

## Coordinated protein sorting, targeting and distribution in polarized cells

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**Abstract** | The polarized distribution of functions in polarized cells requires the coordinated interaction of three machineries that modify the basic mechanisms of intracellular protein trafficking and distribution. First, intrinsic protein-sorting signals and cellular decoding machineries regulate protein trafficking to plasma membrane domains; second, intracellular signalling complexes define the plasma membrane domains to which proteins are delivered; and third, proteins that are involved in cell–cell and cell–substrate adhesion orientate the three-dimensional distribution of intracellular signalling complexes and, accordingly, the direction of membrane traffic. The integration of these mechanisms into a complex and dynamic network is crucial for normal tissue function and is often defective in disease states.

### Epithelial–mesenchymal transition

Phenotypic and functional changes in epithelial cells, usually associated with the loss of cell–cell adhesion and increased cell migration, as cells are induced to become fibroblasts.

Cell polarity is a structural and functional specialization that is ubiquitous in biology. The commonality of polarity across the phyla reflects a fundamental requirement of individuals to localize different activities to distinct regions of cells, especially when individual cells come together to form complex multicellular tissues. The specialized domains of the plasma membrane that result from polarization determine cell orientation, function and fate. For example, polarization enables long-range communication by neurons and short-range communication in the immune system, vectorial transport of ions across epithelial cells and niche-specific orientation of stem-cell division, which specifies the developmental fate of daughter cells.

At first glance, the fact that different cell types exhibit diverse polarized phenotypes implies that a diverse array of specialized machineries has evolved. However, it seems that simple variations of common mechanistic themes result in the unique shapes, asymmetries and functions that characterize polarized cells and tissues. First, intrinsic protein-sorting codes are recognized and segregated by cytoplasmic adaptor complexes that regulate protein trafficking to plasma membrane domains. Second, signalling complexes and scaffolds become differentially associated with the cytosolic face of the membrane, where they define and stabilize the biochemical features of resulting domains. Third, adhesion receptors that detect neighbouring cells and the extracellular matrix (ECM) provide cues that orientate cells in three-dimensional (3D) space.

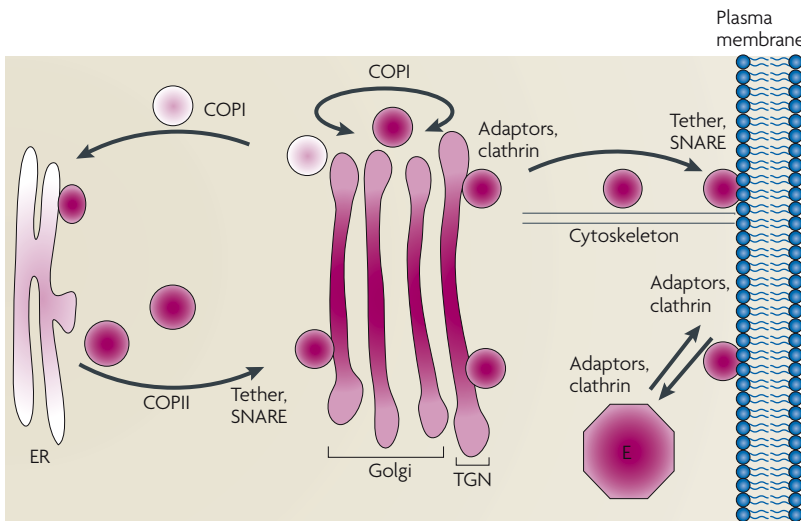
Two considerations support the idea that cell polarity is achieved through the integration of these three conserved molecular mechanisms. First, all eukaryotic cells

share common cellular machineries for post-translational protein trafficking and compartmentalization<sup>1</sup>. Second, cells can adopt different shapes and functions in response to specific physiological contexts. For example, during embryogenesis, a single cell can change its shape and function as it migrates, according to morphogenetic gradients, and can then repolarize on detecting transcriptionally specified cell–cell interactions<sup>2</sup>. In disease states, such as cancer, epithelial cells lose polarity (through epithelial–mesenchymal transition (EMT)), disengage from multicellular interactions, migrate and then reintegrate into a second tissue, in which they undergo structural and functional reorganization to reside at the new site<sup>3,4</sup>. Thus, the dynamics and plasticity of the loss and re-establishment of polarity suggest that common machineries of membrane traffic are used at all times, but are deployed differently depending on the physiological context.

Here, we summarize the basic cellular machineries and biochemical rules that control the delivery of protein components to different plasma membrane domains in a generic polarized cell. We then describe the spatial cues and signalling pathways that organize these basic machineries to produce various cell shapes and functions. Finally, we consider how component asymmetry at the single-cell level is orientated in 3D space to define polarity in complex tissues. We emphasize how these different pathways generate plasticity in the forms of cell polarity, and how defects underlie important pathological states. Although much has been learnt about each of these three mechanisms in isolation, understanding how they are integrated and coordinated into a network remains a central challenge — one that is fundamental

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**Figure 1 | A generic post-translational pathway for protein trafficking to the plasma membrane.** After synthesis in the endoplasmic reticulum (ER), membrane proteins are sorted into vesicles by the coatomer protein complex-II (COPII) machinery and delivered to the Golgi complex by vesicle-tethering and SNARE machineries. Intra-Golgi transport and retrograde transport from the Golgi to the ER are regulated by the COPI machinery. At the *trans*-Golgi network (TGN), proteins are sorted into vesicles by intrinsic sorting motifs and cytoplasmic adaptor complexes, and are transported along cytoskeletal elements to the plasma membrane. Protein delivery to the plasma membrane is mediated by vesicle-tethering and SNARE machineries. Some proteins (for example, ligand-receptor complexes) are internalized through another set of adaptors and delivered to an endosome (E), from which they might be recycled back to the plasma membrane.

membrane curvature<sup>8</sup>. Regardless of how or where transport vesicles are formed, vesicle trafficking between compartments is mediated by the actin and microtubule cytoskeleton<sup>9</sup>, and vesicle fusion with the target plasma membrane is regulated by organelle-specific Rab GTPases, vesicle-tethering complexes<sup>10</sup> and SNAREs<sup>5</sup>. These generic mechanisms are the core machineries of trafficking in all cells, and are modified in polarized cells to sort proteins into separate plasma membrane domains. Below, we focus on how machineries that are involved in the Golgi-plasma membrane-endosome pathways control membrane-protein sorting to different plasma membrane domains in polarized cells.

**Protein sorting in the exocytic and endocytic pathways.** There are three major sites of protein sorting in the exocytic and endocytic pathways: the Golgi complex, the plasma membrane and endosomes (FIG. 1). At each site, proteins can be sorted into separate vesicle carriers on the basis of intrinsic sorting signals and the cellular machineries that recognize those signals. A major component of protein sorting at all three sites is provided by the AP-complex family, which not only recognizes protein-sorting signals but also regulates the assembly of clathrin scaffolds, which sculpt the membrane to form vesicles.

Four AP complexes (AP-1-4) have been identified, and these localize to different membrane compartments between the Golgi complex, the plasma membrane and endosomes<sup>11</sup>. Each AP complex is composed of two large subunits ( $\alpha$ ,  $\gamma$ ,  $\epsilon$ ,  $\delta$  or  $\beta 1$ - $\beta 4$ ; ~100 kDa each), one small subunit ( $\sigma 1$ - $\sigma 4$ ; ~20 kDa) and one medium subunit ( $\mu 1$ - $\mu 4$ ; ~50 kDa). Together with other proteins (such as GGA, AP180 (assembly protein of 180 kDa), epsin-1, epsin-2, EPS15,  $\beta$ -arrestin and ARH) that interact with clathrin, the AP subunits recognize and bind specific amino-acid motifs in the cytoplasmic domain of membrane proteins and cluster these proteins into patches on the membrane by assembling a clathrin cage<sup>8,11</sup>. One of the best-studied interactions is between the AP-2-clathrin complex and the transferrin receptor (TfnR), which occurs on the plasma membrane. The  $\mu$  subunit of AP-2 ( $\mu 2$ ) recognizes a degenerate tetrapeptide cytoplasmic domain sorting motif (YXX $\emptyset$ ; in which  $\emptyset$  represents any hydrophobic amino-acid residue), resulting in the clustering of TfnR into clathrin-coated pits on the plasma membrane<sup>12</sup>. These coated pits then bud into the cytoplasm and are delivered to early endosomes. This sorting motif can be recognized in endosomes, probably by the AP-1-clathrin complex, and the receptor is recycled back to the plasma membrane by an endosome-derived vesicle population<sup>11</sup> (FIG. 2).

In general, protein recycling from the endosome to the plasma membrane can be signal dependent or signal independent. It is difficult to distinguish these two mechanisms in non-polarized cells, which exhibit a single, non-differentiated plasma membrane. However, the situation is different in polarized cells. For example, in epithelial cells and neurons, TfnR and other well-characterized recycling receptors (such as low-density lipoprotein receptor (LDLR)) are localized to a specialized domain of the plasma membrane: the basolateral membrane in epithelia

to understanding organogenesis, tissue function and various pathological states.

**Controlling protein sorting and trafficking**

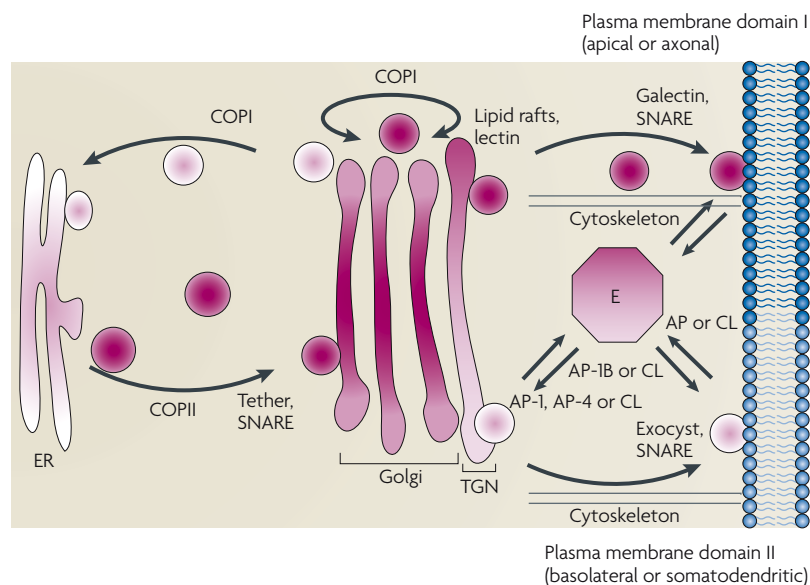
The generic post-translational pathway of membrane-protein trafficking to the plasma membrane involves the sequential transport of proteins between different membrane compartments (for example, the endoplasmic reticulum (ER), the Golgi complex, endosomes and the plasma membrane<sup>1</sup>). Membrane proteins are modified in these membrane compartments, usually through the assembly and processing of complex oligosaccharide chains (FIG. 1). Transport is mediated by vesicular intermediates that bud from one compartment and fuse with the next. Genetics and *in vitro* reconstitution experiments have identified three classes of protein complexes that form these vesicular intermediates<sup>3-7</sup>: coatomer protein complex-II (COPII) complexes are crucial for ER-Golgi trafficking; COPI complexes are crucial for Golgi-ER trafficking and intra-Golgi transport; and the adaptor protein (AP)-clathrin complex (the AP-clathrin complex) is crucial for transport between the Golgi, the plasma membrane and endosomes. Certain proteins of each complex recognize and selectively recruit different cargo proteins into transport vesicles (non-recognized proteins are excluded from vesicles), whereas other complex proteins deform and sculpt the membrane to produce the membrane vesicle or a tubule. The AP-clathrin complex also has accessory proteins that modulate the basic functions of the complex, increasing the complexity of cargo-selection strategies and affecting

**COPII**  
A specific coat protein complex that initiates the vesicle budding process from the endoplasmic reticulum.

**COPI**  
A specific coat protein complex that initiates the vesicle budding process from membranes of the Golgi complex, and is involved in intra-Golgi and Golgi-to-endoplasmic reticulum vesicle trafficking.

**AP-clathrin complex**  
A complex of proteins that comprises adaptor proteins and structural clathrin (which forms a coat that initiates vesicle budding from membranes).

**Rab GTPases**  
A large family of Ras-like GTPases that have key roles in the secretory and endocytic pathways.



**Figure 2 | Superimposing protein sorting demands on the generic trafficking pathway.** In polarized cells, such as epithelia and neurons, protein processing occurs along the generic pathway between the endoplasmic reticulum (ER) and the trans-Golgi network (TGN; see also FIG. 1). However, proteins in the TGN might be sorted into various different vesicles through the recognition of different intrinsic sorting motifs (TABLE 1) and cytoplasmic adaptor complexes. These vesicles are then targeted either directly or indirectly, through an endosome (E), to different plasma membrane domains (designated as domains I and II) along cytoskeletal elements. These cytoskeletal elements might have different orientations (or polarity) relative to the different membrane domains. Vesicle delivery to each plasma membrane domain is mediated by different vesicle-tethering and SNARE complexes. Some proteins are internalized through another set of adaptors and delivered to an endosome, from which they might be recycled back to the original plasma membrane domain, or to the other domain by transcytosis, depending on the presence (or activation or inactivation) of specific protein-sorting motifs. AP, adaptor protein; CL, clathrin; COP, coatomer protein complex.

**Vesicle-tethering complex**  
A large protein complex that localizes to various sites of vesicle delivery in the secretory pathway and facilitates the capture, docking and fusion of specific vesicles with different membranes (for example, the exocyst complex at the plasma membrane).

**SNARE**  
[Soluble N-ethyl-maleimide-sensitive fusion protein attachment-protein receptor]. These proteins comprise a large protein superfamily and mediate the fusion of transport vesicles with membranes.

**Transferrin receptor**  
A membrane protein that binds soluble transferrin, which is required for the cellular import of iron.

and the somatodendritic membrane in neurons<sup>13,14</sup>. In such cases, the recognition of specific targeting information in the cytoplasmic domain of TfnR is essential to maintain the polarized localization of the receptor to the appropriate domain.

**Polarity sorting motifs and adaptors for basolateral proteins.** In polarized cells, cytoplasmic domain-sorting signals are not only required for endocytosis, they are also required for the polarized delivery of newly synthesized receptors and the maintenance of recycling receptors in their appropriate membrane domains<sup>15,16</sup> (FIG. 2). Canonical internalization signals have a role in polarized endocytosis and recycling, and they are assisted by additional sorting signals and decoding adaptor complexes. LDLR is a well-studied example. It has two Tyr-based sorting motifs — a membrane-proximal motif and a C-terminal motif — in its cytoplasmic domain, and these are required for post-Golgi delivery and the maintenance of LDLR to the basolateral or somatodendritic domains<sup>13,14</sup>. The membrane-proximal motif is important for LDLR endocytosis, and although the C-terminal motif is not required for endocytosis, it encodes a signal for sorting to the basolateral or

somatodendritic domains<sup>13,14</sup>. Many other basolateral membrane proteins (TABLE 1) have Tyr-based targeting motifs<sup>16</sup>, although diLeu-based signals have also been identified (diLeu motifs can also be responsible for clathrin-mediated endocytosis)<sup>17</sup>. Other motifs, such as the sorting motif in TfnR<sup>18</sup>, seem to be devoid of crucial Tyr or Leu residues<sup>18,19</sup>.

The observation that many basolateral proteins have a Tyr- or diLeu-based motif for basolateral polarity has long suggested that AP-clathrin complexes are important in one or more steps in specifying protein trafficking in polarized cells. The only AP complex to be identified to date that is definitively associated with basolateral protein trafficking is an epithelial cell-specific variant of the clathrin-associated AP-1 complex (designated AP-1B), in which the ubiquitously expressed  $\mu 1A$  subunit is replaced by a closely related  $\mu 1B$  subunit<sup>20,21</sup>. AP-1B clearly functions to determine the polarity of Tyr-based signals (and some non-Tyr signals, such as that in TfnR), as the absence of  $\mu 1B$  leads to a non-polarized or apically localized protein, a similar effect to that of eliminating the sorting motif. However, it is unlikely that all such signals are decoded by AP-1B. DiLeu-based signals, for example, programme basolateral polarity, but in an AP-1B-independent manner<sup>20,21</sup> (TABLE 1). A second AP complex, AP-4, also has a role in basolateral targeting, although its cargo and signal specificity remain poorly defined<sup>22</sup>.

AP-1B is not expressed in all polarized cells, indicating that other adaptors or protein-sorting pathways exist. Many neurons, for example, do not express  $\mu 1B$ , but protein transport to the somatodendritic plasma membrane domain involves the same targeting signals that are used for transport to the basolateral membrane in most epithelial cells<sup>14</sup>. Hepatocytes also do not express  $\mu 1B$ , but they use the same Tyr-based signals on basolateral proteins (such as LDLR) as  $\mu 1B$ -positive epithelial cells<sup>23</sup>. The identities of these alternative basolateral adaptors remain elusive. Additionally, do AP-1B or any alternative adaptor proteins work in conjunction with clathrin? This is possible, but the removal of clathrin by small interfering (si)RNA has a surprising range of effects on basolateral membrane-protein sorting<sup>24</sup>. Proteins such as VSVG (the major surface-coat protein of the stomatitis virus, which is a Tyr-dependent protein), NCAM (neural cell adhesion molecule, which is Tyr-independent) and CD147 (which is Leu dependent) are delivered in a 1:2 ratio to the basolateral and apical membranes, whereas others are delivered in a 2:1 ratio (TfnR, which is Tyr independent), a 3:1 ratio (epithelial (E)-cadherin, which is diLeu dependent) or are unaffected ( $Na^+/K^+$ -ATPase). By comparison, deletion of both basolateral sorting motifs in LDLR results in the almost complete mistargeting of mutant LDLR to the apical membrane<sup>13</sup>. The observation that some proteins are less affected than others by clathrin knockdown further indicates that there is a degree of redundancy and overlap in sorting signals and decoding elements, only some of which are likely to be clathrin dependent. For example, ankyrins are adaptor proteins that bind to a subclass of membrane proteins (such as E-cadherin and  $Na^+/K^+$ -ATPase) and to the cytoskeletal protein spectrin<sup>25</sup>. Importantly, decreased expression of

Table 1 | **Intrinsic protein sorting codes and the cellular decoding machinery\***

| Protein   | Protein code                     | Decoding machinery |
|---|----------------------------------|--------------------|
| <i>Protein sorting pathway A (apical plasma membrane and axonal membrane)</i>               |                                  |                    |
| Occludin and ERGIC53  | N-linked glycosylation           | Unknown (lectin?)  |
| p75, sucrase-isomaltase and podocalyxin (or GP135)  | O-linked glycosylation           | Unknown (lectin?)  |
| PLAP, THY1 and DAF  | GPI-anchor (oligomerization)     | Lipid raft         |
| Haemagglutinin and neuraminidase  | Transmembrane domain             | Lipid raft         |
| Rhodopsin and Na <sup>+</sup> -dependent bile acid transporter                              | Cytoplasmic domain               | Unknown            |
| Podocalyxin (or GP135)  | Cytoplasmic PDZ domain           | Unknown            |
| <i>Protein sorting pathway B (basolateral plasma membrane and somatodendritic membrane)</i> |                                  |                    |
| LDLR, TfnR, VSVG and AGPR-HI  | Cytoplasmic domain (Tyr based)   | AP-1B              |
| ErB2  | Unknown                          | AP-1B              |
| H <sup>+</sup> /K <sup>+</sup> -ATPase  | Cytoplasmic domain (Tyr based)   | Unknown            |
| FcII B2R  | Cytoplasmic domain (diLeu based) | Unknown            |
| E-cadherin  | Juxtamembrane 40 amino acids     | Ankyrin            |
| CD147   | Cytoplasmic domain (Leu based)   | Unknown            |
| Na <sup>+</sup> /K <sup>+</sup> -ATPase, poly-IgAR and NCAM                                 | Cytoplasmic domain               | Unknown            |

\*Two generic sorting pathways are described that could represent the apical or axonal (pathway A) and basolateral or somatodendritic (pathway B) plasma membrane domains of polarized cells. See the main text for experimental evidence that these proteins belong to different sorting pathways, and for selected references. AP, adaptor protein; E-cadherin, epithelial cadherin; GPI, glycosyl phosphoinositol; LDLR, low-density lipoprotein receptor; NCAM, neural cell adhesion molecule.

**Basolateral membrane**

A domain of the plasma membrane that comprises the basal and lateral membranes. It is orientated towards cell-extracellular matrix (basal membrane) and cell-cell contacts (lateral membrane).

**Somatodendritic membrane**

A part of neuronal cells that comprises the dendritic membranes and soma and excludes the axon.

**Apical membrane**

A domain of the plasma membrane in polarized epithelial cells that is usually orientated on the luminal side of epithelial tubes (for example, the intestine).

**Ankyrin**

A large adaptor protein that was originally found in erythrocytes but is ubiquitously expressed in nucleated cells. Ankyrin binds to various membrane proteins, spectrin and actin-binding proteins.

**Spectrin**

A large protein that comprises subunits (α and β) that form a heterotetramer, (αβ)<sub>2</sub>. Spectrin binds to ankyrin and to the actin cytoskeleton.

**Sucrase-isomaltase**

A glycosidase that comprises activities of a sucrase and an isomaltase. Sucrase-isomaltase is found in the apical membrane of intestinal epithelial cells.

**Glycosyl phosphoinositol**

A modification at the C terminus of membrane proteins that occurs in the endoplasmic reticulum. This modification allows the protein to insert into the outer leaflet of the lipid bilayer.

**Lipid raft**

A membrane subdomain that is enriched in glycosphingolipids, sphingomyelin and cholesterol.

**Transcytosis**

The delivery of transport vesicles between the apical and basolateral membrane domains of polarized cells.

ankyrin B or ankyrin G reduces E-cadherin trafficking from the Golgi to the basolateral membrane<sup>26</sup>. Further studies are required to uncover additional mechanisms and pathways of protein sorting and trafficking.

**Polarized sorting of apical proteins.** The sorting motifs and the cellular machineries that sort proteins to the apical membranes of epithelial cells are different from those that sort proteins to basolateral membranes. In contrast to the cytoplasm-orientated basolateral sorting motif, apical sorting motifs are localized in the extracellular or transmembrane domains of proteins<sup>27</sup> (TABLE 1). Extracellular domain motifs contain N- and O-linked oligosaccharide chains (such as those found in p75 (REF. 28) and sucrase-isomaltase<sup>29</sup>), although it is unclear whether these oligosaccharide chains are directly recognized by sorting machinery or whether they control protein conformation and hence the display of a sorting motif in the amino-acid backbone of the protein.

The membrane-associated signal can be the transmembrane domain itself (as occurs in some viral glycoproteins, such as haemagglutinin and neuraminidase), but the best described signal is the glycosyl phosphoinositol (GPI) lipid anchor<sup>30</sup>. GPI-anchored proteins are sorted into the apical pathway in the Golgi complex. This occurs by GPI-anchored protein oligomerization in lipid rafts<sup>31</sup> that are enriched in glycosphingolipids, sphingomyelin and cholesterol<sup>30</sup>. Interestingly, large clusters of GPI-anchored proteins fail to form in a lectin-resistant Madin-Darby Canine Kidney (MDCK) cell line that missorts GPI-anchored proteins. This suggests that carbohydrates are involved in this clustering event and are necessary for apical sorting<sup>32</sup>. Several other proteins in lipid rafts might have roles in lipid-raft synthesis (such

as FAPP2 (REF. 33)) and stabilization (such as MAL<sup>30</sup>), and in clustering of apical proteins (such as the lipid-raft protein galectin-4 (REF. 34)). It is unknown, however, how apical protein clustering, by lipid rafts or any other mechanism, leads to deformation of the membrane and to the formation of a transport vesicle that can deliver its cargo to the apical plasma membrane.

In general, the cytoplasmic domain of apical proteins is not thought to be important in protein trafficking. The protein rhodopsin, however, is targeted to the apical membrane of photoreceptors by a sorting determinant that is localized to its cytoplasmic C terminus<sup>35</sup>. This sorting determinant interacts with dynein<sup>36</sup>. Importantly, missorting of rhodopsin leads to retinitis pigmentosa (BOX 1).

Basolateral (and somatodendritic) sorting signals are often 'dominant' over apical sorting signals. In other words, if both a functional basolateral and an apical signal are present, basolateral targeting will ensue. Transcytosis of membrane proteins from the basolateral to apical domains can occur if the basolateral signal is inactivated after newly synthesized proteins are included into basolaterally directed transport vesicles (FIG. 2); transcytosis involves endocytosis from one membrane domain, delivery to endosomes, resorting and trafficking to the other membrane domain. For example, the adhesion-signalling protein neuronal-gial (Ng)-CAM has a Tyr-containing, AP-1B-dependent basolateral targeting signal that is inactivated by phosphorylation on reaching the basolateral surface. In the absence of a functional basolateral signal, Ng-CAM is not captured by AP-1B in endosomes after internalization and is instead transcytosed to the apical surface<sup>37</sup>. Variations on this simple theme probably explain other examples of transcytosis (such as the extensively studied polymeric Ig receptor<sup>38</sup>).



Box 1 | When cell polarity goes awry

Various disease states (see table) show that cells can dissolve or reorganize the cellular machinery that generates polarized distributions of proteins<sup>4</sup>. This process can occur at the three layers of regulation of cell polarity that are discussed in the main text.

Mutations in the intrinsic protein-sorting motifs that sort proteins to specific membrane domains are found in genetic disorders of major organs, such as the kidney, lungs, intestine and nervous system<sup>125,131</sup>. These mutations can affect protein transport through the secretory pathway, and can cause missorting of proteins to the wrong membrane domain or cause loss of protein localization at the plasma membrane. In these cases, overall polarity might not be affected, but the key role of the mutated protein in a biological process can result in the loss of cell function.

Mutations in key components that detect extrinsic orientational cues have strong effects on cell organization and function. Many of these proteins are tumour suppressors, including phosphatase and tensin homologue (PTEN)<sup>127</sup>, and many oncogenic mutations activate phosphoinositide 3-kinase (PI3K) or AKT/protein kinase B (PKB) directly. Changes in the distributions of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P<sub>3</sub>) and phosphatidylinositol-3,4-bisphosphate (PtdIns(3,4)P<sub>2</sub>) might have additional effects on the structure and function of cells. Scribble, lethal giant larvae (LGL) and discs large (DLG) were isolated as neoplastic tumour-suppressor mutations in *Drosophila melanogaster*, based on the distinctive 'giant larvae' phenotype of zygotic mutant animals<sup>132</sup>. Little is known about their roles in human disease, but the loss or decreased expression of human homologues of Scribble and LGL have been found in colorectal cancers and malignant melanoma<sup>128</sup>.

Changes in the orientational cues that specify cell polarity occur in a form of reversed cell polarity, termed epithelial-mesenchymal transition (EMT). EMT has an essential role in embryogenesis and homeostasis (during morphogenetic movements, epidermal wound healing and migration of neutrophils in chemotactic gradients) and in cancer-cell metastasis<sup>3</sup>. EMT is characterized by decreased expression of epithelial (E)-cadherin (which is mediated by the expression of transcriptional repressors such as SNAIL, ZEB and some basic helix-loop-helix factors<sup>133</sup>), the loss of cell-cell adhesion and epithelial characteristics, and altered cell-extracellular matrix (ECM) interactions, including ECM remodelling by secreted metalloproteinases and increased cell migration. It is conceivable that the 'forward reaction' can be re-initiated when a metastatic cell reaches the appropriate distal tissue site or niche, resulting in a partly differentiated tumour<sup>3</sup>.

| Disease   | Protein   | Defect  |
|---|---|---|
| Bartter syndrome* (REF. 123)                            | ROMK  | Missorted to apical membrane  |
|   | CICK  | Missorted to basolateral membrane   |
| Liddle syndrome* (REF. 124)                             | βENaC   | Recycling on apical membrane  |
| Cystic fibrosis <sup>125</sup>                          | CFTR (ΔF508)  | ER exit   |
|   | CFTR (C-terminal PDZ)   | Golgi exit  |
| Congenital sucrase-isomaltase deficiency <sup>126</sup> | Sucrase-isomaltase  | Missorted to basolateral membrane   |
| Familial hypercholesterolaemia <sup>23</sup>            | LDLR (FH-Turku)   | Missorted to the apical membrane  |
| Polycystic kidney disease <sup>†</sup>                  | Polycystin-1 and -2   | Growth or mitotic defects; protein mislocalization (for example, Na <sup>+</sup> /K <sup>+</sup> -ATPase, EGFR)   |
| <i>Situs inversus</i> <sup>‡</sup>                      | Left-right dynein   | Cilium motility   |
| Bardet-Biedl syndrome <sup>‡</sup>                      | BBS4, BBS5 and BBS8   | Unknown (protein transport to cilium?)  |
| Nephronophthisis <sup>‡</sup>                           | NPHP1-4   | Unknown   |
| Retinitis pigmentosa <sup>‡</sup>                       | Kinesin-2 (IFT)   | Rhodopsin transport into cilium   |
| Cardiac arrhythmia <sup>25</sup>                        | Ankyrin B   | T-tubule organization of Na <sup>+</sup> /K <sup>+</sup> -ATPase, Na <sup>+</sup> /Ca <sup>2+</sup> exchanger and IP3R complex; stress-induced sudden cardiac death |
| Brugada syndrome <sup>25</sup>                          | Voltage-gated Na <sup>+</sup> channel (E1053K mutation in the ankyrin-G binding site) | Loss of plasma membrane localization, ventricular arrhythmia and sudden cardiac death   |
| CRASH syndrome <sup>25</sup>                            | L1 cell adhesion molecule (S1224L and Y1229H mutations in the ankyrin-B binding site) | Loss of axon and dendrite localization, corpus callosum hypoplasia, retardation and aphasia   |
| Oncogenesis   | PTEN and PI3K <sup>127</sup>  | Activates the AKT/PKB signalling pathway (promotes cell survival, proliferation and growth)   |
|   | Scribble and LGL <sup>128</sup>   | Decreased expression; function is unknown but the defect is found in colorectal cancer and malignant melanoma   |
| Retinitis pigmentosa <sup>129</sup>                     | Rhodopsin   | Missorting to photoreceptor apical membrane   |
| Peutz-Jeghers syndrome <sup>130</sup>                   | LKB1 (PAR4)   | Mutations; overgrowth of cells in the gastrointestinal tract (hamartomas)   |

\*Bartter syndrome and Liddle syndrome contain defects in Na<sup>+</sup> transport. †Polycystic kidney disease, *situs inversus*, Bardet-Biedl syndrome, nephronophthisis and retinitis pigmentosa are ciliopathies<sup>92</sup>. ER, endoplasmic reticulum; LDLR, low-density lipoprotein receptor; PAR4, partitioning-defective-4.

**Sorting in the trans-Golgi network and recycling endosomes.** The sorting of plasma membrane proteins to their appropriate destinations seems to be dependent on the general core mechanisms found in the endocytic and secretory pathways, but protein sorting is subject to slight modifications in polarized cells. In all cells, the endocytic and secretory pathways possess multiple sorting sites. In most cells, however, the sorting sites that are most relevant for generating and maintaining polarity — the *trans*-Golgi network (TGN) and recycling endosomes — are found in the perinuclear cytoplasm (FIG. 2). It is highly likely that signals for apical or basolateral sorting are recognized at either or both of these sites, with the TGN mediating the sorting of newly synthesized proteins in the secretory pathway and recycling endosomes responsible for polarized recycling or transcytosis after endocytosis<sup>37,38</sup>. It also seems likely that newly synthesized membrane proteins could pass through recycling endosomes after leaving the Golgi and before reaching the surface<sup>39</sup>. Thus, recycling endosomes might be a common site for protein sorting; perhaps adaptor complexes in these endosomes select cargo for inclusion in transport vesicles that are destined for specific plasma membrane domains.

#### Getting to, and staying in, membrane domains

Sorting proteins during exit from the Golgi or recycling endosomes is one level of adaptive machinery for generating different protein distributions in polarized cells. However, molecular sorting must be coupled to mechanisms that specify vesicle delivery and fusion to different plasma membrane domains. These mechanisms involve the cytoskeleton, for long-range vesicle delivery, and (at short range) protein complexes at target plasma membrane domains that specify the docking and fusion of vesicles.

**The cytoskeleton in vesicle trafficking.** Most polarized cells have specialized organizations of actin and microtubule cytoskeletons. Meshworks of short actin filaments are localized underneath the entire plasma membrane and might provide linkages through the ankyrin–spectrin complex to membrane proteins<sup>25</sup>. The actin cytoskeleton might have several roles in cell polarity and protein trafficking<sup>40</sup> as it is regulated by CDC42. CDC42 is involved at several crucial control points that regulate cell polarity (see below), including myosin motor proteins, which could regulate vesicle trafficking, and the ankyrin–spectrin complex. Indeed, ankyrin knockdown results in the loss of lateral membrane surface area<sup>41</sup>, perhaps by decreasing protein trafficking from the Golgi (see above) or by structurally destabilizing the basolateral membrane in a manner that is analogous to spectrin deletion in erythrocytes.

Microtubule arrays are normally organized in fibroblasts with the dynamic plus ends at the cortex and the minus ends near the centrosomes, close to the nucleus. In polarized cells, microtubules are generally bundled along the long axis of the cell (apico-basal in epithelia, soma–axon in neurons) with all

the plus ends at the base of the cell or end of the axon, respectively<sup>42,43</sup>. Microtubules seem to regulate the efficiency rather than the fidelity of vesicle delivery to the apical and basolateral membrane in epithelial cells<sup>44</sup>. Plus-end microtubule kinesin motors are involved in apical protein delivery (for example, the delivery of the p75 protein, but not of GPI-anchored proteins)<sup>45</sup>, and are generally involved in the targeting of basolateral<sup>46</sup> and axonal membrane proteins<sup>47</sup> (see the review by Li and Gunderson in this issue).

#### Controlling vesicle fusion at plasma membrane domains.

The fusion of vesicles with the correct membrane domains is a crucial step in the generation of polarity. Two interconnected mechanisms regulate the initial tethering of vesicles to the membrane and then the fusion of vesicles with the target-membrane domain. Members of the Rab family of small GTPases seem to control many stages of vesicle docking and fusion, especially by having a role in tethering vesicles to their target membranes<sup>10</sup>. Vesicle-tethering complexes are found at many stages of the exocytic pathway and are thought to increase the efficiency and specificity of vesicle delivery (FIG. 2). At the plasma membrane, the exocyst complex seems to regulate the docking of a subset of vesicles, including basolateral vesicles in epithelial cells<sup>48</sup>, neurite outgrowths and axonal synapse-assembly domains<sup>49</sup>. The exocyst is a large complex of at least six proteins, some of which are localized on the target plasma membrane and others of which are localized to the transport vesicle, along with a Rab family GTPase that helps to control tethering-complex assembly (RAB8 on basolateral vesicles of epithelia; RAB3 on synaptic vesicles)<sup>50,51</sup>. This suggests that vesicle docking at the plasma membrane might occur through the assembly of an exocyst holocomplex. Another type of vesicle-tethering complex comprises annexins and is present on other plasma membranes, such as the apical membrane of epithelial cells. Annexins bind to membranes in a phosphatidylinositol-3,4-bisphosphate (PtdIns(3,4)P<sub>2</sub>)-dependent and Ca<sup>2+</sup>-dependent manner and self-aggregate. As with components of the exocyst complex, annexins might be present on both vesicles and target membranes<sup>52</sup>. The delivery of some apical proteins in epithelial cells (for example, the delivery of sucrase-isomaltase<sup>53</sup>) requires annexin II, but the full repertoire of proteins that require annexins for plasma membrane delivery is not known.

Fusion of vesicles with a target plasma membrane is mediated by the SNARE complex, which comprises vesicle (v)-SNAREs (such as vesicle-associated membrane protein (VAMP)) and target (t)-SNAREs (the syntaxins)<sup>5</sup>. In polarized epithelial cells, apical and basolateral vesicles contain different v-SNAREs (such as tetanus-insensitive (Ti)-VAMP and VAMP8, respectively<sup>54</sup>), and different t-SNAREs are localized to the apical (syntaxin-3) and basolateral (syntaxin-4) plasma membranes<sup>55,56</sup> (FIG. 2). Loss-of-function or mislocalization of SNAREs leads to a concomitant disruption in the delivery of apical or basolateral vesicles to the plasma membrane<sup>54,57–59</sup>.

#### Trans-Golgi network

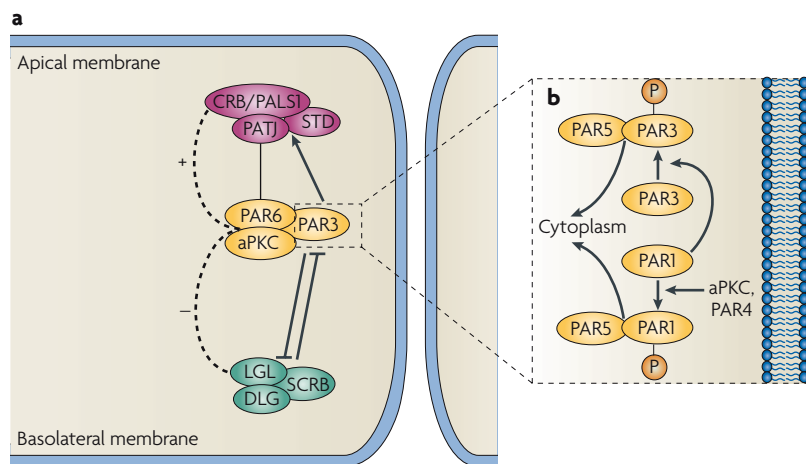
The terminal region of the Golgi complex, in which proteins are sorted and packaged into transport vesicles for delivery to the plasma membrane.

#### Recycling endosome

A membrane compartment in which proteins delivered by transport vesicles are resorted and packaged into vesicles for delivery to different membranes.

#### Exocyst complex

An example of a vesicle-tethering complex that associates with the plasma membrane and regulates the delivery of transport vesicles to the basolateral membrane domain of polarized epithelial cells.



**Figure 3 | Signalling complexes and scaffolds on the cytosolic face of the membrane define and stabilize membrane domains.** **a** | Three signalling complexes (Crumbs, partitioning defective (PAR) and Scribble) associate with the cytoplasmic surface of the plasma membrane around sites of cell adhesion, which demarcates different plasma membrane domains — in this example, the apical membrane (top) and the basolateral membrane (bottom). Crumbs protein (CRB) is a transmembrane protein, but mechanisms of binding of the PAR and Scribble complexes are poorly understood (see the main text). Proteins within each of these three complexes physically interact, as do PATJ (PALS1 (protein associated with LIN-7)-1-associated tight-junction protein) and PAR6 in the Crumbs and PAR complexes. Atypical protein kinase C (aPKC; in the PAR complex) genetically interacts with, and stabilizes, CRB (broken line, +), and phosphorylates and negatively regulates (broken line, –) lethal giant larvae (LGL) in the Scribble complex. Overall, the PAR complexes reinforce the localization and activity of the Crumbs complex (thick arrow), and the PAR and Scribble complexes mutually antagonize each other (inhibition lines). **b** | PAR3 phosphorylation by PAR1 results in the binding of phosphorylated PAR3 and PAR5, and dislocation of PAR3 from the membrane into the cytoplasm. Similarly, PAR1 phosphorylation by aPKC or PAR4 results in the binding of phosphorylated PAR1 and PAR5, and dislocation of PAR1 into the cytoplasm (see the main text for details). SCRIB, Scribble protein; STD, stardust.

of a continuous lipid bilayer. In epithelial cells, this barrier is localized to the boundary between the apical and basolateral membrane domains and comprises the tight junction, which acts as a molecular fence to prevent the free diffusion of proteins from one domain to the other<sup>64</sup>; regulated vesicular transport (transcytosis) is therefore required to bypass the barrier. Diffusion barriers are not always dependent on cell–cell contact, and can be constructed in single cells by the deposition of cytoskeletal scaffolds that selectively recruit high concentrations of membrane proteins, thereby impeding the movement of freely diffusing membrane proteins. Such a barrier occurs at the axon initial segment in neurons and restricts the movement of axonal membrane proteins into the soma, and vice versa<sup>65</sup>. This molecular fence involves localized arrays of actin and ankyrin, and might form the basis of diffusion barriers in other cell types<sup>25</sup>. Other cytoskeletal arrays might restrict membrane-protein diffusion in other systems (such as in the immunological synapse, in the border between a nascent bud and a mother yeast cell, and in the membrane-associated cytosolic ‘necklace’ that is found at the base of cilia).

**Intrinsic membrane domain orientational cues**

Although asymmetry in the localization of plasma membrane proteins is achieved by intrinsic sorting mechanisms, it remains unclear how these pathways are coupled to the overall orientation of cells in a multicellular context. Below, we discuss evidence that intracellular complexes (partitioning defective (PAR), Crumbs and Scribble complexes) provide orientation cues that specify overall cell polarity, shape and other functional specialization (FIGS 3,4).

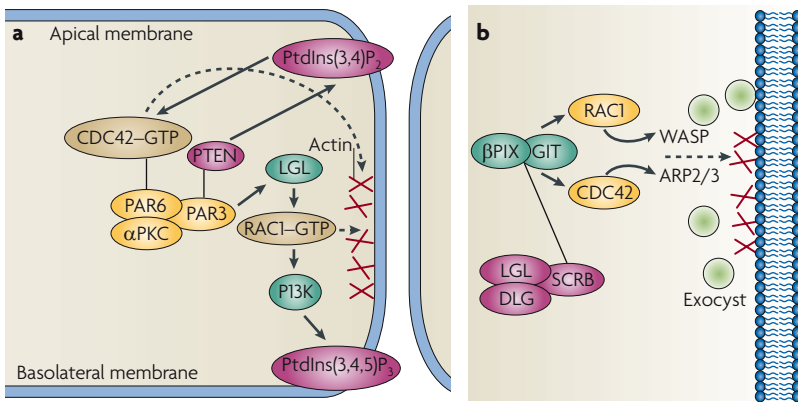
**Protein retention and molecular fences.** Polarized membrane-protein localization can also be achieved by asymmetric stabilization at a plasma membrane domain. Selective stabilization can occur in the absence of direct sorting in the exocytic or endocytic pathways<sup>60</sup>, or as a mechanism for further refining proteins that are sorted in these pathways<sup>61</sup>. Several classes of membrane proteins bind to the cytoplasmic scaffold complex of ankyrin–spectrin, including ion transporters and channels (such as the anion exchanger, Na<sup>+</sup>/K<sup>+</sup>-ATPase, voltage-gated Na<sup>+</sup> channel and Na<sup>+</sup>/Ca<sup>2+</sup>-exchanger), receptors (such as Ins(1,4,5)P<sub>3</sub> receptor, ryanodine receptor and N-methyl-D-aspartate receptor (NMDAR)), and cell-adhesion proteins (such as E-cadherin, L1 (Ng-CAM) and CD44)<sup>25</sup>. In epithelial cells and neurons, ankyrin–spectrin complexes are differentially localized to the basolateral membrane<sup>62</sup>, and to axons and dendrites, respectively<sup>63</sup>. Binding to these scaffolds can recruit membrane proteins that function in the same biological process (such as T-tubule clustering of Na<sup>+</sup>/K<sup>+</sup>-ATPase, IP3R and Na<sup>+</sup>/Ca<sup>2+</sup>-exchanger)<sup>25</sup> and can sequester membrane proteins from the endocytic pathway, thereby increasing their residence time and accumulation<sup>60</sup>.

Another essential feature for the development and maintenance of polarity is a diffusion barrier that separates membrane domains that are otherwise part

**The role of the PAR complex.** Polarization in single cells and in multicellular organisms can be induced by a pathway that involves the Ser/Thr kinase and tumour suppressor LKB1 (the mammalian orthologue of PAR-4, one of six PAR proteins that are required to establish polarity in early *Caenorhabditis elegans* development<sup>66</sup>; see below). Activation of LKB1 in epithelial cells results in apico–basal polarization of single cells, even in the absence of cellular adhesion<sup>67</sup>; inactivation of LKB1 in neurons inhibits axon formation *in vitro* and *in vivo*<sup>68</sup>. Here, we consider the roles of PAR-4 (LKB1) and other PAR proteins in cell polarity in multicellular contexts.

Among the substrates of LKB1 are members of the AMP-activated protein kinase (AMPK) family, which have multiple roles in cells<sup>69</sup>. In addition to being a glucose or energy sensor, AMPK $\alpha$  and AMPK $\beta$  phosphorylate non-muscle myosin light-chain kinase, an activity that is required for the formation of junctional complexes in *Drosophila melanogaster*<sup>70</sup>. AMPK members have several functional homologues: the kinase synapses of amphids defective (SAD), the Ser/Thr kinase PAR1 and the family of ELKL-motif kinases (EMKs; also known as microtubule affinity-regulating kinases (MARKs)), which destabilize microtubules<sup>71</sup>. These functional homologues have important roles in the orientation of polarity in neurons and epithelial cells<sup>69</sup>.

**Immunological synapse**  
The site of interaction between a lymphocyte and an antigen-presenting cell.



**Figure 4 | Roles of signalling complexes on the cytosolic face of the membrane control phosphoinositide distribution and vesicle trafficking.** **a** | The partitioning defective (PAR) complex structurally and functionally interacts with Rho family GTPases, either by direct binding of PAR6 to active CDC42 (CDC42-GTP) or regulation of the guanine nucleotide-exchange factor T-cell-lymphoma invasion and metastasis-1 (TIAM1), which locally activates RAC1 (forming RAC1-GTP). These GTPases locally regulate actin organization. The PAR complex and associated CDC42 and RAC1 also locally regulate phospholipid synthesis through the direct binding of PAR3 to phosphatase and tensin homologue (PTEN), which generates phosphatidylinositol-(3,4)-biphosphate (PtdIns(3,4)P<sub>2</sub>), and through activation of phosphoinositide 3-kinase (PI3K), which generates phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P<sub>3</sub>). PtdIns(3,4)P<sub>2</sub> and PtdIns(3,4,5)P<sub>3</sub> might be localized to different domains of the plasma membrane — in this example, the apical membrane (top) and the basolateral membrane (bottom). **b** | The Scribble complex locally regulates actin organization by Scribble protein (SCRIB) binding to the PAK-interacting exchange-factor-β-G protein-coupled receptor kinase-interactor (βPIX-GIT) complex, which locally regulates CDC42 and RAC1 in activating the actin-polymerization machinery (Wiskott-Aldrich syndrome protein (WASP) or WASP family verprolin-homologous protein (WAVE), and actin-related protein-2/3 (ARP2/3)). Local activity of the βPIX-GIT complex and actin polymerization affect the organization of the exocyst vesicle-tethering complex and vesicle delivery to the plasma membrane.

vesicles at the plasma membrane. The actual mechanisms by which these kinases exert their effects are unclear, as *D. melanogaster* mutants that are defective in AMPK or LKB1 exhibit generalized defects in polarity that affect both the apical and basolateral domains<sup>70</sup>.

In addition to PAR1 and PAR4, the PAR complex comprises three scaffolding proteins with either multiple PDZ domains (PAR3 and PAR6) or 14-3-3 homology (PAR5), and a RING-finger protein<sup>75,76</sup> (PAR2) (FIG. 3). Biochemical studies show that PAR3, PAR6 (which also binds active CDC42 (REF. 77), see below) and atypical protein kinase C (aPKC) form a subcomplex (FIG. 4) that localizes to the apical junctional complex in polarized epithelial cells<sup>78,79</sup> and to the tips of neuron axons<sup>80</sup>. Genetic studies reveal that the PAR3-PAR6-aPKC subcomplex contributes to the establishment and maintenance of apico-basal polarity in embryonic epithelial cells<sup>79,81</sup> and to axon formation in neurons<sup>80,82</sup> (see the review by Iden and Collard in this issue).

**The role of Scribble and Crumbs complexes.** The PAR3-PAR6-aPKC subcomplex interacts functionally with two other complexes that control cell polarity: the Scribble and Crumbs complexes (FIG. 3). The Scribble complex also comprises lethal giant larvae (LGL) and discs large (DLG), regulates the identity of the basolateral membrane, and is localized below the apical junctional complex and along the membrane at cell-cell contacts<sup>79,81</sup>. The Crumbs complex comprises the PDZ-domain-containing proteins PALS1 (protein associated with LIN-7)-1 (also known as stardust (STD)) and PATJ (PALS1-associated tight-junction protein)<sup>83</sup>. Crumbs regulates the identity of the apical membrane<sup>81</sup> and localizes to the apical side of the junctional complex in polarized epithelial cells<sup>81</sup>. Loss of function of either the Crumbs or Scribble complexes results in defects in epithelial polarity that are due to the reduction of the surface area of the apical and basolateral plasma membrane domains, respectively<sup>79,81</sup>. How the Crumbs and Scribble complexes regulate the identity of the apical and basolateral membranes is unknown.

The PAR, Crumbs and Scribble complexes mutually regulate the localization and activity of each other (FIG. 3). Genetic analysis showed that aPKC is required in early *D. melanogaster* development to maintain the presence of the Crumbs complex at the apical membrane, perhaps by direct phosphorylation<sup>84</sup> or by Crumbs binding, through its PDZ-interaction domain, to PAR6 (REF. 85). Later in development, Crumbs is required to stabilize the PAR3-PAR6-aPKC complex at the apical junctional complex<sup>79,81</sup>. aPKC can also phosphorylate PAR3, decreasing the affinity of PAR3 for aPKC and suggesting that the association of PAR3 with PAR6-aPKC is dynamic<sup>86</sup>. This is further supported by the finding that the AMPK family member PAR1 phosphorylates PAR3, causing PAR3 to bind PAR5 and thereby inhibiting the association of PAR3 at the basolateral membrane<sup>87</sup> (FIG. 3). Interestingly, PAR1 itself is phosphorylated by aPKC, which causes PAR1 to bind to PAR5 and results in the inhibition of the association of PAR1 with the cortex at the apical membrane<sup>88</sup>. Finally, a member of the Scribble

Overexpression of PAR1b (also known as EMK1 and MARK2) in MDCK cells results in the partial reorientation of the apical plasma membrane to intercellular lumens on the lateral membrane domain, similar to the orientation of the apical (bile canalicular) membrane in hepatocytes<sup>72</sup>. MDCK apical plasma membrane reorientation is accompanied by a change in microtubule organization, such that the minus ends of microtubules localize to the intercellular lumens instead of to the top of the cells. In addition, trafficking of post-Golgi transport vesicles to the apical surface is redirected into an indirect pathway (vesicles first appear at the basolateral domain and then transcytose to the apical domain), as also occurs in hepatocytes<sup>73</sup>. Because induction of the transcytotic pathway of apical vesicle transport occurs independently of microtubule reorganization, PAR1 probably affects apical protein sorting into vesicles or the delivery of vesicles to the plasma membrane. Interestingly, the PAR1 homologues in budding yeast, Kin1 and Kin2, interact with the machinery for vesicle tethering (the Rab proteins and the exocyst complex) and membrane fusion (the SNAREs)<sup>74</sup>. Thus, both PAR1 and PAR4 might regulate at least two key control points in post-Golgi vesicle delivery: microtubule remodelling and orientation and, hence, the directionality of post-Golgi vesicle transport; and the docking and fusion of

**PDZ domain**

A common structural domain of ~80 amino acids that is found in many signalling proteins. It was first found in PSD95, DLG and ZO1.

**14-3-3**

The characteristic migration pattern of a family of proteins on electrophoretic gels. 14-3-3 proteins bind to kinases, phosphatases and transmembrane receptors.

**RING-finger protein**

A specialized type of zinc finger of 40–60 residues that binds to 2 atoms of zinc, and is involved in mediating protein-protein binding.

**Apical junctional complex**

A collection of cell-cell junctions (tight junctions, adherens junctions and junction-associated proteins) that localize to the apex of the lateral membrane of polarized epithelial cells.



complex, LGL, is also a target for aPKC phosphorylation, and LGL phosphorylation inhibits its localization to the apical cortex<sup>89</sup>. The activities of aPKC, PAR1 and PAR5 have the overall effect of ‘protecting’ the identities of the apical and basolateral membrane domains. This is achieved by maintaining the apical activities of the Crumbs (and PAR) complex by blocking the intrusion of components of the Scribble complex (LGL), and vice versa. The positioning of the PAR3–PAR6–aPKC complex at the crucial apical–basolateral junction might allow it to arbitrate the phosphorylation of components, such as LGL (preventing its apical association) and PAR3, and prevents the PAR complex from encroaching too far into the basolateral domain.

The PAR complex and Crumbs-3, an isoform of Crumbs<sup>90,91</sup>, also colocalize in the primary cilium in vertebrate polarized epithelial cells. The primary cilium is involved in many vertebrate developmental pathways and in tissue homeostasis<sup>92</sup>. Crumbs-3 and the PAR complex are directly bound through PAR6, and PAR5 is also present in the complex<sup>90</sup>. Localization of the complex to the cilium requires association with the microtubule motor protein KIF3A and intact microtubules<sup>90</sup>. Deletion of Crumbs-3 inhibits ciliogenesis<sup>90</sup>, whereas deletion of PAR3 or inhibition of aPKC results in a decrease in cilium length<sup>91</sup>. Although it is not known whether localization of the Crumbs-3–PAR complexes to the primary cilium affects cell polarity, defects in genes for the assembly or function of the primary cilium have been associated with many genetic disorders that are characterized by defects in growth and polarity of epithelial cells, including polycystic kidney disease, left–right asymmetry defects and Bardet–Biedl syndrome<sup>92</sup> (BOX 1).

### Extrinsic membrane domain orientational cues

Although the intrinsic cell mechanisms described above regulate sorting, targeting and separation of proteins to different membrane domains, it is important to note that these domains are not organized arbitrarily on the cell surface, but rather are topologically ordered in 3D space. Here, we consider how extracellular cues provide the template that orientates intracellular mechanisms of domain specification and protein sorting. Cell adhesion, both to the substratum (ECM) and to other cells, is important in establishing the polarized orientation of cells.

In general, individual epithelial cells that are grown in suspension — that is, in the absence of cell–cell and cell–ECM adhesion — do not develop polarity, but instead undergo programmed cell death (anoikis). When grown on a surface, however, even single epithelial cells develop an apico–basal axis of polarity. When this surface has biological relevance, such as a surface of laminin-containing ECM, neurons specifically form an axon<sup>93</sup>, single mammary epithelial cells selectively secrete  $\beta$ -casein from the apical surface<sup>94</sup> and 3D epithelial cysts polarize correctly<sup>95</sup>. In this way, cell–substrate adhesion is coupled not only to post-translational control of membrane traffic and cytoskeletal reorganization, but also to the control of transcriptional activity that specifies the function of the resulting polarized cell types<sup>96</sup>.

Cell–cell contact, mediated by a range of cell–adhesion proteins, is intimately involved in cell polarization<sup>97</sup>. For example, epithelial cell–cell adhesion in suspension culture induces the segregation of basolateral membrane proteins to the cell–cell contacts and induces the trafficking of apical proteins to the free surface<sup>98</sup>. Perhaps the most important protein for cell–cell adhesion and cell polarization is E-cadherin, which is required for epithelial organization in early embryonic development<sup>99</sup>. Homotypic E-cadherin interactions are necessary to form the junctional complexes that maintain the diffusion barrier between the apical and basolateral domains of epithelial cells. They are also required for localization of the exocyst complex<sup>48</sup> and to ensure that basolateral vesicles are delivered<sup>100</sup> to sites of cell–cell adhesion and to the lateral membrane domain. Although cell–cell contacts can help control epithelial cell differentiation<sup>101</sup>, cell–cell adhesion is insufficient to trigger full apico–basal polarity in epithelia and, as discussed above, a set of signals must be supplied following adhesion to ECM.

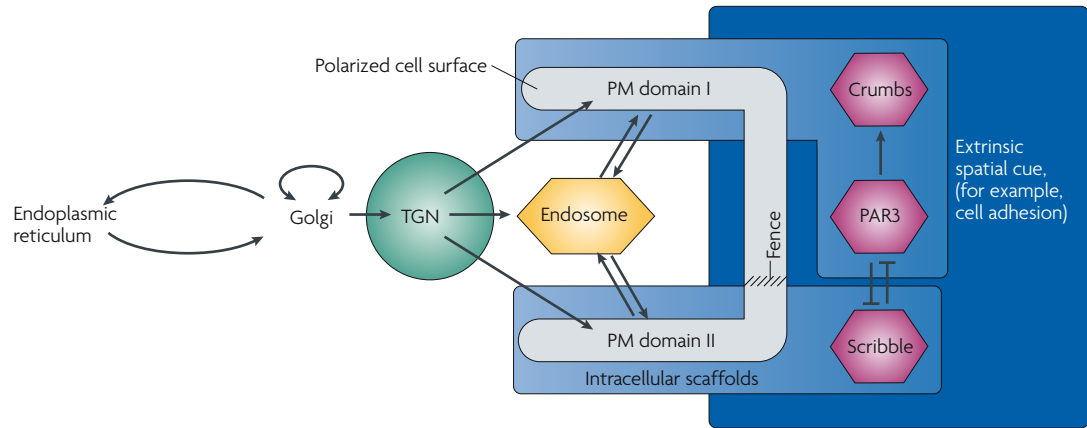
### Integrating extrinsic and intrinsic polarity cues

We have discussed three main layers of regulation that control cell polarity. Intrinsic mechanisms sort membrane proteins into different vesicles (sorting motifs and decoding machinery), and deliver these vesicles to different membrane domains (cytoskeleton-mediated targeting, vesicle tethering through exocyst complexes and annexins) for membrane fusion (SNARE proteins). Protein complexes at the plasma membrane (the PAR, Crumbs and Scribble complexes) control the identity and distribution of functionally and structurally unique plasma membrane domains from the cytosolic face. Extrinsic cues provided by cell adhesion to the ECM and by other cells control the orientation of cell polarity. But how are these different levels of regulation of cell polarity integrated into a single network (FIG. 5)?

### Localization of PAR, Scribble and Crumbs complexes.

As mentioned above, PAR3–PAR6–aPKC complexes are localized at sites that are important for cell-polarity decisions: the boundary between the apical and basolateral membranes of epithelial cells (demarcated by the apical junctional complex), the cilium and the axon tip in neurons (FIG. 3). The localization of PAR3–PAR6–aPKC complexes to these sites could involve direct binding to cell–adhesion complexes or indirect recruitment by other complexes that are locally activated by adhesion at those sites (FIG. 5). Genetic studies in *D. melanogaster* suggest that PAR3 functions upstream of the cell–cell adhesion signal that is mediated by *D. melanogaster* E-cadherin<sup>102</sup>, but membrane proteins that recruit PAR3 to the apical junctional complex in *D. melanogaster* have not been identified. By contrast, in mammalian cells, PAR3 binds directly to the cell–cell adhesion proteins JAM-A<sup>103</sup> and nectin<sup>104</sup>, both of which colocalize with E-cadherin at the apical junctional complex. Protein–protein interactions between the PDZ-domain-containing proteins of the Crumbs complex (PALS1 and PATJ) and proteins of the PAR complex (PAR3 and PAR6) provide links that

**Bardet–Biedl syndrome**  
A complex human genetic disease that is characterized by obesity, retinitis pigmentosa, polydactyly, mental retardation, hypogonadism and renal failure.



**Figure 5 | Polarized cells form from the hierarchical integration of three fundamental mechanisms.** Cell adhesion to the extracellular matrix (ECM) or to adjacent cells provides an extrinsic spatial cue (dark blue) that signals the assembly of intracellular scaffolds (light blue): Crumbs and PAR3 specify the apical domain and Scribble specifies the basolateral domain. Intracellular scaffolds define distinct plasma membrane (PM) domains and separate them by inserting a molecular fence that acts as a diffusion barrier. Finally, protein-sorting codes and cellular decoding machineries distinguish membrane components destined for distinct plasma membrane domains, sorting them into distinct vesicular transporters at the level of the *trans*-Golgi network (TGN) and endosomes. The transport vesicles are equipped to recognize either domain I or II, thus constructing the biochemical heterogeneity that is characteristic of the polarized cell surface (grey). The integration of these mechanisms is important for normal tissue function and is often defective in disease states.

in principle could allow co-recruitment of the PAR and Crumbs complexes to the pre-specified plasma membrane domain<sup>83</sup> (FIG. 4a). PALS1 is also required for the trafficking of E-cadherin to the plasma membrane and thereby the localization of the exocyst complex to the plasma membrane<sup>105</sup>, indicating that complex feedback mechanisms are involved in the organization of these protein complexes at sites of cell–cell adhesion. Interestingly, disruption of one of the exocyst complex subunits, EXO84, results in the loss of Crumbs from the epithelial cell surface of early *D. melanogaster* embryos<sup>106</sup>. This suggests that the exocyst complex has a role in regulating the polarized organization of proteins at the apical junctional complex. Mechanisms involved in the localization of the Scribble complex to plasma membrane domains are poorly understood.

Local recruitment of the PAR and Scribble complexes to the plasma membrane occurs following cell-adhesion-mediated activation of CDC42 and RAC1 due to a complex regulatory network<sup>107</sup> (FIG. 4a). CDC42 and RAC1 are members of the Rho family of small GTPases. Although the precise mechanisms are not clear, loss of activity of RAC1 and CDC42 inhibits the normal polarization of neurons and epithelial cells by causing a lack of axonal differentiation<sup>82</sup> and the misorientation of the apico–basal axis<sup>95,108</sup>, respectively. This feature undoubtedly reflects the fact that components of the PAR complex are linked to the local regulation of CDC42 activity: CDC42 binds and recruits PAR6 to the cortex<sup>77,109</sup>; PAR3 binds the RAC1 guanine nucleotide-exchange factor (GEF) T-cell-lymphoma invasion and metastasis-1 (TIAM1)<sup>110</sup>; and loss of PAR3 inhibits CDC42-induced RAC1 activation in neurons<sup>82</sup>. Thus, the localization and activities of the PAR complex, CDC42 and RAC1, and perhaps the junctional complex itself, seem to be regulated by a positive-feedback loop (FIG. 4a).

**The role of phospholipids in defining membrane domains.** Local asymmetries in the phospholipid content of plasma membrane domains also effect localization of CDC42 and cell polarity (FIG. 4a). Local changes in phosphoinositide synthesis are determined by the relative activities of phosphoinositide 3-kinase (PI3K) and phosphatase and tensin homologue (PTEN). PI3K generates phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P<sub>3</sub>) and PTEN removes the 5' phosphate from PtdIns(3,4,5)P<sub>3</sub> to generate PtdIns(3,4)P<sub>2</sub>. Inhibition of PI3K activity affects the normal polarization of neurons, by causing a lack of axonal differentiation<sup>80</sup>, and of epithelial cells, by causing the misorientation of the apico–basal axis<sup>111</sup>. PtdIns(3,4,5)P<sub>3</sub> is localized to the axon tip of neurons<sup>80</sup>. In polarized epithelial cells, PtdIns(3,4,5)P<sub>3</sub> is enriched in the basolateral plasma membrane<sup>111</sup> and is absent from the apical membrane in which PtdIns(3,4)P<sub>2</sub> is enriched<sup>112</sup> (FIG. 4a).

How might different phospholipids regulate the generation of cell polarity? Interestingly, annexin II, a putative scaffolding protein that is required for the delivery of sucrase-isomaltase to the apical membrane<sup>33</sup>, also binds PtdIns(3,4)P<sub>2</sub> (REF. 113). Artificially induced accumulation of PtdIns(3,4)P<sub>2</sub> on the basolateral membrane causes apical membrane proteins and annexin II to mislocalize to that membrane domain; concomitantly, the former apical membrane is disrupted<sup>112</sup>. Annexin II might in turn recruit other polarity-inducing proteins, such as CDC42 and aPKC (possibly through CDC42 as part of the PAR3–PAR6 complex). However, this cannot be the only mechanism involved in positioning CDC42 and aPKC. Active CDC42 might also be excluded from cell–cell contacts by a homologue of PAC-1, a Rho GTPase-activating protein (RhoGAP) that inactivates CDC-42 at cell–cell contacts in early *C. elegans* embryos<sup>114</sup>. It is interesting, however, that PAR3 also

**GEF**

A protein that is involved in locally activating small GTPases, such as the Rab proteins and members of the Rho GTPase family, by catalysing the exchange of GDP for GTP.

**GTPase-activating protein**

A protein that stimulates the GTPase activity of small GTPases (for example, Rab proteins and Rho family GTPases), leading to their inactivation.

binds PTEN<sup>115,116</sup> (FIG. 4a). This suggests a more complex local regulation of phospholipid synthesis on either side of the apical junctional complex.

How phosphoinositide asymmetry connects to the polarity of membrane proteins is not clear. For example, it is unknown whether differences in the distributions of PtdIns(3,4)P<sub>2</sub> and PtdIns(3,4,5)P<sub>3</sub> have a role in the fidelity of docking and fusion of Golgi and endosome-derived transport vesicles (FIG. 4a). CDC42 and RAC1 activate the ubiquitously distributed Wiskott–Aldrich syndrome protein neural isoform N-WASP and WASP family verprolin-homologous protein-1 (WAVE1), respectively, and together these proteins regulate actin polymerization by the actin-related protein-2/3 (ARP2/3) complex<sup>117</sup>. The mammalian exocyst subunit EXO70 associates with the plasma membrane by binding to PtdIns(3,4)P<sub>2</sub> and PtdIns(3,4,5)P<sub>3</sub>. This membrane association is required for the recruitment of other exocyst components into the complex and the docking and fusion of post-Golgi basolateral transport vesicles with the plasma membrane<sup>118</sup>. Scribble also interacts with proteins that locally regulate CDC42 and RAC1 and regulate vesicle exocytosis. Scribble binds to the PAK-interacting exchange-factor-β-G protein-coupled receptor kinase-interactor (βPIX–GIT) complex<sup>119</sup> (FIG. 4b), of which βPIX is a GEF for CDC42 and RAC1 (REF. 120), and GIT is a GAP<sup>121</sup>. The βPIX–GIT complex regulates Ca<sup>2+</sup>-dependent docking and fusion of vesicles with the plasma membrane, and βPIX–GIT function requires its GEF activity and membrane localization by Scribble<sup>119</sup> (FIG. 4b). Importantly, Scribble mutants in the central nervous system result in the accumulation of vesicles at synaptic boutons and in decreased vesicle release, suggesting that Scribble is important for vesicle docking and fusion with the synaptic membrane<sup>122</sup>. Whether the Scribble–LGL–DLG complex directs local tethering and fusion of vesicles through local regulation of CDC42 and RAC1 activity, and through recruitment of cognate t-SNAREs, requires further study. Thus, control of vesicle trafficking by the exocyst, actin polymerization induced by CDC42 and complexes such as Scribble and βPIX–GIT could reinforce the overall polarized phenotype that is induced by the PAR complex.

Such a mechanism shows how intrinsic cellular machinery for specifying cell polarity is intimately coupled to extrinsic spatial cues; these cues provide a template that coordinates the development of complex tissue organization in 3D space. Indeed, it seems likely that even in single polarized cells, the same intrinsic machinery can be used for different purposes, depending on whether a cell is generating polarity *de novo* or is maintaining a previously formed polarized phenotype. This, in turn, might simply reflect the fact that the spatial cues detected during and following polarity induction are, themselves, different.

**Conclusions**

Three major layers of regulation that control cell polarity have been identified on the basis of genetic, biochemical and cell biological criteria (FIG. 5). Intrinsic mechanisms sort membrane proteins into different post-Golgi or endosomal vesicles and deliver those vesicles to different membrane domains for membrane fusion. Protein complexes at the plasma membrane (the PAR, Crumbs and Scribble complexes) control the identity of plasma membrane domains. Extrinsic cues that are provided by cell adhesion to the ECM and to other cells control the orientation of cell polarity.

Although the basic biochemical rules that govern protein sorting in the exocytic or endocytic pathway have been described, and important signalling complexes that control cell polarity have been identified, many of the details remain poorly understood. How are apical vesicles formed? What is the full repertoire of adaptor proteins that is required for basolateral protein sorting? How are intrinsic protein sorting and trafficking pathways integrated with functions of the Crumbs, PAR and Scribble complexes in specifying membrane-domain identity? How are extrinsic cell-adhesion orientational cues linked to the intracellular machineries that define membrane-domain organization? Answers to these questions will shed new light on many fundamental aspects of normal development and on the mechanisms that underlie the induction and progression of many diseases, such as cancer.

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