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Tug of war - The influence of opposing physical forces on epithelial cell morphology

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Abstract
The shape of a single animal cell is determined both by its internal cytoskeleton and through physical interactions with its environment. In a tissue context, this extracellular environment is made up largely of other cells and the extracellular matrix. As a result, the shape of cells residing within an epithelium will be determined both by forces actively generated within the cells themselves and by their deformation in response to forces generated elsewhere in the tissue as they propagate through cell-cell junctions. Together these complex patterns of forces combine to drive epithelial tissue morphogenesis during both development and homeostasis. Here we review the role of both active and passive cell shape changes and mechanical feedback control in tissue morphogenesis in different systems.

Keywords: Forces, adhesion, actin, myosin, cell shape, epithelial morphogenesis

Introduction

Animal cells are both actively shaped by internally generated forces and passively shaped by external forces that are transmitted across the tissue within which they reside. When grown in isolation, they tend to be spherical as the result of membrane tension and the contractile cortical forces generated in the underlying meshwork of actin and myosin (actomyosin cytoskeleton) that act against the cell's internal hydrostatic pressure (Salbreux et al., 2012)(Fig. 1A). When binding to a two-dimensional (2D) surface, through adhesion molecules such as integrins, adhesion lowers the effective surface tension acting along the cell-substrate interface. As a result cells tend to spread like water droplets on a hydrophilic surface (Fig. 1B). Interestingly, however, because cells match the mechanical properties of their cytoskeleton to that of their environment, this spreading depends on substrate stiffness, so that cells tend to remain rounded when adhering to soft substrates but spread on hard substrates (Discher et al., 2005; Georges and Janmey, 2005). In addition, cell spreading can be aided by active actin polymerisation-based protrusive forces generated at the cell front (Vicente-Manzanares et al., 2009).

Additional levels of forces come into play when cells grow in the context of a three-dimensional (3D) tissue, where they become subject to tissue-level forces. In the main, these are made up of contractile actomyosin-based forces that propagate across cell-cell junctions and the effects of physical confinement. For epithelial cells, it is the cell-cell adhesion molecules cadherins that play the dominant role in mechanically coupling tension-generated forces between neighbouring cells (Fig. 1C) (Maître and Heisenberg, 2013;

Niessen et al., 2011). As a consequence, changes in the shape of one cell will result in passive changes in the shape of its neighbours. If this connectivity is maintained, as is the case for many morphogenetic events during tissue morphogenesis and homeostasis, the change in the form of one set of cells undergoing an active morphogenesis movement is therefore accompanied by a passive change in the shape of the surrounding tissue (Fig. 2)

The molecular and cellular origins of these tissue-level forces have been studied in a variety of model systems. Here we review recent examples in which a complex interplay of active and passive forces, either individually or collectively, shape and sculpt cells and tissues into their final 3D shape. Since animals tend to be constructed from the folding of 2D sheets of cells, as if shaped by origami, epithelial sheets will be our main focus. We will primarily discuss examples from *Drosophila*, whilst highlighting parallels from vertebrate systems wherever possible.

Although many cytoskeleton components are involved in the generation of cell and thus tissue shape and mechanics, including microtubules and intermediate filaments (Booth et al., 2014; Huber et al., 2014), this review will focus on the functions of cadherin-based adhesion molecules which couple the contractile actomyosin cytoskeleton within neighbouring cells, since these appear to play the dominant role in sculpting most animal tissues. These actomyosin-dependent contractile forces can propagate across multiple cells in the plane of the epithelium and are counter-balanced by outward pressures generated during tissue growth. The result of this, as with single cells, is a force balance in which contractile tension and outward pressure combine to generate form.

Active cell shape changes within epithelial tissues

Within cells, contractile force is generated by networks of antiparallel actin and myosin II filaments. To affect changes in cell and tissue form, these structures must then be coupled to the membrane and the outside world. In developing epithelial cells, cell-cell coupling occurs mainly at the apical adherens junctions through E-cadherin, via □-catenin and □-catenin (Halbleib and Nelson, 2006). Although the exact molecular composition of this force transmitting complex has been controversial (Gates and Peifer, 2005; Yamada et al., 2005), recent evidence suggests that □-catenin can undergo force dependent conformational changes that reveal vinculin binding sites to strengthen the linkage of the cadherin/catenin complex with actin filaments (Leerberg et al., 2014; Yao et al., 2014; Yonemura et al., 2010). In many tissues, this can be seen in stress-bearing actomyosin cables that cross cell-cell boundaries (Landsberg et al., 2009; Monier et al., 2010; Röper, 2013). Importantly, as a result of force transmission, these cables tend to align in a single tissue plane. In this way, the local formation of cadherin-coupled actomyosin cables appear to both reflect and to enhance apical-basal cell polarity.

Apical subcellular forces

All epithelial cells have an apical/basal axis, which is often divided into 3 domains. Moving from the outside in: apical, lateral and basal (Fig. 3). Within each domain, different molecular polarity regulators control the local assembly of actin filaments and myosin activation (Escudero et al., 2007; Martin et al., 2009). Through this type of local control, cells can alter the sizes of their apical, lateral and basal domains.

When coupled across cells, these planar forces can be used to drive tissue morphogenesis. Then, because cells tend to conserve mass and volume, if this actomyosin mesh becomes contracted apically or basally, the cell will tend to become wedge shaped. Classically this was attributed to a ‘purse-string’ type mechanism as circum-junctional bundles of actomyosin shorten (Owaribe et al., 1981). This is exemplified by the junctional recruitment of Shroom3 in vertebrates, which recruits F-actin and Rho kinase to activate junctional myosin II to induce the apical constriction required to drive neural tube closure (Hildebrand, 2005; Nishimura and Takeichi, 2008). However, recent advances in microscopy have

revealed that actomyosin dynamics are not limited to these circum-junctional bundles but are also found underlying the plasma membrane within both apical, basal and lateral domains (Gorfinkiel and Blanchard, 2011; Wu et al., 2014). Once myosin begins to become activated, this contractile gel can act to reduce the size of the domain in which they form, Fig. 3A. And when coordinated across a tissue, this can result in buckling. A good example of this is ventral furrow formation in *Drosophila*, and gastrulation in *C. elegans*, where pulsed contractions of apical actomyosin networks drive the loss of the cell apex, leading to tissue folding and invagination (Martin et al., 2009; Roh-Johnson et al., 2012). In order for apical constriction to occur, however, actomyosin generated forces must be properly transmitted to the junction - a process that is poorly understood (Roh-Johnson et al., 2012). Moreover, as the apex undergoes pulses of actomyosin network assembly and constriction, a second process is required to ensure the steady shrinkage of the junction itself (Martin et al., 2009; Roh-Johnson et al., 2012). While this process has been compared to a junctional rachet, in most systems, the rapid turnover of junctional and cytoskeletal machinery will tend to dissipate elastic forces building up in the system within tens of seconds.

While the focus of most research in the field has been on forces generated on individual cells, large-scale changes in tissue shape require coordinated changes in cell shape. Thus, it is the pattern of the constricting cells that dictates the pattern of the indentation (Escudero et al., 2007); a long row of cells undergoing isotropic apical constriction will induce a furrow such as the ventral furrow in *Drosophila* embryos (Martin et al., 2009) or the morphogenetic furrow in eye imaginal discs (Corrigall et al., 2007; Escudero et al., 2007), while a more circular group of cells will lead to a tubular like invagination seen during posterior midgut formation (Sweeton et al., 1991). The exact nature of the coupling will determine the efficiency of the force transmission process in different developmental tissues and processes.

Lateral subcellular forces

While changes in the size of apical/basal domains in a subpopulation of cells in a tissue will tend to lead to tissue folding, changes in the length of the lateral domain will lead to changes in the height or thickness of epithelial cells, Fig. 3A (Cai and Mostov, 2012; Johnston and Sanson, 2011; Keller et al., 2003; Kolahi et al., 2009). Epithelial tissues change in thickness throughout the development of many organisms, such as epithelial thinning during epiboly in vertebrate embryos (Lepage and Bruce, 2010), the flattening of the amnioserosa cells in *Drosophila* (Pope and Harris, 2008) and the cell height transitions in *Drosophila* imaginal discs (Aldaz and Escudero, 2010). In combination with apical constriction, cell shortening generates buckling forces that help to drive more pronounced changes in tissue organisation. This has long been known to be a key element in driving ventral furrow formation in flies (Brodland et al., 2010; Rauzi et al., 2013). Similarly, the shortening due to mitotic rounding drives the buckling that underlies the internalization of trachea pits in flies (Kondo and Hayashi, 2013). And, during gastrulation in ascidians, endoderm invagination is thought to be driven by the active shortening of cells via lateral myosin activation (Sherrard et al., 2010). In most cases, however, the common mechanisms and forces involved in cellular shortening in different contexts are not clear. They could result from lateral actomyosin activity (Wu et al., 2014) or from changes in the length distribution of microtubules (Picone et al., 2010), or through the microtubule-dependent positioning of junctions (Wang et al., 2012). One system in which this process of shortening has been studied in most detail is the *Drosophila* egg chamber. Here, the follicular epithelium undergoes a lateral domain shortening on the anterior half of the egg chamber to form the squamous cells surrounding the nurse cells, while the cells around the oocyte extend their lateral domains to become columnar (Grammont, 2007; Kolahi et al., 2009). Interestingly, in this case the force driving follicle cell flattening is thought to come from the growth of the germline that underlies the apical surface of follicle cells (see later sections). On the other hand, posterior follicle cells can actively counteract this force to remain columnar, by having comparatively higher apical actomyosin activity (Grammont, 2007; Kolahi et al., 2009; Wang

and Riechmann, 2007). This apical constriction force counteracts the cell flattening force from the germline, therefore helping to maintain their columnar cell shape. Recent work has also shown that lateral adhesion can also allow cells to resist lateral membrane shortening. This can be a property of lateral interfacial tension, which is decreased by lateral adhesion molecules (Brodland, 2002). A good example of this is Fasciclin2, an immunoglobulin family cell adhesion molecule that mediates homophilic cell adhesion between the lateral membranes of follicle cells (Szafranski and Goode, 2004). Tao, a serine/threonine kinase, promotes endocytosis of Fasciclin2, to remove it from the lateral surface of cells. This reduction in lateral adhesion is required for the shortening of the lateral domain, and the cuboidal to squamous cell shape transition (Gomez et al., 2012). Conversely, the height of epithelial cells can be increased by a concomitant contraction of the apical and basal cell surfaces (Fig. 3A). This has been proposed to be the mechanism responsible for the cuboidal to columnar cell shape transition in the *Drosophila* wing imaginal disc, where Dpp signalling is thought to localize Rho1 and myosin II activity apically and basally, thus generating a cortical tension anisotropy in the apical/basal plane of the cell (Widmann and Dahmann, 2009). This apical and basal constriction also likely requires an increase in lateral adhesion (decrease in lateral interfacial tension) and/or pressure from the growing epithelium and the encapsulating extracellular matrix (Pastor-Pareja and Xu, 2011) to generate tall cells with a stable form.

Planar polarised subcellular forces

If forces are applied to individual junctions between two cells, these junctions can preferentially shorten or lengthen. In isolation, this type of stochastic anisotropic force application would be expected to make epithelial tissues more fluid by driving changes in the length of cell-cell junctions together with neighbours. However, if there is a mechanism in place to polarize junctions and to maintain their polarity with respect to the global tissue axis during neighbour exchanges, the local shrinkage or expansion of junctions can be used to drive morphogenesis in the plane of the epithelium. The best-studied example of this is during *Drosophila* germ-band extension. Here, actomyosin contractility is polarised to the anterior-posterior (AP) cell interfaces, Fig. 3B (Bertet et al., 2004; Blankenship et al., 2006; Kasza et al., 2014; Simões et al., 2014; Zallen and Wieschaus, 2004). As a result of the contraction coupling via E-cadherin complexes, this drives the contraction and eventual loss of AP cell junctions, leading to polarized cell intercalations, such that originally AP neighbouring cells lose contact, but new junctions along the orthogonal dorso-ventral (DV) cell interfaces are created (Rauzi et al., 2008). Collectively, these directed cell rearrangements drive tissue elongation without there being sustained changes in the shape of individual cells (Fig. 3B, top row). For cells to rearrange, junctional plasticity is essential. It has been observed that shrinking AP interface junctions display a higher turnover of E-cadherin and catenins (Tamada et al., 2012; Warrington et al., 2013), whereas DV interface junctions show an enrichment of Baz/Par3 (Simões et al., 2010), which is important for stabilisation of the newly formed DV junctions. In the *Drosophila* pupal wing, the stabilisation and extension of newly formed junctions after intercalation is dependent on PTEN activity, which also appears to be planar polarised. This selectively reduces PIP3 levels at newly formed junctions to decrease Rok dependent myosin II levels, allowing these new junctions to now extend (Bardet et al., 2013).

In addition to myosin II, which is the principle force generator for actomyosin induced contractility, atypical myosins have also been shown to be important for generating mechanical anisotropy in tissues. Dachs, an atypical myosin in *Drosophila*, which is apically planar polarised by the Fat-Dachsous planar cell polarity pathway (Mao et al., 2006; Rogulja et al., 2008), can lead to local increases in interfacial tension where it is localised (Bosveld et al., 2012; Mao et al., 2011). In the dorsal thorax, this leads to oriented cell rearrangements that shape the epithelium (Bosveld et al., 2012). In the highly proliferative wing imaginal disc, Dachs is polarised to the distal junctions of cells (Mao et al., 2006; Rogulja et al., 2008). This polarised increase in tension of the proximal-distal (PD) cell interfaces is thought to lead to

anisotropic cell growth and timely elongation of cells along the PD axis at the end of interphase, immediately prior to mitotic entry. This results in PD-oriented cell divisions in the centre of the wing pouch and tissue elongation along the PD axis of the wing (Fig. 3B bottom row, Mao et al., 2011; Mao et al., 2013). Oriented cell divisions have also been observed in other developmental systems, such as the cell divisions in the enveloping cell layer (EVL) during zebrafish epiboly (Campinho et al., 2013), where they are thought to function to relax anisotropic tension in the tissue. Thus, timely and complementary localisation of contractile structures and adhesion complexes ensures that the shrinking junctions have higher interfacial tension whereas the extending junctions have lower interfacial tension, which can lead to oriented cell rearrangements in plastic tissues, or oriented cell divisions in highly proliferative tissues. Similar mechanisms of tissue elongation through convergent/extension and oriented cell divisions also exist in vertebrate embryogenesis (as reviewed in Keller, 2006).

Collective cellular forces

As long as cells are mechanically coupled to other cells through cell-cell junctions, forces generated by individual cells can be transmitted across tissues to produce collective multicellular level forces. In addition, supracellular actomyosin cables have been seen in many developmental systems (reviewed in Röper, 2013). These are able to generate transcellular line tension to aid processes such as compartment boundary formation to prevent cell mixing (Landsberg et al., 2009; Monier et al., 2010; Monier et al., 2011). Groups of cells can also be driven to invaginate by a supracellular actomyosin cable around the outer margin of the invaginating cells, as seen during salivary gland formation (Röper, 2012). Such circumferential actomyosin cables, acting as a purse string, are also found during dorsal closure at the leading edge of the dorsal epidermis, acting as a ratchet for the pulsatile shrinkage of the amnioserosa cells (Kiehart et al., 2000; Solon et al., 2009). They are also responsible for wound healing in zebrafish and *Drosophila* (Abreu-Blanco et al., 2012; Martin and Lewis, 1992; Wood et al., 2002) as well as for epiboly in zebrafish (Behrndt et al., 2012). However, it remains to be understood how these tissue level cables are able to collectively generate and transmit force across multiple cells (Röper, 2013).

Cell and tissue growth forces

Growth also plays a major role in shaping tissues. When groups of cells grow and divide and remain adhered together, the collective tissue expansion generates a positive pressure that is rapidly propagated across non-compressible tissues. If there are growth differentials between neighbouring cells, this can generate differential mechanical stress and strain, which modulates cell shape and tissue morphology (Mao et al., 2013; Savin et al., 2011; Shyer et al., 2013). During the early stages of *Drosophila* wing imaginal disc growth, the higher rate of cell proliferation in the centre of the pouch generates global tension anisotropies between the centre and periphery of the disc, with the peripheral cells experiencing higher circumferential tension, Fig. 2A' (Mao et al., 2013). As a result, the peripheral cells become passively stretched circumferentially (black arrows, Fig. 2A') and orient their divisions circumferentially (Legoff et al., 2013; Mao et al., 2013). Interestingly, differential growth had been theorized to generate patterns of compression, cell shape changes and tissue buckling in computational models of the wing disc before differential growth had been measured experimentally (Aegerter-Wilmsen et al., 2007; Aegerter-Wilmsen et al., 2012; Mao et al., 2013; Shraiman, 2005). Recently, elegantly combined theoretical and experimental studies on the growth and form of the chick gut have also attributed differential growth, this time between two connected tissue layers, as the primary cause of the buckling and looping of the chick gut, at multiple layers and scales (Savin et al., 2011; Shyer et al., 2013). Thus, differential growth rates can have profound effects on differential force accumulation leading to autonomous and non-autonomous cell and tissue morphology changes.

Apoptotic forces

In a mechanism similar to localised cell contractions, programmed cell death, or apoptosis (Kerr et al., 1972), can generate active forces to induce cell shape changes and cell shrinkage but with eventual cell loss. During embryonic development apoptosis is essential for sculpting tissue structures, strategically removing cells that are no longer useful, such as the webbing tissue on limb digits (Jacobson et al., 1997). The apoptotic cells also exert mechanical forces on neighbouring cells, which may play a role in the active morphogenesis of tissues surrounding the dying cells, as suggested for hair follicle regression (Stenn and Paus, 2001). In tissue culture monolayers, it has been shown that the apoptotic cell can induce an actomyosin ring in itself and a supra-cellular actomyosin purse string in the neighbouring non-apoptotic cells (Rosenblatt et al., 2001), which drives the extrusion of the apoptotic cell out of the epithelium. The integrity of the epithelium is maintained by junctional remodelling to ensure that any gaps that may have been left by the apoptotic cells are filled. More recently, apoptotic cells in the amnioserosa were shown to play an active mechanical role in regulating the precise dynamics of dorsal closure during *Drosophila* embryogenesis (Toyama et al., 2008). Studies have also shown that when apoptosis is inhibited, neural tube closure in chick embryos is disrupted (Weil et al., 1997), as well as palatal fusion in higher vertebrates (Cecconi et al., 1998; Honarpour et al., 2000). The exact mechanical consequences of apoptosis will depend on the relative rate of apoptosis, compared to surrounding morphogenetic events, and the mechanical properties of the surrounding tissue in transmitting and responding to the effect of the lost cells.

Passive cell shape changes

Although many changes in the shape of cells within developing epithelia result from active internal forces generated by the cell itself, in a tissue context any change in the shape of cells that results from active force generation will be accompanied by passive changes in the shape of neighbouring tissues. As a result, at the level of tissue morphogenesis, all changes depend on a combination of active and passive changes working in concert. Dissecting the cause or effect of cell shape changes can therefore be a challenge in complex developmental systems (Brodland et al., 2010). This is seen clearly in computational models of development, where one must explicitly specify the mechanics of active force generation and the passive response of neighbouring tissue to observe any morphogenesis (Munoz et al., 2007).

The key consideration here is the relative timescale at which passive changes are followed by plastic changes in the structure of the deformed tissue, i.e. the rate at which forces are dissipated. Thus, following a uniaxial stretch (along the x-axis) (Harris et al., 2012), there will be different time-scales over which the applied forces are relaxed. Immediately after a deformation, the tissue behaves elastically – enabling it to return to its initial shape if released. However, over time, various processes will occur within the stretched tissue that will lead to the dissipation of stress and to tissue relaxation. These can include lateral thinning of the tissue in both width (y-axis) and height (z-axis), analogous to the thinning one sees when one stretches gum. Importantly, however, the relaxation of the tissue will not be the same in the two axes. This is because relaxation in the plane of the tissue can be achieved using processes such as neighbour exchange or oriented division to redistribute cell mass. Conversely, changes in z require a change in the preferred cell height. While these processes are poorly understood, it is clear that we need to know more about processes like tissue thinning, neighbour exchange and oriented division to understand the contribution of passive cell behaviours to tissue morphogenesis.

Here we highlight a few morphogenetic processes *in vivo* where elegantly designed experiments have managed to attribute the observed cell and tissue shape changes to be the result of passive responses to forces from surrounding cells and tissues.

Passive responses to localised tissue contractions

During *Drosophila* germband extension, the invaginating mesoderm has been proposed to provide an extrinsic force that drives the passive cell shape changes in the ectoderm, which, along with active cell intercalations, contributes to the anterior-posterior elongation of the germband (Butler et al., 2009). When active cell intercalation is blocked, such as in *Krüppel* mutants, an increase in anterior-posterior cell elongation compensates for the reduced elongation from the lack of cell intercalation, ensuring that the initial rate of extension of the germ-band remains the same. This shows that there is a combination of active and passive processes at play during germband extension.

Localised tissue contractions also has a similar affect during *Drosophila* pupal wing development, where the cells and tissues also undergo complex shape changes, most notably a transient elongation of wing blade cells along the PD axis and overall tissue elongation of the wing blade along the PD axis, Fig. 2B' (Aigouy et al., 2010). Concomitant with these shape changes in the wing blade, the wing hinge, which is connected to the wing blade, contracts. It is thought that the cell (and tissue) shape changes in the wing blade are actually driven by the contraction force of the hinge (Fig. 2B'). Before hinge contraction, tension is similar on all cell junctions. When the hinge contracts, tension increases specifically on those cell junctions oriented close to the PD axis. This suggests that hinge contraction exerts anisotropic forces on the blade, Fig. 2B'. Severing the hinge from the blade reduced cell elongation along the PD axis, and mis-oriented cell divisions and neighbour exchanges, resulting in a less hexagonally packed tissue (Aigouy et al., 2010; Sugimura and Ishihara, 2013).

Passive responses to cell and tissue growth

When one considers shaping tissues as they grow, things become even more complex. For tissues growing as disks or spheres (Hannezo et al., 2014), by analogy with a single cell (Fig. 2A), one can see that tissue shape will be determined by the balance of forces between the outward pressure generated by the increase in cell mass that accompanies growth, and counterbalancing tension of tissue in the periphery. A good example of this is the fly wing pouch (Fig. 2A'). As mentioned above, the circumferential cell elongation of apical cell shape in the periphery of the *Drosophila* wing pouch is a passive consequence of increased radial pressure from the higher proliferation rates in the centre of the wing pouch (Mao et al., 2013). This differential growth (in the plane of the epithelium) leads to higher circumferential tension in the apical junctions of cells around the periphery of the tissue (Legoff et al., 2013; Mao et al., 2013). This leads to circumferential cell stretching (Fig. 2A', black arrows). Thus, although polarised myosin II expression and activity correlates with the longer junctions that are under higher tension in the periphery, this is likely a consequence of a mechanical feedback response to the initial stretch that is induced by differential growth, which serves to counteract the imposed deformation. The planar polarity of Dachs has also been suggested to act as a counteracting force in the periphery of the pouch to prevent cells from being excessively stretched. In fact, peripheral cells lacking myosinII activity (Legoff et al., 2013) or Dachs (unpublished work) are even more stretched, suggesting that the myosin II and Dachs polarisation are not the cause of the cell stretching, but a consequence, serving to prevent excessive deformations induced by the growing pressure from the pouch centre. Again, this is analogous to single cells, where the actomyosin cortex acts to counterbalance the expansion force from the internal cell pressure (Fig. 2A). Computational models and genetic perturbations to induce localised increases in cell proliferation (growth and division) have shown that higher localised growth can be sufficient to induce an increase in junctional tension of neighbouring cells, thereby causing passive cell shape changes (Legoff et al., 2013; Mao et al., 2013). How these initially passive cell shape changes can lead to active cellular and molecular responses remains to be understood in the wing disc.

Within an epithelium, tissue pressure generated through crowding can also be relaxed through the loss of individual epithelial cells via delamination (Fig. 2C'). This has been

described both for vertebrate and invertebrate systems. In both cases, individual cells within crowded tissues lose junctions and delaminate out of the epithelium while still alive. The choice of which cells are to go is not clear and may be stochastic. Once they have delaminated, these cells then die by anoikis as the results of the loss of neighbours – so that they sacrifice themselves to help restore the normal mechanical environment of the whole. Interestingly, the direction by which cells leave will be determined by the relative contractile forces, apically and basally, and by any physical barriers that prevent their delamination in one direction. In addition, this will be affected by the curvature of the tissue. Thus, cells tend to leave from regions of the tissue that are most folded (Eisenhoffer et al., 2012; Marinari et al., 2012). Therefore this process may contribute to the shedding of cells from the tips of microvilli in the human gut (Simons and Clevers, 2011). Since hyperplastic cancers are likely to be overgrown and crowded, it has been argued that the same process could contribute to cancer spread. In this case, the direction of cell delamination will be critical, since cancer cells that leave apically will tend to be lost, while those leaving basally may remain alive within the matrix – making them an ideal substrate for metastasis.

Opposing forces generated by growth and tissue tension also occur in 3D. They have been studied in cysts. The *Drosophila* egg chamber provides a good example of a developing system where the increased growth of an internal tissue region induces the passive stretching of the surrounding cells (Kolahi et al., 2009). The egg chamber consists of an inner cyst of germline lines surrounded by a monolayered follicular epithelium (which is initially cuboidal shaped). Early in oogenesis, the growth of the inner germline cyst is accommodated by cell divisions in the outer follicle epithelium. This balance of growth between the inner cell mass and the outer cell mass is disrupted when the outer follicle cells stop dividing while the inner cyst continues to grow (López-Schier and St Johnston, 2001). This results in the stretching and flattening of the anterior follicle epithelium cells, as they cannot counteract the cyst pressure, due to Notch signalling induced localised adherens junction disassembly (Grammont, 2007; Kolahi et al., 2009), whereas the posterior cells can resist this pressure, and do not flatten (see earlier section).

Passive responses to the extracellular environment

A tissue's extracellular environment can also affect the morphology of the tissue it encapsulates, acting either as a frictional surface for forces to be transmitted and dissipated, or simply as a scaffold for structural support. The exact mechanical role of a tissue's extracellular environment remains to be understood, but in *Drosophila* wing imaginal discs, Collagen IV, a basement membrane component, is required to maintain the correct height of the cells in the epithelium, and therefore the correct tissue morphology (Pastor-Pareja and Xu, 2011). Inhibiting integrin function, which detaches cells from the extracellular matrix (ECM), also causes similar cell shape defects as ECM removal (Domínguez-Giménez et al., 2007). It is also known that different extracellular matrix enzymes are required for imaginal disc eversion and morphogenesis (Pino-Heiss and Schubiger, 1989), as well as tracheal morphogenesis in *Drosophila* (Dong et al., 2014), suggesting that timely regulation of ECM composition is essential for allowing cell and tissue shape changes. Interesting, mechanical models have shown that cell sliding against the vitelline membrane is required for the correct cell shape changes to occur during *Drosophila* ventral furrow invagination (Conte et al., 2008).

Tuning the passive response - mechanical feedback

The few examples above nicely illustrate that in complex multicellular developmental systems, cells can be shaped by active cell autonomously generated forces, or by passive non-autonomously generated forces, and the final cell shape is a fine balance between the variety of forces experienced by the cell. This emphasizes the complexity of the passive response to stretch.

In addition, however, biological cells are not purely passive materials, but can respond actively to applied forces. This phenomenon, known as mechanical feedback, can lead to an auto-regulatory feedback control that ensures the robust sculpting of cells and tissues during developmental growth and morphogenesis. The best evidence for this occurring comes from cells in culture, where they have been shown to respond to stretch by pulling back, and to differentiate according to the mechanical properties of the substrate upon which they grow. While poorly understood, feedback may act at multiple cellular levels and time scales within the cell, leading to, for example, rapid remodelling of cytoskeletal elements, or more delayed changes in gene expression and signalling pathways, and eventual cell fate changes (Chen et al., 1997; Engler et al., 2006).

Mechanical feedback on actomyosin remodelling

One of the methods for cells to response to, or resist, mechanical forces is to re-organise its own force generating apparatus, such as the actomyosin contractile network. In multicellular epithelial systems, there have been only a few examples of causation, where the application of a direct mechanical force has led to actomyosin changes in the cell. During *Drosophila* gastrulation, Snail is a transcription factor expressed in the mesoderm that is required for constriction of the mesoderm (Morize et al., 1998). Mesoderm invagination defects in *snail* mutants can be rescued by mechanical deformations with a micromanipulation needle. This mechanical deformation triggers the redistribution of myosin II to the apical surface of cells, thereby inducing apical constriction (Pouille et al., 2009). Similar myosin II recruitment to the cell cortex was observed by micro-aspiration experiments that deformed the apical surface of *Drosophila* embryo cells (Fernandez-Gonzalez et al., 2009). This is supported by more correlative data in the *Drosophila* wing imaginal disc where the peripheral cells that are under higher circumferential tension due to mechanical stretching also display elevated myosin II levels at their circumferential junctions (Fig. 4 top row, Legoff et al., 2013; Mao et al., 2013). A slightly more direct link for tension induced myosin accumulation along cellular junctions is observed when the relaxation of junctions (reduction in tension), by severing the hinge from the wing blade during *Drosophila* pupal morphogenesis, led to a reduction of myosin accumulation along the PD oriented junctions (Sugimura and Ishihara, 2013). However, whether direct anisotropic epithelial tension can lead to anisotropic accumulation of myosin II remains to be seen.

At the single cell level, re-organisation of actomyosin structures in response to mechanical stimuli have been shown more directly. These include the re-localisation of myosin II and the actin cross linker cortexillin in response to microaspiration in Dictystelium cells (Effler et al., 2006). The molecular mechanisms behind this response are still unclear, but there are suggestions that actin itself could be the mechanosensor, and its affinity for myosin II is increased when actin filaments are stretched (Uyeda et al., 2011). The interaction kinetics between actin and myosins (muscle and nonmuscle) are also known to change depending on mechanical load (Guo and Guilford, 2006; Kovács et al., 2007). The most widely studied actomyosin remodelling response to mechanical forces is in the formation of actin stress fibres, recently reviewed in (Smith et al., 2014). These supra-actin structures, which span across cells, and attach to the extracellular matrix via integrin based focal adhesions, allow cells to sense and respond to dynamic changes in their mechanical environment, via the regulation of molecules such as zyxin, □-actinin, vinculin and talin (Hoffman et al., 2011). It is out of the scope of this review to discuss the plethora of literature in this field, but it will be interesting to see in future years if similar mechanisms exist in epithelial tissues, such as the mobilisation of zyxin from focal adhesions to actin filaments to reinforce the cytoskeleton upon increased mechanical tension (Yoshigi et al., 2005).

Mechanical feedback on adhesion complex remodelling

Actomyosin contractilities are transmitted to other cells via E-cadherin complexes or the ECM via integrins. In order for the mechanically induced increase in actomyosin contractile force to be transmitted efficiently, it seems logical that the strength of the adhesion coupling

complex also needs to be enhanced, or stablised, by forces pulling on the complex. Recent research has shown that slower actin turnover can directly increase E-cadherin recruitment at the membrane, independently of cortical tension (Engl et al., 2014). Since increased myosin II induced contractility can lead to slower actin turnover, this means that increased cortical tension, will also, indirectly, lead to more immobilisation of E-cadherin at the membrane (Fig. 4, bottom row), providing a positive feedback loop between cortical tension and adhesion strength (Engl et al., 2014). This is consistent with other findings where differential stabilisation of F-actin at the apical and lateral domains can lead to differential diffusion properties of cadherins (Wu et al., 2014). This mechanism would act in parallel to tension induced conformational changes in □-catenin that strengthens actin binding to E-cadherin adhesion complexes (Leerberg et al., 2014; Yao et al., 2014; Yonemura et al., 2010). Such mechano-sensitive reinforcement of adhesion strengths at cell-cell adhesion sites are highly similar to the cell-ECM integrin based focal adhesion sites, where mechanical force can induce the growth of focal contacts (Riveline et al., 2001) and also molecular conformational changes in talin to reveal binding sites for vinculin and thus strengthen the linkage to actin filaments (del Rio et al., 2009; Grashoff et al., 2010).

Mechanical feedback on growth

As mentioned above, cell growth can also generate significant physical forces to drive cellular and tissue shape changes. The mechanical feedback control of cell growth and proliferation patterns has been postulated in the *Drosophila* wing imaginal disc, but the exact mechanism remains unknown (Hufnagel et al., 2007; Shraiman, 2005) (Aegerter-Wilmsen et al., 2007; Aegerter-Wilmsen et al., 2012). The theory aims to resolve an apparent contradiction – how uniform growth and proliferation patterns are achieved during the later stages of wing disc growth despite gradients of growth promoting signalling molecules (morphogens). The theory hypothesizes that as a result of the initial morphogen gradient induced growth differential (higher in the centre, lower in the peripheral cells), the peripheral cells become stretched (Mao et al., 2013), while the central cells become compressed. This mechanical stretching of cells, could lead to, via mechanical feedback, an induction of cell growth and proliferation, while compression could induce growth arrest or cell death (Chen et al., 1997; Nelson et al., 2005). Although this theory requires experimental verification in multicellular *in vivo* systems, it is nevertheless an interesting hypothesis for how differential tissue stresses generated by differential tissue growth could be alleviated to achieve mechanical tissue homeostasis. Interesting, this could be regulated through the Hippo signaling pathway, where the nuclear-cytoplasmic translocation of the Yorkie/Yap/Taz transcriptional co-activator changes depending on the cell's mechanical environment (Dupont et al., 2011; Fernández et al., 2011; Sansores-Garcia et al., 2011) (Aragona et al., 2013; Rauskolb et al., 2014; Wada et al., 2011), reviewed recently in (Gaspar and Tapon, 2014). Stretched cells accumulate more nuclear Yorkie/Yap/Taz, which leads to the transcription of many cell growth promoting and cell differentiation target genes, such as cyclin E (Dong et al., 2007), whereas cell compression would achieve the opposite, possibly explaining the phenomenon of contact inhibition of growth (Zhao et al., 2007). The speed and sensitivity of such mechanical feedback responses, which remains to be fully understood, will no doubt be critical for understanding how cells and tissues respond to the constantly changing mechanical forces during organismal developmental growth.

Concluding remarks and future perspectives

As discussed in this review, changes in form of any tissue reflect active force generation by individual cells and the more or less passive response of the neighbours, to which they are connected by cell-cell adhesions. The balance of such opposing forces, and the timescales required to achieve new equilibrium states, will determine the final morphology of the tissue and the rates of morphogenesis. As we know from looking at animals, the results can be extraordinary.

While it is not known how any one tissue is shaped during development, some simple rules are emerging. First, a relatively small number of molecules appear to provide the forces required to drive a host of morphogenetic processes, most notably actomyosin-based tension, and to couple these forces between cells, E-cadherin-based adherens junctions. While this has been the focus of most attention, the response of cells to stress is just as important a player although much less well understood. This will require exploration of the visco-plastic properties of cells and their response to deformation and mechanical stress. Finally, it is clear that many tissues are patterned when still small enough for processes like diffusion and protrusion-mediated signalling to induce symmetry breaking, before growing. For this reason, mass accumulation plays a major role in morphogenesis. When growth is local, it can, over time, generate enough pressure to drive the deformation of surrounding cells. Thus, morphogenesis of a tissue can be seen like the shape of a single cell, a combination of internal pressure, long-range tension and adhesion to extracellular structures, like the ECM and cuticle.

Nevertheless, since tissues are the products of the individual cells that make them, the cells carry with them all the information to drive. For large animals, patterning often starts when the tissue is sufficiently small for processes like diffusion to create cellular and tissue asymmetries. Once tissues have a fixed identity they must begin to take on their final form. As they do so, the patterning information remembered by individual cells is used to guide tissue shape as it unfolds. Gaining an integrated view of morphogenesis therefore requires inter-disciplinary approaches by groups that study the molecular, cellular and tissue-scale physical factors at work over a range of timescales.

Figure Legends

Figure 1. Balance of forces regulates cell shape.
(A) For single animal cells in isolation, cell shape is a balance of the outward expansion forces from the internal cell pressure (blue arrows) and the inward contractile forces generated by the actomyosin cortex (green arrows). This isotropic balance of forces generates a spherical cell. (B) When a cell adheres to a substrate, adhesion generates additional forces which act on the cell to lower the effective surface tension acting along the cell-substrate interface (red arrows). As a result the cell-substrate contact area increases, causing the cell to spread on the substrate. (C) Cell-cell adhesion forces create a similar effect, lowering inter-cellular tension, and increasing the cell-cell contact area.

Figure 2. Active and passive forces regulate cell and tissue shape.
(A) For a single cell, growth of the cell generates an active outward force (red arrows), which causes the passive stretch of the cell membrane (black arrows). This is analogous to a multicellular tissue context (A') when a central tissue region grows faster then surrounding tissues, such as in the *Drosophila* wing disc pouch. This active tissue expansion force (red arrows) causes the passive stretching of surrounding cells (black arrows). (B) If cells are coupled to neighbouring cells through adhesion molecules, the active contraction of some cells (red arrows) can cause their neighbouring cell to become passively stretched (black arrows). (B') The same effect is observed for collective cell contractions (red arrows). As long as the contracting tissue is coupled to the neighbouring tissue, this active contraction force can cause the passive expansion and stretching of the neighbouring tissue (black arrows), such as during *Drosophila* pupal wing morphogenesis. (C) Active cell expansion (red arrows) can lead to the passive shrinkage of neighbouring cells (black arrows) and eventual cell loss through delamination. This is analogous to tissue crowding forces (red arrows) that can be relaxed by the extrusion of individual cells and tissue regions (black arrows), such as in the *Drosophila* notum (C').

Figure 3. Apical, lateral and basal forces regulate cell shape in an epithelium.
(A). The cell shape of an epithelial cell is regulated by the relative sizes of their apical, lateral and basal surfaces. Apical and basal contractions differences will lead to wedge shaped

cells, whereas concomitant apical and basal contractions will increase the cell height. Contraction of the lateral surface and lateral junctions will reduce cell height, whereas increased lateral adhesion (red arrow) will increase cell height. (B). Cell shape in the plane of the epithelium can be regulated by anisotropies in actomyosin contractility and adhesion. Polarized contractility (green junctions and arrows) will cause these junctions to actively shrink, leading to cell shape changes. In proliferative tissues this can lead to oriented cell divisions (bottom row). In non-proliferative tissues, this can lead to cell intercalations (top row). When cell junctions shrink to a 4-way vertex, the orthogonal junctions grow by polarised expression of adhesion molecules, leading to the polarised expansion of these new junctions (red junctions and arrows)

Figure 4. Mechanical feedback on junctional remodelling
Cells can actively remodel their actomyosin and adhesion complexes in response to mechanical forces. Correlative data suggests that in response to external stretching forces (top row), the junctions under higher tension will selectively upregulate myosin II expression to counteract the stretching force and restore the cell to its original geometry. Conversely, when actomyosin contractility increases cortical tension (bottom row), this leads to an increase in adhesion, which acts to expand the shrinking junctions, and restore the cell to its original geometry.

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