OPINION

Transduction of mechanical and cytoskeletal cues by YAP and TAZ

Georg Halder, Sirio Dupont and Stefano Piccolo

Abstract | The physical and mechanical properties of the cellular microenvironment regulate cell shape and can strongly influence cell fate. How mechanical cues are sensed and transduced to regulate gene expression has long remained elusive. Recently, cues from the extracellular matrix, cell adhesion sites, cell shape and the actomyosin cytoskeleton were found to converge on the regulation of the downstream effectors of the Hippo pathway YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif) in vertebrates and Yorkie in flies. This convergence may explain how mechanical signals can direct normal and pathological cell behaviour.

In the past 20 years, the study of signal transduction pathways has been a focus of intense research. The main growth factor pathways are now well characterized, and signalling networks of protein–protein interactions and gene expression programmes that control cell behaviour are starting to be delineated. However, cell behaviour is not only governed by chemical signals. Tissue architecture and mechanical forces are also overarching signals that inform cell decisions. Key elements of such architectural signals are cell–extracellular matrix (ECM) and cell–cell adhesions, the organization of the cytoskeleton and tensional forces that keep individual cells and whole tissues in a certain shape. These elements control cell proliferation, cell migration, stem cell identity, differentiation and cell death. Thus, such geometrical and mechanical signals add an additional dimension to cell signalling that may convey information about global tissue properties, such as tissue size, into the behaviour of individual cells[1–7].

The activity of YAP (Yes-associated protein), TAZ (transcriptional co-activator with PDZ-binding motif; also known as WWTR1) and their fly homologue Yorkie has recently been linked to the nuclear transduction of mechanical and cytoskeletal signals[8–12]. YAP, TAZ and Yorkie are transcriptional cofactors that shuttle between the cytoplasm and the nucleus where they associate with several promoter-specific transcription factors. TEA domain family member (TEAD) transcription factors emerged as the main partners of YAP and TAZ on DNA, although RUNX2, T-box 5 (TBX5) and p73 have also been reported to interact with YAP and TAZ to regulate gene expression[13–15]. YAP, TAZ and Yorkie have key roles in cell proliferation, survival, differentiation, tissue regeneration and organ size determination. Beside these physiological roles, growing evidence links the activity of YAP and TAZ to tumorigenesis, induction of cancer stem cells and chemoresistance[13–16]. Genetic data in mammals indicate that YAP and TAZ perform largely overlapping functions; yet, these closely related proteins may also display specificities in their activity or interacting partners[17].

> “Tissue architecture and mechanical forces are overarching signals that inform cell decisions.”

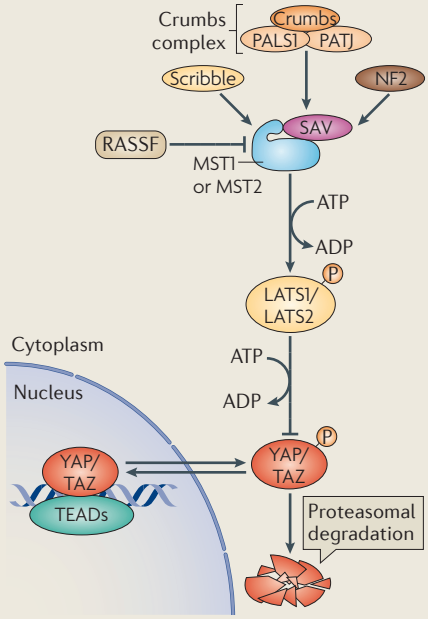
Because of their potent biological relevance in various contexts, the identification of the upstream inputs that regulate YAP, TAZ and Yorkie is the focus of intense research. The best characterized regulators of YAP, TAZ and Yorkie are the components of the Hippo pathway (BOX 1). The Hippo pathway entails the inhibitory activity of mammalian STE20-like protein kinase 1 (MST1) and MST2 (also known as STK4 and STK3, respectively; which are the mammalian homologues of Hippo in *Drosophila melanogaster*) as well as Large tumour suppressor homologue 1 (LATS1) and LATS2 (which are the mammalian homologues of *D. melanogaster* Warts). LATS1 and LATS2 phosphorylate YAP and TAZ (or Yorkie in flies), thereby promoting their cytoplasmic relocalization or degradation[13–15].

This Opinion article focuses on the links between YAP and TAZ (or Yorkie) activity and mechanical signals. Following a brief overview of some of the seminal findings that contributed to the early development of the field of mechanobiology (the field that studies the effect of physical forces such as stretching, compression and shear stress on living systems), we examine how the actomyosin cytoskeleton may be involved in the translation of such mechanical cues into biochemical signals. Next, we compare the mechanisms that are involved in the regulation of YAP and TAZ (or Yorkie) activity by the Hippo pathway and cytoskeletal dynamics. We propose that crosstalk between these parallel inputs integrates signalling, architectural and structural cues at the tissue level. Finally, we discuss examples of how such integration may advance our understanding of physiology and disease.

Mechanical signals control cell fate

Mechanical signals are highly pervasive in biology. Every cell responds to the mechanical properties of its environment, such as the elasticity (or stiffness) of the ECM and traction or compression forces exerted by neighbouring cells[1–7]. Thus, forces are constantly transmitted across cell–ECM and cell–cell adhesion sites. The cell balances these external forces by adjusting the stiffness of its cytoskeleton[18–20]. Reciprocally, forces generated inside the cell by the contraction of the actomyosin cytoskeleton are transmitted across adhesion sites to surrounding structures. Thus, the cytoskeleton

Box 1 | **The Hippo pathway in organ growth control**



The core components of the Hippo pathway are mammalian STE20-like protein kinase 1 (MST1) and MST2 (known as Hippo in *Drosophila melanogaster*)[83–86], their cofactor Salvador (SAV; also known as WW45)[87,88] and Large tumour suppressor homologue 1 (LATS1) and LATS2 (known as Warts in *D. melanogaster*)[89,90] (see the figure). The Hippo pathway becomes activated when MST kinases (or Hippo) phosphorylate (P) LATS proteins (or Warts), which then phosphorylate YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif) (or Yorkie in *D. melanogaster*) on multiple sites[50,91–94]. Phosphorylated YAP and TAZ are excluded from the nucleus and accumulate in the cytoplasm, where they are degraded by the proteasome. When the Hippo pathway is inactivated, YAP and TAZ are dephosphorylated, accumulate in the nucleus and regulate gene transcription together with DNA-binding transcription factors such as TEADs (TEA domain family members)[55,82,95–101].

In *D. melanogaster*, mutations in most of the Hippo pathway components result in overgrown adult structures[13,15,53]. Similarly, mice with conditionally ablated MST1 and MST2 function exhibit overgrowth of organs, including the liver and the heart, and hyperproliferation of the intestinal epithelium as a result of YAP hyperactivation[56,81,102].

The core components of the Hippo pathway are regulated by several inputs, including cell–cell adhesions and apicobasal polarity complexes[13–15,52,53]. Neurofibromin 2 (NF2; also known as Merlin) binds to multiple upstream components of the pathway (including MST1, MST2 and SAV) to promote phosphorylation of LATS1 and LATS2 (REFS 103–105) (see the figure). NF2 deficiency leads to increased YAP activity in the eye lens epithelium and in the liver, which overgrows[106].

Yorkie-dependent overgrowth phenotypes are also observed in *D. melanogaster* mutants of the Discs-large basolateral polarity complex (comprising the proteins Scribble, Discs-large and Lethal giant larvae; not shown in the figure)[107–111]. In mammals, scribble associates with TAZ, LATS1 and MST2 and promotes LATS protein activation, TAZ phosphorylation,TAZ association with the ubiquitin ligase β-TRCP1 and its degradation (not shown in the figure)[16]. Proteins of the crumbs apical-polarity complex (comprising crumbs, PALS1 (protein associated with Lin-7 1) and PATJ (PALS1-associated tight junction protein) interact with YAP and TAZ and regulate the Hippo pathway in both mammals and flies[79,107,112–115]. Finally, RASSF (RAS association domain-containing family) interacts with MST1 and MST2 and negatively regulates the Hippo pathway[116–118].

rapidly senses and adapts to changes in the mechanical properties of the microenvironment. It is thought that this dynamic 'I pull you, you pull me' interplay results in a state of isometric tension within the cytoskeleton that stabilizes the cell shape and enables cells to sense external forces and to respond by regulating their behaviour[3,19,21].

The spatiotemporal context in which mechanical cues function to control cell fate *in vivo* and the molecular components that sense and transduce such signals are still poorly understood. However, in recent years, the field of mechanobiology has witnessed a surge of interest, mainly because of two reasons: first, the increasing appreciation that cell shape, ECM elasticity and cytoskeletal tension have pivotal roles in development, physiology and in the aetiology of many diseases (BOX 2); second, the development of new technologies that allow mechanical cues to be investigated as independent experimental variables.

Control by cell shape. The first indication that cell shape is an important regulator of cell behaviour dates back 30 years ago, when it was shown that gradual changes in substrate adhesiveness regulate cell proliferation and differentiation[22,23]. This was followed by the observation that the degree of cell-shape distortion is itself a fundamental and dose-dependent signal for proliferation control[24–26]. In these studies, microprinted ECM islands of different sizes were engineered to control the extent of cell spreading of a single endothelial cell (FIG. 1a). Strikingly, it was observed that well-spread cells proliferate, whereas cells confined to small adhesive areas do not proliferate and instead undergo apoptosis. To prove that this effect was due to changes in cell shape per se, rather than due to the extent of cell–ECM contact areas, cells were seeded on top of arrays of ECM microdots, so that cells could spread from dot to dot over non-adhesive surfaces, thus developing limited contact with the ECM. Notably, cells that spread across these dots still displayed high levels of proliferation, indicating that cell shape and not the extent of cell–ECM contacts is a determining factor for the control of cell proliferation[24]. Similarly, cell shape has been reported to strongly influence cell fate. For example, cell shape affects the balance between keratinocyte self-renewal and differentiation[26] and the differentiation of human mesenchymal stem cells (MSCs). Indeed, human MSCs differentiate into osteoblasts when allowed to spread, whereas they differentiate into adipocytes when they are confined to a round shape[27]. Thus, these studies show that cell shape per se is a key determinant of cellular behaviour.

Control by ECM elasticity. Each organ of the human body has a distinct rigidity pattern, which is dictated by ECM elasticity and by the three-dimensional shape of the tissue. In addition to highlighting the crucial role of cell shape, studies of MSC differentiation provided a striking example of how cells respond to the stiffness of their surroundings (FIG. 1b). It was shown that *in vitro* MSCs display a 'chameleon-like' behaviour when confronted with ECM substrates of different elasticity. For example, MSCs differentiate into osteoblasts when seeded on a synthetic matrix engineered to have a bone-like stiffness, into myoblasts when grown on ECMs with intermediate stiffness or into neurons and adipocytes when cultured on a soft ECM[28]. Similarly, skeletal muscle stem cells require an ECM substrate that mimics the stiffness of the adult muscle in order to preserve high regenerative capacity when engrafted back into mice[29,30]. Moreover, ECM elasticity exerts effects that are comparable to cell shape in controlling cell proliferation[31,32]. Thus, the varying elastic properties of the different tissues seem to influence tissue regeneration. Taken together, the evidence indicates that, similar to soluble growth factors, cell morphology and the mechanical properties of the cellular microenvironment can affect both cell growth and cell differentiation.

Mechanical forces in multicellular contexts. The role of mechanical cues ultimately needs to be understood in the context of the multicellular organization that characterizes natural tissues. The mammary gland was one of the first model systems in which this was addressed[33,34]. Mammary epithelial cells (MECs) embedded in a soft matrix, such as a recombinant basement membrane or soft collagen gels, grow into spheric epithelial monolayers (called acini) with a central lumen. In these cultures, MECs undergo growth arrest, polarization and, eventually, differentiation[35,36]. Intriguingly, changes in the elasticity of the ECM have profound effects on the development of acinar structures. A stiff ECM causes disruption of the spheric architecture as a result of reduced cell–cell junctions, defective cell polarization, impaired lumen formation, increased MEC proliferation and even cell invasiveness into the surrounding matrix[37,38] (FIG. 1c). These results show that the physical properties of the ECM are essential for the structural integrity and proliferative homeostasis of epithelial tissues.

In vertebrates, cells are mechanically coupled in multicellular aggregates (for example, epithelial sheets) through different cell–cell junctions, including adherens junctions and desmosomes. These intercellular junctions contribute to the overall three-dimensional shape of the multicellular structure and enable the transmission of forces between cells. Thus, intercellular junctions contribute to the generation of tension gradients and compression forces within epithelia that may translate into patterning events. For example, when sheets of endothelial cells are grown on substrates that form different geometrical shapes (for example, squares and annular rings), cells that are located at the centre stop proliferating when reaching a high cell density, whereas more stretched cells at the outer border of the colony and at the tips of edges (that is, at points of higher mechanical stress) continue to proliferate[39]. Similarly, the differentiation of MSC aggregates can be guided by manipulating the distribution of tensional and compressional forces[40].

Mechanical forces and the cytoskeleton

How do cells sense and transduce mechanical cues? The major structures that are involved in mechanosensing are the sites of cell–ECM and cell–cell adhesion. The molecular machineries by which cells attach to the ECM and sense mechanical cues have been studied in depth and are summarized in several excellent reviews[18,19]. By contrast, the role of cell–cell adhesion in mechanosensing has not been investigated in as much detail[41,42]. Here, we briefly introduce the main components of cell–ECM adhesion and how they respond to mechanical cues.

At the molecular level, cell–ECM adhesion is mediated by integrins, which are the main substrate adhesion receptors[19,21]. Integrins, clustered into multiprotein complexes called focal adhesions, provide a direct physical link between the ECM and cytoskeletal adaptors, thereby connecting the ECM with the actin cytoskeleton. The relationship between filamentous actin (F-actin) and the ECM is reciprocal: integrins promote bundling of actin filaments to generate tension within a cell; and, at the same time, the activity of actin regulatory molecules, the rate of actin polymerization and actin spatial organization affect integrin function and thereby the adhesive state of a cell[19,21,43].

Although cell shape is sustained by the organization of the entire cytoskeleton, major emphasis in the mechanotransduction field has been placed on the dynamics of actin microfilaments[19,21], as the contractile forces exerted by F-actin cables and their associated myosin motors are responsible for generating mechanical tension in cells[19,21]. The structure and dynamics of the actomyosin cytoskeleton are regulated by the RHO family of GTPases[1–3]. Inhibition of RHO, myosin, the myosin activity regulating kinases ROCK (RHO-associated kinase) and MLCK (myosin light chain kinase; also known as MYLK) or F-actin polymerization itself, all convert cells into a state of low tensile forces[1–3]. A similar effect is caused by a soft environment or by reducing the area over which a cell can spread[21]. There is indeed a close relationship between the degree of cell spreading and tensile forces, as the actomyosin cytoskeleton exerts higher traction in spread cells than in rounded cells attached to a smaller ECM area[19,21]. However, it is not known whether the ultimate signal that regulates cellular behaviour in response to mechanical cues is the contractility machinery per se or whether contractility is just a means to attain a specific cell shape and cytoskeletal organization[44–46].

Box 2 | A brief overview of mechanobiology

Role in early development

Following fertilization, the first event that limits totipotency of mammalian blastomeres is the morphological process of compaction, whereby cells increase contact with their neighbouring cells and start to stretch. This influences the mitotic spindle positioning in outer cells (which are then committed to the extra-embryonic trophectoderm lineage) and in inner cells (which remain pluripotent). Loss of cell–extracellular matrix (ECM) contact, of cell–cell adhesion or of the mechanosensitive filamentous actin (F-actin) adaptor protein termed talin lead to very early developmental arrest[119–122]. In vertebrates, the three germ layers display different surface tension properties, and this prevents random cell–cell mixing[123]. Gastrulation is a subsequent developmental step that leads to the generation of the body plan and is characterized by an ordered succession of mechanically driven morphogenetic events[124,125,133]. Understanding how these long-range mechanical events concatenate and crosstalk with soluble morphogens remains the holy grail in developmental biology[126].

Role in epithelial–mesenchymal interactions

Organogenesis is often the product of reciprocal interactions between epithelial sheets and their underlying mesenchyme. Mammoto *et al.* recently provided the proof of principle that this crosstalk entails a fundamental mechanical control. In tooth development, the epithelium secretes a combination of soluble attractant and repulsive factors, leading to tight packing of mesenchymal cells. The subsequent change in cell shape (which is cell rounding) drives initial tooth-specific differentiation[127].

Role in the cardiovascular system

Heart morphology and physiology are profoundly influenced by mechanical forces. In zebrafish embryos, interference with shear forces causes heart disorders[128,129], and shear stress associated with the first heart beat has a causal role in the generation of the first haematopoietic progenitors[130,131]. In the vasculature, disturbance of the laminar blood flow can have profound effects on the homeostasis of endothelial cells, as turbulent flow can predispose to atherosclerosis. Excessive haemodynamic stress on the heart ventricular wall causes a vicious cycle of aberrant ECM remodelling, loss of mechanical compliance and myocyte apoptosis, ultimately leading to heart failure[6].

Role in the musculoskeletal system

In the bone, physical load keeps interstitial fluid flowing through the canalicular network, an essential mechanical signal for osteocyte survival[1]. In muscle, alterations in force generation and transmission due to mutations in the ECM or cytoskeletal proteins typically characterizes muscle dystrophies[1].

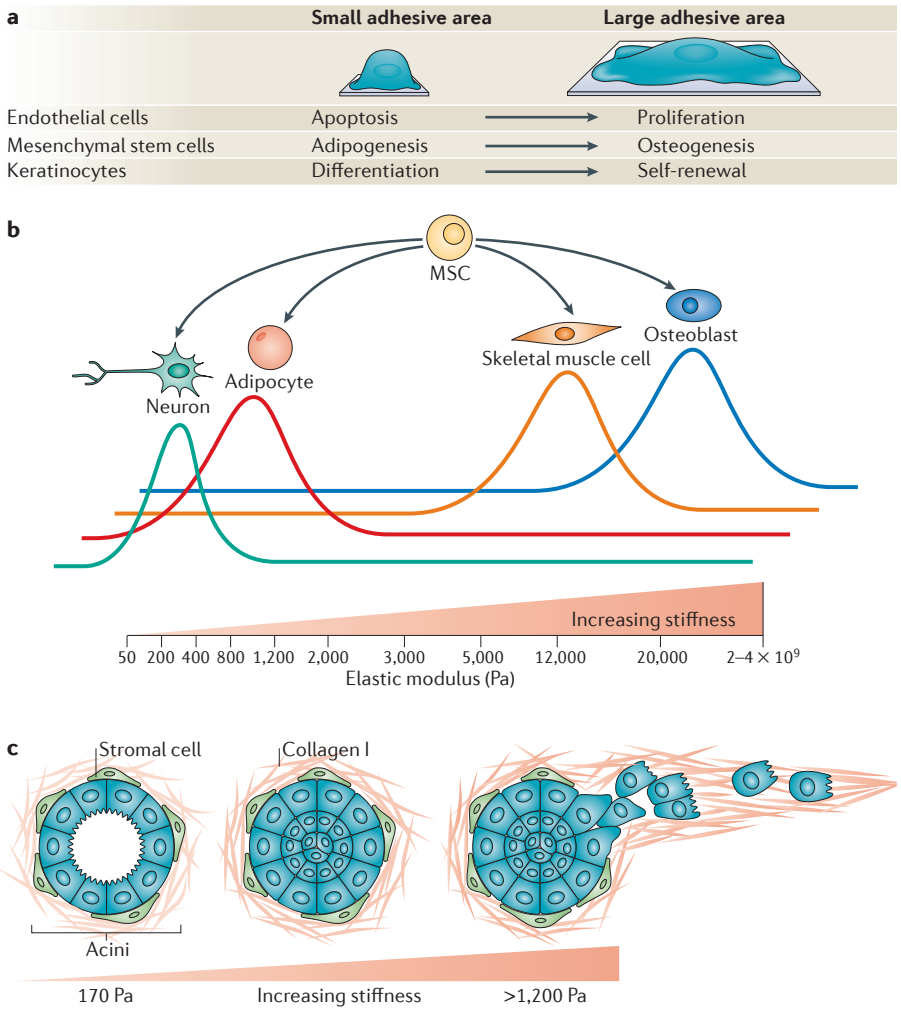


Figure 1 | **Influence of mechanical and physical properties of the ECM on cell behaviour.** **a** | Microprinting techniques enable the design of extracellular matrix (ECM) areas of defined shape and dimensions, on which cells adhere by conforming to the substrate geometry. In endothelial cells, cell geometry is sufficient to change the response to growth factors from apoptosis to proliferation[24,26,49]. In bone marrow-derived mesenchymal stem cells (MSCs), cell geometry regulates the switch in lineage commitment from adipocytes to osteoblasts[27,28]. In keratinocytes, restriction of cell shape induces terminal differentiation[26,49]. **b** | Cells within tissues experience very different degrees of ECM elasticity, ranging from very soft surroundings (such as those found in the brain and adipose tissue) to very stiff and rigid environments (such as those found within bones or at the bone surface). By recapitulating these different ECM elasticities *in vitro*, it was found that MSCs differentiate optimally into neurons, adipocytes, skeletal muscle cells or osteoblasts at elasticities that match the physiological ECM stiffness of their corresponding natural niche (shown as coloured lines, with peaks indicating maximal differentiation)[28]. Similarly, muscle stem cells maintain their self-renewal and regenerative capacities only when expanded on substrates mimicking the elasticity of skeletal muscle *in vitro*[30] (not shown). **c** | Mammary epithelial cells (shown in blue) grown embedded in a soft basement membrane form growth-arrested and well polarized acinar structures[36] (left). Increasing type I collagen concentration and crosslinking, and thus ECM stiffness, compromises tissue organization, inhibits apoptosis and lumen formation and destabilizes adherens junctions (centre), favouring the acquisition of migratory and invasive behaviours[37,38] (right). Pa, Pascal.

YAP and TAZ transduce mechanical cues

Given that the actomyosin cytoskeleton is required for mechanotransduction, how are mechanical signals transduced into biological outcomes and how do these signals ultimately affect gene expression? The transcriptional co-activators YAP and TAZ recently emerged as key mediators of the biological effects that are observed in response to ECM elasticity and cell shape[8,9]. YAP and TAZ localize in the nucleus and are transcriptionally active in cells cultured on a stiff ECM, whereas YAP and TAZ are excluded from the nucleus and functionally inhibited in cells cultured on a soft ECM[8] (FIG. 2). A similar regulation of the activity and nuclear–cytoplasmic shuttling of YAP and TAZ occurs in cells that are grown on micropatterned ECMs with the same stiffness but that show different degrees of cell spreading: cells that are seeded on large fibronectin islands, which enable cell spreading, have active YAP and TAZ, whereas cells that are confined to small adhesive islands have the inactive forms of YAP and TAZ[8,9] (FIG. 2). Importantly, the activity of YAP and TAZ ultimately determines the biological response to mechanical cues. Knockdown of YAP and TAZ in cells grown on large adhesive areas or on a stiff ECM produced a phenotype that is typical for cells grown on small adhesive areas or on soft ECM; vice versa, overexpression of YAP and TAZ was sufficient to 'trick' cells into behaving as they would on a stiff matrix even in the presence of a soft matrix[8].

In agreement with the idea that mechanotransduction is tightly linked to the integrity of the actomyosin cytoskeleton, YAP and TAZ are inactivated when F-actin is disrupted or when RHO is inhibited[8–10,12]. On the other hand, F-actin polymerization resulting from overexpression of the RHO-regulated F-actin nucleator diaphanous correlates with increased activity of YAP and TAZ[8,10]. Finally, blunting of endogenous tensile forces through the inhibition of the myosin regulators ROCK and MLCK or myosin itself results in YAP and TAZ inactivation[8,9]. This leads to cell behaviour similar to that observed in the presence of a soft ECM or following cell size restriction[24,28]. Thus, YAP and TAZ not only respond to mechanical cues, but they are also mediators of mechanical signals.

In line with these data, genetic experiments in fly embryos showed that the expression of actin polymerization antagonists is required to restrain the activity of Yorkie in developing tissues[10,11]. Severe overgrowth of imaginal discs, which are the larval single-cell layer epithelial structures that give rise to wings, legs and other appendages, was shown to be induced by loss of function of capping proteins (leading to excessive growth of F-actin at barbed ends), deletion of adenylyl cyclase-associated proteins (CAPs; leading to increased association of actin monomers into new actin filaments), or by overexpression of an activated version of Diaphanous. These overgrown imaginal discs showed increased levels of Yorkie activity, and their phenotype was strikingly similar to that caused by loss of Hippo signalling

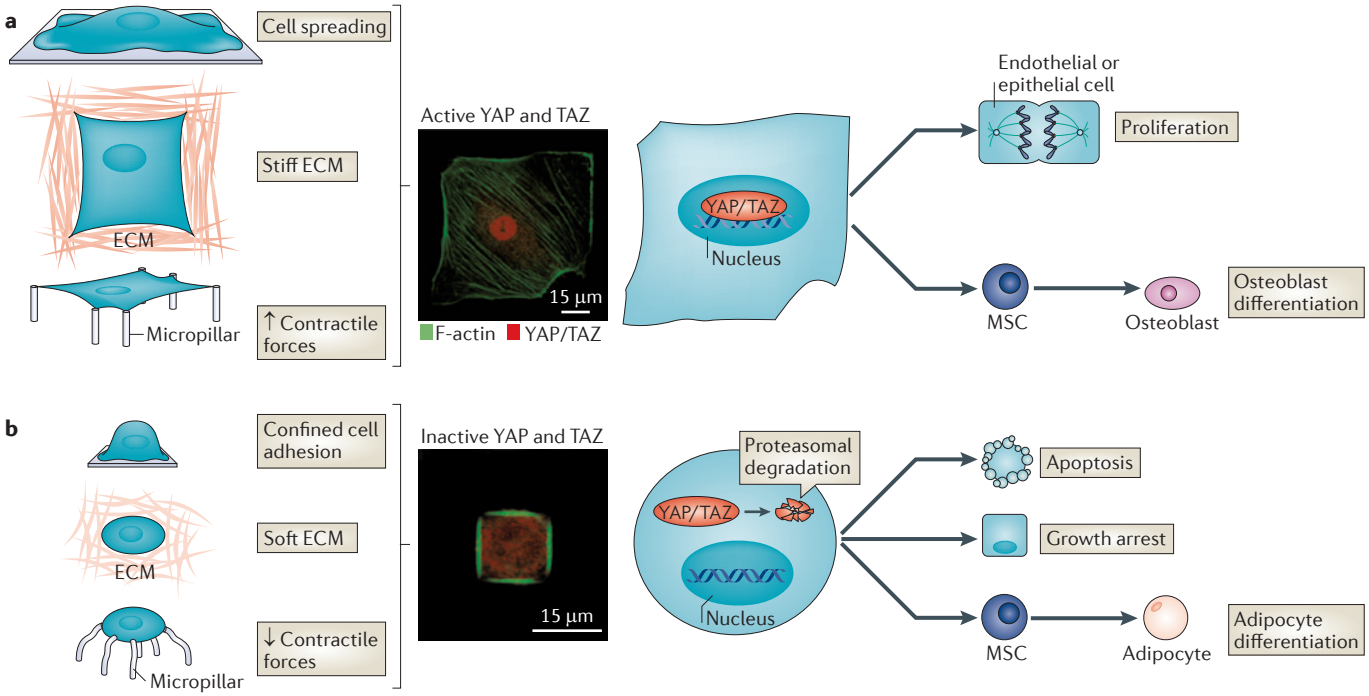


Figure 2 | **YAP and TAZ as sensors and mediators of mechanical inputs from the ECM. a** | The transcriptional regulators YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif) are localized in the nucleus and active under experimental mechanical conditions that favour the development of high intracellular resisting forces, such as an unlimited adhesive area and the subsequent adoption of a spread cell shape, a stiff extracellular matrix (ECM) or stretching between stiff micropillars (left). An immunofluorescence image of an endothelial cell plated on a large, square microprinted fibronectin island (middle panel) is shown. Cells were stained for filamentous actin (F-actin) with phalloidin (green) and for YAP and TAZ (red). In these conditions, YAP and TAZ are required for cells to proliferate (in the endothelium and epithelial cells) or differentiate towards osteoblasts (in mesenchymal stem cells (MSCs)). **b** | In mechanical conditions in which cells develop low contractile forces (for example, when they are grown on small adhesive areas favouring a small cell size, on soft ECM or on top of bendable micropillars), YAP and TAZ are inactivated and relocalize to the cytoplasm. An immunofluorescence image of an endothelial cell plated on a small, square microprinted fibronectin island is shown. Reduced YAP and TAZ activity shifts the cell responses towards apoptosis and growth arrest or diverts differentiation towards cell fates that would be specified on a soft matrix, for example, adipocytes. Experimental up- or downregulation of YAP and TAZ levels attain similar control of cell behaviour irrespective of their mechanical environment (not shown). Images are reproduced, with permission, from REF. 8

activity. Importantly, Yorkie was found to be required for the F-actin-induced overgrowth of imaginal discs[10,11].

Thus, the regulation of YAP, TAZ and Yorkie by F-actin is an evolutionarily conserved phenomenon that is relevant for the control of organ growth *in vivo*.

Control of YAP and TAZ by actomyosin

Although the above discussed studies provide compelling evidence for the relevance of YAP, TAZ and possibly Yorkie as downstream mediators of mechanical cues, these discoveries raise several new questions of how mechanical forces and the F-actin cytoskeleton can regulate these transcriptional effectors.

Integrins are central transducers of mechanical cues from the ECM. As such, integrin signalling clearly occurs upstream of YAP and TAZ activity in physiological settings. However, artificial induction of a spread cell shape in a manner that does not engage integrins, for example, by using poly-Lys-coated substrates, can sustain YAP nuclear localization[12]. Moreover, interfering with key components of focal adhesions, such as focal adhesion kinase (FAK; also known as PTK2) or SRC, does not affect YAP and TAZ responses[12]. Thus, it seems more likely that cell spreading per se and the related changes in F-actin structures downstream of integrin signalling are the most crucial mediators that keep YAP and TAZ active.

Are YAP, TAZ and Yorkie regulated by the levels of G-actin versus F-actin? A well-known example of regulation of a transcriptional cofactor by F-actin is the case of megakaryocytic acute leukaemia protein (MAL; also known as MKL1), which is a transcriptional partner of serum response factor (SRF)[47]. Monomeric G-actin binds to MAL and causes its nuclear exclusion and functional inhibition. After serum stimulation and RHO activation, increased actin polymerization reduces the amount of free G-actin; this condition unleashes MAL, leading to its nuclear uptake and activation of SRF-dependent transcription[48].

However, despite some analogies, current evidence suggests that YAP, TAZ and Yorkie are regulated by a different mechanism. First, cells plated on small ECM islands display increased SRF activity[49] but lower YAP and TAZ activity[8,9]. Second, expression of non-polymerizable G-actin is sufficient to inhibit MAL activity but has no effect on the activity of YAP, TAZ or Yorkie activity *in vitro* or *in vivo*[8] (and G. Halder, unpublished observations). Third, a global increase in F-actin levels, as found in fly mutants for the actin severing protein Cofilin (also known as Twinstar), does not increase Yorkie activity[11]. Altogether these data indicate that YAP, TAZ and Yorkie are not simply regulated by the levels of G-actin. Rather, it is possible that the activity of YAP, TAZ and Yorkie depends on a particular F-actin structure, such as stress fibres or a yet-to-be defined contractile actin network, which is enriched in cells with a spread shape (see below).

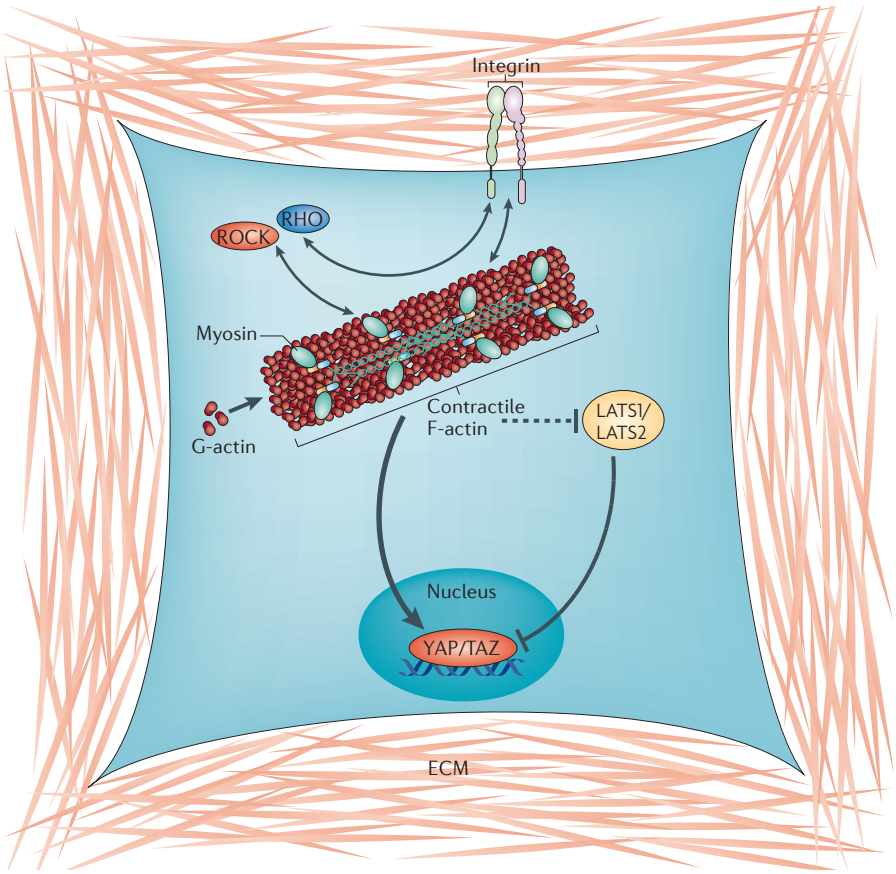


Figure 3 | **A model for the mechanical regulation of YAP and TAZ.** When cells are allowed to spread freely on the extracellular matrix (ECM), or when cells are grown on a stiff ECM, extracellular forces promote cell–ECM adhesions via integrins and the development of intracellular contractile filamentous actin (F-actin) structures containing myosin molecules. This is regulated by bidirectional signalling between RHO, ROCK (RHO-associated kinase), integrins and myosin activity. Contractile F-actin structures in turn sustain YAP and TAZ nuclear localization and activity through unidentified molecular effectors. In addition, F-actin also opposes YAP and TAZ phosphorylation through inhibition of the kinases LATS1 (Large tumour suppressor homologue 1) and LATS2. G-actin, globular actin.

Dual regulation of YAP and TAZ and Yorkie activity by F-actin and Hippo. Current data point to a multilayered and perhaps complex interaction between F-actin polymerization and activation of the Hippo pathway that regulates YAP and TAZ activity (FIG. 3). Most intriguingly, one set of data suggests that mechanical signals regulate YAP and TAZ through an unknown pathway that acts in parallel to the classic kinase cascade of the Hippo pathway. Knockdown of LATS1 and LATS2 was not sufficient to restore YAP and TAZ activity in the presence of the actin polymerization inhibitor latrunculin A or in cells cultured in soft ECM hydrogels[8]. Consistently, the activity of a TAZ mutant that is LATS-insensitive was still inhibited under the same conditions[8]. We thus envision that, in spread cells, F-actin opposes unknown YAP and TAZ inhibitory molecules, in a manner largely independent of LATS1 and LATS2. For example, we can speculate that a specific F-actin structure might either physically sequester this inhibitory factor, or serve as a platform to promote its post-translational modification, in any case preventing it from interacting with YAP and TAZ. Conversely, when cells are seeded on soft substrates or are confined to small areas, this inhibitory factor may be released (or activated) by the remodelling of the actin cytoskeleton. A related possibility is that protein stretching associated with actin contractility[19] may expose, or mask, cryptic protein–protein binding sites in regulators of YAP and TAZ, controlling their competence to interact with other partners of YAP or TAZ or with YAP or TAZ proteins directly. Alternatively, F-actin reorganization and the resulting formation of a spread cell shape may promote activation of a positive cofactor that mediates YAP and TAZ nuclear localization and activity.

Notably, F-actin destabilization or RHO inhibition also activate LATS[9,12], thus contributing to complete nuclear exclusion of YAP and TAZ (FIG. 3). Indeed, preventing YAP phosphorylation by depletion of LATS partially limits the relocalization of YAP to the cytoplasm following mild inhibition of F-actin[9,12]. This suggests that the opposite regulation of YAP and TAZ by the Hippo kinases and the cytoskeleton can rebalance each other (FIG. 3) but only up to a given extent. In fact, the integrity of the actomyosin cytoskeleton is vital for YAP and TAZ activity. As such, an effective F-actin disruption that causes quantitative cytoplasmic retention of YAP or overt TAZ degradation cannot be compensated by LATS knockdown[8]. The same applies to other attempts aiming to revive YAP and TAZ from the Hippo inhibitory signalling branch (S. Dupont, unpublished observation). Indeed, a more complete latrunculin A treatment still leads to YAP nuclear exit and cytoplasmic relocalization, even in LATS-depleted cells (S. Dupont, unpublished observation).

Further evidence supports a model in which mechanical cues initiate both LATS-dependent and LATS-independent signalling. In mammalian cells, a soft substrate enhances the effects of high cell density (which is thought to induce LATS activity[50]) to inhibit YAP and TAZ target gene expression[8]. Moreover, cells placed in suspension (that is, in absence of any cell–ECM contact) deactivate YAP and promote cell death via anoikis, but LATS knockdown only partially rescues cells from this cell death[12]. In *D. melanogaster*, knockdown of capping proteins enhances the overgrowth phenotypes of mutations in Hippo or Warts on cell proliferation[11], again suggesting the existence of a Warts-independent Yorkie inhibitor, whose activity is suppressed following F-actin polymerization. Altogether, these data indicate that mechanical forces regulate the activity of YAP, TAZ and Yorkie through both LATS (or Warts)-dependent and -independent pathways.

It is important to note that mechanical regulation of YAP and TAZ can occur in isolated cells, indicating that this regulatory network can operate cell autonomously and independently of cell–cell contacts. However, in multicellular epithelia the actin cytoskeleton is connected to adherens junctions and tight junctions, and transmission of mechanical forces through these sites is inherent to junctional maturation and function[42,51,132]. Cell–cell junctions are now considered as important sites to regulate the Hippo pathway[13,15,52,53]. Indeed, multiple

positive and negative regulators of YAP, TAZ and Yorkie are associated with adherens junctions or tight junctions, such as crumbs, scribble, PALS1 (protein associated with Lin-7 1), PATJ (PALS1-associated tight junction protein; also known as INADL and MPP5)), NF2 (neurofibromin 2; also known as merlin), kibra, zonula occludens 2 (ZO2), ajuba, zyxin, angiomotin and α-catenin[15]. Some of these molecules bind YAP and TAZ or are associated with LATS kinases[52,54]. However, many of these molecules also display actin-binding or actin-regulatory functions and may thus regulate, or be regulated by, the cytoskeleton. Moreover, contractile F-actin structures can form in response to cadherin engagement[51]. These data raise the possibility that YAP and TAZ regulation by the cytoskeletal pathway also occurs downstream of cell–cell contacts.

Perspectives

The identification of YAP, TAZ and Yorkie as sensors and effectors of mechanical cues may shed light on several biological processes, such as contact inhibition, skin homeostasis, organ size control and cancer, in which the activity of YAP and TAZ or Yorkie is involved.

Cell geometry in contact inhibition of cell proliferation. Cultured cells stop dividing when they reach high cell density, even in the presence of unlimited amounts of growth factors and nutrients. This behaviour is referred to as contact inhibition of cell proliferation and is associated with inactivation and phosphorylation of YAP and TAZ, indicating the involvement of the kinases of the Hippo pathway[50,55]. However, the regulation of YAP and TAZ by contact inhibition may be complex and may involve other pathways besides the known Hippo cascade. Genetic data in mouse embryonic fibroblasts show that the mammalian Hippo kinase homologues MST1 and MST2 are not required for contact inhibition[56]. Furthermore, LATS1 and LATS2 seem to be largely dispensable for YAP inactivation in contact-inhibited keratinocytes[57,58].

Clues about additional regulators of YAP and TAZ may come from the observation that cells reduce their contact area with the substrate as cell density increases. Indeed, contact inhibition entails progressive reduction of the cell area as a consequence of cell crowding, and it was shown that a cell arrests its growth only when its size falls below a crucial threshold[59]. Interestingly, increasing the stiffness of the ECM lowers the sensitivity of a cell to contact inhibition[60], and reduced RHO activity and cytoskeletal rearrangements are remarkably similar between contact inhibited cells and isolated cells seeded on a small ECM island and not allowed to spread[27]. Thus, contact inhibition may be a consequence of reduced cell shape and reduced mechanical forces due to the reduced cell–ECM contact rather than a direct effect of cell–cell contacts.

> “mechanical forces regulate the activity of YAP, TAZ”

Homeostasis in the skin. Mechanical signals have profound effects on skin homeostasis. Decreased expression of integrin[61], reduced cell spreading[26] or culture in suspension[62] cause reduced proliferation and increased keratinocyte differentiation. Consistently, disturbance of mechanical homeostasis has a role in skin tumorigenesis: first, ROCK is activated downstream of oncogenic transformation and contributes to the development of squamous cell carcinomas[63]; second, increased ROCK activity facilitates skin tumour development in mouse models[64].

Notably, the process of epidermal stratification itself can be interpreted as a differentiation process induced by loss of cell–ECM contacts[65]. Interestingly, in proliferating cells of the basal layer YAP is localized in the nuclei, whereas it is excluded to the cytoplasm in differentiating suprabasal cells. Increased levels of nuclear YAP enhance proliferation and cause defective differentiation. By contrast, inactivation of YAP leads to impaired growth and precocious differentiation[58,66,67]. Thus, the pattern of YAP activation in the skin can be correlated with the pattern of cell–ECM interactions.

Skin homeostasis also depends on cell–cell contacts. For example, deletion of α-catenin in the skin leads to increased YAP activation. Yet, such activation remains spatially restricted to the basal layer[57,58], indicating that other signals are essential to locally sustain YAP activation, even when cell–cell adhesions are impaired. Such signals may be cell–ECM contacts and the corresponding cytoskeletal dynamics.

Organ size control. How organ size is regulated has been a long-standing question in developmental biology. How do organs achieve uniform proliferation rates despite the presence of localized sources of growth factors? And how do organs ‘sense’ when they should stop growing? The regulation of YAP, TAZ and Yorkie by mechanical cues may provide an interesting solution to these riddles. For example, in *D. melanogaster*, many of the adult structures develop from imaginal discs, which grow by cell proliferation to reach their ultimate size. The process of disc growth depends on Yorkie expression[13,15]. Interestingly, imaginal discs can compensate for experimentally ablated cells[68], indicating that cells in this tissue can somehow sense the size of the entire imaginal disc and adjust their proliferation rate accordingly. Gradients of morphogen signalling molecules, such as those of Decapentaplegic (DPP) and Wingless, are essential for the growth of imaginal discs; however, these morphogen gradients insufficiently explain how imaginal discs stop growing when they reach their proper size[69].

Computational models that integrate mechanical control with secreted morphogens explain imaginal disc growth better than models based on morphogen gradients alone[70–72]. The morphogens DPP and Wingless drive cell proliferation mainly in the centre of the imaginal disc where their concentration is highest. As cells in the centre are stimulated to proliferate, the expansion of the centre stretches cells that are located more towards the periphery of the imaginal disc[71]. We speculate that such stretching would compensate for low local morphogen concentration, leading to Yorkie activation and sustained Yorkie-dependent cell proliferation. On the other hand, compression of the centre region would favour Yorkie inactivation. Growth is inhibited when the stretching and compression forces are at equilibrium. We propose that in flies carrying mutations in the F-actin inhibitors capping proteins and CAP, this equilibrium is unbalanced, thereby sensitizing cells to Yorkie activation by mechanical forces. The validation of this hypothesis will be an important aim for the field.

Breast cancer as a ‘mechanodisease’. Cancer is a disease characterized by loss of spatial control over cell proliferation. Although this has been classically viewed as a consequence of transformation, new evidence suggests that tissue remodelling is intrinsic to cancer progression and even precedes overt disease[4,5]. Thus, disturbed architectural features of tissues, a hallmark of cancer, may have a causal and perhaps initiating role in tumorigenesis. Aggressive breast lumps are detected because of their stiffness, which is due to extensive deposition of collagen and the recruitment of stromal cells and inflammation[4,73]. Several inputs (for example, genetic and environmental factors) then converge to upregulate the expression of lysyloxydases, which increase

collagen crosslinking (an ECM modification that has also been associated with tumour progression and invasion)[74]. By contrast, 'softening' the tumour microenvironment slows tumour growth and progression[38].

Notably, disturbed tumour rheology correlates well with the effects of TAZ during breast cancer progression. TAZ is stabilized and thus constitutively active in more advanced tumours and is essential and sufficient to endow cancer cells with self-renewal capacity, chemoresistance and tumour-initiating capabilities[16]. Thus, we speculate that the mechanism by which ECM stiffness is linked to tumour progression may be intimately coupled to TAZ, and probably YAP, activity. In addition, matrix stiffness regulates cell polarity[37,75], which is a key factor regulating TAZ stability in breast cancer. TAZ forms a complex with the cell-polarity determinant scribble, and loss of scribble disrupts the inhibitory association of TAZ with the core kinases of the Hippo pathway MST2 and LATS1 (REF. 16). In turn, sustaining YAP and TAZ levels can induce epithelial–mesenchymal transition in epithelial cells[76,77], generating a feed-forward loop in which tissue stiffness, loss of polarity and TAZ itself conspire to sustain TAZ levels and advance tumour progression. Although intriguing, the connection between increased ECM stiffness, TAZ and cancer progression awaits experimental validation.

Conclusions

Mechanical cues may provide a largely unexplored form of positional information, whereby individual cells constantly sense the development of the whole organ or body. YAP, TAZ and Yorkie represent a cellular hub where several pathways and signals converge, including canonical Hippo signalling, cell polarity, EMT and crowd control. Their mechanical regulation could thus set the tone for the responsiveness of a cell to these signals. In turn, YAP and TAZ crosstalk with other signal transduction pathways, including the WNT, transforming growth factor-β (TGFβ) and bone morphogenetic protein (BMP) pathways[78–82]. As such, spatial and temporal regulation of YAP and TAZ by mechanical cues may be regarded as a mechanism that controls the magnitude and duration of growth factor signalling. This may ensure that certain structural and architectural checkpoints are met before further developmental or homeostatic events can occur.

It will be interesting to investigate the relevance of YAP and TAZ in various cell-biological assays that are routinely performed only in specific, and perhaps extreme, mechanical conditions. Indeed, most of our knowledge of cell behaviour stems from growing cells on YAP- and TAZ-activating stiff tissue culture substrates (such as plastic or glass) or, at the other extreme, on YAP- and TAZ- inactivating soft and non-adhesive substrates. For example, culturing cells in soft agar, a classic assay to monitor oncogene-mediated transformation, may entail the bypassing of anchorage-dependent activation of YAP and TAZ for survival and proliferation. Furthermore, the manipulation of mechanical parameters to control YAP and TAZ activity may be crucial for exploiting the full regenerative potential of current stem cell-based therapies.

Despite substantial progress, we still lack answers to fundamental questions in the field of mechanotransduction. Future work is needed to understand the distribution of forces in living tissues and embryos and to develop genetic and experimental approaches to manipulate tissue mechanics. YAP and TAZ may represent a starting point to address these issues: on the one hand, mapping their activity *in vivo* may serve as a direct read-out to visualize the effect of architectural constraints; on the other hand, the identification of the mechanisms linking YAP and TAZ regulation to the cytoskeleton would not only fill a major gap in cell biology but also provide tools to tackle the biological relevance of mechanical forces *in vivo*.

Georg Halder is at the Viaams Instituut voor Biotechnologie Center for the Biology of Disease and the Center for Human Genetics, Katholieke Universiteit Leuven, 3000 Leuven, Belgium.

Sirio Dupont and Stefano Piccolo are at the Department of Biomedical Sciences, University of Padua School of Medicine, viale Colombo 3, 35131 Padua, Italy.

e-mails: Georg.Halder@med.kuleuven.be; sirio.dupont@unipd.it; piccolo@bio.unipd.it

doi:10.1038/nrm3416
Published online 16 August 2012

1. Jaalouk, D. E. & Lammerding, J. Mechanotransduction gone awry. *Nature Rev. Mol. Cell Biol.* **10**, 63–73 (2009).
2. Wozniak, M. A. & Chen, C. S. Mechanotransduction in development: a growing role for contractility. *Nature Rev. Mol. Cell Biol.* **10**, 34–43 (2009).
3. Mammoto, A. & Ingber, D. E. Cytoskeletal control of growth and cell fate switching. *Curr. Opin. Cell Biol.* **21**, 864–870 (2009).
4. Provenzano, P. P. & Keely, P. J. Mechanical signaling through the cytoskeleton regulates cell proliferation by coordinated focal adhesion and Rho GTPase signaling. *J. Cell. Sci.* **124**, 1195–1205 (2011).
5. Butcher, D. T., Alliston, T. & Weaver, V. M. A tense situation: forcing tumour progression. *Nature Rev. Cancer* **9**, 108–122 (2009).
6. Hahn, C. & Schwartz, M. A. Mechanotransduction in vascular physiology and atherogenesis. *Nature Rev. Mol. Cell Biol.* **10**, 53–62 (2009).
7. Discher, D. E., Mooney, D. J. & Zandstra, P. W. Growth factors, matrices, and forces combine and control stem cells. *Science* **324**, 1673–1677 (2009).
8. Dupont, S. *et al.* Role of YAP/TAZ in mechanotransduction. *Nature* **474**, 179–183 (2011).
9. Wada, K.-I., Itoga, K., Okano, T., Yonemura, S. & Sasaki, H. Hippo pathway regulation by cell morphology and stress fibers. *Development* **138**, 3907–3914 (2011).
10. Sansores-Garcia, L. *et al.* Modulating F-actin organization induces organ growth by affecting the Hippo pathway. *EMBO J.* **30**, 2325–2335 (2011).
11. Fernández, B. G. *et al.* Actin-capping protein and the Hippo pathway regulate F-actin and tissue growth in *Drosophila*. *Development* **138**, 2337–2346 (2011).
12. Zhao, B. *et al.* Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis. *Genes Dev.* **26**, 54–68 (2012).
13. Pan, D. The Hippo signaling pathway in development and cancer. *Dev. Cell* **19**, 491–505 (2010).
14. Zhao, B., Li, L., Lei, Q. & Guan, K.-L. The Hippo–YAP pathway in organ size control and tumorigenesis: an updated version. *Genes Dev.* **24**, 862–874 (2010).
15. Halder, G. & Johnson, R. L. Hippo signaling: growth control and beyond. *Development* **138**, 9–22 (2011).
16. Cordenonsi, M. *et al.* The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* **147**, 759–772 (2011).
17. Nishioka, N. *et al.* The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. *Dev. Cell* **16**, 398–410 (2009).
18. Parsons, J. T., Horwitz, A. R. & Schwartz, M. A. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nature Rev. Mol. Cell. Biol.* **11**, 633–643 (2010).
19. Vogel, V. & Sheetz, M. Local force and geometry sensing regulate cell functions. *Nature Rev. Mol. Cell Biol.* **7**, 265–275 (2006).
20. Janmey, P. A. & Miller, R. T. Mechanisms of mechanical signaling in development and disease. *J. Cell. Sci.* **124**, 9–18 (2011).
21. Schwartz, M. A. Integrins and extracellular matrix in mechanotransduction. *Cold Spring Harb. Perspect. Biol.* **2**, a005066 (2010).
22. Folkman, J. & Moscona, A. Role of cell shape in growth control. *Nature* **273**, 345–349 (1978).
23. Spiegelman, B. M. & Ginty, C. A. Fibronectin modulation of cell shape and lipogenic gene expression in 3T3-adipocytes. *Cell* **35**, 657–666 (1983).
24. Singhvi, R. *et al.* Engineering cell shape and function. *Science* **264**, 696–698 (1994).
25. Chen, C. S., Mrksich, M., Huang, S., Whitesides, G. M. & Ingber, D. E. Geometric control of cell life and death. *Science* **276**, 1425–1428 (1997).
26. Watt, F. M., Jordan, P. W. & O'Neill, C. H. Cell shape controls terminal differentiation of human epidermal keratinocytes. *Proc. Natl Acad. Sci. USA* **85**, 5576–5580 (1988).
27. McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriraju, K. & Chen, C. S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* **6**, 483–495 (2004).
28. Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677–689 (2006).
29. Engler, A. J. Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J. Cell Biol.* **166**, 877–887 (2004).
30. Gilbert, P. M. *et al.* Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science* **329**, 1078–1081 (2010).
31. Provenzano, P. P., Inman, D. R., Eliceiri, K. W. & Keely, P. J. Matrix density-induced mechanoregulation of breast cell phenotype, signaling and gene expression through a FAK–ERK linkage. *Oncogene* **28**, 4326–4343 (2009).
32. Klein, E. A. *et al.* Cell-cycle control by physiological matrix elasticity and *in vivo* tissue stiffening. *Curr. Biol.* **19**, 1511–1518 (2009).
33. Keely, P. J. Mechanisms by which the extracellular matrix and integrin signaling act to regulate the switch between tumor suppression and tumor promotion. *J. Mammary Gland Biol. Neoplasia* **16**, 205–219 (2011).
34. Celeste, M. Nelson, M. J. B. Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. *Annu. Rev. Cell Dev. Biol.* **22**, 287–309 (2006).
35. Weigelt, B. & Bissell, M. J. Unraveling the microenvironmental influences on the normal mammary gland and breast cancer. *Semin. Cancer Biol.* **18**, 311–321 (2008).

36. Debnath, J. *et al.* The role of apoptosis in creating and maintaining luminal space within normal and oncogene-expressing mammary acini. *Cell* **111**, 29–40 (2002).
37. Paszek, M. J. *et al.* Tensional homeostasis and the malignant phenotype. *Cancer Cell* **8**, 241–254 (2005).
38. Levental, K. R. *et al.* Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **139**, 891–906 (2009).
39. Nelson, C. M. *et al.* Emergent patterns of growth controlled by multicellular form and mechanics. *Proc. Natl Acad. Sci. USA* **102**, 11594–11599 (2005).
40. Ruiz, S. A. & Chen, C. S. Emergence of patterned stem cell differentiation within multicellular structures. *Stem Cells* **26**, 2921–2927 (2008).
41. Liu, W. F., Nelson, C. M., Pirone, D. M. & Chen, C. S. E-cadherin engagement stimulates proliferation via Rac1. *J. Cell Biol.* **173**, 431–441 (2006).
42. Yonemura, S. Wada, Y., Watanabe, T., Nagafuchi, A. & Shibata, M. α-catenin as a tension transducer that induces adherens junction development. *Nature Cell Biol.* **12**, 533–542 (2010).
43. Gardel, M. L., Schneider, I. C., Aratyn-Schaus, Y. & Waterman, C. M. Mechanical integration of actin and adhesion dynamics in cell migration. *Annu. Rev. Cell Dev. Biol.* **26**, 315–333 (2010).
44. Saez, A., Buguin, A., Silberzan, P. & Ladoux, B. Is the mechanical activity of epithelial cells controlled by deformations or forces? *Biophys. J.* **89**, L52–L54 (2005).
45. Trappmann, B. *et al.* Extracellular-matrix tethering regulates stem-cell fate. *Nature Mater.* **11**, 642–649 (2012).
46. Huebsch, N. *et al.* Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nature Mater.* **9**, 518–526 (2010).
47. Olson, E. N. & Nordheim, A. Linking actin dynamics and gene transcription to drive cellular motile functions. *Nature Rev. Mol. Cell Biol.* **11**, 353–365 (2010).
48. Miralles, F., Posern, G., Zaromytidou, A.-I. & Treisman, R. Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* **113**, 329–342 (2003).
49. Connelly, J. T. *et al.* Actin and serum response factor transduce physical cues from the microenvironment to regulate epidermal stem cell fate decisions. *Nature Cell Biol.* **12**, 711–718 (2010).
50. Zhao, B. *et al.* Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev.* **21**, 2747–2761 (2007).
51. Ganz, A. *et al.* Traction forces exerted through N-cadherin contacts. *Biol. Cell* **98**, 721–730 (2012).
52. Grusche, F. A., Richardson, H. E. & Harvey, K. F. Upstream regulation of the Hippo size control pathway. *Curr. Biol.* **20**, R574–R582 (2010).
53. Genevet, A. & Tapon, N. The Hippo pathway and apico-basal cell polarity. *Biochem. J.* **436**, 213–224 (2011).
54. Zhao, B., Tumaneng, K. & Guan, K.-L. The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. *Nature Cell Biol.* **13**, 877–883 (2011).
55. Ota, M. & Sasaki, H. Mammalian Tead proteins regulate cell proliferation and contact inhibition as transcriptional mediators of Hippo signaling. *Development* **135**, 4059–4069 (2008).
56. Zhou, D. *et al.* Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. *Cancer Cell* **16**, 425–438 (2009).
57. Schlegelmilch, K. *et al.* Yap1 acts downstream of α-catenin to control epidermal proliferation. *Cell* **144**, 782–795 (2011).
58. Silvis, M. R. *et al.* α-catenin is a tumor suppressor that controls cell accumulation by regulating the localization and activity of the transcriptional coactivator Yap1. *Sci. Signal.* **4**, ra33 (2011).
59. Puliafito, A. *et al.* Collective and single cell behavior in epithelial contact inhibition. *Proc. Natl Acad. Sci. USA* **109**, 739–744 (2012).
60. Kim, J.-H. & Asthagiri, A. R. Matrix stiffening sensitizes epithelial cells to EGF and enables the loss of contact inhibition of proliferation. *J. Cell. Sci.* **124**, 1280–1287 (2011).
61. Piwko-Czuchra, A. *et al.* β1 integrin-mediated adhesion signalling is essential for epidermal progenitor cell expansion. *PLoS ONE* **4**, e5488 (2009).
62. Rice, R. H. & Green, H. Relation of protein synthesis and transglutaminase activity to formation of the cross-linked envelope during terminal differentiation of the cultured human epidermal keratinocyte. *J. Cell Biol.* **76**, 705–711 (1978).
63. Lefort, K. *et al.* Notch1 is a p53 target gene involved in human keratinocyte tumor suppression through negative regulation of ROCK1/2 and MRCK kinases. *Genes Dev.* **21**, 562–577 (2007).
64. Samuel, M. S. *et al.* Actomyosin-mediated cellular tension drives increased tissue stiffness and β-catenin activation to induce epidermal hyperplasia and tumor growth. *Cancer Cell* **19**, 776–791 (2011).
65. Watt, F. M. & Fujiwara, H. Cell–extracellular matrix interactions in normal and diseased skin. *Cold Spring Harb. Perspect. Biol.* **3**, a005124 (2011).
66. Zhang, H., Pasolli, H. A. & Fuchs, E. Yes-associated protein (YAP) transcriptional coactivator functions in balancing growth and differentiation in skin. *Proc. Natl Acad. Sci. USA* **108**, 2270–2275 (2011).
67. Camargo, F. D. *et al.* YAP1 increases organ size and expands undifferentiated progenitor cells. *Curr. Biol.* **17**, 2054–2060 (2007).
68. Haynie, J. L. & Bryant, P. J. Intercalary regeneration in imaginal wing disk of *Drosophila melanogaster*. *Nature* **259**, 659–662 (1976).
69. Schwank, G. & Basler, K. Regulation of organ growth by morphogen gradients. *Cold Spring Harb. Perspect. Biol.* **2**, a001669 (2010).
70. Aegerter-Wilmsen, T., Aegerter, C. M., Hafen, E. & Basler, K. Model for the regulation of size in the wing imaginal disc of *Drosophila*. *Mech. Dev.* **124**, 318–326 (2007).
71. Nienhaus, U., Aegerter-Wilmsen, T. & Aegerter, C. M. Determination of mechanical stress distribution in *Drosophila* wing discs using photoelasticity. *Mech. Dev.* **126**, 942–949 (2009).
72. Hufnagel, L., Teleman, A. A., Rouault, H., Cohen, S. M. & Shraiman, B. I. On the mechanism of wing size determination in fly development. *Proc. Natl Acad. Sci. USA* **104**, 3835–3840 (2007).
73. Egeblad, M., Rasch, M. G. & Weaver, V. M. Dynamic interplay between the collagen scaffold and tumor evolution. *Curr. Opin. Cell Biol.* **22**, 697–706 (2010).
74. Erler, J. T. *et al.* Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature* **440**, 1222–1226 (2006).
75. Weaver, V. M. *et al.* Reversion of the malignant phenotype of human breast cells in three-dimensional culture and *in vivo* by integrin blocking antibodies. *J. Cell Biol.* **137**, 231–245 (1997).
76. Lei, Q.-Y. *et al.* TAZ promotes cell proliferation and epithelial–mesenchymal transition and is inhibited by the Hippo pathway. *Mol. Cell. Biol.* **28**, 2426–2436 (2008).
77. Bhat, K. P. L. *et al.* The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma. *Genes Dev.* **25**, 2594–2609 (2011).
78. Varelas, X. *et al.* TAZ controls Smad nucleocytoplasmic shuttling and regulates human embryonic stem-cell self-renewal. *Nature Cell Biol.* **10**, 837–848 (2008).
79. Varelas, X. *et al.* The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-β-SMAD pathway. *Dev. Cell* **19**, 831–844 (2010).
80. Varelas, X. *et al.* The Hippo pathway regulates Wnt/β-catenin signaling. *Dev. Cell* **18**, 579–591 (2010).
81. Heallen, T. *et al.* Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. *Science* **332**, 458–461 (2011).
82. Oh, H. & Irvine, K. D. Cooperative regulation of growth by Yorkie and Mad through *bantam*. *Dev. Cell* **20**, 109–122 (2011).
83. Harvey, K. F., Pfleger, C. M. & Hariharan, I. K. The *Drosophila* Mst ortholog, Hippo, restricts growth and cell proliferation and promotes apoptosis. *Cell* **114**, 457–467 (2003).
84. Wu, S. Huang, J., Dong, J. & Pan, D. *hippo* encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with *salvador* and *warts*. *Cell* **114**, 445–456 (2003).
85. Udan, R. S., Kango-Singh, M., Nolo, R., Tao, C. & Halder, G. Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. *Nature Cell Biol.* **5**, 914–920 (2003).
86. Pantalacci, S., Tapon, N. & Léopold, P. The Salvador partner Hippo promotes apoptosis and cell-cycle exit in *Drosophila*. *Nature Cell Biol.* **5**, 921–927 (2003).
87. Tapon, N. *et al. Salvador* promotes both cell cycle exit and apoptosis in *Drosophila* and is mutated in human cancer cell lines. *Cell* **110**, 467–478 (2002).
88. Kango-Singh, M. *et al.* Shar-pei mediates cell proliferation arrest during imaginal disc growth in *Drosophila*. *Development* **129**, 5719–5730 (2002).
89. Justice, R. W., Zilian, O., Woods, D. F., Noll, M. & Bryant, P. J. The *Drosophila* tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. *Genes Dev.* **9**, 534–546 (1995).
90. Xu, T., Wang, W., Zhang, S., Stewart, R. A. & Yu, W. Identifying tumor suppressors in genetic mosaics: the *Drosophila lats* gene encodes a putative protein kinase. *Development* **121**, 1053–1063 (1995).
91. Dong, J. *et al.* Elucidation of a universal size-control mechanism in *Drosophila* and mammals. *Cell* **130**, 1120–1133 (2007).
92. Huang, J., Wu, S., Barrera, J., Matthews, K. & Pan, D. The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the *Drosophila* homolog of YAP. *Cell* **122**, 421–434 (2005).
93. Oh, H. & Irvine, K. D. *In vivo* analysis of Yorkie phosphorylation sites. *Oncogene* **28**, 1916–1927 (2009).
94. Oh, H. & Irvine, K. D. *In vivo* regulation of Yorkie phosphorylation and localization. *Development* **135**, 1081–1088 (2008).
95. Wu, S., Liu, Y., Zheng, Y., Dong, J. & Pan, D. The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway. *Dev. Cell* **14**, 388–398 (2008).
96. Goulev, Y. *et al.* SCALLOPED interacts with YORKIE, the nuclear effector of the hippo tumor-suppressor pathway in *Drosophila*. *Curr. Biol.* **18**, 435–441 (2008).
97. Zhao, B. *et al.* TEAD mediates YAP-dependent gene induction and growth control. *Genes Dev.* **22**, 1962–1971 (2008).
98. Chan, S. W. *et al.* TEADs mediate nuclear retention of TAZ to promote oncogenic transformation. *J. Biol. Chem.* **284**, 14347–14358 (2009).
99. Peng, H. W., Slattery, M. & Mann, R. S. Transcription factor choice in the Hippo signaling pathway: *homothorax* and *yorkie* regulation of the microRNA *bantam* in the progenitor domain of the *Drosophila* eye imaginal disc. *Genes Dev.* **23**, 2307–2319 (2009).
100. Zhang, L. *et al.* The TEAD/TEF family of transcription factor Scalloped mediates Hippo signaling in organ size control. *Dev. Cell* **14**, 377–387 (2008).
101. Zhang, H. *et al.* TEAD transcription factors mediate the function of TAZ in cell growth and epithelial–mesenchymal transition. *J. Biol. Chem.* **284**, 13355–13362 (2009).
102. Zhou, D. *et al.* Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance. *Proc. Natl Acad. Sci. USA* **108**, e1312–e1320 (2011).
103. Hamaratoglu, F. *et al.* The tumour-suppressor genes *NF2/Merlin* and *Expanded* act through Hippo signalling to regulate cell proliferation and apoptosis. *Nature Cell Biol.* **8**, 27–36 (2006).
104. Genevet, A., Wehr, M. C., Brain, R., Thompson, B. J. & Tapon, N. Kibra is a regulator of the Salvador/Warts/Hippo signaling network. *Dev. Cell* **18**, 300–308 (2010).
105. Yu, J. *et al.* Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded. *Dev. Cell* **18**, 288–299 (2010).
106. Zhang, N. *et al.* The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. *Dev. Cell* **19**, 27–38 (2010).
107. Grzeschik, N. A., Parsons, L. M., Allott, M. L., Harvey, K. F. & Richardson, H. E. Lgl, aPKC, and Crumbs regulate the Salvador/Warts/Hippo pathway through two distinct mechanisms. *Curr. Biol.* **20**, 573–581 (2010).
108. Chen, C.-L., Schroeder, M. C., Kango-Singh, M., Tao, C. & Halder, G. Tumor suppression by cell competition through regulation of the Hippo pathway. *Proc. Natl Acad. Sci. USA* **109**, 484–489 (2011).
109. Doggett, K., Grusche, F. A., Richardson, H. E. & Brumby, A. M. Loss of the *Drosophila* cell polarity regulator Scribbled promotes epithelial tissue overgrowth and cooperation with oncogenic Ras–Raf through impaired Hippo pathway signaling. *BMC Dev. Biol.* **11**, 57 (2011).

110. Sun, G. & Irvine, K. D. Regulation of Hippo signaling by Jun kinase signaling during compensatory cell proliferation and regeneration, and in neoplastic tumors. *Dev. Biol.* **350**, 139–151 (2011).
111. Dow, L. E. & Humbert, P. O. Polarity regulators and the control of epithelial architecture, cell migration, and tumorigenesis. *Int. Rev. Cytol.* **262**, 253–302 (2007).
112. Laprise, P. & Tepass, U. Novel insights into epithelial polarity proteins in *Drosophila*. *Trends Cell Biol.* **21**, 401–408 (2011).
113. Robinson, B. S., Huang, J., Hong, Y. & Moberg, K. H. Crumbs regulates Salvador/Warts/Hippo signaling in *Drosophila* via the FERM-domain protein Expanded. *Curr. Biol.* **20**, 582–590 (2010).
114. Ling, C. *et al.* The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. *Proc. Natl Acad. Sci. USA* **107**, 10532–10537 (2010).
115. Chen, C.-L. *et al.* The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in *Drosophila*. *Proc. Natl Acad. Sci. USA* **107**, 15810–15815 (2010).
116. Ikeda, M. *et al.* Hippo pathway-dependent and -independent roles of RASSF6. *Sci. Signal.* **2**, ra59 (2009).
117. Polesello, C., Huelsmann, S., Brown, N. H. & Tapon, N. The *Drosophila* RASSF homolog antagonizes the hippo pathway. *Curr. Biol.* **16**, 2459–2465 (2006).
118. Ribeiro, P. S. *et al.* Combined functional genomic and proteomic approaches identify a PP2A complex as a negative regulator of Hippo signaling. *Mol. Cell* **39**, 521–534 (2010).
119. Stephens, L. E. *et al.* Deletion of β1 integrins in mice results in inner cell mass failure and peri-implantation lethality. *Genes Dev.* **9**, 1883–1895 (1995).
120. Monkley, S. J. *et al.* Disruption of the talin gene arrests mouse development at the gastrulation stage. *Dev. Dyn.* **219**, 560–574 (2000).
121. Larue, L., Ohsugi, M., Hirchenhain, J. & Kemler, R. E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proc. Natl Acad. Sci. USA* **91**, 8263–8267 (1994).
122. Miner, J. H., Cunningham, J. & Sanes, J. R. Roles for laminin in embryogenesis: exencephaly, syndactyly, and placentopathy in mice lacking the laminin α5 chain. *J. Cell Biol.* **143**, 1713–1723 (1998).
123. Krieg, M. *et al.* Tensile forces govern germ-layer organization in zebrafish. *Nature Cell Biol.* **10**, 429–436 (2008).
124. Davidson, L. A. Epithelial machines that shape the embryo. *Trends Cell Biol.* **22**, 82–87 (2011).
125. Weber, G. F., Bjerke, M. A. & DeSimone, D. W. A mechanoresponsive cadherin–keratin complex directs polarized protrusive behavior and collective cell migration. *Dev. Cell* **22**, 104–115 (2012).
126. Eiraku, M., Adachi, T. & Sasai, Y. Relaxation-expansion model for self-driven retinal morphogenesis: a hypothesis from the perspective of biosystems dynamics at the multi-cellular level. *Bioessays* **34**, 17–25 (2012).
127. Mammoto, T. *et al.* Mechanochemical control of mesenchymal condensation and embryonic tooth organ formation. *Dev. Cell* **21**, 758–769 (2011).
128. Taber, L. A., Voronov, D. A. & Ramasubramanian, A. The role of mechanical forces in the torsional component of cardiac looping. *Ann. NY Acad. Sci.* **1188**, 103–110 (2010).
129. Hove, J. R. *et al.* Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. *Nature* **421**, 172–177 (2003).
130. Adamo, L. *et al.* Biomechanical forces promote embryonic haematopoiesis. *Nature* **459**, 1131–1135 (2009).
131. North, T. E. *et al.* Hematopoietic stem cell development is dependent on blood flow. *Cell* **137**, 736–748 (2009).
132. Liu, Z. et al. Mechanical tugging force regulates the size of cell-cell junctions. *Proc. Natl Acad. Sci. USA* **107**, 9944–9949 (2010).
133. Keller R., Davidson L. A. & Shook D. R. How we are shaped: the biomechanics of gastrulation. *Differentiation* **71**, 171–205 (2003).

Acknowledgements
The authors apologize to colleagues whose work could not be cited owing to space limitations. We thank G. Vozzi and A. Mertz for help with the manuscript. This work was supported by grants from the Flemish Institute for Biotechnology (VIB), the US National Institutes of Health (NIH) and Bob Duncan, American Leather to G.H.; Italian Association for Cancer Research-Principal Investigator grant (PI-AIRC) and Italian Ministry for University and Research (MIUR) grants to S.D.; AIRC Special Program Molecular Clinical Oncology "5 per mille", Human Frontiers Science Programme (HSFP), University of Padua Strategic-grant, Excellence-grant-IIT, Epigenetics Flagship project CNR-Miur to S.P.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Stefano Piccolo's homepage:
http://www.bio.unipd.it/piccolo

ALL LINKS ARE ACTIVE IN THE ONLINE PDF