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Planar cell polarity (PCP) proteins and spermatogenesis

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Abstract

In adult mammalian testes, spermatogenesis is composed of several discrete cellular events that work in tandem to support the transformation and differentiation of diploid spermatogonia to haploid spermatids during the seminiferous epithelial cycle. These include: self-renewal of spermatogonial stem cells via mitosis and their transformation into differentiated spermatogonia, meiosis I/II, spermiogenesis and the release of sperms at spermiation. Studies have shown that these cellular events are under precise and coordinated controls of multiple proteins and signaling pathways. These events are also regulated by polarity proteins that are known to confer classical apico-basal (A/B) polarity in other epithelia. Furthermore, spermatid development is likely supported by planar cell polarity (PCP) proteins since polarized spermatids are aligned across the plane of seminiferous epithelium in an orderly fashion, analogous to hair cells in the cochlea of the inner ear. Thus, the maximal number of spermatids can be packed and supported by a fixed population of differentiated Sertoli cells in adult testes in the limited space of the seminiferous epithelium. In this review, we briefly summarize recent findings regarding the role of PCP proteins in the testis. This information should be helpful in future studies to better understand the role of PCP proteins in spermatogenesis.

Keywords

Testis; spermatogenesis; planar cell polarity proteins; seminiferous epithelial cycle; Sertoli cells; germ cells; Wnt/PCP signaling

1. Introduction

Cell polarity is pivotal in multiple biological events such as directional cell movement, formation and maintenance of cell epithelium, morphogenesis and embryogenesis in fruit flies, worms, invertebrates and vertebrates including rodents and humans (for reviews see [1-6]). Cell polarity usually refers to the apico-basal (A/B) polarity, illustrating the polarized

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arrangement of cells within an epithelium, such as the conspicuous alignment of developing spermatids during spermatogenesis in the testis in which the heads of spermatids are pointing to the basement membrane while their elongating tails toward the tubule lumen (Figure 1 and 2). Besides A/B polarity in epithelial cells, planar cell polarity (PCP) refers to the arrangement of polarized cells within the plane of an epithelium, mostly notably in hair cells of the cochlea in mammals or wing hair cells in insects (Figure 1) [7]. Furthermore, PCP is essential to convergent extension (CE) during embryogenesis, such as gastrulation, in which the tissue narrows (converge) along one axis concomitant with elongation (extension) along a perpendicular axis as the result of polarized cell movement to generate anteroposterior (A/P) axis (Figure 1). Studies have shown that proteins that regulate PCP, similar to proteins that regulate A/B polarity, are well conserved from invertebrates to vertebrates (for reviews, see [7, 8]). Three discrete polarity protein complexes or modules that support A/B polarity: the partitioning defective (PAR)-, the Crumbs (CRB)- and the Scribble (SCRIB)-based modules as well as their corresponding partner proteins are expressed in the testis [9, 10]. The functions of these polarity proteins during spermatogenesis in the testis have recently been reviewed (see [11]). Interestingly, multiple PCP proteins are also expressed in the testis. Furthermore, the polarized alignment of developing spermatids within the plane of the seminiferous epithelium during spermiogenesis mimics the alignment of wing cell hairs in *Drosophila* or the hair cells in the cochlea of the inner, supporting the notion that PCP proteins are probably involved in determining spermatid planar cell polarity during spermiogenesis. Herein, we focus our discussion on the emerging evidence on the significance of PCP proteins in spermatogenesis

2. Planar cell polarity (PCP) proteins

Planar cell polarity (PCP) refers to the precise polarized arrangement of cells within the plane of an epithelium (for reviews, see [12, 13]). Studies have shown that PCP is regulated by multiple PCP proteins that display asymmetric localization in discrete regions of the plasma membrane, and PCP proteins are well conserved from *Drosophila*, *C. elegans*, insects, rodents to humans. PCP proteins are categorized into several classes which include the Core PCP proteins such as Frizzled (Fz), Flamingo (Fmi)/starry night, Disheveled (Dsh), Diego (Dgo), Van Gogh (Vang) (also known as Strabismus) and Prickle (Pk) which localize asymmetrically at the proximal (e.g., Vang, Pk, and Fmi) *versus* distal (Fz, Dsh, Dgo, and Fmi) end of neighboring cells of a cell epithelium based on studies in *Drosophila* (Figure 1A) (for a review, see [14]). Besides the Core PCP proteins, there are also PCP ligands (e.g., Wnt5a), PCP effectors (e.g., Fuzzy), and PCP signaling proteins (e.g., Dshs1). These PCP proteins are known to regulate wing hair, bristle or ommatidial polarity by working in concert with atypical cadherins Fat and Dachshous (Dchs) signaling in *Drosophila* [15-20]. Interestingly, core PCP proteins are mutually interdependent regarding their asymmetric localization since a depletion of any core PCP proteins leads to a loss of asymmetry of all the others (for a review, see [21]). PCP proteins exert their effects via the noncanonical Wnt/PCP pathway which is also an evolutionarily conserved mechanism that confers directional cell polarity necessary for development of tissues and organs such as the formation of neural tube during embryonic development and assembly of filtration units of the developing kidney (for reviews, see [22, 23]). PCP proteins also exert their effects via the

canonical Wnt/PCP pathway involving β -catenin downstream [24, 25]. Studies based on the use of genetic models have illustrated various functions of PCP proteins in rodents, most notably in the development of neural crest and brain development, cardiac development and also hair cells of the inner ear (Table 1), but its role in the testis and spermatogenesis is largely unexplored. It is now known that many of these PCP proteins are expressed by Sertoli and/or germ cells in the rat testis, and one of these PCP proteins such as Vangl2 is known to be involved in regulating blood-testis barrier (BTB) function [26].

3. PCP proteins in the testis

3.1. PCP proteins and BTB

Studies have shown that the BTB is constituted by coexisting actin-based tight junction (TJ), basal ectoplasmic specialization (ES), and gap junction (GJ), as well as the intermediate filament-based desmosome that are found between adjacent Sertoli cells, located near the basement membrane in the seminiferous epithelium. The BTB also divides the epithelium into the basal and adluminal (apical) compartment (for reviews, see [27-30]) (Figure 2). As such, meiosis I/II and post-meiotic spermatid development all take place in a specialized microenvironment of the adluminal compartment. Studies have also shown that the BTB is supported, unlike all other tissue barriers, by an extensive network of actin microfilaments that are bundled and sandwiched in-between the Sertoli cell plasma membrane and the cisternae of endoplasmic reticulum, known as the basal ES (for reviews, see [28, 31]). While the BTB is one of the tightest blood-tissue barriers in the mammalian body, it undergoes continuous remodeling in particular at stage VIII of the epithelial cycle to accommodate the transport of preleptotene spermatocytes connected in clones across the immunological barrier so that they can differentiate into late stage spermatocytes in the adluminal compartment to prepare for meiosis (for a review, see [32]). Interestingly, the events of BTB/basal ES remodeling coincides with the apical ES remodeling at the opposite end of the epithelium at the interface of Sertoli cells and step 19 spermatids to accommodate the release of fully developed spermatids (i.e., spermatozoa) into the tubule lumen at spermiation (for reviews, see [33, 34]). The apical ES is structurally similar to the basal ES except that the actin microfilament network is confined to the Sertoli cell without any noticeable ultrastructures contributed by the spermatids. In short, there are two arrays of actin microfilament bundles at the basal ES vs. just one array of filament bundles at the apical ES. However, the actin microfilament networks at both the apical and basal ES have to be broken down and be re-organized to facilitate their degeneration at stage VIII and reassembly at stage IX, respectively. Several PCP proteins have been found in the adult rat testis. Vangl2, a core PCP protein, has been shown to be expressed in Sertoli cells and germ cells in the rat testis. It is also an integrated component of the BTB in the seminiferous epithelium in virtually all stages of the epithelial cycle, localized at the Sertoli cell-cell interface, and is involved in BTB remodeling [26]. Most notably, a knockdown (KD) of Vangl2 *in vitro* is known to tighten the Sertoli cell TJ-barrier function by promoting the retention of TJ proteins claudin 11 and ZO-1, and also basal ES protein N-cadherin at the Sertoli cell-cell interface [26]. On the contrary, overexpression of Vangl2 in cultured rat Sertoli cells leads to the disruption of Sertoli cell TJ barrier which is caused by a reduced expression of TJ proteins claudin 11 and ZO-1, and also basal ES-protein N-cadherin at the

cell-cell interface, probably through an enhanced protein internalization [26]. The knockdown phenotype in the testis *in vivo* also resembles that *in vitro* in which BTB restructuring is delayed at stage VIII possibly due to a persisted expression of ZO-1 and N-cadherin at the BTB [26].

The findings that PCP proteins, such as Vangl2, are involved in modulating BTB integrity are not entirely unexpected. Studies have shown that Vangl2-expressing MDCK cells fail to form large and mid-size cell aggregates, suggesting that Vangl2 negatively regulates cell adhesion [35], consistent with the findings in the testis in which overexpression of Vangl2 perturbs the Sertoli cell TJ-barrier function [26]. During lymphatic vessel formation, lymphatic endothelial cells (LECs) are known to undergo re-orientation and collective migration into the vessel lumen in which Celsr1 and Vangl2 are recruited from filopodia to the endothelial cell-cell interface, supporting the notion that PCP signaling is involved in the assembly of cell junctions [36]. Similar observations are reported in the kidney. For instance, slit diaphragm (SD), a multiprotein complex between adjacent podocyte foot processes that restricts the passage of proteins into the filtrate [37], is known to undergo continuous remodeling in response to changes in filtration pressure [37, 38]. Studies have shown that a depletion of Vangl2 in HEK293/N cells leads to a retention of SD membrane protein nephrin at the cell contacts through a reduced endocytic activity of nephrin [38]. Moreover, specific deletion of Vangl2 in podocytes increases susceptibility of the kidney to glomerular injury in adult mice receiving anti-glomerular basement membrane (anti-GBM) IgG administered through the tail vein to impair glomerulus function by causing nephritis [39], showing a pivotal role for Vangl2 in the maintenance of glomerular TJ-barrier function in the adult mouse kidney.

The precise mechanism(s) by which Vangl2 regulates TJ functions remain largely unexplored. One possibility is that PCP proteins modulate adhesion protein function via direct interaction at the junctional complexes. In fact, Vangl2 directly binds to N-cadherin and E-cadherin through its prickles-binding domain [40, 41] (Figure 3). Interestingly, both Vangl2-N-cadherin and Vangl2-E-cadherin interactions require the presence of free β -catenin binding site in N-cadherin or E-cadherin and these interactions are competitively regulated by β -catenin [40, 41]. As such, Vangl2 is a negative regulator of cadherin-catenin interactions at cell junctions. Vangl2 has also been shown to bind to N-cadherin when Vangl2 was overexpressed in Sertoli cells [26]. Thus, it is likely that the disruptive effect on the TJ-barrier function following overexpression of Vangl2 in Sertoli cells is mediated through, at least in part, compromised interactions between basal ES components N-cadherin and β -catenin and also E-cadherin and β -catenin due to the binding of cadherins to Vangl2. Studies have also shown that PCP proteins are playing a role in modulating endocytic vesicle-mediated trafficking events in mammalian cells. For example, Celsr1–3, which are seven-pass transmembrane proteins and are the vertebrate homologs of atypical cadherin Flamingo, are involved in the establishment of polarized cell junctions across proximo-distal cell boundaries by recruiting an asymmetric complex consisting of Vangl and Frizzled to the cell membrane [42, 43]. Recently, Celsr1 has been shown to form a punctuate pattern along the junctions of confluent lymphatic endothelial cells (LECs) [36] and to localize to E-cadherin-containing adhesion junctions at the neural plates [44]. Overexpression of Celsr1 in LECs leads to a reduction of VE-cadherin level at the cell

junction, which is mediated by a disrupted recruitment of VE-cadherin to the site of newly formed junctions but not via an enhanced endocytosis of VE-cadherin at the established cell junctions [36]. Meanwhile, overexpression of Vangl2 in HEK293T cells with established cell junctions leads to a rapid internalization of E-cadherin through an increase in endocytosis since introduction of dominant-negative mutant of either Rab5 or dynamin to block endocytosis is able to rescue cell surface expression of E-cadherin [41]. Taken collectively, these findings illustrate that PCP proteins are involved in both cell junction formation and maintenance through their effects on endocytic vesicle-mediated cellular trafficking.

In the testis, BTB undergoes remodeling at stage VIII of the epithelial cycle during which the 'old' BTB above the preleptotene spermatocytes connected in clones undergoes gradual degeneration, which coincides with the gradual assembly of a 'new' barrier behind these spermatocytes (for a review, see [45]). Studies have shown that these events require extensive endocytic vesicle-mediated trafficking so that integral membrane proteins and their associated adaptors from the 'old' BTB site can be rapidly endocytosed and recycled to assemble the 'new' BTB to avoid exhausting the available cell junction proteins in the Sertoli cell (for a review, see [46]). Emerging evidence has supported the role of Vangl2 in this process. First, the expression of Vangl2 at the BTB peaks at stage VIII [26] when BTB remodeling is most intensive, illustrating this surge in Vangl2 expression at stage VIII may be associated with BTB remodeling. This notion indeed is supported by the observation that a knockdown of Vangl2 in the testis in vivo causes retention of ZO-1 and N-cadherin at the BTB [26]. Although it is not clear whether this phenotype is due to a Vangl2-mediated increase in endocytosis, but overexpression of Vangl2 in Sertoli cells with an established TJ-barrier was found to cause a re-distribution of TJ- and basal ES-proteins at the cell-cell interface with these proteins internalized into the cell cytosol [26]. Earlier studies have shown that Vangl2 is able to affect endocytosis likely through Rab5 or dynamin as briefly reviewed above. On the other hand, Rab11, a marker of recycling endosomes, is also regulated by Vangl2. In Vangl2-depleted *Xenopus* embryos, the apical Rab11 becomes largely undetectable in ectodermal cells in which Rab11 switches its localization from near the cell junction to the cytosol [47]. Moreover, injection of Rab11 RNA into the embryos is able to rescue the phenotypes in Vangl2-depleted embryos and to promote normal gastrulation [48], illustrating Vangl2 is involved in regulating gastrulation via the Rab11-dependent vesicular trafficking. It is obvious that much work is needed to define the precise mechanism(s) by which Vangl2 regulates BTB dynamics. Furthermore, the involvement of other PCP proteins such as Celsr, Prickle and Dishevelled in the Vangl2-dependent protein trafficking will also need to be investigated.

3.2. PCP proteins and actin-based cytoskeletal organization

Studies have shown that multiple cellular events during embryonic development, such as neural crest migration, glomerular morphogenesis and gastrulation, involve extensive interactions between PCP proteins and actin cytoskeleton (for reviews, see [49-52]). Furthermore, the signaling molecules, small GTPases, actin or MT cleavage proteins and the signaling cascades involved in PCP-mediated changes in actin and MT organization are known [53-55] (Figure 4). For instance, Wnts, such as Wnt5a, serve as signaling initiators that bind to the Wnt receptors, such as Fzd and Ror, to facilitate the recruitment of Dvl near

the plasma membrane [56-58]. Two downstream signaling cascades that involve small GTPases are subsequently activated. The first cascade begins with the binding of Dvl to Daam1 (Dvl associated activator of morphogenesis1), which in turn activates RhoA and subsequently the activation of ROCK (Rho-associated coiled-coil-containing protein kinase) [59]. Activation of ROCK then leads to phosphorylation of LIMK (LIM kinase) (activation) and SSH (Slingshot homologue) (inhibition), thereby resulting in phosphorylation and inhibition of cofilin to suppress its actin-severing activity [60, 61] (Figure 4). It has also been reported that Dvl-Damm1 inhibits RhoA through activating CDC42, therefore suppressing ROCK activity to facilitate actin turnover [62]. The state of RhoA, either activation or inhibition under PCP signaling may rely on the Wnt ligands upstream since Wnt11/Fzd is active during *Xenopus* CE when RhoA is activated by PCP signaling [63] whereas the inhibition of RhoA is induced through Wnt5a/Fzd during human corneal endothelial cell migration [62]. In addition to exerting its effects through RhoA, Daam1 can also mediate Wnt/Dvl signaling through the actin-binding protein Profilin [64]. For the second cascade, Dvl can activate another small GTPase of the Rho family, Rac1, independent of Rho, which in turn stimulates its downstream effectors such as Jun N-terminal kinase (JNK) and Arp2/3 complex to regulate F-actin organization [59, 65-67]. Studies have shown that signaling activation of PCP proteins requires the proper membrane localization of Dvl (for a review, see [59]). In *Drosophila*, Vang (or Stbm) and Prickle (Pk) forms a complex which interacts with Dsh directly [68]. New evidence suggests that Vangl2 binds to Rac1 directly by recruiting endogenous Rac1 to specific cellular domains to elicit actin microfilament turnover [35]. This finding implies that Vangl2 could probably bypass Dvl-mediated signaling by exerting its effects on actin organization directly. Indeed, Vangl2 binds to Dvl and Rac1 through different domains. The binding of Vangl2 to Rac1 is mediated via the PDZ-binding motif (PBM) of Vangl2 at the C-terminus [35] whereas it interacts with Dvl through its C-terminal cytoplasmic tail independent of the PBM [69] (Figure 5). Thus, it is likely that the effects of Vangl2 on actin cytoskeleton may be the combined efforts of Vangl2-Rac1 and Vangl2-Dvl signaling.

In the rat testis, the ES is an actin-rich ultrastructure, typified by the presence of an array of actin filament bundles that are sandwiched in-between the cisternae of the endoplasmic reticulum and the opposing plasma membrane of Sertoli cell-spermatid (apical ES) or Sertoli-Sertoli cells (basal ES). These actin filament bundles serve as anchors that confer adhesive strength and mechanical support to the ES, which in turn supports spermatid polarity. As such, this unique actin cytoskeletal arrangement supports both junction integrity and spermatid polarity during the epithelial cycle. It was shown that overexpression or knockdown of Vangl2 altered the organization of actin microfilaments in cultured Sertoli cells with an established functional TJ-barrier [26]. For instance, a knockdown of Vangl2 by RNAi *in vivo* led to actin branching at the apical ES through a disruptive spatiotemporal expression of branched actin nucleation protein Arp3 and actin barbed end capping and bundling protein Eps8, which caused apical ES disassembly and defects in spermatid polarity [26]. Interestingly, a knockdown of Vangl2 was found to be accompanied by a concomitant decrease in the expression of Scribble both *in vitro* and *in vivo* [26]. Studies have shown that Scribble, besides a cell polarity protein, is also a PCP protein [70], and a putative binding partner of Vangl2 [26, 71], possibly mediated through the PDZ binding

motifs of Vangl2. Furthermore, a knockdown of the Scribble complex in the testis in vivo which is predominantly expressed at the basal ES/BTB also led to a disruption of the F-actin network at the basal and apical ES, perturbing spermatid adhesion and polarity, displaying a phenotype similar to the Vangl2 knockdown model [10]. It is tempting to speculate that Scribble and Vangl2 may be working in concert, perhaps in conjunction with Rac1, to modulate actin microfilament organization at the ES during spermatogenesis.

3.3. Wnt/PCP signaling and spermatogenesis

Wnt/PCP signaling pathway is known to control a variety of cell processes such as embryogenesis (e.g., neural crest specification, gastrulation), cell proliferation and differentiation, directional cell movements, tumorigenesis and proliferation of stem cells (for reviews, see [72-75]). Wnt/PCP signaling is activated by binding of the wingless integration (Wnt) ligands onto the receptors Frizzleds (Fzds), which in turn activates PCP proteins downstream such as Dvl and Daam1 for signal transduction and amplification through non-canonical pathway (Figure 4). For canonical Wnt pathway, it involves activation of Dvl through Wnt ligand binding onto a Frizzled receptor in the presence of LRP (low density lipoprotein receptor-related protein 1) upstream, which in turn inhibits the β -catenin destruction complex (GSK-3 β (glycogen synthase kinase 3 β)-Axin-APC (adenomatous polyposis coli) downstream. Accumulation of β -catenin in cell cytosol in turn induces transcriptional activation of Wnt target genes [24]. Studies have shown that Wnt proteins are lipid-modified, secreted glycoproteins. Non-canonical Wnt proteins, such as Wnt5a, bind to different Fzd family members, including Fzd3 [76, 77], Fzd5 [58, 78, 79] and Fzd7 [80], and co-receptors such as tyrosine kinase PTK7 and Ror2 [57, 80, 81], to elicit PCP signaling and JNK activation [57, 81]. Several Wnt ligands and signaling components are known to be expressed in the testis [75, 82, 83], but their physiological roles pertinent to spermatogenesis remain to be elucidated. Studies have shown that Wnt5a is expressed in mouse and rat Sertoli cells, and germ cells including spermatogonial stem cells (SSCs) [26, 75]. Potential Wnt5a receptors such as Fzd5, Fzd7 and Ror2 are also expressed in Sertoli cells and stem/progenitor spermatogonia [26, 75], suggesting a role of non-canonical Wnt signaling pathway in Sertoli cell and germ cell function and/or development during spermatogenesis. Indeed, Wnt5a was shown to promote SSC maintenance as a cell-extrinsic factor since Wnt5a-expressing fibroblasts were found to support SSC activity better than those not expressing Wnt5a in culture [75]. Although the mechanism(s) by which core PCP proteins such as Vang and Dvl regulate SSC self-renewal requires further investigation, it is likely that they serve as mediators for signal transduction to activate downstream signaling proteins such as JNK [59, 84]. Wnt4 and Wnt11 are also found in the testis, most notably in Sertoli cells [82, 85]. Interestingly, expression of Wnt4 and Wnt11 are activated by Wilms Tumor gene *Wt1*, which is a Sertoli cell transcription factor. It is known that Sertoli cell-specific KO of *Wt1* leads to a failure of testis cord formation, and progressive loss of Sertoli cells and germ cells [86]. Moreover, following *Wt1* deletion, Sertoli cells undergo subtle morphologic transformation from cubical epithelial cells into mesenchyme-like cells, with a loss of tight junctions and a down-regulation of E-cadherin, Wnt4 and Wnt11 in this genetic model [85], suggesting that *Wt1* may modulate Sertoli cell polarity during testicular development through Wnt4 and Wnt11. Taken together, these findings support a potential

role of the Wnt/PCP signaling pathway in Sertoli cell and germ cell function and development during spermatogenesis.

3.4. Wnt/PCP signaling and germ cell transport

During the epithelial cycle of spermatogenesis, germ cells are being transported across the seminiferous epithelium from the basal compartment toward the luminal edge. However, developing germ cells, in particular post-meiotic spermatids, are not motile cells per se, lacking the ultrastructures as found in other motile cells (e.g., fibroblasts, macrophages, neutrophils) to engage in cell locomotion. Studies in the last decade have shown that several signaling pathways are involved in conferring germ cell transport (for reviews, see [87, 88]). Emerging evidence supports a role of PCP signaling in germ cell transport. For example, following a knockdown of Vangl2 in the testis *in vivo*, elongated spermatids found near the tubule lumen in stage VI-VII tubules underwent premature spermiation; however, spermatids that were located inside the epithelium in stage VI-VII tubules failed to be transported to the tubule lumen since many elongated spermatids were found in stages IX-X tubules, embedded inside the epithelium when spermiation had already occurred [26]. These findings thus support the notion that a loss of Vangl2 impeded spermatid transport. Studies have shown that spermatid adhesion and spermatid transport are supported by actin-and MT-based cytoskeletons (for reviews, see [89, 90]). Indeed, a knockdown of Vangl2 in the testis *in vivo* perturbed the organization of F-actin through changes in the spatiotemporal expression of actin regulatory proteins such as Arp3 and Eps8. For instance, the expression of Eps8 was considerably diminished at the apical ES following Vangl2 knockdown, so that actin microfilament bundles could no longer be maintained at the apical ES to confer spermatid adhesion, leading to their premature release into the tubule lumen. However, it remains to be investigated if Vangl2 knockdown would affect MT organization in the testis. In this context, it is of interest to note that rat testes also express a number of secreted Frizzled-related proteins (sFRP) such as sFRP1 [83], which is known to bind to Wnt proteins and Fz receptors through its cysteine-rich domain (CRD) to modulate Wnt signaling (for a review, see [91]). Overexpression of sFRP1 in Sertoli-germ cell co-cultures using lentivirus led to a considerable decline in phosphorylation of nectin-3 (an apical ES-specific adhesion protein), causing retention of nectin-3-mediated adhesion complex at the apical ES, consistent with findings *in vivo* by administering recombinant sFRP1 protein to the testis that delayed spermiation by increasing the number of stage VIII tubules considerably due to defects in spermiation [83]. In short, sFRP1 regulates spermiation, the final stage of spermatid transport through retention of apical ES adhesion protein nectin-3 [83]. Although it is not known that whether PCP proteins such as Vangl2 is affected in this sFRP1 model, It is tempting to speculate that Vangl2 may be working in concert with sFRPs to modulate spermatid transport and spermiation. However, this possibility must be carefully evaluated in future studies.

4. Concluding remarks and future perspectives

As reviewed herein, PCP proteins have been studied for more than two decades. However, the role of Wnt/PCP signaling in spermatogenesis remains largely unexplored. Recent studies have shown that PCP proteins exert their effects by modulating the organization of

actin microfilaments in mammalian cells and tissues including the testis. It is also known that spermatid transport requires continuous re-organization of actin microfilaments at the apical ES (for a review, see [89]). This process may be regulated, at least in part, by PCP proteins such as Vangl2 on actin cytoskeleton organization. Meanwhile, MTs serve as the 'tracks' for the transport of cellular organelles (e.g., residual bodies, endosomes, phagosomes) with the help of MT motor proteins to serve as engines, working in concert with the actin microfilaments [92, 93]. It is possible that PCP proteins may play a role in MT organization besides its effects on actin microfilaments at the ES during the epithelial cycle. PCP core protein Dvl1 has been shown to regulate axon formation through its direct binding to aPKC, and the Dvl1/aPKC complex allows Dvl1 to affect MT stability through aPKC-mediated signaling [94]. Vangl2 has also been shown to structurally interact with aPKC in *Xenopus* oocytes, and this association is negatively regulated by non-polarized tubulins [95]. Interestingly, both Vangl2 and aPKC are able to affect MT stability [95]. Additionally, both Wnt5a and Dvl are required in the re-orientation of the centrosome and Golgi apparatus during epithelial cell polarization which is a MT-dependent cellular event. And this Wnt/Dvl signal pathway appears to cooperate with Cdc42/Par6/aPKC to promote polarized re-organization of MTs [96, 97]. In the rat testis, a knockdown of Vangl2 has been shown to induce a considerable reduction in the occurrence of meiosis I/II which is a MT-dependent event in stage XIV tubules [26]. Taken collectively, these findings support the notion that PCP proteins are probably involved in MT dynamics in the rat testis. Studies are warranted to examine the likely involvement of PCP proteins in polarized MT organization in the testis.

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Figure 1. Cellular arrangements regulated by planar cell polarity (PCP) proteins

(A) Establishment of PCP regarding insect hair within the plane of a *Drosophila* wing epithelium. Hair are asymmetrically localized at the apical region of epithelial hair cells with PCP proteins Vang, Pk, and Fmi at the proximal end (black), and PCP proteins Fz, Dsh, Dgo, and Fmi (red) at the distal end of neighboring cells. Wing hair are located at the distal end. (B) Vertebrate convergent extension (CE). Under the influence of PCP signaling, tissue of a developing embryo is restructured to narrow along the mediolateral axis (converge) and elongate (extend) along a perpendicular axis called anteroposterior axis through mesodermal cell migration and intercalation mediated through their actin-rich lateral protrusions. Cell cortical zone is outlined by red.

Figure 2. A schematic drawing that shows PCP regarding the alignment of polarized elongate spermatids across the plane of the seminiferous epithelium in a stage VIII tubule

(A) Cross-section of a stage VIII tubule from a frozen adult rat testis in which cell nuclei were visualized by DAPI, supporting the concept of PCP in which polarized step 19 spermatids were aligned orderly across the plane of the seminiferous epithelium. Basement membrane of the tunica propria is annotated by a dashed white-line at the base of the seminiferous epithelium. Heads of the spermatids from a section of the epithelium are encircled in a dashed green-lined box and magnified below, illustrating these are polarized cells with their heads pointing toward the basement membrane and their tails (not visible by DAPI) toward the tubule lumen and the corresponding convex and concave side of a spermatid shown in the red boxed magnified image on the right. In short, these polarized spermatids are orderly aligned across the plane of the seminiferous epithelium and schematically shown in (B). Scale bar, 80 μm , top panel; 40 μm and 20 μm in the green and red boxed inset, respectively. (B) Schematic drawing that illustrates the concept of PCP regarding the alignment of polarized spermatids across the plane of seminiferous epithelium as noted in this stage VIII tubule.

Figure 3. A schematic drawing that illustrates the spatial relationship of different cell junctions and of the actin- and microtubule (MT)-based cytoskeletons across the seminiferous epithelium of adult rat testes

A schematic drawing that illustrates the cross-section of a late stage VII to early stage VIII tubule in the rat testis, showing the seminiferous epithelium which is composed of only Sertoli and germ cells. Spermatogonia, such as type B spermatogonia, that reside near the basement membrane of the tunica propria differentiate from spermatogonial stem cells (SSCs) transform to preleptotene spermatocytes, which are transported across the blood-testis barrier (BTB) during stage VIII of the epithelial cycle to enter the adluminal compartment for differentiation into more advanced primary spermatocytes. The BTB, composed of coexisting actin-based tight junction (TJ), basal ectoplasmic specialization (basal ES) and gap junction (GJ) alongside with intermediate filament-based desmosome, physically divides the seminiferous epithelium into the adluminal (apical) and the basal compartment. Following meiosis I and II, haploid spermatids undergo spermiogenesis and being transported across the epithelium so that they line-up at the luminal edge of the tubule lumen to prepare for spermiation. Schematic drawings that illustrate the detailed structure of apical ES vs. basal ES/BTB are shown in the magnified boxes on the right panel. PCP proteins Vangl2 and Scribble are also shown.

Figure 4. Non-canonical Wnt/PCP signaling pathway in vertebrates

Upon binding of Wnt ligands to receptors such as Frizzled (Fzd) and Ror2, multiple downstream signaling cascades are activated. Dishevelled (Dvl) serves as a cytoplasmic hub for signal transduction. Dvl binds to Daam1 to either activate RhoA directly or inhibit RhoA through Cdc42. Cofilin serves as a downstream effector of the ROCK/LIMK or ROCK/SSH pathway to cause actin rapid reorganization. Actin-binding protein profilin also works downstream of Dvl-Daam1 by binding to monomeric actin, and these profilin-actin complexes are then assembled into rapidly growing actin polymers at the barbed end (or +end) by nucleation proteins (e.g., formin). Dvl can also bind to aPKC [94], the latter of which oppresses MARK2 activity via phosphorylation [98], therefore stabilizing microtubule (MT) network by protecting MT-associated proteins (MAPs) from being phosphorylated by MARK2. Activation of Dvl-Rac1 signaling is independent of Dvl-RhoA which leads to changes in actin dynamics mediated through effectors such as the Arp2/3 complex and the JNK signaling. Vangl2 is working in concert with Prickle and antagonizes Dvl signaling by affecting the cellular localization of Dvl. Vangl2 can also associate with Scribble and Rac1 to modulate actin cytoskeletal function. The binding of Wnt ligands to Wnt receptors can be blocked by antagonists such as sFRPs, which in turn oppresses Wnt/PCP signaling. (see text for details)

Figure 5. Protein-protein interactions through different functional domains of PCP proteins

The establishment and maintenance of PCP is regulated, at least in part, through protein-protein interactions (red solid lines) among PCP proteins. Vangl2 is able to bind to Scribble-based complex component Scribble through PDZ interaction [99]. The C-terminal cytoplasmic domain of Vangl2 also associates with other PCP core proteins Dishevelled and Prickle [68, 69, 100]. It is reported that Dishevelled and Prickle also interact with each other [101]. Another transmembrane receptor Frizzled binds to the PDZ domain of Dishevelled [102] to transmit PCP signals upon interaction with Wnts via its cysteine-rich domain at the N-terminus [103]. Abbreviation used: DEP, Dishevelled-Egl-10-pleckstrin; DIX, Dishevelled and Axin; LRR, Leucine rich repeat; PDZ, PSD-95, Discs-large and ZO-1; PET, Prickle, Espinas, and Testin.

Table 1
Functions of PCP proteins in rodents based on studies of genetic models

Gene	Zygoty	Phenotype	References
Celsr1	Heterozygous	Misoriented inner ear hair cell stereociliary bundles.	[104]
	Homozygous	Perinatal lethality caused by severe neural tube defects.	
Celsr3	Homozygous	Mice died a few hours after birth due to a central ventilation failure, defects in axonal development.	[105]
Dishevelled1	Homozygous	Mice were viable and fertile and having abnormal social behavior.	[106]
Dishevelled2	Homozygous	~ 50% perinatal lethality due to neural crest abnormalities, abnormalities of the vertebral bodies and ribs, survivors showed normal fertility.	[107]
Dishevelled3	Homozygous	Mice died shortly after birth with cardiac anomalies, no neural tube defects and skeletal defects observed, a mild disruption of stereociliary bundles orientation.	[108]
Dishevelled1 and 2	Double heterozygous	Normal stereocilia orientation.	[107, 109]
	Double homozygous	Stereocilia misorientation, shorter and wider cochlear ducts, skeletal malformations in the vertebral ribs.	
Dishevelled1 and 3	Compound heterozygous	<i>Dvl1^{+/-};Dvl3^{+/-}</i> and <i>Dvl1^{+/-};Dvl3^{+/-}</i> mice were viable and fertile	[108]
	Double homozygous	Embryonic lethality between embryonic day (E) 13.5 and E15.5 due to unknown causes, no neural tube defects noted.	
Dishevelled2 and 3	Compound heterozygous	<i>Dvl2^{+/-};Dvl3^{+/-}</i> mice were viable and fertile, ~50% of the embryos in an inbred background displayed heart development; <i>Dvl2^{+/-};Dvl3^{+/-}</i> mice exhibited abnormal head shape, a truncated snout, a shortened and curly tail and cardiac defects; <i>Dvl2^{+/-};Dvl3^{+/-}</i> mice failed to survive beyond E9.5 due to abnormal cardiac development	[108]
	Double homozygous	Mice failed to survive beyond E8.5.	
Frizzled1	Homozygous	Mice were viable and fertile with no orofacial or cardiac anomalies.	[110]
Frizzled2	Homozygous	~ 50% mice died as neonates with cleft palate, survivors displayed failure-to-thrive phenotype.	[110]
Frizzled3	Heterozygous	No noticeable anomalies.	[111]
	Homozygous	Mice died within 30 min of birth with defects in axonal development.	
Frizzled4	Homozygous	Severely reduced arterial network in the heart; A reduction of small arteries in the kidney.	[112]
Frizzled6	Heterozygous	No noticeable anomalies.	[113]
	Homozygous	Mice were viable and fertile but with altered hair patterning over much of the body surface.	
Frizzled1 and 2	Double homozygous	A near absence of cleft palate and with cardiac defects.	[110]
Frizzled2 and 7	Double homozygous	Embryonic lethality at ~E10.5 as a result of vascular development failure.	[114]

Gene	Zygosity	Phenotype	References
Frizzled3&6	Double homozygous	Mice died within minutes of birth with a fully open neural tube and defects in hair bundle orientation in the organ of Corti and the cristae.	[115]
Protein tyrosine kinase 7 (PTK7)	Homozygous	Perinatal lethality due to severe neural tube defects, disrupted orientation of stereociliary bundles of sensory hair cells in the organ of Corti in cochlea.	[116, 117]
Prickle	Homozygous	Embryonic lethality with failure of anterior migration of distal visceral endoderm and mesoderm formation.	[118]
Ror2	Heterozygous	Mice were healthy and fertile.	[57, 119]
	Homozygous	Mice died within 6h after birth with short limbs and tails, malformation of facial structures, respiratory malfunction and cardiac defects.	
Scribble	Homozygous	Severe neural tube defects.	[70, 120]
Vangl1	Heterozygous	<i>Vangl1^{st/+}</i> mice were healthy and fertile	[121]
	Homozygous	<i>Vangl1^{st/st}</i> mice were healthy with no gross anatomical defects, a modest but significant misorientation of stereociliary bundles in all hair cell layers	
Vangl2	Heterozygous	<i>Vangl2^{p/+}</i> mice displayed a characteristic looped tail, reduced female fertility and no neural tube defects observed	[70, 122-126]
	Homozygous	<i>Vangl2^{p/p}</i> , <i>Vangl2^{TM/ TM}</i> , <i>Vangl2^{ATG/ ATG}</i> mice displayed embryonic lethality due to an open neural tube in the hindbrain and spinal region, misoriented hair cell stereociliary bundles in cochlea, severe cardiac defects	
Vangl1 and 2	Compound heterozygous	<i>Vangl1^{st/+}</i> ; <i>Vangl2^{+/+}</i> mice were normal; <i>Vangl1^{st/st}</i> ; <i>Vangl2^{p/+}</i> mice displayed craniorachischisis in >60% of the embryo	[121, 127]
	Double heterozygous	<i>Vangl2^{+/+}</i> ; <i>Vangl1^{st/+}</i> mice were fertile and normal; <i>Vangl1^{st/+}</i> ; <i>Vangl2^{p/+}</i> mice displayed late embryonic lethality due to severe neural tube defects, having curly tails, and a marked reduction in cochlear size, misoriented hair cell stereociliary bundles in cochlea.	
	Double homozygous	<i>Vangl2^{-/-}</i> ; <i>Vangl1^{st/st}</i> mice had severe defects in sensory hair cell polarity and CE.	
Dishevelled3 and Vangl2	Double heterozygous	Some embryos displayed neural tube defects with rotated stereociliary bundles.	[108]
Frizzled1, 2; Vangl2	Compound heterozygous	<i>Fz1^{+/-}</i> ; <i>Vangl2^{p/+}</i> , <i>Fz2^{+/-}</i> ; <i>Vangl2^{p/+}</i> , <i>Fz1^{+/-}</i> ; <i>Fz2^{+/-}</i> ; <i>Vangl2^{p/+}</i> and <i>Fz2^{-/-}</i> ; <i>Vangl2^{p/+}</i> mice had neural tube defects	[110]
PTK7 and Vangl2	Double heterozygous	<i>PTK7^{XST87/+}</i> ; <i>Vangl2^{p/+}</i> mice displayed spina bifida, relatively normal stereociliary bundles of sensory hair cells.	[116]
Scribble and Vangl2	Double heterozygous	<i>Scrib^{CrcII/+}</i> ; <i>Vangl2^{p/+}</i> mice had misoriented stereociliary bundles of sensory hair cells.	[70]
Wnt5a	Heterozygous	No apparent mutant phenotype.	[57, 128]
	Homozygous	Perinatal lethality with a significant shortening of the embryonic anterior-posterior axis, ventricular septal defects, outgrowth defects in the genitals.	
Wnt7a	Homozygous	Mice were viable but sterile with limb abnormalities, alteration in dorsal-ventral polarity.	[129]

Gene	Zygosity	Phenotype	References
Wnt11	Homozygous	Cardiac defects including outflow tract defects and ventricular septal defects.	[130]

* This Table is not intended to be exhaustive. However, the function of major PCP genes using mouse genetic models reported in the literature are covered. Phenotypes summarized herein are focused on the PCP phenotypes and fertility following the inactivation of PCP gene(s).