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Sticky Business: Orchestrating Cellular Signals at Adherens Junctions

Review

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Cohesive sheets of epithelial cells are a fundamental feature of multicellular organisms and are largely a product of the varied functions of adherens junctions. These junctions and their cytoskeletal associations contribute heavily to the distinct shapes, polarity, spatially oriented mitotic spindle planes, and cellular movements of developing tissues. Deciphering the underlying mechanisms that govern these conserved cellular rearrangements is a prerequisite to understanding vertebrate morphogenesis.

Introduction

In order to function as a tissue, epithelial cells must have the right shape and structure to pack together with their neighbors. To undergo self-renewal while maintaining tissue anatomy, simple and stratified epithelia possess a single layer of dividing cells, orienting their mitotic spindles parallel to the underlying basement membrane (Figure 1). In stratified tissues such as the epidermis, a parallel plane of mitoses confines the transiently dividing cells to a single layer. To stratify and execute a program of terminal differentiation, cells must either rotate their mitotic plane 90 degrees and divide asymmetrically, or otherwise weaken cell substratum and cell-cell attachments to exit the basal layer, and migrate toward the skin surface. To repair a skin injury, epidermal sheets at the wound edge must move in an orchestrated manner, as occurs in developmental processes such as dorsal closure in fly embryos. During all of these processes, the exquisite cellular architecture of epithelia is achieved and maintained through dynamic permutations of protein complexes at cell-cell junctions.

In mammals, adhesion between epithelial cells is generally mediated by three types of junctions: tight junctions (TJs), adherens junctions (AJs), and desmosomes, which together constitute the Intercellular Junctional Complex (Figure 2). The complexes contain transmembrane receptors, usually glycoproteins that mediate binding at the extracellular surface and determine the specificity of the intracellular response. The associated cytoplasmic proteins of these receptors structurally link them to the cytoskeleton, thereby establishing molecular lines of communication to other cell-cell junctions and to cell-substratum junctions. The linkage of cell-cell junctions to the cytoskeleton allows single cells of an epithelial sheet to function as a coordinated tissue. Additional companion proteins connect structural and signaling elements, and thus intercellular junctions function to integrate a number of cellular processes ranging from cytoskeletal dynamics to proliferation, transcription, and differentiation.

Without diminishing the importance of other cellular junctions (reviewed in Kowalczyk et al., 1999; Tsukita et al., 2001), recent evidence has uncovered a key role for AJs not only in directing coordinated cellular organization and movements within epithelia, but also in transmitting information from the environment to the interior of cells. AJs are cadherin-dependent adhesive structures that are intricately linked to the actin microfilament network. AJs were originally identified by ultrastructural analysis, which revealed electron dense plaques of closely apposed membranes between epithelial cells. The ancient origins of AJs are likely to extend across the eukaryotic kingdom to include even single-cell organisms such as yeast. While yeast cells have no use for connecting to their neighbors, they do coordinate cytoskeletal dynamics, spindle polarity, and cell polarity, and thus employ many of the same features of AJs in multicellular organisms. During the past few years, elucidation of the assembly, functions, and dynamics of AJs have unveiled crucial roles in governing morphogenetic and patterning processes. Although the molecular and regulatory mechanisms are not fully understood, novel signaling events at AJ-cytoskeletal intersections have been discovered. These insights reveal how defects in AJs can contribute to a plethora of developmental defects and human disease.

Biochemical Organization of Cadherin/Catenin Complexes and Their Links to the Actin Cytoskeleton

The transmembrane core of AJs consists of cadherins, which cluster at sites of cell-cell contact in most solid tissues. E-cadherin, the prototype and best-characterized member of the family, is primarily expressed in epithelia. The extracellular portion of classical cadherins consists of five ectodomains, which bind calcium and adopt a rod-like template for homophilic, albeit relatively weak, interactions with E-cadherin molecules on the surface of neighboring cells. The sequential binding of proteins to the cytoplasmic tail physically bridges the cadherin receptor to the cytoskeleton and other signaling modules and results in a mature AJ (Yonemura et al., 1995; Adams and Nelson, 1998). The multiple levels of protein interaction are potential sites for the exquisite regulation of AJ complexes required during normal development.

The highly conserved, ~150 amino acid cytoplasmic tail of classical cadherins possesses a binding site for either β-catenin or γ-catenin (plakoglobin), members of the superfamily of armadillo repeat proteins (Huber and Weis, 2001; Figure 3). Binding of β-catenin's 12 repeats of 42 amino acid "armadillo" sequences to the cytoplasmic cadherin tail lends structure to the cadherin protein and is required for the transport of the newly

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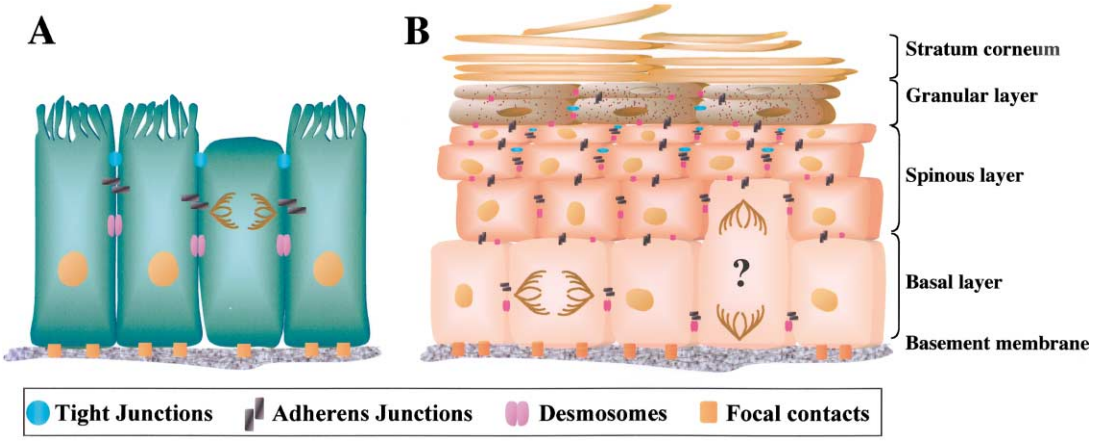

Figure 1. Organization of Simple and Stratified Epithelia

(A) Simple epithelia comprised of one layer of cells attaches to the basement membranes by focal contacts (orange squares) and to adjacent cells via adherens junctions (black rectangles) and desmosomes (pink ovals). Tight junctions (blue circles) contribute to the maintenance of apical-basolateral polarity. The plane of the mitotic spindles aligns perpendicular to the basement membrane allowing lateral expansion of the cells.

(B) The four layers of mammalian epidermis as a model of stratified squamous epithelia. Adherens junctions (black rectangles) and desmosomes (pink ovals) attach cells to each other, and integrins in focal contacts (orange squares) attach cells of the basal layer to the basement membrane. Tight junctions (blue circles) appear in the later spinous layers through the granular layer. Dividing cells of the basal layer have the spindle plane parallel to the basement membrane to allow lateral expansion of the basal layer. In embryonic skin, there are also dividing cells with the mitotic spindle plane perpendicular to the basement membrane, which allows daughter cells to contribute to the suprabasal layers. Whether this mechanism is responsible for detaching a basal cell and inducing terminal differentiation has not been unequivocally established.

synthesized E-cadherin to the plasma membrane (Chen et al., 1999). The affinity for this interaction is increased by phosphorylation of several key serine residues in the cadherin tail, and reduced by phosphorylation of β-catenin Y654, a known site of action for activated growth factor receptor tyrosine kinases (Huber and Weis, 2001). Thus, through posttranslational modifications, the strength of the AJ complex can be tailored and modified to suit the particular needs of the epithelial cell within the context of its tissue.

The ordered structure between E-cadherin and β-catenin or plakoglobin is thought to initiate an association between residues within the N-terminal head domain of the armadillo proteins and α-catenin, a protein capable of binding to F actin binding proteins. α-Catenin is normally found as a homodimer in solution, which

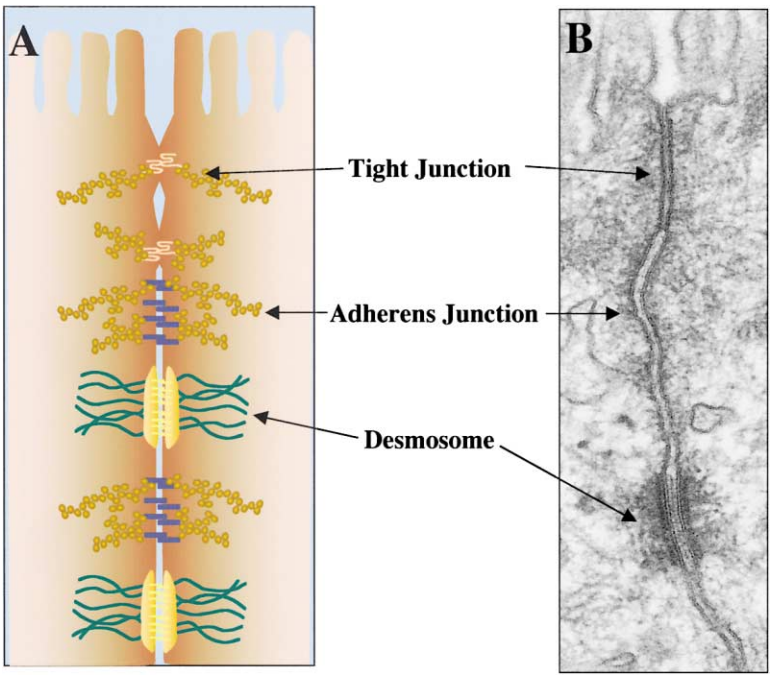

Figure 2. Composition of Three Types of Intercellular Junctions

(A) Diagram of the three major types of intercellular junctions in epithelial cells. Tight junctions are composed of transmembrane proteins linked to the actin cytoskeleton and constitute a physical barrier between the apical and basolateral regions of the cells. Adherens junctions are formed by homophilic interaction of transmembrane cadherins that are linked to the actin cytoskeleton. Desmosomes are formed by interactions between desmosomal cadherins linked to intermediate filaments.

(B) Electron micrograph depicting the ultrastructure of adherens junctions, desmosomes, and tight junctions between two murine intestinal epithelial cells (courtesy of Dr. Amalia Pasolli, The Rockefeller University).

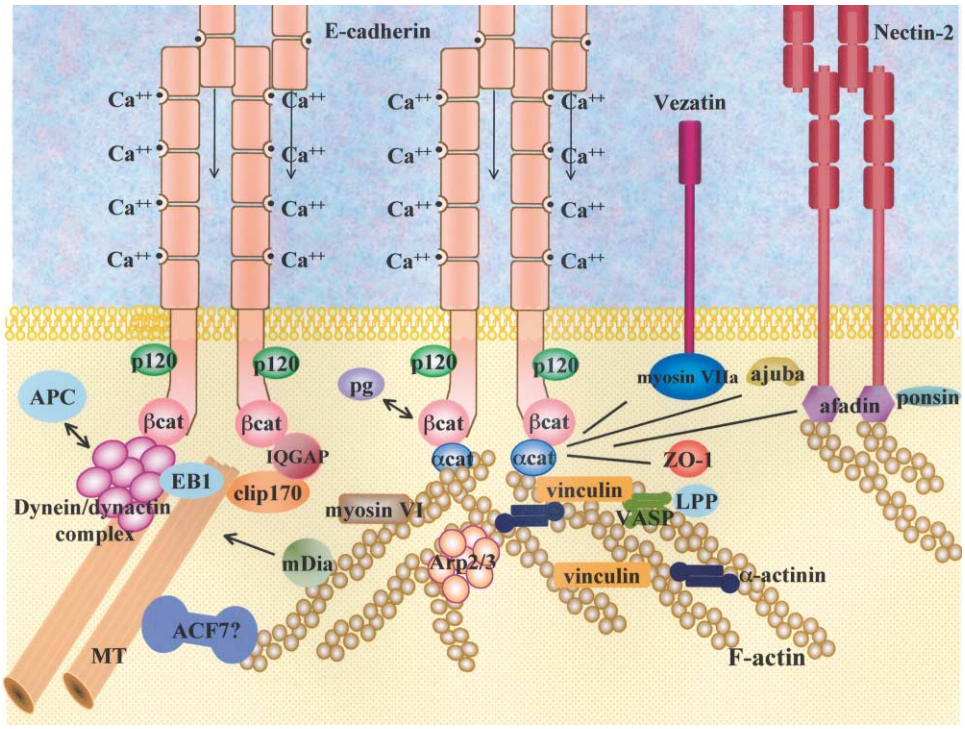

Figure 3. Protein Interactions at Adherens Junctions

E-cadherin-based adhesion junctions can associate with the actin and microtubule cytoskeletons, through associated cytoskeletal proteins. E-cadherin's direct interacting partner, β-catenin, binds to several proteins. It associates with α-catenin and links cadherin/catenin complexes to the actin cytoskeleton. Its ability to interact with some microtubule-associated proteins such as IQGAP, APC, and the dynein/dynactin complex may link E-cadherin to the microtubule network. Double arrows mean that both proteins can compete for the same site.

dissociates to bind the E-cadherin/β-catenin complex at the plasma membrane as a monomer (Koslov et al., 1997). This interaction can be modulated by the association of the E-cadherin/β-catenin complex with other proteins such as IQGAP, which blocks the binding of α-catenin to β-catenin (Kuroda et al., 1998).

α-Catenin is a central player in nucleating the assembly of a number of proteins that link E-cadherin/β-catenin complexes to F actin, a process critical not only for stabilizing intercellular junctions but also for coordinating actin dynamics at these sites (Vasioukhin et al., 2000, 2001). α-catenin can associate with F actin by direct binding through its C-terminal domain, but it can also associate directly with vinculin and zyxin family members, which in turn can bind actin and/or recruit members of the Ena/Vasp families of profilin-actin binding proteins. Biochemical studies have uncovered what appears to be a phospholipid PIP_2 (phosphatidylinositol 4,5-bisphosphate)-mediated interaction between the head and tail domains of vinculin (Johnson and Craig, 1995; Gilmore and Burridge, 1996). This head-tail interaction may block access to vinculin's Vasp and F actin binding sites, providing a potential means of controlling the association between these proteins and AJs. Finally, α-catenin can partner with the protein afadin, which also binds to F actin (Ikeda et al., 1999; Pokutta et al., 2002).

Why do AJs have so many potential binding surfaces for actin? An answer may be found in the diversity of cytoskeletal dynamics required for epithelial cells within tissues to respond to particular environmental cues. Recently, videomicroscopy of calcium-stimulated cells expressing GFP actin or GFP-cadherin has been employed to explore actin-cadherin/catenin movements during epithelial sheet formation (Adams and Nelson, 1998; Vaezi, et al., 2002; Ehrlich et al., 2002). This dynamic process starts when initial cell-cell contacts are formed by the engagement of two opposing E-cadherin/β-catenin complexes at the tips of filopodial and/or lamellopodial projections. The rate-limiting step in epithelial adhesion is the anchoring of cadherin/catenin complexes to the cortical actin cytoskeleton, promoting the clustering and stabilization of AJ proteins to form a punctum visible by fluorescence microscopy (Vasioukhin et al., 2000; Vaezi et al., 2002 and references therein). Following the appearance of this initial stable cluster of AJ proteins, additional adjacent puncta assemble, generating a zipper-like structure, which later "zips" to seal the membranes into epithelial sheets (Figure 4A; Vasioukhin et al., 2000). In vitro, the assembly and sealing of these zippers initiates near the apical surface of the polarized epithelium (Vaezi et al., 2002). A comparable situation may exist during formation of the blastoderm epithelium when AJs concentrate as spots at the apical edge of the lateral membrane and fuse into a circumferential belt during gastrulation (Tepass et al., 2002).

In epithelial cultures, a bundle of radial actin cable fibers organizes on each side of a punctum, and anchors to the underlying cortical actin ring (Figure 4A; Yonemura et al., 1995; Adams and Nelson, 1998; Vaezi et al., 2002). How the actin cables assemble is not yet fully under-

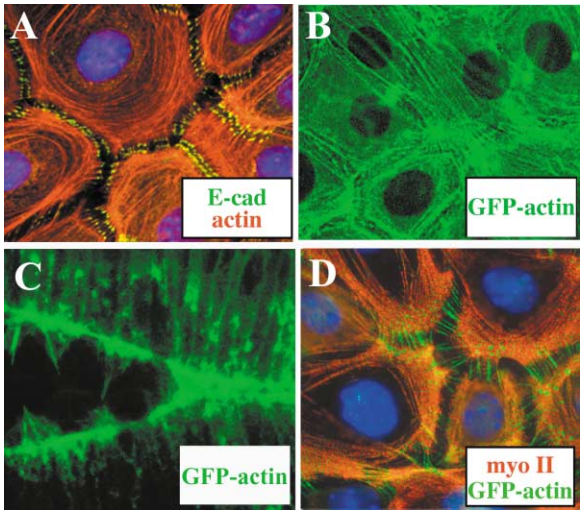

Figure 4. Actin Dynamics during Epithelial Sheet Formation

(A) At the initiation of adherens junction formation in primary mouse keratinocytes, actin-packed filopodia make contact and embed into neighboring cells. At the tips, nascent adherens junctions make contact with and attach to the underlying cortical actin cytoskeleton, forming a punctum, or stable adherens junction. This rate-limiting step increases the probability of forming additional adjacent adherens junctions, and thus the process resembles a zipper (Vasioukhin et al., 2000). E-cadherin (E-cad) is green and actin filaments are labeled with phalloidin in red.

(B) In cells expressing GFP actin, adherens junction-associated actin cables form a continuous cytoskeletal network that spans the sheet, enabling coordinated movements through the epithelium (Vaezi et al., 2002).

(C) Dorsal closure in a GFP actin expressing *Drosophila* embryo showing filopodia extending from leading edges of cells (image courtesy of W. Wood and P. Martin).

(D) The developing epidermal sheet is under tension, due to the fact that the radial actin cables (GFP actin), are linked to the central actomyosin network spanning the cell (decorated here with antibodies against anti-myosin II in red) (Vaezi et al., 2002).

stood. However, members of the zyxin and Vasp/Ena family of proteins can be observed at puncta, which are also sites of active actin polymerization (Vasioukhin et al., 2000). Recently, studies by Bear and colleagues demonstrated that Vasp can function by competing for barbed end actin-capping proteins, keeping the barbed ends open and available for extended actin polymerization (Bear et al., 2002). Although this mechanism on its own is sufficient to explain the actin polymerization seen at puncta sites, Arp2/3 complexes have also been shown to interact with E-cadherin, suggesting an underlying complexity in the dynamics (Yap and Kovacs, 2003). Irrespective of the mechanism, the outcome of actin polymerization and reorganization is the assembly of a uniform network of apical actin cables that span the entire epithelial sheet by virtue of interconnections to AJs (Figure 4B). Thus, by coordinating cytoskeletal rearrangements, individual cells can respond to stimuli as an integrated network or tissue.

Adherens junctions are also integrated into a variety of other cellular processes through associations with other types of intercellular junctions and membrane receptors. Although a detailed description of these interactions is beyond the scope of this review, it is intriguing that most epithelial sheets display closely apposed membranes where AJs alternate with desmosomes (e.g., Vasioukhin et al., 2000; Figure 2). Studies with blocking antibodies revealed that the establishment of AJs is a prerequisite for the formation of desmosomes and other junctions (Gumbiner et al., 1988). Desmosomes are specialized cadherin-mediated cell-cell junctions that attach to the intermediate filament network of keratin polymers, providing internal mechanical strength to epithelial cells (Figure 2; reviewed by Fuchs and Cleveland, 1998; Kowalczyk et al., 1999). While γ-catenin associates preferentially with desmosomal cadherins and β-catenin prefers E-cadherin, the two catenins can substitute for one another when one is missing (Bierkamp et al., 1999; Huelsken et al., 2001). Cadherin also plays an important role as a precursor for the establishment of tight junctions, which can restrict access of certain receptors and nutrients to the apical surface of the epithelium (Tsukita et al., 2001; Figure 2). Additionally, through shared interactions with afadin, AJs associate with homotypic junctions involving nectin-2, a transmembrane protein of the immunoglobulin superfamily (Ikeda et al., 1999; Takahashi et al., 1999). In fact, many additional types of membrane receptor interactions, including connexins (gap junctions), Notch and Delta, vezatin, and receptor tyrosine kinases and phosphatases, are influenced by the intimate cell-cell contacts that are directly or indirectly provided by cadherin-mediated junctions (Figure 3). In this way, AJs not only bring epithelial cells together but also affect the ability of cells to sense and respond to environmental cues.

Regulating Actin Dynamics at AJs Through the Rho Family of Small GTPases

During epithelial sheet formation and morphogenesis, actin rearrangements dramatically alter cellular architecture and motility. Members of the Rho family of small GTPases play a major role in directing actin dynamics and therefore impact profoundly upon these developmental processes. This family includes Cdc42 (which can generate filopodia), Rac (which mediates lamellipodia formation), and Rho (which promote stress fiber formation) (reviewed in Etienne-Manneville and Hall, 2002). Rho family GTPases function as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state. The activation state of these proteins is finely tuned by regulatory proteins such as guanine nucleotide exchange factors (GEFs), which catalyze the exchange of GDP for GTP; GTPase activating proteins (GAPs), which increase the rate of GTP hydrolysis; and guanine dissociation inhibitors (GDIs), which inhibit the release of GDP (reviewed in Etienne-Manneville and Hall, 2002). The ability of Rho GTPases to elicit their effects during development is contingent upon their being active at the right time and place. The fact that nascent AJs are often found at the leading edges of moving cells raises the question of whether these junctions participate in the spatio-temporal regulation of Rho GTPase activity or vice versa.

Thin, filopodial protrusions of membrane filled with bundles of actin at the cell surface have been shown to mediate the formation of epithelial sheets both in vivo, such as during dorsal closure in *Drosophila* embryos,

and in vitro, during calcium-induced adhesion of mouse keratinocyte cultures. Live imaging of GFP actin during dorsal closure in vivo reveals dynamic actin-rich filopodia and lamellipodia at the leading front of the closing epithelium (Figure 4C; Wood et al., 2002; Jacinto et al., 2002), and similar dynamics are displayed in calcium-induced keratinocytes cultured from GFP actin transgenic mice (Vaezi et al., 2002; Vasioukhin et al., 2000). When filopodial protrusions are blocked by inhibiting Cdc42 activity in fly embryos, opposing epithelial sheets fail to zip or close (Jacinto et al., 2002). Conversely, adherens junction assembly seems to result in the recruitment and activation of Cdc42, as illustrated by the behavior of a GFP-tagged substrate that only binds to Cdc42 when it is in its GTP bound, i.e., active state (Kim et al., 2000). If so, it would seem that E-cadherin "primes" the cell's membrane activity, which in turn promotes AJ formation.

The ability of cadherins to influence the polarity of the cell may have its foundation in the capacity of nascent AJs to stimulate Cdc42 activation. In this regard, Cdc42 is known to promote functionality of the PAR/atypical protein kinase C (aPKC) kinase complex, which translocates to apical sites of cell-cell adhesion after calcium stimulation (Izumi et al., 1998). The PAR complex, composed of aPKC, PAR3/ASIP (aPKC specific interacting protein), and PAR6, establishes polarity in a variety of cells and tissues across the eukaryotic kingdom (Lyczak et al., 2002; Wodarz, 2002). In many epithelia, polarity requires the formation of tight junctions (TJs), which are not only adjacent to AJs, but are dependent upon AJs for their formation (Gumbiner et al., 1988). TJ formation is facilitated by a group of membrane proteins, called junctional adhesion molecules (JAMs), which recently were found to bind to members of the PAR complex (Itoh et al., 2001; Ebnet et al., 2001). These findings suggest a model whereby adherens junction formation leads to local activation of Cdc42, which in turn recruits the PAR complex, allows JAMs to bind, and promotes TJ assembly. Thus, through generation of a scaffold for the formation of tight junctions, the E-cadherin/Cdc42/PAR/aPKC pathway may facilitate the physical separation of the apical and basolateral membranes of a polarized cell (Knust and Bossinger, 2002).

Rac1 also seems to play a role in promoting AJ formation, perhaps through its ability to stimulate actin dynamics and cell-cell contacts (Eaton et al., 1995; reviewed by Braga, 2002). In cultured cells, the levels of Rac1-GTP rise following calcium-activated stimulation of cell-cell adhesion (Noren et al., 2001). Live cell microscopy with Rac1-GFP and actin-GFP proteins support this notion and reveal that Rac1 activation correlates with the extensive lamellipodia activity that is subsequently followed by stable AJ formation (Ehrlich et al., 2002; Vaezi et al., 2002). Consistent with this notion is the finding that Tiam 1 (T-lymphoma invasion and metastasis gene 1), a GEF for Rac1, localizes to lamellae and ruffles in motile cells and to sites of cell-cell adhesion in epithelial cells (Braga, 2002; Ehrlich et al., 2002; Lampugnani et al., 2002).

Precisely how the GEFs of Rac1 find their way to sites of cell-cell adhesion is not yet clear. However, initial E-cadherin engagement may be critical to the recruitment process, and in this regard, it may be relevant that homophilic E-cadherin interactions result in the activation of PI3K (reviewed in Yap and Kovacs, 2003). The activation of PI3K at developing AJs may be important for producing phosphatidylinositol lipids that in turn could serve as a localized binding platform for GEFs, such as Tiam1, that have a pleckstrin homology (PH) domain. A functional role for PI3K has been demonstrated through use of its potent inhibitor wortmannin, which blocks the recruitment of Rac1 and disrupts intercellular adhesion (Nakagawa et al., 2001).

Could activated Rac1 have roles at AJs that extend beyond lamellipodial dynamics? One possibility is that Rac might function to stimulate actin polymerization at puncta and participate in actin cable formation (Vasioukhin et al., 2000). While a role for small GTPases in this process has not yet been established, it may be relevant that a member of the Vasp family was recently shown to be recruited to filopodia through a mechanism involving activated Cdc42 (Krugmann et al., 2001). An alternative role for activated Rac1 might be to bind IQGAP, a downstream effector of both activated Cdc42 and Rac1. Rac1-IQGAP interactions might displace IQGAP from β-catenin, thereby freeing β-catenin for association with α-catenin (reviewed by Fukata and Kaibuchi, 2001). Activated Rac1 might also function to recruit rather than displace IQGAP, enabling it to perform one of its functions, such as polarizing microtubules (see below and Figure 3; Gundersen, 2002; Fukata et al., 2002).

In contrast to Cdc42 and Rac1, which seem to be recruited indirectly to AJs, Rho GTPases may partner directly with cadherin-catenin components. *Drosophila* Rho1, the homolog of mammalian RhoA, was recently found to bind to two AJ proteins: α-catenin and p120ctn (Magie et al., 2002). p120ctn is an armadillo protein that binds to the juxtanuclear region of E-cadherin, at a site that does not overlap with the β-catenin binding site (Anastasiadis and Reynolds, 2001; Braga, 2002). In mammalian cells, p120ctn appears to inhibit RhoA (Anastasiadis et al., 2000) and promote activation of Rac and Cdc42 (Noren et al., 2000). In the only functional study to date, RNAi-mediated reduction of p120ctn or α-catenin in fly embryos elicited aberrant localization of Rho1 and defects in adhesion (Magie et al., 2002). Conversely, DE-cadherin and catenin localization was disrupted in Rho1 mutant embryos, which exhibit delays in repair of epithelial wounds and dorsal closure (Figure 5; Magie et al., 2002; Bloor and Kiehart, 2002; Wood et al., 2002).

Whether the activity of Rho has a positive or negative impact upon adhesion is still controversial, but a seemingly positive role for mammalian RhoA in epithelial sheet formation and/or sheet movements has received support from several studies (reviewed by Braga, 2002). Deciphering the role of Rho in adhesion has not been straightforward, and in part this is likely to be a reflection of the many downstream targets of Rho capable of affecting actin dynamics. Several activated RhoA effectors, including PRK2/PKN kinases and the diaphanous-related formins, Dia1 and Dia2, promote cell-cell adhesion in mammalian epithelial cells (Calautti et al., 2002; Sahai and Marshall, 2002). Thus, components of AJs can regulate actin dynamics through many different mechanisms. This level of complexity seems to be required for the dramatic changes in actin organization and polymer-

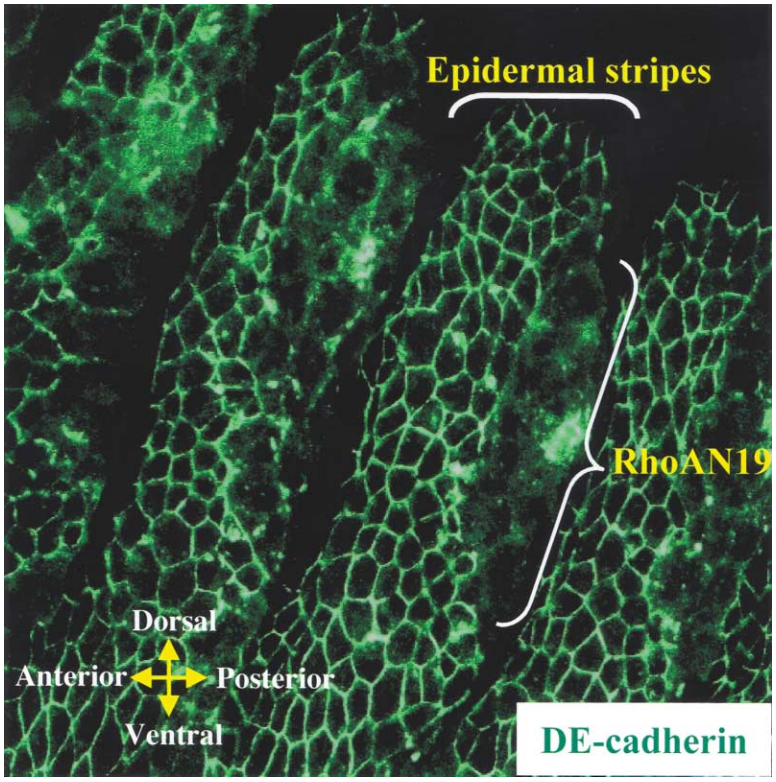

Figure 5. Effect of RhoA Mutants on E-Cadherin Expression during Dorsal Closure of *Drosophila* Embryos

Epidermal stripes of stage 15 *Drosophila* embryos expressing patches of a dominant-negative RhoA mutant (UAS-RhoAN19 under the control of enGal4). Embryos are stained with DE-cadherin antibody (green). The RhoA mutant negatively affects E-cadherin expression on patches of epidermal cells and compromises the integrity of the ventral epidermis. (Image courtesy of J. Bloor and D. Kiehart).

ization during epithelial sheet formation (Vaezi et al., 2002).

Through its indirect ability to activate myosin II, RhoA may also contribute to the generation of tension and contractile forces required for the compaction of cells into a tissue. Synchronization of these forces may be achieved by the association of AJs with the actomyosin cytoskeleton, as can be readily visualized in epithelial sheet formation in vitro (Figure 4D; Vaezi et al. 2002). Furthermore in vivo, fly zipper (zip) embryos mutant for the motor protein non-muscle myosin II, as well as RhoA mutant embryos, often fail to complete dorsal closure (Bloor and Kiehart, 2002 and references therein). During this process, actomyosin cables act as drivers of leading edge cell contractility at early stages. Later, they restrain the leading edge while maintaining a taut epithelial margin as the dorsal epithelial surfaces zip together (Kiehart et al., 2000; Jacinto et al., 2002). In *C. elegans* ectoderm, adherens junctions coordinate the actomyosin contractions that elongate the ovoid embryo into a worm (Costa et al., 1998, Priess and Hirsh, 1986). Thus, dynamic changes in cell shape and tissue movements are coordinated by the dynamic links between actomyosin cables and adherens junctions. Despite the positive roles of RhoA and myosin II, overexpression of a RhoA effector, Rho-associated kinase (Rock) or mutants in myosin light chain phosphatase are paradoxically deleterious (reviewed by Jacinto et al., 2002). Interestingly, in mammalian cells, inhibitors of Rock relax the tension across developing epithelial sheets and accelerate membrane sealing (Sahai and Marshall, 2002), but they also adversely affect the ability of sheets to generate the radial actin cables and coordinate cellular movements (Vaezi et al., 2002). Taken together, these findings suggest the importance of striking the right balance of tension and adhesion in epithelial sheet movements and tissue formation.

A Link Between AJs and Spindle Polarity: Parallels Between Yeast Buds and Adherens Junctions

Unlike actin filaments, microtubules are not required for AJ assembly; however, they do physically associate with adhesive structures. The molecules responsible for the connection between microtubules and AJs are not yet clear. One possible candidate is APC (adenomatous polyposis coli), which binds to β-catenin as well as to the microtubule binding protein EB-1 (Berrueta et al., 1998; Askham et al., 2002). Another potential player is Clip-170, which binds to microtubules as well as to IQGAP (Gundersen, 2002). The actin binding protein ACF-7 localizes to the tips of microtubules at the leading edge of migrating cells, and in response to calcium, it reorganizes with microtubules to sites of cell-cell adhesion (Figure 3; Karakesisoglou et al., 2000). ACF-7 is a unique candidate for directly integrating a microtubule-actin-AJ connection, as it possesses binding sites for both actin filaments and microtubules (Karakesisoglou et al., 2000; Sun et al., 2001). Given its large size (>600 kDa) and localization, ACF-7 may also bind to other proteins, such as dynein-dynactin patches, which form at sites along the actin cortex near developing cell-cell contacts (Figure 3). In this regard, it is interesting that another microtubule binding protein, the motor protein dynein, is not only an organizer of dynein-dynactin patches, but also binds to β-catenin (Ligon et al., 2001).

These putative connections between microtubules

and AJs are particularly fascinating in light of recent evidence that implicates adherens junctions in symmetric and asymmetric cell divisions. The orientation of mitotic spindle during cell division is critical in determining the organization and architecture of cells within epithelial tissues. Whether simple or stratified, epithelia often need to maintain a single layer of symmetrically dividing cells anchored to an underlying basement membrane (see Figure 1). To do so, an expanding epithelium must orient its spindles parallel to the basement membrane. Often in development, however, cells shift their spindle along the apical-basal axis, such that only one daughter cell remains within the plane. Such asymmetric divisions may be able to generate stratified epithelia, or produce new cell types, such as in the formation of a hair follicle (Byrne et al., 1994).

How cells choose their axis of division has been a matter of intense investigation, and recently, AJs have emerged as essential components of the machinery. Lu et al. (2001) discovered that disruption of the adherens junction-associated component E-APC and its binding partner EB-1 in *Drosophila* results in the conversion of symmetric epithelial divisions to asymmetric ones during embryogenesis. Tissue culture studies of EB-1 RNAi-treated cells suggest that this phenotype may result from malformed mitotic spindles, defocused spindle poles, and mispositioned spindles away from the cell center (Rogers et al., 2002).

A survey of the proteins involved in spindle orientation in the single-cell yeast *Saccharomyces cerevisiae* reveals some striking parallels with the cytoskeletal-associated proteins that interact with AJs. In budding yeast, EB1 is a genetic determinant of spindle orientation, which is established through capture of astral microtubules and tethering along the mother-bud axis. Dynein-dynactin complexes are also involved later in this process by maintaining spindle orientation and facilitating spindle movement during mitosis (Theesfeld et al., 1999; Heil-Chapdelaine et al., 2000). The bud site, sometimes referred to as the polarisome (Sagot et al., 2002), contains attachment sites for the astral microtubules (Figure 6). EB1, dynein-dynactin and other microtubule-associated proteins, including Kar9 (Miller and Rose, 1998), a possible APC homolog, contribute to docking the microtubules to the cortical actin and then stabilizing the interaction. Although no ACF7-like homolog has been found in yeast that can directly link together actin and microtubules, many of the other functional homologs involved in the basic process of spindle orientation in unicellular organisms seem to be shared with AJs (Figure 6; compare with Figure 3).

The parallels between AJs and bud sites can be taken one step further, to look at the similarities in actin dynamics and polarized growth. Bud-associated actin cables appear to initiate and grow from the bud along the mother-bud axis (Yang and Pon, 2002). These cables are thought to serve as a polarizing highway for the directional transport of both proteins and RNAs, a process that may also involve the myosin Myo2p (Pruyne et al., 1998). Moreover, they may function together with the cortical basket of actin cables in the mother cell to help guide the astral microtubules into the bud (Theesfeld et al., 1999; Yin et al., 2000). Similarly in mammalian cells, the radial actin cables linked to AJ appear to utilize

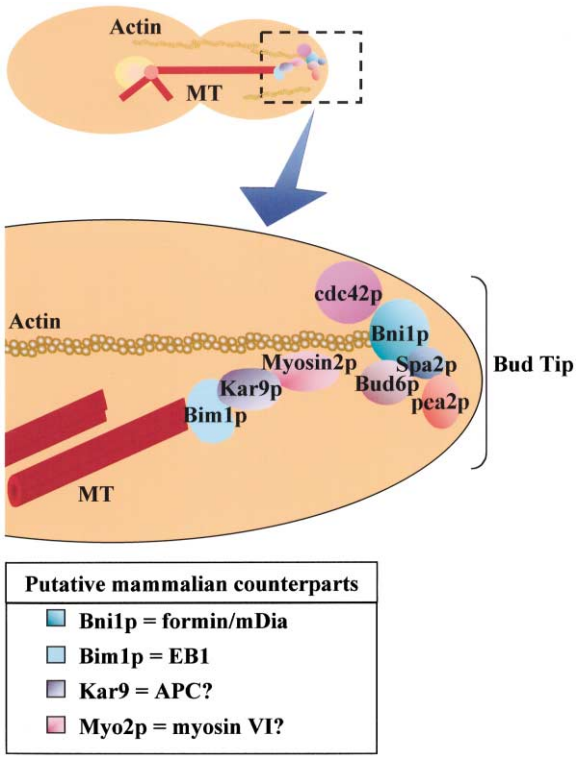

Figure 6. Proteins Associated with Capturing Actin and Microtubules at the Bud Tips of Yeast

The top image shows how both the actin and microtubule cytoskeletons of the mother cell extend into the daughter cell and converge at the bud tip. The middle image shows a magnified view of the bud tip to illustrate the complement of proteins implicated in nucleating actin cable formation at the bud tip and in capturing microtubules at this site. Bud6p, Spa2p, Pea2p and Sph1p, which determine the bud tip site, are distinct from the cadherins and catenins, which initiate the formation of adherens junctions. However, there are marked parallels in the cytoskeletal dynamics and in the associated proteins that are involved at these tips in budding yeast and at adherens junctions in mammalian cells. The table lists possible mammalian counterparts of the yeast proteins.

myosins, in both stabilizing cell-cell adhesion and promoting cellular polarization (Vaezi et al., 2002; Geisbrecht and Montell, 2002). Finally, actin polymerization and cable dynamics at bud sites rely upon the Rho-GTP activated formin Bni1p in yeast. If parallels to AJs hold, this offers a possible function for Rho-GTP and mDia1 at cell-cell junctions in multicellular organisms (Figures 3 and 6; Sahai and Marshall, 2002). While additional work is necessary to truly establish the functional equivalence between a number of these yeast and mammalian proteins, the parallels suggest a tantalizing evolutionary link between the two systems.

AJs and Cell Sorting during Development

During embryogenesis, boundaries often develop between morphologically homogeneous cell populations. It has long been surmised that differential cell affinities orchestrate the formation of tissue boundaries, and cadherins play a central role in this process. Mammalian cadherins now encompass a superfamily of >20 proteins, which are differentially expressed in elaborate pat-

terns. In a now classic experiment, Takeichi and coworkers transfected two of them, E- and P-cadherin, into separate groups of L cells, which normally possess little or no cadherin activity (Nose et al., 1988). The transfected cells preferentially adhered to cells expressing the same cadherin subclass and they developed epithelial sheets. In contrast, untransfected cells associated with mesenchymal cells, which do not express cadherins.

Recent findings suggest that additional transmembrane receptors, particularly the ephrin (Eph) receptor tyrosine kinases, contribute to the sorting specificity of cell populations (reviewed by Kullander and Klein, 2002). Eph receptors comprise a family of receptor tyrosine kinases whose ephrin ligands are also membrane bound. Receptor-ligand interaction and signaling requires direct cell-cell contact, and recently, the function of several Eph receptors and ephrins has been found to depend upon E-cadherin and cytoskeletal dynamics. Thus, in non-epithelial cells, ectopic expression of E-cadherin can induce EphA2 receptor expression, and in epithelial cells, AJs regulate localization of the protein (Orsulic and Kemler, 2000).

Like the cadherins, Eph receptors are expressed in complex patterns during embryonic and postnatal development. However, in contrast to cadherins, Ephr-Eph associations can mediate repulsion or adhesion, depending upon the developmental context (Kullander and Klein, 2002). A particularly intriguing example of this is the EphA7 receptor, which functions with its ligand ephrinA5 in early neural tube closure. An alternatively spliced mRNA encoding a truncated version of EphA7 interferes with EphA7's ability to act in repulsion and instead promotes adhesion (Holmberg et al., 2000). Gene-targeting studies reveal that in the absence of ephrin ligands or receptors, cells otherwise positioned at one place in a tissue now relocalize to distinct sites (Batlle et al., 2002; Kullander and Klein, 2002). Thus, tissue boundaries are sometimes established at interfaces where Eph receptor-expressing cells meet ephrin ligand-presenting cells, reflective of a role for repulsion. Within the confines of these tissues, adhesive forces are critical. While the mechanisms underlying these processes are just beginning to emerge, it seems likely that both cadherin-mediated adhesive affinities and Eph receptors and their ligands will be important in defining and maintaining sorting behavior and boundaries, and in determining positioning, migration, and differentiation within tissues.

Adherens Junctions, Stem Cells, and Early Cell Specification

Recently, cadherins and their close associates have been implicated in providing spatial cues to stem cells. Whether in early development or in adult tissues, stem cells reside in customized niches or microenvironments that contribute to their unique ability to divide asymmetrically to give rise to self and to a daughter with distinct properties. An interesting example of cadherin regulation of stem cells comes from studies on the *Drosophila* ovary (Song et al., 2002). Germ stem cells (GSCs) reside in a niche that is established by the interaction of stem cells with their basement membrane (extracellular matrix) and with neighboring differentiated cells. An asymmetric division of a germ stem cell causes the physical dissociation of one of its daughters from this specialized environment, depriving it of the self-renewing signals and promoting its differentiation. When DE-cadherin is reduced or absent in the *Drosophila* germarium, GSCs no longer interact with the 5–6 cap cells of the ovarian niche, and they differentiate prematurely. How universal is a role for cadherins in maintaining stem cells in their niche, and are they simply the glue that keeps the cell in its microenvironment? Research on sensory organ development and neuroepithelial cell division suggests that AJs may in fact play an active role, by influencing the ability of multipotent cells to divide asymmetrically.

To initiate asymmetric cell divisions, neural precursors of the sensory organ and CNS utilize a planar polarity and an apical-basal polarity cue respectively, regulated by the protein Bazooka, the *Drosophila* homolog of Par3 (Lu et al., 2001). For example in the *Drosophila* CNS, the neural progenitors cells called neuroblasts originate from neuroepithelial cells, which are polarized along the apical-basal axis and divide symmetrically along the planar axis (Figure 7A). In a process involving Notch signaling, neuroblasts delaminate from the neuroectoderm and divide asymmetrically along the apical-basal axis (Jan and Jan, 2001). Some relevant changes associated with this are the loss of cell-cell contacts and the redistribution of proteins required for asymmetric division. The protein Bazooka localizes to the apical membrane and the proteins Pon and Numb to the basal membrane. The expression of inscuteable and its apical targeting through interaction with Bazooka leads to activation of apical-basal spindle cues (Figure 7B; Jan and Jan, 2001). As opposed to neuroblasts, neuroepithelial cells divide symmetrically along the planar axis and segregate Bazooka, Pon, and Numb equally between the two daughter cells. This suggests that other polarity cues may prevent asymmetric division.

What are the molecular cues that cause the spindle plane to rotate and the asymmetric divisions to begin? At present there is no definitive answer, but genetic approaches have suggested that inscuteable is required for asymmetric cell divisions (Kraut et al., 1996; Schober et al., 1999; Wodarz et al., 1999). This said, in a recent study by Rath et al. (2002), some neuroblast lineages were identified that divide asymmetrically in the absence of this gene. The studies of Lu et al. (2001) indicate that when *Drosophila* embryos are treated with RNAi to diminish EB1 or APC levels, the ectodermal cells no longer maintain their polarity, and their divisions become asymmetric and misoriented (Figure 7C). These studies did not involve direct disruption of core adherens junction proteins, and hence it cannot be judged from these studies alone that AJs can override the apical-basal apparatus for spindle positioning. In this context, it is important to consider studies on sensory organ development, where a partial loss of DE-cadherin function and expression of a dominant-negative resulted in defects in the orientation of certain planar asymmetric cell divisions as well as the positioning of Bazooka (Le Borgne et al., 2002).

Taken together, the studies are consistent with a model whereby epithelial cells may utilize two competing sets of polarity cues for spindle positioning: a lateral

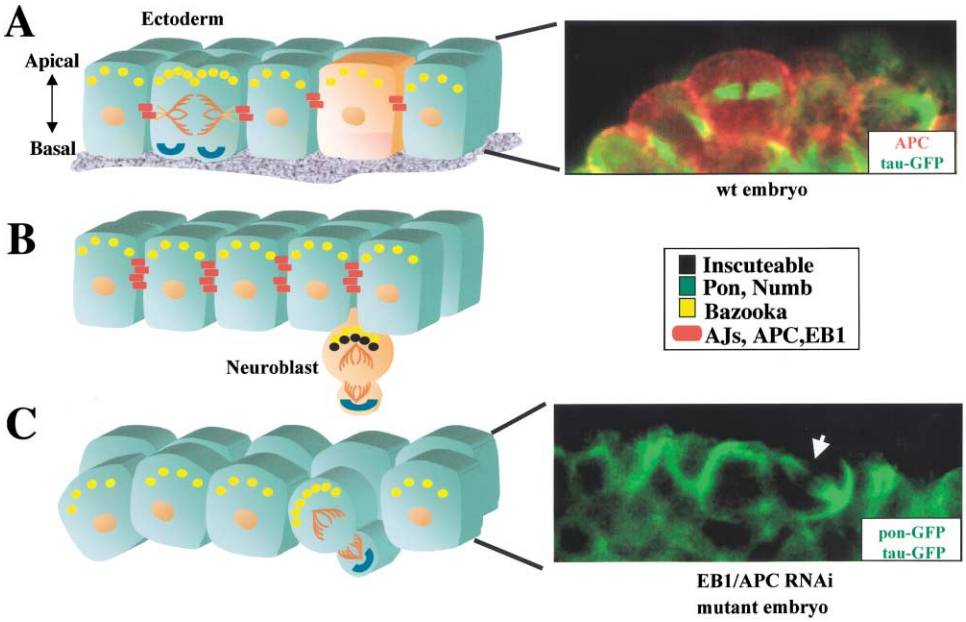

Figure 7. Possible Role for Adherens Junctions in Spindle Orientation

(A) Left image illustrates a symmetrical cell division within the plane of the embryonic ectoderm, which is polarized due to maintenance of adherens junctions and attachment to an underlying extracellular matrix. Bazooka (yellow dots) localizes along the apical membrane of the epithelia while Pons and Numb (blue crescent) localize along the basal membrane. The orange cell depicts a neural-competent ectodermal cell, which will develop into a neuroblast as a consequence of Notch/delta signaling within the ectoderm. Right image shows symmetric division and anterior-posterior spindle orientation in the ectoderm of *Drosophila* embryo expressing a tau-GFP fusion protein to highlight the spindle (Lu et al., 2001). Intercellular contacts are highlighted with antibodies to APC (red), which associates with adherens junctions.

(B) Left image. When a neuroblast develops, and exits the ectoderm, it acquires the inscuteable protein (black dots), which associates with Bazooka, and which is likely to be involved in inducing asymmetric divisions along the apical-basal plane at least in some neural lineages. Adherens junctions are also lost as the cell breaks contacts with its neighbors. Recent evidence suggests that this loss may also contribute to asymmetric cell divisions.

(C) When *Drosophila* ectoderm expresses RNAi for EB1 or APC, adherens junctions and cell polarity is disrupted, and asymmetric divisions are seen (Lu et al., 2001). Shown at right is a section from a pon-GFP, tau-GFP embryo with reduced APC, illustrating the skewed orientation of the mitotic spindle and the enhanced crescent of Pon at the base, where one pole of the spindle is attached (courtesy of F. Roegiers, B. Lu, and Y.N. Jan).

polarity cue mediated by AJs and/or the proteins that it recruits, and an apical-basal polarity cue regulated by Bazooka. If levels of adherens junction proteins are high, a lateral cue would be expected to prevail and cells would divide symmetrically, within the lateral plane. If the levels of AJ proteins are reduced and/or the apical-basal cues are accentuated (e.g., by activator proteins such as inscuteable), then the apical-basal polarity cue might be dominant, inducing asymmetrical divisions and resulting in a cell's exit from its surroundings (Jan and Jan, 2001). This model is tantalizing, as there are many developmental processes such as stem cell activation and differentiation, where changes in cadherin expression and adherens junction dynamics have been observed, at times when rotations in spindle orientation must also be established. Future studies will determine the extent to which adherens junction-cytoskeletal dynamics might be able to overpower the apical-basal cue for asymmetric division.

Several twists on this theme come from the possibility that Bazooka can influence adherens junction formation and this could have a direct impact on the directionality of asymmetric divisions (Lu et al., 2001; Bilder et al., 2003). Another protein that can impact this process is Rap1, which regulates the localization of components of AJs at the apical side of the epithelium of the *Drosophila* wing (Knox and Brown, 2002). The defects in cell shape and morphogenesis of *Drosophila* embryos seen in Rap1 mutants are consistent with the notion that the positioning of adherens junction could play a role in cell mobility and division. Though speculative, such mechanisms could provide a molecular explanation of how each daughter cell in sensory organ development maintains the same asymmetric division as its mother cell (Le Borgne et al., 2002).

One final note is that like the adherens junction proteins, the apical-basal spindle polarity proteins that are membrane-associated seem to have no counterparts in yeast. It seems to be the cytoskeletal dynamics associated with these junctions, rather than the membrane-associated proteins per se, that are conserved.

Regulating Cytoskeletal-AJ Connections and Downregulating Cadherins

Adherens junctions and their associated cytoskeletons must be dynamic to accommodate the tremendous degree of intercellular remodeling that occurs during morphogenesis, tissue homeostasis, and recovery from injury. With a possible role for cadherin levels in regulating such critical processes as spindle positioning, epithelial

sheet movements, and intercellular adhesion, increasing interest has been placed upon how the levels of cadherins and their associates are controlled. Not surprisingly, a number of mechanisms have been implicated, and the regulation appears to be complex and finely tuned.

We have already alluded to the posttranslational modification of Y654 on β-catenin that decreases its affinity to E-cadherin, thereby weakening the stability of AJs (Huber and Weis, 2001). Following this tyrosine kinase-activated modification, the disassembled E-cadherin complexes are subject to endocytosis and ubiquitination-mediated degradation (Fujita et al., 2002). Tyrosine phosphorylation of β-catenin influences a wide variety of developmental processes (Dumstrei et al., 2002 and references therein). In tumorigenesis, where tyrosine phosphorylation levels are unnaturally elevated, excessive β-catenin phosphorylation is accompanied by increased invasiveness (reviewed by Gumbiner, 2000). Taken together, these findings underscore an important and intimate link between growth factor signaling and control of cell-cell contact stability. Like β-catenin, cadherins can also be phosphorylated by tyrosine kinases. Recently, an E3 ligase called Hakai was shown to bind to E-cadherin in a phosphotyrosine-dependent manner causing the shuttling of internalized E-cadherin to the lysosome rather than recycling it back to the plasma membrane (Fujita et al., 2002). Thus, Hakai has the ability to regulate adhesion by modulating the amount of cell surface cadherin.

Interestingly, if the disassembled cadherin-catenin proteins meet the action of tyrosine phosphatases (PTPs) prior to ubiquitination, they are spared, and intercellular adhesion can be restored (Gumbiner, 2000). An example of this is the recent discovery of a PTP that coprecipitates with the endothelial VE-cadherin and reverses its phosphorylation by the tyrosine kinase receptor for VEGF (Nawroth et al., 2002). One possibility is that VEGF-receptor action may loosen cell-cell contacts to modulate transendothelial permeability and to allow blood vessel sprouting and migration during angiogenesis, a response which PTP action might downregulate. However, when endothelial cells are subjected to shear stress, they rapidly anchor both VEGFR-2 and VE-cadherin to the endothelial cytoskeleton, promoting their association and the transduction of shear-stress signals. In this regulatory twist, VE-cadherin's role seems to be critical, as cells lacking this cadherin cannot transduce the signals (Shay-Salit et al., 2002). Additionally, without VE-cadherin, VEGF-mediated cell survival and angiogenesis are compromised. Based upon this example, AJs are likely to play pivotal roles in regulating cellular responses to growth factors and other environmental signals in specific cellular hierarchies during development.

In addition to being direct targets for certain tyrosine kinases, cadherins also interact with a number of other proteins such as p120ctn and Rho GTPases (described above), that can influence their activity and stability. The protein p120ctn has been shown to act both as a positive and negative regulator of cadherin adhesiveness (Anastasiadis and Reynolds, 2001). Nevertheless, recent evidence using a p120ctn-deficient colorectal cell line indicated a crucial role for p120-E-cadherin interaction not only for the proper localization and function of E-cadherin but also an increase of protein expression (Ireton et al., 2002). Another intriguing potential partner for E-cadherin is the presenilin 1 (PS1) protein involved in Alzheimer's disease. Several reports suggest that PS1 directly binds to E-cadherin, although whether this association stabilizes E-cadherin (Baki et al., 2001) or targets E-cadherin for cleavage and AJ disassembly (Marambaud et al., 2002) is not yet clear. Additionally, PS1 facilitates the stepwise phosphorylation of β-catenin that targets it for degradation, and conversely, loss of PS1 leads to stabilization of β-catenin, enabling it to function outside the realm of AJs (Kang et al., 2002). While the implications for Alzheimer's disease remain unknown, PS1 is broadly expressed in epithelia, and these recent findings suggest that its regulation may impact on AJ-cytoskeletal dynamics.

Intricate regulation of the transcription of the E-cadherin gene bestows an added level of sensitivity for controlling E-cadherin levels in tissue morphogenesis. The best-studied element of the E-cadherin promoter is an E-box which binds factors such as Snail, Slug, E12/E47, and SIP to promote transcriptional repression of the E-cadherin gene (Bolos et al., 2003 and references therein). Genetic ablation of snail in mice results in early and striking embryonic abnormalities, including the development of a mesoderm with epithelial characteristics such as AJs and apical-basal polarity (Carver et al., 2001). Conversely, overexpression of snail and its cousin slug result in epithelial to mesenchymal transitions (EMTs) in vitro (Bolos et al., 2003 and references therein). EMTs play a broad role in the normal development of tissues, including kidney and skeletal muscle. Snail has also been implicated in specifying mesodermal fate during gastrulation (Carver et al., 2001, Ciruna and Rossant, 2001). A variety of epithelial cancers show elevation of these repressor proteins, and this correlates with tumor invasiveness (Comijn et al., 2001 and references therein). It will be interesting in the future to see whether changes in the levels of these proteins might influence developmental processes such as epithelial budding or branching morphogenesis, where localized downregulation in adhesive interactions may be required to remodel epithelial junctions.

Direct Participation of Cadherin-Catenins in Signaling Pathways: Adherens Junctions and Beyond

Given the multiple roles of AJs in morphogenesis, it would not be surprising to find communication between AJs and the nucleus. Although the evidence is not yet conclusive, one possible communicating line could be through β-catenin. For nearly 10 years, it was difficult to reconcile that while in *Drosophila* β-catenin was known to be a component of the wingless signal transduction pathway involved in segment polarity, its only known mammalian counterpart at the time, plakoglobin, was a component of intercellular adhesion. When β-catenin was discovered to interact with a new partner, the DNA binding protein Lef-1 (Behrens et al., 1996), the *Drosophila* relative pangolin was quickly placed genetically in the canonical Wnt/Wingless pathway. β-catenin had a newfound role as a transcriptional regulatory protein in both systems (reviewed by van Noort and Clevers, 2002).

Early on, it was recognized that canonical Wnt signaling prevents any excess β-catenin not utilized in AJs from being targeted for ubiquitination and degradation via the proteosome pathway (reviewed by Moon et al., 2002). This stabilization of cytosolic β-catenin allows for the direct interaction of β-catenin with transcription factors of the Lef/Tcf family (Moon et al., 2002). Remarkably, despite little or no sequence identity between E-cadherin and these DNA binding proteins, they both bind to the same site on β-catenin (reviewed by Pokutta and Weis, 2002). Exactly how β-catenin acts to regulate Lef/Tcf activity is not yet clear. In *Drosophila*, Pangolin acts as a repressor, and recent transgenic evidence suggests that Armadillo (β-catenin) might function by exporting Pangolin out of the nucleus to relieve repression and activate downstream target genes (Chan and Struhl, 2002). In mammalian cells, however, Lef1/Tcf proteins often act to transactivate rather than repress genes and they often concentrate in the nucleus of cells upon receipt of a Wnt signal (Merrill et al., 2001 and references therein). In addition, in an in vitro assay with chromatin templates, recombinant β-catenin strongly enhanced binding and transactivation by Lef-1 (Tutter et al., 2001). Taken together, these findings suggest that β-catenin may function both in chromatin remodeling and in nuclear export. As the many different interacting partners of β-catenin are elucidated, the complex mechanisms involved in its actions should become clearer.

While the precise mechanism underlying β-catenin's link to Wnt-mediated transcription remains controversial, the pathway is utilized broadly in development and there is widespread agreement that the consequences of excessive Wnt signaling and constitutive stabilization of β-catenin are frequently tumors and cancers. In this regard, β-catenin differs from E-cadherin and α-catenin, which are mutated or downregulated in a number of epithelial cancers (Conacci-Sorrell et al., 2002). However, it could be that downregulation of E-cadherin could free β-catenin to participate in transcriptional regulation. Since β-catenin's binding sites for E-cadherin and Lef1/Tcf are shared, the level of E-cadherin in cells is likely to impact significantly on the amount of β-catenin that is available for Lef1/Tcf (Gottardi et al., 2001). Conversely, activation of Wnt signaling results in stabilized β-catenin that might directly act on Lef1/Tcf complexes to transcriptionally downregulate key adhesion genes such as E-cadherin or α-catenin. Some evidence for this exists in the mouse brain, where a correlation between Wnt 1 signaling and repressing E-cadherin mRNA expression has been reported (Shimamura et al., 1994).

Conclusions

In closing, epithelial cells have an amazing ability to simultaneously change their shape, polarity, transcriptional agenda, and proliferation status, and they can move through tissues with intricate precision during development and differentiation. AJs appear to be at the crossroads of morphogenetic and patterning processes in tissues that are dependent upon intercellular connections for their development. The molecular mechanisms involved in morphogenesis are not yet well-defined, and much remains to be done to understand how external signals are transmitted through AJs and their neighboring receptors to the cytoskeleton in order to communicate this information to other critical systems within the cell.

While the details are often still fuzzy, the emerging picture suggests that the levels of AJ proteins in cells are central to the fate the cells will adopt. Elevated levels of cadherins may be key determinants in distinguishing epithelial cells from mesenchymal cells, and in establishing and maintaining the proper polarity and spindle orientation of cells within a tissue. Downregulation of cadherin expression during development or differentiation may unmask underlying mechanisms controlling spindle orientation that promote asymmetric cell divisions, a key process in stem cell determination. When downregulation of cadherin expression happens unnaturally, tumor progression and invasion are often a consequence. Finally, complete loss of cadherin results in apoptosis and tissue necrosis (Boussadia et al., 2002). Superimposed on the importance of cadherin levels are the levels of its close associates β-catenin and α-catenin, which expand the repertoire of cellular responses by integrating cytoskeletal networks and transcriptional regulation (van Noort and Clevers, 2002).

With the advancement of genome analyses, the field has been informed by studies encompassing the eukaryotic kingdom, extending even to yeast, which has no need for coordinated cell movements and behavior, but which does need to orient the spindle and control polarity. With advancements in microscopic techniques, it has become possible to monitor adherens junction and cytoskeletal dynamics in living cells and tissues. Thus, many of the tools are now available to tackle the complex process of morphogenesis, a difficult task that until recently seemed insurmountable.

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References

Adams, C.L., and Nelson, W.J. (1998). Cytomechanics of cadherin-mediated cell-cell adhesion. Curr. Opin. Cell Biol. *10*, 572–577.

Anastasiadis, P.Z., and Reynolds, A.B. (2001). Regulation of Rho GTPases by p120-catenin. Curr. Opin. Cell Biol. *13*, 604–610.

Anastasiadis, P.Z., Moon, S.Y., Thoreson, M.A., Mariner, D.J., Crawford, H.C., Zheng, Y., and Reynolds, A.B. (2000). Inhibition of RhoA by p120 catenin. Nat. Cell Biol. *2*, 637–644.

Askham, J.M., Vaughan, K.T., Goodson, H.V., and Morrison, E.E. (2002). Evidence that an interaction between EB1 and p150(Glued) is required for the formation and maintenance of a radial microtubule array anchored at the centrosome. Mol. Biol. Cell *13*, 3627–3645.

Baki, L., Marambaud, P., Efthimiopoulos, S., Georgakopoulos, A., Wen, P., Cui, W., Shioi, J., Koo, E., Ozawa, M., Friedrich, V.L., Jr., and Robakis, N.K. (2001). Presenilin-1 binds cytoplasmic epithelial cadherin, inhibits cadherin/p120 association, and regulates stability and function of the cadherin/catenin adhesion complex. Proc. Natl. Acad. Sci. USA *98*, 2381–2386.

Batlle, E., Henderson, J.T., Beghtel, H., van den Born, M.M., Sancho, E., Huls, G., Meeldijk, J., Robertson, J., van de Wetering, M., Pawson, T., and Clevers, H. (2002). β-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. Cell *111*, 251–263.

Bear, J.E., Svitkina, T.M., Krause, M., Schafer, D.A., Loureiro, J.J., Strasser, G.A., Maly, I.V., Chaga, O.Y., Cooper, J.A., Borisy, G.G., and Gertler, F.B. (2002). Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. Cell *109*, 509–521.

Behrens, J., von Kries, J.P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. (1996). Functional interaction of β-catenin with the transcription factor LEF-1. Nature *382*, 638–642.

Berrueta, L., Kraeft, S.K., Tirnauer, J.S., Schuyler, S.C., Chen, L.B., Hill, D.E., Pellman, D., and Bierer, B.E. (1998). The adenomatous polyposis coli-binding protein EB1 is associated with cytoplasmic and spindle microtubules. Proc. Natl. Acad. Sci. USA *95*, 10596–10601.

Bierkamp, C., Schwarz, H., Huber, O., and Kemler, R. (1999). Desmosomal localization of β-catenin in the skin of plakoglobin null-mutant mice. Development *126*, 371–381.

Bilder, D., Schober, M., and Perrimon, N. (2003). Integrated activity of PDZ protein complexes regulates epithelial polarity. Nat. Cell Biol. *5*, 53–58.

Bloor, J.W., and Kiehart, D.P. (2002). *Drosophila* RhoA regulates the cytoskeleton and cell-cell adhesion in the developing epidermis. Development *129*, 3173–3183.

Bolos, V., Peinado, H., Perez-Moreno, M.A., Fraga, M.F., Esteller, M., and Cano, A. (2003). The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressors. J. Cell Sci. *116*, 499–511.

Boussadia, O., Kutsch, S., Hierholzer, A., Delmas, V., and Kemler, R. (2002). E-cadherin is a survival factor for the lactating mouse mammary gland. Mech. Dev. *115*, 53–62.

Braga, V.M. (2002). Cell-cell adhesion and signalling. Curr. Opin. Cell Biol. *14*, 546–556.

Byrne, C., Tainsky, M., and Fuchs, E. (1994). Programming gene expression in developing epidermis. Development *120*, 2369–2383.

Calautti, E., Grossi, M., Mammucari, C., Aoyama, Y., Pirro, M., Ono, Y., Li, J., and Dotto, G.P. (2002). Fyn tyrosine kinase is a downstream mediator of Rho/PRK2 function in keratinocyte cell-cell adhesion. J. Cell Biol. *156*, 137–148.

Carver, E.A., Jiang, R., Lan, Y., Oram, K.F., and Gridley, T. (2001). The mouse snail gene encodes a key regulator of the epithelial-mesenchymal transition. Mol. Cell. Biol. *21*, 8184–8188.

Chan, S.K., and Struhl, G. (2002). Evidence that Armadillo transduces wingless by mediating nuclear export or cytosolic activation of Pangolin. Cell *111*, 265–280.

Chen, Y.T., Stewart, D.B., and Nelson, W.J. (1999). Coupling assembly of the E-cadherin/β-catenin complex to efficient endoplasmic reticulum exit and basal-lateral membrane targeting of E-cadherin in polarized MDCK cells. J. Cell Biol. *144*, 687–699.

Ciruna, B., and Rossant, J. (2001). FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. Dev. Cell *1*, 37–49.

Comijn, J., Berx, G., Vermassen, P., Verschueren, K., van Grunsven, L., Bruyneel, E., Mareel, M., Huylebroeck, D., and van Roy, F. (2001). The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. Mol. Cell *7*, 1267–1278.

Conacci-Sorrell, M., Zhurinsky, J., and Ben-Ze'ev, A. (2002). The cadherin-catenin adhesion system in signaling and cancer. J. Clin. Invest. *109*, 987–991.

Costa, M., Raich, W., Agbunag, C., Leung, B., Hardin, J., and Priess, J.R. (1998). A putative catenin-cadherin system mediates morphogenesis of the *Caenorhabditis elegans* embryo. J. Cell Biol. *141*, 297–308.

Dumstrei, K., Wang, F., Shy, D., Tepass, U., and Hartenstein, V. (2002). Interaction between EGFR signaling and DE-cadherin during nervous system morphogenesis. Development *129*, 3983–3994.

Eaton, S., Auvinen, P., Luo, L., Jan, Y.N., and Simons, K. (1995). CDC42 and Rac1 control different actin-dependent processes in the *Drosophila* wing disc epithelium. J. Cell Biol. *131*, 151–164.

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

Ehrlich, J.S., Hansen, M.D., and Nelson, W.J. (2002). Spatio-temporal regulation of Rac1 localization and lamellipodia dynamics during epithelial cell-cell adhesion. Dev. Cell *3*, 259–270.

Etienne-Manneville, S., and Hall, A. (2002). Rho GTPases in cell biology. Nature *420*, 629–635.

Fuchs, E., and Cleveland, D.W. (1998). A structural scaffolding of intermediate filaments in health and disease. Science *279*, 514–519.

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

Fukata, M., and Kaibuchi, K. (2001). Rho-family GTPases in cadherin-mediated cell-cell adhesion. Nat. Rev. Mol. Cell Biol. *2*, 887–897.

Fukata, M., Watanabe, T., Noritake, J., Nakagawa, M., Yamaga, M., Kuroda, S., Matsuura, Y., Iwamatsu, A., Perez, F., and Kaibuchi, K. (2002). Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. Cell *109*, 873–885.

Geisbrecht, E.R., and Montell, D.J. (2002). Myosin VI is required for E-cadherin-mediated border cell migration. Nat. Cell Biol. *4*, 616–620.

Gilmore, A.P., and Burridge, K. (1996). Regulation of vinculin binding to talin and actin by phosphatidyl-inositol-4–5-bisphosphate. Nature *381*, 531–535.

Gottardi, C.J., Wong, E., and Gumbiner, B.M. (2001). E-cadherin suppresses cellular transformation by inhibiting β-catenin signaling in an adhesion-independent manner. J. Cell Biol. *153*, 1049–1060.

Gumbiner, B.M. (2000). Regulation of cadherin adhesive activity. J. Cell Biol. *148*, 399–404.

Gumbiner, B., Stevenson, B., and Grimaldi, A. (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.

Gundersen, G.G. (2002). Microtubule capture: IQGAP and CLIP-170 expand the repertoire. Curr. Biol. *12*, R645–647.

Heil-Chapdelaine, R.A., Tran, N.K., and Cooper, J.A. (2000). Dynein-dependent movements of the mitotic spindle in *Saccharomyces cerevisiae* do not require filamentous actin. Mol. Biol. Cell *11*, 863–872.

Holmberg, J., Clarke, D.L., and Frisen, J. (2000). Regulation of repulsion versus adhesion by different splice forms of an Eph receptor. Nature *408*, 203–206.

Huber, A.H., and Weis, W.I. (2001). The structure of the β-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by β-catenin. Cell *105*, 391–402.

Huelsken, J., Vogel, R., Erdmann, B., Cotsarelis, G., and Birchmeier, W. (2001). β-catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell *105*, 533–545.

Ikeda, W., Nakanishi, H., Miyoshi, J., Mandai, K., Ishizaki, H., Tanaka, M., Togawa, A., Takahashi, K., Nishioka, H., Yoshida, H., et al. (1999). Afadin: a key molecule essential for structural organization of cell-cell junctions of polarized epithelia during embryogenesis. J. Cell Biol. *146*, 1117–1132.

Ireton, R.C., Davis, M.A., van Hengel, J., Mariner, D.J., Barnes, K., Thoreson, M.A., Anastasiadis, P.Z., Matrisian, L., Bundy, L.M., Sealy, L., et al. (2002). A novel role for p120 catenin in E-cadherin function. J. Cell Biol. *159*, 465–476.

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

Izumi, Y., Hirose, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kemphues, K.J., and Ohno, S. (1998). An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. J. Cell Biol. *143*, 95–106.

Jacinto, A., Wood, W., Woolner, S., Hiley, C., Turner, L., Wilson, C., Martinez-Arias, A., and Martin, P. (2002). Dynamic analysis of actin cable function during *Drosophila* dorsal closure. Curr. Biol. *12*, 1245–1250.

Jan, Y.N., and Jan, L.Y. (2001). Asymmetric cell division in the *Drosophila* nervous system. Nat. Rev. Neurosci. *2*, 772–779.

Johnson, R.P., and Craig, S.W. (1995). F-actin binding site masked by the intramolecular association of vinculin head and tail domains. Nature *373*, 261–264.

Kang, D.E., Soriano, S., Xia, X., Eberhart, C.G., De Strooper, B., Zheng, H., and Koo, E.H. (2002). Presenilin couples the paired phosphorylation of β-catenin independent of axin: implications for β-catenin activation in tumorigenesis. Cell *110*, 751–762.

Karakesisoglou, I., Yang, Y., and Fuchs, E. (2000). An epidermal plakin that integrates actin and microtubule networks at cellular junctions. J. Cell Biol. *149*, 195–208.

Kiehart, D.P., Galbraith, C.G., Edwards, K.A., Rickoll, W.L., and Montague, R.A. (2000). Multiple forces contribute to cell sheet morphogenesis for dorsal closure in *Drosophila*. J. Cell Biol. *149*, 471–490.

Kim, S.H., Li, Z., and Sacks, D.B. (2000). E-cadherin-mediated cell-cell attachment activates Cdc42. J. Biol. Chem. *275*, 36999–37005.

Knox, A.L., and Brown, N.H. (2002). Rap1 GTPase regulation of adherens junction positioning and cell adhesion. Science *295*, 1285–1288.

Knust, E., and Bossinger, O. (2002). Composition and formation of intercellular junctions in epithelial cells. Science *298*, 1955–1959.

Koslov, E.R., Maupin, P., Pradhan, D., Morrow, J.S., and Rimm, D.L. (1997). α-catenin can form asymmetric homodimeric complexes and/or heterodimeric complexes with β-catenin. J. Biol. Chem. *272*, 27301–27306.

Kowalczyk, A.P., Bornslaeger, E.A., Norvell, S.M., Palka, H.L., and Green, K.J. (1999). Desmosomes: intercellular adhesive junctions specialized for attachment of intermediate filaments. Int. Rev. Cytol. *185*, 237–302.

Kraut, R., Chia, W., Jan, L.Y., Jan, Y.N., and Knoblich, J.A. (1996). Role of inscuteable in orienting asymmetric cell divisions in *Drosophila*. Nature *383*, 50–55.

Krugmann, S., Jordens, I., Gevaert, K., Driessens, M., Vandekerckhove, J., and Hall, A. (2001). Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. Curr. Biol. *11*, 1645–1655.

Kullander, K., and Klein, R. (2002). Mechanisms and functions of Eph and ephrin signalling. Nat. Rev. Mol. Cell Biol. *3*, 475–486.

Kuroda, S., Fukata, M., Nakagawa, M., Fujii, K., Nakamura, T., Ookubo, T., Izawa, I., Nagase, T., Nomura, N., Tani, H., et al. (1998). Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin-mediated cell-cell adhesion. Science *281*, 832–835.

Lampugnani, M.G., Zanetti, A., Breviario, F., Balconi, G., Orsenigo, F., Corada, M., Spagnuolo, R., Betson, M., Braga, V., and Dejana, E. (2002). VE-cadherin regulates endothelial actin activating Rac and increasing membrane association of Tiam. Mol. Biol. Cell *13*, 1175–1189.

Le Borgne, R., Bellaiche, Y., and Schweisguth, F. (2002). *Drosophila* E-cadherin regulates the orientation of asymmetric cell division in the sensory organ lineage. Curr. Biol. *12*, 95–104.

Ligon, L.A., Karki, S., Tokito, M., and Holzbaur, E.L. (2001). Dynein binds to β-catenin and may tether microtubules at adherens junctions. Nat. Cell Biol. *3*, 913–917.

Lu, B., Roegiers, F., Jan, L.Y., and Jan, Y.N. (2001). Adherens junctions inhibit asymmetric division in the *Drosophila* epithelium. Nature *409*, 522–525.

Lyczak, R., Gomes, J.E., and Bowerman, B. (2002). Heads or tails: cell polarity and axis formation in the early *Caenorhabditis elegans* embryo. Dev. Cell *3*, 157–166.

Magie, C.R., Pinto-Santini, D., and Parkhurst, S.M. (2002). Rho1 interacts with p120ctn and α-catenin, and regulates cadherin-based adherens junction components in *Drosophila*. Development *129*, 3771–3782.

Marambaud, P., Shioi, J., Serban, G., Georgakopoulos, A., Sarner, S., Nagy, V., Baki, L., Wen, P., Efthimiopoulos, S., Shao, Z., et al. (2002). A presenilin-1/γ-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions. EMBO J. *21*, 1948–1956.

Merrill, B.J., Gat, U., DasGupta, R., and Fuchs, E. (2001). Tcf3 and Lef1 regulate lineage differentiation of multipotent stem cells in skin. Genes Dev. *15*, 1688–1705.

Miller, R.K., and Rose, M.D. (1998). Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast. J. Cell Biol. *140*, 377–390.

Moon, R.T., Bowerman, B., Boutros, M., and Perrimon, N. (2002). The promise and perils of Wnt signaling through β-catenin. Science *296*, 1644–1646.

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

Nawroth, R., Poell, G., Ranft, A., Kloep, S., Samulowitz, U., Fachinger, G., Golding, M., Shima, D.T., Deutsch, U., and Vestweber, D. (2002). VE-PTP and VE-cadherin ectodomains interact to facilitate regulation of phosphorylation and cell contacts. EMBO J. *21*, 4885–4895.

Noren, N.K., Liu, B.P., Burridge, K., and Kreft, B. (2000). p120 catenin regulates the actin cytoskeleton via Rho family GTPases. J. Cell Biol. *150*, 567–580.

Noren, N.K., Niessen, C.M., Gumbiner, B.M., and Burridge, K. (2001). Cadherin engagement regulates Rho family GTPases. J. Biol. Chem. *276*, 33305–33308.

Nose, A., Nagafuchi, A., and Takeichi, M. (1988). Expressed recombinant cadherins mediate cell sorting in model systems. Cell *54*, 993–1001.

Orsulic, S., and Kemler, R. (2000). Expression of Eph receptors and ephrins is differentially regulated by E-cadherin. J. Cell Sci. *113*, 1793–1802.

Pokutta, S., and Weis, W.I. (2002). The cytoplasmic face of cell contact sites. Curr. Opin. Struct. Biol. *12*, 255–262.

Pokutta, S., Drees, F., Takai, Y., Nelson, W.J., and Weis, W.I. (2002). Biochemical and structural definition of the l-afadin- and actin-binding sites of α-catenin. J. Biol. Chem. *277*, 18868–18874.

Priess, J.R., and Hirsh, D.I. (1986). *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. Dev. Biol. *117*, 156–173.

Pruyne, D.W., Schott, D.H., and Bretscher, A. (1998). Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. J. Cell Biol. *143*, 1931–1945.

Rath, P., Lin, S., Udolph, G., Cai, Y., Yang, X., and Chia, W. (2002). Inscuteable-independent apicobasally oriented asymmetric divisions in the *Drosophila* embryonic CNS. EMBO Rep. *3*, 660–665.

Rogers, S.L., Rogers, G.C., Sharp, D.J., and Vale, R.D. (2002). *Drosophila* EB1 is important for proper assembly, dynamics, and positioning of the mitotic spindle. J. Cell Biol. *158*, 873–884.

Sagot, I., Rodal, A.A., Moseley, J., Goode, B.L., and Pellman, D. (2002). An actin nucleation mechanism mediated by Bni1 and profilin. Nat. Cell Biol. *4*, 626–631.

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

Schober, M., Schaefer, M., and Knoblich, J.A. (1999). Bazooka recruits inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts. Nature *402*, 548–551.

Shay-Salit, A., Shushy, M., Wolfovitz, E., Yahav, H., Breviario, F., Dejana, E., and Resnick, N. (2002). VEGF receptor 2 and the ad-

herens junction as a mechanical transducer in vascular endothelial cells. Proc. Natl. Acad. Sci. USA *99*, 9462–9467.

Shimamura, K., Hirano, S., McMahon, A.P., and Takeichi, M. (1994). Wnt-1-dependent regulation of local E-cadherin and α N-catenin expression in the embryonic mouse brain. Development *120*, 2225–2234.

Song, X., Zhu, C.H., Doan, C., and Xie, T. (2002). Germline stem cells anchored by adherens junctions in the *Drosophila* ovary niches. Science *296*, 1855–1857.

Sun, D., Leung, C.L., and Liem, R.K. (2001). Characterization of the microtubule binding domain of microtubule actin crosslinking factor (MACF): identification of a novel group of microtubule associated proteins. J. Cell Sci. *114*, 161–172.

Takahashi, K., Nakanishi, H., Miyahara, M., Mandai, K., Satoh, K., Satoh, A., Nishioka, H., Aoki, J., Nomoto, A., Mizoguchi, A., and Takai, Y. (1999). Nectin/PRR: an immunoglobulin-like cell adhesion molecule recruited to cadherin-based adherens junctions through interaction with Afadin, a PDZ domain-containing protein. J. Cell Biol. *145*, 539–549.

Tepass, U., Godt, D., and Winklbauer, R. (2002). Cell sorting in animal development: signalling and adhesive mechanisms in the formation of tissue boundaries. Curr. Opin. Genet. Dev. *12*, 572–582.

Theesfeld, C.L., Irazoqui, J.E., Bloom, K., and Lew, D.J. (1999). The role of actin in spindle orientation changes during the *Saccharomyces cerevisiae* cell cycle. J. Cell Biol. *146*, 1019–1032.

Tsukita, S., Furuse, M., and Itoh, M. (2001). Multifunctional strands in tight junctions. Nat. Rev. Mol. Cell Biol. *2*, 285–293.

Tutter, A.V., Fryer, C.J., and Jones, K.A. (2001). Chromatin-specific regulation of LEF-1-β-catenin transcription activation and inhibition in vitro. Genes Dev. *15*, 3342–3354.

Vaezi, A., Bauer, C., Vasioukhin, V., and Fuchs, E. (2002). Actin cable dynamics and Rho/Rock orchestrate a polarized cytoskeletal architecture in the early steps of assembling a stratified epithelium. Dev. Cell *3*, 367–381.

van Noort, M., and Clevers, H. (2002). TCF transcription factors, mediators of Wnt-signaling in development and cancer. Dev. Biol. *244*, 1–8.

Vasioukhin, V., Bauer, C., Yin, M., and Fuchs, E. (2000). Directed actin polymerization is the driving force for epithelial cell-cell adhesion. Cell *100*, 209–219.

Vasioukhin, V., Bauer, C., Degenstein, L., Wise, B., and Fuchs, E. (2001). Hyperproliferation and defects in epithelial polarity upon conditional ablation of β-catenin in skin. Cell *104*, 605–617.

Wodarz, A. (2002). Establishing cell polarity in development. Nat. Cell Biol. *4*, E39–44.

Wodarz, A., Ramrath, A., Kuchinke, U., and Knust, E. (1999). Bazooka provides an apical cue for inscuteable localization in *Drosophila* neuroblasts. Nature *402*, 544–547.

Wood, W., Jacinto, A., Grose, R., Woolner, S., Gale, J., Wilson, C., and Martin, P. (2002). Wound healing recapitulates morphogenesis in *Drosophila* embryos. Nat. Cell Biol. *4*, 907–912.

Yang, H.C., and Pon, L.A. (2002). Actin cable dynamics in budding yeast. Proc. Natl. Acad. Sci. USA *99*, 751–756.

Yap, A.S., and Kovacs, E.M. (2003). Direct cadherin-activated cell signaling: a view from the plasma membrane. J. Cell Biol. *160*, 11–16.

Yin, H., Pruyne, D., Huffaker, T.C., and Bretscher, A. (2000). Myosin V orientates the mitotic spindle in yeast. Nature *406*, 1013–1015.

Yonemura, S., Itoh, M., Nagafuchi, A., and Tsukita, S. (1995). Cell-to-cell adherens junction formation and actin filament organization: similarities and differences between non-polarized fibroblasts and polarized epithelial cells. J. Cell Sci. *108*, 127–142.