Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling

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The epithelial cadherin (E-cadherin)–catenin complex binds to cytoskeletal components and regulatory and signaling molecules to form a mature adherens junction (AJ). This dynamic structure physically connects neighboring epithelial cells, couples intercellular adhesive contacts to the cytoskeleton, and helps define each cell's apical–basal axis. Together these activities coordinate the form, polarity, and function of all cells in an epithelium. Several molecules regulate AJ formation and integrity, including Rho family GTPases and Par polarity proteins. However, only recently, with the development of live-cell imaging, has the extent to which E-cadherin is actively turned over at junctions begun to be appreciated. This turnover contributes to junction formation and to the maintenance of epithelial integrity during tissue homeostasis and remodeling.

Introduction

The presence of apical adherens junctions (AJs) is a defining feature of all epithelial sheets (Fristrom, 1988). AJs are constructed on a foundation of homophilic contacts between epithelial cadherin (E-cadherin) clusters on the surface of adjacent epithelial cells. This adhesion is modified by other adhesion molecules, such as the Nectin–Afadin complex (or Echinoid–Canoe in *Drosophila melanogaster*; Wei et al., 2005; Sawyer et al., 2009) to produce mature junctions. E-Cadherin belongs to the family of classical cadherin adhesion molecules, which facilitate the dynamic regulation of adhesive contacts (for a review of the cadherin family of proteins see Gumbiner, 2005).

E-Cadherins are characterized by long extracellular and cytoplasmic domains. Although the extracellular domain of E-cadherin establishes homophilic interactions between neighboring cells (Gumbiner et al., 1988), its cytoplasmic tail associates with an array of intracellular proteins. These proteins link cell–cell adhesion to the actin–myosin network, vesicle transport, and cell polarity machinery. The best studied of these links is the binding of the cytoplasmic tail of E-cadherin to the Armadillo repeat protein β-catenin, which in turn binds α-catenin, which interacts with actin and several actin-binding proteins (Fig. 1 A; Bershadsky, 2004; Yonemura et al., 2010). Through the action of these intracellular binding partners, E-cadherin contacts modulate actin filament organization at the underlying cortex (Baum and Perrimon, 2001; Perez-Moreno et al., 2003; Drees et al., 2005). Signals generated at cell–cell junctions, such as those in response to changes in cell–cell contact, can also be transduced through the cytoplasmic tail of E-cadherin to the nucleus to alter gene expression (Okada et al., 2007; Balda and Matter, 2009). For example, β-catenin, a component of AJs and a transcriptional coactivator, has been implicated in the transduction of mechanical signals from junctions to the nucleus (Farge, 2003). Moreover, in mouse models for colon cancer, mechanical stimulation leads to β-catenin phosphorylation at the site of its interaction with E-cadherin and increased β-catenin nuclear localization, leading to the transcription of the oncogenes *Myc* and *Twist1*. All of these effects can be prevented by blocking β-catenin phosphorylation using Src kinase inhibitors (Whitehead et al., 2008). Additionally, recent work has implicated E-cadherin, α-catenin, and vinculin in participating in a mechanosensory pathway that allows cells to modulate their actin cytoskeleton in response to applied force (le Duc et al., 2010; Yonemura et al., 2010). These data suggest that AJs can detect changes in cell–cell contacts and mechanical stress.

The basic features of E-cadherin–catenin-based AJs

Junctional E-cadherin–catenin complexes exhibit several important characteristics that are critical for the proper functioning of epithelia. First, homophilic interactions between the extracellular portions of E-cadherin molecules help to provide mechanically strong adhesive links between cells in the tissue. Second, AJs help to define an epithelial cell's apical–basal axis in many systems and, in doing so, act as a reference point for the coordination of cell polarity across the epithelial sheet (Fig. 1 B). Third, individual junctions linking cells in an epithelium can form polarized cortical domains in the plane of the epithelium,

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Abbreviations used in this paper: AJ, adherens junction; aPKC, atypical PKC; E-cadherin, epithelial cadherin; GEF, guanine nucleotide exchange factor.

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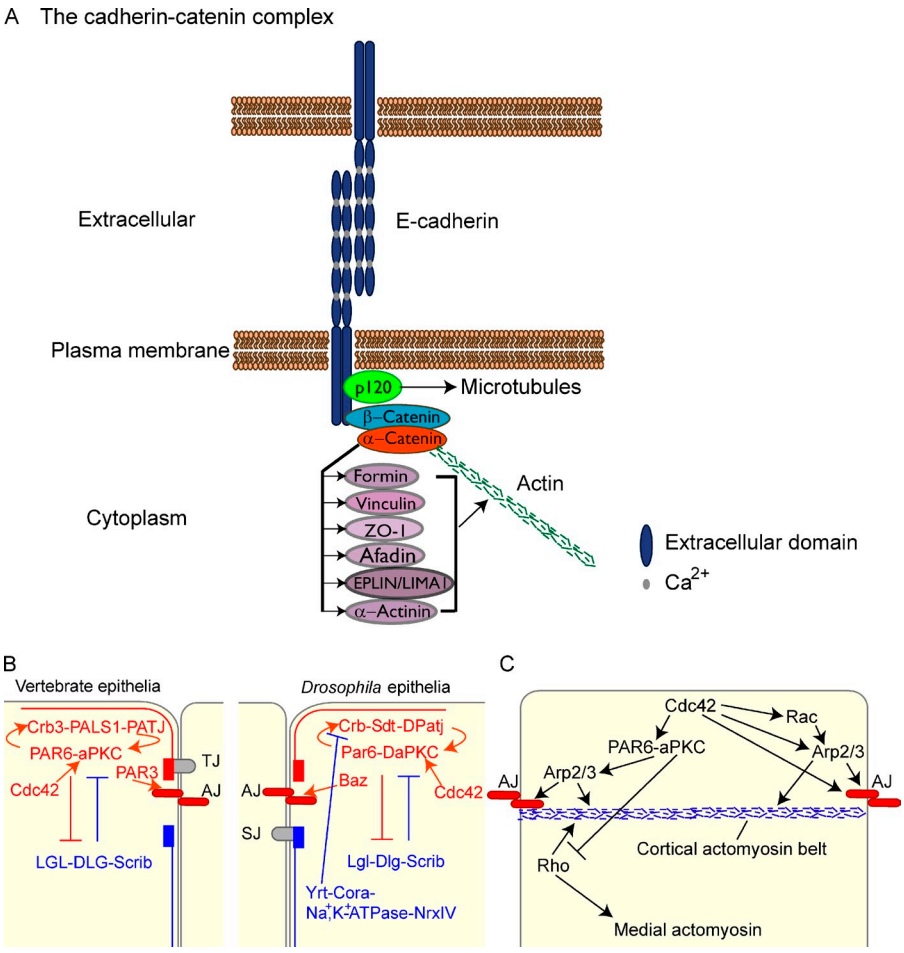

Figure 1. **Factors required to polarize an epithelium.** (A) E-Cadherin can dimerize and form trans-homophilic interactions to form cadherin clusters. Ca^{2+} ions are required to stiffen the extracellular domain and are essential to form homophilic interactions. The E-cadherin intracellular domain contains binding sites for the catenins p120 and β-catenin, thereby forming the cadherin–catenin complex. p120 catenin links cadherin to microtubules and is also important to prevent cadherin endocytosis and degradation. β-Catenin binds α-catenin, which in turn binds actin and several actin-associated proteins, including α-actinin, vinculin, and formin-1. The cadherin–catenin complex also binds many other proteins, including signaling proteins, and cell surface receptors and forms a hub for protein–protein interactions. (B) AJ maturation promotes the assembly of the tight junction (TJ) in vertebrates and the septate junction (SJ) in *Drosophila* epithelial cells, which both function to provide a paracellular diffusion barrier. The AJ is also necessary to form distinct apical and basolateral domains within the cell with conserved protein complexes that are required to establish and maintain these domains. Apical polarity proteins are highlighted in red; basolateral polarity proteins are highlighted in blue. Apical polarity proteins are found throughout the apical domain but are found concentrated just above the AJ (red boxes). Basolateral proteins are found concentrated just below the AJ (blue boxes), and a mutual inhibition between apical and basolateral complexes maintains this apicobasal polarity. (C) The cytoskeleton is also polarized within epithelial cells and several Rho GTPases, and polarity proteins influence the localization and activity of these cytoskeletal structures (Georgiou and Baum, 2010). Other subcellular structures, although not depicted, are also organized along the apicobasal axis, including the centrosome and the Golgi. Baz, Bazooka. Crb, Crumbs. DaPKC, *Drosophila* aPKC. DLG, discs large. LGL, lethal giant larvae. Sdt, Stardust. Yrt, yurt.

a process known as planar cell polarity. Although it is not yet understood how AJs achieve and coordinate these multiple tasks, recent work has begun to reveal many of the underlying molecules and cell biological processes involved. Because these features of epithelia are generic, many of them are likely to be conserved features of epithelia in multicellular animals.

AJ dynamics

A key feature of AJs is that they are dynamic, both when assessed in vivo and in cell culture (Fujita et al., 2002; Pilot et al., 2006; Cavey et al., 2008; de Beco et al., 2009). In fact, the ability for individual AJs to be continually formed and disassembled is vital for the preservation of epithelial integrity because this must be maintained in the face of constant changes in cell packing that accompany changes in tissue organization, cell division, cell death, and delamination. As a result of this plasticity, changes in the length of AJs can release stresses that have accumulated in an epithelium and can accommodate morphogenetic movements—from intercalation to epithelial bending. In addition, the turnover of E-cadherin–mediated adhesions is critical for rapid transitions between epithelial and mesenchymal states (Baum et al., 2008), which because of this turnover, occur unperturbed even in systems in which E-cadherin is ubiquitously overexpressed (Oda and Tsukita, 1999).

Several recent studies have analyzed the turnover of E-cadherin in the context of stable epithelial AJs, in which it can be easily measured (Delva and Kowalczyk, 2009). When E-cadherin turnover was monitored using surface biotinylation and recycling assays in cultured epithelial cells, Le et al. (1999) showed that E-cadherin is actively internalized and recycled back to the plasma membrane via a process that is dependent on clathrin-mediated endocytosis (Fig. 2). Similar observations have been made in vivo, where live imaging of E-cadherin trafficking in the *Drosophila* pupal notum showed that E-cadherin is recycled from the basolateral membrane to AJs (Langevin et al., 2005). In this tissue, dynamin- and actin-dependent endocytosis was shown to be required to remove surface E-cadherin to maintain the position and stability of mature AJs (Georgiou et al., 2008; Leibfried et al., 2008). Recycling requires the exocyst complex for the delivery of E-cadherin to AJs (Langevin et al., 2005; Blankenship et al., 2007). The AJ component β-catenin was shown to directly interact with the Sec10 exocyst

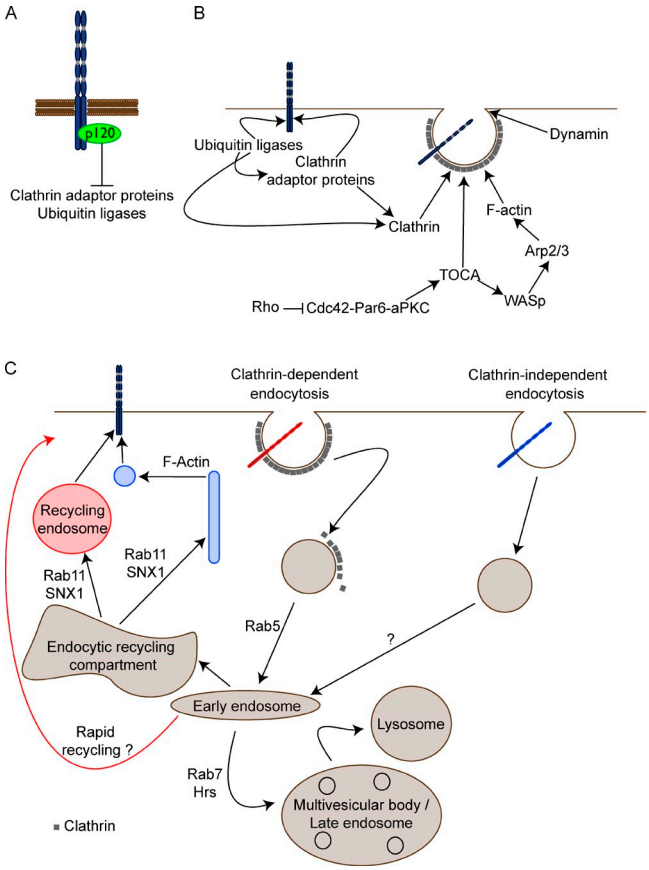

Figure 2. **The regulation of E-cadherin recycling.** (A) p120 catenin inhibits cadherin endocytosis and degradation by preventing the association of adaptor complexes with the cadherin juxtamembrane intracellular region (Fujita et al., 2002; Ishiyama et al., 2010), which prevents cadherin recruitment into clathrin-coated pits. (B) Dissociation between cadherin and p120 allows adaptors, such as AP-2 and β-arrestin, to recruit clathrin and other accessory proteins to promote internalization. Additionally, specific ubiquitin conjugates (E2) and ligases (E3) may act as adaptors for clathrin or as connectors to AP-2 adaptors to activate the clathrin-coated endocytosis machinery. Cdc42–Par6–aPKC, via TOCA proteins and Arp2/3, promotes dynamin-mediated endocytosis. (C) E-Cadherin can undergo either clathrin-dependent (red) or -independent (blue) endocytosis (Delva and Kowalczyk, 2009), and its possible trafficking routes are depicted here together with several proteins that have been shown to have a demonstrated role in E-cadherin trafficking (Lock and Stow, 2005; Palacios et al., 2005; Bryant et al., 2007; Toyoshima et al., 2007). Both trafficking routes converge onto the Rab5-positive early endosome, which sorts its cargo for recycling or degradation. It is not known whether E-cadherin uses the Rab4-dependent rapid recycling route to facilitate its trafficking.

subunit (Langevin et al., 2005), suggesting the possibility that β-catenin can direct exocytosis of AJ components to specific sites on the plasma membrane (Grindstaff et al., 1998; Hsu et al., 1999; Yeaman et al., 2004).

These data suggest that E-cadherin recycling plays a key role in modulating the number and distribution of E-cadherin molecules actively engaged in adhesive interactions between cells. Although the relative contributions of E-cadherin trafficking and diffusion to AJ maintenance have yet to be analyzed in detail in developmental systems, researchers have begun to examine this question in cell culture. Through the use of 2-photon FRAP and fast 3D wide-field fluorescence microscopy in MCF7 and MDCK cells, de Beco et al. (2009) found that most E-cadherin did not diffuse along the membrane in mature junctions. Instead, it was rapidly recycled between internal and plasma membrane pools. Thus, when endocytosis was pharmacologically inhibited, fluorescence recovery at individual junctions was also blocked, suggesting that the majority of E-cadherin membrane redistribution in these cells occurs through recycling via vesicle trafficking (de Beco et al., 2009). Recent work by Hong et al. (2010) suggests a more complex mechanism to maintain AJ homeostasis. By expressing two mutant forms of E-cadherin in epithelial A-431 cells and in CHO cells lacking endogenous cadherin, the authors suggest a three-step process: (1) cadherin is directionally recruited to contact sites, in an energy-dependent process; (2) cadherin forms clusters within the membrane, in part via lateral catenin-dependent association; (3) cadherin is actively removed from these clusters to maintain a dynamic equilibrium. It was noted, however, that clathrin-mediated endocytosis alone did not account for the turnover of cadherin at AJs in this study.

Similarly, experiments in developing animals point to high rates of E-cadherin turnover throughout the life of an AJ, even when actively engaged in strong cell–cell adhesions. In the early *Drosophila* embryo, E-cadherin complexes can be found in clusters with interesting dynamic properties that depend on links with the underlying actin cytoskeleton. Small stable actin patches stabilize E-cadherin microdomains, whereas a dynamic actin network acts in a manner dependent on α-catenin to prevent the lateral movement of adhesive complexes. This suggests a functional separation of E-cadherin turnover and lateral mobility (Cavey et al., 2008).

Regulation of E-cadherin and AJs by GTPases

Although the regulation of AJs varies across experimental systems, in cases in which it has been examined, the formation and maintenance of adhesive cell–cell contacts (Bowers-Morrow et al., 2004) involves an intimate relationship between E-cadherin–mediated AJ protein complexes, the actin cytoskeleton and its regulators, and the Rho family GTPases Rho, Rac, and Cdc42 (Braga, 2002). These interactions occur in both directions, so that whereas Rho family GTPases help to regulate AJ dynamics and to position E-cadherin–based AJs, AJs also modify the activity of these GTPases to alter cell structure and polarity (Fig. 1, B and C). These interactions are discussed in the following sections, during the establishment, maintenance, and remodeling of epithelia.

Rho family GTPases in AJ establishment and maintenance. The establishment of the initial zone of E-cadherin–mediated cell–cell contacts has been shown to require local activation of the Rho family GTPase Rac (Ehrlich et al., 2002; Kovacs et al., 2002; Lambert et al., 2002; Gavard et al., 2004; Hoshino et al., 2004). By driving the formation of actin-based protrusions (Ridley et al., 1992; Braga et al., 1997; Ridley, 2006) that carry E-cadherin (Vasioukhin et al., 2000), Rac can promote the formation of new E-cadherin–based contacts between neighboring cells. Conversely, the establishment of initial contacts between adjacent epithelial cells induces

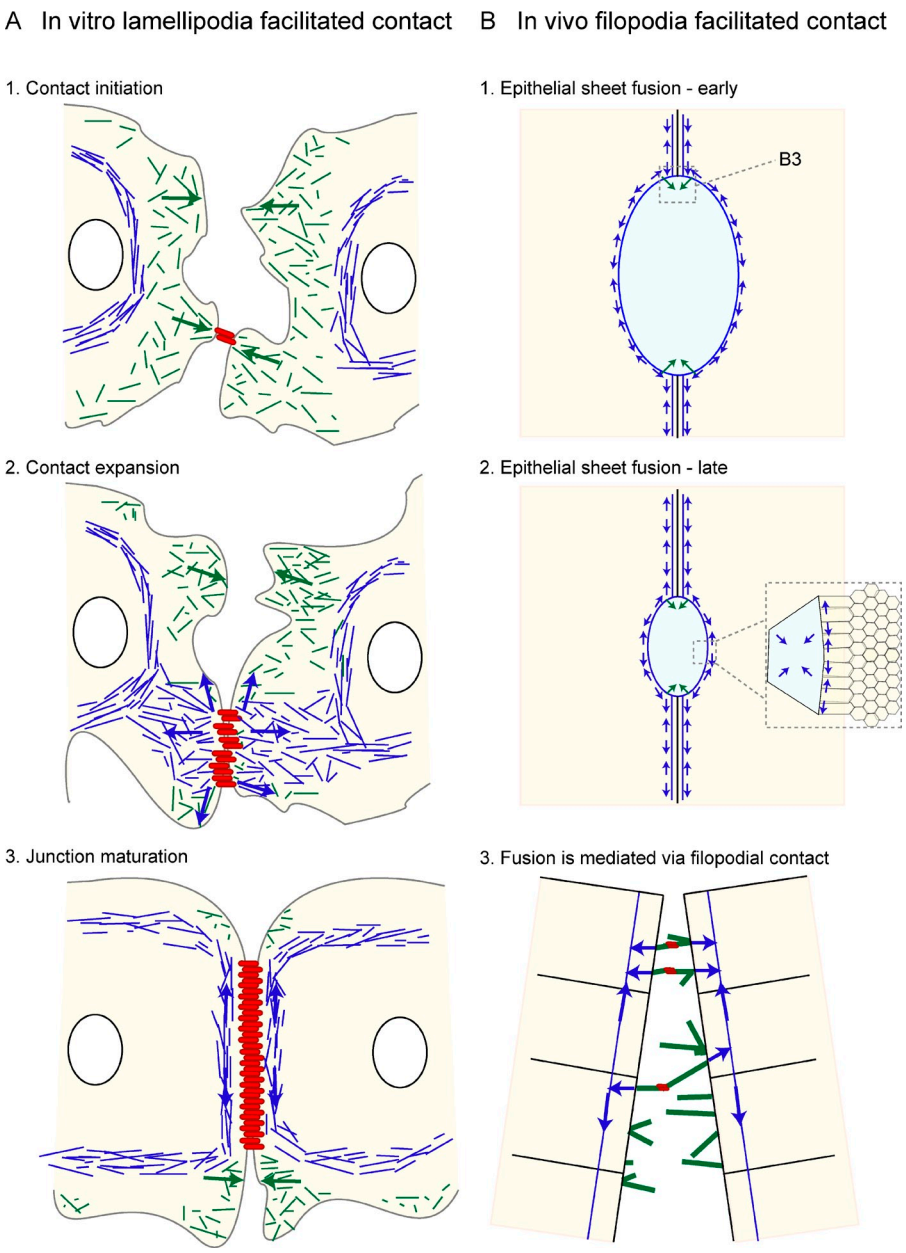

Figure 3. **AJ assembly in vitro and in vivo.** (A, 1) Cell contact and E-cadherin engagement in vitro leads to a remodeling of the actin cytoskeleton (green), promoting lamellipodial and filopodial protrusions via Rac, Cdc42, and Arp2/3 activity. (2) These dynamic protrusions promote further E-cadherin interactions and clustering. The nascent AJs are connected to the circumferential actomyosin cable via contractile actin bundles (blue). (3) Myosin-mediated contraction expands intercellular contact and aligns cadherin–catenin complexes (red bars), leading to the maturation of the junction. (B) Fusion between epithelial sheets in vivo again shows cooperation between dynamic protrusions (green arrows) and actomyosin cables (blue arrows). (1 and 2) An actomyosin cable assembles at the edge of each epithelial sheet, forcing the two sheets together. (3) Individual cells on the leading edge of each epithelial sheet form filopodia (green) that engage with one another, forming cadherin–catenin clusters at the points of contact (red), which are required to seal the two sheets together. In the case of the *Drosophila* embryo during dorsal closure, the ectodermal sheets migrate over a squamous epithelium called the amnioserosa. Here, the apical constriction of amnioserosa cells has been shown to promote dorsal closure (inset). Green arrows represent protrusive activity; blue arrows represent contractile activity.

local membrane remodeling and promotes the formation of lamellipodia (in MDCK cells or IAR-2 cells; Adams et al., 1998; Krendel and Bonder, 1999; Ehrlich et al., 2002) and/or filopodia (in primary mouse keratinocytes; Vasioukhin et al., 2000). In addition, nascent sites of adhesion rich in E-cadherin often appear to be coupled to bundles of actin filaments. These data imply a tight link between de novo contact formation and Rho family GTPase-dependent actin polymerization and/or remodeling (Fig. 3; Adams et al., 1998; Nakagawa et al., 2001; Kovacs et al., 2002; Lambert et al., 2002). Active GTP-bound Rac can also stimulate the activity of phosphatidylinositol 3-kinase (leading to the formation of PIP2) and the activation of Cdc42- and Arp2/3-mediated actin nucleation as well as the recruitment of cortactin, Mena, PAK4, and formin-1 (Vasioukhin et al., 2000; Ehrlich et al., 2002; Kovacs et al., 2002; Kobielak et al., 2003; Rivard, 2009; Wallace et al., 2010); all of which may help to promote an increase in the zone of cell–cell contact.

In vivo studies have come to similar conclusions in supporting a role for actin-based protrusions in intercellular junction formation during development. Filopodia carrying E-cadherin help to bring together the free edges of epithelial sheets during embryonic development in *Caenorhabditis elegans* (Raich et al., 1999), *Drosophila* (Jacinto et al., 2000), and in vertebrates (Fig. 3 B; Brock et al., 1996; Vasioukhin et al., 2000). Also, during *Drosophila* tracheal development, E-cadherin is found accumulating at the tips of filopodia as cell–cell contacts are generated before the fusion of epithelial-based tracheal branches (Tanaka-Matakatsu et al., 1996).

This collaboration between Rho GTPases and AJ components is maintained during AJ maturation, as tight junctions and apical–basal polarity are established through the action of both Rac and Cdc42. Interaction between these activated Rho family GTPases and Par6 leads to the activation of atypical PKC (aPKC), which has been shown to be required for the maturation

of AJs from simple cell–cell adhesions to junctional complexes (Yamanaka et al., 2001). Additionally, TIAM1, a Rac-specific guanine nucleotide exchange factor (GEF), is required for the establishment of functional tight junctions in keratinocytes and in MDCK cells (Takaishi et al., 1997; Chen and Macara, 2005; Mertens et al., 2005). Several other GEFs have also been implicated in E-cadherin cell–cell adhesion, including Tuba (a Cdc42-specific GEF; Otani et al., 2006) and Asef (a Rac GEF; Kawasaki et al., 2003).

In addition, RhoA helps to maintain E-cadherin–mediated adhesion via the action of Dia1 (Sahai and Marshall, 2002) and nonmuscle myosin II (Shewan et al., 2005). A recent paper revealed that two isoforms of myosin II differentially affect junction integrity through different mechanisms, with myosin IIA promoting E-cadherin homophilic adhesion and clustering and myosin IIB supporting the integrity of the apical cortical actin ring (Smutny et al., 2010). Rho activity and actomyosin contractility have also been implicated in cell–cell junctional homeostasis in cell culture systems and in developing animals (Bertet et al., 2004; Dawes-Hoang et al., 2005; Blankenship et al., 2006; Yamada and Nelson, 2007; Abraham et al., 2009; Martin et al., 2009; Rolo et al., 2009; Liu et al., 2010). Rho signaling has additionally been implicated in the disassembly of cell–cell contacts during epithelial–mesenchymal transition, in which active RhoA is important for hepatocyte growth factor– and TGF-β–induced disruption to cadherin contacts (Takaishi et al., 1994; Bhowmick et al., 2001). AJ complex components together with polarity complexes, the balanced activities of Rho, Rac, and Cdc42, and the actomyosin cytoskeleton are, therefore, all required to establish and maintain junctions between adjacent cells in an epithelium.

Rho GTPases, polarity, and regulation of AJ turnover. A role for the apical Par proteins (Par3/Bazooka, aPKC, and Par6) and the Crumbs complex (Crumbs, PALS-1/Stardust, and PATJ/Discs lost) in defining the apical domain of epithelial cells has long been established in a wide variety of systems. Significantly, interactions between these functional modules together with the complexes that define the basolateral domains (the Scribble and Yurt complexes) generate zones of mutual exclusion around AJs that define the apical–basal axis of epithelial polarity (Assémat et al., 2008) and help lead to the formation of a fully differentiated (Müller and Wieschaus, 1996) and properly positioned (Harris and Peifer, 2005) AJ (Fig. 1 B).

Once stable AJs have been established, Cdc42, its associated Par complex components, and the apical Crumbs complex continue to play roles in the regulation of AJ stability by controlling the active turnover of AJ components. This is especially important in tissues undergoing active remodeling. This is most striking when observing the ectoderm of the developing *Drosophila* embryo. In this system, AJs in the relatively stable dorsal ectoderm can be compared with those of the ventral neuroectoderm, where approximately one third of cells within the epithelial sheet delaminate to form neuroblasts (neural stem cells), which occurs in waves and takes 3 h to complete (Campos-Ortega and Hartenstein, 1997). Although most epithelial tissues in mutant embryos lacking zygotic expression of E-cadherin were found to maintain functional cell–cell junctions and apicobasal polarity (Tepass et al., 1996; Uemura et al., 1996), under these conditions, the integrity of the ventral neuroectoderm was lost. Thus, the ventral neuroectoderm requires higher levels of E-cadherin to maintain AJ stability in the face of cell rearrangements than the dorsal epithelium. Because blocking neuroblast specification and delamination within this tissue restores tissue integrity, even in the absence of zygotic E-cadherin (Tepass et al., 1996; Uemura et al., 1996), newly expressed E-cadherin appears to be required to support AJ plasticity and morphogenetic movements within this tissue.

Harris and Tepass (2008) went on to show that Cdc42 and Par proteins regulate the trafficking of AJ components and apical polarity proteins in the ventral ectoderm to maintain AJ stability in the face of cell rearrangements. Once again, AJ integrity was specifically disrupted within the ventral neuroectoderm after a reduction in Cdc42 activity, which was mediated by the expression of a dominant-negative construct or as the result of loss-of-function mutations. Reducing Cdc42 activity also led to a mislocalization of both junction and apical polarity proteins in the ventral ectoderm, including α- and β-catenin, apical Par proteins, Crumbs, and PatJ. Again, all these defects could be restored by blocking neuroblast specification and delamination. Genetic interaction studies suggested that these defects in AJ integrity followed Cdc42-dependent changes in the endocytosis and trafficking of apical polarity proteins, such as Crumbs.

Interestingly, they also showed that the apical Par proteins (Bazooka/Par3, Par6, and aPKC) act together with Cdc42 in the regulation of endocytosis in this system, as loss-of-function mutants for each gene phenocopied the *cdc42* phenotype (Harris and Tepass, 2008). The authors proposed a model in which Cdc42, together with the Par complex, is required to decrease the endocytic uptake of apical proteins and to promote the progression of apical cargo from the early to the late endosome. In line with this need for active membrane recycling to support AJ plasticity, Rab11, a small GTPase required for vesicle recycling, was also found to be required to maintain epithelial integrity in the ventral ectoderm (Roeth et al., 2009).

The connection between Cdc42, apical Par proteins, and junctional endocytosis has also been borne out in work in other systems. In mammalian cell culture, both Rac and Cdc42 activity are required to modulate the actin cytoskeleton to affect E-cadherin endocytosis (Akhtar and Hotchin, 2001; Izumi et al., 2004). Also, a genome-wide RNAi screen in *C. elegans* (Balklava et al., 2007) showed that Cdc42 and Par proteins promote endocytosis. More recently, Par complex proteins were shown to modulate and to be the substrates for dynamin-mediated endocytosis in the *C. elegans* zygote (Nakayama et al., 2009).

In addition, two studies using live imaging and somatic genetic mutant clones to investigate the relationship between Cdc42 and AJs in the developing pupal notum or dorsal thorax of the fly (Georgiou et al., 2008; Leibfried et al., 2008) showed that the loss of Cdc42, Par6, or aPKC function led to AJ breaks and ectopic junctional structures. When using transmission EM to image the electron-dense AJ, a reduction in Cdc42 activity was associated with extensive junctional spreading (Georgiou et al., 2008). Significantly, a similar phenotype was observed when the function of dynamin, a protein known to be required

for the scission of clathrin-coated endocytic vesicles (Hill et al., 2001) was inhibited, implicating a failure of correct endocytosis in these mutants. In this system, in contrast to the *Drosophila* embryo (Harris and Tepass, 2008), Cdc42, Par6, and aPKC appear to promote AJ turnover, raising the possibility that there are tissue-specific roles for the Cdc42–Par6–aPKC complex in the regulation of junction turnover.

Cdc42 is an important regulator of the actin cytoskeleton and is known to bind to and activate WASp, which in turn promotes actin nucleation via the Arp2/3 complex (Takenawa and Miki, 2001; Pollard, 2007). Consistent with these findings, both WASp and components of the Arp2/3 complex were found to be required to maintain AJ integrity in the pupal notum (Georgiou et al., 2008; Leibfried et al., 2008). F-actin dynamics have been shown to be required at multiple stages of clathrin-coated vesicle formation and scission (Yarar et al., 2005), and both WASp and the Arp2/3 complex have previously been implicated as key downstream targets in promoting endocytosis (Sokac et al., 2003; Martin et al., 2006). Additionally, recent evidence from *C. elegans* and in mammalian cells implicated both WASp and the F-BAR domain containing TOCA (transducer of Cdc42-dependent actin assembly) proteins in both membrane trafficking and epithelial morphogenesis (Giuliani et al., 2009; Bu et al., 2010). The TOCA family of proteins regulate actin dynamics via a WASp-interacting SH3 domain and additionally bind to and deform the membrane via a BAR domain, which can trigger the formation of plasma membrane invaginations. This is thought to enable TOCA proteins to promote the internalization of plasma membrane proteins (Itoh et al., 2005). Consistent with this notion, the single *Drosophila* TOCA protein, Cip4, contributes to E-cadherin trafficking downstream of Cdc42 (Leibfried et al., 2008). Therefore, the apical polarity complex Cdc42–Par6–aPKC seems to induce the local activation of WASp and TOCA family proteins to drive dynamin-mediated endocytosis of AJ material and the recycling of E-cadherin complexes (Fig. 2 B). Moreover, this appears to be essential to maintain junction stability and plasticity throughout development, even in relatively stable epithelia.

Cdc42, Par6, and aPKC have additionally been implicated in regulating Rho activity at the junction, providing further evidence of extensive cross talk between Rho GTPases, Par polarity proteins, and the endocytic pathway in maintaining AJs. Work in the *Drosophila* eye has shown that Cdc42–Par6–aPKC-mediated regulation of apical Rho activity is required to maintain AJ integrity and to regulate epithelial cell apical tension (Warner and Longmore, 2009a,b).

AJ remodeling as a driving force for morphogenesis

Organism growth and development is accompanied by complex cell shape changes and movements within epithelia that necessitate apical adhesive junctions that are both strong and plastic. Moreover, changes in AJ length play a critical role in driving many morphogenetic processes, from gastrulation to cell intercalation. Several studies have begun to explore junctional dynamics that accompany and drive these processes.

During *Drosophila* germband extension, the tissue doubles its length and reduces its width by half (Irvine and Wieschaus, 1994) as the result of changes in individual cell–cell contacts that results in cell neighbor exchange within the tissue and is a direct result of junction remodeling (Fig. 4, A and B; Bertet et al., 2004; Zallen and Wieschaus, 2004; Blankenship et al., 2006). Using an E-cadherin GFP fusion protein to label the AJs of all cells within the epithelium, AJ behavior over the course of germband extension revealed a directional remodeling of the junction (Bertet et al., 2004; Blankenship et al., 2006). These cell rearrangements require the polarized distribution of proteins that localize to the cortex at the level of the AJ. Among these, myosin II and F-actin are asymmetrically localized to interfaces that shrink over the course of germband extension (interfaces between anterior and posterior germband cells), whereas E-cadherin, Armadillo/β-catenin, and Bazooka/Par-3 are enriched at interfaces that grow during germband extension (Fig. 4 A; Bertet et al., 2004; Zallen and Wieschaus, 2004; Blankenship et al., 2006). Recent work suggests that the myosin II activator Rho kinase is required to limit Bazooka/Par-3 localization at the cortex, preventing its localization to shrinking junctions (de Matos Simões et al., 2010). These results implicate the actomyosin network in mediating the contraction of junctions over time. Additionally, Rho activity and myosin II have been shown to destabilize AJs, and conversely, Bazooka/Par-3 has been shown to promote AJ stability (Sahai and Marshall, 2002; Harris and Peifer, 2004; Chen and Macara, 2005). Although myosin II can promote neighbor exchange through the contraction of single-cell boundaries, high tension actin–myosin II cables (Fernandez-Gonzalez et al., 2009) spanning multiple pairs of cells are also involved in forming multicellular rosette patterns within the tissue, which resolve in a directional fashion to promote tissue elongation (Fig. 4 B; Blankenship et al., 2006). In addition, Rauzi et al. (2010) showed that anisotropies in cadherin localization at junctions bias the flow of the medial actin–myosin network to induce polarized junctional tension during intercalation. Therefore, asymmetries in cell–cell adhesion and contractility combine to drive intercalation. Similarly, experiments in the fly wing have shown that epithelial cell packing and the generation of polarity in the plane of the epithelium are coupled (Classen et al., 2005) and are remodeled in parallel in response to external forces (Aigouy et al., 2010).

Gastrulation, another major tissue-remodeling event, requires a coordinated apical cell constriction in the mesoderm, which requires the Rho1–Rok1 pathway and myosin II activity (Tan et al., 1992; Barrett et al., 1997; Nikolaidou and Barrett, 2004; Dawes-Hoang et al., 2005). Recently, the *Drosophila* afadin homologue Canoe has been shown to be required to link the actomyosin cytoskeleton to the AJ during mesoderm apical constriction (Sawyer et al., 2009). This apical constriction in the fly mesoderm is thought to be mediated by pulses of actomyosin contractility driven by a medial actomyosin weblike network (Fig. 4 C; Martin et al., 2009). Therefore, during gastrulation, the mesoderm is using a different actomyosin network to that of the ectoderm during germband extension, with the former network forcing apical constriction and the latter using the local cortical enrichment of actin and myosin to drive cell intercalation.

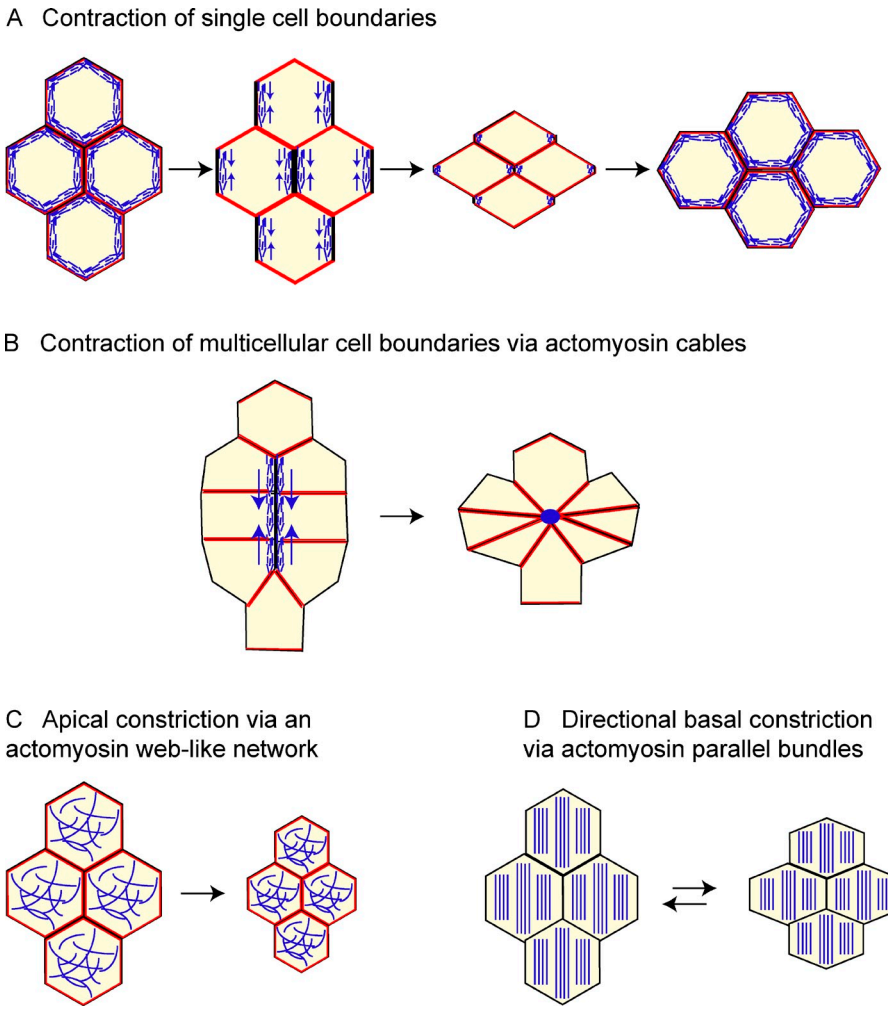

Figure 4. **Different effects of actomyosin-mediated constriction during tissue remodeling.** (A) Cell intercalation requires a polarized redistribution of proteins within the plane of the epithelium to limit remodeling to specific junctions. Before cell intercalation, actin and myosin (blue) as well as E-cadherin, Armadillo/β-catenin, and Bazooka/Par-3 (red) localize uniformly at the cortex. At the onset of cell intercalation, planar symmetry is broken, with F-actin and myosin II concentrating at anteroposterior interfaces (blue). Conversely, Bazooka/Par-3, E-cadherin, and Armadillo/β-catenin accumulate at dorsoventral interfaces (red). This limits actomyosin contractility to the anteroposterior interfaces (blue arrows). (B) The same polarized redistribution of proteins is required to form actomyosin cables that span multiple pairs of cells. Contraction results in the formation of multicellular rosettelike patterns. Blue arrows represent contractile activity. (C) The apical actomyosin medial weblike network (blue) can force apical constriction by shortening all junctions. (D) Basally localized and highly polarized actomyosin parallel bundles (blue) force an oscillating directional constriction at the base of the cell.

It is clear from these experiments in the *Drosophila* embryo that the differential regulation and coordination of medial and junctional actomyosin are critical factors in determining the path of AJ dynamics and morphogenesis. Thus, ectodermal cells appear to actively repress apical constriction mediated via the formation of a medial actomyosin weblike network (Bertet et al., 2009).

The actomyosin weblike network is also required to promote apical constriction in the amnioserosa during *Drosophila* dorsal closure (Gorfinkiel et al., 2009; Solon et al., 2009). The amnioserosa is a squamous epithelium connecting the epidermis of the embryo and is required to guide epidermal tissue rearrangements during embryonic development (Jacinto and Martin, 2001). Additional to Rho activity and the actin cytoskeleton, the Par complex has recently been implicated in regulating apical constriction within this epithelium (David et al., 2010). Each pulse of actomyosin contractility was found to be based on the repeated assembly and disassembly of the actomyosin network. Furthermore, genetic interaction studies suggested that Bazooka/Par3, Par6, and aPKC support myosin activity and determine the rate and duration of contractile pulses.

Oscillating actomyosin contractions have also been observed on basal cell surfaces, in the epithelial follicle cells of the *Drosophila* egg chamber (He et al., 2010). However, rather than a randomly oriented web of actomyosin, as observed apically, basal actomyosin fibers were organized into parallel bundles along the dorsoventral axis (Fig. 4 D). This led to contractions that were directional, leading to a change in cell length along the dorsoventral axis. This contraction, however, is temporary (unlike apical constriction). Here, cells do not change their shape permanently, rather the constriction generates a force that constrains the shape of underlying tissue (He et al., 2010). Basal actomyosin accumulation required Rho activity as well as cadherin-mediated adhesion but, additionally, was subject to regulation through cell–ECM interactions. In summary, a complex picture is emerging, which involves the interplay of interconnecting pathways that determine the location, orientation, and type of force generated to drive specific morphogenetic movements.

Trafficking in mediating cell rearrangements

Intracellular trafficking has been also implicated in regulating cell intercalation in *Drosophila* trachea (Shaye et al., 2008; Shindo et al., 2008). Here, cell intercalation occurs in epithelial tubes, causing tubular elongation with an accompanying reduction in tube circumference. Tracheal cell intercalation relies on the migratory behavior of the leading tip cell of the tracheal branch, which generates a pulling force believed to promote intercalation (Ribeiro et al., 2002). Remarkably, the lumen of

the epithelial tube remains intact, whereas intercellular AJs are remodeled to intracellular junctions once intercalation is complete. Shaye et al. (2008) showed that endocytosis is required for tracheal intercalation through the use of a temperature-sensitive allele of *shibire* (the *Drosophila* dynamin) and a dominant-negative *Rab5*, which markedly reduced dorsal branch intercalation. Conversely, overexpression of a dominant-negative Rab11, a protein required for vesicle recycling (Fig. 2 C), caused inappropriate intercalation. Using a YFP-tagged Rab11 construct, they observed an apical Rab11 accumulation in the dorsal trunk, during stages when cells in other branches intercalate. The authors show that the trunk-specific transcription factor Spalt and its target dRip11, a positive regulator of Rab11 (Li et al., 2007), are necessary and sufficient for the apical Rab11 accumulation observed in the dorsal trunk. Increased Rab11-mediated trafficking led to increased junctional E-cadherin and an inhibition of intercalation. Therefore, there seems to be an asymmetry in tension (mediated by the migratory tip cell) versus E-cadherin–mediated adhesion, as seen in the *Drosophila* embryonic ectoderm (Bertet et al., 2004; Blankenship et al., 2006). Interestingly, pulling forces seem to promote intercalation in all branches unless actively inhibited by Spalt (Ribeiro et al., 2004). In fact, the application of an external force to *Xenopus laevis* explants is sufficient to induce intercalation (Beloussov et al., 2000), suggesting that pulling forces may also provoke a default intercalation response in vertebrate cells, and suggests a conserved mechanism for regulating cell rearrangements. This mechanism involves (a) an asymmetry of tension, which is regulated by myosin, actin filaments, and Rho activity; (b) an asymmetry of adhesion, which is mediated by the cadherins and the endocytosis, recycling, and delivery of cadherin to specific domains within the cell; and (c) polarity proteins, to provide the positional information required to generate this asymmetry.

Conclusions

A common theme emerging from the numerous studies highlighted here, which combine cell biological approaches in cultured cells with genetic approaches, mostly in *Drosophila* and *C. elegans*, is that a surprising molecular complexity is required to maintain homeostasis within an epithelium. Multiple intracellular pathways, which impinge on one another to a great degree, have been implicated in multiple aspects of the establishment and remodeling of epithelial AJs, polarity, and morphology. Furthermore, the same molecular machinery that is required to form and regulate AJs, polarity and the actomyosin cytoskeleton, also appears to play a critical role in driving and coordinating the diverse array of collective cell movements that underlie developmental morphogenesis.

It now seems that AJ regulation requires a constant turnover of AJ proteins, so that even in a stable epithelium, AJs have to be considered as dynamic structures. The inherent plasticity of the AJ appears to be required to maintain junction integrity, as any disruption to the turnover of AJ components leads to a loss of tissue stability. This may imply that junctional complexes have a limited shelf life and require replenishing to maintain adhesion. Alternatively it would be reasonable to assume that, even in stable epithelia that do not undergo any major cell rearrangements, a certain level of reorganization of cell interactions would be required for tissue maintenance and to respond to changes in internal and external mechanical forces over time, especially during processes such as cell division and cell death.

The diverse cell shape changes and movements that are required for complex morphogenetic processes to take place can be stripped down to the regulation of two core mechanical properties: cell–cell adhesion and contractility (Montell, 2008). The asymmetric localization of cortical myosin II and F-actin to shrinking junctions together with the enrichment of E-cadherin and junctional proteins to nonshrinking junctions to drive cell intercalation is a perfect example of this. The localization of a contractile force to specific junctions is required to break tissue homeostasis and force cell movements. Implicated in this process are polarity complexes and Rho GTPases, which are required to asymmetrically localize proteins, remodel the actin cytoskeleton, and pattern and regulate protein trafficking. Additionally, cell–cell adhesion and cortical tension have been implicated in directing tissue organization during several developmental processes in which cell rearrangements, such as cell sorting and compartmentalization, are required (Hayashi and Carthew, 2004; Krieg et al., 2008; Landsberg et al., 2009; Manning et al., 2010; Monier et al., 2010).

It is also apparent that the subcellular localization of the actomyosin cytoskeleton must be tightly regulated to control different cell shape changes. Depending on the tissue and stage of development, constriction can be limited to the medial region of the apex, to junctions in some cells, or to the basal surface in others. Constriction forces can also be focused to a single junction, allowing polarized cell movements to take place. In each case, actomyosin is required, but its localization, organization, and regulation determine its effect. The observation that intercalating epithelial cells have to actively inhibit constriction highlights the fact that these cells possess the ability to form several cytoskeletal structures and carry out numerous cell shape changes. It is the coordinated regulation of the cytoskeleton in all cells within the epithelium that allows complex morphogenetic events to take place.

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References

Abraham, S., M. Yeo, M. Montero-Balaguer, H. Paterson, E. Dejana, C.J. Marshall, and G. Mavria. 2009. VE-Cadherin-mediated cell-cell interaction suppresses sprouting via signaling to MLC2 phosphorylation. *Curr. Biol.* 19:668–674. doi:10.1016/j.cub.2009.02.057

Adams, C.L., Y.T. Chen, S.J. Smith, and W.J. Nelson. 1998. Mechanisms of epithelial cell–cell adhesion and cell compaction revealed by high-resolution tracking of E-cadherin–green fluorescent protein. *J. Cell Biol.* 142:1105–1119. doi:10.1083/jcb.142.4.1105

Aigouy, B., R. Farhadifar, D.B. Staple, A. Sagner, J.C. Röper, F. Jülicher, and S. Eaton. 2010. Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. *Cell*. 142:773–786. doi:10.1016/j.cell.2010.07.042

Akhtar, N., and N.A. Hotchin. 2001. RAC1 regulates adherens junctions through endocytosis of E-cadherin. *Mol. Biol. Cell*. 12:847–862.

Assémat, E., E. Bazellières, E. Pallesi-Pocachard, A. Le Bivic, and D. Massey-Harroche. 2008. Polarity complex proteins. *Biochim. Biophys. Acta*. 1778:614–630. doi:10.1016/j.bbamem.2007.08.029

Balda, M.S., and K. Matter. 2009. Tight junctions and the regulation of gene expression. *Biochim. Biophys. Acta*. 1788:761–767. doi:10.1016/j.bbamem.2008.11.024

Balklava, Z., S. Pant, H. Fares, and B.D. Grant. 2007. Genome-wide analysis identifies a general requirement for polarity proteins in endocytic traffic. *Nat. Cell Biol.* 9:1066–1073. doi:10.1038/ncb1627

Barrett, K., M. Leptin, and J. Settleman. 1997. The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. *Cell*. 91:905–915. doi:10.1016/S0092-8674(00)80482-1

Baum, B., and N. Perrimon. 2001. Spatial control of the actin cytoskeleton in *Drosophila* epithelial cells. *Nat. Cell Biol.* 3:883–890. doi:10.1038/ncb1001-883

Baum, B., J. Settleman, and M.P. Quinlan. 2008. Transitions between epithelial and mesenchymal states in development and disease. *Semin. Cell Dev. Biol.* 19:294–308. doi:10.1016/j.semcdb.2008.02.001

Beloussov, L.V., N.N. Louchinskaia, and A.A. Stein. 2000. Tension-dependent collective cell movements in the early gastrula ectoderm of *Xenopus laevis* embryos. *Dev. Genes Evol.* 210:92–104. doi:10.1007/s004270050015

Bershadsky, A. 2004. Magic touch: how does cell-cell adhesion trigger actin assembly? *Trends Cell Biol.* 14:589–593. doi:10.1016/j.tcb.2004.09.009

Bertet, C., L. Sulak, and T. Lecuit. 2004. Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature*. 429:667–671. doi:10.1038/nature02590

Bertet, C., M. Rauzi, and T. Lecuit. 2009. Repression of Wasp by JAK/STAT signalling inhibits medial actomyosin network assembly and apical cell constriction in intercalating epithelial cells. *Development*. 136:4199–4212. doi:10.1242/dev.040402

Bhowmick, N.A., M. Ghiassi, A. Bakin, M. Aakre, C.A. Lundquist, M.E. Engel, C.L. Arteaga, and H.L. Moses. 2001. Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. *Mol. Biol. Cell*. 12:27–36.

Blankenship, J.T., S.T. Backovic, J.S. Sanny, O. Weitz, and J.A. Zallen. 2006. Multicellular rosette formation links planar cell polarity to tissue morphogenesis. *Dev. Cell*. 11:459–470. doi:10.1016/j.devcel.2006.09.007

Blankenship, J.T., M.T. Fuller, and J.A. Zallen. 2007. The *Drosophila* homolog of the Exo84 exocyst subunit promotes apical epithelial identity. *J. Cell Sci.* 120:3099–3110. doi:10.1242/jcs.004770

Bowers-Morrow, V.M., S.O. Ali, and K.L. Williams. 2004. Comparison of molecular mechanisms mediating cell contact phenomena in model developmental systems: an exploration of universality. *Biol. Rev. Camb. Philos. Soc.* 79:611–642. doi:10.1017/S1464793103006389

Braga, V.M. 2002. Cell-cell adhesion and signalling. *Curr. Opin. Cell Biol.* 14:546–556. doi:10.1016/S0955-0674(02)00373-3

Braga, V.M., L.M. Machesky, A. Hall, and N.A. Hotchin. 1997. The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell–cell contacts. *J. Cell Biol.* 137:1421–1431. doi:10.1083/jcb.137.6.1421

Brock, J., K. Midwinter, J. Lewis, and P. Martin. 1996. Healing of incisional wounds in the embryonic chick wing bud: characterization of the actin purse-string and demonstration of a requirement for Rho activation. *J. Cell Biol.* 135:1097–1107. doi:10.1083/jcb.135.4.1097

Bryant, D.M., M.C. Kerr, L.A. Hammond, S.R. Joseph, K.E. Mostov, R.D. Teasdale, and J.L. Stow. 2007. EGF induces macropinocytosis and SNX1-modulated recycling of E-cadherin. *J. Cell Sci.* 120:1818–1828. doi:10.1242/jcs.000653

Bu, W., K.B. Lim, Y.H. Yu, A.M. Chou, T. Sudhaharan, and S. Ahmed. 2010. Cdc42 interaction with N-WASP and Toca-1 regulates membrane tubulation, vesicle formation and vesicle motility: implications for endocytosis. *PLoS ONE*. 5:e12153. doi:10.1371/journal.pone.0012153

Campos-Ortega, J.A., and V. Hartenstein. 1997. The Embryonic Development of *Drosophila Melanogaster*. Second edition. Springer, Berlin/New York. 405 pp.

Cavey, M., M. Rauzi, P.F. Lenne, and T. Lecuit. 2008. A two-tiered mechanism for stabilization and immobilization of E-cadherin. *Nature*. 453:751–756. doi:10.1038/nature06953

Chen, X., and I.G. Macara. 2005. Par-3 controls tight junction assembly through the Rac exchange factor Tiam1. *Nat. Cell Biol.* 7:262–269. doi:10.1038/ncb1226

Classen, A.K., K.I. Anderson, E. Marois, and S. Eaton. 2005. Hexagonal packing of *Drosophila* wing epithelial cells by the planar cell polarity pathway. *Dev. Cell*. 9:805–817. doi:10.1016/j.devcel.2005.10.016

David, D.J., A. Tishkina, and T.J. Harris. 2010. The PAR complex regulates pulsed actomyosin contractions during amnioserosa apical constriction in *Drosophila*. *Development*. 137:1645–1655. doi:10.1242/dev.044107

Dawes-Hoang, R.E., K.M. Parmar, A.E. Christiansen, C.B. Phelps, A.H. Brand, and E.F. Wieschaus. 2005. *folded gastrulation*, cell shape change and the control of myosin localization. *Development*. 132:4165–4178. doi:10.1242/dev.01938

de Beco, S., C. Gueudry, F. Amblard, and S. Coscoy. 2009. Endocytosis is required for E-cadherin redistribution at mature adherens junctions. *Proc. Natl. Acad. Sci. USA*. 106:7010–7015. doi:10.1073/pnas.0811253106

Delva, E., and A.P. Kowalczyk. 2009. Regulation of cadherin trafficking. *Traffic*. 10:259–267. doi:10.1111/j.1600-0854.2008.00862.x

de Matos Simões, S., J.T. Blankenship, O. Weitz, D.L. Farrell, M. Tamada, R. Fernandez-Gonzalez, and J.A. Zallen. 2010. Rho-kinase directs Bazooka/Par-3 planar polarity during *Drosophila* axis elongation. *Dev. Cell*. 19:377–388. doi:10.1016/j.devcel.2010.08.011

Drees, F., S. Pokutta, S. Yamada, W.J. Nelson, and W.I. Weis. 2005. Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell*. 123:903–915. doi:10.1016/j.cell.2005.09.021

Ehrlich, J.S., M.D. Hansen, and W.J. Nelson. 2002. Spatio-temporal regulation of Rac1 localization and lamellipodia dynamics during epithelial cell-cell adhesion. *Dev. Cell*. 3:259–270. doi:10.1016/S1534-5807(02)00216-2

Farge, E. 2003. Mechanical induction of Twist in the *Drosophila* foregut/stomodeal primordium. *Curr. Biol.* 13:1365–1377. doi:10.1016/S0960-9822(03)00576-1

Fernandez-Gonzalez, R., S. de Matos Simões, J.C. Röper, S. Eaton, and J.A. Zallen. 2009. Myosin II dynamics are regulated by tension in intercalating cells. *Dev. Cell*. 17:736–743. doi:10.1016/j.devcel.2009.09.003

Fristrom, D. 1988. The cellular basis of epithelial morphogenesis. A review. *Tissue Cell*. 20:645–690. doi:10.1016/0040-8166(88)90015-8

Fujita, Y., G. Krause, M. Scheffner, D. Zechner, H.E. Leddy, J. Behrens, T. Sommer, and W. Birchmeier. 2002. Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. *Nat. Cell Biol.* 4:222–231. doi:10.1038/ncb758

Gavard, J., M. Lambert, I. Grosheva, V. Marthiens, T. Irinopoulou, J.F. Riou, A. Bershadsky, and R.M. Mège. 2004. Lamellipodium extension and cadherin adhesion: two cell responses to cadherin activation relying on distinct signalling pathways. *J. Cell Sci.* 117:257–270. doi:10.1242/jcs.00857

Georgiou, M., and B. Baum. 2010. Polarity proteins and Rho GTPases cooperate to spatially organise epithelial actin-based protrusions. *J. Cell Sci.* 123:1089–1098. doi:10.1242/jcs.060772

Georgiou, M., E. Marinari, J. Burden, and B. Baum. 2008. Cdc42, Par6, and aPKC regulate Arp2/3-mediated endocytosis to control local adherens junction stability. *Curr. Biol.* 18:1631–1638. doi:10.1016/j.cub.2008.09.029

Giuliani, C., F. Troglio, Z. Bai, F.B. Patel, A. Zucconi, M.G. Malabarba, A. Disanza, T.B. Stradal, G. Cassata, S. Confalonieri, et al. 2009. Requirements for F-BAR proteins TOCA-1 and TOCA-2 in actin dynamics and membrane trafficking during *Caenorhabditis elegans* oocyte growth and embryonic epidermal morphogenesis. *PLoS Genet.* 5:e1000675. doi:10.1371/journal.pgen.1000675

Gorfinkiel, N., G.B. Blanchard, R.J. Adams, and A. Martinez Arias. 2009. Mechanical control of global cell behaviour during dorsal closure in *Drosophila*. *Development*. 136:1889–1898. doi:10.1242/dev.030866

Grindstaff, K.K., C. Yeaman, N. Anandasabapathy, S.C. Hsu, E. Rodriguez-Boulan, R.H. Scheller, and W.J. Nelson. 1998. Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell*. 93:731–740. doi:10.1016/S0092-8674(00)81435-X

Gumbiner, B.M. 2005. Regulation of cadherin-mediated adhesion in morphogenesis. *Nat. Rev. Mol. Cell Biol.* 6:622–634. doi:10.1038/nrm1699

Gumbiner, B., B. Stevenson, and A. Grimaldi. 1988. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. *J. Cell Biol.* 107:1575–1587. doi:10.1083/jcb.107.4.1575

Harris, K.P., and U. Tepass. 2008. Cdc42 and Par proteins stabilize dynamic adherens junctions in the *Drosophila* neuroectoderm through regulation of apical endocytosis. *J. Cell Biol.* 183:1129–1143. doi:10.1083/jcb.200807020

Harris, T.J., and M. Peifer. 2004. Adherens junction-dependent and -independent steps in the establishment of epithelial cell polarity in *Drosophila*. *J. Cell Biol.* 167:135–147. doi:10.1083/jcb.200406024

Harris, T.J., and M. Peifer. 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

Hayashi, T., and R.W. Carthew. 2004. Surface mechanics mediate pattern formation in the developing retina. *Nature*. 431:647–652. doi:10.1038/nature02952

He, L., X. Wang, H.L. Tang, and D.J. Montell. 2010. Tissue elongation requires oscillating contractions of a basal actomyosin network. *Nat. Cell Biol.* 12:1133–1142. doi:10.1038/ncb2124

Hill, E., J. van Der Kaay, C.P. Downes, and E. Smythe. 2001. The role of dynamin and its binding partners in coated pit invagination and scission. *J. Cell Biol.* 152:309–323. doi:10.1083/jcb.152.2.309

Hong, S., R.B. Troyanovsky, and S.M. Troyanovsky. 2010. Spontaneous assembly and active disassembly balance adherens junction homeostasis. *Proc. Natl. Acad. Sci. USA*. 107:3528–3533. doi:10.1073/pnas.0911027107

Hoshino, T., K. Shimizu, T. Honda, T. Kawakatsu, T. Fukuyama, T. Nakamura, M. Matsuda, and Y. Takai. 2004. A novel role of nectins in inhibition of the E-cadherin-induced activation of Rac and formation of cell-cell adherens junctions. *Mol. Biol. Cell*. 15:1077–1088. doi:10.1091/mbc.E03-05-0321

Hsu, S.C., C.D. Hazuka, D.L. Foletti, and R.H. Scheller. 1999. Targeting vesicles to specific sites on the plasma membrane: the role of the sec6/8 complex. *Trends Cell Biol.* 9:150–153. doi:10.1016/S0962-8924(99)01516-0

Irvine, K.D., and E. Wieschaus. 1994. Cell intercalation during *Drosophila* germband extension and its regulation by pair-rule segmentation genes. *Development*. 120:827–841.

Ishiyama, N., S.H. Lee, S. Liu, G.Y. Li, M.J. Smith, L.F. Reichardt, and M. Ikura. 2010. Dynamic and static interactions between p120 catenin and E-cadherin regulate the stability of cell-cell adhesion. *Cell*. 141:117–128. doi:10.1016/j.cell.2010.01.017

Itoh, T., K.S. Erdmann, A. Roux, B. Habermann, H. Werner, and P. De Camilli. 2005. Dynamin and the actin cytoskeleton cooperatively regulate plasma membrane invagination by BAR and F-BAR proteins. *Dev. Cell*. 9:791–804. doi:10.1016/j.devcel.2005.11.005

Izumi, G., T. Sakisaka, T. Baba, S. Tanaka, K. Morimoto, and Y. Takai. 2004. Endocytosis of E-cadherin regulated by Rac and Cdc42 small G proteins through IQGAP1 and actin filaments. *J. Cell Biol.* 166:237–248. doi:10.1083/jcb.200401078

Jacinto, A., and P. Martin. 2001. Morphogenesis: unravelling the cell biology of hole closure. *Curr. Biol.* 11:R705–R707. doi:10.1016/S0960-9822(01)00414-6

Jacinto, A., W. Wood, T. Balayo, M. Turmaine, A. Martinez-Arias, and P. Martin. 2000. Dynamic actin-based epithelial adhesion and cell matching during *Drosophila* dorsal closure. *Curr. Biol.* 10:1420–1426. doi:10.1016/S0960-9822(00)00796-X

Kawasaki, Y., R. Sato, and T. Akiyama. 2003. Mutated APC and Asef are involved in the migration of colorectal tumour cells. *Nat. Cell Biol.* 5:211–215. doi:10.1038/ncb937

Kobielak, A., H.A. Pasolli, and E. Fuchs. 2003. Mammalian formin-1 participates in adherens junctions and polymerization of linear actin cables. *Nat. Cell Biol.* 6:21–30. doi:10.1038/ncb1075

Kovacs, E.M., M. Goodwin, R.G. Ali, A.D. Paterson, and A.S. Yap. 2002. Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. *Curr. Biol.* 12:379–382. doi:10.1016/S0960-9822(02)00661-9

Krendel, M.F., and E.M. Bonder. 1999. Analysis of actin filament bundle dynamics during contact formation in live epithelial cells. *Cell Motil. Cytoskeleton*. 43:296–309. doi:10.1002/(SICI)1097-0169(1999)43:4<296::AID-CM3>3.0.CO;2-U

Krieg, M., Y. Arboleda-Estudillo, P.H. Puech, J. Käfer, F. Graner, D.J. Müller, and C.P. Heisenberg. 2008. Tensile forces govern germ-layer organization in zebrafish. *Nat. Cell Biol.* 10:429–436. doi:10.1038/ncb1705

Lambert, M., D. Choquet, and R.M. Mège. 2002. Dynamics of ligand-induced, Rac1-dependent anchoring of cadherins to the actin cytoskeleton. *J. Cell Biol.* 157:469–479. doi:10.1083/jcb.200107104

Landsberg, K.P., R. Farhadifar, J. Ranft, D. Umetsu, T.J. Widmann, T. Bittig, A. Said, F. Jülicher, and C. Dahmann. 2009. Increased cell bond tension governs cell sorting at the *Drosophila* anteroposterior compartment boundary. *Curr. Biol.* 19:1950–1955. doi:10.1016/j.cub.2009.10.021

Langevin, J., M.J. Morgan, J.B. Sibarita, S. Aresta, M. Murthy, T. Schwarz, J. Camonis, and Y. Bellaïche. 2005. *Drosophila* exocyst components Sec5, Sec6, and Sec15 regulate DE-Cadherin trafficking from recycling endosomes to the plasma membrane. *Dev. Cell*. 9:365–376. doi:10.1016/j.devcel.2005.07.013

Le, T.L., A.S. Yap, and J.L. Stow. 1999. Recycling of E-cadherin: a potential mechanism for regulating cadherin dynamics. *J. Cell Biol.* 146:219–232.

le Duc, Q., Q. Shi, I. Blonk, A. Sonnenberg, N. Wang, D. Leckband, and J. de Rooij. 2010. Vinculin potentiates E-cadherin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II–dependent manner. *J. Cell Biol.* 189:1107–1115. doi:10.1083/jcb.201001149

Leibfried, A., R. Fricke, M.J. Morgan, S. Bogdan, and Y. Bellaiche. 2008. *Drosophila* Cip4 and WASp define a branch of the Cdc42-Par6-aPKC pathway regulating E-cadherin endocytosis. *Curr. Biol.* 18:1639–1648. doi:10.1016/j.cub.2008.09.063

Li, B.X., A.K. Satoh, and D.F. Ready. 2007. Myosin V, Rab11, and dRip11 direct apical secretion and cellular morphogenesis in developing *Drosophila* photoreceptors. *J. Cell Biol.* 177:659–669. doi:10.1083/jcb.200610157

Liu, Z., J.L. Tan, D.M. Cohen, M.T. Yang, N.J. Sniadecki, S.A. Ruiz, C.M. Nelson, and C.S. Chen. 2010. Mechanical tugging force regulates the size of cell-cell junctions. *Proc. Natl. Acad. Sci. USA*. 107:9944–9949. doi:10.1073/pnas.0914547107

Lock, J.G., and J.L. Stow. 2005. Rab11 in recycling endosomes regulates the sorting and basolateral transport of E-cadherin. *Mol. Biol. Cell*. 16:1744–1755. doi:10.1091/mbc.E04-10-0867

Manning, M.L., R.A. Foty, M.S. Steinberg, and E.M. Schoetz. 2010. Coaction of intercellular adhesion and cortical tension specifies tissue surface tension. *Proc. Natl. Acad. Sci. USA*. 107:12517–12522. doi:10.1073/pnas.1003743107

Martin, A.C., M.D. Welch, and D.G. Drubin. 2006. Arp2/3 ATP hydrolysis-catalysed branch dissociation is critical for endocytic force generation. *Nat. Cell Biol.* 8:826–833. doi:10.1038/ncb1443

Martin, A.C., M. Kaschube, and E.F. Wieschaus. 2009. Pulsed contractions of an actin-myosin network drive apical constriction. *Nature*. 457:495–499. doi:10.1038/nature07522

Mertens, A.E., T.P. Rygiel, C. Olivo, R. van der Kammen, and J.G. Collard. 2005. The Rac activator Tiam1 controls tight junction biogenesis in keratinocytes through binding to and activation of the Par polarity complex. *J. Cell Biol.* 170:1029–1037. doi:10.1083/jcb.200502129

Monier, B., A. Pélissier-Monier, A.H. Brand, and B. Sanson. 2010. An actomyosin-based barrier inhibits cell mixing at compartmental boundaries in *Drosophila* embryos. *Nat. Cell Biol.* 12:60–65. doi:10.1038/ncb2005

Montell, D.J. 2008. Morphogenetic cell movements: diversity from modular mechanical properties. *Science*. 322:1502–1505. doi:10.1126/science.1164073

Müller, H.A., and E. Wieschaus. 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

Nakagawa, M., M. Fukata, M. Yamaga, N. Itoh, and K. Kaibuchi. 2001. Recruitment and activation of Rac1 by the formation of E-cadherin-mediated cell-cell adhesion sites. *J. Cell Sci.* 114:1829–1838.

Nakayama, Y., J.M. Shivas, D.S. Poole, J.M. Squirrell, J.M. Kulkoski, J.B. Schleede, and A.R. Skop. 2009. Dynamin participates in the maintenance of anterior polarity in the *Caenorhabditis elegans* embryo. *Dev. Cell*. 16:889–900. doi:10.1016/j.devcel.2009.04.009

Nikolaidou, K.K., and K. Barrett. 2004. A Rho GTPase signaling pathway is used reiteratively in epithelial folding and potentially selects the outcome of Rho activation. *Curr. Biol.* 14:1822–1826. doi:10.1016/j.cub.2004.09.080

Oda, H., and S. Tsukita. 1999. Dynamic features of adherens junctions during *Drosophila* embryonic epithelial morphogenesis revealed by a Dalpha-catenin-GFP fusion protein. *Dev. Genes Evol.* 209:218–225. doi:10.1007/s004270050246

Okada, T., L. You, and F.G. Giancotti. 2007. Shedding light on Merlin's wizardry. *Trends Cell Biol.* 17:222–229. doi:10.1016/j.tcb.2007.03.006

Otani, T., T. Ichii, S. Aono, and M. Takeichi. 2006. Cdc42 GEF Tuba regulates the junctional configuration of simple epithelial cells. *J. Cell Biol.* 175:135–146. doi:10.1083/jcb.200605012

Palacios, F., J.S. Tushir, Y. Fujita, and C. D'Souza-Schorey. 2005. Lysosomal targeting of E-cadherin: a unique mechanism for the down-regulation of cell-cell adhesion during epithelial to mesenchymal transitions. *Mol. Cell. Biol.* 25:389–402. doi:10.1128/MCB.25.1.389-402.2005

Perez-Moreno, M., C. Jamora, and E. Fuchs. 2003. Sticky business: orchestrating cellular signals at adherens junctions. *Cell*. 112:535–548. doi:10.1016/S0092-8674(03)00108-9

Pilot, F., J.M. Philippe, C. Lemmers, and T. Lecuit. 2006. Spatial control of actin organization at adherens junctions by a synaptotagmin-like protein Btsz. *Nature*. 442:580–584. doi:10.1038/nature04935

Pollard, T.D. 2007. Regulation of actin filament assembly by Arp2/3 complex and formins. *Annu. Rev. Biophys. Biomol. Struct.* 36:451–477. doi:10.1146/annurev.biophys.35.040405.101936

Raich, W.B., C. Agbunag, and J. Hardin. 1999. Rapid epithelial-sheet sealing in the *Caenorhabditis elegans* embryo requires cadherin-dependent filopodial priming. *Curr. Biol.* 9:1139–1146. doi:10.1016/S0960-9822(00)80015-9

Rauzi, M., P.F. Lenne, and T. Lecuit. 2010. Planar polarized actomyosin contractile flows control epithelial junction remodelling. *Nature*. 468:1110–1114. doi:10.1038/nature09566

Ribeiro, C., A. Ebner, and M. Affolter. 2002. In vivo imaging reveals different cellular functions for FGF and Dpp signaling in tracheal branching morphogenesis. *Dev. Cell*. 2:677–683. doi:10.1016/S1534-5807(02)00171-5

Ribeiro, C., M. Neumann, and M. Affolter. 2004. Genetic control of cell intercalation during tracheal morphogenesis in *Drosophila*. *Curr. Biol.* 14:2197–2207. doi:10.1016/j.cub.2004.11.056

Ridley, A.J. 2006. Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. *Trends Cell Biol.* 16:522–529. doi:10.1016/j.tcb.2006.08.006

Ridley, A.J., H.F. Paterson, C.L. Johnston, D. Diekmann, and A. Hall. 1992. The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell*. 70:401–410. doi:10.1016/0092-8674(92)90164-8

Rivard, N. 2009. Phosphatidylinositol 3-kinase: a key regulator in adherens junction formation and function. *Front. Biosci.* 14:510–522. doi:10.2741/3259

Roeth, J.F., J.K. Sawyer, D.A. Wilner, and M. Peifer. 2009. Rab11 helps maintain apical crumbs and adherens junctions in the *Drosophila* embryonic ectoderm. *PLoS ONE*. 4:e7634. doi:10.1371/journal.pone.0007634

Rolo, A., P. Skoglund, and R. Keller. 2009. Morphogenetic movements driving neural tube closure in *Xenopus* require myosin IIB. *Dev. Biol.* 327:327–338. doi:10.1016/j.ydbio.2008.12.009

Sahai, E., and C.J. Marshall. 2002. ROCK and Dia have opposing effects on adherens junctions downstream of Rho. *Nat. Cell Biol.* 4:408–415. doi:10.1038/ncb796

Sawyer, J.K., N.J. Harris, K.C. Slep, U. Gaul, and M. Peifer. 2009. The *Drosophila* afadin homologue Canoe regulates linkage of the actin cytoskeleton to adherens junctions during apical constriction. *J. Cell Biol.* 186:57–73. doi:10.1083/jcb.200904001

Shaye, D.D., J. Casanova, and M. Llimargas. 2008. Modulation of intracellular trafficking regulates cell intercalation in the *Drosophila* trachea. *Nat. Cell Biol.* 10:964–970. doi:10.1038/ncb1756

Shewan, A.M., M. Maddugoda, A. Kraemer, S.J. Stehbens, S. Verma, E.M. Kovacs, and A.S. Yap. 2005. Myosin 2 is a key Rho kinase target necessary for the local concentration of E-cadherin at cell-cell contacts. *Mol. Biol. Cell*. 16:4531–4542. doi:10.1091/mbc.E05-04-0330

Shindo, M., H. Wada, M. Kaido, M. Tateno, T. Aigaki, L. Tsuda, and S. Hayashi. 2008. Dual function of Src in the maintenance of adherens junctions during tracheal epithelial morphogenesis. *Development*. 135:1355–1364. doi:10.1242/dev.015982

Smutny, M., H.L. Cox, J.M. Leerberg, E.M. Kovacs, M.A. Conti, C. Ferguson, N.A. Hamilton, R.G. Parton, R.S. Adelstein, and A.S. Yap. 2010. Myosin II isoforms identify distinct functional modules that support integrity of the epithelial zonula adherens. *Nat. Cell Biol.* 12:696–702. doi:10.1038/ncb2072

Sokac, A.M., C. Co, J. Taunton, and W. Bement. 2003. Cdc42-dependent actin polymerization during compensatory endocytosis in *Xenopus* eggs. *Nat. Cell Biol.* 5:727–732. doi:10.1038/ncb1025

Solon, J., A. Kaya-Copur, J. Colombelli, and D. Brunner. 2009. Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. *Cell*. 137:1331–1342. doi:10.1016/j.cell.2009.03.050

Takaishi, K., T. Sasaki, M. Kato, W. Yamochi, S. Kuroda, T. Nakamura, M. Takeichi, and Y. Takai. 1994. Involvement of Rho p21 small GTP-binding protein and its regulator in the HGF-induced cell motility. *Oncogene*. 9:273–279.

Takaishi, K., T. Sasaki, H. Kotani, H. Nishioka, and Y. Takai. 1997. Regulation of cell–cell adhesion by rac and rho small G proteins in MDCK cells. *J. Cell Biol.* 139:1047–1059. doi:10.1083/jcb.139.4.1047

Takenawa, T., and H. Miki. 2001. WASP and WAVE family proteins: key molecules for rapid rearrangement of cortical actin filaments and cell movement. *J. Cell Sci.* 114:1801–1809.

Tan, J.L., S. Ravid, and J.A. Spudich. 1992. Control of nonmuscle myosins by phosphorylation. *Annu. Rev. Biochem.* 61:721–759. doi:10.1146/annurev.bi.61.070192.003445

Tanaka-Matakatsu, M., T. Uemura, H. Oda, M. Takeichi, and S. Hayashi. 1996. Cadherin-mediated cell adhesion and cell motility in *Drosophila* trachea regulated by the transcription factor Escargot. *Development*. 122:3697–3705.

Tepass, U., E. Gruszynski-DeFeo, T.A. Haag, L. Omatyar, T. Török, and V. Hartenstein. 1996. shotgun encodes *Drosophila* E-cadherin and is preferentially required during cell rearrangement in the neurectoderm and other morphogenetically active epithelia. *Genes Dev.* 10:672–685. doi:10.1101/gad.10.6.672

Toyoshima, M., N. Tanaka, J. Aoki, Y. Tanaka, K. Murata, M. Kyuuma, H. Kobayashi, N. Ishii, N. Yaegashi, and K. Sugamura. 2007. Inhibition of tumor growth and metastasis by depletion of vesicular sorting protein Hrs: its regulatory role on E-cadherin and beta-catenin. *Cancer Res.* 67:5162–5171. doi:10.1158/0008-5472.CAN-06-2756

Uemura, T., H. Oda, R. Kraut, S. Hayashi, Y. Kotaoka, and M. Takeichi. 1996. Zygotic *Drosophila* E-cadherin expression is required for processes of dynamic epithelial cell rearrangement in the *Drosophila* embryo. *Genes Dev.* 10:659–671. doi:10.1101/gad.10.6.659

Vasioukhin, V., C. Bauer, M. Yin, and E. Fuchs. 2000. Directed actin polymerization is the driving force for epithelial cell-cell adhesion. *Cell*. 100:209–219. doi:10.1016/S0092-8674(00)81559-7

Wallace, S.W., J. Durgan, D. Jin, and A. Hall. 2010. Cdc42 regulates apical junction formation in human bronchial epithelial cells through PAK4 and Par6B. *Mol. Biol. Cell*. 21:2996–3006. doi:10.1091/mbc.E10-05-0429

Warner, S.J., and G.D. Longmore. 2009a. Cdc42 antagonizes Rho1 activity at adherens junctions to limit epithelial cell apical tension. *J. Cell Biol.* 187:119–133. doi:10.1083/jcb.200906047

Warner, S.J., and G.D. Longmore. 2009b. Distinct functions for Rho1 in maintaining adherens junctions and apical tension in remodeling epithelia. *J. Cell Biol.* 185:1111–1125. doi:10.1083/jcb.200901029

Wei, S.Y., L.M. Escudero, F. Yu, L.H. Chang, L.Y. Chen, Y.H. Ho, C.M. Lin, C.S. Chou, W. Chia, J. Modolell, and J.C. Hsu. 2005. Echinoid is a component of adherens junctions that cooperates with DE-Cadherin to mediate cell adhesion. *Dev. Cell*. 8:493–504. doi:10.1016/j.devcel.2005.03.015

Whitehead, J., D. Vignjevic, C. Fütterer, E. Beaurepaire, S. Robine, and E. Farge. 2008. Mechanical factors activate beta-catenin-dependent oncogene expression in APC mouse colon. *HFSP J*. 2:286–294. doi:10.2976/1.2955566

Yamada, S., and W.J. Nelson. 2007. Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell–cell adhesion. *J. Cell Biol.* 178:517–527. doi:10.1083/jcb.200701058

Yamanaka, T., Y. Horikoshi, A. Suzuki, Y. Sugiyama, K. Kitamura, R. Maniwa, Y. Nagai, A. Yamashita, T. Hirose, H. Ishikawa, and S. Ohno. 2001. PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex. *Genes Cells*. 6:721–731. doi:10.1046/j.1365-2443.2001.00453.x

Yarar, D., C.M. Waterman-Storer, and S.L. Schmid. 2005. A dynamic actin cytoskeleton functions at multiple stages of clathrin-mediated endocytosis. *Mol. Biol. Cell*. 16:964–975. doi:10.1091/mbc.E04-09-0774

Yeaman, C., K.K. Grindstaff, and W.J. Nelson. 2004. Mechanism of recruiting Sec6/8 (exocyst) complex to the apical junctional complex during polarization of epithelial cells. *J. Cell Sci.* 117:559–570. doi:10.1242/jcs.00893

Yonemura, S., Y. Wada, T. Watanabe, A. Nagafuchi, and M. Shibata. 2010. alpha-Catenin as a tension transducer that induces adherens junction development. *Nat. Cell Biol.* 12:533–542. doi:10.1038/ncb2055

Zallen, J.A., and E. Wieschaus. 2004. Patterned gene expression directs bipolar planar polarity in *Drosophila*. *Dev. Cell*. 6:343–355. doi:10.1016/S1534-5807(04)00060-7