

Cell Polarity in Eggs and Epithelia: Parallels and Diversity

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Cell polarity, the generation of cellular asymmetries, is necessary for diverse processes in animal cells, such as cell migration, asymmetric cell division, epithelial barrier function, and morphogenesis. Common mechanisms generate and transduce cell polarity in different cells, but cell type-specific processes are equally important. In this review, we highlight the similarities and differences between the polarity mechanisms in eggs and epithelia. We also highlight the prospects for future studies on how cortical polarity interfaces with other cellular processes, such as morphogenesis, exocytosis, and lipid signaling, and how defects in polarity contribute to tumor formation.

Introduction

Most animal cells display obvious polarity, indicating the presence of molecular asymmetries. The general steps of cell polarity, including its establishment and transduction, are common irrespective of cell type or organism: a polarity cue, regulation of the cytoskeleton, dedicated polarity proteins, and transduction of polarity information. The initial steps of polarity have mechanistic parallels in different cell types, and PAR proteins exemplify this conservation. Identified through seminal work from Ken Kemphues and colleagues in the *C. elegans* zygote (Kemphues et al., 1988; Suzuki and Ohno, 2006), PAR proteins are conserved and dedicated regulators of polarity in animal cells. Of particular importance are PAR-3, PAR-6, and aPKC (atypical protein kinase C), which form an asymmetrically localized complex in many polarized cells (Suzuki and Ohno, 2006). Although initial work in different organisms suggested a simple view of obligate interactions between PAR proteins, it is now clear that equally important roles are played by complexes containing both PAR and non-PAR proteins and by independent polarity complexes. To illustrate the congruence and diversity of cell polarity mechanisms, we focus on cell polarity in two different cell types: eggs and epithelia.

Establishing Polarity in the *C. elegans* Embryo

The one-celled embryo of *C. elegans* is one of the best understood and most experimentally amenable systems for studying the induction and transduction of polarity. Over the course of 1 hr after fertilization, the oocyte transforms into a highly polarized one-cell embryo that divides asymmetrically to give rise to cells of different sizes, contents, cell-cycle times, and developmental potentials (Figure 1). Where and when does polarity arise? The key event is the negative regulation of actomyosin contractility, which is triggered locally by a sperm cue.

The *C. elegans* oocyte, arrested in prophase of meiosis I, has no developmentally significant polarity (Goldstein and Hird, 1996). After fertilization and the completion of meiosis, the oocyte nucleus

(i.e., the pronucleus) and the sperm pronucleus/centrosome complex are usually found at the presumptive anterior and posterior ends of the cell, respectively. At this time, actin and the non-muscle myosin NMY-2 form a contractile network uniformly over the entire outermost layer, or cortex, of the embryo (Figure 1, left) (Munro et al., 2004). Polarization is blocked by actin inhibitors and myosin knockdowns, demonstrating the importance of actomyosin contractility for polarity establishment (Cowan and Hyman, 2007; Gonczy and Rose, 2005).

The sperm provides a polarity cue at the posterior end of the embryo that locally downregulates cortical contractility (Figure 1, second embryo) (Goldstein and Hird, 1996; Munro et al., 2004). The sperm pronucleus/centrosome complex closely associates with the posterior cortex, inducing local loss of the uniform NMY-2 network (Goldstein and Hird, 1996; Munro et al., 2004). This leads to contraction of the actomyosin cytoskeleton and flow of cortical material (cortical flow) away from the posterior signal, culminating in the strong anterior enrichment of actin foci and NMY-2 (Cowan and Hyman, 2007; Gonczy and Rose, 2005). As a consequence, the posterior cortex appears smooth and quiescent (i.e., with low contractility), while the anterior cortex remains highly contractile (Figure 1, third embryo).

The polarity signal acts at least partially through the negative regulation of the small G protein RHO-1. RHO-1 and the Rho guanine nucleotide exchange factor (GEF) ECT-2 (similar to the human Ect2 protooncogene) are essential for cortical contractility and for anterior enrichment of NMY-2 (Jenkins et al., 2006; Motegi and Sugimoto, 2006; Schonegg and Hyman, 2006). Through an unknown mechanism, the posterior cortex adjacent to the sperm centrosome becomes locally depleted of ECT-2, which presumably prevents the posterior activation and localization of RHO-1 (Motegi and Sugimoto, 2006). RHO-1 activity is also negatively regulated at the anterior by two partially redundant Rho GTPase-activating proteins (RhoGAP), RGA-3 and RGA-4 (Schmutz et al., 2007; Schonegg et al., 2007). Loss of these RhoGAPs leads to hypercontractility of the anterior cortex and

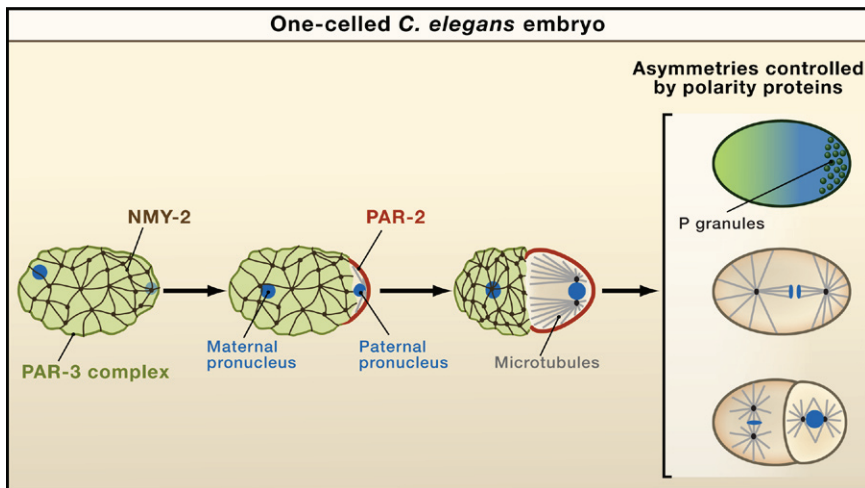


Figure 1. Polarity Induction and Transduction in the *C. elegans* Embryo

(Left) Before polarity induction, the actomyosin cortex (brown lines) is uniformly contractile. The PAR-3 complex (green), which contains PAR-3, PAR-6, and the atypical protein kinase C (aPKC), is distributed uniformly over the outermost layer, or cortex, of the oocyte.

(Middle) The polarity cue, dependent on the centrosome of the sperm and coincident with the growth of microtubules (gray lines), induces loss of actin and the nonmuscle myosin NMY-2 at the posterior of the embryo.

(Right) Movement of the actomyosin cytoskeleton to the anterior drives the PAR-3 complex to localize to the anterior. As the PAR-3 complex leaves, PAR-2 (red) associates with the smooth, quiescent posterior cortex.

(Far Right) PAR proteins control different polarized events in the daughter cells, such as cytoplasmic asymmetries (top), posterior placement of the mitotic spindle (middle), and cell-cycle timing differences in daughter cells (bottom).

excessive anterior movement of NMY-2. Because RHO-1 activates myosin contractility, positive regulation of NMY-2 by active RHO-1 at the anterior could cause anterior movement of the actomyosin cytoskeleton (Motegi and Sugimoto, 2006). Thus, both positive and negative regulation of RHO-1 are important for establishing the sizes of the contractile and quiescent domains.

The polarity cue is currently unknown, but it does depend on the sperm-donated centrosome: embryos with mutations that impair centrosome maturation or with centrosomes ablated by lasers both fail to polarize; further, the induction of polarity is coincident with the growth of microtubules from the centrosome (Cowan and Hyman, 2004; Cuenca et al., 2003; O'Connell et al., 2000; Wallenfang and Seydoux, 2000). Preventing the majority of microtubule growth from the centrosome by RNA interference (RNAi) of tubulin gene expression does not prevent polarity induction, indicating a large mitotic aster is not needed (Cowan and Hyman, 2004; Sonnevile and Gönczy, 2004; Tsai and Ahringer, 2007). However, in embryos with low tubulin levels, polarity induction is delayed until a small aster forms, supporting a requirement for microtubules in the cue (Tsai and Ahringer, 2007). The sperm also appears to deliver to the posterior membrane CYK-4, a Rho family (e.g., Rho, Rac, or Cdc42) GAP (Jenkins et al., 2006). However, its relationship with RHO-1 is unclear because recent work suggests that CYK-4 acts on a Rac GTPase and not RHO-1 (Canman et al., 2008). No polarity phenotypes have yet been reported for Rac mutants, but as there are three *C. elegans* genes encoding Rac proteins, there could be redundancy.

The most important question remaining is the identity of the polarity cue and its mechanism of delivery and reception. One possibility is that microtubules locally deliver a signaling molecule to the cortex, such as a regulator that causes the removal or inhibition of the Rho GEF ECT-2. The current failure to find the polarity cue despite extensive RNAi and mutant screens suggests that redundant mechanisms could induce polarity. Indeed, Zonies et al. (2010) have very recently demonstrated the existence of two redundant pathways that polarize the *C. elegans* zygote, one depending on ECT-2 and the other on PAR-2. These new data combined with new approaches should help to identify the molecules involved.

A Link to Cytokinesis

It is striking that most of the regulators of cell polarity mentioned above are also involved in cytokinesis (i.e., the splitting of a cell into two cells during the late stages of mitosis) (Oegema and Hyman, 2006). This suggests that the embryo has co-opted the cytokinesis machinery for the establishment of polarity. During cytokinesis, actomyosin contractility is inhibited at the poles through cortical interactions with astral microtubules (Foe and von Dassow, 2008; Werner et al., 2007). Perhaps a similar mechanism involving the microtubules nucleated by the sperm causes local downregulation of actomyosin contractility during polarity induction. Given this relationship, it will be interesting to investigate possible roles for other cytokinesis players in polarity induction.

One important process in cytokinesis that has not yet been linked to the induction of polarity in *C. elegans* is lipid signaling. Recently, PPK-1, a PI(4)P-5-kinase that generates the phosphoinositide PIP₂, was found to accumulate at the posterior cortex near the sperm pronucleus approximately at the time of polarity induction (Panbianco et al., 2008). As PIP₂ is important for cytokinesis and regulates the activities of many actin-binding proteins, exploring the possible function of phosphoinositides in polarity induction might also be a fruitful topic of future research.

The PAR Proteins in *C. elegans*

A major consequence of asymmetric regulation of the actin cytoskeleton is the asymmetric localization of many of the PAR proteins and atypical protein kinase C (aPKC). Loss of any of these proteins disrupts cell polarity, usually resulting in two equal-sized cells that divide at the same time and have similar developmental fates (Cowan and Hyman, 2007; Gonczy and Rose, 2005). Asymmetric localization of PAR proteins occurs in two mechanistically distinct phases: establishment and then maintenance (Cuenca et al., 2003; Motegi and Sugimoto, 2006).

PAR-3, PAR-6, and the atypical protein kinase C PKC-3 (the PAR-3 complex) are initially localized uniformly at the cortex, but then they accumulate in the anterior of the cell after polarity induction (Cowan and Hyman, 2007; Gonczy and Rose,

2005). The actomyosin cytoskeleton appears to play a direct role in localizing the PAR-3 complex. A fusion of the PAR-6 protein with green fluorescent protein (GFP-PAR-6) moves anteriorly at the same speed and in register with NMY-2 myosin, and disruption of cortical F-actin prevents cortical association of PAR-3 (Munro et al., 2004; Severson and Bowerman, 2003). Whereas the PAR-3 complex associates with the contractile anterior cortex, PAR-2 (a Ring-finger protein with no clear counterpart outside worms) associates with the expanding quiescent posterior cortex, leading to two non-overlapping PAR polarity domains (Cowan and Hyman, 2007; Gonczy and Rose, 2005). Neither the PAR-3 complex proteins nor PAR-2 are needed for the initial local clearing of ECT-2 or NMY-2, indicating that they act downstream of the response to the polarity cue (Motegi and Sugimoto, 2006). However, they contribute to actomyosin function: PAR-3 positively regulates the speed of anterior cortical movement, *par* mutants show disrupted actin distribution, and PAR-2 negatively regulates NMY-2 cortical association (Cheeks et al., 2004; Munro et al., 2004).

It seems likely that the actomyosin cytoskeleton somehow provides a dynamic binding site for the active PAR-3 complex during the establishment phase. The location of the PAR-3 complex in the cortex closely follows the anterior movement of the contractile actomyosin cortex, even when the latter is hypercontracted in *rga-3/4(RNAi)* embryos in which expression of *rga-3/4* is eliminated by RNAi (Schmutz et al., 2007; Schonegg et al., 2007). This association appears to be dynamic because fluorescence of GFP-PAR-6 recovers rapidly after photobleaching (Cheeks et al., 2004).

The distinct locations of the anterior PAR-3 complex and the posterior PAR-2 domains are maintained through negative regulatory interactions (Cowan and Hyman, 2007; Gonczy and Rose, 2005). First, PKC-3 phosphorylates PAR-2, which excludes it from the anterior cortex (Hao et al., 2006). Reciprocally, PAR-2 at the posterior cortex prevents localization of the anterior PAR complex there, but the mechanism is unknown (Hao et al., 2006; Cowan and Hyman, 2007; Gonczy and Rose, 2005). Second, the small G protein CDC-42 (cell division control protein 42) maintains the polarity of PAR protein localization via positive regulation of the PAR-3 complex (Cowan and Hyman, 2007; Gonczy and Rose, 2005). A recent study, which identified regulators of CDC-42, has shed light on this mechanism (Kumfer et al., 2010). One regulator, CHIN-1, is a GAP protein that localizes to the posterior cortex during the maintenance phase. By inhibiting CDC-42, CHIN-1 is proposed to destabilize anterior PAR complexes that migrate into the posterior, thereby helping to maintain the polarized distribution of proteins. Endocytosis also appears to be important for the activity or localization of PAR polarity proteins in *C. elegans* (Balklava et al., 2007; Nakayama et al., 2009).

Surprisingly, although association of PAR-2 with the posterior cortex normally depends on removal of the PAR-3 complex, PAR-2 can still form a posterior domain in embryos depleted of RHO-1 or ECT-2, in which anterior PAR proteins fail to polarize (Jenkins et al., 2006; Schonegg and Hyman, 2006). In these embryos, NMY-2 asymmetry is also established (Motegi and Sugimoto, 2006; Schonegg and Hyman, 2006). This suggests

that the sperm supplies two polarity signals, one that inhibits RHO-1 activity and another that controls PAR-2 asymmetry independently of RHO-1 and the PAR-3 complex, consistent with Zonies et al. (2010).

Three other PAR proteins (PAR-1, PAR-4, and PAR-5) are also important for embryonic polarity. The Ser/Thr kinase PAR-1 localizes to the posterior cortex and plays a major role in establishing cytoplasmic asymmetries in the one-celled embryo (Gonczy and Rose, 2005). PAR-1 is not needed for the asymmetry of PAR-3, PAR-6, PKC-3, or PAR-2, although their domain sizes are sometimes abnormal in *par-1* mutants (Cuenca et al., 2003). PAR-4 is orthologous to the human tumor suppressor protein, LKB1, which is a master kinase that activates several downstream kinases, including the mammalian PAR-1 orthologs, the MARKs (Lizcano et al., 2004; Watts et al., 2000). Consistent with this role, *C. elegans par-4* mutants display embryonic phenotypes that are similar to but weaker than those of *par-1* mutants (Morton et al., 1992; Watts et al., 2000). Studies in *Drosophila* epithelial cells recently revealed that LKB1 acts in a low-energy polarity pathway (Mirouse et al., 2007). In the stress-resistant dauer larva of *C. elegans*, PAR-4 also has nonpolarity roles, such as the regulation of energy stores and the resistance to oxidative stress, which probably reflect its role in activating other kinases, such as the low-energy sensor, AMPK (Lee et al., 2008; Narbonne and Roy, 2009). PAR-5 is a 14-3-3 protein, which regulates signaling pathways by binding to phosphorylated residues (Morton et al., 2002). Therefore, PAR-5 might cooperate with one or more of the three polarity kinases (PAR-1, PAR-4, PKC-3); indeed, in *Drosophila* PAR-5 binds residues on PAR-3/Bazooka when PAR-1 phosphorylates it (Benton and St Johnston, 2003b).

Cell Polarity Transduction in *C. elegans*

After the establishment of PAR protein asymmetry, the PAR proteins control a number of downstream polarized events (Figure 1, right), including posterior placement of the first mitotic spindle, asymmetries in cytoplasmic protein localization, and differences in cell-cycle timing in the two daughter cells. Protein phosphorylation is likely to be a major mechanism of transduction given that three PAR proteins are kinases; however, only a few direct targets for these kinases are currently known. Asymmetric spindle positioning is controlled by heterotrimeric G protein signaling through a receptor-independent mechanism. This topic was recently reviewed extensively (Gonczy, 2008; Siller and Doe, 2009), and thus we will not cover it here.

Polarity establishment induces a number of cytoplasmic asymmetries in the one-celled embryo, leading to the inheritance of different cytoplasmic contents (Figure 1, right) (Cowan and Hyman, 2007; Gonczy and Rose, 2005). Germline proteins, such as PIE-1, are found in the posterior cytoplasm and on P granules (large RNA/protein granules segregated to germline precursor cells) (Mello et al., 1996). In the anterior cytoplasm, the partially redundant and highly similar CCCH zinc-finger proteins, MEX-5 and MEX-6, are localized in a complementary domain and are required to generate posterior enrichment of germline proteins (Schubert et al., 2000). *par* mutants disrupt asymmetry of both MEX-5/6 and PIE-1; however, loss of PIE-1 does not impair PAR or MEX-5/6 asymmetry, suggesting that

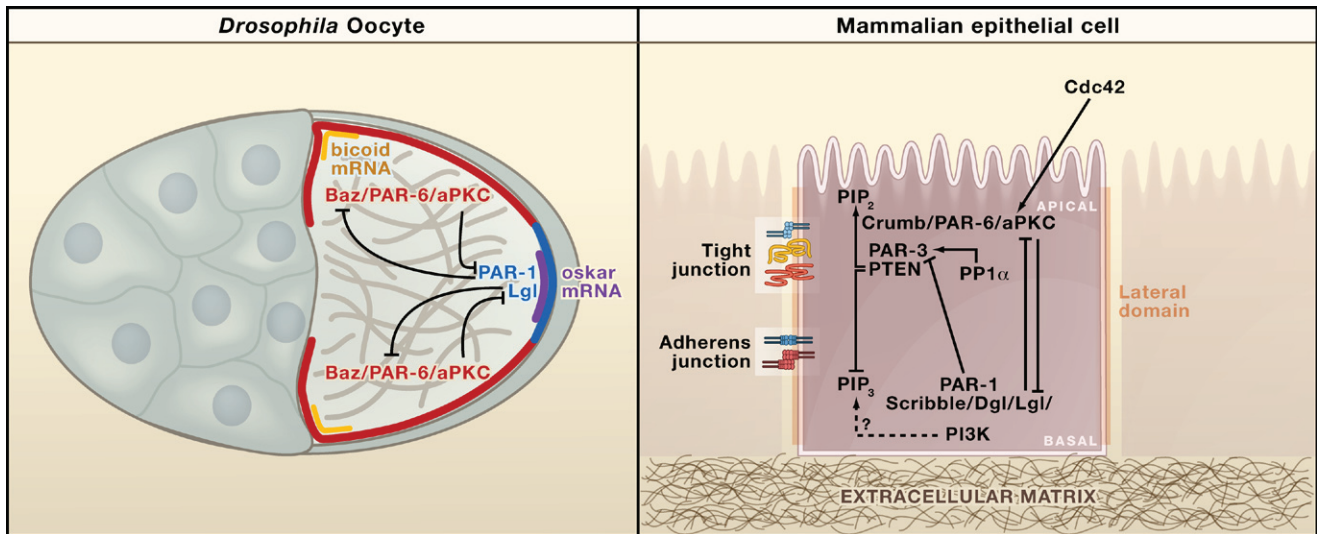


Figure 2. Polarization of the *Drosophila* Oocyte and Mammalian Epithelial Cell

(Left) Unlike the *C. elegans* oocyte, the *Drosophila* oocyte becomes polarized before fertilization with complementary domains of polarity proteins at the cortex. The PAR-3 complex (red), which in *Drosophila* contains Bazooka, PAR-6, and atypical protein kinase C (aPKC), localizes along the anterior and lateral cortex, whereas PAR-1 and lethal giant larvae (Lgl) localize at the posterior (blue). These polarity proteins regulate the organization of the microtubule cytoskeleton so that microtubules are nucleated from the anterior and lateral cortex but not from the posterior. This creates an anterior to posterior gradient of microtubules (gray) with a slight excess of plus ends pointing toward the posterior. This weakly polarized microtubule cytoskeleton then directs the transport of *bicoid* (yellow) and *oskar* (purple) mRNAs to the anterior and the posterior, respectively.

(Right) Maintaining distinct cortical domains in epithelial cells depends on mutually antagonistic interactions between the polarity complexes. The Crumbs complex (Crumb/Stardust/PATJ), which is enriched immediately above where the tight junctions form, interacts with PAR-6 and atypical kinase C (aPKC) at the apical domain to exclude PAR-3 (Bazooka in *Drosophila*) and the Scribble complex. In turn, Scribble excludes aPKC at the lateral domain. These interactions may lead to the asymmetric distribution of phosphatidylinositol phosphates (PIPs), with PIP₃ restricted to the basolateral membrane and PIP₂ concentrated in the apical membrane. To help establish this asymmetry, PTEN (phosphatase and tensin homolog) is localized to the apical junction by PAR-3 (Bazooka), where it converts PIP₃ to PIP₂. In addition, PI3-kinase (PI3K) may be recruited to the lateral membrane by Discs large (Dlg), where it creates PIP₃ by phosphorylating PIP₂.

MEX-5/6 acts downstream of PAR polarity in this pathway (Schubert et al., 2000). MEX-5/6 also affects PAR protein localization, indicating a possible feedback mechanism between PAR and MEX-5/6 (Cuenca et al., 2003). One mechanism for localizing PIE-1 to germ cells is via its degradation in somatic cells after cell division, but how MEX-5/6 and PIE-1 asymmetries were generated in the one-celled embryo before division was not known (DeRenzo et al., 2003).

Two new studies shed light on these mechanisms. The first study shows that reduced mobility of MEX-5 in the anterior cytoplasm compared to the posterior contributes to its cytoplasmic asymmetry (Tenlen et al., 2008). Reduced anterior mobility develops at the time that actin and myosin accumulate in the anterior cortex and depends on NMY-2 being active, which suggests that the actomyosin cytoskeleton or an associated component restricts the mobility of MEX-5. The authors also identified a phosphorylation site on MEX-5 that is not only required for MEX-5 function and asymmetry but also depends on PAR-1 and PAR-4. Neither kinase has been shown to carry out phosphorylation directly, but the site identified by the authors resembles one recently identified for PAR-1 in *Drosophila* (D.St.J., unpublished data). A second study suggests that PIE-1 localization is governed by its conversion between fast- and slow-diffusing forms, and the authors propose that association of PIE-1 with P granules might be related to conversion to the slow form (Daniels et al., 2009). Intriguingly, P granules fail to accumu-

late at the posterior in *mex-5/6* mutants, and MEX-5/6 are required for the loss of P granule epitopes observed in *par-1* embryos (Cheeks et al., 2004; Schubert et al., 2000). Thus, one possible model is that MEX-5/6 inhibits the assembly of P granules in the anterior, thereby limiting PIE-1 diffusion indirectly.

The difference in cell-cycle timing of daughter cells is also regulated by both PAR proteins and MEX-5/6 (Cheeks et al., 2004; Kemphues et al., 1988). At the two-cell stage (Figure 1, right), the anterior AB cell divides 2 min before its posterior sister cell P1. This difference appears to be due to asymmetry in levels of CDC25.1 and PKL-1 (Polo-like kinase) (Budirahardja and Gönczy, 2008; Nishi et al., 2008; Rivers et al., 2008). PAR proteins and MEX-5/6 are not only necessary for anterior enrichment of PLK-1 and CDC-25, but these factors also affect the overall levels of PLK-1 (Budirahardja and Gönczy, 2008; Nishi et al., 2008; Rivers et al., 2008). PLK-1 is required for nuclear accumulation of CDC-25.1, supporting a model whereby higher levels of anterior PLK-1 induce higher levels of nuclear CDC-25.1 in the AB cell compared to the P1 cell. Ultimately, this leads to an earlier cell division time for the AB cell (Rivers et al., 2008). PLK-1 binds to MEX-5 in vitro, suggesting that MEX-5 might control PLK-1 asymmetry by direct association (Nishi et al., 2008). Timing differences are also regulated by DNA replication checkpoints, but it is not yet known how these signals interface with the polarity machinery (Brauchle et al., 2003; Budirahardja and Gönczy, 2008).

Drosophila Oocyte Polarity

Unlike in *C. elegans*, the anterior-posterior axis in *Drosophila* is established prior to fertilization through the polarization of the oocyte, which directs the localization of the maternal determinants *bicoid* and *oskar* messenger RNAs (mRNAs) to opposite poles of the cell (Bastock and St Johnston, 2008). Thus, the polarity does not depend on the site of sperm entry; instead it is induced by an unknown signal from the follicle cells that surround the posterior of the oocyte. Nevertheless, oocyte polarity relies on many of the same PAR proteins as found in *C. elegans* (Figure 2, left) (Bastock and St Johnston, 2008; Becalska and Gavis, 2010; Doerflinger et al., 2010). In particular, PAR-1 accumulates at the posterior cortex in response to the polarity cue, whereas Bazooka (PAR-3), PAR-6, and aPKC localize to the cortex at the anterior and lateral sides of the oocyte (Figure 2) (Doerflinger et al., 2006; Doerflinger et al., 2010). Although *Drosophila* does not have a PAR-2 ortholog, mutually inhibitory interactions between the anterior and posterior PAR complexes appear to maintain these complementary cortical domains by a similar mechanism to that used by the *C. elegans* counterparts. The posterior PAR-1 kinase phosphorylates Bazooka to exclude the Bazooka/PAR6/aPKC complex from the posterior of the oocyte (Benton and St Johnston, 2003b). Conversely, aPKC is thought to exclude PAR-1 from the cortex by phosphorylating a conserved serine in the C-terminal domain of PAR-1 because this has been shown to occur in mammals (Hurov et al., 2004). Furthermore, mutation of the conserved aPKC phosphorylation site causes PAR-1 to distribute over the entire oocyte cortex (Doerflinger et al., 2010).

It is much less clear how the PAR domains are initially established in the oocyte because there is no evidence for a contraction of the cortical actin cytoskeleton toward the anterior as seen in the *C. elegans* zygote. This suggests that the signal from the posterior follicle cells must either recruit PAR-1 to the posterior cortex or exclude or inactivate the Bazooka/PAR6/aPKC complex in this region. One candidate for an upstream transducer of this signal is lethal giant larvae (Lgl) because it localizes to the posterior cortex at approximately the same time as PAR-1; furthermore, Lgl is required for the localization of PAR-1 (Figure 2, left) (Tian and Deng, 2008). Lgl binds directly to the PAR-6/aPKC complex and inhibits aPKC activity (Betschinger et al., 2003; Wirtz-Peitz et al., 2008; Yamanaka et al., 2003). Therefore, this inhibition might allow PAR-1 to localize to the posterior cortex in much the same way PAR-2 excludes the PAR-3 complex to allow PAR-1 localization in the *C. elegans* zygote.

The posterior localization of Lgl cannot entirely explain how anterior-posterior polarity is established in oocytes. First, aPKC is known to phosphorylate Lgl. This phosphorylation appears to negatively regulate the cortical localization of Lgl because a nonphosphorylatable form of Lgl distributes across the entire oocyte cortex and disrupts oocyte polarity (Tian and Deng, 2008). Thus, there is a reciprocal inhibitory relationship between Lgl and the Bazooka/PAR-6/aPKC complex similar to the one between PAR-1 and the Bazooka complex. This makes it very difficult to determine which factor acts upstream of the other to establish the asymmetry in the oocyte. Second, *lgl* null mutants produce a rather weak phenotype in which almost

all oocytes look normal (Li et al., 2008a); in contrast, partial loss-of-function mutations in *par-1* disrupt polarity completely. Therefore, there must be other pathways independent of Lgl that establish the polarized PAR domains.

A key function of the cortical polarity of the oocyte is to establish the anterior-posterior axis by directing the microtubule-dependent localization of *bicoid* and *oskar* mRNAs to the anterior and posterior of the oocyte, respectively (Figure 2, left) (St Johnston, 2005). Unlike the *C. elegans* zygote and *Drosophila* neuroblasts, which must localize cell fate determinants during mitosis, the oocyte is arrested in meiotic prophase, and thus the microtubules are free from the constraints imposed by the mitotic spindle. Instead, the oocyte microtubules form an anterior-posterior gradient in which the minus ends are anchored or nucleated at the anterior and lateral cortex with a majority of plus ends extending toward the posterior pole (Bastock and St Johnston, 2008). This organization of microtubules allows dynein (the minus end directed motor) to transport *bicoid* mRNA to the anterior of the cell, whereas kinesin (the plus end directed motor) transports *oskar* mRNA to the posterior (Brendza et al., 2000; Weil et al., 2006; Zimyanin et al., 2008).

It is not known how the PAR proteins control the polarity of the oocyte microtubule cytoskeleton, but microtubules are anchored at the regions of the cortex that are marked by Bazooka/PAR-6/aPKC complex and are absent from the PAR-1 domain, where the plus ends are enriched. Thus, the Bazooka/PAR-6/aPKC complex may promote nucleation and anchoring of microtubules, or PAR-1 may repress nucleation and stabilize plus ends. Because the Bazooka/PAR-6/aPKC complex and PAR-1 antagonize each other and mutations in one alter the localization of the other, it has been difficult to determine which is the main effector of microtubule organization. However, it is possible to make both complexes active everywhere around the cortex by coexpressing a form of Bazooka that is not inhibited by PAR-1 and a form of PAR-1 that is not inhibited by aPKC. These oocytes show a radially symmetric organization of microtubules that is identical to that seen with the mutant PAR-1 alone. This suggests that PAR-1 plays the major role in organizing the microtubule cytoskeleton (Doerflinger et al., 2010).

Imaging the particles that transport *oskar* mRNA in a kinesin-dependent manner reveals that the oocyte microtubule cytoskeleton is only weakly polarized: approximately 60% and 40% of the microtubules orient with their plus ends toward the posterior and anterior of the oocyte, respectively (Figure 2, left). *oskar* RNA must therefore undergo a biased random walk along a large number of microtubules in order to reach the posterior pole (Zimyanin et al., 2008). Furthermore, the initial polarity that defines the anterior-posterior axis is probably even weaker because the posterior localization of *oskar* mRNA and the subsequent translation of Oskar protein initiates a positive feedback loop that amplifies the polarity and leads to the localization of more mRNA (Zimyanin et al., 2007). Oskar protein also establishes other polarities in the oocyte. For example, the long isoform of Oskar increases clathrin-dependent endocytosis at the posterior by recruiting Rab5, Rab5, Rab7, and Rab11. This is required for the stable

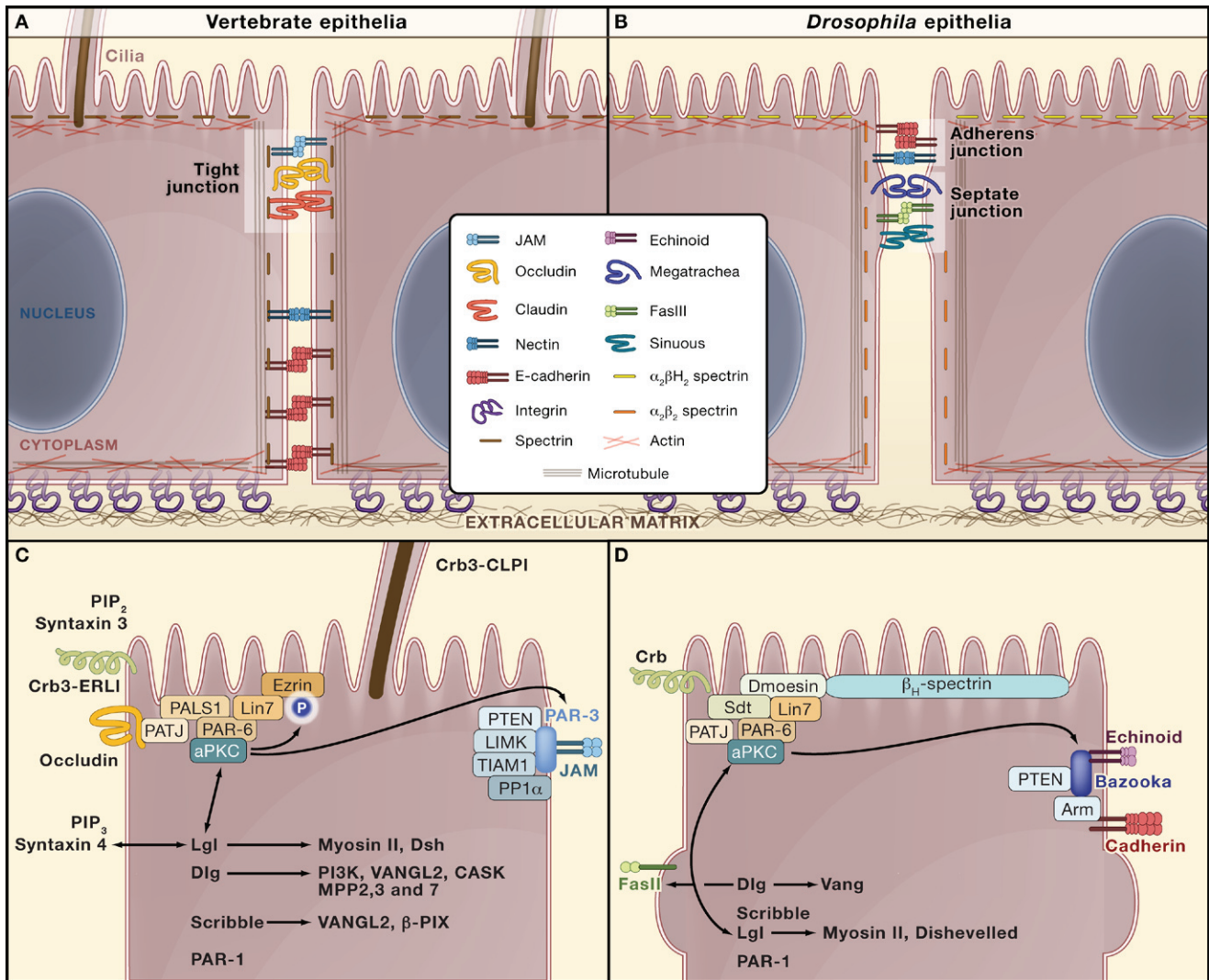


Figure 3. Intercellular Junctions and Polarity Factors in Epithelial Cells

(A) Two typical epithelial cells in vertebrates, showing the cilium and brush border of microvilli at the apical side. The apical tight junction, which functions as a paracellular diffusion barrier, forms as a result of homophilic interactions between Junctional Adhesion Molecules (JAM), Occludins, and Claudins. Adherens junctions, which localize on the lateral side beneath the tight junctions, consist of Cadherins and Nectins.

(B) In *Drosophila* epithelial cells, the arrangement of junctions is reversed, with apical adherens junctions and more basal septate junctions, which form the paracellular diffusion barrier. Epithelial cells in the fly also lack primary cilia and have a polarized spectrin cytoskeleton, with $\alpha_2\beta_{H_2}$ -spectrin apically and $\alpha_2\beta_2$ -spectrin basolaterally.

(C and D) In vertebrates and *Drosophila*, complexes of polarity proteins establish and maintain these local domains inside the epithelial cell. These two diagrams show the locations of the major polarity complexes and important interactions between them in mammalian (C) and *Drosophila* (D) epithelial cells.

anchoring of Oskar through the organization of the cortical actin cytoskeleton (Tanaka and Nakamura, 2008; Vanzo et al., 2007).

Epithelia

Epithelial cells form sheets of cells that function as barriers between compartments, usually the inside and outside of the organism, and they play an essential role in the transport of molecules from one side to the other. For *C. elegans* embryos and *Drosophila* oocytes, the polarity system specifies two complementary cortical domains that distinguish opposite sides of the cell. Epithelial cells are

similarly polarized into apical and basolateral membranes to which they sort different lipids and proteins (Rodriguez-Boulant et al., 2005). They differ from other polarized cell types, however, because they form a series of specialized cell junctions with neighboring cells that are essential for the organization of the epithelium and its function as a paracellular barrier. These junctions, which are positioned along the lateral sides of epithelia cells, increase the complexity of their apical-basal patterning, which comprises at least four distinct cortical domains: the apical domain, the tight junction, the adherens junction, and the basolateral domain (Figure 3).

Structure of Mammalian Epithelial Cells

In mammalian epithelia, such as those formed by Madine Darby canine kidney (MDCK) cells in culture, the apical domain is formed by the brush border of microvilli, which is underlain by a terminal web of actin and spectrin filaments (Figure 3A). These are linked to the plasma membrane by the ERM family proteins Ezrin, Moesin, and Radixin. The primary cilium protrudes from the middle of the apical surface, and it has a distinct membrane and protein composition from the rest of the cell, including the components of several signal transduction pathways.

The boundary between the apical and lateral domains is marked by the tight junctions, which contain a number of homophilic adhesion molecules, such as Occludin, Junctional Adhesion Molecules (JAMs), and the Claudins, which create the barrier to paracellular movement (Figure 3A). These proteins are all clustered by the MAGUK (membrane-associated guanylate kinase-like homology) proteins, ZO-1 and ZO-2, which bind to the cytoplasmic tails of Claudin and Occludin through their N-terminal PDZ domains. Cells that lack ZO-1 and ZO-2 fail to form tight junctions and do not develop high transepithelial resistance to electric current (Umeda et al., 2006). The tight junction has also been proposed to act as a “fence” that prevents the diffusion of membrane proteins between the apical and lateral domains. However, cells lacking ZO-1 and ZO-2 proteins still polarize normally and their membrane proteins still localize correctly to the apical and basolateral lipid bilayers, indicating that tight junctions are dispensable for epithelial polarity and forming the fence.

Adherens junctions assemble beneath the tight junctions and provide the main mechanical link between cells (Figure 3A). Adherens junctions are characterized by the presence of cadherins and their cytoplasmic adaptor proteins, β -catenin and α -catenin, which mediate homophilic adhesion with adjacent cells (Nelson, 2008). Another class of homophilic adhesion molecules, the nectins, also localize to these junctions and link these junctions to the actin cytoskeleton through the adaptor protein Afadin/AF-6 (Nelson, 2008; Takai et al., 2008a).

Finally, contact between the cell and the extracellular matrix or basement membrane is restricted to the basal side of the cell, which is enriched in extracellular matrix receptors, such as the integrins and dystroglycan, and depleted of intercellular adhesion molecules, such as cadherins.

Drosophila and *C. elegans* Epithelia

Epithelial polarity has been extensively studied in *Drosophila*, where the apical-basal axis is organized differently from vertebrates. Most importantly, the arrangement of lateral junctions differs from mammals. Adherens junctions are apical to the septate junctions, which act as the paracellular diffusion barrier in *Drosophila* (Figure 3B). Not all *Drosophila* epithelial cells have this arrangement of junctions because cells in the adult posterior midgut have septate junctions above the adherens junctions, which is more similar to the arrangement in mammals (Baumann, 2001).

Epithelia in *C. elegans* are more similar to those in *Drosophila* than mammals. Although they have a single apical junction that functions as both the adherens junction and the paracel-

lular barrier, cadherin and catenin proteins localize to the apical side of this structure. In contrast, Discs large (Dlg) protein, the coiled-coiled protein AJM-1, and claudins localize slightly more basally (Cox and Hardin, 2004). Furthermore, mutants in Dlg or AJM-1 remove the electron-dense material from the apical junction and disrupt the close apposition of the cell membranes, suggesting that they may be components of a structure that resembles the *Drosophila* septate junction. The cadherin and Dlg complexes appear to provide redundant adhesion activities because adhesion defects are seen in mutants lacking both cadherin and Dlg, but not in animals defective in only a single protein (Segbert et al., 2004).

Epithelial Polarity Cues

Epithelial tissues arise in a variety of ways during development, and it now seems that they use different cues to establish polarity depending on their context. The steps that generate apical-basal polarity are not well characterized for most epithelial tissues, and our understanding of this process is largely based on studies in tissue culture. Experiments with MDCK cells indicate that the development of apical-basal polarity requires both cadherin-dependent cell-cell adhesion and adhesion to the extracellular matrix (Yeaman et al., 1999). Either cell-cell or cell-substrate adhesion is sufficient to define a noncontacting plasma membrane domain that accumulates apical markers and a contacting domain that does not. However, cell-substratum adhesion alone is not sufficient to localize proteins at the basolateral membrane.

Although adhesion to the extracellular matrix does not induce full polarization of MDCK cells in culture, it plays a key role in orienting apical-basal polarity. Cells cultured in suspension form cysts with their apical sides facing outwards, while those cultured in collagen gels form cysts with an internal apical lumen (Wang et al., 1990a). Furthermore, the addition of collagen to cysts that have already polarized can invert this polarity (Wang et al., 1990b). Collagen orients the polarity of MDCK cysts by binding to integrins. However, it does this indirectly by activating the small GTPase Rac, which in turn is necessary for the deposition of laminin (O'Brien et al., 2001; Yeaman et al., 1999; Yu et al., 2005). Thus, laminin is likely to act as the basal polarity cue, although how this signal is transduced to reorient polarity is currently unclear. Interestingly, the reversal of polarity induced by knockdown of Rac or integrins can be rescued by inhibiting RhoA or its downstream effectors, Rho kinase I and Myosin II (Yu et al., 2008). This suggests that Rho and Rac act in alternative polarization pathways with opposite orientations. In support of this view, Rho and Rac promote the development of different types of polarized structures in MDCK cells grown on a substrate (Eisen et al., 2006).

One of the first steps in polarization of MDCK cells is the formation of intercellular adhesions by homophilic adhesion molecules of the nectin family (Sato et al., 2006; Takai et al., 2008b). PAR-3 is initially recruited to the plasma membrane by the binding of its first PDZ domain to the cytoplasmic tails of Nectins 1 and 3, and this association is necessary for the subsequent recruitment of Afadin and Cadherin to these developing adhesion junctions (Figure 2, right) (Ooshio et al., 2007). Very little is known about the next step in polarization, which

is the formation of tight junctions on one side of these initial adhesion sites. However this must be regulated by the pathways that control the orientation of polarity.

Apical-basal polarity is generated in a quite different way in the primary epithelium of *Drosophila*, which forms during cellularization when membrane furrows grow inwards to separate the cortical nuclei of the syncytial blastoderm embryo (which contains only one cell membrane). Polarity is already visible early in the process of cellularization with the partitioning of apical proteins in the membrane above the nucleus and basolateral markers beneath (Mavrakakis et al., 2009). This simple polarity is then extended by the localization of Bazooka to the apico-lateral margins of each cell, where it recruits cadherin to form adherens junctions (Harris and Peifer, 2004; McGill et al., 2009). Bazooka localization depends on both the apical actin network and on dynein-dependent transport of Bazooka along apical-basal microtubules, which are nucleated from the centrosomes immediately above the nuclei (Harris and Peifer, 2005). Thus, the primary polarity cue seems to be provided by apical actin and apical-basal microtubules, rather than by any extrinsic signal from cell adhesion. Therefore, this polarity cue depends on the geometry of the cytoskeleton that develops in the fertilized egg. Microtubules also appear to play a second role in the establishment of apical-basal polarity in particular cases. For example, the epithelial-specific polarity proteins Crumbs and Stardust are targeted to the apical cortex of the *Drosophila* embryonic ectoderm and follicle cells by dynein-dependent localization of their mRNAs (Horne-Badovinac and Bilder, 2008; Li et al., 2008b).

Although PAR-6, aPKC, and Bazooka (PAR-3) form a complex in many polarized cells, they do not colocalize in the primary epithelium in *Drosophila* as they do in the *C. elegans* embryo and *Drosophila* oocyte. Instead, PAR-6 and aPKC accumulate above Bazooka at the level of the apical cortex (Figure 3D), where they are required to stabilize the adherens junctions during gastrulation and germband extension (Harris and Peifer, 2007; Hutterer et al., 2004). The apical localization of PAR-6 and aPKC requires Bazooka and activated Cdc42-GTP, but the underlying mechanism that targets them to the apical cortex is currently unclear. One clue comes from recent work examining how PAR-6 is recruited to the apical surfaces of the first cells with apical-basal polarity in the early *C. elegans* embryo. A screen for mutants that lead to a uniform cortical distribution of PAR-6 identified PAC-1, which is a conserved Cdc42 GTPase-activating protein that promotes the formation of inactive Cdc42-GDP (Anderson et al., 2008). PAC-1 is recruited to sites of cell-cell contact, and its localization is complementary to that of PAR-6. This suggests that the activation of PAC-1 at sites of cell-cell adhesion restricts active Cdc42-GTP to the noncontacting membrane domain, where Cdc42-GTP recruits and activates the PAR-6/aPKC complex. Interestingly, CDC42 is also important for maintaining polarity in the *C. elegans* embryo, where it positively regulates PAR-3. PAC-1 orthologs exist in many other species, including mammals and *Drosophila*, raising the possibility that they function similarly in the embryonic epithelia of other organisms.

The secondary epithelia form in a significantly different way than the primary epithelia of early embryos, as they arise later in development from groups of cells that undergo a mes-

enchymal to epithelial transition. This process has not been studied in detail in vivo, but it is more likely to resemble the behavior of MDCK cells in culture than the formation of primary epithelia. For example, secondary epithelia in the *Drosophila* embryo, such as the midgut and dorsal vessel, require a basal cue to polarize (most probably laminin). In contrast, the follicular epithelium that surrounds the developing *Drosophila* oocyte requires apical cues, basal cues, and cell-cell adhesion to polarize completely (Tanentzapf et al., 2000; Yarnitzky and Volk, 1995).

Although the formation of adherens junctions normally plays a critical role in the establishment of epithelial polarity, in certain cases cells can polarize in the complete absence of cell-cell adhesion. In a human intestinal epithelial cell line in culture, activation of the PAR-4 ortholog, LKB1, induces single cells to polarize with a typical apical actin-rich brush border surrounded by an apical-lateral domain marked by ZO-1 and a basal domain contacting the substrate (Baas et al., 2004). Most importantly, this polarization is sufficient to trigger the sorting of apical and basolateral proteins into discrete domains, which normally does not occur with cell-substrate adhesion alone.

Recent studies indicate that this domain architecture is produced by a distinct epithelial polarity pathway that operates only under low-energy conditions (Lee et al., 2007; Mirouse et al., 2007). The main sensor of cellular energy levels is the AMP-dependent protein kinase (AMPK), which is activated by the LKB1 kinase and the high levels of AMP that accumulate when ATP levels fall. When cultured on sugar-free medium, *Drosophila* epithelial cells that lack either LKB1 or AMPK lose their polarity and over-proliferate. In contrast, under well-fed conditions, these mutant cells have normal polarity. Furthermore, single mammalian cells in culture can be induced to polarize simply by activating this pathway by blocking glucose uptake (Lee et al., 2007). Interestingly, the only function of AMPK in low-energy polarity is to activate Myosin II by phosphorylating its regulatory light chain (MRLC) because a phosphomimetic version of the MRLC rescues the polarity defects of *ampk* and *lkb1* mutant cells and polarizes single mammalian cells in culture. The ability of the low-energy pathway to polarize isolated cells in the absence of cell-cell adhesion suggests that the polarity cue is provided by cell-substrate adhesion. Consistent with this, the extracellular matrix receptor Dystroglycan, and extracellular matrix component Perlecan are specifically required for the apical-basal polarity of *Drosophila* follicle cells under conditions of energetic stress (Mirouse et al., 2009).

In summary, it appears that epithelial cells use different cues to establish apical-basal polarity in different contexts or conditions, even though this results in a very similar final polarity. This suggests that downstream polarity factors form a self-organizing system that establishes polarity in response to a variety of initial asymmetries.

Epithelial Polarity Complexes

Despite its increased complexity, apical-basal polarity in epithelia depends on the same set of polarity proteins as in the *C. elegans* zygote and the *Drosophila* oocyte because mutating or decreasing expression of PAR-1 (EMK1 or MARK1-2 in mammals), Bazooka (PAR-3 in mammals), PAR-6, or aPKC disrupts

epithelial polarity in both *Drosophila* and mammals (Suzuki and Ohno, 2006). Furthermore, the spatial relationship between the PAR proteins is maintained; PAR-3, PAR-6, and aPKC localize apically, whereas PAR-1 labels the basolateral membrane domain. However, unlike more simple oocytes and one-celled zygotes, epithelial cells require additional polarity complexes and deploy their existing PAR proteins in novel combinations (Figure 4).

The Crumbs Complex

One important difference between epithelia and more simple polarized cell types, such as oocytes, is the expression of the transmembrane protein Crumbs (Crb1-3 in vertebrates), which organizes one of the key polarity complexes in epithelial cells (Figures 3C, 3D, and 4). This apically localized complex consists of Crumbs/Crb1-3, the MAGUK protein Stardust (PALS1/MPP5 in vertebrates), PATJ, and Lin7 (Assémat et al., 2008; Tepass et al., 2001). Mutation of *Drosophila crumbs* abolishes the cell's apical domain, whereas overexpression of Crumbs (or only its transmembrane domain and cytoplasmic tail) expands the apical domain at the expense of the lateral domain. Similar results were found in MDCK cells overexpressing Crb3 (Lemmers et al., 2004; Roh et al., 2003; Wodarz et al., 1995). This function depends on two conserved domains in the cytoplasmic tail of Crumbs and its vertebrate orthologs, a membrane proximal FERM-binding domain and a C-terminal ERLI motif (Izaddoost et al., 2002; Klebes and Knust, 2000). The FERM-binding domain recruits β_H -spectrin to the apical side of the cell in *Drosophila*, perhaps by binding Dmoesin. This domain also binds to the FERM protein Yurt/YMO/EP, which limits Crumbs activity (Laprise et al., 2006; Médina et al., 2002). The ERLI motif of Crumbs/Crb3, on the other hand, is necessary for its association with Stardust/PALS1/MPP5 and thus for formation of the complex (Assémat et al., 2008). In addition, a splice variant of Crb3, which has the amino acids CLPI at its C terminus instead of an ERLI motif, plays a specific role in the formation of the primary cilium, perhaps through an interaction with importin- β (Figure 3C) (Fan et al., 2007).

Although Crumbs/Crb3, Stardust/PALS1, and PATJ mark the apical domain, they become strongly enriched at the apical margin of the lateral domain immediately above or abutting the region where the tight junctions form in vertebrate epithelia (Figure 2, right) (Makarova et al., 2003; Shin et al., 2005). Indeed, the Crumbs (Crb) complex seems to be a crucial determinant of tight-junction formation because the expression of Crb3 induces tight-junction formation in mammary MCF10A cells that do not normally form these junctions (Fogg et al., 2005). Furthermore, PALS1 and PATJ are required for the timely formation of tight junctions in MDCK cells and for lumen formation in MDCK cysts in three-dimensional culture (Shin et al., 2005; Straight et al., 2004). The Crb complex might serve as a scaffold for the recruitment of multiple tight-junction proteins because PATJ interacts with both ZO-3 and Claudin1 and is required for their localization (Michel et al., 2005). PATJ also binds to Angiomotin, which forms a complex with the Cdc42-GAP and RICH1. These proteins are both required for tight-junction maintenance, perhaps through the regulation of endocytosis (Wells et al., 2006).

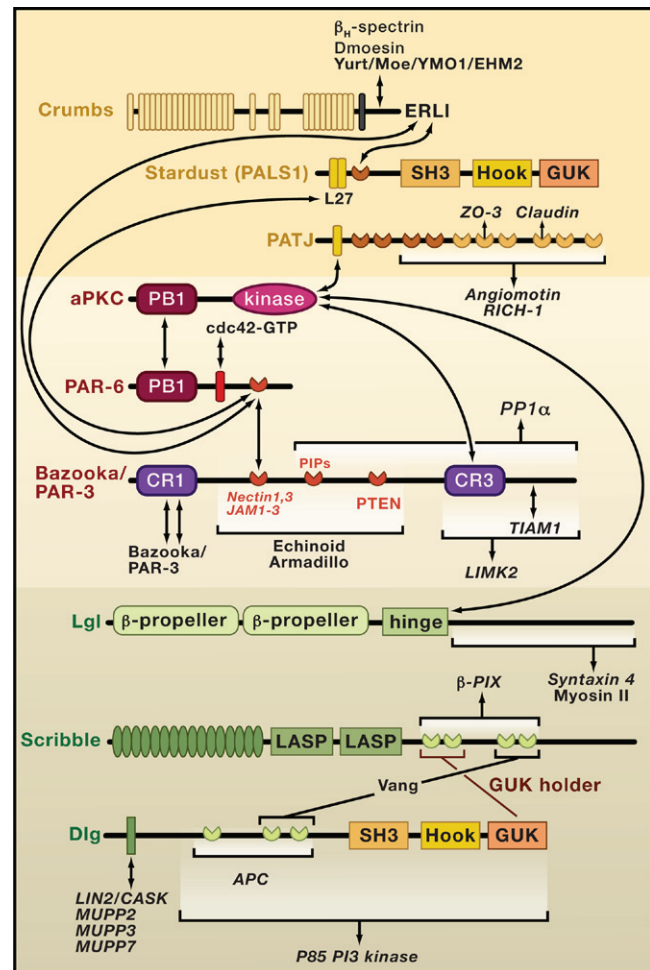


Figure 4. Three Main Polarity Complexes in Epithelial Cells

Domain architecture of the Crumbs, PAR-3, and Scribble complexes, along with key factors that interact with them.

(A) The Crumbs complex (yellow), which in *Drosophila* consists of Crumbs (Crb1-3 in vertebrates), Stardust (PALS1 in vertebrates), and PATJ, is crucial for forming the apical domain and tight junctions in epithelial cells.

(B) The PAR-3 complex (red and purple) in *Drosophila* consists of Bazooka (PAR-3 in vertebrates), PAR-6, and atypical kinase C (aPKC). PAR-6 and aPKC (atypical protein kinase C) are also key components of the apical Crumbs complex. Bazooka/PAR-3 positions the most apical junction to establish the boundary between the apical and lateral domains.

(C) The Scribble complex (green), which in *Drosophila* consists of Scribble, lethal giant larvae (Lgl), and Discs large (Dlg), localizes to the lateral membranes, where it excludes apical proteins and is required for forming septate junctions.

Interactions conserved between *Drosophila* and vertebrates are shown in bold; those identified only in vertebrates or only in *Drosophila* are in italicized or regular font, respectively. PDZ domains are shown as three-quarter circles.

In *Drosophila* epithelial cells, Crumbs and Stardust show a similar enrichment at the boundary between the apical and lateral membrane domains, suggesting that they define an analogous marginal domain to that in vertebrate epithelial cells (Tepass et al., 2001). This region lacks tight junctions, however, and the function of Crumbs and Stardust is to organize the adherens junctions into a continuous band immediately below this marginal zone. As in mammals, PATJ colocalizes with Crumbs and Stardust in *Drosophila*. However, in *Drosophila* its

function has been analyzed only in the eye where it is required for photoreceptor morphogenesis (Nam and Choi, 2006; Richard et al., 2006). The Crb complex is not required for epithelial polarity in *C. elegans*, although, as in other organisms, it localizes to a similar marginal zone, where it plays a redundant role in recruiting the Dlg-1/AJM complex (Segbert et al., 2004).

PAR-3, PAR-6, and aPKC in Epithelial Cells

Early work in different organisms suggested a simple view of obligate interactions between Bazooka/PAR-3, PAR-6, and aPKC as found in the *C. elegans* embryos and *Drosophila* oocytes; however, it is now clear that these PAR proteins also interact with non-PAR proteins (Figure 4), and these complexes have equally important roles in the establishment, maintenance, and transduction of polarity. As is the case in the *Drosophila* primary epithelium, BAZ/PAR-3 does not strictly colocalize with PAR-6 and aPKC in fully polarized epithelia. Most PAR-6 and aPKC localize to the apical membrane and the marginal zone, whereas BAZ/PAR-3 is localized slightly more basally at the level of the adherens junctions in flies (Figures 3C and 3D) (Afonso and Henrique, 2006; Harris and Peifer, 2005; Nam and Choi, 2003; Satohisa et al., 2005; Totong et al., 2007).

PAR-6 and aPKC colocalize with Crb, Stardust/PALS1, and PATJ, and there is increasing evidence that PAR-6 and aPKC are key components of this apical complex. First, PAR-6 interacts directly with PALS1, PATJ, and the C-terminal ERL1 motif of Crb3, and both PAR-6 and aPKC coprecipitate with components of the Crb complex in mammals and *Drosophila* (Assémat et al., 2008). Second, aPKC can phosphorylate two conserved threonine residues in the cytoplasmic tail of Crumbs in vitro, which are essential for Crumbs activity in vivo (Sotillos et al., 2004). Finally, aPKC has recently been shown to regulate Ezrin at the apical side of polarized human intestinal cells in culture, thereby facilitating the formation of the apical cytoskeleton (Wald et al., 2008). Thus, the activity of aPKC in association with the Crumbs complex seems to be a key determinant of apical identity, both through the recruitment and activation of downstream apical complexes and through the inhibition of basolateral determinants. The apical Crumb/PAR-6/aPKC complex is probably also regulated by the binding of active Cdc42 to PAR-6 because Crumbs, PAR-6, and aPKC delocalize when Cdc42 activity is reduced, leading to defects in actin organization, endocytosis, and adherens junction remodeling (Georgiou et al., 2008; Harris and Tepass, 2008; Hutterer et al., 2004; Leibfried et al., 2008).

PAR-3/Bazooka

Most PAR-3 localizes slightly basal to the Crumbs complex, where it positions the most apical junction in epithelial cells to establish the boundary between the apical and lateral domains. It is now clear that this function of PAR-3 depends on its interaction with proteins other than PAR-6 and aPKC (Figures 3C and 3D). In mammalian cells, the third PDZ domain and the C-terminal region of PAR-3 bind to the Rac exchange factor TIAM1 to regulate Rac activity. Decreasing expression of TIAM1 in keratinocytes or MDCK cells causes similar defects in tight junctions as PAR-3 depletion (Chen and Macara, 2005; Mertens et al., 2005). By regulating Rac, PAR-3 probably recruits a ring of F-actin to the cell cortex adjacent to the adherens junctions

(Chen and Macara, 2005). PAR-3 can also regulate the actin cytoskeleton through direct binding of its C-terminal domain to LIM kinase 2 (Chen and Macara, 2006). LIM kinases phosphorylate cofilin, which inhibits its actin-severing activity. The binding of PAR-3 to LIM kinase 2 prevents this phosphorylation, resulting in increased cofilin activity.

PAR-3 is recruited to the cytoplasmic side of tight junctions as they form, and this localization requires at least three different domains of the protein (Figure 2, right). The first PDZ domain interacts with the cytoplasmic tails of JAM1-3, which are also required for tight-junction formation and may recruit PAR-3 to the correct position (Ebnet et al., 2001; Itoh et al., 2001). The second PDZ domain binds to phosphatidylinositol phosphates (PIPs) in the lipid bilayer (Wu et al., 2007). Finally, the N-terminal CR1 domain of PAR-3 or Bazooka oligomerizes to form helical filaments, and this oligomerization is necessary for efficient localization to the apical junctions in vertebrate and *Drosophila* epithelia (Benton and St Johnston, 2003a; Feng et al., 2007; Mizuno et al., 2003). These results suggest that PAR-3 is recruited to the membrane by interactions with PIPs and junctional proteins, where it then oligomerizes to form a scaffold for tight-junction assembly.

Once PAR-3 localizes to tight junctions, it binds downstream proteins that help maintain its localization and activity. It recruits protein phosphatase 1 α (PP1 α) to tight junctions, which may preserve PAR-3 activity by removing inhibitory phosphorylation mediated by aPKC and PAR-1 (Figure 2, right) (Traweger et al., 2008). In addition, the third PDZ domain of PAR-3 binds to the lipid phosphatase PTEN (Phosphatase and Tensin homolog), which converts phosphatidylinositolide 3,4,5 P₃ (PIP₃) to PIP₂ (Wu et al., 2007). This presumably maintains high PIP₂ levels at the membrane so that PAR-3 can remain anchored there.

The *Drosophila* PAR-3 ortholog Bazooka plays a similar role in the formation of the apical zonula adherens because it localizes before and independently of cadherin. Furthermore, Bazooka is required for the coalescence of the spot adherens junctions into a continuous belt (Harris and Peifer, 2004; McGill et al., 2009). The cortical localization of Bazooka requires its binding to phosphatidylinositides in the plasma membrane, and this is mediated by a conserved region near the C terminus of Bazooka rather than PDZ2 (Krahn et al., 2010). The PDZ domains of Bazooka bind to the C termini of Armadillo (β -catenin) and the *Drosophila* Nectin-like protein Echinoid, and this may allow Bazooka to cluster Cadherin and Echinoid adhesion complexes to form the adherens junction (Wei et al., 2005). Like mammalian PAR-3, Bazooka also recruits PTEN, which may help to establish the apical enrichment of PIP₂ (Pinal et al., 2006; von Stein et al., 2005). Finally, Bazooka is required for adherens junction localization of the synaptotagmin-like, PIP₂-binding protein, Bitesize, which in turn recruits the ERM protein, Moesin. This is important to stabilize the adherens junctions and to direct the formation of a continuous belt of actin around the apical cell cortex (Pilot et al., 2006).

The Scribble Complex

In addition to the Crumbs and PAR-3 complexes, epithelial polarity in *Drosophila* depends on a third polarity complex, consisting of Scribble, Lgl, and Dlg (Figure 4). Cells that lack

functional versions of any of these proteins lose their polarity and usually over-proliferate (Bilder et al., 2000; Bilder and Perrimon, 2000). All three proteins localize to the lateral membrane, where they are required for the lateral exclusion of apical proteins and for the coalescence of the spot adherens junctions into a zonula adherens immediately above them (Figures 2 [right] and 3D). In addition, mutants in any of these three genes abolish the formation of the septate junctions, where Dlg and Scribble accumulate.

The Scribble ortholog, LET-413, appears to play a similar role in epithelial polarity in *C. elegans* because apical components spread into the lateral membrane in *let-413* mutants and the apical junctions are mislocalized (Bossinger et al., 2004; Cox and Hardin, 2004). However, *C. elegans* does not have a Lgl homolog, and the Dlg ortholog plays a specific role in the assembly of the basal region of the apical junction and binds to AJM-1 directly (Cox and Hardin, 2004). This suggests that Dlg plays a conserved role in paracellular barrier formation but not in the maintenance of epithelial polarity.

The mammalian orthologs of the Scribble complex proteins, Dlg/SAP97, Lgl1 and 2, and Scribble, localize to the lateral membrane of epithelial cells, and this depends on cadherin and actin (Figure 3C). However, their functions are not clearly defined because small interfering RNAs against them have only mild effects on epithelial polarity (Laprise et al., 2004; Qin et al., 2005; Stucke et al., 2007; Yamanaka et al., 2006). This weak effect could be due to partial gene silencing or could be a consequence of redundancy with other orthologs; mammals possess two Scribble, two Lgl, and seven Dlg homologs.

The idea that Scribble, Dlg, and Lgl form a complex derives from the observations that mutations in these genes produce identical phenotypes, and the proteins show complete or partial colocalizations, which are interdependent (Bilder, 2004). There is no evidence yet that Scribble, Dlg, and Lgl interact directly, although Lgl2 coimmunoprecipitates with Scribble in HEK293 and MDCK cells. Furthermore, the GUK-holder protein is a possible link between Dlg and Scribble (Albertson and Doe, 2003; Kallay et al., 2006). It should also be noted that these proteins function independently of each other in *C. elegans* and in other *Drosophila* tissues. Therefore, Scribble, Dlg, and Lgl may perform related but separate functions as components of distinct protein complexes in epithelial cells.

The phenotypes of *scribble*, *lgl*, and *dlg* mutants suggest that they specify the lateral membrane domain by excluding more apical polarity complexes from this region. The mechanisms underlying this process are still largely unknown, although several hypotheses have been proposed for the function of Lgl. First, Lgl may help define the lateral membrane by interacting with the t-SNARE syntaxin-4 to target secretion of basolateral proteins to this region of the plasma membrane (Müsch et al., 2002). However, very little Lgl coimmunoprecipitates with syntaxin-4 from MDCK cell lysates. Instead, the majority of Lgl protein associates with the actin cytoskeleton through the direct binding of its C-terminal domain to Myosin II, suggesting that Lgl specifies the lateral cortex by regulating Myosin II activity (Betschinger et al., 2005). Finally, Lgl binds directly to the PAR-6/aPKC complex to inhibit aPKC activity in both

mammals and *Drosophila*, and this may be its major function in epithelial polarity (Betschinger et al., 2003; Yamanaka et al., 2003).

Dlg and Scribble interact with a variety of other proteins that function in processes distinct from epithelial polarity, such as planar polarity in inner-ear epithelial cells and the migration of astrocytes (Humbert et al., 2008). However, two classes of Dlg1-binding partners are good candidates for factors that help mediate its polarity function. First, Dlg1 associates directly with the SH2 domains of the PI3K p85 regulatory subunit to recruit it to the lateral membrane (Laprise et al., 2004). This may be important for the establishment of the apical-basal asymmetry of PIPs. Second, Dlg1 associates through its L27N domain with a number of related MAGUK family proteins, including Lin2/CASK, MPP2, MPP3, and MPP7 (Bohl et al., 2007). MPP7 colocalizes with Dlg1 to the lateral membrane, and gene silencing of either MPP7 or Dlg1 delays the formation of functional tight junctions, suggesting that they function together in this context (Stucke et al., 2007). By contrast, CASK colocalizes with Dlg1 along the basal membrane, indicating that the interactions between other MAGUK proteins and Dlg1 might be mutually exclusive.

Antagonistic Interactions

The maintenance of polarity in oocytes, one-celled zygotes, and epithelia depends on antagonistic interactions between the polarity complexes that define distinct cortical domains (Figure 2, right). However, the nature of these interactions is more complicated in epithelial cells than in simpler cells. First, there are more distinct cortical domains in epithelial cells. Second, PAR-3 (or Bazooka in *Drosophila*) and PAR6/aPKC do not colocalize as they do in the embryos, and thus these factors are unlikely to function together as the major apical determinant. Indeed, studies in *Drosophila* indicate that mutual antagonism between the apical Crumbs complex and the lateral Scribble complex plays a central role in defining distinct apical and lateral domains in epithelial cells (Bilder et al., 2003; Tanentzapf and Tepass, 2003). Overexpression of Crumbs mimics the loss of function phenotype of mutations in *scribble* group genes, and *crumbs* and *stardust* null mutants are partially rescued by reducing the levels of any Scribble complex component. The mechanism for this mutual antagonism is not known, but the discovery that PAR-6 and aPKC are components of the Crb complex suggests that aPKC inhibits the Scribble complex by phosphorylation of Lgl, which then prevents Lgl from associating with the apical cortex (Betschinger et al., 2003; Hutterer et al., 2004; Plant et al., 2003). Lgl also associates with the PAR-6/aPKC complex and inhibits this complex's interaction with other partners, its association with the cortex, and probably its kinase activity (Wirtz-Peitz and Knoblich, 2006; Yamanaka et al., 2003). This suggests a mutual antagonism whereby Lgl inhibits aPKC laterally, and the Crumbs complex (Crumbs/PAR-6/aPKC) inhibits Lgl apically. These antagonistic interactions appear to be conserved in vertebrate epithelia. For example, overexpression of aPKC or Crbs3 or gene silencing of Lgl enlarges the apical domain at the expense of lateral domain; in contrast, overexpression of Lgl enlarges the lateral domain and counteracts the effects of aPKC overexpression (Chalmers et al., 2005; Müsch et al., 2002; Yamanaka et al., 2006).

Both the Crumbs and Scribble complexes are required to position Bazooka (PAR-3) and the adherens junctions between them. This presumably occurs by the Crumbs and “Scribble” complexes excluding these factors apically and laterally, respectively. Recently, it was found that apical exclusion requires aPKC to phosphorylate the CR3 domain of Bazooka, which disrupts the Bazooka-aPKC interaction (Morais de Sá et al., 2010). This phosphorylation is not sufficient to exclude Bazooka apically because it is phosphorylated in other polarized cell types, such as neuroblasts, where Bazooka still forms a complex with aPKC and PAR-6. In epithelia, however, the Crb complex out-competes Bazooka for binding to the PDZ domain of PAR-6 to disrupt the second link between Bazooka and PAR-6/aPKC. This displaces Bazooka laterally to define the apical/lateral boundary. Indeed, this appears to be a major function of Crb because a form of Bazooka that is not excluded from the apical domain produces a very similar phenotype to *crumbs* mutants.

It is less clear how the Scribble complex defines the basal extent of the Bazooka domain. One possibility is that it acts through PAR-1 by recruiting this kinase to the lateral cortex, where PAR-1 can then phosphorylate and exclude Bazooka. In support of this view, Lgl is required for the cortical localization of PAR-1 in the *Drosophila* oocyte (although it is not known whether this is also the case in epithelia), and gene silencing of PAR-1 or mutating the PAR-1 phosphorylation sites in Bazooka leads to the lateral spreading of Bazooka and the Cadherin adhesion complex in epithelia (Bayraktar et al., 2006; Benton and St Johnston, 2003b; Tian and Deng, 2008).

These antagonistic interactions can be only part of the story because *Drosophila* embryos that lack both the Crumbs and Scribble complexes eventually form polarized epithelia with apical adherens junctions, indicating the existence of redundant pathways for establishing apical-basal polarity (Bilder et al., 2003; Tanentzapf and Tepass, 2003). Indeed, it was recently shown that Yurt, Neurexin IV, Coracle, and the α subunit of the Na⁺/K⁺ ATPase function as a second group of lateral proteins that antagonize the activity of the apical Crumbs complex during midembryogenesis in *Drosophila*, and this pathway may depend on the direct binding of Yurt to Crumbs (Laprise et al., 2009). Furthermore, the mammalian Yurt ortholog, EPB41L5, appears to play a similar role in defining the lateral membrane in MDCK cells. Nevertheless, there are still other polarity complexes that are not yet identified because *Drosophila* embryos that lack both Scribble and Yurt still form polarized epithelia by the end of embryogenesis.

Epithelial Polarity Transduction and PIP₂/PIP₃ Asymmetry

Very little is known about how cortical polarity is transduced to regulate the organization of the cytoskeleton and the polarization of trafficking pathways in epithelial cells, but a few details are beginning to emerge. As discussed above, mammalian PAR-3 seems to play a key role in organizing actin at the apical junction through its interactions with TIAM1 and Lim kinase 2 (Chen and Macara, 2005, 2006). In *Drosophila*, both aPKC and PAR-1 are required for the normal apical-basal organization of microtubules. aPKC plays a role in the inactivation of the api-

cal centrosomes in *Drosophila* primary epithelium, and lateral PAR-1 is required for the stabilization of microtubules in epithelial follicle cells (Doerflinger et al., 2003; Harris and Peifer, 2007). However, how these localized polarity proteins regulate the microtubule cytoskeleton is currently unknown.

One likely candidate for the link between cortical polarity and polarized secretion is the generation of an apical-basal asymmetry in the distribution of phosphatidylinositol phosphates on cytosolic side of the plasma membrane. As MDCK cells polarize in three-dimensional culture, PIP₂ concentrates at the apical membrane, and PIP₃ becomes restricted to the basolateral membrane (Gassama-Diagne et al., 2006; Martin-Belmonte et al., 2007). These two phospholipids play a key role in defining the two complementary membrane domains because the addition of exogenous PIP₃ to the apical surface induces the mistargeting of basolateral proteins to this domain and the formation of protrusions. Furthermore, the addition of PIP₂ to the basolateral surface induces the mislocalization of apical markers. Indeed, PIP₂ is proposed to be the main determinant of apical identity because it recruits Annexin 2 to the apical domain, which subsequently recruits active Cdc42-GTP, where it binds and activates the PAR-6/aPKC complex. It is worth noting, however, that this is not true for all epithelial cells because the most apical domain in *Drosophila* retinal epithelium is marked by high levels of PIP₃ rather than PIP₂ (Pinal et al., 2006).

It is not completely clear how the PIP₂/PIP₃ asymmetry arises. However, PTEN, which converts PIP₃ into PIP₂, localizes apically in MDCK cells, and its depletion by RNAi inhibits apical lumen formation (Martin-Belmonte et al., 2007). As mentioned earlier, both *Drosophila* Bazooka and human PAR-3 bind to PTEN, and this interaction may recruit PTEN to the apical junctions. In addition, PI3-kinase, which phosphorylates PIP₂ to generate PIP₃, may be recruited to and activated at the lateral membrane by Dlg in response to Cadherin-dependent cell-cell adhesion. PI3-kinase could contribute to asymmetry by providing a source of PIP₃ laterally (Laprise et al., 2004). Thus, this PIP₂/PIP₃ asymmetry probably depends on PAR-3 and Dlg, placing it downstream of cortical polarity complexes (Figure 2, right). However, the strong effects of adding PIP₂ or PIP₃ to the opposite membrane suggest that this pathway may also feed back to regulate the polarity complexes.

These results raise the question of how the PIP₂/PIP₃ asymmetry and/or the cortical polarity complexes control the targeting of cellular components to the apical versus basolateral membrane domains. A number of different mechanisms can direct specific proteins and lipids to either the apical or basolateral membrane domains, including polarized exocytosis, polarized transcytosis (carrying cargo across the cell), and polarized retention by cortical anchors. A key step for polarized exocytosis and transcytosis is the fusion of vesicles carrying either apical or basolateral cargoes with the appropriate membrane domain. Vesicle fusion depends on a specific interaction between complementary v-SNARE complexes on the vesicle membrane and t-SNARE complexes on the target membrane. The apical and basolateral membranes in polarized epithelial cells are marked by different t-SNAREs that contain either syntaxin-3 or syntaxin-4, respectively (Low et al., 1996). Further-

more, the mislocalization of syntaxin-3 to the basolateral membrane results in the inappropriate delivery of apical proteins to this domain and blocks the formation of polarized MDCK cysts in three-dimensional culture (Sharma et al., 2006). Therefore, it will be interesting to determine how the localization of syntaxin-3 and syntaxin-4 relates to the PIP_2/PIP_3 asymmetry.

Another candidate for a link between the PIP_2/PIP_3 asymmetry and polarized secretion is the exocyst. First identified in yeast, this eight-subunit complex is required for polarized exocytosis (He and Guo, 2009). The Sec3 and Exo70 subunits of the yeast exocyst localize to the plasma membrane of the bud tip and serve as docking sites for exocytic vesicles that are associated with the other exocyst subunits. Furthermore, recruitment of Sec3 to the membrane requires its binding to PIP_2 and active Cdc42 in yeast, and Exo70 binds to PIP_2 in both yeast and mammals. Thus, the exocyst could couple the polarized distribution of PIP_2 to the exocytosis of apical proteins. In support of this view, mutations in several components of the *Drosophila* exocyst disrupt apical secretion in the photoreceptors of the eye. In addition, mutations in the exo84 subunit produce a very similar embryonic phenotype to mutations in the apical determinant Crumbs (Beronja et al., 2005; Blankenship et al., 2007). However, these results are inconsistent with studies in mammalian cells, where antibody inhibition and overexpression experiments suggest a specific role for the exocyst in basolateral secretion (Fölsch, 2005). Clearly, more work is needed to understand the role of the exocyst in coupling phosphoinositides to polarized secretion, but these first experiments provide strong motivation for further investigations.

Prospects

In the last few years, considerable progress has been made in identifying and characterizing the cell polarity complexes and how these complexes interact with each other to define different cortical domains in both simple cells, such as *C. elegans* zygotes and *Drosophila* oocytes, and more complex systems, such as epithelial cells. There is increasing evidence, however, for redundancy in the mechanisms that generate polarity in both eggs and epithelia. For example, *Drosophila* embryos that lack components of both the Crumbs and Scribble complexes still form clusters of polarized epithelial cells by the end of embryogenesis (Bildler et al., 2003; Tanentzapf and Tepass, 2003). Therefore, neither complex is absolutely essential for epithelial polarity, and other unidentified factors can probably compensate when the Crumbs and Scribble complexes are absent. The recent discovery of several proteins that are specifically required for the polarity of epithelial cells only under conditions of low energy reveals another level of redundancy in both of these systems. This suggests that loss-of-function screens probably missed a number of polarity factors, and new approaches are required to identify polarity proteins that are not essential under standard conditions.

Another major open question in the field is how the cortical polarity complexes regulate all of the other polarized properties of a cell. Although a number of downstream effectors of polarity complexes have been identified, little is known about how these factors control polarized secretion and endocytosis or the organization of the actin and microtubule cytoskel-

etons. Furthermore, the underlying organization of polarized cells can vary greatly, even though all cells are probably all polarized by the same core machinery. For example, secretory epithelia, such as those of the mammary gland or pancreas, must polarize their vesicle trafficking pathways to secrete large amounts of material apically; in contrast, absorptive epithelia in the gut direct most trafficking in the opposite direction. Therefore, there is likely to be a degree of plasticity in relationships between polarity complexes and their downstream effects on the organization of the cell.

Although most studies in epithelial cells have focused on the basic mechanisms that generate polarity, one question that is becoming increasingly more important is how modifications of this machinery change cell behaviors to drive morphogenesis. Bazooka displays a planar polarization around the margin of *Drosophila* ectodermal cells as they undergo the cell intercalation movements that drive germ band extension. It is tempting to speculate that this relocation of Bazooka plays an instructive role in orienting the intercalation to extend the germ band along the anterior-posterior axis (Zallen and Wieschaus, 2004). Simple epithelia can adopt flat (i.e., squamous), cuboidal, or columnar morphologies, and the tissue often changes from one type to another during the course of development. Because these changes involve alterations in the relative sizes of the apical and lateral domains, modifications in the activities of core polarity complexes may also drive shape changes in epithelial sheets.

Furthermore, most malignant tumor cells have lost their polarity, and this is likely to play a role in both their escape from normal proliferation control and metastasis. The role cell polarity proteins play in oncogenesis has not been widely investigated. However, recent results suggest that several different mechanisms can contribute to the development of tumors, including defects in asymmetric cell division and disruption of cortical polarity (Januschke and Gonzalez, 2008; Tanos and Rodriguez-Boulan, 2008). In addition, the polarity protein LKB1 (the PAR-4 ortholog) is a bona fide human tumor suppressor gene. Mutations in LKB1 were first identified as the cause of Peutz-Jeghers syndrome, an autosomal dominant disorder in which patients develop benign hamartomas and a high frequency of carcinomas (tumors of epithelial origin) (Alessi et al., 2006). This tumor suppressor function of LKB1 might be related to its role in the pathway that polarizes epithelial cells under conditions of low energy (Lee et al., 2007; Mirouse et al., 2007). Most tumors are likely to undergo periods of energetic stress as they outgrow the local blood supply. Furthermore the vast majority of cancer cells produce the majority of their ATP by the inefficient process of glycolysis, rather than oxidative respiration (the Warburg effect), which may lead to lower ATP levels. Thus, disruption of the LKB1/AMPK pathway may disrupt polarity and enhance metastasis in tumor cells when ATP levels are low.

Despite the recent advances in our understanding of cell polarity, much still needs to be learned about the basic mechanisms that polarize both eggs and epithelial cells and how changes in polarity relate to other processes, such as morphogenesis, proliferation control, and metastasis. Nevertheless, this field is rapidly progressing, and the next few years are likely to be exciting and productive times for researchers studying cell polarity.

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