

Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis

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Abstract | Embryonic morphogenesis requires the execution of complex mechanisms that regulate the local behaviour of groups of cells. The orchestration of such mechanisms has been mainly deciphered through the identification of conserved families of signalling pathways that spatially and temporally control cell behaviour. However, how this information is processed to control cell shape and cell dynamics is an open area of investigation. The framework that emerges from diverse disciplines such as cell biology, physics and developmental biology points to adhesion and cortical actin networks as regulators of cell surface mechanics. In this context, a range of developmental phenomena can be explained by the regulation of cell surface tension.

Morphogen

A diffusing substance that induces different cell fates through the formation of a concentration-dependent gradient from a localized source.

Planar polarity

The structural asymmetry of cells in the plane of a tissue. It characterizes the direction of cell elongation, cell division, cell movement and differentiation.

The morphogenesis of biological tissues underlies several developmental processes in plants and animals and is required for the basic organization of embryos and for organ formation. Despite the tremendous diversity of shapes in multicellular organisms, all animals derive from a set of basic body plans that indicate rules of construction that are compatible with the continuous appearance of new forms as variations on a few themes. Previous studies on morphogenesis have identified conserved signalling pathways in animals and general principles that concern the modes of action of such pathways during pattern formation. For example, concentration-dependent morphogen gradients transform a homogeneous field of cells into discrete domains, each characterized with a defined sub-programme of morphogenesis and differentiation^{1,2}. Cells — such as trichomes in the epithelial cells of the fly wing and stereocilia in hair cells of the mammalian inner ear — also receive planar polarity information that is required for their morphogenesis, division or motility in the plane of a tissue³.

During morphogenesis, each cell must process and mechanically respond to this flow of patterning and directional information to participate effectively in tissue remodelling and organism shaping. The repertoire of cell behaviours is limited to processes such as cell division, cell death, cell growth, cell migration and cell-shape changes. Cell shape and morphogenesis are governed by cell mechanics, which explains how a set of intracellular and extracellular forces controls

the cell structure. Studying the mechanics of cell shape is therefore important to extend our understanding of morphogenesis.

Biological tissues have two contradictory properties: they have a robust architecture that is required for their stability and resistance to stress, yet they exhibit a remarkable plasticity that allows remodelling. Tissue homeostasis depends on the balance between the robustness and plasticity of tissues and relies on unique features of cell mechanics. For example, intercellular adhesion is required for cell interactions and the formation and stabilization of polarized epithelial layers. Adhesion was also shown to control cell sorting⁴, a remarkable example of tissue reorganization whereby cells separate from each other in two immiscible populations based on differential adhesion properties. Disruption of this homeostasis is an important step in the formation of several types of cancer.

Here, we review recent data from various fields of cell biology to propose a framework for the control of cell shape based on the concept of surface tension. Surface tension is an equilibrium property and thus cannot describe *per se* the course of events in a morphogenetic process. Nonetheless, this framework accounts for the organization of groups of cells during development. This may stem from the fact that the system is driven into a succession of temporary equilibrium states in response to two sets of constraints that change slowly over time: internal physical cell properties and external physical constraints that act on cells.

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Plasticity

The ability to undergo a persistent deformation.

Tissue homeostasis

The property of biological tissues to remain structurally and functionally stable in a physiological environment.

Sorting

The ability of intermixed, adhesive and mobile cell populations to separate into immiscible adjacent tissues.

Surface tension

The free-energy change when the surface of a medium is increased by a unit area. Strictly speaking, the term 'interfacial tension' should be used instead of 'surface tension' when the liquid adjoins another liquid or a solid. For simplicity, we use the term 'surface tension' in this article.

Tissue surface tension

The apparent surface tension of a tissue, caused by cohesive interactions (adhesion) between cells. Increasing adhesion in a tissue increases tissue surface tension.

Intercellular surface tension

The apparent surface tension of two cells that are in contact, caused by the opposite effects of cortical tension and intercellular adhesion. In contrast to the case of tissue surface tension, increased adhesion lowers intercellular surface tension.

Cortical tension

The apparent cell surface tension due to the contractile microfilaments of the cell cortex and their interaction with the membrane.

Affinity

The tendency of cells with similar developmental origins to aggregate.

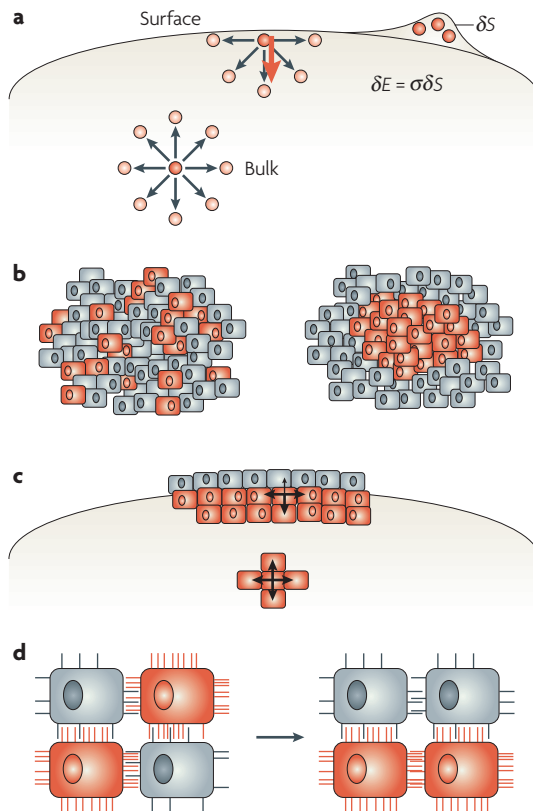


Figure 1 | Analogy between fluids and tissues.

a | Molecular explanation of surface tension in a liquid. In the bulk of a liquid, molecules are, on average, equilibrated by interacting cohesive forces from surrounding molecules (black arrows). At the surface of the liquid, the molecules experience an imbalance of force, with a resultant force (red arrow) that tends to push them away from the surface, thereby introducing a surface tension. The energetic cost δE for a change δS of the liquid surface area is simply related to the surface tension σ . **b** | Cells become sorted and aggregate according to the expression levels of cadherins at their surface. Red cells, which express more cadherins and therefore form stronger cell–cell contacts than grey cells, are surrounded by the grey cells. **c** | A red cell at the interface between the two cell populations (top) has less favourable interactions with similar cells than a red cell in the tissue interior (bottom). This results in tissue surface tension. By adopting a spherical shape, the red tissue minimizes the interface area with the surrounding grey tissue. **d** | Sorting behaviour of grey cells that express a given cadherin at their surface and red cells that express a different cadherin. The preferential association of cells that express the same cadherins cause cell sorting according to the differential adhesion hypothesis.

The morphogenetic processes that are described in this Review develop over medium (minute) to long time-scales (minutes to hours). It has been shown that, in the case of these medium-to-long timescale processes, the elastic response of the cell — which predominantly occurs over short timescales (seconds) — can be neglected and cells behave like viscous fluids with an equilibrium shape that is dictated by surface tension^{5,6}.

First, we present a historical perspective on tissue surface tension during morphogenesis. We then focus on local cell behaviours and the control of intercellular surface tension by adhesion and cortical tension. We also detail how spatial patterns of cell shape that arise in tissues can be explained in this context, and how cell shape controls tissue geometry.

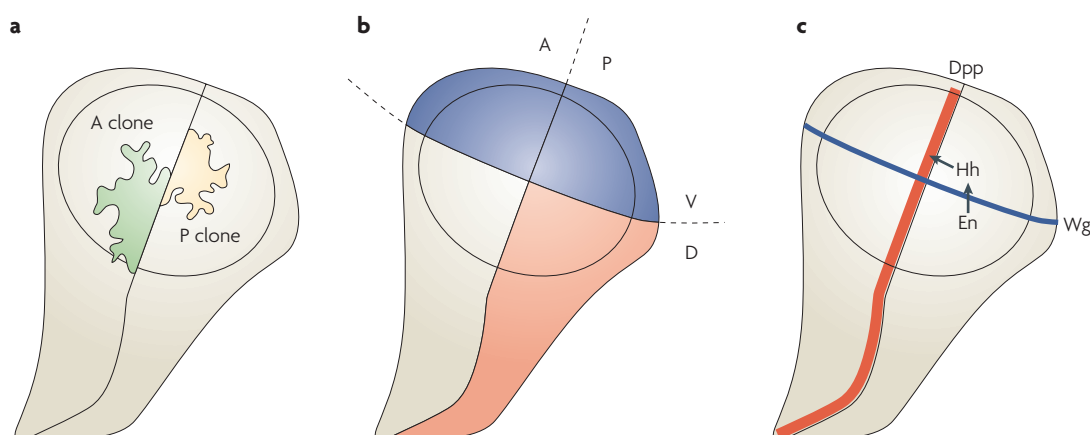
Tissue surface tension and cell sorting

Cell shape and the geometry of cell aggregates show striking similarities with fluids and soap bubbles, as observed nearly 100 years ago by D'Arcy Thompson in his book *On Growth and Form*⁷. The suggested general principles that underlie the control of tissue organization were based on surface tension properties. However, surface tension might refer to different biological entities, for example, a group of cells in a tissue or an individual cell⁸. In this section, we introduce the concept of surface tension as a global tissue property that is controlled by cell–cell interactions. The tissue is simply viewed as a collection of cells with generic properties (adhesion) without consideration of their individuality (cell shape and mechanics).

The concept of surface tension in groups of cells. A liquid droplet, such as an oil droplet in water, is spherical (FIG. 1a); with this shape, it minimizes its contact area with the surroundings and, therefore, minimizes its surface energy. Increasing the surface area of the drop requires an energy δE , which is proportional to the area increment δS , such that $\delta E = \sigma \delta S$, in which σ is the surface tension of the liquid. The coefficient σ has the dimension of energy per unit surface area and is expressed in Joules m^{-2} . The physical significance of liquid surface tension can be related to the cohesive interactions between molecules in a liquid (Van der Waals forces, hydrogen bonds, ionic interactions⁹; FIG. 1a, arrows). In the bulk of a liquid, the intermolecular forces that function on a molecule are, on average, balanced. However, these forces are no longer equilibrated at the surface of the liquid — the molecule loses interactions with similar molecules and gains interactions with dissimilar molecules. Surface-tension forces arise from this imbalance of short-distance intermolecular forces, with the resultant force tending to bring surface molecules back to the bulk, thus tensing the surface (FIG. 1a, red arrow). Surface tension causes molecular sorting at the boundary between two different liquids.

Cells in a tissue are remarkably similar to molecules in a liquid¹⁰ such that, to a good approximation, tissues behave like fluids. First, cells tend to aggregate in clusters in which the surface area of contact with the surrounding environment is minimized. Second, different cell populations can become sorted into two phases like immiscible fluids (FIG. 1b). By analogy to liquids, it is possible to define a tissue surface tension, which can explain cell aggregation and cell sorting⁴. In the 1940s and 1950s, Holtfreter and colleagues showed that tissues separate by affinity¹¹, a term used by Holtfreter in reference to analogous phase-separation phenomena in chemistry. In a remarkable series of experiments,

Box 1 | Developmental compartments and cell sorting



Compartments are immiscible groups of cells that are separated by a smooth tissue boundary. Compartments were discovered more than 30 years ago in the *Drosophila melanogaster*⁹³ wing imaginal discs (and, later, in embryos) through the observation that clones of cells would grow with wiggly borders but failed to cross an imaginary line that bisected the tissue. At compartment borders, cells become sorted and form a long, smooth boundary. Compartments were also shown to exist in vertebrates⁹⁴ and serve two purposes. First, they keep groups of cells in distinct positions and stabilize building blocks in the embryo, such as in segments in the *D. melanogaster* ectoderm and in rhombomeres of the vertebrate central nervous system. Second, the compartment boundary is able to induce a specific cell fate from a distance, such as through the production of a morphogen gradient (for example, Decapentaplegic (Dpp) and the Wnt protein Wingless (Wg)).

The cellular mechanisms of compartment formation have been particularly studied in *D. melanogaster*. The developing wing imaginal disc is a columnar epithelial layer in which two sets of compartments are set aside and sequentially yield four quadrants in the epithelium: the anterior (A) and posterior (P) compartments are formed first (see figure, panel a), followed by the dorsal (D) and ventral (V) compartments (see figure, panel b). Defined sets of selector genes are expressed in each compartment: Engrailed (En) in the posterior compartment and Apterous in the dorsal compartment so that each quadrant of the tissue keeps a certain identity. The maintenance of compartment boundaries requires continuous signalling at the boundaries: the Hedgehog (Hh) family of signalling molecules maintains the anterior–posterior boundary in conjunction with signalling by the transforming-growth factor- β (TGF β)-family growth factor Dpp⁹⁵, whereas Notch signalling is required for the stability of the dorsal–ventral boundary^{96,97} (see figure, panel c). The immiscibility of cells at compartment boundaries is believed to reflect different cell affinities in each compartment and cell sorting at the boundaries.

Rhombomere

An iterative subdivision of the vertebrate hindbrain along the antero-posterior axis.

Selector gene

A gene that specifies (selects) a developmental pathway, as opposed to a 'realisator' gene that executes downstream cellular responses (e.g. adhesion).

Cell adhesion molecule

A transmembrane protein on the cell surface that binds to other cell adhesion molecules on the surface of another contacting cell or to the extracellular matrix.

Homophilic association

Trans-association of similar cell adhesion molecules at contacting cell surfaces.

Heterophilic association

Trans-association of different cell adhesion molecules at contacting cell surfaces.

Nurse cells

Polyploid cells in the *Drosophila melanogaster* ovary that are connected by cytoplasmic bridges and feed the oocyte.

they dissociated tissues such as the prospective nervous system and the future ectoderm into single cells and mixed them. Upon re-aggregation, cells sorted spontaneously into separate tissues like immiscible liquids. By analogy to molecular sorting in a liquid, cell sorting requires the cohesion and mobility of cells. In the bulk of a tissue, cells are stabilized by cohesive forces that are mediated by cell–cell adhesion molecules. Although cells in embryos are adhesive, they are capable of rearrangements and can be mobile.

The differential adhesion hypothesis. About ten years after the seminal work of Holtfreter, Steinberg formulated the differential adhesion hypothesis (DAH), also known as the thermodynamic hypothesis, which proposes that differences in intercellular adhesion guide tissue segregation, mutual envelopment and the sorting of embryonic tissues¹². Recent evaluation of the DAH has shown that tissue surface tension, as measured by specific surface tensiometers¹³, increases linearly with the expression level of adhesion molecules such as cadherins¹⁴. The DAH relies on the assumption that the homophilic association of cadherins is distinct from heterophilic associations (FIG. 1c,d); this hypothesis has now been confirmed experimentally^{14,15}. Computational

simulations of the DAH have further supported its importance in cell sorting^{16,17}. The relative tissue surface tension between two groups of cells dictates that the least cohesive group surrounds (or envelopes) the other one. Note, however, that the binding specificity between cadherin molecules is not sufficient to fully account for cell sorting.

Cell sorting can occur between cells that express different cadherins with promiscuous binding specificities¹⁸, and sometimes occurs with equally strong heterophilic and homophilic association as assayed *in vitro*¹⁹. This suggests that adhesion specificity and the strength of cell association are not fully captured by extracellular binding of cadherins, and that kinetic parameters might also control cell adhesion and cell sorting. The role of actin dynamics and organization in adhesion has recently been re-emphasized²⁰, and is likely to be essential to improve the understanding of cell adhesion and, therefore, cell sorting.

With this caveat in mind, predictions of the DAH have been amply confirmed by experiments in cell cultures (see REF. 21 for an example) and in developing organisms^{22,23}. In the *Drosophila melanogaster* ovary, the oocyte and follicular epithelial cells (which express a higher level of E-cadherin than do nurse cells) come

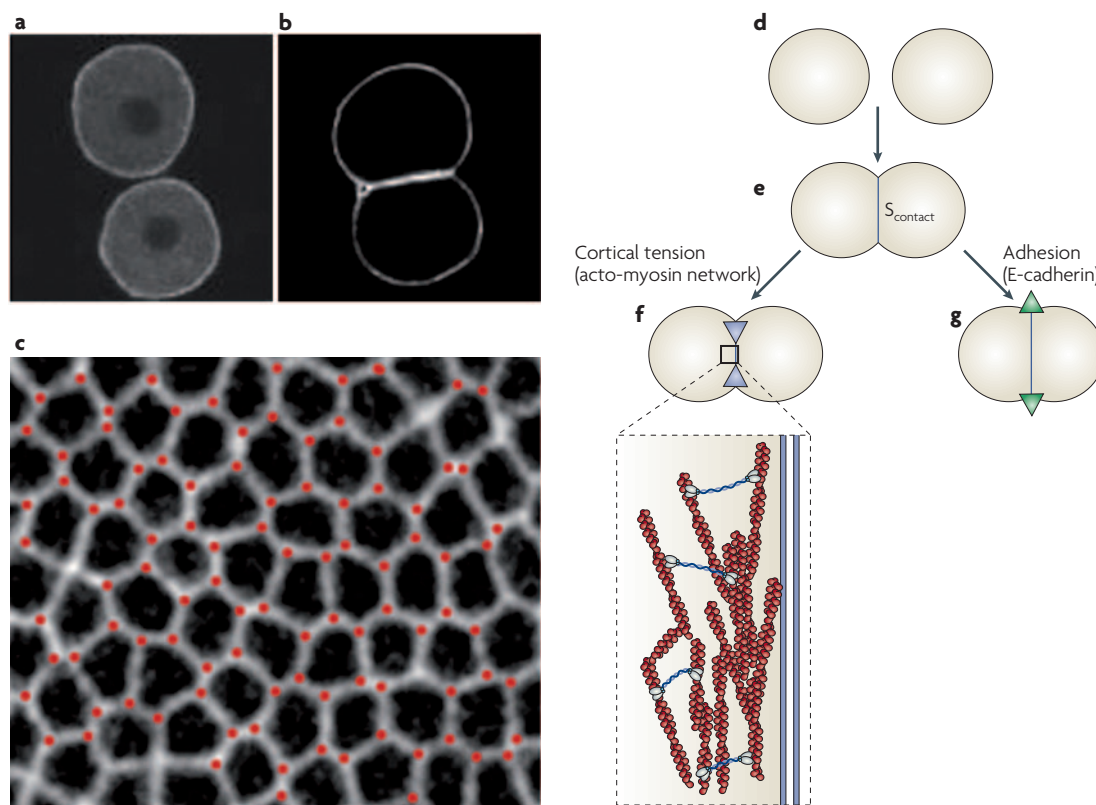


Figure 2 | Control of cell shape by adhesion and cortical tension. Cell–cell contacts change the shape of cells such that they are no longer spherical. Single cells, such as mouse embryonic cells at the two-cell stage with a β -catenin mutation that disrupts E-cadherin-mediated adhesion⁴³, are spherical (**a**), whereas wild-type adhering cells have a different shape (**b**). Cells in an epithelium have a polygonal shape (**c**). The apical section of adhering *Drosophila melanogaster* cells marked with actin–phalloidin is more often hexagonal and pentagonal with many three-fold vertices (marked with a red dot). When two single cells (**d**) are brought into contact (**e**), they change form according to intercellular surface tension that controls the length of the surface of contact (S_{contact}). Intercellular surface tension results from the opposite effects of cortical tension (**f**) — exerted by an actomyosin contractile network — that reduces the surface of contact, and of adhesion (**g**) that increases the surface of contact. The contractile actin (red) network is controlled by myosin-II (grey) and tethered at the plasma membrane (blue). Parts **a** and **b** modified with permission from REF. 43 © (2004) The Company of Biologists.

into contact, ensuring that the oocyte is positioned at the posterior pole of the egg chamber. The existence of developmental compartments (BOX 1) has long been proposed to be a manifestation of cell sorting that is driven by differential adhesion. Cells from adjacent compartments do not mix and form a smooth boundary. For example, the cell-surface adhesion molecules Tartan and Capricious affect cell sorting at the dorsal–ventral boundary following overexpression of these proteins²⁴. Moreover, overexpression of E-cadherin in a clone of cells can distort the anterior–posterior boundary²⁵ and cause the sorting of cells that overexpress E-cadherin from their neighbours. The cadherin Cad99C is upregulated in anterior cells along the anterior–posterior boundary; however, the loss of Cad99C does not affect the boundary. Redundant sets of cell adhesion molecules might be required for the stability of compartment borders. Alternatively, global tissue adhesive properties (that is, those proposed by the DAH) may not fully account for the stability of compartment boundaries (see below).

Cell surface tension
The apparent surface tension of a cell.

Cell shape and cell surface tension

Groups of cells in a tissue behave in a similar manner to a fluid, with the global shape of tissues being dictated by tissue surface tension. Can we similarly explain cell shape in terms of surface tension? Isolated cells, similar to bubbles or liquid droplets, are often spherical, which minimizes their surface area (FIG. 2a). Despite the cell complexity, we can consider that cell shape is controlled by cell surface tension to a large extent.

However, it is important to recognize that cells are different in many respects from liquid drops. Contrary to liquids, the cell surface and interior do not form a homogeneous liquid material and the cell surface area cannot be increased efficiently by moving molecules from its interior (cytoplasm). However, the plasma membrane is decorated by microscopic wrinkles and folds, which constitute a significant reservoir of molecules that are readily available for a change in its apparent surface area and its surface of contact with other cells. Although the surface tension of a liquid is constant, apparent cell surface tension can depend on the cell surface area²⁶. The cell surface energy

is not restricted to cell surface tension and includes elastic terms. The control of cell shape involves an organization of the total plasma membrane into an effective surface of contact with the extracellular environment (contacting cells, extracellular matrix, medium and so on).

Shaping isolated cells by cortical tension. Let us first consider the simple case of a cell that does not have any contacts with other cells. The application of a mechanical force (for example, by aspiration) can smoothen the corrugated plasma membrane of the cell, thereby increasing its apparent surface area. Surface tension forces that oppose membrane smoothening depend first on the density of the cortical actin network and on its dynamic interaction and connections with the plasma membrane²⁷. Surface tension forces also depend on membrane expansibility — the ability to increase the surface area of the lipid bilayer (for a review see REF. 28). The cortical tension (characterized by Evans and Yeung²⁹) can be measured when observing cell deformation within a conical micropipette²⁶ or by pulling membrane nanotubes, also called tethers²⁸.

The cell cortex is able to contract in response to myosin molecular motors that move on actin filaments. Stronger contractility in one location compared with others results in the deformation of cell contours. This is how the cytokinetic furrow ingresses before cell division: a band of actin and myosin-II molecules forms around the waist of the cell and generates local tension³⁰. A tight control of cortical contractility is thus required to control cell shape.

The visco-elastic response of the cell to tension also participates in cell shaping³¹. Flows of cortical components³² or membrane protrusions (blebs)³³ can be driven by cortex contractility or breakage, and are important for cell locomotion or cytokinesis. Myosin-II also has a role in the deformability of the cortex. Macroscopically, myosin-II can render an F-actin network more fluid through its motor activity *in vitro*³⁴. At the molecular level, it was shown that myosin-II enhances longitudinal fluctuations within an actin gel³⁵. *In vivo*, myosin-II remodels actin filaments during cytokinesis in cell cultures and zebrafish^{36,37}. The Rho kinase **Rok**, myosin-II and an integral F-actin cortical network are required for anaphase cell elongation by polar relaxation of the cortex³⁸. Before the asymmetric division of the *Caenorhabditis elegans* unicellular embryo, the acto-myosin cortex contracts and flows away from the sperm microtubule-organizing centre towards the anterior pole. Cortical flows transport other cortex-associated proteins, such as the polarity proteins **PAR-3** and **PAR-6**, and protein kinase C (PKC-3), which in turn stabilize the flow by modulating the cortical tension³⁹. This supports the idea that local relaxation of the cortical tension generates its own flow, as previously postulated⁴⁰.

Cortical tension thus tends to minimize the cell surface area and is an important ingredient of cell surface tension and the shape of isolated cells.

Adhesion controls the shape of contacting cells. When cells are in contact, they tend to minimize their total surface area, similar to bubbles (FIG. 2b,c). Organization rules for the geometry of bubbles in clusters at equilibrium

were stated by Plateau in 1873 (REF. 41). In an ideal two-dimensional foam, bubble edges meet at three-fold vertices with 120° angles, which corresponds to a fully hexagonal network (although pentagons and heptagons also exist, FIG. 2c). This geometrical order optimizes packing and minimizes the surface energy. Recent observations have confirmed this idea in epithelial cells in several organisms^{15,42}. In the case of contacting cells, we shall refer to intercellular surface tension to describe cell shape adequately.

What is the role of adhesion in the control of intercellular surface tension and cell shape? For example, E-cadherin-mediated adhesion increases the surface of contacts between cells⁴³ (FIG. 2a–c,f). The homophilic interaction complexes between E-cadherin molecules of two apposed cell membranes are stabilized by the adaptor proteins **β-catenin** and **α-catenin**, and actin filaments^{44–46} (FIG. 3). Recent work has emphasized the fact that adhesion does not simply rely on cadherin interactions but also, and most importantly, on the dynamic interactions with cortical actin and on actin organization *per se*^{20,47,48}. At E-cadherin adhesive contacts, the plasma membrane of the cells is flattened microscopically. An increase of adhesion molecules favours the cell surface contacts and, therefore, reduces the apparent intercellular surface tension. Note that, in contrast to tissue surface tension, which is positively controlled by adhesion, intercellular surface tension is negatively regulated by adhesion.

Interplay between adhesion and cortical tension. For two adhering contacting cells, the increase of cortical tension due to the formation of a contractile acto-myosin network at the zone of contact (FIG. 2f) reduces the contact surface. Thus, adhesion and cortical tension have opposite effects on intercellular surface tension and on the extent of cell contact (FIG. 2e,f). Yet, adhesion and cortical tension are not independent because both are supported by actin filaments. E-cadherin concentrates at cell–cell contacts through active processes that involve the actin cytoskeleton (reviewed in REF. 46), which in turn reorganizes during the formation of cell–cell contacts^{49,50}. Cortical tension exerts its effects on the cell surface by a tethering mechanism that may involve adherens junctions (AJs) as illustrated during apical constriction⁵¹. The nature of this anchoring mechanism should be evaluated in the light of recent studies that revisited the E-cadherin–catenin–actin conundrum, which revealed that actin does not interact directly with homophilic E-cadherin complexes^{20,47}. Instead, **α-catenin** is in dynamic equilibrium between the E-cadherin–**β-catenin** complex and F-actin, thereby ensuring anchoring and plasticity⁴⁷ (FIG. 3).

The dynamics of cell–cell contacts requires an interplay between adhesion and cortical tension but the underlying mechanisms must be resolved further. At its core, this will require further investigation of how actin filaments are organized and dynamically remodelled at adhesive contacts. The fact that **α-catenin** competes with the actin nucleator ARP2/3 complex for binding to F-actin²⁰ suggests that the extent of actin branching could influence adhesion.

Visco-elasticity

The mechanical response properties of a material in response to a load. Elasticity refers to the ability to recover from a deformation. Viscosity describes a resistance to a flow due to molecular interactions.

Adherens junction

Molecular complexes that are formed by cadherins and associated molecules stabilized by the actin cytoskeleton. They connect two contacting cells.

Apical constriction

Reduction of the apparent apical surface area in an epithelial cell. Apical constriction drives tissue bending.

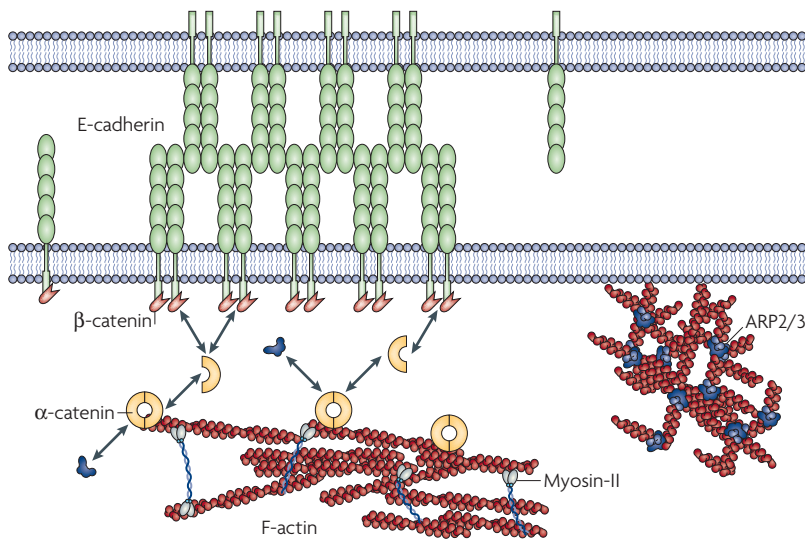


Figure 3 | Molecular interplay between cortical tension and adhesion. Intercellular adhesion is controlled by the *trans*-association of E-cadherin (green) *cis*-dimers that form homophilic complexes in the extracellular space. E-cadherin complexes are stabilized by cortical actin filaments (red). This stabilization requires α -catenin (yellow) shuttling between β -catenin (orange), which is bound to the cytoplasmic tail of E-cadherin, and F-actin. α -catenin exists both as a monomer and as a dimer. α -catenin does not bind to F-actin and β -catenin simultaneously²⁰, and this dynamic association might mediate remodelling of adhesion complexes. α -catenin dimers bind to F-actin and compete out the actin nucleator and branching factor ARP2/3 complex (blue)⁴⁷. This suggests that adhesion complexes may regulate the organization of actin filaments at the cortex. Cortical tension exerted by F-actin and myosin-II (grey) requires anchoring with the plasma membrane, such as with adhesion complexes in epithelial cells⁵¹.

Tissue morphogenesis

The morphogenesis of tissues during embryonic development depends mainly on intrinsic forces that drive local changes in cell shape, although external forces could also be important. Several tissue reorganization events can be explained in terms of the spatial regulation of intercellular surface tension and cell shape.

Apical constriction and tissue bending. Apical constriction is an important driving force for tissue bending and invagination in several developmental contexts, such as *D. melanogaster* mesoderm invagination (FIG. 4a,c,d) and neurulation in vertebrates (FIG. 4b,c,d; BOX 2). Changes in apical–basal cell length and external constraints might also be important for tissue bending⁵². Apical constriction requires the apical positioning of an acto-myosin tensile system at cell junctions that increases surface tension locally through the upregulation of cortical tension by myosin-II. The surface of contacts between constricting cells is reduced apically (FIG. 4d). The apical upregulation of myosin comprises an activation and an anchoring step (FIG. 4e).

Myosin-II is activated by phosphorylation of the regulatory light chain (Myo-IIRLC) on two conserved serines by Rok (FIG. 4e). Rok is required for apical constriction in flies⁵¹ and mice⁵³ and it is activated in most cases by the Rho GTP exchange factor **RhoGEF2** (REFS 51,54–56) and **Rho**⁵⁷. Further evidence for the role of Rho in apical constriction comes from the implication of the RhoGAP Cv-c during tracheal invagination in *D. melanogaster*,

and p190RhoGAP during vertebrate neural tube closure⁵⁸. In amphibians, Rho appears to be dispensable for apical constriction, which is dependent on the small RhoGTPase, Rap1 (REF. 59). Rap1 has also been implicated in mesoderm invagination in *D. melanogaster*⁶⁰, which suggests that multiple small GTPases might contribute to the apical activation of myosin-II.

How is myosin-II activation concentrated apically? This process, which has mostly been studied in flies (in particular during mesoderm invagination), involves apical activation and apical anchoring of RhoGEF2. RhoGEF2 is activated by the G_{α} protein $G_{\alpha12\alpha13}$ –Concertina (**Cta**) pathway^{55,56}. $G_{\alpha12\alpha13}$ –Cta activation is spatially restricted through the apical secretion of the ligand **FOG**⁵¹ that activates an as-yet-unidentified receptor (FIG. 4e). In *D. melanogaster* cultured cells, RhoGEF2 binds to the microtubule (MT) plus-end protein end-binding-1 (EB1); $G_{\alpha12\alpha13}$ –Cta in the GTP-active state releases this association⁶¹. Astral MTs are concentrated apically and plus ends extend towards the apical cell cortex. This suggests that RhoGEF2 activation requires an MT plus-end-dependent search in the cytoplasm and apical release by $G_{\alpha12\alpha13}$ –Cta^{56,61} (FIG. 4f). During fly mesoderm invagination, RhoGEF2 is anchored at the plasma membrane in the apical junctional area (FIG. 4f) through the apically targeted transmembrane protein T48 (REF. 56). This model is partly conserved in mammals. $G_{\alpha13}$ binds to p115RhoGEF and catalyses nucleotide exchange on Rho⁶². In vertebrates, the role of RhoGEF2 in apical constriction has not been demonstrated but both RhoGEF2 and $G_{\alpha12\alpha13}$ –Cta are required for gastrulation and regulate cortical remodelling^{63,64}.

Apical activation of myosin-II is not sufficient for constriction and requires a properly organized actin network^{51,65} (FIG. 4e). The Abelson kinase **Abl** and RhoGEF2 are both required for apical actin reorganization during mesoderm invagination in *D. melanogaster* independently of myosin-II (REF. 65). Abl localizes apically and inhibits the actin regulator ENA/VASP. In vertebrates, many mutants that affect actin integrity, such as Abl, show defects in neural tube closure^{66–70}. The actin-binding protein Shroom, which localizes at apical junctions, is both necessary and sufficient for apical constriction in vertebrates^{59,67,71}. Constriction is accompanied by changes in the morphology of the apical cell surface: the initially highly convoluted surface of contact becomes straight, which is consistent with an upregulation of cortical tension⁷¹. A detailed understanding of how actin remodelling (for example, bundling, branching and turnover) is required for apical cortical tension is lacking. Myosin-II may contribute to actin remodelling and dynamics as suggested from recent work on cytokinesis^{36,72}. *In vitro* studies also suggest the possibility that myosin-II may alter the visco-elastic properties of the contractile actin network^{34,35}.

Connections with the plasma membrane are required for the contractile actin–myosin-II network to exert isotropic mechanical tension to the plasma membrane. In *D. melanogaster*, apical constriction in mesodermal cells requires acto-myosin tethering to AJs. A recent report⁵⁶ highlights the complex regulation of AJs during apical

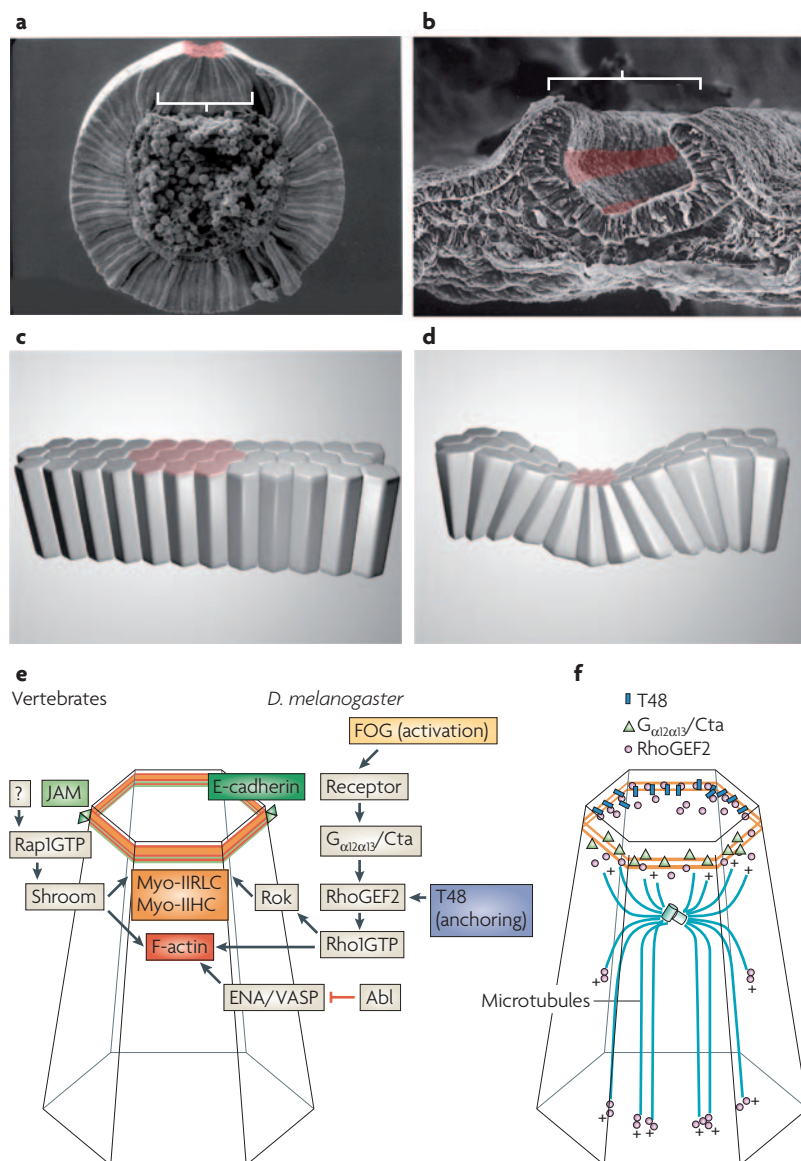


Figure 4 | Apical constriction and tissue bending. **a** | Mesoderm invagination in the *Drosophila melanogaster* embryo involves furrowing of the ventral epithelium and specific cell-shape changes in apical constriction (pink) of invaginating cells (square bracket). **b** | Vertebrate neurulation (for example, in the chick) proceeds by bending of the neuro-epithelium (square bracket) and apical constriction in defined regions (pink). **c** | Schematic representation of apical constriction (pink) in epithelial cells. **d** | Schematic representation of bending associated with apical constriction of the epithelial tissue. **e** | Apical constriction requires the formation of an apical contractile acto-myosin network (orange) that spans the junctional area (green) and interacts with E-cadherin complexes at the adherens junctions in *D. melanogaster* and junctional adhesion molecules (JAMs) at tight junctions in vertebrates. In flies, apical constriction involves activation (phosphorylation) of myosin-II regulatory light chain (Myo-IIRLC) by the $G_{\alpha 12\alpha 13}$ /Cta, RhoGEF2, the Rho small GTPase Rho1 and Rho kinase (Rok). The ligand FOG activates this pathway via an unknown receptor. T48 is a transmembrane protein that binds and localizes RhoGEF2. In vertebrates, the Rho small GTPase Rap1 and Shroom activate Myo-IIRLC apically. The reorganization of actin filaments by Shroom in vertebrates and by the kinase Abl in *D. melanogaster* is also important in this process. **f** | RhoGEF2 is a central regulator of apical constriction that is anchored apically by the transmembrane protein T48. RhoGEF2 binds the plus (+) ends of microtubules. Apical $G_{\alpha 12\alpha 13}$ /Cta releases this association. Myo-IIHC, myosin-II heavy chain. Image in panel **a** courtesy of D. Sweeton and E. Wieschaus, Princeton University, New Jersey, USA. Image in panel **b** reproduced courtesy of K. Tosney, University of Miami, Florida, USA.

constriction, with a disassembly and reassembly that is controlled by RhoGEF2. Apical constriction could potentially involve upregulation of surface tension by inhibition of adhesion. This has never been reported in natural apical constriction processes. However, clones of cells that are devoid of the cell adhesion molecule Echinoid do constrict their apices^{73,74}.

Junction remodelling and intercalation. Anisotropic modulations of cell surface tension have also been shown to control cell-shape changes that result in cell intercalation during convergent extension movements. Cell intercalation was discovered in studies of the antero-posterior elongation of the *Xenopus laevis* mesoderm⁷⁵. Elongation is driven by the dorsal convergence of mesenchymal cells that intercalate medio-laterally by polarized protrusive activity and cell crawling⁷⁵. Intercalation also occurs in epithelial tissues such as the *D. melanogaster* germband^{76–78} (FIG. 5a,b). Epithelial intercalation is not produced by polarized cell migration but through a spatio-temporally ordered process of cell-junction remodelling (FIG. 5c). Cell intercalation produces neighbour exchange in approximately 30 minutes: first, cells that are predominantly hexagonal lose (on average) an interface with their neighbour and, later, gain a new interface. Thus, during intercalation, cells change shape; for example, hexagons produce pentagons or tetragons. This loss and gain of junctional interfaces is not spatially random, but is irreversible and is polarized in the plane of the tissue (FIG. 5c,d): type-1 junctions that separate antero-posterior neighbours shrink and produce a type-2 configuration, and a new junction is formed perpendicularly, producing the type-3 junction. Junction remodelling driving intercalation has also been reported during epithelial tube extension of the *D. melanogaster* tracheal system^{79,80}. In this example, junction remodelling is probably controlled by external forces that pull on intercalating groups of cells.

However, intercalation is not dependent on external forces that act at the tissue boundaries during germband elongation⁷⁷. Local effectors of junction remodelling have been identified, supporting the idea that local modulation of intercellular surface tension drives cell intercalation. First, myosin-II is specifically upregulated in shrinking type-1 junctions and is required for junction remodelling^{77,78} (FIG. 5e,f), probably through the upregulation of cortical tension (FIG. 5g). Second, the polarity protein PAR-3 (which controls E-cadherin stabilization^{48,81} and thus intercellular adhesion), along with E-cadherin and β -catenin, exhibit a polarized localization that is complementary to myosin-II (REFS 78,82) (FIG. 5f). This suggests that adhesion might be locally downregulated in type-1 junctions (FIG. 5h), thereby also contributing to an upregulation of surface tension during shrinkage of type-1 to type-2 junctions. In the *D. melanogaster* tracheal system, the junctional protein polychaetoid (Pyd; also known as ZO-1) is also required for junction remodelling⁸³. Whether adhesion and/or cortical tension are regulated by ZO-1 remains unclear. Thus, the sub-cellular regulation of intercellular surface tension can control dynamic patterns of cell-shape changes, thereby driving cell intercalation and tissue extension.

Box 2 | Tissue invagination and apical constriction

During gastrulation, the different germ layers are formed. In some cases, the changes that occur reflect developmentally controlled global differences in tissue surface tension and cell sorting, such as in the hydra. However, germ-layer formation is controlled by cell involution or tissue bending and invagination, which was proposed to require local cell-shape changes by apical constriction⁹⁸. Apical constriction is accompanied by an expansion of the basal–lateral surface that produces bottle-shaped cells in cross-sections^{99–103}. These local cell-shape changes cause local instabilities in tissue shape and contribute to invagination.

Tissue invagination of epithelial cells controls endoderm formation in *Caenorhabditis elegans*¹⁰⁴, *Drosophila melanogaster*, sea urchins^{100,105,106} and amphibians^{99,107} but also controls mesoderm formation in *D. melanogaster* and neurulation in vertebrates through bending of the dorsal neuro–epithelium. In *D. melanogaster*, apical constriction in epithelial layers promotes developmental processes such as embryonic segmentation¹⁰⁸, tracheal placode¹⁰⁹, salivary gland invagination^{55,110,111} and epithelium folding in imaginal discs⁵⁵. In vertebrates and invertebrates, apical constriction promotes wound healing. Apical constriction occurs in most cases in epithelial cells of the ectoderm and neuroectoderm, but also in non-epithelial cells, such as during ingression of the *C. elegans* endoderm and mesoderm¹⁰⁴. Apical constriction is both an ancestral and general cell-shape change that is important for tissue morphogenesis. Different simulations that model visco-elastic forces⁹⁸ or active deformation and elastic responses⁵² have further supported this notion.

Emergent patterns

The regulation of intercellular surface tension can be controlled in groups of cells to produce simple or more elaborate geometric cell patterns in a tissue.

Reconsidering developmental compartment boundaries.

Apart from the cell sorting model (FIG. 1; BOX 1), a different mechanism — the fence model — was proposed to explain the formation and stabilization of compartment boundaries at the cellular level⁸⁴. This model states that specific mechanical properties of cells at the boundaries prevent cell mixing through the formation of a stiff barrier that cells cannot cross. This is supported by recent evidence that F-actin and ENA/VASP accumulate together with myosin-II at the *D. melanogaster* developing wing dorsal–ventral compartment boundary in response to localized, non-transcriptional Notch signaling⁸⁵. Furthermore, myosin-II prevents cell mixing at the boundary⁸⁴. Although only a subcellular cortical domain of boundary cells accumulates myosin-II and F-actin, the cumulative, supra-cellular effect is an effective increase in myosin-II along the entire dorsal–ventral border and the upregulation of tissue surface tension. The tension contributed by each boundary cell is likely to prevent the addition and stabilization of new cells at the boundary and also reduces the area of contact between compartments. In this context, the boundary-specific surface tension is an emergent property that stems from the contribution of each boundary cell. The dorsal–ventral compartment boundary physically behaves like the type-1 junction during *D. melanogaster* intercalation: it reduces its total surface through the upregulation of myosin-II. Note that PAR-3 is also downregulated at the dorsal–ventral compartment boundary similar to the type-1 junction^{78,84}. Dorsal closure of the *D. melanogaster* epithelium is also driven by a contractile acto-myosin network that contributes to reduce the length of the dorsal epithelium boundary⁸⁶.

Intercalation

A process whereby cells exchange neighbours in a polarized fashion in the plane of a tissue during convergent extension movements.

Convergent extension

Bilateral symmetrical movement of cells that converge towards a given axis, thereby contributing to the joint extension of the tissue. Convergent extension is driven by intercalation and polarized migration.

Germband

Region of the embryo (also called ventral plate) where the body of the embryo will develop. The rest of the cells in the blastoderm become part of a membrane (the serosa) that forms the yolk sac.

Hierarchical geometric patterns controlled by adhesion.

The retina of insects is a highly ordered and regular structure. The *D. melanogaster* eye is composed of ~700 units called ommatidia. The adult eye derives from a mono-layered epithelium, the eye imaginal disc, which grows during larval life. Following an initial stage during which epithelial cells divide and remain unpatterned, a morphogenetic wave sweeps across the eye imaginal disc and produces evenly spaced photoreceptor cell clusters that are composed of two inner and six outer photoreceptors; these cells form the seed around which other epithelial cells are subsequently recruited to form ommatidia⁸⁷. During early metamorphosis in the pupa, ommatidia mature through the sequential recruitment of four lens-secreting cone cells and two primary pigment cells (IPC) that form an aperture ring. Finally, a large population of undifferentiated and loosely arranged interommatidial pigment cells (IPCs) undergoes a series of cell movements, cell-shape changes and cell death that culminates in the formation of a highly regular hexagonal lattice around each ommatidium, thereby optically insulating them and completing the formation of the eye (FIG. 6a,b).

During the morphogenesis of ommatidia, retinal cells change shape, which suggests a precise regulation of cell surface tension. Recent work showed that the differential expression of N-cadherin in cone cells and of E-cadherin in all retinal cells (FIG. 6c) causes distinct morphological changes compared with situations where all cells express similar levels of cadherins (FIG. 6d,e): the contact surface of the cone cell with IPC is reduced, whereas the contacts between cone cells increase as a result of increased adhesion mediated by N-cadherin at these interfaces¹⁵ (FIG. 6c–d). From the perspective of cell surface tension negatively regulated by adhesion, we can explain cell sorting of the cone cell cluster and the specific morphology of cells within this cluster.

Primary pigment cells and cone cells form a higher order structure that expresses the immunoglobulin (Ig)-domain cell adhesion molecule **Hibris**, whereas IPCs express the Ig-domain cell adhesion molecule **Roughest** (FIG. 6b). Hibris-expressing cell aggregates do not fully separate from IPCs and are instead maintained in small, regular aggregates because of heterophilic interaction between Hibris and Roughest⁸⁸ (FIG. 6b). The stronger adhesion between IPCs and 1PCs compared with inter-IPC adhesion causes the increased area of contact between IPCs and 1PCs and cell elongation (FIG. 6b). This envelopment mechanism is accompanied by the formation of the higher order hexagonal shape of ommatidial clusters.

Rosette formation. Another striking example of higher order structures during tissue morphogenesis is the formation of epithelial cell rosettes during *D. melanogaster* germband extension⁸². Rosettes arise in the intercalating region of the epithelium and have various structures. In the most common case, rosettes include five cells, but rosettes can occasionally include six or even seven cells. These rosettes form and later resolve as germband elongates. Rosettes also arise as a result of planar polarized junction remodelling, in particular the polarized enrichment of

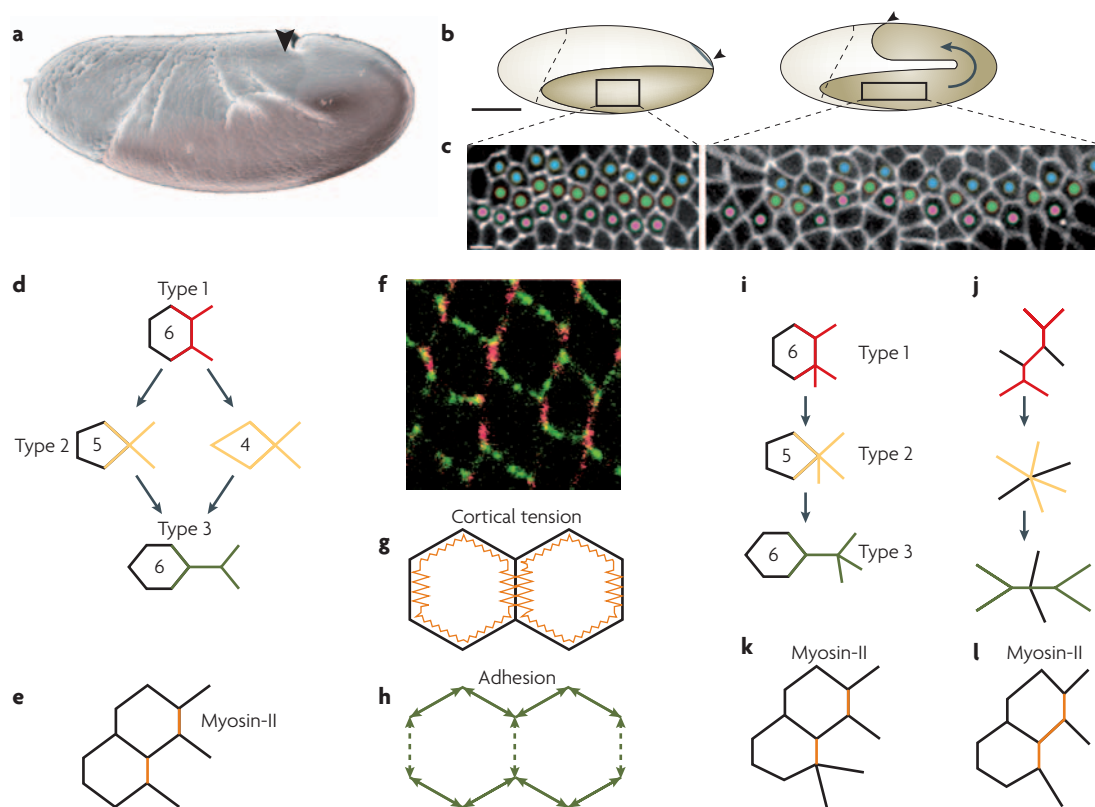


Figure 5 | Dynamic cell patterns during intercalation. Antero-posterior elongation of the *Drosophila melanogaster* germband epithelium (shown on a scanning electron micrograph (a) and schematic diagram (b)) is driven by intercalation. The posterior end (arrowheads in a and b) moves towards the anterior (left) on the dorsal side (top). During intercalation, epithelial cells (which have been marked with E-cadherin–green fluorescent protein and then labelled in different colours) exchange neighbours within 30 minutes (c). Cell intercalation depends on the irreversible change in the geometry of cell contacts, with shrinkage of type-1 junctions (red) to the type-2 configuration (yellow) and regrowth of type-3 junctions (green)⁷⁷ (d). This planar polarized remodelling of cell contacts is driven by the polarized enrichment of myosin-II (orange) in type-1 junctions (e, f) and the downregulation of PAR-3 (shown in green in part f). Polarized remodelling of cell surface tension by an upregulation of cortical tension (orange) (g) and a downregulation of adhesion (green) (h) in shrinking junctions drives cell intercalation. Irregularities in the packing of cells (i, j) and in the distribution of myosin-II (k, l) during polarized junction remodelling causes the clustering of more than four cells in the type-2 configuration and the formation of transient five-fold (i) or six-fold (k) rosettes⁸². Parts b and c modified with permission from REF. 77 © (2004) Macmillan Publishers Ltd.

myosin-II and F-actin in shrinking junctions, and the corresponding downregulation of PAR-3. In this context, the formation of type-2 junctions⁷⁷ produces the simplest rosette that includes four cells (FIG. 5d). Two scenarios can be envisaged for the formation of rosettes. They result from the existence of an as-yet-unknown rosette-specific pathway that might control their number, structure and position. Alternatively, they could simply result from the irregularity of the tissue (for instance, the presence of up to 25% pentagons⁸⁹) that occasionally brings together more than four cells during type-1 junction shrinkage and formation of the type-2 configuration (FIG. 5i–l).

Control of geometric order

Although interacting cells can form ordered patterns such as a perfectly regular hexagonal lattice (for example, in the *D. melanogaster* retina), most tissues also contain several pentagons and heptagons that contribute to an apparent geometric disorder (FIG. 2b). Topological

changes that involve remodelling of cell–cell contacts can be accompanied with a change in geometric disorder. During cell intercalation in *D. melanogaster*, tetragonal, pentagonal and heptagonal cells increase at the expense of hexagonal cells, thus producing an increased disorder⁸⁹. Conversely, in the developing *D. melanogaster* pupal wing, the initially disordered tissue (45% hexagons) becomes highly ordered, with up to 80% hexagons⁹⁰. This ordering of the tissue is controlled by the planar-cell polarity pathway and requires intercellular junction shrinkage and regrowth, and E-cadherin recycling through the endocytic pathway. The two previous examples concern non-proliferating epithelia, in which adhesion and cortical tension are prominent parameters that are able to control geometric order by modulating cell shape and contacts.

Geometric patterns in proliferating epithelia do not follow the same rules⁴². Through cell divisions, adherent cells in a proliferating epithelium are able to form new

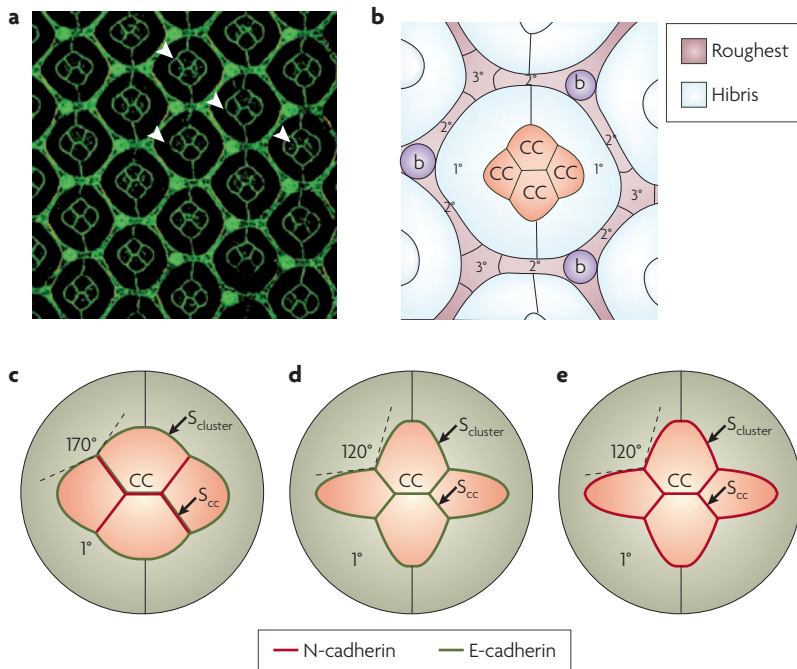


Figure 6 | Building hierarchical cell patterns with adhesion. **a** | The fly retinal epithelium is a highly ordered structure (marked with E-cadherin). Ommatidial units (arrowheads) are assembled in a regular honeycomb hexagonal pattern. **b** | Ommatidia are separated by a belt of secondary (2°) and tertiary (3°) pigment cells (pink) that envelops clusters of primary (1°) pigment cells (light blue) around cone cells (CC; orange). This engulfment process requires heterophilic association between Hibris in 1° cells and Roughest in 2° and 3° cells. Heterophilic adhesion causes cell elongation in 2° cells. **b**, bristle group. **c–e** | Cone cells adopt a specific conformation through the upregulation of N-cadherin (red) in CC, whereas all cells express E-cadherin (green). This upregulation causes a minimization of the surface of the CC cluster ($S_{cluster}$) and an increase of the surface of contact between CC (S_{cc}). Upon uniform adhesion when N-cadherin is removed from CC (**d**) or by overexpression of N-cadherin in all cells (**e**), the surface of the CC cluster increases and CC reduce their surface of contact. The angles between CC become smaller and the geometry of the CC cluster changes. Image in panel **a** courtesy of R. Carthew, Northwestern University, Illinois, USA. Part **b** modified with permission from REF. 88 © (2005) Elsevier.

interfaces^{42,91}. Gibson *et al.*⁴² showed mathematically and confirmed experimentally that in metazoans, a few cycles of topology changes by cell division result in an equilibrium topology in which ~50% of cells are hexagonal with an asymmetric distribution of pentagons and heptagons,

regardless of the initial conditions. Strikingly, the final topology is irregular but not random. This provides an alternative mode of geometric ordering and shows the importance of history in this context. In this case, the concept of surface tension — an equilibrium property — is not relevant to describe the course of events such as cell divisions, which are out of equilibrium by nature.

Conclusions

The integration of disciplines from physics (experimental and modelling) to cell biology and developmental biology is important to address in new ways how patterns arise in living organisms, based on the fundamental properties of cell surfaces. We propose a cellular framework of morphogenesis based on a consideration of intercellular surface tension. Cell-shape changes and cell contacts are remodelled by the opposite actions of cortical tension and adhesion. In this context, we can explain tissue bending and elongation and the formation of various emergent cell patterns at the multicellular level.

Obviously, this framework cannot explain all aspects of morphogenesis. It will be increasingly important to take kinetic parameters into account to understand the course of events during cell-shape changes and tissue morphogenesis. The fact that cell sorting cannot be simply explained by the strength of interaction between cadherins^{18,92} substantiates this idea. At the cellular level, the visco-elastic properties of the cortex should be further studied and taken into account to explain the dynamics of cell shape and cell interactions on short and long timescales. This will require, for example, investigations of actin dynamics and organization in the control of adhesion, cortical tension and visco-elastic properties of the cortex.

Developmental processes are extremely organized. This feature often masks the fact that, upon closer inspection and when looking at temporal sequences, the systems are not perfectly regular and often somewhat disordered. This might reflect the limit of a tight genetic control, but emphasizes the robustness of developmental processes because they can cope with noise. It will be important to further investigate the relationship between fluctuations at the cellular level and tissue order during morphogenesis to discover the origin of cell fluctuations and how assemblies of cells cope with them to produce regular patterns.

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Competing interests statement

The authors declare no competing financial interests.

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