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

Buzz Baum[1] and Marios Georgiou[2]

[1]Medical Research Council Laboratory for Molecular Cell Biology, University College London, London WC1E 6BT, England, UK
[2]School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, England, UK

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.

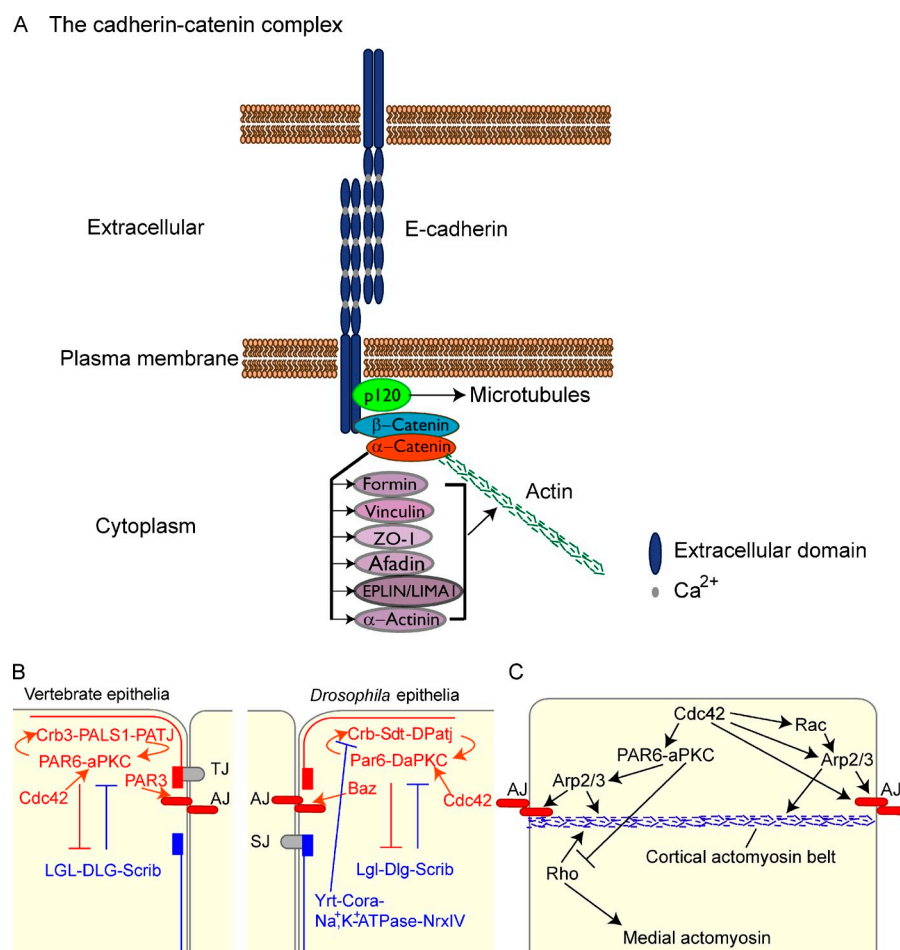## The basic features of E-cadherin–catenin-based AJs

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,

Correspondence to Marios Georgiou: marios.georgiou@nottingham.ac.uk
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   The cadherin-catenin complex

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
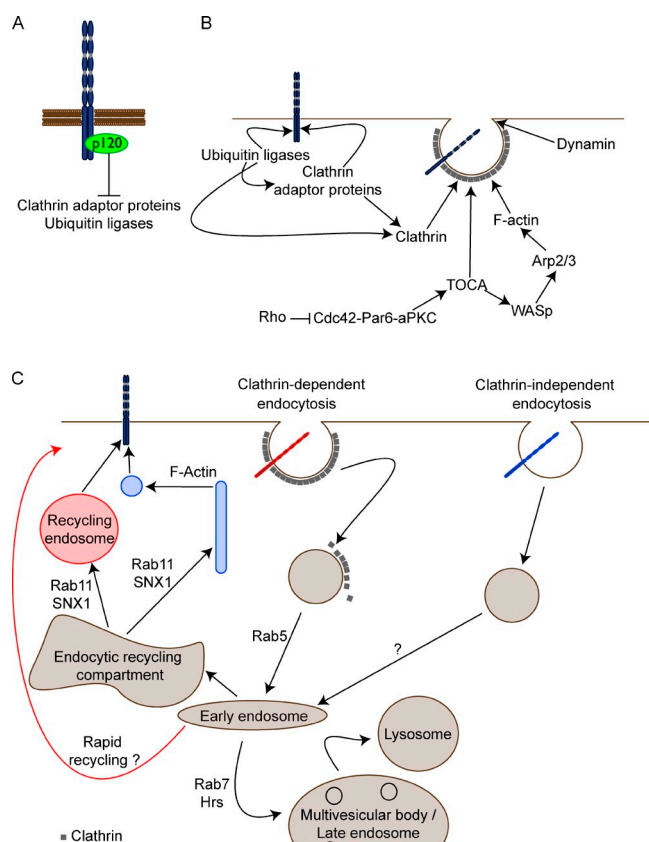
Figure 2. **The regulation of E-cadherin recycling.** (A) p120 catenin inhibits cadherin endocytosis and degradation by preventing the association of adaptor complexes with the cadherin juxtamembrane intracellular region (Fujita et al., 2002; Ishiyama et al., 2010), which prevents cadherin recruitment 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).

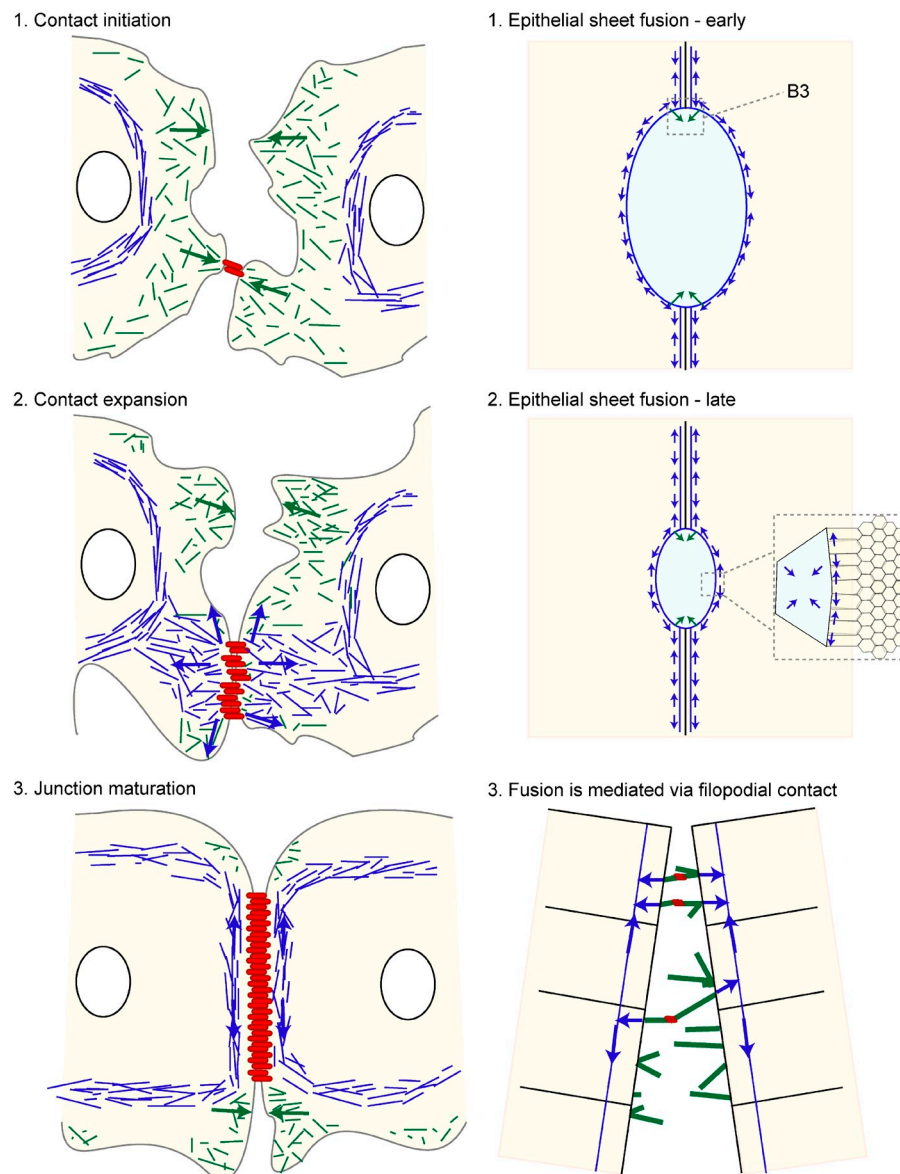## Regulation of E-cadherin and AJs by GTPases

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.

A   In vitro lamellipodia facilitated contact

1. Contact initiation

2. Contact expansion

3. Junction maturation

B   In vivo filopodia facilitated contact

1. Epithelial sheet fusion - early

B3

2. Epithelial sheet fusion - late

3. Fusion is mediated via filopodial contact

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 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).
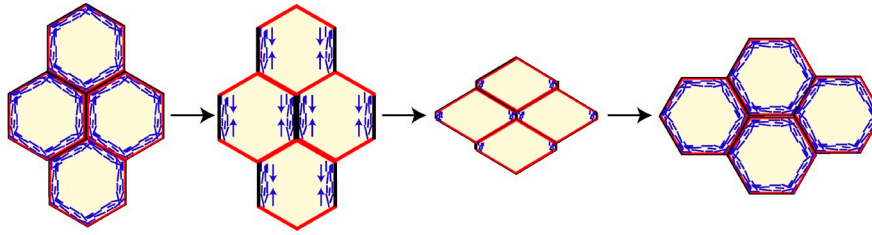
AJ remodeling as a driving force for morphogenesis

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.
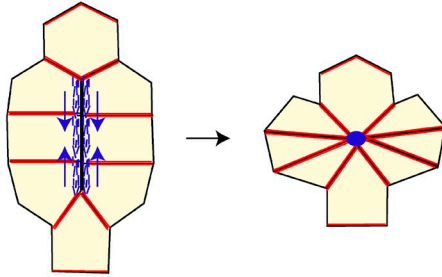
During *Drosophila* germband extension, the tissue doubles its length and reduces its width by half (Irvine and Wieschaus, 1994) as the result of changes in individual cell–cell contacts that results in cell neighbor exchange within the tissue and is a direct result of junction remodeling (Fig. 4, A 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.
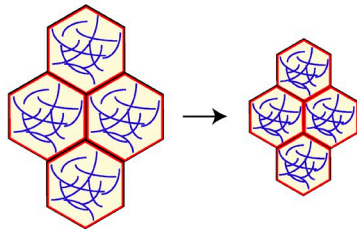
A Contraction of single cell boundaries

B Contraction of multicellular cell boundaries via actomyosin cables

C Apical constriction via an actomyosin web-like network

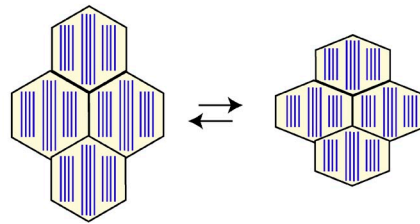## D Directional basal constriction via actomyosin parallel bundles

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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