CADHERIN-MEDIATED CELL-CELL ADHESION IN THE TESTIS

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TABLE OF CONTENTS

1. ABSTRACT

During spermatogenesis, the differentiating germ cells migrate across the seminiferous epithelium while maintaining close contact with the surrounding Sertoli cells via specialized actin-based adherens junctions (ectoplasmic specializations) and intermediate filament-based anchoring junctions (desmosome-like junctions). Although this migration is essential for correct completion of spermatogenesis, the mechanisms that regulate these anchoring junctions are largely unknown, and most of our knowledge of cell-cell adhesion in testis is based on earlier studies in epithelial tissues. In most epithelia, members of the cadherin superfamily play key roles in intercellular adhesion. Cadherins are calcium-dependent cell-cell adhesion molecules mediating numerous homotypic cell-cell interactions. Until recently there has been controversy about the presence and localization of cadherins in the testis, but now there is increasing evidence that various types of cadherins are expressed in this organ, which underscores their importance in testicular functions. Here, we review the expression patterns, regulatory mechanisms and possible roles of the cadherin-mediated cell-cell adhesion in the testis, and particularly during spermatogenesis. Moreover, attention is paid to additional molecular adhesion complexes in the testis, to associated signaling pathways and to cell adhesion-related innovative ways for male contraception.

2. INTRODUCTION: JUNCTION DYNAMICS DURING SPERMATOGENESIS

Mammalian spermatogenesis is probably the most exquisite example of a continuously synchronized and spatially organized sequence of cell proliferation, differentiation, translocation and morphogenesis. Unlike oogenesis, spermatogenesis relies on a population of true stem cells that are direct descendants of primordial germ cells, and which are capable of self-renewal and production of progeny for differentiation into spermatozoa. These progenitor cells, known as type-A spermatogonia, are located at the basal lamina of the seminiferous tubule, where they continuously proliferate by mitotic cell division (Figure 1). Some of these daughter cells, called type-B spermatogonia, stop proliferating and in a subsequent phase they undergo drastic changes in chromatin configuration, as they transform into primary spermatocytes. It is at this stage that meiosis begins and a single primary spermatocyte gives rise to four round, haploid spermatids. During meiosis, the preleptotene spermatocytes detach from the basal lamina at the preleptotene-leptotene transition and start migrating towards the lumen of the seminiferous tubule. Spermiogenesis, the final phase of morphogenesis of haploid spermatids into spermatozoa, is accompanied by extrusion of the cytoplasm, extensive nuclear condensation and the formation of a sperm tail. These specialized elongated spermatozoa are released into the lumen of the

Table 1. Functional classification of cell-cell junctions in the seminiferous epithelium

Junction class	Junction type	Localization
Class I: Occluding junctions	Tight junctions	Inter-Sertoli cell junctions (blood-testis barrier)
Class II: Anchoring junctions		
- IIA. With actin filament attachment sites	Adherens junctions (AJ)	Inter-Sertoli and Sertoli-germ cell junctions
	Ectoplasmic specializations (ES)	Inter-Sertoli and Sertoli-spermatid junctions
	Tubulobulbar complexes (TBC)	Junctions between Sertoli cells and elongated spermatids
- IIB. With intermediate filament attachment sites	Desmosome-like junctions	Inter-Sertoli and Sertoli-germ cell junctions
Class III: Communicating junctions	Gap junctions	Inter-Sertoli and Sertoli-germ cell junctions

seminiferous tubule, finally leaving the testis and passing into the epididymis for storage and further maturation.

During this highly organized program of extensive changes, the migrating germ cells maintain close contact with the surrounding Sertoli cells. The specific interactions between Sertoli cells and germ cells are absolutely crucial for the production of normal spermatozoa. During gametogenesis, Sertoli cells provide developing germ cells with essential structural support via adhesion junctions, and with nutritive support in the form of secreted factors. Evidently, successful migration of germ cells requires the existence of a well-organized system of cell-cell adhesion junctions. A characteristic feature of this system is the dynamic breakage and reformation of cell junctions between Sertoli cells and developing germ cells, a process which allows germ cell migration while maintaining the integrity of the testis. Ultrastructural studies of the seminiferous epithelium identified many morphologically diverse junctions (Figure 1) that mediate inter-Sertoli cell contacts besides contacts between Sertoli cells and germ cells. The cell-cell adhesion structures involved can be divided into three major types: occluding, anchoring and communicating junctions (Table 1).

1) Occluding or tight junctions play an essential role in the compartmentalization of the testis by sealing adjacent Sertoli cells together, and thereby preventing the diffusion of ions and solutes through the paracellular pathway. This barrier, also called the blood-testis barrier (BTB), divides the seminiferous tubule into a basal and an adluminal compartment, and in this way creates a unique separated microenvironment (lumen) in which germ cells can differentiate without risk of autoimmune reactions. A second function of tight junctions is the formation of a morphological and functional boundary between the apical and basolateral surface domains of the cell, a boundary that creates and maintains cell surface polarity (1, 2).

2) Anchoring junctions interconnect cytoskeletal proteins of adjacent cells, creating a strong intercellular network that maintains tissue integrity. Two types of intercellular anchoring junctions are known in epithelia: desmosomes (3) and adherens junctions (4). Desmosomes use intermediate filaments as anchoring sites, while adherens junctions are linked to the actin cytoskeleton. Ultrastructural studies have shown that the testis is equipped with both types of junctions: intermediate filament-anchored desmosome-like junctions, and a modified type of adherens junction called the ectoplasmic specialization (ES), as described in more detail below (section 4.2). Another type of actin-anchored junction, the

tubulobulbar complexes (TBC), is found between Sertoli cells and the elongated spermatids (5).

3) Communication between cells is achieved via gap junctions. These are aggregated channels that connect two adjacent cells and permit the exchange of small regulatory proteins and ions. These communicating junctions are found not only between neighboring Sertoli cells, but also between Sertoli and germ cells (6, 7).

Both adherens junctions and desmosomes have been well documented in epithelia. Over the last decades we have gained considerable insight into their composition, and recently we started to understand their regulation. Cadherins are single-pass transmembrane proteins that appear to play a crucial role in the formation of both types of intercellular junctions. Cadherins form a bridge between the two apposing cell membranes by means of Ca^{2+}-dependent homophilic binding of their extracellular domains. Here, we will focus mainly on the role of cadherin-mediated adhesion in spermatogenesis. Although cadherin-mediated adhesion has been extensively studied in epithelial tissues, much controversy exists in the literature regarding the expression and functions of cadherins and their associated proteins in the testis as outlined below.

3. CADHERIN-MEDIATED CELL-CELL ADHESION

Cadherins comprise a large family of transmembrane or membrane-associated glycoproteins that mediate specific cell-cell adhesion in a Ca^{2+}-dependent manner, functioning as key molecules in the morphogenesis of a variety of organs. The family consists of at least six major subfamilies, namely classical cadherins I and II, desmosomal cadherins (desmocollins and desmogleins), protocadherins and a variety of cadherin-related molecules (8). Many aspects of the three-dimensional structure of cadherins have been elucidated, and several reviews summarize these findings (9-13). In brief, most cadherins are integral membrane glycoproteins consisting of an intracellular domain, a single-pass transmembrane domain and an extracellular domain. Common to all of the family members is a number of so-called cadherin repeats in the extracellular domain, each containing about 110 amino acid residues (AA) and designated EC. A His-Ala-Val sequence in the aminoterminal EC1 repeat of E-cadherin was found to serve as a cell adhesion recognition (CAR) sequence (14). Calcium ions are absolutely essential for cadherin function, as they intercalate between the extracellular cadherin domains and rigidify the multidomain structure (15, 16). In the absence of calcium, cadherins appear to be very vulnerable to proteolytic degradation (17).
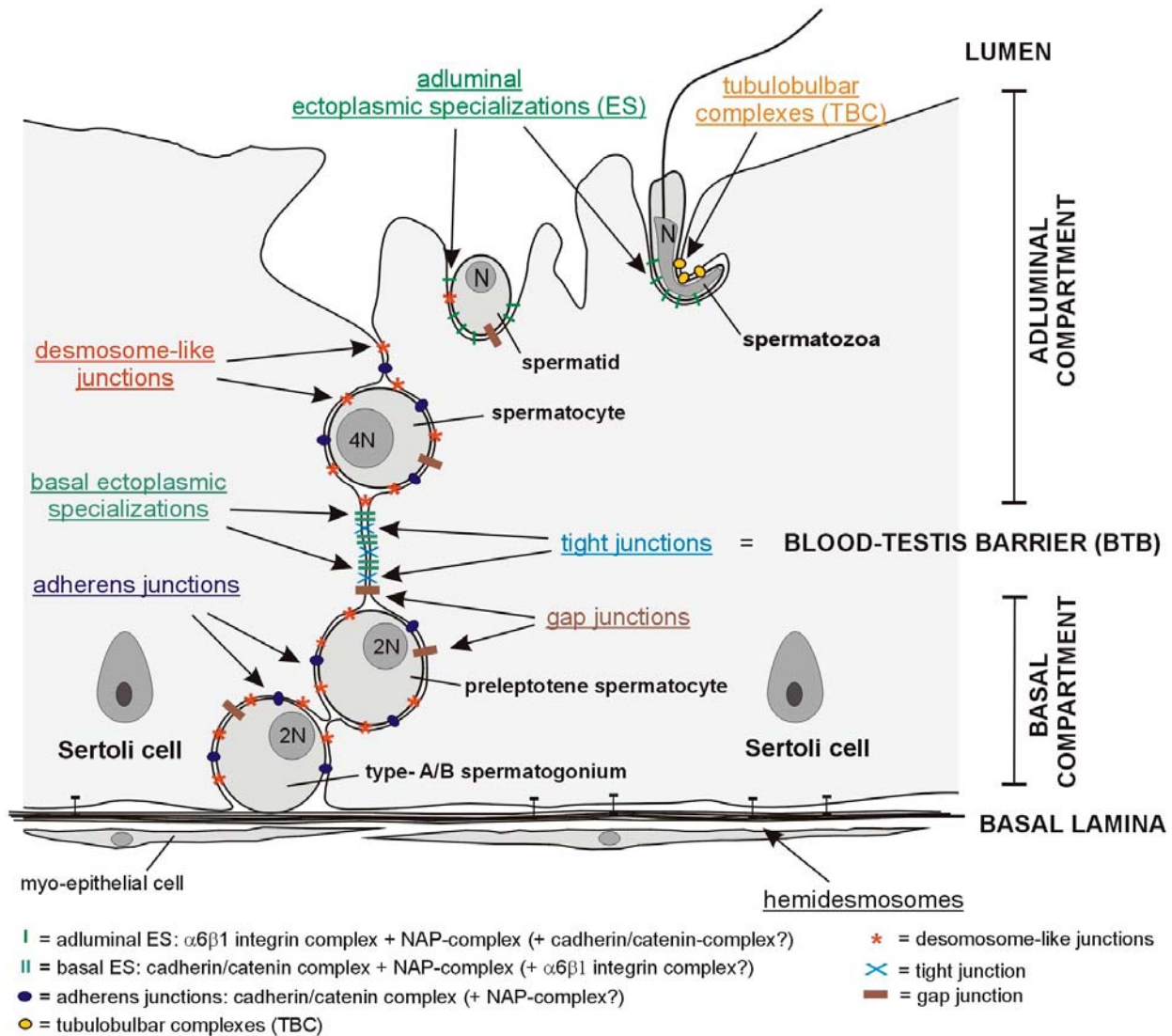
Figure 1. Schematic overview of cell junctions and their dynamics during spermatogenesis. Part of a seminiferous tubule is shown. In addition to occluding (tight) junctions and communicating (gap) junctions, different forms of anchoring junctions are known to regulate cell-cell adhesion in the testis. Desmosome-like anchoring junctions use the intermediate filaments as attachment sites. This is in contrast to ectoplasmic specialization (basal and adluminal ES) and tubulobulbar complexes (TBC), which are actin-based anchoring junctions.

The molecular details of the homophilic binding between the cadherin ectodomains are still a matter of debate. Tomschy et al. (18) suggested a two-step association mechanism in which lateral *cis* interactions (strand dimer) between the aminoterminal ends of cadherin ectodomains occur first and are a prerequisite for a second antiparallel, adhesive *trans* interaction (adhesion dimer). On the basis of the crystal structure of the aminoterminal domain of N-cadherin, Shapiro et al. (19) proposed a first model for homophilic cadherin binding. In this model *cis* bonds are mediated by the exchange of aminoterminal beta-strands between adjacent protomers and the trans dimerization appeared to be determined by a highly conserved tryptophan side chain (Trp2) in the cadherin EC1 domain, which is binding to a hydrophobic pocket comprising the CAR sequence. Specificity of homophilic adhesion by the various cadherins can be ascribed to evolutionarily conserved changes in this CAR sequence. Alternating *cis* and *trans* interactions then form an 'endless' zipper-like structure. However, a later study of the same group (20) and the crystal structure of the combined EC1 plus EC2 domains of E-cadherin (16, 21) could not confirm this hypothesis. Recently a novel model for cadherin-mediated adhesion has been proposed on the basis of the crystal structure of the entire ectodomain of C-cadherin of *Xenopus* (22). Here, a novel type of *cis*-interaction is suggested between Trp2 of the EC1 of one protomer and a hydrophobic pocket within the EC2 of a second adjacent protomer. This *cis*-orientated interface places protomers, emanating from the same cell surface, in a front-to-back arrangement, such that a continuous line of molecules is formed rather than a discrete dimer
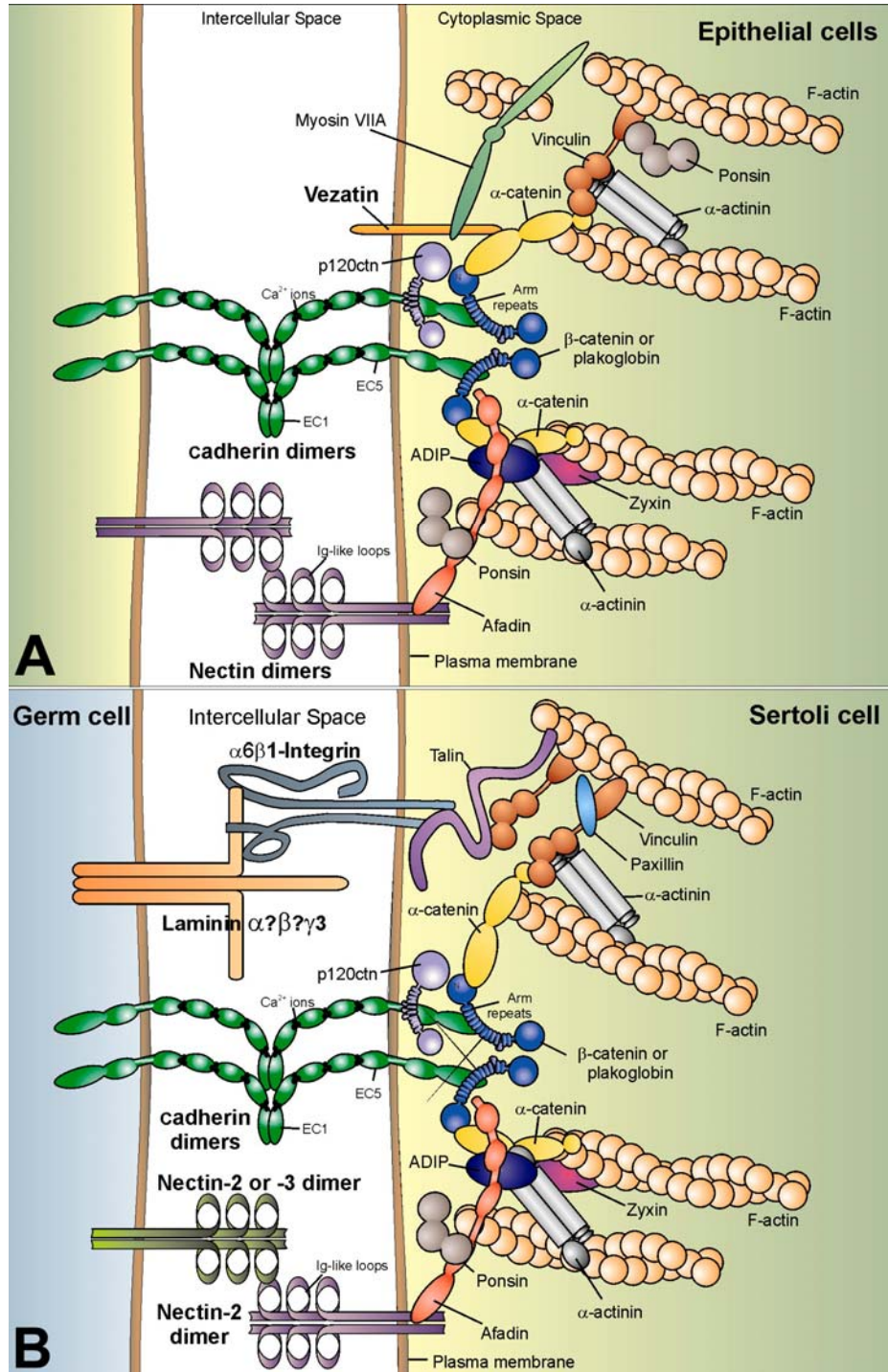
Figure 2.Dissection of adherens junctions. (A) In epithelia, three different protein complexes are known to localize at the adherens junctions: the cadherin/catenin complex, the nectin/afadin/ponsin-complex (NAP-complex) and the vezatin/myosin complex (modified after ref. (204)). (B) In the testis, a specialized actin-based anchoring junction can be found between Sertoli cells and between Sertoli cells and germ cells, called the ectoplasmic specialization. Three different protein complexes may be involved in the formation of these specialized anchoring junctions: the cadherin/catenin complex, the NAP-complex and an alpha-6 beta-1 integrin/laminin gamma-3 complex.

(Figure 2A). *Trans* or juxtaposed interactions between ectodomains were proposed to imply the beta-strand exchange assigned to the *cis* interaction in earlier work (13, 22).

Cadherin-mediated adhesion is crucial for the sorting out of cells during morphogenesis and organogenesis. Cadherins mediate cell-cell adhesion preferentially through homophilic cell-cell interactions, i.e. cells expressing the same type of cadherin adhere to each other, whereas cells expressing different types of cadherins adhere weakly or segregate from each other (23, 24). The role of cadherin-mediated adhesion in cell sorting processes was demonstrated by straightforward *in vitro* assays: two populations of non-adherent cells, each transfected with a different cadherin, sort out according to the type of cadherin expressed (25, 26). Homophilic adhesion enables the sorting out of cells according to predetermined patterns, establishing specific cell populations that form the basis for morphogenesis and organogenesis.

Two major types of cadherin-dependent cell-cell adhesion mechanisms have been extensively studied: the cadherin/catenin complex, which is the major functional unit of the adherens junctions, and desmosomal cell-cell adhesion. Below, we will elaborate on these junctions, their intrinsic and associated proteins and the roles they may play in the testis.

4. ADHERENS JUNCTIONS

4.1. The adherens junction dissected
Adherens junctions were originally defined, on the basis of ultrastructural analysis, as closely apposed plasma membrane domains reinforced by a dense cytoplasmic plaque to which actin microfilaments attach (27). As mentioned above, considerable research has been conducted on cadherin-mediated adhesion in epithelia, and much of our discussion of the data regarding the testis is based on the findings in epithelial tissues. Three protein complexes are found at the classical adherens junction in epithelia: the cadherin/catenin cell-cell adhesion complex, the nectin/afadin/pontin complex and the vezatin/myosin VIIA complex. The cadherin/catenin complex represents the major structural and functional unit at the adherens junctions, and is responsible mainly for homotypic cell-cell adhesion (28). The nectin/afadin/ponsin complex is found at homotypic cell-cell adhesion sites, but it is also present at synapses and at heterotypic cell junctions, e.g. Sertoli cell-spermatid junctions during spermiogenesis (29).

4.1.1. The cadherin/catenin complex: the major functional unit of adherens junctions in epithelia
The transmembrane core of the cadherin/catenin complex consists of a **classical cadherin**, with E(pithelial)-cadherin as the best characterized member (30, 31). The 120-kDa mature E-cadherin protein is composed of an extracellular domain with five tandemly arranged cadherin repeats (EC1-5), a single-pass transmembrane domain and a carboxyterminal cytodomain. Like all type-I classical cadherins, the extracellular domain binds Ca^{2+} and interacts in a homophilic way with E-cadherin molecules on the surface of neighboring cells. E-cadherin's function in intercellular adhesion requires its indirect linkage to the actin cytoskeleton through cytoplasmic binding to catenins (32, 33) (Figure 2).

The cytoplasmic tail of classical cadherins is highly conserved and possesses a binding site for **beta-catenin** or **plakoglobin** (gamma-catenin), both members of the armadillo protein family (34). Beta-catenin binds via its armadillo repeats directly to a carboxy-terminal core region of 30 AA residues containing a cluster of eight serine residues. Phosphorylation of this serine cluster within the cytoplasmic domain of E-cadherin enhances intercellular adherence and the affinity of E-cadherin for beta-catenin (35, 36). The beta-catenin binding site overlaps recently identified PEST sequences, which are associated with ubiquitin/proteasome degradation (37). In that way, binding to beta-catenin structures the cytoplasmic domain of E-cadherin and prevents its degradation (34). Besides its role in cadherin-mediated adhesion, beta-catenin is also an important player in the Wnt/Wingless signal transduction pathway, a highly conserved pathway involved in a large variety of developmental processes (38). This will be elaborated in section 4.4.

Binding of E-cadherin to beta-catenin or plakoglobin triggers the association of **alpha-catenin** with the amino-terminal head domain of these armadillo-proteins. By binding to F-actin, either directly through their carboxy-terminus, or indirectly, e.g. by binding to the actin-binding molecules alpha-actinin, vinculin (39) or zyxin (40), Alpha-catenins provide the necessary link to the actin cytoskeleton. Three different alpha-catenins, which are highly similar at the structural level but differ in expression pattern, are known. Apart from the ubiquitously expressed alphaE-catenin (41) and the neural alphaN-catenin (42, 43), a new alpha-catenin has recently been identified, alphaT-catenin (44). This novel alpha-catenin is expressed predominantly in heart and testis, but is also detectable in skeletal muscle and brain, and at low levels in some other tissues. *In vitro*, alphaN- and alphaT-catenin can substitute for the adhesive functions of alphaE-catenin (44, 45). However, their restricted expression patterns suggest that each one has specific functions.

A third member of the armadillo protein family, **p120ctn**, can also bind the cytoplasmic tail of E-cadherin. At cadherin-based junctions, p120ctn binds directly to a juxtamembrane cytoplasmic domain of classic cadherins, and together with the p120ctn phosphorylation status, this interaction modulates the adhesive strength of the junction (46, 47). More recently, a role for p120ctn in stabilization of cadherin junctions was convincingly demonstrated (48, 49). Furthermore, p120ctn is found within the nucleus of some cell types (50), where it may modulate gene expression by binding to transcription factors such as Kaiso, a new member of the BTB (Broad complex, Tramtrak, Bric à brac)/POZ (Pox virus and zinc finger) protein family of transcriptional repressors implicated in development and cancer (51). Thus, like beta-catenin, p120ctn is a protein that may play dual functions within the cell.

4.1.2. The nectin/afadin/ponsin complex (NAP-complex)
Recently, another membrane protein, **nectin,** and its associated protein l-afadin were found to be concentrated at adherens junctions. Nectin is a Ca^{2+}-independent immunoglobulin-like intercellular adhesion molecule, and comprises a family of at least four members,

all of which, with the exception of nectin-4, have two or three splice variants. All the members of this family have an extracellular region containing three Ig-like domains, a single transmembrane region and a cytoplasmic region. Nectin-3 is the most abundant form in the testis, but almost exclusively expressed by spermatids, whereas lower amounts of nectin-2 isoforms are expressed by most testicular cell types (52). A heterotransdimer of nectin-2 on the Sertoli cell membrane and nectin-3 on the spermatid membrane has been proposed (29). Male nectin-2 knockout mice are infertile, probably due to interference with a late stage of germ cell development (53). Indeed, they show tubules with normal numbers of apparently normal germ cells, but spermatozoa are morphologically quite aberrant. A conserved four-residue motif in the carboxy-terminal part of the cytoplasmic region of nectins interacts with the PDZ domain of l-afadin, an actin filament-binding protein, which connects nectin with the actin cytoskeleton. **Afadin** has two splice-variants, l-afadin and s-afadin. l-Afadin is the larger splice-variant and contains two Ras-association (RA) domains, a forkhead-associated (FHA) domain, a DIL-domain, a PDZ domain, three proline-rich (PR) domains and an F-actin-binding domain (54, 55). The shorter form, s-afadin, lacks the F-actin-binding domain and is probably not positively involved in cell-cell adhesion. **Ponsin** is an afadin-binding protein that is also localized at the adherens junctions, and which is able to bind vinculin (56, 57). Interestingly, the NAP complex interacts directly with the cadherin-based adhesion complex through afadin and alpha-catenin (57-59). Another indirect link between the two adhesion complexes has recently been found: ADIP (afadin DIL-domain-interacting protein) can simultaneously bind afadin and alpha-actinin (60) **(**Figure 2A).

Several lines of evidence indicate that the nectin/afadin and the cadherin/catenin systems are physically and functionally associated, and that these systems cooperate in organizing adherens junctions (29, 61). It has been postulated that when contact is made between two migrating cells, the nectin-based junctions are formed more rapidly than the cadherin-based junctions, which would imply that the nectin/afadin system plays a role in cell recognition. The NAP-complex may be involved in the dynamic formation and disruption of various types of intercellular adhesion. In addition, this complex may play an important role not only in homotypic junctions, because it also constitutes an important adhesion system in heterotypic junctions (between different types of cells), as in the case of the Sertoli cell-spermatid junctions dynamically formed and broken during spermiogenesis (29).

4.1.3. The vezatin/myosin VIIA complex

Vezatin is another transmembrane protein, discovered as a myosin-VIIA binding protein, which is localized at the adherens junctions (62). Its recruitment to the cadherin/catenin complex is dependent on alpha-catenin, but it is not yet clear whether this interaction is direct or indirect. Vezatin is presently considered the link between the cadherin/catenin complex and the actinomyosin-based contractile system. It is proposed that

the forces generated by myosin-VIIA and its linkage to the cadherin/catenin complex might create a tension between the plasma membrane and the actin cytoskeleton. This tension is believed to strengthen cell-cell adhesion between neighboring cells (63).

4.2. Adherens junctions in the testis: Does the cadherin/catenin complex exist in the testis?

During spermatogenesis, differentiating germ cells migrate from the basement membrane to the lumen of the seminiferous tubule, where they maintain close contact with the nourishing Sertoli cells. Little is known about the mechanisms involved in this translocation, but they obviously must include a dynamic and well-organized intercellular adhesion mechanism. Successful migration of the germ cells is characterized by active breakage and reformation of the Sertoli-germ cell junctions, to allow germ cell migration while maintaining the integrity of the testis. Ultrastructural studies of the testicular intercellular junctions performed over the previous decades have shown that the testis is equipped with a modified type of adherens junction, called the ectoplasmic specialization (ES) (64). The Sertoli cell ES is an important and unique junctional structure found within the basal compartment (between pairs of Sertoli cells, at the BTB) and within the adluminal compartment of the seminiferous epithelium (between Sertoli cells and elongating spermatids). The ES has originally been defined as hexagonally packed non-contractile actin filaments sandwiched between the Sertoli cell plasma membrane and the underlying endoplasmic reticulum (65). Despite the fact that some excellent reviews (66-69) deal with ES junctions, our knowledge about the molecular composition and regulation of these specialized testis-specific adherens junctions is still limited.

Since the early 1990s, studies targeting the role of cadherin-mediated adhesion in the testis have proliferated. These studies have raised questions regarding the existence of the cadherin/catenin cell-cell adhesion complex in the testis, and its involvement in the formation of the ES. Morphological observations suggest that the specialized actin-based cell-cell adhesion junctions in the testis may employ a functional unit different from that of the adherens junctions in epithelial cells. Indeed, recent studies have proposed that the apical ES may be largely regulated by interplay of alpha-6 beta-1 integrins, binding to a non-basement membrane laminin comprising the gamma3 chain and to focal adhesion complex-associated proteins (70-72), possibly in combination with the NAP-complex (52) instead of the cadherin/catenin complex. The most extensively studied integrin in the testis is alpha-6 beta-1, being largely restricted to Sertoli cells (73), whereas the laminin gamma3 chain is almost exclusively restricted to the apical compartment of the testis (72). Laminin-12 is composed of alpha2, beta1 and gamma-3 chains (74), but the partner laminin chains of gamma3 in the apical ES of the testis remain to be identified. In contrast, some *in vitro* data revealed that the testis may indeed use the cadherin/catenin complex as a functional unit to regulate actin-based adhesion. Although so far no experiments have been performed *in vivo*, two functional studies have shown that an anti-N-cadherin antibody can inhibit *in vitro*

adhesion between Sertoli cells and germ cells (75, 76). It is therefore essential to settle the controversy of the presence *versus* absence of a functional cadherin/catenin complex in the testis.

There is much disagreement in the literature even regarding the expression and localization of classical cadherins in the testis. A recent study, using a reverse transcriptase-polymerase chain reaction with degenerated primer pairs, showed that at least 7 classical cadherins were present at the mRNA level in developing and maturing testis, a diversity that underscores their possible importance in spermatogenesis and other testicular functions (77, 78). Few studies, however, have localized specific cadherins in postnatal testis. Immunostaining and Northern blotting both indicated that E-, N-, and P-cadherin are expressed in unique patterns in both the developing and the mature testis. In the seminiferous tubule, N-cadherin appears to be associated within the adluminal compartment at the heads of elongating spermatids and within the basal compartment at inter-Sertoli and Sertoli-germ cell junctions (71, 78-83). This is in contrast to P-cadherin, which localizes specifically to the junctions of the peritubular myoid cells (84). There is less consistency concerning E-cadherin in the testis. Whereas some authors reported that E-cadherin was not at all expressed in testis (79, 80, 85), other and more recent studies have shown otherwise (83, 86-88). A hypothesis has been put forward that E-cadherin expression may be restricted to developing and early postnatal testis of mammals, while N-cadherin expression is restricted to mature testis. Further research is needed to elucidate this matter.

The expression of the cadherin-associated catenins is also poorly understood in the testis. It appears that within the basal compartment of the seminiferous epithelium all known catenins (alpha-catenin, beta-catenin and p120ctn) are expressed at the inter-Sertoli junctions (basal ES) and also at junctions between Sertoli cells and spermatogonia or primary spermatocytes (non-ES sites, probably at desmosome-like junctions), where they colocalize with classic cadherins (78, 83). The expression of catenins in the adluminal compartment of the seminiferous epithelium and the involvement of cadherin-mediated adhesion in spermiation remains controversial. Recently, Chapin and coworkers have revealed the presence of beta-catenin near the luminal edge at spermiation (89, 90), and also p120 catenin has been localized at the apical ES (89, 91). In contrast, others reported that catenins were not detectable in the adluminal compartment of the seminiferous tubule. The latter suggests that N-cadherin in the adluminal compartment may serve a signaling rather than a structural function (71, 80). It is possible that much of the discrepancy that exists in the literature regarding the localization of cadherins and catenins is due to differences in the specificities of the antibodies used by the different investigators, e.g. the pan-cadherin antibody used by Mulholland et al. for immunoelectron microscopy (71, 80) can recognize members of the cadherin superfamily other than N-cadherin (83).

In conclusion, there is enough evidence to conclude that the proteins of the cadherin/catenin complex are expressed in the seminiferous tubule and that within the basal compartment of the seminiferous epithelium they are localized at inter-Sertoli cell junctions and at junctions between Sertoli cells and spermatogonia or primary spermatocytes (basal ES as well as non-ES sites). The presence of the cadherin/catenin complex within the adluminal compartment of the seminiferous tubule remains, however, highly controversial. It is possible that the molecular composition of the ES changes during development of the germ cells. It has been suggested (83, 89, 92) that the basal ES in the testis resemble the classical cadherin-based junction, while the adluminal ES (between Sertoli cells and elongated spermatids) is largely regulated by an integrin-based adhesion complex in combination with the NAP-complex. It is also possible that the three complexes (cadherin/catenin complex, NAP-complex and integrin based complex) work side by side to form a specialized, very dynamic and well-regulated cell-cell adhesion system (Figure 2B).

The precise cytoskeletal attachment site for the 'classic' cadherin/catenin complex in the testis is another issue that remains very controversial. Notably, studies using immunofluorescent and electron microscopy suggest that the classic cadherin/catenin complex, an actin-based junctional complex in other epithelia, uses intermediate filaments as attachment sites in the seminiferous epithelium. It was found that (classic) cadherins and beta-catenin colocalize with the intermediate filament-based cytoskeleton at the desmosome-like junctions (71, 78). Nevertheless, it is not known whether the desmosomal cadherins (desmogleins, desmocollins) and their associated proteins, which are the structural proteins of desmosomes in other epithelia, are expressed in the testis (also see subsection 5.3). This contrasts with other reports that clearly demonstrated, using immunoprecipitation experiments on cell lysates prepared from Sertoli-germ cell cocultures, that N-cadherin is structurally associated with beta-catenin and alpha-catenin, and crosslinking experiments showed that the N-cadherin complex is linked with the actin cytoskeleton, rather than with the vimentin-based or microtubule-based cytoskeletons (83). These latest findings are consistent with earlier immunohistochemical evidence that N-cadherin and beta-catenin are colocalized at the actin-based ES (71, 78, 89). It has recently been suggested that the cadherins/catenin within the seminiferous tubule are indeed largely actin-based, but that a small amount of classical cadherins (e.g. E-cadherin) is also structurally linked to vimentin-based intermediate filaments via vimentin-associating adaptors, such as zyxin, axin and WASP (93). Collectively, it becomes clear that the N-cadherin/catenin complex in the testis is largely an actin-based structural unit. Yet, other cadherin-based adherens junction structures may exist side-by-side, using intermediate filaments as their attachment site. Clearly, much work remains to be done to define the detailed biochemical and molecular compositions of the ES in the testis.

Another type of actin-based junction, the tubulobulbar complex (TBC), is found between Sertoli cells and elongated spermatids (maturation steps 18 and 19). Nothing is known regarding its function, but this complex appears before the spermatids are released into the lumen of the seminiferous tubule, and probably plays a crucial role in spermiation (94, 95). TBC consists of two structural elements: a tubular structure and a balloon-like terminal bulbar structure (5). The precise molecular composition of TBC is still unknown. The possible role of cadherin-mediated adhesion in this type of actin-based cell-cell junction remains unexplored.

4.3. Regulation of adherens junction assembly and disassembly in the testis

Adherens junctions must be dynamic in order to respond to the needs of the cells during morphogenesis, tissue homeostasis and tissue injury. The disassembly and reformation of the testis-specific adherens junctions is one of the key events during spermatogenesis because germ cells must translocate from the basal to the adluminal compartment of the seminiferous epithelium. Although the underlying mechanisms that regulate the restructuring of these junctions during the process of germ cell migration remain largely unknown, current studies have shown that a wide range of signaling molecules, which include kinases, phosphatases and growth factors, regulates adherens junction dynamics.

4.3.1. Phosphorylation status

Processes mediating the phosphorylation status of the cadherin/catenin complex are important to its assembly, maintenance and disassembly (96, 97). Kinases and phosphatases maintain a highly dynamic phosphorylation status, and the balance between the two opposing activities provides a rapid and reversible mechanism for regulating cell-cell adhesion. What follows is a summary of some of the phosphorylation pathways known to influence cadherin-mediated cell-cell adhesion.

Serine/threonine kinases are implicated in the regulation of cadherin function. For instance, the beta-catenin-binding site of E-cadherin can be phosphorylated by two serine/threonine kinases, casein kinase II (CKII) and glycogen synthase kinase-3beta (GSK3beta), which increases E-cadherin/beta-catenin interaction and strengthens cell-cell adhesion (35, 98). It has also been shown that N-cadherin is constitutively phosphorylated on serine residues (99). On the other hand, serine phosphorylation of p120ctn has been implicated as a negative regulator of cell-adhesion in Colo-205 and HT-29 cells, where cell adhesion is significantly increased by p120ctn mutants or by treatment of cells with serine kinase inhibitors (100).

Far more is known about the tyrosine phosphorylation status of the cadherin/catenin complex. While maintenance of a basic tyrosine phosphorylation level of the E-cadherin cytoplasmic domain is necessary for functional cell-cell adhesion (101), increased phosporylation of beta-catenin and plakoglobin results in the disassembly of the complex (102, 103). Tyrosine phosphorylation of beta-catenin by several receptor and non-receptor tyrosine kinases reduces the strength of binding between E-cadherin and beta-catenin. Balancing this occurs by action of several protein tyrosine phosphatases that dephosphorylate beta-catenin or maintain it in a dephosphorylated state, favoring its association with E-cadherin and alpha-catenin, and thereby stimulating cell-cell adhesion (103). Another component of the cadherin/catenin complex that has been considered a main target of tyrosine kinases and phosphatases is p120ctn. Although multiple putative tyrosine phosphorylation sites were identified in p120ctn (104), and phosphorylation increases the affinity of p120ctn for E-cadherin, the exact role of p120ctn in the regulation of adherens junctions is not clear since different authors have suggested either negative or positive effects (47, 105).

Kinases that modulate the tyrosine phosphorylation status of the cadherin/catenin complex include the cytoplasmic Src family tyrosine kinases and transmembrane tyrosine kinase receptors of growth factors such as EGF, HGF, PDGF, TGFalpha and CSF-1 (106). Treatment of cells with epidermal growth factor (EGF) or overexpression of the EGF-receptor (EGF-R) increases tyrosine phosporylation of cadherin-associated proteins, and leads to dissociation of the complex from the actin cytoskeleton and diminution of cell adhesion (107). Plakoglobin and beta-catenin have both been shown to associate with various members of the EGF-R family (108, 109). Tyrosine kinases of the Src family are involved in the transduction of intracellular signals by their association with the cytoplasmic domains of membrane tyrosine kinase receptors (110). Src-induced tyrosine phosphorylation of the N-cadherin/catenin complex can lead to the loss of cell adhesive capacity (111). The same holds true for the E-cadherin/catenin complex (106, 112). It has been demonstrated by immunohistochemical techniques that Src is localized at the site of the ES between Sertoli cells and elongated spermatids in a stage-specific manner. Fyn is another member of the Src family of tyrosine kinases that is expressed in Sertoli cells. It was recently found to be enriched at the ES between adjoining Sertoli cells and between Sertoli cells and spermatids (113).

Fer tyrosine kinase is a non-Src family cytoplasmic protein tyrosine kinase that is associated with the increased tyrosine phosphorylation of beta-catenin, plakoglobin and p120ctn in N-cadherin/catenin complexes in the testis (92, 114). A testis-specific form of Fer kinase, Fer*T*, was found to be restricted to spermatocytes at the pachytene stage of meiotic prophase (115). Surprisingly, mice devoid of any Fer/Fer*T* protein-tyrosine kinase activity are viable and fertile, and exhibit apparently normal spermatogenesis (116).

Given the large variety of tyrosine kinases that appear to regulate cadherin function, it is not surprising that protein tyrosine phosphatases have also been implicated as cadherin regulators (97). Protein tyrosine phosphatases, reported to be associated with the cadherin/catenin complex, comprise the homophilically adhering receptor-type protein tyrosine phosphatase PTP mµ (117, 118), PTP

kappa (119), PTP lambda (120), and DEP1 (121), the transmembrane leukocyte antigen-related protein LAR-PTP (122), and the cytoplasmic hematopoietic cell phosphatase SHP-1 (123).

4.3.2. Small GTP-binding proteins

Small GTP-binding proteins (small GTPases) are known to regulate a variety of biological and cellular functions, most notably vesicle and membrane trafficking, reorganization of the actin cytoskeleton, cell movement, transcriptional regulation and apoptosis. Small GTPases, inactive when associated with GDP, are activated by exchange of GDP by GTP. A priori, the GTPases of the Rho subfamily, such as Rho, Rac and Cdc42, can mediate adherens junction formation by changing actin cytoskeleton dynamics (124-126). Rac1 has been shown to concentrate at the newly formed cell-cell contacts and to regulate the rate of initiation and strengthening of cell-cell adhesion in MDCK epithelial cells (127). Both Rac and Cdc42 can promote cell-cell adhesion by inhibiting IQGAP, a protein that directly interacts with beta-catenin resulting in dissociation of alpha-catenin from the E-cadherin/beta-catenin complex (128, 129). On the other hand, Rac activation failed to induce recruitment of cadherin to the adherens junction in human keratinocytes, in contrast to its action in MDCK cells, which suggests that the effect of Rho family GTPases on cell-cell adhesion is cell-type dependent. What may be even more intriguing is that sustained Rac activation in MDCK can specifically remove cadherin from newly formed stable cell-cell contacts in a dose- and time-dependent manner (130). High cytosolic levels of p120ctn contribute to activation of Rac and Cdc42 whereas RhoA is inhibited (reviewed in ref. (131)). Binding of p120ctn to the juxtamembranous domain of classic cadherins lowers its affinity for small GTPases. More research is needed to resolve these apparently conflicting reports concerning the effects of Rho GTPases on adherens junction protein recruitment (132). Immunohistochemical analysis has shown that there is intense staining of Rac1 at the basal compartment of the seminiferous tubule. Cdc42 seems to be largely associated with spermatocytes and Sertoli cells, and the most intense staining was detected nearby the head of the elongated spermatids (90, 133).

Besides the small GTPases of the Rho subfamily, other GTPases may also be involved in junction dynamics. For instance, it was found that Rab8B was upregulated several fold at both the mRNA and protein levels during junction assembly in Sertoli cell cultures (134). Moreover, Rab8B may not function in maintenance of adherens junctions, because Sertoli cell Rab8B expression declined to its basal level after junction assembly, suggesting that this GTPase may be important in orchestrating the events that take place during junction assembly. However, evidence that would link Rab8B physically to the adherens junctions is still lacking.

Two members of the G12 subfamily of heterotrimeric G proteins, G-alpha12 and G-alpha13, were recently shown to interact directly, in their active GTP-bound form, with the cytoplasmic domain of E-cadherin, and to cause release of beta-catenin from the adherens junction (135). The mechanism by which the interaction between E-cadherin and beta-catenin is abrogated is not based solely on competition. Rather, Galpha12 activation seems to result in the activation of the non-receptor tyrosine kinase Src, which lead to beta-catenin phosphorylation and subsequent dissociation from E-cadherin (136).

4.3.3. Other regulatory mechanisms

Several studies have shown that the cadherins expressed by reproductive tissues are responsive to hormonal stimuli, as exemplified by the **oestrogen-**induced upregulation of N-cadherin in the mouse testis. Noteworthy is that targeted disruption of the oestrogen receptor gene in male mice causes abnormal spermatogenesis and infertility (137). Furthermore, it has been demonstrated that suppression of testicular **testosterone** levels in adult rats causes the premature detachment of round step-8 spermatids from the seminiferous epithelium. This led to the hypothesis that androgens regulate adhesion between Sertoli cells and round spermatids (138). *In vitro* data have demonstrated that the binding of round spermatids to Sertoli cells and the production of N-cadherin by Sertoli cells are stimulated in a concentration-dependent manner by testosterone, but only in the presence of follicle-stimulating hormone (FSH) (76, 139). As an N-cadherin-specific antibody can block androgen-stimulated adhesion between Sertoli cells and isolated round spermatids *in vitro*, N-cadherin is likely to be involved in this process (76). However, *in vivo* proof of the importance of androgenic regulation of cadherin-mediated adhesion in round spermatid adhesion is lacking. The detachment of round step-8 spermatids as a consequence of lower testicular testosterone levels in the treated rats is not due to absence of ectoplasmic specializations and no unusual structures were noted at the sites of the spermatid-Sertoli cell junctions where the ES showed a normal morphology (140).

Testin, originally identified in Sertoli cell-enriched culture medium, is a testosterone-responsive Sertoli cell secretory product (141). Testin is a 36-kDa protein, occurring as two molecular variants. Testin II differs from testin I by having three additional N-terminal AA, probably as a result of alternative posttranslational processing of the protein (141, 142). In adult rats, testin is associated with the Sertoli cell membrane. Immunogold electron microscopy studies demonstrated that testin was abundantly localized on the Sertoli cell side of the ectoplasmic specializations contacting developing spermatids. Biochemical analyses have shown that testin binds to the Sertoli cell surface via its interaction with a receptor-like protein, possibly an adherens junction-associated protein such as cadherin or nectin (143, 144). Interestingly, depletion of germ cells of the adult rat testis by X-radiation or by treatment with busulfan or lonidamine, which disrupt actin-based Sertoli-spermatid junctions, induces a drastic surge in testicular expression of testin. These results suggest that the disruption of actin-based anchoring junctions induces testin expression, but may also indicate that junctions are disrupted as a result of the testin induction. In any case, testin levels can

apparently be used to monitor the integrity of adherens junctions in the testis. Although testin is structurally related to the cysteine protease superfamily, it seems that it is devoid of any protease or anti-protease activity (145). Taken collectively, these results demonstrate that testin is a component of the ectoplasmic specialization in the testis, that its expression is inversely correlated with germ cell occurrence but positively correlated with the rate of junctional complex turnover, suggesting that it may be an adherens-junction associated signaling molecule (66).

It has been shown that even under steady state conditions of cadherin expression, at least one pool of cadherin is subjected to **endocytosis** and recycles back to the cell surface via a post-Golgi endosomal pathway (146). Such a regulated recycling pathway provides a mechanism for the dynamic modulation of the cadherin/catenin complex and cell-cell adhesion. The role of phospoinositides as regulators of membrane traffic is well established (147). Male mice deficient in inositol polyphosphate 5-phosphatase (Inpp5b) have reduced fertility and defective cell adhesion in the testis (148). The loss of function of Inpp5b results in the appearance in Sertoli cells of cytosolic vacuoles containing N-cadherin and beta-catenin. These vacuoles are coated with actin and contain proteins, which are normally concentrated at the Sertoli cell surface, suggesting that the vacuoles may represent swollen endosomal structures formed due to an early block in endocytosis. Inpp5b probably functions in the endocytosis and recycling of the plasma membrane in the testis, thereby affecting cell adhesion to neigboring cells and resulting in concomitant, premature release of germ cells from the mutant seminiferous epithelium (148).

The level of cadherin expression is tightly regulated and forms another level of complexity in adherens junction dynamics. Several nuclear factors such as Snail, SIP-1, Slug and E12/E47 are known to repress cadherin transcription by binding to the cadherin promoter sequences. The exact interplay between these factors and their specific roles in adherens junction dynamics in various tissues and organs is still unclear (30, 149, 150).

4.4. Wnt signaling in the testis

Cadherins are not simply biological glue that sticks cells together. It is thought that cadherins also play a role in the transduction of signals through the plasma membrane in order to influence morphogenetic programs controlling the structural and functional integrity of tissues like epithelia (97). The nature of the biochemical routes regulated by cadherins is largely unexplored and it is most probable that other proteins that associate with cadherins are involved in these signaling processes. Catenins are good candidates to function in cadherin-mediated signaling.

In addition to playing a role in cadherin-mediated cell-cell adhesion, beta-catenin is also a crucial element of the Wnt signaling pathway. This signaling pathway is involved in a large variety of developmental processes. In this facet of the beta-catenin function, cytoplasmic beta-catenin is part of the APC (adenomatous polyposis coli) multiprotein complex. In absence of Wnt ligand, beta-

catenin is normally phosphorylated by the serine/threonine kinase GSK3beta and directed to the ubiquitin proteasome pathway for degradation. Activation of the transmembrane Frizzeled receptor through the binding of Wnt results in the activation of Dishevelled, which in turn inhibits the phosphorytation of cytoplasmic beta-catenin by GSK3beta. This leads to stabilization of beta-catenin and translocation to the nucleus, where it associates with transcription factors of the LEF-1/TCF family (151), thereby regulating the transcription of LEF-1/TCF responsive genes (152-154). A comprehensive overview of the latter has been made available by Dr. R. Nusse at URL: http://www.stanford.edu/~rnusse/pathways/targets.html. Translocation of beta-catenin to the nucleus is observed in various tumors. Indeed, nuclear beta-catenin induces the transcriptional activation of several genes implicated in cancer development as they modulate growth, differentiation or invasion. A possible nuclear function for the p120ctn armadillo protein in complex with the transcription factor Kaiso has been mentioned above (4.1.1).

Little is known regarding the involvement of the Wnt canonical signal transduction pathway in spermatogenesis, but it seems that it plays a crucial role in the development of the testis and in sex determination. Hormones secreted by the fetal gonads control the differentiation of the reproductive tract. A specific testicular factor in the male fetus is required for the regression of the Müllerian duct, the precursor of oviducts, uterus and part of the vagina in females. This factor, called the anti-Müllerian hormone (AMH), is a homodimeric glycoprotein secreted by the Sertoli cells of the testis (reviewed in (155)). Beta-catenin may be involved in AMH-signaling, as AMH secretion induces *in vitro* and *in vivo* stabilization and accumulation of cytoplasmic beta-catenin in peri-Müllerian mesenchymal cells, a phenomenon which is correlated with increased apoptosis in the regressing Müllerian duct (156). Furthermore, beta-catenin is also detected in the nucleus, co-localized with LEF1. This probably leads to changes in mesenchymal gene expression and determines cell fate during regression of the Müllerian duct.

Another example of the interaction between sex hormones and catenins involves the male-specific hormones AMH, insulin-3 and testosterone, the latter of which stimulates differentiation of the Wolffian duct (precursor of epididymis and vas deferens). The expression of these hormones is regulated by an orphan nuclear hormone receptor, steroidogenic factor 1(SF-1) (157, 158). It has been demonstrated that stabilization of beta-catenin enhances SF-1-mediated transcription, which in turn increases hormone secretion (159).

5.   DESMOSOMAL CELL-CELL ADHESION

Desomosomes are button-like intercellular contacts found particularly in tissues subjected to mechanical stress (e.g. epithelia and the myocardium). They rivet the cells together by anchoring the intermediate filament cytoskeleton to the cell junctions, in which
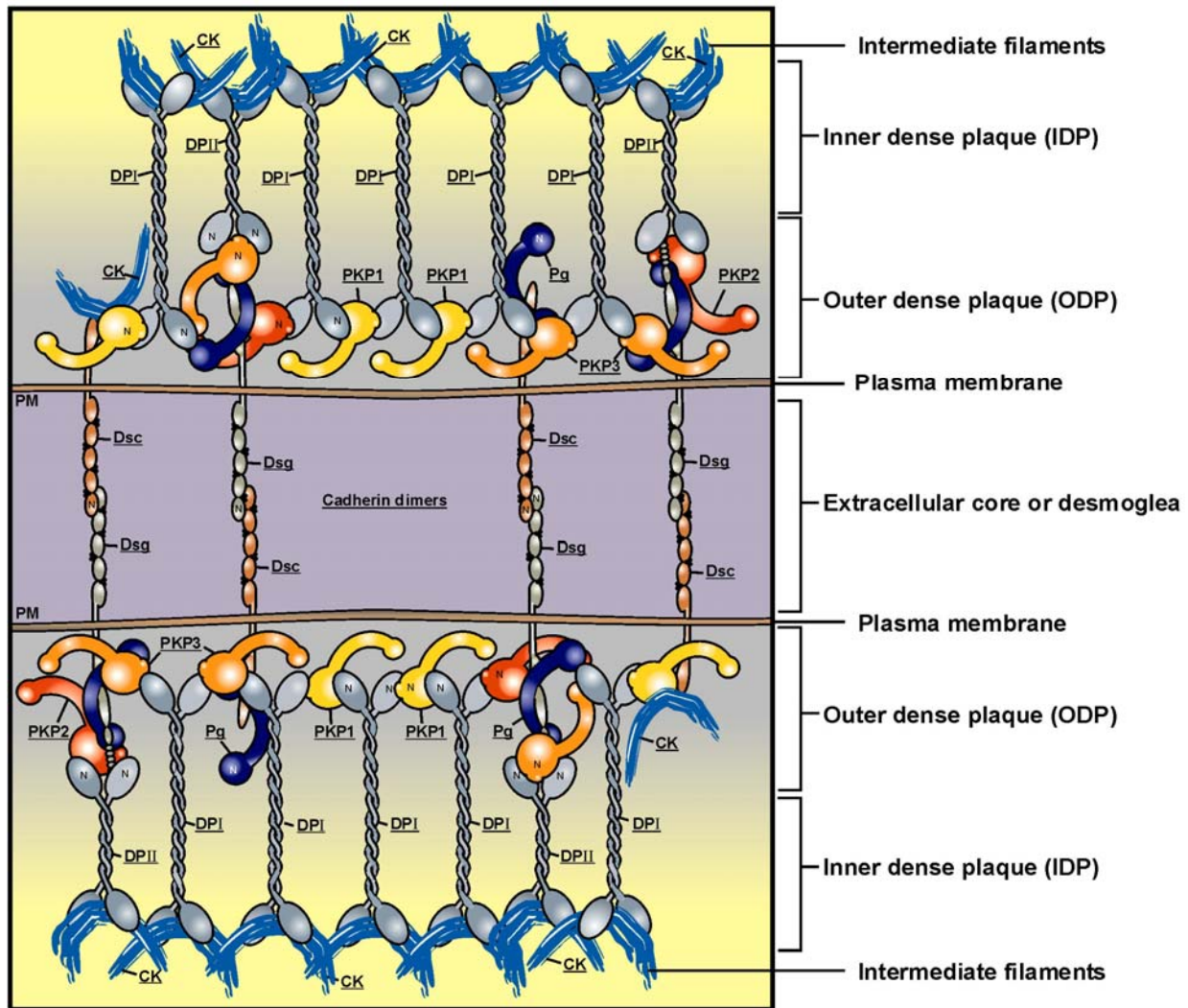
Figure 3. At the electron-microscopic level, desmosomes reveal a common symmetric ultrastructure composed of disc-like sandwiches of electron-dense and electron-lucent layers. The desmosomal plaque consists of an extracellular core or desmoglea, and two intracellular electron-dense discs that are orientated parallel to the plasma membrane: the outer dense plaque (ODP) and a less dense inner plaque (IDP). The latter joins the desmosomal plaque to the intermediate filaments. Desmosomes are highly organized structures composed of members of three protein families: desmosomal cadherins in two classes: desmogleins (Dsg) and desmocollins (Dsc), armadillo proteins comprising plakoglobin (Pg) and plakophilins (PKP) 1-3, and desmoplakins (DPI and DPII) (modified after ref. (180)). CK, cytokeratin; N, aminoterminal domain.

desmosomal cadherins mediate homotypic cell-cell adhesion. The importance of this structural function is evidenced by gene knockout experiments, and by the occurrence of diseases in which tissue integrity is comprised by gene defects or auto-immune antibodies targeting desmosomal components (160).

5.1. Molecular architecture of desmosomes

At the electron-microscopic level, desmosomes reveal a symmetric ultrastructure of disc-like sandwiches of electron-dense and electron-lucent layers. The desmosomal plaque consists of an extracellular core or desmoglea that corresponds to the extracellular space between the two apposing cell membranes, and two intracellular electron-dense discs that are oriented parallel to the plasma

membrane: the outer dense plaque (ODP) and a less dense inner plaque (IDP) (161, 162). The latter joins the desmosomal plaque to the intermediate filaments. Desmosomes are highly organized structures composed of members of three protein families: cadherins, armadillo proteins and plakins (Figure 3).

Desmoglea, the protein-rich midline between two cells, represent a zipper-like adhesive interface established by desmosomal cadherins from apposing cell-surfaces. Desmosomal cadherins are further sub-divided into **desmogleins** (Dsg) and **desmocollins** (Dsc) (8, 11). The extracellular domains of desmogleins and desmocollins are composed of a membrane-proximal anchor domain and four Ca^{2+}-binding cadherin-repeats of about 110 AA. The

major difference between desmocollins and desmogleins resides in their cytoplasmic domains. Specific for desmogleins is the extended intracellular domain containing a proline-rich linker region, followed by a repeated unit of 29 AA (RUD) and a glycine-rich Dsg-terminal domain (DTD). But both types of desmosomal cadherins can bind members of the armadillo protein family, which are indispensable for their linkage to the cytoskeleton (163-165).

Plakoglobin is an important component of the desmosomal outer plaque, where it mediates the binding of desmosomal cadherins to the cytoskeleton via their binding to plakins. Plakoglobin is highly homologous with the adherens junction protein beta-catenin (164). But, despite their high homology, plakoglobin and beta-catenin are distributed differently: plakoglobin is localized at both the desmosomes and adherens junctions, whereas endogeneous beta-catenin is mainly restricted to the adherens junctions, and is normally not present in the desmosomal plaque (166, 167). Binding of plakoglobin to Dsg requires amino acids within Arm repeat 1-4 and sequences at the carboxy-terminus, whereas efficient Dsg-binding to plakoglobin depends on the complete Arm-repeat domain (168-171). The overlap of this desmosomal cadherin binding site of plakoglobin with its alpha-catenin binding site may explain the lack of beta-catenin in the desmosomes. A deletion of the plakoglobin C-terminal end induces an increase in desmosome size, suggesting a role for plakoglobin in an unknown mechanism controlling desmosomal size (172).

Plakophilins are also armadillo proteins present in the outer plaque of the desmosomes. Three plakophilins are known to date, PKP1, PKP2 and PKP3 (173-177), and they all exhibit dual localization, at the desmosomes and in the nucleus. Plakophilins are composed of an amino-terminal head domain that mediates the interaction with different desmosomal proteins (Dsgs, Dscs, plakoglobin, plakins, intermediate filaments) (178-180), and a carboxy-terminal domain containing 10 Arm repeats separated by three conserved short insertions. On the basis of their primary sequence and the organization of their armadillo repeats, plakophilins show highest homology to p120ctn (47). Besides their important role in the formation of desmosomes, it has been suggested recently that plakophilins are also involved in actin filament dynamics (181). Little is known about the nuclear functions of PKPs.

The **plakin** proteins represent a family of very large cytolinker proteins that mediate various functions in the cross-talk between the cytoskeletal networks (182). Plakins are composed of a coiled coil rod-like central domain of variable length, flanked on both sides by globular terminal domains (183). They bind to the intermediate filaments with their carboxy-terminus (184-187). In that way they provide the desmosomal plaque with the necessary link to the cytoskeleton. Four family members (desmoplakin, periplakin, envoplakin and plectin) have been localized to desmosomal cell-cell contacts. Desmoplakin is the most prominent desmosomal plaque protein, and is required for assembly of the desmosomes and their association with the intermediate filaments.

5.2. Desmosome-like junction in the testis

Desmosome-like junctions between Sertoli cells and various germ cells (spermatogonia, spermatocytes and non-elongated spermatids) have been observed by electron microscopy in the adult rat testis. These junctions appear to be attached to the intermediate filaments in the cytosol of the Sertoli cells (188).

Wine and Chapin (89) have demonstrated by immunohistochemistry the presence of desmoglein around the spermatids. Desmoglein appears to be associated most prominently with the convex side of elongating spermatids. Using tyramide amplification, desmoglein immunoreactivity can be seen in all cells of the seminiferous epithelium. Another interesting observation, confirmed by immuno-electron microscopy, is that desmoglein was present on spermatids that had been released from the epithelium.

Human desmoglein 4 has recently been identified and characterized. RT-PCR on multiple tissue cDNA samples demonstrated that desmoglein 4 has a very specific tissue expression pattern. The gene was highly expressed in the testis, prostate and the skin, and was less abundant in salivary gland, but back then no antibodies were available for analyzing the localization at the protein level (189). There is genetic evidence for two additional desmoglein genes, designated desmoglein 5 and 6 (190). Detailed expression studies may clarify whether these desmogleins are also expressed in the testis (190).

There is so far a paucity of data regarding expression patterns of desmocollins, plakoglobin, plakophilins or plakins in the testis.

5.3. Regulation of desmosomal adhesion

Many studies suggest that desmosome assembly is dependent upon the prior formation of adherens junctions. Blocking the formation of adherens junctions, by treatment of cultured keratinocytes with an antibody against the extracellular domain of E-cadherin, inhibits the formation of desmosomes (191-193). In addition, the introduction of a dominant-negative mutant of N-cadherin has been shown to delay desmosomal formation in cultured keratinocytes (194). Furthermore, E-cadherin may regulate the expression of desmosomal proteins (195). However, adhesion mediated by desmosomal cadherins can take place in the absence of any of the classical cadherins, and in some cells the requirement for prior adherens junction formation can be bypassed by downstream signals in the protein kinase C (PKC) pathway. Apparently, classical cadherin-mediated adhesion facilitates the formation of desmosomes by initiating an intracellular signaling pathway. The PKC protein family comprises several isoforms of related serine/threonine-specific, phospholipid-dependent kinases. Activation of PKC, by treatment with 12-O-tetradecanoyl-13-acetate, causes desmosome formation even in cells grown in low Ca^{2+}-containing medium or in cells lacking alpha-catenins (45, 196). These results suggest that PKC signaling pathways are involved in the formation and maintenance of the desmosomal plaque. However, PKC also mediates secretion of urokinase-type

plasminogen activator (uPA) and expression of its receptor (uPAR). Binding of uPA to uPAR activates the plasmin proteolytic system, which may then digest the extracellular domains of desmosomes (196). Together, these findings suggest that PKC mediates the formation and dissociation of desmosomes, and increases desmosomal turnover.

Furthermore, Rho-like GTPase and Fyn tyrosine kinase are implicated not only in the regulation of the adherens junction assembly, but also in the regulation of desmosomal adhesion (197). Thus the regulation of adherens junctions by effectors such as growth factors profoundly affects, even if indirectly, the regulation of desmosome formation. All these data support the presence of cross-talk between adherens junctions and desmosomes.

6. MALE CONTRACEPTION BY PERTURBING THE SERTOLI-GERM CELL JUNCTIONS

The most widely used device for male contraception today is the condom, a barrier method that was developed in the 18th century. Compared to contraceptive methods used by females, such as the contraceptive pill, the condom has a relatively high failure rate. Nonreversible sterilization procedures, such as vasectomy, have been available for some time. This procedure, however, is often associated with surgical complications, unwanted immunological consequences and other pathological conditions. For the past several decades, the development of new, safer, reversible contraceptives for human males has focused largely on hormonal approaches to interfere with the hypothalamus-pituitary-testicular axis, and on immunological approaches based on development of vaccines directed against specific spermatozoal antigens in order to interfere with motility of sperm cells or with sperm-egg interactions. Because hormones such as testosterone cause other physiological changes in addition to their effects in the testis, their prolonged inhibition may have undesirable side effects. Alternatively, the use of a vaccine in either man or woman could have undesirable immunological consequences. In view of these shortcomings, more innovative approaches should be developed (198, 199). Perturbing the Sertoli-germ cell junctions and consequently inducing early detachment of undifferentiated germ cells may be a better approach of inducing safe and reversible aspermatogenesis (66, 67, 69).

Lonidamine is an anti-cancer drug that associates with biological membranes, causing conformational changes that disrupt the respiratory process in cells containing condensed mitochondria, such as tumor cells sensitized by X-irradiation, and certain types of germ cells. In rats, lonidamine causes vacuolization and retraction of the apical cytoplasm in Sertoli cells, thereby inducing release of immature spermatids into the tubular lumen (200). In vitro, lonidamine can disturb the assembly of Sertoli-germ cell anchoring junctions (201). By immunocytochemical and ultrastructural studies performed on two different cell lines, it has been shown that treatment with lonidamine induced a remarkable rearrangement of the actin cytoskeleton with disappearance of the stress fibers (202). Still, the precise cascade of events leading to disruption of cell-cell adhesion by this drug is not known. However, high doses

of lonidamine are toxic and their antispermatogenic effects are irreversible; modifications that eliminate these side effects will be necessary if it is to be developed into a novel male contraceptive.

Lonidamine's effects on the Sertoli cell cytoskeletal network somehow activate the expression of testin, which appears to be a very useful marker for monitoring the loss of integrity of Sertoli-germ cell anchoring or communicating junctions (see section 4.3.3). Based on this unusual feature of testin, it was used to screen different newly synthesized analogs of lonidamine for their ability to disrupt Sertoli-germ cell junctions and induce premature release of germ cells into the tubular lumen. In this way two new chemical entities, AF-2364 and AF-2785, have been shown to specifically deplete spermatids from the seminiferous epithelium without any noticeable toxicity (203).

7. CONCLUSIONS AND PERSPECTIVES

In this review we have summarized the recent developments in the study of cadherin-mediated cell-cell adhesion in the testis and their significance in spermatogenesis. Although it has been proven that cadherin-mediated adhesion plays a crucial role in cell-cell adhesion in epithelia, little is known about the expression and regulation of cadherins in the testis. The biochemical and molecular architecture of anchoring junctions in the testis, such as the ES, TBC and desmosome-like junctions, remain largely unclear. More resources need to be committed to investigating the biochemical composition of these junctions, and how the constituent proteins interact with each other to regulate anchoring junction restructuring. We have described some intriguing evidence and models to illustrate that cadherin-mediated adhesion may play a crucial role in spermatogenesis. Besides this, we referred to many different pathways and sets of proteins that may regulate cadherin-mediated adhesion, such as phosphatases, kinases and small GTPases. Better understanding of cell-cell interactions during the migration of the differentiating germ cells will shed more light on the complex processes of spermatogenesis, fertility and aspermatogenesis, and provide a framework upon which new, safer male contraceptives could be developed.

8. ACKNOWLEDGMENTS

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