Apical-basal polarity and the control of epithelial form and function.

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Abstract

Epithelial cells are the most common cell-type in all animals, forming the sheets and tubes that compose most organs and tissues. Apical-basal polarity is essential for epithelial cell form and function, as it determines the localisation of the adhesion molecules that hold the cells together laterally and the occluding junctions that act as barriers to paracellular diffusion. Polarity must also target the secretion of specific cargoes to the apical, lateral or basal membranes and organise the cytoskeleton and internal architecture of the cell. Apical-basal polarity in many cells is established by conserved polarity factors that define the apical (Crumbs, Stardust/PALS1, aPKC, Par-6, CDC42), junctional (Par-3) and lateral (Scribble, Dlg, LGL, Yurt and RhoGAP19D) domains although recent evidence indicates that not all epithelia polarise by the same mechanism. Research has begun to reveal the dynamic interactions between polarity factors and how they contribute to polarity establishment and maintenance. Elucidating these mechanisms is essential to better understand the roles of apical-basal polarity in morphogenesis and how defects in polarity contribute to diseases like cancer.

[H1] Introduction

Most animal tissues are composed of epithelial cells that adhere to each other to form sheets or tubes that act as barriers between compartments. A hallmark of epithelial cells is that they are divided into three distinct domains: an apical domain, often containing specialised structures such as microvilli or cilia (facing the outside or lumen in simple epithelia), a lateral domain that adheres to the adjacent cells in the epithelium and a basal domain that contacts the basement membrane. This apical-basal polarisation underlies all aspects of epithelial biology (**Figure 1**). The function of epithelia as barriers to fluids and pathogens depends on the correct positioning of the adhesion molecules that form impermeable cell-cell junctions (tight junctions in vertebrates, septate junctions in insects) in the lateral membrane. Polarised exocytosis needs to be directed apically or basolaterally to ensure that cargoes, such as ion channels, growth factors, receptors and extracellular matrix proteins, are secreted on the appropriate side of the epithelium. During development, morphogenetic movements transform

epithelial sheets into more complex tissues. This involves changes in the relative sizes of the apical, lateral and basal domains, often driven by polarised acto-myosin. Some cells undergo an epithelial to mesenchymal transition (EMT) and become migratory, such as the vertebrate neural crest and the *Drosophila* mesoderm. Conversely, mesenchymal cells can undergo the reverse transformation and become epithelial (MET). Indeed, endodermal cells in many animals undergo EMT, migrate and then re-epithelialize to form the intestine[1]. Finally, some human diseases involve disruptions to epithelial polarity. Most notably, 80% of cancers arise from epithelial tissues (carcinomas) and their metastasis often depends on partial and reversible alterations in polarity. Thus, elucidating the mechanisms that polarise epithelial cells is essential for understanding many aspects of developmental, cell and tumour biology.

Most core epithelial polarity factors were identified over twenty years ago through genetic screens in *C. elegans* and *Drosophila*[2–4]. Removing any of these proteins from most *Drosophila* epithelia disrupts apical-basal polarity and leads to a loss of epithelial organisation[3,5–10]. The situation in vertebrates is more complicated, as knockdowns or knockouts of these conserved factors often produce only mild polarity defects, such as a delay in the development of tight junctions and transepithelial resistance in epithelial monolayers; the formation of cysts in 3D culture with multiple (but polarised) lumens instead of a single lumen; knockout mice that die from defects in non-epithelial tissues[11–14]. In many cases, this may be due to redundancy between the multiple paralogues of each polarity factor[15]. It is worth bearing in mind, however, that not all epithelia polarise by the same mechanism (See Supplementary Box 1). With this in mind, this review will describe recent work revealing how classical epithelial polarity factors define specific domains of the cell membrane and how they control other polarised cellular functions. We will then consider how positive and negative interactions between polarity factors maintain polarity and how polarity is established in the first place. Finally, we will discuss how polarity factors are modulated to drive morphogenesis and how defects in polarity contribute to disease.

[H1] Apical polarity factors

The identity of the apical domain is defined by a conserved set of apical polarity factors: atypical protein kinase C (aPKC; PKCι and PKCζ in mammals), Par-6, Cdc42 and the Crumbs complex, consisting of the transmembrane protein Crumbs and the MAGUK scaffolding protein Stardust (PALS1), which in turn recruits PATJ and LIN7 (**Figure 2**)[16,17]. With the exception of Patj and Lin7, mutants in any of these proteins lead to loss of the apical domain and unpolarised cells in both the *Drosophila* embryonic epithelium and the follicular epithelium[3,5–10]. aPKC is considered to be the main effector of apical identity because it phosphorylates the junctional and lateral polarity factors Par-3, Lgl, Par-1 and Yurt to exclude them from the apical domain[18–26].

[H2] The control of aPKC activity

The activity of aPKC depends on most other apical polarity factors (**Figure 3)**. First, it forms a complex with Par-6 through an interaction between their PB1 domains that is essential for both its activation and localisation[27]. Like other Protein Kinase C enzymes, aPKC contains a pseudosubstrate domain that blocks its active site and Par-6 binding displaces this domain to relieve repression[28]. The pseudosubstrate domain is also a polybasic domain that interacts with the plasma membrane phosphoinositides, PI4P and PI4,5P2, thereby helping to recruit aPKC to the plasma membrane once bound to Par-6[29]. Par-6 binding does not lead to full activation of aPKC *in vivo*, however, as measured by its ability to phosphorylate and exclude one of its key lateral substrates, Lgl. This requires two additional polarity factors, Cdc42 and Crumbs. The semi-CRIB domain of Par-6 binds to active Cdc42-GTP to recruit the Par-6/aPKC complex to the apical membrane in a process that requires the WD40 protein MORG-1 in mammals. This increases aPKC activity in some assays[9,30–34]. Furthermore, binding to Cdc42-GTP changes the structure of the Par-6 PDZ domain so that it now recognises the C-terminal ERLI motif of Crumbs, leading to its full activation and retention at the apical membrane[35].

Although this model of stepwise activation of aPKC is attractive, the role of Crumbs in aPKC anchoring and activation remains an open question. Crumbs has been referred to as the apical determinant, because the apical domain is often lost in *Drosophila crumbs* mutants, whereas over-expression of Crumbs expands the apical domain[36–40]. However, it is not essential for apical domain formation in all cells. In *C. elegans*, a triple knockout of all three Crumbs orthologues has no effect on viability or epithelial polarity[41]. In the *Drosophila* embryo, only tissues that are actively changing shape require Crumbs to maintain apical-basal polarity. Most epithelia are correctly polarised in mice that lack CRB2 or CRB3, although their morphogenesis is disrupted[42–45]. Similarly, loss of Crumbs from the *Drosophila* salivary gland or pupal wing causes defects in cell packing and secretion but does not disrupt the localisation of aPKC and Par-6[46,47]. The idea that most Par-6 and aPKC are not bound to Crumbs is further supported by recent super-resolution imaging in CACO-2 cells and the mouse and human intestine, which reveals that CRB3 shows almost no co-localisation with aPKC or PAR-6[48]. It therefore seems unlikely that Crumbs is required to activate aPKC, although it may act redundantly with another factor or only be required for maximal activity.

Whether or not it is required for aPKC activation, Crumbs is essential for the localisation and anchoring of the other apical factors in *Drosophila*. Although it is found throughout the apical domain, Crumbs is concentrated above the most apical cell-cell junction (the adherens junction in insect cells and the tight junction in vertebrates) in a region called the sub-apical or marginal zone[49]. For *Drosophila* Crumbs and Zebrafish Crb2a and 2b, this enrichment is caused by homophilic adhesion between the extracellular domains of Crumbs molecules in adjacent cells, which retains them in the subapical region where cell membranes are apposed[50–52]. The most widely expressed mammalian Crumbs orthologue,

CRB3, lacks the long extracellular domain of its *Drosophila* counterpart and has none of the features required for homophilic adhesion[53,54]. Nevertheless, CRB3 and its binding partners are highly enriched just above the tight junctions of mammalian epithelial cells, in the vertebrate marginal zone (VMZ)[55].

Although aPKC is essential to prevent lateral factors from invading the apical domain, few of its identified substrates directly control the structure or specific functions of this domain. aPKC has been found to phosphorylate the tight junction proteins, JAM-A and Occludin to promote junction maturation and formation of the permeability barrier[56,57]. In addition, aPKC activity restricts the apical endocytosis of Crumbs[58,59]. It has been proposed that aPKC prevents Crumbs endocytosis by phosphorylating its cytoplasmic tail[60]. However, a non-phosphorylatable version of Crumbs is homozygous viable and localises to the apical membrane at normal levels, suggesting that phosphorylation of other substrates must be responsible[61]. aPKC also plays a tissue-specific role in inhibiting the endocytosis of VEGF receptors in the mouse angiogenic endothelium, in this case by phosphorylating the clathrin-associated sorting protein DAB2[62].

[H1] The apical/lateral junction

The most apical cell-cell junction defines the boundary between the apical and lateral domains of epithelial cells and constitutes a distinct domain that acts as a hub for signalling and mechanical interactions between cells. In *Drosophila*, the position of the apical adherens junction is determined by the localisation of Par-3 (termed 'Bazooka' in flies)[5,63,64]. PAR-3 is also required for the formation of the apical tight junction in vertebrate epithelia, but whether it plays a role in positioning the junction has not been investigated and it seems dispensable for tight junction formation in some cases[11,13,14,65].

In addition to PAR-3, tight junction formation depends on the Nectin family of cell-cell adhesion proteins, Afadin and the Zonula occludens proteins, ZO-1 and ZO-2. These large MAGUK family proteins scaffold the assembly of Claudins into strands and interact with components of the tight junctions, adherens junctions and actin-binding proteins[66–68]. Simultaneous knock-down of ZO-1, ZO-2 and Afadin prevents the localisation of PAR-3 to the tight junctions in Eph4 cells, whereas knock-down of only ZO-1 and ZO-2 has a weaker effect, suggesting that they all act redundantly to recruit PAR-3[69]. This function may be indirect since ZO-1 and ZO-2 are also required to recruit another family of MAGUK proteins, MAGI 1-3. These in turn bind a complex of the p53-binding protein, ASPP2, and the N-terminal Ras association domain proteins, RASSF7-10, which are mutually dependent on PAR-3 for recruitment to tight junctions (**Figure 2)**[69–71].

This complex network of interactions has recently been cast in a new light with the demonstration that both ZO proteins and *Drosophila* Bazooka/Par-3 form phase-separated condensates[72–75]. ZO-1 and

ZO-2 form condensates largely through the oligomerisation of their PDZ3-SH3-GUK domains. They concentrate other tight junction proteins, including Claudins, Occludin, Cingulin and Afadin, as well as directly linking to the actin cytoskeleton[72]. *Drosophila* Bazooka/Par-3 can also phase separate through the oligomerisation of its N-terminal CR1 domain, which is enhanced by the binding of PDZ3 to the PB1 domain of Par-6[73,76–78]. It is therefore tempting to speculate that PAR-3 and the ZO proteins act together to form phase-separated condensates that organise tight junctions and link them to the cytoskeleton. Many of the same proteins interact with *Drosophila* Bazooka/Par-3*,* including the Nectin, ZO-1 and Afadin orthologues (Echinoid, Pyd and Canoe), the sole fly MAGI protein and the RASSF8/ASPP2 complex, suggesting that a similar phase-separated condensate scaffolds the apical Adherens junction in flies[79–81]. These condensates may be anchored to the plasma membrane through an interaction between a conserved C-terminal motif in Bazooka/Par-3 and PI4,5P2 and PI3,4,5P3[82–85].

PAR-3 positions and stabilises the apical junction by anchoring junctional adhesion molecules in place; regulating their delivery and endocytosis and controlling their interactions with the underlying actin cytoskeleton. In mammals, PAR-3 binds to the Junctional Adhesion Molecules (JAM1-3), as well as the Nectins[65,86–88]. Similarly, *Drosophila* Bazooka binds to the Nectin-like adhesion molecule Echinoid, to β-catenin (which interacts directly with the E-cadherin cytoplasmic tail), and with the C-terminus of E-cadherin itself to stabilise Cadherin complexes in Adherens junctions[79,89]. However, many effects of Bazooka/Par-3 on junctional stability are likely to be indirect and occur through the regulation of the actin cytoskeleton, as discussed below.

[H1] The basolateral domain

The main polarity factors that define the basolateral domain are Scribble (SCRIB), Discs large (DLG) and Lethal (2) giant larvae (LGL), often referred to as the Scribble complex. *Drosophila* Lgl does not form a complex with Scribble and Dlg, since artificially clustering Scribble recruits Dlg but not Lgl[90]. Instead, Lgl interacts directly with the basolateral membrane through electrostatic interactions between its central polybasic domain and the membrane lipids PI4P and PIP2[91,92]. Lgl is prevented from binding to the apical domain by aPKC, which phosphorylates three conserved serines in this polybasic domain, introducing negative charges that disrupt the interaction with Phosphoinositides[20,21,91]. The requirement of Dlg and Scribble for the lateral localisation of Lgl is therefore presumably indirect and may reflect their function in protecting Lgl from aPKC phosphorylation[93]. SCRIB may recruit LGL directly in vertebrates, however, as LLGL2 has been reported to bind to the N-terminal LRR repeat region of SCRIB and a truncated SCRIB construct containing the LRR repeats and the LAPSDa domain mislocalises to the apical domain of colon carcinoma cells and recruits LLGL2[15,94].

Dlg and Scribble do form a complex, with Dlg binding to the membrane and recruiting Scribble in most epithelia (**Figure 2**)[15,93]. Like aPKC and Lgl, Dlg contains a polybasic domain that binds phosphoinositides and this is sufficient to target it to the lateral membrane[95]. However, Dlg's N-terminal PDZ domains can also mediate its lateral localisation, probably through binding lateral adhesion molecules[95]. How Dlg interacts with Scribble is less clear. Guk-holder (Guk) has been proposed to act as a bridge between the two proteins in *Drosophila* by binding to the Dlg GUK domain and the Scribble PDZ1 domain and mammalian SGEF also binds to these two domains[96,97]. However, neither an intact Dlg GUK domain nor the Scribble PDZ domains are required for Scribble localisation[93,96]. Finally, the N-terminal L27 domain of vertebrate DLG1 has been shown to dimerise, raising the possibility that oligomerisation or even phase separation may play a role in assembling the basolateral polarity complex[98–100].

The principal function of Scribble, Dlg and Llg is to antagonise the apical polarity factors, as discussed below. However, they also play specific roles in regulating the architecture of the lateral domain that differ between *Drosophila* and vertebrates. In flies, Dlg and Scribble are required for the formation of pleated septate junctions, which perform an analogous function to tight junctions as barriers to paracellular diffusion[101,102]. Septate junctions are not present in all epithelia and form late in development as the epithelium matures. Septate junctions contain the claudin homologues Sinuous, Megatrachea and Kune-kune, the Na^+/K^+-ATPase and the scaffolding proteins Coracle and Varicose. These are initially uniformly distributed in the lateral membrane, but coalesce into a continuous band just below the adherens junction and assemble into immobile complexes[103]. The septate junction proteins fail to localise in *dlg* and *scrib* mutants, but still become immobile, suggesting that Dlg and Scribble play an essential role in positioning the junctions and forming a continuous barrier, but are not required for junction assembly.

Mammals contain multiple paralogues of Scribble (Scribble, Erbin, Lano and Densin), Discs large (DLGL-5), and Lgl (LLGL1 and 2) raising the issue of redundancy in their functions in apical-basal polarity. Indeed, knock downs or knock outs of single proteins have weak effects on polarity and often give unrelated phenotypes[12,104–107]. Scribble mutants were first identified because they disrupt planar cell polarity, which is orthogonal to the apical-basal axis, leading to defects in the morphogenesis of the neural tube and lung and misorientation of the cochlear bundles of the ear[108–111]. Both Scribble and Erbin can also act as tumour suppressors by repressing the MAP kinase pathway[112–116].

Although single knock outs often have little effect on apical-basal polarity, triple knock outs of Scribble, Erbin and LANO almost completely disrupt the polarity of colon carcinoma cells, a function that depends on their leucine-rich repeats and LAP-specific domains LAPSDA and LAPSDB, but not on

the PDZ domains[15]. Interestingly, knockouts of both LLGL1 and LLGL2 have a weaker effect and do not affect the apical localisation of the Crumbs complex.

Several other polarity factors contribute to defining the lateral domain. Apical exclusion by aPKC restricts Par-1 laterally where it plays an important role in organising and stabilising the apical-basal arrays of microtubules that characterise epithelial cells and in restricting the lateral extent of Par-3[117–120]. Yurt, septate junction proteins and the Cdc42GAP, RhoGAP19D also localise laterally and contribute to the antagonistic interactions that maintain polarity domains, as discussed below.

[H1] Antagonism between polarity complexes

The relative sizes and positions of the apical, junctional and lateral domains are maintained by antagonistic interactions between apical and lateral polarity complexes (**Figure 4)**. As mentioned earlier, aPKC is thought to be the key factor that phosphorylates and excludes lateral polarity factors from the apical domain. This view has recently been challenged by evidence suggesting that aPKC's kinase activity is not essential for the maintenance of apical domain identity. Mutations in the aPKC kinase domain that severely compromise its activity *in vitro* do not disrupt the maintenance of the apical domain in the *Drosophila* follicular epithelium[6]. Furthermore, treating follicle cells with a specific aPKC inhibitor did not affect Lgl exclusion from the apical domain, whereas treatments with this inhibitor and an inhibitor for another Cdc42-dependent kinase, Pak1, led to the apical mislocalisation of Lgl[121]. These results therefore suggested that aPKC functions redundantly with Pak1 to phosphorylate and exclude lateral polarity factors. However, an alternative explanation for these data is that the missense mutation and inhibitor do not completely abolish aPKC activity *in vivo*. Using a different approach, Hannaford et al. mutated the ATP binding pocket of aPKC to generate an analogue-sensitive form, *aPKC*^{as}, and found that treatment of *aPKC*^{as} homozygous follicle cells with a nonhydrolyzable ATP analogue reproduced the loss of polarity seen with *aPKC* null mutations[122]. These results suggest that aPKC is not redundant with another kinase and that almost all its activity *in vivo* must be abrogated to disrupt its biological function.

The maintenance of polarity is often described as an antagonism between aPKC and Lgl, and this is certainly true for aPKC. However, although Lgl and Par-3 have been proposed to act as inhibitors of aPKC, both are aPKC substrates that bind its active site with high affinity[9,123,124]. Thus, they may compete with other substrates when aPKC activity is limiting but are not efficient inhibitors per se, although Lgl can be turned into an efficient inhibitor by mutating two of its three aPKC phosphorylation sites[125]. One way to reconcile these conflicting views is if Lgl acts to buffer aPKC activity by competitively inhibiting aPKC's phosphorylation of other substrates when aPKC has only basal activity[126]. This would keep aPKC from exerting its effects in the cytoplasm and lateral membrane, but

not at the apical membrane where it is fully activated by the association of Par-6 with Cdc42 and Crumbs. Further support for the view that Lgl does not efficiently inhibit aPKC comes from recent results that indicate that Scribble and Dlg are required to protect Lgl from aPKC phosphorylation at the lateral membrane through an unknown mechanism[90,93].

A second tier of lateral inhibition of apical polarity factors involves the Cdc42GAP, RhoGAP19D (ARHGAP21 and 23 in humans), which is recruited to the lateral membrane by Cadherin adhesion complexes and acts to exclude active Cdc42 from this domain[127]. This therefore helps to restrict aPKC activity to the apical domain by preventing its lateral activation by Cdc42-GTP. Although loss of RhoGAP19D leads to the lateral recruitment of some Cdc42 effectors (such as the myotonic dystrophy kinase-related CDC42-binding kinase; MRCK), Par-6 and aPKC are not mislocalised laterally and instead accumulate at higher levels apically, leading to apical domain expansion. This indicates that there is another mechanism that prevents lateral aPKC activity, which could be inhibition by Scribble, Dlg or Lgl or the absence Crumbs to anchor and activate the Par-6/aPKC complex.

The *Drosophila* FERM domain protein, Yurt (mosaic eyes and YMO1/EPB41L5 in vertebrates) provides a third tier of lateral inhibition. Yurt is restricted to the lateral domain by aPKC phosphorylation of its FA domain, which inhibits its oligomerisation, localisation and activity[25,128,129]. Loss of Yurt leads to an expansion of the apical domain at the expense of lateral, indicating that it acts to repress apical domain formation, possibly by binding to the Crumbs complex[129–131]. In the late-stage *Drosophila* embryo, Yurt also functions in parallel with the septate junction proteins, Coracle, Neurexin IV and the Na^+/K^+-ATPase to exclude Crumbs from the lateral domain and prevent apical domain expansion[131]. These activities may substitute for Scribble, Dlg and Lgl during the late stages of epithelial polarisation, but other unidentified factors seem to fulfil this function at even later stages of embryogenesis. These additional tiers of lateral inhibition have only been observed in the *Drosophila* embryo and it is unclear whether they operate in other organisms.

A key function of the inhibitory interactions between polarity factors is to position the apical junction and thus set the boundary between the apical and lateral domains. This depends on inhibitory signals from both apical and lateral polarity factors. aPKC phosphorylates Bazooka/Par-3 to disrupt the latter's interaction with both aPKC and Stardust, while the Crumbs C-terminal PDZ binding domain outcompetes Bazooka/Par-3 for binding to Par-6[18,19,26,132]. This breaks the interaction between Bazooka/Par-3 and the Par-6/aPKC complex, excluding Par-3 from the apical domain. In parallel, the lateral polarity factor Par-1 phosphorylates two conserved sites in Par-3 to exclude it from the lateral domain[120]. One of these sites falls in the Par-3 N-terminal oligomerisation domain and might therefore act by blocking the phase-separation of Par-3 condensates that position the apical adherens junctions.

[H1] Polarising the rest of the cell

Although we know a great deal about how polarity factors regulate each other to mark different cortical domains, much less is known about how this information is transmitted to the rest of the cell so that cellular functions are co-ordinately polarised along the apical-basal axis. Some progress has been made over the last few years, particularly in understanding how the polarity proteins control the organisation of the actin cytoskeleton to provide the appropriate structural and mechanical support to each domain.

[H2] Apical actin organisation

The apical surfaces of many epithelial cells are covered by actin-rich protrusions, such as microvilli and stereocilia, which are linked to a terminal web of actin and myosin[133]. Crumbs seems to play an important role in organising the apical cytoskeleton. As well as the C-terminal PDZ-binding motif that interacts with Stardust/PALS1 and Par-6, the Crumbs intracellular domain contains a FERM-binding motif (FBM) that binds to Moesin and therefore links Crumbs to the cortical actin network[134,135]. The FBM also helps to recruit *Drosophila* β_H-spectrin to the apical domain, possibly through Moesin, and this leads to the formation of a distinct apical membrane-associated spectrin cytoskeleton[136,137]. Two other FERM domain proteins also interact with the Crumbs FBM: Expanded, which links Crumbs to the Hippo pathway to control cell growth[138,139] and Yurt, which acts to limit the size of the apical domain by activating myosin when it is not excluded by aPKC phosphorylation[25,130,140,141]. *Drosophila* Crumbs also indirectly recruits the RHOGEF Cysts to the subapical region where the latter activates Rho to promote the formation of the junctional actomyosin[142]. Proximity labelling in Madin-Darby canine kidney (MDCK) cells reveals that several other proteins are concentrated in the VMZ near CRB3 and PALS1, including the actin binding proteins HOMER1-3, making these good candidates for downstream effectors of the CRB3/PALS1 complex in organising apical actin[55].

Like Crumbs, Cdc42 plays a significant role in organising apical actin, in addition to its function in aPKC activation and anchoring. One key target of Cdc42 is the myotonic dystrophy-related Cdc42-binding chain kinase (MRCK; *Drosophila* Gek), which phosphorylates the myosin regulatory light chain to activate non muscle myosin (NMYII)[127,143]. This increases cortical tension at the apical surface and has been proposed to play a role in separating PAR-3 at the tight junction from apical PAR-6 and aPKC[143]. Cdc42 also regulates actin polymerisation, particularly during endocytosis, through N-WASP and the TOCA family of actin-nucleation factors[144,145]. Indeed, Cdc42 promotes the apical endocytosis of E-cadherin, thereby helping to restrict the adherens junctions to the top of the lateral domain[58,146].

Just beneath the apical domain, the coupling of adherens junctions to actin is essential for their function as the major mechanical link between cells. Their association with the cytoskeleton is largely mediated

by mechanosensitive interactions between α-catenin and actin that link the junction to the underlying circumferential actin belt, although the Nectin-binding protein Afadin may reinforce this interaction[147,148]. Similarly, the mechanosensitive ZO1-2 proteins link tight junctions to the actin cytoskeleton[149,150]. PAR-3 contributes to this actin organisation by repressing RAC activity through the binding and inhibition of the RACGEF TIAM1 (Sif in flies) and this has been proposed to prevent filopodia formation[11,151–153]. PAR-3 also recruits GIRDIN, which somehow strengthens the junctions, possibly by reinforcing their linkage to actin and antagonising the activity of apical aPKC[154–157].

[H2] Formation of apical-basal microtubule arrays

Epithelial cells typically contain apical-basal arrays of microtubules that play important roles in nuclear positioning, the dynein-dependent transport of exocytic cargoes to the apical side of the cell and the kinesin-dependent transport of basal cargoes[158,159]. In polarising cells, microtubules are initially nucleated by the centrosome. In fully differentiated epithelial cells, microtubules are nucleated from the apical cortex by noncentrosomal microtubule organising centres (ncMTOCs) containing the microtubule minus end binding protein CAMSP3 (invertebrate Patronin), the F-actin and microtubule-binding spectraplakin MACF (*Drosophila* Shortstop*, C. elegans* VAB-10AB), Katanin and WDR62[160–163]. How the ncMTOCs are localised apically is not known, but studies in *Drosophila* follicle cells suggest this depends on an interaction between the spectrin repeats of Shortstop and apical β_H-spectrin as well as its actin-binding activity[158]. On the other hand, the apical ncMTOCs in the *C. elegans* intestinal epithelium derive in part from the apical migration and subsequent dissolution of the centrosome, which deposits its components on the apical cortex in a process that depends on PAR-3[164]. Pard3 has also been implicated in apical centrosome positioning in the zebrafish neuroepithelium and Bazooka/Par-3 recruits centrosomes to the cortex of the early *Drosophila* blastoderm cells that lack aPKC through a positive feedback loop involving Par-1[165,166].

[H2] Cilia positioning

In most mammalian epithelia, the centrosome migrates apically to form the basal body that produces the primary cilium. Primary cilia are major signalling centres for many pathways and are particularly important in Hedgehog signal transduction (reviewed[167]). Motile cilia mediate directional fluid flow, important in processes such as mucus clearance, left/right body patterning and cerebrospinal fluid movement.

Planar cell polarity pathways play a key role in centriole positioning along the planar tissue axis, through modulation of the actin cytoskeleton (reviewed[168]). As well as its potential role in apical positioning of the centrosome, PAR-3 has been implicated in the planar polarisation of basal bodies at the posterior side of apico-lateral junctions within the zebrafish floorplate, due to its interaction with Vangl[169].

However, the role of canonical apical polarity proteins in basal body positioning and ciliogenesis remains an open question and apical proteins and actin must be cleared from the site of basal body membrane docking, a process involving RAB19[170]. Nevertheless, CRB-3 and PAR complex proteins are necessary for ciliogenesis in MDCK cells, with the PAR complex linking CRB-3a to the KINESIN-2 motor protein and the axoneme[171,172]. Interestingly, a cilia-specific splice variant lacking the PDZ-binding ERLI motif, CRB-3b, localises to the cilium via IMPORTIN-B1, where it has an isoform-specific role in ciliogenesis[173]. Analysis of *crumbs* mutant zebrafish suggested a role for Crumbs protein in intraflagellar transport and cilia length regulation[174,170].

[H2] Division Orientation and Cell Fate

Epithelial cells usually divide with their spindles parallel to the plane of the epithelium to ensure that both daughter cells remain within the epithelial monolayer. This is achieved by localising a complex containing LGN (*Drosophila* Pins), NUMA (*Drosophila* Mud) and dynein to the lateral cortex, where the latter exerts pulling forces on the astral microtubules to position the centrosomes and mitotic spindle in the correct plane (reviewed[175]). The mechanisms that recruit the spindle orientation complex to the lateral membrane differ between epithelial cell-types. In *Drosophila* follicle cells and the zebrafish neuroepithelium, the Dlg Guk domain binds directly to Lgn/Pins to recruit the spindle orientation complex laterally[176,177]. In MDCK cells, E-cadherin recruits LGN/PINS to the lateral cortex[178]. Pins is dispensable in the *Drosophila* imaginal discs, however, where the spindles seem to be oriented by the recruitment of NUMA/Mud to tricellular junctions[179,180].

In many epithelia, the balance between planar and nonplanar divisions regulates tissue integrity, morphogenesis and cell fate (reviewed[175]). In the mouse epidermis, LGN, NUMA and Dynactin orient basal progenitor spindles obliquely or vertically to generate suprabasal cells, which then differentiate due to the asymmetric segregation of NOTCH signalling[181]. The role of apical and basolateral domain inheritance during nonplanar divisions is still not well understood, especially in vertebrates, and different mechanisms may operate in different epithelia. For example, asymmetric inheritance of PAR-3, NOTCH signalling components and structural features such as the basal process contribute to cell fate outcome in the vertebrate neuroepithelium (reviewed[182,183]). Earlier studies of the mouse cortex suggested that apical domain inheritance correlated with progenitor fate. However, *in vivo* imaging in zebrafish and chick neuroepithelia revealed that the apical domain is usually inherited by the neuronal daughter and that inheritance of the basal process could specify the other daughter as a progenitor, which then rapidly re-establishes its apical domain following division[184,185]. The asymmetric segregation of PAR-3 to the apical daughter localises Mindbomb to this cell, thereby restricting Notch signalling (and therefore progenitor status) to the basal daughter[186].

Once cells have committed to neural fate, the apical domain must be lost to enable neurons to delaminate from the luminal surface. An interesting mechanism of apical domain loss has been found in the chick and mouse (but not zebrafish), where an actomyosin contraction of the apical endfoot abscises the whole apical domain, leaving the primary cilium at the luminal surface[187].

[H2] Vesicle trafficking

The targeted exocytosis of ion channels, transporters, receptors, junctional proteins and secreted factors to either the apical or basolateral membrane is essential for epithelial function. While the signals that direct cargo protein sorting into the correct post-Golgi carriers are well-characterised, much less is known about what determines where these exocytic vesicles fuse with the plasma membrane and how this is controlled by the polarity network[188,189]. As mentioned above, post-Golgi vesicles containing apical and basal cargoes are transported along polarised microtubules to the correct side of the cell and many apically-secreted proteins then pass through Rab11-positive apical recycling endosomes on the way to the apical membrane. In the final transport step, Myosins, particularly Myosins I and V, move the vesicles through the cortical actin and the exocyst then tethers them to the plasma membrane[190]. The vesicles ultimately fuse with the target membrane in a process that is mediated by the interaction between v-SNARES on the vesicles and the corresponding t-SNARES on the plasma membrane[191]. In both the *Drosophila* salivary gland and photoreceptor cells, Crumbs plays an important in role in targeting the secretion of apical membrane proteins, at least in part by stabilising Myosin V, which interacts with several Rab proteins that control vesicle trafficking from the Golgi to the apical membrane[47,192].

In mammalian cells, it has been proposed that polarised exocytosis depends on specific v-SNARE complexes on the vesicles that pair with either apical SYNTAXIN 3-containing t-SNARE complexes or basal SYNTAXIN 4 t-SNARE complexes[193–197]. The localisation of SYNTAXIN 3 depends on a specific N-terminal motif, but how these SYNTAXINs are targeted to the correct membrane is not known[193]. Furthermore, this cannot be the complete story, as not all apical cargoes require SYNTAXIN 3 and *Drosophila* and *C. elegans* do not contain clear orthologues of either SYNTAXIN 3 or 4[198].

An alternative model is that the specificity of vesicle fusion is determined by plasma membrane tethering by the conserved exocyst complex, which binds to the vesicles, PI4,5P2 in the plasma membrane and the SNARE complexes[199,200]. In support of this view, a C-terminal leucine-rich domain of PAR-3 binds directly to the exocyst, targeting the exocytosis of E-cadherin to the lateral membrane and PAR-3 deficient mammalian tissue culture cells accumulate cadherin in intracellular vesicles[201]. Exocyst mutants also disrupt E-cadherin exocytosis in the *Drosophila* pupal notum[202]. However, the exocyst also associates with PAR-6 in the mammalian neuroepithelium and mutants in the exocyst subunit Exo84 lead to the loss of the apical domain in the *Drosophila* embryo[202,203]. Thus, the exocyst

is required for the exocytosis of both apical and basolateral proteins and therefore cannot fully explain polarised secretion. Although interactions with the exocyst may enhance secretion of specific cargoes at the certain locations, it may be required for the fusion of all exocytic vesicles.

A third possibility is that differences in the lipid composition of the apical and basolateral membranes determine where different types of vesicle can fuse. PI(4,5P)2 is enriched at the apical side of epithelial cells, whereas PIP(3,4,5)P3 is mainly basolateral. Furthermore, adding PI(4,5)P2 to the basal side of MDCK cells induces the basolateral domain to adopt apical characteristics, whereas depletion of PI(3,4,5)P3 reduces the lateral domain, leading to the proposal that PI(4,5)P2 and P(I3,4,5)P3 act as apical and basolateral membrane determinants respectively[204,205]. This phosphoinositide asymmetry could be driven by the lateral production of PIP(3,4,5)P3 by PI(4,5)P3 kinase downstream of E-cadherin adhesion and DLG and its apical inhibition by Crumbs[206–208]. In addition, the third PDZ domain of PAR-3 recruits the lipid phosphatase PTEN to the apical junction where it converts PIP(3,4,5)P3 to PI(4,5)P2, which may provide a barrier to PIP(3,4,5)P3 diffusion into the apical domain[209,210]. The control of membrane identity is not so simple, however, as PI(4,5)P2 is present laterally, albeit at lower levels than apically. Furthermore, PI(3,4,5)P3 is unlikely to play any major role in defining the lateral domain in *Drosophila*, as loss of PI(4,5)P3 kinase has no effect on epithelial polarity[84]. It has recently been proposed that PI(3,4)P2 and PI(4,5P)2 act together as the apical membrane determinant, although the former is normally associated with endocytic vesicles and recycling endosomes[211]. To further complicate matters, glycosphingolipids are essential for apical domain formation in the *C. elegans* intestine[212]. Thus, membrane lipids are probably important in the control of apical versus basolateral exocytosis, but there is currently no clear consensus on their roles.

[H1] Establishing polarity

Primary epithelia derive their polarity from the first epithelium that forms during the development of the embryo, whereas secondary epithelia become polarised as a result of a mesenchymal to epithelial transition. There are multiple mechanisms by which epithelial cells polarise, depending on the environment and the origin of the epithelium.

[H2] Polarisation of early embryos

The polarity of most *Drosophila* epithelia is established during the cellularisation of the early embryo to form the cellular blastoderm epithelium, which will give rise to all ectodermal epithelia[213,214]. Cellularisation occurs in an apical to basal direction, as membranes grow basally from the surface of the embryo to surround each nucleus **(Figure 5a-c)**. The first sign of epithelial polarity is the coalescence of spot adherens junctions into a zonula adherens near the top of the newly-formed lateral membrane. This is driven by the apical localisation of Bazooka/Par-3, which positions the spot adherens junctions and promotes their coalescence[215]. Bazooka itself is localised by multiple overlapping

mechanisms. It is anchored to the apical actin cytoskeleton in a process that depends on the Afadin orthologue, Canoe, and its regulator Rap1[216,217]. It is excluded from the lateral domain by Par-1, whose localisation in turn depends on Scribble and Dlg[218–220]. Thirdly, dynein transports lateral Bazooka aggregates apically by along the microtubules that extend from the apical centrosome[64,221].

Par-6 and aPKC are recruited to the apical membrane during cellularisation in a process that requires Bazooka and active Cdc42, which is itself restricted apically by lateral RhoGAP19D[9,64,127]. Slightly later, Rab11 and the exocyst mediate the apical secretion of Crumbs, which binds Stardust to prevent its endocytosis[4,222,223]. These apical polarity factors are not involved in the initial formation of the apical junction, but are required to maintain a continuous belt of Bazooka and adherens junctions when the cells start moving during gastrulation[42,101,165,224]. Thus, polarity in this system derives from cell-cell adhesion, which localises RhoGAP19D, Dlg and Scribble laterally, as well as apical actin and centrosomes at the non-contacting surface of the cell. However, it does not involve any basal cues, as the cells are still open to the egg cytoplasm on their basal sides[225].

In contrast, the polarisation of the early blastomeres in *C. elegans* is driven entirely by the recruitment of the Cdc42GEF, PAC-1 (the RhoGAP19D orthologue) to the contacting cell surfaces by cadherin, thereby limiting active Cdc42 to the non-contacting cell surface, where it recruits PAR-6 and aPKC[226,227]. The situation is less clear in early mouse blastomeres, where the first sign of polarity is the accumulation of actin and myosin on the non-contacting cell surface, driven by a pathway involving phospholipase-C, conventional PKC and RhoA[228]. This actomyosin network is necessary, but not sufficient for the subsequent apical localisation of the canonical apical factors, PAR-6 and aPKC, which also requires transcription activated by TFAP2C and TEAD4[229]. Human embryo polarisation has since also been found to require PKC signalling[230]. While both the worm and mammalian blastomeres polarise in the absence of a clearly defined basal domain, it is worth noting that these cells are not yet epithelial and the apical domain forms before the apical junction.

[H2] Internally polarising epithelial tubes

Some epithelial tubes and cysts form through epithelisation within the centre of an initially solid organ primordium. In these cases, the contacting surface rather than the contact-free surface becomes apical and the orientation of polarity depends on a basal cue from the extracellular matrix (ECM). This process has been extensively studied using *in vitro* 3D cultures of vertebrate epithelial cells, such as MDCK or mammary acini seeded in ECM matrix. In these cultures, apical factors such as PODOCALYXIN and aPKC are initially localised externally, but then relocalise to the apical membrane initiation site (AMIS) on the opposite side of the cell, eventually forming cysts with an internal apical domain surrounding a central lumen[231–233] (**Figure 5d-i**). In embryonic cell cultures and *in vivo* systems such as the zebrafish

neural tube and mammalian epiblast, apical polarisation also occurs *de novo* at the centre of the organ primordium, but without an initial external localisation of apical proteins[234,235].

The formation of an internal apical domain depends on ECM-mediated signalling in all these systems; inhibition or knockdown of laminin, integrin-β1 or RAC1 reverses polarity, and the cells develop an external apical surface[233,234,236–240]. This suggests that an ECM cue overrides external polarisation. The polarity reversal caused by loss of integrins or RAC activity can be partially rescued by adding exogeneous laminin[238,241] and almost fully rescued by RHOA-ROCK inhibition[240,242]. Thus, integrin and RAC are required both for laminin deposition around the cyst and to inhibit RHOA/ROCK-induced actomyosin accumulation, which seems to trigger apical domain formation. This link between actomyosin contractility and the localisation of PAR6/aPKC resembles that seen in early mouse blastomeres (as discussed above[228,229]). Integrin has been proposed to signal through FAK and p190RHOGAP to inhibit RHO, which then enables the relocalisation of PODOCALYXIN to the future apical side via PKCbII phosphorylation of the PODOCALYXIN /NHERF/EZRIN complex[243]. How this relocalisation is directed to the appropriate membrane is not yet known. A study of mammary acini suggests that integrin is required downstream of basement membrane deposition, independently of RAC1, to position the plus ends of microtubules via ILK, therefore enabling endocytic removal of apical proteins from the external surface[233]. Together, this research suggests that either redundant or alternate ECM signalling pathways establish polarity in different epithelia.

Whilst the ECM appears to be responsible for the overall axis of apical-basal polarity, the localisation of the AMIS is determined by other mechanisms. Cell division plays a dominant role in AMIS positioning: apical and junctional proteins localise to the cleavage furrow in cultured epithelial cells[244] and in the zebrafish neural rod[245]. Consistent with this, misoriented divisions result in ectopic or fragmented apical and junctional protein localisation at a tissue level and the formation of multiple lumens in culture without an overt loss in polarity at a cellular level (e.g.[244–250]). The midbody has been implicated in AMIS formation, acting as a landmark for the trafficking of apical vesicles as well as pre-assembled apical structures called apicosomes, through the targeting of RAB11A and its interacting protein FIP5[251–254] (**Figure 5**). The formation of the midbody has therefore been proposed as a symmetry-breaking event during *de novo* polarisation[251]. However, inhibiting cell division rescues the division misorientation phenotypes in the zebrafish neural rod[245,247,248,255]. Together, this suggests that midbody localisation is sufficient but not necessary for AMIS localisation and that another mechanism is involved (see supplementary box 2).

In line with predictions from the zebrafish neural rod (supplementary box 2), cadherin-mediated early cell-cell adhesions appear necessary and sufficient to direct AMIS localisation in mouse embryonic stem cells (mESCs) in 3D culture, independently of cell division[256]. This fits with research showing

that mature N-cadherin/ β-catenin complexes are necessary for apical protein accumulation in the chick neural tube[257] and that immobilized cadherin in combination with ECM signalling can direct the apical-basal axis of single hepatocytes[258]. The AMIS defines the membrane fusion site for apical proteins such as Crumbs, which then act to displace junctional proteins laterally, separating the apical and junctional domains (**Figure 6c**) and allowing the apical secretion of luminal determinants from the Pre-apical patch (PAP, **Figure 5g**). In dividing epithelial progenitor cells, the tethering of the midbody and AMIS aligns cell division with the formation of the apical domain, allowing a coherent epithelial structure to arise alongside the cell divisions necessary for tissue growth. The tight junction-associated protein Cingulin binds to both FIP5 and to the midbody so might act as a tether, aligning AMIS protein targeting with the midbody[259]. An alternative pathway involves RAB35, which binds to the cytoplasmic tail of PODOCALYXIN to target vesicles to the cleavage site[260]. Whether these pathways act in parallel or in series is unclear, but several additional RAB proteins are also involved in the trafficking of apical membrane proteins to the AMIS[261–263]. A recent study in Caco-2 cells demonstrates that the transmembrane aminopeptidase CD13 acts upstream of RAB11A and RAB35 to both initiate the internalisation of apical proteins from the basal membrane and to localise RAB35 at the AMIS to enable apical vesicle docking[264].

Internally polarising epithelia appear to require a basolateral domain to orient the apical-basal axis of the cell. This induces the localisation of apical components to the AMIS, the position of which is determined by both division dependent and independent mechanisms (Supplementary box 2). This is the opposite way round from primary epithelia in *Drosophila,* which polarise apical-first. As discussed in Supplementary Box 1, the enterocytes of the *Drosophila* midgut polarise from basal to apical as they integrate into the intestinal epithelium from the basal side (Supplementary box 1). Since enterocytes also share several other features with vertebrate epithelia, they may provide a better model for some aspects of polarity establishment in these systems.

[H1] Roles during Morphogenesis

Epithelial cell polarity is intimately linked to tissue morphogenesis, as previously reviewed[265,266]. Much morphogenesis occurs via the spatiotemporal regulation of junctional and cytoskeletal components such as cadherins and non-muscle myosin II (NMYII), to drive changes in cell-cell adhesion and cell shape, such as constriction. For example, NMYII-mediated apical constriction is sufficient to initiate hydrodynamic flows deep into the tissue[267] and the spatial modulation of myosin mediates global morphogenetic changes due to the mechanical interactions between cells[268]. Apical-basal polarity proteins regulate morphogenesis indirectly by controlling the localisation of junctional and cytoskeletal components, but also play more direct roles. For example, the apical protein Crumbs has been implicated in assembling apico-lateral junctions and releasing cell-cell adhesions during *de novo* lumen formation[269], disassembling junctions during EMT[270,271], re-localising PAR-3 during adherens junction

assembly[26] and regulating cell constriction through both inhibition[272,273] and activation[141,274] of NMYII (**Figure 6c**). The context-specific regulation of apical-basal polarity proteins is therefore key to their effects on morphogenesis, as described below, with a focus on Crumbs.

[H2] Cell constriction and tissue folding

Epithelial folding is a common morphogenetic process that generates 3D tissues such as epithelial tubes, the blueprint for many organs. In combination with other cell shape changes, such as basal relaxation and cell wedging, actomyosin-mediated cell constriction is a major driver of tissue folding, often (but not exclusively) at the apical cell cortex. Recent advances in optogenetics have confirmed the sufficiency of apical actomyosin contractility to mediate folding. For example, activating NMYII via optogenetically-activated Rho1 signalling induced ectopic tissue invagination on the dorsal side of the *Drosophila* embryo[275], whilst maintaining high NMYII activity at the basal ends of gastrulating mesoderm cells prevented ventral furrow formation, demonstrating that tissue folding requires both basal NMYII relaxation and apical constriction[276].

The relationship between the regulation of actomyosin activity, junctional remodelling and apical polarity proteins is an active area of research. Crumbs protein is thought to regulate actomyosin during morphogenesis in a context-specific manner. For example, Crumbs is anisotropically localised at the edge of the *Drosophila* salivary gland placode, with low levels at the outer membranes and high levels at the inner membranes of the boundary cells[272]. This asymmetry arises from the homophilic adhesion between Crumbs molecules in the boundary cells with the higher levels of Crumbs in the inner placode cells[272]. Crumbs negatively regulates Rho kinase (ROCK) localisation by increasing its K_{off} on inner cell membranes. This restricts the formation of a supracellular actomyosin cable to the outer edge of the placode and enables tube budding[272,277] (**Figure 6a**).

The complex interplay between its binding partners determines whether Crumbs promotes or inhibits NMYII-mediated apical constriction. In particular, Yurt and Moesin compete for binding to Crumbs FERM binding domain (FBD), but have opposite effects on the actin cytoskeleton. For example, the Crumbs FBD negatively regulates actomyosin within the amnioserosa, presumably by binding Moesin, to control the formation of a supracellular actomyosin cable during *Drosophila* dorsal closure[273]. However, Yurt binding to the Crumbs FBD at the apical membrane of the *Drosophila* embryonic epidermis and follicular epithelium activates NMYII, causing apical constriction[141]. The same study found that the aPKC and Pak1 kinases have opposing effects on Crumbs/Yurt-mediated apical constriction: aPKC phosphorylates Yurt, thereby preventing its interaction with Crumbs and inhibiting apical constriction, whereas Pak1 activates PP2a to dephosphorylate Yurt, promoting its interaction with Crumbs and apical constriction[141] (**Figure 6b**).

Tissue folding can occur independently of NMYII contractility. For example, folding of the *Drosophila* dorsal epithelium during gastrulation is driven by the basal relocalisation of Bazooka/Par-3 and adherens junctions, presumably caused by a decrease in Par-1, which normally excludes Bazooka from the lateral domain[278]. However, junctional repositioning can also be linked to NMYII activity. During *Drosophila* mesoderm invagination, Snail-mediated Bazooka downregulation causes adherens junction disassembly, whilst apical NMYII contractility reorganises junctions, repositioning Bazooka apically[279].

[H2] Downregulation of apical junctions and adhesions

Crumbs can also downregulate, rather than build, apical junctions. For example, high levels of CRB2 are required for gastrulating cells to ingress in the mouse embryo, possibly by promoting NMYII activity in neighbouring cells to induce basal extrusion[270]. CRB2 secreted from dorsally-located Nestin-positive radial glial cells in the mouse spinal cord causes the delamination of adjacent dorsal ventricular cells to induce the dorsal collapse of the spinal canal[271]. As discussed in supplementary box 2, the proposed Crumbs-mediated resolution of cell-cell adhesions between cells on either side of the organ primordium is important in allowing lumen inflation of hollowing epithelial tubes[269].

[H2] Interaction of apico-basal and planar cell polarity

Planar cell polarity is the asymmetric organisation of proteins within the epithelial plane and is orthogonal to apico-basal polarity. These two polarity axes interact, for example, through the planar polarisation of apical-basal polarity factors. This plays an important role in morphogenesis since it allows another axis of control over cell shape change. For example, the anisotropy of Crumbs in the salivary gland, discussed above, mediates the planar polarisation of ROCK that drives salivary placode tube budding[277]. PAR -3, in particular, has been found to reciprocally interact with PCP components in epithelia. For example, Bazooka/Par-3 is planar polarised by PCP signalling in the *Drosophila* eye disc epithelium and sensory organ precursors (SOPs)[280,281]. In the latter, Meru protein (an SOP-specific homologue of human RAS association domain family 9 and 10) recruits Bazooka to the posterior cortex to control the asymmetric segregation of cell fate determinants. During *Drosophila* germ band extension, planar-polarised ROCK activates NMYII and excludes Bazooka from contracting anterior-posterior cell edges, leading to higher levels of Bazooka on expanding dorsal-ventral edges, where it increases Cadherin adhesion to promote cell intercalation[282].

Pard3 is also planar polarised within vertebrate neuroepithelia, such as in the zebrafish floorplate, where it mediates basal body positioning, as mentioned[169]. In the *Xenopus* neuroepithelium, PAR-3 associates with the PCP component Prickle-3, recruiting it apically and promoting its interaction with Vangl2[283]. Knocking down PAR-3 function inhibits neural tube closure without altering apical-basal polarity, suggesting the importance of this interaction for morphogenesis[283].

[H1] Disruption of apico-basal polarity in Disease

Given the importance of apico-basal polarity in the establishment, morphogenesis and function of all epithelia, it is not surprising that disruptions in polarity are implicated in a range of defects and diseases (Supplementary Table 1). Here, we will focus on a few key examples where the localisation or function of canonical apical-basal polarity proteins can be directly linked to disease.

[H2] Developmental diseases

Polarised exocytosis plays an important role in mediating apical-basal polarisation and mutants that disrupt this process generate developmental defects. For example, mutations in *myosin-5B*, *syntaxin-3* or *syntaxin binding protein-2* disrupt the apical delivery of RAB11A and RAB8A vesicles, causing microvillus inclusion disease (reviewed[284]). Whilst the disease mechanisms are still under debate, mis-localisation of basolateral proteins to apical membranes and vice versa also occurs in polycystic kidney disease, characterised by increased cell proliferation, luminal fluid secretion and the formation of ectopic cysts (reviewed[285]).

Mutations in the basolateral protein Scribble have been implicated in neural tube defects (NTDs) due to reduced convergence extension and apical constriction[110,111]. Some of these defects reflect Scribble's role in localising PCP proteins such as VANGL, which can cause NTDs even if only 16% of neural plate cells are affected[286]. Rare *scribble* mutations are associated with human NTDs and are thought to act by mislocalising PAR-3 and VANGL proteins[287]. However, *scribble* mutations also affect the localisation of apical junctional proteins, so defects in apical-basal polarity may contribute to the phenotype[111]. A small cohort of cranial NTD cases have a higher rate of rare deleterious *PAR-3* variants, suggesting a link between defective apicolateral junction formation and NTDs[288].

[H2] Cancer

Apical-basal polarity proteins are often dysregulated in cancer and the link between apical-basal polarity alterations and downstream signalling pathways is well reviewed[289]. At a cellular level, epithelial-mesenchymal transitions are classically evoked as hallmarks of cancer. However, an emerging concept is that this relationship is not hardwired and that a partial and reversible transitions between these states may correlate best with metastatic potential (**Figure 7**).

For metastatic cells to delaminate, apical-basal polarity and/or cell junctions must be disrupted to allow cells to move out of the epithelium. Loss of apical junction proteins such as PAR-3 has been linked to tumorigenesis and metastasis[290–292] and a progressive but reversible loss of apical-basal polarity was observed in preinvasive breast cancer lesions, alongside luminal collapse[293]. The pro-metastatic factor, TGF-β phosphorylates PAR-6 to induce junctional degradation and EMT, in parallel to its effects on

SMAD signalling[294–296]. As well as loss of polarity proteins, reversals in apical-basal polarity of the nucleus-centrosome axis have been associated with EMT[297] and inverted polarisation of epithelial cell clusters results in motile behaviour[243]. An unusual example of collective cancer cell migration is the invasion of peritoneal tissue by metastasising colorectal cancer cells. This is driven by clusters of epithelial-like cells with inverted polarity, formed by budding from primary tumours, which undergo apically-led collective invasion of the ECM (**Figure 7a**)[298,299]. This demonstrates that epithelial characteristics do not need to be lost for metastasis to occur. In fact, the maintenance of some epithelial characteristics is essential for secondary tumour formation (**Figure 7**). For example, individual circulating tumour cells retain an EZRIN-rich pole despite being non-adherent or migratory[300]. An emerging concept is that a partial transition towards the mesenchymal state (partial EMT), resulting in cells with both mesenchymal and epithelial characteristics, may play a central role in cancer metastasis, enabling collective migration and facilitating invasion (reviewed[301,302]). This has recently been demonstrated in mouse mammary tumour cells, which collectively contribute to lung metastasis in their partial, but not full, EMT state[303]. If cells in an intermediate epithelial to mesenchymal state have higher metastatic potential, this could explain why downregulation (rather than complete loss) of polarity-linked proteins such as the CDC42 inhibitor ARHGAP10 are associated with poor prognosis[304,305] and why polarity proteins such as PAR-3 have both tumour suppressor and promoter functions in different contexts[306].

[H1] Conclusions and perspective

Much of our current understanding of apical-basal polarity comes from studies of a small number of epithelia in *Drosophila.* Whilst the fundamental structural and barrier functions of epithelia appear conserved in different tissues and species, differences in subcellular junctional arrangement, requirement for canonical polarity factors and mode of polarisation are starting to emerge. Studies of internally polarising epithelial tubes highlight that, even within one type of epithelium, there appear to be multiple, overlapping mechanisms of polarisation (ECM-mediated basal to apical relocalisation of apical proteins, midbody-dependent apical protein tethering and division independent localisation of the AMIS). This points towards redundancy but also suggests a requirement for polarisation to align with the cell movement and proliferation necessary to form complex 3D structures. Studies of the *Drosophila* midgut demonstrate a different subcellular organisation and mode of polarisation from other *Drosophila* epithelia, which might better model ECM-mediated polarisation of internal vertebrate epithelia. This difference also raises the question of whether epithelial origin (e.g. endoderm vs ectoderm), function (e.g. barrier vs. structural) or position within the organism (e.g. internal vs external) influences epithelial differences between tissues.

Understanding the complex and context-specific relationships between apical-basal polarity protein networks is still evolving. The diverse morphogenetic functions of a single protein such as Crumbs

demonstrate the importance of the precise spatiotemporal regulation of apical-basal polarity proteins and their downstream binding partners in mediating normal physiology. Alterations to this balance can disrupt development, through defects in processes such as apical and basal protein targeting. A more direct link between individual apical-basal polarity proteins and disease is seen in cancer, where metastatic cells have both epithelial and mesenchymal characteristics, enabling both migration and colonisation. Understanding how these intermediate polarisation states intersect with cell signalling and whether this differs between epithelial types might inform the most appropriate therapeutic approach.

Glossary

Basement membrane comprises a layer of extracellular matrix between epithelia and other tissue layers.

Epithelial to mesenchymal transition (EMT) is the progressive and reversible loss of epithelial characteristics such as cell-cell adhesion and apico-basal polarity to form a migratory, mesenchymal cell with front-rear polarity.

The **Neural crest** is a population of multipotent migratory cells originating from the dorsal neural tube of vertebrates. Neural crest cells give rise to diverse cell types, including peripheral nervous system cells such as cranial nerves, cartilage and bone.

The *Drosophila* **follicular epithelium** is a monolayered epithelium encasing developing oocytes within the egg chambers.

PAR-3 is a scaffolding protein associated with the AMIS and with apicolateral junctions. Nomenclature varies between species. We use the following nomenclature: Mammals; PAR-3, Zebrafish; Pard3, *Drosophila*; Bazooka/Par-3, *Xenopus, C. elegans*; Par-3.

Hippo signalling regulates cell division and promotes apoptosis, therefore controlling tissue growth.

Filopodia are protrusions from the plasma membrane containing actin filaments and are used by cells to ‘probe’ their environment and to promote migration.

Importin-β1. Carrier protein usually known for mediating trafficking to the nucleus.

Notch signalling regulates transcription via binding of ligands such as DELTA and the subsequent release of the NOTCH INTRACELLULAR DOMAIN into the nucleus. It has wide ranging effects,

dependent on the context. For example, NOTCH activation promotes proliferation in the neural epithelium but is found in differentiating cells during epidermal development.

Mindbomb is an E3 ligase that regulates ligand availability in signalling cells to enable NOTCH activation in neighbouring cells.

The **exocyst** is a protein complex that tethers vesicles to the plasma membrane prior to fusion.

Apical Membrane Initiation site (AMIS) is a term initially coined in mammalian epithelial cysts in three-dimensional culture. It describes a transient structure, marked by scaffolding and junctional proteins such as PAR-3, ZO-1 and CINGULIN, that marks the site where apical vesicles will fuse to begin forming the apical domain.

The **midbody** is a tubulin-rich structure present at the mid-point between dividing cells just before abscission.

$\mathbf{K_{off}}$ is the dissociation rate constant for a protein

Snail proteins are zinc-finger transcription factors that act to repress expression of genes such as *E-CADHERIN*. They are known for their role in mediating EMT.

Radial glial cells are bipolar cells that span the whole apical to basal width of the neural epithelium and give rise to neurons and glial cells.

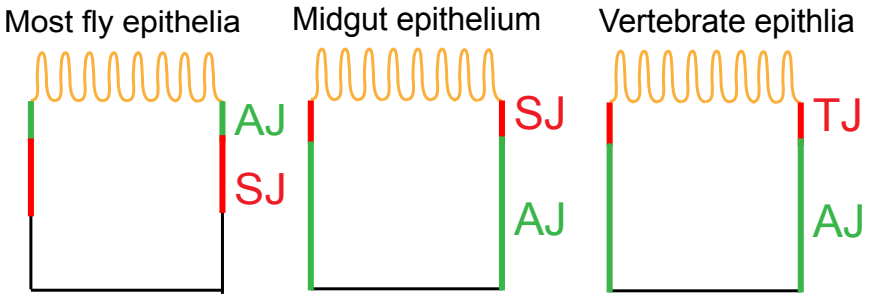
Sensory organ precursors are progenitor cells in the *Drosophila* peripheral nervous system that divide asymmetrically within the plane of the epithelium and give rise to the sensory bristles.

Germ band extension is the process that occurs to elongate the body axis of early *Drosophila* embryos. The epithelial tissue simultaneously converges along the dorsal-ventral axis and extends along the anterior-posterior axis.

The **floorplate** lines the medioventral part of the neural tube. It is an important signalling domain in dorsal-ventral patterning of the spinal cord.

SMAD proteins transduce **transforming growth factor β (TGF-β)** signalling, which has wide-ranging effects including on cell growth, differentiation and apoptosis and promotes EMT.

Supplementary Box 1: An alternative polarity system in the *Drosophila* midgut



In typical *Drosophila* epithelia, such as the follicle cell epithelium, the adherens junctions form at the top of the lateral domain. By contrast, the midgut epithelium forms apical septate junctions above more basal adherens junctions. This arrangement resembles that seen in mammalian epithelia where the occluding tight junctions, which are analogous to the septate junctions, form above the adherens junctions.

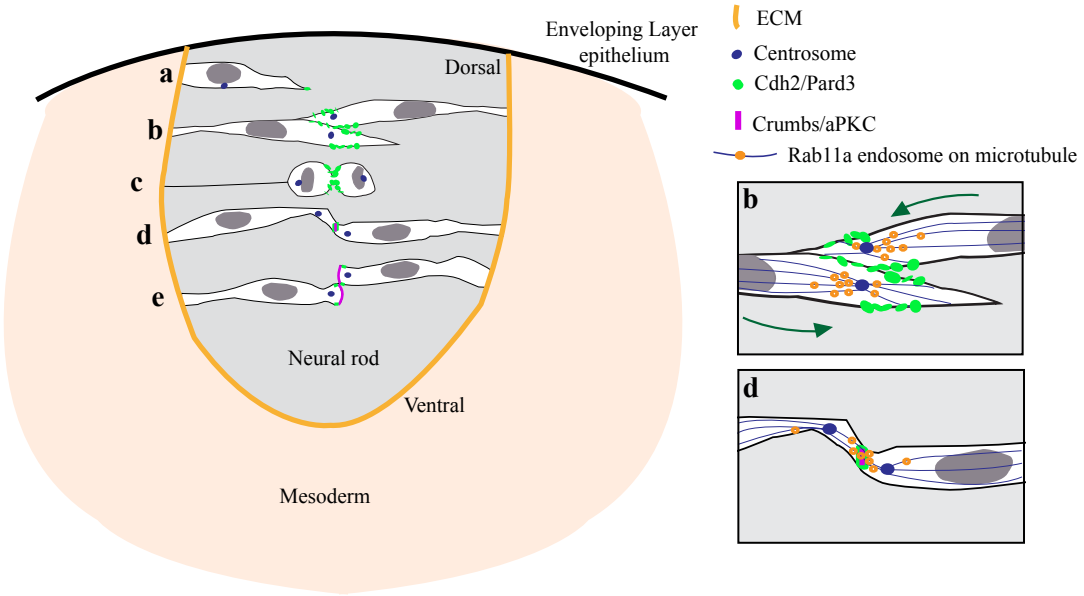
Most *Drosophila* epithelia form apical adherens junctions above the occluding septate junctions, which is the opposite way round from vertebrate epithelia. Not all epithelia are the same in *Drosophila*, however, and the midgut epithelium forms apical septate junctions above lateral adherens junctions, a similar arrangement to vertebrates[225,321,322]. Since one of the key functions of the polarity system is to position the apical junction, this reversed arrangement implies that polarity is specified differently in the midgut. Indeed, neither Crumbs nor Bazooka/Par-3 are required for enterocyte polarity[321]. Other polarity factors localise to similar positions to other fly epithelia: aPKC and Par-6 localise to the apical cortex and Scribble, Dlg and Lgl mark the smooth septate junctions, which are distinct from the pleated septate junctions in other tissues. Surprisingly, null mutants in all these factors have no discernible effect on apical-basal polarity[321]. Furthermore, like many vertebrate epithelia, the polarity of the midgut depends on integrin adhesion to the ECM, since mutants in Talin and Kindlin give rise to unpolarised cells that fail to integrate into the epithelium or form septate junctions. Thus, the enterocytes of the midgut seem to use a different system to polarise than other fly epithelia.

This raises the question of why the polarity system in the midgut is so different. One possibility is that it is the only endodermal tissue in the fly. In support of this view, work on the Cnidarian *Nematostella vectentis*, which only has two germ layers, ectoderm and endomesoderm, has revealed that the ectodermal epithelium requires aPKC and Par-6 to polarise whereas the endomesoderm does not[323]. An alternative possibility is that the polarity system depends on the direction in which the cells polarise, as the enteroblasts are born beneath the epithelial monolayer and polarise in a basal to apical direction as they integrate into the epithelium and differentiate as enterocytes. During their integration, the enteroblasts/pre-enterocytes develop a preformed apical compartment with a brush border beneath the septate junction between the overlying enterocytes[324,325]. They therefore form an apical domain before

they have a free apical surface in a process that resembles that of lumen formation in cysts of mammalian epithelial cells in 3D culture (Supplementary Box 2).

The gut endoderm derives from the blastoderm epithelium that polarises using the canonical polarity system, but the cells undergo an epithelial to mesenchymal transition, in which the GATA transcription factor serpent turns off crumbs expression leading to the disassembly of the adherens junctions[326]. The cells then migrate into the embryo before repolarising and forming apical septate junctions above lateral spot adherens junctions, a process that depends on laminins secreted by the underlying visceral mesoderm[225,327]. Thus, the gut cells polarise by two different mechanisms at different stages of their development. The observation that there are two types of epithelia with different polarity systems in *Drosophila* raises the question of whether this is also the case in other organisms, particularly given that the midgut more closely resembles many vertebrate epithelia in its junctional arrangement and the role of the ECM as a polarity cue.

Supplementary box 2: Division-independent polarisation in the zebrafish hindbrain



Schematic showing zebrafish neuroepithelial (NE) cell polarisation. **a** | NE progenitor cells extend away from the ECM. **b** | Cells interdigitate around the tissue midline, upregulate Cdh2 and localise Pard3 puncta. Centrosomes migrate to this midline region, organising a mirror-symmetric microtubule cytoskeleton, which traffics Rab11a endosomes away from the basally localised ECM towards the centrosome (green arrows). **c** | Mirror-symmetric progenitor cell divisions occur near the tissue midline and junctional proteins localise to the cleavage furrow. **d** | Sister cells remain attached, localising junctional proteins at the midline and Rab11a endosomes accumulate (presumably at the midbody). Apical proteins such as Crumbs are secreted at the midline. **e** | Apical proteins displace junctional proteins basally, allowing cell-cell separation across the midline and the formation of an apicolateral junctional belt.

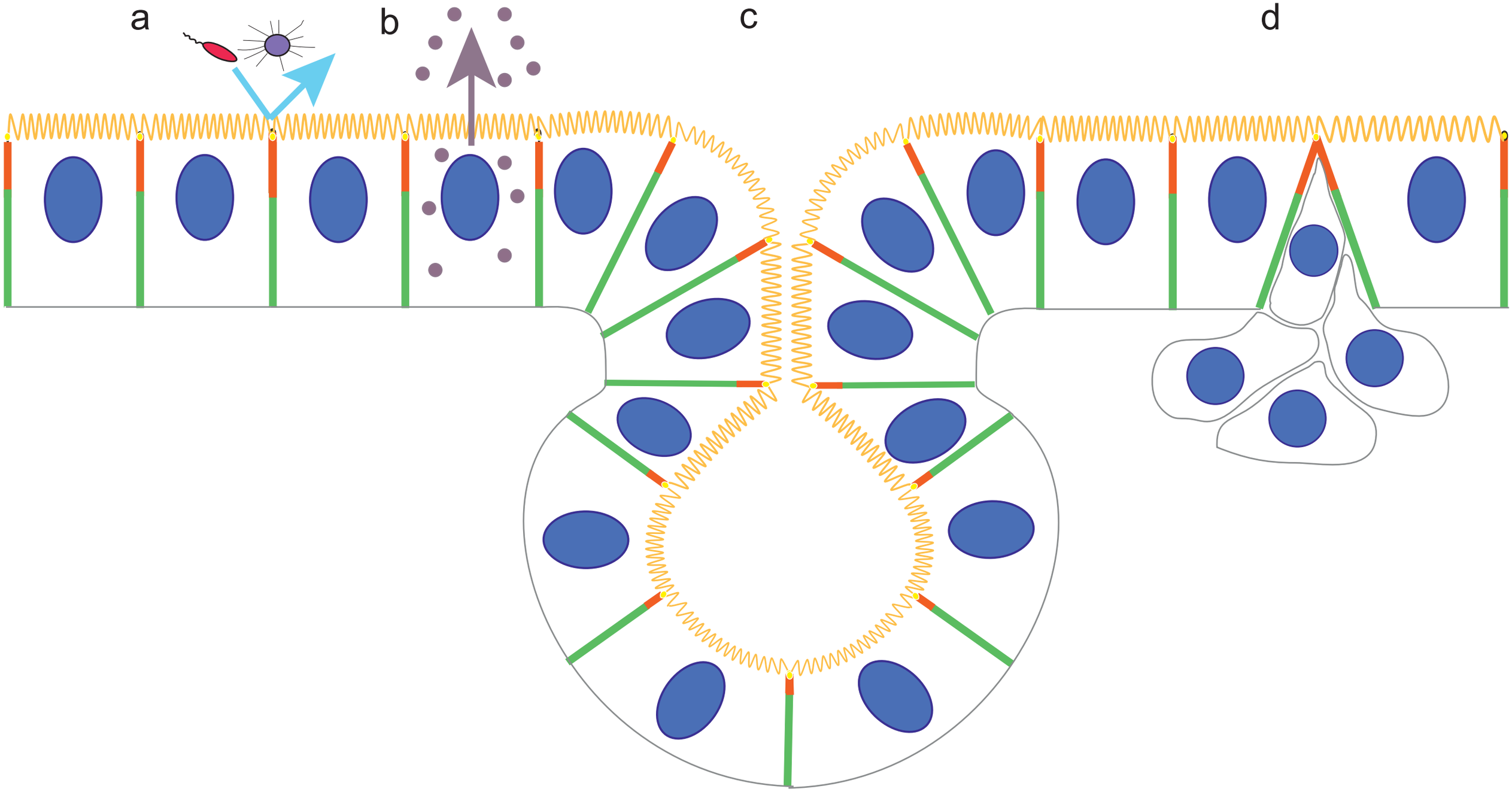
Subcellular live imaging of zebrafish neuroepithelial cells demonstrated that the early deposition of junctional proteins such as Pard3 and N-cadherin occurs close to the centre of the organ primordium, defining the AMIS in advance and independently of cell division[239,328]. Coincident with this deposition of junctional proteins, the centrosome also localises to the tissue centre and acts to organise the microtubule cytoskeleton around this point, allowing the accumulation of Rab11a recycling endosomes at the AMIS[9,10]. The localisation of both Rab11a and Pard3 is dependent on the microtubule cytoskeleton, suggesting that centrosome localisation is key to defining the site of both the AMIS and of apically-trafficked vesicles. However, it is still not clear which acts as the earliest symmetry-breaking event of this system; junctional deposition or centrosome localisation[239,329]. Later in development, at the zebrafish neural rod stage, the polarised apical midline initially comprises a mix of apical proteins such as aPKC and Crumbs and junctional proteins such as Pard3 and N-cadherin[239,269], as well as actin[330]. The Mpp5a scaffold protein and the GTPase Rab11a are necessary to displace the junctional proteins laterally, enabling the separation of adhesions between contralateral cells across the centre of the developing zebrafish neural rod, whilst simultaneously assembling two separate apical junctional belts laterally, both necessary for lumen inflation via hollowing[269]. The mechanism mediating junctional displacement is thought to be the same Crumbs/aPKC-mediated segregation of junctional proteins to the apicolateral border previously described during the polarisation of the *Drosophila* ectodermal and follicular epithelia[26]. The process of apicolateral junctional assembly also appears comparable with externally polarising epithelia; puncta of scaffolding proteins such as Pard3 and ZO-1 progressively coalesce to form 2 separate, opposing junctional belts[269], similar to the process of spot adhesion assembly in the *Drosophila* blastoderm[64,331,215] and likely dependent on Mpp5a/Rab11a mediated Crumbs and E-cadherin exocytosis[269,332].

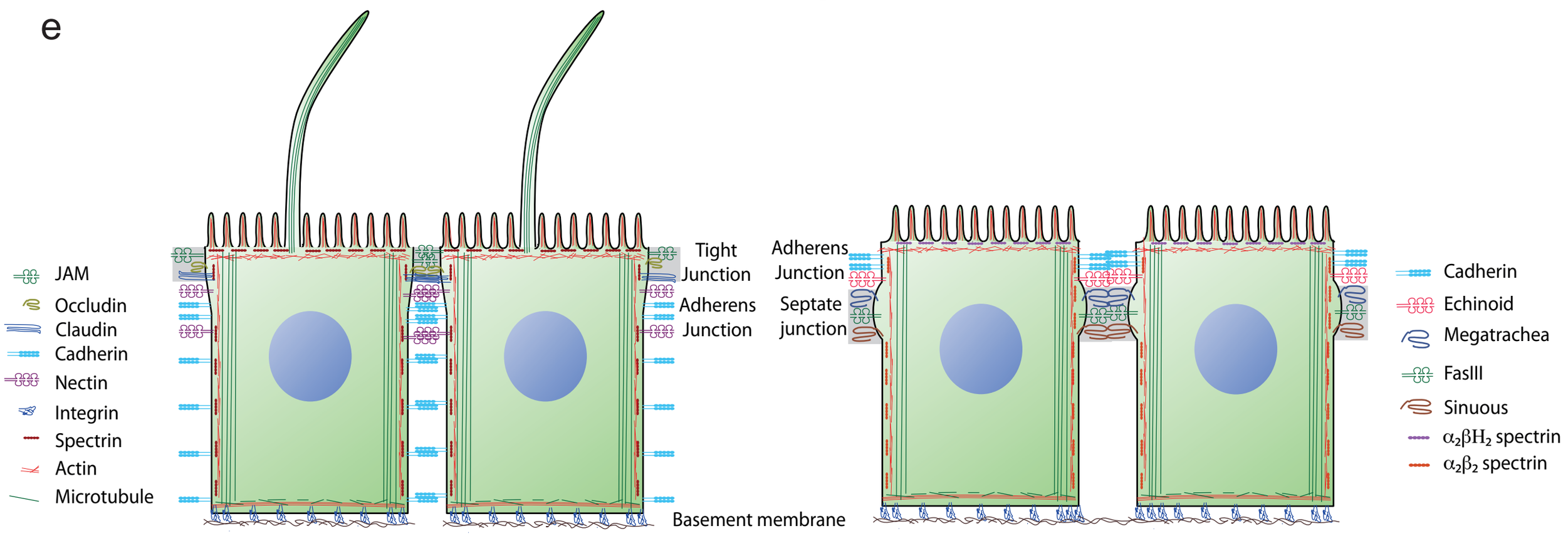
Supplementary Table 1: Apico-basal polarity and disease

Apico-basal polarity linked defect	Examples of Disease	Clinical presentation	Associated mutations and apico-basal polarity changes	Reference
Canonical polarity protein loss or reversal	Carcinoma	various, e.g. luminal filling and aberrant tissue growth, tumourogenesis, metastasis	Many canonical apico-basal polarity proteins are downregulated or lost in epithelial cancers, which dysregulates downstream signalling pathways such as HH, K-Ras, hippo pathway, mTOR, EMT factors and epithelial junction signalling proteins. Polarity defects also result in cellular defects such as spindle misorientation. Various degrees of polarity reversal and partial EMT transitions have been associated with metastatic capability.	289,301
	Inflammatory bowel disease	Crohn's disease and Ulcerative colitis. Loss of cell polarity and epithelial barrier function leads to pathogens entering the intestinal lumen, causing gut inflammation.	Dysregulation of canonical polarity protein levels such as in DLG1, DLG5, scribble and PAR complex. Mispolarisation of membrane proteins.	307
Ciliopathies	Many diseases are associated with ciliopathies associated with primary cilia. E.g. polycystic kidney disease, retinitis pigmentosa, Joubert syndrome	various	Primary cilium absence or dysfunction can occur as a result of an overall defect in apico-basal polarity (see examples below). This causes defects in many major signalling pathways, such as SHH, WNT PCP, notch, hippo, mTOR, GPCR	
	Many diseases are associated with ciliopathies associated with motile cilia. E.g. hydrocephalus, situs inversus, chronic respiratory problems and infertility	various	As discussed in the text, cilia formation depends on correct apico-basal polarity of the cell. Whether there is a direct link between apico-basal polarity defects and motile ciliopathies is not clear.	167,308ferret

Centriole and spindle disruption	Microcephaly	Reduced head and brain size and intellectual disability.	Several centrosome-associated gene mutations are linked to microcephaly. The most common are *ASPM* and *WDR62.* Reduction in these proteins causes centriole duplication defects, division orientation defects and disrupts the apical complex. Premature delamination and differentiation of neurons occurs, leading to a reduction in cell number and smaller brains. The mechanistic link between apical defects, centriolar proteins and microcephaly is an important topic for investigation	309,310
Protein trafficking and membrane insertion defects	Polycystic Kidney Disease	Common genetic disorder leading to fluid filled renal cyst formation and a reduction in kidney function over time.	Reduction in normally functioning Polycystin-1 and 2 proteins. Mispolarisation of basolateral transporters, channels and receptors to apical membranes and vice versa (e.g. NKCC1 and EGFR) in renal epithelia.	285
	cystic fibrosis	Relatively common genetic disorder leading to a build-up of mucus in organs such as the lungs and digestive system	*CFTR* mutation. CFTR is not trafficked to membranes and accumulates in the cytoplasm. Defective chloride channels.	285,311
	Bartter's syndrome	Group of rare kidney diseases that cause salt imbalance and impair kidney function.	Mutations in *SLC12A1, KCNJ1, CLCNKB, BSND* or *CLCNKA.* Mispolarisation of basolateral transporters, channels and receptors to apical membranes and vice versa (e.g. ROMK and ClC-Kb)	285,312
	Congenital sucrase-isomaltase deficiency	Rare condition preventing the processing of sucrose and maltose sugars.	Mutations in *SI* gene. Sucrase-isomaltase mispolarisation to the basolateral membrane	285,313
	Dent’s disease	Rare kidney disease preventing reabsorption in the proximal tubules of men and chronic kidney disease	Mutations in *CLCN5* or *OCRL* genes. H+-ATPase transporter mispolarisation to the apical membrane	285,314
	familial hypercholesterolemia	Common genetic disorder leading to high cholesterol and heart disease	Pathogenic gene variants for *LDLR, APOB* or *PCSK9.* Mispolarisation of LDLR to the apical membrane.	285,315

	retinitis pigmentosa	Group of genetic disorders that lead to retina degeneration, loss of night vision and peripheral vision	Mutations in many different genes, including *CRB, RPE65* and *PRPF31*. Mispolarisation of Rhodopsin to the apical membrane.	285,316,317
	Liddle's syndrome	Rare genetic disorder leading to hypertension	*SCNN1B* or *SCNN1G* mutations. Defective apical membrane recycling of epithelial sodium channels.	285,318
	Microvillus inclusion disease	Rare genetic disorder causing intractable diarrhoea in newborns	*MYO5B, STXBP2* or *STX3* mutations. Defective apical delivery of RAB11a and RAB8A vesicles in intestinal epithelial cells	319,284
	Trichohepatoenteric syndrome	Rare genetic disorder causing intractable diarrhoea in newborns	*TTC37* or *SKIV2L* mutations. Defective trafficking of apical transport proteins in intestinal epithelial cells	319
Planar and apico-basal polarity dysregulation	Neural Tube closure Defects	Range of open neural tube defects, depending on the neural tube closure point affected. E.g. open and closed spina bifida. Symptoms include paralysis and incontinence	The genetics of neural tube defects is not well understood. However, there is a link to mutations in genes affecting convergence and extension, such as scribble. Scribble is thought to be important in the correct localisation of VANGL and PAR-3. There may also be a direct link between *PAR-3* mutations and NTDs.	110,111,287
	Other cortical defects	various	Scribble mutations have also been implicated in defective cortical development and associated diseases.	320

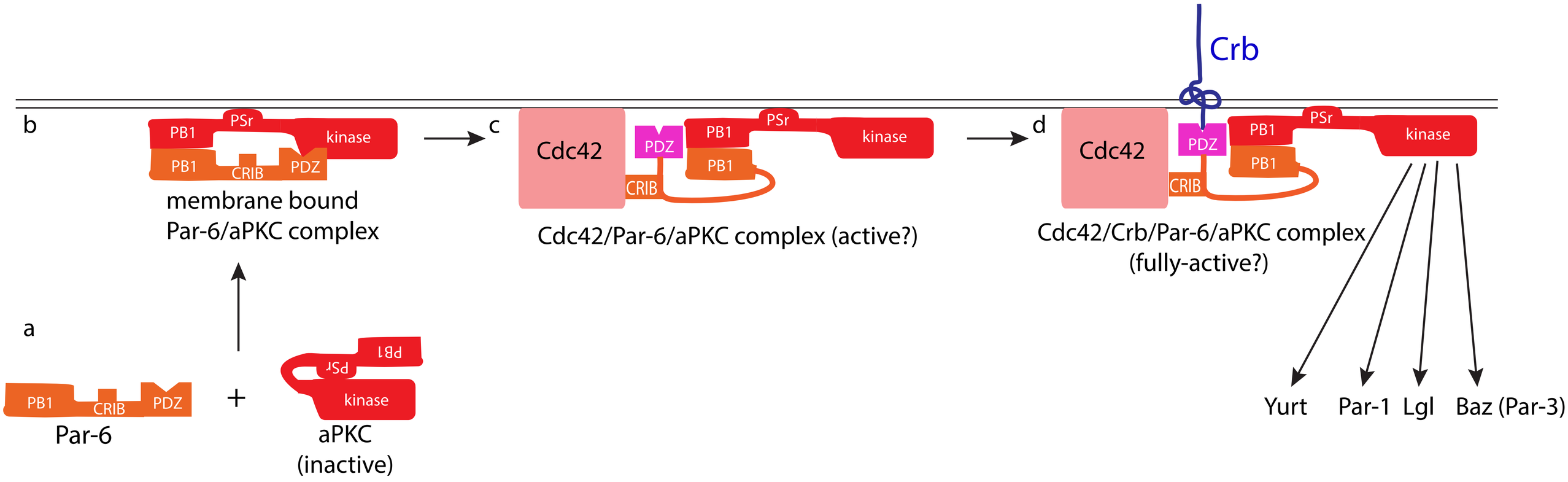


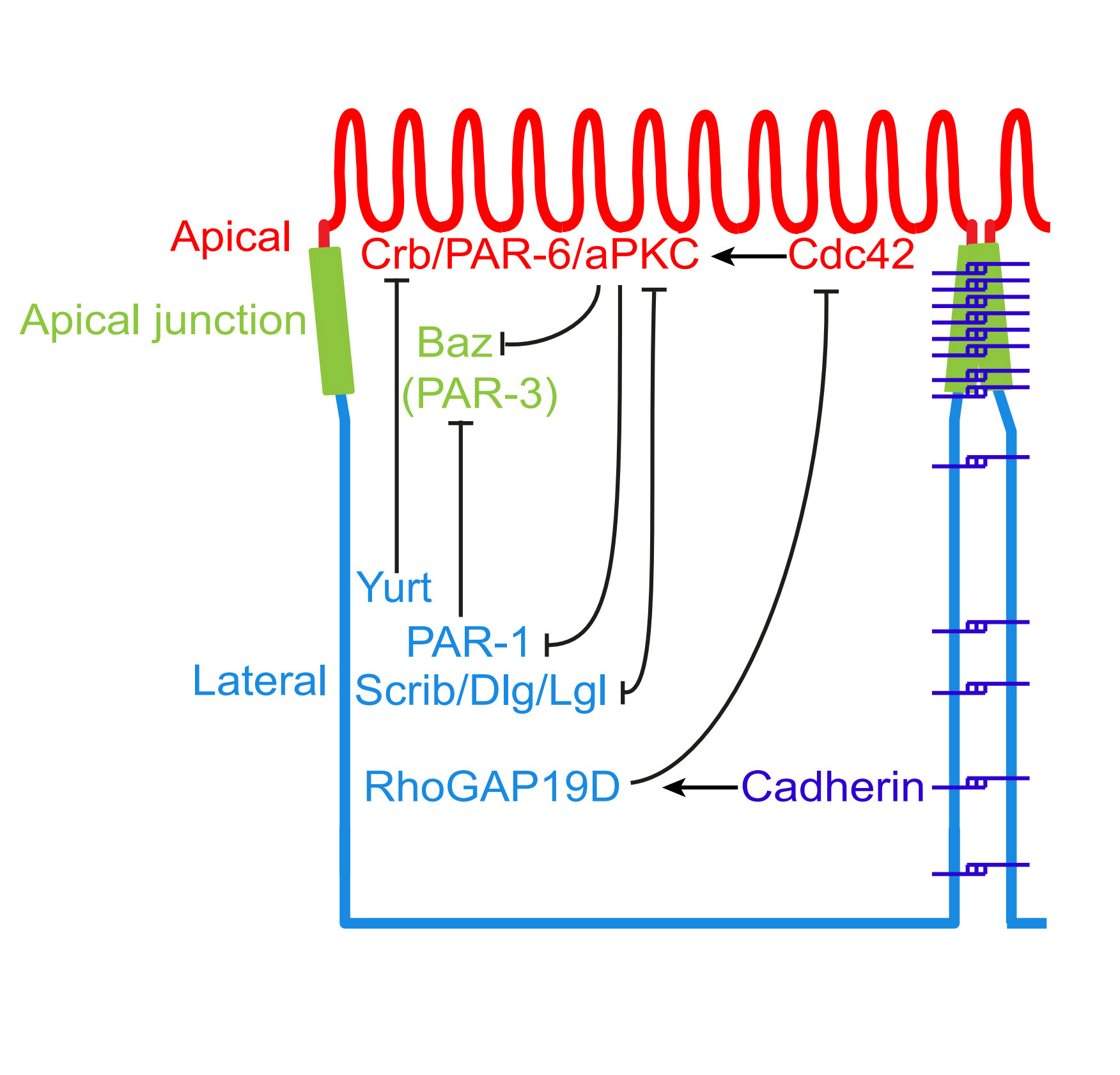


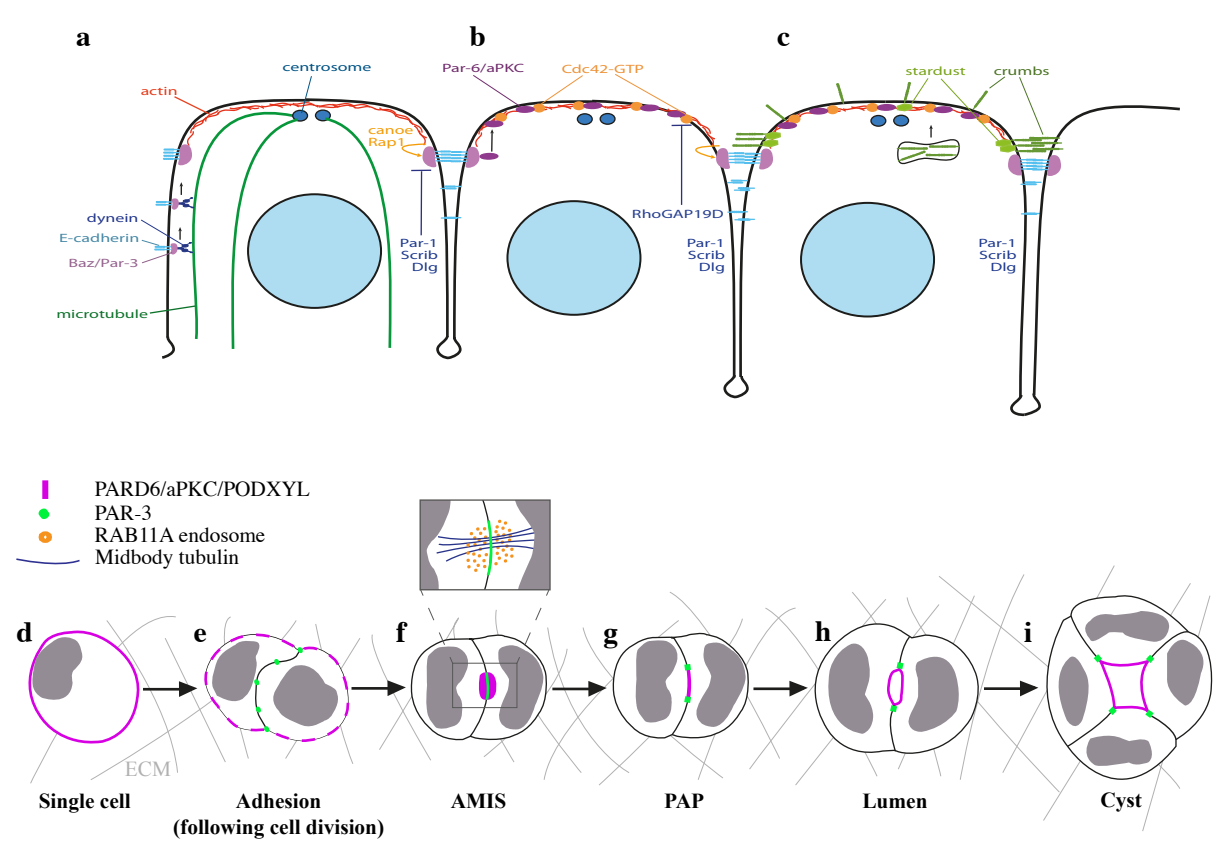
Vertebrate epithelium

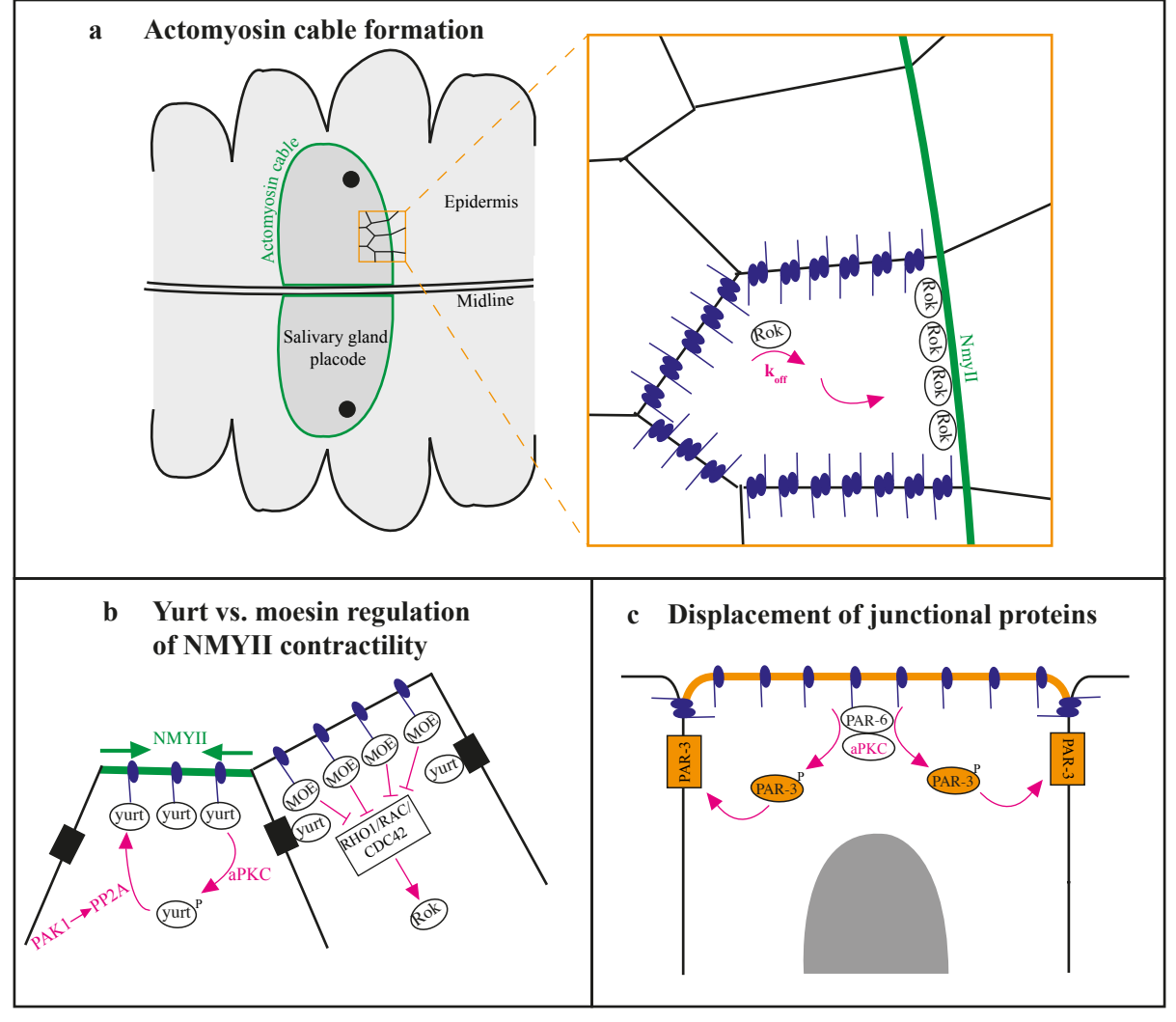
Insect epithelium

Crumbs/Crb1-3
Cdc42
FBM
Moesin, Ezrin, Radixin
Yurt/Lulu2
Expanded
βH-spectrin?
Actin
Morg1
ERLI
Par-6
PB1
Semi-CRIB
PDZ
PBM
L27
L27
PDZ
SH3
Hook
GUK
PatJ
Lin7
aPKC
PB1
pseudosub polybasic D
Kinase
PBM
PI4P, PIP2 (PM)
Oligomerisation phase separation
Par-3/Baz
CR1
PDZ
PDZ
PDZ
CR3
TIAM1/Sif
PIP2, PIP3 (PM)
JAM1-3
Nectin1 and 3
E-cadherin
PTEN
Echinoid
β-catenin
ASPP2
RASSF8 &10
MAGI1-3
Oligomerisation phase separation
Dimerisation
ZO-1
PDZ
PDZ
PDZ
SH3
U5
GUK
IDR
IDR
IDR
Claudin
YAP1
Nectin
JAM-A
ZO-2 & 3
Afadin
Occludin
Actin
VANG1
CASK
Phospholipids
Dlg
PDZ
PDZ
PDZ
SH3
Hook
GUK
Gukh, SGEF
APC
β-catenin
β2-spectrin
Scribble
LAPSD
LAPSD
PDZ
PDZ
PDZ
PDZ
aPKC
PI4P, PIP2 (PM)
NMYII
Lgl
WD40
WD40
WD40
WD40
polybasic D


b
PB1
PSr
kinase
PB1
CRIB
PDZ
membrane bound
Par-6/aPKC complex
c
Cdc42
PDZ
PB1
PSr
kinase
CRIB
PB1
Cdc42/Par-6/aPKC complex (active?)
d
Crb
Cdc42
PDZ
PB1
PSr
kinase
CRIB
PB1
Cdc42/Crb/Par-6/aPKC complex
(fully-active?)
Yurt
Par-1
Lgl
Baz (Par-3)
a
PB1
CRIB
PDZ
Par-6
+
PB1
PSr
kinase
aPKC
(inactive)


Apical
Crb/PAR-6/aPKC
Cdc42
Apical junction
Baz
(PAR-3)
Yurt
PAR-1
Lateral
Scrib/Dlg/Lgl
RhoGAP19D
Cadherin


a
b
c
actin
centrosome
canoe
Rap1
dynein
E-cadherin
Baz/Par-3
microtubule
Par-1
Scrib
Dlg
Par-6/aPKC
Cdc42-GTP
RhoGAP19D
stardust
crumbs
PARD6/aPKC/PODXYL
PAR-3
RAB11A endosome
Midbody tubulin
d
e
f
g
h
i
ECM
Single cell
Adhesion
(following cell division)
AMIS
PAP
Lumen
Cyst


a
Actomyosin cable formation
Actomyosin cable
Epidermis
Midline
Salivary gland placode
Rok
k off
NmyII
b
Yurt vs. moesin regulation of NMYII contractility
NMYII
yurt
MOE
RHO1/RAC/ CDC42
aPKC
PAK1→PP2A
yurt P
Rok
c
Displacement of junctional proteins
PAR-3
PAR-6
aPKC
PAR-3 P

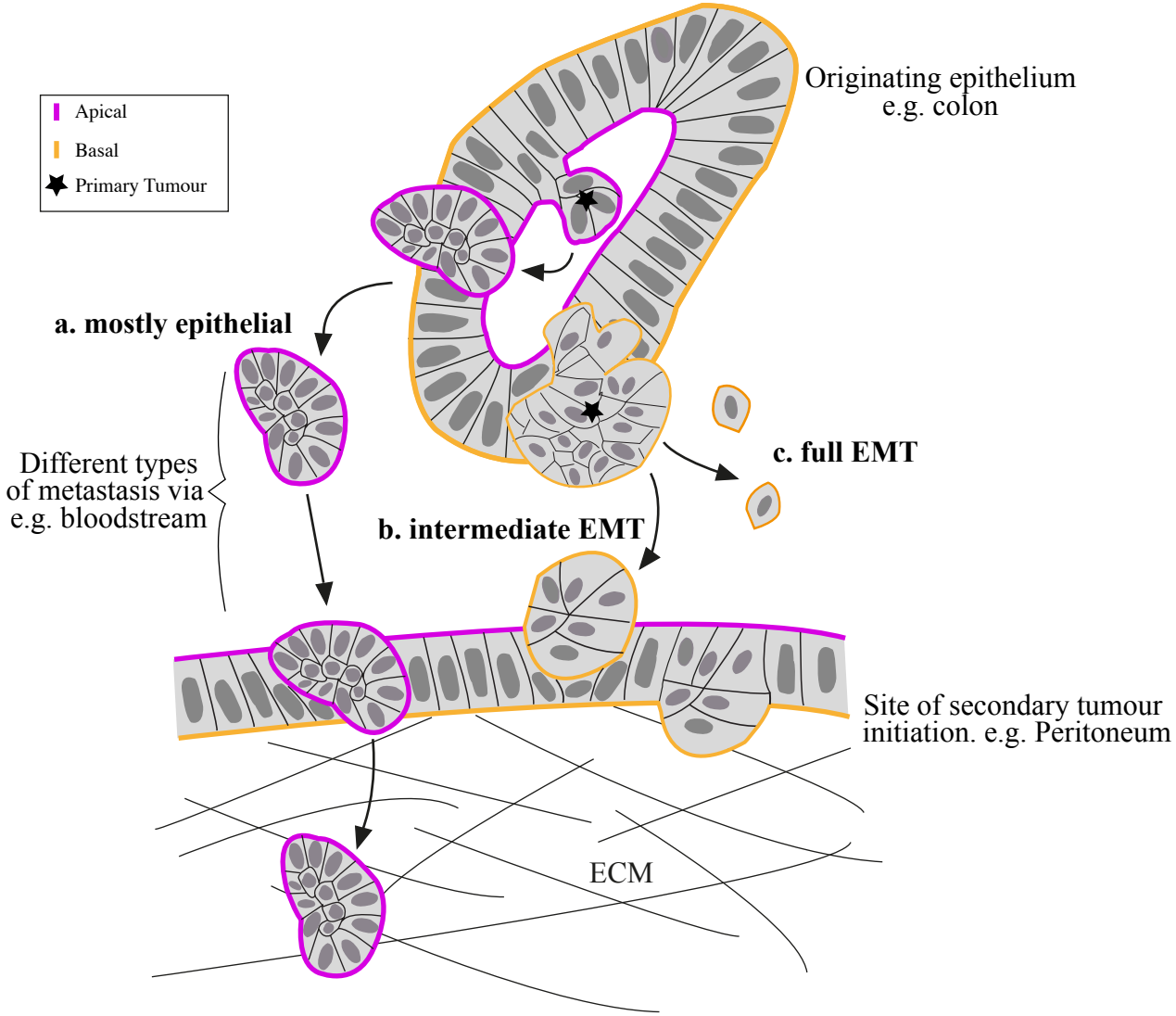

Apical
Basal
Primary Tumour
Originating epithelium
e.g. colon
a. mostly epithelial
Different types
of metastasis via
e.g. bloodstream
b. intermediate EMT
c. full EMT
Site of secondary tumour
initiation. e.g. Peritoneum
ECM

Figure Legends

Figure 1: The structure and functions of simple epithelia

The lateral sides of epithelial cells (green) adhere to each other through homophilic adhesion molecules, such as E-cadherin, which often becomes concentrated in adherens junctions (orange) in the apical region of the lateral membrane. **a** | In vertebrate epithelia, the tight junction (pink) at the top of the lateral domain acts as a barrier to paracellular diffusion, preventing the movement of large molecules and pathogens across the epithelium. The equivalent junction in insects, the septate junction, forms below the adherens junction in all epithelia except the midgut. **b** | Epithelia target the exocytosis of specific secreted or transmembrane proteins to either the apical or basolateral domain of the cell. This is important to ensure that receptors face the compartment that contains their ligands and that ion channels and pumps are in the correct membrane to control the composition of the luminal milieu. **c** | As epithelial sheets undergo morphogenesis to form more complex structures such as tubes, the relative sizes of the apical, lateral and basal domains must change. **d** | During development, some epithelial cells undergo an epithelial to mesenchymal transition (EMT) by losing their apical-basal polarity and becoming migratory, such as the neural crest cells that delaminate from the dorsal margins of the neural tube. Cancers derived from epithelial tissues often show a similar partial loss of apical-basal polarity to become metastatic. **e |** The organisation of vertebrate and invertebrate epithelia showing the main proteins that form intercellular junctions and adhesions to the basement membrane and the arrangement of the actin and microtubule cytoskeletons.

Figure 2: The domain structure of the main epithelial polarity factors

The diagram shows the domain structure of the Crumbs complex (orange), the Par-6/aPKC complex (ochre), the factors that organise the apical junction, PAR-3 and ZO-1 (magenta) and the lateral factors, Scribble, Discs large and Lgl (brown). The extracellular domain of Crumbs is not shown. The arrows show the protein-protein interactions between the polarity factors and with their downstream effectors mentioned in the text. Interactions that have not been mapped to a specific domain have been excluded. PM; plasma membrane

Figure 3: The regulation of aPKC activity by other apical polarity factors

a | The pseudosubstrate domain of aPKC interacts with its active site to inhibit kinase activity. **b** | The binding of the Par-6 PB1 domain to aPKC's PB1 domain releases the pseudosubstrate domain, which then acts as a polybasic lipid-binding domain to recruit the complex to the plasma membrane. **c** | Par-6/aPKC is not active, however, and activation requires the binding of the semi-CRIB domain of Par-6 to active Cdc42-GTP at the membrane. **d** | The binding of Cdc42 to Par-6 induces a conformational change in the latter's PDZ domain that allows it to recognize the C-terminal ERLI motif of Crumbs, thereby anchoring the Par-6/aPKC complex to the plasm membrane and perhaps further increasing aPKC kinase activity.

Figure 4: Antagonistic interactions between polarity factors.

A diagram showing the antagonistic interactions between polarity factors that maintain the identity and control the size of the apical, junctional and lateral domains.

Figure 5: Establishing polarity

a-c | **Steps in the polarisation of the *Drosophila* cellular blastoderm epithelium**. At the beginning of cellularisation, actin is enriched apically and the microtubules grow basally from apical centrosomes. **a** | Bazooka (Baz/Par-3) and associated E-cadherin complexes are localised to the apical margin of the lateral membrane by three different mechanisms: dynein transports Bazooka apically along microtubules, Par-1 excludes Bazooka from the lateral membrane and apical actin recruits Bazooka through Canoe and Rap1. **b** | The Par-6/aPKC complex associates with Bazooka. aPKC is then activated by Cdc42-GTP, which is inhibited laterally by RhoGAP19D, leading to the phosphorylation of Bazooka. This triggers the dissociation of Par-6/aPKC from Bazooka and their apical localisation. **c** | aPKC activity induces the apical exocytosis of Crumbs through a pathway that depends on the Rab11 positive recycling endosome, and Crumbs is then stabilised in the apical membrane by binding to Stardust. Crumbs becomes concentrated in the marginal zone above the adherens junctions through homophilic adhesion with Crumbs in the adjacent cell.

d-h | **Epithelial cyst formation.** **d** | Apical factors are initially localised on the external cell membrane. **e** | Following cell division, PAR-3 puncta localise to the cell-cell interface and apical factors begin to be lost from the external cell membrane due to signalling from the ECM (grey lines). **f** | PAR-3 localises to the cleavage furrow and RAB11A endosomes accumulate around the midbody. Apical proteins such as PODOCALYXIN and PAR-6 are localised around the AMIS. **g** | Apical proteins fuse with the apical membrane and PAR-3 is displaced. **h** | A lumen opens. **i** | Further cell divisions and lumen expansion result in polarised cyst formation.

Figure 6: Context-specific roles of Crumbs in morphogenesis.

a | High levels of Crumbs (blue) on inner membranes of *Drosophila* salivary placode cells increase the k_{off} of Rok whilst low levels of Crumbs on outer membranes allow Rok accumulation, which enables NMYII cable formation. **b** | Yurt and Moesin compete for binding to the FRB domain of Crumbs. Yurt enhances NMYII contractility, and this is inhibited by aPKC phosphorylation of Yurt and promoted by PAK1/PP2 dephosphorylation of Yurt. Moesin represses NMYII contractility through its inhibition of the RHO/Rok pathway. **c** | During apico-lateral junction formation, junctional proteins such as PAR-3 are initially localised at the apical-most cell membrane. Crumbs delivery to the apical membrane then enables the translocation of junctional proteins to the apico-lateral junctions via aPKC phosphorylation of PAR-3.

Figure 7: Partial EMT and metastasis

Schematic of different types of metastasising carcinomas, each at different levels along the epithelial to mesenchymal spectrum. **a)** Tumour spheres with inverted polarity (TSIPs), such as those found in metastasising colorectal cancer. These cells bud away from the apical surface and retain most of their epithelial features, with a clear outer apical surface and adhesions between cells. They enter the peritoneal cavity either via the bloodstream or via invasion through the digestive wall. They then invade the ECM of e.g. the peritoneum via their apical surface. **b)** Delaminating cells that have undergone partial EMT, such that they have lost their apico-basal polarity but retained their cell-cell adhesions. This allows them to invade tissue as collectives, facilitating the MET necessary for colonisation. **C)** Delaminating cells that have undergone full EMT and migrate as single cells. Whilst these cells can also invade tissue via basal specialisations, they are less likely to generate secondary metastasis.

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