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Desmosomes in the testis

Moving into an unchartered territory

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Desmosomes are cell-cell junctions that link to cytoplasmic intermediate filaments, and they are known to mediate robust and stable adhesion in organs such as the skin and heart. Desmosomes are also present between apposing Sertoli cells at the blood-testis barrier, and between Sertoli cells and all germ cells up to, but not including, step 8 spermatids in the seminiferous epithelium. Unfortunately, they remain to be one of the least studied cell junction types in the seminiferous epithelium of the mammalian testis. In this article, we briefly discuss how kinases and the actin cytoskeleton relate to the study of desmosomes in the testis. It is hoped that this information is used to initiate more studies on the biology of the desmosome in the future.

Key words: desmosome, cell junction, testis, Sertoli cell, germ cell, spermatogenesis

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Adhesion between Sertoli cells, as well as between Sertoli and germ cells, in the seminiferous epithelium of the mammalian testis is essential for spermatogenesis. Sertoli cells are highly polarized, 'nurse-like' epithelial cells that extend upwards from the basement membrane which essentially allows these cells to support between 30 to 50 developing germ cells at any one time throughout the entire seminiferous epithelial cycle.[1,2] Another important Sertoli cell feature that is critical for spermatogenesis is the blood-testis barrier (BTB), an ultrastructure comprised of co-existing and mutually interacting junction types [i.e., tight junctions (TJs), basal ectoplasmic specializations (ESs), desmosomes and gap junctions] that basically maintains epithelial cell polarity and integrity[3-5] (**Fig. 1**). The BTB is believed to cyclically restructure so that spermatocytes can enter the adluminal compartment of the seminiferous epithelium, and this is carried out in part by an array of molecules, which include junctional proteins, cytokines, proteases/protease inhibitors, hormones and endocytic/trafficking proteins.[6-8] It is behind the BTB in the adluminal compartment that germ cells complete meiosis; thereby developing into mature elongated spermatids. Throughout spermatogenesis, migrating germ cells must also remain attached to Sertoli cells via desmosomes or ESs up until the time they are released from the seminiferous epithelium at late stage VIII of the epithelial cycle when Sertoli cell-spermatid junctions are promptly disassembled.[9]

Numerous studies from the past two decades have described many important structural molecules that constitute Sertoli-Sertoli and/or Sertoli-germ cell adhesion in the mammalian testis; these include, but are not limited to, classic cadherins, protocadherins, nectins, integrins, junctional adhesion molecules and the coxsackie and adenovirus receptor (CAR).[6,10,11] For instance, studies have reported N-cadherin to be a basal ES protein functioning in the maintenance of BTB integrity but also in its restructuring which is needed for spermatocytes to traverse this barrier. We arrive at this conclusion because cytokines (e.g., transforming growth factor-β, TGF-β) can trigger Sertoli cell N-cadherin endocytosis in vitro via a clathrin-dependent mechanism,[12,13] which can 'loosen' or disassemble at least in part the basal ES. Unfortunately, there are relatively fewer studies that investigate the biology behind

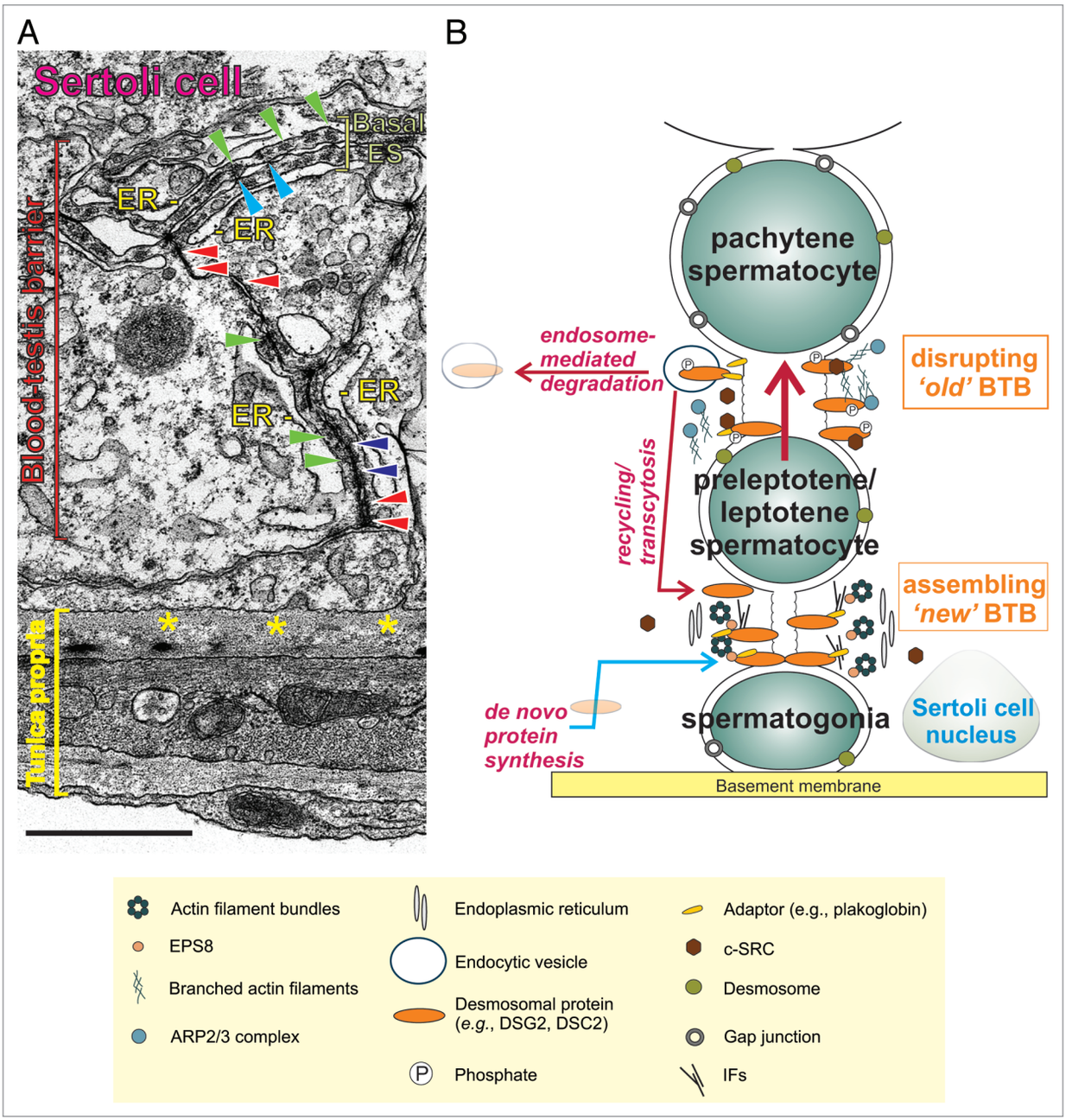

Figure 1. Morphological features of the desmosome and its intimate relationship with TJs, basal ESs and gap junctions that together constitute the BTB in the mammalian testis. (A) This is an electron micrograph of a cross-section of the seminiferous tubule from an adult rat testis. It shows the seminiferous epithelium, which is composed of Sertoli and developing germ cells resting on the basement membrane (a modified type of extracellular matrix, see yellow asterisks) of the tunica propria. Desmosomes are seen between two Sertoli cells (see red arrowheads); they are typified by the presence of electron dense material. Basal ESs, on the other hand, are typified by the presence of actin filament bundles (see green arrowheads) sandwiched in between cisternae of endoplasmic reticulum (ER) and the Sertoli cell plasma membrane. Basal ESs co-exist with either TJs ("kisses" between apposing Sertoli cell plasma membranes, see blue arrowheads) or gap junctions (see purple arrowheads). Thus, desmosomes are critical structural components that constitute BTB function. Bar = 1 μm. (B) This is a schematic drawing of the seminiferous epithelium illustrating the relative location of the BTB. Desmosomal proteins (e.g., DSG2, DSC2, IFs) at the BTB may be phosphorylated by c-SRC, thereby inducing their internalization via endocytic vesicles. Internalized proteins may be degraded, thereby destabilizing the "old" BTB to facilitate the transit of preleptotene spermatocytes. Additionally, desmosomes also serve as a platform for signal transduction events. For instance, c-SRC-mediated protein phosphorylation may further destabilize the BTB. Internalized proteins may also be recycled and trafficked to the "new" BTB to establish new TJ-fibrils. Coupled with de novo synthesis of TJ proteins, the integrity of the immunological barrier can be maintained during the transit of preleptotene spermatocytes.

desmosomes in the testis. Consequently, our understanding of the spermatogenic process and the maintenance of fertility remain incomplete without additional information on this junction type. In this article, we discuss a few key points as they relate to the biology of desmosomes in the seminiferous epithelium, in particular why this junction type should be investigated in future studies, what are some of the more important open questions that can be addressed through basic experiments and how this information can be used to develop safe non-hormonal male contraceptives. For in-depth background information, interested readers are asked to refer to references 14–17.

Desmosomes were originally studied in the testis by the late Lonnie Russell who described these intermediate filament-based structures as 'desmosome-like' (alternatively coined as 'desmosome-gap') because ultrastructurally they did not seem to resemble the robust and Ca^{2+}-independent (i.e., hyper-adhesive) desmosomes that were found in the skin or heart.[18] Today, we know that desmosome-like junctions, which are found between Sertoli cells at the BTB and between Sertoli cells and all germ cells up to (but not including) step 8 (elongating) spermatids (**Fig. 1**), are comprised of many of the same proteins (e.g., desmogleins, desmocollins, plakoglobin, plakophilins and desmoplakins) that constitute desmosomes in other organs;[14] and because of this, they should be called as such. Moreover, desmogleins and desmocollins, either alone or with connexins (gap junction proteins), cannot form functional hemichannels. Irrespective of these important and recent findings, a key difference between desmosomes in the skin, for example, and desmosomes in the testis may be the rate at which these junctions are restructured in vivo. This may ultimately control the extent of their organization because it makes little physiological sense for rapidly migrating cells (i.e., germ cells) to assemble robust and stable adhesive contacts. (In this context, it is worth noting that adhesion conferred by desmosomes in vitro is considerably weaker than that conferred by apical ESs;[19] this is in contrast to other epithelia where desmosomes reinforce adherens junctions.[20]) In addition, the organization, as well as the regulation, of different junction types within an epithelium is highly variable among different tissues. For example, Sertoli-Sertoli and Sertoli-germ cell desmosomes co-exist with gap junctions; and Sertoli-Sertoli cell desmosomes at the BTB are further juxtaposed with TJs and basal ESs[5,6] (**Fig. 1**). As such, other junction types may directly or indirectly affect the adhesive function of desmosomes in the testis. To summarize, these are just two ways in which testicular desmosomes may differ from conventional desmosomes, but at this point additional research is needed to define more concretely how testicular desmosomes differ from their counterpart. For example, it is not known if mature desmosomes between Sertoli cells or between Sertoli and germ cells are hyper-adhesive. Culturing Sertoli cells in low Ca^{2+} medium should provide new insights.

Besides functioning in cell-cell adhesion, desmosomes also provide important platforms for signal transduction events that downstream control many different aspects of cell function because several protein kinases have been found to associate with desmosomal proteins. For instance, conventional protein kinase C (PKC) family members are known to phosphorylate desmosomal proteins.[21-23] While this can affect desmosome assembly and disassembly because phosphorylation per se can elicit discrete changes in protein-protein interactions and protein localization,[16] it can also result in more global changes in cell function. In the testis, the process of germ cell differentiation may be regulated in part by transient and cyclic changes in adhesion and de-adhesion which occur as germ cells traverse the seminiferous epithelium throughout spermatogenesis. In other words, as Sertoli cells re-establish interactions with newly migrated germ cells in vivo, 'nurse-like' Sertoli cells may produce signals to stimulate germ cell differentiation/development—thereby balancing proliferation and apoptosis which essentially determines the number of germ cells that Sertoli cells can physiologically support within the confines of the seminiferous epithelium. Desmosomes may also prevent aberrant proliferation of germ cells, which might otherwise culminate in testicular tumorigenesis, and these hypotheses should be tested in future studies. Furthermore, SRC, a non-receptor tyrosine kinase, is also an important player in desmosome dynamics, as was recently shown in Sertoli cells in vitro. We previously reported that simultaneous knockdown of desmoglein-2 (DSG2) and desmocollin-2 (DSC2) in cultured Sertoli cells by RNA interference (RNAi) resulted in an increase in the rate at which CAR (a transmembrane protein with semi-defined roles in TJ and adherens junction dynamics, it links indirectly to actin filaments[24,25]) was endocytosed and that this was possibly mediated by SRC.[14] SRC is well known to control cell proliferation, adhesion and migration, and actin dynamics;[26,27] it also co-immunoprecipitates with DSG2 and CAR in the testis[14,28] (**Fig. 1**). These results illustrate that crosstalk between junctional proteins converges downstream on a network of kinases that may facilitate endocytic vesicle-mediated protein trafficking events. These results also provide a novel starting point for addressing how kinases regulate desmosome dynamics in the seminiferous epithelium. For example, it would be important to determine whether TGF-β or interleukin-1α (IL-1α, see discussion below) triggers internalization of desmosomal cadherins in Sertoli-germ cell co-cultures, whether this affects Sertoli-germ cell adhesion, and finally whether a decrease in SRC (by RNAi, expression of dominant-negative SRC, or possibly by treatment with a specific SRC family inhibitor) blocks these cellular events.

Conventionally speaking, desmosomes are known to attach to intermediate filaments (IFs, **Fig. 1**); however, recent studies have also unexpectedly implicated actin and actin-associated proteins (e.g., RHOA, SRC) in desmosome dynamics.[29-32] A good example of the involvement of the actin cytoskeleton in desmosome dynamics is evidenced by the loss of adhesion between keratinocytes (i.e., acantholysis) that were earlier treated with pemphigus vulgaris (PV, an autoimmune skin blistering disorder) IgG;[33] here, the actin network was found to be severely disrupted. At the cellular level, PV IgG binds to the DSG3 ectodomain, resulting in its clathrin- and dynamin-independent endocytosis and consequently in desmosome

destabilization.[34] Moreover, PV IgG was found to target RHOA. Specifically, RHOA activation abrogated PV IgG-induced desmosome disassembly.[35] In support of these findings, a disruption in actin polymerization also resulted in a striking increase in DSG3 internalization,[33] illustrating that stable desmosomal adhesion relies largely on the intactness of the actin cytoskeleton. In the testis, actin filaments are confined largely to the apical and basal ES where they are oriented unipolarly, hexagonally packed and not branched (**Fig. 1**). Thus, desmosomes at the BTB are under the control of the actin cytoskeleton, whose remodeling from late stage VIII to XI of the seminiferous epithelial cycle facilitates junction disassembly and germ cell movement across the barrier. Interestingly, a recent study has demonstrated IL-1α to profoundly disrupt the Sertoli cell actin cytoskeleton in vitro and in vivo.[36,37] Because PV pathogenesis has been linked to a disruption of the actin cytoskeleton and desmosomes, and several ILs were found to be elevated in sera from individuals with PV; it is important that the levels of different desmosomal proteins be investigated following IL-1α treatment. The involvement of SRC in these cellular events should also be investigated. On a final note, IFs did not appear to associate with desmosomes in germ cells, suggesting that they may associate weakly with actin filaments to facilitate germ cell movement across the seminiferous epithelium.[35]

Herein, we have briefly highlighted two potentially important directions (i.e., the roles of kinases and the actin cytoskeleton) in the study of desmosomes in the future. Their study is, and will continue to be, of great significance to our field because it will help us better understand how a Sertoli cell can maintain adhesion with another Sertoli cell, as well as with several germ cells, in the seminiferous epithelium. After all, this forms the basis of spermatogenesis, and a disruption in desmosomal adhesion may lead to transient or even permanent sterility, as this would eradicate most germ cells from the seminiferous epithelium. From a clinical perspective, testicular desmosomes may be a unique and appealing target for non-hormonal contraceptive development, and their adhesion may be easy to perturb if we can determine how they differ from desmosomes in other organs. For example, targeting desmosomes within developing germ cells, which do not appear to link to IFs, may be an interesting approach that may result in germ cell sloughing from the seminiferous epithelium as this would leave Sertoli cells largely unscathed. It is hoped that more studies are initiated to better understand the biology of desmosomes in the testis.

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