Appendix I. Intercellular mechanotransduction during multicellular morphodynamics

Abstract

Multicellular structures are held together by cell adhesions. Forces that act upon these adhesions play an integral role in dynamically re-shaping multicellular structures during development and disease. Here, we describe different modes by which mechanical forces are transduced in a multicellular context: (a) indirect mechanosensing through compliant substratum, (b) cytoskeletal "tug-of-war" between cell-matrix and cell-cell adhesions, (c) cortical contractility contributing to line tension, (d) stresses associated with cell proliferation, and (e) forces mediating collective migration. These modes of mechanotransduction are recurring motifs as they play a key role in shaping multicellular structures in a wide range of biological contexts. Tissue morphodynamics may ultimately be understood as different spatiotemporal combinations of a select few multicellular transformations, which in turn are driven by these mechanotransduction motifs that operate at the bicellular to multicellular length scale.

Reprinted from Kim, J.-H., L. J. Dooling, and A. R. Asthagiri from *Journal of Royal Society Interface* (2010)

Introduction

The remarkable dynamism of multicellular structures is in full display during development and continues in adult tissues, such as the intestinal epithelium (1) and the mammary gland (2). Meanwhile, disruptions in multicellular morphology, and consequently tissue function, play a major role in diseases such as cancer (3). Elucidating the forces that form and re-shape multicellular structures is integral to our understanding of development and disease and has clear implications for biomedical applications, such as tissue engineering.

Transformations in multicellular structure are achieved through mechanical forces that act upon cell adhesions. Cells adhere to their neighbors and to the surrounding extracellular matrix (ECM). Significant advances have been made in our understanding of the molecular composition of adhesions (4-5) and their mechanosensitivity (6). Acting as mechanosensors and as an interface for force transmission, cell adhesions play a pivotal role in regulating single-cell behaviors, such as rolling (7), spreading and migration (8), survival and proliferation (9), and differentiation (10-11).

Multicellular morphodynamics, however, is not the simple consequence of cell autonomous responses to local forces. Local forces are transmitted over longer length scales and propagate their effects at a mesoscopic level. In this review, we discuss different modes by which mechanical forces are transduced in a multicellular context, ranging from bicellular interactions to larger tissue-scale structures (figure 1). Here, we

use the term “mechnotransduction” broadly to include both this transmission and re-distribution of mechanical forces and the interconversion of mechanical forces and biochemical signals.

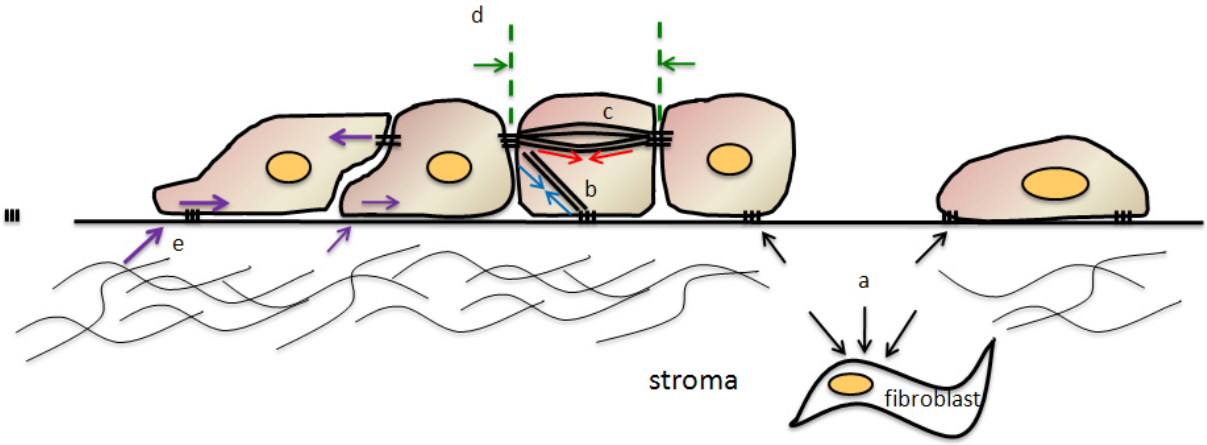

Figure 1: Modes of force transmission in multicellular systems. In a multicellular context, several intercellular mechanotransduction motifs can be identified: (a) indirect mechanosensing through compliant substratum (black arrows), (b) cytoskeletal “tug-of-war” between cell-matrix and cell-cell adhesions (blue arrows), (c) cortical contractility contributing to line tension (red arrows), (d) compressive stresses (green arrows) acting on the planes represented by the dashed lines and resulting from the proliferation of neighboring cells, and (e) forces mediating collective migration (purple arrows) including traction forces, such as those depicted at the leading edge, and tension that is propagated through cell-cell contacts.

1. Indirect cell-cell mechanosensing through a compliant extracellular matrix

An emerging mechanism for cell-cell communication involves exerting and sensing traction forces on the ECM. When a cell contracts, it pulls on its surroundings through integrin-mediated adhesions. This allows the cell to sense the mechanical response of its environment and react appropriately (12-13). As a result, the physical properties of the matrix, in particular its compliance, have a significant effect on cell behaviors such as spreading (14-15), migration (16-18), proliferation (19), and differentiation (10-11). In *in vitro* studies of contractility, substrates of varying compliance are commonly prepared using synthetic polymers, such as polyacrylamide, by varying the extent of crosslinking while keeping the adhesive ligand composition constant (17). Fluorescent beads can be embedded within these substrates, and their displacements are measured to produce a map of the traction forces (20).

It is becoming increasingly apparent that contractile forces generated against the ECM not only influence the behavior of individual cells but also play a role in governing how cells interact with each other. As a cell contracts on a compliant substrate, it produces stress and strain that can be sensed by its neighbors, thus providing a mechanical pathway for cell-cell communication even in the absence of direct contact. Reinhart-King *et al.* (21) have demonstrated this concept by investigating how substrate compliance influences the contact and migratory behaviors of pairs of bovine aortic endothelial cells. Using traction force microscopy, they show that the distance over which a cell significantly deforms its substrate decreases with increasing substrate stiffness, and they postulate that this distance represents the maximum range of ECM-mediated cell-cell mechanosensing. On soft surfaces, two cells that collide remain in

contact throughout the duration of the experiment, most likely because the soft substrate prevents them from generating enough traction force to break the cadherin bonds formed at the cell-cell junction. Conversely, cellular collisions on stiff surfaces are very elastic and cells remain in contact for short durations before migrating away from one another. On substrates of intermediate compliance, a pair of cells repeatedly forms a contact and breaks it. As a result, they exhibit a lower dispersion than isolated cells and fail to migrate beyond the measured distance of significant substrate deformation. This behavior suggests that even after contact is broken, the cells still communicate mechanically through the matrix and that the substrate compliance influences cell-cell interactions.

Cell-cell communication mediated by the ECM has also been observed between human mesenchymal stem cells (hMSC) on fibrin (22). In this case, the communication is believed to involve the strain-stiffening property of nonlinear elastic matrices. The strain produced by cell contraction stiffens the substrate by several orders of magnitude thereby changing the microenvironment of nearby cells. This results in an alignment and elongation of hMSCs cultured on such substrates.

The two previous examples demonstrate how contractile forces generated on the ECM may be responsible for influencing the interactions between cells cultured *in vitro* on compliant substrates. Similar behavior may be observed at the tissue level as well. Epithelial and endothelial cells are often separated from underlying stromal cells by a basement membrane consisting of proteins, such as laminin and collagen. The presence

of stromal cells significantly alters the mechanical properties of the ECM through contractility and matrix remodeling. Elson and colleagues have shown that fibroblasts compress and stiffen collagen gels *in vitro* (23), and that the mechanical properties of the tissue vary with fibroblast concentration (24). These effects can be sensed by the basal surface of the epithelium and endothelium, and may play an important role in tissue homeostasis, development, and tumor progression (25-26).

2. Direct cell-cell interactions and their mechanical interplay with cell-matrix adhesions

While cells are capable of communicating indirectly with each other through the ECM, as cells get close enough to interact directly using cell-cell adhesion receptors, such as cadherins, various short-range modes of crosstalk unfold between cell-cell and cell-matrix adhesions. The differential adhesion paradigm considers the antagonistic interplay between cell-cell and cell-matrix interactions at the level of the cell surface. Steinberg and colleagues observed this antagonism during the transition between aggregation and spread phenotypes of multicellular clusters (27). Cells with minimal cadherin expression level exhibited low cohesivity and a spread phenotype even on substrata that are only moderately adhesive. However, increasing cell-cell cohesivity by raising cadherin expression reverts this spread phenotype and promotes aggregation. Their results demonstrate that tissue spreading is the outcome of a competition between cell-cell cohesivity and cell-substratum adhesivity (figure 2a).

In addition to this antagonism at the level of the cell surface, cell-cell and cell-matrix adhesions are also coupled mechanically through their joint affiliation with the cytoskeleton. At the molecular level, actin cables associate with adherens junctions at cell-cell contacts and provide a physical mechanism for cell-generated contractile forces to act upon cell-cell adhesions. This non-muscle myosin-mediated tension at sites of cell-cell adhesion is necessary for the formation and maturation of cell-cell contacts, which are destabilized upon loss of myosin-generated contractility (28-29). However, excessive contractile forces can compromise cell-cell adhesions (29). Precisely how much contractile force is imposed upon cell-cell adhesions will depend on the level of cell-matrix adhesions, which are also linked to the actin cytoskeleton (figure 2b). In situations where cell-matrix adhesions are enhanced, as observed upon hepatocyte growth factor (HGF) treatment and on stiff substrates, they are better able to withstand contractile forces, while cell-cell adhesions are compromised, thereby promoting cell scatter (29). Consistent with these observations, cells are better able to form multicellular aggregates and undergo tissue-like compaction on a compliant substratum than on stiff substrates (30). Furthermore, using mammary epithelial cells cultured in 3D matrix, Weaver and colleagues showed that increasing matrix stiffness elevates ROCK-generated contractility and FA formations among mammary epithelial cells, in turn weakening adherens junctions and disrupting organized acinar structures (31). In this manner, cell-generated contractile forces mediate a “tug-of-war” between cell-cell adhesions and cell-matrix adhesions that has implications for multicellular organization in both two- and three-dimensional contexts. It is important to note, however, that these forces at cell adhesions may also induce changes in gene expression that contribute to cell scatter. For example,

enhanced adhesion-mediated signaling on stiff surfaces may lead to gene expression patterns facilitating the loss of cell-cell contacts and cell scatter as observed in epithelial-mesenchymal transition (EMT).

The crosstalk between cell-cell and cell-matrix interactions can also promote spatial gradients in mechanical stresses within multicellular structures. Cells at the periphery of a cluster extend their free edge into the surrounding ECM and exert greater traction forces through their adhesions to the matrix than cells in the interior of the cluster. In contrast, interior cells are surrounded by neighbors, and the contractile forces generated within these cells are imposed upon their neighbors through cell-cell contacts. By measuring the deflection of vertical elastomeric micropillars, Chen and colleagues directly quantified the gradient in traction forces in multicellular clusters and correlated this gradient to spatial patterns in proliferation (figure 2c) (32). The introduction of the cytoplasmic-deletion mutant of VE-cadherin, which is defective in linking cadherin to the actin cytoskeleton, ablated the spatial gradient in traction forces and the pattern in cell cycle activity across cell clusters.

In addition to the distribution of traction forces within multicellular aggregates, the level of soluble growth factors, such as epidermal growth factor (EGF), also play an important role in shaping spatial patterns in proliferation. We recently have demonstrated that in epithelial clusters, cadherin-dependent contact-inhibition is enforced only below a critical threshold level of EGF (33). Thus, only when the growth-promoting activity of EGF dips below a threshold, cell-cell contact is able to inhibit effectively the

proliferation of cells in the interior of a cluster, leading to a spatial pattern in proliferation. When EGF concentration is raised above this threshold, epithelial cells exhibit contact-independent, uniform proliferation. Intriguingly, this threshold amount of EGF is tunable: augmenting cell-cell interactions increases the EGF threshold at which the system transitions from contact-inhibited to contact-independent proliferation. Thus, it is evident that crosstalk between hormonal/growth factor pathways and the physical distribution of traction forces is involved in regulating patterns in cell proliferation in epithelial clusters.

The maturation of cell-cell contacts in epithelial sheets can be accompanied by the recruitment of the focal adhesion protein, vinculin, from sites of cell-matrix adhesions to cell-cell junctions. This change in vinculin localization leads to the reorganization of stress fibers associated with focal adhesions at cell-substratum interfaces into cortical bundles that run parallel with cell-cell contacts (34). During epidermal stratification, cortical actin bundles further polarize into the apical plane and form a continuous cytoskeletal network spanning the entire epithelial sheet. Coordinated tension developed through these apical actin cables enables cells to slide under neighboring cells by transiently disrupting their cell-substratum interactions (35).

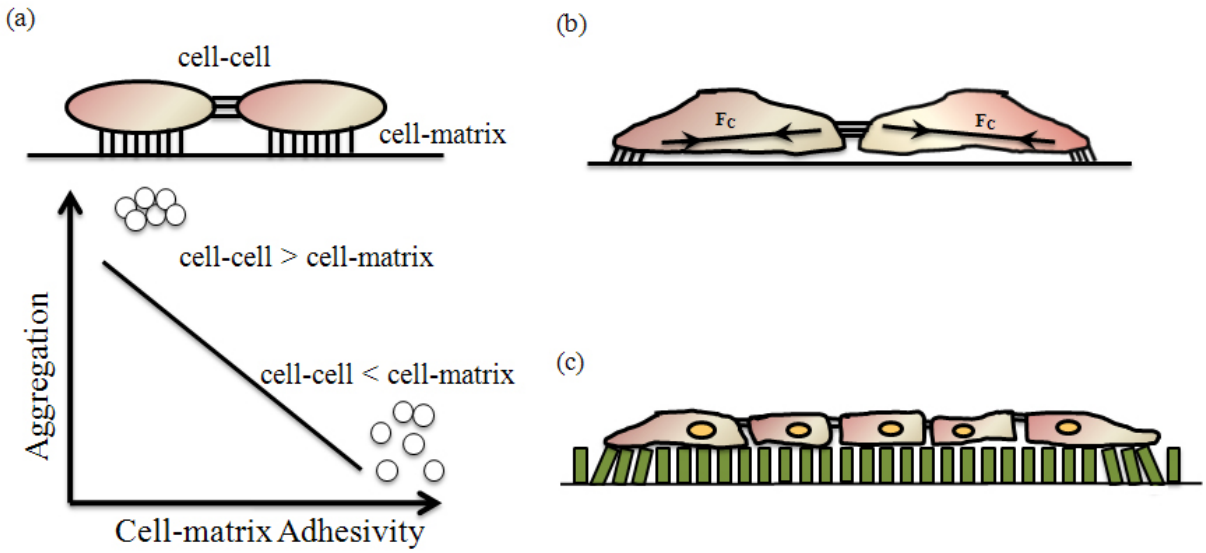

Figure 2: Crosstalk between cell-matrix and cell-cell adhesions. (a) The differential adhesion paradigm states that aggregation is preferred when cell-cell cohesivity outweighs cell-matrix adhesivity, while a spread phenotype is promoted when cell-matrix adhesivity dominates. (b) Cell-generated contractile forces mediate a "tug-of-war" between cell-cell and cell-matrix adhesions. (c) A multicellular implication of (b) is depicted in two-dimensional cell culture on elastomeric micropillars. Peripheral cells exhibit high traction forces on the substrate resulting in the bending of the pillars while cells in the interior of the cluster dissipate cell-generated contractile forces against their neighbors.

3. Cortical contractility and line tension along cell-cell contacts

Cell-generated contractile forces along cortical actin structures in the apical region of epithelial cells also contribute to line tension along cell-cell interfaces. This

line tension plays a significant role in cellular rearrangements during processes such as intercalation in response to external and internal forces, in shaping and sizing cells in growing epithelial sheets and in maintaining multicellular compartments (figure 3).

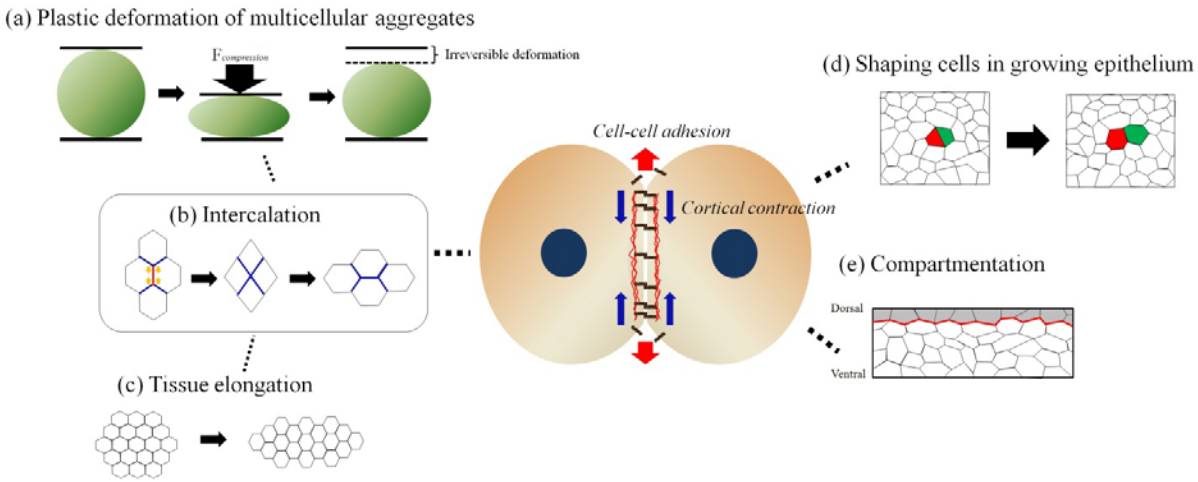

Figure 3: Cortical contractility and interfacial line tension as a recurring motif in multicellular morphodynamics. Cell-generated contractile forces acting along the cortical actin structures are counterbalanced by cell-cell adhesion, contributing to the development of line tension at the interface between cells. (a) This line tension presents an energy barrier to cellular rearrangements under externally applied forces, giving rise to irreversible deformation of multicellular aggregates. (b) Internal asymmetry in local cortical tension drives intercalation involving the collapse of dorsal/ventral junctions (indicted by the red line) followed by the addition of anterior-posterior contacts (indicated by blue lines). (c) Collective cell intercalation propels tissue-wide change in morphology such as tissue elongation. (d) In a growing epithelium, cell division predominantly results in daughter cells sharing an edge and cleaving at a side rather than

a vertex, posing “geometric rules” governing distribution of cell shapes. (e) Anisotropic line tension developed at the boundary of two cell populations (indicated by red line) is responsible for the maintenance multicellular compartmentation such as dorsal-ventral (DV) demarcation of wing imaginal discs.

3a. Line tension as an energy barrier for plastic deformation

At a macroscopic scale, line tension is involved in the plasticity of multicellular aggregates (i.e., irreversible shape change of the aggregates) exposed to external compressive load (36). Line tension provides an energy barrier for cellular rearrangements within the aggregates. Cell aggregates under high compressive stress overcome this barrier and undergo not only elastic cell shape change, but also cellular rearrangements involving shuffling of cells (intercalation). These cellular rearrangements persist even after the imposed external stress is removed, rendering a plastic deformation (figure 3a). In contrast, in a low stress regime where line tension is not overcome, aggregates exhibit only cell shape changes through spontaneous membrane fluctuations, and when the external force is removed, the original aggregate shape is recovered.

3b. Line tension in intercalation

Asymmetric line tension provides the driving force for intercalation during germband elongation in *Drosophila* embryos (37). In this process, the epithelial tissue elongates along the anterior/posterior axis through the intercalation of cells along the dorsal/ventral axis. This process involves the shrinkage of dorsal/ventral contacts (v-junctions) followed by the establishment of new cell-cell contacts parallel to the anterior-

posterior axis (t-junctions) (figure 3b). Myosin-II is preferentially localized at v-junctions (38), and this localization corresponds to greater tension at v-junctions than along t-junctions as quantified by local laser ablation and the consequent recoil speed of the cell-cell interface (37). This asymmetry in local cortical tension drives the tissue-wide change in morphology (figure 3c). Furthermore, this tension increases as the v-junction collapses, suggesting that cortical elasticity is also a critical factor.

3c. Line tension in shaping cells in growing epithelium

During the intercalation process described above, the number of epithelial cells remains fixed and the predominant activity involves the relative shuffling in position of cells within the epithelium. In other situations, the epithelium undergoes a significant change in cell number while the relative position of cells does not change markedly. An important feature of such growing epithelial sheets is the distribution of polygonal cell shapes. While most cells are hexagonal, there are also significant numbers of cells with a shape that ranges from quadrilateral to octagon. Gibson *et al.* (39) show that simple "geometric rules" of epithelial cell divisions are sufficient to predict the distribution of polygonal shapes in the developing epithelial wing primordium of *Drosophila melanogaster*. These rules were based on observations such as the following: The vast majority of epithelial divisions (94%) result in daughter cells that share an edge, and cell divisions tend to cleave a side rather than a vertex (figure 3d). A Markov model based on these and other geometric rules predicts that a growing epithelial sheet reaches a distribution of polygonal shapes consistent with that observed in developing wings. In fact, the predicted distribution of cell shapes matches that observed in epithelial tissues

from vertebrate, arthropod, and cnidarian organisms, suggesting that a common set of geometric division rules governs the shapes of epithelial cells in growing tissues throughout the metazoa.

It should be noted, however, that the distribution of polygonal shapes is not the only feature of interest in a growing epithelium. For example, the average size of a cell increases as cell number increases, and cells occasionally desorb or delaminate from the epithelium. Furthermore, the geometric constraints of cell divisions likely arise from mechanical forces and biophysical properties, such as membrane elasticity, contractility and cell-cell adhesion. This raises the question of how these forces and biophysical properties shape cells within a growing epithelium. Farhadifar *et al.* (40) examined this issue by developing a model in which the positions of vertices in a growing epithelium are determined by the minimization of energy associated with the contractility of the cortical actin-myosin network, line tension along apical junctions and cell surface elasticity. Their model predictions of frequency of cell delamination, cell area and polygonal shapes matched those in the developing *Drosophila* wing disc only for specific ranges of parameter values. These results suggest that the biophysical properties of epithelial cells are wired to give rise to the observed cell shapes in growing epithelial tissues. It would be interesting to determine whether these parameter values are also necessary to give rise to the geometric rules of cell divisions used in the Gibson model.

3d. Line tension in compartmentation

Anisotropic line tension is involved not only in local re-shuffling of neighboring cells during intercalation (figure 3b), but also in maintaining long range barriers between two cell populations (figure 3e). This role of partitioning cell populations was first suggested in the context of dorsal-ventral (DV) demarcation of wing imaginal discs (41-42). More recently, the magnitude of anisotropic line tensions has been directly measured and computationally modeled in anterior-posterior (AP) demarcation of wing imaginal discs (43-44), and eliminating this line tension has been shown to compromise the re-establishment of anterior-posterior compartmentation following cell divisions at this interface during *Drosophila* embryonic development (45). Consistent with the idea that anisotropic line tension may be a recurring motif for maintaining cell compartments, the above studies span AP and DV compartmentation in *Drosophila* wing discs and AP compartmentation in *Drosophila* embryonic development.

3e. Contractility and cell-cell adhesion: opposing contributions to line tension?

In the above models of line tension along cell-cell junctions, contractility opposes cell-cell adhesion (figure 3). However, there is some evidence that contractility can influence the endocytosis of cell adhesion receptors (46) while planar cell polarity (PCP) proteins regulate the exocytosis and recycling of cell adhesion proteins (47). Furthermore, in the case of cell adhesion to the extracellular matrix, contractility is essential to forming and maintaining focal adhesions; in a similar manner, contractile forces are involved in promoting the maturation of cell-cell adhesions (48). Thus, it remains an open question to what extent cortical contractility and cell-cell adhesion ought to be viewed as independent opposing contributions to line tension.

4. Forces associated with cell behaviors

The loss, accrual and movement of cells due to apoptosis, proliferation, and migration, respectively, can generate local forces on direct neighbors and even propagate to affect tissue morphology at a mesoscopic scale.

4a. Forces associated with apoptosis

The extrusion of apoptotic cells from an epithelial sheet has been observed in the context of various developmental processes and is essential to maintain the integrity of the epithelium and its barrier function. Rosenblatt *et al.* (49) showed that an apoptotic cell within an epithelial layer rapidly develops an actomyosin ring around its periphery and signals to its neighboring cells to induce actin cable formation at the interface between the apoptotic cell and neighboring live cells (figure 4a). Rho-mediated contraction of these actin cables pulls neighboring cells toward the apoptotic cell and extrudes the apoptotic cell out of its parental epithelia, rapidly sealing the opening that could have been left by the removal of the dead cell. In fact, selective blocking of Rho activity in neighboring cells aborted the extrusion of the apoptotic cells completely, disrupting the integrity of epithelia. Thus, apoptotic force involves not only an autonomous contractile force in a cell undergoing the death, but also collective force developed among live cells surrounding the apoptotic cell.

Such forces involved in the extrusion of apoptotic cells also propagate through cell-cell interactions to affect the long-range morphology of tissues. An example involves dorsal closure of the *Drosophila* embryo. During this process, an elliptical opening in the dorsal epidermis is occupied by the amnioserosa and is covered by two dorsally migrating epithelial leading edges with the two flanks advancing along the dorsal midline (figure 4b). A precise coordination of forces, including the contractility of the amnioserosa, contributes to sheet migration and dorsal closure (50) . The apoptosis of amnioserosa cells contributes significantly to the contractility of this tissue and thus the rate of dorsal closure (51). By quantitatively comparing the recoiling velocity of the leading edge of lateral epidermis upon laser ablation in wild type and apoptotic mutants, it was estimated that apoptosis of aminoserosa cells accounts for approximately one-third to one-half of the net force developed at the leading edge of lateral epidermis. The contractile forces involved in extruding apoptotic cells may be transmitted by cell-cell contacts to the lateral epidermis, contributing the force needed for dorsal migration of lateral epithelia and fusion.

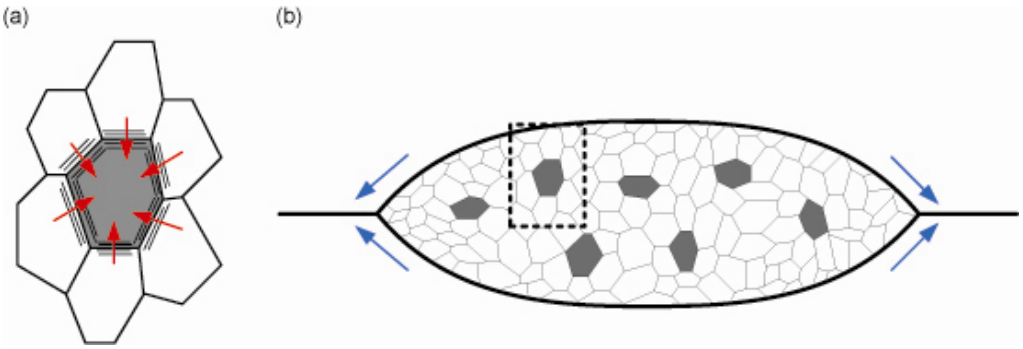

Figure 4: Forces associated with apoptosis and their implications for *Drosophila* dorsal closure. (a) An actomyosin ring forms in both the cell undergoing apoptosis (grey) and its live neighbors. Contractile forces along this actomysin ring generate a force (red arrows) that pulls neighboring cells into the space occupied by the extruded cell and prevents gaps in the epithelium. (b) This force associated with apoptosis contributes to dorsal closure in the *Drosophila* embryo. The contractile forces generated by apoptotic cells (grey) in the amnioserosa (AS) contribute to tension (blue arrows) along the leading edge of the lateral epithelia tissues (LET).

4b. Mechanical stresses imposed by proliferation

In a growing tissue in which cellular rearrangements are restricted in the time scale of cell division, mechanical stresses imposed by an increase in cell mass (i.e., proliferation) are not fully released and thus rapidly accumulate in a local environment. One of the phenotypic features of rapidly growing tissue is that cell spreading against underlying substrate decreases with increasing cell density. Restricted cell spreading further correlates with decrease in stress fiber formation, which in turn destabilizes focal adhesions. Consistent with these changes, when plated on varying sizes of adhesive patterns consisting of micropillars, cells grown on smaller islands exhibited significantly reduced cytoskeletal tension and contraction force (52). In addition, accumulation of mechanical stresses accompanying aforementioned events has been correlated with cell cycle arrests in high density culture (53).

Notably, coupled with other microenvironmental factors, force induced by proliferation plays a central role in patterning multicellular behaviors in the context of developmental processes (figure 5). Patterning and growth regulation of *Drosophila* wing imaginal discs involves the gradients of morphogens including Decapentaplegic (Dpp). However, while it is clear how reduced morphogen concentration far from the source would halt cell proliferation at the edge of a developing tissue, how cell proliferation and tissue growth stop near the morphogen source remained unclear. Shraiman (54) theoretically showed that at the region of high morphogen concentration, mechanical stresses rapidly accumulate as a result of a high rate of proliferation. This accumulated mechanical stress in turn inhibits morphogen-induced proliferation. Thus, once cell proliferation ceases at the edge of a developing tissue due to low morphogen concentration, continued proliferation near the morphogen source would escalate the local mechanical stress and stop the growth of tissue as a whole. Thus, mechanical stresses would serve as a local negative regulator of growth, thereby affecting growth patterns and organ size (55).

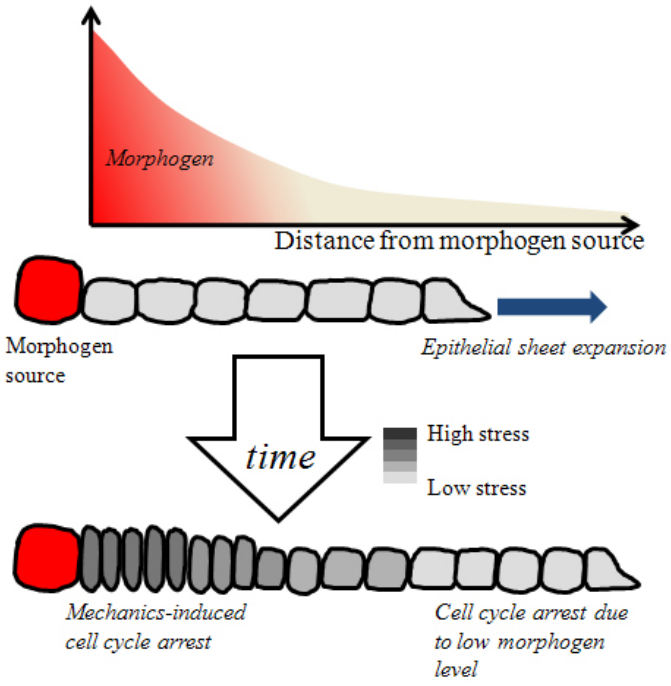

Figure 5. Role of proliferation-induced mechanical stresses in growth patterning and organ size determination. During development of *Drosophila* wing imaginal discs, spatial gradients in morphogens regulating cell growth are established by their localized secretion and transport from source cells (red cell). The graph depicts the steady-state gradient in morphogen concentration as a function of distance from the morphogen source. Below the graphs, grayscale color gradients in the cells indicate the level of mechanical stress due to crowding. At early stages of the development (top cell array), cells proliferate uniformly, expanding the epithelium without local accumulation of mechanical stresses. Later, cell growth ceases at the edge of the epithelium due to low morphogen level (bottom cell array), and mechanical stresses accumulate near the morphogen source as a result of imbalanced growth. It is postulated that this mechanical

stress may desensitize cells to the locally high levels of growth factor and lead to mechanics-induced cell cycle arrest (Shraiman 2005).

4c. Forces driving collective migration

The mechanics of migration in single cells have been widely studied, revealing the importance of protrusive forces that drive the extension of the leading edge of the cell and contractile forces that detach the trailing edge and pull the cell body forward (56). However, less is known about the mechanics of sheets and strands of cells moving together, a process known as collective migration. Given that these cells not only adhere to the surrounding matrix but also remain in contact with each other through cell-cell adhesion proteins, such as cadherins, one would expect the interplay between mechanical forces involved in cell-cell and cell-matrix adhesion to play a major role in the behavior of such systems. Understanding how collective migration forces are generated and transmitted between cells has important implications in disease and physiology.

Collective migration is a key phenomenon in tissue morphogenesis and is widely observed in developing organisms (57-58). Wound healing is a classic example of collective motility, and *in vitro* assays of this process have provided a powerful model system to study the movement of two-dimensional cell sheets. Other examples include border cell motility during *Drosophila* ovary development (59-60) and branching morphogenesis in mammary epithelia (61).

Two important questions arise concerning how the forces that lead to wound closure are generated. The first is whether wound healing is driven predominantly by proliferation within the monolayer that pushes it forward or whether cell migration propels the healing process by pulling the sheet into the wound. The emerging consensus appears to be that cell migration at the healing front is the key driver with proliferation helping to maintain the monolayer (62). Several studies have suggested a leader-follower model wherein the leading cells at the wounded edge migrate and pull along the trailing cells. For example, in both IAR-2 and MDCK epithelial sheets exposed to model wounds, leaders temporarily lose their epithelial character and develop lamellipodia and focal adhesions that protrude into the wound (63-64). Leader-follower behavior is also observed in a wounded endothelial monolayer in the presence of fibroblast growth factor (65).

Rho-dependent cytoskeleton reorganization appears to play a significant role in the leader-follower model of wound healing. Omelchenko *et al.* (2003) note that leader cells disassemble their cortical actin cables upon wounding and reorient filaments perpendicular to the advancing front. Follower cells maintain their cortical actin cables but exhibit radial, rather than tangential, cell-cell contacts with leaders, indicating that tension is generated by the leader cells. Pojade *et al.* (2007) observe similar behavior in leader cells and also note the development of a supracellular actin belt in follower cells that may transmit force as in the purse-string wound closure mechanism.

If we accept the notion that wound healing is driven primarily by cell migration rather than proliferation, a second question that arises is where the traction forces necessary for migration are generated. In the simplest leader-follower model, traction forces would be generated by the first row of cells (i.e., the leaders) so that followers need only to release their attachments and be pulled forward. Recent findings, however, suggest that this is not the case and that instead, the traction forces involved in propelling wound healing may be generated by cells much farther into the monolayer (66). Thus, in growing MDCK sheets, significant traction forces are observed far away from the leading edge. Furthermore, a force balance shows that the tensional stress is propagated into and accumulates within the sheet, suggesting long-range transmission of forces from the leading edge into the interior of a growing epithelial sheet (67). This view may be supported by the observation of cryptic lamellipodia protruding from submarginal cells in the direction of the wound as well as the ability for these cells to compensate for a loss of motility in the first row of the advancing edge (68-69).

The observations from these studies suggest that collective sheet migration and wound healing may occur by different modes depending on the tissue environment. In one extreme, cells within a monolayer may behave nearly autonomously and generate their own motile forces (70). While leader cells may be present in such cases, they act primarily to guide or polarize their followers to move in the direction of the wound. In the other extreme, leader cells may exert enough force to physically drag follower cells behind them (71). Most observations appear to suggest a mode in which both behaviors are important. As a result, the migration of each cell arises from its own traction forces

as well as the forces exerted by its neighbors. The relative strength of these forces could depend on a number of factors, including monolayer size and density, the strength of cell-cell adhesions, the matrix over which the sheet is migrating, and the presence of soluble factors. Such behavior would be consistent with the recent hypothesis that collective morphogenic movements are controlled *in vivo* by modular mechanical properties (72).

5. Concluding remarks

The modes of force propagation described in this review are recurring motifs as they contribute to morphodynamics across several distinct multicellular contexts. An intriguing possibility is that these and other force transmission modalities may enable a well-defined set of multicellular transformations. Indeed, seemingly diverse morphological patterns observed *in vivo* may be an outcome of different coupling and executions of these common motifs. For example, diverse epithelial morphogenetic phenotypes observed during dorsal closure and germband extension in the *Drosophila* embryo and during convergence of the zebrafish trunk neural ectoderm are simply quantitative combinations of cellular deformation and intercalation (Blanchard *et. al.* 2009). The rapidly growing interest in dynamical imaging of development in several model organisms should add to these findings and provide a more complete description of possible multicellular transformations. Tissue morphodynamics may ultimately be understood as different spatiotemporal combinations of a select few multicellular transformations, which in turn are driven by a small group of mechanotransduction motifs that operate at the bicellular to multicellular length scale.

References

1. van der Flier LG & Clevers H (2009) Stem Cells, Self-Renewal, and Differentiation in the Intestinal Epithelium. *Ann Rev Physiol* 71(1):241-260
2. Nelson CM & Bissell MJ (2006) Of extracellular matrix, scaffolds, and signaling: Tissue architecture regulates development, homeostasis, and cancer. *Ann Rev Cell Dev Bi* 22(1):287-309
3. Debnath J & Brugge JS (2005) Modelling glandular epithelial cancers in three-dimensional cultures. *Nat Rev Cancer* 5(9):675-688
4. Zaidel-Bar R, Itzkovitz S, Ma'ayan A, Iyengar R, & Geiger B (2007) Functional atlas of the integrin adhesome. *Nat Cell Biol* 9(8):858-867
5. Zamir E & Geiger B (2001) Molecular complexity and dynamics of cell-matrix adhesions. *J Cell Sci* 114(20):3583-3590
6. Geiger B, Spatz JP, & Bershadsky AD (2009) Environmental sensing through focal adhesions. *Nat Rev Mol Cell Biol* 10(1):21-33
7. Chang K-C, Tees DFJ, & Hammer DA (2000) The state diagram for cell adhesion under flow: Leukocyte rolling and firm adhesion. *Proc Natl Acad Sci USA* 97(21):11262-11267
8. Beningo KA, Dembo M, Kaverina I, Small JV, & Wang Y-L (2001) Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts. *J Cell Biol* 153(4):881-888
9. Chen CS, Mrksich M, Huang S, Whitesides GM, & Ingber DE (1997) Geometric control of cell life and death. *Science* 276(5317):1425-1428

10. Engler AJ, *et al.* (2004) Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J Cell Biol* 166(6):877-887

11. Engler AJ, Sen S, Sweeney HL, & Discher DE (2006) Matrix Elasticity Directs Stem Cell Lineage Specification. *Cell* 126(4):677-689

12. Discher DE, Janmey P, & Wang Y-l (2005) Tissue Cells Feel and Respond to the Stiffness of Their Substrate. *Science* 310(5751):1139-1143

13. Vogel V & Sheetz M (2006) Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Biol* 7(4):265-275

14. Engler A, *et al.* (2004) Substrate compliance versus ligand density in cell on gel responses. *Biophys J* 86(1):617-628

15. Yeung T, *et al.* (2005) Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cell Motil Cytoskel* 60(1):24-34

16. Lo C-M, Wang H-B, Dembo M, & Wang Y-L (2000) Cell Movement Is Guided by the Rigidity of the Substrate. *Biophys J* 79(1):144-152

17. Pelham RJ & Wang Y-l (1997) Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci USA* 94(25):13661-13665

18. Peyton SR & Putnam AJ (2005) Extracellular matrix rigidity governs smooth muscle cell motility in a biphasic fashion. *J Cell Physiol* 204(1):198-209

19. Wang H-B, Dembo M, & Wang Y-L (2000) Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am J Physiol Cell Physiol* 279(5):C1345-1350

20. Dembo M & Wang Y-L (1999) Stresses at the Cell-to-Substrate Interface during Locomotion of Fibroblasts. *Biophys J* 76(4):2307-2316

21. Reinhart-King CA, Dembo M, & Hammer DA (2008) Cell-Cell Mechanical Communication through Compliant Substrates. *Biophys J* 95(12):6044-6051

22. Winer JP, Oake S, & Janmey PA (2009) Non-Linear Elasticity of Extracellular Matrices Enables Contractile Cells to Communicate Local Position and Orientation. *PLoS ONE* 4(7):e6382

23. Wakatsuki T, Kolodney MS, Zahalak GI, & Elson EL (2000) Cell Mechanics Studied by a Reconstituted Model Tissue. *Biophys J* 79(5):2353-2368

24. Marquez J, Genin G, Pryse K, & Elson E (2006) Cellular and Matrix Contributions to Tissue Construct Stiffness Increase with Cellular Concentration. *Ann Biomed Eng* 34(9):1475-1482

25. Grinnell F (2003) Fibroblast biology in three-dimensional collagen matrices. *Trends in Cell Biology* 13(5):264-269

26. Lopez JI, Mouw JK, & Weaver VM (2008) Biomechanical regulation of cell orientation and fate. *Oncogene* 27(55):6981-6993

27. Ryan PL, Foty RA, Kohn J, & Steinberg MS (2001) Tissue spreading on implantable substrates is a competitive outcome of cell-cell vs. cell-substratum adhesivity. *Proc Natl Acad Sci USA* 98(8):4323-4327

28. Conti MA, Even-Ram S, Liu C, Yamada KM, & Adelstein RS (2004) Defects in cell adhesion and the visceral endoderm following ablation of nonmuscle myosin heavy chain II-A in mice. *J Biol Chem* 279(40):41263-41266

29. de Rooij J, Kerstens A, Danuser G, Schwartz MA, & Waterman-Storer CM (2005) Integrin-dependent actomyosin contraction regulates epithelial cell scattering. *J Cell Biol* 171(1):153-164

30. Guo WH, Frey MT, Burnham NA, & Wang YL (2006) Substrate rigidity regulates the formation and maintenance of tissues. *Biophys J* 90(6):2213-2220

31. Paszek MJ, *et al.* (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8(3):241-254

32. Nelson CM, *et al.* (2005) Emergent patterns of growth controlled by multicellular form and mechanics. *Proc Natl Acad Sci USA* 102(33):11594-11599

33. Kim J-H, Kushiro K, Graham NA, & Asthagiri AR (2009) Tunable interplay between epidermal growth factor and cell-cell contact governs the spatial dynamics of epithelial growth. *Proc Natl Acad Sci USA* 106(27):11149-11153

34. Maddugoda MP, Crampton MS, Shewan AM, & Yap AS (2007) Myosin VI and vinculin cooperate during the morphogenesis of cadherin cell cell contacts in mammalian epithelial cells. *J Cell Biol* 178(3):529-540

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

36. Marmottant P, *et al.* (2009) The role of fluctuations and stress on the effective viscosity of cell aggregates. *Proc Natl Acad Sci USA* 106(41):17271-17275

37. Rauzi M, Verant P, Lecuit T, & Lenne PF (2008) Nature and anisotropy of cortical forces orienting Drosophila tissue morphogenesis. *Nat Cell Biol* 10(12):1401-1410

38. Lecuit T & Lenne PF (2007) Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nat Rev Mol Cell Biol* 8(8):633-644

39. Gibson MC, Patel AB, Nagpal R, & Perrimon N (2006) The emergence of geometric order in proliferating metazoan epithelia. *Nature* 442(7106):1038-1041

40. Farhadifar R, Roper JC, Aigouy B, Eaton S, & Julicher F (2007) The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. *Curr Biol* 17(24):2095-2104

41. Major RJ & Irvine KD (2005) Influence of Notch on dorsoventral compartmentalization and actin organization in the Drosophila wing. *Development* 132(17):3823-3833

42. Major RJ & Irvine KD (2006) Localization and requirement for Myosin II at the dorsal-ventral compartment boundary of the Drosophila wing. *Dev Dyn* 235(11):3051-3058

43. Landsberg KP, *et al.* (2009) Increased cell bond tension governs cell sorting at the Drosophila anteroposterior compartment boundary. *Curr Biol* 19(22):1950-1955

44. Vincent JP & Irons D (2009) Developmental biology: tension at the border. *Curr Biol* 19(22):R1028-1030

45. Monier B, Pelissier-Monier A, Brand AH, & Sanson B (2010) An actomyosin-based barrier inhibits cell mixing at compartmental boundaries in Drosophila embryos. *Nat Cell Biol* 12(1):60-65; sup pp 61-69

46. Sahai E & Marshall CJ (2002) ROCK and Dia have opposing effects on adherens junctions downstream of Rho. *Nat Cell Biol* 4(6):408-415

47. Classen AK, Anderson KI, Marois E, & Eaton S (2005) Hexagonal packing of Drosophila wing epithelial cells by the planar cell polarity pathway. *Dev Cell* 9(6):805-817

48. Yamada S & Nelson WJ (2007) Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. *J Cell Biol* 178(3):517-527

49. Rosenblatt J, Raff MC, & Cramer LP (2001) An epithelial cell destined for apoptosis signals its neighbors to extrude it by an actin- and myosin-dependent mechanism. *Curr Biol* 11(23):1847-1857

50. Kiehart DP, Galbraith CG, Edwards KA, Rickoll WL, & Montague RA (2000) Multiple forces contribute to cell sheet morphogenesis for dorsal closure in Drosophila. *J Cell Biol* 149(2):471-490

51. Toyama Y, Peralta XG, Wells AR, Kiehart DP, & Edwards GS (2008) Apoptotic force and tissue dynamics during Drosophila embryogenesis. *Science* 321(5896):1683-1686

52. Tan JL, *et al.* (2003) Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc Natl Acad Sci USA* 100(4):1484-1489

53. Liu WF, Nelson CM, Pirone DM, & Chen CS (2006) E-cadherin engagement stimulates proliferation via Rac1. *J Cell Biol* 173(3):431-441

54. Shraiman BI (2005) Mechanical feedback as a possible regulator of tissue growth. *Proc Natl Acad Sci USA* 102(9):3318-3323

55. Hufnagel L, Teleman AA, Rouault H, Cohen SM, & Shraiman BI (2007) On the mechanism of wing size determination in fly development. *Proc Natl Acad Sci USA* 104(10):3835-3840

56. Lauffenburger DA & Horwitz AF (1996) Cell Migration: A Physically Integrated Molecular Process. *Cell* 84(3):359-369

57. Friedl P & Gilmour D (2009) Collective cell migration in morphogenesis, regeneration and cancer. *Nat Rev Mol Cell Biol* 10(7):445-457

58. Rørth P (2009) Collective Cell Migration. *Ann Rev Cell Dev Bi* 25(1):407-429

59. Montell DJ (2003) Border-cell migration: the race is on. *Nat Rev Mol Cell Biol* 4(1):13-24

60. Rørth P (2002) Initiating and guiding migration: lessons from border cells. *Trends Cell Biol* 12(7):325-331

61. Ewald AJ, Brenot A, Duong M, Chan BS, & Werb Z (2008) Collective Epithelial Migration and Cell Rearrangements Drive Mammary Branching Morphogenesis. *Dev Cell* 14(4):570-581

62. Gov NS (2007) Collective cell migration patterns: Follow the leader. *Proc Natl Acad Sci USA* 104(41):15970-15971

63. Omelchenko T, Vasiliev JM, Gelfand IM, Feder HH, & Bonder EM (2003) Rho-dependent formation of epithelial "leader" cells during wound healing. *Proc Natl Acad Sci USA* 100(19):10788-10793

64. Poujade M, *et al.* (2007) Collective migration of an epithelial monolayer in response to a model wound. *Proc Natl Acad Sci USA* 104(41):15988-15993

65. Vitorino P & Meyer T (2008) Modular control of endothelial sheet migration. *Gene Dev* 22(23):3268-3281

66. Trepat X, *et al.* (2009) Physical forces during collective cell migration. *Nat Phys* 5(6):426-430

67. Ladoux B (2009) Biophysics: Cells guided on their journey. *Nat Phys* 5(6):377-378

68. Farooqui R & Fenteany G (2005) Multiple rows of cells behind an epithelial wound edge extend cryptic lamellipodia to collectively drive cell-sheet movement. *J Cell Sci* 118(1):51-63

69. Fenteany G, Janmey PA, & Stossel TP (2000) Signaling pathways and cell mechanics involved in wound closure by epithelial cell sheets. *Curr Biol* 10(14):831-838

70. Bindschadler M & McGrath JL (2007) Sheet migration by wounded monolayers as an emergent property of single-cell dynamics. *J Cell Sci* 120(5):876-884

71. Friedl P, Hegerfeldt Y, & Tusch M (2004) Collective cell migration in morphogenesis and cancer. *Int J Dev Biol* 48:441-449

72. Montell DJ (2008) Morphogenetic Cell Movements: Diversity from Modular Mechanical Properties. *Science* 322(5907):1502-1505