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Review

# Cell polarity proteins and spermatogenesis☆

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ABSTRACT

When the cross-section of a seminiferous tubule from an adult rat testes is examined microscopically, Sertoli cells and germ cells in the seminiferous epithelium are notably polarized cells. For instance, Sertoli cell nuclei are found near the basement membrane. On the other hand, tight junction (TJ), basal ectoplasmic specialization (basal ES, a testis-specific actin-rich anchoring junction), gap junction (GJ) and desmosome that constitute the blood-testis barrier (BTB) are also located near the basement membrane. The BTB, in turn, divides the epithelium into the basal and the adluminal (apical) compartments. Within the epithelium, undifferentiated spermatogonia and preleptotene spermatocytes restrictively reside in the basal compartment whereas spermatocytes and post-meiotic spermatids reside in the adluminal compartment. Furthermore, the heads of elongating/elongated spermatids point toward the basement membrane with their elongating tails toward the tubule lumen. However, the involvement of polarity proteins in this unique cellular organization, in particular the underlying molecular mechanism(s) by which polarity proteins confer cellular polarity in the seminiferous epithelium is virtually unknown until recent years. Herein, we discuss latest findings regarding the role of different polarity protein complexes or modules and how these protein complexes are working in concert to modulate Sertoli cell and spermatid polarity. These findings also illustrate polarity proteins exert their effects through the actin-based cytoskeleton mediated by actin binding and regulatory proteins, which in turn modulate adhesion protein complexes at the cell–cell interface since TJ, basal ES and GJ utilize F-actin for attachment. We also propose a hypothetical model which illustrates the antagonistic effects of these polarity proteins. This in turn provides a unique mechanism to modulate junction remodeling in the testis to support germ cell transport across the epithelium in particular the BTB during the epithelial cycle of spermatogenesis.


**Contents**



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## 1. Introduction

Cell polarity refers to asymmetry in cell shape, and asymmetrical distribution of proteins, macromolecules and/or intracellular organelles epithelial cells, which are crucial to cell function and development in particular during embryogenesis (for reviews, see Refs. [1–3]). Cell polarity is also essential to multiple biological processes, including cell differentiation, proliferation and migration, as well as cellular and molecular transports in unicellular and multicellular organisms (for a review, see Ref. [4]). Apico-basal polarity is regulated by three evolutionarily conserved cell polarity protein complexes or modules (for reviews see Refs. [2,4–6]), namely the Crumbs (CRB) complex, the partitioning defective (Par) complex and the Scribble complex. In mammals, the CRB complex is composed of CRB and two key protein partners called protein associated with Lin-7 1 (PALS1) and PALS1-associated tight junction protein (PATJ). The Par complex consists of Par3 and Par6, and two key partner proteins including atypical protein kinase C (aPKC) and small GTPase Cdc42. The Scribble complex is composed of Scribble and two partner proteins Lethal giant larvae (Lgl) and Discs large (Dlg). CRB and Par complexes localize at the apical domain near the tight junction (TJ) of an epithelium usually adjacent to one another, whereas Scribble complex is restricted to the basolateral domain near the basal lamina. The CRB- and Par-based complexes *vs.* the Scribble complex are mutually exclusive relative to their localization and/or activation via phosphorylation, and since each of these complexes recruits its own binding partners, creating a relatively large multi-protein complex, thereby conferring apico-basal polarity.

In the testis, Sertoli and germ cells are being arranged in a highly polarized fashion to support spermatogenesis efficiently, so that the maximal number of developing germ cells can be packed in the limited space of the seminiferous epithelium. First, adjacent Sertoli cells near the basement membrane (a modified form of extracellular matrix, similar to the basal lamina in other epithelia (for reviews, see Refs. [7,8])) create the blood-testis barrier (BTB) which divides the seminiferous epithelium into the basal and apical (adluminal) compartments. Spermatogonial stem cells (SSCs), undifferentiated and differentiated spermatogonia, and preleptotene spermatocytes reside in the basal compartment, whereas spermatocytes, including leptotene, zygotene, pachytene and diplotene spermatocytes, and all post-meiotic spermatids (*e.g.*, round, elongating and elongated spermatids) reside in the adluminal compartment, displaying distinctive polarity regarding their restrictive localization. Second, Sertoli cell nuclei, phagosomes and Golgi apparatus are also found near the basal region of the Sertoli cell whereas residual bodies and early phagosomes, and the cytoplasmic processes that support developing spermatids are all limited to the adluminal region, displaying strict polarity. Third, the most obvious cell polarity in the testis is the restrictive arrangement of developing spermatids during spermiogenesis in which the heads of spermatids are all pointing toward the basement membrane with their elongating/elongated tails point toward the tubule lumen to allow the maximal number of spermatids to be packed in the epithelium. These unique and orderly morphological features thus support the notion that polarity proteins are being utilized to support Sertoli cell and spermatid polarity. Earlier studies have suggested that Sertoli cell and spermatid polarity are supported by a testis-specific and actin-rich anchoring junction known as the ectoplasmic specialization (ES). The ES is found at the Sertoli cell–cell interface known as the basal ES which coexists with the actin-based tight junction (TJ) and gap junction (GJ) and the intermediate filament-based desmosome to constitute the BTB *vs.* the apical ES restricted to Sertoli-spermatid (step 8–19 in the rat testis) (for reviews, see Refs. [9–13]). Interestingly, studies on the role of polarity proteins in spermatogenesis are not found in the literature until the late 2000s when the Par-based complex was described in the testis [14], to be followed by studies in the Scribble- [15] and CRB-complex [16], and other Par-based proteins such as Par5 (also known as 14-3-3) [17] and Cdc42 [18]. Herein, we provide a timely update on the role of polarity proteins in spermatogenesis.

## 2. CRB complex

Crumbs, a transmembrane protein, was initially identified as an apical polarity determinant in *Drosophila* [19–21]. *Drosophila* Crumbs (CRB), Stardust (Sdt) and Discs lost (Dlt) create the *Drosophila* CRB complex. In mammals, three CRB orthologues, namely CRB1, CRB2 and CRB3 are known to date [22], with the small 24 kDa CRB3 being the only member, also an integral membrane protein, found in the testis [14,16]. PALS1 and PATJ are the mammalian orthologues of Sdt and Dlt, respectively [23,24] and they are also expressed by Sertoli and germ cells in the rat testis [14,16].

### 2.1. CRB

CRB1, CRB2 and CRB3 are transmembrane proteins. A CRB polypeptide consists of an extracellular domain from its N-terminus, a transmembrane domain and a cytoplasmic tail at its C-terminus (Fig. 1). Unlike CRB1 and CRB2 which contain large extracellular domain including laminin A/G like domains and several epidermal growth factor (EGF)-like domains, CRB3 found in the testis has a very short extracellular domain (for a review, see Refs. [25]) with a Mr of 24 kDa [16]. The highly conserved intracellular domain of CRB proteins contains of a protein 4.1/ezrin/radixin/moesin (FERM)-binding domain and a PSD-95/Discs large/ZO-1 (PDZ)-binding domain. CRB3 has an additional Src homology domain 3 (SH3)-binding domain not found in other CRB proteins [22] (Fig. 1). CRB1 is exclusively expressed in the eye and central nervous system [26]. Mutations of human CRB1 lead to Leber congenital amaurosis (LCA), retinitis pigmentosa type 12 (RP12) [27–29]. CRB1 knockout study illustrates that CRB1 is essential to maintain adherens junctions (AJs) between photoreceptor cells and Müller glial cells when exposed to light [30]. CRB2 is restricted to retina, brain and kidney [31,32], but its function is less known. Recent knockout study has shown that deletion of CRB2 leads to embryonic lethality by 12.5E due to disrupted polarity of