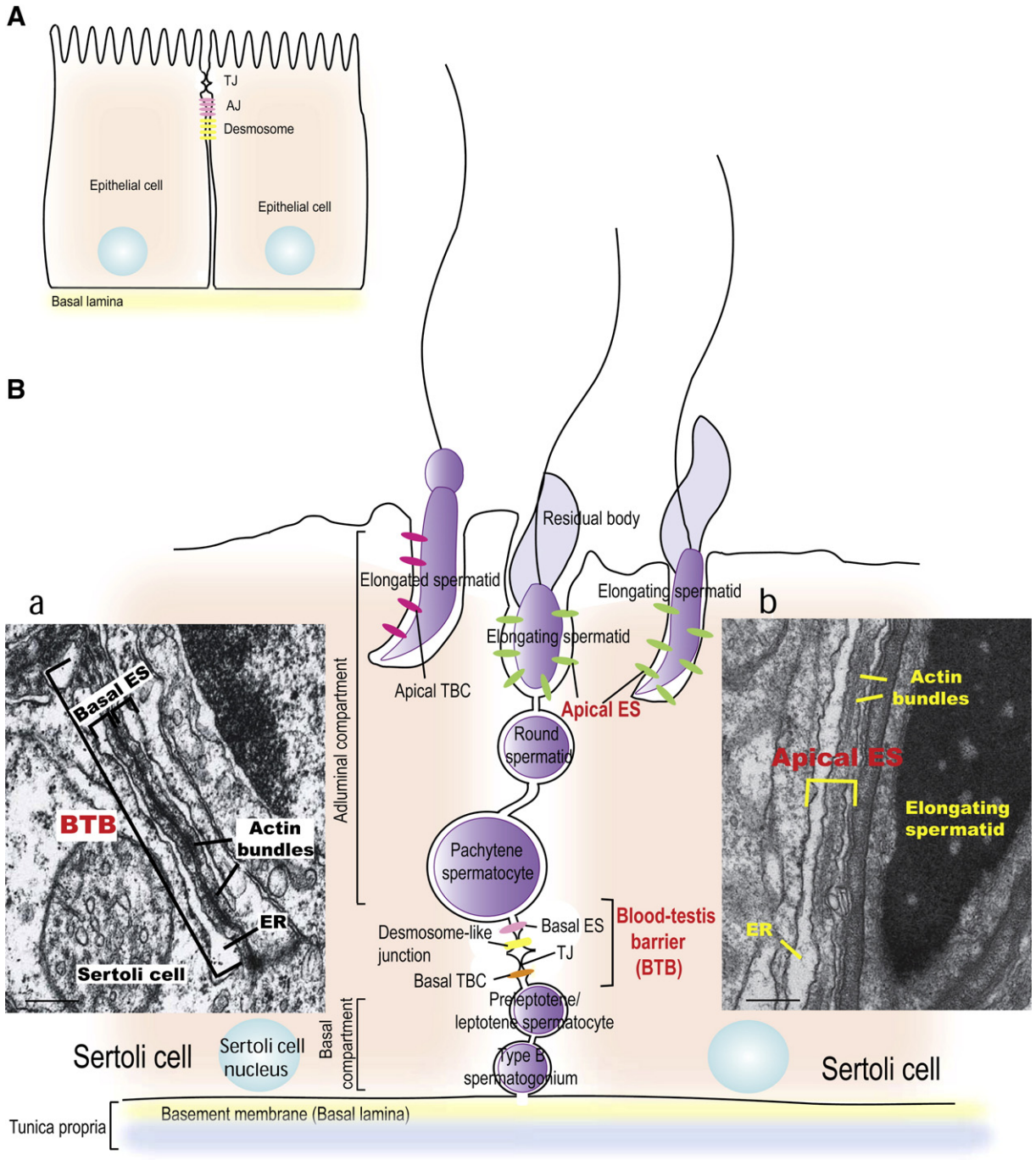

Fig. 1. Schematic drawing illustrating some of the junctions found in the seminiferous epithelium of adult rat testes versus other epithelia. The ultrastructures of the basal and apical ectoplasmic specialization (ES) are also shown in electron micrographs. (A) In most epithelial cells, the tight junctions (TJs), adherens junctions (AJs) and desmosomes are distinctly segregated and located at or near the apical end of the cells, away from the basal lamina. These junctions, in turn, constitute the junctional complex. This is in sharp contrast to the relative position of these junctions in the seminiferous epithelium (see B). (B) The seminiferous epithelium is physically divided into the basal compartment and adluminal compartment by the blood–testis barrier (BTB), which is created by adjacent Sertoli cells near the basement membrane (basal lamina) (see the left panel in B). The BTB, in turn, is constituted by TJ, basal ectoplasmic specialization (ES), basal tubulobulbar complex (TBC) (both are testis-specific AJs) and desmosome-like junctions intermixed and located in close proximity to the tunica propria which is subdivided into the acellular zone: basement membrane (a modified form of extracellular matrix, also called basal lamina) and collagen fibril network layer, and the cellular zone: peritubular myoid cell layer and the lymphatic vessel. Junction types at the BTB, so as the apical ES and apical TBC are highlighted. Note that AJ, desmosome-like junctions and gap junctions are found between Sertoli and germ cells at different stages of germ cell development during the seminiferous epithelial cycle of spermatogenesis, but they are not labeled here. The inset (a) in panel B is an electron micrograph showing the cross-section of the seminiferous epithelium of an adult rat testis illustrating the BTB between two Sertoli cells. Basal ES, which is typified by the presence of actin filament bundles sandwiched between the Sertoli cell plasma membrane and cisternae of the endoplasmic reticulum (ER), at the BTB, present on both sides of the two Sertoli cells, is also shown. The inset (b) in panel B is also an electron micrograph showing the ultrastructural features of the apical ES between Sertoli cell and an elongating spermatid. Similar to the basal ES, apical ES is typified by the presence of actin filament bundles sandwiched between the Sertoli cell plasma membrane and cisternae of the ER. However, these distinct ultrastructures are restricted only to the Sertoli cell side and not found at the spermatid side. Bar in (a) and (b) is 0.2 and 0.3 μm, respectively.

19) based on changes in the morphology of the acrosome, sperm head, and sperm tail (Fig. 2).

In the testis, the best-characterized AJ is the ES [12,13,35–39]. ES is a testis-specific cell–cell actin-based AJ. Two types of ES are found in testes namely the basal ES and the apical ES. Basal ES is restricted to adjacent Sertoli cells at the BTB while apical ES is limited to Sertoli cells and elongating spermatids (step 8 and beyond) [12,13,31,32,40,41]. Instead of the apical ES, cell adhesion between Sertoli cells and step 1–7 spermatids is conferred by AJs and desmosome-like junctions.

ES is a unique structure in the testis [36,39,41]. First, the basal ES is intermixed with TJs, basal TBC and desmosome-like junctions at the BTB adjacent to the basement membrane, which is in contrast to other epithelia where these junction types are distinctively segregated [29] (Fig. 1). Second, although apical ES has no ultrastructural features of TJ, several TJ proteins (e.g., coxsackie and adenovirus receptor, CAR and junctional adhesion molecule-C, JAM-C) are recently shown to be integral membrane components of the apical ES [42–44]. In addition, focal adhesion complex (FAC) proteins, which are normally restricted to cell–matrix interface, such as integrin α6β1–laminin-333 complex is found at the apical ES [31,45,46]. Connexin 43, a gap junction protein is localized in the seminiferous epithelium at the basal and apical ES [47]. The presence of TJ (e.g., CAR, JAM-C), FAC (e.g., integrins, laminins) gap junction (e.g., connexin 43) and AJ (e.g., nectins, afadins) proteins at the apical ES was thought to be important to facilitate the extensive junction restructuring events during germ cell movement in spermatogenesis [31]. Thus, even though apical ES is the only junction type at the developing spermatid–Sertoli cell interface, it contains the best features of other junctions that are needed to confer adhesion (e.g., AJ), communication (e.g., GJ), cell movement (e.g., FAC) and cell polarity (TJ).

In adult rat testes, the number of Sertoli cells, about 40 million, remains relatively unchanged throughout the entire adulthood since they cease to divide by day 15 post-partum [48,49]. Each Sertoli cell structurally and nutritionally supports

Table 1
Integral membrane proteins, their peripheral proteins, and cancer/testis antigens (CT) pertinent to AJ dynamics in the testis during the seminiferous epithelial cycle of spermatogenesis*

Integral membrane/cell surface proteins	Location	Peripheral proteins
N-Cadherin, E-cadherin	Apical and basal ES but mostly at the basal ES	α, β, and γ catenins, protein kinase G, nitric oxide synthases, Src, p-Src, p-ERK, WASP, Rab 8, Cdc42, IQGAP1, dynamin-2, soluble guanylate cyclase, $p120^{ctn}$, Fer kinase, MTMR2, axin, zyxin
α6β1-Integrin, β2-integrin	Mostly apical ES	ILK, FAK, p-FAK, PKB, PAK, paxillin,inculin,PI 3-K, PTEN, $p130^{Cas}$, MMP-2, MT1-MMP, TIMP-2, c-Src, Rho B, LIMK1, ROCK1,
Laminin α3β3γ3**	Restricted to apical ES	c-Src, p-FAK
Not known***	Apical and basal TBC	dynamin 3, cofilin
Nectin-2,-3	Mostly apical ES, also present in basal ES	afadin
CAR	Apical and basal ES	Src, β-catenin, vinculin
JAM-C	Apical ES	Par3, Par6, aPKC, Cdc42, Pals1, PATJ
Cancer/testis antigens+	**Expression during germline development**	**Functions**
$TPTE^{++}$	Primary and secondary Spermatocytes	PTEN-related tyrosine phosphatase [208]
TPX1	Spermatocytes	Spermatocytes binding to Sertoli cells [209]
SCP1	Spermatocytes	Components of the synaptonemal complex [210]
SPA17	Spermatocytes, round spermatids	Cell–cell adhesion function between germ cell and Sertoli cells [211]

*This list is not intended to be exhaustive due to the page limit, however, it serves as a guide for investigators in the field based on recent findings, and additional information can be found in the following original research articles [22,42–45,57,74,76–78,82,86–88,94–97,114,118,123–128,212]; **, laminin-333 is restricted to elongating and elongated spermatids, it does not possess a transmembrane domain [45] and the protein(s) that anchors laminin on spermatid surface remains to be identified; ***, the integral membrane proteins for tubulobulbar complex (TBC) is currently not known; +, selected cancer/testis antigens (CTA) that are expressed only in selected cancers and developing germ cells pertinent to cell adhesion and/or germ cell development are included. ++, TPTE is included in this list since earlier studies have shown that PTEN is part of the integrin/PAK/PKB complex that regulates apical ES function and TPTE is a member of the PTEN family [82]. aPKC, atypical protein kinase C; CAR, coxsackie and adenovirus receptor; FAK, focal adhesion kinase; ILK, integrin-linked kinase; IQGAP1, IQ motif containing GTPase activating protein 1; JAM-C, junctional adhesion molecule-C; LIMK1, LIM kinase 1; MMP-2, matrix metalloprotease-2; MT1-MMP, membrane-type 1-matrix metalloprotease; MTMR2, myotubularin-related protein2; $p120^{ctn}$, p120 catenin; $p130^{Cas}$, protein encoded by Crk-associated protein; PAK, p21-activated kinase; Pals1, protein associated with Lin seven 1; Par3, partitioning-defective 3; Par6, partitioning-defective 6; PATJ, Pals1 associated tight junction protein; p-ERK, phospho-extracellular signal-regulated kinase; p-FAK, phospho-focal adhesion kinase; PI3K, phosphatidylinositol 3-kinase also known as phosphoinositide 3-kinase; PKB, protein kinase B; p-Src, phospho-Src; PTEN, phosphatases and tensin homolog deleted on chromosome ten; ROCK1, Rho-associated protein kinase 1; SCP1, synaptonemal complex protein 1; SPA17, sperm autoantigenic protein 17; TIMP2, tissue inhibitor of metalloproteinase 2; TPTE, transmembrane phosphatases with tensin homology; TPX1, testis specific protein 1; WASP, Wiskott–Aldrich syndrome protein.

30–50 germ cells at different stages of their development [50]. By DNA flow cytometry, it is known that more than 60% of the germ cells in the seminiferous epithelium of adult rat testes are composed of spermatids at step 8 and beyond [51,52]. Thus, the apical ES between Sertoli cell and developing spermatids is the principal AJ type in the seminiferous epithelium. Furthermore, junctions such as desmosome-like junctions and gap junctions disappear once the apical ES is detected at the spermatid–Sertoli cell interface [13,53]. Thus the apical ES is also regarded as the multi-functional cell adhesion apparatus in the testis.

The apical ES is typified by a layer of hexagonally packed actin filament bundles sandwiched between the Sertoli cell plasma membrane and cisternae of the endoplasmic reticulum, encircling almost the entire head region of the elongating/elongated spermatids [40] (Fig. 1). A few hours prior to spermiation, the apical ES is replaced by apical TBC which is restricted only to the concave side of the elongated spermatid heads [54–57]. Thus, the apical ES and apical TBC do not co-exist in the testes. Interestingly, there is no distinctive ultrastructure on the spermatid side of the apical ES under electron microscope (see Fig. 1B-b). For the basal ES, the ultrastructures are the same as of the apical ES except that actin filament bundles and cisternae of endoplasmic reticulum are present on both sides of the two adjacent Sertoli cells. In addition, they always co-exist with TJ, alongside with desmosome-like junctions and gap junctions (GJ) at the BTB [58–61].

2.1. Basal ES

The intimate association of the basal ES and TJs at the BTB has made the study of the basal ES difficult. Antibody that stains a basal ES protein is difficult to distinguish from TJ-associated

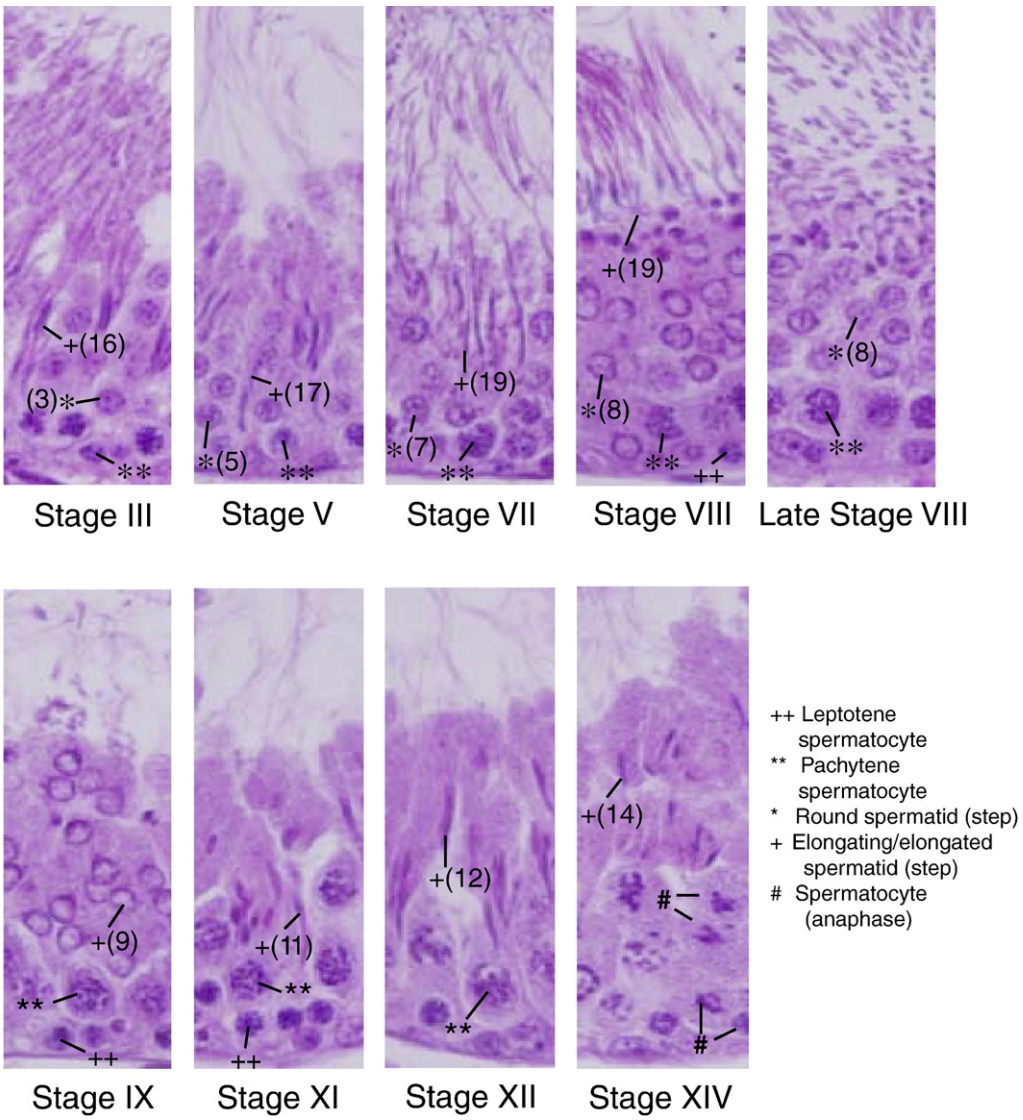

Fig. 2. Cross-sections of adult rat testes illustrating representative stages of the seminiferous epithelial cycle of spermatogenesis. In rats, a total of 14 stages of I to XIV can be defined in a complete epithelial cycle based on the unique association between Sertoli cells and germ cells at different stages of their development. Paraffin sections of testes were stained with hematoxylin and eosin showing specific stages of the epithelial cycle. The Arabic numbers in the diagram indicate spermatids at different stages of spermiogenesis. In rats, a total of 19 steps of spermatids can be defined during spermiogenesis.

proteins because they are both located at the BTB. However, studies in TJs and AJs in multiple epithelia where TJs and AJs are morphologically segregated have allowed investigators in the field to dissect the basal ES from TJs at the BTB. For instance, occludin, claudins and JAMs are putative TJ integral membrane proteins in virtually all other epithelia [1,62–66]. Thus investigators can safely assume that these proteins are the constituent components of the TJs at the BTB. Indeed, occludin, claudins, JAM-A and ZO-1 are found to localize at the BTB in the seminiferous epithelium [22,67–70]. Furthermore, biochemical studies have shown that these are putative TJ proteins since they are restricted to Sertoli cells in vivo and in vitro [13,21,67,68,71]. On the other hand, putative AJ proteins such as N- and E-cadherins, catenins, nectins and afadins are also localized to the seminiferous epithelium at the BTB site [22,72–76]. Thus, cadherins, catenins, nectins and afadins are thought to be components of the basal ES. However, studies by immunohistochemistry and/or immunofluorescent microscopy have also detected cadherins and nectins at the apical ES [76–78] and in some cases such as N-cadherin, it displays stage-specific staining pattern, being present in stages I through VII, but becomes very weak at stage VIII [77]. These findings were further strengthened by studies using primary Sertoli cells culture in recent years. For instance, it is known that Sertoli cells establish functional TJ-permeability barrier in vitro, mimicking the BTB in vivo based on physiological barrier assays (e.g., quantifying the transepithelial electrical resistance, TER; polarized secretion of Sertoli cell products) [79–81] and electron microscopy studies [82–84]. Also, the production of these AJ proteins (e.g., N-cadherin, β-catenin, $p120^{ctn}$) and their localization are tightly associated with the Sertoli–Sertoli cell interface and the assembly of functional BTB barrier [74,78,85].

2.2. Apical ES

Recent studies have shown that the apical ES is a hybrid anchoring junction type having the properties of AJ, focal contact, TJ, and gap junction (GJ). For instance, α6β1 integrin is the first identified apical ES protein in adult rat testes [86,87] as well as a component of the basal ES [88]. In most epithelia, integrins are the receptors of laminins and collagens which are restricted to the cell–matrix focal contacts and being used to facilitate cell migration (e.g., fibroblasts and macrophages) [89–91]. Subsequent studies identified laminin γ3 chain as the first non-basement membrane laminin chain found in adult mouse testes [92], possibly at the apical ES site. Additional biochemical studies have identified laminin γ3 to be a putative spermatid product which forms a bona fide complex with β1 integrin residing in Sertoli cells [93]. Further studies have shown that laminin γ3, together with laminin α3 and β3 forms a functional integrin binding protein. This illustrates that laminin α3β3γ3 is the functional ligand residing in spermatids that form a putative protein adhesion complex with α6β1-integrin receptor residing in Sertoli cells [45]. Additionally, many regulatory proteins at the focal contacts are also found at the apical ES. These include integrin-linked kinase (ILK), focal adhesion kinase (FAK), p-FAK, Src, vinculin, paxillin and others [82,88,94–97] (Table 1). These studies suggested that the testis is adopting the junctional apparatus that is used in cell migration at focal contacts to facilitate junction restructuring events and germ cell movement during spermatogenesis.

On the other hand, coxsackie- and adenovirus receptor (CAR) is a known integral TJ protein in many epithelia [98,99]. Recently, it is suggested that CAR is being used to mediate viral and cellular migration, traversing the TJ-barrier [100,101]. As such, migration of neutrophils across the microvascular endothelial TJ-barrier to the site of inflammation was thought to be mediated by CAR [101]. In rat testes, CAR is a putative product of Sertoli [44] and germ cells [43,44] and is found at the Sertoli–Sertoli and Sertoli–germ cell interfaces in vitro [44]. These findings seemingly suggest that CAR might also be used to facilitate germ cell migration via a yet-to-be defined mechanism since both Sertoli and germ cells are equipped with this novel cell migration-related TJ-protein [102].

In short, the apical ES is an efficient and novel cell adhesion apparatus. However, it is also equipped with component proteins usually restricted to focal contacts, TJs, and gap junctions. It is tempting to speculate that this dynamic AJ type perhaps is utilizing some features of these other junctions (e.g., TJ) to confer the needed functionality, such as cell polarity for spermatid orientation during spermatogenesis. For the past decade, different laboratories including ours have put much effort to understand the biochemical and molecular nature of this cell adhesion apparatus with some success. It was shown that Adjudin, (1-(2,4-dichlorobenzyl)-1H-indazole-3-carbohydrazide), is able to induce dislodgement of elongate spermatids as early as 6.5 h in 50% of the seminiferous tubules after a single dose of 50 mg/kg b.w. by gavage [103,104]. By day 4, virtually all the tubules examined had become devoid of spermatids [32,104,105]. While there is no specific organ uptake of Adjudin following its administration by gavage [103], these results illustrate that the apical ES, being one of the tightest cell adhesion apparatus in adult testes [106], can become preferentially disrupted following treatment with Adjudin [107], illustrating that it is the target of Adjudin. This also explains the significant effects of Adjudin treatment to adult rats in depleting spermatids from the seminiferous epithelium. Interestingly, the cell adhesion between spermatogonia and Sertoli cells at the basal compartment is virtually undisrupted [103,104,108]. It is obvious that much research and effort are needed to delineate the architecture and biochemical composition of the ES in the testis since this is a prime candidate of intervention to transiently disrupt male fertility. Apart from Adjudin, suppression of intratesticular androgen level by using testosterone-estradiol (TE) implants [95, 109, 110] also leads to a disruption of apical ES and the subsequent detachment of elongating spermatids from the seminiferous epithelium [109,110]. Interestingly, in the androgen suppression model, the BTB remains intact indicating that junction restructuring is limited to the apical ES, without affecting the BTB integrity [75]. These models are useful for studying AJ dynamic in vivo. As such, they are also distinct from other commonly used models to study junction dynamic, such as the in vitro calcium switch model in which TJ integrity is also compromised.

3. Role of kinases and phosphatases in AJ dynamics in the testis

In mammalian cells, about 30% of the cellular proteins are phosphorylated, illustrating the importance of phosphoproteins in physiological processes [111]. It is known that tyrosine phosphorylation of junction-associated proteins plays a crucial role in junction assembly at both the blood–brain barrier [112] and the BTB [29]. Studies have shown that members of Src protein tyrosine kinase family are localized at AJs [113]. In the testis, Src and its activated form, p-Src, are both localized to the basal ES and apical ES in the seminiferous epithelium, with p-Src more predominantly located at the apical ES [95,114]. β-Catenin becomes highly phosphorylated in Src transfected cells indicating it is a putative substrate of Src protein kinases [115]. Indeed Src, carboxyl-terminal Src kinase (Csk), and casein kinase 2(CK2) are components of both Sertoli and germ cells and are physically associated with β-catenin in rat testes [114]. Besides Src and p-Src, Csk was also shown to localize to the apical ES in the seminiferous epithelium of rat testes [114]. Studies from our laboratory have also demonstrated the presence of myotubularin related protein-2 (MTMR2), a member of the myotubularin family and putative lipid/protein phosphatase in Sertoli and germ cells [116,117]. Its expression level is induced when functional Sertoli cell TJs are being assembled in vitro [116], and MTMR2 apparently forms a functional protein complex with c-Src to regulate Sertoli–germ cell adhesion [118]. These findings have illustrated that kinases and phosphatases are integral components of apical ES and basal ES. By using various protein tyrosine phosphatase inhibitors (PTPi), it was shown that both β-catenin and ZO-1 could be tyrosine phosphorylated and are putative substrates of tyrosine kinases [119]. Also, vanadate (a specific PTPi) can induce TJ-permeability disruption in MDCK cells in vitro by increasing the cellular phosphoprotein content [120]. This is consistent with recent studies demonstrating vanadate indeed perturbed the Sertoli cell TJ-permeability barrier in vitro [121]. Furthermore, these changes in TJ-permeability barrier in MDCK cells coincided with an increase in the phospho-Tyr immunofluorescence at the site of the TJ and with redistribution of F-actin, E-cadherin and ZO-1 [120]. More importantly, these changes can be blocked in MDCK and/or Sertoli cells by using a protein tyrosine kinase (PTK) inhibitor (PTKi), such as tyrphostin A25 [121], although to a significantly lesser extent when a Ser/Thr protein kinase inhibitor, such as staurosporine, was used [120]. Studies in MDCK cells have shown that the assembly, opening, and re-sealing of the TJ-permeability barrier correlate with the phosphorylation of occludin on the Ser/Thr residues [122]. Consistent with these earlier observations, the disruption of anchoring junction at the Sertoli–germ cell interface (particularly with elongating/elongated spermatids) induced either by androgen suppression [75,118] or by Adjudin [123] was shown to associate with an increase in phosphorylation of β-catenin or LIM kinase 1 (LIMK1 also called *l*in-11 *i*sl-1 *m*ec3 *k*inase *1*). The physiological significance of these studies remains to be fully elucidated and that additional studies are needed to expand these observations, such as by immunohistochemistry and/or immunofluorescent microscopy to assess changes in the cellular localization of β-catenin and LIMK1 versus their phosphorylated forms during androgen-induced and Adjudin-induced AJ disruption. Nonetheless, these findings strongly suggest that the assembly and maintenance of AJs (e.g., ES) and TJs are regulated by the phosphorylation status of cellular proteins at the Sertoli–Sertoli and Sertoli–germ cell interface even though the identities for most of these proteins remain to be deciphered. The use of gene profiling technique coupled with appropriate gene chips specifically designed for phosphoproteins could be helpful in this area of research. Taking these results collectively, it is increasingly clear that a decline in cellular phosphoprotein content favors the assembly and maintenance of Sertoli–Sertoli TJ and Sertoli–germ cell anchoring junctions, whereas an increase in cellular phosphoprotein content perturbs junction integrity.

Until now, a major proportion of research on the role of kinases and phosphatases in junction dynamics in the testis was focused on identifying the kinases and phosphatases, as well as their binding partners that were originally identified in other epithelia and/or endothelia [22,42,44,45,57,74,77,78,82,86–88,94–97,114,118,123–128] (Table 1). Few functional studies are available in the literature except it was shown that the use of a c-Src inhibitor (e.g., PP1, $C_{16}H_{19}N_5$) administered locally to the testis could induce unexpected loss of round and early spermatids, but not elongating/elongated spermatids from the seminiferous epithelium, without increasing germ cell apoptosis when assessed by the TUNEL assay [114]. These results thus suggest that a disruption of the intrinsic c-Src kinase activity led to a disruption of desmosome-like junction and perhaps AJ but not apical ES, implicating that these junctions in the seminiferous epithelium are differentially regulated. These findings also suggest that a disruption of the key kinases and/or phosphatases that are crucial to Sertoli–germ cell adhesion can lead to germ cell exfoliation in the testis. However, it is known that the deletion of c-Src [129], Csk [130] or FAK [131] (all are non-receptor protein tyrosine kinases found in the seminiferous epithelium associated with at least one of the known integral membrane proteins that confer cell adhesion or TJ function) can lead to postnatal lethality, embryonic lethality at E10 (post-coitus) and E8.5 (post-coitus), respectively. Thus the precise functional role of these genes/proteins in junction dynamics in the testis can only be studied by using testis- or Sertoli cell-specific knockouts or by using inhibitors that can be delivered to the testes specifically, and behind the BTB to exert its action in the seminiferous epithelium.

4. Proteases and protease inhibitors: their role in spermatogenesis and AJ dynamics

The role of proteases and protease inhibitors in spermatogenesis and male reproduction is known for decades and this subject area has been covered in recent reviews [13,132,133]. For instance, the formation of acrosome during spermiogenesis at the heads of elongating spermatids is an important cellular event, and the proteolytic enzyme acrosin in the acrosome is essential for fertilization [134]. Indeed, recent studies have been conducted to tackle the acrosin/acrosome for male contraception [135,136]. Furthermore, it has been demonstrated that

ubiquitination and the endocytic pathway are being used by Sertoli cells for intracellular processing of unwanted proteins as well as spontaneous degeneration of germ cells as a result of apoptosis [137–139]. This is not entirely unexpected that proteolysis is used by the testes to maintain the cellular homeostasis in the seminiferous epithelium since around 75% of the developing germ cells undergo spontaneous degeneration during spermatogenesis [140,141]. For instance, Sertoli cells cease to divide by day 15 post-partum in rats and the number of Sertoli cells in the testes remains relatively stable throughout the whole adulthood at around 40 millions [48,49]. Each Sertoli cell can only support about 30–50 developing germ cells both structurally and nutritionally [50,142], and thus many of the developing germ cells must undergo spontaneous degeneration during the seminiferous epithelial cycle in order to maintain the appropriate number of germ cells that can be supported by the Sertoli cells. As such, Sertoli cells are phagocytic cells known to serve as scavengers in cleaning up apoptotic and degenerating germ cells in the epithelium [143–145]. In this section, we discuss the latest findings regarding the role of proteases and protease inhibitors on AJ dynamics in the seminiferous epithelium.

4.1. The homeostasis of proteases and protease inhibitors in the seminiferous epithelium and junction dynamics

The first observation illustrating the involvement of proteases in junction restructuring at the Sertoli–germ cell interface was reported by Mruk et al. [146]. It was shown that the assembly of Sertoli–germ cell anchoring junctions in Sertoli-germ cell cocultures in vitro was associated with an induction of protease activities when media from the apical and basal compartments of the bicameral unit were collected for specific protease assays [146]. Subsequent studies have also illustrated the involvement of protease inhibitors in anchoring and TJ assembly between Sertoli cells as well as Sertoli and germ cells [147–150]. For instance, tissue inhibitor of metalloproteases-1 (TIMP-1) was shown to facilitate anchoring junction assembly in Sertoli–germ cell cocultures in a cell adhesion assay [147] and protease inhibitors were also shown to promote the TJ-permeability barrier assembly in Sertoli cells cultured in vitro [148]. Nonetheless, the precise mechanism(s) by which proteases and protease inhibitors take part in junction restructuring during spermatogenesis is virtually unknown. However, in studies using an in vivo model of BTB restructuring induced by cadmium chloride [126,151], it was shown that the steady-state protein level of cathepsin C (a cysteine protease) at the time of BTB disruption and germ cell loss from the epithelium was significantly induced, which was also accompanied by an induction of cystatin C (a cysteine protease inhibitor) as well as α_2-macroglobulin (a non-specific protease inhibitor). Furthermore, this induced production of α_2-macroglobulin during cadmium-induced junction restructuring in the seminiferous epithelium was mediated via the c-Jun N-terminal protein kinase (JNK) signaling pathway [126]. These findings also illustrate that if a specific inhibitor against JNK can be delivered to the testes behind the BTB, it can somehow intervene with the homeostasis of proteases/protease inhibitors in the epithelium, eliciting germ cell loss from the seminiferous epithelium and may potentially serve as a male contraceptive.

4.2. Proteases/protease inhibitors and ES dynamics

Studies have shown that proteases and protease inhibitors are important regulators of apical ES dynamics [13]. For instance, it is known for years that membrane-type 1-matrix metalloprotease (MT1-MMP) is an integral membrane protein [152] which forms a complex with tissue inhibitor of metalloproteases-2 (TIMP-2). This complex in turn serves as the receptor for pro-matrix metalloprotease-2 (pro-MMP-2) for the formation of activated MMP-2 [153,154]. It was found that MT1-MMP was localized in the apical compartment of the seminiferous epithelium [155]. Subsequent studies have shown that MT1-MMP indeed co-localized with MMP-2 and TIMP-2 as well as β1-integrin [93], illustrating that this protein complex can be used to induce cleavage of the cell adhesion complex at the apical ES at spermiation. Consistent with this postulation, pre-administration of rats with a specific inhibitor of MMP-2 intratesticularly prior to treatment of adult rats with Adjudin by gavage could effectively block the Adjudin-induced spermatid loss from the epithelium [93]. Collectively, these results illustrate that spermiation may indeed be initiated via an activation of the MMP-2, which in turn causes cleavage of the integral membrane proteins at the apical ES. This thus facilitates the release of spermatozoa (fully developed spermatids) into the seminiferous tubule lumen. This possibility should be vigorously investigated in future studies.

4.3. An emerging concept

Recent studies have shown that α6β1 integrin residing in Sertoli cells form a bona fide cell adhesion complex with laminin α3β3γ3 residing in elongating/elongated spermatids [45], which is a major cell adhesion complex at the apical ES. Other studies have demonstrated that proteolytic fragments of integrins and/or laminin chains can serve as biologically active fragments to regulate cellular processes. For instance, fragments of laminins could enhance cell migration [156,157]. Furthermore, peptide fragments from the cell-binding domain of laminin α and β1 chain were shown to inhibit Sertoli cell cord structure formation [158]. These other studies thus suggest that proteolytic fragments generated at the apical ES might indeed exert biological effects either locally or remotely from the apical ES site. Consistent with this postulation, a recent study has shown that by using a blocking antibody against one of the laminin-333 chains at the apical ES indeed perturbs BTB dynamics and modulates the steady-state levels of proteins at the BTB [45]. It is possible that proteolytic fragments of laminins and integrins released during spermiation elicit BTB restructuring to facilitate preleptotene spermatocyte migration which occur at stage VIII of the epithelial cycle. This hypothesis must be vigorously tested in future experiments by identifying the putative biological fragments from either laminin-333 or α6β1-integrin. If this is true, this provides the first clue as how the events of spermiation and the transient "opening" (or

Table 2
Components of the CRB and Par complexes in mammals and their corresponding homologues in *Drosophila**

Component proteins (Mammals)	*Drosophila* homologue	References
The CRB complex		
CRB3**	CRB	[172,180]
Pals1	Sdt	[186]
PATJ	DmPATJ	[180,183]
The Par complex		
Par3/ASIP	Bazooka	[196,213]
Par6	DmPar6	[170]
aPKC	DaPKC	[214–216]

*References listed here are the original articles that identified the corresponding polarity proteins in mammals versus their *Drosophila* counterparts.
**aPKC, atypical protein kinase C; ASIP, aPKC isotype-specific interacting protein; CRB, Crumbs; CRB3, Crumbs3; DaPKC, *Drosophila* atypical protein kinase C; DmPar6, *Drosophila* partitioning-defective 6; DmPATJ, *Drosophila* Pals1 associated tight junction protein; Pals1, protein associated with Lin seven 1; Par3, partitioning-defective 3; Par6, partitioning-defective 6; PATJ, Pals1 associated tight junction protein; Sdt, stardust.

restructuring) of the BTB to permit the transit of preleptotene/leptotene spermatocytes across the barrier are coordinated.

5. Regulation of junction dynamics through endocytosis and recycling of integral membrane proteins

As mentioned above, AJ dynamics in the testis are regulated by multiple factors. However, it remains unknown how these factors are working in concert to affect AJ dynamics since the seminiferous epithelium is undergoing continuous junction restructuring to facilitate germ cell movement throughout the epithelial cycle, and rapid turnovers of junctional proteins are expected. Recent studies have suggested that endocytosis is an important mechanism in regulating junction dynamics [4,5]. In general, proteins are internalized through clathrin-coated pits, caveolae or actin-coated vacuolae. Internalized proteins are then sorted in common recycling endosomes where they are either returned to the plasma membrane through recycling endosomes or degraded in lysosomes or proteosomes [5]. At present, the underlying mechanism(s) that precisely regulates the sorting of the endosomes to recycle back to cell surface or intracellular degradation at the lysosomes remain unclear. Studies have shown that E-cadherin undergoes continuous recycling [159,160] and various cytokines and growth factors are shown to speed up protein endocytosis [5,161].

Recently, our laboratory has shown that C-type natriuretic peptide (CNP) regulates BTB dynamics by disrupting the TJ-barrier integrity and overexpression of CNP in Sertoli cells accelerates internalization of junction proteins, such as N-cadherin [162]. In addition, Rab GTPases, such as Rab8B [73] and Rab4A [163], that are involved in vesicle transport, recognition and docking are also found in the testis. It has been demonstrated that Rab4A interacts with α- and β-catenins and there is an increase in their association during disassembly of Sertoli–germ cell adhesion [163]. Dynamin II, a large GTPase involved in vesicle shedding from plasma membrane, was found in the testis and associated with cadherin- and occludin-based protein complexes. It was suggested that dynamin II might be involved in the disengagement of TJ-based and basal ES-based protein complexes at the BTB to facilitate the transit of preleptotene/leptotene spermatocytes across the barrier [127]. The apical TBC, another testis-specific AJ between Sertoli cells and elongated spermatids which appears just a few hours before spermiation, was suggested to take part in internalization of junctional proteins [164]. Collectively, these results suggest that endocytosis may play an active role in regulating AJ dynamics in the testis, which should be vigorously investigated in future studies.

6. Cell polarity and vesicle transport

Conventionally, epithelial and endothelial cell polarity was thought to be conferred by TJs. For instance, apical–basal polarity of epithelial cells is conferred by the differential distribution of

Table 3
Domains/motifs found in polarity proteins and the corresponding interacting partners

Component proteins	Domains	Interacting partners	References
CRB3	PDZ-binding motif (amino acids ERLI)*	Pals1, PATJ, Par6	[172,180,217]
	FERM	/	
Pals1	L27N	PATJ	[183]
	L27C	mLin-7	[186,218]
	PDZ	CRB1, CRB3	[172,183]
	U1 region	Par6	[219,220]
	GUK	/	
	4.1B	/	
	SH3	/	
	/	Rich1, Amot	[175]
PATJ	L27N	Pals1	[180,183]
	PDZ	ZO-3, claudin-1	[189]
	/	Amot, Rich1	[175]
Par-3	C-terminal	Tiam1, LIMK2	[198,199]
	PDZ	Par-6, nectin-1/3, JAM-A/C, VE-cadherin	[169,178,206,221,222]
	aPKC binding	aPKC	[196]
	/	Rich1, Amot	[175]
Par-6	CRIB, PDZ	Cdc42/Rac1	[169,170]
	PDZ	Par-3, Pals1, mLgl, CRB3	[169,170,217,219,220,223]
	PB1	aPKC	[171]
	/	VE-cadherin	[178]
aPKC	KD	mLgl, Par-3	[196,223]
	PB1	Par-6	[171]

* 4.1B, 4.1 binding; Amot, angiomotin; aPKC, atypical protein kinase C; CRB3, Crumbs3; CRIB, Cdc42/Rac interacting binding; FERM, juxtamembrane protein 4.1/ezrin/radixin/moesin; GUK, guanylate kinase; JAM-A/C, junctional adhesion molecule-A/C; KD, kinase domain; L27, Lin-2/Lin-7; LIMK2, Lim kinase 2; mLgl, mammalian Lethal giant larvae; mLin-7, mammalian Lin-7; Pals1, protein associated with Lin seven 1; Par3, partitioning-defective 3; Par6, partitioning-defective 6; PATJ, Pals1 associated tight junction protein; PDZ, postsynaptic density-95/Discs Large/zona occludens-1; PB1, phagocyte oxidase/Bem1; SH3, Src-homology 3; Tiam1, T-lymphoma invasion and metastasis; VE-cadherin, vascular endothelial-cadherin; ZO-3, zonula occludens-3; /, not known.

cellular proteins and macromolecules between the apical and basolateral membrane domains which in turn are physically separated by the TJs (known as the "fence" function). TJs also serve as a selectively permeable barrier to the paracellular diffusion of solutes (known as the "gate" function) [1,165–168]. Initial genetic and biochemical studies in *Drosophila melanogaster* and *Caenorhabditis elegans* have identified two conserved multiprotein complexes known to be associated with TJs in determining cell polarity in mammalian cells namely the Crumbs (CRB) and the partitioning-defective (Par) complexes [167–174] (Tables 2 and 3) (Fig. 3). Surprisingly, recent studies have implicated that polarity proteins are probably involved in vesicle transport of transmembrane proteins, such as E-cadherin, and are associated with AJ proteins in addition to TJs [175–179]. Besides, these polarity proteins were recently shown to be present at junctional plaques on the heads of elongated spermatids (i.e. the apical ES site) in the testis where TJs do not exist [42]. These emerging results suggest that polarity protein complexes are associated with AJs and play a role in AJ dynamics [13, 32]. Herein, we briefly discuss the current status of research on these polarity protein complexes, and the areas of research that deserve attention.

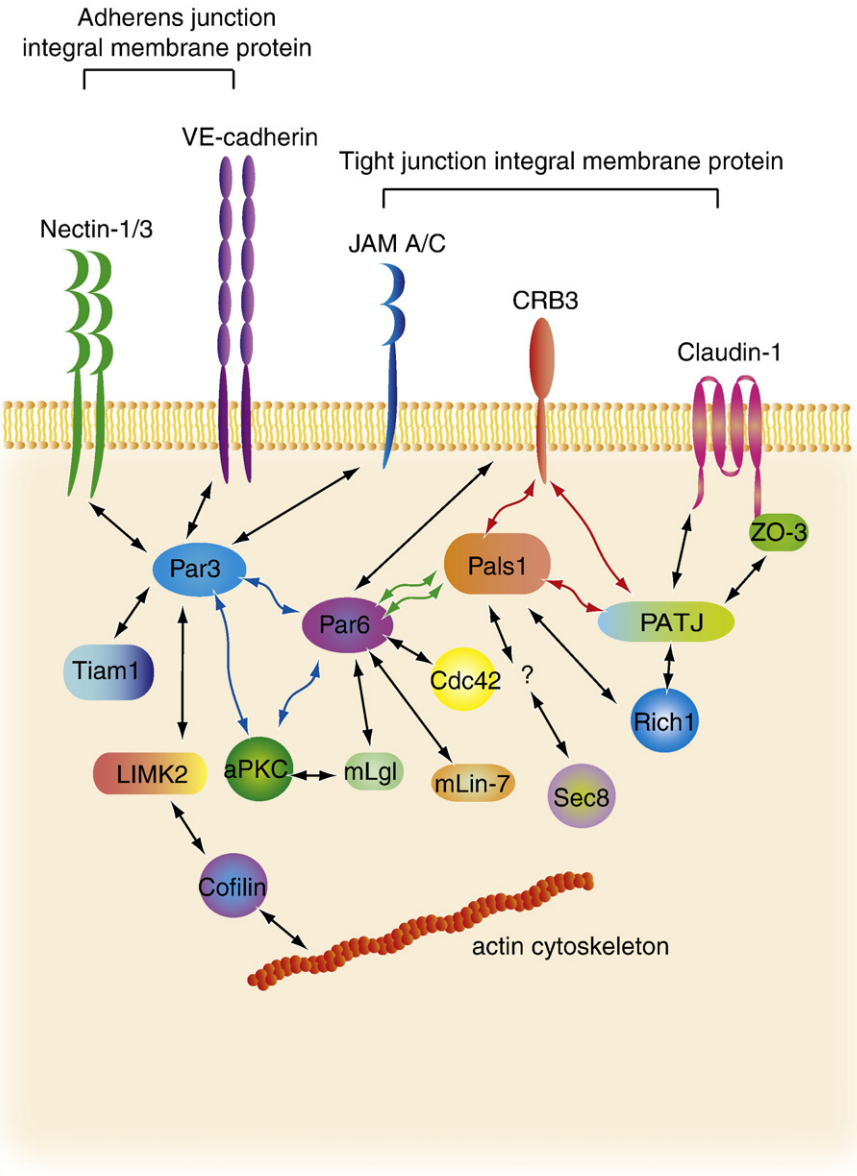

Fig. 3. A schematic drawing illustrating some of the known interactions between cell polarity proteins with various adaptors and integral membrane proteins. This diagram summaries most of the known interacting partners of cell polarity proteins at both tight junction (TJ) and adherens junction (AJ) based on studies in different epithelia and endothelia. Interacting partners are indicated with double arrowheads. Component proteins in the crumb (CRB) and partitioning-defective (Par) complexes are shown in red and blue arrows, respectively. Interaction of the two complexes is via protein associated with Lin-7 1 (Pals1) and partitioning defective 6 (Par6) which is shown in two green arrows. Cell polarity proteins are currently thought to target TJ and/or AJ via interaction with integral membrane proteins such as nectins and junctional adhesion molecules (JAMs), or via peripheral adaptors such as zonula occludens-3 (ZO-3). While most of these interactions are identified in either epithelial or endothelial cells, the precise interaction of these proteins in the testis remains unknown.

6.1. The Crumbs (CRB) complex

In mammalian cells, the CRB3/Pals1/PATJ complex was identified as the homologue of the *Drosophila* CRB/Stardust/DmPATJ complex (Table 2). Three mammalian CRB isoforms were identified but only CRB1 and CRB3 have been functionally studied [168,172,180–182]. Similar to *Drosophila* CRB, CRB1 is a transmembrane protein and it is known to form a complex with Pals1 and PATJ (Table 3) (Fig. 3) and colocalizes to the TJs. However, it is predominantly expressed in the eye and brain [183,184]. This leads to the identification of CRB3 in epithelial cells in subsequent studies. In contrast to *Drosophila* CRB and mammalian CRB1, CRB3 has a small extracellular domain [172,180,185]. Nevertheless, CRB3 has a conserved cytoplasmic domain for interactions with Pals1 and PATJ [172,180].

Pals1, protein associated with Lin-7 1, is a membrane-associated guanylate kinase (MAGUK) protein [186]. mLin-7 and possibly its interacting partners are involved in endosomal sorting by targeting endocytosed proteins to basolateral membrane domain instead of lysosomes for degradation [187]. Similar to other MAGUK proteins, such as zonula occludens (ZO), Pals1 has a guanylate kinase (GUK) domain without any catalytic but protein interaction functions [168,188]. Pals1 also contains the postsynaptic density-95/Discs Large/zona occludens-1 (PDZ) domain which mediates its interaction with CRB1 and CRB3 [172,183].

The third member of the CRB complex is the Pals1 associated tight junction (PATJ) protein. PATJ is a scaffolding protein with 10 PDZ domains [180,183]. Besides Pals1, PATJ also physically interacts with ZO-3 and claudin-1 via the PDZ domains [189]. Recent studies have identified CRB3, Pals1 and PATJ in both Sertoli and germ cells as well as the association of Pals1 with JAM-C at the apical ES (Wong and Cheng, unpublished observations), illustrating their potential role in maintaining spermatid orientation in the seminiferous epithelium.

The CRB complex is important for TJ formation and conferring cell polarity [167,168]. Overexpression of either CRB3 or a dominant negative form of Pals1 which disrupts the association between endogenous CRB3 and Pals1 delayed TJ formation, as well as disrupted the apical–basal polarity[173]. In MDCK and Caco2 cells, reduction of PATJ by RNAi resulted in defects in TJ formation and mislocalization of occludin and ZO-3, respectively [190,191].

The loss of Pals1 expression was shown to delay TJ formation and cell polarization without perturbing AJ formation [192]. Likewise, AJs are not significantly affected in cells with overexpression of CRB3 or PATJ knockdown [173,190,191]. Thus it was thought that the CRB complex was strictly involved in TJ formation and cell polarization but not AJ dynamics until recently when Pals1 was shown to regulate E-cadherin trafficking in mammalian cells [177]. In more severe Pals1 knockdown cells compared to the previous study, apart from pronounced TJ defects, a defect in AJs was also observed [177,192]. For instance, E-cadherin was not effectively translocated to the cell surface although its expression was not down-regulated. E-Cadherin remained inside the Pals1 knockdown cells in puncta-like structures probably due to the disruption of E-cadherin exocytosis; and Pals1 re-expression could correct all the defects [177]. These findings thus suggest that Pals1 plays a direct role in regulating E-cadherin trafficking.

6.2. The partitioning-defective (Par) complex

The Par proteins were first identified as regulators for anterior–posterior polarity of *Caenorhabditis elegans* zygote [193–195]. Subsequently, mammalian Par homologues were identified and found to form an evolutionarily conserved complex with Cdc42 and atypical protein kinase C (aPKC) involved in regulating epithelial cell polarity (Table 2). Par3 or aPKC isotype-specific interacting protein (ASIP) was the first Par homologue identified in mammals [196]. Additional research has demonstrated the importance of Par3 in promoting TJ formation [197–199]. Par3 regulates TJ assembly through binding directly to the Rac exchange factor Tiam1 [199]. It is also involved in actin dynamics by inhibiting Lim kinase 2 (LIMK2) which, in turns, regulate cofilin (an actin severing protein) phosphorylation and its inactivation [198].

Mammalian Par6 was identified as a key adaptor which links Par3 to activated Cdc42/Rac1 and aPKC [169,170]. The involvement of Par6, Cdc42 and aPKC in TJ formation is well defined [169–171,200]. Unlike the CRB complex which seems to be more static, the Par complex is a highly dynamic complex. It has been shown that the asymmetric distribution of Par proteins in complementary membrane domains is mediated by actinomyosin dynamics and mutual molecular interactions through phosphorylation by aPKC [201,202]. Existence of several isoforms of each protein, such as Par3 and Par6, also suggests the dynamic nature of the Par complex; and it may also play other roles apart from TJ formation and cell polarization since the function for some of these isoforms remains to be defined [169,170,196,203].

In *Drosophila*, Bazooka (the mammalian Par3 homolog) is crucial to epithelial cell polarity, which likely exerts its effects upstream of AJ formation and maintenance [204,205]. In contrast to *Drosophila*, the involvement of Par complex in mammalian AJ is not well established until recently when Par3/ASIP was shown to be a component of AJs [196].

Par3 was found to colocalize with AJ proteins nectin-3, afadin and β-catenin in neuroepithelial cells of embryonic telencephalon at E13.5 day post-coitus where TJs are absent [206,207]. Par3 binds directly to nectin-1 and-3 but not nectin-2 and this interaction was thought to be important to target Par3 to AJ [206]. A recent study has reported a distinct Par complex which is associated with another AJ protein vascular endothelial cadherin (VE-cadherin) in endothelial cells. A direct association was found among VE-cadherin, Par3 and Par6 but not aPKC, indicating the absence of aPKC in this complex. Besides, overexpression of VE-cadherin but not platelet-endothelial cell adhesion molecule 1 (PECAM1) disrupted TJs possibly due to sequestration of Par3 and Par6 from TJs. This further strengthens the possibility of two complexes which are associated with the TJs and AJs respectively [178]. The functional role of the Par complex at AJs is not clear and whether or not it resembles the function of Pals1 in regulating AJ protein trafficking remains to be elucidated.

Some recent studies that probe the role of CRB and Par complexes in regulating AJs in the testis are of interest. For instance, during spermatogenesis, developing spermatids must orient themselves properly using the apical ES, so that the heads of the elongating/elongated spermatids are pointing toward the basement membrane until they are released into the tubule lumen at spermiation. We have noticed that in adult rats treated with Adjudin, elongating spermatids were misaligned apparently before detachment of these cells could take place (Wong and Cheng, unpublished observations). Furthermore, recent studies have shown that Par3/Par6/aPKC/Cdc42 is a crucial cell polarity complex downstream of JAM-C, which in turn is an integral membrane protein of the apical ES. JAM-C$^{-/-}$ mice were infertile in which round spermatids failed to differentiate into elongating spermatids and orient properly in the seminiferous epithelium. In addition, the actin bundles failed to form properly at the apical ES [42]. Thus, it is important to examine if the CRB and Par are important polarity complexes that confer spermatid orientation during the seminiferous epithelial cycle in particular if these protein complexes are associated with other integral membrane proteins at the apical ES.

7. Concluding remarks and future perspectives

In this review, we discuss the biology and the unusual features of ES in AJ dynamics in the seminiferous epithelium. It is increasingly clear that junction dynamics in the testis are regulated by the intriguing interactions between kinases and phosphatases, proteases and protease inhibitors, and perhaps the kinetics of protein endocytosis, recycling/and or endocytic degradation. Taking advantage of the unique biology of the seminiferous epithelium and the models that are available to study junction dynamics in the testis, the seminiferous epithelium serves as a good model to investigate AJ dynamics. In particular, models are being established (e.g., the Adjudin model) in which a drug was shown to target apical ES specifically without significant impacts on the BTB integrity at the time of ES restructuring [12,13]. Although ES per se does not possess TJ ulstrastructures, several TJ proteins are found to be the integral components of apical ES. In light of these findings, we propose to call ES as an "atypical adherens junction type". Interestingly, since the presence of CRB, Par, and their interacting partner proteins have recently been identified in Sertoli and/or spermatids at the ES (Wong and Cheng, unpublished observations), it is now possible to use the seminiferous epithelium as a model to study some of the functions of cell polarity proteins in AJs. This is physiologically significant since cell polarity proteins are thought to relate to the establishment of TJs only. Knockdown or overexpression of these genes in previous experiments resulted in delay in TJ, but not AJ assembly. There are also emerging evidence that the CRB/Par polarity complexes are linked to the vesicle sorting machinery. Clearly, several missing players are yet-to-be identified. For instance, repeated attempts failed to find interactions between Pals1 and Sec8 [177], illustrating missing adaptor(s) remains to be identified. It is observed that many of the polarity proteins have numerous isoforms, illustrating the possibility that different isoforms are participating in different cellular processes. On the other hand, as discussed herein, spermatogenesis that involves extensive junction restructuring, cell division and differentiation of germ cells in the testis resembles some of the features of tumorigenesis as manifested by the transient expression of cancer/testis antigens (e.g., TPX1, SPA17). This area of research should be carefully evaluated in future studies.

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