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"Forced to communicate: integration of mechanical and biochemical signaling in morphogenesis"

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Abstract

Morphogenesis is a physical process that requires the generation of mechanical forces to achieve dynamic changes in cell position, tissue shape, and size as well as biochemical signals to coordinate these events. Mechanical forces are also employed by the embryo to transmit detailed information across space and detected by target cells leading to downstream changes in cellular properties and behaviors. Indeed, forces provide signaling information of complementary quality that can both synergize and diversify the functional outputs of biochemical signaling. Here we discuss recent findings that reveal how mechanical and biochemical signaling are integrated during morphogenesis and the possible context-specific advantages conferred by the interactions between these signaling mechanisms.

Introduction: force generation and detection in morphogenesis

Morphogenesis occurs across a range of time scales and physical space, requiring the coordinated interplay of a host of different cell behaviors. Although ligand-based biochemical signaling elicits cellular responses during tissue morphogenesis, the mechanical

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References and recommended reading

Notable, recently-published papers have been highlighted in references as being of *special interest or **outstanding interest

forces generated by cells downstream of this signaling ultimately mold tissues. However, these forces can also be detected by cells leading to biochemical and mechanical signal propagation within and between cells, that not only regulate cellular behavior and fate changes, but also coordinate and diversify the functional outputs of biochemical signaling to propel morphogenesis. Here we focus on this particular form of mechanical signaling in development and examine *in vivo* examples where forces are utilized by cells to transmit information to other cells with unique advantages.

Cells and tissues can generate and transmit forces by several general mechanisms, but all of these begin with the cytoskeleton [1]. Actin polymerization generates pushing force during the establishment of cellular protrusions, and tension is generated when non-muscle myosin II (MyoII) binds to filamentous actin (F-actin) and hydrolyzes ATP to convert chemical energy into mechanical movement [2]. Forces generated by actomyosin contractility are transmitted across tissues through adhesion molecules that allow individual cellular forces to be translated into global changes in tissue shape. Adherens junctions (AJs) vary in their size and composition, but are mediated by classic cadherins that connect to the actin cytoskeleton intracellularly through binding to β-catenin, which in turn binds α-catenin (Fig. 1A). Under contractility-generated tension, α-catenin undergoes conformational changes to recruit vinculin, which connects to F-actin [3], resulting in maturation and growth of the AJ and recruitment of additional F-actin (Fig. 1A') [3,4,5]. This mechanosensory function allows the AJ to react dynamically to other cells and actomyosin contractility while mechanically coupling the intracellularly-generated force with surrounding cells. Whereas most of the force generation for morphogenesis has been thought to derive from the actomyosin cytoskeleton, microtubules can also generate forces within cells, and this is coordinated by cell-signaling to regulate cell shape and epithelial morphogenesis in *Drosophila* [6–8].

In addition to cell-cell adhesion, cell-extracellular matrix (ECM) adhesion is critical to convey or buffer the transmission of forces across tissues during morphogenesis [9,10]. Physical coupling of cells to the ECM at focal adhesions (FA) is critical for cellular reorganization and movement, but the ECM is also an instructional biochemical signal received through integrin receptors to modulate downstream signaling cascades and control a variety of cell behaviors during development [9]. FAs are large multiprotein signaling hubs that include heterodimeric integrin receptors, which recruit intracellular adaptors including talin and vinculin, linking the FA to F-actin, and focal adhesion kinase (FAK) and SRC kinase, which can activate numerous downstream pathways (Fig. 1B, B'). Similar to α-catenin in AJs, physical force exerted by actomyosin contractility mechanically induces a conformational change in TALIN, allowing increased actin binding and greater FA stability [11]. The strength of cell-ECM adhesions is also modulated by the stiffness of the ECM wherein stiffer substrates allow cells to adhere more strongly and exert higher tension. Integrin/ECM binding therefore allows the detection of distinct types and compositions of ECM and organizes the formation of signaling complexes with the actomyosin cytoskeleton [10]. In this way cells effectively convert mechanical information into biochemical signals and through changes in actomyosin contractility, can reciprocally remodel ECM, resulting in a host of cellular and tissue-level changes; this form of mechanosensation has been reviewed extensively elsewhere [9,12], and will not be the focus of this review, which will instead focus on the ways that cells utilize force to communicate with other cells. In addition to

providing a biochemical ligand for physical or chemical signaling into cells, ECM may generate forces signaling to cells; for example, hydration of chondroitin sulfate proteoglycan in the vegetal epithelium of sea urchins results in differential expansion and bending of a bilayered cell sheet [13].

One important consequence of mechanically sensitive signaling at FAs and AJs is the biochemical activation of the Yes Associated Protein (YAP) and WW Domain Containing Transcription Regulator 1 (TAZ), leading to changes in transcription that impact cell proliferation and differentiation. YAP and TAZ, initially identified as Yorkie in Drosophila (Yki/YAP/TAZ), are transcriptional effectors of the Hippo (Hpo/MST1/2) kinase signaling cascade [14]. When this pathway is active, Hpo/MST1/2 kinases bind to the Sav/SAV1 adapter protein and phosphorylate Wts/LATS1/2 kinases to activate them. In turn, the Wts/LATS1/2 serine/threonine kinases phosphorylate Yki/YAP/TAZ and prevent them from entering the nucleus and activating transcription (Fig. 1C). Inactivation of the Hippo pathway results in Yki/YAP/TAZ accumulation within the nucleus, where they bind to TEAD transcription factors to drive target gene expression and promote cell proliferation and survival (Fig. 1C'). Mechanical change detected by FAs and AJs under actomyosin-generated contractility is a key force-sensing signaling mechanism that leads to activation of Yki/YAP/TAZ and transcriptional changes. Cell junctions serve as a site of assembly for Hippo pathway members and loss of AJ components can lead to increased YAP nuclear localization in different contexts [14]. In contrast, activation of YAP at FAs involves FAK and SRC activation of PI3K, leading to inhibition of LATS1/2 and nuclear accumulation of YAP [15]. Forces transmitted through FAs can also mechanically alter nuclear shapes and stretch nuclear pores to allow active nuclear YAP import [16]. Generally, the ability of Hippo signaling to measure junctional changes depends on actomyosin contractility, and thereby provides a central pathway for translating physical information into biochemical information in the form of gene expression changes.

In addition to the described mechanisms of force detection at cell junctions, dedicated mechanosensors can detect forces within a tissue. For example, PIEZO proteins are mechanically sensitive ion channels that are critical for mechanosensation in multiple contexts in development by sensing crowding forces to induce cell extrusion and control cell density [17], and to regulate stem cell proliferation and differentiation [18,19]. Although the mechanisms by which PIEZO transduces a biochemical signal are still under active investigation, it is clear that they can function through Ca^{2+} signaling [18], or by impacting YAP/TAZ function (Fig. 1D, D') [19,20].

While mechanical forces can be detected and translated into biochemical signaling, it is also the case that biochemical signaling pathways that regulate morphogenesis have outcomes that generate forces. For example, EPH/EPHRIN signaling often regulates actomyosin contractility [21,22]; mitogenic signals such as WNT and SHH increase cell number, and chemoattractant pathways such as FGF increase cellular aggregation, both of which lead to increased cell density to generate compression forces. That these forces are transmitted throughout a tissue with both directional and magnitude information and detected by other cells suggests that these forces may be utilized as signal transducers downstream of biochemical signals. Here we review recent discoveries that connect biochemical signaling

with mechanical signaling, focusing particularly on those cases where mechanical forces mediate biochemical signaling to regulate morphogenesis. These studies support the idea that force is not only detected during development, but that it is actively employed to transmit and convey biochemical signaling information in a manner that provides unique advantages.

Mechanical signals coordinate physical information with cellular differentiation and proliferation

Several recent papers have demonstrated that forces can signal to couple cell position within a tissue with cell fate specification, thereby coordinating physical information and cellular differentiation (Fig. 2A). While it is known that the stemness of epidermal progenitors can be manipulated by altering cell shape or ECM stiffness [23,24], how mechanical changes are employed to enable specific cell fate decisions has remained unclear. Totaro et al. recently demonstrated that cell shape and ECM rigidity regulates YAP/TAZ, which in turn regulate Notch signaling and downstream differentiation within the epidermis. In epidermal stem cells experiencing higher mechanical forces from either cytoskeletal or ECM rigidity, YAP nuclear localization inhibits Notch signaling, promoting epidermal stemness [25]. Conversely, low mechanical force inhibits YAP/TAZ, thus releasing Notch signaling, promoting differentiation [25]. Interestingly, YAP/TAZ increase expression of several Notch ligands, including DLL1 and DLL3, which stimulate Notch activity in neighboring cells [25,26]. Importantly, these same ligands likely inhibit differentiation of basal cells through their cis-regulation, thus maintaining a layer of basal progenitors, and efficient differentiation of suprabasal cells [25,26].

Mechanical signals are integrated to coordinate boundary formation and cell differentiation during rhombomere formation in the developing hindbrain. Rhombomeres are developmentally transient blocks of neuroepithelial cells that give rise to distinct structures in the vertebrate hindbrain. Boundaries between rhombomeres are formed as a result of signaling between Eph receptor tyrosine kinases and their signaling partner, the Ephrins; in zebrafish, alternating rhombomeres express EphA4 and Ephrin-B3 such that EphA4/Ephrin-B3 signaling only occurs at the rhombomere boundary [27–30]. Boundary cells express molecular markers that distinguish them from non-boundary cells, provide proliferating progenitors and organize spatially-restricted neurogenesis within segments [31–33], and are involved in boundary straightening through the formation of actomyosin-cable like structures at rhombomeric boundaries [34]. Disruption of either actomyosin contractility or Eph/Ephrin signaling disrupts boundary sharpness [34,35]. Interestingly, increased tension from actomyosin contractility at rhombomere boundaries creates this positional information, which impacts boundary cell identity [22,36]. EphA4 loss of function results in reduced actomyosin contractility at rhombomere boundaries and a loss of boundary cells in EphA4-expressing rhombomere segments, indicating that EphA4 signaling generates positionally-specific tension to simultaneously specify rhombomere separation and cell identity specification [22]. This increased tension at rhombomere boundaries promotes Taz nuclear localization and downstream activation of boundary markers [22,36] in EphA4-expressing boundary cells. When Yap/Taz pathway components are disrupted, border cell marker

expression is lost [22]. Yap also maintains the proliferative capacity and the progenitor potential in boundary cells, with neurogenesis coinciding with Yap downregulation as daughter cells exit the boundary domain [36]. Together these data elucidate a complete pathway linking boundary formation and maintenance through Eph/Ephrin signaling to downstream cell fate decisions by a mechanical signaling intermediary. Interestingly, as Notch pathway components are also expressed in boundary cells and have been shown to regulate neurogenesis in rhombomeres, it is intriguing to postulate that integration of mechanical signals and Notch activation may similarly exist here as in the example above to preserve progenitor state in boundary cells.

In many contexts, changes in force can be interpreted as morphogenetic signals to rapidly remodel and differentiate specialized cell types that further contribute to organ development and function (Fig. 2B). Shear force due to blood flow is detected during outflow tract (OFT) valve development in zebrafish, coupling the positions of highest shear force due to blood flow with positional specification of smooth muscle differentiation that results in valve morphogenesis [20]. In the regions of the OFT with the smallest diameter, where shear stress is highest, Piezo mechanosensitive channels detect this shear force, resulting in spatially-restricted expression of Klf2 and Notch signaling within the valve endothelium, and Yap1 activation and differentiation of the underlying smooth muscle. In the atrioventricular heart valve, Klf2a and Notch signaling activity are also high in regions experiencing high blood flow [37,38], though the structure of this valve, and the forces it experiences are somewhat different. Indeed, it is notable that Klf2 expression and Notch signaling are commonly mechanosensitive to blood flow during development [39,40], supporting them as common nodes in pathways converting mechanical to biochemical signaling information in a spatially-restricted manner.

Intra-organ communication is necessary to coordinate the growth and position of discrete, but interdependent structures (Fig. 2C). In zebrafish heart development, Wnt8a signaling is critical for promoting cardiomyocyte formation and its overexpression results in increased atrial and decreased ventricle myocardial size [41]. Interestingly, this effect is mirrored by changes in the size of the underlying atrial and ventricular endocardium [42]. Expansion of the myocardium places the endocardial cells under tension, which is sensed by junctional Cadherin-5 (VE-cadherin), resulting in nuclear Yap1 localization and increased proliferation of endocardial cells to compensate for myocardial overgrowth. These data reveal that tension generated by tissue growth can signal to neighboring tissues allowing the coordination of tissue-intrinsic growth rates.

Chemical signals modulate cell polarity, adhesion, and tissue deformability to signal mechanically

The emergence of coordinated collective cell behaviors requires the detection, coupling, and propagation of forces across groups of cells. Tissue rheology, or the way in which tissues mechanically react, arises from the contractility of the cells composing the tissue, the ECM, and the strength of the cell-cell contacts within a tissue. Viscoelasticity determines the deformability of the tissue and permissibility for cellular arrangement in response to

inductive signals. Modulation of these properties within a tissue allows for regulated deformation and shaping of a tissue.

Chemical signals can guide morphogenesis by tuning tissue mechanics and viscoelasticity through control of adhesion, cortical contractility, and associated cell polarity. This was recently demonstrated in the developing mouse pharyngeal arch, which is composed of a mesenchymal core surrounded by a single layer of epithelium, and undergoes extensive outgrowth and shape changes throughout development. Tao et al. demonstrated that, in the mesenchyme, WNT5a activates PIEZO1 to induce oscillations in cortical tension in the middle portion of the developing arch, resulting in reduced tissue viscoelasticity and increased cell intercalation to drive arch elongation [43]. In *Wnt5a* mutant mice the shape of the mandibular arch is disrupted with diminished cortical oscillations and a decrease in oriented cell intercalation, suggesting that WNT5a coordinates mandibular cell behaviors through control of cell polarity and cytoskeleton tension [43]. This study therefore demonstrates a mechanism by which chemical signals impact tissue mechanics to enable proper morphogenesis.

Signaling by WNT5a through the ROR2 receptor is also critical during angiogenesis, where it coordinates endothelial cell behavior by activating CDC42 and stabilizing vinculin at the AJ [44]. This results in mechanocoupling between endothelial cells and their collective polarization, which is necessary for their proper migration. Therefore, non-canonical WNT signaling tunes the sensitivity of endothelial cells to junctional force to modulate their behavior. Detection and sensitivity of cells to forces is often tuned by biochemical signaling pathways, thereby allowing these pathways to influence the cellular outcomes upon experiencing a given force. As in heart development, shear force from blood flow is critical for vessel reorganization during angiogenesis, such that the direction and strength of flow dictates endothelial cell polarization and migration. Endothelial non-canonical WNT signaling is required for the detection of shear force, and modulates sensitivity to this force in order to select which vessels undergo normal pruning [45]. Interestingly, VEGFR3 signaling also influences sensitivity of endothelial cells to shear stress from flow, indicating that in this context multiple biochemical pathways converge to regulate sensitivity to a force-based signal [46]. Differences in VEGFR3 levels may be a major determinant of differences in sensitivity to shear stress by vascular endothelial cells, compared with lymphatic endothelial cells, which have a higher sensitivity to shear stress allowing detection of lower flow rates and therefore the remodeling of these different vascular cell types at different force reception set-points [46].

Mechanical modulation of chemical signaling by cell density and crowding forces

As morphogenesis progresses, changes in tissue shape and cell organization can concurrently reshape the spatial distribution of signaling molecules (Fig. 3A). For instance, villi formation in the developing chick gut as a result of mechanical buckling of the endodermal epithelium distorts the SHH signaling gradient from the epithelium, concentrating the signal at the tip of each villus to activate high threshold response genes in

the mesenchyme that ultimately determine the location of intestinal stem cells [47]. This suggests that tissue mechanical forces can actively modulate signaling pattern via emerging cellular organization. This idea is consistent with recent findings in developing chick feather buds, which arise in the midline of the dorsal skin as regularly spaced mesenchymal aggregates beneath epidermal placodes, with subsequent new buds formed laterally in a spatiotemporal manner. Feather bud development is initiated as a result of MyoII-dependent mesenchymal contraction that amplifies randomly formed small cell clusters into larger aggregates [48]. Condensed mesenchyme in turn compresses the overlying epithelium and mechanically induces nuclear accumulation of β-catenin to initiate the follicle genetic program [48,49]. This mesenchymal contraction also concentrates and upregulates local FGF20 signaling from the epithelium to further promote mesenchymal condensation [50,51]. Simultaneously, condensed mesenchyme begins to express BMP4, which diffuses and inhibits epithelial *Fgf20* expression neighboring the condensate [50]. Tissue mechanical forces thus help shape FGF20 and BMP4 expression pattern with altering peaks and troughs of FGF20 and BMP4 signaling activities, which function as the activator and inhibitor respectively in a Turing reaction-diffusion system [52] to establish the formation of feather buds repetitively at a regular interval.

Formation of repetitive structures can also be achieved through molecular oscillators, such as in the vertebrate presomitic mesoderm (PSM). In this model, cyclic activation of Notch and WNT pathways and corresponding signaling responsive genes generate periodic travelling waves of signaling activation and instruct the formation of segmented structures called somites [53]. Interestingly, when PSM cells are dissociated and scrambled in primary cell culture, they continue to oscillate and produce waves of Notch signaling [54]. However, this phenomenon is only maintained when the cell density is above certain threshold. The system exhibits a quorum sensing behavior involving YAP, which functions as a checkpoint to only allow full Notch signaling when a certain cell crowding threshold is reached. Intriguingly, quorum sensing via YAP can be modulated by cell shapes and actin-dependent mechanical forces, raising the possibility that signaling oscillation during somite formation is regulated by mechanical inputs associated with changes in crowding-force [54]. It will be interesting to determine if such an excitable density detection system similarly functions in other developmental contexts involving cell condensation, such as in the feather bud example above, to govern local activation of specific signaling cascades and generation of signaling waves.

Mechanical force as a long-range intermediary signal to regulate morphogenesis

While paracrine signaling is only effective over a relatively short distance of 50–100 μm (spanning 5–10 cells) due to rapid signal dilution and decay in its intensity [55–57], mechanical forces can be directionally transmitted over a longer distance and function as a long-range morphogenetic signal downstream of a localized biochemical stimulus (Fig. 3B). One example demonstrating mechanical signaling over distance is the regulation of zebrafish body elongation by the tail organizer [58]. Bmp signaling from the tail organizer is postulated to promote an ordered anterior-to-posterior cell flow in the tail bud that

contributes to body elongation [59,60]. When Bmp signaling is perturbed, a cell-to-cell relay of disturbed cell motion in the tail bud results in a mechanical transmission of cellular jamming that travels posterior-anteriorly, resulting in disorganized cell motion outside the Bmp signaling range [58]. This hints at a mechanism whereby signaling ligands may induce directional movement of cells outside the signaling range by propagating mechanical signals through neighboring cells.

How then do cells propagate mechanical signals over distance without dampening force transmission? A recent paper addressed this question by studying *Drosophila* endoderm morphogenesis, a MyoII-dependent process involving invagination of endoderm primordium moving posterior-anteriorly [61]. Importantly, while Rho1/MyoII activation is initiated by secreted Fog/GPCR signaling, a wave of MyoII actomyosin contractility continually propagates endoderm invagination anteriorly along the dorsal epithelium without Fog signal propagation. As MyoII can be activated in response to mechanical stimuli, such as increased cellular tension [62–64], cellular forces associated with epithelial buckling trigger apical spreading in unbuckled cells at the anterior edge of the furrow and activate MyoII in these cells and their subsequent buckling, thus cyclically amplifying the travelling mechanical wave. Interestingly, sequential activation of MyoII is also observed in other developmental contexts, such as the mechanical interaction between the invaginating endoderm and extending germband in *Drosophila* and the zippering process during neural tube closure in *Ciona intestinalis* [65,66], all suggesting that mechanical forces can act as a long-range signal and as a second messenger to regulate morphogenesis at a distance. Future work will determine whether such mechanisms can also control other cell behaviors, such as differentiation, proliferation, and polarity in this and other developmental processes.

Conclusions and perspectives

Cells may have evolved to actively utilize force as an extracellular second messenger to transduce information between cells with several advantages. The recent studies that we describe above give insight into this idea, and present explanations of the advantages that might be achieved by employing mechanical signals: these signals can coordinate growth of organs, specify cell fate with respect to tissue architecture, modulate chemical signals, and act over longer distances than biochemical signals. Importantly, mechanical force is a multiparameter signal; whereas a biochemical signal detected at a single point has only a magnitude value, mechanical force is a vector quantity, encoding both magnitude and directional information. This property makes mechanical forces particularly compelling for providing information to organize directed cell behaviors such as cell polarity and cell migration. Multiple biochemical signals may therefore converge to collate and convert information from multiple cellular inputs into a mechanical force signal that can be transmitted in a coordinated fashion. As mechanical signals have been historically challenging to observe, increasingly integrating techniques such as atomic force microscopy and laser ablation with genetic and biochemical approaches as well as the application of new techniques such as the application of oil droplets, or magnetic beads to measure and apply forces, will be transformative in further understanding the interplay between mechanical and biochemical signals during development [67,68].

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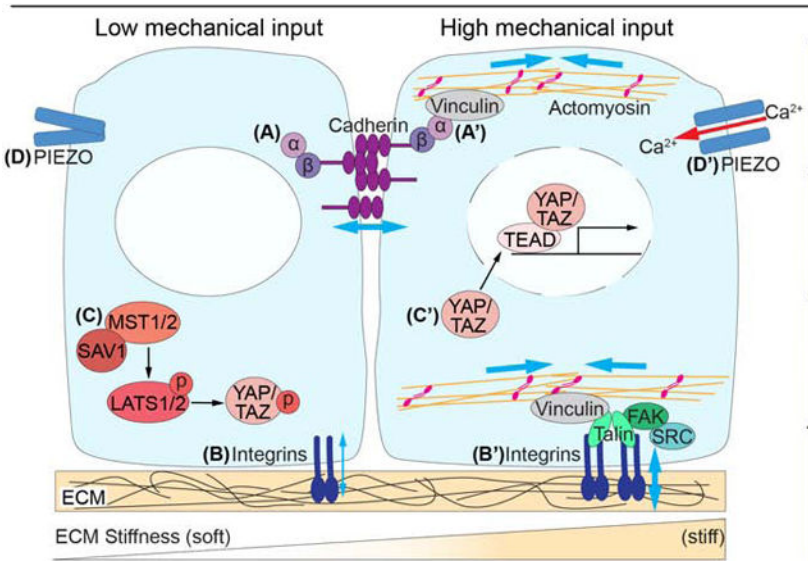

Mechanism	Example Ref.
A, A') Adherens Junctions	3, 4, 5, 42
B, B') Focal Adhesions	9, 10, 11, 12, 13, 25
C, C') Hippo/Yap/ Taz Pathway	14, 15, 16, 25, 36
D, D') Dedicated Mechanosensors	17, 18, 43

Figure 1. Mechanisms of mechanosensation.

Mechanical forces are sensed and transmitted across cells and tissues through a variety of mechanisms. (**A**) Cadherins, bound intracellularly to β-catenin, which in turn binds α-catenin, make up adherens junctions. (**A'**) Under tension, generated by actomyosin contractility, α-catenin recruits the actin binding protein vinculin. The mechanosensory function of adherens junctions allows the mechanical coupling of adjacent cells. (**B**) Focal adhesions, composed of Integrins, couple cells to the ECM providing cells with both mechanical and biochemical information. (**B'**) Under tension a series of intracellular adaptors are recruited to focal adhesions, including FAK, SRC, Talin and Vinculin, linking the focal adhesions to actomyosin. (**C**) The Hippo/YAP/TAZ pathway is a critical mechanosensitive signaling pathway. When there is low mechanical input MST1/2 kinases bind SAV1, phosphorylating LATS1/2 kinases which in turn phosphorylate YAP/TAZ, preventing them from entering the nucleus. (**C'**) When there is high mechanical input Hippo signaling is inactive, allowing YAP/TAZ to translocate to the nucleus, where they bind to TEAD transcription factors, driving target gene expression. (**D**) Dedicated mechanosensors, such as PIEZO proteins, can also detect forces within a tissue. (**D'**) When sensing crowding forces, PEIZO channels undergo a conformational change, enabling a calcium influx into the cell to impact downstream signaling.

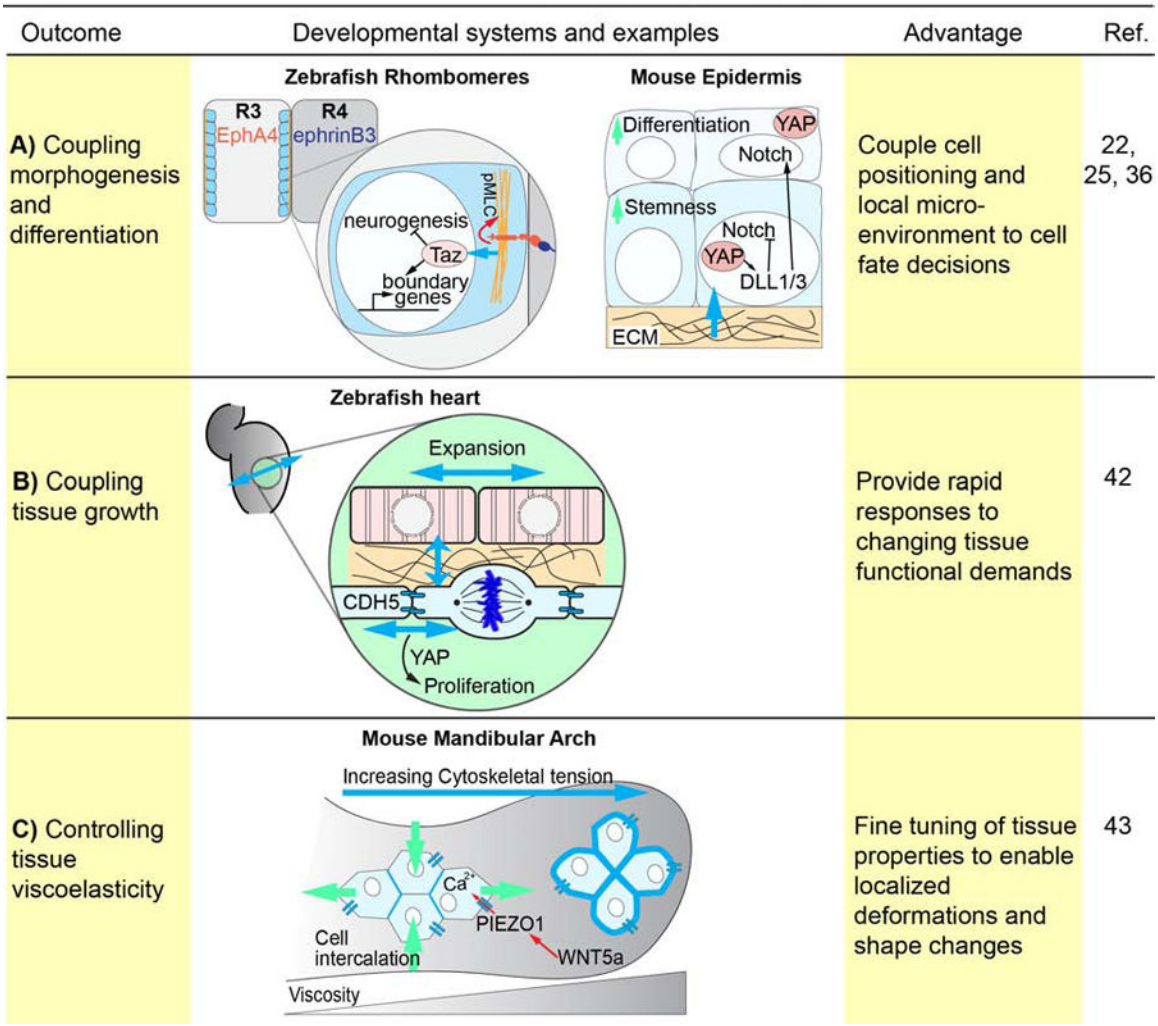

Figure 2. Integration of mechanical and biochemical signaling during morphogenesis.

Mechanical and biochemical signaling can be integrated to affect various morphogenetic outcomes including tissue growth and cellular differentiation. (**A**) In developing zebrafish rhombomeres morphogenesis is coupled to cellular differentiation through Eph/Ephrin signaling generated actomyosin contractility, which in turn activates Yap/Taz signaling in boundary cells. Additionally, YAP/TAZ mechanotransduction inhibits NOTCH signaling in the developing mouse epidermis to maintain epidermal stemness in basal cells, while promoting differentiation of the suprabasal layer. (**B**) In the zebrafish heart myocardial and endocardial growth are coupled through tension sensing via VE-cadherin and Yap1 to regulate cell proliferation. (**C**) Mechanical and biochemical signaling can also be integrated to modulate tissue viscoelasticity as demonstrated in the developing mandibular arch, where WNT5a acts upstream of YAP and Piezo1 to coordinate cellular polarity and force oscillations in the middle arch to diminish tissue rigidity, enabling cell intercalations.

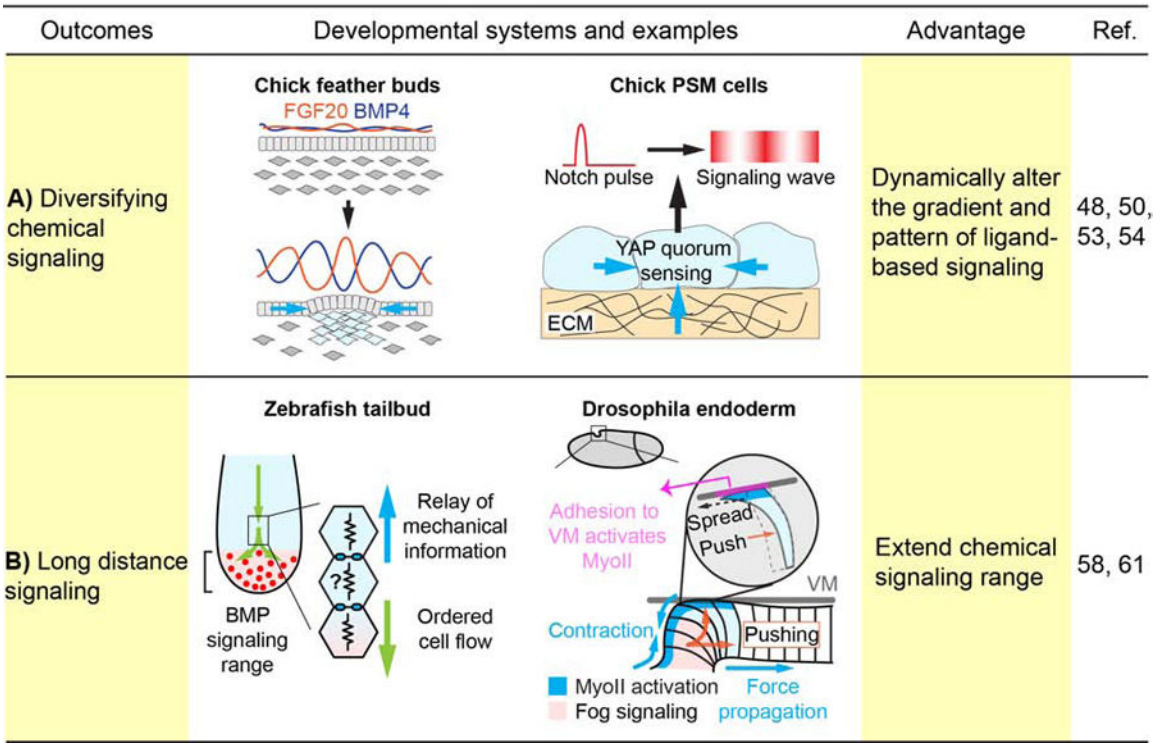

Figure 3. Mechanical modulation of chemical signaling.

Tissue mechanical forces can modulate the gradient and pattern of biochemical signaling. (**A**) In the chick feather buds, condensing mesenchyme contracts overlying epithelium to concentrate local FGF20, and at the same time secrets BMP4 that diffuses and inhibits neighboring FGF20 expression, resulting in a Turing-like pattern. In the chick presomitic mesoderm (PSM), YAP integrates mechanical information from the substrate and cell density to transform signaling pulses into oscillations and waves. (**B**) Tissue forces can also function as a second messenger downstream of a biochemical source to relay its instructive signal across space. For instance, in the zebrafish tailbud, the anterior-to-posterior cell flow is modulated by mechanical signals transmitted from cell to cell and thus beyond the range of Bmp signaling in the tail organizer. Similarly, during Drosophila endoderm invagination, although the initial MyoII activation is initiated by Fog signaling, the subsequent traveling wave of MyoII activation and apical contraction is independent from Fog signaling and is induced by cyclic forward pushing of buckling cells and apical spreading of edge cells along the vitelline membrane (VM).