Mapping Cellular Polarity Networks Using Mass Spectrometry-based Strategies

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Abbreviations

aPKC, atypical protein kinase C; APEX, engineered ascorbate peroxidase; BioID, proximity-dependent biotin identification; Dlg, Discs Large; (FERM)-binding motif (FBM); GST, glutathione S-transferase, HRP, horseradish peroxidase; Lgl, Lethal Giant Larva; MAGUK, membrane-associated guanylate kinase; MS/MS, mass spectrometry; MST1/2, microtubule affinity-regulating kinases 1/2; PATJ, Pals1-associated tight junction protein; PCP, Planar Cell Polarity; PDZBM, PDZ binding

motif; LRR, leucine-rich repeat; Stdt, Stardust; STRAD, STe-20 Related ADaptor; Tobacco Etch Virus (TEV) protease; Yeast-2 hybrid (Y2H).

Abstract

Cell polarity is a vital biological process involved in the building, maintenance and normal functioning of tissues in invertebrates and vertebrates. Unsurprisingly, molecular defects affecting polarity organization and functions have a strong impact on tissue homeostasis, embryonic development and adult life, and may directly or indirectly lead to diseases. Genetic studies have demonstrated the causative effect of several polarity genes in diseases, however much remains to be clarified before a comprehensive view of the molecular organization and regulation of the protein networks associated with polarity proteins is obtained. This challenge can be approached head-on using proteomics to identify protein complexes involved in cell polarity and their modifications in a spatio-temporal manner. We review the fundamental basics of mass spectrometry techniques and provide an in-depth analysis of how mass spectrometry has been instrumental in understanding the complex and dynamic nature of some cell polarity networks at the tissue (apico-basal and planar cell polarities) and cellular (cell migration, ciliogenesis) levels, with the fine dissection of the interconnections between prototypic cell polarity proteins and signal transduction cascades in normal and pathological situations. This review primarily focuses on epithelial structures which are the fundamental building blocks for most metazoan tissues, used as the archetypal model to study cellular polarity. This field offers broad perspectives thanks to the ever-increasing sensitivity of mass spectrometry and its use in combination with recently developed molecular strategies able to probe *in situ* proteomic networks.

Introduction

Epithelial cells form complex tridimensional structures acting not only as barriers against external physical, chemical and biological threats but also as filters allowing the regulated transport of nutriments between the internal and external compartments of the organism. During tissue and organ morphogenesis, epithelial cells adhere to neighboring cells and to the supporting substrates, and adopt a highly organized structure with an apical domain in contact with the external environment. Apico-basal polarity describes the underlying molecular mechanisms of this lateral axis organization, and has been studied for several decades from the molecular, functional and genetic viewpoints, in a plethora of organisms. Epithelial cell membranes are divided into apical and basolateral compartments which are segregated by adherens (AJs) and tight (TJs) junctions formed by complex networks of cell adhesion molecules, intracellular adaptors, enzymes and cytoskeletal proteins (FIGURE 1A) [1]. Cell junctions are made of cell surface core components such as E-cadherin in AJs and claudins and occludin in TJs, which are associated with intracellular machineries connecting these membrane structures to the cytoskeleton and signaling networks. This finely tuned apico-basal axis orientation is organized and maintained by a set of asymmetrically distributed polarity protein complexes which have mainly been identified by genetic screens [2; 3]. Studies in *Drosophila melanogaster* have indeed pinpointed three evolutionarily conserved polarity complexes, the PAR (partitioning defective), CRUMBS and SCRIB complexes [4] which localize apically (PAR, CRUMBS) or basolaterally (SCRIB) and act antagonistically to maintain apico-basal polarity in the fly (FIGURE 1B) [5]. Each complex is composed of multiple subunits containing PDZ domain containing proteins acting as scaffolding molecules (PAR-3, PAR-6, SCRIB, Stardust, Discs Large),

small GTPases (CDC42) and protein kinases (atypical PKC, aPKC). The Par complex is the best characterized of these complexes at the molecular level; it contains the PAR-3 and PAR-6 PDZ proteins, and aPKC [1]. Other PDZ proteins (PAR-6, MAGUKs) bind to the cell surface molecule Crumbs, the major component of another cell polarity complex, through a well conserved carboxyl-terminal PDZ binding motif (PDZBM)[6]. The SCRIB complex is genetically described as having a causative role in apico-basal polarity and epithelial homeostasis in *Drosophila*. Several components of this complex (i.e. SCRIB, Discs Large (DLG), and Lethal Giant Larvae (LGL)) behave as strong tumor suppressor genes[3; 7; 8] and are evolutionarily conserved, from invertebrates to humans. The exact molecular composition of the PAR and SCRIB complexes were later studied using proteomic-based strategies which will be described in this review.

Planar Cell Polarity (PCP) is the second major, though more poorly characterized process, organizing epithelial sheets and their apical structures perpendicular to the apico-basal axis (FIGURE 1A). The importance of this cellular program is demonstrated by mutations in PCP genes which disrupt the stereotyped hair orientation on *Drosophila* wings and the alignment of the stereocilia in the mouse inner ear. Genetic studies initially performed in *Drosophila* identified a set of core PCP genes responsible for the establishment of planar cell polarity [9]. These genes encode integral membrane molecules such as Frizzled, Van Gogh, Flamingo as well as cytoplasmic proteins such as Dishevelled, Prickle and Diego. In the *Drosophila* epithelial system, core PCP proteins have a strikingly asymmetrical distribution in cells along a proximal-distal gradient. The protein complex comprising Van Gogh, Flamingo and Prickle is localized at the proximal sides whereas Frizzled, Flamingo, Dishevelled and Diego proteins are localized at the distal sides (FIGURE 1C). During

early gastrulation, a subset of core PCP molecules controls a phenomenon called convergent-extension which contributes to the formation of the antero-posterior axis of the embryo [10]. In addition to the crucial role of PCP in the correct execution of developmental events, increasing numbers of studies detail its importance in the proper organization and function of adult tissues and organs. Likewise, loss-of-function mutations in PCP genes lead to multiple diseases including neural tube defects [11] and polycystic kidney disease[12]. Recently, downregulation or, more frequently, upregulation of PCP genes has been associated with cancer progression either by promoting growth, resistance to cell death and drug treatment, or metastasis [13].

Much of the knowledge and understanding of apico-basal and planar cell polarities has been obtained through genetic screens, mainly in invertebrates (*D. melanogaster, C. elegans*). This led to the discovery of master genes devoted to the establishment and maintenance of epithelial polarities, and has highlighted how cell polarity defects can have a strong impact in cellular, tissue, and organ and organism homeostasis. A deeper understanding of these cellular programs has been obtained using protein-based strategies, especially to identify protein associated networks, but also the post-translational regulation of cell polarity proteins.

Over the last decade, mass spectrometry has been critical in elucidating new polarity protein components and complexes, as well as in revealing unexpected modes of action of these proteins. Various strategies have been used over the years to prepare samples for mass spectrometry analysis in order to purify high quality protein complexes amenable to mass spectrometry analysis. Sample preparation methods are largely dictated by the biological question addressed, which we will detail in the review. One of the main advantages of mass spectrometry is that the functional

hypothesis is no longer answered by a binary yes/no response, but by a multi-dimensional response. Unlike genetic methods and yeast two-hybrid assays, which test the direct interaction between a specific bait with a partner (prey), proteomics analysis provides more complex information.

This review describes how mass spectrometry has contributed to identify apico-basal and planar cell polarities networks, as well as other molecular networks involved in cell polarity processes occurring at the cellular level during cell migration and ciliogenesis. This review is organized in sections which focus on the following topics:

- Basis of mass spectrometry and techniques used to purify and concentrate protein complex populations amenable to mass spectrometry analysis.
- Specific examples of how mass spectrometry has allowed the molecular dissection of cell polarity protein networks involved in cell polarity (including apico-basal polarity, planar cell polarity, cell migration and ciliogenesis).
- Examples of how mass spectrometry has fueled new fields of research by connecting different types of cell polarity.

Protein identification by mass spectrometry

The term ‘proteomics’ was created over 25 years ago, with the aim of providing a complete characterization of all proteins (‘proteome’) expressed by a given genome [14; 15]. The term ‘proteoform’ was then used to define a specific molecular form of a protein product arising from a specific gene (including products differing due to genetic variations, alternatively spliced RNA, and post-translational modifications)[16].

Proteomics aims to elucidate the properties and functions of proteins by defining their amino acid sequences, post-translations modifications and protein interactions, amongst other parameters. Ideally, when carried out at a large scale, proteomics offers an integrated view of a complex biological process or cellular network [17]. The human genome contains approximately 20,000 genes, with more than 50% of them giving rise to alternative splice variants. However the total number of proteins expressed, including isoforms produced by splice variants, non-pathological and pathological amino-acid changes and post-translational modifications, is currently greater than 1 million. In addition, the numbers of copies of each protein in the ~230 human cell types can range from zero to 10,000,000. The proteome catalyzes and essentially controls all cellular processes and constitutes about 50-60% of the dry mass of a cell. Further layers of complexity are added by cellular processes which modify proteins such as phosphorylation, ubiquitination, degradation, protein localization and protein-protein interactions [18].

Mass spectrometry devices measure the mass-to-charge ratio of gas phase ions. Mass spectrometers have three main components, which include an ion source, a mass analyzer, which separates ionized substrates according to their ratio, and a detector. In proteomics applications, two main types of soft ionisation techniques are used to generate protein and peptide ions, which include matrix assisted laser desorption ionization (MALDI) and electro-spray ionization (ESI). Nano-electrospray (nanoES) is the ionization method of choice to generate additional partial sequence information to complement the mass information [19]. The ions follow a trajectory established by the Lorentz force in electric or magnetic field. The electrodynamic ion funnel allows the manipulation and focusing of ions in a pressure regime, which are then analyzed using mass analyzers. Different mass analyzers can be associated

with each ionization approach. The mass analyzer is the unit of the mass spectrometer dedicated to manipulating ionized masses and separating them based on mass-to-charge ratios, and distributing them to the detector. The main analyzers used in proteomics are the quadrupole (Q), the time of flight (TOF), the ion-trap (IT) and the Orbitrap (OB). A technical dissection of the component units of mass spectrometry devices is provided in the following review [20]. High-throughput proteomics rely on two main strategies: bottom-up or top-down approaches.

All bottom-up proteomics approaches require protein digestion by a sequence-specific enzyme such as trypsin which cleaves after basic amino acids (lysine or arginine) (FIGURE 2)[21]. The peptides thus generated are then separated by reverse phase chromatography, and ionized directly using ESI or indirectly using MALDI, after which the ionized peptides are introduced into the vacuum of a mass spectrometer. The ions are manipulated and analyzed using different mass analyzers such as Q and OB as a quadrupole-orbitrap mass spectrometer (Q-Exactive; Thermo Scientific). However, other types of mass spectrometers are also used for data acquisition. Usually the acquisition method used is automated data dependant acquisition (DDA) also known as tandem mass spectrometry. DDA involves multiple steps switching from full scan MS which measures the mass-to-charge ratios of ionized peptides, to a series of MS/MS acquisition according to data dependent criteria. MS and MS/MS spectrum are then analyzed by softwares (such as MaxQuant and Perseus software) which identify the peptides [22; 23; 24]. These search engines attribute each MS and MS/MS spectrum to the peptide of a given protein, thereby allowing the protein content of the sample to be identified and/or a particular post-translational modification (PTM) modification to be precisely mapped within a protein sequence. In targeted proteomics, the DDA is limited to a list of peptides and

their fragmented ions. Peptides of known mass-to-charge ratio (*m/z*) (parents ions) are selected in the first quadrupole, the peptide is then fragmented and several fragment ions are monitored over time using a quadrupole (for single reaction monitoring analysis ; SRM) or a high resolution mass analyzer such as an Orbitrap (for parallel reaction monitoring ; PRM)[25]. These transitions (parent ion / fragment ions) are multiplexed and their specificity is checked against a spectral library using dedicated software packages such as SkyLine [26]. A third acquisition method called data-independent acquisition (DIA) is also available for bottom-up proteomics. DIA is a method in which all ions within a selected *m/z* range (5-25 m/z) are fragmented and analyzed all together in MS/MS [25; 27; 28]. This DIA strategy has quickly expanded over the last few years because of the availability of next-generation mass spectrometers with high resolution and accurate mass (HR/AM). As for PRM, DIA analysis requires spectral libraries derived from auxiliary DDA data to provide protein identification and quantification. New software tools applied to DIA analysis are proposed by different groups such as Skyline and open SWATH[29].

In contrast, top-down proteomics does not require any prior proteolytic digestion. Top-down proteomics allows the identification and relative quantification of unique proteoforms by direct analysis of whole proteins[30]. Proteins are typically ionized by electrospray ionization and analyzed using HR/AM mass spectrometry for mass measurement. These whole proteins are then fragmented by direct ion dissociation in the gas phase using electron-capture dissociation (ECD) or electron-transfer dissociation (ETD). The main advantages of the top-down approach include the ability to detect degradation products, sequence variants, and combinations of post-translational modifications. Software dedicated to top-down proteomics still need to be developed, except for the ProsightPC initially described by Kelleher team[31]. The

main disadvantage of top-down proteomics is its limitation to relative simple mixtures and to small proteins (10-30 kDa). The latter limitation can be overcome using middle-down proteomics which consists in limiting protein digestion in order to yield relatively larger peptides (ideally above 3 kDa). Fewer peptides are generated in comparison to bottom-up proteomics (peptides from 0.7 to 3 kDa); the complexity of the digested products is thus reduced which allows better proteome coverage for proteins of higher molecular weight, which cannot be analysed by top-down proteomics. To date, most of the middle-down proteomics studies have been limited to very specific applications[32].

Given the importance of dynamic regulation of protein expression and the modifications of proteins, it is crucial to be able to monitor the dynamics of the proteomes. Different quantitative proteomics approaches have been developed. Quantitative proteomics is currently mainly based on bottom-up strategies using DDA, DIA and targeted acquisition. Numerous methods allowing relative as well as absolute quantification are available. Quantitative proteomics can be performed by two major approaches: one using stable isotopes and one named the "label-free" method. Stable isotope-based quantitative proteomics allows the identification of equivalent peptides using the specific increase in mass due to the addition of mass tags with stable isotopes as ^{13}C, ^{15}N and ^{18}O. Several strategies (ICAT, ICPL, iTRAQ, TMT, SILAC) involving stable isotopes have been developed using metabolic or chemical labelling[33]. The classical workflow consists of mixing labelled samples containing the heavy mass tags and the light samples with no tags. The relative abundance of peptides and proteins is revealed at the MS level (ICAT, ICPL, SILAC, AQUA) or at the MS/MS level (iTRAQ, TMT). Alternatively, the quantitative proteomics approach named “label-free” allows the relative abundance of proteins to

be determined between several samples and/or conditions by directly measuring the difference of abundance of different peptides/proteins by LC-MS/MS. This method is based on the measurement of the spectral intensities of the precursor ions in MS and the integration of these intensities through the chromatographic elution profile. This approach is possible thanks to the remarkable improvements of mass spectrometry devices over the last few years and to the strong correlation between the intensities of the ions and their abundance in the process of ionization by electrospray. The 'label-free' method requires a good precision of the detected masses, an important spectral resolution and precise and reproducible times of retention for the eluted peptides. The relative abundance of peptides and proteins is revealed at the MS level and the identification at the MS/MS level. A large number of softwares are available for "label-free" proteomics analysis. The most popular ones are MaxQuant developed by J. Cox and M. Mann [22; 24] and Progenesis QI (Waters). In targeted proteomics, SRM, PRM and DIA, absolute quantification methods use synthetic AQUA peptides in which amino acids containing stable isotopes are incorporated into the peptide. AQUA peptides are used as internal standards allowing the absolute quantification of proteins[34]. FIGURE 2 depicts the main steps of sample preparation for mass spectrometry analysis. Over the past two decades, several strategies have been used to purify protein complexes amenable to mass spectrometry analysis. These strategies each have their specific advantages and limitations, which we will describe in the following sections and is summarized in (TABLE 1).

Purifying protein complexes using peptide pull down strategies

The peptide pull-down technique is commonly used to identify interactions occurring on a limited sequence of a protein of interest. Synthetic peptides or recombinant protein fragments mimicking the sequence of interest used as baits are fused for

example to glutathione S-transferase (GST)) or not and subsequently bound covalently or non-covalently to agarose beads. Peptide pull-down strategies have been extensively used for intracellular sequences of membrane proteins [35; 36] the purification of which in large quantities is difficult due to low expression levels and poor solubility [37]. Peptides can be synthesized with biotin modifications and subsequently precipitated with streptavidin beads to purify proteins from cell lysates and identify interacting proteins[38]. This method allows the rapid identification of interactors; however it is limited to peptides and relies on limited linear motives. Peptide pull-down methods have for instance been used for the identification of protein interactors of the cytosolic regions of membrane proteins such as G protein–coupled receptors (GPCRs)[36]. Another advantage of this approach is the possibility to easily generate a mutant for a specific binding domain. For example, PDZ domains bind to a three amino acid hydrophobic motif generally found at the C-terminus of proteins. Mutation of this motif usually leads to a complete loss of interaction with PDZ domains. Using a peptide encompassing the C-terminus of VANGL2, it has been possible to identify two PDZ domain-containing proteins, SCRIB and SNX27, the interaction of which is disrupted with the mutant peptide[38].

Tandem Affinity Purification tag (TAP-tag) of Proteins

In 1999, Bertrand Séraphin's group designed a novel method to purify protein complexes under native conditions [39]. This protocol has been extensively used in yeast[40] and later in human cells focusing on TNFα/NF-κB signaling[41]. The tag consists of two IgG binding domains followed by a calmodulin binding domain, separated by a site of recognition for the Tobacco Etch Viruses (TEV) protease. This two-step purification method allows the rapid purification of proteins complexes under native conditions with low amount of background proteins. Despite the versatility of

the TAP-tag protocol, the size of the tag (~25kDa) may hinder some interactions. For this reason, several groups have optimized the original protocol by developing various combinations of tags to reduce the hindrance of the tag. One example is a combination of two streptavidin binding domains followed by a calmodulin binding domain (~10kDa), which has been successfully used to decipher the molecular mechanisms of Wnt and Sonic Hedgehog signaling pathways [42; 43].

Single-step protein purification

Over the last ten years, mass spectrometry has become increasingly sophisticated and has allowed the development of novel purification methods. For example, several groups have used Flag-tagged proteins[44; 45] to identify weakly expressed proteins and their associated protein complexes and to characterize proteins complexes with lower affinity. This strategy however yields a high rate of false positive hits, which can be identified as such and disregarded if appropriate controls are used. A list of the common “contaminant” proteins (http://crapome.org/) has been generated and can be used to rapidly discriminate false positives from real partners[46]. The advantages of the single-step purification methods are the small size of the tag, which limits steric hindrance for interacting proteins, as well as the rapidity of the purification compared to TAP-tag protocol, thus circumventing the use of time-consuming techniques which may lead to a loss of associated proteins.

Due to the recent emergence of nanobody technology and the availability of GFP-TRAP affinity reagents, several groups have taken advantage of the fluorescent properties of GFP to confirm the correct localization of GFP tagged proteins, prior to purification and mass spectrometry analysis[47]. The main advantage of nanobody purification for protein complexes is the extremely low size of this molecule (~10kDa)

resulting in limited contamination of peptides generated from nanobodies after trypsin digestion, rending mass spectrometry analysis of the purified protein complex more efficient [48]. However, caveats to tagging proteins with a GFP tag include the possible mislocalization of the fusion protein, hindering protein-protein interactions, and possible protein misfolding.

Purifying endogenous protein complexes using antibody immunoprecipitation

The development of high affinity antibodies directed against proteins of interest allows the purification of large amounts of endogenous proteins and their associated partners amenable to mass spectrometry identification. The success of this method is highly dependent on the quality of the antibodies - in terms of affinity and specificity. The main advantage of this method is that the number of false positives is limited, as the targeted protein is not artificially overexpressed, and that it is localized in its natural subcellular compartment. This technique has been successful in describing the molecular components of the SCRIB complex and their role in cell migration [49], and in characterizing the role of endogenous binding partners of VANGL2 and their functional importance in basal breast cancer progression[50]. One major drawback of this approach is that when the protein of interest is expressed at very low levels, the MS identification of co-purified proteins is difficult. These limitations are counterbalanced by the constant improvement in sensitivity of mass spectrometry devices and the development of high affinity antibodies.

Proteomics techniques defining the near neighborhood of a given protein of interest

With the recent development of new technologies based on proximal biotinylation and of highly sensitive MS devices, it is now possible to go a step further and explore

larger protein complexes, and even to exhaustively characterize a specific cellular compartment. These methods take advantage of enzymes such as the biotin protein ligase BirA, the engineered ascorbate peroxidase (APEX), or the horseradish peroxidase HRP to promote the biotinylation of proteins in the immediate neighborhood of a protein of interest in living cells. Biotinylated proteins are subsequently isolated by affinity purification and identified by mass spectrometry analysis.

Over the last few years, several techniques have been developed. The first technique exploits the capacity of a bacterial biotin ligase (BirA) to covalently biotinylate proteins in living cells. A BirA mutant called proximity-dependent biotin identification (BioID) was designed and fused to proteins of interest for expression in cells [51]. BioID has a significantly lower affinity for biotinoyl-adenylate (bio-AMP), an intermediate of the biotinylation reaction, which is released from the binding pocket of BirA to the immediate neighborhood. Recently, a smaller form of BioID called BioID2 [52] has been developed. This second generation enzyme is more efficient to label proteins with lower concentrations of biotin. Yet, one major drawback of this technique is the rate of the biotinylation reaction which can take up to 24 hours, rendering this technique unsuitable to decipher the transient activation of signaling pathways. Nevertheless, this approach has allowed the identification of molecular components of focal adhesions[53]. By probing several known components of the focal adhesion complex, Dong et al. were able to define different layers of neighborhood proteins (proximal to the membrane, intermediate layer, F-actin layer)[53]. The datasets obtained with this approach correlate closely with cellular imaging data using high resolution microscopy [54]. Other studies fused the biotin ligase to cell polarity components used as baits (E-cadherin [55], ZO-1 [56], occludin and Claudin-4 [57]) and expressed in

polarized MDCK cells allowing the identification of known as well as novel protein networks [55].

Another recently developed biotinylation approach uses a modified peroxidase, ascorbate peroxidase (APEX) first developed for electromicroscopy studies[58; 59]. The labeling radius of biotinylation has been estimated to be less than 20nm and the half-life of the biotin-phenoxyl radical is shorter than that of bio-AMP rending the labelling radius narrower. An optimized form of the APEX enzyme (APEX2) has recently been developed with greater enzymatic stability and activity[60]. As for BioID, the main advantage of this approach is that labelling of the proximal proteins occurs in living cells which decreases the rate of false positive as compared to other affinity purification-based strategies. Several studies have successfully used APEX biotinylation, notably to describe the protein composition of confined cellular compartments such as cilia[61] or mitochondria[58; 62]. In other studies, this approach was used to determine the spatiotemporal recruitment of proteins during the activation of signaling pathways such as the GPCR signaling[63; 64].

Optimization of the biotinylation protocol has been recently described using a common horseradish peroxidase (HRP) available in routine in laboratories. This method uses the properties of the HRP to proximally label proteins at the cell surface. In contrast to the APEX system, mature HRP needs to form disulfide bonds in order to be active, restricting its use to the extracellular environment[65]. HRP has a higher intrinsic peroxidase activity than APEX2 and can be used either with the membrane permeable biotin-phenol or with a membrane non-permeable version of the biotin-phenol called BxxP which harbors a polar polyamide linker. This approach allows the proteomics analysis of extracellular environments and it was recently employed to determine the composition of the proteome within the neuronal synaptic cleft[65].

Proteomics approaches to analyze PAR functions

In 1983, work pioneered by Ken Kemphues and Jim Priess using a screen for mutants defective in cell polarity in *C. elegans* identified the *par* genes . The mutants failed to form the endoderm because of defective partitioning of protein components between anterior and posterior poles of the early embryos[66; 67]. PAR proteins, with the exception of PAR-2, are highly conserved in model organisms including *C. elegans*, *Drosophila* and mammals [68]. PAR family proteins are involved in epithelial morphogenesis and contain two serine-threonine protein kinases, PAR-1 and PAR-4 (also known as STK11 or LKB1), a phospho- protein PAR-5 (a 14-3-3 isoform) and two scaffold proteins containing PDZ domains, PAR-3 and PAR-6. Atypical PKC (aPKC) and CDC42 were demonstrated as crucial downstream polarity effector proteins in this pathway. In vertebrates, multiple copies of the *PAR* genes have been identified, three for *PAR-6*, two for *PAR-3* and *aPKC*, and they are thought to have different functions. Recently, only PAR-3L, and not PAR-3, has been shown to be involved in mammary gland stem cell maintenance through binding and suppression of LKB1 kinase activity[69]. At the cellular level, PAR proteins are asymmetrically localized in polarized epithelial cells[68]. The apical domain is enriched in CDC42 and PAR-6/aPKC, whereas cell-cell junctions are enriched in PAR-3 and PAR-1. During polarization, PAR-3 acts as a crucial landmark, which localizes at the apical portion of cell-cell contacts, and then recruits other components of the adherens junctions. Therefore it acts as a transient docking site for PAR-6/aPKC and Crb/CDC42[70; 43; 69] and allows apico-basal polarity to be established.

Mass spectrometry methods have been used to comprehensively analyze the human PAR protein complexes in 2004[71], with the aim to determine whether the PAR orthologues have common or different protein partners. TAP-tagged fusion proteins

of the PAR “core members”, including PAR-1, PAR-3, PAR-4, PAR-5, PAR-6A, PAR-6B, PAR-6C and PKCλ[39] were independently expressed, resulting in the identification of a total of 60 proteins, which include partners directly or indirectly linked to cell polarity such as Hensin and mSpaghetti (also known as MLCK-like protein) [72]. More than 50 novel interactors were identified, some of which, such as the 14-3-3 phospho-protein scaffolds, were present in more than one distinct complex[71].

Furthermore, immunoprecipitation of FLAG-tagged PAR-3s, followed by mass spectrometry analysis, identified the serine-threonine protein phosphatase PP1 (predominantly the alpha isoform) as a binding partner of PAR-3[73]. PAR-3 is phosphorylated by PAR-1 which leads to dissociation from aPKC and association with 14-3-3 and PAR-3 at the basolateral membrane[74; 75]. Dephosphorylation of PAR-3 by PP1α frees 14-3-3, which in turn restores the association of PAR-3 with aPKC, and therefore the relocalization of a functional complex to tight junctions[73]. Mass spectrometry analysis allowed the identification of Serine 144 and Serine 885 of PAR-3 as residues that are dephosphorylated by PP1. This data confirmed previously published work, highlighting the critical role of PAR-3 phosphorylation. Accordingly, expression of a mutant of PAR-3 deficient in Serine 144 phosphorylation results in epithelial cell polarity defects[75]. Together these data provide a clear understanding of the molecular mechanism controlling PAR-3 localization and maintenance at the tight junctions of epithelial cells (FIGURE 3).

The LKB1 homologue, PAR-4, was originally ascribed a role in asymmetric cell division in *C. elegans*[67]. LKB1 is described as a “master” kinase of epithelial polarity establishment[76] through its association with the STRAD-α or -β pseudo-kinases and the adaptor MO25. LKB1 is able to phosphorylate up to 14 downstream kinases, including AMPK or PAR-1, which in turn phosphorylate various downstream

components such as RAPTOR or PAR-3. LKB1 and its regulatory subunits STRAD localize at the adherens junctions in an E-cadherin dependent manner[77], controlling local AMPK activation and MLC phosphorylation, which in turn leads to constriction of the apical cytoskeleton[78]. In mammals, *LKB1* acts as a tumor suppressor and is responsible for the Peutz-Jegher syndrome, an autosomal dominant inherited disorder causing gastrointestinal hamartomatous polyposis and increased predisposition to cancer in colon, breast, ovarian, pancreatic and lung tissues. *LKB1* is also somatically mutated in lung or cervical carcinoma and synergizes with human papilloma viruses as its loss promotes disease progression [79]. Yeast two hybrid screens have allowed the first identification of the pseudo-kinases STRADs as interacting partners of LKB1[80]. Upon binding to STRADs, LKB1 becomes active and autophosphorylates: mass spectrometry confirmed the existence of two previously identified phosphorylation sites (Threonines 336 and 363) and detected two novel autophosphorylation sites on Threonines 185 and 402, which correlate with increased LKB1 catalytic activity (FIGURE 3)[80]. A year later, an immunopurification procedure followed by mass spectrometry analysis led to the identification of LKB1-associated proteins, including MO25, which stabilizes LKB1 in a LKB1-STRADs-MO25 tripartite complex[81]. To further understand the molecular components associated with LKB1, two independent studies used mass spectrometry analysis of the purified LKB1 protein complex, and identified HSP90 as a chaperone protein protecting LKB1 from proteasomal degradation [82 83]. In a later study, Hans Clevers's team showed that the full activation of the kinase activity of LKB1 by STRADs results in the remodeling of the actin cytoskeleton, leading to the formation of an apical brush border in isolated epithelial cells. These data suggest that LKB1 behaves as a master regulator of apico-basal polarity in epithelial cells[76]. Although downstream

signaling of LKB1 is well defined, the mechanisms of regulation and activation of this serine/threonine kinase remains elusive and deserve further investigation.

Proteomics applied to study the Crumbs complex

The second conserved polarity complex is the Crumbs complex. The *crumbs* (*crb*) gene, originally identified in *Drosophila* [84], was first shown to be important in the establishment of epithelial polarity in 1990s[85]. Four proteins are considered to be the core components of the *Drosophila* Crumbs complex: CRB, Stardust (SDT), PATJ (protein associated with tight junctions or Pals1-associated tight junction protein) and Lin-7 [86]. CRB is a type-I transmembrane protein containing two highly conserved regions in its 37-amino-acid cytoplasmic domain which are crucial for its polarity functions: the C-terminal PDZ-binding motif and the FERM-binding domain [86]. The PDZ-binding motif is responsible for its interaction with PAR-6 and its partner aPKC, as well as MAGUKs and SDT [87; 88; 89; 90; 91; 92]. The juxtamembrane Four-point-one, Ezrin, Radixin, Moesin (FERM)-binding motif (FBM) has been reported to bind the FERM domain proteins Yurt and Moesin (Moe)[93; 94]. CRB was shown to be linked to Hippo signaling as FLAG-tagged YAP and TAZ bind to Crumbs polarity partners identified by mass spectrometry analysis, including PALS1, PATJ (or INADL), MUPP1, LIN7C, and AMOT[95]. The WW-domain and PDZ-binding motifs of YAP/TAZ are required for binding to the multiple members of the Crumbs complex[95]. YAP/TAZ interaction with Crumbs is required to suppress TGF-β signaling, and consequently important for TGFβ-mediated epithelial-to-mesenchymal transition[95]. At the molecular level, YAP/TAZ retains phospho-SMAD in the cytosol, thus suppressing TGF-β signaling. In *Drosophila*, the Crumbs complex is linked to the Hippo pathway by binding to the FERM protein, Expanded, leading to its recruitment at the apical membrane of the peripodial epithelium of imaginal discs[96]. Recently, a siRNA screen

identified RNF146 and Tankyrases (TNKS1 and TNKS2) as regulators of PALS1 subcellular localization. A proteomics approach using the BioID methodology has contributed to revealing the underlying molecular mechanism whereby RNF146 and TNSK2 target AMOTL2 for ubiquitin-dependent degradation and therefore blocking the inhibition of AMOTL2 over PALS1, which then relocates apically and stabilizes the Crumbs complex[97]. This finding is consistent with a previously published study which used single-step purification followed by mass spectrometry, and determined that YAP is associated with AMOTL2 and promotes inhibition of Hippo signaling[98]. The link between the Crumbs complex and Hippo or TGF-β signaling to maintain apico-basal polarity is well characterized, however a more in-depth understanding of the role of each member of the Crumbs complex is still needed.

Dissecting the molecular components of the SCRIB complex

Scribble was initially identified at the septate junctions of *Drosophila* epithelial cells where it controls the distribution of apical proteins such as Crumbs[99]. Scribble behaves as a tumour suppressor by regulating cell polarity and growth, together with Lgl and Dlg[99]. The Scribble complex comprising these three gene products acts by antagonizing the spread of the apical domain promoted by the PAR complex[100]. Conversely, aPKC, bound to PAR6 and CDC42, acts downstream of Crumbs and PALS1, and phosphorylates LGL. This event leads to the exclusion of the Scribble complex from the apical membrane and to its relocalization to the basolateral one. The Scribble complex has also been shown to play a role in *Drosophila* neuroblast cell size and mitotic spindle asymmetry[101]. Human Scribble (or SCRIB) is composed of 16 amino-terminal leucine rich repeat (LRRs) and 4 carboxy-terminal PDZ protein

domains, as is the case for *Drosophila* Scribble, and is involved in the regulation of cell adhesion, cell shape and cell polarity[82]. SCRIB was first identified through its interaction with E6, an oncoprotein encoded by human papillomaviruse-16 (HPV-16). Infection of epithelial cells by HPV-16 results in lesions of cutaneous and mucosal epithelia, genital tissues and upper respiratory tracts. As E6 associates with an ubiquitin ligase (E6AP) in infected cells, it promotes ubiquitination and proteosomal degradation of its associated proteins. To identify E6 targets, a GST-E6AP has been used as bait and affinity-purified SCRIB was identified by mass spectrometry [102]. Mechanistically, E6 harbors a PDZ-binding motif allowing its interaction with the PDZ domain-3 of SCRIB and its recruitment close to E6AP, which promotes SCRIB degradation. Expression of E6 in epithelial cells results in reduced SCRIB levels (as well as the level of other PDZ proteins able to interact with the E6 PDZ-binding motif), subsequently altering the integrity of cell-cell junctions[103]. The first attempt to identify the proteins endogenously associated to SCRIB was performed using immunoprecipitation of proteins extracted from non-transformed mammary epithelial MCF10A cells followed by mass spectrometry. SCRIB was very efficiently co-purified with ARGHEF7, also known as β-PIX, a RhoGEF which directly interacts with the first and third SCRIB PDZ domains through its PDZ binding motif[49; 104]. The SCRIB/β-PIX complex is associated with the serine-threonine kinase PAK, and it was shown to be essential for cell survival when localized within the adherens junctions[105]. In neuronal cells, SCRIB anchors ARGHEF7/β-PIX at the plasma membrane, controlling calcium dependent exocytosis[49]. SCRIB also interacts via its PDZ domains with the C-terminal of Thyrotropin Stimulating Hormone Receptor, a GPCR, and controls its trafficking to the plasma membrane via the ARGHEF7/β-PIX-GIT-ARF6 pathway[106]. Other studies have shown that SCRIB localizes adenomatous polyposis coli (APC) at

the adherens junctions and regulates cell cycle[107], and the phosphatase PHLPP1 at the plasma membrane, which negatively regulates AKT signaling[108]. SCRIB is a potent inhibitor of other signaling pathways in polarized epithelial cells (FIGURE 4). For example, SCRIB recruits members of the Hippo pathway to adherens junctions and targets TAZ for degradation through ubiquitination by β-TRCP[109]. A single step purification of SCRIB followed by mass spectrometry showed its interaction with SHOC2 and M-RAS and its inhibitory role on ERK activation[110]. Conversely, in migrating astrocytes, SCRIB promotes CDC42 activity at the leading edge and controls Golgi apparatus orientation and directed cell migration[111]. SCRIB is also involved in the orientation of lamellipodia and directs cell migration of endothelial cells through the control of the RAB7-dependent endocytic machinery responsible for α5-integrin internalization and degradation[112]. We have also shown that the SCRIB/β-PIX complex controls PAK subcellular localization at the leading edge of breast cancer cells to promote cell migration[113]. More recently, a report has demonstrated that the subtle subcellular localization of SCRIB at the plasma membrane is required for its activity in animals[114]. Indeed, transgenic mice overexpressing a mutant form of SCRIB (mutation P305L within LRR13) previously shown to be unable to reach the plasma membrane [115; 116] display multifocal hyperplasia of mammary glands and develop mammary tumors following the mislocalization of PTEN in the cytosol and activation of the AKT/mTOR/S6K signaling pathway[117]. In other mouse models, as is the case in Drosophila [118], downregulation of SCRIB synergizes with oncogenes (Myc or Ras) in the promotion of tumorigenic transformation of epithelial cells [119; 120]. However, the analysis of public datasets shows that SCRIB overexpression is also correlated with an increased risk of patient relapse[108]. Indeed, SCRIB can also behave as an oncogenic protein in certain circumstances[121]. Since its first

identification in 2000[99], several attempts have been made to identify the SCRIB-associated protein network. Yeast two-hybrid screens have mostly resulted in the identification of direct interactors of the SCRIB PDZ domains [103; 106; 111]. The TAP-tag approach identified protein complexes associated with SCRIB and showed that it forms a ternary complex with a member of the PCP pathway (VANGL1) and NOS1AP distinctly from the ARGHEF7/β-PIX-GIT-PAK complex previously characterized by our group[49]. Clinical data showed that, in breast cancer, increased expression of VANGL1 and SCRIB is correlated with poor patient prognosis, suggesting that SCRIB may have protumoral functions. Accordingly, targeting VANGL1, NOS1AP or SCRIB expression by siRNAs decreased breast cancer cell migration[108]. In summary, several methods, including endogenous IP, TAP-tag or single-step purification methods, have been used to decipher the network of proteins associated to SCRIB in different cellular models, yielding similar and conflicting results [*49; 108; 110*] (FIGURE 5). Recently, two independent groups used proteomics to define the composition of the multi-molecular structures that connect integrins and the actin cytoskeleton in focal adhesions. The first group, led by Ed Manser, used a BioID strategy using as baits PAXILLIN, a cytoplasmic focal adhesion protein, and KIDLIN2 which directly binds to β1-integrin. Mass spectrometry analysis of purified proteins reveals the molecular composition of focal adhesions and identified several previously uncharacterized proteins[53]. The second group, led by Martin Humphries, assembled and compared all available datasets generated by multiple mass spectrometry-based proteomics reports [117; 122; 123; 124; 125] in order to identify the composition of the multi-molecular structures linking integrins to the actin cytoskeleton. In both studies, several known SCRIB interactors, including ARGHEF7/β-PIX and GIT1, previously known to participate with SCRIB in cell

motility, have been identified[53 38; 113]. However, SCRIB was absent from the list of focal adhesion proteins, confirming our previous data obtained by immunofluorescence and confocal analysis[113] raising the important issue of the spatio-temporal association between SCRIB and the GIT-ARGHEF7/β-PIX during cell migration.

Mass spectrometry-based determination of planar cell polarity networks

Since the first description of PCP in 1982 [126], most studies have used a combination of genetic approaches to determine the organization of the PCP pathway and only a few have attempted to do this at the proteomics level. Here, we will mostly focus on two components of PCP signaling which we recently investigated using proteomics: the cytoplasmic PRICKLE1 [44; 127 128] and the four transmembrane protein VANGL2 [38; 50]. These core PCP components have been also recently involved in cancer, where their high expression correlates with poor prognosis [13]. In order to understand their involvement in cancer, we and others have used proteomics to identify partners regulating their functions. Single-step purification of Flag-tagged forms of PRICKLE followed by mass spectrometry has identified several previously unknown interactors of PRICKLE1 and 2 [44]. One of them was the serine-threonine kinase, MINK1. MINK1 was previously shown to be required for PCP signaling in *Drosophila* [129] and to regulate convergent-extension in Xenopus [130]. However, the underlying mechanism of action was not known. We showed that PRICKLE1 is phosphorylated by MINK1, which leads to its relocalization to the plasma membrane via the endocytic pathway and contributes to its asymmetrical distribution during convergent-extension in *Xenopus* and PCP signaling in *Drosophila* ommatidia[44]. Mass spectrometry analysis

of PRICKLE1 also identified the MINK1 phosphorylation site which was functionally characterized. Indeed, expression of a PRICKLE1 mutant unable to be phosphorylated by MINK1 phenocopied PRICKLE1 loss in *Xenopus*[44]. The PRICKLE-MINK1 interaction is evolutionary conserved and, in breast cancer cells, promotes cancer cell migration[131]. In addition to its binding to MINK1, PRICKLE1 also interacts with ARGHAP21/24 [128], mTORC2 [127], and PHLDB2 [132] which contribute to basal breast and gastric cancer cell dissemination (FIGURE 6). At the signaling level, PRICKLE1 controls the spatio-temporal activation of downstream events by inhibiting Rho signaling and restricting Rac1 activation at the edge of cancer cells [128]. It also contributes to local activation of AKT and promotes remodeling of the actin cytoskeleton[127]. Finally, through its interaction with PHLDB2, it regulates CLASP2 localization at the plus-end of microtubules to promote focal adhesion turnover and β1-integrin internalization[127 132]. Together these data demonstrate the importance of PRICKLE1 as a molecular platform able to spatially control downstream signaling cascades[133; 134]. Other components of PCP signaling have also been studied by proteomics. SCRIB, a known interactor of VANGL1 and VANGL2, was genetically characterized for its involvement in the PCP pathway during mouse embryonic development[135]. As seen before, the SCRIB interactome was extensively and mostly characterized in epithelial and breast cancer cells[38; 49; 108; 110]. Although SCRIB acts as regulator of apico-basal polarity and PCP, little is known about the subset of SCRIB partners involved in the two processes and their potential interactions. In the study by Anastas et al., two distinct SCRIB complexes (VANGL/SCRIB/NOS1AP and SCRIB/ARGHEF7/GIT/PAK) were found to control cell motility[108; 111; 112; 113; 136] and cancer progression [108] (FIGURE 6). More recently, attempts have been made to identify and characterize the interactome of VANGL2. Genetic studied showed that

Vangl/Strabismus interacts with Prickle in *Drosophila*[137; 138] and *Xenopus*[139]. We used yeast-two hybrid to identify proteins bound to the mammalian VANGL2 PDZ binding sequence[38]. Among the positive clones, we identified SCRIB and SNX27 . SNX27 is known to control the retrograde route of GPCR trafficking at the plasma membrane[140] and we found that, indeed, it is responsible for plasma membrane localization of VANGL2 [38]. Thanks to the generation of a high affinity and specific antibody targeting VANGL2[141], we were able to immunoprecipitate endogenous VANGL2 from breast cancer cells at levels amenable to MS identification. This led to the first identification of the endogenous interaction between VANGL2 and VANGL1, which requires the four transmembrane domains of these partners. Surprisingly, no PDZ proteins were found in the VANGL2 complex, which might be due to the IP procedure and/or to the low affinity of PDZ interactions. Instead, we identified a novel VANGL2 interactor, p62/SQSTM1 (Sequestosome-1) which acts synergistically with VANGL2 in regulating convergent-extension *in Xenopus*[50]. p62/SQSTM1 was shown to bind directly to the VANGL2 C-terminal region independently from the PDZ-binding sequence. *VANGL2* and *p62/SQSTM1* genes are upregulated in basal breast cancer and are markers of poor prognosis. The specific interaction between VANGL2 and p62/SQSTM1 occurs in endocytic vesicles of breast cancer cells and results in the recruitment of JNK, leading to its activation and to JNK-dependent cell proliferation[50] (FIGURE 6). PTK7 is a PCP tyrosine kinase receptor functionally linked to VANGL2 in the mouse [142; 143]. However, no interaction has so far been observed between the two receptors. Using a myc-tagged version of PTK7 expressed into the animal pole of *Xenopus* embryos, the Borchers lab was able to identify by mass spectrometry RACK1 (receptor of activated protein kinase C 1) as a cytosolic binding partner of PTK7. RACK1 is involved in the membrane localization of Dishevelled, a PDZ protein

involved in PCP [144]. The authors subsequently showed that RACK1, like PTK7, is required for *Xenopus* neural tube closure. Interestingly, RACK1 also directly interacts with VANGL2 in *Zebrafish* and regulates PCP-related functions by localizing VANGL2 at the plasma membrane [145]. Much work is still needed to clarify the organization and regulation of protein networks associated with the numerous PCP components.

Proteomics of the primary cilia

Primary cilia are non-motile microtubule-based structures that are protruding from the cell body of almost all vertebrate cells. They develop from the mother centriole of the centrosome and have an antenna-like organization at the apical membrane of polarized epithelial cells (FIGURE 3). These structures concentrate membrane receptors able to respond to diverse stimuli including soluble ligands, mechanical and thermal stress, and act as sensors to fluid flow [146]. Identification of protein components of primary cilia is important to understand their organization, signal transduction properties and their involvement in diseases called ciliopathies. Over the past 10 years, several groups have tempted to identify ciliary proteins by using a combined procedure of primary cilia isolation through successive steps of sucrose gradient centrifugation and detergent solubilization, followed by mass spectrometry analysis. This strategy led to the identification of ~200 putative cilia-related proteins in human bronchial epithelial cells[147], then of more than 2,000 proteins in rod and cone photoreceptor sensory cilia [148]. The mouse kidney collecting duct cell line (IMCD3) which behaves as a polarized epithelial cell was also used as a model system to identify more than 2,900 ciliary proteins. However, known non-ciliary proteins were also identified in this study, questioning the specificity of the experimental procedure. Nevertheless, using a subtractive procedure combined with

protein correlation profiling, the authors were able to generate a refined list of 195 confirmed primary cilia proteins[149]. Another study based on protein correlation profiling coupled to a semi-quantitative technique based mass spectrometry (SILAC : stable isotope labelling by amino acids in cell culture) identified SCRIB in centrosomes[150], which confirmed previous findings[111]. However, SCRIB has not been identified as a cilia resident protein so far. Interestingly, VANGL2, a partner of SCRIB, plays a role in the localization and orientation of primary cilia, and directional fluid flow involved in embryonic development[151]. In the mouse, VANGL2 interacts with the Bbs8 and Ift20 ciliary proteins in a complex required for PCP signaling in the cochlea [152]. TAP-tag strategy has been used to identify interactions between intraflagellar transport proteins and endocytic components in cilia in *zebrafish* [153]. The group of M. Nachury used a TAP-tag strategy to connect Arf-like GTPases to cargo entry into primary cilia[154]. More recently the same group used the APEX technique to characterize the proteins composition of primary cilia using a domain of NPHP3 as bait, which specifically localized in cilia. This approach generated a list of 622 putative ciliary proteins, including LKB1 and its partner STRADβ[61]..

Future directions

The rapidly increasing number of studies using mass spectrometry has resulted in the discovery of protein complexes associated with major polarity proteins and has been instrumental in deciphering some of their functions, which is expected to further continue thanks to novel protein purification methods such as proximity biotinylation (BioID, APEX) and access to more sensitive mass spectrometers. One of the best characterized polarity complexes which is centered on SCRIB has benefited from a combination of several purification and mass spectrometry improvements over the

last 15 years, leading to a comprehensive picture of the network at the steady state (FIGURE 5)[49; 108; 110]. However, there is currently no information about the composition, compartmentalization and spatio-temporal regulation of this protein complex during the different steps of epithelial morphogenesis and transformation. This issue is, in our opinion, of particular importance considering the multiple cellular localizations (adherens junctions, leading edge, centrosomes) of SCRIB and their now well established functional importance. This also applies to other cell polarity proteins (PAR, LKB1,…) in normal and pathological situations. We believe that the combination of proximity biotinylation techniques with CRISPR/Cas9 gene editing and proximity ligation assay (PLA)[155] may be key in deciphering and validating the composition of cell polarity interactomes in cells or tissues. PLA is an oligonucleotide-conjugated antibodies-based technique of particular interest to confirm low affinity direct interactions (for example PDZ interactions) which are not amenable to regular co-immunoprecipitations. Furthermore, PLA is suitable for the exploration of direct protein-protein interactions in tissues[156].

Recently, Norbert Perrimon's group used APEX labeling in live Drosophila tissues to map the sub-proteomes of mitochondria in different physiological conditions [62].This study demonstrates the feasibility of identifying proteomes (and surely, in the future, protein networks) *in vivo* in a more comprehensive manner than other procedures relying on TAP-tag techniques [157] .

Recent improvements of proximity biotinylation techniques may also help to assess the organization of polarity complexes, especially at the plasma membrane. A derivative of the APEX strategy based on the more sensitive HRP was recently developed to identify protein complexes present in the neuronal synaptic cleft[65]. By taking advantage of the inactivity of HRP in reducing intracellular compartments, it is

possible to specifically biotinylate proteins in secretory and extracellular compartments. Furthermore, the use of a biotin-phenol derived substrate unable to cross the plasma membrane will specifically target the extracellular domains of membrane proteins. This approach should be able to address yet answered issues such as the organization of membrane cell polarity receptors and co-receptors upon ligand stimulation in different physiological and pathological situations [13]. A recent alternative to this method relies on high affinity HRP-coupled antibodies, avoiding the need to ectopically express HRP-fused proteins in cells, and allowing the direct identification of endogenous protein complexes including in fixed patient tissues samples [158].

Two independent groups have recently optimized the APEX technology using anti-biotin antibodies instead of streptavidin to purify trypsin-digested biotinylated proteins and have shown that this variant technique provides additional information, quantitatively and qualitatively (spatial and topological organization of protein complexes) compared to the original method. [159; 160].

Mass spectrometry can also provide important information about post-translational modifications the identification and function of which are poorly understood in cell polarity complexes, with a few exceptions[44; 73; 161; 162],. Notably, it has recently been published that ciliary proteins contained high levels of non-canonical phosphopeptides of unknown function[163]. Obviously, many questions remain to be answered before obtaining a comprehensive picture of the cell polarity processes but the development of novel sensitive mass spectrometry techniques should allow us tackle this exciting challenge.

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References

1. Margolis, B. & Borg, J. P. (2005). Apicobasal polarity complexes. *J Cell Sci* **118**, 5157-9.
2. Suzuki, A., Yamanaka, T., Hirose, T., Manabe, N., Mizuno, K., Shimizu, M., Akimoto, K., Izumi, Y., Ohnishi, T. & Ohno, S. (2001). Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures. *J Cell Biol* **152**, 1183-96.
3. Bilder, D. (2004). Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors. *Genes Dev* **18**, 1909-25.
4. Khursheed, M. & Bashyam, M. D. (2014). Apico-basal polarity complex and cancer. *J Biosci* **39**, 145-55.
5. Bilder, D. & Perrimon, N. (2000). Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* **403**, 676-80.
6. Pocha, S. M. & Knust, E. (2013). Complexities of Crumbs function and regulation in tissue morphogenesis. *Curr Biol* **23**, R289-93.
7. Plant, P. J., Fawcett, J. P., Lin, D. C., Holdorf, A. D., Binns, K., Kulkarni, S. & Pawson, T. (2003). A polarity complex of mPar-6 and atypical PKC binds, phosphorylates and regulates mammalian Lgl. *Nat Cell Biol* **5**, 301-8.
8. Yamanaka, T., Horikoshi, Y., Sugiyama, Y., Ishiyama, C., Suzuki, A., Hirose, T., Iwamatsu, A., Shinohara, A. & Ohno, S. (2003). Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity. *Curr Biol* **13**, 734-43.
9. Zallen, J. A. (2007). Planar polarity and tissue morphogenesis. *Cell* **129**, 1051-63.
10. Tada, M. & Heisenberg, C. P. (2012). Convergent extension: using collective cell migration and cell intercalation to shape embryos. *Development* **139**, 3897-904.
11. Gao, B. (2012). Wnt regulation of planar cell polarity (PCP). *Curr Top Dev Biol* **101**, 263-95.
12. Harris, P. C. & Torres, V. E. (2009). Polycystic kidney disease. *Annu Rev Med* **60**, 321-37.
13. Daulat, A. M. & Borg, J. P. (2017). Wnt/Planar Cell Polarity Signaling: New Opportunities for Cancer Treatment. *Trends Cancer* **3**, 113-125.
14. Fraser, C. M., Gocayne, J. D., White, O., Adams, M. D., Clayton, R. A., Fleischmann, R. D., Bult, C. J., Kerlavage, A. R., Sutton, G., Kelley, J. M., Fritchman, R. D., Weidman, J. F., Small, K. V., Sandusky, M., Fuhrmann, J., Nguyen, D., Utterback, T. R., Saudek, D. M., Phillips, C. A., Merrick, J. M., Tomb, J. F., Dougherty, B. A., Bott, K. F., Hu, P. C., Lucier, T. S., Peterson, S. N., Smith, H. O., Hutchison, C. A., 3rd & Venter, J. C. (1995). The minimal gene complement of Mycoplasma genitalium. *Science* **270**, 397-403.
15. Swinbanks, D. (1995). Government backs proteome proposal. *Nature* **378**, 653.

16. Smith, L. M. & Kelleher, N. L. (2013). Proteoform: a single term describing protein complexity. *Nat Methods* **10**, 186-7.
17. Blackstock, W. P. & Weir, M. P. (1999). Proteomics: quantitative and physical mapping of cellular proteins. *Trends Biotechnol* **17**, 121-7.
18. Harper, J. W. & Bennett, E. J. (2016). Proteome complexity and the forces that drive proteome imbalance. *Nature* **537**, 328-38.
19. Wilm, M. & Mann, M. (1996). Analytical properties of the nanoelectrospray ion source. *Anal Chem* **68**, 1-8.
20. Nolting, D., Malek, R. & Makarov, A. (2017). Ion traps in modern mass spectrometry. *Mass Spectrom Rev*.
21. Zhang, Y., Fonslow, B. R., Shan, B., Baek, M. C. & Yates, J. R., 3rd. (2013). Protein analysis by shotgun/bottom-up proteomics. *Chem Rev* **113**, 2343-94.
22. Cox, J. & Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol* **26**, 1367-72.
23. Paulo, J. A. (2013). Practical and Efficient Searching in Proteomics: A Cross Engine Comparison. *Webmedcentral* **4**.
24. Tyanova, S., Temu, T., Sinitcyn, P., Carlson, A., Hein, M. Y., Geiger, T., Mann, M. & Cox, J. (2016). The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nat Methods* **13**, 731-40.
25. Vidova, V. & Spacil, Z. (2017). A review on mass spectrometry-based quantitative proteomics: Targeted and data independent acquisition. *Anal Chim Acta* **964**, 7-23.
26. MacLean, B., Tomazela, D. M., Shulman, N., Chambers, M., Finney, G. L., Frewen, B., Kern, R., Tabb, D. L., Liebler, D. C. & MacCoss, M. J. (2010). Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* **26**, 966-8.
27. Hu, A., Noble, W. S. & Wolf-Yadlin, A. (2016). Technical advances in proteomics: new developments in data-independent acquisition. *F1000Res* **5**.
28. Bilbao, A., Varesio, E., Luban, J., Strambio-De-Castillia, C., Hopfgartner, G., Muller, M. & Lisacek, F. (2015). Processing strategies and software solutions for data-independent acquisition in mass spectrometry. *Proteomics* **15**, 964-80.
29. Rost, H. L., Rosenberger, G., Navarro, P., Gillet, L., Miladinovic, S. M., Schubert, O. T., Wolski, W., Collins, B. C., Malmstrom, J., Malmstrom, L. & Aebersold, R. (2014). OpenSWATH enables automated, targeted analysis of data-independent acquisition MS data. *Nat Biotechnol* **32**, 219-23.
30. Kelleher, N. L. (2004). Top-down proteomics. *Anal Chem* **76**, 197A-203A.
31. Taylor, G. K., Kim, Y. B., Forbes, A. J., Meng, F., McCarthy, R. & Kelleher, N. L. (2003). Web and database software for identification of intact proteins using "top down" mass spectrometry. *Anal Chem* **75**, 4081-6.
32. Cristobal, A., Marino, F., Post, H., van den Toorn, H. W., Mohammed, S. & Heck, A. J. (2017). Toward an Optimized Workflow for Middle-Down Proteomics. *Anal Chem* **89**, 3318-3325.
33. Chahrour, O., Cobice, D. & Malone, J. (2015). Stable isotope labelling methods in mass spectrometry-based quantitative proteomics. *J Pharm Biomed Anal* **113**, 2-20.
34. Gerber, S. A., Rush, J., Stemman, O., Kirschner, M. W. & Gygi, S. P. (2003). Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. *Proc Natl Acad Sci U S A* **100**, 6940-5.
35. Becamel, C., Alonso, G., Galeotti, N., Demey, E., Jouin, P., Ullmer, C., Dumuis, A., Bockaert, J. & Marin, P. (2002). Synaptic multiprotein complexes associated with 5-HT(2C) receptors: a proteomic approach. *EMBO J* **21**, 2332-42.
36. Maurice, P., Daulat, A. M., Broussard, C., Mozo, J., Clary, G., Hotellier, F., Chafey, P., Guillaume, J. L., Ferry, G., Boutin, J. A., Delagrange, P., Camoin, L. & Jockers, R. (2008). A generic approach for the purification of signaling complexes that specifically interact with the carboxyl-terminal domain of G protein-coupled receptors. *Mol Cell Proteomics* **7**, 1556-69.

37. Daulat, A. M., Maurice, P., Froment, C., Guillaume, J. L., Broussard, C., Monsarrat, B., Delagrange, P. & Jockers, R. (2007). Purification and identification of G protein-coupled receptor protein complexes under native conditions. *Mol Cell Proteomics* **6**, 835-44.
38. Belotti, E., Polanowska, J., Daulat, A. M., Audebert, S., Thome, V., Lissitzky, J. C., Lembo, F., Blibek, K., Omi, S., Lenfant, N., Gangar, A., Montcouquiol, M., Santoni, M. J., Sebbagh, M., Aurrand-Lions, M., Angers, S., Kodjabachian, L., Reboul, J. & Borg, J. P. (2013). The human PDZome: a gateway to PSD95-Disc large-zonula occludens (PDZ)-mediated functions. *Mol Cell Proteomics* **12**, 2587-603.
39. Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M. & Seraphin, B. (1999). A generic protein purification method for protein complex characterization and proteome exploration. *Nat Biotechnol* **17**, 1030-2.
40. Gavin, A. C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J. M., Michon, A. M., Cruciat, C. M., Remor, M., Hofert, C., Schelder, M., Brajenovic, M., Ruffner, H., Merino, A., Klein, K., Hudak, M., Dickson, D., Rudi, T., Gnau, V., Bauch, A., Bastuck, S., Huhse, B., Leutwein, C., Heurtier, M. A., Copley, R. R., Edelmann, A., Querfurth, E., Rybin, V., Drewes, G., Raida, M., Bouwmeester, T., Bork, P., Seraphin, B., Kuster, B., Neubauer, G. & Superti-Furga, G. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415**, 141-7.
41. Bouwmeester, T., Bauch, A., Ruffner, H., Angrand, P. O., Bergamini, G., Croughton, K., Cruciat, C., Eberhard, D., Gagneur, J., Ghidelli, S., Hopf, C., Huhse, B., Mangano, R., Michon, A. M., Schirle, M., Schlegl, J., Schwab, M., Stein, M. A., Bauer, A., Casari, G., Drewes, G., Gavin, A. C., Jackson, D. B., Joberty, G., Neubauer, G., Rick, J., Kuster, B. & Superti-Furga, G. (2004). A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. *Nat Cell Biol* **6**, 97-105.
42. Angers, S., Thorpe, C. J., Biechele, T. L., Goldenberg, S. J., Zheng, N., MacCoss, M. J. & Moon, R. T. (2006). The KLHL12-Cullin-3 ubiquitin ligase negatively regulates the Wnt-beta-catenin pathway by targeting Dishevelled for degradation. *Nat Cell Biol* **8**, 348-57.
43. Lui, T. T., Lacroix, C., Ahmed, S. M., Goldenberg, S. J., Leach, C. A., Daulat, A. M. & Angers, S. (2011). The ubiquitin-specific protease USP34 regulates axin stability and Wnt/beta-catenin signaling. *Mol Cell Biol* **31**, 2053-65.
44. Daulat, A. M., Luu, O., Sing, A., Zhang, L., Wrana, J. L., McNeill, H., Winklbauer, R. & Angers, S. (2012). Mink1 regulates beta-catenin-independent Wnt signaling via Prickle phosphorylation. *Mol Cell Biol* **32**, 173-85.
45. Goudreault, M., D'Ambrosio, L. M., Kean, M. J., Mullin, M. J., Larsen, B. G., Sanchez, A., Chaudhry, S., Chen, G. I., Sicheri, F., Nesvizhskii, A. I., Aebersold, R., Raught, B. & Gingras, A. C. (2009). A PP2A phosphatase high density interaction network identifies a novel striatin-interacting phosphatase and kinase complex linked to the cerebral cavernous malformation 3 (CCM3) protein. *Mol Cell Proteomics* **8**, 157-71.
46. Mellacheruvu, D., Wright, Z., Couzens, A. L., Lambert, J. P., St-Denis, N. A., Li, T., Miteva, Y. V., Hauri, S., Sardiu, M. E., Low, T. Y., Halim, V. A., Bagshaw, R. D., Hubner, N. C., Al-Hakim, A., Bouchard, A., Faubert, D., Fermin, D., Dunham, W. H., Goudreault, M., Lin, Z. Y., Badillo, B. G., Pawson, T., Durocher, D., Coulombe, B., Aebersold, R., Superti-Furga, G., Colinge, J., Heck, A. J., Choi, H., Gstaiger, M., Mohammed, S., Cristea, I. M., Bennett, K. L., Washburn, M. P., Raught, B., Ewing, R. M., Gingras, A. C. & Nesvizhskii, A. I. (2013). The CRAPome: a contaminant repository for affinity purification-mass spectrometry data. *Nat Methods* **10**, 730-6.
47. Bouguenina, H., Salaun, D., Mangon, A., Muller, L., Baudelet, E., Camoin, L., Tachibana, T., Cianferani, S., Audebert, S., Verdier-Pinard, P. & Badache, A. (2017). EB1-binding-myomegalin protein complex promotes centrosomal microtubules functions. *Proc Natl Acad Sci U S A* **114**, E10687-E10696.
48. Verdier-Pinard, P., Salaun, D., Bouguenina, H., Shimada, S., Pophillat, M., Audebert, S., Agavnian, E., Coslet, S., Charafe-Jauffret, E., Tachibana, T. & Badache, A. (2017). Septin 9_i2 is

downregulated in tumors, impairs cancer cell migration and alters subnuclear actin filaments. *Sci Rep* **7**, 44976.

49. Audebert, S., Navarro, C., Nourry, C., Chasserot-Golaz, S., Lecine, P., Bellaiche, Y., Dupont, J. L., Premont, R. T., Sempere, C., Strub, J. M., Van Dorsselaer, A., Vitale, N. & Borg, J. P. (2004). Mammalian Scribble forms a tight complex with the betaPIX exchange factor. *Curr Biol* **14**, 987-95.
50. Puvirajesinghe, T. M., Bertucci, F., Jain, A., Scerbo, P., Belotti, E., Audebert, S., Sebbagh, M., Lopez, M., Brech, A., Finetti, P., Charafe-Jauffret, E., Chaffanet, M., Castellano, R., Restouin, A., Marchetto, S., Collette, Y., Goncalves, A., Macara, I., Birnbaum, D., Kodjabachian, L., Johansen, T. & Borg, J. P. (2016). Identification of p62/SQSTM1 as a component of non-canonical Wnt VANGL2-JNK signalling in breast cancer. *Nat Commun* **7**, 10318.
51. Roux, K. J., Kim, D. I., Raida, M. & Burke, B. (2012). A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *J Cell Biol* **196**, 801-10.
52. Kim, D. I., Jensen, S. C., Noble, K. A., Kc, B., Roux, K. H., Motamedchaboki, K. & Roux, K. J. (2016). An improved smaller biotin ligase for BioID proximity labeling. *Mol Biol Cell* **27**, 1188-96.
53. Dong, J. M., Tay, F. P., Swa, H. L., Gunaratne, J., Leung, T., Burke, B. & Manser, E. (2016). Proximity biotinylation provides insight into the molecular composition of focal adhesions at the nanometer scale. *Sci Signal* **9**, rs4.
54. Kanchanawong, P., Shtengel, G., Pasapera, A. M., Ramko, E. B., Davidson, M. W., Hess, H. F. & Waterman, C. M. (2010). Nanoscale architecture of integrin-based cell adhesions. *Nature* **468**, 580-4.
55. Van Itallie, C. M. & Anderson, J. M. (2014). Architecture of tight junctions and principles of molecular composition. *Semin Cell Dev Biol* **36**, 157-65.
56. Van Itallie, C. M., Aponte, A., Tietgens, A. J., Gucek, M., Fredriksson, K. & Anderson, J. M. (2013). The N and C termini of ZO-1 are surrounded by distinct proteins and functional protein networks. *J Biol Chem* **288**, 13775-88.
57. Fredriksson, K., Van Itallie, C. M., Aponte, A., Gucek, M., Tietgens, A. J. & Anderson, J. M. (2015). Proteomic analysis of proteins surrounding occludin and claudin-4 reveals their proximity to signaling and trafficking networks. *PLoS One* **10**, e0117074.
58. Hung, V., Zou, P., Rhee, H. W., Udeshi, N. D., Cracan, V., Svinkina, T., Carr, S. A., Mootha, V. K. & Ting, A. Y. (2014). Proteomic mapping of the human mitochondrial intermembrane space in live cells via ratiometric APEX tagging. *Mol Cell* **55**, 332-41.
59. Rhee, H. W., Zou, P., Udeshi, N. D., Martell, J. D., Mootha, V. K., Carr, S. A. & Ting, A. Y. (2013). Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. *Science* **339**, 1328-1331.
60. Lam, S. S., Martell, J. D., Kamer, K. J., Deerinck, T. J., Ellisman, M. H., Mootha, V. K. & Ting, A. Y. (2015). Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nat Methods* **12**, 51-4.
61. Mick, D. U., Rodrigues, R. B., Leib, R. D., Adams, C. M., Chien, A. S., Gygi, S. P. & Nachury, M. V. (2015). Proteomics of Primary Cilia by Proximity Labeling. *Dev Cell* **35**, 497-512.
62. Chen, C. L., Hu, Y., Udeshi, N. D., Lau, T. Y., Wirtz-Peitz, F., He, L., Ting, A. Y., Carr, S. A. & Perrimon, N. (2015). Proteomic mapping in live Drosophila tissues using an engineered ascorbate peroxidase. *Proc Natl Acad Sci U S A* **112**, 12093-8.
63. Lobingier, B. T., Huttenhain, R., Eichel, K., Miller, K. B., Ting, A. Y., von Zastrow, M. & Krogan, N. J. (2017). An Approach to Spatiotemporally Resolve Protein Interaction Networks in Living Cells. *Cell* **169**, 350-360 e12.
64. Paek, J., Kalocsay, M., Staus, D. P., Wingler, L., Pascolutti, R., Paulo, J. A., Gygi, S. P. & Kruse, A. C. (2017). Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling. *Cell* **169**, 338-349 e11.

65. Loh, K. H., Stawski, P. S., Draycott, A. S., Udeshi, N. D., Lehrman, E. K., Wilton, D. K., Svinkina, T., Deerinck, T. J., Ellisman, M. H., Stevens, B., Carr, S. A. & Ting, A. Y. (2016). Proteomic Analysis of Unbounded Cellular Compartments: Synaptic Clefts. *Cell* **166**, 1295-1307 e21.
66. Kemphues, K. (2000). PARsing embryonic polarity. *Cell* **101**, 345-8.
67. Kemphues, K. J., Priess, J. R., Morton, D. G. & Cheng, N. S. (1988). Identification of genes required for cytoplasmic localization in early C. elegans embryos. *Cell* **52**, 311-20.
68. Goldstein, B. & Macara, I. G. (2007). The PAR Proteins: Fundamental Players in Animal Cell Polarization. *Developmental Cell* **13**, 609-622.
69. Huo, Y. & Macara, I. G. (2014). The Par3-like polarity protein Par3L is essential for mammary stem cell maintenance. *Nat Cell Biol* **16**, 529-37.
70. Izumi, Y., Hirose, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kemphues, K. J. & Ohno, S. (1998). An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of Caenorhabditis elegans polarity protein PAR-3. *J Cell Biol* **143**, 95-106.
71. Brajenovic, M., Joberty, G., Kuster, B., Bouwmeester, T. & Drewes, G. (2004). Comprehensive proteomic analysis of human Par protein complexes reveals an interconnected protein network. *J Biol Chem* **279**, 12804-11.
72. Martin, A. C., Kaschube, M. & Wieschaus, E. F. (2009). Pulsed contractions of an actin-myosin network drive apical constriction. *Nature* **457**, 495-9.
73. Traweger, A., Wiggin, G., Taylor, L., Tate, S. A., Metalnikov, P. & Pawson, T. (2008). Protein phosphatase 1 regulates the phosphorylation state of the polarity scaffold Par-3. *Proc Natl Acad Sci U S A* **105**, 10402-7.
74. Benton, R. & St Johnston, D. (2003). Drosophila PAR-1 and 14-3-3 inhibit Bazooka/PAR-3 to establish complementary cortical domains in polarized cells. *Cell* **115**, 691-704.
75. Hurd, T. W., Fan, S., Liu, C. J., Kweon, H. K., Hakansson, K. & Margolis, B. (2003). Phosphorylation-dependent binding of 14-3-3 to the polarity protein Par3 regulates cell polarity in mammalian epithelia. *Curr Biol* **13**, 2082-90.
76. Baas, A. F., Kuipers, J., van der Wel, N. N., Batlle, E., Koerten, H. K., Peters, P. J. & Clevers, H. C. (2004). Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell* **116**, 457-66.
77. Sebbagh, M., Santoni, M. J., Hall, B., Borg, J. P. & Schwartz, M. A. (2009). Regulation of LKB1/STRAD localization and function by E-cadherin. *Curr Biol* **19**, 37-42.
78. Lee, J. H., Koh, H., Kim, M., Kim, Y., Lee, S. Y., Karess, R. E., Lee, S. H., Shong, M., Kim, J. M., Kim, J. & Chung, J. (2007). Energy-dependent regulation of cell structure by AMP-activated protein kinase. *Nature* **447**, 1017-20.
79. Wingo, S. N., Gallardo, T. D., Akbay, E. A., Liang, M. C., Contreras, C. M., Boren, T., Shimamura, T., Miller, D. S., Sharpless, N. E., Bardeesy, N., Kwiatkowski, D. J., Schorge, J. O., Wong, K. K. & Castrillon, D. H. (2009). Somatic LKB1 mutations promote cervical cancer progression. *PLoS One* **4**, e5137.
80. Baas, A. F., Boudeau, J., Sapkota, G. P., Smit, L., Medema, R., Morrice, N. A., Alessi, D. R. & Clevers, H. C. (2003). Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. *EMBO J* **22**, 3062-72.
81. Boudeau, J., Baas, A. F., Deak, M., Morrice, N. A., Kieloch, A., Schutkowski, M., Prescott, A. R., Clevers, H. C. & Alessi, D. R. (2003). MO25alpha/beta interact with STRADalpha/beta enhancing their ability to bind, activate and localize LKB1 in the cytoplasm. *EMBO J* **22**, 5102-14.
82. Boudeau, J., Deak, M., Lawlor, M. A., Morrice, N. A. & Alessi, D. R. (2003). Heat-shock protein 90 and Cdc37 interact with LKB1 and regulate its stability. *Biochem J* **370**, 849-57.
83. Nony, P., Gaude, H., Rossel, M., Fournier, L., Rouault, J. P. & Billaud, M. (2003). Stability of the Peutz-Jeghers syndrome kinase LKB1 requires its binding to the molecular chaperones Hsp90/Cdc37. *Oncogene* **22**, 9165-75.

84. Jurgens, G., Wieschaus, E., Nusslein-Volhard, C. & Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle inDrosophila melanogaster : II. Zygotic loci on the third chromosome. *Wilehm Roux Arch Dev Biol* **193**, 283-295.
85. Tepass, U., Theres, C. & Knust, E. (1990). crumbs encodes an EGF-like protein expressed on apical membranes of Drosophila epithelial cells and required for organization of epithelia. *Cell* **61**, 787-99.
86. Bulgakova, N. A. & Knust, E. (2009). The Crumbs complex: from epithelial-cell polarity to retinal degeneration. *Journal of Cell Science* **122**, 2587-2596.
87. Bachmann, A., Schneider, M., Theilenberg, E., Grawe, F. & Knust, E. (2001). Drosophila Stardust is a partner of Crumbs in the control of epithelial cell polarity. *Nature* **414**, 638-43.
88. Hong, Y., Stronach, B., Perrimon, N., Jan, L. Y. & Jan, Y. N. (2001). Drosophila Stardust interacts with Crumbs to control polarity of epithelia but not neuroblasts. *Nature* **414**, 634-8.
89. Krahn, M. P., Buckers, J., Kastrup, L. & Wodarz, A. (2010). Formation of a Bazooka-Stardust complex is essential for plasma membrane polarity in epithelia. *J Cell Biol* **190**, 751-60.
90. Morais-de-Sa, E., Mirouse, V. & St Johnston, D. (2010). aPKC phosphorylation of Bazooka defines the apical/lateral border in Drosophila epithelial cells. *Cell* **141**, 509-23.
91. Walther, R. F. & Pichaud, F. (2010). Crumbs/DaPKC-dependent apical exclusion of Bazooka promotes photoreceptor polarity remodeling. *Curr Biol* **20**, 1065-74.
92. Wodarz, A., Hinz, U., Engelbert, M. & Knust, E. (1995). Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of Drosophila. *Cell* **82**, 67-76.
93. Laprise, P., Beronja, S., Silva-Gagliardi, N. F., Pellikka, M., Jensen, A. M., McGlade, C. J. & Tepass, U. (2006). The FERM protein Yurt is a negative regulatory component of the Crumbs complex that controls epithelial polarity and apical membrane size. *Dev Cell* **11**, 363-74.
94. Medina, E., Williams, J., Klipfell, E., Zarnescu, D., Thomas, G. & Le Bivic, A. (2002). Crumbs interacts with moesin and beta(Heavy)-spectrin in the apical membrane skeleton of Drosophila. *J Cell Biol* **158**, 941-51.
95. Varelas, X., Samavarchi-Tehrani, P., Narimatsu, M., Weiss, A., Cockburn, K., Larsen, B. G., Rossant, J. & Wrana, J. L. (2010). The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-beta-SMAD pathway. *Dev Cell* **19**, 831-44.
96. Chang, Y., Klezovitch, O., Walikonis, R. S., Vasioukhin, V. & LoTurco, J. J. (2010). Discs large 5 is required for polarization of citron kinase in mitotic neural precursors. *Cell Cycle* **9**, 1990-7.
97. Campbell, C. I., Samavarchi-Tehrani, P., Barrios-Rodiles, M., Datti, A., Gingras, A. C. & Wrana, J. L. (2016). The RNF146 and tankyrase pathway maintains the junctional Crumbs complex through regulation of angiomotin. *J Cell Sci* **129**, 3396-411.
98. Zhao, B., Li, L., Lu, Q., Wang, L. H., Liu, C. Y., Lei, Q. & Guan, K. L. (2011). Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein. *Genes Dev* **25**, 51-63.
99. Bilder, D., Li, M. & Perrimon, N. (2000). Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. *Science* **289**, 113-6.
100. Bilder, D., Schober, M. & Perrimon, N. (2003). Integrated activity of PDZ protein complexes regulates epithelial polarity. *Nat Cell Biol* **5**, 53-8.
101. Albertson, R. & Doe, C. Q. (2003). Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry. *Nat Cell Biol* **5**, 166-70.
102. Huang, L., Kinnucan, E., Wang, G., Beaudenon, S., Howley, P. M., Huibregtse, J. M. & Pavletich, N. P. (1999). Structure of an E6AP-UbcH7 complex: insights into ubiquitination by the E2-E3 enzyme cascade. *Science* **286**, 1321-6.
103. Nakagawa, S. & Huibregtse, J. M. (2000). Human scribble (Vartul) is targeted for ubiquitin-mediated degradation by the high-risk papillomavirus E6 proteins and the E6AP ubiquitin-protein ligase. *Mol Cell Biol* **20**, 8244-53.
104. Lim, K. Y. B., Godde, N. J., Humbert, P. O. & Kvansakul, M. (2017). Structural basis for the differential interaction of Scribble PDZ domains with the guanine nucleotide exchange factor beta-PIX. *J Biol Chem* **292**, 20425-20436.

105. Frank, S. R., Bell, J. H., Frodin, M. & Hansen, S. H. (2012). A betaPIX-PAK2 complex confers protection against Scrib-dependent and cadherin-mediated apoptosis. *Curr Biol* **22**, 1747-54.
106. Lahuna, O., Quellari, M., Achard, C., Nola, S., Meduri, G., Navarro, C., Vitale, N., Borg, J. P. & Misrahi, M. (2005). Thyrotropin receptor trafficking relies on the hScrib-betaPIX-GIT1-ARF6 pathway. *EMBO J* **24**, 1364-74.
107. Takizawa, S., Nagasaka, K., Nakagawa, S., Yano, T., Nakagawa, K., Yasugi, T., Takeuchi, T., Kanda, T., Huibregtse, J. M., Akiyama, T. & Taketani, Y. (2006). Human scribble, a novel tumor suppressor identified as a target of high-risk HPV E6 for ubiquitin-mediated degradation, interacts with adenomatous polyposis coli. *Genes Cells* **11**, 453-64.
108. Anastas, J. N., Biechele, T. L., Robitaille, M., Muster, J., Allison, K. H., Angers, S. & Moon, R. T. (2012). A protein complex of SCRIB, NOS1AP and VANGL1 regulates cell polarity and migration, and is associated with breast cancer progression. *Oncogene* **31**, 3696-708.
109. Cordenonsi, M., Zanconato, F., Azzolin, L., Forcato, M., Rosato, A., Frasson, C., Inui, M., Montagner, M., Parenti, A. R., Poletti, A., Daidone, M. G., Dupont, S., Basso, G., Bicciato, S. & Piccolo, S. (2011). The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* **147**, 759-72.
110. Young, L. C., Hartig, N., Munoz-Alegre, M., Oses-Prieto, J. A., Durdu, S., Bender, S., Vijayakumar, V., Vietri Rudan, M., Gewinner, C., Henderson, S., Jathoul, A. P., Ghatrora, R., Lythgoe, M. F., Burlingame, A. L. & Rodriguez-Viciana, P. (2013). An MRAS, SHOC2, and SCRIB complex coordinates ERK pathway activation with polarity and tumorigenic growth. *Mol Cell* **52**, 679-92.
111. Osmani, N., Vitale, N., Borg, J. P. & Etienne-Manneville, S. (2006). Scrib controls Cdc42 localization and activity to promote cell polarization during astrocyte migration. *Curr Biol* **16**, 2395-405.
112. Michaelis, U. R., Chavakis, E., Kruse, C., Jungblut, B., Kaluza, D., Wandzioch, K., Manavski, Y., Heide, H., Santoni, M. J., Potente, M., Eble, J. A., Borg, J. P. & Brandes, R. P. (2013). The polarity protein Scrib is essential for directed endothelial cell migration. *Circ Res* **112**, 924-34.
113. Nola, S., Sebbagh, M., Marchetto, S., Osmani, N., Nourry, C., Audebert, S., Navarro, C., Rachel, R., Montcouquiol, M., Sans, N., Etienne-Manneville, S., Borg, J. P. & Santoni, M. J. (2008). Scrib regulates PAK activity during the cell migration process. *Hum Mol Genet* **17**, 3552-65.
114. Chen, B., Zheng, B., DeRan, M., Jarugumilli, G. K., Fu, J., Brooks, Y. S. & Wu, X. (2016). ZDHHC7-mediated S-palmitoylation of Scribble regulates cell polarity. *Nat Chem Biol* **12**, 686-93.
115. Legouis, R., Jaulin-Bastard, F., Schott, S., Navarro, C., Borg, J. P. & Labouesse, M. (2003). Basolateral targeting by leucine-rich repeat domains in epithelial cells. *EMBO Rep* **4**, 1096-102.
116. Navarro, C., Nola, S., Audebert, S., Santoni, M. J., Arsanto, J. P., Ginestier, C., Marchetto, S., Jacquemier, J., Isnardon, D., Le Bivic, A., Birnbaum, D. & Borg, J. P. (2005). Junctional recruitment of mammalian Scribble relies on E-cadherin engagement. *Oncogene* **24**, 4330-9.
117. Feigin, M. E., Akshinthala, S. D., Araki, K., Rosenberg, A. Z., Muthuswamy, L. B., Martin, B., Lehmann, B. D., Berman, H. K., Pietenpol, J. A., Cardiff, R. D. & Muthuswamy, S. K. (2014). Mislocalization of the cell polarity protein scribble promotes mammary tumorigenesis and is associated with basal breast cancer. *Cancer Res* **74**, 3180-94.
118. Humbert, P. O., Grzeschik, N. A., Brumby, A. M., Galea, R., Elsum, I. & Richardson, H. E. (2008). Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module. *Oncogene* **27**, 6888-907.
119. Zhan, L., Rosenberg, A., Bergami, K. C., Yu, M., Xuan, Z., Jaffe, A. B., Allred, C. & Muthuswamy, S. K. (2008). Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma. *Cell* **135**, 865-78.

120. Dow, L. E., Elsum, I. A., King, C. L., Kinross, K. M., Richardson, H. E. & Humbert, P. O. (2008). Loss of human Scribble cooperates with H-Ras to promote cell invasion through deregulation of MAPK signalling. *Oncogene* **27**, 5988-6001.
121. Hawkins, E. D., Oliaro, J., Ramsbottom, K. M., Newbold, A., Humbert, P. O., Johnstone, R. W. & Russell, S. M. (2016). Scribble acts as an oncogene in Emu-myc-driven lymphoma. *Oncogene* **35**, 1193-7.
122. Kuo, J. C., Han, X., Hsiao, C. T., Yates, J. R., 3rd & Waterman, C. M. (2011). Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for beta-Pix in negative regulation of focal adhesion maturation. *Nat Cell Biol* **13**, 383-93.
123. Horton, E. R., Byron, A., Askari, J. A., Ng, D. H., Millon-Fremillon, A., Robertson, J., Koper, E. J., Paul, N. R., Warwood, S., Knight, D., Humphries, J. D. & Humphries, M. J. (2015). Definition of a consensus integrin adhesome and its dynamics during adhesion complex assembly and disassembly. *Nat Cell Biol* **17**, 1577-87.
124. Schiller, H. B., Friedel, C. C., Boulegue, C. & Fassler, R. (2011). Quantitative proteomics of the integrin adhesome show a myosin II-dependent recruitment of LIM domain proteins. *EMBO Rep* **12**, 259-66.
125. Schiller, H. B., Hermann, M. R., Polleux, J., Vignaud, T., Zanivan, S., Friedel, C. C., Sun, Z., Raducanu, A., Gottschalk, K. E., Thery, M., Mann, M. & Fassler, R. (2013). beta1- and alphav-class integrins cooperate to regulate myosin II during rigidity sensing of fibronectin-based microenvironments. *Nat Cell Biol* **15**, 625-36.
126. Gubb, D. & Garcia-Bellido, A. (1982). A genetic analysis of the determination of cuticular polarity during development in Drosophila melanogaster. *J Embryol Exp Morphol* **68**, 37-57.
127. Chiu, C. W., Monat, C., Robitaille, M., Lacomme, M., Daulat, A. M., Macleod, G., McNeill, H., Cayouette, M. & Angers, S. (2016). SAPCD2 Controls Spindle Orientation and Asymmetric Divisions by Negatively Regulating the Galphai-LGN-NuMA Ternary Complex. *Dev Cell* **36**, 50-62.
128. Zhang, L., Luga, V., Armitage, S. K., Musiol, M., Won, A., Yip, C. M., Plotnikov, S. V. & Wrana, J. L. (2016). A lateral signalling pathway coordinates shape volatility during cell migration. *Nat Commun* **7**, 11714.
129. Paricio, N., Feiguin, F., Boutros, M., Eaton, S. & Mlodzik, M. (1999). The Drosophila STE20-like kinase misshapen is required downstream of the Frizzled receptor in planar polarity signaling. *EMBO J* **18**, 4669-78.
130. Lee, R. H., Iioka, H., Ohashi, M., Iemura, S., Natsume, T. & Kinoshita, N. (2007). XRab40 and XCullin5 form a ubiquitin ligase complex essential for the noncanonical Wnt pathway. *EMBO J* **26**, 3592-606.
131. Daulat, A. M., Bertucci, F., Audebert, S., Serge, A., Finetti, P., Josselin, E., Castellano, R., Birnbaum, D., Angers, S. & Borg, J. P. (2016). PRICKLE1 Contributes to Cancer Cell Dissemination through Its Interaction with mTORC2. *Dev Cell* **37**, 311-25.
132. Lim, B. C., Matsumoto, S., Yamamoto, H., Mizuno, H., Kikuta, J., Ishii, M. & Kikuchi, A. (2016). Prickle1 promotes focal adhesion disassembly in cooperation with the CLASP-LL5beta complex in migrating cells. *J Cell Sci* **129**, 3115-29.
133. Daulat, A. M. & Borg, J. P. (2016). When mTORC2-AKT signaling meets cell polarity. *Cell Cycle*, 1-2.
134. Zhang, L. & Wrana, J. L. (2016). Regulation of Rho GTPases from the lateral sides of migrating cells. *Small GTPases*, 1-4.
135. Montcouquiol, M., Rachel, R. A., Lanford, P. J., Copeland, N. G., Jenkins, N. A. & Kelley, M. W. (2003). Identification of Vangl2 and Scrb1 as planar polarity genes in mammals. *Nature* **423**, 173-7.
136. Sun, T., Yang, L., Kaur, H., Pestel, J., Looso, M., Nolte, H., Krasel, C., Heil, D., Krishnan, R. K., Santoni, M. J., Borg, J. P., Bunemann, M., Offermanns, S., Swiercz, J. M. & Worzfeld, T. (2017). A reverse signaling pathway downstream of Sema4A controls cell migration via Scrib. *J Cell Biol* **216**, 199-215.

137. Bastock, R., Strutt, H. & Strutt, D. (2003). Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during Drosophila planar polarity patterning. *Development* **130**, 3007-14.
138. Rawls, A. S. & Wolff, T. (2003). Strabismus requires Flamingo and Prickle function to regulate tissue polarity in the Drosophila eye. *Development* **130**, 1877-87.
139. Jenny, A., Darken, R. S., Wilson, P. A. & Mlodzik, M. (2003). Prickle and Strabismus form a functional complex to generate a correct axis during planar cell polarity signaling. *EMBO J* **22**, 4409-20.
140. Temkin, P., Lauffer, B., Jager, S., Cimermancic, P., Krogan, N. J. & von Zastrow, M. (2011). SNX27 mediates retromer tubule entry and endosome-to-plasma membrane trafficking of signalling receptors. *Nat Cell Biol* **13**, 715-21.
141. Belotti, E., Puvirajesinghe, T. M., Audebert, S., Baudelet, E., Camoin, L., Pierres, M., Lasvaux, L., Ferracci, G., Montcouquiol, M. & Borg, J. P. (2012). Molecular characterisation of endogenous Vangl2/Vangl1 heteromeric protein complexes. *PLoS One* **7**, e46213.
142. Lu, X., Borchers, A. G., Jolicoeur, C., Rayburn, H., Baker, J. C. & Tessier-Lavigne, M. (2004). PTK7/CCK-4 is a novel regulator of planar cell polarity in vertebrates. *Nature* **430**, 93-8.
143. Lhoumeau, A. C., Puppo, F., Prebet, T., Kodjabachian, L. & Borg, J. P. (2011). PTK7: a cell polarity receptor with multiple facets. *Cell Cycle* **10**, 1233-6.
144. Wehner, P., Shnitsar, I., Urlaub, H. & Borchers, A. (2011). RACK1 is a novel interaction partner of PTK7 that is required for neural tube closure. *Development* **138**, 1321-7.
145. Li, S., Esterberg, R., Lachance, V., Ren, D., Radde-Gallwitz, K., Chi, F., Parent, J. L., Fritz, A. & Chen, P. (2011). Rack1 is required for Vangl2 membrane localization and planar cell polarity signaling while attenuating canonical Wnt activity. *Proc Natl Acad Sci U S A* **108**, 2264-9.
146. Reiter, J. F. & Leroux, M. R. (2017). Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol Cell Biol* **18**, 533-547.
147. Ostrowski, L. E., Blackburn, K., Radde, K. M., Moyer, M. B., Schlatzer, D. M., Moseley, A. & Boucher, R. C. (2002). A proteomic analysis of human cilia: identification of novel components. *Mol Cell Proteomics* **1**, 451-65.
148. Liu, Q., Tan, G., Levenkova, N., Li, T., Pugh, E. N., Jr., Rux, J. J., Speicher, D. W. & Pierce, E. A. (2007). The proteome of the mouse photoreceptor sensory cilium complex. *Mol Cell Proteomics* **6**, 1299-317.
149. Ishikawa, H., Thompson, J., Yates, J. R., 3rd & Marshall, W. F. (2012). Proteomic analysis of mammalian primary cilia. *Curr Biol* **22**, 414-9.
150. Jakobsen, L., Vanselow, K., Skogs, M., Toyoda, Y., Lundberg, E., Poser, I., Falkenby, L. G., Bennetzen, M., Westendorf, J., Nigg, E. A., Uhlen, M., Hyman, A. A. & Andersen, J. S. (2011). Novel asymmetrically localizing components of human centrosomes identified by complementary proteomics methods. *EMBO J* **30**, 1520-35.
151. Borovina, A., Superina, S., Voskas, D. & Ciruna, B. (2010). Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia. *Nat Cell Biol* **12**, 407-12.
152. May-Simera, H. L., Petralia, R. S., Montcouquiol, M., Wang, Y. X., Szarama, K. B., Liu, Y., Lin, W., Deans, M. R., Pazour, G. J. & Kelley, M. W. (2015). Ciliary proteins Bbs8 and Ift20 promote planar cell polarity in the cochlea. *Development* **142**, 555-66.
153. Omori, Y., Zhao, C., Saras, A., Mukhopadhyay, S., Kim, W., Furukawa, T., Sengupta, P., Veraksa, A. & Malicki, J. (2008). Elipsa is an early determinant of ciliogenesis that links the IFT particle to membrane-associated small GTPase Rab8. *Nat Cell Biol* **10**, 437-44.
154. Liew, G. M., Ye, F., Nager, A. R., Murphy, J. P., Lee, J. S., Aguiar, M., Breslow, D. K., Gygi, S. P. & Nachury, M. V. (2014). The intraflagellar transport protein IFT27 promotes BBSome exit from cilia through the GTPase ARL6/BBS3. *Dev Cell* **31**, 265-78.
155. Soderberg, O., Gullberg, M., Jarvius, M., Ridderstrale, K., Leuchowius, K. J., Jarvius, J., Wester, K., Hydbring, P., Bahram, F., Larsson, L. G. & Landegren, U. (2006). Direct observation of individual endogenous protein complexes in situ by proximity ligation. *Nat Methods* **3**, 995-1000.

156. Smith, M. A., Hall, R., Fisher, K., Haake, S. M., Khalil, F., Schabath, M. B., Vuaroqueaux, V., Fiebig, H. H., Altiok, S., Chen, Y. A. & Haura, E. B. (2015). Annotation of human cancers with EGFR signaling-associated protein complexes using proximity ligation assays. *Sci Signal* **8**, ra4.
157. Roncagalli, R., Cucchetti, M., Jarmuzynski, N., Gregoire, C., Bergot, E., Audebert, S., Baudelet, E., Menoita, M. G., Joachim, A., Durand, S., Suchanek, M., Fiore, F., Zhang, L., Liang, Y., Camoin, L., Malissen, M. & Malissen, B. (2016). The scaffolding function of the RLTPR protein explains its essential role for CD28 co-stimulation in mouse and human T cells. *J Exp Med* **213**, 2437-2457.
158. Bar, D. Z., Atkatsh, K., Tavarez, U., Erdos, M. R., Gruenbaum, Y. & Collins, F. S. (2018). Biotinylation by antibody recognition-a method for proximity labeling. *Nat Methods* **15**, 127-133.
159. Kim, D. I., Cutler, J. A., Na, C. H., Reckel, S., Renuse, S., Madugundu, A. K., Tahir, R., Goldschmidt, H. L., Reddy, K. L., Huganir, R. L., Wu, X., Zachara, N. E., Hantschel, O. & Pandey, A. (2018). BioSITe: A Method for Direct Detection and Quantitation of Site-Specific Biotinylation. *J Proteome Res* **17**, 759-769.
160. Udeshi, N. D., Pedram, K., Svinkina, T., Fereshetian, S., Myers, S. A., Aygun, O., Krug, K., Clauser, K., Ryan, D., Ast, T., Mootha, V. K., Ting, A. Y. & Carr, S. A. (2017). Antibodies to biotin enable large-scale detection of biotinylation sites on proteins. *Nat Methods* **14**, 1167-1170.
161. Xie, J., Han, M., Zhang, M., Deng, H. & Wu, W. (2018). PP5 (PPP5C) is a phosphatase of Dvl2. *Sci Rep* **8**, 2715.
162. Yoshihara, K., Ikenouchi, J., Izumi, Y., Akashi, M., Tsukita, S. & Furuse, M. (2011). Phosphorylation state regulates the localization of Scribble at adherens junctions and its association with E-cadherin-catenin complexes. *Exp Cell Res* **317**, 413-22.
163. Hardmanmm, G., Perkins, S., Ruan, Z., Kannan, N., Brownridge, P., Byrne, D. P., Eyers, P. A., Jones, A. R. & Eyers, C., E. (2017). Extensive non-canonical phosphorylation in human cells revealed using strong-anion exchange-mediated phosphoproteomics. In *bioRxiv preprint*. bioRxiv preprint.

Figure legends

Figure 1: Key features of the epithelial polarity program

A) Schematic representation of the Apico-Basal Polarity (ABP) and Planar Cell Polarity (PCP) processes. B) Subcellular localization of prototypic ABP proteins, Crumb, PAR and SCRIB protein complexes, indicating their reciprocal inhibition. C) Subcellular localization of PCP proteins in the *Drosophila* wing epithelium showing their asymmetric localization at either the proximal or distal cell membranes.

Figure 2: Main steps of MS sample preparation and analysis

Complex protein samples derived from tissues or cultured cell lines are fractionated and purified for mass spectrometry analysis. Proteins can be subjected to separation with acrylamide gels or processed directly (shotgun proteomics). Enzymatic digestion can be used to generate peptides, which can be separated and individually analyzed using the mass spectrometer. Search engines are used to analyze the generated data for each peptide and compared to information available from different protein databases, which allows the identification of proteins.

Figure 3: Key findings from MS-based studies revealing the underlying cellular mechanisms of action of the PAR protein complex

Subcellular localization of PAR-4/LKB1 and PAR-3, members of the PAR protein complex. MO25 is needed to fully activate LKB1 and for its interaction with either STRAD-α or STRAD-β and consequent signaling activity. PAR-4/LKB1 activity is stabilized following interaction with CDC37 and HSP90. Proteomic approaches have identified the PAR-4/LKB1 complex in the primary cilia of epithelial cells. The PAR-3 complex is localized at the apical side of epithelial cells and is phosphorylated by PAR-1 which in turn liberates aPKC and promotes 14-3-3 binding. PP1 dephosphorylates PAR-3 and restores PAR-3 association with aPKC.

Figure 4: Role of the SCRIB complex in epithelial polarity and cell migration

Basolaterally-localized SCRIB is associated with the highlighted proteins and acts to decrease AKT, HIPPO and ERK signaling. In migrating cells, SCRIB is localized at

the leading edge of migrating cells and promotes activation of small G-proteins (RAC1, CDC42 or ARF6) in order to enhance cell motility.

Figure 5: Mass spectrometry-based analysis of the SCRIB protein complex

Schematic representation of the result of successive proteomics approaches used to identify protein complexes associated with SCRIB. Each dashed circle encompasses proteins identified by different experimental approaches. Cross-validation of known SCRIB-associated proteins has been possible as well as the elucidation of novel components of the SCRIB protein complex.

Figure 6: Mechanistic insights into the roles of VANGL and PRICKLE1 during cancer cell progression

VANGL-1/-2 and PRICKLE1 are localized at the leading edge of migratory cancer cells. Their association with various downstream associated proteins allows them to contribute to cell migration by decreasing RhoA activation, increasing AKT signaling and remodeling focal adhesions. VANGL2 associates with p62/SQSTM1 in endosomes to activate JNK signaling.

Table

Table 1:

Experimental Approach	*Main features*	*Major limitations*
Peptide Pull-down	Based on chemically synthesized peptides; allows the purification of protein complexes from diverse origins (cultured cells or tissues); current method of choice for determining protein-protein interactions; different immobilization techniques are applicable (covalent interactions using biotinylation and non-covalent interactions employing 6x His or GST-tags).	Solubility and size render certain synthetic peptides incompatible with this experimental procedure.
Tandem Affinity Purification	Successfully applied in the purification of membrane protein complexes; associated with low levels of non-specific protein binding.	Potential spatial interference or non-specific protein binding with protein tags (false positives may be detected); lengthy two-step purification protocol may lead to the loss of low affinity proteins.
Single-Step purification	Successfully applied in the purification of membrane and cytosolic protein complexes; straight-forward one-step purification protocol.	Potential spatial interference or non-specific binding with protein tag (false positives may be detected); high rate of background protein binding.
Endogenous Immunoprecipitation	Purification of endogenous complexes is possible; associated with a low rate of false positives.	Reliance on high-affinity antibodies and good expression levels of endogenous proteins.
BioID	Applied to purify proteins in the neighbourhood of a protein of interest; successfully used to identify low-affinity protein-protein interactions.	Protein identification may include proteins in the close spatial neighbourhood of the given protein of interest and not necessarily strongly associated with the protein of interest; protein labelling can occur during all stages of protein turnover of the given protein of interest.
APEX	Used to purify proteins in the neighbourhood of a protein of interest; used to identify low affinity protein-protein interactions; purification of proteins deriving from a specific subcellular compartment is possible; spatiotemporal identification of protein complexes is possible.	Protein identification may include proteins in the close spatial neighbourhood of the given protein of interest and not necessarily associated with the protein of interest.

Figure1

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FIGURE 1: Key features of the epithelial polarity programm

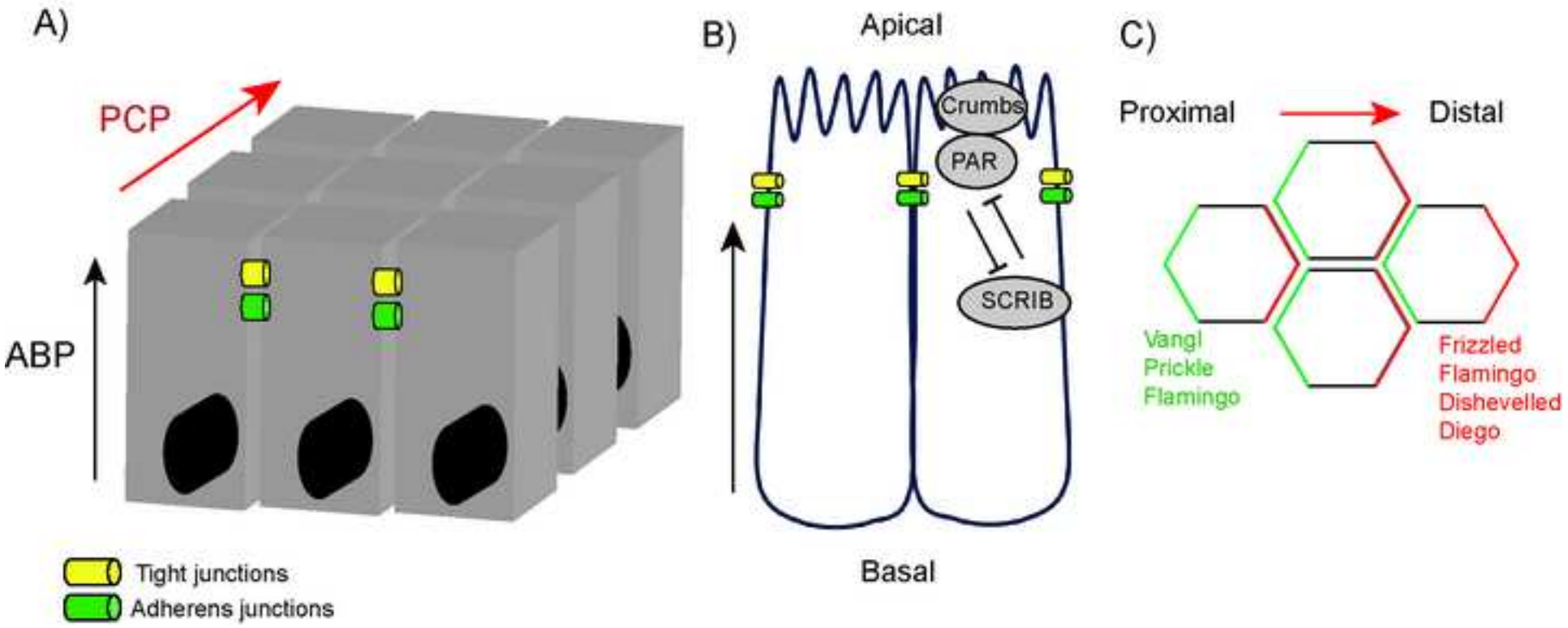

Figure2

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FIGURE 2: Pipeline of mass spectrometry sample preparation

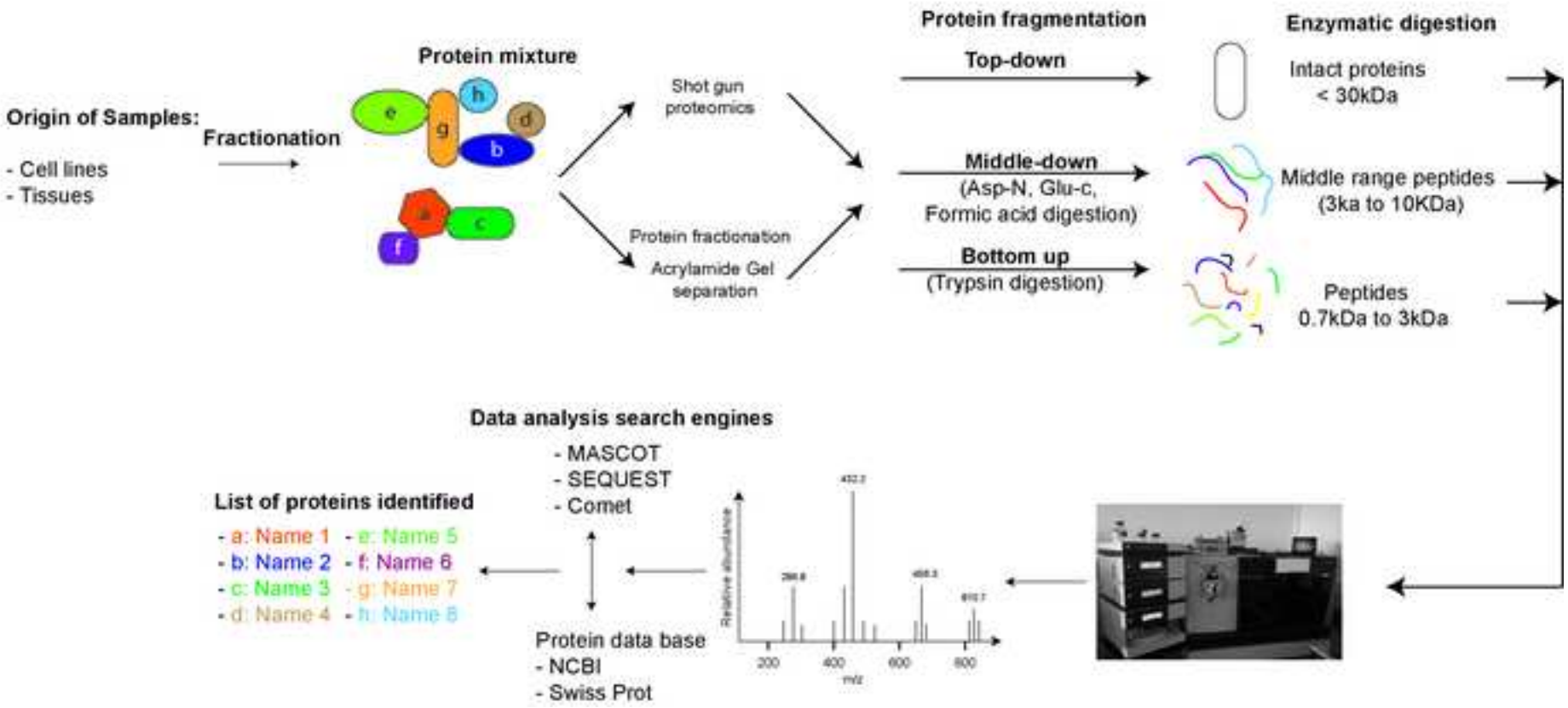

Figure3

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FIGURE 3: Key findings from MS-based studies revealing the underlying cellular mechanisms of the PAR protein complex

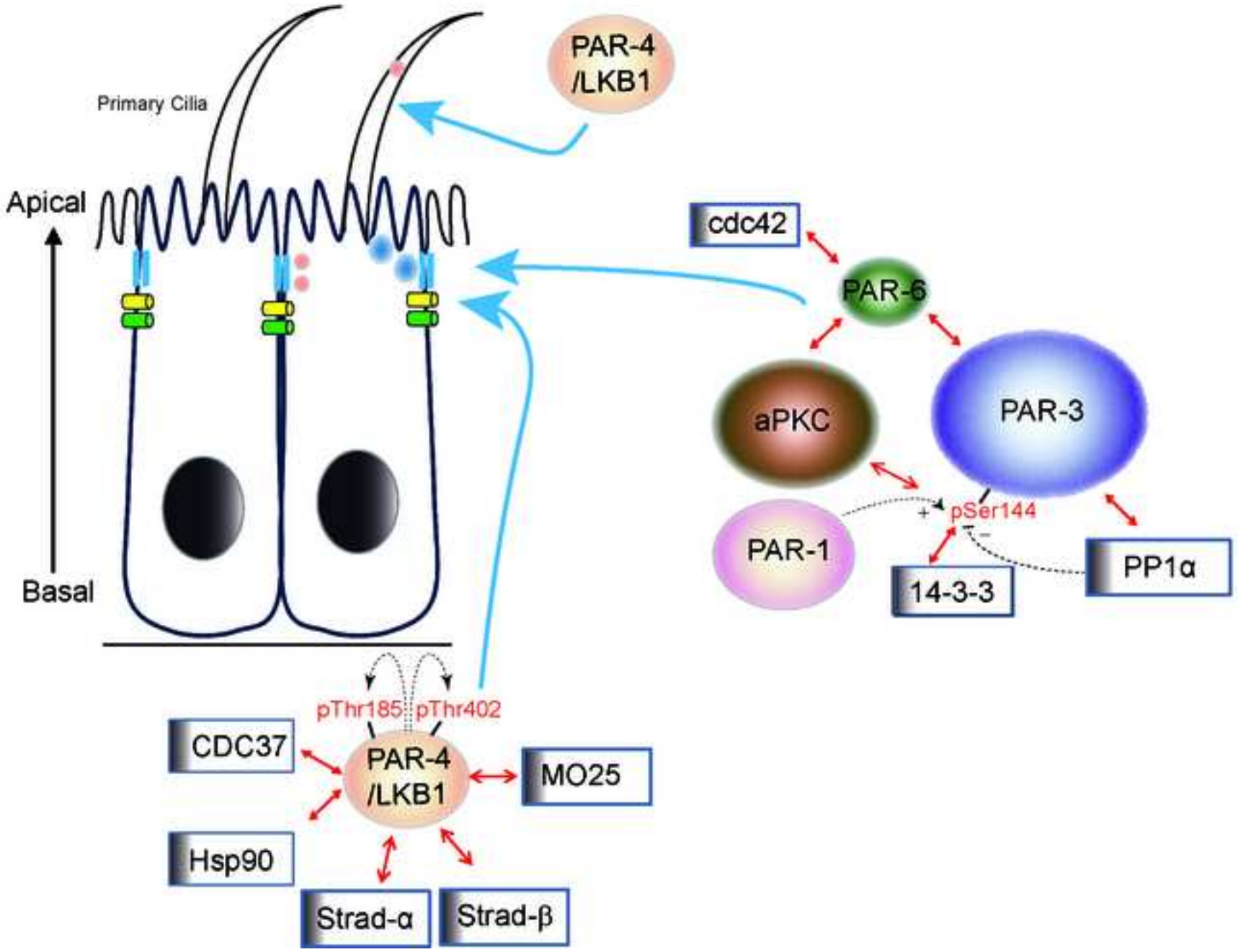

Figure4

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FIGURE 4: Role of the SCRIB complex in epithelial polarity and cell migration

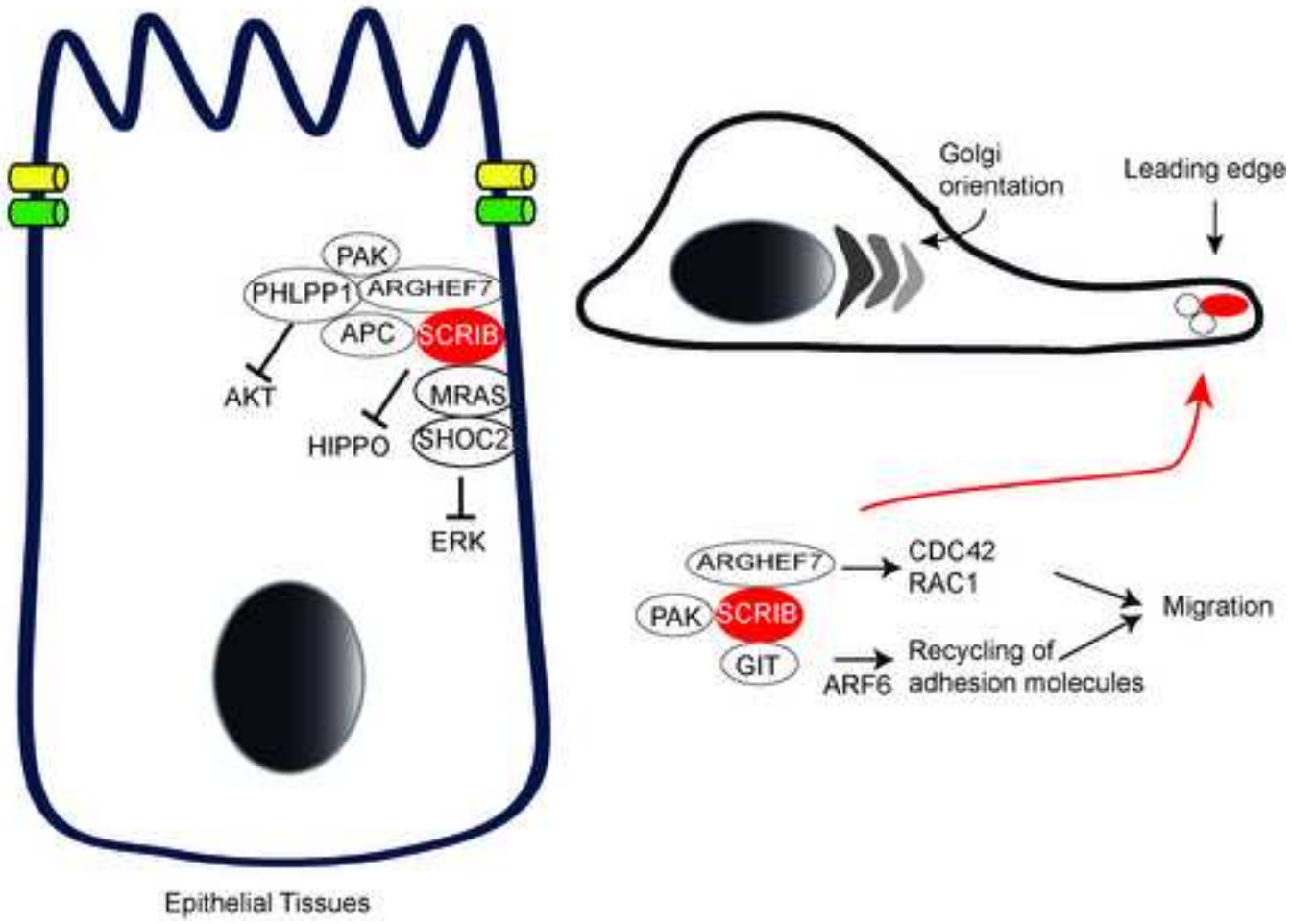

Figure5

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FIGURE 5: Mass spectrometry-based analysis of the SCRIB protein complex

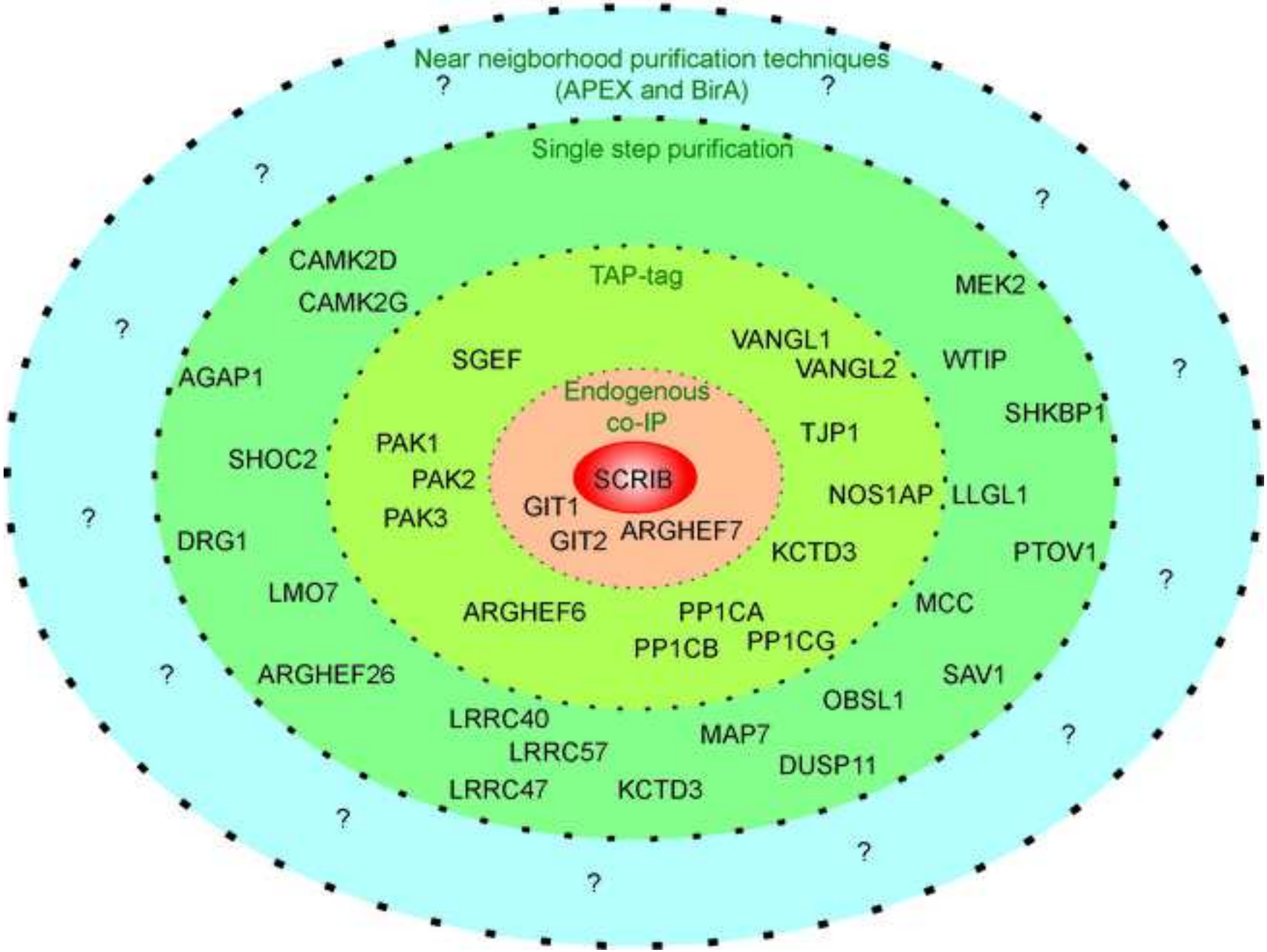

Figure6

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FIGURE 6: Mechanistic insights into the roles of VANGL and PRICKLE1 during cancer cell progression