Chapter 5

Male stem Cell Niche and Spermatogenesis in the *Drosophila* testis — A Tale of Germline-Soma Communication

Fani Papagiannouli

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/58756

1. Introduction

A fundamental question in biology is how communication and exchange of short-range signals shape the microenvironment for setting up functional tissues. In all adult tissues and organs harboring stem cells, tissue homeostasis and repair relies on the proper communication of stem cells and their differentiating daughter cells with the local tissue microenvironment that homes them [1, 2]. Stem cell research has made outstanding contributions on the factors that maintain stem cells or drive them to generate differentiated daughter cells. The use of stem cells in the development of cell-based medicine and in repairing malformed, damaged or aging tissues demands a better understanding of stem cells at a molecular level and of how they behave in their physiological context.

The basic principles controlling stem cell self-renewal versus differentiation are strikingly conserved during evolution and their regulatory logic is often very similar among homologous stem cell niches. Since the signaling pathways and their regulatory circuits are highly complex in the mammalian system with significant molecular redundancy, they are often difficult to study. Therefore, using a simpler model system such as the *Drosophila* testis allows us to elucidate the underlying cellular and molecular mechanisms of stem cell maintenance and differentiation in a straightforward way.

The *Drosophila* testis provides an excellent system to study *in vivo* how two closely apposed cell types communicate and coordinate their reciprocal interaction. Recent advances in spermatogenesis have shown that testis morphogenesis is achieved through the physical contact and diffusible signals exchanged between the germline and the somatic cell populations [3]. Moreover, the *Drosophila* testis provides a powerful system to study germline-soma

communication as it is possible to identify the different cell populations with specific markers, study them within the context of their wild type surrounding and trace them after genetic manipulations [2, 4]. Although several signaling molecules, cytoskeletal and other factors have been so far identified, many aspects of the coordination of these events remain unsolved. Using well-established genetic tools, cell-type specific markers and imaging techniques we can manipulate cell function in a spatio-temporal specific way within the germline-soma micro-environment and decode how signal transmission and polarity are established, maintained and coordinated on the mechanistic level. Therefore, elucidating the mechanisms and factors that regulate these processes is crucial for understanding cell communication and coordination *per se,* which is a prerequisite for the therapeutic applications in other stem cell systems and in various tissue contexts.

The proposed chapter gives an overview of the *Drosophila* male stem cell niche and its importance as a model system for understanding stem cell function. The chapter starts with an introduction to the system, focusing on the importance of soma-germline communication, mutual coordination and progressive co-differentiation. As next, follows the role of the stem cell niche and signaling pathways in balancing stem cell maintenance and differentiation. The specification and positioning of the stem cell niche is discussed, in view of recent data in the field, which put the way we understand stem cell niche establishment and maintenance into a new perspective. Finally, the role of septate junctions and cortical polarity components in the somatic lineage is presented, together with open questions and challenges of the current research in the field.

2. The *Drosophila* testis

Organogenesis of the *Drosophila* testis, a structure first made by the coalesce of germ cells and somatic gonadal cells in late embryogenesis, proceeds continuously throughout embryonic and larval stages, to reach maturation in adult stages. The embryonic gonad results from the coalescence of the germ cells that completed migration and the somatic gonadal precursors (SGPs). SPGs are mesodermal cells specified in bilateral clusters within the *eve* domain of abdominal parasegments [5] 10 to 13 [6-9]. The development of male and female gonads already differs at the time of gonad coalescence. In the male gonads three SGP populations are identifiable by their different gene expression: the posterior-SGPs, the posterior male-specific SGPs which die by apoptosis in females [6] and the anterior-SGPs which will give rise to the hub, the core of the testicular niche which will recruit and organize the anterior-most germ cells to become germline stem cells (GSCs) [10]. Therefore, it becomes evident that the different SGP populations joining the male gonad orchestrate testis morphogenesis since the germ cells represent a uniform population at that time. The SGPs are specified initially through the function of Zinc-finger homeodomain protein 1 (Zfh-1) within the cluster of the lateral mesoderm (PS2-14) which work together the homeobox protein Tinman to promote germ cell migration to the lateral mesoderm. Subsequently, Zfh-1 restriction in PS10-13 correlates with the specification of these cells as SPGs.

http://dx.doi.org/10.5772/58756

The first signs of testis organogenesis are detected in 1st instar larvae (L1) and a testis with a mature stem cell niche and all premeiotic stages is detected at 3rd instar larvae (L3). The *Drosophila* testis contains two types of stem cells: the germline stem cells (GSCs) and the somatic cyst stem cells (CySCs). Each GSC is surrounded by two somatic cyst stem cells (CySCs) and both types of stem cells are maintained through their association to the hub cells, a cluster of non-dividing cells forming the niche organizer. Upon asymmetric cell division, each GSC produces a new GSC attached to the hub and a distally located gonialblast (Gb), whereas each CySC pair divides to generate two CySCs remaining associated with the hub and two distally located post-mitotic daughter somatic cyst cells (SCCs) [1, 11]. Upon asymmetric stem cell division, each GSC produces a new GSC attached to the hub and a distally located gonialblast, whereas each CySC pair divides to generate two CySCs and two somatic cyst cells (SCCs) [1, 12]. GSCs divide asymmetrically with the mitotic spindle orientated perpendicular to the hub [13, 14]. After division the GSC remains in contact with the hub and inherits the mother centriole whereas the gonialblast, inherits the daughter centriole and initiates differentiation [15]. However, upon starvation-or genetically-induced GSC loss, the GSC population can be renewed both by symmetric renewal and de-differentiation of transient amplifying spermatogonia, which repopulate the niche and reestablish contact to the hub [16].The gonialblast divides mitotically four more times to give rise to 16 interconnected spermatogonial cells, forming a cyst surrounded by the two SCCs (Fig.1). As germ cells enter their differentiation program of four transient amplifying divisions followed by pre-meiotic gene expression and meiotic divisions, the SCCs grow enormously in size, elongate and wrap the germ cells creating cysts [17] outside "sealed" by extracellular matrix (ECM) [18]. After the growth phase, the spermatocytes undergo meiosis and differentiate into elongated spermatids.

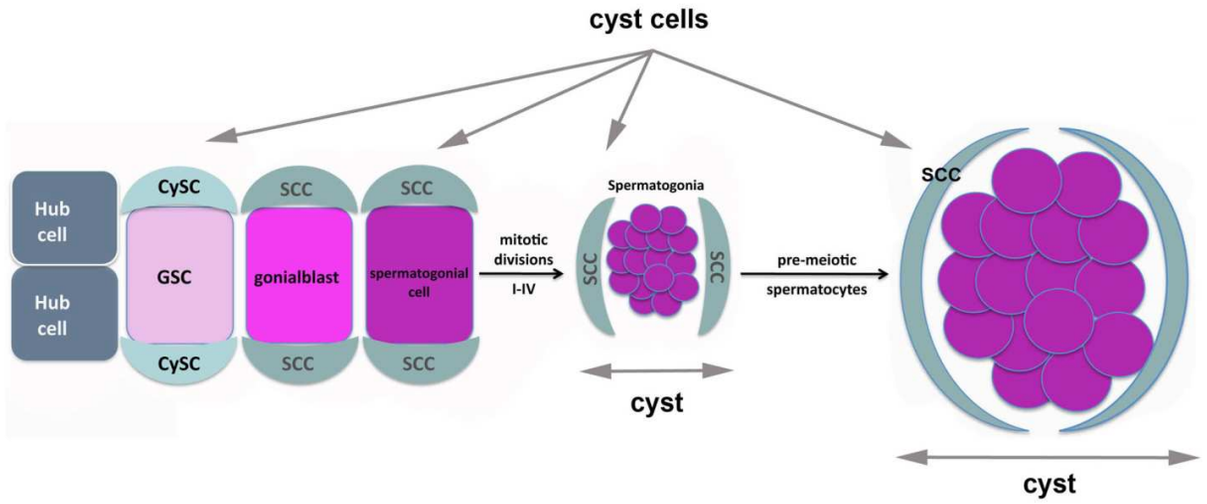

Figure 1. Diagram depicting early spermatogenesis in *Drosophila*. GSC: germline stem cell, CySC: somatic cyst stem cell, SCC: somatic cyst cell. For simplicity reasons CySCs and SCCs are collectively called cyst cells. Testicular cysts comprise of a pair of cyst cells flanking the germline (GSCs, spermatogonia or spermatocytes).

Testis organogenesis is completed during pupal stages. For the formation of a mature testis and a functional reproductive tract, the *Drosophila* testis contacts the seminal vesicle growing

out of the genital disc during metamorphosis. The outer sheath of the male reproductive tract develops from two populations of cells: the pigment cells of the testis and the precursors of smooth muscle cells from the genital disc [19]. First, the muscle progenitor cells of the genital disc contact the basal surface of the pigment cells of the testis. Then, migration of muscle and pigment cells proceeds in opposite directions until the gonad and the seminal vesicle have each acquired an inner layer of muscle tissue and an outer layer of pigment cells [19]. It is the addition of the acto-myosin sheath, which gives to the adult testis its characteristic coiled-shape. The pigment cells are responsible for the yellow color of the testis sheath and seminal vesicle [17]. *wnt2*, expressed in the SGPs, is required for the correct development of pigment cells [19], and in *wnt2* mutant embryos pigment cells are not specified and *Sox100B* is not expressed in pigment cell precursors [20, 21].

2.1. Cyst cells: The safeguards of the Germline

Critical for testis differentiation and morphogenesis is the cyst microenvironment created by the cyst cells (CySCs and SCCs) that enclose the germline cells, accompany them throughout their differentiation steps up to sperm individualization and maintain cyst integrity and architecture [22, 23]. Although it is well established that soma-germline physical contact is critical for the cell communication and for promoting their mutual development and differentiation [3], it remains so far elusive how these tightly packed cysts coordinate adhesion and cell shape changes with signaling and membrane addition on a mechanistic level.

The thin and squamous cyst cells lack the columnar epithelial structure of e.g. the ovarian follicular epithelium, which caught the attention of scientists analyzing apico-basal polarity many years ago. For this reason, several questions concerning cyst cell architecture, apical-basal polarity and sub-cellular localization of cytoskeletal proteins such as Dlg, Integrin and Talin remained unclear. Preliminary data show that cyst cells are polarized with an inner-apical surface phasing the germline (Fig. 2E; arrowheads) [22] and an outer-basal surface surrounded by ECM [18]. Critical cytoskeletal and polarity components localize at cyst cells, such as Rho1, Bazooka (Baz), Fasciclin II (FasII), Integrin-linked kinase (ILK), βPS-Integrin (encoded by the *myospheroid* gene) (Fig. 2B-F'), as well as the septate junction proteins Dlg, Scrib and Lgl (Fig.3 A-D). Moreover, cyst cells are able to extend projections in between the germline spermatogonia (small insets of Fig.3 A-C) and spermatocytes (Fig.2 C-C', E-F'; yellow arrowheads), similar to what was previously observed in the embryonic gonads [24]. On the morphological level, the orientation of the SCCs flanking the germ cells changes in comparison to their mother CySCs via a not yet uncovered mechanism. The two CySCs flanking the same GSC are arranged parallel to the testis anterior-posterior axis (A/P) and attach to the hub whereas their post-mitotic daughter SCCs change their orientation perpendicular to the A/P testis axis (Fig.1). During terminal differentiation, the two cyst cells of the same cyst acquire different identities followed by morphological changes [25]: the forward SCC becomes the "head cyst cell" (HCC) onto which all 64 spermatid heads are anchored shortly after meiosis, and the posterior one becomes the much larger "tail cyst" (TCC) that surrounds the spermatid tails of 1.8 mm length [26]. This results in creating polarized cysts across the testis A/P axis and

http://dx.doi.org/10.5772/58756

towards the direction (A→P) of differentiation. The HCC finally is engulfed by cells of the terminal epithelium to allow coiling of the spermatid bundles towards the testis base [27].

So far the main evidence for cyst cell (CySCs and SCCs) function came from the analysis of individual signal transduction pathways that establish a cross talk between the soma and the germline. In this chapter recent findings critically affecting germline-soma communication and coordination will be highlighted, with emphasis on the role of cytoskeletal and scaffolding components such as integrins and adaptor proteins, ECM and the septate junction components. Interestingly, the *Drosophila* testis cyst cells show striking similarities with the Sertoli cells, the supportive cells of the mammalian germline, in terms of cytoskeletal and scaffolding components [2]. Moreover, the genes presented in this study show high degree of conservation to their vertebrate homologues [18, 23]. Accordingly, although we use *Drosophila* spermatogenesis as a model for its powerful genetic tools, accessible imaging and the wealth of underlying prior knowledge on which to built on, the regulatory mechanisms discovered in the *Drosophila* testis provide paradigms for regulatory strategies in spermatogenesis and allow us to discern the complexity of niche and testis homeostasis in other organisms and stem cell systems in other tissues, which will eventually advance the basic knowledge required for stem cell applications.

2.2. Niche Homeostasis: Signaling regulation of stemness vs. differentiation

Tissue specific stem cells are the lifetime source of many types of differentiated cells. They reside in microenvironments, the stem cells niches that have an important role in stem cell behavior [28]. Gamete development requires a coordinated soma-germ line interaction that keeps the balance between germline stem cell renewal and differentiation. The balance between stem cell identity and differentiation at the *Drosophila* testicular niche results from signals exchanged among the hub, GSCs and CySCs. The Janus-kinase transducer and activator of transcription (JAK-STAT) pathway was the first signaling pathway found to regulate GSC and CySC maintenance in the *Drosophila* testis [29, 30]. The hub cells secrete the ligand Unpaired (Upd), which activates the JAK-STAT pathway in adjacent GSCs and CySCs [29-31]. In the absence of JAK-STAT signaling the GSCs differentiate and are unable to self-renew, whereas ectopic expression of *upd* in the germline greatly expands the population of GSCs and CySCs in adult as well as in the larval testis [29, 30]. In GSCs, STAT is required so that E-cadherin (E-cad) maintains the connection of the GSC to the hub and ectopic E-cad partially rescues the maintenance of STAT-depleted GSCs [32]. Another STAT target in GSCs is *chickadde*, the homologue of the *Drosophila* profilin. Chic is required cell autonomously to maintain GSCs by facilitating GSC-hub contact possibly via E-cad whereas Chic in the SCCs is affecting germ cell enclosure and restricting trans-amplifying (TA) spermatogonial divisions [33]. When GSCs divide, their daughter cells displaced from the hub are thought to receive lower levels of hub-derived signals and therefore differentiate. In CySCs, STAT is critical for maintaining their stem cell character and the activation of targets essential for their identity such as *zfh-1* and *chinmo* [32, 34]. *zfh-1* is expressed predominantly in CySCs and their immediate SCCs, and ectopic expression in late SCCs outside the niche leads in accumulation of GSC-and CySCs-like cells which fill in the whole testis. Similarly, *chinmo* is expressed in

comparable levels in CySCs and early SCCs, is required for CySCs and not GSC renewal, and ectopic expression causes accumulation of GSCs-and CySCs-like cells. Furthermore, *zfh-1* and *chinmo* are not expressed in GSCs meaning that STAT can activate distinct downstream cascades in the GSC vs. CySCs. *ken* and *barbie* (*ken*) is another gene necessary and sufficient to promote CySC identity, yet in a STAT independent manner and with similar ectopic phenotypes like *zfh-1* and *chinmo* [35]. At the same time, Suppressor of cytokine signaling 36E (Socs36E) suppresses Jak-Stat signaling in the CySCs preventing them from outcompeting the GSCs and thereby maintains the proper balance of GSCs and CySCs, in a manner that depends on the adhesion protein integrin [36].

Interestingly, very recent findings revealed that the Hedgehog (Hh) ligand secreted from the hub cells activates the Hh signaling in CySCs (and not in the GSCs) with critical function in CySC maintenance [37-40]. Hh overexpression leads in increased number of CySCs, identified as Zfh-1 positive cyst cells outside the niche, which can still proliferate in contrast to the normal post-mitotic SCCs. Furthermore, rescue of STAT depleted testis by Hh signaling activation in the CySCs can rescue the CySCs but GSC and germline maintenance is still impaired, as these Zfh-1 positive CySCs are not able to induce the GSC over-proliferation phenotype observed in SCCs ectopic Zfh-1 activation [38]. This suggests that [1] *zfh-1* expression relies on inputs from both Hh and JAK-STAT signaling pathways and that [2] apart from Zfh-1 other STAT regulated factors are necessary for allowing the CySC-to-GSC communication, which promotes GSC maintenance.

Notably, BMP seems to be the primary pathway leading to GSC self-renewal in the *Drosophila* testis [41-44]. BMP ligands and the BMP modulator *magu*, are expressed in the hub and CySCs that serve as the GSC niche and their loss results in reduced GSC numbers and *bam* derepression, whereas the hub and CySCs remain unaffected [42-44]. This could also suggest that expansion of GSC population by the JAK-STAT signaling could be due to its activation in the CySCs that consequently leads to enhanced expression of BMP ligands from CySCs [32] that finally drive GSC expansion. The BMP pathway is also negatively regulated in the course of testis morphogenesis along embryonic-larval-adult stages via Smurf (SMAD ubiquitination regulatory factor) [45]. High BMP levels are required at the initial steps of niche establishment when the hub cells attract the nearby germ cells to become GSCs in late embryogenesis up to early 3rd instar larval stages. Apparently, BMP signaling is spatially and temporally downregulated in stem cells and early germline cells in late 3rd instar larval and pupal testes through Smurf proteolytic activity. The described BMP downregulation seems to be critical for the normal decrease in stem cell number during pupal development, for restricting TA spermatogonia proliferation and control of the testis size. This dynamic regulation indicates the requirement for fine trimming the BMP signaling intensity during subsequent developmental stages and might even suggest a difference between establishment vs. maintenance of certain cell populations across different stages. Yet, another recent story revealed that GSC characteristics can be maintained over time even after ablating the CySC and SCCs [46]. Without CySCs and SCCs, early germ cells away from the hub failed to initiate differentiation and maintained their GSC-like characteristics. Therefore, it becomes evident that the interactions between different stem cell populations and how one stem cell population influences the other

http://dx.doi.org/10.5772/58756

can be indeed very complex. Finally, antagonistic functions between the *Drosophila* β-catenin Armadillo (Arm) and the microRNAs-(miR-) 310-313 suggest that modulation of the Wingless signaling activity is important to buffer germ cell and somatic differentiation in the *Drosophila* testis [47].

Critical for germ cell differentiation is the expression of *bag of marbles (bam)* and *benign gonial cell neoplasm (bgcn)* in dividing spermatogonial cells in order to regulate their proliferation [48]. *bam* transcription is negatively regulated by the cooperation of the Glass bottom boat (Gbb) and Decapentaplegic (Dpp) signaling pathways emanating from the hub and CySCs to maintain the GSC identity [42]. Bam is required cell autonomously in TA spermatogonia to stop proliferation and enter the spermatocyte differentiation program [49]. The switch from TA proliferation to differentiation is mediated by translational control: Mei-P26 facilitates the accumulation of Bam in TA cells whereas Bam and Bcgn bind *mei-P26* 3′ untranslated region and repress translation of mei-P26 in late TA cells. Thus, germ cells progress through subsequent regulatory states that is: from a Mei-P26 on/Bam off to a Bam on/Mei-P26 off state.

Another signaling pathway restricting GSC proliferation is mediated by Epidermal Growth Factor Receptor (EGFR), whose inactivation in SCCs leads to an expansion of male GSCs [50]. In *Drosophila* testis, the major ligand of the EGFR pathway, Spitz (Spi) is secreted from the germline cells to stimulate the EGFR on cyst cells (CySCs and SCCs) [25]. Removal of either *spi* or *stet* from the germline cells, or removal of the EGFR from the cyst cells resulted in increased division frequencies of GSCs but did not affect the division frequencies of CySCs, suggesting that EGF signaling downregulates GSC divisions. Likewise, Raf, an EGFR downstream component, is required in SCCs to limit GSC expansion [51-53]. In testes mutated for the *rhomboid* homologue *stet*, the germ cells fail to associate with SCCs. Furthermore, germ cells recruit CySCs via the ligand Spitz, which binds to EGFR, and acts through the nucleotide exchange factor Vav to regulate the activity of Rac1, a downstream component of the EGFR pathway. Taken together, EGF signaling from the germline cells produces differential Rac-and Rho-activities across the cyst cells that leads to a directional growth of the cyst cells around the germline cells [25]. Finally, Zero population growth (Zpg), the *Drosophila* gap junction Innexin 4, is localized to the spermatogonia surface, primarily on the sides adjacent to SCCs [54] and is required for the survival and differentiation of early germ cells in both sexes [55, 56].

3. The male stem cell niche: Specification and positioning

The somatic cells of the hub form the organizing center, a cluster of non-dividing cells, at the anterior part of the embryonic male gonad originating, as already discussed, from SGPs [10]. However, not only the hub but also the cyst cells are specified from the SGPs and the common origin between hub and CySCs has been shown by lineage tracing experiments [57]. This is further supported by the fact that both cell types can be traced using the same cell markers such as Zfh-1 and Traffic Jam (TJ) [25]. Hub cell fate vs. cyst cell fate is specified prior to gonad

coalesce in a subset of somatic gonadal precursor cells (SGPs) upon Notch signaling activation [57]. In a next step, the *abdominal A (abd-A)* and *Abdominal B (Abd-B) Hox* genes promote the distinct identities of the SGP clusters: anterior SGP identity (PS10-11) is specified by *Abd-A* and repressed by *Abd-B*, a combination of *Abd-A* and *Abd-B* specifies the posterior SPGs (PS12) and *Abd-B* alone specifies the male-specific [58] SPGs (PS13) [9, 10, 20, 59]. Thus, *Abd-A* and *Abd-B* pattern the A/P axis of the formed gonad. Moreover, *Abd-B* can control the correct hub positioning by upregulating the tyrosine-kinase *sevenless (sev)* in the ms-SGPs. Sev is activated by the Boss ligand emanating from the primordial germ cells to represses ectopic hub differentiation [60] whereas the Epidermal growth factor receptor (EGFR) signaling represses hub formation in the rest of the SGPs [61]. Specification of CySCs vs. hub cell fate relies as well on the antagonistic function of *lines (lin)* and *brother of odd with entrails limited (bowl).* Bowl is a zinc finger transcription factor required in the hub cells and its antagonist Lin is a cytoplasmic protein with catalytic activity whereas Drumstick (Drm) competes with Lin for binding to Bowl [25, 62]. This regulatory network was supported by analysis of mutant phenotypes: *bowl* mutant gonads had fewer hub cells, *lines* mutant gonads had increased number of hub cells, whereas *lines* depleted CySCs acquired some hub-like properties and markers [57]. Once specified, the hub cells are able to recruit the anterior-most germ cells to become the germline stem cells (GSCs) [63], giving rise to the male stem cell niche [64].

We have discussed how the posteriorly expressed Hox genes *AbdA* and *AbdB* promote the distinct identities of the SGP clusters in the embryonic male gonad and how the diffusible signals and physical contact of germ and somatic cells keep the balance between stem cell renewal and differentiation in the larval and adult testis. However, it is interesting to understand how the male stem cell niche is maintained from its initial specification up to the adult stages and how this morphogenetic process is coordinated. In order to ensure normal niche function in the *Drosophila* testis, the hub cells not only need to be properly specified but also need to be correctly placed. Integrin-mediated adhesion is important for maintaining the correct position of the embryonic hub cells during gonad morphogenesis. In the absence of integrin-mediated adhesion, the hub cells still form a cluster, but instead of remaining at the anterior part of the gonad they migrate to the middle part of the developing gonad [65]. Disruption of integrin-mediated adhesion in adult testis by knocking down *talin*/*rhea,* an integrin-binding and essential focal adhesion protein of the Integrin-cytoskeleton link [66, 67], results in GSC loss and gradual hub disappearance, a phenotype, which becomes more severe as adult males age [67]. As in *talin*-depleted adult testis the hub is progressively lost, the signals that normally emanate from the hub to instruct stem cell renewal are absent, driving the balance between stem cell maintenance and differentiation towards more differentiation and progressive stem cell loss [65]. A similar hub displacement phenotype is observed by depleting adult testis of Lasp [68], an actin-binding protein. From the vertebrate system we know that Lasp interacts genetically with Integrin [69] and in blood platelets Lasp requires Integrin for its proper localization to the cytoskeleton [70]. Moreover, expression levels of Integrin and Talin are critical for occupation of the niche as CySCs with enhanced integrin-mediated adhesion are able to compete and displace their neighboring GSCs [36].

http://dx.doi.org/10.5772/58756

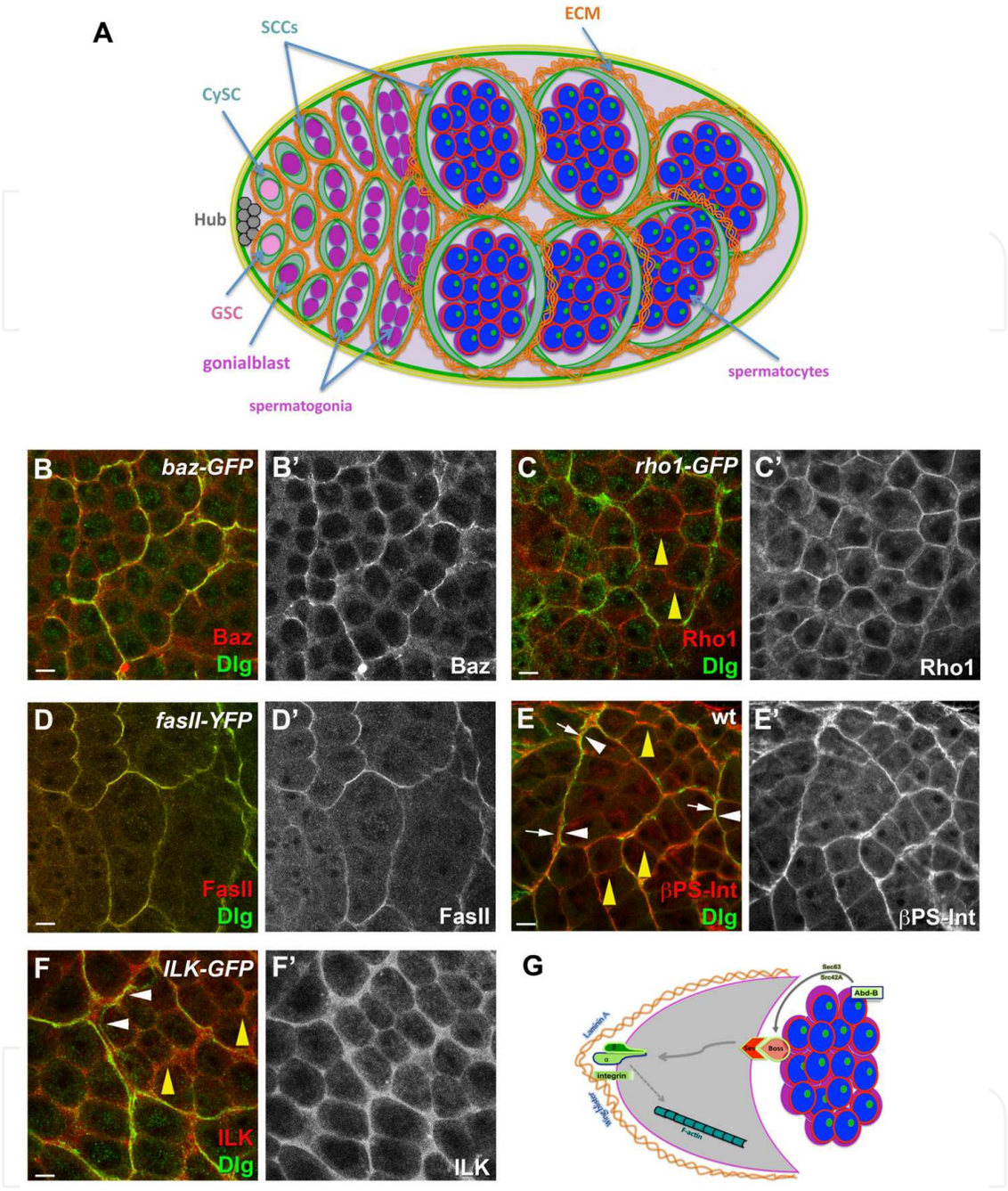

Figure 2. Somatic cyst cells are thin, elongated cells with apical and basal surfaces surrounded by ECM. (A) Schematic diagram of early *Drosophila* spermatogenesis. Somatic cyst cells (SCC) are thin, squamous cells and wrap the germline creating cysts surrounded by ECM (orange). (B-F') Components of cyst cells (red) co-stained with Dlg (green). Here, only the spermatocyte region is shown. Baz (B, B') and FasII (D, D') co-localize with Dlg. Rho1 and Dlg decorate the SCCs but do not co-localize (C, C'). In SCCs Dlg and Integrin are not co-localizing, with Dlg being apical (inner side; white arrowheads) and PS-Integrin more basal (facing outside; white arrows) (E, E'). ILK decorates the SCC cytoplasm and Dlg decorates SCCs facing the germline (white arrowheads) (F, F'). Yellow arrowheads in (C), (E) and (F) show SCC cellular projections growing in between the germ cells. Testes are oriented anterior left. Scale Bar: 10 mm. (G) Schematic diagram depicting a close up of a spermatocyte cyst with key players involved in niche positioning (for simplicity only one somatic cyst cell is shown). Within the spermatocytes, red line indicates the spermatocyte nuclear membrane, green dots illustrate Abd-B in the nucleolus and blue represents the nucleoplasm.

3.1. Some function, different mechanisms: How the Boss/Sev-AbdB cross-talk regulates niche positioning and integrity

As already mentioned, the Boss/Sev signaling pathway plays an important role in hub positioning in the *Drosophila* embryonic male gonads by preventing ectopic niche differentiation in the posterior gonadal somatic cells. *Abd-B*, upstream of this cascade, activates *sev* in the posterior SGPs [60] and consistent with the fact that weak *Abd-B* mutant alleles result in hub expansion and integrity defects in embryonic gonads [10]. A very recent study revealed a new role for the posterior *Hox* gene *Abd-B* in the larval and adult testis. Analysis of the role of the Hox protein Abd-B in the *Drosophila* testis revealed that Abd-B present in the germline spermatocytes acts upstream of the Boss/Sev pathway to regulate hub positioning and integrity, which finally leads to loss of Integrin and Actin localization in the neighboring cyst cells [18]. Analysis of the genetic interactions of *Abd-B* with *integrin* and focal adhesion proteins, revealed that male stem cell niche positioning is regulated by a number of factors, which link Integrin to the extracellular matrix (ECM) and actin filaments. Interestingly, the incorrect placement of the niche in *Abd-B* depleted testes, results in cell non-autonomous centrosome mispositioning and reduced GSC divisions, leading to a dramatic reduction of the pre-meiotic stages of the adult testis, a hallmark of aging in testis [14, 71].

Taken together these studies show that the same players, AbdB, Boss, Sev and Integrin, are used in larval stages to preserve hub positioning and integrity after the initial establishment at embryonic stages but using a slightly variable mechanism: (a) In embryonic gonads, *Abd-B* from the male-specific SGPs regulates *sev* expression in the same cells, whereas Boss signals from the germ cells signals to the Sev expressing cells to ensure that the niche develops in the anterior region of the gonad [60]. Integrin is also required in the somatic cells of the embryonic gonads for anterior positioning of the hub [65]. (b) In larval testes, Abd-B regulates the same process from the germline spermatocytes and via the Boss/Sev pathway controls integrin localization in the neighboring SCCs. This expression switch of Abd-B from the somatic to the germline lineage not only highlights that the mechanism of Abd-B dependent hub positioning is different between embryonic and larval stages but also raises the interesting questions of why and how Abd-B changes its expression and thus the mechanism of hub positioning. During adult stages when testis morphogenesis is completed with the addition of the actomyosin sheath originating from the genital disc [19], hub positioning and integrity is regulated by Sev, Boss [60] and Integrin [68] whereas Abd-B regulates hub positioning from a different cell type in comparison to embryonic and larval stages which is this time the cells of the actomyosin sheath, originating from the genital disc. It seems that the occurrence of new cell types and cell interactions in the course of testis organogenesis made it necessary to adapt the whole stem cell system to the new cellular conditions by reusing the same main players of niche positioning in an alternative manner.

3.1.1. Boss mediates, in a Dynamin-and Src-dependent way, germline-soma signaling in larval testis

Drosophila Boss is an atypical G-protein coupled receptor membrane protein that was first identified as a ligand of the Sevenless (Sev) tyrosine kinase involved in eye differentiation. Previous studies in the eye showed that upon binding of the transmembrane protein Boss to

http://dx.doi.org/10.5772/58756

its receptor Sev, Boss becomes internalized in the *sev*-expressing cell (Cagan et al., 1992; Kramer, 1993; Kramer et al., 1991] whereas in the fat body, in response to stimulation by glucose, Boss becomes enclosed in internalized vesicles (Kohyama-Koganeya et al., 2008]. In the *Drosophila* testis, Boss is found in the germline spermatocytes, primarily in vesicles (Fig. 3G), whereas Sev localizes in the cyst cells enclosing them. Abd-B performs its function by affecting Boss internalization in the germline, as Boss is lost from internalized vesicles in *Abd-B* depleted testes [18]. Expression of activated Sev in cyst cells of Abd-B depleted testes could fully rescue the phenotype, meaning the Boss exerts its function via Sev activation. Similarly, a partial rescue of hub positioning and integrin localization was observed by expressing the *shibire* (*shi*) gene [72, 73], which is critical for the endocytic uptake of receptors from the plasma membrane [74, 75] in spermatocytes of *Abd-B* depleted testes. This further suggested that Boss functions in a dynamin-dependent way for its endocytic recycling.

In order to elucidate how the Hox transcription factor Abd-B affects Boss localization, genes directly regulated by Abd-B in the *Drosophila* testis were identified by mapping Abd-B binding sites *in vivo* using the DNA adenine methyltransferase identification (DamID) technology [76-79]. This analysis resulted in the identification of 1804 Abd-B binding regions in larval testes, which are associated with 2771 genes. To determine over-representation of GO terms, GO terms were grouped using their annotated Biological Process and subsequently the over-representation of GO term groups among the identified genes was analyzed [18]. Since Abd-B controls signaling between the germline and somatic lineage by regulating genes required for Boss receptor recycling or trafficking, further analysis focused on genes involved in trafficking processes. Two genes, one encoding the non-receptor tyrosine kinase Src oncogene at 42A (Src42A) and another one encoding the putative signal recognition binding protein Sec63, were identified as potential mediators of Boss function in the larval testis. In support of a direct regulatory interaction between *src42A* and Abd-B in the larval testis, *src42A* mRNA levels [80] were found to be significantly downregulated in spermatocytes of $AbdB^{RNAi}$*::T100* animals (with *T100-GAL4* driving expression of UAS-$AbdB^{RNAi}$ in germline spermatocytes), and likewise the activity of the protein tyrosine kinase Src42A was dramatically reduced [18]. Importantly, functional analysis revealed that *src42A* depleted testes mimic the loss of *Abd-B* function: in contrast to wild-type testes, Boss protein was not detected in vesicles, the hub was mispositioned and βPS-integrin was not properly localized in somatic cyst cells of *src42A* depleted testes. Same results were obtained for *sec63*.

4. Dlg, Scrib & Lgl: New functions in the *Drosophila* testis

The *discs large (dlg), scribble (scrib) and lethal* [2] *giant larvae (lgl)* genes were initially identified in *Drosophila* as tumor suppressor genes (TSGs) whose mutations lead to neoplastic transformation, such as imaginal disc overgrowth and brain tumors [81-84]. Mutant flies die after an extended larval life as "giant" larvae without pupariation. In these tumors the overproliferating cells lose their typical epithelial apico-basal polarity, fail to organize an epithelial monolayer and terminally differentiate [84-86]. Therefore, all three TSGs are additionally classified as "cell polarity genes" [83, 84, 87-89]. Since their initial discovery, *dlg, scrib* and *lgl* have been

recognized as having important roles also in other forms of polarity as well as in regulation of the actin cytoskeleton, cell signaling and vesicular trafficking [86, 90].

Dlg belongs to the MAGUK (membrane-associated guanylate kinases) protein family, a class of scaffolding proteins that recruit signaling molecules into localized multimolecular complexes [83, 91]. Dlg localizes at the cytoplasmic side of septate junctions between adjacent epithelial cells (the equivalent of vertebrate tight junctions), as well as in neuromuscular junctions (NMJs). It contains three PDZ domains involved in protein-protein interactions with membrane or cytoskeletal proteins, an SH3 domain and a GUK domain. Scrib is also a septate junctional protein of the LAP protein family, containing four PDZ domains and leucine-rich repeats (LRRs) [85, 87, 91, 92]. Lgl is a cytosolic protein containing two WD40 motifs, involved in protein-protein interactions [87]. Lgl can bind to non-muscle myosin II and to the cytoskeleton matrix, along the baso-lateral portion of the plasma membrane of epithelial cells to affect cell polarization [93]. All three proteins, often referred to as the Dlg-polarity module, are highly conserved in sequence among different species and growing evidence suggests that they are functionally conserved to a large degree since the vertebrate homologues can rescue the polarity defects and tumorous overgrowth of the respective *Drosophila* mutants [94-96].

4.1. Dlg, Scrib & Lgl: Multitasking proteins in common pathways in various tissues

Research over several years, defined *dlg, scrib* and *lgl* as key players in numerous tissues contents and malignancies at different time points throughout development, and revealed their multitasking role in: polarity and septate junction establishment; nervous system and brain development; organ development; cancer initiation, progression and metastasis; and mechanism of cooperation with various signaling pathways (Ras, Salvador-Warts-Hippo, Dpp, JNK, Wg, EGFR etc) [22, 97-104]. Some of their common modes of action across different tissues and organisms are analyzed below.

4.1.1. Polarity establishment in various cellular contexts

The Dlg polarity module works in cooperation with the Crumbs-(Crb, Pals1 & Patj) and the Par-(Bazooka/Par3, Par6, αPKC) polarity complexes to control polarity in several tissues. In epithelial cells, polarity is established in a finely balanced process involving cooperative and antagonistic interactions among the apical Par-and Crumbs-complexes and the basolateral Dlg-complex, which restrict the activity of each complex to its specific membrane domain [85, 86]. In neuroblast asymmetric cell division Dlg, Scrib and Lgl cooperate with the Par and Inscutable-Pins complexes whereas microtubules induce Pins & Gαi cortical polarity through Dlg and Khc-73 interactions [86, 105, 106]. In the *Drosophila* ectoderm, phosphorylation of αPKC is required for Lgl to establish the lateral domain and to prevent apical Lgl recruitment. Lgl homologues genetically interact with Par components to regulate apicobasal polarity in *Xenopus* and MDCK epithelial cells, and in partitioning cell fate determinants in *C.elegans* [85, 90, 91, 107]. Finally, the Dlg polarity module has critical functions also in *Drosophila* dorsal closure formation, in patterning anterior and posterior follicle cells, in wound healing processes, in planar cell polarity, in formation of synapses and in NMJs together with other polarity, scaffolding and receptor complexes [86, 102, 108].

http://dx.doi.org/10.5772/58756

4.1.2. Vesicle and membrane trafficking

Several pieces of evidence suggest that Dlg, Scrib and Lgl are involved in vesicle and membrane trafficking [86, 102]: i) Dlg and Strabismus (VanGogh) form a complex that allows membrane deposition during cellularization in *Drosophila* embryos [109] ii) Dlg regulates membrane proliferation of the subsynaptic reticulum (SSR) in NMJs by binding the t-SNARE protein Gtaxin [110, 111] iii) Dlg and Lgl genetically interact with Exo84 which is required for membrane addition [112] iv) the yeast Lgl homologues Sro7p and Sro77p interact directly with Exo84p and Sec9p traggicking components [113], v) mammalian Lgl binds Syntaxin-4 (t-SNARE) to direct protein trafficking [114], and vi) mammalian Scrib regulates exocytosis by binding to the β–Pix-GIT1 complex [115].

4.1.3. Gene regulation and signaling output

Recent studies associate Dlg, Scrib and Lgl with transcriptional response and signaling output since they can regulate the shuttling of critical components between junctional complexes and the nucleus. Such a shuttling mechanism has been described for the Dlg and Scrib vertebrate homologues [116, 117]. In *Drosophila* salivary glands, Lgl together with non-muscle myosin regulate in the cytoplasm access to chromatin modifiers, remodeling and transcription factors necessary for salivary gland degeneration [118]. In wild type salivary glands, chromatin remodeling factors are localized in the nucleus to bind chromatin whereas in the absence of Lgl they accumulate in the cytoplasm and the cortical nuclear zone but cannot bind to chromatin to regulate secondary gene expression [118].

Taken together, Dlg, Scrib and Lgl emerge as dynamic cytoskeletal components which affect polarity, cell structure and behavior by directing the trafficking of proteins to proper plasma membrane surfaces of the cell, and by organizing and stabilizing supramolecular adhesion and signaling complexes through their action as scaffolding adaptor molecules [83-86, 89-91, 109, 111].

4.2. Dlg, Scrib & Lgl in testis somatic cells promote cyst cell function & testis homeostasis

Septate junctions are primary candidates for cyst integrity and coordination, as apart from acting as sealing junctions in epithelia and neurons by mediating cell-cell adhesion, they act as scaffolding networks together with multiple pathways to promote organ morphogenesis [120]. Although the function of Dlg, Scrib and Lgl as TSGs has been intensively studied, their role in testis development has been largely overlooked, as mutations in their coding genes do not result in testis tumors. Moreover, the fact that testes lack an easy to study columnar epithelium, which facilitates analysis of apicobasal polarity genes, didn't favor the analysis of these genes in this stem cell system for many years. The last years a number of studies addressed the role of *scrib*, *dlg* and *lgl* scaffolding proteins in the *Drosophila* male gonad, testis architecture and homeostasis [22-24, 119, 121]. Prompted by the observation that the septate junction protein Scrib [122] is expressed in the newly formed embryonic *Drosophila* gonads [88], Scrib dynamics in the embryonic gonads was investigated [24]. During gonad formation Scrib forms a polygonal network around the germ cells and is present primarily in the somatic

gonadal cells, the so-called gonadal mesoderm, that surrounds them. Scrib synthesis in the gonadal mesoderm is cell autonomous, since analysis of agametic gonads and pseudo-gonads made of aggregated germ cells revealed that Scrib in the germ cells requires a direct contact to the gonadal mesoderm [24].

As Dlg, Scrib and Lgl act cooperatively in several tissue contexts [23, 84], their function during male gonad and testis development was analyzed in a comparable way [22, 119]. This work revealed that cell autonomous *scrib* and *dlg* expression in the gonadal mesoderm affects critically the internal structure of the gonads by establishing the intimate contacts of the germ cells to the gonadal mesoderm [24, 119]. At later stages, *dlg*, *scrib* and *lgl* expression in the hub, CySCs and SCCs (Fig.3 A-C) is indispensable for testis development and homeostasis, as depletion of these genes results in extremely small testes with reduced number of germline stem cells and impaired differentiation (Fig.3 E-H). Moreover, Dlg localization in CySCs establishes a tight connection between GSCs and CySCs, and thereby preserves the niche architecture. In late SCCs *dlg* expression is critical for their survival, growth, expansion and for maintaining the integrity of the cysts [22]. This is supported by the observation that the Eya-positive SCCs present in the wild-type testes (Fig.3I; arrowheads) are lost in *dlg* testes (Fig.3J) and die due to apoptosis [22]. Similar to *dlg*, *lgl* testes also lose Eya-positive SCCs (Fig.3L), whereas in *scrib* testes late SCCs are still present (Fig.3K; arrowheads) but the size of these Eya-positive nuclei and of overall testis size is significantly reduced [119]. In contrast to the overgrowth phenotypes observed in imaginal discs and brain hemispheres, the extensive defects in *dlg, scrib* and *lgl* mutant testes underline the importance of the somatic lineage in the establishment of a tight soma-germline adhesion and cyst integrity, which is a prerequisite for a functional male stem cell niche and proper testis differentiation [2, 23, 119].

Another striking finding was the formation of wavy and ruffled plasma membrane upon *dlg* over-expression in somatic cyst cells capping the spermatocyte cysts. Up to now, there is no mechanism describing how cyst cells in *Drosophila* testis grow enormously, elongate and ensheath the germ cells of spermatogonial and spermatocyte cysts or how spermatid differentiation and individualization is guided by the polarized head and tail SCC. From other systems we know that Dlg regulates membrane proliferation in a subset of NMJs in a dose-dependent fashion [123] and is an important player in the process of polarized membrane insertion during cellularization [109, 124-126].

Another way to interpret this result would be to consider that Dlg regulates the intensity of germ cell encapsulation through the Egfr pathway, which is the major signaling pathway active at the microenvironment of the spermatogonial cysts [50, 51]. Membrane ruffling, detected in somatic cells upon *dlg* over-expression, is highly reminiscent of the formation of lammellipodia-like structures, formed upon up-regulation of Rac1 in SCCs [53]. Rac1 is a downstream component of the Egfr pathway and acts antagonistically to Rho in order to regulate germ cell encapsulation; moreover, Rho activation perturbates TJ function in various experimental systems [129]. It has already been shown that Dlg regulates membrane proliferation in a subset of NMJs in a dose-dependent fashion [123] and is an important player in the process of polarized membrane insertion during cellularization [109,

http://dx.doi.org/10.5772/58756

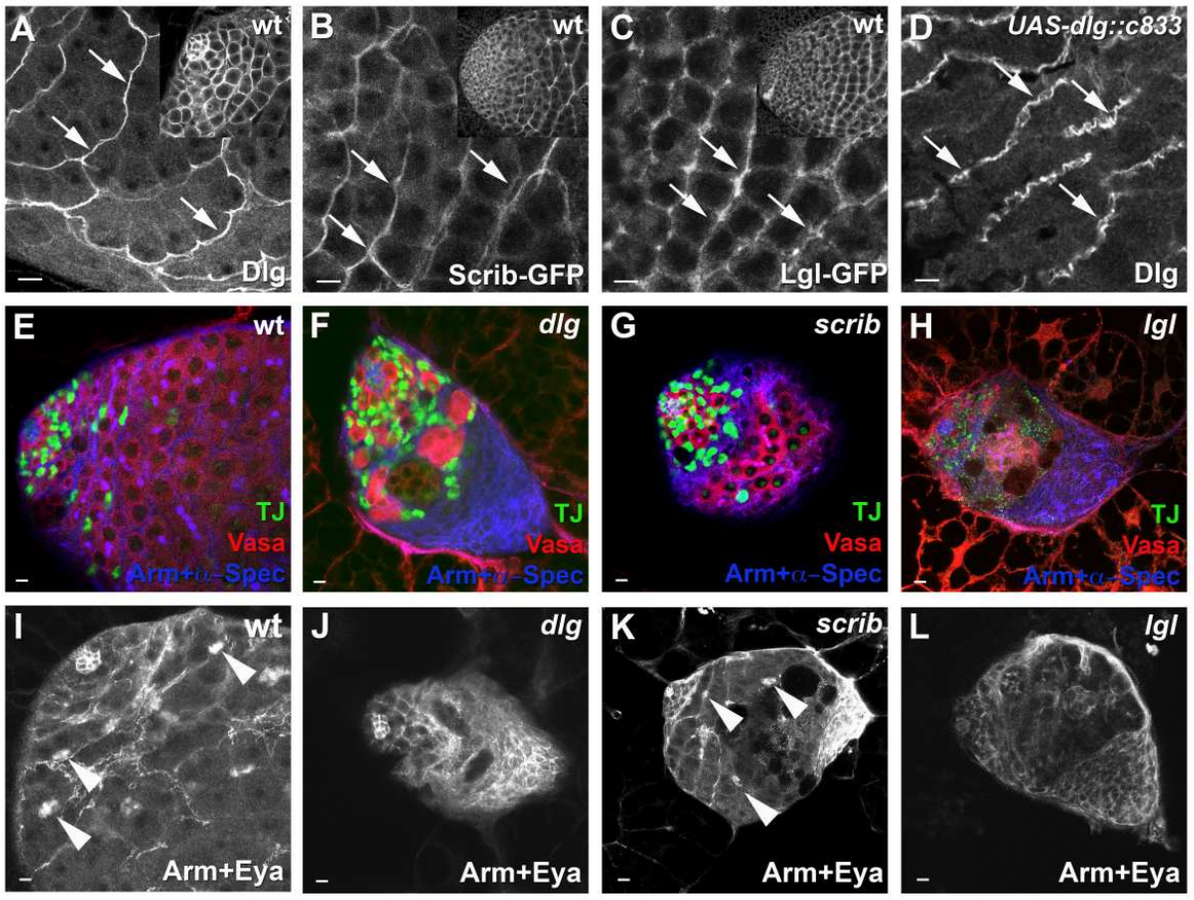

Figure 3. Dlg, Scrib and Lgl in the somatic lineage have critical functions in niche architecture, testis differentiation and homeostasis. (A-C) Dlg, Scrib and Lgl localize in somatic hub, somatic stem and cyst cells in *Drosophila* testis. (D) Dlg overexpression leads to ruffled membranes of somatic cyst cells, showing that Dlg promotes somatic cyst cell growth and membrane addition. (E-H) *dlg, scrib* and *lgl* mutant testes are extremely small, with reduced number of germline stem cells and impaired differentiation with only few spermatogonial cysts. Traffic Jam (TJ) marks the somatic stem cell and cyst cell nuclei, Vasa the germline, Armadillo (Arm) the hub and somatic stem and cyst cells, a-Spectrin the fusome growing through the interconnected spermatogonia and spermatocytes. (I-L) In *dlg* and *lgl* testes late somatic cyst cells are lost as no Eyes Absent (Eya)-positive cyst cells are observed and the tight connection between the cyst cells and the germline is lost. In *scrib* testes Eya-positive somatic cyst cells are present, however testes are small and underdeveloped. Arrows point at the somatic cyst cell membrane. Arrowheads point at Eya-positive late somatic cyst cells. Testis hub is oriented towards the left. Scale Bar: 10mm

124-126]. The fact that membrane proliferation is also involved in mechanisms such as tissue spreading and cell surface extensions, including membrane ruffles [127, 128] and combined with our results on SCCs membrane ruffling upon Dlg overexpression it can be suggested that polarized membrane insertion, mediated by Dlg, might conduct SCCs growth, expansion and spreading over the germ cells of testicular cysts.

5. Conclusions and future perspectives

Cell polarity and signaling are fundamental biological processes that impact stem cell function, cancer, cell migration, tissue morphogenesis and response to pathogenic infections. Growing

scientific evidence suggests that these processes are intimately linked. Moreover, shuttling of signaling complexes into specific intracellular regions happens via their recruitment in subcellular domains guided by polarity scaffolds. The microenvironment of the male testis cysts, built by the cyst cell-germline intimate connection, provides an ideal model system to investigate how soma-germline adhesion and cell morphological changes are coordinated with cell communication and exchange of short-range signals.

So far the main evidence for cyst cell (CySCs and SCCs) function came from the analysis of individual signal transduction pathways that establish a cross-talk between the soma and the germline. Now we know that cyst cells are crucially important for soma-germline cyst integrity, overall rigidity and for setting up a functional cyst microenvironment. To this end, it is important (a) to investigate the requirement of the somatic lineage, the cyst cells, as safeguard of germline function, and (b) to characterize the local soma-germline communication within the cysts with focus on how polarity scaffolds and signaling platforms promote this. Resolving the basic features of cyst's microenvironment and soma-germline coordination will allow the study of more complex questions in the future such as long-range signaling at the level of cyst-cyst communication. Moreover, the use of a combination of genetic, genomic and high-resolution microscopy techniques to approach these questions will enable us to adapt tools, already successfully established in other tissues and model systems (such as FRAP, FRET and organ cultures) to the *Drosophila* testis.

Acknowledgements

The author wishes to thank the *Drosophila* community for providing generously fly stocks and antibodies and apologizes to all whose work has not been sited due to space limitations.

Author details

Fani Papagiannouli*

Centre for Organismal Studies (COS), University of Heidelberg, Germany

References

[1] Fuller MT, Spradling AC. Male and female Drosophila germline stem cells: two versions of immortality. Science. 2007;316(5823):402-4.

[2] Papagiannouli F, Lohmann I. Shaping the niche: Lessons from the Drosophila testis and other model systems. Biotechnol J. 2012.

[3] Walker MR, Patel KK, Stappenbeck TS. The stem cell niche. J Pathol. 2009;217(2): 169-80.

[4] Papagiannouli F, Mechler BM. Modeling tumorigenesis in Drosophila: current advances and future perspectives. In: Cheng Y, editor. "Future Aspects of Tumor Suppressor Genes": InTech publications; 2013. p. 97-128.

[5] Lang D, Lu MM, Huang L, Engleka KA, Zhang M, Chu EY, et al. Pax3 functions at a nodal point in melanocyte stem cell differentiation. Nature. 2005;433(7028):884-7.

[6] DeFalco TJ, Verney G, Jenkins AB, McCaffery JM, Russell S, Van Doren M. Sex-specific apoptosis regulates sexual dimorphism in the Drosophila embryonic gonad. Dev Cell. 2003;5(2):205-16.

[7] Riechmann V, Rehorn KP, Reuter R, Leptin M. The genetic control of the distinction between fat body and gonadal mesoderm in Drosophila. Development. 1998;125(4): 713-23.

[8] Moore LA, Broihier HT, Van Doren M, Lehmann R. Gonadal mesoderm and fat body initially follow a common developmental path in Drosophila. Development. 1998;125(5):837-44.

[9] Boyle M, DiNardo S. Specification, migration and assembly of the somatic cells of the Drosophila gonad. Development. 1995;121(6):1815-25.

[10] Le Bras S, Van Doren M. Development of the male germline stem cell niche in Drosophila. Dev Biol. 2006;294(1):92-103.

[11] Yamashita YM, Fuller MT, Jones DL. Signaling in stem cell niches: lessons from the Drosophila germline. J Cell Sci. 2005;118(Pt 4):665-72.

[12] Wong MD, Jin Z, Xie T. Molecular mechanisms of germline stem cell regulation. Annu Rev Genet. 2005;39:173-95.

[13] Yamashita YM, Jones DL, Fuller MT. Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. Science. 2003;301(5639):1547-50.

[14] Cheng J, Turkel N, Hemati N, Fuller MT, Hunt AJ, Yamashita YM. Centrosome misorientation reduces stem cell division during ageing. Nature. 2008;456(7222): 599-604.

[15] Yamashita YM, Mahowald AP, Perlin JR, Fuller MT. Asymmetric inheritance of mother versus daughter centrosome in stem cell division. Science. 2007;315(5811): 518-21.

[16] Sheng XR, Matunis E. Live imaging of the Drosophila spermatogonial stem cell niche reveals novel mechanisms regulating germline stem cell output. Development. 2011;138(16):3367-76.

[17] Fuller MT. Spermatogenesis. Arias MBAM, editor. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 1993. 71-147 p.

[18] Papagiannouli F, Schardt L, Grajcarek J, Ha N, Lohmann I. The hox gene abd-B controls stem cell niche function in the Drosophila testis. Dev Cell. 2014;28(2):189-202.

[19] Kozopas KM, Samos CH, Nusse R. DWnt-2, a Drosophila Wnt gene required for the development of the male reproductive tract, specifies a sexually dimorphic cell fate. Genes Dev. 1998;12(8):1155-65.

[20] DeFalco T, Camara N, Le Bras S, Van Doren M. Nonautonomous sex determination controls sexually dimorphic development of the Drosophila gonad. Dev Cell. 2008;14(2):275-86.

[21] Nanda S, DeFalco TJ, Loh SH, Phochanukul N, Camara N, Van Doren M, et al. Sox100B, a Drosophila group E Sox-domain gene, is required for somatic testis differentiation. Sex Dev. 2009;3(1):26-37.

[22] Papagiannouli F, Mechler BM. discs large regulates somatic cyst cell survival and expansion in Drosophila testis. Cell Res. 2009;19(10):1139-49.

[23] Papagiannouli F, Mechler BM. discs large in the Drosophila testis: An old player on a new task. Fly (Austin). 2010;4(4).

[24] Marhold J, Papagiannouli F, Li M, Patel A, Mechler BM. Requirements for scribble expression in newly formed gonads of Drosophila embryos. Gene Expr Patterns. 2003;3(2):143-6.

[25] Zoller R, Schulz C. The Drosophila cyst stem cell lineage: Partners behind the scenes? Spermatogenesis. 2012;2(3):145-57.

[26] Lindsley DL, Tokuyasu KT. Spermatogenesis. In: Ashburner M, Wright TRF, editors. Genetics and Biology of Drosophila. 2D. 2nd ed. New York: Academic Press; 1980. p. 225-94.

[27] Tokuyasu KT, Peacock WJ, Hardy RW. Dynamics of spermiogenesis in Drosophila melanogaster. II. Coiling process. Z Zellforsch Mikrosk Anat. 1972;127(4):492-525.

[28] Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978;4(1-2):7-25.

[29] Tulina N, Matunis E. Control of stem cell self-renewal in Drosophila spermatogenesis by JAK-STAT signaling. Science. 2001;294(5551):2546-9.

[30] Kiger AA, Jones DL, Schulz C, Rogers MB, Fuller MT. Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. Science. 2001;294(5551):2542-5.

[31] Zeng X, Singh SR, Hou D, Hou SX. Tumor suppressors Sav/Scrib and oncogene Ras regulate stem-cell transformation in adult Drosophila malpighian tubules. J Cell Physiol. 2010;224(3):766-74.

[32] Leatherman JL, Dinardo S. Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in Drosophila testes. Nat Cell Biol. 2010;12(8):806-11.

[33] Shields AR, Spence AC, Yamashita YM, Davies EL, Fuller MT. The actin-binding protein profilin is required for germline stem cell maintenance and germ cell enclosure by somatic cyst cells. Development. 2014;141(1):73-82.

[34] Flaherty MS, Salis P, Evans CJ, Ekas LA, Marouf A, Zavadil J, et al. chinmo is a functional effector of the JAK/STAT pathway that regulates eye development, tumor formation, and stem cell self-renewal in Drosophila. Dev Cell. 2010;18(4):556-68.

[35] Issigonis M, Matunis E. The Drosophila BCL6 homolog Ken and Barbie promotes somatic stem cell self-renewal in the testis niche. Dev Biol. 2012;368(2):181-92.

[36] Issigonis M, Tulina N, de Cuevas M, Brawley C, Sandler L, Matunis E. JAK-STAT signal inhibition regulates competition in the Drosophila testis stem cell niche. Science. 2009;326(5949):153-6.

[37] Michel M, Kupinski AP, Raabe I, Bokel C. Hh signalling is essential for somatic stem cell maintenance in the Drosophila testis niche. Development. 2012;139(15):2663-9.

[38] Amoyel M, Sanny J, Burel M, Bach EA. Hedgehog is required for CySC self-renewal but does not contribute to the GSC niche in the Drosophila testis. Development. 2013;140(1):56-65.

[39] Zhang Z, Lv X, Jiang J, Zhang L, Zhao Y. Dual roles of Hh signaling in the regulation of somatic stem cell self-renewal and germline stem cell maintenance in Drosophila testis. Cell Res. 2013;23(4):573-6.

[40] Zhang Z, Pan C, Zhao Y. Hedgehog in the Drosophila testis niche: what does it do there? Protein & cell. 2013.

[41] Shivdasani AA, Ingham PW. Regulation of stem cell maintenance and transit amplifying cell proliferation by tgf-beta signaling in Drosophila spermatogenesis. Curr Biol. 2003;13(23):2065-72.

[42] Kawase E, Wong MD, Ding BC, Xie T. Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing bam transcription in the Drosophila testis. Development. 2004;131(6):1365-75.

[43] Schulz C, Kiger AA, Tazuke SI, Yamashita YM, Pantalena-Filho LC, Jones DL, et al. A misexpression screen reveals effects of bag-of-marbles and TGF beta class signaling on the Drosophila male germ-line stem cell lineage. Genetics. 2004;167(2):707-23.

[44] Zheng Q, Wang Y, Vargas E, DiNardo S. magu is required for germline stem cell self-renewal through BMP signaling in the Drosophila testis. Dev Biol. 2011;357(1):202-10.

[45] Chang YJ, Pi H, Hsieh CC, Fuller MT. Smurf-mediated differential proteolysis generates dynamic BMP signaling in germline stem cells during Drosophila testis development. Dev Biol. 2013;383(1):106-20.

[46] Lim JG, Fuller MT. Somatic cell lineage is required for differentiation and not maintenance of germline stem cells in Drosophila testes. Proc Natl Acad Sci U S A. 2012;109(45):18477-81.

[47] Pancratov R, Peng F, Smibert P, Yang S, Jr., Olson ER, Guha-Gilford C, et al. The miR-310/13 cluster antagonizes beta-catenin function in the regulation of germ and somatic cell differentiation in the Drosophila testis. Development. 2013;140(14): 2904-16.

[48] Gonczy P, Matunis E, DiNardo S. bag-of-marbles and benign gonial cell neoplasm act in the germline to restrict proliferation during Drosophila spermatogenesis. Development. 1997;124(21):4361-71.

[49] Insco ML, Bailey AS, Kim J, Olivares GH, Wapinski OL, Tam CH, et al. A self-limiting switch based on translational control regulates the transition from proliferation to differentiation in an adult stem cell lineage. Cell Stem Cell. 2012;11(5):689-700.

[50] Kiger AA, White-Cooper H, Fuller MT. Somatic support cells restrict germline stem cell self-renewal and promote differentiation. Nature. 2000;407(6805):750-4.

[51] Tran J, Brenner TJ, DiNardo S. Somatic control over the germline stem cell lineage during Drosophila spermatogenesis. Nature. 2000;407(6805):754-7.

[52] Schulz C, Wood CG, Jones DL, Tazuke SI, Fuller MT. Signaling from germ cells mediated by the rhomboid homolog stet organizes encapsulation by somatic support cells. Development. 2002;129(19):4523-34.

[53] Sarkar A, Parikh N, Hearn SA, Fuller MT, Tazuke SI, Schulz C. Antagonistic roles of Rac and Rho in organizing the germ cell microenvironment. Curr Biol. 2007;17(14): 1253-8.

[54] Tazuke SI, Schulz C, Gilboa L, Fogarty M, Mahowald AP, Guichet A, et al. A germline-specific gap junction protein required for survival of differentiating early germ cells. Development. 2002;129(10):2529-39.

[55] Gilboa L, Forbes A, Tazuke SI, Fuller MT, Lehmann R. Germ line stem cell differentiation in Drosophila requires gap junctions and proceeds via an intermediate state. Development. 2003;130(26):6625-34.

[56] Bohrmann J, Zimmermann J. Gap junctions in the ovary of Drosophila melanogaster: localization of innexins 1, 2, 3 and 4 and evidence for intercellular communication via innexin-2 containing channels. BMC Dev Biol. 2008;8:111.

[57] Dinardo S, Okegbe T, Wingert L, Freilich S, Terry N. lines and bowl affect the specification of cyst stem cells and niche cells in the Drosophila testis. Development. 2011;138(9):1687-96.

[58] Redline RW, Williams AJ, Patterson P, Collins T. Human HOX4E: a gene strongly expressed in the adult male and female urogenital tracts. Genomics. 1992;13(2):425-30.

[59] DeFalco T, Le Bras S, Van Doren M. Abdominal-B is essential for proper sexually dimorphic development of the Drosophila gonad. Mech Dev. 2004;121(11):1323-33.

[60] Kitadate Y, Shigenobu S, Arita K, Kobayashi S. Boss/Sev signaling from germline to soma restricts germline-stem-cell-niche formation in the anterior region of Drosophila male gonads. Dev Cell. 2007;13(1):151-9.

[61] Kitadate Y, Kobayashi S. Notch and Egfr signaling act antagonistically to regulate germ-line stem cell niche formation in Drosophila male embryonic gonads. Proc Natl Acad Sci U S A. 2010;107(32):14241-6.

[62] Hatini V, Green RB, Lengyel JA, Bray SJ, Dinardo S. The Drumstick/Lines/Bowl regulatory pathway links antagonistic Hedgehog and Wingless signaling inputs to epidermal cell differentiation. Genes Dev. 2005;19(6):709-18.

[63] Sheng XR, Posenau T, Gumulak-Smith JJ, Matunis E, Van Doren M, Wawersik M. Jak-STAT regulation of male germline stem cell establishment during Drosophila embryogenesis. Dev Biol. 2009;334(2):335-44.

[64] Casper A, Van Doren M. The control of sexual identity in the Drosophila germline. Development. 2006;133(15):2783-91.

[65] Tanentzapf G, Devenport D, Godt D, Brown NH. Integrin-dependent anchoring of a stem-cell niche. Nat Cell Biol. 2007;9(12):1413-8.

[66] Cram EJ, Schwarzbauer JE. The talin wags the dog: new insights into integrin activation. Trends Cell Biol. 2004;14(2):55-7.

[67] Tanentzapf G, Martin-Bermudo MD, Hicks MS, Brown NH. Multiple factors contribute to integrin-talin interactions in vivo. J Cell Sci. 2006;119(Pt 8):1632-44.

[68] Lee S, Zhou L, Kim J, Kalbfleisch S, Schock F. Lasp anchors the Drosophila male stem cell niche and mediates spermatid individualization. Mech Dev. 2008;125(9-10): 768-76.

[69] Suyama R, Jenny A, Curado S, Pellis-van Berkel W, Ephrussi A. The actin-binding protein Lasp promotes Oskar accumulation at the posterior pole of the Drosophila embryo. Development. 2009;136(1):95-105.

[70] Traenka J, Hauck CR, Lewandrowski U, Sickmann A, Gambaryan S, Thalheimer P, et al. Integrin-dependent translocation of LASP-1 to the cytoskeleton of activated platelets correlates with LASP-1 phosphorylation at tyrosine 171 by Src-kinase. Thromb Haemost. 2009;102(3):520-8.

[71] Boyle M, Wong C, Rocha M, Jones DL. Decline in self-renewal factors contributes to aging of the stem cell niche in the Drosophila testis. Cell Stem Cell. 2007;1(4):470-8.

[72] Kitamoto T. Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons. J Neurobiol. 2001;47(2):81-92.

[73] Rogat AD, Miller KG. A role for myosin VI in actin dynamics at sites of membrane remodeling during Drosophila spermatogenesis. J Cell Sci. 2002;115(Pt 24):4855-65.

[74] Urrutia R, Henley JR, Cook T, McNiven MA. The dynamins: redundant or distinct functions for an expanding family of related GTPases? Proc Natl Acad Sci U S A. 1997;94(2):377-84.

[75] McPherson PS, Kay BK, Hussain NK. Signaling on the endocytic pathway. Traffic. 2001;2(6):375-84.

[76] van Steensel B, Henikoff S. Identification of in vivo DNA targets of chromatin proteins using tethered dam methyltransferase. Nat Biotechnol. 2000;18(4):424-8.

[77] van Steensel B, Delrow J, Henikoff S. Chromatin profiling using targeted DNA adenine methyltransferase. Nat Genet. 2001;27(3):304-8.

[78] Vogel MJ, Peric-Hupkes D, van Steensel B. Detection of in vivo protein-DNA interactions using DamID in mammalian cells. Nat Protoc. 2007;2(6):1467-78.

[79] Choksi SP, Southall TD, Bossing T, Edoff K, de Wit E, Fischer BE, et al. Prospero acts as a binary switch between self-renewal and differentiation in Drosophila neural stem cells. Dev Cell. 2006;11(6):775-89.

[80] Toledano H, D'Alterio C, Loza-Coll M, Jones DL. Dual fluorescence detection of protein and RNA in Drosophila tissues. Nat Protoc. 2012;7(10):1808-17.

[81] Gateff E SH. Developmental capacities of benign and malignant neoplasms of Drosophila. Wilhelm Roux' Archiv. 1974;176(23-65).

[82] Mechler BM, McGinnis W, Gehring WJ. Molecular cloning of lethal(2)giant larvae, a recessive oncogene of Drosophila melanogaster. EMBO J. 1985;4(6):1551-7.

[83] Woods DF, Hough C, Peel D, Callaini G, Bryant PJ. Dlg protein is required for junction structure, cell polarity, and proliferation control in Drosophila epithelia. J Cell Biol. 1996;134(6):1469-82.

[84] Bilder D, Li M, Perrimon N. Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science. 2000;289(5476):113-6.

[85] Humbert P, Russell S, Richardson H. Dlg, Scribble and Lgl in cell polarity, cell proliferation and cancer. Bioessays. 2003;25(6):542-53.

[86] Elsum I, Yates L, Humbert PO, Richardson HE. The Scribble-Dlg-Lgl polarity module in development and cancer: from flies to man. Essays in biochemistry. 2012;53:141-68.

[87] Wodarz A. Tumor suppressors: linking cell polarity and growth control. Curr Biol. 2000;10(17):R624-6.

[88] Li M, Marhold J, Gatos A, Torok I, Mechler BM. Differential expression of two scribble isoforms during Drosophila embryogenesis. Mech Dev. 2001;108(1-2):185-90.

[89] Goode S, Perrimon N. Inhibition of patterned cell shape change and cell invasion by Discs large during Drosophila oogenesis. Genes Dev. 1997;11(19):2532-44.

[90] Harris BZ, Lim WA. Mechanism and role of PDZ domains in signaling complex assembly. J Cell Sci. 2001;114(Pt 18):3219-31.

[91] Bilder D. PDZ proteins and polarity: functions from the fly. Trends Genet. 2001;17(9): 511-9.

[92] Mathew D, Gramates LS, Packard M, Thomas U, Bilder D, Perrimon N, et al. Recruitment of scribble to the synaptic scaffolding complex requires GUK-holder, a novel DLG binding protein. Curr Biol. 2002;12(7):531-9.

[93] Strand D, Jakobs R, Merdes G, Neumann B, Kalmes A, Heid HW, et al. The Drosophila lethal(2)giant larvae tumor suppressor protein forms homo-oligomers and is associated with nonmuscle myosin II heavy chain. J Cell Biol. 1994;127(5):1361-73.

[94] Thomas U, Phannavong B, Muller B, Garner CC, Gundelfinger ED. Functional expression of rat synapse-associated proteins SAP97 and SAP102 in Drosophila dlg-1 mutants: effects on tumor suppression and synaptic bouton structure. Mech Dev. 1997;62(2):161-74.

[95] Grifoni D, Garoia F, Schimanski CC, Schmitz G, Laurenti E, Galle PR, et al. The human protein Hugl-1 substitutes for Drosophila lethal giant larvae tumour suppressor function in vivo. Oncogene. 2004;23(53):8688-94.

[96] Dow LE, Brumby AM, Muratore R, Coombe ML, Sedelies KA, Trapani JA, et al. hScrib is a functional homologue of the Drosophila tumour suppressor Scribble. Oncogene. 2003;22(58):9225-30.

[97] Moreau MM, Piguel N, Papouin T, Koehl M, Durand CM, Rubio ME, et al. The planar polarity protein Scribble1 is essential for neuronal plasticity and brain function. J Neurosci. 2010;30(29):9738-52.

[98] Brumby AM, Goulding KR, Schlosser T, Loi S, Galea R, Khoo P, et al. Identification of Novel Ras-Cooperating Oncogenes in Drosophila melanogaster: A RhoGEF/Rho-Family/JNK Pathway Is a Central Driver of Tumorigenesis. Genetics. 2011;188(1): 105-25.

[99] Brumby AM, Richardson HE. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. EMBO J. 2003;22(21):5769-79.

[100] Brumby AM, Richardson HE. Using Drosophila melanogaster to map human cancer pathways. Nat Rev Cancer. 2005;5(8):626-39.

[101] Cordero JB, Macagno JP, Stefanatos RK, Strathdee KE, Cagan RL, Vidal M. Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter. Dev Cell. 2010;18(6):999-1011.

[102] Humbert PO, Grzeschik NA, Brumby AM, Galea R, Elsum I, Richardson HE. Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module. Oncogene. 2008;27(55): 6888-907.

[103] Pagliarini RA, Xu T. A genetic screen in Drosophila for metastatic behavior. Science. 2003;302(5648):1227-31.

[104] Grzeschik NA, Parsons LM, Allott ML, Harvey KF, Richardson HE. Lgl, aPKC, and Crumbs regulate the Salvador/Warts/Hippo pathway through two distinct mechanisms. Curr Biol. 2010;20(7):573-81.

[105] Wirtz-Peitz F, Knoblich JA. Lethal giant larvae take on a life of their own. Trends Cell Biol. 2006;16(5):234-41.

[106] Albertson R, Doe CQ. Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry. Nat Cell Biol. 2003;5(2):166-70.

[107] Humbert PO, Dow LE, Russell SM. The Scribble and Par complexes in polarity and migration: friends or foes? Trends Cell Biol. 2006;16(12):622-30.

[108] Thomas U, Ebitsch S, Gorczyca M, Koh YH, Hough CD, Woods D, et al. Synaptic targeting and localization of discs-large is a stepwise process controlled by different domains of the protein. Curr Biol. 2000;10(18):1108-17.

[109] Lee OK, Frese KK, James JS, Chadda D, Chen ZH, Javier RT, et al. Discs-Large and Strabismus are functionally linked to plasma membrane formation. Nat Cell Biol. 2003;5(11):987-93.

[110] Gorczyca D, Ashley J, Speese S, Gherbesi N, Thomas U, Gundelfinger E, et al. Postsynaptic membrane addition depends on the Discs-Large-interacting t-SNARE Gtaxin. J Neurosci. 2007;27(5):1033-44.

[111] Chen K, Featherstone DE. Discs-large (DLG) is clustered by presynaptic innervation and regulates postsynaptic glutamate receptor subunit composition in Drosophila. BMC Biol. 2005;3:1.

[112] Blankenship JT, Fuller MT, Zallen JA. The Drosophila homolog of the Exo84 exocyst subunit promotes apical epithelial identity. J Cell Sci. 2007;120(Pt 17):3099-110.

[113] Zhang X, Wang P, Gangar A, Zhang J, Brennwald P, TerBush D, et al. Lethal giant larvae proteins interact with the exocyst complex and are involved in polarized exocytosis. J Cell Biol. 2005;170(2):273-83.

[114] Musch A, Cohen D, Yeaman C, Nelson WJ, Rodriguez-Boulan E, Brennwald PJ. Mammalian homolog of Drosophila tumor suppressor lethal (2) giant larvae interacts

with basolateral exocytic machinery in Madin-Darby canine kidney cells. Mol Biol Cell. 2002;13(1):158-68.

[115] Audebert S, Navarro C, Nourry C, Chasserot-Golaz S, Lecine P, Bellaiche Y, et al. Mammalian Scribble forms a tight complex with the betaPIX exchange factor. Curr Biol. 2004;14(11):987-95.

[116] Petit MM, Meulemans SM, Alen P, Ayoubi TA, Jansen E, Van de Ven WJ. The tumor suppressor Scrib interacts with the zyxin-related protein LPP, which shuttles between cell adhesion sites and the nucleus. BMC Cell Biol. 2005;6(1):1.

[117] Carr HS, Cai C, Keinanen K, Frost JA. Interaction of the RhoA exchange factor Net1 with discs large homolog 1 protects it from proteasome-mediated degradation and potentiates Net1 activity. J Biol Chem. 2009;284(36):24269-80.

[118] Farkas R, Kucharova-Mahmood S, Mentelova L, Juda P, Raska I, Mechler B. Cytoskeletal proteins regulate chromatic access of BR-C transcription factor and Rpd3-Sin3A histone deacetylase complex in Drosophila salivary glands. Nucleus. 2011:(in print).

[119] Papagiannouli F. The internal structure of embryonic gonads and testis development in Drosophila melanogaster requires scrib, lgl and dlg activity in the soma. Int J Dev Biol. 2013;57(1):25-34.

[120] Yi P, Johnson AN, Han Z, Wu J, Olson EN. Heterotrimeric G proteins regulate a noncanonical function of septate junction proteins to maintain cardiac integrity in Drosophila. Dev Cell. 2008;15(5):704-13.

[121] Papagiannouli F, Mechler BM. Refining the role of Lgl, Dlg and Scrib in tumor suppression and beyond: Learning from the old time classics. In: Cheng Y, editor. "Tumor Suppressor Genes": InTech publications; 2012. p. 181-220.

[122] Bilder D, Perrimon N. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature. 2000;403(6770):676-80.

[123] Budnik V, Koh YH, Guan B, Hartmann B, Hough C, Woods D, et al. Regulation of synapse structure and function by the Drosophila tumor suppressor gene dlg. Neuron. 1996;17(4):627-40.

[124] Lecuit T, Wieschaus E. Polarized insertion of new membrane from a cytoplasmic reservoir during cleavage of the Drosophila embryo. J Cell Biol. 2000;150(4):849-60.

[125] Dudu V, Pantazis P, Gonzalez-Gaitan M. Membrane traffic during embryonic development: epithelial formation, cell fate decisions and differentiation. Curr Opin Cell Biol. 2004;16(4):407-14.

[126] Strickland LI, Burgess DR. Pathways for membrane trafficking during cytokinesis. Trends Cell Biol. 2004;14(3):115-8.

[127] Lecuit T, Pilot F. Developmental control of cell morphogenesis: a focus on membrane growth. Nat Cell Biol. 2003;5(2):103-8.

[128] Albertson R, Riggs B, Sullivan W. Membrane traffic: a driving force in cytokinesis. Trends Cell Biol. 2005;15(2):92-101.

[129] Fischer A, Stuckas H, Gluth M, Russell TD, Rudolph MC, Beeman NE, et al. Impaired tight junction sealing and precocious involution in mammary glands of PKN1 transgenic mice. J Cell Sci. 2007;120(Pt 13):2272-83.

IntechOpen