Mechanical mechanisms of morphogenesis as potential substrates for evolutionary change

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Abstract

The first quarter of this century has seen a resurgence of interest in the mechanical and physical mechanisms that drive cellular behaviors in the context of morphogenesis. Far from being a new discovery, the fact that the material properties of cells and the physical forces that they exert ad experience must play decisive roles in development, was an important part of the field of experimental embryology well over a century ago. Following the birth of molecular biology, and the development of live imaging approaches that can capture the dynamics of both cellular properties and materials, and the activity of genes and gene products, the current manifestation of this field promises to link mechanical and molecular genetic mechanisms. Here we review recent advances in understanding the relationships between mechanical and molecular genetic mechanisms, and suggest paths forward that could yield answers to the pressing questions of whether and evolutionary forces act not only on functional morphologies, but also on the mechanical forces that create them.

Keywords

Physics; forces; cytoskeleton; genetics; materials; development

Introduction

Much like a bust is carved through sequences of tool-born forces against marble or a house built from the fastening of wooden posts, multicellular organisms are constructed through specific actions of removing, adding, connecting, and refiguring the materials that constitute them. The result of these transformations during development, what we consider here as morphogenesis, is the generation of forms – segments, organs, limbs, connective tissues, etc. – that organize the body and give organisms the ability to reproduce, move, communicate, and absorb nutrients, among other essential physiological tasks. The diversity of ways that extant multicellular species engage their environments reflects the long evolutionary history of these form-generating processes.

It has been appreciated for over a century that physical forces and material properties of cells and tissues–akin to those of marble or wood–play a central role in multicellular development. In the 1860s, only a few years after the publication of Darwin's *On the Origin of Species* [1], the embryologist Wilhelm His constructed "mechanical" models of early-stage chick embryos in which he deformed lead plates to approximate their emerging form [2] (**Figure 1**).

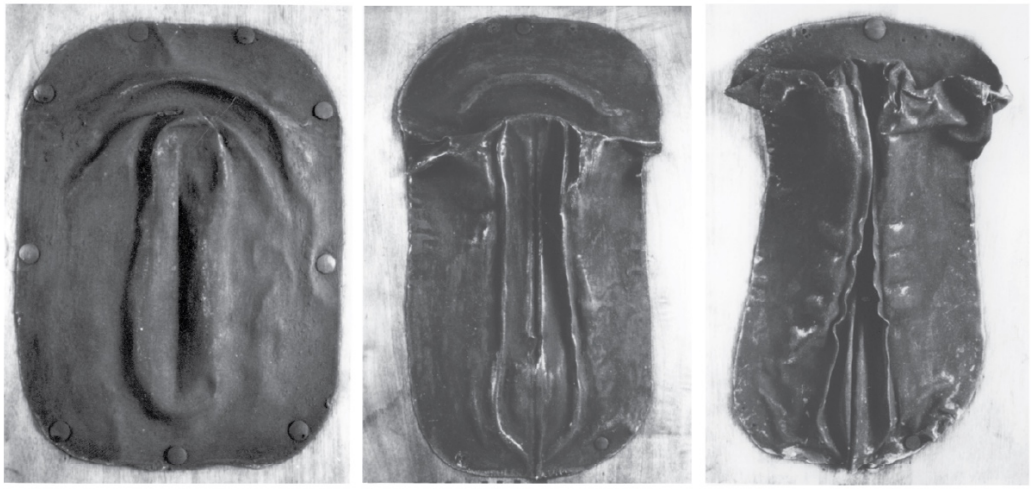

Figure 1. Wilhelm His' developmental series of the early chick embryo in which development is modeled as the physical deformation of a pliable lead plate (circa 1866-67). Reprinted with permission from the Anatomisches Museum Basel.

His also collaborated with the mathematician Eduard Hagenbach to mathematically model the chick embryo as a sheet with zones of differential elasticity [3] (**Figure 2**). His was a proponent of the cytoplasm as the locus of morphogenetic "anlagen," [4] meaning that he considered that the rudimentary or foundational contributors to morphogenesis, were to be found in the cytoplasm of cells. His' approach would influence Wilhelm Roux's program of developmental mechanics [5,6] as well as D'Arcy Thompson's *On Growth and Form* decades later [7,8]. Even though embryology played a central role in nineteenth century conceptions of species transformation [9], questions of ontogeny would become increasingly siloed from those of phylogeny in subsequent decades [10].

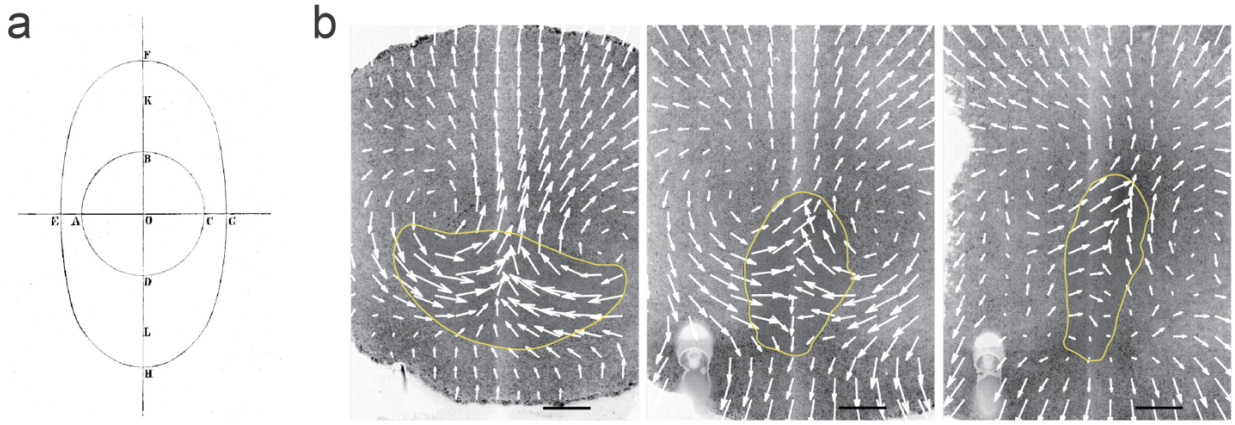

Figure 2. Mathematical and physical descriptions of early chick development. At these stages, the chick embryo is a relatively flat, round disk. **a** Schematic of the chick embryo from "Change in Form of a Thin, Incompletely Elastic Plate, of Which Different Parts Grow Unequally" by Eduard Hagenbach (title translation from [2], published in Wilhelm His' *Untersuchungen über die erste Anlage des Wirbelthierleibes: die erste Entwickelung des Hühnchens im Ei* [3]) **b** Three chronologically consecutive stages (Hamburger Hamilton stages [11]HH1, HH2 and HH3 from left to right) of a gastrulating chick embryo. The regions outlined in yellow undergo convergent extension and ingression to generate the tissue flows indicated by the white arrows (velocity). Scale bars = 500 μm. Modified from Figure 1 in [12].

By the end of the twentieth century, the physical and material processes of form-generation as a causal force in development and morphogenesis had become sidelined. The rise of genetics and molecular biology infused mainstream evolutionary and developmental biology, which became nearly synonymous with developmental genetics, with explanatory frameworks that reduced development and evolution to the action of individual or networks of genes [13–15]. The mechanics of form-building was by-and-large understood to be explained entirely by genetic "programming" and natural selection on genetic variation [16–18].

Given this history, the first decades of twentieth century evolutionary developmental biology, or "evo-devo," leveraged the gene as the pivot point between scales and broader evolutionary relationships. Indeed, some of the most highly cited papers on the topic of the evolution of morphogenesis–specifically of animal eyes and vertebrate limbs–build their explanations around the presence of the same individual "master" genes or gene families across species [19–21].[1] This molecular orientation, which has continued to permeate contemporary research, has also skewed the narrative of evo-devo's history towards the identification and cloning of conserved body patterning genes across diverse phyla [22].

From its inception, however, evo-devo included morphogenetic perspectives. The physical aspect of form-generation was a central interest of John Tyler Bonner, who studied morphogenesis in cellular slime molds and organized the catalyzing 1981 Dahlem Workshop on Evolution and Development [23–25]. Bonner was heavily influenced by D'Arcy Thompson and embryologists of the mid-twentieth century who embraced explanations of development that accounted for dynamic, form-generating processes emerging at the level of cells and tissues [15,26]. Placing morphogenesis more squarely in an evolutionary context, Pere Alberch–who participated in the 1981 workshop along with like-minded colleagues such as Stephen J. Gould–

[1] We determined this using the citation metric from a Web of Science database we created by searching for "evolution" and "morphogenesis" in the Zoological Record, CABI, and Biosis Index databases. These databases are curated by experts trained in the various disciplines and topics each covers. The papers cited here were in the top five most cited papers among our search results.

advocated for the dynamic interactions of cells and the morphogenetic rules that emerge from them as the prime constraints driving evolutionary transformation [27,28].

Within the past fifteen years, there has been a resurgence of interest in the physics of development [29]. This growing literature has made clear that while the transcription of a gene or the subcellular localization of its protein products can set the parameters for or "trigger" physical transformations of tissue, many such transformations can be accounted for irrespective of these triggering events [16]. There has also been a more recent recognition within contemporary biology of the limitations of genetic reductionism in explanations of biological phenomena [28,30,31]. The fact that this has had to be re-stated in the current decade highlights the persistent perception of genetic explanations as universal, and the mechanistic rigor that continues to be attributed to them [13]. However, this emerging literature has been largely limited to explaining processes at particular temporal and spatial scales of biological organization, and currently leaves many open questions as to how the generation of form is subject to evolutionary change.

How do mechanical constraints drive not only the emergence of form in individuals but also the evolution of morphologies? Mechanics and geometries, which arise from the physio-chemical properties of cell-material (adhesion, cytoskeletal tensions, cytoplasmic viscosity, etc.), are elements of development that may be subject to natural selection just as much as variants of gene sequences. The dynamics of these properties, as the evolutionary biologist Keith Thomson has put it, "add their own layer of causal influence in evolution" [17]. Our position is not that genes are dispensable for the evolution of morphogenesis. As we discuss, they produce proteins that generate, catalyze, and regulate the material used to build biological structures at every length scale. Naturally, constraints emerge from the properties of available materials–properties that are indeed contributed to by DNA sequence and regulatory variations–and their respective interactions, generating an allowed space of morphological variations [32]. Many genes–the suite of *Drosophila melanogaster* segmentation genes, for example–also provide essential cues or set transcriptional profiles in motion. But the cellular or sub-cellular mechanistic processes caused

126 by such genes must be integrated with mechanical, or physical, causes of morphogenesis across
127 scales at high temporal resolution.[2] We propose that environment-specific parameters together
128 with corresponding fitness contributions of a structure determine whether a morphology is
129 stabilized or diversified across evolutionary time.

130 Here, we review recent literature, primarily drawn from studies of Metazoa, that point
131 towards two promising avenues for broadening the field's perspective beyond genetic
132 reductionism and further integrating temporal and spatial scales in studies of morphogenesis and
133 evolution. Our first focus is literature that is beginning to couple gene expression with the physics
134 of the cell and tissue, or organismic, scale. This literature suggests that the mechanics of cells
135 and tissues can regulate transcription, suggesting not only a direct relationship between forces
136 that organisms experience at the cell or tissue scale with regulation at the genetic, molecular
137 scale, but also that the causal connection between the two is bidirectional. This area of
138 investigation requires us to be specific in the language we use to describe the exact role genes
139 play in our explanations of morphogenesis.[3]

140 We then turn to phenomena that are difficult to detect with the traditional approaches of
141 evolutionary or developmental genetics. The production of cell shape, cytoskeletal meshes, and
142 differential adhesion, for example, all require genes to produce the raw materials they are
143 constructed from. But we cannot understand how these materials come together to produce
144 functional architectures simply by detecting the presence of gene products. Nor is it sufficient to
145 limit our assessment of causation to the elimination or substitution of nucleic acid or amino acid
146 sequences.[4] Rather, we need to spatially describe the emergence of form at high temporal

[2] Palmquist et al. have outlined two kinds of reductionism commonly used in contemporary developmental biology: temporal and length-scale reductionism. Recognizing the limits of these two kinds of reductionism is key for expanding our explanatory frameworks [31].

[3] The ambiguity of the "gene" has existed since the term was first introduced in the early 1900s [174]. Decades later T.H. Morgan wrote that there was still "no consensus opinion among geneticists as to what the genes are" [13]. Today, we know an immense amount about the architecture and activities of genes, yet these distinctions are still often glossed over in the catch-all notion of the "gene."

[4] Laura Nuño de la Rosa and Gerd Müller have articulated nicely how biological causality has been traditionally attributed in developmental genetics: "Developmental genetics typically perceives development as a sequence of gene

147 resolution. As we discuss, a wide range of morphologies across phyla can be produced from
148 highly conserved genes and their products–actin, non-muscle myosin, tubulin, cadherins, etc..
149 What matters for the phenotype is how, where, and when these products are assembled in
150 specific physical and mechanical contexts. Further, form at one time point in development can
151 itself have a regulative role in determining the emergence of form at the next time point. In sum,
152 forms that emerge from dynamics at the molecular scale can themselves regulate development
153 without the need for information from genes.

154 Together, these foci suggest two approaches to identifying substrates other than gene
155 sequences, and the molecular products that they encode, for potential evolutionary change.
156 Whereas the first involves making causal connections between genes, molecules and the
157 mechanics of cellular collectives, the second shifts attention to the tissue and organ levels of
158 biological organization, where the mechanics of multicellular form-building emerge. In the second
159 approach, seeking an exclusively genetic "cause" is likely to be fruitless, as these phenomena
160 cannot be explained at the genetic scale alone.

161

162 ## 1. Mechanical control of gene expression

163

164 In this section, we argue that morphologies are not simply the end products of genetic
165 information but instead play an instructive or permissive role in gene expression, potentially
166 influencing evolution. To support this idea, we draw attention to recent studies that link the
167 mechanical states of cells and tissues to changes in gene expression. Specifically, we highlight
168 evidence showing that mechanical forces are crucial in metazoan development, activating the first

expressions revealed through an interventionist methodology wherein induced genetic alterations yield discrete phenotypic changes. This procedure traces a chain of events from perturbation to consequence, attributing causal significance to the altered gene in the developmental process. From an organismic perspective, this approach has significant limitations: while it identifies certain causal elements, it fails to elucidate the complex suite of interactions that determine the final phenotypic outcomes." [p.3 in 28].

zygotic genes that determine germ layers and early cell types. For clarity, we do not include an extensive discussion focused on many different types of dedicated mechanosensory proteins, as these issues have been well covered by other authors [33–38]. The role of mechanosensory molecules has also been discussed in detail elsewhere [39–42]. Instead, we emphasize how the morphological state of tissues actively drives expression patterns of a wide range of genes, illustrating a widespread reciprocal relationship between form and function.

1.1 Tissue scale morphogenesis

Over the past few decades, it has become clear that cells constantly sense and respond to their mechanical environment in predictable ways [43]. For example, outside the context of embryology, the role of substrate stiffness in guiding neuronal growth, neural crest migration, and stem cell fate has been well established [44–46]. Developing neurons and glial cells are highly sensitive to the stiffness of the extracellular matrix, using this mechanical information to regulate processes including proliferation, differentiation and axonal growth [47,48]. These functions are essential for shaping proper morphogenetic outcomes, including the formation of the brain, spinal cord, and motor neurons. Studies of these phenomena, conducted primarily in isolated cells, have largely examined how intracellular signaling pathways respond to artificially induced mechanical stress or substrate stiffness. While these investigations have provided valuable insights into cellular signaling, they do not address how cells respond to the mechanical forces that arise naturally during tissue morphogenesis. These forces, unlike external manipulations, emerge as a direct consequence of cell behaviors themselves. Moreover, the dynamic interplay between mechanically altered cell behavior and the evolving geometry of the tissue, both in time and space, remains poorly understood.

Across animals, mechanical forces play a pivotal role during embryogenesis, shaping the entire embryo through a combination of local cellular movements and specialized structures

spanning multiple cells [49–55]. Key stages of early development, such as gastrulation, are marked by dramatic, large-scale morphogenetic movements. Collective motion of cells generates forces which act at a tissue scale, determining the future identities of different embryonic regions. Much like the deformations crafted in Wilhelm His' lead-sheet models (**Figure 1**), asymmetrical tension within an embryo-wide contractile actomyosin ring drives the formation of the primitive streak in gastrulating quail embryos [56]. This asymmetry in tissue contractility not only shapes embryonic structure but also regulates the expression of early fate-determining genes across distinct regions of the embryo. Perturbing this asymmetric contractility leads to the formation of additional ectopic embryos, underscoring the critical role of geometric force patterns, rather than just the presence or absence of mechanical forces, in guiding gene expression [57].

The evolutionary diversification of embryogenesis may be linked to how mechanical forces interface with gene expression. In chick embryos, alterations in cell-autonomous behaviors and supracellular actomyosin cables modified stereotypical tissue morphologies, producing gastrulation patterns resembling those of evolutionarily distinct vertebrate groups, such as amphibians, reptiles, and fishes [58]. Echoing Hagenbach's earlier efforts (**Figure 2**), Serra and colleagues [12] proposed a minimal mechanochemical model that, by varying only initial conditions and a single cellular behavior, accurately predicted tissue flows during gastrulation across these vertebrate groups. It is striking that such morphological diversity can arise from a limited set of signaling nodes that couple tissue mechanics to cellular behavior. Connecting such changes in tissue mechanics, and thereby gastrulation modes [see 59], with species-specific spatial patterns of downstream gene expression is a promising avenue for further investigation.

One of the most studied molecular nodes in force-dependent germ layer specification is β-catenin, known for its dual role as a junctional protein and a transcription factor [reviewed in 60]. Across metazoans, β-catenin mediates mechanical regulation of gene expression to specify germ layer identity [61–66]. This general role appears to be both ancient and conserved across animals, with functional evidence for this hypothesis emerging from studies in cnidarians [67] and

ctenophores [68]. However, the specific ways that β-catenin is linked to tissue mechanics, and the downstream signaling pathways thus triggered, show some evolutionary divergence.

In the clawed frog *Xenopus laevis*, embryonic β-catenin activity patterns are critical for dorsal-ventral axis patterning [69–71], tissue movements associated with the formation of the blastopore [72], and early mesodermal identity [73]. In embryos of the quail *Coturnix japonica*, β-catenin activity shares functional similarity to that in amphibians, in that it partially determines early mesodermal identity [57]. In contrast to *X. laevis*, however, inhibiting β-catenin activity in the quail embryo does not disrupt tissue flows leading to the formation of the primitive streak [57]. This divergence of β-catenin activity in regulating the formation of equivalent structures in amphibians and avian embryos suggests that while β-catenin retains a central role in force-mediated gene-expression in vertebrates, its functional outcomes may have changed across evolutionary lineages.

Another example of divergence in β-catenin activity is found in invertebrate deuterostomes, notably echinoderms and ascidians. In echinoderms, β-catenin activity regulates endoderm and mesoderm specification in a dose-dependent manner along the primary embryonic axis, with its peak activity restricted to the vegetal pole [63,74]. Studies on sea urchin embryos (echinoderms) show that endomesoderm specification is governed by the fine spatiotemporal control of β-catenin activity and degradation along the animal-vegetal axis [75,76]. In sea squirts (ascidians), β-catenin activity-mediated endomesoderm specification occurs at the 8-cell stage, significantly earlier than in echinoderms [62]. In contrast to the gradient of β-catenin activity observed in echinoderms, in studied ascidians a binary on-off switch between the 16 and 32 cell stages determines endodermal and ectodermal fates [62] . To our knowledge, these examples lack a mechanical explanation for the observed divergence in β-catenin activity. Investigating how force-dependent β-catenin activity emerges in these animals using an experimental and theoretical framework similar to that of the previously highlighted studies[12,56–58,77,78][77,78],

including *in vitro* experiments comparing beta-catenin activity and function, could provide valuable insights into the evolutionary mechanisms shaping morphogenesis.

1.2 Force transmission in the nucleus

The force-induced nuclear translocation of molecules is often regarded as an indicator of their involvement in gene expression. β-catenin, when mechanically activated, translocates to the nucleus to drive the expression of both the canonical Wnt pathway and of non-canonical target genes [60,79]. In this framework, the genome is viewed as a static repository, where genes become available for transcription upon the right stoichiometric assembly of transcription factors, co-factors, and complexes. However, recent evidence suggests that the spatial organization of the genome itself plays a crucial role in regulating gene expression, adding another layer of complexity to this process.

Eukaryotic genomes are organized into complex three-dimensional architectures spanning multiple length scales. This organization ranges from smaller structural elements, such as DNA loops and domains, to larger structures like entire chromosomes [reviewed in 80]. The condensation state, or spatial configuration, of chromatin determines the accessibility of genes to transcription factors, thereby regulating gene expression [81]. Gene expression patterns concomitant with changing structural organization of the genome are therefore more appropriately considered as probability functions rather than binary on/off switches [82,83]. In this section, we discuss how forces experienced at the cell and subcellular scale–including those generated by the physics of genetic material itself– can regulate gene expression.

During tissue morphogenesis, cells take up diverse morphologies resulting from a variety of stress and strain generated by surrounding tissues. These mechanical states can be transitory [84] or long-lasting [85,86]. We speculate that altered genome structure during such periods might drive specific gene expression patterns or even enable differentiation of cell types. Wei and

colleagues showed that the angle of applied stress on Chinese Hamster Ovary cells determined the degree of chromatin stretching [87]. This impact of the stress angle was mediated by the initial orientation of pre-existing actin stress fibers in the cells. In addition, the stress angle and duration directly controlled expression levels of a target gene.

Looking at mechanics at the subcellular scale, Matsushita and colleagues showed that human mesenchymal cells undergoing differentiation into osteoblasts exhibit gradual changes in nuclear viscoelastic properties [88]. These changes are driven by alterations in chromatin condensation and DNA packing, which collectively shape the chromatin meshwork—a key determinant of nuclear viscoelasticity. A growing body of evidence [89–92] suggests that the physical nature of the nuclear genome is “fluid-like”, and thus constantly coupled to the mechanical state of the cell. Keizer and colleagues [91] directly measured the physical dynamics of a single genomic locus by applying magnetic point forces. Combining single-molecule imaging with different regimes of pulling and recoil, they found that weak (near-piconewton) forces could generate relatively large-scale movements of a genomic locus. It is likely that extracellular stress and strain, even when partially transmitted to the nucleus, may influence gene expression by repositioning a genomic region to make it more accessible to transcription factors.

While chromatin rearrangement may explain temporary transcriptomic responses to force, stable gene expression and cellular differentiation are likely to require more permanent alterations. Tissue morphogenesis is a highly dynamic process where forces experienced by a cell constantly evolve. Do patterns of gene expression change with similar temporal dynamics? Some stem cells retain substrate stiffness ‘memory’ and commit to fate specific programs even when transferred to a substrate with different stiffness [44,93]. Molecular mediators of such mechanical ‘memory’ include microRNAs and YAP/TAZ pathway members [35,93,94]. One example of this kind of ‘memory” is provided by studies of mesenchymal stem cells (MSCs). When cultured on stiff substrates, MSCs expressed higher levels of the microRNA *miR-21* than when cultured on soft substrates. If these cells were first grown on stiff substrate, and then transferred

to a soft substrate, the expression profile associated with the stiff substate persisted for days [94]. Knockdown of *miR-21* after initial growth on stiff substrate was sufficient to erase the transcriptional profile associated with stiff substrates [94]. In another study of mechanical 'memory" in MSCs using deformable hydrogels, initial culturing on stiff substrates increased YAP levels in the nucleus, and this nuclear YAP persisted for ten days after substrate softening [93] .

In addition to changing methylation patterns, nuclear heterochromatin undergoes persistent force-specific changes. Exogenous forces acting on cells can decondense the chromatin and increase protein diffusivity within the nucleoplasm [95]. Changes in chromatin and protein mobility can persist tens of minutes after removal of exogenous forces [95].

Similar modulation of the physical properties of chromatin might facilitate the stabilizing effects shown by Damodaran and colleagues [96]. They observed that compressive forces acting on mouse fibroblasts cause nuclear import of HDAC3, which removes acetylation marks on histones and thereby condenses the chromatin towards a quiescent state [96]. Additionally, they observed that the initial geometry of the fibroblast cells was correlated with the transcriptional response to the compressive force [96]. During embryonic development, similar cell types often assume different geometries [97–99] as tissues undergo dramatic changes in form. For example, different regions of a blastoderm epithelium will adopt different shapes at opposite poles of a major body axis, or form tissue folds required for gastrulation. A promising avenue for further research could be to determine whether specific geometries of groups of cells can also yield disparate stable gene-expression outcomes because of physical pressures on chromatin (**Figure 3)**, possibly resulting in progressive or terminal differentiation in response to large-scale tissue movements.

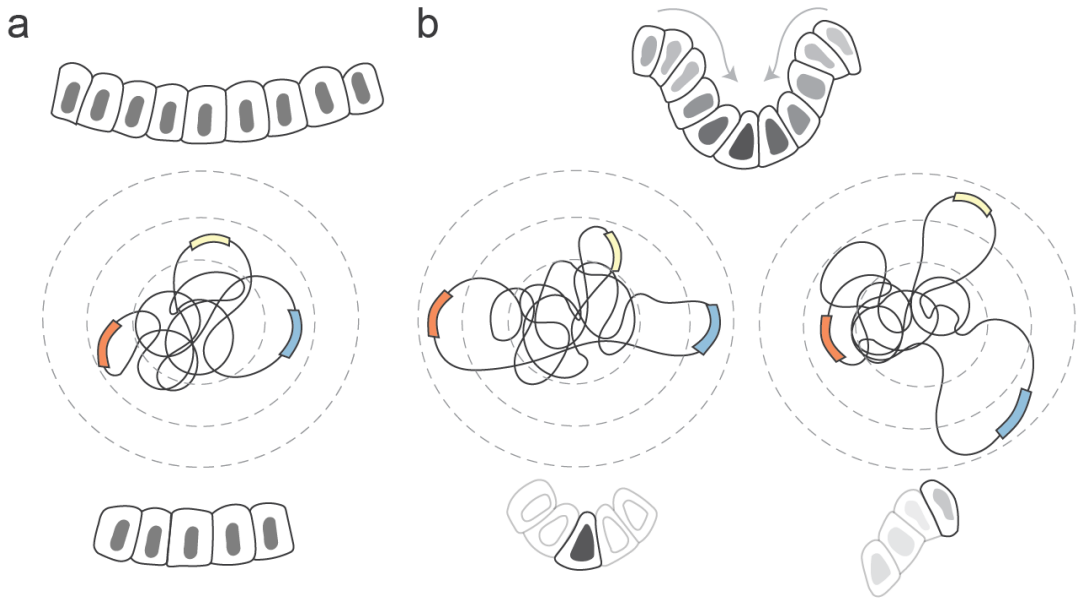

Figure 3. Coupling mechanical states with differential gene expression. An epithelial tissue state (top row) and a corresponding hypothetical chromatin architecture (middle row) that gives rise to differential gene expression in particular cells (highlighted, bottom row). In tissues undergoing a mechanical transition from uniform stress/strain (**a**) to differential stress/strain (**b**) chromatin architecture may change according to the local forces experienced by the tissue. Under non-uniform stress, a range of chromatin architectures could emerge from varying local mechanical states (two such cases shown in **b**). Accessibility of specific genes to transcription factors could then vary across cells, resulting in differential gene expression. Nuclear shading is indicative of gene expression states: colored boxes correspond to the same three genic regions across states, and concentric dotted rings demarcate zones of chromatin accessibility (increasing from inside out).

Just as the availability of transcription factor binding sites is carefully regulated, the response of chromatin architecture, and thus gene expression, to mechanical forces may also be carefully tuned. Sun and colleagues [100] showed that through changes in H3K9 methylation, cells can interpret both the frequency and magnitude of cyclic forces, translating these mechanical cues into varying levels of gene expression. Interestingly, they found that gene expression responses are not directly proportional to the magnitude or frequency of the applied forces, nor are they consistently linked to the extent of chromatin deformation. Instead, the absence of a clear correlation between these factors suggests a more nuanced regulatory mechanism that determines the optimal balance of mechanical cues driving specific transcriptional responses.

Collectively, these studies illuminate how mechanical forces can act as potent regulators of gene expression, directing transcriptional outcomes through changes in chromatin accessibility, nuclear architecture, and the strategic relocation of transcriptional machinery. Extending this body of work to the developmental context will perhaps shed light on how large-scale morphogenetic movements and evolving mechanical landscapes shape tissue- and stage-specific gene expression programs. Deploying techniques including live non-toxic live staining, specific pharmacological inhibition and force inference modelling, should allow future studies to capture the emergent properties of multicellular systems—where dynamic, global changes in cell shape and nuclear mechanics intersect with localized signaling events to drive robust developmental outcomes. Understanding how these mechanical cues are sensed, transduced, and ultimately manifested in stable or transient gene expression patterns will be critical for determining the extent to which these fundamental processes are conserved or diverge across evolutionary lineages, thus offering insights into the origins of the diversity of developmental strategies observed in nature.

2. Conserved morphogenetic materials across cells, tissues, and species

Cells exist in myriad shapes that perform diverse functions individually as well as collectively. The conserved mechanics of the cytoskeleton enable the emergence of tissue-level properties that are fundamental to development and physiology. By bridging the gap between individual cell behaviors and emergent tissue properties, the cytoskeleton ensures the complexity and functionality of multicellular systems across diverse contexts. In this section, we focus particularly on the physics of whole integrated cells and tissues as well as the cytoskeleton, which is fundamental to crucial cell architectures and behaviors.

Understanding the physical dynamics of form requires imaging cells and tissues with high temporal resolution. In the last several decades, much work looking at physical properties and geometries of cells and tissues during embryogenesis has been limited to model species where labels for live imaging of membranes, cytoskeleton, and adhesion molecules are readily available. These model species span very few phyla, and achieving the goal of uncovering empirical laws of collective cell behavior will thus require broadening the list of taxa studied. As more of these tools are developed for a wider range of species, the emergent biophysical dynamics from studies of model organisms can be contextualized within a comparative evolutionary framework**.** Nevertheless, the great extent of conservation of cytoskeletal components and their regulators across the cellular tree of life [101] allows us to generate hypotheses as to the general biophysical mechanisms involved in regulating cytoskeletal dynamics during multicellular morphogenesis and development.

2.1 Coordinating behaviour at the tissue level: regulation of actin dynamics via intercellular adhesion

For coordinated movement at the tissue level, the internal cellular meshwork of a tissue needs to be physically coupled. In animals, this is often achieved through intercellular adherens junctions formed through cadherin family adhesion molecules [102] , including E-cadherin [103]. The intracellular domain of E-cadherin is mechanically coupled to cortical actin, leading to emergence of mechanical continuity across tissues like epithelial sheets [103]. This mechanical continuity is crucial for coordinated activity, force transmission across larger spatial scales, and tissue integrity during several morphogenetic processes.

Biological processes can exhibit remarkable similarities in their underlying mechanisms, even when they occur in vastly different contexts. For example, dorsal closure in *D. melanogaster* embryos, neural tube closure in chordate embryos, and wound healing both embryonically and

postembryonically occur in very different biological contexts, yet they all deal with a gap closure, making them topologically equivalent [104–107]. In all these cases, cadherin-mediated mechanical coupling between the cells lining the gap enables the formation of a supracellular actin cable [104,105,108]. Eventually, the gap is closed by the tightening of the actin cable in a manner analogous to the drawing of purse strings [104,105]. These processes highlight how conserved mechanical principles can drive diverse biological events.

While gap closure involves cells functioning together at a common spatial site, coordinated cell behavior mediated by mechanical feedback can also guide cells to different locations to perform their functions. In some well-studied collective migration phenomena, migrating cells come to a halt or change direction upon collision, reminiscent of the trajectory of collision between rigid bodies. This process is referred to as contact inhibition of locomotion (CIL) [109]. The tactile feedback that cells receive through CIL is crucial, for example, for proper migration of neural crest cells in *X. laevis* embryos, embryonic hemocytes in *D. melanogaster*, and during adult mammalian wound healing, among other processes [110–112].

Much like repelling magnetic beads in a confined area assume a spatially uniform distribution, CIL enables hemocyte (primary immune cells) dispersal, potentially enabling them to survey the body effectively [111] . On collision, the intercellular adhesion molecules in the region of contact in the colliding cells drive synchronous reorganization of the actin, resulting in the formation of transient stress fibers that guide cellular repulsion [111]. The kinematics of cellular repulsion mediated through CIL, acting at the timescale of a few minutes, resembles that of colliding ice pucks occurring at a millisecond timescale.

While migrating cells avoid each other during CIL, in different developmental contexts cells also exhibit coordinated movement, showcasing another layer of cellular cooperation [113] . During collective migration, establishing cellular polarity (i.e. a leading edge and a trailing edge of the migrating cluster) is critical for directed migration, and this is achieved through asymmetric localization of actomyosin and its regulators at the two ends of the group of migrating cells [114].

In the case of collective migration, the cluster of cells must establish polarity much like a single migrating cell, but at a larger spatial scale [115]. The implication of this in several cases is the emergence of distinct leader and follower cell phenotypes [115,116]. Border cell migration in the *D. melanogaster* egg chamber provides a convenient system to investigate inter-cellular cytoskeletal coordination, as the migrating cluster in this case consists of fewer than ten cells [117]. Only the leader cell forms prominent protrusions, while moesin, a protein that links cortical actin and the plasma membrane, helps curb protrusive activity in the follower cells [118,119]. Disruption of moesin activity in the follower cells frees cortical actin to form protrusions, adversely affecting the collective motility of the cell cluster [119]. E-cadherin binding between the migrating cluster and the underlying nurse cells (which act as a substrate for migration of the border cell cluster) promotes forward-directed protrusions in the leader cell. The directionality is transmitted to the follower cells through E-cadherin-mediated mechanical coupling between the border cells [118]. This provides an illustration of how adherens junctions mechanically coupled to the intracellular cytoskeleton enable a migrating cell cluster to behave as one large migrating cell [118,119].

Different instances of collective cell migration can display unique, process-specific features, including variation in the number of cells involved in the collective migration. For example, *D. melanogaster* border cell migration involves approximately ten cells, wherein the motile border cells collectively carry a pair of immotile polar cells [117]. In contrast, during zebrafish and insect epiboly, thousands of cells move to cover the yolk in the absence of distinct leader cells [52,120]. In other cases, collectives of very different sizes can nonetheless share motile principles, including the presence of leader and follower cells. Zebrafish lateral line primordium migration, for instance, involves distinct leader and follower cell fate, where the leader cells have higher expression levels of receptors that can bind to the chemotactic cue, than other cells in the collective. The remaining cells then follow the leader cells through mechanical coupling between the two types of cells [121]. *D. melanogaster* embryonic tracheal

branching involves many fewer cells in the collective but is nonetheless also achieved by migration involving distinct leader and follower cells. The tip cells (leaders), named for their location in the tracheal branch, can sprout new branches, while stalk cells (followers) follow the tip cell and complete the formation of tracheal tubes through proliferation and intercalation [122].

2.2 Sensing mechanical stress at the cellular level: mechanosensitive ion channels

One of the ways that cells sense external mechanical stress is by deploying the Piezo family of mechanosensitive ion channels [123]. First identified in mice as a pair of paralogous genes (Piezo1 and Piezo2), Piezo homologs have since been characterized in *D. melanogaster*, *Caenorhabditis elegans*, chickens, and other species [124–126].

Piezo 1 is critical for vasculature formation during angiogenesis in mice, zebrafish and humans [127–130]. The Piezo1 ion channel responds to shear stress caused by blood flow by opening and allowing an influx of Ca^{2+} ions, which act as a key signal for endothelial cell alignment and migration [128]. Cell lines deficient in Piezo1 activity exhibit weak stress-induced Ca^{2+} entry, and mice lacking Piezo1 are embryonic lethal, with most dying between embryonic days 9.5 and 11.5, a critical window for vascular development [128]. Furthermore, the activity of Piezo1 is tied to actin dynamics, such that actin configuration at the membrane can determine the sensitivity of Piezo1 [128].

Piezo2 is expressed in sensory neurons [124], and has well-studied neuronal roles in mechanosensation [see for example 131–133]. Piezo2 can also play roles in sensing mechanical stimuli in non-neuronal cells. In the urinary bladder, for example, they are expressed in non-neuronal urothelial cells and are involved in sensing the stretch of the bladder [131]. Piezo2 aids in touch sensation in a specialized non-neuronal skin cell type called Merkel cells [133–136], and in chondrocytes, Piezo2 is involved in sending the mechanical strain experienced by joint cartilage

[137] . Suggestions that evolution of mechanosensory capabilities could involve evolutionary changes in Piezo2 expression arise from work by Schneider and colleagues, who revealed that ducks possess more Piezo2-expressing mechanoreceptors in their trigeminal ganglia, which innervate the bill, tongue, and mouth cavity, than chickens [138]. Because ducks are thought to rely more heavily on tactile stimulation for feeding than chickens, expression levels of Piezo2 could potentially be a component of the underlying mechanistic basis for evolutionary change in tactile sensitivity between these birds. Electrophysiological recordings demonstrated a strong correlation between Piezo2 expression and the presence of mechanosensitive neurons with intermediate and slow mechanically activated (MA) currents [138], which could enhance tactile awareness. Comparative analyses across six duck species revealed a strong correlation between the fraction of Piezo2-expressing neurons in the trigeminal ganglia and the species' use of tactile feedback for foraging [139]. This expansion of Piezo2-expressing neurons was coincident with a reduction in the number of nociceptive neurons, suggesting a potential trade-off that enhances tactile abilities in species less dependent on visual or olfactory cues [138,139]. Piezo2 regulates the activity of RhoA, a regulator of actin cytoskeleton [140]. Membrane tension influences the activation of Piezo channels, while membrane curvature is inversely correlated with the number of Piezo channels embedded in the membrane [141,142]. Since cortical actin plays a key role in regulating both membrane tension and curvature, it may work in conjunction with Piezo channels to facilitate mechanosensation.

2.3 Viscoelasticity – a thermodynamic view of the cytoskeleton

On mixing oil and water, the immiscible droplets separate to form homotypic aggregates. This aggregation minimizes surface tension as the surface area of the aggregate is lower than the sum of individual droplet areas [143]. The interfacial tension that exists at the boundary between oil and water is also minimized by homotypic aggregation. Heterotypic cell populations

also often segregate into homotypic spatial clusters, a process referred to as cell sorting [144–146]. The striking similarity between cell sorting and surface tension-driven homotypic aggregation has led many researchers to explore the cellular basis for surface tension. In 1963, Malcolm Steinberg [147] proposed that differential adhesion between cell types was the major driver of cell sorting, while Albert Harris [148] argued in 1976 that it was differential cell contractility that drove the sorting. Subsequent experiments demonstrated that intercellular adhesion molecules like cadherins are mechanically coupled to the intracellular actomyosin cytoskeleton [149–151]. This coupling means that changes in actomyosin contractility can directly influence cell adhesion strength and vice versa [149] . Since contractility generates tension at adhesion sites, it affects how cells adhere and sort within tissues, integrating both Steinberg's and Harris's theories.

Just as intercellular adhesion and contractility influence cell sorting through surface tension-like mechanisms, they also contribute to the mechanical properties of individual cells, determining their response to external forces. An elastic object under shear stress deforms and regains its initial shape on removal of the applied stress, while liquids flow when shear stress is applied [143]. Empirical studies, including those described in the following section, have shown that cells behave like elastic solids on short timescales, resisting deformation under shear stress, while flowing like viscous liquids at longer timescales. The contribution of the cytoskeleton to the viscoelastic properties of the cell has been studied by measuring changes in viscoelasticity as result of cytoskeletal perturbations [152,153]. Experiments on early-stage *D. melanogaster* embryos have provided further insights into cytoskeleton-driven viscoelastic properties of biological cells. In one such study, analyzing the trajectories of micrometer-sized injected fluorescent beads revealed that the early syncytial embryo exhibited predominantly viscous behavior with microtubules contributing to the viscous modulus [154]. To compare these viscoelastic properties with those of the later stage blastoderm embryo, magnetic beads were injected and then pulled by an external field [155]. This experiment revealed that while the

cytoplasm at this stage is largely viscous, as at the earlier stage of development, the cortex is elastic in its response to applied external force. The later stage cortex exhibited an elastic response over a timescale of four minutes, while deformations lasting longer than four minutes yielded a viscous response. The viscosity of the cytoplasm turned out to be 1000-fold higher than that of water [155]. These experiments on early *D. melanogaster* embryos provide insight into the connection between cytoskeletal components and time-dependent viscoelastic cellular responses to external forces.

Precise measurements of viscoelastic properties like those observed in the fly embryos discussed above, are limited by scalability. To study morphogenetic processes at larger spatial scales involving thousands of cells, alternative methods are needed to extract the mechanical forces driving collective cell movement over time. In studies of epithelial sheet dynamics in the gastrulating *D. melanogaster* embryo, physics-inspired models of epithelial sheets enabled researchers to estimate membrane tension and internal cell pressures entirely from geometrical properties like membrane curvature [156–158]. These estimates can in turn lead to inference of forces driving tissue scale morphogenetic movements during development [77,156–158]. Descriptions of tissue level phenomena purely in terms of the viscoelastic properties of the component cells is an approach that is applicable to larger spatial scales.

Thermodynamic descriptions of gases originated well before we knew about their microscopic constitution [159]. Yet, it was not necessary to understand the atomic nature of gases to establish quantitative empirical laws relating macroscopic properties like volume, pressure and temperature, which were sufficient to build the steam engine that powered much of the industrial revolution [159,160]. Statistical mechanics, the branch of physics that describes physical systems in terms of their constituents, has shown how thermodynamic laws emerge from microscopic interactions in the Avogadro limit [159]. Similar in spirit, the study of morphological processes calls for both microscopic and macroscopic views of the system, depending on the spatial scale

of the phenomenon and the nature of the “mechanistic” explanation sought. At larger spatial scales, purely microscopic descriptions like quantifying molecular gene products may prove insufficient to explain morphogenesis. As the physicist P.W. Anderson famously stated, 'More is different' [161], meaning that emergent phenomena are far more than the sum of their parts, and a reductionist approach offers only limited insight. We posit that for the study of morphogenesis, there is no single, absolute definition of the units or scale of explanatory mechanism that constitutes “the real mechanism.” Rather, much like very detailed biomechanical analysis of bird flight has limited utility for understanding flocking, the spatial scale of the phenomenon under study must dictate the level of detail needed in describing the agents in a collective phenomenon [162,163].

3. Future Directions

In conclusion, we address two broad classes of limitations inherent in current approaches and suggest possible avenues forward through these challenges. We believe that finding ways to overcome these challenges could lead to a deeper understanding not only of cellular mechanics, but of the role of these parameters in morphological evolution.

3.1 Developing systematic approaches to finding mechanical drivers of morphogenesis

The mechanical mechanisms regulating morphogenesis that we have discussed here, were primarily elucidated using what a geneticist would call a “candidate approach.” This approach is essentially one of taking an educated guess as to the nature of a particular mechanism based on prior knowledge of the mechanism operating in other contexts, and it has been highly successful in the traditional fields of molecular genetics, developmental genetics, and

565 especially evolutionary developmental biology. In some of our examples [e.g. 45,48,54], particular
566 physical or mechanical properties were observed or quantified, noted to correlate with specific
567 morphogenetic processes, and hypothesized to be causally related to those processes.
568 Subsequent experiments were aimed at perturbing the physical or mechanical parameters and
569 assessing the impact on the morphogenetic process. In other examples [e.g. 3,44,50],
570 researchers generated hypotheses about relevant mechanical or physical forces based on the
571 perceived similarity of morphogenetic processes to physical behaviors characterized in other
572 contexts.

573 While the "candidate gene" approach is useful in contexts where there is little a priori
574 knowledge of genetic regulatory mechanisms, geneticists also seek and prize so-called "unbiased
575 approaches" that uncover relevant genes by assessing the impacts of systematically perturbing
576 many genes, either at random or chosen based on a broad unifying characteristic (e.g.
577 transcription factors). Is there a way to discover mechanical mechanisms of morphogenesis using
578 an unbiased approach? This is more challenging than performing a genetic screen, because there
579 is no one-to-one mapping of mechanical parameters to genes or even specific biomolecules.
580 Further, the "variables" of morphogenesis are ontologically distinct from, and not as cleanly
581 defined as, those of a genetic screen (Table 1).

582

	Traditional genetic screen	**Systematic mechanical analysis**
Variables are	genes	emergent cell / tissue phenomena
	abstract entities (not directly observable)	observable descriptions of dynamic form
	cleanly transferable	emerge from locally specific environments
Perturbations	can be unbiased	cannot be completely unbiased (need to be situated in specific context)

583
584 **Table 1.** The disparate nature of "variables" and "perturbations" in the context of traditional genetic screens,
585 and our proposed approach of systematic mechanical analysis.
586

However, one approach to understanding the nature of this mapping could be to perform a forward genetic screen and assess the impacts on mechanical parameters already known to be regulators of morphogenetic processes of interest. Data from such studies may also allow us to understand the genetic basis of the evolution of mechanical properties. Whether the emergence of significantly different mechanical properties between species is highly polygenic, or may be explained through evolutionary changes in relatively few loci, remains an open question. A systematic mechanical analysis conceptually analogous to a targeted genetic screen could consist of developing morphogenetic assays for involvement of the classically recognized Newtonian physical forces (for example, quantifying forces experienced or exerted by cells), and systematically testing for their correlation with or involvement in a developmental process of interest. This has proven fruitful in studies of morphogenesis in highly disparate contexts, including fly wings, tunicate embryos, and plant leaves [164–167]. Applying this approach across taxa is essential to understand not just the individual mechanical mechanisms of each case study, but more importantly, the range of processes that they can regulate, and their interdependencies during development.

A third approach could attempt to screen for mechanical mechanisms based on morphological output. Such an approach could test the hypothesis that, for example, similar morphologies are achieved through a conserved set of physical mechanisms. There may be an opportunity here for machine learning approaches to construct a collection of topologically similar cell or tissue morphologies (e.g. the branches of plant roots or neural dendrites, or the tubes of renal or bronchial systems) using image data that could span broad phylogenetic ranges, potentially pointing towards common underlying mechanical principles across broad domains of life.

We suggest that by pursuing one or more of these approaches going forward, the field may be able to shift our understanding from the level of individual case studies to determining the extent of pattern and predictability in the mechanical control of morphogenesis. However, the bottleneck to this approach will likely be, as is the case for traditional forward genetic screens, the

efficiency and throughput of phenotyping. The phenotypes often quantified in mechanical studies of development – for example, tissue flows assessed or material properties of cells – are usually obtained with low-throughput approaches like high-resolution live imaging or atomic force microscopy. New and creative methods for obtaining mechanical and material phenotypes at scale will need to be developed. Recent attempts to infer cellular forces from light microscopy data [e.g. 77,157,158] are an example of one promising approach to overcoming this challenge.

3.2 Detecting natural variation in the mechanics of morphogenesis between and within species

Much of our understanding of the mechanisms discussed here has been obtained from exogenous mechanical or material perturbations of morphogenesis. However, as briefly mentioned in section 1.1 above, how the scale of these perturbations relates to the endogenous scales of tissue forces and properties is unknown. Obtaining at least biologically relevant boundary conditions, if not precise system-specific measurements, for these properties will be important if we wish to understand whether and how they may contribute to the evolutionary process.

We suggest three potential roads forward. The first is to, as suggested in 3.1 above, develop new methods to measure relevant properties in live, intact tissue systems, or creative inference of these properties from existing static or dynamic data. The second is to compare these properties and mechanisms between taxa across a range of phylogenetic distances. Such comparisons may allow us to understand what determines the degree of conservation of mechanical mechanisms, and to what extent they may be predicted based on evolutionary relationships, as can sometimes be done for molecular functions [168]. Third, to determine the extent of standing variation and thus potential evolvability of these mechanisms, genotyped collections of populations of a single species [169,e.g. 170–173] could be screened to quantify

637 the variance of the key parameters governing the mechanical mechanisms that regulate
638 morphogenesis.
639

Statement on AI/LLM use

No content was generated using artificial intelligence. ChatGPT 4.0 (OpenAI) was used to check for grammatical errors, improve language flow and readability of parts of the text. Scite_(scite.ai) was used for mining references and topic-based literature searches. All AI-suggested references and content were manually checked for accuracy.

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