© 2021. Published by The Company of Biologists Ltd | Journal of Cell Science (2021) 134, jcs248757. doi:10.1242/jcs.248757

REVIEW

SUBJECT COLLECTION: POLARITY

How cells tell up from down and stick together to construct multicellular tissues – interplay between apicobasal polarity and cell–cell adhesion

Claudia G. Vasquez*,[1], Eva L. de la Serna*,[1] and Alexander R. Dunn[1,2,3,‡]

ABSTRACT

Polarized epithelia define a topological inside and outside, and hence constitute a key evolutionary innovation that enabled the construction of complex multicellular animal life. Over time, this basic function has been elaborated upon to yield the complex architectures of many of the organs that make up the human body. The two processes necessary to yield a polarized epithelium, namely regulated adhesion between cells and the definition of the apicobasal (top–bottom) axis, have likewise undergone extensive evolutionary elaboration, resulting in multiple sophisticated protein complexes that contribute to both functions. Understanding how these components function in combination to yield the basic architecture of a polarized cell–cell junction remains a major challenge. In this Review, we introduce the main components of apicobasal polarity and cell–cell adhesion complexes, and outline what is known about their regulation and assembly in epithelia. In addition, we highlight studies that investigate the interdependence between these two networks. We conclude with an overview of strategies to address the largest and arguably most fundamental unresolved question in the field, namely how a polarized junction arises as the sum of its molecular parts.

KEY WORDS: Adhesion, Crosstalk, Polarity

Introduction

Multicellularity allows organisms to grow larger, move faster and perform more complicated biological functions than their unicellular competitors. Due to these immense advantages, multicellularity is estimated to have evolved 16–22 times across the tree of life, though only once in metazoans (animals) (Sebé-Pedrós et al., 2017). In animals, two key features that define the architecture of epithelial tissues are the induction of a defined top–bottom, or apicobasal, axis in individual cells and the formation of regulated adhesions between neighboring cells. These two features allow cells to collectively define an inside and an outside, the core topology that defines metazoans (Butterfield, 2009) and that typifies the earliest steps in modern metazoan embryogenesis. The purpose of this Review is to succinctly encapsulate what is currently known about how apicobasal polarity and cell–cell adhesion occur, and how they coordinate to generate the fundamental building block of animal tissues: the polarized epithelium.

[1]Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA. [2]Biophysics Program, Stanford University, Stanford, CA 94305, USA. [3]Stanford Cardiovascular Institute, Stanford School of Medicine, Stanford, CA 94305, USA.
*These authors contributed equally to this work

[‡]Author for correspondence (alex.dunn@stanford.edu)

C.G.V., 0000-0002-3030-4828; E.L.d.l.S., 0000-0003-0166-8750; A.R.D., 0000-0001-6096-4600

Apicobasal polarity is established and maintained via three main polarity protein complexes: the apically localized Crumbs complex, the apical–lateral-localized Par complex, and the basolateral-localized Scribble complex (Fig. 1A). Cell–cell adhesion is mediated by multiple and less strictly defined molecular assemblies. In chordates, particularly important adhesion complexes are adherens junctions (AJs) and tight junctions (TJs), which together enable cells to assemble into sheets that form a semi-permeable barrier between tissue compartments. These adhesion complexes are vertically organized in the cells with TJs assembled apical to AJs, a spatial organization that suggests interplay of the AJs and TJs with each other and the polarity complexes (Fig. 1A).

Here, we provide brief introductions to both cell polarity and intercellular adhesion biology and their roles in epithelial tissues, which we hope will be helpful to experts in one field but not the other, and for newcomers to both. We then focus on the relatively small number of studies that examine how these two subsystems interact with each other, with the anticipation that much remains to be discovered in this area. In so doing, we highlight several open challenges, all of which revolve around one, unifying question: how does the architecture of a simple epithelium arise from the sum of its molecular parts?

Evolutionary origins of animal multicellularity

Our current understanding is that most metazoans rely on polarity and junctional complexes to establish apicobasal polarity and maintain cell–cell adhesion; however, homologs of the polarity and adhesion proteins appeared in unicellular organisms well before the advent of metazoans (Fahey and Degan, 2010; Murray and Zaidel-Bar, 2014) (Fig. 1B). Studying the emergence of these components provides insight into how unicellular organisms evolved into multicellular animals. Choanoflagellates, close relatives of metazoans (Fig. 1B), have predicted homologs for polarity proteins and even cadherin molecules, which are canonically involved in cell–cell adhesion; however, in some cases the functions of these proteins are different than in metazoans (Sebé-Pedrós et al., 2017). For example, although there are 20–30 predicted cadherins in choanoflagellate genomes, many or most are thought to be used for feeding rather than cell–cell adhesion (Abedin and King, 2008; Sebé-Pedrós et al., 2017). In other cases, the usage of polarity and adhesion proteins is more similar to that in metazoans: while choanoflagellates are often unicellular, many species have a multicellular life stage (Brunet et al., 2019; Dayel et al., 2011). Some, such as *Choanoeca flexa*, go as far as forming cup-shaped cellular sheets (Brunet et al., 2019). These sheets can undergo a form of apical constriction that inverts the polarity of the sheet, allowing the colony to feed or swim more efficiently. A contractile actomyosin network powers apical constriction, similarly to many metazoan epithelial tissue folding events (Brunet et al., 2019).

Journal of Cell Science (2021) 134, jcs248757. doi:10.1242/jcs.248757

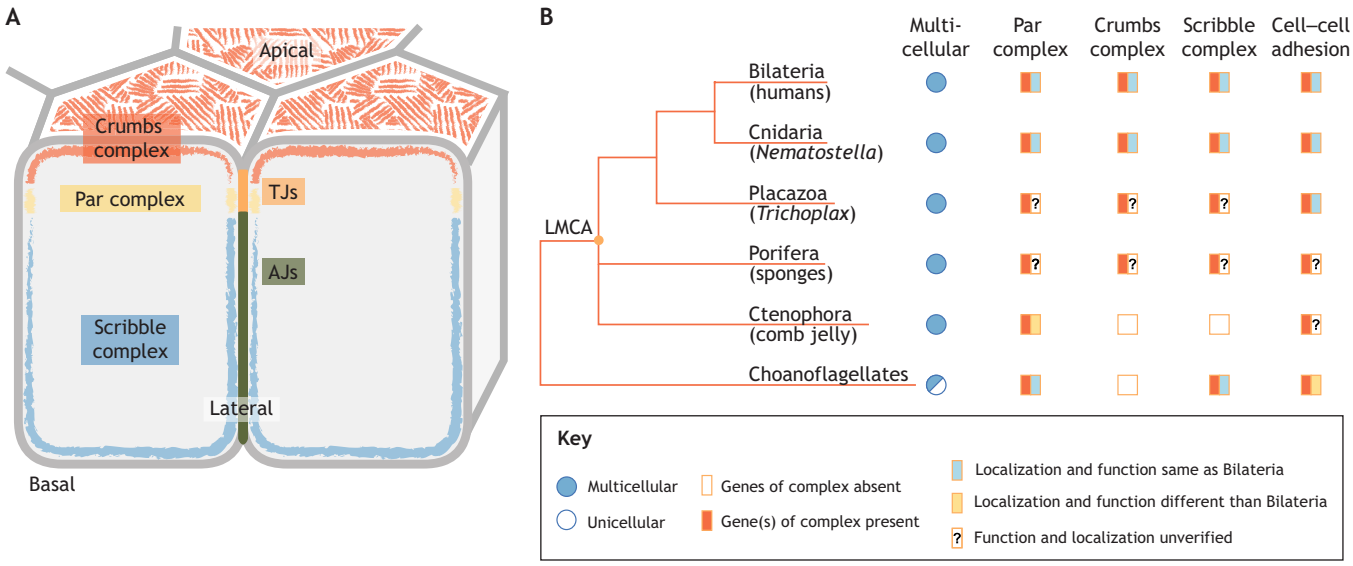

Fig. 1. Apicobasal polarity complexes and their evolutionary origins. (A) Localization of apicobasal polarity complexes (Crumbs, Par and Scribble) and junctional complexes (TJs and AJs) in a vertebrate epithelial cell. Together, these complexes establish apical and basal cellular domains. (B) Phylogenetic relationship between multicellular animals and unicellular eukaryotes, with additional information regarding the role of orthologs in epithelial polarity and adhesion. LMCA, last metazoan common ancestor.

Even within the metazoans, there is considerable variability in how polarity and adhesion complexes are deployed. In contrast to the canonical organization and dependency on polarity complexes in many well-studied metazoan tissues (Fig. 1A), polarization of the epithelium in the adult *Drosophila* midgut does not depend on Par or Crumbs complex proteins (Chen et al., 2018). In the ctenophore (comb jelly) *Mnemiopsis leidyi*, Par proteins are likewise asymmetrically localized in early embryos but not in later-stage epithelial tissues, indicating that asymmetric Par localization is not necessarily required to maintain tissue polarity (Salinas-Saavedra and Marindale, 2020). Interestingly, *M. leidyi* Par proteins retain the capability to localize asymmetrically when injected into the cnidarian *Nematostella vectensis*, illustrating that the *M. leidyi* Par proteins themselves retain the ability to self-segregate (Salinas-Saavedra and Marindale, 2020).

The function and organization of intercellular adhesion complexes likewise varies widely across metazoans. Perhaps most prominently, in chordates, claudin- and occludin-based TJs are apical to cadherin-based AJs, whereas in other metazoans the TJ-analog septate junctions are positioned basally to AJs (Farquhar and Palade, 1963; Lane and Skaer, 1980; Noirot-Timothée et al., 1978). These and other examples illustrate that the interrelated functions and interactions of adhesion and polarity complexes are nuanced, and likely to depend on species and tissue type. Here, we focus on what has been learned from studies of epithelia in commonly studied model organisms, with the understanding that important details are likely to vary across phyla and even across tissues in the same organism.

Apicobasal polarity complexes

Cell polarity refers to asymmetrical localization of distinct proteins within a cell and is the basis for diverse tissue architectures and functions. As the linings of tissues, epithelial cell sheets rely on the establishment of top–bottom, or apicobasal, polarity of proteins to form a polarized tissue with a defined inside and outside. In this section, we briefly describe the core components of the three complexes that establish apicobasal polarity and mechanisms of regulation of these complexes (Fig. 2A). These complexes, their regulation and their biochemical interactions have been reviewed in detail previously (Campanale et al., 2017; Flores-Benitez and Knust, 2016; Pickett et al., 2019; Tepass, 2012). Here, we briefly introduce the composition of these complexes and then discuss general concepts of regulation.

The Crumbs complex

The Crumbs complex is the most apically localized complex and plays a critical role in apicobasal polarity maintenance, as well as regulation of cell shape via apical organization and AJ positioning (Bulgakova and Knust, 2009; Letizia et al., 2013). The core of the complex has four proteins: Crumbs (Crb in *Drosophila*; CRB1, CRB2 and CRB3 in mammals), Stardust (Sdt in *Drosophila*; PALS1 in mammals), Patj (also known as INADL) and Lin7 (also known as in Veli in *Drosophila* and as LIN7A or MALS1 in vertebrates) (Fig. 2A).

Crb is the only transmembrane protein of the apical protein regulators (Box 1), while Sdt is an intracellular scaffolding protein that directly interacts with Crb (Tepass, 1996). Loss of Sdt in *Drosophila* whole-eye clones results in the same defects as loss of Crb in *Drosophila* photoreceptor cells: the rhabdomeres malform into split or enlarged structures (Hong et al., 2003; Nam and Choi, 2003). In contrast, Patj and Lin7 play more nuanced roles. In *Drosophila* wing imaginal discs, Lin7 is recruited to the apical membrane by directly binding to Sdt (Bachmann et al., 2004). In *Drosophila*, Patj also binds to Sdt (Bachmann et al., 2004; Bulgakova et al., 2008). Neither Patj nor Lin7 is required for polarization of *Drosophila* early embryonic epithelia, but photoreceptor cells with mutant Lin7 exhibit a retinal degeneration phenotype similar to that of Crb mutants despite exhibiting a normal morphology (Bachmann et al., 2008). However, Patj and Lin7 are both essential to establish proper polarization in Madin–Darby canine kidney (MDCK) cells (Straight et al., 2006) (Fig. 2B). This varying dependency on Patj and Lin7 for proper polarization highlights the nuanced usage of the same basic polarity toolbox in different epithelial tissues.

Journal of Cell Science (2021) 134, jcs248757. doi:10.1242/jcs.248757

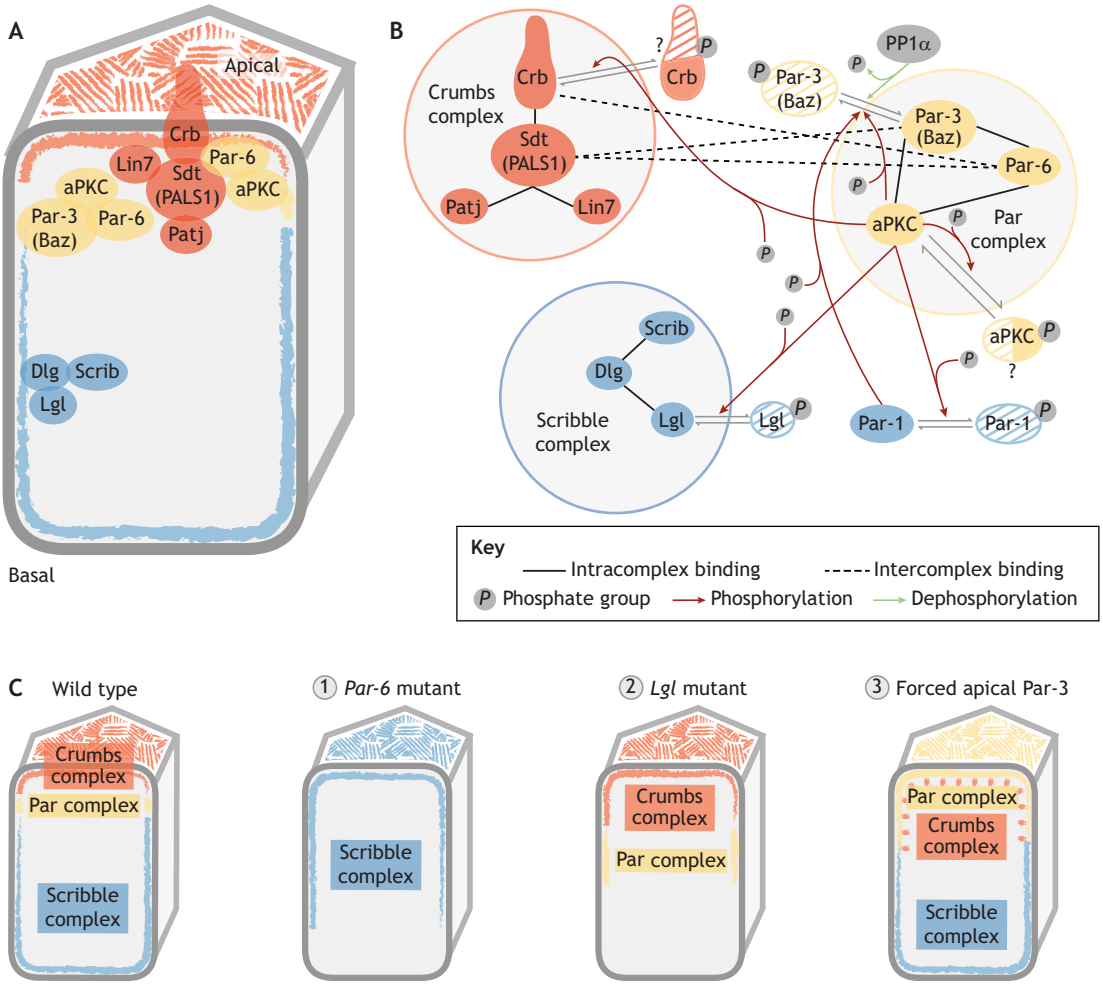

Fig. 2. Mechanisms of apicobasal intra- and inter-complex regulation. (A) Primary components of the Crumbs (red), Par (yellow) and Scribble (blue) complexes, and their localization in a polarized cell. (B) Interactions within and between polarity complexes. The schematic shows known binding interactions within and between polarity complexes. Kinases and phosphatases modulate polarity protein interactions and localization. Canonical polarity complexes (indicated with solid shading) are thought to localize to their respective cellular domains as shown in A. Phosphorylated forms of the proteins are indicated with hatched lines and a phosphate group label. (C) Schematics depicting how mutations in or mislocalization of a component of one polarity complex affect the localization of the other complexes. Wild-type cells show the Crumbs complex apical to the Par complex and the Scribble complex localized basolaterally. In cells mutant for *Par-6*, components of the Scribble complex are no longer restricted to the basolateral domain (1). Conversely, cells mutant for *Lgl* display an expansion of the Crumbs and Par complex domains (2). Forced apical localization of Par-3 disrupts apical localization of the Crumbs complex (3).

The Par complex

The Par complex is composed of three cytoplasmic proteins: Par-3 [known as PAR-3 in *Caenorhabditis elegans*, PARD3 in vertebrates and Bazooka (Baz) in *Drosophila*], atypical protein kinase C (aPKC) and Par-6 (also known as PARD6 in mammals, of which PARD6-alpha is the primary isoform) (Fig. 2A). Par-3/Baz (used here to refer to both the vertebrate and *Drosophila* homologs) and Par-6 are scaffolding proteins, while aPKC is a kinase. Par-3/Baz in particular is highly conserved in metazoans (Atwood et al., 2007; Fahey and Degan, 2010; Hutterer et al., 2004; Kuchinke et al., 1998). All three are essential in establishing polarity in a wide variety of systems, as evidenced by the disruption of apicobasal polarity establishment when any of the three components are silenced or functionally impaired (Hutterer et al., 2004; Krahn et al., 2010; Nance et al., 2003). The small GTPase Cdc42 is also often considered part of the Par complex, as its activation is essential to regulate the activity of Par-6 (Yamanaka et al., 2001).

A series of intricate interactions between the Par complex components are required for the establishment of polarity (Fig. 2B). First, Par-3/Baz, aPKC and Par-6 bind to each other to form the canonically defined Par complex, which localizes to the apical portion of the lateral (hereafter, apical–lateral) domain (Fig. 2A). There, Par-6 can then interact with active, membrane-associated Cdc42. This relieves Par-6-induced inhibition of aPKC, which in turn allows aPKC to phosphorylate Par-3/Baz (Atwood et al., 2007; Hutterer et al., 2004; Yamanaka et al., 2001). aPKC-mediated phosphorylation of Par-3/Baz weakens its interactions with both aPKC and Sdt, thus promoting the dissociation of Par-3/Baz from the Par complex and banishing it from the apical–lateral domain (Krahn et al., 2010; Morais-de-Sá et al., 2010). Additionally, in *Drosophila* follicular epithelia, Crb and Sdt appear to play additional roles in banishing Baz from the apical membrane by preventing Baz–Par-6 interactions (Morais-de-Sá et al., 2010). Once dissociated, Par-3/Baz localizes to the lateral domains, while Par-6–aPKC remains apically with the Crumbs complex (McGill et al., 2009; Morais-de-Sá et al., 2010; Müller and Wieschaus, 1996). Interestingly, there is evidence that aPKC and Par-6 may also participate in organizing the Crumbs complex (Nam and Choi,

Journal of Cell Science (2021) 134, jcs248757. doi:10.1242/jcs.248757

Box 1. The puzzling role of the Crumbs extracellular domain

In *Drosophila*, the transmembrane protein Crb consists of a short intracellular tail domain and a large extracellular domain. The extracellular domain was initially thought to be dispensable for the role of Crb in apical polarity establishment (Wodarz et al., 1995). However, studies now show that the extracellular domain appears to both facilitate homophilic binding between adjacent cells and is itself essential for cell polarization and embryonic morphogenesis in *Drosophila* embryos (Das and Knust, 2018; Klebes and Knust, 2000; Letizia et al., 2013). In mammals, there are three CRB proteins: CRB1, CRB2 and CRB3. Whereas CRB1 and CRB2 each have large extracellular domains that contain EGF-like and laminin-like binding domains, CRB3 lacks most of this extracellular domain (Vacca et al., 2015). Despite this difference, the cytoplasmic domain of CRB3 is essential to establish polarity in some cell types (Fan et al., 2007; Gao et al., 2016; Roh et al., 2003). Interestingly, the Crb extracellular domain is sufficient to induce the aggregation of both cultured *Drosophila* S2 cells (Letizia et al., 2013) and mammalian cells exogenously expressing zebrafish isoforms of Crb2a or Crb2b (Zou et al., 2012). *In vivo*, the extracellular domain of Crb2a is required for photoreceptor adhesion in zebrafish (Zou et al., 2012), suggesting that in some systems Crb isoforms may play a role in cell–cell adhesion.

2003; Sotillos et al., 2004; St Johnston and Ahringer, 2010) (Fig. 2B).

The Scribble complex and Par-1

The Scribble complex was initially identified and described in *Drosophila*. It is a basolaterally localized complex composed of the Scribble (Scrib), Discs large (Dlg) and Lethal giant larvae (Lgl) proteins (Bilder et al., 2000; Bilder and Perrimon, 2000; Woods and Bryant, 1991) (Fig. 2A). Although not a member of the Scribble complex, the kinase Par-1 (also known as Par1/MARK kinases in vertebrates) also localizes to the basolateral domain in epithelial cells and is likewise required for polarity induction. Although outside the scope of this Review, it is interesting to note that mammalian central nervous system synapses contain many Scribble complex protein homologs that play important roles in synapse formation and function, for instance the Dlg homolog PSD-95 (also known as DLG4) (Chen et al., 2015; Cho et al., 1992; Nair et al., 2013). Moreover, all three Scribble complex components act as tumor suppressors in simple epithelia, as loss of any one of these proteins results in unregulated cell proliferation, emphasizing the importance of apicobasal polarity for normal pathology (Bilder et al., 2000; Woods and Bryant, 1991). The Scribble complex is also reutilized by cells to orient asymmetric cell divisions and to build neuronal synapses (reviewed in Bonello and Peifer, 2019).

In *Drosophila*, the simultaneous expression of *scrib*, *lgl* and *dlg* genes is required for the proper localization of Scrib, Lgl and Dlg (Bilder et al., 2000). Although it is known that basolateral localization of Lgl requires binding to Dlg and Scrib, the underlying mechanisms are only beginning to come to light (Bilder et al., 2000; Daulat et al., 2018; Khoury and Bilder, 2020; Strand et al., 1994). At least in *Drosophila*, Lgl localization is dependent on both Dlg and Scrib (Khoury and Bilder, 2020). However, this requirement is not bidirectional, as Dlg can localize to the cell cortex independently of Lgl or Scrib, and Scrib localization only requires Dlg (Khoury and Bilder, 2020). Thus, Dlg appears to be at the top of this recruitment hierarchy, followed by Scrib, and then Lgl. Nevertheless, the entire Scribble complex is required for proper specification of the basolateral domain (Khoury and Bilder, 2020) (Fig. 2B).

Positive and negative regulation of polarity proteins

Polarity complexes establish and maintain cell polarity through localization antagonism and phosphorylation (Fig. 2B).

Mutual antagonism

A prevalent model of apicobasal polarity establishment and maintenance centers on the idea of mutual antagonism, whereby the improper accumulation of basolateral polarity proteins at the developing apical domain is prevented by the presence of apical polarity complexes, and vice versa. Loss of proteins involved in this antagonism results in misregulation and expansion of cellular apical domains upon the loss of the basolateral domains, or vice versa. For example, Par-6 is required to restrict Lgl to the basolateral domain, and, consequently, *Drosophila* embryos from *par-6* mutant germline clones exhibit Lgl localization throughout the apical–basal axis (Hutterer et al., 2004) (Fig. 2C, panel 1). Conversely, Lgl expression is required to restrict Par-6 localization to the apical–lateral region at the boundary of the apical and basolateral membranes (Hutterer et al., 2004) (Fig. 2C, panel 2). Scrib itself is required to restrict components of the Crumbs complex to the apical membrane, and, accordingly, in *scrib* mutants, the Crumbs complex localizes unrestrictedly along the apical and basolateral membranes (Bilder and Perrimon, 2000). Furthermore, expression of Par-3/Baz that is targeted to the apical membrane is sufficient to expand the apical–lateral identity into the apical membrane, displacing typically apically localized proteins like the Crumbs complex from the apical membrane (Ruch et al., 2017) (Fig. 2C, panel 3). Thus, there is a clear intercomplex antagonism to promote proper segregation of apical and basolateral components, thereby establishing and maintaining cellular polarity.

Phosphorylation

Kinase and phosphatase activities are critical for the regulation of polarity complexes. Par-3/Baz protein interactions and localization are known to be tightly regulated by its phosphorylation state. aPKC-mediated phosphorylation of Par-3/Baz weakens the interaction between aPKC and Par-3/Baz, resulting in Par-3/Baz dissociation (Morais-de-Sá et al., 2010). Par-1 phosphorylation of Par-3/Baz prevents the protein from aggregating basally and also disrupts Par-3/Baz binding to the rest of the Par complex (aPKC and Par-6) (Benton and St Johnston, 2003; Hurov et al., 2004). The phosphatase PP1α (Flapwing in *Drosophila*) binds to and dephosphorylates cytoplasmic Par-3/Baz, enabling Par-3/Baz to reassociate with aPKC. Here, PP1α may work to balance Par-1 kinase activity and the reformation of the Par complex (Traweger et al., 2008).

aPKC also maintains polarity by restricting basolateral proteins, such as Lgl and Par-1, to the basolateral domain through their phosphorylation (Hurov et al., 2004; Hutterer et al., 2004). Phosphorylation of Lgl by aPKC prohibits its accumulation at the apical membrane and instead promotes its localization to the basolateral domain with the rest of the Scribble complex. Lgl phosphorylation by aPKC in turn antagonizes aPKC localization at the basolateral cortex (Hutterer et al., 2004). Despite this mutual antagonism between Lgl and aPKC, ectopic apical localization of the entire Scribble complex is insufficient to evict aPKC from the apical domain, perhaps reflecting the inability of the Scribble complex to protect Lgl from phosphorylation by aPKC (Khoury and Bilder, 2020).

Negative regulation of aPKC kinase activity also plays a role in polarity stabilization. Par-6 is thought to further stabilize apical–lateral localization of the Par complex by inhibiting aPKC kinase activity (Suzuki et al., 2004; Yamanaka et al., 2001). However, this

Journal of Cell Science (2021) 134, jcs248757. doi:10.1242/jcs.248757

inhibitory role may vary between model systems, as Par-6 can activate aPKC kinase activity in some circumstances (Etienne-Manneville and Hall, 2001; Graybill et al., 2012).

Finally, aPKC also phosphorylates itself and can also phosphorylate Crb (Sotillos et al., 2004) (Fig. 2B). Although the precise effects of Crb phosphorylation *in vivo* are not well understood, a recent study with *Drosophila* Crb mutants has shown that Crb phosphorylation does not appear to contribute to proper Crb localization at the apical membrane in *Drosophila* embryonic epithelia (Cao et al., 2017). It will be interesting to further investigate the functions of these poorly understood phosphorylation events, such as whether these also occur in mammalian models and whether Par-1 exhibits any reciprocal kinase activity towards aPKC.

Cell–cell adhesion complexes

In this section, we will discuss the molecular themes involved in cell–cell adhesion complexes. Due to length and scope limitations, in this Review we do not discuss several important classes of cell–cell adhesions, notably desmosomes (Broussard et al., 2020) and gap junctions (Goodenough and Paul, 2009). While there are a variety of complexes, cell–cell adhesion complexes are made up of two core components (Fig. 3A): (1) transmembrane receptor proteins and (2) scaffolding cytoplasmic proteins that directly or indirectly link the transmembrane proteins to the cytoskeleton. Below we outline essential examples from both classes.

Adherens junctions

AJs are cell–cell adhesion complexes that mechanically link adjacent cells. The cadherin–catenin complex is the well-studied core of the AJ (Fig. 3B; Box 2). In epithelial cells, E-cadherin (also known as DE-cadherin in *Drosophila*, encoded by *shotgun*; CDH1 in vertebrates) binds to p120-catenin (encoded by *p120ctn* in *Drosophila*, *CTNND1* in vertebrates) and β-catenin (encoded by *armadillo* in *Drosophila*, *CTNNB1* in vertebrates), with the latter in turn binding to α-catenin (encoded by *CTNNA1* and *CTNNA2* in vertebrates). α-Catenin binds to F-actin, connecting the cell–cell junction to the cytoskeleton (Fig. 3B). In addition, α-catenin acts as a scaffolding protein to recruit many proteins, including ZO-1 (also known as TJP1) and afadin (Canoe in *Drosophila*). In some cell types, such as endothelial cells, cadherins can additionally bind to a third catenin, plakoglobin (also known as γ-catenin or JUP), which

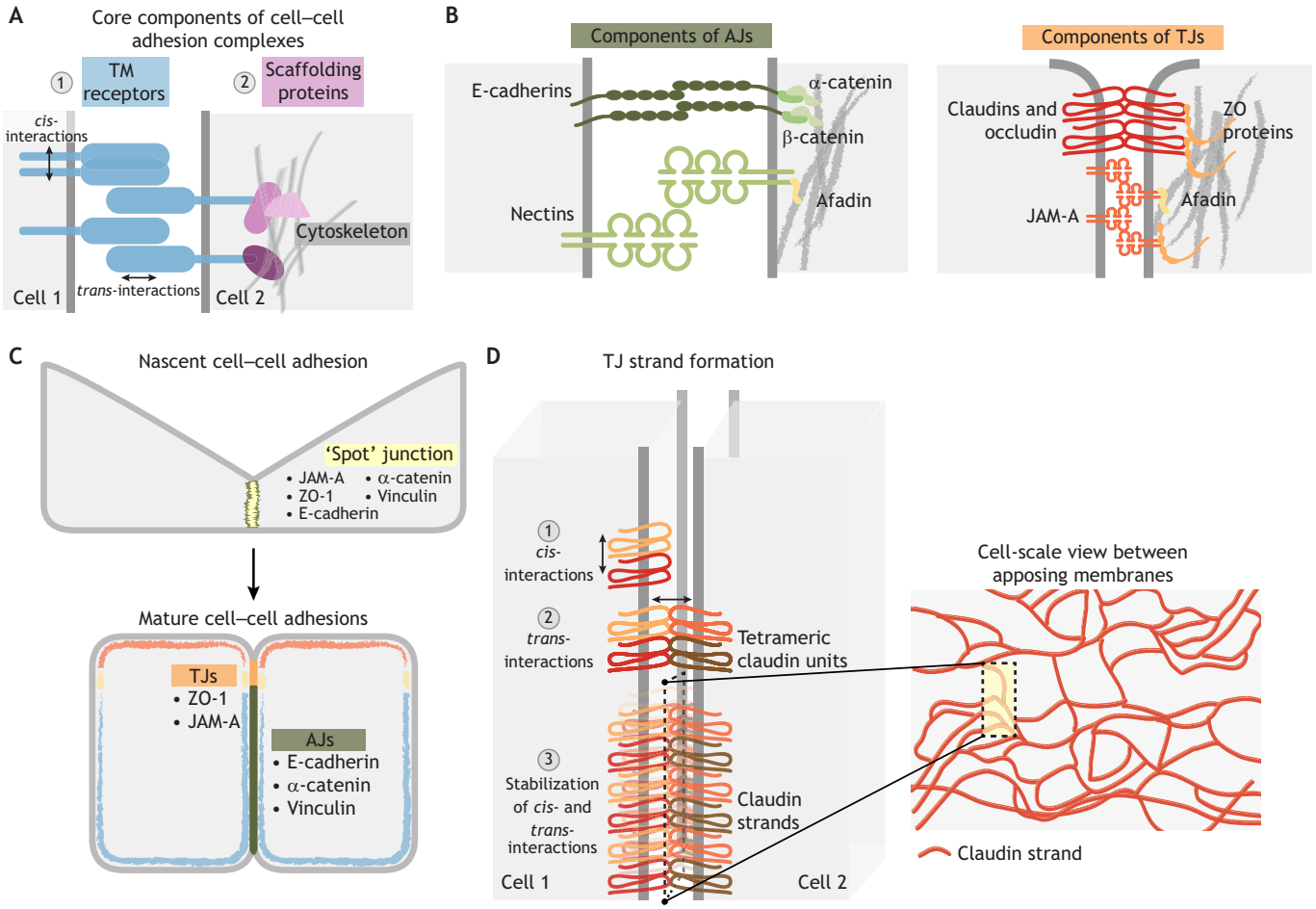

Fig. 3. Components and organization of cell–cell adhesion complexes. (A) Cell–cell adhesion complexes are composed of (1) transmembrane (TM) receptors containing extracellular domains that bind to adjacent cells and intracellular domains that bind to (2) cytoplasmic scaffolding proteins, which can also bind to the cytoskeleton. *Cis*- and *trans*-interactions of TM receptors can drive the assembly of cell–cell adhesion complexes, where *cis*-interactions are between molecules of the same cell (cell 1) and *trans*-interactions are between molecules from adjacent cells (cells 1 and 2). (B) Schematic illustration of AJ (left) and TJ (right) components. (C) Reorganization of junctional proteins as cell–cell junctional complexes mature from spot junctions (top) to mature adhesion complexes (bottom) (D) Assembly of adhesive TJ strands occurs in a stepwise process. TJ strands assemble by *cis*-dimerization of claudin molecules (1), followed by *trans*-interactions between claudin dimers of adjacent cells to form tetrameric units (2). These units continue to assemble, forming long strands of claudin molecules between adjacent cells (3 and right-hand panel).

Journal of Cell Science (2021) 134, jcs248757. doi:10.1242/jcs.248757

Box 2. Mechanosensing at cell–cell adhesions

Cell–cell adhesions are exquisitely responsive to mechanical forces, a property that allows these cellular linkages to reinforce in response to tensile stresses that might otherwise tear the tissue apart. Reductionist *in vitro* work shows that some junctional proteins are responsive to force. Notably, the binding strength of the cadherin–catenin complex to F-actin displays catch-bond behavior – its affinity for F-actin increases in response to tensile force over a certain force range (Buckley et al., 2014). The interactions of α-catenin with its binding partners are also regulated by mechanical tension: *in vitro* studies indicate that force drives a large change in the conformation of α-catenin that exposes a cryptic binding site for vinculin, an F-actin binding protein (Yao et al., 2014), and possibly afadin (Pokutta et al., 2002). Vinculin itself forms a directional catch bond with F-actin, indicating that not only is the interaction force-sensitive but also the interaction depends on the polarity of F-actin (Huang et al., 2017). Like α-catenin, ZO-1 displays force-dependent alterations in conformation (folded and stretched) that depend on actomyosin-generated tension (Spadaro et al., 2017). The stretched conformation of ZO-1 is thought to sequester the transcription factor ZONAB at junctions, inhibiting its pro-proliferative activity (Spadaro et al., 2017). These and other studies suggest that tensile stress can actually strengthen cell–cell junctions and may have an instructive role in organizing junctional complexes.

mediates an indirect linkage to the intermediate filament cytoskeleton (reviewed in Kowalczyk and Green, 2013). A less studied AJ adhesion complex is the nectin–afadin complex. Nectin family proteins are a class of single transmembrane domain cell adhesion proteins. Nectins can mediate homotypic and heterotypic cell–cell adhesion via their three immunoglobulin-like extracellular domains that can bind to themselves, other nectin family proteins and nectin-like proteins (Mandai et al., 2015). The cytoplasmic domain of nectins binds to the cytoplasmic scaffolding protein afadin (Takahashi et al., 1999). Like α-catenin, afadin binds directly to actin filaments (Mandai et al., 1997), and in addition, can also bind to α-catenin (Pokutta et al., 2002) and tight junction proteins (ZO-1 and JAM-A) (Ooshio et al., 2010; Severson et al., 2009) (Fig. 3B). Consequently, afadin appears to have roles in organizing actin filaments and E-cadherin-mediated cell–cell adhesion via binding to α-catenin (Sakakibara et al., 2020). A recent study of human intestinal biopsies and Caco-2 cells indicates that nectins, rather than cadherins, may be the primary mediators of mechanical coupling of cells in the intestinal epithelium; using super-resolution microscopy, the authors resolved nectin localization to a band 100–300 nm apical of an E-cadherin band and showed that the nectin band colocalized with a circumferential actin filament belt (Mangeol et al., 2019, preprint). It will be interesting to see whether these findings are applicable to other systems and to determine the physiological conditions that promote nectin- versus cadherin-based adhesions.

Tight junctions

TJs are cell–cell adhesion complexes necessary for establishing and maintaining epithelial barrier function of tissues. In addition, TJ barriers are implicated in restricting the mixing of apical and basal lipids within the plasma membrane, acting as a membrane fence (Mandel et al., 1993; Zihni et al., 2016). TJs were first identified in electron microscopy (EM) images of slices of rat intestines and are characterized by a narrowing, or tightening, of the intercellular gap between adjacent plasma membranes apical to the AJs (Farquhar and Palade, 1963). The proteins that mediate the narrowing of these gaps arrange themselves in 10 nm strands (Staehelin et al., 1969) and are typically composed of TJ transmembrane proteins, claudin family proteins, occludin, tricellulin (also known as MARVELD2), as well as junctional adhesion molecules (JAMs) and a variety of cytoplasmic scaffolding proteins (Fig. 3B; Box 2).

General mechanisms of assembly of cell–cell adhesion complexes

Although the protein complexes described above localize to distinct domains along lateral cell–cell contacts, during *de novo* assembly of cell–cell contacts, the roles of AJ and TJ components are more nuanced. While E-cadherin and JAM-A have been identified at nascent cell–cell interfaces, nectins may have a role in recruiting these molecules. In interfaces between nectin-1-coated beads and nectin-1-expressing MDCK cells, nectin-1–afadin complexes are recruited prior to E-cadherins (Honda et al., 2003), and nectin-1 *trans* interactions are required to recruit JAM-A to MDCK cell–cell contacts (Fukuhara et al., 2002). At least in some systems, the normal recruitment of cadherin–catenin complexes and TJ proteins depends on essential interactions between nectin and afadin (Sato et al., 2006). Afadin, in turn, is suggested to recruit ZO-1 to these nascent cell–cell contacts. In fact, knockdown of afadin in MDCK cells impairs both AJ and TJ formation in epithelial monolayers (Ooshio et al., 2010). Importantly, at these initial stages, ZO-1 'moonlights' as an AJ protein during the establishment of cell–cell adhesion, localizing with canonical AJ components, and subsequently re-localizes with its TJ transmembrane binding partners as the junction matures (Ikenouchi et al., 2007; Rajasekaran et al., 1996; Yonemura et al., 1995) (Fig. 3C). The strength of the binding interaction between ZO-1 and actin is thought to tune the permeability of epithelial tissues: in MDCK cells expressing engineered ZO-1 proteins with a catalog of strong and weak actin filament binding sites, weak binding between actin filaments and ZO-1 optimally promotes stabilization of the TJ structure by templating and aligning claudins into strands (Belardi et al., 2020). This suggests that carefully tuned interactions between proteins within TJs operate together to generate TJ function; *in vitro* experiments that measure force-dependent binding of ZO-1 to actin provide a possible means of testing this hypothesis.

As junctions mature, E-cadherin clusters form and further drive cell–cell adhesion (Strale et al., 2015; Truong Quang et al., 2013; Wu et al., 2015). E-cadherin molecules form both *trans*-interactions between cells, as well as *cis*-interactions between cadherins on the same cell (Harrison et al., 2011; Satoh-Horikawa et al., 2000) (Fig. 3A). E-cadherin clusters in turn pack further and are thought to amplify cell–cell adhesion, suggesting that the initial clustering of cadherin molecules increases adhesive strength (reviewed in Yap et al., 2015). While the exact role of cadherin clustering in static tissues is unclear, recent work has demonstrated that E-cadherin clusters are required for proper elongation of the body axes of *Drosophila* and *Xenopus* embryos (Huebner et al., 2021; Levayer and Lecuit, 2013).

Other transmembrane components of cell–cell junctions also form clusters, including nectins (Indra et al., 2013), JAM-A, claudins and occludins. *Cis*-dimerization of the extracellular domain of JAM-A is required for JAM-A enrichment at cell–cell contacts and for subsequent *trans*-interactions between adjacent cells (Mandell et al., 2004). Similarly, claudins are thought to form *cis* dimers that bind to another dimer on the opposing cell to yield a *trans* tetramer. Subsequently, these tetramers assemble into strands linking the adjacent cells (Hempel et al., 2020) (Fig. 3D).

Another proposed mechanism of cell–cell junction assembly is the generation of condensates by ZO-1 proteins, which *in vitro* and in cells can spontaneously condense into micrometer-sized clusters (Beutel et al., 2019; Schwayer et al., 2019). This condensation is

Journal of Cell Science (2021) 134, jcs248757. doi:10.1242/jcs.248757

driven by multivalent and reversible interactions, which *in vitro* result in a liquid–liquid phase separation (Beutel et al., 2019). It is intriguing to speculate that weak, multivalent interactions such as those exhibited by ZO-1 contribute to the formation of the stratified structures seen in many cell–cell adhesion complexes. Relatedly, a recent review has examined the likelihood of various junctional scaffolding proteins to undergo phase separation based on their protein sequences (Rouaud et al., 2020). Here, in addition to ZO-1, the authors suggest that the TJ adaptor protein cingulin and the apicobasal-specifying proteins Par-3/Baz, Patj and Scrib are all predicted to display phase-separating behavior. In contrast, catenins (α-catenin, β-catenin and p120-catenin), afadin and other apicobasal-specifying proteins such as Par-6, aPKC and Dlg are not predicted to form condensates (Rouaud et al., 2020).

Crosstalk between polarity and cell–cell adhesion

Establishment and maintenance of both apicobasal polarity and cell–cell adhesion are multistep processes that often occur concurrently in epithelial cells. However, the crosstalk between these two systems is arguably underexplored considering its probable biological importance. Below, we discuss some examples where polarity and junctional proteins interact with and influence each other.

Role of polarity components in influencing the assembly of cell–cell adhesion complexes

The Crumbs complex

One of the first identified instances of crosstalk between epithelial polarity proteins and cell–cell adhesion proteins was the requirement of certain polarity proteins for the localization of junctional proteins to cell–cell contacts. For example, expression and apical localization of the apical determinant Crumbs is required for lateral localization of *Drosophila* DE-cadherin to cell–cell contacts and subsequent assembly of AJs in the *Drosophila* embryonic ectoderm (Grawe et al., 1996; Klebes and Knust, 2000; Tepass, 1996). This interplay of Crumbs with cell–cell contacts extends to mammalian cells as well, as Crumbs isoforms (CRB1, CRB2 and CRB3) are essential for the formation of mature TJs (Tilston-Lünel et al., 2016).

The Par complex

The Par complex components also influence AJ and TJ formation. In MDCKs, Par-3 plays a critical role in TJ development, as Par-3-knockdown mutants exhibit delayed TJ formation (Horikoshi et al., 2009). In the *Drosophila* embryonic epithelium, Baz accumulates in small cytoplasmic clusters, and its presence is required to recruit AJ components to lateral domains of cells (Harris and Peifer, 2005; McGill et al., 2009; Müller and Wieschaus, 1996) (Fig. 4A). Additionally, while Baz is not required for the assembly of *Drosophila* DE-cadherin clusters, it affects their size, as depletion of Baz markedly decreases the size of such clusters (Truong Quang et al., 2013). In addition, aPKC also plays a critical role in resolving AJs and TJs at nascent cell–cell contacts in mouse epithelial cells (MTD1-A) (Suzuki et al., 2002). Here, initial contacts between cells are mediated by spot-like junctions that contain Par-3, E-cadherin, ZO-1, JAM-A, claudin-1 and occludin. As the spot contacts mature, the apical junctional complexes develop to contain claudin-1, Par-3 and aPKC (Suzuki et al., 2002). One possibility is that aPKC is recruited to maturing junctions and is involved in TJ maturation, perhaps by phosphorylating JAM-A, which is known to be an essential step in TJ maturation (Iden et al., 2012).

Interactions between Par-3 and afadin appear to be particularly relevant for AJ formation. In MDCK cells assembling cell–cell adhesions, Par-3 is not only necessary for proper AJ and TJ formation, but may also facilitate nectin-based adhesion development at the lateral domain (Ooshio et al., 2010). Indeed, Par-3-knockdown cells retain normal nectin-1 localization to the cell–cell junctions, but do not have colocalized afadin, indicating that Par-3 may be somehow necessary for the formation of afadin–nectin adhesion complexes. Conversely, nectin-1 and Par-3 show undisturbed localization to the

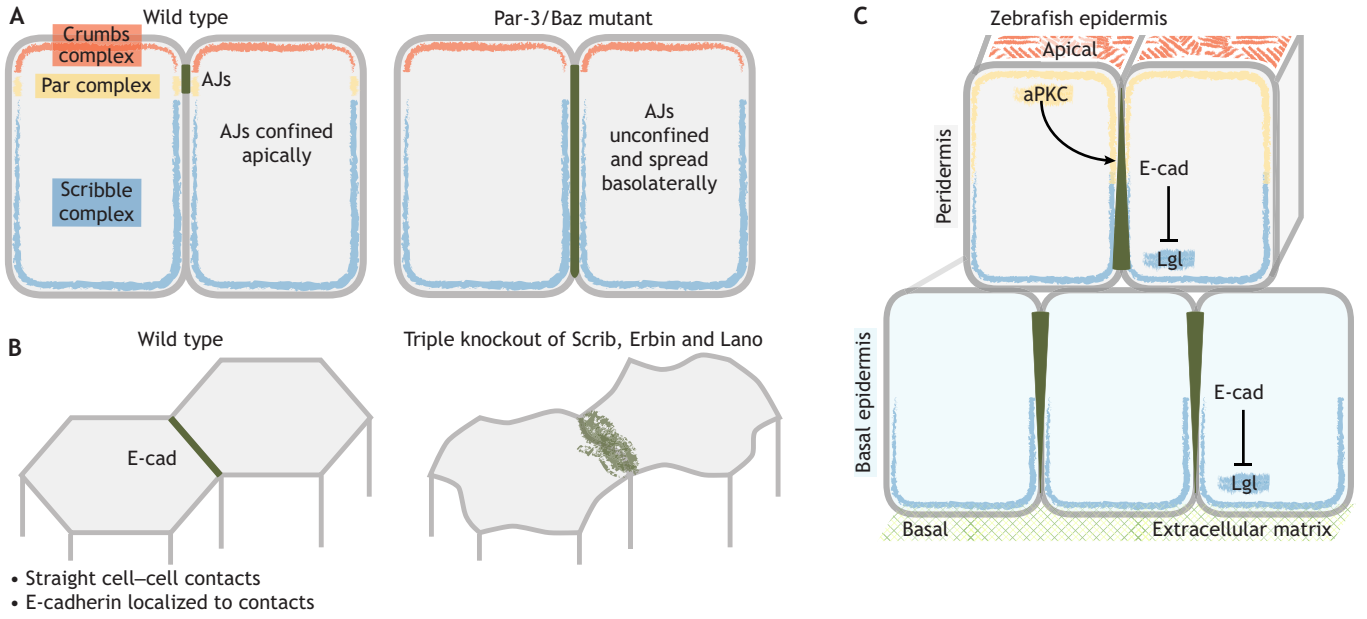

Fig. 4. Examples of crosstalk between apicobasal polarity determinants and cell–cell adhesions. (A) In most early *Drosophila* embryonic tissues, AJ components localize along the apical–lateral membrane (left). In *baz* mutants, AJs are no longer restricted apically and spread basolaterally (right). (B) In wild-type mammalian cells, E-cadherin (E-cad) localizes specifically to cell–cell contacts. In cells lacking Scrib and its homologs (Erbin and Lano), E-cad localization is more dispersed and cell–cell contacts are wavy. (C) In the zebrafish epidermis, a multilayered epithelium, apical localization of aPKC in the peridermis establishes a gradient of E-cad. The gradient of E-cad is required for basal restriction of Lgl.

lateral cell–cell domain in afadin-knockdown cells, indicating that afadin may not be necessary for Par-3 and nectin-1 interactions at cell adhesion sites (Ooshio et al., 2010).

Recent work has found that Shank2, a major scaffolding protein involved in the neuronal postsynaptic density, is implicated in polarity and cell–cell contact crosstalk (Boeckers et al., 1999; Sasaki et al., 2020). In MDCK cells, Shank2 binds to aPKC, and the resulting complex colocalizes to maturing TJs. Once localized, Shank2 dissociates from aPKC and binds to the small GTPase Rap1. The Shank2–Rap1 complex recruits and activates Cdc42, enabling Cdc42 to act in a positive feedback loop and indirectly activate aPKC. Additionally, Shank2–Rap1 complexes can also recruit afadin to TJs, which subsequently recruits ZO-1 (Sasaki et al., 2020). Therefore, additional polarity proteins might be involved in apicobasal polarity and cell–cell junction establishment. Indeed, a recent study has leveraged quantitative proximity proteomics and imaging of MDCK cells to demonstrate that the Crumbs complex defines an apical zone that is molecularly distinct from the apical TJ complexes, and also identified a network of scaffolding proteins, including PALS1, Patj, HOMER1, HOMER2 and HOMER3, that establish the apical–lateral organization (Tan et al., 2020). It will be interesting to see whether further affinity and mass spectrometry-based screening efforts will identify additional components required for apicobasal polarity and cell–cell junction establishment.

The Scribble complex

Basolateral proteins are also required to localize and position junctional adhesion proteins. In the early *Drosophila* embryo, Scrib is not only required for proper localization of junctional proteins, but also for proper relative placement of AJs and septate junctions (an invertebrate analog of TJs) in the developing epithelium (Bilder and Perrimon, 2000; Zeitler et al., 2004). Additionally, both Scrib and Dlg are critical for the assembly of AJ puncta and their retention in the lateral plasma membrane within *Drosophila* embryonic epithelia (Bonello et al., 2019; Bonello and Peifer, 2019). In mammals, Scrib and two other LAP proteins (characterized by leucine-rich repeats and PDZ domains), Erbin and Lano (encoded by *LRRC1* in vertebrates), are functionally redundant, and cells that are deficient in all three have disorganized TJs and AJs, suggesting that the functions of Scrib and its related proteins are indeed conserved and critical for proper localization of junctional complexes (Choi et al., 2019) (Fig. 4B).

In summary, these studies demonstrate that polarity proteins have conserved instructive roles in positioning cell–cell adhesion complexes in epithelia. While the linkage between polarity signaling and adhesion formation have been explored at the genetic level, it remains unclear what determines the binding of one partner to another. For example, what determines whether Par-3 binds to its complex partners (aPKC and Par-6) or TJ components such as JAM-A and nectins? Some of these decisions might be determined by post-translational modifications, with phosphorylation being only one of several possibilities.

Role of junction components in influencing polarity

ZO-1 and ZO-2

Junctional proteins also have a role in regulating epithelial polarity. In MDCK cells and mouse epithelial cells (MTD1-A and EpH4) where individual ZO proteins are depleted, epithelial polarity is maintained (Fanning et al., 2012; Ikenouchi et al., 2012; Umeda et al., 2006). However, MDCK epithelial cells that do not express any ZO-1 or ZO-2 (also known as TJP2) display severe apicobasal polarity defects including, but not limited to, mislocalization of Par-3 and aPKC, in addition to the lack of proper AJs or TJs (Otani et al., 2019). Interestingly, MDCK cells do not require the TJ barrier function to establish polarity, as cells absent of the five most highly expressed claudins still polarize (Otani et al., 2019).

JAM-A, nectin and afadin

JAM-A and nectin-1 are also involved in the crosstalk between polarity and junctional protein complex networks. The cytoplasmic domain of JAM-A binds to Par-3 (Ebnet et al., 2001; Itoh et al., 2001). JAM-A localizes to nascent cell–cell junctions prior to the localization of Par-3 or aPKC (Suzuki et al., 2002), and JAM-A-mediated recruitment of the Par–aPKC complex is required for the subsequent maturation of nascent cell–cell junctions into mature AJs and TJs (Iden et al., 2012). Once localized to the nascent junctions, phosphorylation of JAM-A by aPKC is necessary for TJ maturation (Iden et al., 2012). Experiments using L cells, mouse fibroblasts that express low levels of cell–cell adhesion proteins, have shown that exogenous expression of nectin-1 and JAM-A is sufficient to instruct polarization and segregation of AJs and TJs along the lateral cortex (Yamada et al., 2013). Furthermore, in these L cells, polarization of AJs and TJs (i.e. TJs apical to AJs) depends on the expression of ZO-1 and afadin, as well as the presence of the C-terminal cytoplasmic domains of nectin-1 and JAM-A (Yamada et al., 2013).

Although not required for establishment of apicobasal polarization, afadin and JAM-A are required for planar cell division in epithelial tissues, which is essential for maintenance of a single polarization axis in the tissue (Gao et al., 2017; Tuncay et al., 2015). In kidney renal tubules, absence of afadin results in misorientation of cell division and abnormal tubule morphogenesis (Gao et al., 2017). In MDCK cells, JAM-A also plays an instructive role in orienting cell division in the plane of the epithelium (Tuncay et al., 2015).

E-cadherin

Loss of maternally provided E-cadherin in *Drosophila* embryos results not only in defects in cell–cell adhesion, but also in loss of polarity (Cox et al., 1996). Another example of reciprocal feedback between polarity and cell adhesion proteins occurs in the zebrafish epidermis, a multilayered epithelium. In this system, E-cadherin localization is polarized along lateral cell–cell contacts. In the periderm, the outermost facing layer of the epidermis, E-cadherin is enriched basally to apically, while in the basal epidermis, the layer just basal to the periderm, E-cadherin is enriched apically to basally (Arora et al., 2020) (Fig. 4C). The establishment and maintenance of these E-cadherin gradients depend on the localization of aPKC to the apical domain of the periderm. Furthermore, E-cadherin itself is required to restrict aPKC localization and controls the levels of the basolateral protein Lgl (also known in zebrafish as Llgl1) (Arora et al., 2020). The identities of the molecular intermediaries involved in the regulation of E-cadherin localization by aPKC, and vice versa, remain unknown.

It is striking that our review of the literature has uncovered only a few examples of how junctional adhesion complexes regulate cell polarity. One possible explanation is that, in general, polarity complexes act upstream of adhesion assembly. Nevertheless, it is plausible that the relative paucity of results in this area instead suggests the presence of important gaps in our knowledge of how epithelial tissues are constructed.

Future perspectives

Although it is clear that junctional and polarity complexes exhibit causal, functional relationships, a full understanding of how their

individual functions are coordinated remains elusive. Below, we outline several general questions regarding how an organized cell–cell junction arises from its molecular parts, and we suggest possible experimental strategies for addressing these questions.

How are polarity and adhesion complexes organized at the submicrometer scale?

Numerous studies suggest the presence of intricate spatial relationships within and between the protein complexes that make up adhesion and polarity complexes (Fanning et al., 1998; Mangeol et al., 2019, preprint; Tan et al., 2020). With the rapid advancement of EM and image analysis techniques, we are now on the cusp of visualizing the ultrastructure of cell–cell contacts with molecular resolution (Chakraborty et al., 2020; Heinrich et al., 2021; Hoffman et al., 2020; Müller et al., 2021). In this regard, EM, which is not limited by the number of fluorescent tags, can provide an unbiased window into the cell and the 'molecular sociology' of its processes (Mahamid et al., 2016). Coupling EM with substrate micropatterning (Engel et al., 2021) and reconstitution techniques to guide cells to form specific interfaces will further aid in visualizing the spatial layout of cell–cell contacts at molecular resolution.

How do the protein–protein interactions within polarized junctions evolve in space and time?

Advances in proximity-labeling techniques and mass spectrometry (MS) have enabled what is probably a near-complete enumeration of a generic polarity and junctional protein interaction network (Daulat et al., 2018; Pires and Boxem, 2017). However, thus far, MS-based strategies have been used primarily to achieve a static view of the protein–protein interaction networks at cell–cell junctions. Going forward, time-resolved proximity labeling and MS techniques, such as TurboID and APEX, provide a potentially powerful means of establishing how these interaction networks change as a function of cell state (Cho et al., 2020a,b; Nguyen et al., 2020). Furthermore, advances in structural MS techniques, such as *in vivo* crosslinking-MS, limited proteolysis-MS and hydrogen exchange-MS, may soon provide insight into changes in protein conformation and interactions as junctions mature or remodel (Branon et al., 2018; Cho et al., 2020a). A critical challenge will be to determine how such interaction networks can be used to infer the mechanisms that underlie specific subcellular processes, for example the induction of polarity or the segregation of TJ and AJ components.

How do cell–cell junctions build themselves?

Insight into the molecular-level architecture of junctional complexes does not necessarily translate into a mechanistic understanding of how the micrometer-scale organization that typifies cell–cell junctions arises. The increasing accessibility of three-dimensional live-cell imaging (e.g. light-sheet microscopes) provides a potentially powerful means of visualizing the mechanisms of assembly of junctional and polarity complexes, and, importantly, their interrelations in space and time (Kono et al., 2019; Mangeol et al., 2019 preprint). Continued improvements in microscopy techniques may be particularly helpful in addressing the role of biomolecular condensates in establishing cell–cell junctions and cell polarity. Many junctional and polarity proteins have been suggested to have the capacity to phase separate, although this has been explored thus far for only a few junctional proteins, notably ZO-1 and Par-3 (Beutel et al., 2019; Liu et al., 2020; Schwayer et al., 2019). The possible presence and function of molecular condensates and related, higher-order assemblies (Korkmazhan et al., 2021) at cell–cell junctions is thus an attractive target for future investigations.

When will we know enough?

Given the considerable complexity of the systems discussed in this Review, it is reasonable to wonder whether truly predictive mechanistic models are even possible. Reconstitution assays are unique in their ability to determine whether a given set of molecular components is sufficient to recapitulate a given biological phenomenon, and thus provide a powerful means of testing a proposed model. In cell adhesion biology, *in vitro* reconstitutions have for the most part been limited to individual components, for example the cadherin ectodomains (Harrison et al., 2011) or, more recently, claudin-4 (Belardi et al., 2018). For polarity proteins, experiments using liposomes or immobilized lipids have revealed that Baz utilizes its PDZ domains to directly bind to and interact with phosphatidic acid (Yu and Harris, 2012). It is likely that more complex reconstitution strategies, for example supported lipid bilayer systems, as used to probe signaling at the immune synapse (Huang et al., 2019), may prove useful in testing the sufficiency of extant mechanistic models. In this regard, it is telling that the spontaneous polarization of the apical and basal polarity complexes has not, to the authors' knowledge, been recapitulated in an *in vitro* setting. 'Minimal cell' models provide an alternate means of building up complexity. For example, expression of Par-3, Par-6 and aPKC in otherwise apolar *Drosophila* S2 cells has been used to demonstrate that these three factors, along with endogenous Lgl, can recapitulate polarization (Kono et al., 2019). Finally, semi-reconstituted systems can, in some cases, capture the best of both worlds. In one noteworthy example, isolated hepatocytes that were seeded on E-cadherin-functionalized supported lipid bilayers polarized and formed hemi-lumens facing the glass surface, providing powerful evidence that, in this system, polarization is cell intrinsic (Zhang et al., 2020). As these examples illustrate, bottom-up strategies retain the capacity to surprise and illuminate. Looking forward, we are hopeful that these and other strategies will ultimately enable the elucidation of the molecular mechanisms by which cells, tissues and animals arise from their molecular parts.

Acknowledgements

We thank Dunn lab members Dr Christina Hueschen, Mr Elgin Korkmazhan and Mr Vipul Vachharajani for their feedback on the initial versions of this manuscript. We also thank the three referees for their careful reviews, which greatly contributed to improving this Review.

Competing interests

The authors declare no competing or financial interests.

Funding

Our work in this area is supported by the National Institutes of Health (R35-GM130332 to A.R.D.), a Howard Hughes Medical Institute Faculty Scholar Award (to A.R.D.), the National Science Foundation Graduate Research Fellowship Program under grant DGE-1656518 (to E.L.d.l.S.), and the Stanford University EDGE Fellowship Program (to E.L.d.l.S.). Deposited in PMC for release after 12 months.

References

Abedin, M. and King, N. (2008). The premetazoan ancestry of cadherins. *Science* **319**, 946-948. doi:10.1126/science.1151084

Arora, P., Dongre, S., Raman, R. and Sonawane, M. (2020). Stepwise polarisation of developing bilayered epidermis is mediated by aPKC and E-cadherin in zebrafish. *Elife* **9**, e49064. doi:10.7554/eLife.49064

Atwood, S. X., Chabu, C., Penkert, R. R., Doe, C. Q. and Prehoda, K. E. (2007). Cdc42 acts downstream of Bazooka to regulate neuroblast polarity through Par-6–aPKC. *J. Cell Sci.* **120**, 3200-3206. doi:10.1242/jcs.014902

Bachmann, A., Draga, M., Grawe, F. and Knust, E. (2008). On the role of the MAGUK proteins encoded by Drosophila varicose during embryonic and postembryonic development. *BMC Dev. Biol.* **8**, 55. doi:10.1186/1471-213X-8-55

Bachmann, A., Timmer, M., Sierralta, J., Pietrini, G., Gundelfinger, E. D., Knust, E. and Thomas, U. (2004). Cell type-specific recruitment of Drosophila Lin-7 to distinct MAGUK-based protein complexes defines novel roles for Sdt and Dlg-S97. *J. Cell Sci.* **117**, 1899-1909.

Belardi, B., Son, S., Vahey, M. D., Wang, J., Hou, J. and Fletcher, D. A. (2018). Claudin-4 reconstituted in unilamellar vesicles is sufficient to form tight interfaces that partition membrane proteins. *J. Cell Sci.* **132**, jcs221556. doi:10.1101/309856

Belardi, B., Hamkins-Indik, T., Harris, A. R., Kim, J., Xu, K. and Fletcher, D. A. (2020). A weak link with actin organizes tight junctions to control epithelial permeability. *Dev. Cell* **54**, 792-804.e7. doi:10.1016/j.devcel.2020.07.022

Benton, R. and St Johnston, D. (2003). Drosophila PAR-1 and 14-3-3 inhibit Bazooka/PAR-3 to establish complementary cortical domains in polarized cells. *Cell* **115**, 691-704. doi:10.1016/S0092-8674(03)00938-3

Beutel, O., Maraspini, R., Pombo-Garcia, K., Martin-Lemaitre, C. and Honigmann, A. (2019). Phase separation of zonula occludens proteins drives formation of tight junctions. *Cell* **179**, 923-936. doi:10.1016/j.cell.2019.10.011

Bilder, D. and Perrimon, N. (2000). Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* **403**, 676-680. doi:10.1038/35001108

Bilder, D., Li, M. and Perrimon, N. (2000). Cooperative regulation of cell polarity and growth by drosophila tumor suppressors. *Science* **289**, 113-116. doi:10.1126/science.289.5476.113

Boeckers, T. M., Kreutz, M. R., Winter, C., Zuschratter, W., Smalla, K. H., Sanmarti-Vila, L., Wex, H., Langnaese, K., Bockmann, J., Garner, C. C. et al. (1999). Proline-rich synapse-associated protein-1/cortactin binding protein 1 (ProSAP1/CortBP1) is a PDZ-domain protein highly enriched in the postsynaptic density. *J. Neurosci.* **19**, 6506-6518. doi:10.1523/JNEUROSCI.19-15-06506.1999

Bonello, T. T. and Peifer, M. (2019). Scribble: a master scaffold in polarity, adhesion, synaptogenesis, and proliferation. *J. Cell Biol.* **218**, 742-756. doi:10.1083/jcb.201810103

Bonello, T. T., Choi, W. and Peifer, M. (2019). Scribble and Discs-large direct initial assembly and positioning of adherens junctions during the establishment of apical-basal polarity. *Development* **146**, dev180976. doi:10.1242/dev.180976

Branon, T. C., Bosch, J. A., Sanchez, A. D., Udeshi, N. D., Svinkina, T., Carr, S. A., Feldman, J. L., Perrimon, N. and Ting, A. Y. (2018). Efficient proximity labeling in living cells and organisms with TurboID. *Nat. Biotechnol.* **36**, 880-887. doi:10.1038/nbt.4201

Broussard, J. A., Jaiganesh, A., Zarkoob, H., Conway, D. E., Dunn, A. R., Espinosa, H. D., Janmey, P. A. and Green, K. J. (2020). Scaling up single-cell mechanics to multicellular tissues - the role of the intermediate filament-desmosome network. *J. Cell Sci.* **133**, jcs228031. doi:10.1242/jcs.228031

Brunet, T., Larson, B., Linden, T., Vermeij, M., McDonald, K. and King, N. (2019). Light-regulated collective contractility in a multicellular choanoflagellate. *Science* **366**, 326-334. doi:10.1126/science.aay2346

Buckley, C. D., Tan, J., Anderson, K. L., Hanein, D., Volkmann, N., Weis, W. I., Nelson, W. J. and Dunn, A. R. (2014). Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force. *Science* **346**, 1254211. doi:10.1126/science.1254211

Bulgakova, N. A. and Knust, E. (2009). The Crumbs complex: from epithelial-cell polarity to retinal degeneration. *J. Cell Sci.* **122**, 2587-2596. doi:10.1242/jcs.023648

Bulgakova, N. A., Kempkens, O. and Knust, E. (2008). Multiple domains of Stardust differentially mediate localisation of the Crumbs-Stardust complex during photoreceptor development in Drosophila. *J. Cell Sci.* **121**, 2018-2026. doi:10.1242/jcs.031088

Butterfield, N. J. (2009). Modes of pre-Ediacaran multicellularity. *Precambrian Res.* **173**, 201-211. doi:10.1016/j.precamres.2009.01.008

Campanale, J. P., Sun, T. Y. and Montell, D. J. (2017). Development and dynamics of cell polarity at a glance. *J. Cell Sci.* **130**, 1201-1207. doi:10.1242/jcs.188599

Cao, H., Xu, R., Shi, Q., Zhang, D., Huang, J. and Hong, Y. (2017). FERM domain phosphorylation and endogenous 3'UTR are not essential for regulating the function and subcellular localization of polarity protein Crumbs. *J. Genet. Genomics* **44**, 409-412. doi:10.1016/j.jgg.2017.08.002

Chakraborty, S., Jasnin, M. and Baumeister, W. (2020). Three–dimensional organization of the cytoskeleton: A cryo–electron tomography perspective. *Protein Sci.* **29**, 1302-1320. doi:10.1002/pro.3858

Chen, X., Levy, J. M., Hou, A., Winters, C., Azzam, R., Sousa, A. A., Leapman, R. D., Nicoll, R. A. and Reese, T. S. (2015). PSD-95 family MAGUKs are essential for anchoring AMPA and NMDA receptor complexes at the postsynaptic density. *Proc. Natl. Acad. Sci. USA* **112**, E6983-E6992. doi:10.1073/pnas.1517045112

Chen, J., Sayadian, A.-C., Lowe, N., Lovegrove, H. E. and Johnston, D. S. (2018). An alternative mode of epithelial polarity in the Drosophila midgut. *PLoS Biol.* **16**, e3000041. doi:10.1371/journal.pbio.3000041

Cho, K. O., Hunt, C. A. and Kennedy, M. B. (1992). The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. *Neuron* **9**, 929-942. doi:10.1016/0896-6273(92)90245-9

Cho, K. F., Branon, T. C., Rajeev, S., Svinkina, T., Udeshi, N. D., Thoudam, T., Kwak, C., Rhee, H. W., Lee, I. K., Carr, S. A. et al. (2020a). Split-TurboID enables contact-dependent proximity labeling in cells. *Proc. Natl. Acad. Sci. USA* **117**, 12143-12154. doi:10.1073/pnas.1919528117

Cho, K. F., Branon, T. C., Udeshi, N. D., Myers, S. A., Carr, S. A. and Ting, A. Y. (2020b). Proximity labeling in mammalian cells with TurboID and split-TurboID. *Nat. Protoc.* **15**, 3971-3999. doi:10.1038/s41596-020-0399-0

Choi, J., Troyanovsky, R. B., Indra, I., Mitchell, B. J. and Troyanovsky, S. M. (2019). Scribble, Erbin, and Lano redundantly regulate epithelial polarity and apical adhesion complexScribble, Erbin, and Lano in cell polarity. *J. Cell Biol.* **218**, 2277-2293. doi:10.1083/jcb.201804201

Cox, R. T., Kirkpatrick, C. and Peifer, M. (1996). Armadillo is required for adherens junction assembly, cell polarity, and morphogenesis during Drosophila embryogenesis. *J. Cell Biol.* **134**, 133-148. doi:10.1083/jcb.134.1.133

Das, S. and Knust, E. (2018). A dual role of the extracellular domain of Drosophila Crumbs for morphogenesis of the embryonic neuroectoderm. *Biol Open* **7**, bio031435. doi:10.1242/bio.031435

Daulat, A. M., Puvirajesinghe, T. M., Camoin, L. and Borg, J. P. (2018). Mapping cellular polarity networks using mass spectrometry-based strategies. *J. Mol. Biol.* **430**, 3545-3564. doi:10.1016/j.jmb.2018.05.023

Dayel, M. J., Alegado, R. A., Fairclough, S. R., Levin, T. C., Nichols, S. A., McDonald, K. and King, N. (2011). Cell differentiation and morphogenesis in the colony-forming choanoflagellate Salpingoeca rosetta. *Dev. Biol.* **357**, 73-82. doi:10.1016/j.ydbio.2011.06.003

Ebnet, K., Suzuki, A., Horikoshi, Y., Hirose, T., Meyer Zu Brickwedde, M. K., Ohno, S. and Vestweber, D. (2001). The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM). *EMBO J.* **20**, 3738-3748. doi:10.1093/emboj/20.14.3738

Engel, L., Vasquez, C. G., Montabana, E. A., Sow, B. M., Walkiewicz, M. P., Weis, W. I. and Dunn, A. R. (2021). Lattice micropatterning for cryo-electron tomography studies of cell-cell contacts. *J. Struct. Biol.* **213**, 107791. doi:10.1016/j.jsb.2021.107791

Etienne-Manneville, S. and Hall, A. (2001). Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell* **106**, 489-498. doi:10.1016/S0092-8674(01)00471-8

Fahey, B. and Degan, B. M. (2010). Origin of animal epithelia: insights from the sponge genome. *Evol. Dev.* **12**, 601-617. doi:10.1111/j.1525-142X.2010.00445.x

Fan, S., Fogg, V., Wang, Q., Chen, X. W., Liu, C. J. and Margolis, B. (2007). A novel Crumbs3 isoform regulates cell division and ciliogenesis via importin beta interactions. *J. Cell Biol.* **178**, 387-398. doi:10.1083/jcb.200609096

Fanning, A. S., Jameson, B. J., Jesaitis, L. A. and Anderson, J. M. (1998). The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J. Biol. Chem.* **273**, 29745-29753. doi:10.1074/jbc.273.45.29745

Fanning, A. S., Van Itallie, C. M. and Anderson, J. M. (2012). Zonula occludens-1 and -2 regulate apical cell structure and the zonula adherens cytoskeleton in polarized epithelia. *Mol. Biol. Cell* **23**, 577-590. doi:10.1091/mbc.e11-09-0791

Farquhar, M. and Palade, G. (1963). Junctional complexes in various epithelia. *J. Cell Biol.* **17**, 375-412. doi:10.1083/jcb.17.2.375

Flores-Benitez, D. and Knust, E. (2016). Dynamics of epithelial cell polarity in Drosophila: how to regulate the regulators? *Curr. Opin. Cell Biol.* **42**, 13-21. doi:10.1016/j.ceb.2016.03.018

Fukuhara, A., Irie, K., Nakanishi, H., Takekuni, K., Kawakatsu, T., Ikeda, W., Yamada, A., Katata, T., Honda, T., Sato, T. et al. (2002). Involvement of nectin in the localization of junctional adhesion molecule at tight junctions. *Oncogene* **21**, 7642-7655. doi:10.1038/sj.onc.1205875

Gao, Y., Lui, W. Y., Lee, W. M. and Cheng, C. Y. (2016). Polarity protein Crumbs homolog-3 (CRB3) regulates ectoplasmic specialization dynamics through its action on F-actin organization in Sertoli cells. *Sci. Rep.* **6**, 28589. doi:10.1038/srep28589

Gao, L., Yang, Z., Hiremath, C., Zimmerman, S. E., Long, B., Brakeman, P. R., Mostov, K. E., Bryant, D. M., Luby-Phelps, K. and Marciano, D. K. (2017). Developing renal tubules orient cell division via Afadin to position the tubule lumen. *Development* **144**, 3511-3520.

Goodenough, D. A. and Paul, D. L. (2009). Gap junctions. *Cold Spring Harb Perspect Biol* **1**, a002576. doi:10.1101/cshperspect.a002576

Grawe, F., Wodarz, A., Lee, B., Knust, E. and Skaer, H. (1996). The Drosophila genes crumbs and stardust are involved in the biogenesis of adherens junctions. *Development* **122**, 951-959. doi:10.1242/dev.122.3.951

Graybill, C., Wee, B., Atwood, S. X. and Prehoda, K. E. (2012). Partitioning-defective protein 6 (Par-6) activates atypical protein kinase C (aPKC) by pseudosubstrate displacement. *J. Biol. Chem.* **287**, 21003-21011. doi:10.1074/jbc.M112.360495

Harris, T. J. and Peifer, M. (2005). The positioning and segregation of apical cues during epithelial polarity establishment in Drosophila. *J. Cell Biol.* **170**, 813-823. doi:10.1083/jcb.200505127

Harrison, O. J., Jin, X., Hong, S., Bahna, F., Ahlsen, G., Brasch, J., Wu, Y., Vendome, J., Felsovalyi, K., Hampton, C. M. et al. (2011). The extracellular architecture of adherens junctions revealed by crystal structures of type I cadherins. *Structure* **19**, 244-256. doi:10.1016/j.str.2010.11.016

Heinrich, L., Bennett, D., Ackerman, D., Park, W., Bogovic, J., Eckstein, N., Petruncio, A., Clements, J., Pang, S., Xu, C. S. et al. (2021). Whole-cell organelle segmentation volume electron microscopy. *Nature* [Epub]. doi:10.1038/s41586-021-03977-3

Hempel, C., Protze, J., Altun, E., Riebe, B., Piontek, A., Fromm, A., Lee, I. M., Saleh, T., Günzel, D., Krause, G. et al. (2020). Assembly of tight junction strands: Claudin-10b and Claudin-3 form homo-tetrameric building blocks that polymerise in a channel-independent manner. *J. Mol. Biol.* **432**, 2405-2427. doi:10.1016/j.jmb.2020.02.034

Hoffman, D. P., Shtengel, G., Xu, C. S., Campbell, K. R., Freeman, M., Wang, L., Milkie, D. E., Pasolli, H. A., Iyer, N., Bogovic, J. A. et al. (2020). Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells. *Science* **367**, eaaz5357. doi:10.1126/science.aaz5357

Honda, T., Shimizu, K., Kawakatsu, T., Yasumi, M., Shingai, T., Fukuhara, A., Ozaki-Kuroda, K., Irie, K., Nakanishi, H. and Takai, Y. (2003). Antagonistic and agonistic effects of an extracellular fragment of nectin on formation of E-cadherin-based cell-cell adhesion. *Genes Cells* **8**, 51-63. doi:10.1046/j.1365-2443.2003.00616.x

Hong, Y., Ackerman, L., Jan, L. Y. and Jan, Y. N. (2003). Distinct roles of Bazooka and Stardust in the specification of Drosophila photoreceptor membrane architecture. *Proc. Natl. Acad. Sci. USA* **100**, 12712-12717. doi:10.1073/pnas.2135347100

Horikoshi, Y., Suzuki, A., Yamanaka, T., Sasaki, K., Mizuno, K., Sawada, H., Yonemura, S. and Ohno, S. (2009). Interaction between PAR-3 and the aPKC-PAR-6 complex is indispensable for apical domain development of epithelial cells. *J. Cell Sci.* **122**, 1595-1606. doi:10.1242/jcs.043174

Huang, D. L., Bax, N. A., Buckley, C. D., Weis, W. I. and Dunn, A. R. (2017). Vinculin forms a directionally asymmetric catch bond with F-actin. *Science* **357**, 703-706. doi:10.1126/science.aan2556

Huang, W. Y. C., Alvarez, S., Kondo, Y., Lee, Y. K., Chung, J. K., Lam, H. Y. M., Biswas, K. H., Kuriyan, J. and Groves, J. T. (2019). A molecular assembly phase transition and kinetic proofreading modulate Ras activation by SOS. *Science* **363**, 1098-1103. doi:10.1126/science.aau5721

Huebner, R. J., Malmi-Kakkada, A. N., Sarıkaya, S., Weng, S., Thirumalai, D. and Wallingford, J. B. (2021). Mechanical heterogeneity along single cell-cell junctions is driven by lateral clustering of cadherins during vertebrate axis elongation. *Elife* **10**, e65390. doi:10.7554/eLife.65390.sa2

Hurov, J. B., Watkins, J. L. and Piwnica-Worms, H. (2004). Atypical PKC phosphorylates PAR-1 kinases to regulate localization and activity. *Curr. Biol.* **14**, 736-741. doi:10.1016/j.cub.2004.04.007

Hutterer, A., Betschinger, J., Petronczki, M. and Knoblich, J. A. (2004). Sequential roles of Cdc42, Par-6, aPKC, and Lgl in the establishment of epithelial polarity during Drosophila embryogenesis. *Dev. Cell* **6**, 845-854. doi:10.1016/j.devcel.2004.05.003

Iden, S., Misselwitz, S., Peddibhotla, S. S., Tuncay, H., Rehder, D., Gerke, V., Robenek, H., Suzuki, A. and Ebnet, K. (2012). aPKC phosphorylates JAM-A at Ser285 to promote cell contact maturation and tight junction formation. *J. Cell Biol.* **196**, 623-639. doi:10.1083/jcb.201104143

Ikenouchi, J., Umeda, K., Tsukita, S. and Furuse, M. (2007). Requirement of ZO-1 for the formation of belt-like adherens junctions during epithelial cell polarization. *J. Cell Biol.* **176**, 779-786. doi:10.1083/jcb.200612080

Ikenouchi, J., Suzuki, M., Umeda, K., Ikeda, K., Taguchi, R., Kobayashi, T., Sato, S. B., Stolz, D. B. and Umeda, M. (2012). Lipid polarity is maintained in absence of tight junctions. *J. Biol. Chem.* **287**, 9525-9533. doi:10.1074/jbc.M111.327064

Indra, I., Hong, S., Troyanovsky, R., Kormos, B. and Troyanovsky, S. (2013). The adherens junction: a mosaic of cadherin and nectin clusters bundled by actin filaments. *J. Invest. Dermatol.* **133**, 2546-2554. doi:10.1038/jid.2013.200

Itoh, M., Sasaki, H., Furuse, M., Ozaki, H., Kita, T. and Tsukita, S. (2001). Junctional adhesion molecule (JAM) binds to PAR-3: a possible mechanism for the recruitment of PAR-3 to tight junctions. *J. Cell Biol.* **154**, 491-497. doi:10.1083/jcb.200103047

Khoury, M. J. and Bilder, D. (2020). Distinct activities of Scrib module proteins organize epithelial polarity. *Proc. Natl. Acad. Sci. USA* **430**, 201918462.

Klebes, A. and Knust, E. (2000). A conserved motif in Crumbs is required for E-cadherin localisation and zonula adherens formation in Drosophila. *Curr. Biol.* **10**, 76-85. doi:10.1016/S0960-9822(99)00277-8

Kono, K., Yoshiura, S., Fujita, I., Okada, Y., Shitamukai, A., Shibata, T. and Matsuzaki, F. (2019). Reconstruction of Par-dependent polarity in apolar cells reveals a dynamic process of cortical polarization. *Elife* **8**, e45559. doi:10.7554/eLife.45559.039

Korkmazhan, E., Tompa, P. and Dunn, A. R. (2021). The role of ordered cooperative assembly in biomolecular condensates. *Nat. Rev. Mol. Cell Biol.* **22**, 647-648. doi:10.1038/s41580-021-00408-z

Kowalczyk, A. P. and Green, K. J. (2013). Structure, function, and regulation of desmosomes. *Prog. Mol. Biol. Transl. Sci.* **116**, 95-118. doi:10.1016/B978-0-12-394311-8.00005-4

Krahn, M. P., Bückers, J., Kastrup, L. and Wodarz, A. (2010). Formation of a Bazooka–Stardust complex is essential for plasma membrane polarity in epithelia. *J. Cell Biol.* **190**, 751-760. doi:10.1083/jcb.201006029

Kuchinke, U., Grawe, F. and Knust, E. (1998). Control of spindle orientation in Drosophila by the Par-3-related PDZ-domain protein Bazooka. *Curr. Biol.* **8**, 1357-1365. doi:10.1016/S0960-9822(98)00016-5

Lane, N. J. and Skaer, H. I. (1980). Intercellular junctions in insect tissues. In *Adv Insect Phys*, Vol. 15 (ed. M. J. Berridge, J. E. Treherne and V. B. Wigglesworth), pp. 35-213: Academic Press.

Letizia, A., Ricardo, S., Moussian, B., Martín, N. and Llimargas, M. (2013). A functional role of the extracellular domain of Crumbs in cell architecture and apicobasal polarity. *J. Cell Sci.* **126**, 2157-2163.

Levayer, R. and Lecuit, T. (2013). Oscillation and polarity of E-cadherin asymmetries control actomyosin flow patterns during morphogenesis. *Dev. Cell* **26**, 162-175. doi:10.1016/j.devcel.2013.06.020

Liu, Z., Yang, Y., Gu, A., Xu, J., Mao, Y., Lu, H., Hu, W., Lei, Q.-Y., Li, Z., Zhang, M. et al. (2020). Par complex cluster formation mediated by phase separation. *Nat. Commun.* **11**, 1-18.

Mahamid, J., Pfeffer, S., Schaffer, M., Villa, E., Danev, R., Cuellar, L. K., Förster, F., Hyman, A. A., Plitzko, J. M. and Baumeister, W. (2016). Visualizing the molecular sociology at the HeLa cell nuclear periphery. *Science* **351**, 969-972. doi:10.1126/science.aad8857

Mandai, K., Nakanishi, H., Satoh, A., Obaishi, H., Wada, M., Nishioka, H., Itoh, M., Mizoguchi, A., Aoki, T., Fujimoto, T. et al. (1997). Afadin: A novel actin filament-binding protein with one PDZ domain localized at cadherin-based cell-to-cell adherens junction. *J. Cell Biol.* **139**, 517-528. doi:10.1083/jcb.139.2.517

Mandai, K., Rikitake, Y., Mori, M. and Takai, Y. (2015). Nectins and nectin-like molecules in development and disease. *Curr. Top. Dev. Biol.* **112**, 197-231. doi:10.1016/bs.ctdb.2014.11.019

Mandel, L. J., Bacallao, R. and Zampighi, G. (1993). Uncoupling of the molecular 'fence' and paracellular 'gate' functions in epithelial tight junctions. *Nature* **361**, 552-555. doi:10.1038/361552a0

Mandell, K. J., McCall, I. C. and Parkos, C. A. (2004). Involvement of the junctional adhesion molecule-1 (JAM1) homodimer interface in regulation of epithelial barrier function. *J. Biol. Chem.* **279**, 16254-16262. doi:10.1074/jbc.M309483200

Mangeol, P., Massey-Harroche, D., Bivic, A. L. and Lenne, P.-F. (2019). Nectins rather than E-cadherin anchor the actin belts at cell-cell junctions of epithelia. *bioRxiv*, 809343. doi:10.1101/809343

McGill, M. A., McKinley, R. F. and Harris, T. J. (2009). Independent cadherin-catenin and Bazooka clusters interact to assemble adherens junctions. *J. Cell Biol.* **185**, 787-796. doi:10.1083/jcb.200812146

Morais-de-Sá, E., Mirouse, V. and St Johnston, D. (2010). aPKC phosphorylation of bazooka defines the apical/lateral border in Drosophila epithelial cells. *Cell* **141**, 509-523. doi:10.1016/j.cell.2010.02.040

Müller, H. A. and Wieschaus, E. (1996). armadillo, bazooka, and stardust are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in Drosophila. *J. Cell Biol.* **134**, 149-163. doi:10.1083/jcb.134.1.149

Müller, A., Schmidt, D., Xu, C. S., Pang, S., D'Costa, J. V., Kretschmar, S., Münster, C., Kurth, T., Jug, F., Weigert, M. et al. (2021). 3D FIB-SEM reconstruction of microtubule-organelle interaction in whole primary mouse β cells. *J. Cell Biol.* **220**, e202010039. doi:10.1083/jcb.202010039

Murray, P. S. and Zaidel-Bar, R. (2014). Pre-metazoan origins and evolution of the cadherin adhesome. *Biol. Open* **3**, 1183-1195. doi:10.1242/bio.20149761

Nair, D., Hosy, E., Petersen, J. D., Constals, A., Giannone, G., Choquet, D. and Sibarita, J. B. (2013). Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95. *J. Neurosci.* **33**, 13204-13224. doi:10.1523/JNEUROSCI.2381-12.2013

Nam, S.-C. and Choi, K.-W. (2003). Interaction of Par-6 and Crumbs complexes is essential for photoreceptor morphogenesis in *Drosophila*. *Development* **130**, 4363-4372. doi:10.1242/dev.00648

Nance, J., Munro, E. M. and Priess, J. R. (2003). *C. elegans* PAR-3 and PAR-6 are required for apicobasal asymmetries associated with cell adhesion and gastrulation. *Development* **130**, 5339-5350. doi:10.1242/dev.00735

Nguyen, T. M. T., Kim, J., Doan, T. T., Lee, M. W. and Lee, M. (2020). APEX proximity labeling as a versatile tool for biological research. *Biochemistry* **59**, 260-269. doi:10.1021/acs.biochem.9b00791

Noirot-Timothée, C., Smith, D. S., Cayer, M. L. and Noirot, C. (1978). Septate junctions in insects: comparison between intercellular and intramembranous structures. *Tissue Cell* **10**, 125-136. doi:10.1016/0040-8166(78)90011-3

Ooshio, T., Kobayashi, R., Ikeda, W., Miyata, M., Fukumoto, Y., Matsuzawa, N., Ogita, H. and Takai, Y. (2010). Involvement of the interaction of afadin with ZO-1 in the formation of tight junctions in Madin-Darby canine kidney cells. *J. Biol. Chem.* **285**, 5003-5012. doi:10.1074/jbc.M109.043760

Otani, T., Nguyen, T. P., Tokuda, S., Sugihara, K., Sugawara, T., Furuse, K., Miura, T., Ebnet, K. and Furuse, M. (2019). Claudins and JAM-A coordinately regulate tight junction formation and epithelial polarity. *J. Cell Biol.* **218**, 3372-3396. doi:10.1083/jcb.201812157

Pickett, M. A., Naturale, V. F. and Feldman, J. L. (2019). A polarizing issue: diversity in the mechanisms underlying Apico-basolateral polarization In Vivo. *Annu. Rev. Cell Dev. Biol.* **35**, 285-308. doi:10.1146/annurev-cellbio-100818-125134

Pires, H. R. and Boxem, M. (2017). Mapping the polarity interactome. *J. Mol. Biol.* **430**, 3521-3544. doi:10.1016/j.jmb.2017.12.017
Pokutta, S., Drees, F., Takai, Y., Nelson, W. J. and Weis, W. I. (2002). Biochemical and structural definition of the l-Afadin- and actin-binding sites of α-catenin. *J. Biol. Chem.* **277**, 18868-18874. doi:10.1074/jbc.M201463200
Rajasekaran, A. K., Hojo, M., Huima, T. and Rodriguez-Boulan, E. (1996). Catenins and zonula occludens-1 form a complex during early stages in the assembly of tight junctions. *J. Cell Biol.* **132**, 451-463. doi:10.1083/jcb.132.3.451
Roh, M. H., Fan, S., Liu, C. J. and Margolis, B. (2003). The Crumbs3-Pals1 complex participates in the establishment of polarity in mammalian epithelial cells. *J. Cell Sci.* **116**, 2895-2906. doi:10.1242/jcs.00500
Rouaud, F., Sluysmans, S., Flinois, A., Shah, J., Vasileva, E. and Citi, S. (2020). Scaffolding proteins of vertebrate apical junctions: Structure, functions and biophysics. *Biochim. Biophys. Acta Biomembr.* **1862**, 183399.
Ruch, T. R., Bryant, D. M., Mostov, K. E. and Engel, J. N. (2017). Par3 integrates Tiam1 and phosphatidylinositol 3-kinase signaling to change apical membrane identity. *Mol. Biol. Cell* **28**, 252-260. doi:10.1091/mbc.e16-07-0541
Sakakibara, S., Mizutani, K., Sugiura, A., Sakane, A., Sasaki, T., Yonemura, S. and Takai, Y. (2020). Afadin regulates actomyosin organization through αE-catenin at adherens junctions. *J. Cell Biol.* **219**, e201907079. doi:10.1083/jcb.201907079
Salinas-Saavedra, M. and Marindale, M. Q. (2020). Par protein localization during the early development of Mnemiopsis leidyi suggests different modes of epithelial organization in the Metazoa. *eLife* **9**, e54927. doi:10.7554/eLife.54927
Sasaki, K., Kojitani, N., Hirose, H., Yoshihama, Y., Suzuki, H., Shimada, M., Takayanagi, A., Yamashita, A., Nakaya, M. A., Hirano, H. et al. (2020). Shank2 binds to aPKC and controls tight junction formation with Rap1 signaling during establishment of epithelial cell polarity. *Cell Rep* **31**, 107407. doi:10.1016/j.celrep.2020.02.088
Sato, T., Fujita, N., Yamada, A., Ooshio, T., Okamoto, R., Irie, K. and Takai, Y. (2006). Regulation of the assembly and adhesion activity of E-cadherin by nectin and afadin for the formation of adherens junctions in Madin-Darby canine kidney cells. *J. Biol. Chem.* **281**, 5288-5299. doi:10.1074/jbc.M510070200
Satoh-Horikawa, K., Nakanishi, H., Takahashi, K., Miyahara, M., Nishimura, M., Tachibana, K., Mizoguchi, A. and Takai, Y. (2000). Nectin-3, a new member of immunoglobulin-like cell adhesion molecules that shows homophilic and heterophilic cell-cell adhesion activities. *J. Biol. Chem.* **275**, 10291-10299. doi:10.1074/jbc.275.14.10291
Schwayer, C., Shamipour, S., Pranjic-Ferscha, K., Schauer, A., Balda, M., Tada, M., Matter, K. and Heisenberg, C.-P. (2019). Mechanosensation of tight junctions depends on ZO-1 phase separation and flow. *Cell* **179**, 937-952.e18. doi:10.1016/j.cell.2019.10.006
Sebé-Pedrós, A., Degnan, B. M. and Ruiz-Trillo, I. (2017). The origin of Metazoa: a unicellular perspective. *Nat Rev Gen* **18**, 498-512. doi:10.1038/nrg.2017.21
Severson, E. A., Lee, W. Y., Capaldo, C. T., Nusrat, A. and Parkos, C. A. (2009). Junctional adhesion molecule A interacts with Afadin and PDZ-GEF2 to activate Rap1A, regulate beta1 integrin levels, and enhance cell migration. *Mol. Biol. Cell* **20**, 1916-1925. doi:10.1091/mbc.e08-10-1014
Sotillos, S., Díaz-Meco, M. T., Caminero, E., Moscat, J. and Campuzano, S. (2004). DaPKC-dependent phosphorylation of Crumbs is required for epithelial cell polarity in Drosophila. *J. Cell Biol.* **166**, 549-557. doi:10.1083/jcb.200311031
Spadaro, D., Le, S., Laroche, T., Mean, I., Jond, L., Yan, J. and Citi, S. (2017). Tension-Dependent Stretching Activates ZO-1 to Control the Junctional Localization of Its Interactors. *Curr. Biol.* **27**, 3783-3795.e8. doi:10.1016/j.cub.2017.11.014
St Johnston, D. and Ahringer, J. (2010). Cell polarity in eggs and epithelia: parallels and diversity. *Cell* **141**, 757-774. doi:10.1016/j.cell.2010.05.011
Staehelin, L. A., Mukherjee, T. M. and Protoplasma, A. W. W. (1969). Freeze-etch appearance of the tight junctions in the epithelium of small and large intestine of mice. *Protoplasma* **67**, 165-184. doi:10.1007/BF01248737
Straight, S. W., Pieczynski, J. N., Whiteman, E. L., Liu, C.-J. and Margolis, B. (2006). Mammalian Lin-7 stabilizes polarity protein complexes. *J. Biol. Chem.* **281**, 37738-37747. doi:10.1074/jbc.M607059200
Strale, P. O., Duchesne, L., Peyret, G., Montel, L., Nguyen, T., Png, E., Tampé, R., Troyanovsky, S., Hénon, S., Ladoux, B. et al. (2015). The formation of ordered nanoclusters controls cadherin anchoring to actin and cell-cell contact fluidity. *J. Cell Biol.* **210**, 333-346. doi:10.1083/jcb.201410111
Strand, D., Raska, I. and Mechler, B. M. (1994). The Drosophila lethal(2)giant larvae tumor suppressor protein is a component of the cytoskeleton. *J. Cell Biol.* **127**, 1345-1360. doi:10.1083/jcb.127.5.1345
Suzuki, A., Hirata, M., Kamimura, K., Maniwa, R., Yamanaka, T., Mizuno, K., Kishikawa, M., Hirose, H., Amano, Y., Izumi, N. et al. (2004). aPKC acts upstream of PAR-1b in both the establishment and maintenance of mammalian epithelial polarity. *Curr. Biol.* **14**, 1425-1435. doi:10.1016/j.cub.2004.08.021
Suzuki, A., Ishiyama, C., Hashiba, K., Shimizu, M., Ebnet, K. and Ohno, S. (2002). aPKC kinase activity is required for the asymmetric differentiation of the premature junctional complex during epithelial cell polarization. *J. Cell Sci.* **115**, 3565-3573. doi:10.1242/jcs.00032
Takahashi, K., Nakanishi, H., Miyahara, M., Mandai, K., Satoh, K., Satoh, A., Nishioka, H., Aoki, J., Nomoto, A., Mizoguchi, A. et al. (1999). Nectin/PRR: an immunoglobulin-like cell adhesion molecule recruited to cadherin-based adherens junctions through interaction with Afadin, a PDZ domain-containing protein. *J. Cell Biol.* **145**, 539-549. doi:10.1083/jcb.145.3.539
Tan, B., Yatim, S. M. J. M., Peng, S., Gunaratne, J., Hunziker, W. and Ludwig, A. (2020). The mammalian crumbs complex defines a distinct polarity domain apical of epithelial tight junctions. *Curr. Biol.* **30**, 2791-2804.e6. doi:10.1016/j.cub.2020.05.032
Tepass, U. (1996). Crumbs, a component of the apical membrane, is required for zonula adherens formation in primary epithelia of Drosophila. *Dev. Biol.* **177**, 217-225. doi:10.1006/dbio.1996.0157
Tepass, U. (2012). The apical polarity protein network in *Drosophila* epithelial cells: regulation of polarity, junctions, morphogenesis, cell growth, and survival. *Annu. Rev. Cell Dev. Biol.* **28**, 655-685. doi:10.1146/annurev-cellbio-092910-154033
Tilston-Lünel, A. M., Haley, K. E., Schlecht, N. F., Wang, Y., Chatterton, A. L. D., Moleirinho, S., Watson, A., Hundal, H. S., Prystowsky, M. B., Gunn-Moore, F. J. et al. (2016). Crumbs 3b promotes tight junctions in an ezrin-dependent manner in mammalian cells. *J. Mol. Cell Biol.* **8**, 439-455. doi:10.1093/jmcb/mjw020
Traweger, A., Wiggin, G., Taylor, L., Tate, S. A., Metalnikov, P. and Pawson, T. (2008). Protein phosphatase 1 regulates the phosphorylation state of the polarity scaffold Par-3. *Proc. Natl. Acad. Sci. USA* **105**, 10402-10407. doi:10.1073/pnas.0804102105
Truong Quang, B. A., Mani, M., Markova, O., Lecuit, T. and Lenne, P. F. (2013). Principles of E-cadherin supramolecular organization in vivo. *Curr. Biol.* **23**, 2197-2207. doi:10.1016/j.cub.2013.09.015
Tuncay, H., Brinkmann, B. F., Steinbacher, T., Schürmann, A., Gerke, V., Iden, S. and Ebnet, K. (2015). JAM-A regulates cortical dynein localization through Cdc42 to control planar spindle orientation during mitosis. *Nat. Commun.* **6**, 8128. doi:10.1038/ncomms9128
Umeda, K., Ikenouchi, J., Katahira-Tayama, S., Furuse, K., Sasaki, H., Nakayama, M., Matsui, T., Tsukita, S. and Furuse, M. (2006). ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation. *Cell* **126**, 741-754. doi:10.1016/j.cell.2006.06.043
Vacca, B., Barthélémy-Requin, M., Burcklé, C., Massey-Harroche, D. and Bivic, A. L. (2015). The Crumbs3 complex. In *Cell Polarity 1: Biological Role and Basic Mechanisms* (ed. K. Ebnet), pp. 51-63. Cham: Springer International Publishing.
Wodarz, A., Hinz, U., Engelbert, M. and Knust, E. (1995). Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of Drosophila. *Cell* **82**, 67-76. doi:10.1016/0092-8674(95)90053-5
Woods, D. F. and Bryant, P. J. (1991). The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. *Cell* **66**, 451-464. doi:10.1016/0092-8674(81)90009-X
Wu, Y., Kanchanawong, P. and Zaidel-Bar, R. (2015). Actin-delimited adhesion-independent clustering of E-cadherin forms the nanoscale building blocks of adherens junctions. *Dev. Cell* **32**, 139-154. doi:10.1016/j.devcel.2014.12.003
Yamada, T., Kuramitsu, K., Rikitsu, E., Kurita, S., Ikeda, W. and Takai, Y. (2013). Nectin and junctional adhesion molecule are critical cell adhesion molecules for the apico-basal alignment of adherens and tight junctions in epithelial cells. *Genes Cells* **18**, 985-998. doi:10.1111/gtc.12091
Yamanaka, T., Horikoshi, Y., Suzuki, A., Sugiyama, Y., Kitamura, K., Maniwa, R., Nagai, Y., Yamashita, A., Hirose, T., Ishikawa, H. et al. (2001). PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex: PAR-6 regulates aPKC in cell polarization. *Genes Cells* **6**, 721-731. doi:10.1046/j.1365-2443.2001.00453.x
Yao, M., Qiu, W., Liu, R., Efremov, A. K., Cong, P., Seddiki, R., Payre, M., Lim, C. T., Ladoux, B., Mège, R. M. et al. (2014). Force-dependent conformational switch of α-catenin controls vinculin binding. *Nat. Commun.* **5**, 4525. doi:10.1038/ncomms5525
Yap, A. S., Gomez, G. A. and Parton, R. G. (2015). Adherens junctions revisualized: organizing cadherins as nanoassemblies. *Dev. Cell* **35**, 12-20. doi:10.1016/j.devcel.2015.09.012
Yonemura, S., Itoh, M., Nagafuchi, A. and Tsukita, S. (1995). Cell-to-cell adherens junction formation and actin filament organization: similarities and differences between non-polarized fibroblasts and polarized epithelial cells. *J. Cell Sci.* **108**, 127-142. doi:10.1242/jcs.108.1.127
Yu, C. G. and Harris, T. J. (2012). Interactions between the PDZ domains of Bazooka (Par-3) and phosphatidic acid: in vitro characterization and role in epithelial development. *Mol. Biol. Cell* **23**, 3743-3753. doi:10.1091/mbc.e12-03-0196
Zeitler, J., Hsu, C. P., Dionne, H. and Bilder, D. (2004). Domains controlling cell polarity and proliferation in the Drosophila tumor suppressor Scribble. *J. Cell Biol.* **167**, 1137-1146. doi:10.1083/jcb.200407158
Zhang, Y., De Mets, R., Monzel, C., Acharya, V., Toh, P., Chin, J. F. L., Van Hul, N., Ng, I. C., Yu, H., Ng, S. S. et al. (2020). Biomimetic niches reveal the minimal cues to trigger apical lumen formation in single hepatocytes. *Nat Mat* **79**, 1-15. doi:10.1038/s41563-020-0662-3
Zihni, C., Mills, C., Matter, K. and Balda, M. (2016). Tight junctions: from simple barriers to multifunctional molecular gates. *Nat. Rev. Mol. Cell Biol.* **17**, 564-580. doi:10.1038/nrm.2016.80
Zou, J., Wang, X. and Wei, X. (2012). Crb apical polarity proteins maintain zebrafish retinal cone mosaics via intercellular binding of their extracellular domains. *Dev. Cell* **22**, 1261-1274. doi:10.1016/j.devcel.2012.03.007