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MOLECULAR MECHANISMS OF EPITHELIAL MORPHOGENESIS

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■ **Abstract** Epithelial morphogenesis comprises the various processes by which epithelia contribute to organ formation and body shape. These complex and diverse events play a central role in animal development and regeneration. Recently, the characterization of some of the molecular mechanisms involved in epithelial morphogenesis has provided an abundance of new information on the role and regulation of the cytoskeleton, cell-cell adhesion, and cell-matrix adhesion in these processes. In this review, we discuss our current understanding of the molecular mechanisms driving cell shape changes, cell intercalation, fusion of epithelia, ingression, egression, and cell migration. Our discussion is mostly focused on results from *Drosophila* and mammalian tissue culture but also draws on the insights gained from other organisms.

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INTRODUCTION

Adequate cell adhesive properties are required for most developmental processes generating a multicellular organism. In particular, cell adhesion is critical to the formation of coherent sheets of cells or epithelia (Gumbiner 1996). Subsequent complex changes in the organization of epithelial sheets, which include changes in cell shape and cell motility, eventually generate the diversity of body shapes found in metazoans. Thus understanding the mechanisms that drive the cellular rearrangement of epithelial sheets is fundamental to understanding morphogenesis.

Epithelial morphogenesis is a multistep process, each part of which needs to be carefully coordinated and integrated in order to achieve the appropriate biological outcome. First, diverse cell fates are specified by an array of transcription factors, which differentiate the various cell groups that are to undergo the cellular changes (Leptin 1995). Second, extracellular signals trigger specific morphogenetic events within a whole epithelial sheet or within particular subsets of cells (Montell 2001, Oda & Tsukita 2001). In a third step, the responding cells execute a morphogenetic program, which requires the precise coordination of the cells' cytoskeletal and adhesive properties (Gumbiner 1996). Finally, patterns of cell proliferation and cell death can contribute to epithelial morphogenesis (Conlon & Raff 1999, Vaux & Korsmeyer 1999).

Overall, we are far from a coherent picture of even a simple morphogenetic process that fully integrates the information from these various steps. Although developmental biology has been successful in establishing the epistatic relationship of transcription factors and signal transduction molecules required for cell fate determination and initiation of morphogenesis, much less is known about the nature and function of the actual effectors of morphogenesis. Indeed, even when cytoskeletal and cell adhesion regulators have been implicated in a particular process, it is not clear how these molecules operate. Cell biology, on the other hand, has successfully characterized the function of proteins or protein complexes in individual cells but rarely provides information on the relative physiological relevance of these observations for a particular morphogenetic process.

In this review, we classify morphogenetic events into groups that potentially employ similar molecular mechanisms, keeping the focus on the actual effectors of shape changes. We distinguish four major types of epithelial morphogenetic mechanisms: (*a*) cell shape changes; (*b*) cell intercalation, ingression, egression, and fusion; (*c*) cell migration; and (*d*) cell division and cell death. The basis of our classification is as follows: First, in cell shape changes, individual cells reorganize their cytoskeleton, which causes a remodeling of epithelial shape as a whole, but does not involve changes in cell position within an epithelial sheet. Second, cell intercalation, ingression, egression, and fusion of epithelia require, in addition to the reorganization of the cytoskeleton, a breakdown and rebuilding of the cadherin-mediated cell-cell adhesion system and changes in cell position relative to other cells in an epithelium. Third, cell migration of epithelia requires, in addition to cytoskeletal remodeling, integrin-mediated cell-matrix adhesion. Lastly, we have

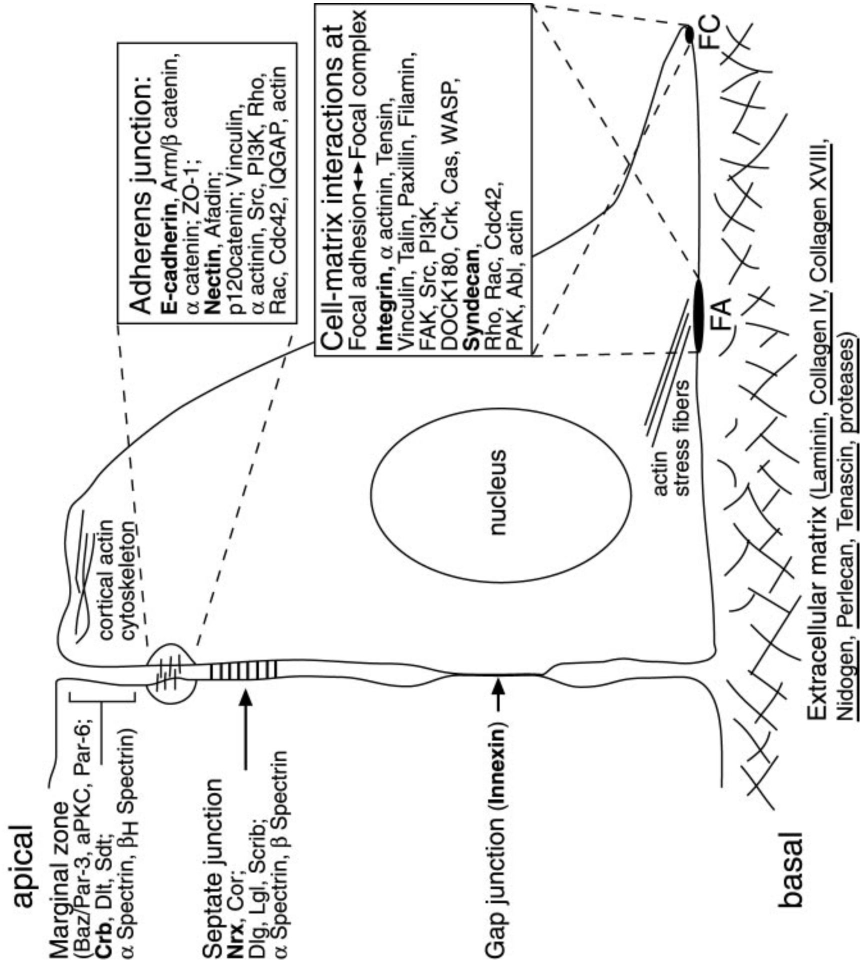
grouped cell division and cell death together because the spatial and temporal regulation of these processes in some cases plays an important role in shaping tissues.

EPITHELIAL STRUCTURE

Epithelia serve two main functions: protection from the environment and the separation of two different chemical milieus. An epithelium is a laterally coherent sheet of cells with distinct apical-basal polarity. Cells or cell aggregates lacking these criteria are defined as mesenchymal. Although mesenchymal cells are sometimes polarized, the orientation of their polarization is generally random. In contrast, epithelial cells are all polarized in the same manner, and they are interconnected by junctions that link them to each other (Fristrom 1988). Every epithelial cell is subdivided into an apical and a basolateral membrane domain. The apical surface of epithelia faces either the exterior environment, in the case of epidermal epithelia, or the luminal space, in the case of internal epithelia. The basolateral domain is further subdivided into a basal domain mediating cell-matrix adhesion and a lateral domain involved in cell-cell adhesion. Similarly, the apical domain is divided into the free apical domain and a domain where a neighboring cell is contacted (Figure 1). This region is called the marginal zone in *Drosophila* and is presumably related to the tight junction in vertebrates, which is found at the same location (Tepass et al. 2001). The simplest classification of epithelia is by cell proportions: A columnar epithelium features tall cells, a cuboidal epithelium features cells that are as high as they are wide, and a squamous epithelium consists of very flat cells. Most epithelia are single-layered, but a few adult epithelia are multilayered such as the stratified skin of mammals.

Primary epithelia form early in embryogenesis either by compaction of loosely adherent cells at the blastula stage or by cellularization, a process best analyzed in insects in which a syncytium is subdivided into individual cells by membrane formation around cortically localized nuclei. Secondary epithelia arise by a mesenchymal-to-epithelial transition of mesenchymal cells. Mesenchymal cells are themselves derived from primary epithelia that have undergone an epithelial-to-mesenchymal transition during gastrulation (Fristrom 1988, Tepass et al. 2001).

Several types of junctions mediate cell-cell and cell-matrix contact in epithelia. The most relevant junctions for epithelial morphogenesis are the adherens junction and the hemiadherens junction. The adherens junction is a generally circumferential junction located just basal to the apical-basolateral boundary. Its major function is to link the actin cytoskeleton of neighboring cells. The hemiadherens junction is a spot-like junction connecting the actin cytoskeleton of a cell to the basement membrane (Figure 1) (Tepass & Hartenstein 1994, Tepass et al. 2001). Adhesion of adherens and hemiadherens junctions is mediated by cadherin and integrin, respectively (discussed below). Note that we do not discuss desmosomes and hemidesmosomes because these structures are absent in invertebrates (Hynes & Zhao 2000).


apical
Marginal zone
(Baz/Par-3, aPKC, Par-6;
Crb, Dlt, Sdt;
α Spectrin, β_H Spectrin)
Septate junction
Nrx, Cor;
Dlg, Lgl, Scrib;
α Spectrin, β Spectrin
Gap junction (**Innexin**)
basal
cortical actin
cytoskeleton
nucleus
actin
stress fibers
FA
FC
Adherens junction:
E-cadherin, Arm/β catenin,
α catenin; ZO-1;
Nectin, Afadin;
p120catenin; Vinculin,
α actinin, Src, PI3K, Rho,
Rac, Cdc42, IQGAP, actin
Cell-matrix interactions at
Focal adhesion↔Focal complex
Integrin, α actinin, Tensin,
Vinculin, Talin, Paxillin, Filamin,
FAK, Src, PI3K,
DOCK180, Crk, Cas, WASP,
Syndecan,
Rho, Rac, Cdc42,
PAK, Abl, actin
Extracellular matrix (Laminin, Collagen IV, Collagen XVIII,
Nidogen, Perlecan, Tenascin, proteases)

In addition to the adherens and hemiadherens junction, three other junctional complexes exist in *Drosophila*: the gap junction, septate junction, and the marginal zone (Figure 1). Gap junctions form intercellular channels allowing the transmission of ions and small molecules between cells; they are not thought to play a major role in cell adhesion or morphogenesis (Phelan & Starich 2001).

The septate junction of insects lies immediately basal to the adherens junction and is characterized by an array of electron-dense septae that probably mediate the function of septate junctions as permeability barriers (Tepass et al. 2001). Septate junctions form only after most morphogenetic events have taken place (Tepass & Hartenstein 1994). Three proteins, Discs large (Dlg), Lethal giant larvae (Lgl), and Scribble (Scrib), which localize basal to the adherens junction at early embryonic stages and later at the septate junction, have been proposed to form a biochemical complex that controls and refines the segregation of apical and basolateral membrane domains (Bilder et al. 2000, Bilder & Perrimon 2000).

Finally, a structure called the marginal zone lies apical to the adherens junction (Figure 1). It is defined by the accumulation of two protein complexes, the Crumbs (Crb), Stardust (Sdt), Discs lost (Dlt) complex and the Bazooka (Baz)/Par-3, atypical protein kinase C (aPKC), Par-6 complex. These complexes are important for the generation of epithelial polarity (Tepass et al. 2001). In vertebrates, the Baz/Par-3 complex localizes to tight junctions and is necessary for their formation (Tepass et al. 2001). Similar to the marginal zone, the tight junction is found apical to the adherens junction in vertebrates and separates the apical and basolateral membrane domains (Fesenko et al. 2000). In *Drosophila*, the Baz/Par-3 complex is also needed for the establishment of the fully differentiated adherens junction (Müller & Wieschaus 1996). Mutations in genes coding for the Dlg, the Crb, and the Baz/Par-3 complex all disrupt epithelia formation but appear to do so because

Figure 1 Schematic of an idealized epithelial *Drosophila* cell. The septate junction starts to form late in embryonic development, at stage 14/15 of *Drosophila* embryogenesis. Basally, a basement membrane assembles from some ECM components during late embryogenesis. In addition, focal adhesions presumably develop into hemiadherens junctions during differentiation. Proteins participating in the formation and regulation of adherens junctions and cell-matrix interactions, the two predominant determinants of epithelial morphogenesis, are shown in boxes on the right. The function of most of these proteins in cadherin- or integrin-mediated adhesion in *Drosophila* is hypothetical and inferred from studies in other systems. No mutants have been described for α catenin, collagen XVIII, crk, cas, FAK, nectin, nidogen, p120catenin, IQGAP, filamin, paxillin, perlecan, syndecan, talin, and tensin. Transmembrane proteins are printed in bold type, cytoplasmic proteins in plain type, and secreted proteins are underlined. Only a selection of proteins with their most common name is shown. Several genes have paralogs in the *Drosophila* genome, e.g., there are three Rac genes, two collagen type IV genes, five α integrin genes, etc. FA, focal adhesion; FC, focal complex.

they are required for the initial generation of epithelial polarity and junctions; however, their contribution to epithelial morphogenesis has not yet been clarified.

Adhesive Properties of Epithelial Cells

During morphogenesis, epithelia undergo extensive rearrangements in response to extracellular signals. This requires the coordinated regulation of cell-cell adhesion, cell-matrix adhesion, and the cytoskeleton. The two predominant adhesion mechanisms are cadherin-mediated cell-cell adhesion at the adherens junction and integrin-mediated cell-matrix adhesion on the basal side of cells. Both adhesion systems share common regulators such as tyrosine kinases and Rho family GTPases. In addition, they share structural components such as vinculin and α actinin and, of course, actin, to which they are connected. Because both vinculin and α actinin compete for regulators and for the pool of free actin, they are presumably tightly co-regulated (Schmidt et al. 1993).

Results from vertebrate tissue culture have established that cadherins mediate Ca^{2+}-dependent homophilic interactions between two cells and are prevalent at, but not restricted to, the adherens junction (Gumbiner 2000). Cadherins are thought first to form *cis*-dimers with other cadherins in the same membrane, then *trans*-dimers by interdigitating with *cis*-dimers on opposite membranes (Fukata & Kaibuchi 2001). The cadherin protein consists of several extracellular cadherin domains that mediate the homophilic interaction, and two cytoplasmic domains, one binding to β catenin and the other binding to p120 catenin (p120ctn), an important regulator of cadherin clustering. Cadherins are anchored to the actin cytoskeleton by α catenin, which binds to β catenin. β catenin acts either as an adaptor linking cadherins to the actin cytoskeleton or as part of the Wingless/Wnt signal transduction pathway (Fukata & Kaibuchi 2001, Gumbiner 2000). Vinculin also helps to anchor cadherins to the actin cytoskeleton and appears to be important for organizing cadherin into circumferential adherens junctions (Watabe-Uchida et al. 1998).

Integrins are heterodimeric transmembrane receptors consisting of an α subunit noncovalently associated with a β subunit. They link the extracellular matrix (ECM) to the actin cytoskeleton at structures called hemiadherens junctions in differentiated tissues. Integrin function is best characterized in migrating mesenchymal cells, which contain two main types of adhesion sites, the focal adhesion and focal complex sites. Focal adhesions are large, elongated structures often located halfway between the nucleus and the migrating edge of a cell. They provide strong adhesion to the substrate and are anchored to bundles of actin microfilaments, called actin stress fibers (Geiger et al. 2001). Focal adhesions appear to be the precursors of adhesion sites found in differentiated tissues such as the hemiadherens junction or muscle attachment site (Kano et al. 1996, Turner et al. 1991). In contrast, focal complexes are small, dot-like transient complexes present in lamellipodia or filopodia. Focal complexes are associated with cell migration and can serve as precursors for focal adhesions (Geiger et al. 2001). Importantly, it has recently been shown that focal complexes generate the propulsive force for cell migration in fibroblasts (Beningo et al. 2001).

Integrins bind to virtually all major constituents of the ECM. Extracellular ligands of integrins include collagens and laminins, and fibronectin in vertebrates. Several proteins, including α actinin, talin, tensin and filamin, connect integrins to the actin cytoskeleton intracellularly (Kreis & Vale 1999). Other diffusely localized proteins also play an important role in adhesion. Among these are the immunoglobulin-like proteins, which mediate cell-cell adhesion, and the proteoglycans, which can mediate both cell-cell and cell-matrix adhesion (Hynes & Zhao 2000).

Cytoskeletal Organization of Epithelial Cells

For a detailed description of cytoskeletal organization and regulation, the reader is referred to another review in this volume (Welch & Mullins 2002). In epithelia, not only the membrane, but also the underlying cortical actin cytoskeleton is polarized. One protein that appears to play a particularly important role in the polarization of the actin cytoskeleton is spectrin. Spectrin molecules are tetrameric actin cross-linking proteins having two α and two β subunits. In *Drosophila*, three different spectrin subunits, α, β, and β_H, assemble into two isoforms exhibiting non-overlapping distributions (Dubreuil & Yu 1994, Thomas & Kiehart 1994). $\alpha_2\beta_2$ spectrin localizes to the basolateral membrane where it forms a complex with ankyrin, which anchors the cortical actin cytoskeleton to the plasma membrane (Dubreuil & Yu 1994). In contrast, $\alpha_2\beta_{H2}$ spectrin associates with the apical domain (Thomas & Kiehart 1994).

The cortical actin cytoskeleton contains many bipolar assemblies of nonmuscle myosin II molecules that can slide actin filaments over each other, thus mediating local contractions of actin filament bundles (Small et al. 1999). The polarized spectrin network may help to recruit regulators of actomyosin contraction to the apical or basolateral domain, respectively.

Lastly, microtubules act mainly during mitosis, vesicle transport, and stabilization of the cytoarchitecture (Gelfand & Bershadsky 1991). They also contribute to some morphogenetic processes such as cell migration, even though their effect appears to be indirect. For example, inhibition of the motor domain of kinesin inhibits cell migration in fibroblasts (Rodionov et al. 1993). Indeed, it has been shown that microtubules are targeted to and stabilized at cell-matrix adhesion sites (Kaverina et al. 1998) and that microtubules promote the turnover of these sites (Kaverina et al. 1999), thereby indirectly contributing to cell migration. However, cell migration is possible without microtubules in other systems (Euteneuer & Schliwa 1984, Kaverina et al. 2000).

CYTOSKELETAL REMODELING IN EPITHELIA

One of the most common events associated with epithelial morphogenesis is reorganization of cell shape. Cell shape changes refer to the modification in dimension of one or more sides of the cells in an epithelial sheet. These changes depend on the

concerted reorganization of the cytoskeleton and are often mediated by differential actomyosin contractility. Cell shape changes can occur along several sides of a cell as during flattening or elongation of an epithelial sheet, or they can be restricted to one side of a cell as during the first step of invagination.

Cell Shape Changes

A common cell shape change is the shortening of the lateral sides of a cell along the apical-basal axis. This event results in the flattening of the epithelial sheet such that the epithelium increases its apical and basal surface at the expense of its lateral surfaces (Figure 2*A*). Clear examples of flattening are found in epiboly of frog embryos and during the formation of the wing blades in insects (Fristrom 1988). The reverse process, columnarization, is more unusual. An example of columnarization of epithelial cells is found in a subpopulation of follicle cells during *Drosophila* oogenesis (Zarnescu & Thomas 1999). Little is known about the mechanisms regulating the substantial amount of cytoskeletal reorganization underlying flattening or columnarization. Columnarization is disrupted in follicle cells mutant for β_H spectrin, meaning that the follicle cells overlying the oocyte remain cuboidal (Zarnescu & Thomas 1999). In such mutants, the apical spectrin membrane skeleton consisting of $\alpha_2\beta_{H2}$ spectrin is absent and the adherens junction is disrupted (Zarnescu & Thomas 1999), implying that apical constriction contributes to columnarization. During flattening, the surface area increases on the apical and the basal side. Although the basal side could theoretically grow at the expense of the lateral sides, the apical membrane has a noticeably different composition, and therefore an increase in apical surface probably requires the transport of newly synthesized components. In addition, the adherens junctions must adjust to the increased circumference of the cell. Electron micrograph studies have shown that adherens junctions in columnar cells display an increased height compared with squamous cells, suggesting that adherens junctions might be able to stretch to some extent during flattening (Fristrom 1988).

Shortening along the planar axis of cells causes anisotropic changes in an epithelial sheet, i.e., a shortening along one side and concomitant elongation along the other side (Figure 2*B*). This process has been observed in morphogenetic events such as germ band retraction in the *Drosophila* amnioserosa, *Caenorhabditis elegans* ectoderm elongation, and *Drosophila* egg elongation (Bateman et al. 2001, Frydman & Spradling 2001, Priess & Hirsh 1986, Schöck & Perrimon 2002). In both the elongation of the *C. elegans* ectoderm, which transforms the ovoid embryo into the final worm, and the conversion of a round egg into an elongated egg by follicle cells during *Drosophila* oogenesis, prominent actin bundles lie parallel to the axis of cell shortening (Bateman et al. 2001, Frydman & Spradling 2001, Priess & Hirsch 1986). In follicle cells, the actin bundles are localized basally and the filament ends colocalize with βPS integrin. Mutating βPS integrin or disrupting the polarity of actin bundles causes defects in egg elongation, which suggests involvement of these actin bundles in egg elongation (Bateman et al. 2001). In *C. elegans* embryos, actin filament bundles are localized at the apical cortex and appear to

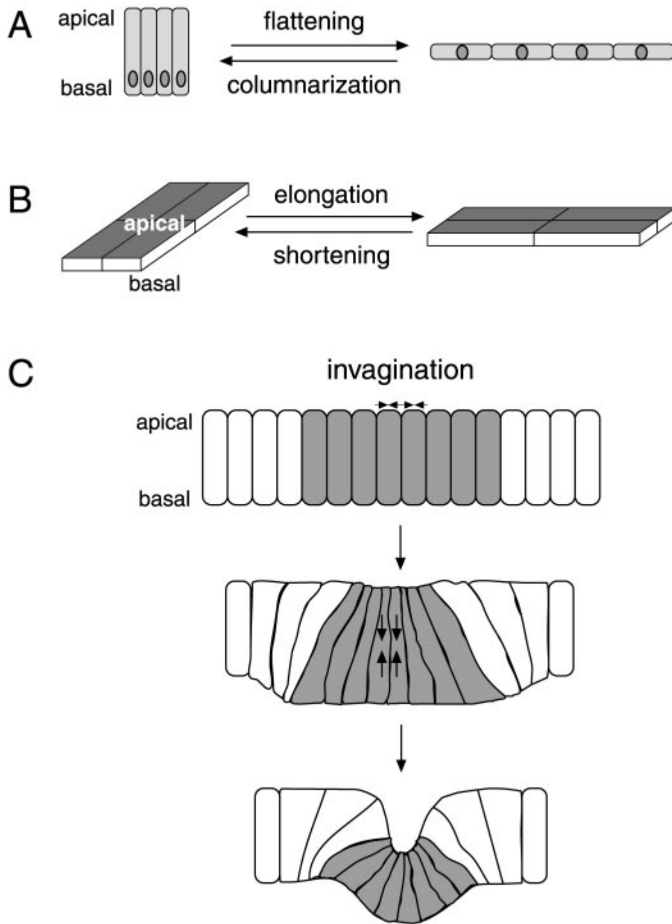


Figure 2 Cell shape changes. (*A*) Flattening and columnarization of epithelial cells lead to shortening and elongation along the apical-basal axis, respectively. These events result from isotropic changes in surface area. (*B*) Elongation along the planar axis results in anisotropic changes in surface area. (*C*) Invagination as illustrated by ventral furrow formation in *Drosophila*. Apical constriction causes increased columnarization and formation of a shallow groove. Presumably because of the continued constriction of the cell apices, shortening along the apical-basal axis then results in inward movement and furrow formation. Invaginating cells are shown in gray. Arrows indicate the major cell shape changes of two cells located in the middle.

connect with the adherens junctions that encircle each ectodermal cell (Costa et al. 1998, Priess & Hirsh 1986). Indeed, mutations in α catenin, β catenin, and cadherin disrupt the elongation of *C. elegans* embryos completely. In these mutants, actin filament bundles are detached from adherens junctions, supporting the model that actomyosin contraction is the principal force during elongation (Costa et al.

1998). Consistent with this, treating embryos with cytochalasin D, which disrupts actin filaments, completely inhibits their elongation (Priess & Hirsh 1986).

Lastly, there is a group of more complex cell shape changes leading to a bending of epithelial sheets. The most important of those is invagination, which describes the inpocketing or furrow formation at a specific region of an epithelium, with the apical surface being the concave side (Figure 2*C*). It is the first step of gastrulation in most animals and also initiates the formation of many internal organs such as the gut, insect trachea, and salivary glands. A related process is evagination, which describes the outpocketing or ridge formation within an epithelium such that the apical surface is the convex side of the pocket (Fristrom 1988). It is much less frequent but occurs, for example, during budding in Hydra (Graf & Gierer 1980). Finally, folds can arise through a passive response to extrinsic forces. One such example is the ciliary body of avian eyes. Here, at a specific stage of development, regular radial folds form. These folds arise from an increase in intraocular pressure and can be stimulated artificially even in the presence of the actin fiber inhibitor, cytochalasin D, and the microtubule inhibitor, colchicine (Bard & Ross 1982).

Cytoskeletal Remodeling in *Drosophila* Invagination

The invagination process during *Drosophila* gastrulation is characterized by the formation of a ventral furrow and allows insight into the molecular basis of apical constriction within epithelial cells. Ventral furrow formation in *Drosophila* embryos occurs in a stripe about 10 cells wide along the ventral midline (Figure 2*C*). It begins with a flattening of cell apices, followed by a synchronous slow constriction of the apical plasma membrane. Subsequently, cells randomly constrict faster resulting in formation of a shallow groove and a squeezing of the cells' contents to the basal side. This constriction event also causes further columnarization and basal extension of the invaginating cells (*middle panel*, Figure 2*C*). Subsequently, because the cell apices remain constricted, cell shortening along the apical-basal axis causes further basal extension and deepening of the furrow (Costa et al. 1993, Oda & Tsukita 2001). Finally, as the closing of the furrow occurs, the invaginated mesodermal cells undergo an epithelial-to-mesenchymal transition. The whole gastrulation process from apical flattening to complete furrow invagination takes less than 20 min (Leptin & Grunewald 1990).

In the first step of invagination, *twist* and *snail*, which encode a transcription factor and a transcriptional repressor, respectively, determine mesodermal cell fate. Twist activates most mesodermal genes such as N-cadherin, whereas Snail represses ectodermal genes such as E-cadherin (Oda et al. 1998). Differential cadherin expression is later required for the epithelial-to-mesenchymal transition, which starts with the degradation of E-cadherin at the time of ventral furrow closure (Oda et al. 1998). Interestingly, in electron micrographs of epithelial sections, vesicles are visible close to degrading adherens junctions, possibly implying endocytic recycling in this process (Oda et al. 1998). The down-regulation of E-cadherin is conserved in vertebrates, where *snail* mutant mice display less

E-cadherin down-regulation in the mesoderm than in wild-type mice (Carver et al. 2001). However, this switch from E- to N-cadherin contributes to only the epithelial-to-mesenchymal transition, not to the morphogenetic movements of ventral furrow formation, as N-cadherin mutants in *Drosophila* have no obvious defects in gastrulation (Iwai et al. 1997).

twist and *snail* also regulate the expression of *folded gastrulation* (*fog*), a putative secreted molecule, that together with the maternally supplied *concertina* (*cta*), a G protein α subunit, appears to be necessary for the correct timing of the initiation of gastrulation. This view is supported by the observation that random constrictions still occur in *fog* mutants (Costa et al. 1994), whereas synchronous initiation of constrictions is disrupted in this mutant (Oda & Tsukita 2001).

Apical constriction in invagination seems to be mediated by actomyosin contractility because nonmuscle myosin II relocalizes to the apical ends of constricting cells (Young et al. 1991). In addition, strong apical staining of the cortical cytoskeleton protein β_H spectrin was observed in ventral furrow cells (Thomas & Kiehart 1994). Furthermore, expression of a dominant-negative form of the GTPase RhoA during gastrulation inhibits ventral furrow formation completely, and embryos that lack *DRhoGEF2* activity, a guanine nucleotide exchange factor for RhoA, do not gastrulate (see below) (Barrett et al. 1997, Häcker & Perrimon 1998). *DRhoGEF2* is also required for shape changes induced by ectopic *fog* expression, i.e., apical constrictions induced by ectopic *fog* on the dorsal side of the embryo are absent in a *DRhoGEF2* mutant (Barrett et al. 1997), thus placing *DRhoGEF2* activity downstream of *fog* and *cta*. One possibility is that DRhoGEF2 is localized apically and therefore activates RhoA only locally, which would then trigger apical constriction by inducing actomyosin contractility. In this case, DRhoGEF2 might be recruited by β_H spectrin, whose apical localization becomes particularly strong during ventral furrow formation (Thomas & Kiehart 1994). This, however, still does not explain invagination, because apical constriction results only in a shallow groove. Additionally, DRhoGEF2 may activate RhoA along the lateral sides, resulting in apicobasal shortening of the invaginating cells.

Rho-Family GTPases as Regulators of Cytoskeletal Remodeling

GTPases are the most ubiquitous regulators of cytoskeletal reorganization, cell-cell adhesion, and cell-matrix adhesion. In particular, they seem to be an integral part of the mechanism that restricts cytoskeletal reorganization to distinct intracellular sites. Prominent members of the Rho-family GTPases are RhoA, Rac1, and Cdc42. GTPases in general bind GTP or GDP and have GTP-hydrolyzing activities. They rapidly cycle between a GTP-bound active state and a GDP-bound inactive state. The switch between the active and inactive states of these GTPases depends on three classes of regulators: the GTPase-activating proteins (GAPs), the guanine nucleotide dissociation inhibitors (GDIs), and the guanine nucleotide exchange factors (GEFs). GAPs stimulate the comparatively weak intrinsic hydrolyzing activity of the GTPases, converting them more rapidly to the GDP-bound inactive

state. GDIs bind to the GDP-bound GTPases, retaining them in the inactive state. Finally, the GEFs facilitate the exchange of GDP for GTP, converting the GTPases back into their active state (Hall 1998).

Once activated at the right time and place, Rho-family GTPases interact with their effectors, which trigger cytoskeletal rearrangements. Activation of RhoA stimulates assembly of actin filaments into actin stress fibers and also induces actomyosin-based contractility within cells (Chrzanowska-Wodnicka & Burridge 1996). In fibroblasts, activation of Rac and Cdc42 induces actin polymerization in lamellipodia and filopodia, structures that participate in cell migration and polarity determination, respectively (Nobes & Hall 1999). One effector of RhoA is the serine/threonine kinase, Rho-associated kinase (ROCK). ROCK directly or indirectly phosphorylates the myosin regulatory light chain, causing increased contraction of actin stress fibers, which, in fibroblasts, results in increased anchorage to the ECM (Schoenwaelder & Burridge 1999). Downstream of Rac are effectors that regulate actin polymerization or inhibit actin contractility. The serine/threonine kinase p21-activated kinase (PAK) is one such Rac target. It can inhibit myosin light chain kinase, thereby inhibiting actin contractility (Sanders et al. 1999). A Cdc42 effector is Wiskott-Aldrich syndrome protein (WASP), which induces actin polymerization and filopodia formation (Fukata & Kaibuchi 2001).

There is ample evidence that Rho-family GTPases shuttle from the cytosol, where they are probably inactive, to specific membrane sites where they activate their effectors (Fukata & Kaibuchi 2001, Geiger et al. 2001). These membrane sites can be adherens junctions, cell-matrix adhesion sites, or intracellular membranes involved in vesicle targeting. How do GTPases get to these specific intracellular sites and how are they retained there? An answer may be found in the relative abundance of GTPase regulatory proteins such as GEFs and GAPs versus GTPases: There are many more GAPs and GEFs than GTPases. For example, the *Drosophila* genome contains about 20 Rho-family GAPs and 20 Rho-family GEFs, but only 6 Rho-family GTPases (Settleman 2001). Importantly, several GEFs have protein-protein interaction domains such as PDZ domains that have been implicated in protein localization to the cell membrane (Bilder 2001). Thus one likely model is that GEFs are localized to specific membrane sites via PDZ domains and subsequently recruit the GTPases to these sites. In support of this mechanism, co-immunoprecipitation experiments demonstrate that PDZ-GEF1, a GEF for Rap1 GTPase (de Rooij et al. 1999), binds to β catenin and colocalizes with β catenin at adherens junctions in Madine-Darby canine kidney (MDCK) epithelial cells (Kawajiri et al. 2000). Furthermore, Tiam1, which is a Rac-GEF, contains both a PDZ and a Pleckstrin homology (PH) domain and is localized to adherens junctions (Hordijk et al. 1997). In this case, the PH domain, but not the PDZ domain, is required for membrane targeting (Stam et al. 1997). Interestingly, Tiam1 localizes to adherens junctions in epithelial MDCK cells but relocalizes to lamellipodia when motile behavior is induced in these cells (Sander et al. 1998), demonstrating that Rac can be recruited to different subcellular locations depending on the state of the cell. Therefore, GAPs and GEFs are likely to provide spatial specificity in

addition to determining the activity state of GTPases. As GEFs often contain more than one protein-protein interaction domain, it is possible that they recruit not only the GTPases but also their downstream effectors. Further studies will demonstrate whether GEFs can indeed assemble protein complexes containing both GTPases and their targets.

REMODELING OF CELL-CELL ADHESION IN EPITHELIA

A number of epithelial morphogenetic events depend on the breakdown and reconstitution of the cadherin-mediated cell-cell adhesion system. Regulation of the cells' adhesive properties, together with the coordinated reorganization of their cytoskeleton, is associated with morphogenetic processes such as cell intercalation, ingression, egression, and cell sheet fusion.

Cell Intercalation

Cell intercalation is defined as the insertion of individual cells between other cells. This process is observed both in mesenchyme and epithelia (Figure 3*A*, *B*). The best-described intercalation process is that of mesodermal cells during *Xenopus* gastrulation, a process that has been termed convergent extension (Keller et al. 1992, Shih & Keller 1992). Convergent extension can be regarded as a well-coordinated cell movement of polarized mesenchymal cells by which cells located along the mediolateral axis intercalate in between cells along the anteroposterior axis, thus resulting in an elongation of the embryo along its anteroposterior axis. This process starts with initially unpolarized cells, which then extend lamellipodia preferentially in a medial and lateral direction, thereby producing bipolar cells. These bipolar cells interdigitate with adjacent cells resulting in intercalation (Figure 3*A*) (Shih & Keller 1992). Intercalation also occurs within epithelia as observed during germ band extension in *Drosophila* and notochord formation in ascidians (Irvine & Wieschaus 1994, Munro & Odell 2002). In contrast to mesenchymal cells, intercalation within an epithelium requires the reorganization of tight and adherens junctions while ensuring that the permeability barrier is maintained. Intriguingly, in a recent analysis of ascidian notochord formation, it was proposed that epithelial intercalation is basically similar to mesenchymal intercalation. The authors suggested that basolateral protrusions between other cells gradually replace the adherens junctions of two neighboring cells with the intruding one (Munro & Odell 2002). In support of this model, the interior basolateral edges of notochord epithelial cells extend actin-containing lamellipodia over the lateral sides of adjacent cell neighbors (Munro & Odell 2002).

Some observations suggest that cadherins are required for the movements of cell intercalation and take on a role similar to integrins during cell migration. In particular, a specific amount of C-cadherin appears to be required for mesenchymal intercalation in *Xenopus*, where disruption of C-cadherin function, which is ubiquitously expressed at gastrula stages, affects convergent extension (Brieher

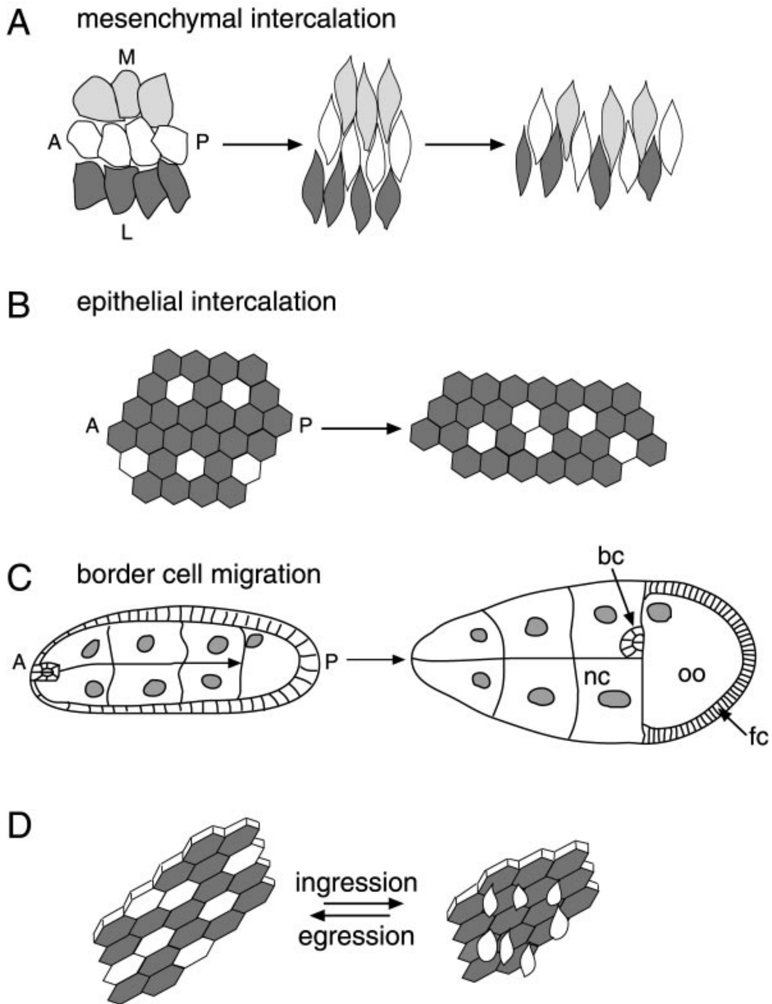


Figure 3 Cell intercalation. (*A*) Mesenchymal intercalation results in anteroposterior elongation and mediolateral shortening. In the first step, unpolarized cells extend lamellipodia mostly in a medial and lateral direction. The resulting bipolar cells then migrate on the surfaces of adjacent cells as indicated. (*B*) Epithelial intercalation. A few cells have been labeled in white to follow their movements. (*C*) Border cell migration in *Drosophila*. The border cell cluster (bc) consists of two epithelial polar cells, surrounded by four to eight partially mesenchymal rosette cells. They migrate in a cluster toward the oocyte (oo) after ingression in between the nurse cells (nc). Only the rosette cells are migratory. Note that at the same time, the follicle cells (fc) covering the nurse cells become extremely squamous and therefore, have not been drawn in the second panel, whereas the follicle cells covering the oocyte columnarize. (*D*) During ingression, cells leave the epithelium. During egression, mesenchymal cells join the epithelium, resulting in an enlargement of the epithelium. A, anterior; L, lateral; M, medial; P, posterior.

& Gumbiner 1994, Lee & Gumbiner 1995). In addition, a dominant-negative protocadherin was found to disrupt convergent extension movements in *Xenopus* (Kim et al. 1998). In contrast to classical cadherins, to which C-cadherin belongs, protocadherins do not contain intracellular catenin-binding sites and are therefore probably not anchored to the cytoskeleton (Kim et al. 1998, Usui et al. 1999). In *Drosophila*, the protocadherin Flamingo was found to be involved in signaling through the planar cell polarity branch of the Wnt pathway (Chae et al. 1999, Usui et al. 1999), and appears to form a complex with the Wingless receptor Frizzled and the downstream adaptor protein Dishevelled (Dsh) (Shimada et al. 2001). The *Xenopus* protocadherin presumably has a similar function because it was recently demonstrated that the directionality of mesenchymal intercalation in *Xenopus* is mediated by the Dsh protein (Wallingford et al. 2000). Dsh binds to RhoA via an adaptor protein and activates RhoA, which then presumably reorganizes cell polarity, thereby generating bipolar cells ready for intercalation (Habas et al. 2001).

Studies of egg formation in *Drosophila* have also provided a number of insights on the role of cadherins. During egg formation, 16 germline cells develop into 15 nurse cells and 1 oocyte and are surrounded by an epithelial layer of somatic follicle cells (Figure 3*C*). Within this cluster of 16 germ cells, the oocyte is always positioned at the most posterior end of the egg chamber (Godt & Tepass 1998). Interestingly, the location of the oocyte depends upon cadherin expression. First, most E-cadherin expression is found between follicle cells and the oocyte, and this enhanced expression is required for positioning the oocyte posteriorly. Second, in mosaic egg chambers, which possess both wild-type and E-cadherin mutant follicle cells, the oocyte always localizes next to the wild-type follicle cells regardless of their position (Godt & Tepass 1998). Such a sorting mechanism due to differential cadherin expression had already been demonstrated in tissue culture experiments (Nose et al. 1988, Steinberg & Takeichi 1994). However, it is unclear if these sorting mechanisms are related to intercalation, because sorting appears to occur passively owing to differential adhesiveness rather than via lamellipodia-mediated motility.

Another cellular process that occurs during *Drosophila* oogenesis, border cell migration, requires cadherins and appears to be more closely related to intercalation (Figure 3*C*). There, 6 to 10 follicle cells ingress into the egg, thereby forming the border cell cluster at the anterior end of the egg. This cluster, which partially retains epithelial polarity, then migrates in between the nurse cells toward the oocyte (Montell 2001). Mutations in integrins do not appear to affect border cell migration. In contrast, E-cadherin is required in both border cells and nurse cells for border cell migration suggesting that migration is mediated by homophilic cadherin interaction (Niewiadomska et al. 1999). To allow a movement of border cells in between the nurse cells, cadherin complexes should be turned over relatively quickly. Indeed, mosaic analysis has shown that E-cadherin is required only for migration, and not for stable cell-cell adhesion within the border cell cluster. In partially mutant border cell clusters, E-cadherin mutant follicle cells trail the migrating wild-type cells but are still attached to the leading cells (Niewiadomska et al. 1999).

The cytoskeletal machinery of this type of migration could be shared with integrin-mediated migration processes. In support of this notion, downstream factors such as Rac and the GEF-like protein DOCK180 are used in border cell migration and in integrin-mediated migration of carcinoma cells (Duchek et al. 2001, Gu et al. 2001, Montell 2001).

Fusion of Epithelia, Ingression and Egression

The morphogenetic processes of epithelial fusion, ingression, and egression are reminiscent of the process of cell intercalation in that they all depend on modulation of the cadherin-mediated cell-cell adhesion system. They differ, however, in that cells involved in these processes undergo a mesenchymal-to-epithelial transition, or vice versa. For example, during cell sheet fusion, epithelial sheets merge into one continuous epithelium. Cells at the leading edge of the cell sheet, which are the cells that contact cells of the opposite sheet, are partially mesenchymal because they lack adherens junctions at their free edge. During ingression and egression, individual cells or a group of cells leave or join an epithelium, respectively (Figure 3*D*). This is slightly counterintuitive, but the term ingression was originally coined for the movement of primary mesenchyme cells into the blastocoel cavity.

Fusion of epithelia is a common phenomenon that is used in a variety of processes, including wound healing, where cell sheet fusion is required to repair the lesioned area (Radice 1980); fusion of epithelial sheets derived from separate imaginal discs during metamorphosis, an event necessary to form the adult epidermis in holometabolous insects (Fristrom & Fristrom 1993); dorsal closure, which involves the fusion of ectodermal cells at the dorsal midline in *Drosophila* embryos (Stronach & Perrimon 1999); ventral enclosure in the *C. elegans* embryo (Williams-Masson et al. 1997); and *Drosophila* tracheal tube fusion, during which a continuous lumen forms from neighboring tracheal branches (Tanaka-Matakatsu et al. 1996). Early reports on various fusion processes using electron microscopy consistently describe the presence of filopodia, originating from cells that are located at the tip of a tubule or at the leading edge of a cell sheet. Interestingly, it was observed that these filopodia establish contact with the cells that they will fuse to prior to the joining event (Fristrom 1988).

Several studies have provided new insights into the function of these filopodia and the role of E-cadherin (Jacinto et al. 2000, Raich et al. 1999, Tanaka-Matakatsu et al. 1996). In trachea, E-cadherin is required for tube fusion and is found at the tips of filopodia in thin tracheal branches shortly before fusion. Interestingly, E-cadherin expression in this system is regulated by the zinc finger transcription factor Escargot. In *escargot* mutants, thin branches overmigrate without fusing, a phenotype that can be rescued by expressing E-cadherin in these cells (Tanaka-Matakatsu et al. 1996). Thick tracheal branches, however, fuse in the absence of filopodia (Tanaka-Matakatsu et al. 1996). During ventral enclosure in *C. elegans*, epithelial sheet fusion is initiated by filopodia, and α catenin is recruited to these filopodia and required for the fusion event (Raich et al. 1999). Similarly, during

Drosophila dorsal closure, filopodia extend from the leading edge of the epithelium before fusing. Dominant-negative Cdc42 inhibits filopodia formation but not fusion and causes an inappropriate alignment of the joining cells at the dorsal midline (Jacinto et al. 2000).

Thus E-cadherin is required for fusion, whereas filopodia appear to be required for the correct alignment and guidance of cell sheets or tubules that are going to fuse but not for the fusion process itself. Finally, the function of the Cdc42 GTPase in dorsal closure is in agreement with a tissue culture wound closure assay, in which dominant-negative Cdc42 inhibits the polarity of migration, but not migration itself (Nobes & Hall 1999).

Ingression of cells or cell groups out of an epithelium occurs in parallel with an epithelial-to-mesenchymal transition, as during ingression of primary mesenchymal cells into the blastocoel in sea urchins (Katow & Solursh 1980). During this process, the first step is the dissolution of the basement membrane below the leaving cells. Then the apical ends of the ingressing cells constrict into long necks, leading to a bulging of the basal ends toward the blastocoel. Eventually, junctions are lost and the necks of these now mesenchymal cells retract, with the resulting gaps being closed by apical processes from neighboring epithelial cells (Katow & Solursh 1980). Therefore sea urchin mesenchyme ingression could be considered as a variation of invagination, with the difference that cell-cell adhesion is disrupted early in the ingressing cells and pocket formation is therefore prevented. During ingression of border cells (Figure 3*C*), the two central polar cells retain their polarized epithelial character, but the surrounding rosette cells become at least partially mesenchymal.

An example of egression of cells into an epithelium occurs during epiboly in urodeles (Holtfreter 1943). In these cases, egression contributes substantially to the spreading of the cell sheet, which in other organisms is entirely mediated by flattening. In both ingression and egression, adherens junctions must first be dissolved and then reformed to reestablish an intact epithelium. In contrast to intercalation, leaving or joining cells in addition undergo an epithelial-to-mesenchymal transition, or vice versa.

Regulation of Cadherin-Mediated Cell-Cell Adhesion

Although cadherin is required for most of the cellular processes described above, it is unclear how cell-cell adhesion is controlled in these events. Many insights on the role of cadherins and their regulation have been obtained from experiments with epithelia forming in tissue culture, in particular with MDCK cells. We discuss several of these cadherin regulatory mechanisms even though it is not yet clear which might apply to any given morphogenetic process.

E-cadherin has a half-life of 5–10 h in confluent tissue culture (Gumbiner 2000). Transcriptional regulation of cadherin expression can therefore be important for long-term changes. In cell culture, quantitative differences in cadherin expression influence the strength of adhesion and the sorting behavior of cells

(Steinberg & Takeichi 1994), and the presence of different types of cadherins also influences the specificity of cell-cell interactions (Nose et al. 1988). Similar mechanisms, e.g., a switch in cadherin expression, are also used in vivo during epithelial-to-mesenchymal transition (Carver et al. 2001, Oda et al. 1998), and cadherin expression is switched on before tracheal fusion in tip cells (Tanaka-Matakatsu et al. 1996). In all of these cases, however, cadherin still has to cluster and engage in homophilic interactions that require additional regulation. Moreover, in many instances, morphogenetic processes such as cell intercalation during *Drosophila* germ band extension occur so quickly (about one hour) that regulation of expression is largely excluded.

One pathway quickly regulating cadherin clustering could again involve GTPases. Rac1 is needed for cadherin clustering at sites of cell-cell contact in MDCK cells and, in contrast to RhoA, Rac1 localizes to adherens junctions (Takaishi et al. 1997). Also, upon adherens junction formation, active RhoA (RhoA-GTP) is down-regulated, whereas the amount of Rac1-GTP and Cdc42-GTP increases (Noren et al. 2001), arguing for a mutually antagonistic role of Rac1/Cdc42 and RhoA during cadherin engagement similar to their role during focal adhesion formation.

Where could Rac1 act? An important protein influencing cadherin clustering is IQGAP1, which is an effector of Rac1/Cdc42 (Kuroda et al. 1998). It localizes to adherens junctions by direct interaction with cadherin and β catenin. IQGAP1 down-regulates cadherin adhesiveness, probably by dissociating α catenin from the cadherin-catenin complex (Kuroda et al. 1998). By binding to IQGAP1, activated Rac1 appears to prevent IQGAP1 from dissociating α catenin (Fukata et al. 2001). However, the importance of IQGAP1 has recently been questioned because IQGAP1 knock-out mice are viable and exhibit only mild gastric hyperplasia (Li et al. 2000). One possibility is that two other IQGAPs that exist in mice play redundant roles (Fukata & Kaibuchi 2001).

Another regulator of the cadherin-catenin complex is p120ctn, which is the only protein to date found to bind to the juxtamembrane domain (JMD) of cadherin (Yap et al. 1998). The cadherin JMD is required for clustering of cadherins, as cells expressing JMD deletion mutants are only weakly adherent (Yap et al. 1998). Recent work indicates that p120ctn can shuttle between the cytoplasm and the adherens junction. When in the cytosol, p120ctn increases motile behavior; however, when bound to the JMD, it fosters cadherin adhesiveness (Grosheva et al. 2001, Noren et al. 2000). Shuttling of p120ctn could be regulated by tyrosine phosphorylation, which affects p120ctn as well as cadherin and β catenin. Tyrosine kinases such as Src have been shown to bind directly or indirectly to the cadherin-catenin complex (Daniel & Reynolds 1997). Keratinocytes lacking both Src and Fyn exhibit decreased tyrosine phosphorylation of β catenin and p120ctn, as well as cell-cell adhesion defects (Calautti et al. 1998), implying an important role for these tyrosine kinases in cell-cell adhesion.

Cell-cell adhesion can also be disrupted by cleavage and subsequent release of the ectodomain of cadherin by metalloproteinases (Lochter et al. 1997). Addition

of the ectodomain to MDCK cells is sufficient to induce disruption of cell-cell adhesion and invasion (Noe et al. 2001), indicating that the disruption of cell adhesion is from competition of the cadherin ectodomain with intact cadherins.

Finally, the normal turnover of adherens junctions requires recycling of cadherins by endocytosis, which is markedly higher in subconfluent cells compared with cell layers having stable adherens junctions (Le et al. 1999), which again provides regulatory potential. Up-regulation of endocytosis may facilitate epithelial-to-mesenchymal transitions, as increased numbers of vesicles were found close to degrading adherens junctions in mesodermal cells shortly after *Drosophila* invagination (Oda et al. 1998).

REMODELING OF CELL-MATRIX ADHESION IN EPITHELIA

During cell migration, the free edges of laminar or tubular epithelia exhibit a partially mesenchymal phenotype allowing them to migrate on the underlying substrate. Such a mechanism is important for wound closure and the formation of many tubular organs and requires cell-matrix adhesion in addition to cytoskeletal remodeling.

Cell-matrix adhesion is largely mediated by integrins whose main function in epithelial morphogenesis is during cell migration. Integrins are also major components of stable adhesions to the basement membrane in hemiadherens junctions, but basement membranes form late in embryogenesis upon completion of tissue differentiation. This implies that hemiadherens junctions are more important for stabilization of tissues (Tepass & Hartenstein 1994). In support of this idea, it was shown that the basement membrane is actually digested by collagenases to allow morphogenetic movements occurring later in development, such as those found during insect metamorphosis (Fessler et al. 1993). In addition, analysis of the phenotype associated with *Drosophila* integrin mutations indicates that these proteins are not involved in cell shape changes and intercalation. For example, *Drosophila* embryos that are devoid of βPS integrin encoded by the *myospheroid* gene undergo normal gastrulation. In these mutant embryos, only the adhesion between the ectoderm and the mesoderm during germ band extension is affected (Roote & Zusman 1995). Because the only other β integrin in *Drosophila* is expressed exclusively in the midgut (Yee & Hynes 1993), the observed defects in the *myospheroid* mutant correspond to a complete functional absence of integrins during gastrulation.

Cell Migration

Epithelia migrate either as a sheet, as during wound closure, or as a tube. Examples of the latter are distal tip cell migration during *C. elegans* gonad formation, tracheal development in *Drosophila*, and kidney development in vertebrates. Common to

all is their migration on an ECM substrate using integrin as a receptor (Boube et al. 2001, Lee et al. 2001, Wallner et al. 1998). The importance of integrins in distal tip cell migration was demonstrated by expression of a dominant-negative β integrin transgene, which resulted in migration defects (Lee et al. 2001). Furthermore, during tracheal development in *Drosophila*, the visceral tracheal branch migrates along the visceral mesoderm. Loss of αPS1 or βPS integrin in this branch prevents migration along the visceral mesoderm (Boube et al. 2001).

Epithelial cells at the migration front have a partially mesenchymal character. This dual nature can be observed in closing wounds or in the *Drosophila* amnioserosa during germ band retraction. These leading edge cells are connected to the epithelial sheet with adherens junctions, but form lamellipodia and membrane ruffles on the migration front (see Figure 1) (Radice 1980, Schöck & Perrimon 2002). In systems such as distal tip cell migration, which consist only of a few cells, migration is presumably mediated by an individual cell, with the other cells of the epithelium being dragged behind. In support of this notion, expression of a dominant-negative β integrin in the distal tip cell results in gonadal migration defects (Lee et al. 2001). During wound healing, it is thought that up to three rows of cells contribute to migration; wound closure was only inhibited when three rows of cells next to the wound edge were injected with dominant-negative Rac, but not when only the first row was injected (Fenteany et al. 2000). Similarly, in a wound closure assay in *Xenopus* tadpoles, migration of the first row of cells results in a small gap between the first and second rows, which is closed by lamellipodia formation and migration of the second row of cells. These lamellipodia often extend below the first cell row and therefore are sometimes not visible (Radice 1980). How many cell rows participate may depend on the size of the wound.

Another interesting question regarding tubule migration is whether the tubule lumen is retained throughout the migration process. Tubules feature a continuous lumen during tracheal tube formation in *Drosophila* (Manning & Krasnow 1993) but not during kidney tubule formation as analyzed using MDCK cells as a model. Kidney tubule formation cannot be analyzed in vivo, but MDCK cells form a tubule network if they are grown in a three-dimensional collagen gel and are induced with hepatocyte growth factor. In these conditions, basic epithelial organization is maintained throughout tubule formation, but the tubule lumen forms de novo after a chain of cells migrates/grows out of a spherical monolayer of MDCK cells (Pollack et al. 1998) (Figure 4*A*). An important difference potentially influencing these two tubule formation mechanisms is that in the MDCK system, proliferation is required for tubules to form, whereas *Drosophila* trachea form without any cell divisions (Davies et al. 1995, Manning & Krasnow 1993).

One of the best-analyzed epithelial migration processes on a molecular level is distal tip cell migration. During this process, the two gonad arms in *C. elegans* hermaphrodites migrate and undergo two 90° turns, resulting in two U-shaped gonad arms (Figure 4*B*). Many genes involved in this process have been uncovered over the years. Particularly interesting is the finding that mutations in Rac and genes of the Crk-DOCK180 complex exhibit migration defects (Reddien & Horvitz 2000,

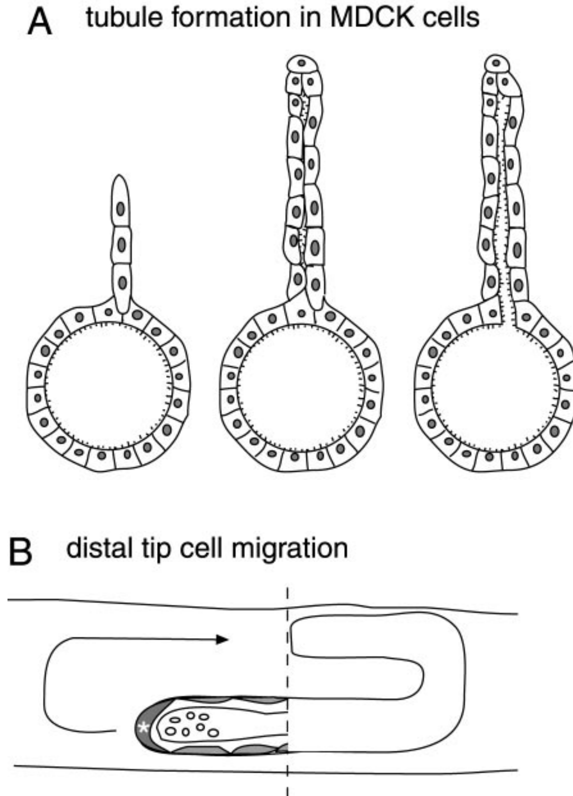


Figure 4 Cell migration. (*A*) Tubule formation of MDCK cells grown in a three-dimensional collagen gel (adapted from Pollack et al. 1998). The lumen forms de novo in a chain of cells after these cells have migrated. (*B*) Diagram of distal tip cell migration in *C. elegans* hermaphrodites. Migration of the two arms of the gonad occurs in three steps between the second and fourth larval instar. Distal tip cell migration begins with a straight migration, as shown on the left, then two 90° turns occur. The right panel shows the completed migration. The star indicates the distal tip cell, the only somatic gonad cell not dividing during the migration. Somatic gonad cells are shown in gray.

Wu & Horvitz 1998). The Crk-DOCK180 complex also regulates migration in carcinoma cells (see below). However, mutations in Crk or DOCK180 affect mostly distal tip cell pathfinding and to only a small degree migration itself. One possible explanation for this result is redundancy, since a double mutant with lesions in two of three *rac* genes in *C. elegans* showed significantly increased migration defects (up from 6 to 24%) (Lundquist et al. 2001). Furthermore, two ADAM metalloproteinases, GON-1 and MIG-17, are needed for migration (Blelloch & Kimble 1999, Nishiwaki et al. 2000). *gon-1* mutants display a complete block in

migration, which can only be rescued by expression of *gon-1* in the distal tip cell (Blelloch & Kimble 1999), whereas *mig-17* mutants are associated with pathfinding defects (Nishiwaki et al. 2000). It is not yet known if these metalloproteinases activate a guidance signal or simply digest the ECM.

Regulation of Integrin-Mediated Cell-Matrix Adhesion

Integrin signaling is bidirectional, with the ECM reorganizing the cytoskeleton and the cytoskeleton influencing ECM assembly. In a simple scenario, the "outside-in" model, integrins in their inactive state are present at the plasma membrane, binding neither to the cytoskeleton nor to the ECM. Upon binding to the ECM, integrins may change their conformation, thus resulting in clustering and activation of nonreceptor kinases such as Src. Src in turn may enable integrins to bind to the actin cytoskeleton (Schoenwaelder & Burridge 1999). In most cases, a certain threshold of both ligand ligation and clustering of integrins has to be reached before fully functional cell-matrix adhesions are formed (Miyamoto et al. 1995). Importantly, it was recently shown not only that the size of focal adhesions and the force exerted at these sites are linearly related (Balaban et al. 2001) but also that force application induces growth of focal adhesions (Riveline et al. 2001). If local forces generate stronger focal adhesions during development, this could elegantly explain how strong adhesive structures such as muscle attachment sites arise over time.

At the core of integrin involvement in migration is the balance between stable focal adhesions and transient focal complexes. Both focal complex formation and focal adhesion turnover are required for cell migration. RhoA promotes stable focal adhesions, whereas Rac1 promotes focal complex formation (Geiger et al. 2001). Therefore, migration can be promoted by inhibiting the RhoA pathway or by activating the Rac1 pathway (Rottner et al. 1999, Sanders et al. 1999).

One player potentially upstream of both pathways is the nonreceptor tyrosine kinase Src, which plays a pivotal role in reducing stable adhesions and promoting motility. Cells lacking the three Src family members, Src, Fyn, and Yes, are unable to migrate (Klinghoffer et al. 1999). Using vitronectin-coated beads held on top of a fibroblast cell by a laser trap, it was shown that integrin linkage to the cytoskeleton is stronger in Src-deficient cells than in wild-type cells, whereas integrin-matrix adhesion is not affected by Src (Felsenfeld et al. 1999). This indicates that Src promotes migration by enhancing the turnover of focal adhesions in this context. Src could function by phosphorylating focal adhesion kinase (FAK), which is also required for cell motility (Ilic et al. 1995, Parsons et al. 2000). Cells lacking Src kinases, which display a complete absence of FAK phosphorylation, form normal focal adhesions in the same way as FAK null cells do (Ilic et al. 1995, Klinghoffer et al. 1999). Therefore, FAK phosphorylation is likely involved in the turnover of focal adhesions.

Src also appears to be involved in induction of the Rac1 pathway, because Src is recruited into focal complexes and necessary for their formation (Timpson

et al. 2001). Src could phosphorylate p130Cas (Cas) (Harte et al. 1996), which can then form a complex with Crk, and thereby induce cell migration (Klemke et al. 1998). Cas and Crk are both adaptor proteins containing SH2 and/or SH3 domains. The Cas-Crk complex activates Rac1 through DOCK180 (Kiyokawa et al. 1998, Nolan et al. 1998). DOCK180 is not a classical GEF, but it binds to Rac1 and causes Rac1 activation (Kiyokawa et al. 1998). The Cas-Crk-DOCK180 complex also localizes to lamellipodia (Kiyokawa et al. 1998, Klemke et al. 1998). More recently, biochemical purification of extending and retracting lamellipodia revealed that the Cas-Crk complex is assembled only in extending lamellipodia, and at the same time, Rac is activated only in extending lamellipodia (Cho & Klemke 2002).

Another important nonreceptor kinase downstream of integrins is phosphatidylinositol (3)-kinase (PI3K), which induces cell migration and invasion (Shaw 2001, Shaw et al. 1997). PI3K phosphorylates inositol lipids, generating, among others, the lipid phosphatidylinositol (3,4,5)-trisphosphate [PI(3,4,5)P_3] (Jones et al. 2000). The PH domain of the Rac-GEF, Tiam1, binds preferentially to PI(3,4,5)P_3 (Rameh et al. 1997), suggesting a mechanism whereby Rac-GEFs could be recruited to lamellipodia where Rac could be activated, thus possibly explaining the migratory phenotype induced by PI3K.

In contrast to cadherins, which interact only with other cadherins, integrins adhere to a variety of ECM substrates, each one eliciting a different response. For example, cell motility and activation of the Cas-Crk-DOCK180 complex are particularly strong on laminin-10 and laminin-11, which both contain the laminin $\alpha 5$ chain, but not on fibronectin (Gu et al. 2001). It was further shown that induction of cell migration through laminin is concomitant with a preferential phosphorylation of Cas instead of FAK, whereas the reverse was observed by plating the cells on fibronectin (Gu et al. 2001).

The fact that distal tip cells in *C. elegans* use the same protein complex downstream of integrins as invasive carcinoma cells to regulate migration implies a wide phylogenetic conservation of these mechanisms. It also suggests that other regulatory mechanisms demonstrated for cadherin- or integrin-mediated adhesion in human tissue culture will be conserved in some epithelial morphogenetic processes.

CELL DIVISION AND DEATH

Studies in a number of systems have shown that, in general, cell divisions are downregulated or shut off before the initiation of large-scale morphogenetic changes. For example, the complex cell shape changes that occur during gastrulation are incompatible with cell divisions in *Drosophila* (Foe et al. 1993). The kinase Tribbles, which induces Cdc25 degradation, is expressed exclusively in the future mesoderm of the *Drosophila* embryo to prevent cell divisions while gastrulation occurs (Grosshans & Wieschaus 2000, Mata et al. 2000, Seher & Leptin 2000). In

other instances, however, cell divisions contribute to morphogenesis. For example, cell proliferation is required for kidney development and, in its absence, tubules do not elongate, although branching is initiated (Davies et al. 1995). Another example of the contribution of cell proliferation to morphogenesis is during wound healing where cell divisions substantially accelerate reepithelialization of wounds (Werner et al. 1994).

Cell death is sometimes employed during late stages of development in morphogenesis, for example, during digit formation in mammals (Merino et al. 1999) and head involution in *Drosophila* embryos (Nassif et al. 1998). One interesting issue is how phagocytosis of apoptotic epithelial cells could occur without destroying the permeability barrier of epithelia. Recently, studies in both the chick embryonic epithelium and MDCK cells have shown that apoptotic cells are extruded apically following basolateral actomyosin contraction in neighboring cells, such that the membrane permeability barrier is retained. Injection of C3 toxin, which inhibits RhoA, into neighboring cells was found to disrupt the extrusion process. This process appears to be regulated by the apoptotic cells themselves, which at a very early stage induce actomyosin contraction in neighboring cells (Rosenblatt et al. 2001).

CONCLUSIONS

Analysis of morphological processes reveals the amazing plasticity of epithelia and the ease with which epithelial cells can change their shape, intercalate, and migrate. In this review, we have classified the main morphogenetic processes involving epithelia. Much remains to be done to understand these processes in the first place and to clarify the relationship between them.

The first challenge is to assign the correct regulatory mechanisms and the sequence of events for specific, well-defined morphogenetic processes. Even this task might prove daunting considering that the cadherin regulation mechanisms discussed largely refer to E-cadherin in MDCK cells, whereas the *Drosophila* genome alone contains 17 open reading frames with cadherin repeats (Hynes & Zhao 2000). Similarly, the results on integrin regulation largely refer to a subset of integrin receptors in fibroblasts interacting with fibronectin in the ECM.

The next challenge lying ahead will be to integrate the various events in order to understand the multistep process of epithelial morphogenesis. One topic not considered in this review is the integration of signal transduction pathways with morphogenesis. Although it has been shown that signal transduction cascades modulate and coordinate cytoskeletal reorganization, and vice versa (Stronach & Perrimon 1999), details about the spatial and temporal connection of extracellular signals and intracellular remodeling during morphogenesis are still rare.

Nevertheless, progress in genome biology, as well as novel techniques from the cell biology field, which are currently applied to study morphogenetic processes, have dramatically accelerated the pace of discovery. Particularly encouraging in this respect are the advances in microscopy and green fluorescent protein

techniques, which already allow live imaging of protein localization during morphogenetic processes and may soon allow visualization of protein-protein interactions in vivo.

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