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Review

Sertoli–germ cell junctions in the testis: a review of recent data

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Spermatogenesis is a process that involves an array of cellular and biochemical events, collectively culminating in the formation of haploid spermatids from diploid precursor cells known as spermatogonia. As germ cells differentiate from spermatogonia into elongated spermatids, they also progressively migrate across the entire length of the seminiferous epithelium until they reach the luminal edge in anticipation of spermiation at late stage VIII of spermatogenesis. At the same time, these germ cells must maintain stable attachment with Sertoli cells via testis-unique intermediate filament- (i.e. desmosome-like junctions) and actin- (i.e. ectoplasmic specializations, ESs) based cell junctions to prevent sloughing of immature germ cells from the seminiferous epithelium, which may result in infertility. In essence, both desmosome-like junctions and basal ESs are known to coexist between Sertoli cells at the level of the blood–testis barrier where they cofunction with the well-studied tight junction in maintaining the immunological barrier. However, the type of anchoring device that is present between Sertoli and germ cells depends on the developmental stage of the germ cell, i.e. desmosome-like junctions are present between Sertoli and germ cells up to, but not including, step 8 spermatids after which this junction type is replaced by the apical ES. While little is known about the biology of the desmosome-like junction in the testis, we have a relatively good understanding of the molecular architecture and the regulation of the ES. Here, we discuss recent findings relating to these two junction types in the testis, highlighting prospective areas that should be investigated in future studies.

Keywords: testis; Sertoli cell; germ cell; cell junctions

1. INTRODUCTION

Spermatogenesis is a highly regulated and complex process that initiates shortly after birth under the regulation of follicle-stimulating hormone, luteinizing hormone, testosterone and oestradiol 17β and continues until old age in males (de Kretser & Kerr 1988; Kerr *et al.* 2006). It involves four key cellular events, namely (i) spermatogoniogenesis (a continuous process that involves division of type A spermatogonia, which maintains a pool of stem cells, and the production of type B spermatogonia whose fate is to develop into spermatozoa), (ii) spermatocyte differentiation, (iii) spermiogenesis (a process in which spermatids undergo morphogenesis to become mature and motile spermatozoa) and (iv) spermiation (the release of elongated spermatids or spermatozoa, the end-product of spermatogenesis) into the lumen of the seminiferous epithelium (Holstein *et al.* 2003). Besides developing germ cells, the seminiferous epithelium is also composed of a somatic constituent: the Sertoli cell, a 'nurse-like' cell known to provide nutritional and structural support to developing germ cells (Bardin *et al.* 1988; Griswold & McLean 2006). As germ cells differentiate and travel progressively towards the tubule lumen throughout spermatogenesis, Sertoli and germ cells form dynamic associations within the seminiferous epithelium defined as stages of the seminiferous epithelium, and 14 distinct stages (denoted by roman numerals) have been described in the rat (Leblond & Clermont 1952; Clermont 1972). Spermiogenesis, on the other hand, involves different morphological changes (i.e. development of polarity, condensation of chromosomes and formation of the acrosome, tail and residual body) within spermatids, and this process is divided into 19 steps in the rat. These transformations, in turn, are accompanied by distinct changes in spermatid position within the seminiferous epithelium (Leblond & Clermont 1952).

Throughout spermatogenesis, developing germ cells remain in close contact with Sertoli cells, which is essential for their development. Crucial to these cell–cell interactions in the seminiferous epithelium is the production of several molecules such as hormones, growth factors, proteases, protease inhibitors and components of the extracellular matrix (ECM)

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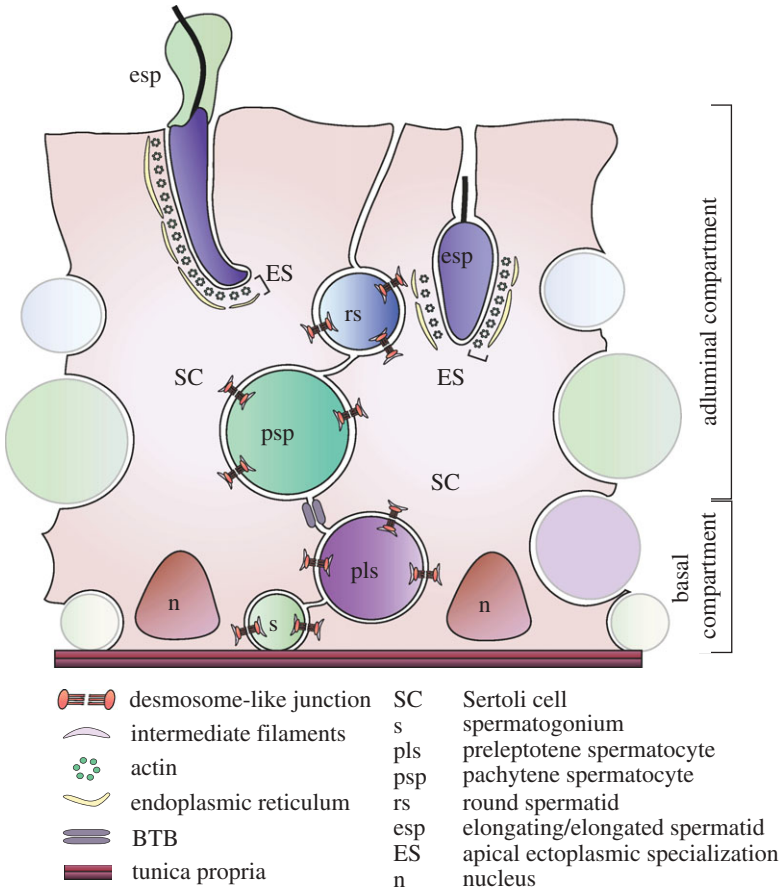

Figure 1. Different cell–cell adhesion junctions in the adult rodent testis. This schematic drawing illustrates anchoring junctions found between Sertoli and germ cells. A cross section of the seminiferous epithelium, showing two Sertoli cells sitting atop the tunica propria with germ cells at different stages of development (i.e. spermatogonia, preleptotene spermatocytes, round and elongating spermatids). The BTB physically divides the seminiferous epithelium into basal and adluminal compartments, and it forms the immunological barrier. Desmosome-like junctions are present between Sertoli cells and all germ cells up to, but not including, step 8 spermatids (see also figure 2), whereas the apical ES is found between Sertoli cells and germ cells at step 8 of development and beyond (see also figure 3).

by both Sertoli and germ cells, and there are several reports to support the direct role of both cell types in the maintenance of spermatogenesis (Cheng & Mruk 2002; Mruk & Cheng 2004). Additionally, Sertoli cells are known to contribute to the formation of the blood–testis barrier (BTB), an impermeable barrier that divides the seminiferous epithelium into basal and adluminal compartments, thereby sequestering post-meiotic germ cell development from the systemic circulation (Dym & Fawcett 1970; Mruk & Cheng 2004; Hedger & Hales 2006) (figure 1). This is important because spermatozoa and their cell-surface antigens appear long after 'self' tolerance is established, and a compromise in BTB function would result in the host producing antibodies against its own sperm. Ultrastructurally, the BTB is composed of coexisting tight junctions (TJs), ectoplasmic specializations (ESs), desmosome-like junctions and gap junctions, and these junctions are known to function collectively in the maintenance of BTB integrity, which is essential for spermatogenesis and fertility. Throughout spermatogenesis, these junctions undergo remodelling to facilitate the transit of preleptotene spermatocytes across the BTB starting at late stage VIII of the seminiferous epithelial cycle. At the same time, Sertoli–germ cell junctions, namely desmosome-like junctions and ESs pachytene spermatocytes, are also restructured to promote the progressive migration of developing germ cells towards the tubule lumen (figure 1). In this review, we discuss briefly recent findings, as well as challenges and prospects for future studies, relating to Sertoli–germ cell desmosome-like junctions and ESs.

2. SERTOLI–GERM CELL ANCHORING JUNCTIONS

As briefly discussed above, two types of testis-unique anchoring junctions are present in the seminiferous epithelium: desmosome-like junctions and ESs. Desmosome-like junctions (defined as such in the testis only because they lacked characteristics of true desmosomes found in other organs such as the heart and skin; Holthofer *et al.* 2007) are found between Sertoli cells at the BTB, as well as between Sertoli and all germ cells up to, but not including, step 8 spermatids. On the other hand, the basal ES is found between Sertoli cells coexisting with desmosome-like junctions at the BTB, whereas the apical ES is found between Sertoli cells and all elongating/elongated spermatids

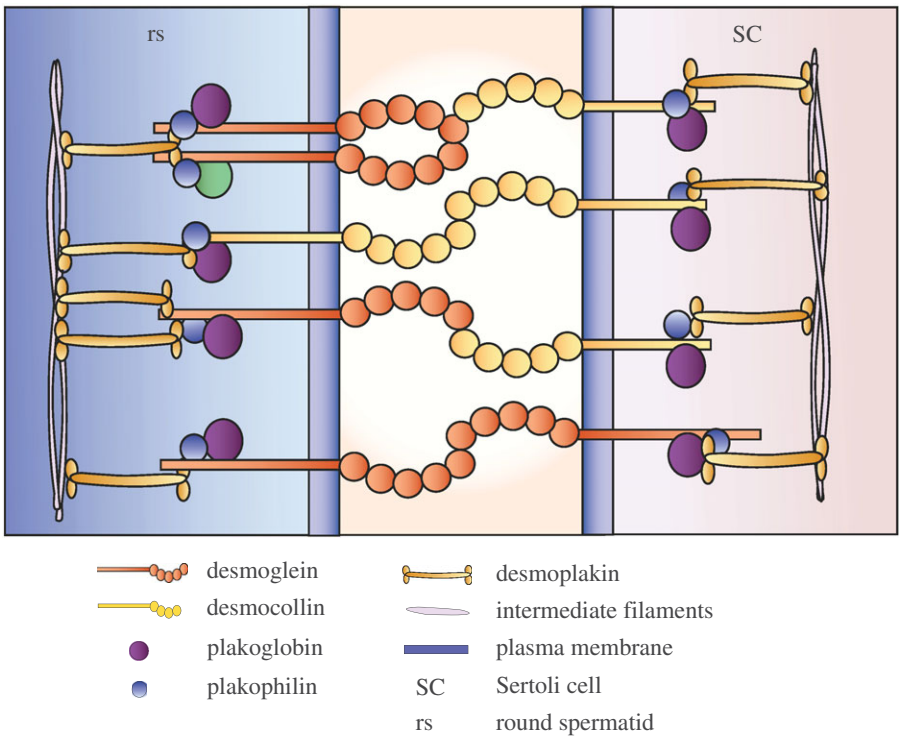

Figure 2. Desmosome-like junction in the testis. This schematic drawing is based on a recently completed study from our laboratory (Lie *et al.* in press), and it generalizes the different types of proteins found at the Sertoli–germ cell desmosome-like junction. In this study, desmoglein, desmocollin, plakoglobin, plakophilin, desmoplakin and vimentin were all shown to be expressed by the testis. Additional studies are now underway to better understand the regulation of desmosome-like junctions.

(Russell 1993) (figure 1). In this context, several important observations should be emphasized. First, the Sertoli–germ cell desmosome-like junction and the apical ES do not coexist in the seminiferous epithelium. This is in contrast to all other cell types where desmosomes cofunction and coexist with adherens junctions (AJs) to mediate adhesion. Second, it is not completely known at this point why the desmosome-like junction would need to be replaced by the apical ES, except that the latter junction type facilitates spermatid orientation, and its presence within the seminiferous epithelium correlates precisely with spermatid elongation and the acquisition of polarity. Finally, both junction types are known to mediate stable adhesion throughout spermatogenesis. For instance, perfusion of testes with a hypertonic fixative solution did not affect the integrity of desmosome-like junctions between Sertoli and germ cells, but adjacent regions of cell contact were clearly damaged (Russell 1977). Adhesion mediated by the ES was shown to be equally robust. When the force required to detach different types of germ cells (i.e. spermatocytes, pre-step 8 and step 8 spermatids) from Sertoli cells *in vitro* was measured with a micropipette pressure transducing system, step 8 spermatids were shown to exhibit the strongest adhesive force (Wolski *et al.* 2005), suggesting that Sertoli–germ cell adhesion is strengthened as germ cells approach the tubule lumen in anticipation of spermiation at late stage VIII. In the remainder of this section on Sertoli–germ cell anchoring junctions, we discuss the desmosome-like junction and the ES in greater detail, highlighting important insights from *in vitro* and *in vivo* model systems that have helped to expand our knowledge of their biology and regulation in the testis.

(a) *Desmosomes/desmosome-like junctions*

Desmosomes are cell junctions mediating stable and robust adhesion between epithelial cells via the intermediate filament cytoskeleton. They are prominent in organs subjected to mechanical stress (i.e. heart and skin), but they are also found elsewhere (i.e. liver, kidney and testis) (Holthofer *et al.* 2007). Ultrastructurally, desmosomes appear to be composed of two components: (i) an extracellular space filled with electron-dense material (i.e. the desmoglea) with distinct midline and (ii) two dense cytoplasmic plaques (i.e. inner and outer dense plaques), and desmosomes lacking these components are assumed to be simple, immature and not hyper-adhesive (see discussion below) (Garrod *et al.* 2005; Holthofer *et al.* 2007; Scothern & Garrod 2008). The molecular backbone of desmosomes is composed of integral membrane proteins of the cadherin family, namely desmogleins (Dsg) and desmocollins (Dsc), which in contrast to classic cadherins (e.g. E- or N-cadherin) interact both homo- and heterotypically to mediate cell adhesion (Delva *et al.* 2009). Desmosomal cadherins are then linked to the intermediate filament network via armadillo (i.e. plakoglobin and plakophilins) and plakin (i.e. desmoplakins) family proteins to form a multi-protein complex (Holthofer *et al.* 2007) (figure 2). Desmosome assembly in confluent cell cultures *in vitro* has been shown to require Ca^{++}, but as desmosomes mature, they become Ca^{++}-independent

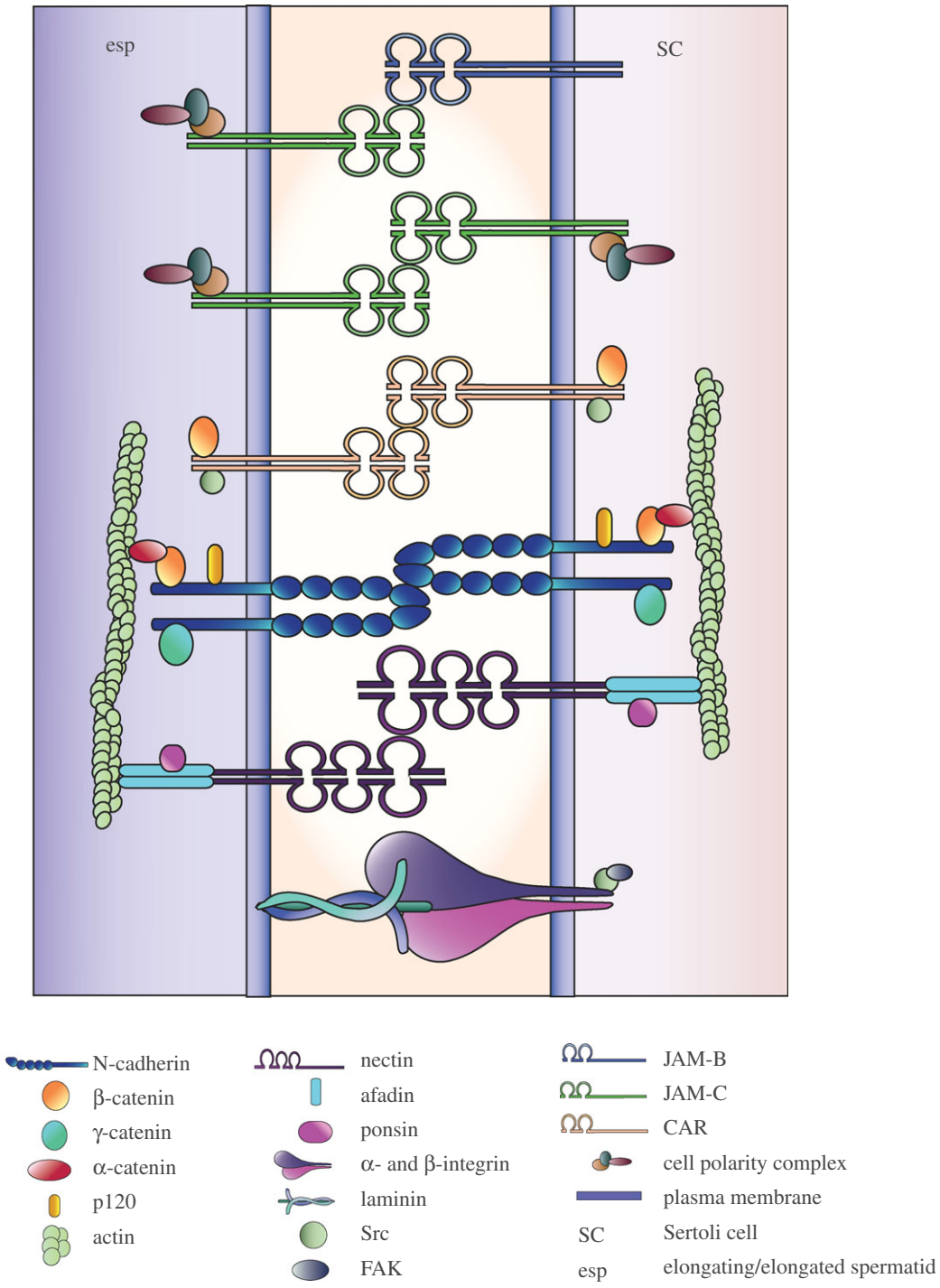

Figure 3. Apical ES. This illustration depicts several proteins that have been described to exist at the apical ES, namely the cadherin–catenin, nectin–afadin, α6β1 integrin–laminin α3β3γ3 and JAM–Par/CAR multi-protein complexes (see also table 1). The apical ES is unique with hybrid-like characteristics because it is composed of several proteins that are generally found in focal contacts and TJs. For simplicity, only proteins that were discussed in the text are included in this figure.

and resistant to disruption even by chelation of Ca^{++} ions (i.e. hyper-adhesive), in turn facilitating strong and stress-resistant adhesion. Recent evidence indicates that desmosomes also function outside of cell adhesion as important hubs to organize and regulate signalling events relating to cell proliferation, differentiation, migration and morphogenesis (Garrod & Chidgey 2008).

Recent studies have shown protein phosphorylation to play a critical role in the regulation of desmosome function, and some interesting results have emerged in recent years (Yin & Green 2004). In most cases, but not all, phosphorylation of cell junction-associated proteins, namely those of the TJ and AJ, is followed by a compromise in junction function (Gonzalez-Mariscal *et al.* 2008; Mruk *et al.* 2008; Nelson 2008). For instance, Tyr phosphorylation of Dsg-2 and plakoglobin in response to epidermal growth factor (EGF) treatment resulted in weakened adhesion in keratinocytes and A431 cells (epithelial cell carcinoma) (Gaudry *et al.* 2001; Yin *et al.* 2005). In line with these findings, blocking EGF receptor (EGFR) function in

SCC68 cells (oral squamous cell carcinoma) by using either an EGFR tyrosine kinase inhibitor or blocking antibody recruited Dsg-2 and Dsc-2 to cell–cell contact sites, thereby enhancing adhesion (Lorch *et al.* 2004). Desmosome-mediated cell adhesion was also strengthened following treatment of epithelial cells with sodium pervanadate, a Tyr phosphatase inhibitor, thereby inducing hyper-adhesion (Garrod *et al.* 2008). Moreover, protein kinase C (PKC), a family of Ser/Thr kinases, has been implicated in desmosome function (Wallis *et al.* 2000). Specifically, phosphorylation of desmosome plaque proteins by PKCα was demonstrated to switch desmosomes from a state of Ca^{++}-independence (i.e. hyper-adhesion) to one of Ca^{++}-dependence (Wallis *et al.* 2000). Interestingly, PKCα localized by confocal microscopy to the plasma membrane in Madin–Darby canine kidney (MDCK) cells and keratinocytes with Ca^{++}-dependent desmosomes (Garrod & Kimura 2008), and knockdown of PKCα by RNA interference (RNAi) increased the number of cells with hyper-adhesive desmosomes (Wallis *et al.* 2000). Likewise, in plakophilin-2-deficient cells, PKCα failed to associate with desmoplakin, thereby affecting the delivery of desmoplakin to nascent desmosomes (Bass-Zubek *et al.* 2008). These data clearly reveal protein kinases such as PKCα to be critical for desmosome regulation.

Although the presence of desmosome-like structures in the testis has been known for over three decades (Russell 1977), our understanding of their biology and regulation is still surprisingly poor. A striking feature of desmosome-like junctions in the testis is that they lack a clearly defined dense midline, which is characteristic of conventional desmosomes, suggesting that these structures are Ca^{++}-dependent and not likely to mediate robust adhesion. However, this observation is seemingly in disagreement with the *in vivo* study discussed previously, which showed that desmosome-like junctions were unaffected following use of a hypertonic buffer for perfusion (Russell 1977; Russell & Peterson 1985). Additional biochemical and cellular studies would be needed to address this disparity in morphological observations. Based on a recently completed study from this laboratory, we know that several desmosomal genes are expressed by the testis and that functional desmosome-like junctions are assembled between Sertoli cells *in vitro* (Lie *et al.* in press). If desmosome-like junctions in the testis are indeed Ca^{++}-dependent and not hyper-adhesive, a logical next step would be to investigate their function *in vitro* in the presence of selective PKC, as well as other kinase inhibitors. Moreover, desmosome-like junctions in the testis have been shown to share ultrastructural features of gap junctions (Russell 1993). This is underscored by the recent finding that plakophilin-2 (a cytoplasmic protein of the armadillo family that links desmosomal cadherins to intermediate filaments) structurally associates with connexin 43 (a transmembrane protein of the gap junction that is widely expressed), and that connexin 43, in turn, interacts with constituent proteins of the BTB (i.e. N-cadherin and ZO-1) and plays a role in the maintenance of BTB integrity (Li *et al.* 2009). In support of these results, there is at least one report that describes coimmunoprecipitation and colocalization of connexins with TJ proteins (i.e. occludin, claudin and ZO-1) in endothelial cells (Nagasawa *et al.* 2006). Interestingly, inhibition of gap junction function by 18β-glycyrrhetinic acid or oleamide adversely affected barrier function as demonstrated by the measurement of transepithelial electrical resistance across the cell epithelium (Nagasawa *et al.* 2006). In light of these engaging results, it is important that future studies investigate signalling events that mediate crosstalk between different junction types in the testis.

(b) *Ectoplasmic specializations*

(i) *Cadherin–catenin multi-protein complex*

The cadherin–catenin multi-protein complex is the best studied actin-based adhesion unit in epithelial and endothelial cells (table 1); hence, it is discussed first in this section, which focuses on ES dynamics. Although very well known for its role in cell adhesion, the cadherin–catenin protein complex also functions in other cellular processes, i.e. in the regulation of the cytoskeleton and cell polarity, control of cell division and tumour suppression. Cadherins are a large family of Ca^{++}-dependent transmembrane proteins that can be further categorized into five distinct subfamilies: (i) type I and type II, (ii) desmosomal, (iii) atypical (note: atypical cadherins are GPI-anchored and do not link to the cytoskeleton), (iv) protocadherins and (v) cadherin-like. Catenins (i.e. α-, β- and γ-catenins), on the other hand, are cytoplasmic proteins that bind to the C-terminus of type I/II and desmosomal cadherins, thereby regulating cadherin-mediated adhesion via the actin and intermediate filament (as is the case for desmosomes/desmosome-like junctions) cytoskeletons (figures 2 and 3). In this section, we aim our discussion on type I cadherins because they have been studied most extensively in the testis.

A number of different cadherins have been identified in the testis, and although this was an arguable topic in the past, it is now well established that N-cadherin is present at the apical ES (Wine & Chapin 1999; Johnson & Boekelheide 2002; Lee *et al.* 2003, 2004) (table 1, figure 3). As in other epithelial cells that use conventional AJs for adhesion, the ES is regulated by various mechanisms and constantly remodelled. For instance, both N- and E-cadherin are known to be expressed by Sertoli and germ cells (Lee *et al.* 2003), and *in vitro* and *in vivo* studies have demonstrated their steady-state levels to be regulated by cytokines and testosterone (Lee *et al.* 2003; Yan *et al.* 2008*a*). Generally speaking, the turnover of AJs, including the ES, is achieved in part by the endocytosis of cadherin, followed by its recycling back to the cell surface (Yan *et al.* 2008*a*; Delva & Kowalczyk 2009). Indeed, a recent study demonstrated an increase in the kinetics of N-cadherin (as well as in two TJ proteins: occludin and JAM-A) internalization via a clathrin-dependent pathway when Sertoli cells were cultured in the presence of transforming growth factor-β2 (Yan *et al.* 2008*b*). The addition of testosterone was also shown to enhance the rate of N-cadherin endocytosis, as well as to promote its recycling back to the Sertoli cell

Table 1. Different AJ, focal contact and TJ proteins/multi-protein complexes present at the apical ES. This table summarizes different proteins/multi-protein complexes, as well as their interacting partners, present at the apical ES. Each protein/multi-protein complex listed here is classified as an AJ, focal contact or TJ protein, illustrating that the apical ES is a hybrid-like junction composed of diverse proteins. For additional background information, readers are asked to refer to the following review articles: Cheng & Mruk (2002), Mruk & Cheng (2004) and Yan *et al.* (2007). aPKC, atypical protein kinase C; CAR, coxsackievirus and adenovirus receptor; Src, protein tyrosine kinase of the transforming gene of Rous sarcoma virus; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; ILK, integrin-linked kinase; IQGAP1, IQ motif containing GTPase-activating protein; JAM-C, junctional adhesion molecule-C; LIMK1, lin-11 isl-1 mec3 kinase 1; MMP-2, matrix metalloprotease-2; MT1-MMP, membrane-type 1-MMP; MTMR2, myotubularin-related protein 2; NOS, nitric oxide synthase; $p130^{Cas}$, p130 Crk-associated protein; PAK, p21-activated kinase; Par3, partitioning defective 3; Par6, partitioning defective 6; PATJ, Pals1-associated tight junction protein; *p*-FAK, phosphorylated FAK; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PKG, protein kinase G; PRKG, cGMP-dependent protein kinase; *p*-Src, phosphorylated Src; PTEN, phosphatase and tensin homologue deleted on chromosome 10; RA175, IGSF4A/RA175/TSLC1/SynCAM/SgIGSF/Necl2; ROCK1, Rho-associated protein kinase 1; sGC, soluble guanylate cyclase; TIMP-2, tissue inhibitor of metalloproteases-2 and WASP, Wiskott–Aldrich syndrome protein.

protein/multi-protein complex	interacting proteins	reference(s)
AJ		
N-cadherin–β-catenin	PKG, α-catenin, β-catenin, NOS, c-Src, Cdc42, IQGAP1, dynamin-2, sGC, *p*-$p120^{ctn}$, *Fer* kinase, zyxin, axin, WASP, β1-integrin, α4-integrin, cortactin, MTMR2, *p*-Src, *p*-ERK, Rab 8	Wine & Chapin (1999), Chapin *et al.* (2001), Johnson & Boekelheide (2002), Chen *et al.* (2003), Lau & Mruk (2003), Lee *et al.* (2004, 2005), Lui *et al.* (2005), Zhang *et al.* (2005), Lie *et al.* (2006) and Sarkar *et al.* (2006)
nectin–afadin	ponsin, αT-catenin, sGC	Ozaki-Kuroda *et al.* (2002), Sarkar *et al.* (2006) and Goossens *et al.* (2007)
focal contact		
α6β1-integrin–laminin α3β3γ3	ILK, c-Src, *p*-Src, FAK, *p*-FAK, PI3K, PKB, PAK, ERK, vinculin, paxillin, cofilin, gelsolin, PTEN, $p130^{Cas}$, Rho B, ROCK1, LIMK1, MMP-2, MT1-MMP, TIMP-2, N-cadherin	Palombi *et al.* (1992), Salanova *et al.* (1995), Chapin *et al.* (2001), Mulholland *et al.* (2001), Lui *et al.* (2003), Siu *et al.* (2003, 2005), Siu & Cheng (2004), Beardsley *et al.* (2006) and Yan & Cheng (2006)
tight junction (TJ)		
JAM-C	JAM-B, Par3, Pals1, Par6, Cdc42, PKCλ, PATJ, RA175 (associated via Par3)	Gliki *et al.* (2004), Fujita *et al.* (2007) and Wong *et al.* (2008)
CAR	c-Src, vinculin, α-catenin, β-catenin	Wang *et al.* (2007)
aPKC–Par3/Par6 and Crb–Pals1–PATJ	JAM-C, Src, RA175	Gliki *et al.* (2004), Fujita *et al.* (2007) and Wong *et al.* (2008)

surface. These findings seemingly illustrate that movement of preleptotene spermatocytes across the BTB is facilitated by cytokine-mediated internalization of ES and TJ proteins that resulted in the transient disassembly of the BTB, whereas testosterone promoted its assembly after spermatocytes crossed the barrier and entered into the adluminal compartment of the seminiferous epithelium. Interestingly, the Cdc42-atypical protein kinase C (aPKC)–Par6 multi-protein complex known to function in cell polarity has been shown to regulate AJ dynamics in epithelial cells in *Drosophila* by controlling E-cadherin endocytosis via actin regulatory proteins (i.e. WASp, Arp2/3 and dynamin) (Georgiou *et al.* 2008; Leibfried *et al.* 2008). In essence, loss of Cdc42, aPKC or Par6 was shown to result in AJ disruption, and Cdc42 in particular was thought to function with the Par complex to slow down the entry of proteins into the endocytic pathway (Harris & Tepass 2008) (see also table 1). This finding is supported to some extent by another study which demonstrated that homophilic interaction of E-cadherin molecules activates Rho GTPases, which in turn mediates cadherin–actin association, and this can protect E-cadherin from endocytosis (Izumi *et al.* 2004). A next step would be to investigate how polarity proteins present at the BTB take part in TGF-β3- and testosterone-mediated endocytosis of N-cadherin, occludin and JAM-A in light of published results that Par6 immunoreactivity was weakest at the BTB at stage VIII when preleptotene spermatocytes cross the barrier (Wong *et al.* 2008). Whether a mechanism similar to the one described in *Drosophila* is at work in the seminiferous epithelium remains to be examined in future studies.

(ii) *Nectin–afadin multi-protein complex*

Nectins (nectins 1–4) and nectin-like molecules (Necls, Necls1–5) comprise a small family of Ca^{++}-independent, immunoglobulin (Ig)-like molecules known to have roles in cell adhesion, proliferation, differentiation, cell survival, migration and cell polarity (Takai & Nakanishi 2003; Takai *et al.* 2003, 2008*c*; Irie *et al.* 2004). The major difference between nectins and Necls lies in their ability to bind afadin, an F-actin binding protein (Takai *et al.* 2008*b*); nectins bind afadin, but Necls do not. The assembly of nectin-based adhesive structures is a

complex, multi-step process. Nectins are known to first form homo-*cis* dimers, followed by the assembly of homo- or hetero-*trans* dimers, and the existence of nectin 1–nectin 3, nectin 1–nectin 4 and nectin 2–nectin 3 hetero-*trans* dimers has been reported (Takai *et al.* 2008*a*,*b*). These *trans*-interactions then activate other proteins, namely Cdc42, Rac1 and Rap1 GTPases via Src (Fukuhara *et al.* 2004; Fukuyama *et al.* 2005; Kawakatsu *et al.* 2005), followed by the phosphorylation of FGD1-related Cdc42-guanine nucleotide exchange factor and Vav2, guanine nucleotide exchange factors specific for Cdc42 and Rac1, respectively (Fukuhara *et al.* 2004; Kawakatsu *et al.* 2005). This is followed by the recruitment of cadherins, which also promote the assembly of the AJ (Ogita & Takai 2008).

As mentioned above, nectins and Necls are not simply cell adhesion molecules. They also play an important role in cell movement (Takai *et al.* 2003, 2008*b*). Studies have shown that nectins 1 and 3 (Sakamoto *et al.* 2006) and Necl-5 (Ikeda *et al.* 2004; Kakunaga *et al.* 2004) associate with integrin αvβ3, and that integrin αvβ3 activation was essential for nectin-mediated AJ assembly (Sakamoto *et al.* 2006). However, logical reasoning would suggest integrin activation to support cell junction disassembly and cell migration—and not cell adhesion. This implies that nectin-bound integrin αvβ3 would have to be inactivated or downregulated to mediate cell adhesion. While this mechanism is unique in its ability to regulate cell adhesion, it remains to be known whether nectins bind to additional integrin family members.

All four members of the nectin family are present in the testis, but only two of them—nectin 2 and nectin 3—are found at the apical ES, with nectin 2 and nectin 3 residing on the Sertoli cell and spermatid cell surface, respectively (Ozaki-Kuroda *et al.* 2002) (table 1, figure 3). Studies have shown that heterotypic interactions between these two nectins are essential for apical ES function, as well as for the proper development and positioning of elongated spermatids within the seminiferous epithelium (Ozaki-Kuroda *et al.* 2002; Toyama *et al.* 2008). Nectin 2 and nectin 3 knockout mice displayed abnormalities in actin distribution and defects in spermatid morphology, including irregularities in nuclear shape and mislocalization of mitochondria (Bouchard *et al.* 2000; Ozaki-Kuroda *et al.* 2002; Inagaki *et al.* 2006). These malformations resulted in male sterility (Ozaki-Kuroda *et al.* 2002), illustrating the importance of nectin-mediated Sertoli cell–spermatid adhesion for spermatogenesis and fertility. It would be interesting to know whether nectin 2 and/or nectin 3 interacts with the integrin–laminin multi-protein complex (see discussion below) at the apical ES to regulate adhesion (figure 3).

(iii) *Integrin–laminin multi-protein complex*

Generally speaking, integrins are well-studied proteins of the focal contact (a type of actin-based cell junction that connects a cell to the ECM) and hemidesmosome (a type of intermediate filament-based cell junction that connects a cell to the ECM) (Margadant *et al.* 2008; Geiger *et al.* 2009), whereas laminins are constituents of the basement membrane (Miner & Yurchenco 2004; Miner 2008). Both protein families are known to have an important role in cell adhesion, but also in cell migration and invasion during metastasis (Barczyk *et al.* 2009; Durbeej 2009; Huveneers & Danen 2009; Moser *et al.* 2009), and at least eight integrins have been reported to interact with laminins to produce 'inside-out' and 'outside-in' signals at the level of the cell–ECM (Huveneers & Danen 2009; Moser *et al.* 2009). For example, interaction of α3β1 integrin with laminin α3β2γ2 is known to regulate Src via focal adhesion kinase (FAK) (see also table 1), in turn promoting Rac1 activation and keratinocyte movement (Choma *et al.* 2007). Another integrin (i.e. α6β4 integrin) was also reported to coimmunoprecipitate with Rac1 from extracts of keratinocytes (Sehgal *et al.* 2006), illustrating the critical role of integrin in cell migration.

In the testis, the focal contact is absent from the Sertoli cell–ECM interface, and the only adhesive structure mounting Sertoli cells to the tunica propria is the hemidesmosome (Wrobel *et al.* 1979). While β1 integrin staining was recently observed at the hemidesmosome (Yan *et al.* 2008*a*), the α6β1 integrin–laminin α3β3γ3 multi-protein complex did not localize to this junction type in the testis. Instead, it was shown to localize at the opposite end of the seminiferous epithelium (i.e. at the apical ES), with α6β1 integrin and laminin α3β3γ3 being expressed by Sertoli cells and elongated spermatids, respectively (Siu & Cheng 2004; Yan & Cheng 2006) (table 1, figure 3). Interestingly, laminin β3 domain I and laminin γ3 domain IV recombinant protein fragments were recently shown to reduce the level of occludin at the Sertoli cell barrier and β1 integrin at the hemidesmosome, probably revealing precise coordination of junction restructuring during spermiation and transit of preleptotene spermatocytes across the BTB at stage VIII of the seminiferous epithelial cycle via signalling that also involved the hemidesmosome (Yan *et al.* 2008*a*). These findings are in line with at least another report demonstrating that cleavage of laminin α3β3γ2 by matrix metalloprotease 2 (MMP-2) or membrane type 1 metalloprotease (MT1-MMP) produces a γ2 fragment of 80 kDa that can facilitate migration of human breast epithelial cells (Giannelli *et al.* 1997; Koshikawa *et al.* 2000). In a separate study, however, the reverse was reported: proteolytic cleavage of the α3 chain of laminin α3β3γ2 by plasmin was shown to support hemidesmosome assembly, resulting in decreased cell motility (Goldfinger *et al.* 1998). Taken collectively, these findings reveal that laminin fragments elicit a broad repertoire of biological effects to regulate different aspects of cell function.

On a final note, a recent study has reported basolateral colocalization of Par1 with laminin in MDCK cells (Masuda-Hirata *et al.* 2009). Par1 was also reported to regulate the localization of dystroglycan, a laminin receptor essential for basement membrane formation. Interestingly, knockdown of Par1 by RNAi upregulated the secretion of β1 and γ1 chains (Masuda-Hirata *et al.* 2009), illustrating that polarity

proteins take active part in ECM organization, which is required for epithelial cell polarity. Overexpression of a dominant-negative Rac1 mutant also resulted in the accumulation of extracellular laminin (O'Brien *et al.* 2002; Yu *et al.* 2005). The connection between key polarity determinants and ECM proteins at the apical ES in the testis is not yet known but well worth investigating because elongated spermatids express both Par3 and Par6, and laminin (Yan & Cheng 2006; Fujita *et al.* 2007; Wong *et al.* 2008) (table 1, figure 3). The role of the α6β1 integrin–laminin α3β3γ3 multi-protein complex in coordinating cell movement (i.e. spermiation) and germ cell polarity should also be addressed.

(iv) *JAM–Par/CAR multi-protein complex*

Although TJs are restricted to the BTB in the seminiferous epithelium, TJ proteins have been shown to localize at the apical ES, and these include junctional adhesion molecule (JAM), coxsackievirus and adenovirus receptor (CAR) and Par proteins (table 1, figure 3). It is also worthy to note that these proteins are expressed by elongating/elongated spermatids, even though the ES, as well as its unique ultrastructural characteristics (e.g. hexagonally arranged actin filament bundles and cisternae of endoplasmic reticulum), has never been described to exist within germ cells. JAMs (i.e. JAM-A, -B, -C, -D and JAML) are single-span transmembrane proteins of the Ig superfamily known to mediate homo- and heterophilic interactions with diverse transmembrane ligands, including CAR and integrin (Bazzoni *et al.* 2000; Ostermann *et al.* 2002; Bazzoni 2003; Zen *et al.* 2005; Mirza *et al.* 2006; Luissint *et al.* 2008). In addition to facilitating cell adhesion, JAMs are also understood to function in the migration of cells across the endothelium (Martin-Padura *et al.* 1998; Chavakis *et al.* 2004; Bradfield *et al.* 2007; Weber *et al.* 2007). The expression of all three JAMs has been reported in the testis: both JAM-A and -B are present in Sertoli cells at the BTB, and in round and elongated spermatids (Gliki *et al.* 2004; Shao *et al.* 2008), whereas JAM-C is only found at the apical ES where it probably functions in Sertoli cell–spermatid adhesion and germ cell positioning and polarization (Gliki *et al.* 2004). JAM-A is also present in spermatozoa and essential for sperm motility, as supported by a study using JAM-A$^{-/-}$ mice (Shao *et al.* 2008). Interestingly, Zen and colleagues reported JAM-C to be a component of the desmosome (i.e. JAM-C colocalized precisely with desmoplakin, a cytoplasmic protein of the desmosome), but not the TJ, in intestinal epithelial cells, as well as to participate in the transepithelial migration of leucocytes (Zen *et al.* 2004). This led the authors to speculate that desmosomes, similar to TJs, may also need to be opened transiently to allow leucocytes to cross the epithelium. This is apparently in line with a recently completed study by our laboratory which demonstrated that knockdown of Dsg-2 and Dsc-2 by RNAi in Sertoli cells *in vitro* downregulated the steady-state level of JAM-A, as well as ZO-1 (Lie *et al.* in press). These findings were corroborated by a functional *in vitro* experiment that showed a partial compromise in barrier function when TER was quantified across the epithelium of Dsg-2 and Dsc-2 silenced-Sertoli cells, revealing that desmosomes contribute to barrier function possibly by opening up TJs during the passage of preleptotene spermatocytes. JAMs are also known to interact with the Par–aPKC–Cdc42 multi-protein complex, which plays an important role in TJ dynamics (Assemat *et al.* 2008) (see also table 1), illustrating extensive but yet-to-be understood crosstalk among different junction types.

CAR, on the other hand, was initially characterized as a cell-surface protein required for the entry of coxsackie B and adenoviruses into cells (Coyne & Bergelson 2005). Subsequently, CAR was reported to be a component of the TJ complex and a regulator of TJ assembly when it was shown to colocalize with occludin and to coimmunoprecipitate ZO-1 (Cohen *et al.* 2001; Coyne *et al.* 2004; Excoffon *et al.* 2004; Mirza *et al.* 2005; Raschperger *et al.* 2006). CAR has also been assigned a role in cell adhesion (Cohen *et al.* 2001; Philipson & Pettersson 2004). In human airway epithelia, CAR staining overlapped with that of β-catenin, and immunoprecipitation revealed a direct interaction between these two proteins (Walters *et al.* 2002). Similar results were also reported in the testis, and CAR was shown to be expressed by both Sertoli and germ cells (Wang *et al.* 2007) (table 1, figure 3). Furthermore, homophilic interactions are known to underlie CAR function, but as mentioned above, there are at least two reports in the literature illustrating a heterophilic interaction with JAML (Zen *et al.* 2005; Luissint *et al.* 2008). Generally speaking, loss of CAR expression results in weakened cell adhesion, thereby promoting cell migration (Okegawa *et al.* 2001; Bruning & Runnebaum 2004; Matsumoto *et al.* 2005). The role of CAR in the testis is being actively investigated in light of its possible role in the movement of preleptotene spermatocytes across the BTB. However, it will be several years before reproductive biologists have a clearer understanding of the biochemical and molecular events that underlie germ cell movement in the seminiferous epithelium of the testis.

3. FUTURE PERSPECTIVES

Although the biology of the desmosome-like junction and ES is not yet clearly understood, the number of proteins present at these two structures has increased systematically. Recently, carcino-embryogenic antigen-related cell adhesion molecule (CEACAM6) was described as a novel component of the ES in the rat testis (Kurio *et al.* 2008), and it is likely that other important cell adhesion proteins will be identified in the years to come. However, the challenges that remain are those that address desmosome-like junction and ES regulation in the testis. For instance, are desmosomal cadherins endocytosed during germ cell migration which involves extensive restructuring of Sertoli–germ cell junctions? What is the identity of the signal that initiates desmosome-like junction disassembly and dissolution in late step 7 spermatids, followed by apical ES assembly in step 8 spermatids?

Is the signal a polarity-related protein kinase? Finally, is there any crosstalk between cadherin–catenin, nectin–afadin, integrin–laminin and JAM–Par/CAR multi-protein complexes at the apical ES, and how do they contribute to one of the most important events taking place in the seminiferous epithelium: spermiation at late stage VIII of the seminiferous epithelial cycle? With recent technological advancements in cell and molecular biology such as RNAi and fluorescence recovery after photobleaching, we expect the next few decades to be an exciting time in the field as reproductive biologists find answers to some of the most important questions in testis biology.

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