Mechanobiology of collective cell behaviours

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Abstract | The way in which cells coordinate their behaviours during various biological processes, including morphogenesis, cancer progression and tissue remodelling, largely depends on the mechanical properties of the external environment. In contrast to single cells, collective cell behaviours rely on the cellular interactions not only with the surrounding extracellular matrix but also with neighbouring cells. Collective dynamics is not simply the result of many individually moving blocks. Instead, cells coordinate their movements by actively interacting with each other. These mechanisms are governed by mechanosensitive adhesion complexes at the cell–substrate interface and cell–cell junctions, which respond to but also further transmit physical signals. The mechanosensitivity and mechanotransduction at adhesion complexes are important for regulating tissue cohesiveness and thus are important for collective cell behaviours. Recent studies have shown that the physical properties of the cellular environment, which include matrix stiffness, topography, geometry and the application of external forces, can alter collective cell behaviours, tissue organization and cell-generated forces. On the basis of these findings, we can now start building our understanding of the mechanobiology of collective cell movements that span over multiple length scales from the molecular to the tissue level.

Border cells
In *Drosophila melanogaster*, a cluster of six to eight migratory cells migrating in the egg chamber from the follicular epithelium towards the oocyte.

How living cells are able to sense their environment and adequately respond by adjusting their shape, migration, proliferation, differentiation potential and survival remains an open question. In tissues, cells do not exist in isolation but are surrounded by other cells as well as the extracellular matrix (ECM), through which they can migrate. During morphogenesis and tissue repair, such multicellular assemblies rearrange or actively migrate to ensure the proper formation and repair of organs. These behaviours are tightly biomechanically regulated and depend on the coupling between neighbouring cells[1].

Collective migration (crawling) is particularly important during development, as exemplified by migration of border cells in *Drosophila melanogaster*[2], migration in the primordium of the zebrafish lateral line[3] and specification of the anterior–posterior axis in mice (see Supplementary information S1 (box)). During these processes, cohesive cell groups display front-to-back polarity with the specification of polarized leading cells at the front edge that coordinate the migration. The organization of these polarized cell clusters may be seen as a scaled-up version of a single cell, a 'giant migrating cell'. Similar mechanisms of collective cell migration also seem to be employed by cancer cells during metastasis[4–6]. Collective cell rearrangements are driven by coordinated contractile processes of entire epithelial sheets — driven by contractions of an actomyosin belt[7] — which are as important as collective migration for tissue shaping. An example of such rearrangements is epiboly during gastrulation in zebrafish embryos (see Supplementary information S1 (box)). Importantly, the two mechanisms, collective migration and actomyosin-driven contractions, are not mutually exclusive and can coexist, as is the case during epithelial wound closure, for example[8].

Bearing in mind the complex 3D topological organization and constant remodelling of the ECM and the cell–cell environment, *in vivo* analysis of cell collectives is difficult. Moreover, even though techniques have recently been developed to measure forces *in vivo*[9,10], the precise analysis of mechanical forces in biological phenomena remains challenging. Thus, *in vitro* approaches are particularly useful for multiscale analysis (from molecular to multicellular levels). Complementary to experiments, various physical models have been developed to understand collective cell behaviours (for a complete perspective, see REFS 11,12 and Supplementary information S2 (box)).

Single cell deformation and movement are complex processes that rely on actin polymerization, cell contractility and dynamics of adhesion complexes at the ECM interface: cells apply traction forces to the substrate through the formation of adhesive structures and the

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transmission of internal forces produced within their cytoskeleton[13–15] (FIG. 1a). These endogenous forces can be modulated by numerous external factors such as matrix physical properties[16], external forces[17] and biochemical factors[18]. In addition to cell–ECM coupling, neighbouring cells (and their cytoskeletons) are also mechanically coupled by various junctions (BOX 1; Supplementary information S3,S4 (boxes)), and this intercellular coupling adds another layer of complexity[19] (FIG. 1b). Fortunately, many collective behaviours can be reproduced *in vitro* (FIG. 1c); we now know that these processes require, and are regulated by, mechanical forces exerted by individual cells on their underlying substrate[20], stresses at the cell–cell contacts[21], cell contractions[7] and cellular protrusions[22], as well as the physical properties of the cellular environment, including its stiffness, topography and porosity[23,24]. In this Review, we discuss our current understanding, based on these *in vitro* systems, of the role of mechanical forces and cell coupling in collective cell rearrangement and migration. We focus here on cadherin-based adherens junctions (BOX 1), as the mechanobiology of these junctions has been most extensively studied. Other junctions, namely, tight junctions and desmosomes, are briefly discussed in Supplementary information S3 (box) and S4 (box), respectively.

Mechanisms of collective motion

One of the most intriguing questions regarding collective cell behaviours is how cells are coordinated to form multicellular systems at the mesoscale. Two main mechanisms that support collective dynamics are at play: polarized collective cell migration and coordinated contractility of actin cables.

Studying collective cell behaviours. As stated in the 1960s by M. Abercrombie[25], "it is a well-known principle that epithelium will not tolerate a free edge. Given a suitable substrate, a free edge will advance by movement of the epithelial sheet behind it". Hence, wound healing is a particularly good model to study collective cell behaviours, and various assays have been developed to mimic wound-healing processes *in vitro* (BOX 2). Quantifying the dynamics of collective cell behaviours (FIG. 1c) requires the measurement of physical parameters such as speed, forces and stresses. These measurements are very challenging — measurement of collective motion requires simultaneous recording of the displacement of each single cell over the entire population, which is difficult to achieve. To overcome some of these difficulties, particle image velocimetry (PIV) has been used to map local displacements within cellular subpopulations, thereby providing velocity fields of multicellular assemblies without relying on the trajectories of individual cells[26] (FIG. 1c). From these velocity fields, the spatial correlation of cellular movements can be calculated together with correlation lengths, which provide information on the range over which movements are coordinated within the tissue[27]. These measurements provide information about the local ordering of cells, which is a good descriptor of the polarity of cell clusters as well as of dynamic cellular flows in the sheet (FIG. 1c).

Collective cell migration in epithelial cells. Epithelial cell monolayers are useful model systems to characterize collective cell behaviours and cell–cell interactions. When an epithelial monolayer is exposed to an empty edge, it starts to move into that empty space, forming a protruding, finger-like structure encompassing tens of cells in diameter, within which cells acquire migratory behaviour[26]. Cells at the tip of these structures, called leader cells, form large lamellipodia and maintain strong connections via cadherin-based adhesions with follower cells, dragging a small cluster of the followers along (FIG. 1c). The establishment of the migrating leading front observed *in vitro* shares strong similarities with *in vivo* developmental processes[2,3,28].

The leading edge is a polarized structure in which each single cell has a velocity vector pointing in the migrating direction of the leader[29]. Actin staining reveals the assembly of thick actin bundles over multiple cells (the actin belt), connecting the leaders to the followers along the edges of these finger-like structures and showing pluricellular mechanical coupling[26] (FIG. 1c). In addition, the mechanical perturbation of leader cells using laser ablation strongly supports the role of leader cells in providing local guidance cues for follower cells.

Cell motion and multicellular coordination of cellular movements occur not only at the migrating front but also in the bulk of cell monolayers (FIG. 1c). Of note, cell movements occur at low Reynolds number[30] (meaning that viscous drag of the surrounding medium, which tends to slow down the movement, dominates over inertia, which tends to maintain the movement once it has started) and are reminiscent of fluid dynamics. Nevertheless, cell behaviours in the bulk of the sheet cannot be described just by simple laminar flows: the cells often display coordinated swirling motions extending over tens of cells, which cause 'chaotic' motion within monolayers[31]. The unexpected diversity of these movements arises partly from the complex mechanical behaviour of biological tissues (that is, cells can deform or exchange neighbours) and their active nature (that is, cells can modify their contractility or their adhesive properties). Consequently, living tissues can be seen as complex viscoelastic materials that behave as solids on short timescales and as liquids on long timescales[32,33]. They actively respond to mechanical stimuli exerted by their environment by adjusting their actomyosin network and adhesion complexes to fine-tune contractility and forces they apply on their surroundings[19,34].

To add to the complexity, other cell biological processes occurring within the tissue can disturb the dynamic organization of cell monolayers (FIG. 1c). For example, cell division can generate a dipolar flow field, whereby the tissue flow is pushed outwards in the axis in which the bodies of the two daughter cells are connected[35]. The appearance of new daughter cells can 'stir' the tissue and lead to ordered flows over large distances away from the division site. In addition, cell extrusion events are preceded by a coordinated, long-range flow of cells towards the eventual location of the extrusion. The group of cells that participates in these coordinated

Lateral line
A system of sense organs found in fish, allowing the detection of movement, vibration and pressure gradients in the water.

Traction forces
Forces exerted by cells on their underlying surfaces during adhesion or migration.

Velocity fields
Maps of the flows of tissues by measuring the displacement field of natural tracers inside the tissues between successive images.

Reynolds number
Dimensionless quantity used in hydrodynamics that represent the ratio between inertial forces and viscous forces. Laminar flows occur at low Reynolds number, whereas turbulent flows appear at high Reynolds number.

Cell extrusion
An expulsion of apoptotic, non-apoptotic or transformed cells from a cell monolayer (apically or basally).

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flows induces defects in the physical arrangement of cell monolayers[36]. This misalignment of cells causes considerable bending of cells, leading to high compressive stresses that are sufficient to trigger the extrusion of a nearby cell.

The computation of velocity fields using the PIV method (see above)[37] helps with understanding the physical nature of tissues through the characterization of their collective movements, including the coordination and the degree of cell–cell rearrangements. For instance,

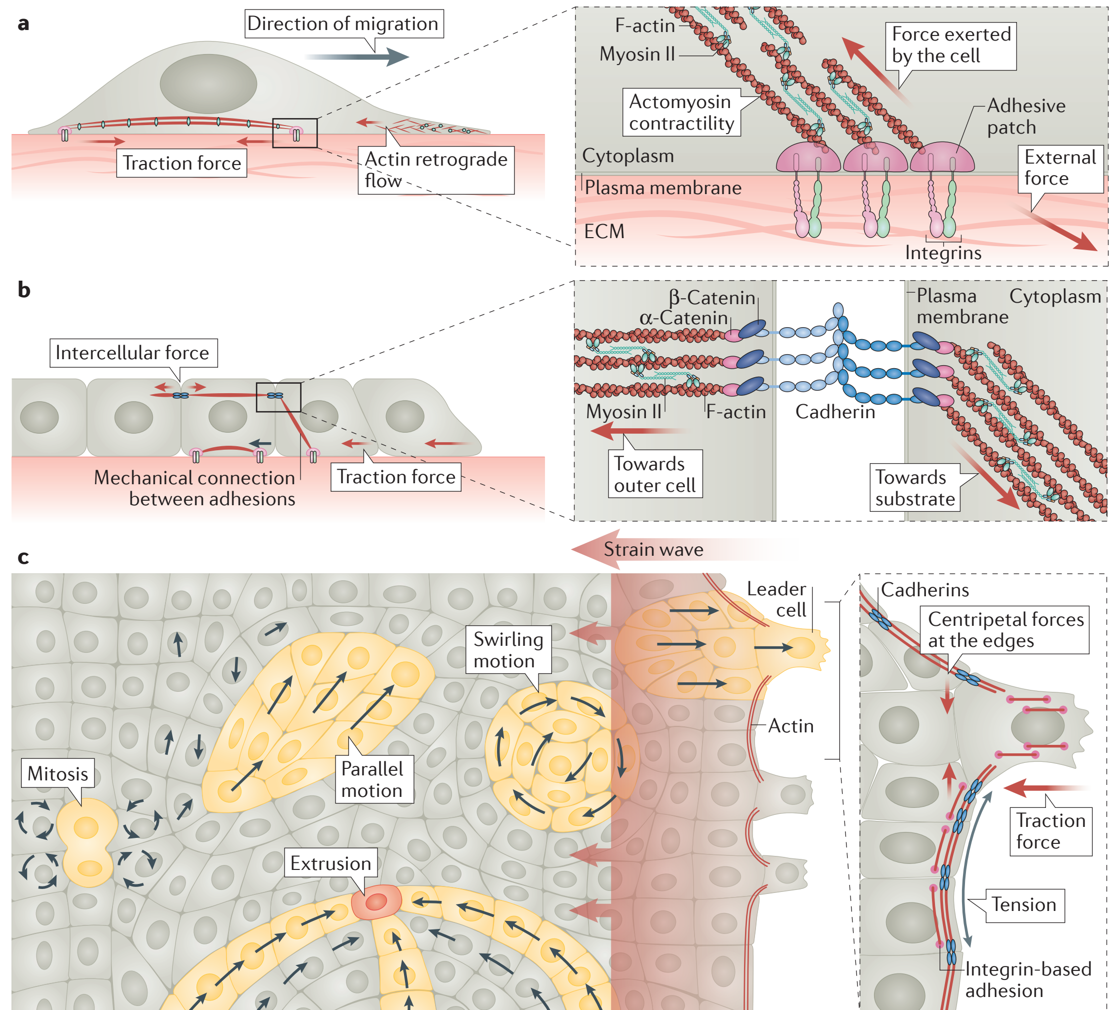

Figure 1 | **Cell movements from single to collective dynamics. a** | Single cells move by exerting traction forces (driven by actomyosin contractility) on the extracellular matrix (ECM) at the integrin-based adhesions (inset) both at the front and at the back. The protrusive activity at the front is driven by actin polymerization and retrograde actin flows. **b** | In the case of collective cell movements, the external substrate with which the individual cell interacts is either the ECM or the neighbouring cells. Cell–cell and cell–ECM adhesions are mechanically connected. Transmission of intercellular forces results from the coupling of cell–cell junctions to actomyosin (mainly mediated by mechanosensitive cadherin complexes; inset), while traction forces propagate deep into the monolayer (with leader cells exerting the highest traction forces, helping to orient the followers) **c** | As in single cells, cells in collectively migrating monolayers extend lamellipodia at their contact with the substrate at the edge[26] but also underneath other cells in the bulk (cryptic lamellipodia; not shown)[42], leading to large-scale polarization. Leader cells at the edge of the monolayer organize followers into finger-like structures, within which cells are coupled by actin cables to preserve the mechanical integrity of the leading edge[68]. Local displacements within the tissue are mapped by particle image velocimetry (PIV), which allows a complete analysis of cellular movements, including the magnitude and the orientational field of velocity vectors (shown by black arrows)[26,27]. Coordinated movements can be deduced from the spatial correlation of these velocity vectors: the larger the velocity correlation length, the more cells 'feel' the interactions with distant neighbours (the stronger the coordination). In addition to simple laminar flows, swirling motions of cell clusters and vortices are also observed[31]. Other events, such as cell division and extrusion, can also alter tissue flows. Strain and velocity waves propagate from the edge towards the bulk of cellular monolayers. These waves are driven by mechano-biochemical signals[75]. Leader cells at the edge of the migrating tissue adhere and migrate over the substrate through focal adhesion assembly and the extension of a large lamellipodium. Actomyosin-based structures (contractile belts) reminiscent of the ones observed in epithelial gap closure[107] are formed along the finger-like structures that emerge from the leader cells[68] and, more generally, along concave regions of the migrating front. These contractile cables are connected between cells through adherens junctions and can also be coupled to focal adhesions[44].

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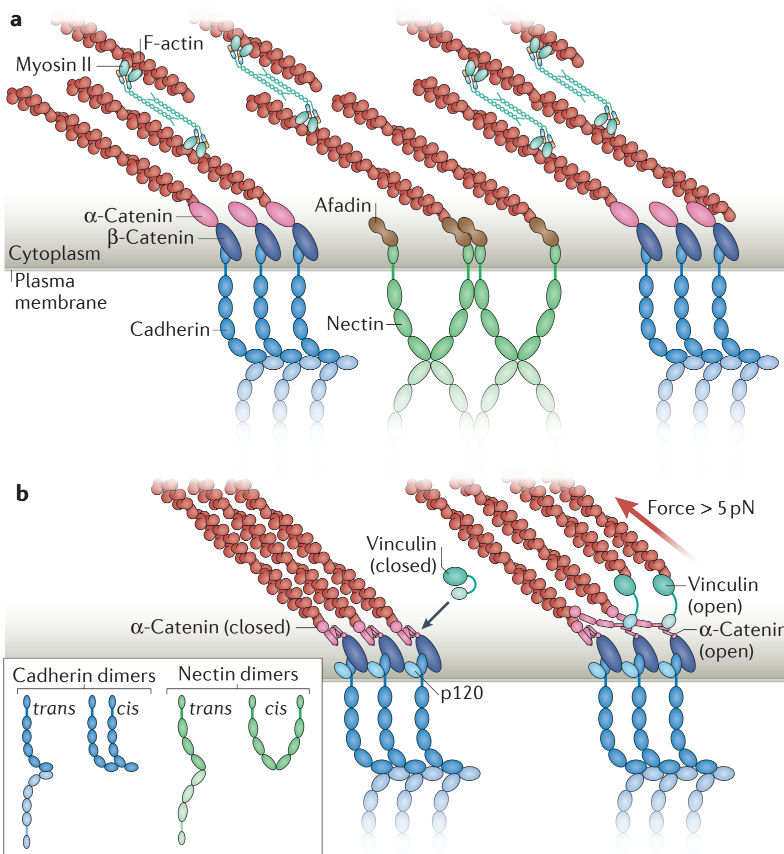
Box 1 | **Cell–cell coupling at adherens junctions**

Adherens junctions are dynamic structures that are remodelled and turned over in response to cell-autonomous and environmental changes such as mechanical stresses[43,93]. They are the primary sites of intercellular mechanical coupling. They are populated by two families of adhesion molecules: cadherins[43] and nectins[142], which mediate adhesion of neighbouring cells via their ectodomains, while their intracellular regions interact with actomyosin (see figure part **a**). Functional cadherin receptors result from the association of the cadherin (E-cadherin in epithelia) with cytoplasmic p120 (also known as catenin δ1), α-catenin and β-catenin, which are responsible for the association of cadherin receptors with F-actin[93]. This coupling is required for both efficient cell–cell adhesion and force transmission[80]. The establishment of adherens junctions results from a complex interplay between interactions of cadherin ectodomains in *cis* and in *trans*, their clustering and/or oligomerization[143–148] and dynamic molecular interactions of the F-actin-binding proteins α-catenin and vinculin with F-actin, which depend on myosin II-generated tension[93].

Cadherins are the major force-bearing proteins in adherens junctions[34,43,93]. Their complexes are tension-adaptive, multi-protein assemblies that respond to external load and tensile force produced by intracellular actomyosin[78,79,89,99,145,149] and allow morphogenesis[34,43] and collective cell migration *in vitro*[31,54,118] and *in vivo*[150,151] to proceed. Nectins, although recognized as obligatory components of adherens junctions, are less studied[142]. They also dimerize in *cis* and in *trans*[152], but unlike cadherins, nectin *trans* dimers are unable to support strong cell–cell adhesion and can form homophilic and heterophilic interactions with related family members, which can be an inbuilt mark of junctional polarization. Nectins and cadherins form distinct microclusters co-aligned along actin bundles[153]; however, nectin–nectin interactions in *trans* could initiate or strengthen cadherin *trans* interactions[142,154].

The adaptation of the cadherin complexes to forces is mediated by a force-dependent conformational switch in α-catenin: when stretched (at forces of ~5 pN or greater), α-catenin molecules unfold, which triggers vinculin binding and inhibits α-catenin refolding after force is released (see figure part **b**). Physiologically relevant forces may also stabilize vinculin in an open conformation that is able to bind F-actin, as well as enhance α-catenin binding to F-actin, thereby contributing to junctional mechanosensitivity.



monolayers of human skin[38,39] and of Madin–Darby canine kidney[26,39] epithelial cells both show velocity correlation extending over 10–12 cells. The emergence of such large-scale coordination remains to be clarified at the molecular level, but it involves mechanical, biomechanical and biochemical signalling: building up of intercellular stress through cell–cell contacts[40], co-alignment of the traction forces of individual cells[41], formation of cryptic lamellipodia[42] and activation of signalling by RHO GTPases at cell–cell contacts[43]. Importantly, this long-range coordination vanishes when cadherin-based adhesions are disrupted, highlighting the crucial role of adherens junctions in biomechanical cell coupling.

Contraction of an actin belt. The formation of contractile actin cables spanning multiple cells is an alternative mechanism to collective, active cell migration for driving collective cell displacement. For example, constricting actomyosin rings are observed during zebrafish epiboly (Supplementary information S1 (box)) as well as during wound healing and closure of epithelial gaps[7,8,44,45]. Also *in vivo*, a contractile actomyosin ring-like structure (referred to as a purse-string structure) assembles and contracts at the wound margins [46]. The contraction of the actomyosin cable progressively brings the marginal cells close together to seal the gap. These actomyosin rings are reminiscent of actin cables observed at the leading edge of migrating monolayers (FIG. 1c).

Actomyosin structures are linked between neighbouring cells through adherens junctions[47] or tight junctions[48] (BOX 1; Supplementary information S3 (box)). Adherens junctions are particularly well suited for mechanical coupling of cells, as they also serve as signalling centres that recruit RHO GTPases and regulate the actomyosin assembly[43] to propagate tension. In *in vitro* experiments in which epithelial gaps close over non-adherent patches, the closure of the wound is driven solely by actomyosin purse-string contractility. In classical wound-healing assays and other experiments mimicking the closure of epithelial gaps (see BOX 2 for details), epithelial cells display both active cellular protrusions and actin cable formation along the void[49,50] (see also below). The cable part is contractile and under tension, as shown by laser ablation experiments[50], and it shares strong similarities with the purse-string structures previously described *in vivo*.

The role of cell–cell interactions in coordinated dynamics. The coordinated movements of cells are known to strongly depend on the interactions between cells through cell–cell junctions[31,37,51,52]. Supporting the role of cell–cell junctions in mediating collective behaviours, migrating fibroblast-like cells, which, in contrast to epithelial cells, do not form strong cadherin-based junctions, have a correlation length of only 20 μm (approximately two cell diameters); this is ten times smaller than described for epithelial cells[37]. Although three types of intercellular junction — adherens junctions (BOX 1; FIG. 1b), tight junctions (Supplementary information S3 (box)) and desmosomes (Supplementary information S4 (box)) — are necessary to maintain the cohesiveness and physical

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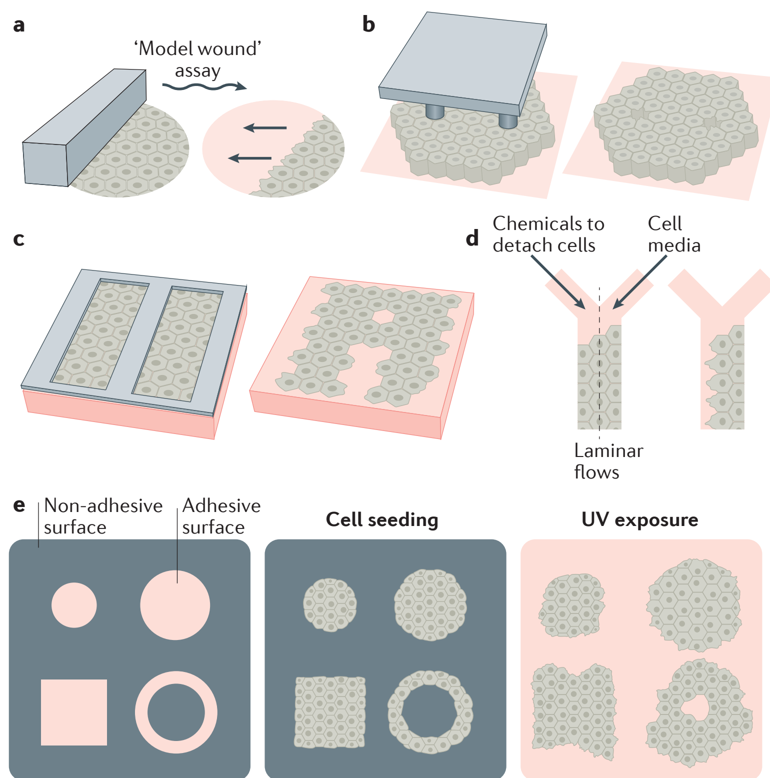
Box 2 | *In vitro* experimental methods to study collective cell behaviours

Cells migrate when exposed to an empty space, and there are several techniques that allow the controlled generation of free edges in the cell monolayer. First, a microfabricated barrier can be used to physically confine cells. Upon removal of this barrier, cells are unconfined and free to migrate[26,49,155]. The barrier is often made of silicone elastomer (polydimethylsiloxane, PDMS) by soft lithography techniques. Various types of micropatterned barriers can be produced, including slabs, micropillars and microstencils (see figure, parts **a–c**). In addition to micropatterns, microfluidic devices have been developed that allow a localized application of cell-detaching chemicals to selectively remove cells cultured in microchannels, creating a locally free edge (see figure, part **d**)[156]. A recent approach used photo-switchable surfaces to allow collective cell migration from precisely confined cell sheets after the removal of a non-adherent polymer layer. In this technique, a non-adherent surface is prepared by covalent binding of polymer brushes to glass substrates via a photocleavable group, which is sensitive to UV light. Cells seeded on these micropatterned substrates are first able to adhere only inside the confined adhesive geometries, and the confinement can then be released by irradiation with UV light (see figure, part **e**). These different methods can be easily combined with time-lapse videomicroscopy to observe cell movements, measure flows and forces and extract quantitative parameters, and they have been successfully used to study epithelial cell migration and endothelial wound closure[157].



integrity of epithelial cell sheets[53], only adherens junctions are crucial to maintain coordinated cell behaviour. Accordingly, the disruption of E-cadherin (also known as cadherin 1)-based adhesions in epithelial cells — without affecting close cell packing[31,54] — leads to highly uncoordinated motion at the single cell level, thereby leading to lower migration velocity of the groups.

Migration of cells that exhibit 'weak' cell–cell adhesion can be organized by means other than stable cadherin junctions. A notable example is the phenomenon of contact inhibition of locomotion (CIL), whereby two colliding cells change direction after coming into contact[55]. This repulsion is driven by cell repolarization in the opposite directions upon contact. CIL has been used to explain the collective migration of loose clusters of neural crest cells[56,57], where it involves the formation of N-cadherin (also known as cadherin 2) contacts[58]. In the context of more-adhesive cells, mechanisms governing CIL likely also operate but are restrained by strong cell–cell contacts ('frustrated' CIL). Indeed, myoblastic or endothelial cells in doublets show polarization reminiscent of CIL, at the same time maintaining cadherin-mediated adhesions[59,60]. Still, it remains to be clarified whether these mechanisms contribute to collective migration in epithelia.

Cryptic lamellipodia
Short lamellipodia forming under neighbouring cells in a migrating monolayer.

Neural crest cells
In vertebrates, a group of migrating cells that arises from the border between the neural plate and the non-neural ectoderm.

Cell density is also an important regulator of collective cell dynamics[61–63]. The average velocity of epithelial cells seeded in a dish stabilizes only after cell monolayers reach confluence and decreases as density further increases, leading to a switch from a fluid-like to a more solid-like state[64]. This decrease in cell dynamics is reminiscent of a phenomenon called 'cell jamming' (REF. 65) — as cell density increases, each cell within the population becomes increasingly trapped by its neighbours. Hence, the friction between cells increases, leading to reduced motion but at the same time greater correlation of motion (larger clusters of cells that migrate together)[66]. The increase in cluster size leads to a decrease in cellular rearrangements, and the system eventually becomes 'frozen'. However, the phenomenon of cell jamming is far from understood, and cell density may not be the (only) relevant parameter that results in jamming. Accordingly, recent studies indicate that relative changes in cell–cell adhesion and cortical tension or the crosstalk between cell–cell and cell–ECM interactions could be sufficient to explain jamming–unjamming transitions in cellular assemblies[63,65,67].

Forces in collective cell behaviours

The characterization of forces remains crucial to understand how cells interact with their environment. Various techniques have been developed to infer forces and stresses *in vivo* and *in vitro* at the molecular, cellular and tissue scales (FIG. 2), and we now have a much better understanding of their contributions to collective cell behaviours.

Forces exerted on the ECM and propagated across cellular assemblies. Mechanical traction forces exerted by cells on their substrate are classically measured by plating cells on soft, deformable substrates (FIG. 2B). In the context of monolayer migration over a free edge, traction force measurements reveal that leader cells exert high traction forces[68], as expected, although follower cells, even at positions far away from the leading edge, are shown to exhibit considerable traction forces[69]. This is correlated with the formation of larger focal adhesions by the leading cell than by the followers[26,69,70]. Maximal traction forces are localized at the tip of leader-cell positions and directed from the tip of the finger towards its base (opposite to the direction of migration), whereas at the back of followers within finger-like structures, opposite retraction forces are observed on average. This suggests that these multicellular structures

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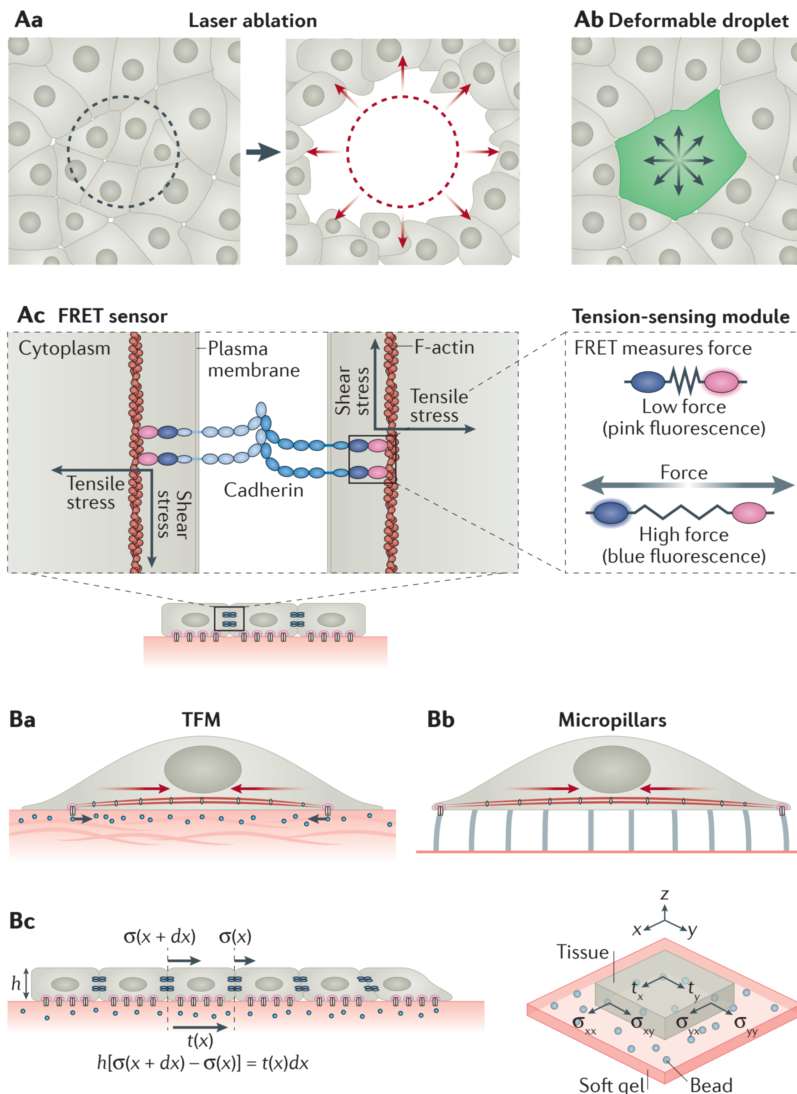

Figure 2 | **Methods of force measurement. A** | Measuring intercellular tensions. **Aa** | Laser ablation has been extensively used to estimate tensions[169]. After ablation, the immediate recoil velocity of the structure is measured (red arrows), but the inferred tension is the product of the velocity and the viscosity coefficient of the surrounding environment, which is usually unknown. As such, this method can be used to measure only relative tensions within cell structures. **Ab** | Promising methods such as optical tweezers[10] or deformable particles[9] have also been developed to capture intercellular forces *in vivo*. In the latter, liquid droplets can be embedded to measure local pressures inside tissues. The pressure can be extracted from the deformation of a droplet of defined rigidity. **Ac** | Förster resonance energy transfer (FRET)-based tension sensors have been developed to measure cell–cell tension[94]. Briefly, a polypeptide of known stiffness is inserted in a particular domain of a protein to measure the tension experienced by this protein. This approach has revealed in a direct manner that cadherins have an important role in the transmission of mechanical forces at cell–cell junctions *in vivo*[110]. **B** | Measuring traction forces (red arrows). **Ba** | Traction forces are classically measured by plating cells on soft, deformable gels in which small markers (such as beads) are embedded to track deformation patterns (an approach known as traction force microscopy (TFM))[170]. By inverting the displacement field of these markers, the force field can be calculated[69]. **Bb** | As an alternative method, cells are grown on arrays of micron-sized flexible polydimethylsiloxane (PDMS) pillars, deflection of which gives direct information on local forces exerted by cells[171]. **Bc** | From TFM experiments, the intercellular stresses in a monolayer, σ, are inferred in 1D or 2D configurations. In 2D these stresses can be tensile or compressive, σ_{xx} and σ_{yy}, in the normal direction of the intercellular junctions or correspond to shear forces, σ_{xy} and σ_{yx}, in the tangential direction and can be deduced from cell traction forces (t_x, t_y) using force–balance relation[66]. *h* denotes the average height of the monolayer.

present mechanical signatures similar to those of single migrating cells, such as a force contraction dipole. Though leader cells exert large traction forces that could destabilize cell–cell contacts, they maintain stable cadherin-based adhesions with the following cells[26]. This is facilitated by the presence of the actin cables along the leading edge; these structures generate mechanical forces directed inwards to preserve the integrity of the finger-like structures (FIG. 1c).

Traction force measurements have also been used to analyse epithelial wound closure[44], and these *in vitro* experiments helped to understand closing of finite gaps *in vivo*[71]. As a wound closes, epithelial cells exhibit two types of traction force: forces pointing away from the gap, as classically observed during cell migration, and forces pointing towards the gap. Interestingly, these forces can be respectively attributed to lamellipodial protrusions and actomyosin cable contraction[44,72]. At late stages of gap closure, the purse-string mechanism takes over, with traction forces mostly pointing towards the gap. This reveals spatiotemporal cooperation between these two processes, producing efficient wound closure.

Forces exerted at cell–cell contacts. The measurements of cellular traction forces on the underlying substrate can be used to calculate the forces at cell–cell junctions or, more generally, the intercellular stress within cell monolayers, in an approach often called 'monolayer stress microscopy' (REFS 66,73) (FIG. 2Bc). Migrating cell sheets exert the largest forces at their edges (FIG. 1c), but important forces exerted on the substrate are also observed far away from the edge. The transmission of forces depends on cell interactions not only with the substrate but also with neighbouring cells (FIG. 2Bc). This leads to a 'tug of war' between the two types of adhesion — signals from the substrates tend to induce migration of cells away from each other, whereas a stronger mechanical input from cell–cell interactions maintains the cohesion of cell sheets. Analysis of the mechanical balance of these forces within migrating cell sheets shows that the intercellular stress accumulates within the sheet from the edge to the interior, leading to higher tension on cell–cell junctions[31,69]. Migrating cell sheets can thus be seen as tissues under tension. This long-range cooperative process is driven by the transmission of forces through cell–cell junctions (FIG. 1b). This tensile stress state in epithelia has also been observed *in vivo* using laser ablation experiments[74]. The measurements of stress (or stress rate) inferred from traction forces (FIG. 2Bc) and strain (or strain rate) obtained from velocity maps can be used to reveal the rheology of cell monolayers. Interestingly, a linear relationship has been found between monolayer tension and strain, reminiscent of the behaviour of elastic materials[75] (as for a spring, in which force is proportional to deformation). This surprising finding suggests that stress dissipation (through cell–cell rearrangements, for example) either does not substantially contribute to mechanical properties of cell monolayers or is compensated for by active forces generated by the cells. In addition, the measurements of stress transmission within cellular monolayers have revealed that cells preferentially

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migrate in a collective manner along defined directions dictated by their mechanical interactions. Within the monolayer, intercellular interactions experience two types of stress: shear and normal stress (tension or compression generated by cell pulling or pushing, respectively) (FIG. 2Ac). It appears that cells tend to migrate along stress orientations that minimize shear stresses and thus may limit potential neighbour exchanges[66], and the disruption of cell–cell junctions reduces this correlation between the orientation of stresses and cellular motions. Even though the importance of stress-guided collective movements has not yet been addressed *in vivo*, these mechanisms can help explain cell differentiation and pattern formation in multicellular assemblies[76,77].

Mechanical coupling between cells is also characterized by the emergence of mechanical waves of velocity and stress during collective cell migration. Upon the release of a physical obstacle, the spreading and migration of cell monolayers lead to cellular movements and force transmission from cell to cell that progressively penetrate deeper into the monolayer, towards its centre[75] (FIG. 1c). The appearance of these waves may be partly explained by pulling forces of front cells on their neighbours, leading to strain, which is propagated and results in the build-up of mechanical tension due to the active response of the cytoskeleton.

At the molecular level, the transmission of mechanical signals through cell sheets (at least for epithelia and endothelia) occurs mainly through adherens junctions, which have been shown to be mechanosensitive[78–80] (BOX 1). High-throughput screening assays have indeed identified cadherins and other junctional proteins as major players in collective migration[40,51] (see the following section for more details).

Mechanisms of cell coupling

A major challenge in understanding collective cell behaviours is to link these behaviours to the action and dynamics of molecular cell components. Various cellular components, including the elements of the cytoskeleton, as well as components of cell–ECM and intercellular adhesions, are mechanically active and/or mechanosensitive, and they contribute to the mechanics of collective cell behaviours by providing necessary force for motion and mechanical coupling between cells.

Internal force generation and its roles in cell motion. The cytoskeleton is an active material that generates mechanical work while consuming energy from ATP hydrolysis (whereas adhesions are mostly passive systems allowing force transmission). Actomyosin is the major driving force for single and collective cell migration. Traction forces, which propel cells, are mainly generated by myosin II motors, whereas the polymerization of polar actin filaments generates pushing forces on cell membranes, thereby enabling the leading edge of the cell to protrude[81]. Actomyosin applies forces on cell–ECM adhesions and intercellular junctions, enabling cells to migrate through the substrate and pull on their neighbours, respectively[19] (FIG. 1a,b). Cell-generated forces have been shown to be regulated by various components, including mechanosensitive channels, actomyosin cytoskeleton and adhesion complexes between cells and their substrate, as well as intercellular adhesions[82–84]. These various components are themselves mechanosensitive and are important for the regulation of collective cell behaviours: density fluctuations within cell monolayers can generate areas of lower density, leading to cell stretching and the activation of mechanosensitive channels and subsequently to increased cell proliferation[84]; reduced activity of actomyosin promotes streamlined movements of cell monolayers, thereby preventing the formation of swirls and reducing neighbour exchanges[31] and also leading to the disappearance of the mechanical waves propagating into the monolayer[75]; finally, actomyosin-based forces can be exerted at both sites, cell–matrix and cell–cell adhesions, and their mechanical crosstalk can regulate cell traction forces, the shape of cell assemblies and even cell scattering[85–87].

Cadherin-dependent force transmission and sensing. The molecular link between cadherins and F-actin is essential for the cohesion of cell ensembles, and perturbing this coupling suppresses cell coordination during collective cell migration[31,54]. This link is mechanosensitive, and central to this property are two junctional proteins that bind F-actin: α-catenin and vinculin. As observed at the single molecule level[88,89] and in cells[79,90–92], α-catenin unfolds under force, thereby enabling the recruitment of vinculin (BOX 1). Upon binding to α-catenin, vinculin may also unfold, abolishing a head-to-tail auto-inhibition and favouring its association with F-actin[88,89,93]. The binding of α-catenin to F-actin is itself governed by a catch bond[78], which could also contribute to force sensing[93]. It has been shown — using tension sensors designed for Förster resonance energy transfer (FRET) — that E-cadherin[94], α-catenin[92] and vinculin[95] are under tension at cell–cell contacts. However, there is no consistency between molecular tension in E-cadherin and E-cadherin engagement at junctions, as stretched E-cadherin is also detected outside cell–cell junctions[94]. There is also no timely concordance between α-catenin opening and vinculin recruitment[92], nor is there concomitant opening of α-catenin and vinculin[95], suggesting a complex molecular mechanism of force-sensing along with the coexistence of multiple molecular architectures of the adhesion complexes. This architecture may vary in different cellular contexts in relation to the organization of junctional actin bundles[95] (FIG. 3Aa,Ab). For example, in 'immature' adherens junctions (also called focal adherens junctions) of epithelial[96], endothelial[97] or myogenic cells[98], where traction forces applied on cadherins may be high[95,99], actin bundles are oriented perpendicular to the junctions. However, in mature epithelial junctions (zonulae adherens), contractile actomyosin bundles run adjacent to a ring of E-cadherin, and the contractile tension is parallel to the cell–cell contact[34,97]. These contractile bundles may apply shearing forces on E-cadherin complexes and serve to protect them from normal pulling forces, which are generated by the intracellular actomyosin network[100]. As such, zonulae adherens are not passive integrators of force but instead are active mechanical structures

Confluence
In cell culture, the state in which 100% of the surface is covered by cells; also called 100% confluence.

Cortical tension
The force per unit length exerted on a part of the cortex, which is a thin layer mainly composed of actin-based proteins and myosin attached to the cell membrane, by the network around it.

Focal adhesions
Integrin-mediated adhesion structures formed at the cell–ECM interface, at the anchorage points of stress fibres.

Optical tweezers
Highly focused laser beams that attract small objects to the centre of the beam.

Stress
The force per unit area.

Strain
A measure of deformation representing the length change in a body relative to a reference length.

Shear
Local stresses exerted tangentially to a defined surface.

Normal stress
Forces perpendicular to a defined surface, such as a cell–cell interface.

Catch bond
A noncovalent bond that shows an increased lifetime with increasing amounts of tensile force applied to the bond.

Förster resonance energy transfer
(FRET). When applied to optical microscopy, a method allowing the determination of the distance (or dynamic changes in the proximity) between two fluorescent molecules within several nanometres.

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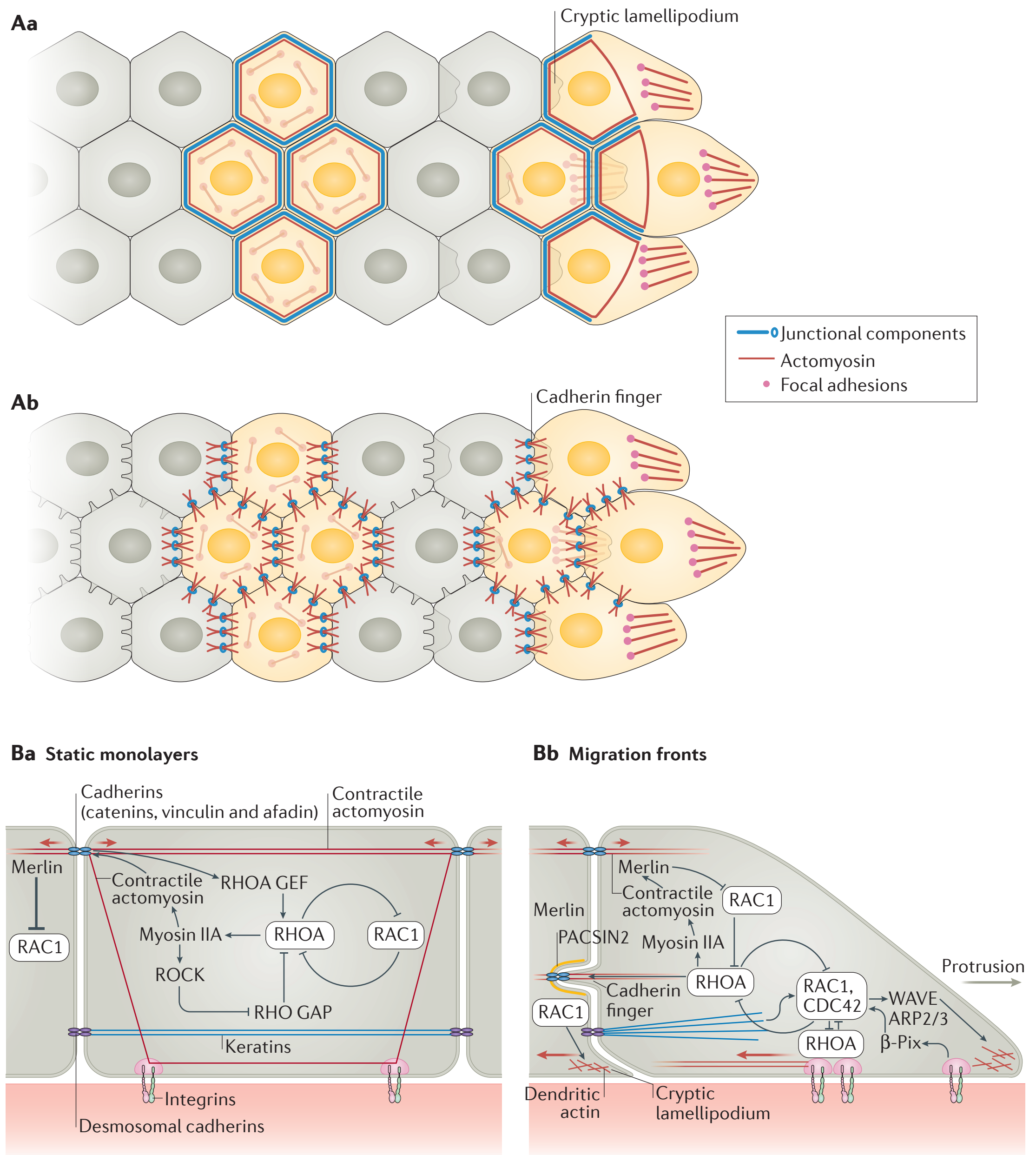

Figure 3 | **Molecular coupling at intercellular contacts.** Topologies of intercellular junctions, focal adhesions, contractile actin fibres and protrusive lamellipodia in migrating monolayered epithelia (part **Aa**) and in endothelium-like cell monolayers (part **Ab**). For clarity, tight junctions, desmosomes and intermediate filaments are not represented. Major molecular players regulating cell–cell coupling in cohesive monolayers (**Ba**) and governing coordinated cell polarization in collective migration (**Bb**). Positive-feedback loops between cadherin engagement and RHOA activity, involving the recruitment of RHO GEF (guanine exchange factor) and inhibition of RHO GAP (GTPase activating protein) by the activity of RHO-associated protein kinase (ROCK) allow the activation of myosin II, thereby ensuring high stability of zonulae adherens[34,104,105]. At the same time, RAC1 activity is inhibited in a Merlin-dependent manner (part **Ba**). At the free edges of cellular monolayers, integrin engagement by the extracellular matrix (ECM) activates a PI3K–RAC1–CDC42 pathway, dendritic actin polymerization (through the activity of Wiskott–Aldrich syndrome protein family member (WAVE) and actin-related protein 2/3 (ARP2/3) and lamellipodial protrusion in leading cells. β-Pix is a major GEF downstream of early integrin activation[28,172,173]. At the rear of the lamellipodium, RHOA is activated by more-mature integrin complexes, promoting myosin II activity and generation of traction forces[108]. Cell–cell contacts at the rear of leading cells provide instructive cues to maintain high levels of active RHOA and low levels of RAC1 through a positive-feedback loop between cadherin engagement, activation of RHO GEFs, RHOA activation and increased tension reinforcing adherens junctions[28,34,59,60]. At their front, upon Merlin release from cell-cell contact, follower cells extend reduced lamellipodia (cryptic lamellipodia) in a RAC1-dependent manner (REF. 39). In endothelial cells, additional structures known as cadherin fingers extend from the rear of leading cells and are engulfed by the following cells. Protein kinase C and casein kinase substrate in neurons protein 2 (PACSIN2) might be involved in the extension of these fingers[111,112] (part **Bb**). Horizontal red arrows indicate vectors of forces applied at cell–ECM and cell–cell contacts.

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Box 3 | **Regulation of mechanical cell coupling by junctional components**

Junctional cadherins exist in various isoforms, including the best-known epithelial (E)-cadherin but also neuronal (N)-cadherin, placental (P)-cadherin and vascular–endothelial (VE)-cadherin (also known as cadherins 1, 2, 3 and 5, respectively), as well as cadherin 6, cadherin 7 and cadherin 11 (REF. 158). E-cadherin and P-cadherin are expressed in compact tissues such as skin, mammary gland and kidney epithelia, and VE-cadherin is expressed in endothelial cells that show a milder degree of intercellular compaction[159]. N-cadherin is expressed in neuronal and mesenchymal cells, which are more loosely connected to each other. N-cadherin is upregulated in invasive cancer cells in combination with a downregulation of E-cadherin following epithelial–mesenchymal transition (EMT)[159]. Switching from E-cadherin to N-cadherin is also observed in neural crest cells before EMT[58] and is involved in their contact inhibition of locomotion (CIL) behaviours[56]. E-cadherin[31,37,51,52], P-cadherin[40,160] and VE-cadherin[112] all contribute to force transmission during collective cell migration *in vitro* in cells that express them. However, they may contribute differently to building and sustaining intercellular stress[40]. Their ectodomains have been shown to differentially resist force, with E-cadherin resisting more than N-cadherin[161]. Different responses to force have been noted for P-cadherin and E-cadherin in normal breast epithelial (MCF10A) cells, in which P-cadherin responds to the level of force, whereas E-cadherin is more sensitive to the rate at which the intercellular stress builds up[40]. It was subsequently shown that P-cadherin may sustain higher tension as a result of activating the β-Pix–CDC42 signalling pathway, and P-cadherin expression has been linked to the invasive potential of cancer cells[160]. In addition to homotypic junctions, heterotypic contacts have also been described. One example is a mechanically active heterotypic N-cadherin–E-cadherin adhesion between cancer-associated fibroblasts (CAFs) and cancer cells that is important in driving cancer cell invasion[162]. In contrast to CIL, where N-cadherin homotypic contacts are unstable, heterotypic N-cadherin–E-cadherin contacts are stable and withstand similar forces to homophilic E-cadherin junctions. They are similarly mechanosensitive, engaging similar tension-regulated α-catenin–vinculin interactions. These heterotypic adhesions repolarize CAFs away from the cancer cells and enable them to guide cancer cell migration by regulating cancer cell trajectories and leader–follower patterns.

In addition to cadherins, other junctional proteins are implicated in regulating mechanical coupling of cells. For example, in *Drosophila melanogaster*, Canoe (afadin in vertebrates), which interacts with another component of adherens junctions, nectins (BOX 1), has been implicated in maintaining tissue integrity during morphogenesis and collective cell migration[163–165]. In mice, the absence of afadin is associated with the detachment of actomyosin from the zonulae adherens, leading to developmental defects that indicate that afadin may have similar roles in vertebrates[166,167]. *In vitro*, it has been reported that Madin–Darby canine kidney epithelial cells presenting increased contractility at zonulae adherens show elevated levels of junctional afadin[101]. Removal of afadin alters cell shape and favours large-scale movement across the monolayer, suggesting that afadin acts as a robust protein scaffold that maintains zonulae adherens architecture and tissue cohesion[101]. Interestingly, nectins and afadin are recruited to the N-cadherin–E-cadherin interface between cancer cells and CAFs, and CAF repolarization is afadin dependent[162]. In addition, tight junctions and their constituents zonula occludens proteins ZO1, ZO2 and ZO3 (REF. 101), as well as desmosomes and associated intermediary filaments[168], have been reported to regulate intercellular mechanocoupling and cell polarization (see also Supplementary information S3,S4 (boxes)).

displaying contractile tension parallel to the cell–cell contact[34,101]. The contractility of junctions is an important determinant of their function and has been shown to be important for morphogenesis[102,103]. Zonulae adherens have been shown to be under tension in static monolayers *in vitro*[91,101]. This contractility is sustained by an ensemble of molecular components that are recruited to the junctional cortex in response to E-cadherin (FIG. 3Ba). This includes the components of RHOA signalling and activation of myosin II (REFS 34,104,105). In addition, proteins that control actin polymerization and branching are important for the maintenance of effective contractile tension and mechanosensing at cell–cell junctions[34,91,106]. Mechanical cell–cell coupling is also regulated by key constituents of junctions (including cadherin subtypes), and their expression markedly affects junctional mechanobiology and monolayer cohesion (BOX 3).

Cell–cell coupling, intercellular tension and coordinated cell polarization. An important aspect of cell migration is cell polarization, which involves specification of front and back. This polarization needs to be propagated throughout the migrating monolayer to ensure collective, organized motion. An interesting consideration is the contribution of cell–cell adhesions and the force transmission at these contacts to cell polarization during collective cell behaviours[107].

The front polarization of cells at the leading edge of monolayers and the formation of migratory protrusions are induced by the presence of the free edge (lack of junctions), whereas their rear is instructed and maintained by tension-resistant intercellular cadherin adhesions[28]. At the edges of cellular colonies, integrin engagement activates RAC1-dependent protrusions (in a mechanism involving the guanine exchange factor β-Pix (also known as RHO guanine nucleotide exchange factor 7)) (FIG. 3Bb), in a process equivalent to the activation of migration in single cells[108]. This pathway is also responsible for the polarization of the leading cells during anterior vascular endoderm migration in mouse embryos[109]. RHOA activated by focal adhesions at the rear of the lamellipodium is then responsible for myosin II activation and the generation of traction forces[108]. During the migration of single cells, RHOA is activated at the rear of the cell, leading to disassembly of focal adhesions, which is needed for efficient cell migration. RAC1 activity is reduced at the cell rear by the combined effects of inhibition by high tension and cross inhibition by RHOA[108], leading to the establishment of opposing gradients of RAC1 and RHOA activity along the front-to-rear axis. Similar gradients have been proposed to drive the migration of front cells during collective cell migration[28] (FIG. 3Bb). Cell–cell contacts at the rear of leading cells may provide additional instructive cues to maintain high levels of active RHOA and low levels of RAC1, through the positive-feedback loop between cadherin engagement and activation of RHOA at adherens junctions[28,34,59,60]. Thus, intercellular stress mediated by cell–cell junctions is critical for maintaining the front-to-rear polarization of leader cells in the first row of the collectively migrating sheet. Mechanical coupling mediated by cadherin-based cell–cell junctions also

Cancer-associated fibroblasts
(CAFs). Stromal fibroblasts closely associated with primary tumour cells and participating in the neoplastic process.

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establishes extended vectorial signalling throughout the monolayer, involving coordinated, polarized activation of RAC1 and RHOA on a multicellular-length scale, which is essential for collective cell migration[52,68,110]. The first demonstration of such a multicellular polarization has been produced by the observation of cryptic lamellipodia that extend in the direction of migration by cells distant from the edge and that potentially guide the migration of cell clusters[42].

However, how symmetric cell–cell junctions can support asymmetric signal transduction whereby one cell can direct the movement of its follower remains elusive. Two recent studies[39,111] have provided clues about two alternative molecular strategies for propagating RAC1 and RHOA gradients throughout a sheet (FIG. 3Bb). In epithelial cells, Merlin, a Hippo pathway regulator and tumour suppressor protein belonging to the ERM family, has been reported to shuttle from adherens junctions to the cytoplasm, coordinating collective migration of monolayers spanning tens of cells in diameter. In a stationary monolayer, Merlin localizes to cell–cell junctions and contributes to RAC1 inhibition. During migration, Merlin relocalizes to the cytoplasm in follower cells owing to the intercellular pulling force developed by the leading cell, contributing to polarized activation of RAC1 and formation of cryptic lamellipodia; these events are then coordinated on a multicellular-length scale[39].

Recent studies revealed that apart from canonical cadherin-based cell–cell junctions, cells in migrating monolayers (at least in endothelial sheets) form additional cadherin-based structures, which appear as fingers linking the back of one moving cell to the front of its follower in a tension-dependent manner[111] (FIG. 3Ab,Bb). These 'cadherin fingers' are present throughout the monolayer, even hundreds of micrometres into the cell sheet, in strands of cells that display sequential polarization within the monolayer. These structures are extended from the rear of leading cells and are engulfed by followers at their front, thereby generating opposite plasma membrane curvatures in the two cells[111]. In follower cells, engulfment of cadherin fingers occurs along with the formation of a lamellipodia-like zone with the low actomyosin and high dendritic actin polymerization typical of RAC1 activation. Interestingly, local activation of RHOA was sufficient to trigger the formation of cadherin fingers at the rear of leading cells. An asymmetric recruitment of membrane-bending and curvature-sensing BAR domain-containing proteins such as protein kinase C and casein kinase substrate in neurons protein 2 (PACSIN2) to the front of follower cells has been described[111,112]. These proteins, recruited asymmetrically at junctions, are good candidates to instruct RAC1 and RHOA activity gradients. Interestingly, PACSIN2 inhibits internalization of cadherin complexes from the membranes at trailing ends of endothelial cells and is important for endothelial monolayer integrity[112]. Although very different, the two mechanisms may contribute — perhaps to different extents in different contexts — to the coupling of cell polarization at the level of an entire monolayer.

Hippo pathway
Also known as the Salvador–Warts–Hippo pathway, this pathway controls organ size in animals through the regulation of cell proliferation and apoptosis.

ERM family
A protein family that is named for three closely related proteins: ezrin, radixin and moesin.

BAR domain
A highly conserved protein dimerization domain that occurs in many proteins involved in cellular membrane dynamics. The BAR domain is banana-shaped, binds to membranes and is capable of sensing membrane curvature by binding preferentially to curved membranes.

Regulation by physical constraints

In vivo, collective cell behaviours occur under a broad range of external constraints. These include external forces such as shear flows on endothelial cells[113] or contractile forces of surrounding cells[114], confinement[23], such as that imposed by tissue architecture or growth, physical properties of the ECM and ECM remodelling[20]. In this section, we discuss the impact of some of these physical constraints on collective cell dynamics and the importance of cell–cell interactions in responding to them. We focus here on *in vitro* studies that aim to reproduce *in vivo* conditions in more-controlled environments, thereby helping to predict the emergence of overlooked or hidden features not apparent in *in vivo* studies.

Confinement. The use of micropatterning techniques[31,115,116] (BOX 2), such as stripes of ECM printed at various widths, helped to impose well-controlled confinement, enabling the study of how this constraint, which has well-established roles *in vivo* (for example, during neural crest migration or cancer cell invasion[23,117]), affects collective behaviours. It has been revealed that confinement increases the migration speed of epithelial cell sheets, in particular for geometries smaller than the expected correlation length for the cell type measured under unconfined conditions (FIG. 4a). Under high confinement, velocity vectors mostly point in the direction of migration, leading to the disappearance of swirling motions with a high ordering in the direction of migration and an increase in the velocity of the cell front. Cell polarity and actin fibres are also preferentially oriented in the direction of movement under these conditions.

However, when cell monolayers are constrained without any free space to expand, they can still display surprising dynamics. For example, when a confluent epithelium is constrained in a circular adherent pattern, the dimensions of which are smaller than the correlation length of the unconstrained epithelium, the coordination of cell motions leads to the establishment of a persistent whole-tissue rotational motion[118]. This persistent rotation may be reminiscent of coordinated rotational movements of morphogenetic events such as the formation of epithelial acini[119] or the elongation of *D. melanogaster* egg chambers[120]. This collective behaviour is substantially altered when E-cadherin-based contacts are blocked, in cancerous cells and in cells that have undergone epithelial–mesenchymal transition (EMT)[118,119], underlining the essential role of cell–cell junctions in maintaining the collective behaviour. The large-scale coordination is also reduced as cell density increases over time, because displacements are gradually frozen in a mechanism reminiscent of cell jamming. Other studies have shown surprising oscillatory movements of epithelial cells plated on circular confined substrates. These oscillations correspond to phases in which outward cell movements of the whole epithelial monolayer alternate with inward movements in the radial direction of the pattern[64,121]. These oscillations may share similarities with the propagation of mechanical waves during monolayer expansion described above (FIG. 1c). A plausible mechanism for the oscillations may involve coordination between cell

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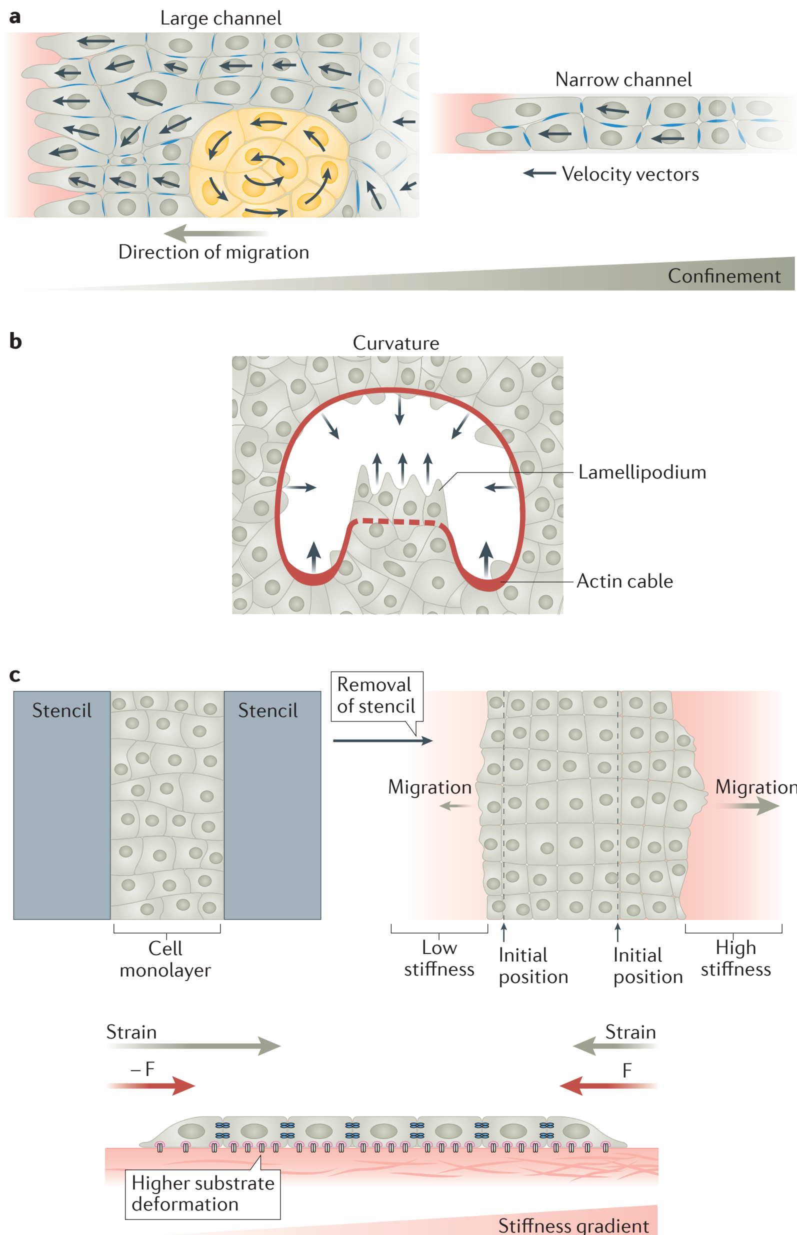

Figure 4 | **Physical constraints influencing collective cell behaviours. a** | Confinement into stripes of various widths induces changes in flow in the bulk of the migrating monolayer[23,31]. Vortices and rotational movements are observed in wide channels, whereas narrow channels favour parallel coordinated and polarized movements in the longitudinal direction of the stripe. **b** | Substrate geometry influences the mode of collective migration. In areas of positive curvature (for example, the tips of finger-like structures pointing into the gap), cells predominantly move by active crawling[72]. In areas of negative curvature (that is, where the gap bows into the tissue), prominent actomyosin cables are formed. Actomyosin contractility and active cell crawling operate additively in gap closure, leading to faster tissue velocity in regions of negative curvature. **c** | Coupling of cells in cell collectives promotes durotactic motion[54], whereby cell colonies move collectively more quickly in the direction of high stiffness (top panel), which is not the case for single cells under similar conditions. This collective durotactic motion can be explained by the impact of force transmission across the monolayers (bottom panel). Soft edges (on the left) present similar traction forces to stiff edges (on the right). Consequently, substrate deformations are larger on the soft side than on the stiff side (since a force of equal amplitude will deform a soft material more than a stiff one), inducing a net movement towards the high stiffness. This simple explanation is confirmed by an integrated model that couples substrate stiffness, focal adhesion dynamics and actin polymerization (see REF. 54 for further explanation).

contractility and polarity to enable synchronization of cellular deformations, that is, compression and extension, over the entire monolayer. However, molecular details of how this coordination could be achieved are lacking.

Curvature sensing and collective cell migration. The migrating front of cell sheets spontaneously displays large shape fluctuations associated with changes in the curvature of the edge of the cell sheet[122]. Such shape changes are associated with cytoskeletal reorganization that can promote the assembly of either cellular protrusions or contractile actomyosin cables[8,68,123] (FIG. 4b). As expected, forward protrusions of cell monolayers that can spontaneously emerge during migration or be induced by micropatterning techniques (BOX 2) are associated with the formation of leader cells[124] that exert large traction forces directed towards the bulk of the collective (FIG. 1c).

Microfabricated stencils made of pillars of various geometries[49] (BOX 2) have also been used to probe the relationship between collective cell migration and the curvature of monolayer edges. This revealed that negative curvature is related to actin cable assembly and purse-string-based closure, whereas positive curvature favours cell migration and the formation of leader cells[72] (FIG. 4b). The speed at which cells along the gap edge move depends on the local curvature with cells at concave edges (negative curvature) moving in more quickly than those at convex edges (positive curvature). Force measurements and the analysis of the distribution of cell–ECM adhesions revealed that forces coming from lamellipodia and actin cable contractility are additive in concave regions, leading to faster migration, whereas in convex regions, cell migration is the only force that pushes the monolayer forward. This reveals unexpected coupling between these mechanisms and a regulatory role of actomyosin cables to coordinate epithelial gap closure. *In vivo*, the interplay between the two mechanisms may be important to regulate cell migration processes that occur on surfaces with heterogeneous properties in terms of adhesiveness, stiffness or topography[125–127]. For instance, the re-epithelialization that restores tissue integrity during wound healing requires the migration of cells over damaged areas with spatially disorganized ECM composition. Whereas cells migrate with lamellipodial protrusions over adherent substrates, the absence of ECM induces concave cell edges associated with the formation of contractile actin bundles[122].

Physical properties of the substrate. The development of deformable substrates to measure cell traction forces led to the discovery that living cells sense and respond to substrate stiffness[128]. The impact of substrate stiffness on single cell migration has been reported *in vitro*[16], and recently *in vivo*[129], in the context of embryonic brain development, where axon growth is guided towards softer tissue. Thus, it is not surprising that physical properties of the substrates are also important for collective cell motion.

On deformable soft substrates, the stress is likely transmitted not only through the cell–cell interactions but also through the substrate itself, which may not be

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the case on extremely stiff surfaces traditionally used for *in vitro* experiments, such as glass or plastic. Hence, substrate deformability feeds into the mechanical cross-talk between cell–cell and cell–substrate adhesions, modulating collective movements. Indeed, cells can detect and respond to substrate strains created by the traction stresses of a neighbouring cell, having the ability to communicate through the underlying deformable matrix[130,131]. This may in turn affect intercellular forces and affect cell coordination during collective behaviours. Accordingly, it has been shown that on soft, deformable substrates, cell coordination is increased, and cells are able to migrate in larger groups. This has been further linked to long-distance substrate deformations[131].

Traction forces and contractility of epithelial cell colonies increase with substrate stiffness[52,132]. On substrates composed of local anisotropic stiffness, the migration of epithelial colonies (as well as the scattering of individual cells during EMT) is promoted in the direction of the highest stiffness[133]. Similarly, the behaviour of cells within 3D cellular aggregates varies with substrate stiffness: on stiff substrates, cells disperse from aggregates, covering the surface, whereas on soft substrates, the aggregates are maintained and can even coalesce to form multicellular tissue-like structures[134,135]. These observations may be explained by mechanical competition between forces exerted at cell–substrate and cell–cell adhesion sites, whereby excessive traction forces on the substrate generated on stiff substrates can compromise cell–cell adhesion and may reduce cohesiveness and promote cell scattering. However, these effects will clearly differ between cells, depending on adhesion type, architecture of the adhesions and cell contractility characteristic of the cell type (and cell state), and they may not be relevant in all situations.

Another important phenomenon linked to sensing substrate stiffness is durotaxis — the ability of cells to follow gradients of ECM stiffness — which is well established for single cells[16,136]. Recent experiments have also reported collective durotaxis of epithelial monolayers[54]. In wounds performed on a substrate with a stiffness gradient, cell monolayers expanded more on the stiffer side (FIG. 4c). This long-range mechanism was shown to involve the transmission of cell-generated forces across the cell collective, as the inhibition of myosin II blocked this durotactic expansion. Similarly, perturbation of cell–cell contacts by knocking down α-catenin also abrogated durotaxis. Interestingly, durotaxis emerges in cell collectives, even if isolated constituent cells are unable to durotax, suggesting that mechanical cell coupling by cell–cell adhesions has a large impact on the overall mechanics of migration. The changes in the mechanical properties of the substrate modulate the cell–substrate adhesions, which in turn may affect the cell–cell adhesions. This mechanical feedback appears to be crucial to understand tissue shape and dynamics. When looking at *in vivo* tissues, it turns out that the elasticity of environment can vary by orders of magnitude between tissues from brain (hundreds of pascals) to bones (~GPa). Thus, collective cell dynamics may differ considerably from one tissue to another.

Conclusions and perspective

Cellular coordination, which is at the basis of various phenomena, most prominently including tissue shaping during morphogenesis, is a mechanoregulated, multiscale process integrating events on the molecular, cellular and multicellular scales that can be observed at a wide range of timescales, from milliseconds to days. The continued development of microfabricated tools (BOX 2) over the past 10 years and the accompanying development of *in vitro* cellular models (Supplementary information S2 (box)) have enabled the links between cellular mechanics and the underlying molecular pathways to be studied for the first time. Despite being limited in terms of complexity, these *in vitro* approaches, owing to their amenability and ease of manipulation, support molecular, biochemical, physical and mechanical analyses on cellular and multicellular scales. Accordingly, they have provided valuable knowledge of the various scales of cell coordination and can now support or inspire more complex analysis in *in vivo* studies at the tissue or organism scale. This is illustrated, for example, by a recent study describing neural crest collective cell migration *in vivo*[23] that follows a mode of regulation described previously for epithelial cells migrating in confined geometries *in vitro*[31]. Future discoveries are sure to follow in various *in vivo* scenarios, provided that physical and mechanical parameters can be reproducibly measured and analysed in these situations.

The next frontier for the application of the *in vitro* models is to investigate the impact of extrinsic forces on the mechanics and biomechanical responses of cell collectives. There are numerous physiologically relevant forces that could be investigated, including traction forces generated by the migration or deformation of other tissues and pushing forces generated by cell division, intercalation and delamination. It will also be interesting to study in more detail the role of physical properties of the substrate in driving collective cell behaviours. For instance, the recent insights into the responsiveness of cell collectives to stiffness and the process of collective durotaxis can help to take a different view on studies of morphogenesis, wound healing and cancer cell invasion. As an example, differential deposition and composition of ECM during morphogenesis[20,137] may lead to changes in substrate stiffness that can regulate coordination between cells in collective processes during tissue shaping. Matrix deposition during wound healing can also be associated with changes in stiffness[138,139], which in turn may promote coordinated collective migration for re-epithelialization. Moreover, the impact of substrate stiffness on coordinated cellular responses could be an important factor in the increased tumour invasion associated with matrix stiffening[140,141].

Lastly, it is important to consider that cells and cell collectives respond also to other non-mechanical-based cues and gradients, such as gradients of diffusible factors, adhesive cues or electrical fields, eliciting chemotactic, haptotactic or galvanotactic cell responses, respectively. Fortunately, the *in vitro* model systems are by now sophisticated enough to reproduce all these combinations of gradients to gain a more complete insight into collective cell behaviours and how they shape tissues, organs and whole organisms.

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1. Friedl, P. & Gilmour, D. Collective cell migration in morphogenesis, regeneration and cancer. *Nat. Rev. Mol. Cell Biol.* **10**, 445–457 (2009).
2. Bianco, A. *et al.* Two distinct modes of guidance signalling during collective migration of border cells. *Nature* **448**, 362–365 (2007).
3. Lecaudey, V., Cakan-Akdogan, G., Norton, W. H. & Gilmour, D. Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development* **135**, 2695–2705 (2008).
4. Nabeshima, K., Inoue, T., Shimao, Y., Kataoka, H. & Koono, M. Cohort migration of carcinoma cells: differentiated colorectal carcinoma cells move as coherent cell clusters or sheets. *Histol. Histopathol.* **14**, 1183–1197 (1999).
5. Einenkel, J., Braumann, U. D., Horn, L. C., Kuska, J. P. & Hockel, M. 3D analysis of the invasion front in squamous cell carcinoma of the uterine cervix: histopathologic evidence for collective invasion per continuitatem. *Anal. Quantitative Cytol. Histol.* **29**, 279–290 (2007).
6. Giampieri, S. *et al.* Localized and reversible TGFβ signalling switches breast cancer cells from cohesive to single cell motility. *Nat. Cell Biol.* **11**, 1287–1296 (2009).
7. Behrndt, M. *et al.* Forces driving epithelial spreading in zebrafish gastrulation. *Science* **338**, 257–260 (2012).
8. Abreu-Blanco, M. T., Verboon, J. M., Liu, R., Watts, J. J. & Parkhurst, S. M. *Drosophila* embryos close epithelial wounds using a combination of cellular protrusions and an actomyosin purse string. *J. Cell Sci.* **125**, 5984–5997 (2012).
9. Campas, O. *et al.* Quantifying cell-generated mechanical forces within living embryonic tissues. *Nat. Methods* **11**, 183–189 (2014).
In this paper, the authors develop a method to measure the mechanical forces that cells exert while building tissues. They insert calibrated oil droplets in living embryos in contact with other cells. When cells push and pull on an oil droplet, they deform it, and this deformation provides a direct readout of the pressure they exert.
10. Bambardekar, K., Clement, R., Blanc, O., Chardes, C. & Lenne, P. F. Direct laser manipulation reveals the mechanics of cell contacts *in vivo*. *Proc. Natl Acad. Sci. USA* **112**, 1416–1421 (2015).
11. Hakim, V. & Silberzan, P. Collective cell migration: a physics perspective. Reports on progress in physics. *Phys. Soc.* **80**, 076601 (2017).
12. Marchetti, M. C. *et al.* Hydrodynamics of soft active matter. *Rev. Modern Phys.* **85,** 1143 (2013).
13. Parsons, J. T., Horwitz, A. R. & Schwartz, M. A. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat. Rev. Mol. Cell Biol.* **11**, 633–643 (2010).
14. Kanchanawong, P. *et al.* Nanoscale architecture of integrin-based cell adhesions. *Nature* **468**, 580–584 (2010).
15. Sarangi, B. R. *et al.* Coordination between intra- and extracellular forces regulates focal adhesion dynamics. *Nano Lett.* **17**, 399–406 (2017).
16. Lo, C. M., Wang, H. B., Dembo, M. & Wang, Y. L. Cell movement is guided by the rigidity of the substrate. *Biophys. J.* **79**, 144–152 (2000).
17. Sniadecki, N. J. *et al.* Magnetic microposts as an approach to apply forces to living cells. *Proc. Natl Acad. Sci. USA* **104**, 14553–14558 (2007).
18. Saxena, M. *et al.* EGFR and HER2 activate rigidity sensing only on rigid matrices. *Nat. Mater.* **16**, 775–781 (2017).
19. Ladoux, B., Mège, R. M. & Trepat, X. Front-rear polarization by mechanical cues: from single cells to tissues. *Trends Cell Biol.* **26**, 420–433 (2016).
20. Cetera, M. *et al.* Epithelial rotation promotes the global alignment of contractile actin bundles during *Drosophila* egg chamber elongation. *Nature Commun.* **5**, 5511 (2014).
During the elongation of the *D. melanogaster* egg chamber into an ellipsoidal shape, the follicular epithelial cells undergo a collective migration that causes the egg chamber to rotate within its surrounding basement membrane. This study demonstrates that collective rotation plays a critical role in building up the actin-based component of a corset that forms during the elongation, in which actin bundles in the epithelium and fibrils in the basement membrane are all aligned perpendicular to the elongation axis.
21. Bertet, C., Sulak, L. & Lecuit, T. Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* **429**, 667–671 (2004).
22. Dona, E. *et al.* Directional tissue migration through a self-generated chemokine gradient. *Nature* **503**, 285–289 (2013).
23. Szabo, A. *et al.* *In vivo* confinement promotes collective migration of neural crest cells. *J. Cell Biol.* **213**, 543–555 (2016).
Combining computational and experimental approaches, the authors show that the *in vivo* collective migration of neural crest cells depends on confinement and geometrical constraints. They demonstrate that such confinement promotes directional migration of cell groups *in vivo*.
24. Haeger, A., Krause, M., Wolf, K. & Friedl, P. Cell jamming: collective invasion of mesenchymal tumor cells imposed by tissue confinement. *Biochim. Biophys. Acta* **1840**, 2386–2395 (2014).
25. Abercrombie, M. & Ambrose, E. J. The surface properties of cancer cells: a review. *Cancer Res.* **22**, 525–548 (1962).
26. Poujade, M. *et al.* Collective migration of an epithelial monolayer in response to a model wound. *Proc. Natl Acad. Sci. USA* **104**, 15988–15993 (2007).
27. Deforet, M. *et al.* Automated velocity mapping of migrating cell populations (AVeMap). *Nat. Methods* **9**, 1081–1083 (2012).
28. Mayor, R. & Etienne-Manneville, S. The front and rear of collective cell migration. *Nat. Rev. Mol. Cell Biol.* **17**, 97–109 (2016).
29. Reffay, M. *et al.* Orientation and polarity in collectively migrating cell structures: statics and dynamics. *Biophys. J.* **100**, 2566–2575 (2011).
30. Purcell, E. M. Life at low Reynolds number. *Am. J. Phys.* **45**, 3–11 (1977).
31. Vedula, S. R. K. *et al.* Emerging modes of collective cell migration induced by geometrical constraints. *Proc. Natl Acad. Sci. USA* **109**, 12974–12979 (2012).
In this study, the authors show that collective cell migration can be regulated by confinement. Cells migrate more quickly on narrow lines of the size of a single cell than on larger stripes of several cell diameters, and the mode of migration varies depending on the width of the micropatterned lines, consistent with different patterns of force transmission through cell–cell junctions.
32. Foty, R. A. & Steinberg, M. S. The differential adhesion hypothesis: a direct evaluation. *Dev. Biol.* **278**, 255–263 (2005).
33. Ranft, J. *et al.* Fluidization of tissues by cell division and apoptosis. *Proc. Natl Acad. Sci. USA* **107**, 20863–20868 (2010).
34. Lecuit, T. & Yap, A. S. E-Cadherin junctions as active mechanical integrators in tissue dynamics. *Nat. Cell Biol.* **17**, 533–539 (2015).
35. Rossen, N. S., Tarp, J. M., Mathiesen, J., Jensen, M. H. & Oddershede, L. B. Long-range ordered vorticity patterns in living tissue induced by cell division. *Nature Commun.* **5**, 5720 (2014).
In this study, the authors analyse the dynamic movements of endothelial cells induced by cell division. Cell divisions induce long-range reorganization of cells together with the formation of vortex patterns that extend far away from the division site.
36. Saw, T. B. *et al.* Topological defects in epithelia govern cell death and extrusion. *Nature* **544**, 212–216 (2017).
This paper shows that epithelial cells are elongated and closely packed, which means that they can spontaneously align in a similar way to the molecules in nematic liquid crystals. Accordingly, compressive stresses induced by oriented ordering and defects in the epithelium provide a physical trigger for cell extrusion and death.
37. Petitjean, L. *et al.* Velocity fields in a collectively migrating epithelium. *Biophys. J.* **98**, 1790–1800 (2010).
38. Vedula, S. R. K. *et al.* Epithelial bridges maintain tissue integrity during collective cell migration. *Nat. Mater.* **13**, 87–96 (2014).
39. Das, T. *et al.* A molecular mechanotransduction pathway regulates collective migration of epithelial cells. *Nat. Cell Biol.* **17**, 276–287 (2015).
In this study, the authors show that the protein Merlin is involved in coordinating motility forces from the edge into the bulk of the monolayer.
40. Bazellieres, E. *et al.* Control of cell-cell forces and collective cell dynamics by the intercellular adhesome. *Nat. Cell Biol.* **17**, 409–420 (2015).
The paper analyses the role of molecules at desmosomes, tight junctions and adherens junctions involved in cell–cell interactions in the establishment of forces and stresses during collective cell migration. It also shows that E-cadherins and P-cadherins respond to the level of force or to the rate at which the intercellular stress builds up, respectively, which allows for a particularly efficient control of mechanosensation at cadherin-based junctions.
41. Basan, M., Elgeti, J., Hannezo, E., Rappel, W. J. & Levine, H. Alignment of cellular motility forces with tissue flow as a mechanism for efficient wound healing. *Proc. Natl Acad. Sci. USA* **110**, 2452–2459 (2013).
42. Farooqui, R. & Fenteany, G. Multiple rows of cells behind an epithelial wound edge extend cryptic lamellipodia to collectively drive cell-sheet movement. *J. Cell Sci.* **118**, 51–63 (2005).
43. Takeichi, M. Dynamic contacts: rearranging adherens junctions to drive epithelial remodelling. *Nat. Rev. Mol. Cell Biol.* **15**, 397–410 (2014).
44. Brugues, A. *et al.* Forces driving epithelial wound healing. *Nat. Phys.* **10**, 684–691 (2014).
45. Vedula, S. R. K. *et al.* Mechanics of epithelial closure over non-adherent environments. *Nat. Commun.* **6**, 6111 (2015).
46. Clark, A. G. *et al.* Integration of single and multicellular wound responses. *Curr. Biol.* **19**, 1389–1395 (2009).
47. Wood, W. *et al.* Wound healing recapitulates morphogenesis in *Drosophila* embryos. *Nat. Cell Biol.* **4**, 907–912 (2002).
48. Tamada, M., Perez, T. D., Nelson, W. J. & Sheetz, M. P. Two distinct modes of myosin assembly and dynamics during epithelial wound closure. *J. Cell Biol.* **176**, 27–33 (2007).
49. Anon, E. *et al.* Cell crawling mediates collective cell migration to close undamaged epithelial gaps. *Proc. Natl Acad. Sci. USA* **109**, 10891–10896 (2012).
50. Cochet-Escartin, O., Ranft, J., Silberzan, P. & Marcq, P. Border forces and friction control epithelial closure dynamics. *Biophys. J.* **106**, 65–73 (2014).
51. Vitorino, P. & Meyer, T. Modular control of endothelial sheet migration. *Genes Dev.* **22**, 3268–3281 (2008).
52. Ng, M. R., Besser, A., Danuser, G. & Brugge, J. S. Substrate stiffness regulates cadherin-dependent collective migration through myosin-II contractility. *J. Cell Biol.* **199**, 545–563 (2012).
53. Gumbiner, B. Generation and maintenance of epithelial cell polarity. *Curr. Opin. Cell Biol.* **2**, 881–887 (1990).
54. Sunyer, R. *et al.* Collective cell durotaxis emerges from long-range intercellular force transmission. *Science* **353**, 1157–1161 (2016).
In this study, the authors show that collective cell migration can be regulated by substrate stiffness, with cells moving towards stiff substrates — cells on a stiffer substrate have more resistance, enabling them to generate higher traction forces, so they win the tug of war with cells on the softer part. They further show that even cells that do not show durotaxis as single cells can durotax as a collective.
55. Abercrombie, M. & Heaysman, J. E. Observations on the social behaviour of cells in tissue culture: I. Speed of movement of chick heart fibroblasts in relation to their mutual contacts. *Exp. Cell Res.* **5**, 111–131 (1953).
56. Carmona-Fontaine, C. *et al.* Contact inhibition of locomotion *in vivo* controls neural crest directional migration. *Nature* **456**, 957–961 (2008).
57. Theveneau, E. *et al.* Chase-and-run between adjacent cell populations promotes directional collective migration. *Nat. Cell Biol.* **15**, 763–772 (2013).
In this paper, the authors show the importance of multiple cellular interactions of different cell types for collective cell migration. They study the interactions between neural crest cells and placode cells, an epithelial tissue, identifying a mechanism based on a coupling between chemotaxis and mechanical forces that promotes the correct migration of neural crest cells.
58. Bahm, I. *et al.* PDGF controls contact inhibition of locomotion by regulating N-cadherin during neural crest migration. *Development* **144**, 2456–2468 (2017).
59. Desai, R. A., Gao, L., Raghavan, S., Liu, W. F. & Chen, C. S. Cell polarity triggered by cell-cell adhesion via E-cadherin. *J. Cell Sci.* **122**, 905–911 (2009).
60. Ouyang, M. *et al.* N-Cadherin regulates spatially polarized signals through distinct p120ctn and β-catenin-dependent signalling pathways. *Nat. Commun.* **4**, 1589 (2013).

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61. Kocgozlu, L. *et al.* Epithelial cell packing induces distinct modes of cell extrusions. *Curr. Biol.* **26**, 2942–2950 (2016).
62. Park, J. A., Atia, L., Mitchel, J. A., Fredberg, J. J. & Butler, J. P. Collective migration and cell jamming in asthma, cancer and development. *J. Cell Sci.* **129**, 3375–3383 (2016).
63. Garcia, S. *et al.* Physics of active jamming during collective cellular motion in a monolayer. *Proc. Natl Acad. Sci. USA* **112**, 15314–15319 (2015).
64. Deforet, M., Hakim, V., Yevick, H., Duclos, G. & Silberzan, P. Emergence of collective modes and tri-dimensional structures from epithelial confinement. *Nat. Commun.* **5**, 3747 (2014).
This paper shows that cellular confinement of epithelial cells *in vitro* can lead to spontaneous modes of collective oscillatory movements. A simple mathematical model, in which cells are described as persistent random walkers that adapt their motion to their neighbours, with an additional term that mimics cell adhesion, captures the essential characteristics of these oscillatory modes.
65. Sadati, M., Qazvini, N. T., Krishnan, R., Park, C. Y. & Fredberg, J. J. Collective migration and cell jamming. *Differentiation* **86**, 121–125 (2013).
66. Tambe, D. T. *et al.* Collective cell guidance by cooperative intercellular forces. *Nat. Mater.* **10**, 469–475 (2011).
67. Bi, D. P., Lopez, J. H., Schwarz, J. M. & Manning, M. L. A density-independent rigidity transition in biological tissues. *Nat. Phys.* **11**, 1074–1079 (2015).
68. Reffay, M. *et al.* Interplay of RhoA and mechanical forces in collective cell migration driven by leader cells. *Nat. Cell Biol.* **16**, 217–223 (2014).
In this paper, the authors analyse the mechanical role of leader cells during collective cell migration. They measure the forces exerted by finger-like structures composed of multiple cells at the migrating front and correlate the mechanical forces with RHOA activity.
69. Trepat, X. *et al.* Physical forces during collective cell migration. *Nat. Phys.* **5**, 426–430 (2009).
70. du Roure, O. *et al.* Force mapping in epithelial cell migration. *Proc. Natl Acad. Sci. USA* **102**, 2390–2395 (2005).
71. Jacinto, A., Martinez-Arias, A. & Martin, P. Mechanisms of epithelial fusion and repair. *Nat. Cell Biol.* **3**, E117–E123 (2001).
72. Ravasio, A. *et al.* Gap geometry dictates epithelial closure efficiency. *Nat. Commun.* **6**, 7683 (2015).
To analyse epithelial gap closure, the authors experimentally implemented microscopic gaps of defined geometries and curvatures into confluent epithelial sheets. Their study clarifies the roles of two gap-closing mechanisms — cell migration and actomyosin-based contractile cable dynamics — and describes how the relative contributions of the two mechanisms are affected by gap geometry.
73. Nier, V. *et al.* Inference of internal stress in a cell monolayer. *Biophys. J.* **110**, 1625–1635 (2016).
74. Hutson, M. S. *et al.* Forces for morphogenesis investigated with laser microsurgery and quantitative modeling. *Science* **300**, 145–149 (2003).
75. Serra-Picamal, X. *et al.* Mechanical waves during tissue expansion. *Nat. Phys.* **8**, 628–634 (2012).
76. Nelson, C. M. *et al.* Emergent patterns of growth controlled by multicellular form and mechanics. *Proc. Natl Acad. Sci. USA* **102**, 11594–11599 (2005).
77. Warmflash, A., Sorre, B., Etoc, F., Siggia, E. D. & Brivanlou, A. H. A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. *Nat. Methods* **11**, 847–854 (2014).
78. Buckley, C. D. *et al.* The minimal cadherin-catenin complex binds to actin filaments under force. *Science* **346**, 1254211 (2014).
In this paper, the authors use single molecule assays to analyse cadherin–α-catenin complexes and their interaction with actin filaments under force. They show that tension is required to stabilize a linkage between the cadherin–catenin complex and actin filaments, and they clarify how the cadherin–catenin complex could interact directly with the actin cytoskeleton in cells.
79. Yonemura, S., Wada, Y., Watanabe, T., Nagafuchi, A. & Shibata, M. α-Catenin as a tension transducer that induces adherens junction development. *Nat. Cell Biol.* **12**, 533–542 (2010).
80. Ladoux, B. *et al.* Strength dependence of cadherin-mediated adhesions. *Biophys. J.* **98**, 534–542 (2010).
81. Blanchoin, L., Boujemaa-Paterski, R., Sykes, C. & Plastino, J. Actin dynamics, architecture, and mechanics in cell motility. *Physiol. Rev.* **94**, 235–263 (2014).
82. Plotnikov, S. V., Pasapera, A. M., Sabass, B. & Waterman, C. M. Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. *Cell* **151**, 1513–1527 (2012).
83. Gupta, M. *et al.* Adaptive rheology and ordering of cell cytoskeleton govern matrix rigidity sensing. *Nat. Commun.* **6**, 7525 (2015).
84. Gudipaty, S. A. *et al.* Mechanical stretch triggers rapid epithelial cell division through Piezo1. *Nature* **543**, 118–121 (2017).
85. Kaliman, S., Jayachandran, C., Rehfeldt, F. & Smith, A. S. Novel growth regime of MDCK II model tissues on soft substrates. *Biophys. J.* **106**, L25–28 (2014).
86. de Rooij, J., Kerstens, A., Danuser, G., Schwartz, M. A. & Waterman-Storer, C. M. Integrin-dependent actomyosin contraction regulates epithelial cell scattering. *J. Cell Biol.* **171**, 153–164 (2005).
87. Mertz, A. F. *et al.* Cadherin-based intercellular adhesions organize epithelial cell-matrix traction forces. *Proc. Natl Acad. Sci. USA* **110**, 842–847 (2013).
88. Maki, K. *et al.* Mechano-adaptive sensory mechanism of α-catenin under tension. *Sci. Rep.* **6**, 24878 (2016).
89. Yao, M. *et al.* Force-dependent conformational switch of α-catenin controls vinculin binding. *Nat. Commun.* **5**, 4525 (2014).
90. Barry, A. K. *et al.* α-Catenin cytomechanics — role in cadherin-dependent adhesion and mechanotransduction. *J. Cell Sci.* **127**, 1779–1791 (2014).
91. Acharya, B. R. *et al.* Mammalian diaphanous 1 mediates a pathway for E-cadherin to stabilize epithelial barriers through junctional contractility. *Cell Rep.* **18**, 2854–2867 (2017).
92. Kim, T. J. *et al.* Dynamic visualization of α-catenin reveals rapid, reversible conformation switching between tension states. *Curr. Biol.* **25**, 218–224 (2015).
93. Mège, R. M. & Ishiyama, N. Integration of cadherin adhesion and cytoskeleton at adherens junctions. *Cold Spring Harb. Perspect. Biol.* **9**, a028738 (2017).
94. Borghi, N. *et al.* E-Cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. *Proc. Natl Acad. Sci. USA* **109**, 12568–12573 (2012).
95. Bertocchi, C. *et al.* Nanoscale architecture of cadherin-based cell adhesions. *Nat. Cell Biol.* **19**, 28–37 (2017).
96. Vasioukhin, V., Bauer, C., Yin, M. & Fuchs, E. Directed actin polymerization is the driving force for epithelial cell-cell adhesion. *Cell* **100**, 209–219 (2000).
97. Twiss, F. *et al.* Vinculin-dependent cadherin mechanosensing regulates efficient epithelial barrier formation. *Biol. Open* **1**, 1128–1140 (2012).
98. Causeret, M., Taulet, N., Comunale, F., Favard, C. & Gauthier-Rouviere, C. N-Cadherin association with lipid rafts regulates its dynamic assembly at cell-cell junctions in C2C12 myoblasts. *Mol. Biol. Cell* **16**, 2168–2180 (2005).
99. le Duc, Q. *et al.* Vinculin potentiates E-cadherin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II-dependent manner. *J. Cell Biol.* **189**, 1107–1115 (2010).
100. Ladoux, B., Nelson, W. J., Yan, J. & Mège, R. M. The mechanotransduction machinery at work at adherens junctions. *Integr. Biol.* 1109–1119 (2015).
101. Choi, W. *et al.* Remodeling the zonula adherens in response to tension and the role of afadin in this response. *J. Cell Biol.* **213**, 243–260 (2016).
102. Mason, F. M., Xie, S., Vasquez, C. G., Tworoger, M. & Martin, A. C. RhoA GTPase inhibition organizes contraction during epithelial morphogenesis. *J. Cell Biol.* **214**, 603–617 (2016).
103. Martin, A. C. & Goldstein, B. Apical constriction: themes and variations on a cellular mechanism driving morphogenesis. *Development* **141**, 1987–1998 (2014).
104. Acharya, B. R. & Yap, A. S. Pli selon pli: mechanochemical feedback and the morphogenetic role of contractility at cadherin cell-cell junctions. *Curr. Top. Dev. Biol.* **117**, 631–646 (2016).
105. Priya, R. & Yap, A. S. Active tension: the role of cadherin adhesion and signaling in generating junctional contractility. *Curr. Top. Dev. Biol.* **112**, 65–102 (2015).
106. Jodoin, J. N. *et al.* Stable force balance between epithelial cells arises from F-actin turnover. *Dev. Cell* **35**, 685–697 (2015).
107. Begnaud, S., Chen, T., Delacour, D., Mège, R. M. & Ladoux, B. Mechanics of epithelial tissues during gap closure. *Curr. Opin. Cell Biol.* **42**, 52–62 (2016).
108. Lawson, C. D. & Burridge, K. The on-off relationship of Rho and Rac during integrin-mediated adhesion and cell migration. *Small GTPases* **5**, e27958 (2014).
109. Omelchenko, T. *et al.* β-Pix directs collective migration of anterior visceral endoderm cells in the early mouse embryo. *Genes Dev.* **28**, 2764–2777 (2014).
110. Cai, D. *et al.* Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. *Cell* **157**, 1146–1159 (2014).
This paper illustrates the importance of E-cadherin during border cell migration in *D. melanogaster*, showing that E-cadherin adhesions maintain the cohesion and polarity of the group through mechanical feedback.
111. Hayer, A. *et al.* Engulfed cadherin fingers are polarized junctional structures between collectively migrating endothelial cells. *Nat. Cell Biol.* **18**, 1311–1323 (2016).
This paper shows the formation of cadherin fingers along moving endothelial cell monolayers, which are cadherin-rich protrusions that are extended from leading migrating cells and engulfed by follower cells to guide collective migration.
112. Dorland, Y. L. *et al.* The F-BAR protein pacsin2 inhibits asymmetric VE-cadherin internalization from tensile adherens junctions. *Nat. Commun.* **7**, 12210 (2016).
113. Davies, P. F. Flow-mediated endothelial mechanotransduction. *Physiol Rev.* **75**, 519–560 (1995).
114. Solon, J., Kaya-Copur, A., Colombelli, J. & Brunner, D. Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. *Cell* **137**, 1331–1342 (2009).
115. Segerer, F. J., Thuroff, F., Piera Alberola, A., Frey, E. & Radler, J. O. Emergence and persistence of collective cell migration on small circular micropatterns. *Phys. Rev. Lett.* **114**, 228102 (2015).
116. Rolli, C. G. *et al.* Switchable adhesive substrates: revealing geometry dependence in collective cell behavior. *Biomaterials* **33**, 2409–2418 (2012).
117. Friedl, P., Locker, J., Sahai, E. & Segall, J. E. Classifying collective cancer cell invasion. *Nat. Cell Biol.* **14**, 777–783 (2012).
118. Doxzen, K. *et al.* Guidance of collective cell migration by substrate geometry. *Integr. Biol.* **5**, 1026–1035 (2013).
119. Tanner, K., Mori, H., Mroue, R., Bruni-Cardoso, A. & Bissell, M. J. Coherent angular motion in the establishment of multicellular architecture of glandular tissues. *Proc. Natl Acad. Sci. USA* **109**, 1973–1978 (2012).
120. Haigo, S. L. & Bilder, D. Global tissue revolutions in a morphogenetic movement controlling elongation. *Science* **331**, 1071–1074 (2011).
121. Notbohm, J. *et al.* Cellular contraction and polarization drive collective cellular motion. *Biophys. J.* **110**, 2729–2738 (2016).
122. Grasso, S., Hernández, J. A. & Chifflet, S. Roles of wound geometry, wound size, and extracellular matrix in the healing response of bovine corneal endothelial cells in culture. *Am. J. Physiol. Cell Physiol.* **293**, C1327–C1337 (2007).
123. Klarlund, J. K. Dual modes of motility at the leading edge of migrating epithelial cell sheets. *Proc. Natl Acad. Sci. USA* **109**, 15799–15804 (2012).
124. Rausch, S. *et al.* Polarizing cytoskeletal tension to induce leader cell formation during collective cell migration. *Biointerphases* **8**, 32 (2013).
125. Watt, F. M. & Fujiwara, H. Cell-extracellular matrix interactions in normal and diseased skin. *Cold Spring Harb. Perspect. Biol.* **3**, a005124 (2011).
126. Goodwin, K. *et al.* Basal cell-extracellular matrix adhesion regulates force transmission during tissue morphogenesis. *Dev. Cell* **39**, 611–625 (2016).
127. Paszek, M. J. *et al.* Tensional homeostasis and the malignant phenotype. *Cancer Cell* **8**, 241–254 (2005).
128. Pelham, R. J. & Wang, Y. L. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl Acad. Sci. USA* **94**, 13661–13665 (1997).
129. Koser, D. E. *et al.* Mechanosensing is critical for axon growth in the developing brain. *Nat. Neurosci.* **19**, 1592–1598 (2016).
130. Reinhart-King, C. A., Dembo, M. & Hammer, D. A. Cell-cell mechanical communication through compliant substrates. *Biophys. J.* **95**, 6044–6051 (2008).

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131. Angelini, T. E., Hannezo, E., Trepat, X., Fredberg, J. J. & Weitz, D. A. Cell migration driven by cooperative substrate deformation patterns. *Phys. Rev. Lett.* (2010).
132. Saez, A., Buguin, A., Silberzan, P. & Ladoux, B. Is the mechanical activity of epithelial cells controlled by deformations or forces? *Biophys. J.* **89**, L52–L54 (2005).
133. Saez, A., Ghibaudo, M., Buguin, A., Silberzan, P. & Ladoux, B. Rigidity-driven growth and migration of epithelial cells on microstructured anisotropic substrates. *Proc. Natl Acad. Sci. USA* **104**, 8281–8286 (2007).
134. Guo, W. H., Frey, M. T., Burnham, N. A. & Wang, Y. L. Substrate rigidity regulates the formation and maintenance of tissues. *Biophys. J.* **90**, 2213–2220 (2006).
135. Douezan, S., Dumond, J. & Brochard-Wyart, F. Wetting transitions of cellular aggregates induced by substrate rigidity. *Soft Matter* **8**, 4578–4583 (2012).
136. Raab, M. *et al.* Crawling from soft to stiff matrix polarizes the cytoskeleton and phosphoregulates myosin-II heavy chain. *J. Cell Biol.* **199**, 669–683 (2012).
137. Williams, C. M., Engler, A. J., Slone, R. D., Galante, L. L. & Schwarzbauer, J. E. Fibronectin expression modulates mammary epithelial cell proliferation during acinar differentiation. *Cancer Res.* **68**, 3185–3192 (2008).
138. Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C. & Brown, R. A. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* **3**, 349–363 (2002).
139. Chao, C. Y. *et al. In vivo* and *ex vivo* approaches to studying the biomechanical properties of healing wounds in rat skin. *J. Biomechan. Engineer.* **135**, 101009–101008 (2013).
140. Levental, K. R. *et al.* Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **139**, 891–906 (2009).
141. Odenthal, J., Takes, R. & Friedl, P. Plasticity of tumor cell invasion: governance by growth factors and cytokines. *Carcinogenesis* **37**, 1117–1128 (2016).
142. Takai, Y., Miyoshi, J., Ikeda, W. & Ogita, H. Nectins and nectin-like molecules: roles in contact inhibition of cell movement and proliferation. *Nat. Rev. Mol. Cell Biol.* **9**, 603–615 (2008).
143. Wu, Y., Kanchanawong, P. & Zaidel-Bar, R. Actin-delimited adhesion-independent clustering of E-cadherin forms the nanoscale building blocks of adherens junctions. *Dev. Cell* **32**, 139–154 (2015).
144. Truong Quang, B. A., Mani, M., Markova, O., Lecuit, T. & Lenne, P. F. Principles of E-cadherin supramolecular organization *in vivo. Curr. Biol.* **23**, 2197–2207 (2013).
145. Hong, S., Troyanovsky, R. B. & Troyanovsky, S. M. Binding to F-actin guides cadherin cluster assembly, stability, and movement. *J. Cell Biol.* **201**, 131–143 (2013).
146. Strale, P. O. *et al.* The formation of ordered nanoclusters controls cadherin anchoring to actin and cell-cell contact fluidity. *J. Cell Biol.* **210**, 333–346 (2015).
147. Wu, Y., Vendome, J., Shapiro, L., Ben-Shaul, A. & Honig, B. Transforming binding affinities from three dimensions to two with application to cadherin clustering. *Nature* **475**, 510–513 (2011).
148. Harrison, O. J. *et al.* The extracellular architecture of adherens junctions revealed by crystal structures of type I cadherins. *Structure* **19**, 244–256 (2011).
149. Thomas, W. A. *et al.* α-Catenin and vinculin cooperate to promote high E-cadherin-based adhesion strength. *J. Biol. Chem.* **288**, 4957–4969 (2013).
150. Niewiadomska, P., Godt, D. & Tepass, U. DE-cadherin is required for intercellular motility during *Drosophila* oogenesis. *J. Cell Biol.* **144**, 533–547 (1999).
151. Bruce, A. E. Zebrafish epiboly: spreading thin over the yolk. *Dev. Dyn.* **245**, 244–258 (2016).
152. Harrison, O. J. *et al.* Nectin ectodomain structures reveal a canonical adhesive interface. *Nat. Struct. Mol. Biol.* **19**, 906–915 (2012).
153. Troyanovsky, R. B., Indra, I., Chen, C. S., Hong, S. & Troyanovsky, S. M. Cadherin controls nectin recruitment into adherens junctions by remodeling the actin cytoskeleton. *J. Cell Sci.* **128**, 140–149 (2015).
154. Honda, T. *et al.* Antagonistic and agonistic effects of an extracellular fragment of nectin on formation of E-cadherin-based cell-cell adhesion. *Genes Cells* **8**, 51–63 (2003).
155. Nikolic, D. L., Boettiger, A. N., Bar-Sagi, D., Carbeck, J. D. & Shvartsman, S. Y. Role of boundary conditions in an experimental model of epithelial wound healing. *Am. J. Physiol. Cell Physiol.* **291**, C68–C75 (2006).
156. Murrell, M., Kamm, R. & Matsudaira, P. Tension, free space, and cell damage in a microfluidic wound healing assay. *PLoS ONE* **6**, e24283 (2011).
157. van der Meer, A. D., Vermeul, K., Poot, A. A., Feijen, J. & Vermes, I. A microfluidic wound-healing assay for quantifying endothelial cell migration. *Am. J. Physiol. Heart Circ. Physiol.* **298**, H719–H725 (2010).
158. Gul, I. S., Hulpiau, P., Saeys, Y. & van Roy, F. Evolution and diversity of cadherins and catenins. *Exp. Cell Res.* **358**, 3–9 (2017).
159. van Roy, F. Beyond E-cadherin: roles of other cadherin superfamily members in cancer. *Nat. Rev. Cancer* **14**, 121–134 (2014).
160. Plutoni, C. *et al.* P-Cadherin promotes collective cell migration via a Cdc42-mediated increase in mechanical forces. *J. Cell Biol.* **212**, 199–217 (2016).
161. Chu, Y. S. *et al.* Prototypical type I E-cadherin and type II cadherin-7 mediate very distinct adhesiveness through their extracellular domains. *J. Biol. Chem.* **281**, 2901–2910 (2006).
162. Labernadie, A. *et al.* A mechanically active heterotypic E-cadherin/N-cadherin adhesion enables fibroblasts to drive cancer cell invasion. *Nat. Cell Biol.* **19**, 224–237 (2017).
This paper shows that cancer-associated fibroblasts (CAFs) form heterotypic E-cadherin–N-cadherin contacts with tumour cells, exerting a physical force on cancer cells that enables their collective invasion.
163. Sawyer, J. K. *et al.* A contractile actomyosin network linked to adherens junctions by Canoe/afadin helps drive convergent extension. *Mol. Biol. Cell* **22**, 2491–2508 (2011).
164. Sawyer, J. K., Harris, N. J., Slep, K. C., Gaul, U. & Peifer, M. The *Drosophila* afadin homologue Canoe regulates linkage of the actin cytoskeleton to adherens junctions during apical constriction. *J. Cell Biol.* **186**, 57–73 (2009).
165. Mandai, K. *et al.* Afadin: a novel actin filament-binding protein with one PDZ domain localized at cadherin-based cell-to-cell adherens junction. *J. Cell Biol.* **139**, 517–528 (1997).
166. Ikeda, W. *et al.* Afadin: a key molecule essential for structural organization of cell-cell junctions of polarized epithelia during embryogenesis. *J. Cell Biol.* **146**, 1117–1132 (1999).
167. Zhadanov, A. B. *et al.* Absence of the tight junctional protein AF-6 disrupts epithelial cell-cell junctions and cell polarity during mouse development. *Curr. Biol.* **9**, 880–888 (1999).
168. Weber, G. F., Bjerke, M. A. & DeSimone, D. W. A mechanoresponsive cadherin-keratin complex directs polarized protrusive behavior and collective cell migration. *Dev. Cell* **22**, 104–115 (2012).
169. Mayer, M., Depken, M., Bois, J. S., Julicher, F. & Grill, S. W. Anisotropies in cortical tension reveal the physical basis of polarizing cortical flows. *Nature* **467**, 617–621 (2010).
170. Dembo, M., Oliver, T., Ishihara, A. & Jacobson, K. Imaging the traction stresses exerted by locomoting cells with the elastic substratum method. *Biophys. J.* **70**, 2008–2022 (1996).
171. Tan, J. L. *et al.* Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc. Natl Acad. Sci. USA* **100**, 1484–1489 (2003).
172. Yamaguchi, N., Mizutani, T., Kawabata, K. & Haga, H. Leader cells regulate collective cell migration via Rac activation in the downstream signaling of integrin β1 and PI3K. *Sci. Rep.* **5**, 7656 (2015).
173. Osmani, N., Peglion, F., Chavrier, P. & Etienne-Manneville, S. Cdc42 localization and cell polarity depend on membrane traffic. *J. Cell Biol.* **191**, 1261–1269 (2010).

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