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Review

Cell polarity and cytoskeletons—Lesson from the testis

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ABSTRACT

Cell polarity in the adult mammalian testis refers to the polarized alignment of developing spermatids during spermiogenesis and the polarized organization of organelles (e.g., phagosomes, endocytic vesicles, Sertoli cell nuclei, Golgi apparatus) in Sertoli cells and germ cells to support spermatogenesis. Without these distinctive features of cell polarity in the seminiferous epithelium, it is not possible to support the daily production of millions of sperm in the limited space provided by the seminiferous tubules in either rodent or human males through the adulthood. In short, cell polarity provides a novel mean to align spermatids and the supporting organelles (e.g., phagosomes, Golgi apparatus, endocytic vesicles) in a highly organized fashion spatially in the seminiferous epithelium during the epithelial cycle of spermatogenesis. This is analogous to different assembling units in a manufacturing plant such that as developing spermatids move along the "assembly line" conferred by Sertoli cells, different structural/functional components can be added to (or removed from) the developing spermatids during spermiogenesis, so that functional spermatozoa are produced at the end of the assembly line. Herein, we briefly review findings regarding the regulation of cell polarity in the testis with specific emphasis on developing spermatids, supported by an intriguing network of regulatory proteins along a local functional axis. Emerging evidence has suggested that cell cytoskeletons provide the tracks which in turn confer the unique assembly lines in the seminiferous epithelium. We also provide some thought-provoking concepts based on which functional experiments can be designed in future studies.

Contents

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1. Introduction

In the mammalian testis, developing germ cells, in particular elongating/elongated spermatids, and Sertoli cells display remarkable cell polarity throughout the seminiferous epithelial cycle of spermatogenesis in rodents and humans [1,2]. For instance, the heads of all elongating spermatids align almost perpendicularly to the basement membrane with their tails point to the tubule lumen (Fig. 1). On the other hand, Sertoli cell nuclei, Golgi apparatus, tight junctions (TJs) and basal ectoplasmic specialization (basal ES, a testis-specific actin-rich adherens junction (AJ) type) are located near the base of Sertoli cells, almost adjacent to the basement membrane of the tunica propria [1,3–5]. This strict orientation of developing spermatids and Sertoli cell-based ultrastructures thus facilitate the maximal number of spermatids to be packed within the confined and limited space of the seminiferous tubules and they can also be supported by a fixed population of Sertoli cells functionally in the testes [3,6]. As such, ~30–50 million *vs.* ~200–300 million of sperm [7–9] can be produced daily in each adult rodent or human male through spermatogenesis during adulthood. The concept of apico-basal polarity conferred by the concerted efforts of the three polarity protein complexes is known for decades, which is essential to embryogenesis and morphogenetic development of multiple organs include the testis, and to support multiple cellular functions [10–15]. However, the presence of polarity proteins in the testis that are crucial to spermatogenesis was not reported until 2008 when the Par (partitioning-defective)-based polarity complex of Par3/Par6/aPKC (atypical protein kinase C)/Cdc43 was first identified in the rat testis [16]. Thereafter, the presence of Scribble/Dlg1 (Discs large 1)/Lgl2 (Lethal giant larvae 2) polarity complex [17], to be followed by the Crb3 (Crumbs homolog-3)-based complex [18], in the testis were reported in subsequent years. The reason that we sought to examine the likely involvement of cell polarity in spermatogenesis stems from the initial observation that when adult Sprague-Dawley rats treated with a single dose of adjudin, a male contraceptive under investigator in our laboratory [19,20], by oral gavage were found to induce considerable disarray in the alignment of elongating/elongated spermatids across the seminiferous epithelium [16]. For instance, the heads of developing spermatids no longer aligned perpendicularly to the basement membrane but deviated by as much 90° to 180° from the intended orientation versus spermatids in normal testes [16]. Furthermore, this defect in spermatid polarity occurred within ~6–12 h, at least 12–24 h before extensive exfoliation of germ cells from the seminiferous epithelium took place in the testis [16]. These findings thus implicate that there may be a functional relationship between spermatid polarity and the underlying cytoskeletons that confer spermatid adhesion. When the actin microfilament organization at the Sertoli-spermatid interface, which is known as the apical ectoplasmic specialization (ES), was examined by electron microscopy, considerable truncation of actin microfilaments was detected [16]. As such, F-actin no longer capable of supporting spermatid adhesion and spermatid orientation, leading to their eventual premature release from the testis. This concept that relates the function of polarity proteins and the involvement of cytoskeletal organization in mammalian cells and tissues is indeed supported by studies in other epithelia [21–23].

In the earlier report investigating the role of Par-based polarity protein complex on Sertoli cell function, it was shown that a knockdown of Par3 or Par6 by RNAi indeed perturbed the localization of adhesion proteins at the Sertoli cell–cell interface [16]. For instance, Par3 or Par6 knockdown induced re-distribution of JAM-A (Junctional Adhesion Molecule-A, also known as JAM-1, a TJ integral membrane protein [24]) and α-catenin (a basal ES adaptor protein [25,26]) so that these proteins no longer localized tightly to the Sertoli cell–cell interface to support the Sertoli TJ-permeability barrier function [16]. These findings also implicated that the F-actin organization in Sertoli cells had been disrupted since both TJ- and basal ES-based adhesion proteins utilize F-actin for attachment. The concept that polarity proteins exert their regulatory role in conferring spermatid polarity in the testis through changes in cytoskeletal organization is supported by two subsequent reports when the role of Scribble [17] and Crb3 [18] in the testis was examined. For instance, a knockdown of Scribble/Dlg1/Lgl2 was found to promote the organization of F-actin at the Sertoli cell basal ES/BTB, making the BTB "tighter" [17], whereas Crb3 knockdown perturbed actin organization at the apical and basal ES, disrupting the Sertoli cell BTB function, making the Sertoli cell TJ-barrier "leaky" [18]. Taking these data collectively, in normal testes, the Par3/Par6- and the Crb3-based polarity complex confer apical and basal ES integrity, promoting spermatid and Sertoli cell adhesion and polarity by organizing actin microfilaments at the ES to support adhesion and polarity function (Fig. 2). On the other hand, the Scribble/Dlg1/Lgl2 polarity complex promotes apical and basal ES disruption (Fig. 2) during the epithelial cycle of spermatogenesis. In short, the functionality of the Par3/Par6- and Crb3-based polarity complexes and the Scribble-based polarity complex are antagonistic, and they mediate their regulatory effects through changes in actin microfilament organization at the ES as noted in Fig. 2.

2. Ectoplasmic specialization (ES), cell polarity and cytoskeletal function in the testis

It is noted that ES is a testis-specific *atypical* adherens junction (AJ) since it is constituted by proteins usually found at the TJ (tight junction) (e.g., JAM-A, JAM-C), gap junction (e.g., connexin 43), and even focal adhesion complex (FAC, also known as focal contact which is restricted to the cell-extracellular matrix interface) (e.g., p-FAK-Tyr397 (phosphorylated/activated focal adhesion kinase phosphorylated at Tyr-397 from the N-terminus), p-FAK-Tyr407), as well as putative AJ adhesion protein complexes (e.g., nectin-afadin) [27,28]. On the other hand, some of the best studied AJ adhesion protein complexes found in other epithelia, such as N-cadherin/ß-catenin are only stage-specifically expressed at the apical ES at the Sertoli-spermatid interface, such as at stage VII-early VIII tubules [29]. However, N-cadherin/ß-catenin remains conspicuously expressed at the basal ES at the BTB in virtually all stages of the epithelial cycle [29,30], utilizing actin for attachment as found in other epithelia [30]. In the mammalian testis, ES is restricted to the Sertoli-spermatid (step 8–19 or 8–16 spermatids in rat or mouse testes, respectively) interface in the adluminal compartment designated apical ES [31–33]. Once apical ES appears in stage VIII tubules between Sertoli cells and step 8 spermatids, it replaces all other anchoring junctions at the site, including desmo-

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