Apicobasal polarization: epithelial form and function

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The structure and function of epithelial sheets generally depend on apicobasal polarization, which is achieved and maintained by linking asymmetrically distributed intercellular junctions to the cytoskeleton of individual cells. Recent studies in both *Drosophila* and vertebrate epithelia have yielded new insights into the conserved mechanisms by which apicobasal polarity is established and maintained during development. In mature polarized epithelia, apicobasal polarity is important for the establishment of adhesive junctions and the formation of a paracellular diffusion barrier that prevents the movement of solutes across the epithelium. Recent findings show that segregation of ligand and receptor with one on each side of this barrier can be a crucial regulator of cell–cell signaling events.

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Abbreviations
SAR subapical region
SJ septate junction
TJ tight junction
ZA zonula adherens

Introduction

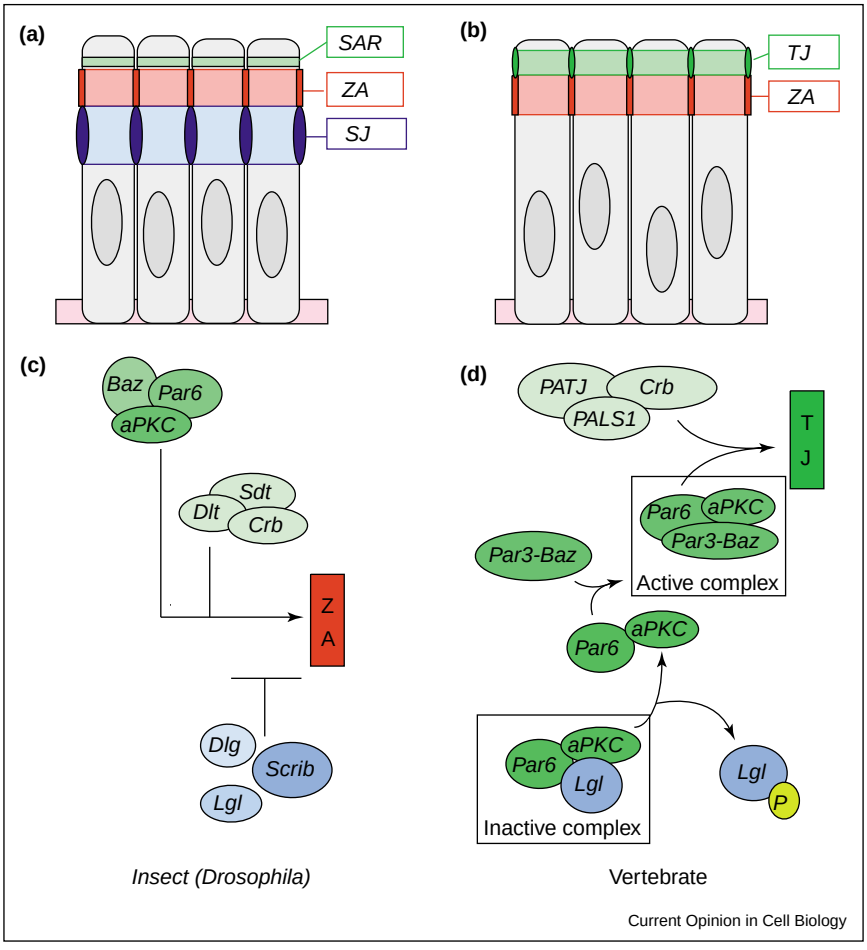
The regulated association of cells in epithelial sheets serves multiple functions in development, including barrier formation and control of tissue architecture. In most cases the functionality of an epithelium requires polarization of each component cell along its apicobasal axis. There is surprising evolutionary conservation of the core molecular mechanisms underlying cell polarization among animals [1–3]. However, studies of *Drosophila* and vertebrate epithelia illustrate that these core mechanisms often operate within the context of significantly different epithelial architecture. In *Drosophila*, epithelial cells exhibit an apically localized cell–cell adhesive belt known as the zonula adherens (ZA), and a more basal junctional complex known as the septate junction (SJ). Just apical to the *Drosophila* ZA lies the subapical region (SAR), which has an organizing role in epithelial polarization but is not known to function as a site of cell–cell junctions [2,3] (Figure 1a). Contrasting with *Drosophila*, vertebrate epithelial cells lack SJs and instead exhibit tight junctions (TJs), cell–cell adhesive structures that lie apical to the vertebrate ZA in a position analogous to the *Drosophila* SAR [2] (Figure 1b). The apical TJ complexes between vertebrate epithelial cells serve an organizing role in epithelial polarization and establish a paracellular diffusion barrier that restricts the movement of solutes across the cell layer [4,5]. This barrier effectively segregates the epithelium and surrounding media into immiscible apical and basolateral compartments. In *Drosophila*, SJs appear to fulfill a similar paracellular barrier role to the vertebrate TJs [6,7•], albeit with the functional barrier lying basal to the ZA.

Despite differences in the distribution of cell–cell junctions, conserved sets of polarity proteins govern apicobasal polarization in both *Drosophila* and vertebrate epithelia. Recent studies have exploited *Drosophila* for genetic analyses and vertebrate research has capitalized on excellent epithelial-cell culture systems and biochemical analyses. The two fields have converged to implicate integrated activity of three protein groups in epithelial polarity control: Par3/Par6/aPKC (the Baz–Par3 complex), Crumbs/Discs lost/Stardust (the Crb complex), and Lethal giant larvae/Discs large/Scribble (the Lgl group) [2,3,8•,9••,10••,11•]. Although biochemical studies support the existence of Baz–Par3 and Crb protein complexes in both vertebrates and *Drosophila*, the evidence does not presently support the classification of the Lgl group as a protein complex. This review will first consider some of the most recent advances in understanding the apicobasal polarization of *Drosophila* and vertebrate epithelia. We will then go on to discuss some potential implications of the polarized paracellular diffusion barrier for the regulation of cell signaling events.

Epithelial apicobasal polarity in *Drosophila*

The proteins that regulate the polarity of *Drosophila* epithelial cells fall into three categories based on similarities in both function and subcellular localization during polarization of the embryonic blastoderm epithelium (reviewed in [2,3]). All three categories are functionally required for morphogenesis and stabilization of the ZA during embryonic development, despite their different subcellular localizations. Proteins of the Baz–Par3 protein complex associate with the SAR and the apical plasma membrane and regulate early phases of ZA assembly [3,12,13], whereas proteins of the Lgl group localize to the basolateral plasma-membrane domain and are likely to play a slightly later role in ZA formation [3,14,15]. Lastly, proteins of the Crb complex localize apical to the

Figure 1



Distribution and activity of polarity complexes in *Drosophila* and vertebrate epithelia [2,3]. **(a)** *Drosophila* epithelial cells exhibit two principal sets of junctions: adherens junctions, which form the ZA (red), and SJs (blue). Proteins of the Lgl group localize at or below the level of the SJ. The SAR (green) lies apical to the ZA in a position analogous to the vertebrate TJ. Proteins of the *Drosophila* Crb and Baz–Par3 complexes localize to the SAR, or marginal zone. **(b)** Vertebrate epithelia exhibit a ZA (*red*), as well as a slightly more apical TJ complex (*green*) [2]. Both the vertebrate Crb and Baz–Par3 complexes localize to the TJ, which is perhaps consistent with a conserved function of the vertebrate TJ and *Drosophila* SAR. **(c)** Model for the activity of polarity protein complexes in *Drosophila* epithelial polarization and ZA morphogenesis, adapted from [8•,9••]. **(d)** Model for the activity of polarity protein complexes in mammalian epithelia, adapted from [10••,11•]. Here, mLgl binds and inactivates Par6/aPKC in the lateral plasma membrane domain, but dissociates upon phosphorylation by aPKC. Par6/aPKC is then free to bind Par3 and form an active complex [11•] that mediates TJ morphogenesis through interactions between Par6 and PALS1 of the Crb complex [10••].

ZA and regulate later phases of ZA maturation and stabilization [3,16–18]; this occurs at least in part via Crb-mediated recruitment of the actin-binding protein Dmoesin and components of the apical Spectrin-based membrane cytoskeleton [19]. Although it is has been widely accepted that each of these three protein groups participates in cell polarization, it has only recently become clear how they might collaborate to produce a unified network that governs epithelial polarization in diverse organismal systems.

The transmembrane protein Crb is considered to be a crucial apical determinant on the basis of its ability to confer apical-membrane identity to basolateral membranes [20]. In *Drosophila*, *crb* misexpression causes apicalization of cells and consequently gross defects in epithelial organization. A mutant screen for enhancers of the *crb* misexpression phenotype identified *lgl*, suggesting a negative regulatory interaction between the Crb and Lgl complexes in epithelial polarization [9••]. This notion is reinforced by the converse observation that the *crb* loss-of-function phenotype, embryonic epithelial disintegration, is partially rescued by concomitant loss of *dlg*, *lgl* or *scrib*, and that the *sdt* mutant phenotype, which is similar to that of *crb*, is partially rescued by mutations in *lgl* or *dlg* [8•,9••]. Together, these experiments show that basolateral Lgl group proteins counteract the apicalizing activity of the Crb complex (Figure 1c), although the precise

mechanism of interaction remains poorly resolved. To date, there are no documented biochemical interactions between members of the Lgl and Crb groups in *Drosophila*, which may indicate that the Lgl group antagonizes apical polarization independent of Crb activity or indirectly regulates the Crb complex through interactions with Baz/Par6/aPKC [8•,9••]. Indeed, Lgl has been shown to bind Par6/aPKC independent of Baz in both *Drosophila* neuroblasts [21] and mammalian epithelia [11•,22], suggesting a regulatory mechanism by which Lgl complex proteins could antagonize the formation or activity of the Baz/Par6/aPKC complex in lateral membrane domains.

Studies of *Drosophila* embryonic epithelia are thus beginning to reveal a genetic hierarchy by which the Baz–Par3, Crb, and Lgl groups integrate to regulate ZA morphogenesis and epithelial cell polarity. However, phenotypic analysis indicates that this system only operates in a narrow temporal window [9••] that follows the actual initiation of apicobasal polarity during earlier developmental stages. In *Drosophila*, early embryonic development is syncytial and the blastoderm epithelium forms *de novo* through a process known as cellularization. Following fertilization, zygotic nuclei undergo 13 syncytial mitoses (to number ~5000), migrate to the surface plasma membrane, and subsequently become encased by polarized invaginations of the plasma membrane known as furrow canals. During cellularization, the furrow canals expand as a result of polarized insertion of newly synthesized plasma membrane [23], and adjacent cells begin to form polarized cell–cell contacts [24]. Hence, some aspects of apicobasal polarity are in effect before the completion of cellularization.

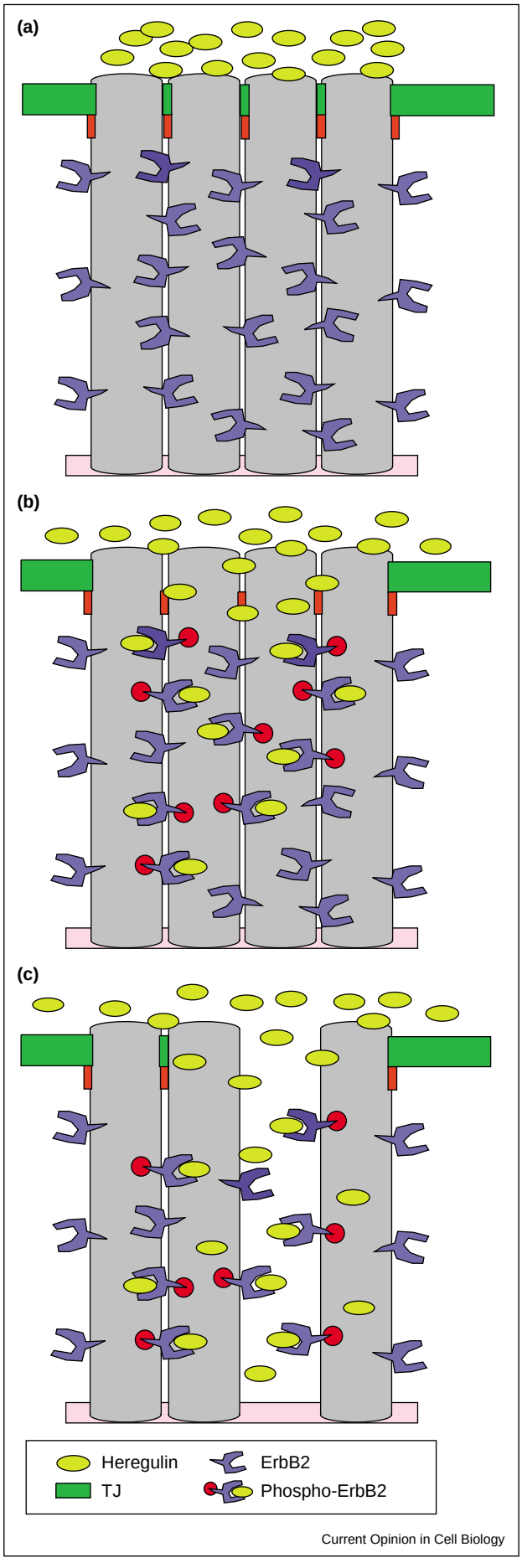
Analysis of large chromosomal aberrations reveals that relatively few genes are zygotically required for the process of cellularization, indicating that a large portion of the initial polarity machinery is provided by maternal contributions. Among the few genes zygotically required for cellularization is the newly described locus, *slow as molasses* (*slam*) [25•,26]. Slam protein associates with the plasma membrane and shows a polarized distribution in the furrow canals. Perhaps more importantly, *slam* loss-of-function specifically impairs growth of the basolateral plasma membrane domain and disrupts polarized localization of important junctional proteins such as Armadillo/β-Catenin and the PDZ protein encoded by *discs lost* (*dlt*, a member of the Crb complex). These and additional findings imply that *slam* plays an early and essential role in the specification of distinct membrane domains that form during cellularization, perhaps by mobilizing an inert maternally-supplied polarity apparatus [25•]. A remaining question is how directly Slam controls the localization of proteins such as Dlt, which is itself implicated in the establishment of polarity and discrete apical and lateral plasma-membrane domains [27]. Future studies into the molecular mode of action of *slam* and other loci zygotically required for cellularization should provide crucial insight into links between the initial polarity cues and later-acting networks that govern ZA formation and stabilization.

Epithelial apicobasal polarity in mammals

Investigations of apicobasal polarization in mammalian epithelia have revealed striking parallels with *Drosophila*. Vertebrate epithelial cells lack SJs, but instead feature TJs in a region analogous to the *Drosophila* SAR [2,3]. Members of the vertebrate Par3–Baz complex (Par3/Par6/aPKC) localize to the TJ [28,29] and members of both the Par3–Baz and Crb complexes are implicated in TJ formation [10••]. Although the homologous fly complexes are known to colocalize and interact genetically, experiments in mammalian cell culture document direct physical interaction between these protein complexes on the basis of co-immunoprecipitation of PALS1 (the homologue of *Drosophila sdt*) with Par6. On a functional level, overexpression of Par6 inhibits the TJ localization of PALS1, and expression of dominant-negative PATJ (a member of the vertebrate Crb complex) causes mislocalization of aPKC away from the TJ in MDCK cells [10••]. These results reveal direct interactions between the Crb/PALS1/PATJ and PAR3/PAR6/aPKC complexes during cell polarization and TJ formation, outlining a mechanism by which Crb could act through PALS1 to recruit the Par3–Baz complex to the apical TJ complex of vertebrate epithelia (Figure 1d).

Just as Par3–Baz complexes localize to the TJ (an apical domain analogous to the insect SAR), vertebrate homologues of the *Drosophila* Lgl group are excluded from the TJ and localize to the lateral plasma membrane in polarized epithelial cells [11•,29,30]. Despite its basolateral localization, recent reports indicate that mammalian Lgl (mLgl) can bind Par6β and aPKC exclusive of Par3 in immunoprecipitates of several different types of tissue culture cells [11•,22]. These results document the existence of at least two distinct Par6/aPKC complexes in epithelial cells: 'active' complexes of Par3/Par6/aPKC, and 'inactive' complexes of mLgl/Par6/aPKC. Intriguingly, basolateral mLgl does not normally co-localize with Par6β/aPKC in polarized MDCK cells, but does transiently co-localize with Par6β/aPKC during the early stages of cell repolarization induced by calcium [11•]. During this transient co-localization, mLgl becomes phosphorylated (perhaps by aPKC [11•,22]) and then dissociates from the complex, permitting formation of the 'active' Par3/Par6/aPKC complex and the subsequent events leading to TJ morphogenesis [11•] (Figure 1d). These findings suggest a general model for polarity control remarkably similar to what has been observed in *Drosophila*: opposing apical and basolateral protein groups position cell–cell junctions at the boundary between apical and basolateral plasma membrane domains. It is interesting to note that, in *Drosophila*, Lgl also binds Par6/

Figure 2



aPKC independently of Baz–Par3 and becomes phosphorylated by aPKC during neuroblast polarization [21]. Par6/aPKC is also clearly shown to regulate mLgl by phosphorylation in non-epithelial mammalian cell lines [22]. Whether similar interactions between Par6/aPKC and Lgl also occur in *Drosophila* epithelial cells remains unknown, but seems quite probable.

Apicobasal polarity: signaling implications

It is clear that a great deal of cellular energy is invested in epithelial cell polarization, but to what end? A well-established role of the polarity apparatus is to position the junctional complexes that maintain tissue integrity and act as scaffolds for transmembrane adhesion and signal transduction molecules [1–3]. For example, one recent analysis elegantly demonstrates that Wnt and BMP signaling govern hair follicle morphogenesis in mammalian epithelia by locally modulating a switch from E- to P-cadherin expression in epithelial TJs [31]. A less-considered but equally crucial role for the polarity apparatus may be to delimit separate apical and basolateral microenvironments within an epithelium. The immiscibility of apical and basolateral plasma membrane domains together with the diffusion barrier properties of some junctional complexes allows for targeted secretion of extracellular signals to the apical or basal compartments of an epithelium. This possibility presents an intriguing new level of complexity in cell–cell communication.

Some of the earliest studies to hint at the importance of apicobasal polarization in cell–cell signaling came from analysis of EGFR signaling during vulva induction by the Lin-3/TGF-α ligand in *C. elegans*. Genetic analysis has identified several additional genes required for EGFR function in *C. elegans*, many of which seem to function in basolateral localization of the receptor [32,33]. Vertebrates also feature a family of EGFR-like receptors, known as ErbB1–4. These receptors also show polarized subcellular distribution in epithelia, and in some cases are known to generate qualitatively distinct signaling activities depending on their localization [34]. Binding of ligands such as Heregulin or EGF stimulates the heterodimerization and phosphorylation of ErbB receptors, initiating a downstream signaling cascade [35]. Surprisingly, analysis of cultured human airway epithelia reveals that the ligand Heregulin is secreted into the apical

The paracellular diffusion barrier regulates Heregulin activation of ErbB2 in mammalian epithelia. **(a)** In control epithelium, Heregulin is found in the apical extracellular space. The transmembrane receptor ErbB2 is confined to basolateral membranes below the TJs and thus the receptor is inactive. **(b)** When the paracellular diffusion barrier is compromised by addition of calcium to the culture media, apical Heregulin is able to activate ErbB2, leading to its phosphorylation and activation of signal transduction. **(c)** When epithelial integrity is disrupted by wounding, apical ligand and basolateral receptors come into contact, stimulating receptor activation and a proliferative wound-healing response [36••].

extracellular space above the TJ level, whereas its receptor ErbB2 is localized to the basolateral plasma membrane domain below the TJ. Analysis of ErbB2 activation in control airway epithelia suggests that apical Heregulin cannot activate ErbB2, even when exogenous ligand is applied to the apical epithelial surface (Figure 2a). Conversely, addition of Heregulin to the basolateral side of cultured airway epithelia stimulates high levels of receptor activation and hence epithelial remodeling. Finally, when the paracellular diffusion barrier is compromised by calcium addition, apically applied ligand can access and activate basolateral ErbB2, demonstrating that physical segregation of receptor and ligand normally prevents receptor activation (Figure 2b) [36••].

These observations explain how airway epithelia can constitutively express a mitogenic ligand but still feature a low level of receptor activation, but do not address the biological significance of a ligand/receptor pair that is physically segregated under normal conditions. The answer to this apparent paradox could lie in the delicate nature of airway epithelia and their vital function in presenting a barrier to microbial or viral attack. When pathogens or irritants physically compromise epithelial integrity, apical Heregulin could move into the basolateral space and activate ErbB2 to stimulate an immediate wound response [36••] (Figure 2c). This model is largely borne out by the ability of antibodies that block the functions of ErbB2 and Heregulin to abolish the proliferative wound-healing response of epithelia injured *in vitro* [36••].

The finding that basolateral application of Heregulin induces severe epithelial abnormalities demonstrates that dysplastic disease states could arise from physical disruption of the epithelial paracellular diffusion barrier. Consistent with this idea, it was recently reported that infection of mammalian epithelial cells with *Heliobacter pylori* causes disruption of the paracellular diffusion barrier, among other effects [37••]. The long-term implications of *H. pylori* infection may include gastric carcinoma and peptic ulcer disease. Some aspects of these disease states could result from the inappropriate movement of solutes between apical and basolateral aspects of infected epithelia. It is also interesting to consider whether ligand/receptor segregation plays a role in developmental cell–cell signaling. A major distinction between vertebrate and insect epithelia is the relative position of the paracellular diffusion barrier. In *Drosophila*, genetic studies suggest that the epithelial diffusion barrier is mediated by the SJ basal to the ZA [6,7•]. We note that flies mutant for the SJ-localized polarity proteins *dlg*, *scrib* and *lgl* exhibit a dramatic overproliferation of imaginal disc epithelial cells [38]. Conceivably, this phenotype could result from disruption of the paracellular barrier and enhanced cell proliferation due to mixing of a mitogenic ligand/receptor pair that is segregated under normal conditions.

Conclusions

Recent studies demonstrate that conserved molecular machinery governs apicobasal polarization of *Drosophila* and vertebrate epithelial cells. In both systems, a delicate balance between competing protein complexes defines the position of cell–cell junctions at the interface between apical and basolateral plasma membrane domains. One of the consequences of apicobasal polarization is establishment of a paracellular diffusion barrier mediated by the vertebrate TJ or the SJ in *Drosophila*. Recent studies demonstrate that segregation of ligand/receptor pairs to either side of this barrier may represent an important new level of regulation in cell–cell signaling events.

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