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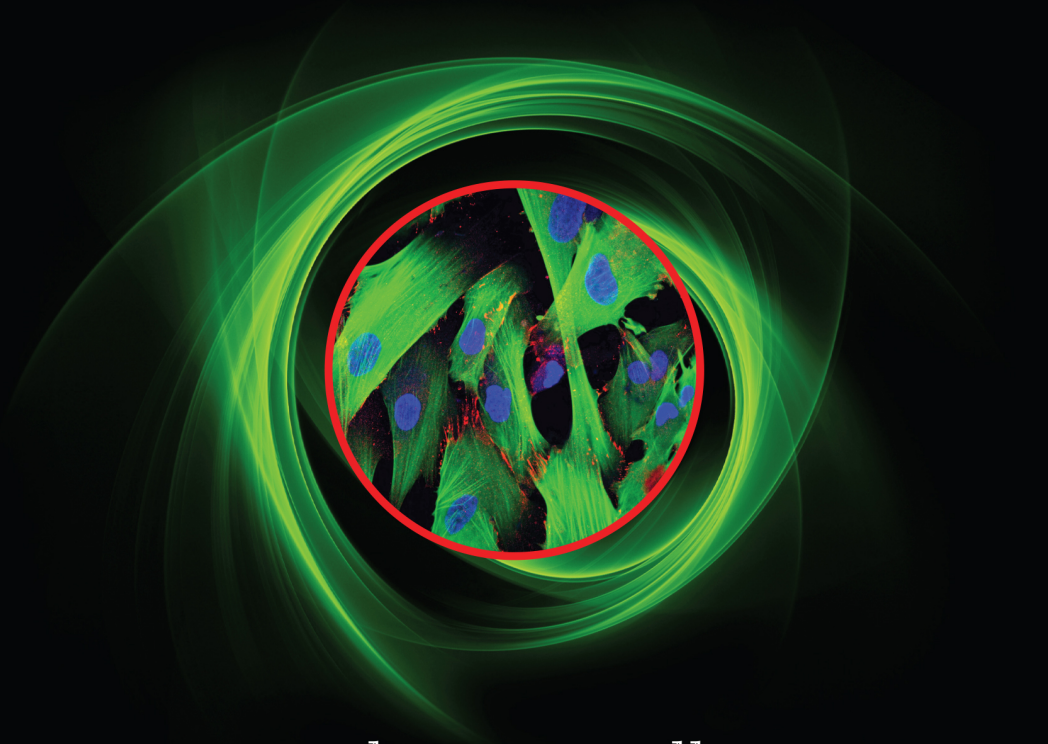
Cadherins

Types, Structure and Functions



Jonathan McWilliam
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TYPES, STRUCTURE AND FUNCTIONS

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EDITOR

nova
science publishers
New York

In: Cadherins
Editor: Jonathan McWilliam
ISBN: 978-1-53618-077-0
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Chapter 2

CADHERIN-MEDIATED CELL ADHESION WITHIN THE SEMINIFEROUS TUBULES

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ABSTRACT

Cadherins (Cadh) are key-molecules in Adherens junctions (AJs). They are multiprotein complexes mediating cell-cell adhesion, and particularly important to shape cell polarity, provide plasticity and maintain architectural integrity. Cadh, a large superfamily of cell surface glycoproteins, present a unique extracellular region domain folding like the immunoglobulin domains. They are found in a wide array of species and a multitude of tissues, including the testis. In the mammalian testis, the seminiferous tubules represent a unique type of epithelium-like tissue,

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composed of two different cellular populations: the Sertoli somatic cells and the spermatogenic cells. Different sorts of cell-to-cell attachments connect adjacent Sertoli cells and Sertoli to germ cells. The overall arrangement of junctions forms the blood-testis barrier. These connections offer an immune-privileged environment to the developing germ cells, and the nutritional and metabolic support to germ cells while offering particular plasticity to the tubular structure. They allow the migration of differentiated germ cells from the basal towards the adluminal compartment while providing a tight-fitting barrier for paracellular translocation of molecules and particles. Between adjacent Sertoli cells, various types of homotypic adherens junctions exists, while heterotypic junctions are present between Sertoli and spermatogonia (basolateral junctions) or spermatid heads (apical junctions). Intercellular N-cadherin connections, anchored in cytoplasmic plaques involving (but not limited to) actin filaments, form different morphological types of AJs. All sorts of AJs work together with tight and gap junctions to form the blood-testis barrier. The integrity of the different adherens junctions are critical for the spermatogenic process and the production of viable spermatozoa. In this chapter, we propose to review and discuss the structure of the cadherin-mediated junctions in the seminiferous tubules and their function in male fertility.

Keywords: N-cadherin, Sertoli cells, germ cells, cell-cell connections, seminiferous tubules, spermatogenesis, fertility

ABBREVIATIONS

ADIP	afadin-and alpha-actinin-binding protein
aES	apical ectoplasmic specialization
AJs	adherens junctions
arp3	actin-related protein 3
N-WASP	neural Wiskott-Aldrich syndrome protein
AXPC	axial protocadherin
bES	basal ectoplasmic specialization
BTB	blood-testis barrier
Cadh	cadherins
CLMP	CXADR-like membrane protein precursor
CRB3	protein crumbs homolog 3 precursor

Ds	dachsous protein
Dsc	desmocollin
Dsg	desmoglein
Dvl	dishevelled
EC	extracellular cadherin
EGF	endotheliun growth factor
ERK1/2	extracellular signal-regulated kinase 1/2
ES	ectoplasmic specialization
FAK	focal adhesion/adherens kinase
Fmi	flamingo protein
Fz	Frizzled protein (receptor)
GJs	gap junctions
ILK	Integrin-linked kinase
JAM	junctional adhesion molecule
LMO7	LIM domain only protein 7
MAG	myelin associated glycoprotein
MAPK	mitogen-activated protein kinase
Pcdh	protocadherins
SPD	spermatids
SPG	spermatogonium
Src	proto-oncogene tyrosine-protein kinase
TBC	tubulobulbar complexes
TGF-β	transforming growth factor beta
TJs	tight junctions
TNF	tumour necrosis factor
WASP	Wiskott-Aldrich syndrome protein
Yes	tyrosine protein kinase
ZO	zonula occludens (protein)

INTRODUCTION

Epithelial cells found in many organs are characterized by their arrangement into cohesive sheets [1]. In tissue, strong cell-cell linkage is

achieved by the called cell junctions and mediated by transmembrane proteins that are anchored intracellularly to the cytoskeleton [2, 3]. In vertebrates, cell junctions are classified into three major categories based on their physiological role: anchoring junctions, occluding junctions and communicating junctions [4]. Anchoring junctions are multiprotein complexes. They are critical components in the development and homeostasis of higher eukaryotic organisms allowing the adhesion of cells to each other and to large insoluble proteins of the extracellular matrix [5]. They are essential to shape cell polarity, provide plasticity and maintain architectural integrity helping the tissue to survive to inflicted mechanical stress [6]. At the anchoring junction complexes, cadherins play a crucial role as transmembrane adhesion proteins linking the cytoskeleton inside the cell to adjacent cell structures. When bonded to actin, the Cadh complex forms adherens junctions (AJs) while when bonded to intermediate filaments, it forms desmosomes [3]. The second category, named as occluding junctions, includes tight junctions (TJs) that create a physical barrier between adjacent epithelial cells controlling the movement and transport between the apical and basal layers of epithelia [7]. The last category of cell-cell junctions includes the gap junctions (GJs), a channel-like structure made up of proteins that allow the selective transport of material having molecular weight under 1000 Da between cells [4].

AJs are actin or intermediate filaments-based junctions. Cadherins (Cadh) and nectins are key-molecules in AJs. The expression of Cadh in the mammalian testis has been described in some reports. However, much controversy still exists regarding its localization and functions particularly on their involvement in the formation of the testis-specific adherent junctions. In this chapter, we intend to review the role of cadherin-mediated junctions in the seminiferous tubules and present a short description of AJs dynamics during spermatogenesis. The complexity of the structural junctions of the cells in the seminiferous tubules made it challenging to present a clear and concise description of the junction complexes existing there. The fact that those junctions interconnect, driving some conflicting reports on the literature, hardened this task. TJs and GJs overlap and integrate the adherence areas. The changes with time

in the nomenclature of testicular junctions only add a defy to it. The information presented in this chapter does not exhaust the theme addressed herein but intends to serve as an opening for those starting on the topic.

JUNCTIONAL COMPLEXES IN THE SEMINIFEROUS TUBULES

In adult testicular cross-sections, the parenchyma shows a typical architecture: multiple segments of seminiferous tubules containing polarized Sertoli cells and the germinal epithelium, surrounded by a basement membrane and species-specific number of myoid cells layers; the tubules are separated by an interstitium of diverse density where Leydig cells embed [8].

In the mammalian testis, the seminiferous tubules represent a unique type of epithelium-like tissue [9], composed of two different cellular populations [10, 11]:

- The Sertoli somatic cells, in a basal position, are laterally anchored to each other. They provide nutrition and support to the developing spermatogenic cells, to which they are connected by multiple cell-to-cell attachment structures. These cells orchestrate the development and differentiation of germ cells by providing structural and metabolic support to germ cells and maintaining an immune-protective environment.
- The germ cell population, which can be found in different developmental stages, organized according to a regular cellular association (named stages of the seminiferous epithelium). Cells originating from a differentiated spermatogonium form a syncytium, maintained interconnected by intercellular bridges that derive from incomplete cytokinesis. This morphofunctional feature is of paramount importance to the synchronous development of germ cells and the success of spermatogenesis.

Sperm production occurs regularly in the post-pubertal testicular seminiferous tubules, according to a temporal species-specific pattern. To evolve normally, spermatogenesis demands an intense and complex interplay between germ cells and Sertoli cells as well as between the Sertoli and Leydig cells. Spermatogenesis aims to produce a genetically unique, haploid male cell from an originally diploid germ cell. For spermatogenesis to succeed, the developing germ cells depend on a tightly controlled environment, provided by the Sertoli cells [12]. In particular, seminiferous tubules are immune-privileged spaces that ensure the survival of advanced haploid germ cells (spermatids) that would otherwise be seen as foreign (non-self) by the immune system [13, 14]. The production and survival of haploid spermatozoa are paramount in male fertility.

Within the seminiferous tubules, Sertoli cells form an epithelial barrier, one of the tightest known blood-tissue barriers in the mammalian body. Named as the blood-testis barrier (BTB), it has been long recognized as crucial to protect the developing germ cells from the self-immune system [14]. BTB fosters the integrity of the seminiferous epithelium and the functional interconnections between the somatic and germ cells.

For long, it was considered the tight junctions between Sertoli cells composed BTB. Nonetheless, BTB is far more complex than initially thought. Today it is accepted that it does not rely exclusively on epithelial TJs, but rather represents a multifunctional system of junctions [15-17]. Therefore, the creation of an immune-privileged space in the seminiferous tubules is only one aspect of a larger role played by the junctional complexes in the seminiferous tubules. Whether or not the AJs should be included within the BTB junctions remained controversial. More recently, it has been demonstrated that, unlike other existing blood-tissue barriers, in the blood-testis barrier tight junctions (TJs) coexist and intermix with adherens junctions (AJs) and gap junctions (GJs) (Table 1), that work in concert to maintain BTB integrity and support spermatogenesis [14, 18-20].

The spermatogenic process is characterized by a continuously synchronized and spatially organized sequence of cell proliferation, differentiation, translocation and morphogenesis [21], whose success

depends on the support and coordination of Sertoli cells. The spatial arrangement of germ cell within the tubules, resulting in the BTB particular junctional system, is crucial to spermatogenesis. Spermatogenesis depends on the intimate crosstalk between germ cells and Sertoli cells. It drives the nutritional and development support for each stage of the spermatogenic process, the timely transport of developing germ cells from near the basement membrane to the adluminal compartment of seminiferous tubules, as fully developed spermatids [12]. This arrangement demands a continuous remodelling of the junctional system to cope with the proliferation and differentiation of the germ cells and the transmigration of developing cells from one compartment to the other. The disassembly and reassembly of the local junctions when the germ cells move through the tubular compartments help to maintain the integrity of the barrier [18]. The different junctional complexes work together [16], as in a canal or barrage sluice lock to allow the passage of a boat through sections of different water levels. The plasticity of this system is crucial to male fertility, allowing the migration of differentiated germ cells from the basal towards the adluminal compartment, while at the same time providing a tight-fitting barrier for the paracellular translocation of molecules and particles (hence maintaining the strength of the blood-testis barrier) [10, 11].

As stated by Hess and Vogl [15], Sertoli cell anatomy embodies one of the most complex, three-dimensional structures in biology. Sertoli cells are polarized columnar cells that extend the entire height of the seminiferous epithelium, creating a barrier that protects and supports generations of developing non-somatic cells by creating a complex system of intricate membrane associations that is different between basal and apical locations [15].

Table 1. Classification of intercellular junctions within the seminiferous tubules [21-23]

Functional characterization		**Occluding junctions**	**Anchoring junctions**				**Communicating junctions**
			linked to actin filaments			linked to intermediated filaments	
Type of junctions		Tight junctions	Classic Adherens junctions	Ectoplasmic specializations	Tubulo-bulbar complexes	"Desmosome-like junctions"	Gap Junctions
Links Sertoli cells to	Adjacent Sertoli cells	✓	✓	✓		✓	✓
	Germ cells		✓ [stem SPG]			✓	✓
	Spermatids (SPD)			✓	✓ [elongated SPD]	✓ [round SPD]	
Transmembrane proteins		Occludins; Claudins; JAM (A&B); CAR; CLMP; CRB3	Cadherins (N-E-P); Nectins (2-3) JAM (B-C)	Cadherins (N-E-P); Nectins (2-3); JAM-C; Vezatin	Clathrins	Desmogleins; Desmocollins; Connexins	Connexins
Adaptor proteins		ZO (2-3); MAGI; Cingulin	Catenin (α, β, γ..) Catenins Afadin	Catenins, Afadin, ZO-1	Dynamins (2-3); Cofilin; arp3; N-WASP	Plakophilins; Plakoglobins; Desmoplakins; Plectins	ZO-1
Main role		Compartmentalization of the testis	Tissue integrity				Communication

Within the different junctional complexes existing in the seminiferous tubules, AJs are the anchoring junctions connecting the cytoskeleton of adjacent cells, providing a robust tissue architecture. Testicular AJs link adjacent Sertoli cells, germ cells to Sertoli cells and germ cells to each other. This kind of structure provides mechanical support to the epithelium and allows germ cells movements through the different compartments of the tubules, besides playing a determinant role in germ cell morphogenesis and differentiation [24].

In the testis, classical cadherin-mediated AJs exists only between Sertoli cells and spermatogonia in the stem cells niche [16]. These junctions co-exist, however, with a modified type of adherens junctions called the ectoplasmic specialization (ES), and the tubulobulbar complexes (TBC), in a time- and cycle dependent pattern. ES established between adjacent Sertoli cells can be found in the basal compartment, and ES established between Sertoli cells and elongating spermatids were described within the adluminal compartment [21, 25, 26]. ES are unique, testis-specific AJs involving actin filaments, endoplasmic reticulum, and microtubules beneath the plasma membrane of Sertoli cells [19]. ES is expressed in the basal compartment (basal ES; bES), where it is intermixed with TJs, basal TBC and "desmosome-like" junctions at the BTB adjacent to the basement membrane, as well as in the adluminal compartment (apical ES), which engage both several TJ proteins (e.g., JAM-C) and focal adhesion complex (FAC) proteins [27]. Apical ES (aES) is the only junction type at the developing spermatid–Sertoli cell interface and seems to gather in one junctional type the ability to confer adhesion, communication, and cell movement and polarization [27]. TBC seems to be formed before spermiation, between Sertoli cells and the elongating spermatids. TBC are testis-specific actin-related endocytic structures that form between Sertoli cells and elongated spermatids at the point where the ectoplasmic specializations begin to disassemble [19]. All those types of junctions act in concert and show dynamic changes all through the gonad development as well as in a cyclic pattern during spermatogenesis [15, 28].

Domke [29], based on molecular studies, defends that due to their particular features, it is incorrect to use the name "desmosome-like junctions". Previous studies [9], showed that the AJs in seminiferous tubules contain N-cadherin connected to different adaptors (α- and β-catenin, plakoglobin, proteins p120, p0071 and a protein of the striatin family and, in rodents, also the proteins ZO-1 and myozap). According to the pattern and disposition of the junctions, the authors propose the use of the names "*areae adhaerentes*" for the larger N-Cadherin mediate junctions, and "*cribelliform junctions*" for the small clusters of strainer-like intercellular junctions perforated by cytoplasm-to-cytoplasm channels, localized close to the apical SE, found in bovine seminiferous tubules [9]. Notwithstanding, at present, no substantial evidence supports one or the other opposing opinion. Therefore, we retain the designation of "desmosome-like" junctions in this chapter.

CADHERINS

Cadherins are cell surface glycoproteins usually associated with cell-cell adhesion and recognition processes occurring in animal tissues. Cadh were discovery in the 1970s after unexpected observations of Masatoshi Takeichi in Japan [30]. Takeichi´ studies showed that cells interact using two adhesion systems, calcium-dependent and calcium-independent and that calcium protects the calcium-dependent adhesion molecule from proteolysis [31]. The name "cadherins" was introduced by Takeichi and stands for "calcium cell-cell adhesion molecules" [32]. Cadh forms a superfamily of more than one hundred of calcium-dependent membrane proteins [33]. Cadh are found both in vertebrates and invertebrates in a wide array of species and tissues [34, 35].

Cadh are intrinsic membrane proteins with an N-terminal extracellular region (ectodomain), followed by a single-pass α-helix transmembrane domain and a C-terminal intracellular region [36]. The extracellular cadherin (EC) domain consisting of about 110 residues folded into a sandwich of seven β-strands forming two β-sheets is the structural

hallmark of Cadh family members [37, 38]. Cadh present from 2 to 34 ECs in a plethora of structural arrangements [38]. Ca^{2+} bounds to sites between adjacent EC domains inhibiting a free rotation around their linker peptides, thereby stabilizing cadherin domains together into rigid rods [39]. The number and homology of EC domains determine de division of Cadh superfamily of proteins into subfamilies: the classical (type I and II) Cadh, the desmosomal Cadh, the protocadherins (clustered and non-clustered Cadh), and the atypical Cadh (FAT, FAT-like or Dachsous and the large Cadh) [33, 40-42].

Classical Cadh do much more than hold cells together. They control cell movements underlying morphogenesis, changes in cell polarity, cell structure, and also mediate several intracellular signalling processes to control both cytoplasmic organization and motile behaviours of cells as well as changes in gene expression to control cell differentiation and growth and tissue architecture [41, 43]. Additionally, Cadh present physiological regulation properties in tissues, in a barrier and synaptic mechanisms [43]. The classical cadherins are subdivided into types I and II based on sequence homologies (e.g., E-, N-cadherins in type I, and VE-cadherin, in type II). They are involved in cell-to-cell adhesion and are concentrated at the adherens junctions. Classical (and desmosomal) Cadh ectodomain contains five EC domains usually designated as EC1 – EC5 beginning with the N-terminus of the molecule. These Cadh ectodomains usually form Ca^{2+}-dependent interactions with the Cadh ectodomains of adjacent cells (homophilic interactions) [36]. The cytoplasmic domain of classical Cadh links to catenins and, through catenins, to actin cytoskeleton filaments and intracellular signalling proteins [37, 43, 44].

Desmosomal cadherins [desmocollin (Dsc) or desmoglein (Dsg)] are typically found in desmosomes, intercellular junctions of epithelia and cardiac muscle. They are characterised by the enhanced stability and adhesive strength [45]. The types of desmosomal cadherins vary with the tissue, accounting for the tissue-specificity of desmosomes. Moreover, the role of these cadherins goes beyond those of cell-to-cell adhesion, and have been connected to intracellular signal transduction pathways controlling cell proliferation and differentiation [45].

Protocadherins (e.g., α-, β- and γ-Pcdhs; PAPC; AXPC) are the largest Cadh subfamily. Their structure is similar to type I classical cadherins but they possess an extracellular domain composed of six to seven EC repeats lacking conserved sequence elements present in classical cadherins. Additionally, the intracellular domain of protocadherins (Pcdh) is structurally diverse and do not interact with catenins, but instead with other proteins such as Fyn-kinase [46]. Only vertebrates expressed Pcdh, mostly in the nervous system, but can also be expressed elsewhere [34, 47]. Yet, their functions remain unclear. It has been hypothesized that non-clustered Pcdh promote cell motility and migration, mediate dendrite ramification (axon growth and patterning) and dendritic self-avoidance (self- and non-self-recognition of neurons). They also may regulate synapse dynamics [48], thereby participating in the neural circuit assembly.

The atypical Cadhs Dachsous (Ds), Fat and Flamingo (Fmi) control the cellular and tissue organisation by regulating growth and planar cell polarity signalling [40, 49]. Ds and Fat have 27 and 34 ECs, respectively [50]. Fmi is the only cadherin with a seven-pass, rather than a single, transmembrane domain, and also possess an extracellular sequence including nine (Drosophila) or eight (mouse and human) ECs [41, 49, 51]. This group of planar polarity proteins further includes the Wingless (or Wnt) armadillo (the prototype of the family that includes β-catenin and plakoglobin), Frizzled (Fz) and Dishevelled (Dvl). A recently identified human homologue (EGFL2) has nine cadherin repeats [40, 42].

CADHERIN IN THE JUNCTIONAL COMPLEXES OF THE SEMINIFEROUS TUBULES

AJs are actin or intermediate filaments-based junctions. Cadherins have classically been considered the primary adhesive unit at adherens junctions. By linking to underlying actin belts via catenins, Cadh confers resistance to the intercellular adhesion. Cadh also engages in numerous functions other than their participation in the adhesive complex in AJs,

including cell-cell recognition, cytoskeletal organization, signal transduction, acquisition of cell polarity and growth control [41]. In multiple body structures, Cadh often cooperate with nectins in AJs. The association between the nectin and cadherin systems is physically mediated by afadin, α-catenin, and their binding proteins, namely ponsin, LMO7, ADIP, vinculin, or α-actinin [52].

In the seminiferous tubules, AJs are junctional complexes based on N-Cadherin anchored in a diversity of cytoplasmic plates. Seminiferous tubules AJs (whether ES, TBC or the formerly named "desmosome-like" junctions) are composed of integral-membrane proteins, adaptors, and signalling molecules. The integral membrane proteins in testicular AJs encompass cadherins, nectins, and integrins. These molecules use β-catenin, α-actinin, afadin, vinculin, or cofilin as adaptors to the cellular cytoskeleton of actin (ES and TBC) and other intermediate filaments (vimentin in the "desmosome-like junctions") or microtubules (TBC) [53].

Cadherins Presence in the Seminiferous Tubules

Different cadherins have been identified in the testicular tissue in some species. Classical Cadh have been identified within the seminiferous tubules of fetal, immature and adult testis, each exhibiting a unique expression profile during the testicular development [54-57]. The time- and spatially orchestrated changes of the gonad development as well as with the stage of the spermatogenesis in mature tubules suggest a crucial role to be played by these molecules in the testicular homeostasis. Accordingly, Cadh inhibition with specific antibodies in the adult gonad entrains the disassembly of the tubular architecture and leads to the progressive loss of germ cells [19]. It has also been proposed cadherins to play an essential role during collective cell migration [58], by acting as a mechanosensor connected with the contractile actomyosin cytoskeleton via α-catenin and vinculin [58, 59]. This role is mediated through conformational changes in α-catenin under tension that expose vinculin binding sites [59, 60]. Vinculin recruitment to cell–cell junctions increases the duration of the

interaction between Cadh-catenin complex and the actin cytoskeleton, fostering AJs stabilization during intercellular tensive stress [60].

The expression of E-Cadh seems to be restricted to the fetal and immature testis before the formation of BTB [19, 55]. It has been proposed to be involved in the migration of primordial germ cells and the gonad differentiation, particularly in the formation of testicular cords and the survival of germ cells [61]. However, its presence after puberty has been elusive. It has been shown that E-Cadh is only expressed by spermatogonial stem cells of adult mice [62] and rats [63], and peripubertal sheep [64].

Early in the nineties, N-Cadh was found to be expressed by both the Sertoli and germ cells and recognized as one of the molecules participating in the cellular junctions at the seminiferous epithelium [65]. It is now acknowledged that N-Cadh is present in testes at all the developmental stages. Its expression and localization obey a tight spatiotemporal regulation [55, 57]. In the post-pubertal seminiferous tubules, N-Cadh (colocalized with β-catenin and p120) displays a stage-specific immunostaining pattern. It locates in the basal compartment of the seminiferous tubules, close but independently of the basal ectoplasmic specializations [19, 66], suggesting its participation in the "desmosome-like" junctions. It was also found in association with spermatogonia, spermatocytes and the heads of elongated spermatids [66, 67]. Albeit N-Cadh was also located around the round spermatids, evidence showed that the junctional complexes here lack plectin or β-catenin adaptor proteins, suggesting that a different junctional complex is formed after the dismantling and reassembly of the Sertoli-germ cell junction as spermatocytes develop into round spermatids [54] and migrates into the adluminal compartment. This finding supported the hypothesis that the N-Cadh/β-catenin complexes, anchored to the actin network, regulates cell adhesive function together with the "desmosome-like" junctions linking Sertoli and other germ cells [68].

Besides, N-Cadh is present at either the basal and apical ES. In the former, the anchoring complex is established with some proteins [69] usually seen in the focal adhesion complex at the cell-matrix interface

(such as vinculin, FAK, claudins and ZO-1). Thereby, basal ES may be considered as a hybrid cell-cell anchoring junction [20]. Conversely, apical ES is also considered to be a hybrid intercellular actin-based AJs that integrates N-cadh/catenin complexes [70], yet it is composed of proteins customarily found within tight junctions and focal contacts, such as α6β1 integrin or the nectin/afadin complex [69]. Moreover, its relation with the typical actin filaments is limited to the Sertoli cell side [71].

The expression of Cadh other than the N-cadherin are controversial and no consensual information is retrieved from studies using immune-based techniques; also, more recent reports often contradict results from previous ones. This controversy might be explained by the differences in the antibodies used for detection. Additionally, the existence of species particularities or the existence of a short, transitory expression pattern may also account for the reported differences in respect to the expression of cadherins or its adaptors as well as the unavailability of appropriate antibodies against specific Cadh epitopes. Below we present a summary of the data provided or validated by gene expression studies.

Cdh-6 immunostaining was also described in the adult rat seminiferous tubules. It is invariably colocalized with p120 at Sertoli-round spermatid desmosome-like junctions [54], and the heads of elongated spermatids, in a transient stage-related pattern. Contrasting with N-Cadh, no catenins other than p120 were found associated with Cadh-6. Still, these findings were not confirmed in the studies by Domke [23].

P-cadherin has been localized in the testis, but only in the junctions between adjacent peritubular myoid cells [21], and therefore will not be addressed in this chapter.

More recently, VE-Cadh has been localized in the seminiferous tubules of adult mice, exclusively in germ cells, in contrast to what has been described so far for the other Cadhs in the seminiferous tubules [72]. VE-Cadh has been located in round spermatids and in early elongating spermatids, before the formation of apical ES, for which Berruti and colleagues [72] defend that its role may be related to the spermatid acquisition of polarity or the orientation inside the seminiferous tubule. As VE-Cadh is completely internalized in elongated spermatids, these authors

further argue that it may not a component of a fully established aES. In the same study, Berruti and colleagues [72] suggested that VE-Cadh could be involved in the junctions linking Sertoli cells and round spermatids ("desmosome-like" junctions).

Cadherin Adaptors and Scaffold Proteins

In canonic AJs, β-catenin bridges Cadh to α-catenin, which in turn conjugates the cadh/catenin complex to the actin cytoskeleton or vimentin filaments in mature Sertoli cells [68]. α-catenin is crucial to the strength of AJs, and facilitates the linkage of the Cadh-catenin complex to the actin cytoskeleton [68] if the interaction is under tension [59].

Membrane-associated β-catenin and p120 catenin partnering with cell-specific cadherins (including E-Cadh and N-Cadh) were localized in the fetal gonads at the time of gonad differentiation [73]. In the adult testis, expression of Cadh-associated catenins (β-catenin, α-catenin and p120) have been associated with basal ES, at the junctions between Sertoli cells and spermatogonia [21], primary spermatocytes [19, 54], but also in tight junctions between adjacent Sertoli cells [16]. The N-Cadh-β-catenin complex at the basal ES is crucial for BTB maintenance [74]. In the "desmosome-like" junctions, Cadh-catenin complex also integrates γ-catenin (known also as plakoglobin) [9]. Even though the complex N-Cadh-β-catenin has been evidenced at the apical ES, the existence of α-catenin has not been demonstrated there, suggesting that β-catenin may interact with an alternative adaptor in those particular junctions [68].

Besides its participation in the cadherin/catenin complexes, α-catenin may also bind to afadin, vinculin and zyxin, to link with the actin cytoskeleton [27, 75]. Nectins anchors to other proteins, such as catenins or afadin, thereby clustering Cadhs and stabilizing the AJs [76]. In the testis, this arrangement between the nectin/afadin and the cadherin/catenin complex allows for the crosstalk between the two types of adhesion complexes [24], fostering a type of AJs that are at the same time strong and easily remodeled [76]. This interaction is particularly significant for the

later stages of the development of spermatids and during the assembly and disassembling of various types of intercellular adhesion [21].

It has been shown that in the testis, the assembly of AJs and GJs are interdependent. The connection between the two types of junctions is provided by the fact that N-Cadh associated β-catenin colocalize with connexin 43, which favours a close physical and functional association between AJs and GJs [77]. Similarly, afadin and catenins mediate interactions with ZO proteins, thus providing another chain to the link between TJs and AJs [78].

There were also additional peripheral proteins regulating the actin dynamics, such as zyxin, axin and WASP (Wiskott-Aldrich syndrome protein), shown to be structurally linked with N-cadh/β-catenin complexes in the seminiferous tubules [19, 79], suggesting that N-cadh/β-catenin complexes may also indirectly link vimentin-based intermediate filaments via these adaptors. Additionally, c-Src protein and c-Yes have been identified as proteins peripheral to N-cadherin [27], and proposed as possible mediators of the regulatory system that modulates the dynamics of the Sertoli–germ cell AJs.

The studies by Domke [23] failed to evidence desmocollin or desmoglein in the seminiferous tubules of different species.

Alternative Adaptors and Scaffolds for N-Cadherin in the Seminiferous Tubules

In the seminiferous tubules, adaptors other than the catenins have been related to apical ES (aES), a hybrid anchoring junction type sharing the properties of the different junctional complexes found in the basal compartment of the seminiferous tubules [27]. In aES, N-cadh has been associated to α6-β1 integrin, linking to γ3-laminin [21, 80] that together with laminin α3 and β3 forms a functional integrin binding protein in elongating spermatids [27]. The α6-β1 integrin is expressed in Sertoli cells, while α3/β3/γ3-laminins are expressed in the elongating spermatids [81]. In aES integrin-laminin complexes connecting to the actin

cytoskeleton, the strength of the junction is lower compared to the bES, coping with the changes in the shape of elongating spermatids and facilitating an easy disengagement of the cells at spermiation.

This integrin-laminin functional complex is supported by the co-existence of other peripheral regulatory proteins, such as the integrin-linked kinase (ILK), focal adhesion kinase (FAK), p-FAK, Src, vinculin, and paxillin [27].

Interactions between the cadherin- and the integrin-mediated AJs may occur through different adaptors and scaffold (e.g., p120, α- or γ-catenin, vinculin) or effector proteins (e.g., non-receptor tyrosine kinases such as Src and Yes, or Focal Adherens Kinases – FAK, phosphatases) [82]. Integrin and Cadh mediated junctions share several common signaling components, e.g., vinculin or FAT [83]. Also, data from other tissues showed that Necls (nectin-like molecules) interact with nectins and associates with integrins and Growth Factor Receptor [78]. α3-β1 integrin associates with Cadh-mediated AJs in multiple tissues. Tyrosine phosphorylation of β-catenin, mediated by α3-β1 integrin via the tetraspanin interaction, shifts the molecule from the Cadh complexes. Freed, β-catenin becomes available to act in other pathways such as the Wnt. Also, through the phosphorylation of α-catenin, modulates the Cadh complex connection to the actin cytoskeleton [84]. Integrins and Cadh complexes are also intrinsically linked to each other via their binding to the actin-myosin network [83].

JUNCTION DYNAMICS AND SPERMATOGENESIS

Through spermatogenesis, developing germ cells cross the BTB and pass from the basal into the adluminal tubular compartment to finally be released as fully maturated spermatids into the lumen of the tubules (spermiation). The main events requesting the turnover of AJs are the transport of pre-leptotene/leptotene spermatocyte across the BTB, the

movement of round spermatids back and forth the seminiferous epithelium, and spermiation [16, 85].

During this process, the junctional complexes mediating cell to cell interaction in the seminiferous tubules (either between Sertoli cells and between the Sertoli and germ cells) suffer changes and are remodelled, on a precise spatiotemporal dependent regulation [86]. The trans-compartmental migration implies the disassembly and reassembly not only of the TJs but also of the AJs up- and downstream the migrating cells. To the perfect synchrony of the junctional remodelling, it is of utmost importance the existence of functional crosstalk as well as a fine-tuned mechanism of recycling within and between compartments, a phenomenon intimately dependent on GJs [16]. Similarly, before spermiation, the apical ES suffers rearrangements, a transitory AJs complex forms – the TBC -, in a process tightly coordinated. The TBC has been associated with a "recycling device" allowing the Sertoli cell to reuse structures and molecules belonging to former/changed junctional complexes. Signals originating in one type of junctions will determine the changes in the other junctional complexes elsewhere in the cell, which is achieved by changes in membrane trafficking, cytoskeleton association or binding affinity [82].

The assembling-disassembling of AJs is a dynamic and stringently regulated process, responding to intra- or extracellular stimuli [75]. The regulation of the AJs may be achieved through transcriptional and post-transcriptional mechanisms directed to the adhesion complex as well as to the actin cytoskeleton. The transcriptional mechanism regulates the *de novo* production of any element of the junctional complexes. In a structure like the seminiferous tubules, where an immense number of cells develop and need to migrate across and out of the tubules, the homeostasis of the process should not depend exclusively on de synthesis of new molecules to rebuild the junctions, the posttranslational mechanisms allowing the recycling and trafficking of molecules to assemble new AJs predominate [16]. A cell can control the protein activity and its interactions at a given place via posttranslational modifications (e.g., glycosylation, ubiquitination, proteolysis, and phosphorylation) [87].

AJs dynamics in the seminiferous tubules are mainly regulated by protein kinases and phosphatases that phosphorylate the AJ structural proteins [88], thereby breaking down the complex N-Cadh-β-catenin and consequently its connection to the actin cytoskeleton. Changes in the linkage to the actin cytoskeleton would also assist germ cell propulsion into a new location.

It has been proposed that the SRC family of non-receptors protein kinases are essential players in the remodelling of the junctional complexes during intratubular cell migration, representing a critical hub controlling junctional remodelling. Among those non-receptor proteins, c-Src and c-Yes are present in either Sertoli and germ cells. They trigger the phosphorylation of adhesion cell proteins [85, 89]. In the seminiferous tubule, c-Src structurally associates with p-FAK (Focal Adhesion Kinase) and the integrin-laminin complex. On the other hand, c-Yes interacts with occludin, FAK, N-cadherin, β- and γ-catenin, β1-integrin as well as with actin (via the Epidermal Growth Factor receptor-8) [90]. An increase in tyrosine residues of proteins phosphorylation at the cadherin/catenin complexes drives a loss of interaction with the actin cytoskeleton, and thereby to the loss of intercellular adhesion. However, these molecules regulate far more than the dynamics of AJs, the availability of the junctional proteins or the actin interaction. It has been shown that they also play a role in the endocytic vesicle-mediated protein trafficking [91] in Sertoli cells, that allows the recycling of molecules of the junctional complexes during the restructuring of AJs. Endosome vesicles with internalized cadh or their adaptor proteins might be transported across the Sertoli cell to where the new junctions are assembled [85], even if in a different tubular compartment, or be sent to degradation. c-Src and c-Yes seems to have opposing roles in the outcome of disassembled proteins, the c-Yes driving the molecules towards the endocytic vesicles trafficking mechanism (transcytosis/recycling), whereas c-Src fosters their lysosomal degradation [85, 91].

On the other hand, the reversible ubiquitination of adhesion molecules will also foster degradation of the molecules (in proteasomes), namely occludins, Cadhs and β-catenin, disassembling TJs and AJs. Ubiquitinating

and deubiquitinating enzymes have been identified in the seminiferous tubules, and propose to contribute to changes in the permeability of junctional complexes [92].

Several molecular regulators have been implicated in the AJs dynamics, namely cytokines (e.g., TGF-β or TNF) and hormones (e.g., testosterone). Androgens are important determinants of spermatogenesis. It has been shown that testosterone plays a crucial role during the restructuring of the junctional complex in the seminiferous tubules [93], by mediating the mechanisms of endocytosis, transcytosis and recycling of the membrane-linked proteins that participate in the intercellular interface of tubular junctions [16]. In particular, testosterone actions on the AJs dynamics are exerted thought nonclassical signalling cascades, via c-Src and MAP-Kinase [86, 89]. Testosterone-induced phosphorylation of EGF receptor II, activates SRC family proteins [89], which promote the recycling of integrated membrane proteins. Contrastingly, cytokines enhance endosome-mediated degradation. Moreover, ERK1/2 activation by the TGF-β3 receptor induces AJs disruption, in a MAPK-mediated pathway that recruits peripheral adaptors. Those molecules (e.g., ZO-1 and α- and γ-catenin) allow the linkage of the TJs- and AJs-integral membrane proteins, which do not physically interact with each other, thereby contributing to the coordinated junctional remodelling in the seminiferous tubules [92, 94].

An additional candidate participating in the AJs remodelling is the Nitric Oxide (NO)-cGMP signalling pathway [95], which regulates β-catenin availability to partner with N-Cadherin [96], driving the dissolution of the AJ structural protein complexes.

CONCLUSION

In the seminiferous tubules, Cadherins are important actors in the interplay of the AJs, TJs and GJs that together form the blood-testis-barrier. BTB is one of the most complex form of cell-to-cell connection,

and a fundamental structure requesting a finely tuned control to support the development and protection of the spermatogenic epithelium. This brief review intent to highlight the role of N-Cadh in testicular AJs and its role in the junctional dynamics. Limited literature is available at the topic, and most references address the role of AJs as a complement for the TJs in BTB. Much remains elusive regarding the mechanisms regulating AJs during spermatogenesis, and on how they behave during the germ cells movement across the epithelium. A few research groups are now working with hypothetical models which may prove useful to design future functional experimental studies whether to support the quest for male fertility and contraceptive purposes. The ever-growing pace of the science undoubtedly will soon provide new insights on Cadhs and AJs in the seminiferous tubules.

REFERENCES

[1] Honda, H. 2017. "The world of epithelial sheets." *Dev Growth Differ* 59 (5):306-316. doi: 10.1111/dgd.12350.

[2] Obrink, B. 1986. "Epithelial cell adhesion molecules." *Exp Cell Res* 163 (1):1-21. doi: 10.1016/0014-4827(86)90554-9.

[3] Wei, Q., and H. Huang. 2013. "Insights into the role of cell-cell junctions in physiology and disease." *Int Rev Cell Mol Biol* 306:187-221. doi: 10.1016/B978-0-12-407694-5.00005-5.

[4] Alberts, B., A. Johnson, J. Lewis, D. Morgan, M. Raff, K. Roberts, and P. Walter. 2015. "Cell junctions, Cell adhesion and the extracelular matrix." In *Molecular Biology of the Cell*, edited by G. S. Lewis and E. Zayatz, 1035-1090. New York: Garland Science.

[5] LaFlamme, S. E., and P. A. Vincent. 2006. "Cell Junctions, Structure, Function, and Regulation." *Reviews in Cell Biology and Molecular Medicine*. doi: 10.1002/3527600906.mcb.200300165.

[6] Niessen, C. M. 2007. "Tight junctions/adherens junctions: basic structure and function." *J Invest Dermatol* 127 (11):2525-32. doi: 10.1038/sj.jid.5700865.

[7] Pollard, T. D., W. C. Earnshaw, J. Lippincott-Schwartz, and G. T. Johnson. 2017. "Chapter 30 - Cellular Adhesion." In *Cell Biology (Third Edition)*, edited by Thomas D. Pollard, William C. Earnshaw, Jennifer Lippincott-Schwartz and Graham T. Johnson, 525-541. Elsevier.

[8] Ni, F. D., S. L. Hao, and W. X. Yang. 2019. "Multiple signaling pathways in Sertoli cells: recent findings in spermatogenesis." *Cell Death Dis* 10 (8):541. doi: 10.1038/s41419-019-1782-z.

[9] Domke, L. M., S. Rickelt, Y. Dörflinger, C. Kuhn, S. Winter-Simanowski, R. Zimbelmann, R. Rosin-Arbesfeld, H. Heid, and W. W. Franke. 2014. "The cell-cell junctions of mammalian testes: I. The adhering junctions of the seminiferous epithelium represent special differentiation structures." *Cell Tissue Res* 357 (3):645-65. doi: 10.1007/s00441-014-1906-9.

[10] Lara, N. L. M., G. M. J. Costa, G. F. Avelar, S. M. S. N. Lacerda, R. A. Hess, and L. R. de França. 2018. "Testis Physiology—Overview and Histology." In *Encyclopedia of Reproduction (Second Edition)*, edited by Michael K. Skinner, 105-116. Oxford: Academic Press.

[11] Lara, N. L. M., G. F. de Avelar, G. M. J. Costa, S. M. S. N. Lacerda, R. A. Hess, and L. R. de França. 2018. "Cell–Cell Interactions—Structural." In *Encyclopedia of Reproduction (Second Edition)*, edited by Michael K. Skinner, 68-75. Oxford: Academic Press.

[12] Goldberg, E., and B. R. Zirkin. 2018. "Spermatogenesis: Overview." In *Encyclopedia of Reproduction (Second Edition)*, edited by Michael K. Skinner, 13-18. Oxford: Academic Press.

[13] Stanton, P. G. 2016. "Regulation of the blood-testis barrier." *Semin Cell Dev Biol* 59:166-173. doi: 10.1016/j.semcdb.2016.06.018.

[14] Mao, B., M. Yan, L. Li, and C. Y. Cheng. 2018. "Blood-Testis Barrier." In *Encyclopedia of Reproduction (Second Edition)*, edited by Michael K. Skinner, 152-160. Oxford: Academic Press.

[15] Hess, R. A., and A. W. Vogl. 2015. "Sertoli cell anatomy and cytoskeleton." In *Sertoli Cell Biology (Second Edition)*, edited by Michael D. Griswold, 1-55. Oxford: Academic Press.

[16] Yan Cheng, C., and D. D. Mruk. 2015. "Biochemistry of Sertoli cell/germ cell junctions, germ cell transport, and spermiation in the seminiferous epithelium." In *Sertoli Cell Biology (Second Edition)*, edited by Michael D. Griswold, 333-383. Oxford: Academic Press.

[17] Cheng, C. Y., and D. D. Mruk. 2009. "Regulation of blood-testis barrier dynamics by focal adhesion kinase (FAK): an unexpected turn of events." *Cell Cycle* 8 (21):3493-9. doi: 10.4161/cc.8.21.9833.

[18] Mruk, D. D., and C. Y. Cheng. 2015. "The Mammalian Blood-Testis Barrier: Its Biology and Regulation." *Endocr Rev* 36 (5):564-91. doi: 10.1210/er.2014-1101.

[19] Vazquez-Levin, M. H., C. I. Marín-Briggiler, J. N. Caballero, and M. F. Veiga. 2015. "Epithelial and neural cadherin expression in the mammalian reproductive tract and gametes and their participation in fertilization-related events." *Dev Biol* 401 (1):2-16. doi: 10.1016/j.ydbio.2014.12.029.

[20] Yan, H. H. N., D. D. Mruk, W. M. Lee, and C. Y. Cheng. 2008. "Cross-Talk between Tight and Anchoring Junctions—Lesson from the Testis." In *Molecular Mechanisms in Spermatogenesis*, edited by C. Yan Cheng, 234-254. New York, NY: Springer New York.

[21] Goossens, S., and F. van Roy. 2005. "Cadherin-mediated cell-cell adhesion in the testis." *Front Biosci* 10:398-419. doi: 10.2741/1537.

[22] Ravel, C., and S. Jaillard. 2011. "[The Sertoli cell]." *Morphologie* 95 (311):151-8. doi: 10.1016/j.morpho.2011.07.118.

[23] Domke, L. M. 2018. *Molecular and ultrastructural characteristics of adhering junctions and cytoskeletons in cells of mammalian testes.* Doctor of Natural Sciences Dissertation, Combined Faculty of Natural Sciences and Mathematics, Ruperto Carola University, Germany.

[24] Lui, W.-Y., D. D. Mruk, W. M. Lee, and C. Y. Cheng. 2003. "Adherens Junction Dynamics in the Testis and Spermatogenesis." *Journal of Andrology* 24 (1):1-14. doi: 10.1002/j.1939-4640.2003.tb02627.x.

[25] Berruti, G., and C. Paiardi. 2014. "The dynamic of the apical ectoplasmic specialization between spermatids and Sertoli cells: the

case of the small GTPase Rap1." *Biomed Res Int* 2014:635979. doi: 10.1155/2014/635979.

[26] Toyama, Y., M. Maekawa, and S. Yuasa. 2003. "Ectoplasmic specializations in the Sertoli cell: new vistas based on genetic defects and testicular toxicology." *Anat Sci Int* 78 (1):1-16. doi: 10.1046/j.0022-7722.2003.00034.x.

[27] Wong, E. W., D. D. Mruk, and C. Y. Cheng. 2008. "Biology and regulation of ectoplasmic specialization, an atypical adherens junction type, in the testis." *Biochim Biophys Acta* 1778 (3):692-708. doi: 10.1016/j.bbamem.2007.11.006.

[28] França, L. R., R. A. Hess, J. M. Dufour, M. C. Hofmann, and M. D. Griswold. 2016. "The Sertoli cell: one hundred fifty years of beauty and plasticity." *Andrology* 4 (2):189-212. doi: 10.1111/andr.12165.

[29] Domke, L. M. 2019. "The cell-cell junctions of mammalian testes-a summary." *Cell Tissue Res*. doi: 10.1007/s00441-019-03150-3.

[30] Takeichi, M. 2018. "Historical review of the discovery of cadherin, in memory of Tokindo Okada." *Dev Growth Differ* 60 (1):3-13. doi: 10.1111/dgd.12416.

[31] Takeichi, M. 1977. "Functional correlation between cell adhesive properties and some cell surface proteins." *J Cell Biol* 75 (2 Pt 1):464-74. doi: 10.1083/jcb.75.2.464.

[32] Yoshida-Noro, C., N. Suzuki, and M. Takeichi. 1984. "Molecular nature of the calcium-dependent cell-cell adhesion system in mouse teratocarcinoma and embryonic cells studied with a monoclonal antibody." *Dev Biol* 101 (1):19-27. doi: 10.1016/0012-1606(84)90112-x.

[33] Colás-Algora, N., and J. Millán. 2019. "How many cadherins do human endothelial cells express?" *Cell Mol Life Sci* 76 (7):1299-1317. doi: 10.1007/s00018-018-2991-9.

[34] Nollet, F., P. Kools, and F. van Roy. 2000. "Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members." *J Mol Biol* 299 (3):551-72. doi: 10.1006/jmbi.2000.3777.

[35] Oda, H., and M. Takeichi. 2011. "Evolution: structural and functional diversity of cadherin at the adherens junction." *J Cell Biol* 193 (7):1137-46. doi: 10.1083/jcb.201008173.

[36] Pokutta, S., and W. I. Weis. 2007. "Structure and mechanism of cadherins and catenins in cell-cell contacts." *Annu Rev Cell Dev Biol* 23:237-61. doi: 10.1146/annurev.cellbio.22.010305.104241.

[37] Angst, B. D., C. Marcozzi, and A. I. Magee. 2001. "The cadherin superfamily: diversity in form and function." *J Cell Sci* 114 (Pt 4):629-41.

[38] Hulpiau, P., I. S. Gul, and F. van Roy. 2016. "Evolution of Cadherins and Associated Catenins." In *The Cadherin Superfamily: Key Regulators of Animal Development and Physiology*, edited by Shintaro T. Suzuki and Shinji Hirano, 13-37. Tokyo: Springer Japan.

[39] Shapiro, L., and W. I. Weis. 2009. "Structure and biochemistry of cadherins and catenins." *Cold Spring Harb Perspect Biol* 1 (3):a003053. doi: 10.1101/cshperspect.a003053.

[40] Wheelock, M. J., and K. R. Johnson. 2003. "Cadherins as modulators of cellular phenotype." *Annu Rev Cell Dev Biol* 19:207-35. doi: 10.1146/annurev.cellbio.19.011102.111135.

[41] Halbleib, J. M., and W. J. Nelson. 2006. "Cadherins in development: cell adhesion, sorting, and tissue morphogenesis." *Genes Dev* 20 (23):3199-214. doi: 10.1101/gad.1486806.

[42] Brasch, J., O. J. Harrison, B. Honig, and L. Shapiro. 2012. "Thinking outside the cell: how cadherins drive adhesion." *Trends Cell Biol* 22 (6):299-310. doi: 10.1016/j.tcb.2012.03.004.

[43] Gumbiner, B. M. 2016. "Classical Cadherins." In *The Cadherin Superfamily: Key Regulators of Animal Development and Physiology*, edited by Shintaro T. Suzuki and Shinji Hirano, 41-69. Tokyo: Springer Japan.

[44] Heuberger, J., and W. Birchmeier. 2010. "Interplay of cadherin-mediated cell adhesion and canonical Wnt signaling." *Cold Spring Harb Perspect Biol* 2 (2):a002915. doi: 10.1101/cshperspect.a002915.

[45] Chidgey, M., and D. Garrod. 2016. "Desmosomal Cadherins." In *The Cadherin Superfamily: Key Regulators of Animal Development and Physiology*, edited by Shintaro T. Suzuki and Shinji Hirano, 159-193. Tokyo: Springer Japan.

[46] Junghans, D., I. G. Haas, and R. Kemler. 2005. "Mammalian cadherins and protocadherins: about cell death, synapses and processing." *Curr Opin Cell Biol* 17 (5):446-52. doi: 10.1016/j.ceb.2005.08.008.

[47] Sano, K., H. Tanihara, R. L. Heimark, S. Obata, M. Davidson, T. St John, S. Taketani, and S. Suzuki. 1993. "Protocadherins: a large family of cadherin-related molecules in central nervous system." *EMBO J* 12 (6):2249-56.

[48] Hayashi, S., and M. Takeichi. 2015. "Emerging roles of protocadherins: from self-avoidance to enhancement of motility." *J Cell Sci* 128 (8):1455-64. doi: 10.1242/jcs.166306.

[49] Fulford, A. D., and H. McNeill. 2019. "Fat/Dachsous family cadherins in cell and tissue organisation." *Curr Opin Cell Biol* 62:96-103. doi: 10.1016/j.ceb.2019.10.006.

[50] Sharma, P., and H. McNeill. 2013. "Fat and Dachsous cadherins." *Prog Mol Biol Transl Sci* 116:215-35. doi: 10.1016/B978-0-12-394311-8.00010-8.

[51] Usui, T., Y. Shima, Y. Shimada, S. Hirano, R. W. Burgess, T. L. Schwarz, M. Takeichi, and T. Uemura. 1999. "Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled." *Cell* 98 (5):585-595.

[52] Fujiwara, T., A. Mizoguchi, and Y. Takai. 2016. "Cooperative Roles of Nectins with Cadherins in Physiological and Pathological Processes." In *The Cadherin Superfamily: Key Regulators of Animal Development and Physiology*, edited by Shintaro T. Suzuki and Shinji Hirano, 115-156. Tokyo: Springer Japan.

[53] Lee, N. P., and C. Y. Cheng. 2004a. "Adaptors, junction dynamics, and spermatogenesis." *Biol Reprod* 71 (2):392-404. doi: 10.1095/biolreprod.104.027268.

[54] Johnson, K. J., and K. Boekelheide. 2002. "Dynamic testicular adhesion junctions are immunologically unique. II. Localization of classic cadherins in rat testis." *Biol Reprod* 66 (4):992-1000. doi: 10.1095/biolreprod66.4.992.

[55] Munro, S. B., and O. W. Blaschuk. 1996. "A comprehensive survey of the cadherins expressed in the testes of fetal, immature, and adult mice utilizing the polymerase chain reaction." *Biol Reprod* 55 (4):822-7. doi: 10.1095/biolreprod55.4.822.

[56] Byers, S. W., S. Sujarit, B. Jegou, S. Butz, H. Hoschutzky, K. Herrenknecht, C. MacCalman, and O. W. Blaschuk. 1994. "Cadherins and cadherin-associated molecules in the developing and maturing rat testis." *Endocrinology* 134 (2):630-9. doi: 10.1210/endo.134.2.7507830.

[57] Rode, K., H. Sieme, P. Richterich, and R. Brehm. 2015. "Characterization of the equine blood-testis barrier during tubular development in normal and cryptorchid stallions." *Theriogenology* 84 (5):763-72. doi: 10.1016/j.theriogenology.2015.05.009.

[58] Ebnet, K., D. Kummer, T. Steinbacher, A. Singh, M. Nakayama, and M. Matis. 2018. "Regulation of cell polarity by cell adhesion receptors." *Semin Cell Dev Biol* 81:2-12. doi: 10.1016/j.semcdb.2017.07.032.

[59] Padmanabhan, A., M. V. Rao, Y. Wu, and R. Zaidel-Bar. 2015. "Jack of all trades: functional modularity in the adherens junction." *Curr Opin Cell Biol* 36:32-40. doi: 10.1016/j.ceb.2015.06.008.

[60] Collins, C., and W. J. Nelson. 2015. "Running with neighbors: coordinating cell migration and cell-cell adhesion." *Curr Opin Cell Biol* 36:62-70. doi: 10.1016/j.ceb.2015.07.004.

[61] Piprek, R. P., M. Kolasa, D. Podkowa, M. Kloc, and J. Z. Kubiak. 2019. "Tissue-specific knockout of E-cadherin (Cdh1) in developing mouse gonads causes germ cells loss." *Reproduction*. doi: 10.1530/REP-18-0621.

[62] Tolkunova, E. N., A. B. Malashicheva, E. V. Chikhirzhina, E. I. Kostyleva, W. Zeng, J. Luo, I. Dobrinski, A. Hierholzer, R. Kemler, and A. N. Tomilin. 2009. "E-cadherin as a novel surface marker of

spermatogonial stem cells." *Cell and Tissue Biology* 3 (2):103-109. doi: 10.1134/S1990519X09020011.

[63] Zhang, Y., H. Su, F. Luo, S. Wu, L. Liu, T. Liu, B. Yu, and Y. Wu. 2011. "E-cadherin can be expressed by a small population of rat undifferentiated spermatogonia in vivo and in vitro." *In Vitro Cellular & Developmental Biology - Animal* 47 (8):593. doi: 10.1007/s11626-011-9446-z.

[64] Zhang, Y., S. Wu, F-h. Luo, Baiyinbatu, L-h. Liu, T-y. Hu, B. Yu, G-p. Li, and Y-j. Wu. 2014. "CDH1, a Novel Surface Marker of Spermatogonial Stem Cells in Sheep Testis." *Journal of Integrative Agriculture* 13 (8):1759-1765. doi: 10.1016/S2095-3119(13)60689-9.

[65] Newton, S. C., O. W. Blaschuk, and C. F. Millette. 1993. "N-cadherin mediates Sertoli cell-spermatogenic cell adhesion." *Dev Dyn* 197 (1):1-13. doi: 10.1002/aja.1001970102.

[66] Johnson, K. J., and K. Boekelheide. 2002. "Dynamic testicular adhesion junctions are immunologically unique. I. Localization of p120 catenin in rat testis." *Biol Reprod* 66 (4):983-91. doi: 10.1095/biolreprod66.4.983.

[67] Andersson, A. M., K. Edvardsen, and N. E. Skakkebaek. 1994. "Expression and localization of N- and E-cadherin in the human testis and epididymis." *Int J Androl* 17 (4):174-80. doi: 10.1111/j.1365-2605.1994.tb01239.x.

[68] Lee, N. P., D. Mruk, W. M. Lee, and C. Y. Cheng. 2003. "Is the cadherin/catenin complex a functional unit of cell-cell actin-based adherens junctions in the rat testis?" *Biol Reprod* 68 (2):489-508. doi: 10.1095/biolreprod.102.005793.

[69] Mruk, D. D., and C. Y. Cheng. 2004. "Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis." *Endocr Rev* 25 (5):747-806. doi: 10.1210/er.2003-0022.

[70] Lie, P. P., C. Y. Cheng, and D. D. Mruk. 2011. “The biology of the desmosome-like junction a versatile anchoring junction and signal transducer in the seminiferous epithelium.” *Int Rev Cell Mol Biol* 286:223-69. doi: 10.1016/B978-0-12-385859-7.00005-7.

[71] Mok, K-W., P. P. Y. Lie, D. D. Mruk, J. Mannu, P. P. Mathur, B. Silvestrini, and C. Y. Cheng. 2013. “The Apical Ectoplasmic Specialization-Blood-Testis Barrier Functional Axis is A Novel Target for Male Contraception.” In *Biology and Regulation of Blood-Tissue Barriers*, edited by C. Yan Cheng, 334-355. New York, NY: Springer New York.

[72] Berruti, G., M. Ceriani, and E. Martegani. 2018. “Dynamic of VE-cadherin-mediated spermatid-Sertoli cell contacts in the mouse seminiferous epithelium.” *Histochem Cell Biol* 150 (2):173-185. doi: 10.1007/s00418-018-1682-9.

[73] Fleming, A., N. Ghahramani, M. X. Zhu, E. C. Délot, and E. Vilain. 2012. “Membrane β-catenin and adherens junctions in early gonadal patterning.” *Developmental Dynamics* 241 (11):1782-1798. doi: 10.1002/dvdy.23870.

[74] Islam, R., H. Yoon, B. S. Kim, H. S. Bae, H. R. Shin, W. J. Kim, W. J. Yoon, Y. S. Lee, K. M. Woo, J. H. Baek, and H. M. Ryoo. 2017. “Blood-testis barrier integrity depends on Pin1 expression in Sertoli cells.” *Sci Rep* 7 (1):6977. doi: 10.1038/s41598-017-07229-1.

[75] D'Souza-Schorey, C. 2005. “Disassembling adherens junctions: breaking up is hard to do.” *Trends Cell Biol* 15 (1):19-26. doi: 10.1016/j.tcb.2004.11.002.

[76] Miyoshi, J., and Y. Takai. 2007. “Nectin and nectin-like molecules: biology and pathology.” *Am J Nephrol* 27 (6):590-604. doi: 10.1159/000108103.

[77] Li, M. W. M., D. D. Mruk, and C. Y. Cheng. 2013. “Gap Junctions and Blood-Tissue Barriers.” In *Biology and Regulation of Blood-Tissue Barriers*, edited by C. Yan Cheng, 260-280. New York, NY: Springer New York.

[78] Togashi, H. 2016. "Differential and Cooperative Cell Adhesion Regulates Cellular Pattern in Sensory Epithelia." *Front Cell Dev Biol* 4:104. doi: 10.3389/fcell.2016.00104.

[79] Lee, N. P., D. D. Mruk, A. M. Conway, and C. Y. Cheng. 2004. "Zyxin, axin, and Wiskott-Aldrich syndrome protein are adaptors that link the cadherin/catenin protein complex to the cytoskeleton at adherens junctions in the seminiferous epithelium of the rat testis." *J Androl* 25 (2):200-15. doi: 10.1002/j.1939-4640.2004.tb02780.x.

[80] Lee, N. P., and C. Y. Cheng. 2004b. "Ectoplasmic specialization, a testis-specific cell-cell actin-based adherens junction type: is this a potential target for male contraceptive development?" *Hum Reprod Update* 10 (4):349-69. doi: 10.1093/humupd/dmh026.

[81] Wu, S., M. Yan, R. Ge, and C. Y. Cheng. 2019. "Crosstalk between Sertoli and Germ Cells in Male Fertility." *Trends Mol Med.* doi: 10.1016/j.molmed.2019.09.006.

[82] Weber, G. F., M. A. Bjerke, and D. W. DeSimone. 2011. "Integrins and cadherins join forces to form adhesive networks." *J Cell Sci* 124 (Pt 8):1183-93. doi: 10.1242/jcs.064618.

[83] Mui, K. L., C. S. Chen, and R. K. Assoian. 2016. "The mechanical regulation of integrin-cadherin crosstalk organizes cells, signaling and forces." *J Cell Sci* 129 (6):1093-100. doi: 10.1242/jcs.183699.

[84] Chattopadhyay, N., Z. Wang, L. K. Ashman, S. M. Brady-Kalnay, and J. A. Kreidberg. 2003. "alpha3beta1 integrin-CD151, a component of the cadherin-catenin complex, regulates PTPmu expression and cell-cell adhesion." *J Cell Biol* 163 (6):1351-62. doi: 10.1083/jcb.200306067.

[85] Xiao, X., Y. Yang, B. Mao, C. Y. Cheng, and Y. Ni. 2019. "Emerging Role for SRC family kinases in junction dynamics during spermatogenesis." *Reproduction.* doi: 10.1530/REP-18-0440.

[86] Lui, W.-Y., and C. Y. Cheng. 2013. "Transcriptional Regulation of Cell Adhesion at the Blood-Testis Barrier and Spermatogenesis in the Testis." In *Biology and Regulation of Blood-Tissue Barriers*, edited by C. Yan Cheng, 281-294. New York, NY: Springer New York.

[87] Bertocchi, C., M. Vaman Rao, and R. Zaidel-Bar. 2012. "Regulation of adherens junction dynamics by phosphorylation switches." *J Signal Transduct* 2012:125295. doi: 10.1155/2012/125295.

[88] Chen, Y. M., N. P. Lee, D. D. Mruk, W. M. Lee, and C. Y. Cheng. 2003. "Fer kinase/FerT and adherens junction dynamics in the testis: an in vitro and in vivo study." *Biol Reprod* 69 (2):656-72. doi: 10.1095/biolreprod.103.016881.

[89] Chojnacka, K., and D. D. Mruk. 2015. "The Src non-receptor tyrosine kinase paradigm: New insights into mammalian Sertoli cell biology." *Mol Cell Endocrinol* 415:133-42. doi: 10.1016/j.mce.2015.08.012.

[90] Xiao, X., D. D. Mruk, F. L. Cheng, and C. Y. Cheng. 2013. "c-Src and c-Yes are Two Unlikely Partners of Spermatogenesis and their Roles in Blood-Testis Barrier Dynamics." In *Biology and Regulation of Blood-Tissue Barriers,* edited by C. Yan Cheng, 295-317. New York, NY: Springer New York.

[91] Xiao, X., Y. Ni, C. Yu, L. Li, B. Mao, Y. Yang, D. Zheng, B. Silvestrini, and C. Y. Cheng. 2018. "Src family kinases (SFKs) and cell polarity in the testis." *Semin Cell Dev Biol* 81:46-53. doi: 10.1016/j.semcdb.2017.11.024.

[92] Lui, W. Y., and C. Y. Cheng. 2007. "Regulation of cell junction dynamics by cytokines in the testis: a molecular and biochemical perspective." *Cytokine Growth Factor Rev* 18 (3-4):299-311. doi:10.1016/j.cytogfr.2007.04.009.

[93] Meng, J., R. W. Holdcraft, J. E. Shima, M. D. Griswold, and R. E. Braun. 2005. "Androgens regulate the permeability of the blood-testis barrier." *Proc Natl Acad Sci U S A* 102 (46):16696-700. doi: 10.1073/pnas.0506084102.

[94] Yan, H. H., and C. Y. Cheng. 2005. "Blood-testis barrier dynamics are regulated by an engagement/disengagement mechanism between tight and adherens junctions via peripheral adaptors." *Proc Natl Acad Sci U S A* 102 (33):11722-7. doi: 10.1073/pnas.0503855102.

[95] Mruk, D. D., O. Sarkar, and P. P. Mathur. 2008. "Nitric Oxide-cGMP Signaling: Its Role in Cell Junction Dynamics During Spermatogenesis." *Current Medicinal Chemistry - Immunology, Endocrine & Metabolic Agents)* 8 (1):28-35. doi: 10.2174/18715 2208783790741.

[96] Lee, N. P., D. D. Mruk, C. H. Wong, and C. Y. Cheng. 2005. "Regulation of Sertoli-germ cell adherens junction dynamics in the testis via the nitric oxide synthase (NOS)/cGMP/protein kinase G (PRKG)/beta-catenin (CATNB) signaling pathway: an in vitro and in vivo study." *Biol Reprod* 73 (3):458-71. doi: 10.1095/biolreprod. 105.040766.

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