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► **To cite this version:**

Éric Bailly, Alexandra Walton, Jean-Paul Borg. The planar cell polarity Vangl2 protein: From genetics to cellular and molecular functions. *Seminars in Cell and Developmental Biology*, inPress, 10.1016/j.semcdb.2017.10.030 . hal-01785416

**HAL Id: hal-01785416**

**<https://hal.science/hal-01785416>**

Submitted on 15 May 2018

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**Title :**

The Planar Cell Polarity Vangl2 protein: from genetics to cellular and molecular functions.

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## **Abstract**

Planar cell polarity (PCP) refers to the capacity of a tissue, typically, but not exclusively, an epithelium, to transmit directional information across the tissue plane such that its cellular constituents can differentiate, divide or move in a coordinated manner and along a common axis, generally orthogonal to the apical-basal axis. PCP relies on a core module of highly conserved proteins originally identified in *Drosophila* which can act intra- and extracellularly. In this review, we focus on the vertebrate ortholog of one of these core PCP components, namely the Vangl2 protein. After a brief historical perspective, we discuss novel cellular settings for which a cellular Vangl2 requirement has been recently documented, with a particular emphasis on adult tissues that rely on Vangl2 for the maintenance of their regenerative capacity or their physiological functions. Finally we compile the most recent data about Vangl2 interacting proteins.

### *Historical perspective of Vangl2 functions in PCP*

Vangl2 is a vertebrate counterpart of the *Drosophila melanogaster* Vang Gogh (Vang)/Strabismus (Stbm) protein, discovered nearly two decades ago by virtue of its central role in planar cell polarity (PCP) [1,2]. Pioneering genetic studies conducted in this organism identified a total of six so-called core PCP components, which in addition to Vang/Stbm included two seven-pass transmembrane proteins, Frizzled (Fz) and Flamingo (Fmi)/Starry Night (Stan), and three cytoplasmic factors, Dishevelled (Dsh), Prickle (Pk) and Diego (Dgo) [3]. This set of core PCP proteins appears remarkably well conserved as orthologs can be easily recognized throughout the animal kingdom with very few exceptions [4,5].

Vertebrates possess a second Vang/Stbm ortholog, Vangl1, that is not found in *Drosophila* or *C. elegans*. Vangl1 and Vangl2 are closely related and exhibit a large number of common interactors. The present review mainly focuses on Vangl2 as genetic studies support the view that the two paralogs have to a large extent overlapping functions notably in the context of mouse development [6,7].

Vangl2 is a membrane-embedded protein characterized by the presence of four internal and contiguous transmembrane domains in the amino-terminal half (Figure 1a). The protein exhibits no canonical signal peptide and its amino- and carboxy-terminal regions are predicted to be exposed to the cytosol (Figure 1b). This topology is supported by immunofluorescence data obtained with intact, i.e. non-permeabilized cells, showing the non-accessibility of an epitope tag introduced into the N- or C-terminal regions of Vangl1 whereas the same tag is readily accessible to antibodies when inserted in the predicted extracellular loops located between the first and second and between the third and fourth transmembrane domains of Vangl1 [8]. Besides the presence of two Ser/Thr phosphorylation clusters in the cytoplasmic N-terminal region of Vangl2 [9], the functional contribution of this region remains poorly characterized. This contrasts with the long intracellular C-terminal region which harbors interaction domains for multiple protein partners, including the two PCP components Dvl and Pk [10,11]. A *trans* Golgi network (TGN) export signal and a conserved PDZ binding motif (PBM) also map to this region [2,12]. It is also in this region that two missense mouse mutations causing a *looptail* phenotype have been mapped [10].

A salient feature of the core PCP module is the capacity of its components to segregate in a stereotyped and highly asymmetric manner, first along the apical-basal polarity axis and second, but most importantly, in a specified direction of the epithelial sheet [13]. This is best illustrated in the case of the developing *Drosophila* wing epithelium, an experimental setting that has tremendously contributed to our understanding of planar polarity organization and PCP-mediated regulatory mechanisms [14]. The power of this model stems from the fact that

morphological manifestations of planar polarity can be directly read out through the stereotyped positioning and orientation of single actin-rich cell appendages, the trichomes that each cell of the wing epithelium assemble at its distal edge (Figure 2a). Trichome assembly along the proximal-distal axis is preceded by a dramatic cellular redistribution of the core PCP proteins from a uniform apicolateral to a highly asymmetric localization along the same axis. During this dynamic process Fz, Dsh and Dgo accumulate at the distal side, while Vang/Stbm and Pk concentrate on the opposite, i.e. proximal region (Figure 2a). This cellular asymmetry is thought to result from an intricate series of antagonistic and stimulatory interactions between the two complexes. The reciprocal inhibitory interactions that operate intracellularly between Fz-Dsh-Dgo and Vang-Pk are intercellularly counterbalanced by stabilizing interactions occurring between the Fmi-Fz complex of one cell and the Fmi-Vang complex of its immediately adjacent neighbor (Figure 2b). Such intercellular interactions serve at least two functions: the bridging of neighboring cells and the propagation of PCP signal from cell to cell [15]. These spatially integrated feedback-loops also account for both the interdependent localization property and the extreme dosage-sensitivity of the whole core PCP module, and explains in particular why loss or overexpression of any of the core components randomize the localization and polarity of the other core proteins. They also provide a molecular basis for how the transmembrane core PCP actors can function both in a non-cell autonomous and cell autonomous manner. It is worth noting here that similar asymmetric localization patterns of core PCP components have been described in planar polarized vertebrate epithelia, from mouse tissues such as cochlear inner ear [16,17], epidermis [11], limb [9] or trachea [19], to *Xenopus* larval skin [20].

The mechanisms governing the asymmetric distribution of the core PCP factors are progressively being unraveled. Yet a pending question remains regarding the symmetry-breaking cues that orient planar polarity in a particular direction [21]. Earlier studies hypothesized that the global PCP module comprising the protocadherins Fat (Ft) and Dachshous (Ds) and the Golgi-associated Four-jointed (Fj) kinase could play an instructive role in providing the directionality to the core PCP module. This scenario however was ruled out after a genetic analysis performed in *Drosophila* unambiguously demonstrated that the Ft-Ds module acts in parallel to rather than upstream of the core PCP pathway [22]. Other obvious candidates for external cues directing PCP orientation are the secreted Wnt ligands as PCP and the Wnt-Fz canonical pathways share at least two components: the Fz receptor itself which acts as a Wnt receptor in the canonical Wnt cascade and its direct partner Dsh. Data obtained in the *Drosophila* wing epithelium [23] and the mouse limb bud [9] lend strong support to this hypothesis [24,25].

Several vertebrate models have been exploited to study PCP functions, including mouse, *Xenopus* and zebrafish embryos. Remarkably in all these animal models, loss of Vangl2 function is invariably associated with the occurrence of neural tube defects (NTDs), the most severe form of which is the craniorachischisis phenotype with a complete opening of the neural tube from the midbrain-hindbrain boundary up to the tail tip [26]. NTDs result from disrupted convergent-extension (CE) movements that normally take place at the gastrulation and neurulation stage of embryogenesis. Early studies of Vangl2 in mammals have benefited from a spontaneous *Looptail* (*Lp*) mutant mouse line isolated nearly seventy years ago by Strong and Hollander [27], long before the mutation could be mapped to the *Vangl2* gene [28]. The *Lp* encoded protein harbors a S464N substitution that confers a semi-dominant looped tail phenotype to heterozygous mice while *Lp/Lp* homozygous embryos die *in utero* with a craniorachischisis phenotype [27]. The *Lp* allele has been used to document several genetic interactions of Vangl2, in particular with the apicobasal polarity protein Scribble [29,30]. Using the planar polarized stereociliary bundles of hair cells in the mouse inner ear as a readout, Montcouquiol et al. could document extensive orientation anomalies, i.e. PCP defects, in the cochlea of *Lp* mouse mutants [26]. Consistent with the *Lp* phenotype in the mouse model, familial or sporadic cases of human NTDs have been reported in the literature for individuals harboring spontaneous heterozygous missense mutations in the *VANGL2* locus [31,32].

#### *New PCP-dependent cellular functions of Vangl2*

The recent years have seen the emergence of novel mammalian models addressing Vangl2 functions beyond conventional PCP functions (table 1). The mouse developing tooth, for instance, has been shown to require Vangl2 at an early step of its morphogenetic program, when a signaling center has to emerge and organize before instilling its differentiation program to the whole dental epithelium [33]. Another model is provided by the mouse tongue [34]. The dorsal epithelium of this organ is covered by several thousands of papillae whose morphological asymmetry present a degree of local order high enough such that a stereotyped macroscopic pattern of polarization can be deduced, thereby allowing PCP defects to be quantitatively assessed. Using conditional knockout constructs of *Vangl1* and *Vangl2* in the tongue epithelium, Wang et al. observed strong papillae orientation defects in the tongue of *Vangl1*, *Vangl2* double mutant mice [34], a phenotype that they found more penetrant than in the context of a *Fz3*, *Fz6* double mutation [35]. Whether this new role of Vangl1 and Vangl2 also requires their spatial segregation along the plane of the mouse lingual epithelium has not been addressed yet and will certainly deserve further immunolocalization studies.

Other mouse adult organs have caught the attention of PCP investigators, with the rationale that Vangl2 might contribute repair and homeostatic functions after birth (Table 1). Consistent with this idea, organs such as the brain, lung or kidney have been found to express substantial levels of Vangl2 [36]. In the case of adult kidney, induction of Vangl2 expression and of other PCP components has been observed in response to glomerular injury [37]. In this experimental setting, Rocques et al. could provide evidence that podocyte-specific ablation of Vangl2 exacerbates glomerular lesions [37], thereby arguing in favor of a protective role of Vangl2 in this context. Similar conclusions have been reached with the lung [38]. As for the kidney [39], branching morphogenesis of the lung takes place late in the development and critically depends on Vangl2 [40]. Recent findings indicate that the lungs of adult mice with slightly compromised Vangl2 function due to a heterozygous *Vangl2<sup>Lp/+</sup>* genetic background display larger alveolar spaces than wild type lungs, defects that persist as the animals age and are associated with impaired lung functions. Although the possibility that the altered lung function seen in *Vangl2<sup>Lp/+</sup>* adult mice stems from earlier embryonic defects has not been formally excluded, the authors favor the hypothesis that these defects reflect a disrupted regenerative capacity of the *Vangl2<sup>Lp/+</sup>* lung tissue [38].

Using the mouse cornea as another model to investigate PCP functions, it has been reported that Vangl2 is highly expressed in the adult tissue [41]. Furthermore, targeted *Vangl2* inactivation strongly impairs *in vitro* directional migration and alignment properties of corneal epithelial cells [41]. These results have been confirmed by *in vivo* data demonstrating a dramatic decrease of the characteristic centripetal migration of corneal cells from the periphery to the center of the cornea in the cornea of *Vangl2* KO mice. Because cornea is constantly replenished by cells coming from the limbus periphery, the migratory defects caused by the absence of Vangl2 is likely to have adverse repercussions on the regenerative capacity of this tissue. Evidence for a role of Vangl2 in adult tissues has also been obtained in studies of the male and female reproductive systems. In rat testes, Vangl2 expression in Sertoli and germ cells appears to play a critical role in the polarity, maturation and transport of spermatids during spermatogenesis [42] while periodic expression of Vangl2 in the mouse uterus has been functionally linked to proper crypt formation and blastocyst implantation and development [43]. The contribution of Vangl2 to the planar polarized ciliary beating in mono- and multi-ciliated cells is now well established [44]. While first demonstrated in embryonic contexts, this Vangl2 function has been extended to postnatal events in the brain [45] as well as in the trachea [19].

*Vangl2 trafficking: a long path to the surface*

Vangl2, like its fly counterpart Vang/Stbm, localizes to the plasma membrane in planar polarized epithelial tissues [9,16,18]. Our knowledge of the regulatory factors responsible for the trafficking of Vangl2 from its site of biosynthesis, i.e. the endoplasmic reticulum (ER) to the place where PCP signaling is supposed to occur, i.e. the cell surface, is slowly progressing. Two genetic studies [46,47] focusing on mouse lines with PCP-related phenotypes have independently isolated mutant alleles of *Sec24b*, a gene that encodes a component of the coat protein complex II (COPII) involved in the ER-to-Golgi transport [48]. The finding that *Sec24b* genetically interacts with the dominant negative *Vangl2 looptail* mutant provided a first hint that the two genes might function in the same pathway. A role for Sec24b in the vesicular trafficking of Vangl2 has been demonstrated by elegant biochemical experiments showing that the loading of Vangl2 cargos onto vesicles could be recapitulated in an *in vitro* reaction assay containing recombinant Sec24b [46]. As expected, targeting of Vangl2 to the cell surface is impaired upon genetic inactivation of *Sec24b* [46,47,49].

In a search for small GTP-binding proteins that might regulate further downstream events of Vangl2 trafficking, Guo et al. [12] could ascribe an important role to the small GTPase Arfrp1 and the clathrin adaptor complex (AP-1) in Vangl2 export from the Trans-Golgi Network (TGN). Downregulation of either protein was found to induce a similar phenotype characterized by a massive accumulation of Vangl2 in a juxtannuclear compartment that colocalized with the TGN marker Golgin 97. This work has also provided evidence for the existence of a basolateral sorting tyrosine-based motif (YYXXF) in the C-terminal cytosolic domain of Vangl2 that is absolutely required for the interaction of Vangl2 with the  $\mu$ 1 subunit of AP-1 and for its TGN export. The model proposed by these authors is that the membrane recruitment of AP-1 by Arfrp1 exposes a domain of  $\mu$ 1 adaptin that allows the interaction and thereby the loading of Vangl2 into transport cargos [12]. The finding that localization of Frizzled 6 and Celsr1, two other components of the mammalian core PCP module, is insensitive to siRNA-mediated silencing of Arfrp1 and the  $\mu$ 1 adaptin is interesting as it suggests that distinct transmembrane PCP proteins use specific sorting machineries to ensure their polarized delivery to the right site. The TGN sorting mechanism of Vangl2 appears evolutionarily conserved as polarized localization of Vang/Stbm in *Drosophila* requires orthologs of Arfrp1 and AP-1 [50] and because the YYXXF sorting motif identified in Vangl2 is also found in Vang/Stbm [12].

Fluorescence recovery after photobleaching (FRAP) experiments performed in the *Drosophila* wing model [51] or more recently in the mouse oviduct [52] support the notion that cellular asymmetry of the core PCP proteins is achieved through their rapid turnover at the plasma membrane followed by a progressive stabilization at specialized subdomains or

punctae [51]. Along the same lines, Vangl2 dynamically redistributes to anterior cell edges at the time mesodermal cells undergo CE movements in zebrafish or *Xenopus* embryos [53,54]. Yet our current understanding of the molecular mechanisms involved in the dynamic behavior of Vangl2 is still limited. Biochemical evidence for Vangl2 endocytosis has been reported using cell surface biotinylation of ectopically expressed Vangl2 in HEK293T cells [55]. In this assay, a small amount of the overexpressed Vangl2 pool could be detected among the cell surface labeled proteins, a fraction that markedly increased when dominant-negative forms of two well-established endocytic regulators namely Rab5 and dynamin were co-expressed [55]. Other trafficking regulatory factors like GIPC1 and SNX27 are likely to play an active role in Vangl2 recycling at the plasma membrane. Both factors harbor a PDZ domain that shows a robust yeast two-hybrid interaction with the PBM of Vangl2 [56,57]. GIPC1, in a complex with the actin motor Myosin IV, has been proposed to regulate the pool of Vangl2 at the plasma membrane, possibly by powering one of its internalization steps [56]. SNX27, on the other hand, is an early endosome-associated retromer component that may promote the retrieval and/or recycling of endocytosed Vangl2 back to the plasma membrane or to the TGN compartment [58]. Other proteins such as Rack1 or Syndecan 4 (Sdc4) have also been postulated to participate in the control of some undefined Vangl2 trafficking events based on their physical [59] or genetic interactions [60,61] with Vangl2. Vangl2 and Sdc4 both contain a C-terminal PBM, but whether this motif is required for their interaction, for instance via a third unidentified partner with multiple PDZ domains remains to be addressed.

### *Molecular organization and partners of Vangl2*

Over the past 15 years, many Vangl2 interactors have been isolated through a variety of methods (see table 2). While Vangl2 interacting proteins like Scribble [16,57,62], GIPC1 [56,57], Ror2 [9], p62/SQSTM1 [63], AP-1 [12], MCC [64] or more recently Nrdp1 [65] have been validated by independent functional studies, others need to be further characterized. Nevertheless the list of Vangl2 interacting proteins provided in Table 2 already gives us an idea of the large spectrum of regulatory mechanisms exploited or contributed by Vangl2 under normal or pathological conditions.

Not surprisingly, and excluding Vangl2 itself and Vangl1, four Vangl2 interacting proteins, namely Dvl [10,66], Fz3 [16], Pk1 [11,67] and the vertebrate Fmi homolog Celsr1 [18] are core PCP components whose fly orthologs also assemble in large complexes with Vang/Stbm [14]. These interactions are therefore likely reflecting ancestral binding properties of the core PCP components. We and others have provided biochemical evidence that Vangl2 can self-interact and also form hetero-oligomers with Vangl1 [36,68]. Moreover, we could

demonstrate that Vangl1-Vangl2 hetero-oligomers required neither the N-terminal nor the C-terminal cytoplasmic domains of Vangl2, as a fragment of Vangl2 encompassing its four transmembrane domains still coimmunoprecipitated with Vangl1 [36]. The ability of Vangl1 and Vangl2 to hetero and homo-oligomerize echoes the supramolecular organization of fly core PCP complexes into signalosome-like structures as recently reported [69]. It is currently unclear whether Vangl1 and/or Vangl2 are endowed with paralog-specific functions or structural properties, but if this turned to be the case and as depicted in Figure 3, the composition, stoichiometry and relative abundance of such oligomers could have important functional implications.

The conserved PDZ interaction that Scribble entertains with Vang/Stbm/Vangl proteins [16,62,70], although still poorly understood from a mechanistic point of view, is nevertheless thought to be functionally important [36]. Considering the pivotal role of Scribble in the maintenance of apico-basal organization in polarized epithelia [62], this scaffold protein appears ideally positioned to serve as a molecular link between apico-basal polarity regulators and PCP factors (Figure 3) as proposed by a recent study [71]. Scribble, however, is not the only PDZ protein to bind the PBM of Vangl2 and at least six other PDZ proteins have been shown to do so as well. These include GIPC1 [56] and SNX27 [57] whose possible function in Vangl2 trafficking has been discussed above. The multi-PDZ domain MAGI-3 protein has been proposed to bridge Frizzled4 and Vangl2 to promote Rac1-dependent activation of JNK signaling [72]. DLG/SAP97 and PSD95, on the other hand, are two members of the MAGUK protein family that bind the PBM of Vangl2 in very diverse cellular contexts [57,73,72]. These adaptor proteins are widely used by cell surface receptors, notably in neuronal cells, for their targeting to specialized membrane domains. Vangl2 indeed functions in neuronal cells and in particular in the postsynaptic compartment where it is found to be strongly enriched. A recent study indicates that the PDZ interaction between Vangl2 and the postsynaptic protein PSD95 is not absolutely required for the synapse-associated distribution of Vangl2 and suggests that at that site Vangl2 might serve to control the clustering of postsynaptic molecules [75]. The last PDZ protein to robustly interact with the PBM of Vangl2 is PDZRN3 [57]. However, whether this E3 ubiquitin ligase targets Vangl2 for polyubiquitination in a PBM-dependent manner is currently unknown but will certainly warrant further investigations.

Among the other Vangl2 interactors, Ror2, stands out as this single transmembrane receptor tyrosine kinase is strongly suspected to translate a mouse Wnt5a proximo-distal (PD) gradient into a global directional cue through a graded phosphorylation of Vangl2 that promotes its own asymmetric localization [9]. Ryk, another RTK member with a

nonfunctional kinase domain seems to operate through a very similar mechanism [76]. MCC is a PDZ protein that binds Vangl2 in a manner similar to Dvl, i.e. through the same unconventional internal PDZ binding motif of Vangl2 and functional data support a role of MCC in the Wnt5a-mediated PCP pathway, with MCC acting downstream of Ror2 and Ryk and upstream of RhoA/JNK [64]. Potentially acting in the same signaling pathway, Dact1 [77] and Sestd1 [78], have also been shown to interact with Vangl2. Although the mechanism is not entirely clear, it has been proposed that Dact1 and SestD1 act in a complex downstream of Vangl2 to activate a Rho GTPase branch of the PCP signaling pathway. Interestingly, Vangl2 has also been reported to directly bind Rac1 and RhoA [79]. *In vitro* as well as *in vivo* functional data from the same study led the authors to propose that this might allow Vangl2 to recruit active Rac1 to adherens junctions where the Rho GTPase would affect cell adhesion and the actin cytoskeleton. E-Cadherin is a major component of AJs, in epithelial cells and Vangl2 expression has been shown to control its internalization in different cellular contexts [55]. Among the many functions of Vangl2, internalization clearly appears as a recurrent theme as this PCP component has been found to interact with and enhance the endocytosis of an ever growing list of PCP and non-PCP proteins, including Frizzled 3 [80], the E- and N-Cadherin AJ components [55,81], the podocyte protein nephrin [82] or the matrix metalloproteinase MMP14 [83].

A newcomer to the list of Vangl2 interactors is the p62/SQSTM1 autophagy receptor [63] that has been recently co-purified with VANGL2 upon immunoprecipitation with a highly specific monoclonal antibody [55]. A previously uncharacterized small region (amino-acid 346-388) in p62/SQSTM1 was shown to be necessary and sufficient for the interaction with the cytoplasmic C-terminal region of VANGL2. The usual autophagy partners of p62/SQSTM1 such as LC3 were undetectable in the immunopurified fraction, indicating that the VANGL2-p62/SQSTM1 complex had no functional relationship with an autophagic process. In this study we propose that this complex constitutes a platform for the recruitment and activation of JNK, an established PCP effector. Importantly, blocking the formation of this complex with a dominant negative p62/SQSTM1 peptide strongly reduced the tumorigenic potential of human breast cancer cells engrafted in the mouse fat pad. Remarkably, the magnitude of the VANGL2-p62/SQSTM1 interaction exhibited an inverse correlation with the occurrence of cell junctions, strongly suggesting that JNK activation by the Vangl2-p62/SQSTM1 platform is strongly enhanced following polarity loss events as frequently observed during tumorigenesis (Figure 4).

Nrdp1, a RING-finger E3 ubiquitin ligase frequently downregulated in aggressive forms of glioblastomas, has been recently shown to physically interact with Vangl1 and

Vangl2 possibly through a coiled coil interaction with these two paralogs [65]. Published biochemical data also revealed that Nrdp1 does not promote polyubiquitination of Vangl1 and Vangl2, despite the fact that it can bind the two proteins. Instead, this E3 ligase was found to stimulate the polyubiquitination of Dvl in a Vangl-dependent manner and through K63-ubiquitin linkages [65]. The model proposed by the authors posits that Vangl1/2-stimulated and Nrdp1-dependent polyubiquitination of Dvl1 prevents Dvl binding to phosphatidic acid and consequently the plasma membrane recruitment of Dvl1, thereby blocking the signals lying downstream of Dvl. Nrdp1 therefore behaves as a negative regulator of PCP transduction. While, as already mentioned, Vangl2 does not seem to be a substrate for this E3 ligase, it will be interesting to investigate whether this enzyme targets other regulatory proteins in a similar, i.e. Vangl2-dependent manner.

### *Concluding remarks*

Vang/Stbm and its vertebrate counterparts, Vangl1 and Vangl2 have, over the years, caught the attention of more and more laboratories. Arguably, our current understanding of these key PCP molecules has greatly improved [84,85] and will hopefully continue to do so. For instance, it is now very clear that, contrary to the initial general belief, the PCP functions fulfilled by these two paralogs are not limited to embryonic developmental morphogenetic event but are frequently maintained and exploited throughout or over a long period of the adult life [38,41,43,38,52,86]. Other recent major advances in our conceptual understanding of PCP establishment mechanisms have come from the elucidation of a global cue used by some tissues to coordinately elongate in a proximo-distal direction [24,25] or from the recent use of sophisticated quantitative imaging techniques to address the fundamental question regarding the composition of the core protein complex *in vivo* [69].

Yet, we still have a lot to learn. For instance, we all would like to know more about how Vangl1/2 proteins reach their final destination, are targeted for internalization recycling or destruction, whether they also make higher-order assemblies, where and with whom. Another long standing and still unresolved issue relates to their status: should we really conceive them as *bona fide* membrane receptors for secreted ligands, and if not, what other proteins could do the job? While Ror2 has already been proposed to serve such a role [9], the list is likely to grow in the next decade. A new question then immediately comes to mind: how many complexes are these two paralogs able to form at the cell surface and/or in intracellular compartments and with which stoichiometry? Finally, as their role in tumorigenesis is getting clearer [63,65,87,88] could these PCP molecules be of any help for

diagnosis or therapeutic purpose? With all these questions, we have no doubt that many discoveries, and maybe also some surprises, are ahead of us.

## **Acknowledgements**

We would like to sincerely apologize to our colleagues whose contributions were not cited in this review due to manuscript length limitation. JP Borg's lab is funded by La Ligue Nationale Contre le Cancer (Label Ligue J.P.B.), Institut Paoli-Calmettes, Assistance Publique - Hôpitaux de Marseille (fellowship to A.W.), and SIRIC (INCa-DGOS-Inserm 6038). JPB is a scholar of Institut Universitaire de France.



Organ (species)	Vangl2 mutation	Phenotype	Molecular/cellular Pathway	References
Tooth (mouse)	RNA interference	Retarded tooth germ growth	ND	[33]
Tongue (mouse)	<i>Vangl2<sup>CKO/-</sup>;K14-Cre</i> (viable)	papillae orientation defects	ND	[34]
Kidney (mouse)	<i>Vangl2<sup>Lp/Lp</sup></i> (embryonic lethal)	Reduced kidney size, branching and glomerular maturation	ND	[39]
	<i>Vangl2<sup>Ex4/Flox</sup>; Pod-Cre</i> (Podocyte-targeted Vangl2 KO) (viable)	Normal size Glomerular maturation delay Reduced Podocyte recovery after injury	ND	[37]
Lung (mouse)	<i>Vangl2<sup>Lp/Lp</sup></i> (embryonic lethal)	Branching defects	ND	[40]
	<i>Vangl2<sup>Lp/+</sup></i>	Alveolar enlargement Impaired adult lung function	ND	[38]
Cornea (mouse)	<i>Vangl2<sup>Flox/Flox</sup>; Le-Cre</i> (Cornea-targeted Vangl2 KO)	Centripetal cell migration defects	JNK pathway	[41]
Uterus (mouse)	<i>Vangl2<sup>Flox/Flox</sup>; Prg-Cre</i>	Misdirected epithelial evaginations Defective crypt formation and blastocyst implantation	Wnt5a-ROR1/2 pathway	[43]
Testis (rat)	RNA interference	15 disrupted spermatid transport and polarity	Actin microfilament polarization	[42]

Skin (mouse)	<i>Vangl2<sup>Lp/Lp</sup></i>	Mis-angled hair follicles	Celsr1 dependent asymmetric localization	[18]
Brain (mouse)	<i>Vangl2<sup>Lp/Lp</sup></i> (embryonic lethal)	Ciliary orientation and alignment defects	-	[45]
Trachea (mouse)	<i>Vangl2<sup>Lp/Lp</sup></i>	Misoriented motile cilia in multiciated tracheal cells	Planar polarization of MT-associated basal bodies	[19]

Table 1: *Vangl2*-associated mutant phenotypes beyond conventional PCP functions

Abbreviations used throughout the table:

ND: Not Determined

<b>Vangl2 Partners</b>	<b>Method(s)</b>	<b>Interacting part of Vangl2</b>	<b>Vangl1 Interaction</b>	<b>Functional relevance</b>	<b>references</b>
Dvl1, Dvl2 et Dvl3	Y2H GST pull-down co-IP	unconventional internal PBM requiring Ser464	Yes	Proper signaling during neural tube closure	[10] [66]
Prickle	Y2H GST pull-down Affinity purification/ MS	Internal C-terminal domain (85 amino acids)	Yes	Fz/Dsh localization inhibition Membrane Vangl2 localization	[11] [67]
Celsr1/Fmi	Co-IP	ND	Yes	Interdependent asymmetric localization	[18]
Frizzled 3	co-IP	ND	ND	ND	[16]
Scribble	Y2H Peptide pull down Co-IP	C-terminal PBM	Yes	Vangl2 promigratory activity.	[16] [57] [62]
MAGI-3	pull-down Co-IP	C-terminal PBM	ND*	JNK signaling cascade.	[72]
GIPC1	Y2H co-IP	C-terminal PBM	ND*	Regulates Vangl2 trafficking	[56] [57]
SNX27	Y2H Co-IP	C-terminal PBM	ND*	ND	[57] [58]
PDZNR3	Y2H	C-terminal PBM	ND*	ND	[57]
DLG	Y2H	C-terminal PBM	ND*	plasma membrane formation during cellularization	[73]
PSD-95	co-IP	C-terminal PBM	ND*	postsynaptic Vangl2 localization	[74]
MCC	co-IP	C-terminal region	ND	Convergent extension	[64]
VANGL1	Affinity-purification/ MS Co-IP	region encompassing the four TM domains	Yes	ND	[36] [68]
VANGL2	Co-IP	ND	Yes	ND	[36] [68]
p62/SQSTM1	Affinity purification/ MS	C-terminal region	No	JNK-dependent cancer cell proliferation	[63]

Vangl2 Partners	Method(s)	Interacting part of Vangl2	Vangl1 Interaction	Functional relevance	references
Ryk	Genetic interaction co-IP	ND	ND	Increased Vangl2 stability	[76]
ROR2	Co-IP in Wnt5a stimulated cells.	ND	ND	WNT5A-ROR2 dependent stimulation of VANGL2 phosphorylation	[9]
Bbs8	co-IP	ND	ND	Vangl2 localization	[89]
Ift20	co-IP	ND	ND	Vangl2 localization	[89]
E-Cadherin	Co-IP	C-terminal region	ND	E-Cadherin internalization	[55]
N-Cadherin	co-IP	C-terminal region	ND	N-Cadherin internalization and synapse formation	[81]
AP-1 ( $\mu$ 1 subunit)	Y2H GST pull-down	YYXXF sorting signal (C-ter region)	ND	TGN export of Vangl2	[12]
Rac1	Co-IP	PBM-dependent	ND	adherens junctions formation	[79]
RhoA	Co-IP	ND	ND	adherens junctions formation	[79]
Dact1	Co-IP pulldown assays	ND	ND	Morphogenetic events at primitive streak	[77]
SestD1	Co-IP	ND	ND	Unknown	[78]
Rack1	Y2H co-IP	ND	ND	Vangl2 membrane localization	[59]
Nrdp1	Co-IP	C-terminal region	Yes	Dvl Ubiquitination	[65]

**Table 1:** A list of VANGL2 interacting proteins.

Abbreviations used throughout the table

PBM: PDZ Binding Motif

ND: Not Determined

PBM: PDZ Binding Motif

TM: Transmembrane domain

ND\*: Not Determined but expected based on highly conserved PBM of Vangl1 and Vangl2

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## Figure legends

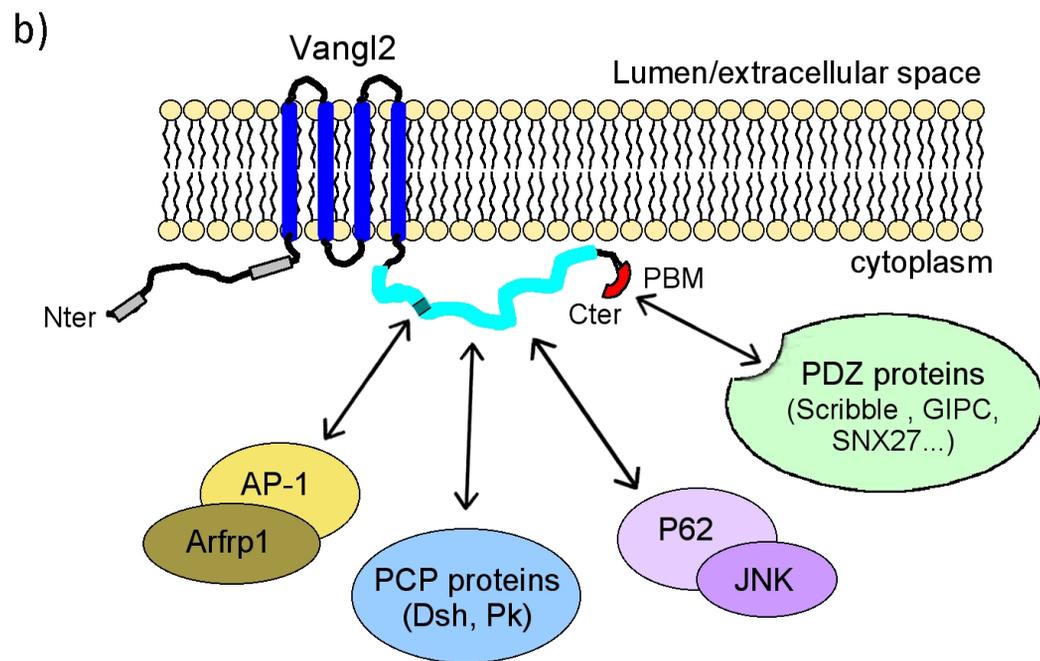
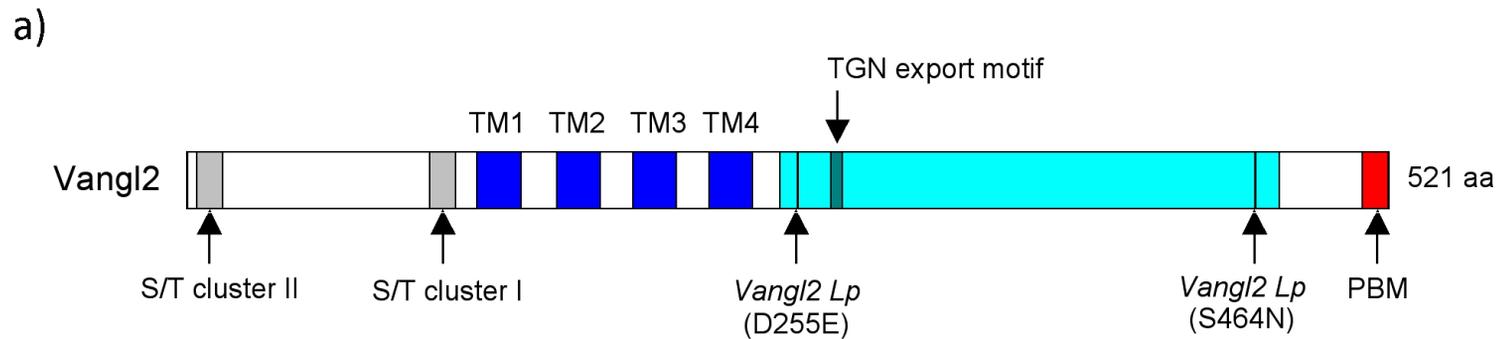
**Figure 1. Structural and topological organization of Vangl2.** (a) Schematics depicting the structural organization of Vangl2 with the positions of its main domains such as the four transmembrane (TM) domains (dark blue), two Ser/Thr phosphorylation site clusters (grey), the Dvl/Pk interacting region (cyan), the C-terminal PDZ binding Motif (PBM) domains (red) or the TGN sorting motif (grey box in the C-terminal region). Positions of the two Lp mutations are also indicated by arrows. (b) Schematic representation of Vangl2 topology.

Some of the many protein interactions that Vangl2 and possibly Vangl1 as well can make, notably with the other core PCP components and the trafficking regulatory protein SNX27. Note that Scribble has been shown to interact with both Vangl1 and Vangl2.

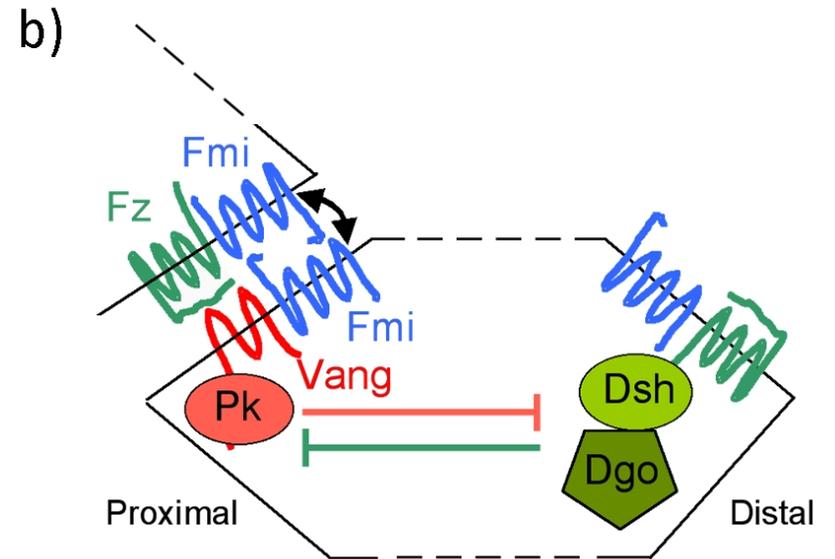
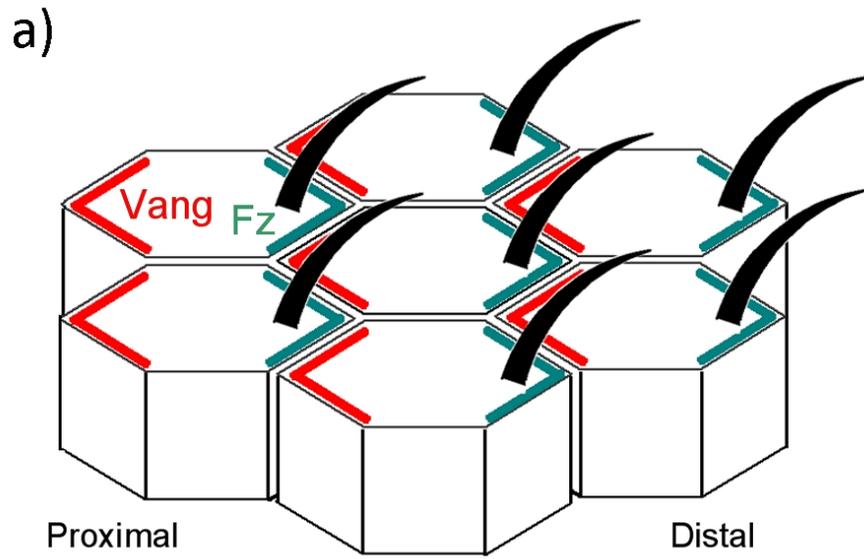
**Figure 2. Asymmetric distribution and antagonistic relationships of core PCP proteins in *Drosophila* wing epithelial cells.** (a) Schematic representation of the asymmetric distribution of the fruit fly core PCP proteins in the wing epithelium. The distal positioning and tilting of each trichome is also depicted in each cell. The proximally accumulated Vang/Stbm-Pk complex is in red while the distally enriched Fr-Dsh complex is shown in green. (b) Schematics depicting the positive (double black arrows) intercellular interactions and the antagonizing intracellular interactions between opposing complexes formed by Vang/Stbm-Pk on the proximal pole and by Fz/Dsh on the distal edge of the cells.

**Figure 3. A model of paralog-specific protein interactions and complex composition.** In this model, we speculate on the possible functional consequences of having Vangl1 (green pies) and Vangl2 (red pies) complexes of various composition, stoichiometry and paralog specific interactors. We arbitrarily opted for a scenario whereby Vangl1 and Vangl2 make hexamers based on previous report [69]. In this model and depending on which isoform is expressed in a given cell type, Vangl1 and Vangl2 can form homohexamers (a) and (b) or heterohexamers (c), (d) and (e). To further elaborate on this scenario, we postulate that Scribble (black triangles) can interact with both isoforms while p62/SQSTM1 (blue triangles) has a Vangl2-specific interaction associated with proliferative and pro-migratory properties. Finally we assume that the subunit ratio between Vangl1 and Vangl2 in the complexes reflects the relative concentration of each paralog.

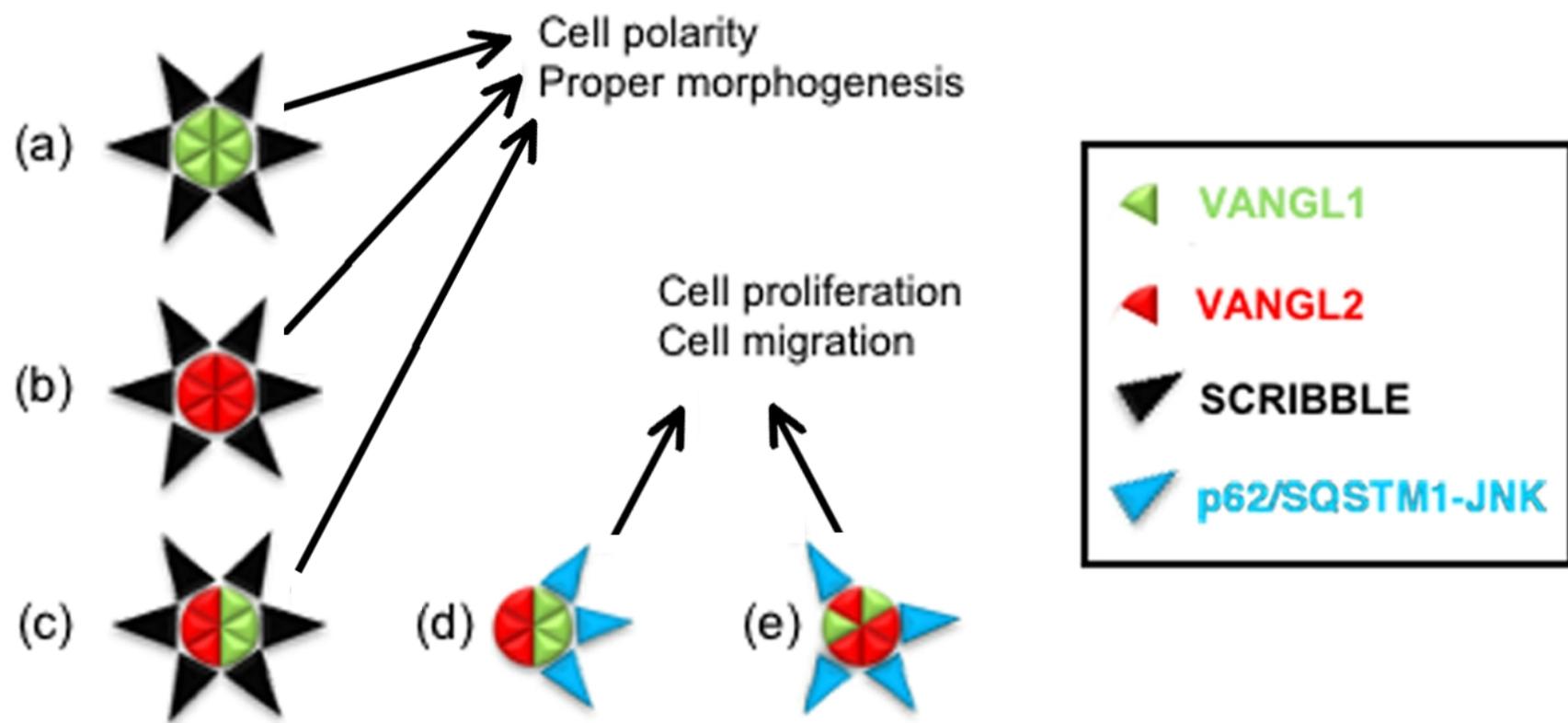
**Figure 4. Model of epithelial cell transformation with concomitant upregulation of Vangl2.** Here we further elaborate at the tissular level on the molecular model presented in Figure 2. Our model depicts the consequences of a Vangl2 up-regulation following an oncogenic event in an epithelium that originally expresses both isoforms and in which Scribble-dependent apical-basal and PCP signaling are strongly disrupted by a sudden excess of Vangl2. This model is largely inspired based on our recently published data [63].



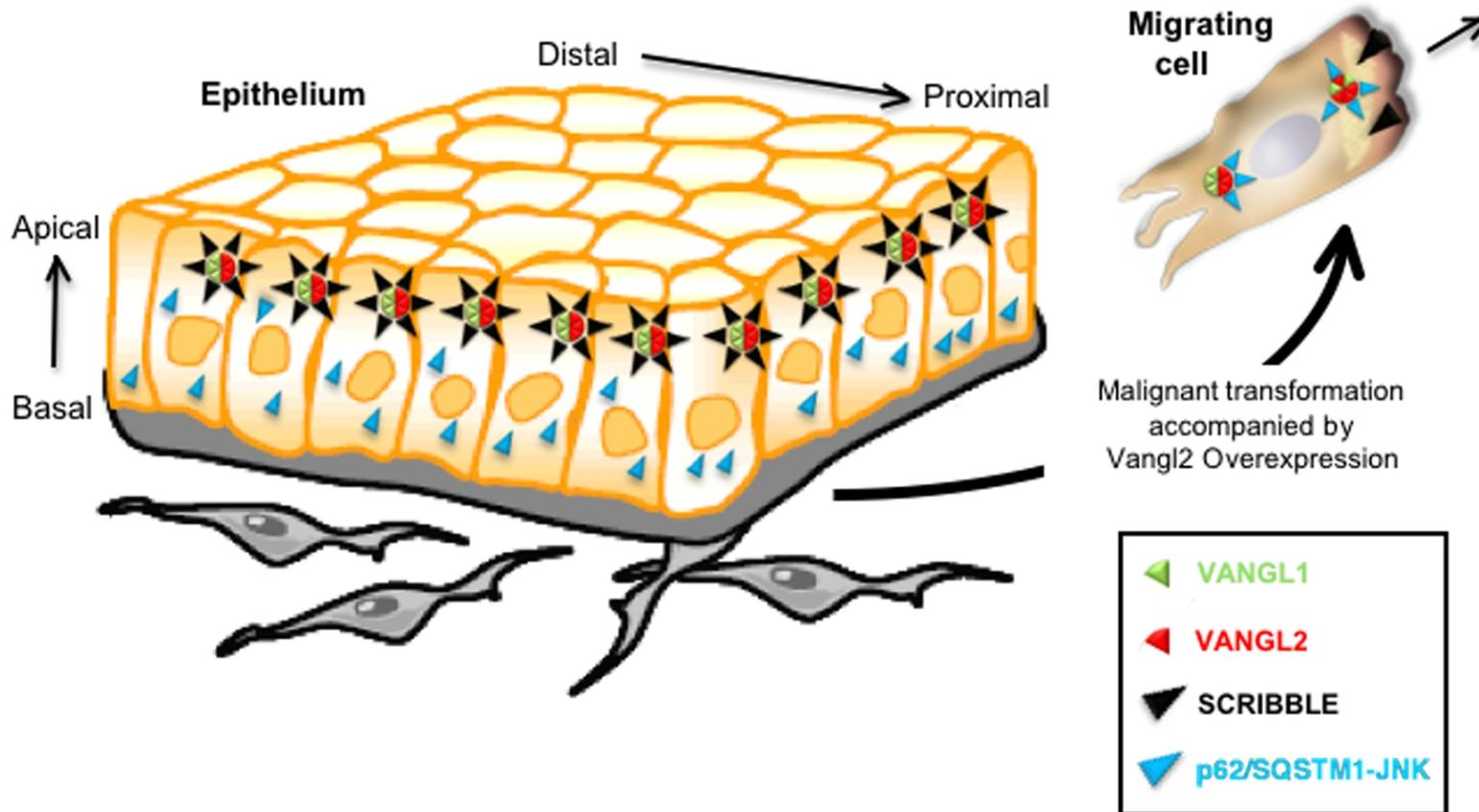
Bailly et al. Figure 1



**Bailly et al. Figure 2**



Bailly et al. Figure 3



Bailly et al. Figure 4