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Commentary

Morphomechanics: An Extended View

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Morphomechanics is based on the idea that living matter can mechanically self-organize into forms without the need for a pre-pattern, as recently supported by the physics of active matter. Here, an extended view is proposed that integrates bioelectricity and differentiation waves as the mechanisms by which cells measure mechanical stress and couple morphogenesis and cell differentiation, respectively. Morphomechanics is a largely unexplored approach, which, however, could deeply transform our way of seeing nature.

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Introduction

The idea of living matter as an active and intrinsically ordered entity begins to sound like a robust scientific statement. Controversial from the very beginnings of embryology as a research field[1][2], it has now opened a new avenue at the interface between physics and biology[3]. This emerging field is concerned with the study of large ensembles of entities that consume chemical energy to self-propel or to exert mechanical forces, called active matter (e.g., [4]). The definition includes biological systems across scales (e.g., cytoskeletal components, cells, whole organisms) and some artificial systems. Some of the main experimental models are biopolymers and biofilms, from a biological origin, and Janus particles, from an artificial origin[5][6][7].

Their distinguishing feature is that, as long as there is an energy supply, they are in permanent activity, i.e., at a far from equilibrium state. This condition confers on them a rich variety of large-scale patterns and behaviors not available at equilibrium (e.g., see Fig. 1 in [8]). Powered from within, they do not require an external factor that drives them to a new state, like those formed by passive entities (e.g., Rayleigh–Bénard system), but they will do it spontaneously. However, this intrinsic capacity of generating order can be externally *harnessed* for the system to acquire reproducibility and robustness. This is an important point for designing materials and for understanding biological forms.

Contrary to previous models, the physics of active matter seems to capture the essence of the living (note that some physical models widely applied to morphogenesis, such as, for example, the differential adhesion hypothesis[9], assume the system is at equilibrium). In the future, it could provide the theoretical framework for understanding the role of mechanical, molecular, and electrical signals in the generation of biological forms, transforming our fragmented views into a theory of embryogenesis[10].

Morphomechanics is an approach to the study of morphogenesis in living systems that, contrary to the genetic – or bioelectric – program for development, is formulated on the idea of living matter as an active medium[11][12][13]. In the present work, it is shown that morphomechanics: 1) is supported by recent findings in active liquid crystals, 2) can integrate bioelectrical signals as the mechanism by which cells measure mechanical stress, and 3) in combination with the differentiation waves, can be extended to integrate cell differentiation.

At the core of morphomechanics

Living matter may behave, to some extent, as a liquid crystal. Liquid crystals are materials formed by rod-like particles (e.g., cytoskeletal filaments and cells under some circumstances) that can flow like a liquid and still keep a long-range directional and/or orientational order, like a solid, hence the name. A characteristic feature of anisotropic liquid crystals is the formation of topological defects, i.e., regions at which the long-range directional and/or orientational order is disrupted[4][8][14]. These defects display characteristic geometries depending on whether the rod-like particles are polarized

(polar liquid crystals) or non-polarized (nematic liquid crystals) (Fig. 1). Importantly, these defects are loci of high mechanical stress[15].

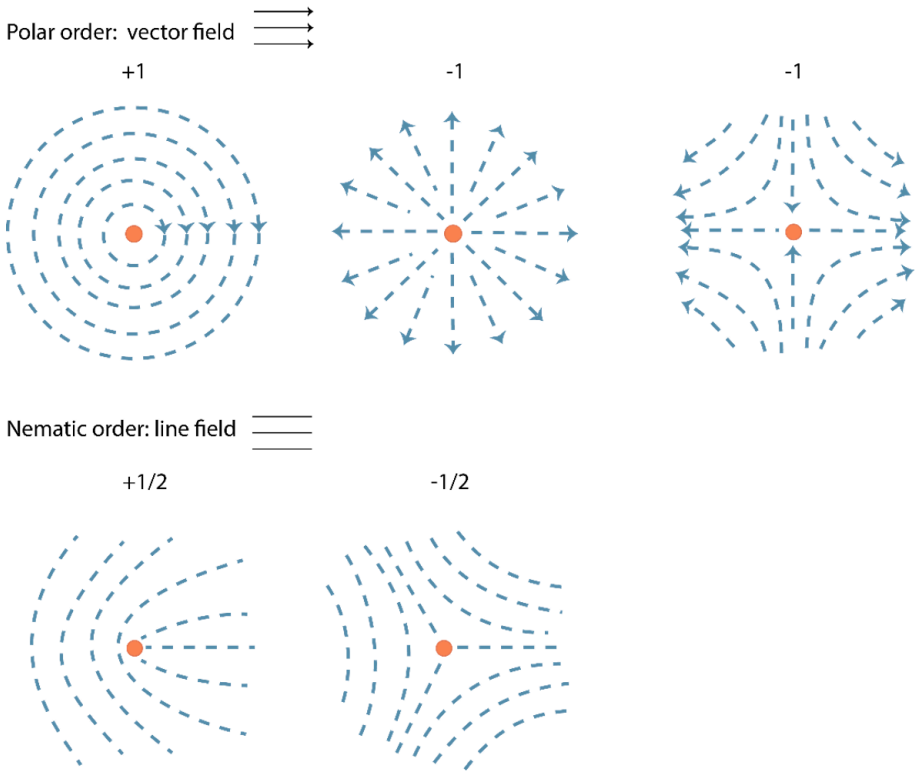

Figure 1. Types of topological defects in polar and nematic liquid crystals (redrawn from [7]).

There is growing evidence of the existence of topological defects in biological systems across length scales[16][17]. Remarkably, some studies have shown a correlation between topological defects and some morphogenetic events, suggesting they could represent *mechanical* organizing centers. For example, in *Hydra* regeneration, a pair of +1 defects marks the location of the prospective mouth and foot (i.e., the body axis). Furthermore, the position of the tentacles surrounding the mouth is specified by a pair of -1/2 defects at the base and a +1 defect at the prospective tip[18] (Fig. 2). Topological defects could spatially coincide with the classical organizers of early embryogenesis, traditionally defined in molecular terms only[19].

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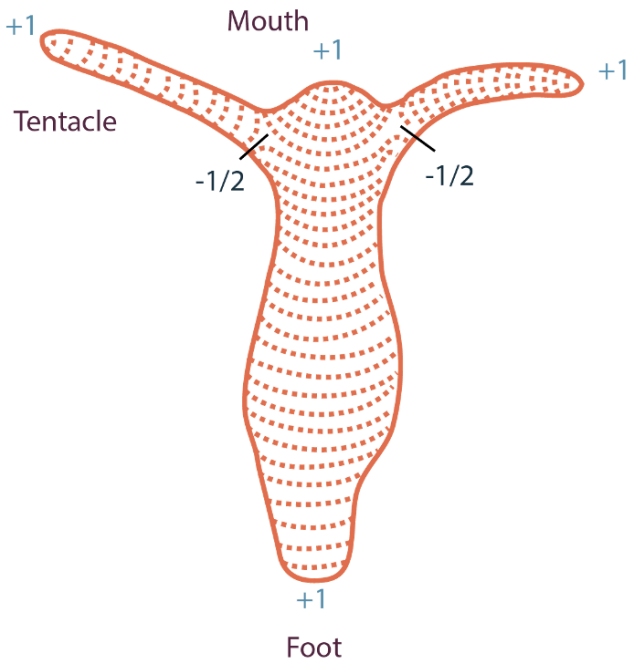

Figure 2. Schematic illustration of the nematic orientation field formed by actin fibers in *Hydra*. Note the correspondence between the topological defects and the body plan (redrawn from [18]).

The presence of topological defects in living systems indicates that the body of theoretical work developed for understanding the behavior of liquid crystals can be useful to explain biological forms, once the *activity* component is incorporated into the models. [20] have theoretically tested if topological defects can drive morphogenesis. The authors have shown that a thin film of a confined active polar liquid crystal, which could represent a cell monolayer, is unstable to the formation of protrusions at the location of +1 defects. This has been experimentally observed in myoblast cultures[21]. The forces leading to these out-of-plane protrusions only appear if the liquid crystal is extensile (i.e., the entities extend along their long axis); if contractile (i.e., the entities contract along their long axis), the film remains flat. Similarly, [22] have shown that a transition from a 2D to a 3D nematic layer is only theoretically possible under extensile activity. According to the authors, this result stresses the relevance of extensile forces as an underlying mechanism of epithelial morphogenesis. Modelled as a shell (i.e., a thin film with a spherical shape), +1/2 defects in a nematic crystal lead to the formation of protrusions and shell elongation – resembling organoid elongation – under extensile and contractile activity, respectively.

The relevance of extensile forces for a layer to grow in a third dimension could help to better understand the evolution of the metazoan body plan. It has been observed that some cell monolayers behave as extensile liquid crystals (e.g., neural progenitor cells), whereas others are contractile (e.g., NIH 3T3 mouse embryonic fibroblasts). It has been observed that Madin-Darby Canine Kidney (MDCK) cells switch from extensile to contractile activity when E-cadherin expression is knocked out[23]. The results indicate that when these epithelial cells cannot attach to each other, they strongly attach to the substratum and contract themselves, i.e., they behave like loose mesenchymal cells. This leads to a contractile monolayer. However, when cells form an epithelium, the net forces from neighbors and substrate interactions allow them to extend along their long axis, and the monolayer is extensile.

A major transition during the evolution of the metazoan body plan leading to the eumetazoans was coincident with the acquisition of the membrane protein Van Gogh/Strabismus and the enzyme peroxsidasin[24]. The former is involved in the planar cell polarity pathway, by which cells become

anisotropic in shape. The latter is involved in the formation of the basement membrane, i.e., true epithelial sheets. That is, the emergence of eumetazoans was coincident with the capability of cells to form active nematic liquid crystals with extensile activity.

When activity at the lower scale is considered, matter is capable of *mechanically* self-organizing into forms without the need for any external factor (e.g., a chemical or electrical pre-pattern). Under certain conditions, the active stresses generated at topological defects can bring about certain morphological motifs common in embryogenesis. At the cell scale, however, the possibility of a response to topological defects could increase the capacity of living matter for mechanical self-shaping. According to Beloussov and co-workers[25][26], this could be accomplished as follows:

> Whenever a change is produced in the amount of local stress applied to a cell or local region of tissue (regardless of whether this change in force comes from a neighbouring part of the embryo or has been exerted by an experimenter), the cells or tissue will respond by actively generating forces directed toward the restoration of the initial stress value, but as a rule, overshooting it.

A cell (or tissue) with *fixed edges* that is stretched or compressed by an external force will expand or contract, respectively, in order to restore its initial stress value with an overshoot, i.e., it will exceed it. Generally, this overshoot will mechanically perturb the surrounding cells, and so on, thereby leading to sustained morphogenesis.

It has been theoretically demonstrated that the hyper-restoration hypothesis is part of a more general principle that includes two other behaviors: growth response and stretch activation. In the former, the cell (or tissue) behaves as expected by the hyper-restoration response, but without an overshoot. In the stretch activation, the cell (or tissue) behaves opposite to the hyper-restoration response: it will contract if stretched and expand if compressed. At the steady state, it will remain at a higher stress[27]. According to [27], these responses could be related to differences in the duration of the mechanical stimulus. For example, blood pressure exerted on vessels increases slowly (in the order of days), whereas cutting a membrane under tension will induce a recoil of the margins surrounding the cut in a matter of seconds.

Morphomechanics provides a way for unifying the wide variety of cell behaviors observed in embryogenesis. They could be classified into two categories: those that decrease mechanical stress and those that increase it. Under stretching, cell behaviors that decrease stress include, for example, division, intercalation, growth, elongation, or recruitment. For increasing it: contraction, migration, apoptosis, or extrusion. Those cell behaviors that decrease stress under tension will increase it under compression, and *vice versa.* As stressed by [27], the duration of the mechanical stimulus could guide cells to select which mechanical response to adopt. Within each response, cells could also select among several behaviors. For this purpose, they could be guided by the magnitude of the mechanical stimulus.

It has been shown that myoblasts are extruded at +1/2 defects in cultured monolayers[15]. This extrusion is due to the accumulation of high compressive stress at these defects. This observation is in agreement with the morphomechanic view: cell extrusion under compression will restore the initial stress value of the tissue.

Harnessing defects

If not externally forced or trapped by a structural inhomogeneity, topological defects in passive liquid crystals tend to disappear as they collide and annihilate each other, and the long-range orientational order is eventually restored. Contrarily, the addition of activity to these theoretical models has shown the formation of a chaotic flow of self-propelled defects which are spontaneously and continually created and destroyed, a state called active turbulence. To construct something upon it, either biological or artificial, it would be necessary to harness this potential of mechanical self-shaping.

Researchers have proposed several ways of harnessing active turbulence (e.g. [23][7]). Here, two techniques that would be present in biological systems will be briefly discussed. One of them is confinement. Most of the studies on liquid crystals have been carried out in two dimensions. In order to elucidate its relevance for understanding biological forms, the approach has been extended to three-dimensional confinements. For example, when confined to a spherical shape, an active nematic film of microtubules and molecular motors displays four +1/2 defects that oscillate between two tetrahedral configurations[28]. The frequency of this oscillation can be tuned by changing the concentration of ATP (i.e., its activity). By decreasing the surface tension of the vesicle that encapsulated the film, these defects lead to the formation of four filopodia-like protrusions (Fig. 3).

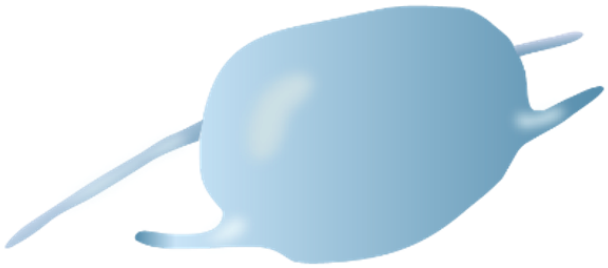

Figure 3. Filopodia-like protrusions formed at +1/2 defects in an active nematic film (redrawn from [28]).

Another technique is activity patterning. Experimental and theoretical studies have shown that, when activity is confined to a specific region, rather than homogeneously distributed, defects are created and trapped at these regions. Activity patterns have been created by engineering light-sensitive cytoskeletal components. For example, kinesin motor proteins form dimers capable of pulling on microtubules when binding to them. [29] engineered a molecule in which a kinesin motor was fused to an optically-dimerizable iLID protein, i.e., kinesin dimers formed only upon illumination. An activity pattern can be induced in a nematic system formed by microtubules and these light-activatable kinesin motors by illuminating specific regions. By this technique, the authors controlled the formation, movement, and fusion of topological defects.

In another study, [30] regulated the activity of a nematic system formed by actin filaments and light-sensitive myosin motors. These engineered motors consisted of myosin XI catalytic heads and a lever arm containing the light-sensitive LOV2 domain. This lever arm unfolds upon illumination, which increases the sliding velocity of the motor protein on actin filaments. The authors have shown, theoretically and experimentally, that +1/2 defects created within an activation area are deflected when they approach the boundaries, i.e., they are trapped. They have also shown how a low activity stimulation can be used to guide the motion of +1/2 defects along desired trajectories. Activity patterning offers a more direct way of regulating the formation and flow of topological defects than other techniques.

Measuring mechanical stress

Morphomechanics is based on the assumption that cells are capable of measuring the magnitude and duration of different mechanical forces. Here, it is suggested that they could perform these measurements by using electrical signals. The lipid membrane is electrically charged in *all* cells. This charge results from a difference in the concentration of negatively (Cl^-) and positively charged ions (Na^+, Ca^{2+}, K^+) between its intracellular and extracellular sides. Non-excitatory cells possess an excess of negatively charged ions in their interior, i.e., a negative membrane voltage (V_{mem}). Among the cellular components involved in the regulation of this voltage, it has been shown that cells possess *mechanically* activated ion channels[31][32][33]. These channels are pore-forming proteins inserted in the cell membrane that change their configuration upon mechanical stimuli. This configurational change opens the pore, which alters the V_{mem} by facilitating the influx of ions. Some of the identified channels are present in specific tissues (e.g., PIEZO2: neural), but others have been found in a wide variety of tissues (e.g., PIEZO1)[34]. They are not only involved in the regulation of physiological conditions of adult tissues but also in their embryonic development (e.g., [35][36]). This is a relatively recent finding in vertebrates[34] so there is not too much detail about their number, structure, mechanism of activation, or role in embryogenesis. Here, some characteristics of these force-sensing molecules that may provide cells with the ability to finely measure and respond to changes in their membrane mechanics will be commented on.

Mechanically activated ion channels are *primary* transducers of mechanical stress as they are both directly and quickly activated (on the millisecond timescale) by forces applied to the cell membrane. For example, PIEZO1 channels purified and reconstituted in a lipid bilayer are capable of generating electric currents when the membrane is mechanically perturbed, i.e., they are directly activated by forces transmitted from the surrounding lipids[34]. Although there is controversy about to what

extent mechanically activated ion channels are intrinsically sensitive to mechanical forces (force-from-lipids model) or whether they require the interaction of other cellular components (force-from-filament model), present data seem to suggest these are not mutually exclusive mechanisms. A general view is emerging in which *inherent* force-sensing ion channels can be *modulated* by other cellular components[37]. For example, PIEZO1 is more sensitive to mechanical pulling when attached to the extracellular matrix[38] (for other modulators, see [32][33]).

These channels present differences in their activation profile, threshold, and ion selectivity, which may be related to their different structures. For example, PIEZO1 is activated by stretching, compression, shear, or pillar deflection (forces applied at the cell-matrix interface), whereas TRPV4 only generates rapid electrical currents by pillar deflection[33]. They also display different activation thresholds. The pressure needed for half-maximal activation of OSCA channels (OSCA1.1 and OSCA 1.2) is two-fold higher than that for PIEZO1, i.e., OSCA evokes *high-threshold* currents under stretching[39]. Regarding the duration of the mechanical stimulus, TMEM63 needed stimuli of 1s to 2s, whereas OSCA channels were activated by a stimulus of 500 ms[39]. In general terms, the currents evoked by mechanically activated ion channels are proportional to the intensity of the applied force, so a higher change in the V_{mem} would be indicative of a higher change in mechanical stress. PIEZO1 is a non-selective cation channel that allows the flow of Na^+ and Ca^{2+}. OSCA channels are also non-selective cation channels with some Cl^- permeability, and TREK channels are permeable to K^+[32].

Using micro-patterned cultures of EpH4 mouse mammary epithelial cells, [40]have shown how differences in mechanical stress lead to the formation of a bioelectrical gradient in which cells located at areas of high tension are more depolarized (more positive V_{men}) than those located in areas of low tension. In a square culture, areas of high tension form at the corners; in a sinusoidal culture, at convex areas[41]. This bioelectrical gradient was generated by connexin-43 hemichannels, which opened preferentially at areas under tension. This change in V_{mem} gave rise to an increase in cell proliferation via Yap/Taz signaling.

Differentiation waves: coupling morphogenesis and cell differentiation

There is a general agreement that mechanical forces are relevant in morphogenesis; however, it is considered that they are downstream of gene regulatory networks, which are the drivers of this process. In an alternative view, a bioelectrical pre-pattern is added on top of molecular pre-patterns[42][43], but this new layer of information would not alter the general view that pre-patterns are *indispensable* to generate a form.

When living matter is conceived as a mechanically active medium, common morphological motifs of embryos can arise by mechanical interactions only, i.e., pre-patterns are *dispensable*[44][10][20]. Conjointly with strong evidence in support of the capability of mechanical forces to change gene expression (e.g., [45]), a different view emerges in which mechanical forces can drive morphogenesis and molecular pre-patterns may be downstream rather than upstream of them. Under this framework, pre-patterns would not provide *instructions* as commonly assumed, but would harness the self-shaping potential of living tissues[46][47][48]. It is important to stress that morphomechanics is focused on morphogenesis, and therefore, it is compatible with the idea that gene regulatory networks can drive other developmental processes. According to morphomechanics, morphogenesis and cell differentiation could be uncoupled.

There is some evidence in support of this idea. In the cnidarian *Nematostella vectensis*, [49] have shown that the invagination of the blastula during gastrulation is uncoupled from the differentiation of the resulting inner layer into endoderm. This invagination is mediated by the apical constriction of cells at the animal pole via Wnt/Planar Cell Polarity. Blocking this pathway inhibits the invagination of the blastula, but not its differentiation into endoderm. Contrarily, blocking Wnt/ß-catenin inhibited endodermal differentiation, but not tissue invagination.

Gastruloids are three-dimensional cell aggregates capable of elongating and differentiating into the three germ layers and some of their derivatives after a pulse of Wnt activation, mimicking the spatiotemporal organization of a gastrula. When they are cultured in suspension, this organization is reflected at the molecular level only, i.e., gastruloids express key markers of specific tissues, but they do not reproduce the corresponding morphology[50][51]. For example, they express neural markers along the longitudinal axis, but these cells do not form a tube. Cells expressing somite markers appear on both sides of the longitudinal axis and at the expected developmental time; however, they

do not form somite-like structures (i.e., hollow epithelial spheres). That is, morphogenesis and cell differentiation can be uncoupled in this system[52].

Here it is suggested that these two processes could be coupled in embryonic development by the differentiation waves. The latter are mechanical waves that travel throughout an epithelium (Figure 4). They are generated by a structure called the "cell state splitter," which is located at the apical side of epithelial cells[53][54][55]. This organelle consists of a ring of microfilaments, a ring of intermediate filaments, and a mat of microtubules connecting both rings. The cell state splitter is a bistable structure that can display two states: contracted or expanded. The former is mediated by the contraction of the microfilament ring. The latter, by the polymerization of microtubules (Figure 5).

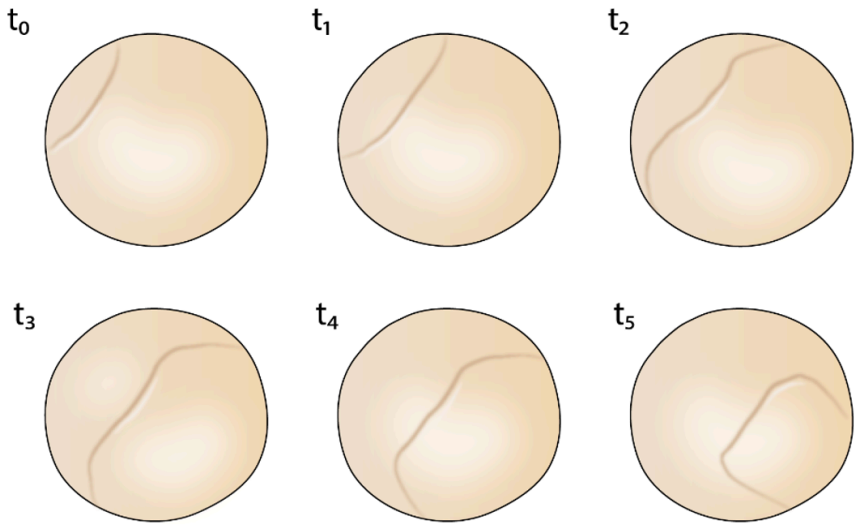

Figure 4. Propagation of a contraction wave in an axolotl gastrula. This wave is visible as a deep furrow on the surface of the gastrula (i.e., ectoderm). The drawings are based on a sequence of time-lapse images with an interval of 1 hour. This wave goes across one hemisphere only and determines the differentiation of the neural ectoderm. An expansion wave goes across the other hemisphere, which determines the differentiation of the epithelial ectoderm (redrawn from [56]) (t: time).

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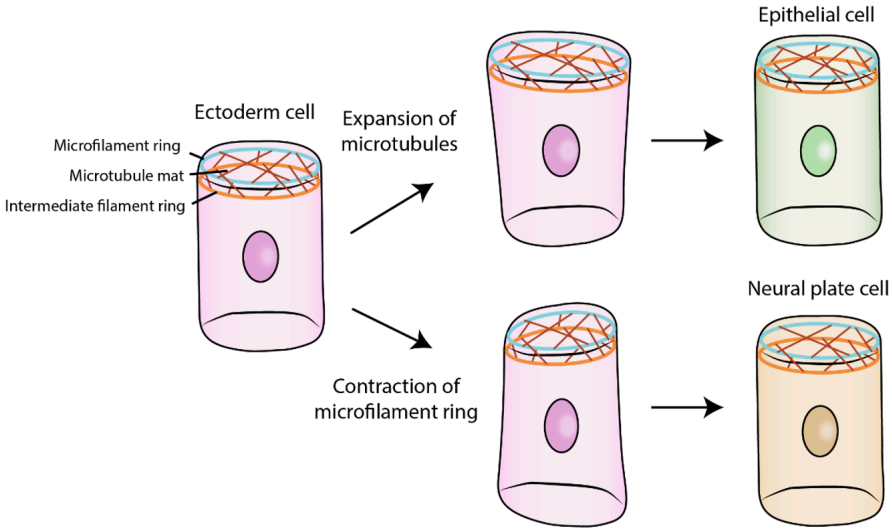

Figure 5. Schematic representation of the cell state splitter at the apical side of an ectoderm cell in the axolotl gastrula. The cell state splitter can expand or contract, which triggers the transcription of a different set of genes. Ectoderm cells that propagate an expansion wave differentiate into epithelial ectoderm, and those that propagate a contraction wave differentiate into neural ectoderm. Differentiation waves induce transient mechanical deformations; however, during their differentiation, these two cell types will develop different shapes (not depicted) (redrawn from [57]).

Before a differentiation wave starts, the contraction force exerted by the microfilament ring is in equilibrium with the expansion force exerted by the microtubules. However, this equilibrium is unstable: an *external force* enhancing either the contraction or the expansion of the cell state splitter will let it adopt the corresponding stable state. Once the cell state splitter contracts or expands, it sends a signal to the nucleus that triggers the transcription of a set of genes (i.e., cell differentiation). Triggering the same state in neighboring cells via cell-cell attachments, the initial response leads to the formation of a mechanical wave of cell contraction/expansion that will travel throughout the epithelial sheet. At the same time, the signal sent to the nucleus feedbacks to the cell state splitter, returning it to a new equilibrium state. Now the cell is ready to be part of another differentiation wave.

The intermediate filaments ensure the metastability of the system by buffering the cell state splitter from small random fluctuations. The initial stimulus should be strong enough to be able to switch the cell state splitter to one of its stable states. Each differentiation wave triggers the transcription of a different set of genes, and therefore, cells undergoing different sequences of contraction and expansion waves will follow different cell fates[53][54][55].

Accordingly, differentiation waves (contractile or extensile) would be initiated at topological defects and will propagate across the epithelium depending on the presence of other defects, as the same cell cannot participate in more than one wave at a time. Cells at different locations of the embryo will receive different combinations of contraction and expansion waves and at different timings. They could use this to know which set of genes to express and at what time. Simultaneously, they will undergo different regimes of mechanical stress. By measuring the type, magnitude, and duration of these mechanical stimuli by the V_{mem}, they will know if they should restore their initial stress value – with or without overshoot – or increase it, and which cell behavior to use to perform this task. This will lead to changes in shape.

Bioelectrical waves traveling along the embryo have also been observed[58]. In case they were coincident, these electrical waves could be caused by the differentiation waves. Differentiation waves expand as a transient, elastic cell deformation that expands or contracts the cell state splitter, but these deformations could also stimulate, to some extent, the mechanically activated ion channels. This would generate an electrical wave. In this case, these pairs of waves could be related, at least, in four ways. Bioelectrical waves may: 1) be a side effect of the differentiation waves, without a specific

role; 2) provide redundant information for increasing the robustness of the system; 3) provide additional information; 4) be part of the signal sent to the nucleus by the cell state splitter.

Coming back to *Nematostella vectensis*,[59] have shown that both tissue invagination by apical constriction (morphogenesis) and endoderm specification (cell differentiation) are mechanically coupled, as suggested in the present work. This coupling would be mediated by the existence of a mechanically activable site (Y654) in the ß-catenin molecule, which regulates endoderm specification[59]. However, it is unknown if this coupling involves a differentiation wave.

When mouse gastruloids are embedded in matrigel (an extracellular matrix surrogate), they are able to form trunk-like structures with a morphologically recognizable neural tube, somites, and a gut – i.e., they undergo both morphogenesis and cell differentiation[60]. A key difference between embedded and non-embedded gastruloids is the lack of an epithelium in the latter[52]. This system highlights the relevance of the epithelium in morphogenesis, as expected from active nematics. As suggested in *Hydra* regeneration[18], cell movements during gastrulation could be guided by a *nematic orientation field* formed by the epithelium. The lack of morphogenesis in suspended gastruloids would be expected, as this field would be absent.

Discussion

Morphomechanics does not deny that genes have played a fundamental role in the emergence of biological forms. It does not deny the existence of developmental programs. Morphomechanics challenges the common view that living matter needs to be *instructed* to give rise to forms. This is the consequence of conceiving it as a passive and non-intrinsically ordered entity, i.e., like a piece of "play doh"[1][2]. The standard view assumes that gene regulatory networks *give form* to living matter; they contain the blueprint of the organism. In an alternative view, this blueprint is contained in a bioelectric code, which governs gene regulatory networks[42][43] (Figure 6).

When matter is formed not by passive, but by active entities capable of transforming energy into mechanical work, large-scale patterns *spontaneously* arise from *mechanical* interactions only, without the need for an external factor (i.e., a pre-pattern) (Figure 6). In a liquid crystal state, rod-like active entities form flows with a large-scale orientational order that is disrupted by topological defects[4][8][14]. These defects are foci of active mechanical stress able to lead morphogenesis[20][21] (Figure 4). The correlation in *Hydra* regeneration between topological defects of different charges and its body plan suggests that nematic flows may constitute a morphogenetic field that guides embryogenesis[18].

In a self-organizing system, patterns can emerge by random interactions of identical entities, but the addition of more controllable conditions and heterogeneities would not annul this emergence, but rather would potentiate it[47]. Contrary to natural, non-living entities, cells can regulate the initial/boundary conditions and parameters of the laws relevant to their material properties. Within the context of active nematics, for example, cells can regulate the number of topological defects by modulating the level of activity[15]. A patterned activity could also specify the location in which a defect will appear, as well as its trajectory[29][30]. The introduction of heterogeneities in the type of activity (extensile or contractile) leads to phase separation[23].

By harnessing self-organization using gene regulatory networks, cells could generate complex, functional forms in a reproducible way[46][47][48]. However, these developmental programs are not as usually conceived. In analogy with man-made artefacts, it is commonly thought that gene regulatory networks (or bioelectrical signals) *encode instructions* to form an organism, and they *control* the spatiotemporal organization of cells. However, in self-organizing natural systems, there is not a central entity imposing order, but it emerges from the *collective* interaction of entities at the lower level, in a bottom-up direction (e.g., a nematic orientation field)[47]. To better understand this point, it may be helpful to compare artificial and natural buildings. Artificial buildings are constructed by builders under the guidance of an architect (a centralized control), who has designed a blueprint that contains the details about how the building will look. Contrarily, in nature, social insects construct complex buildings without following any blueprint or the instructions of an "architect", but they emerge from the collective interaction of insects (a decentralized control), which respond to some environmental cues in a specific way (low-level rules) (e.g., [61]).

Organisms could be constructed this way. Like social insects, cells could also construct rules that guide their response to environmental cues. According to morphomechanics, a tendency to restore their initial stress value – with or without an overshoot – or to increase it, depending on the

magnitude and duration of the mechanical stimuli[27], could increase the ability of biological systems to mechanically self-organize. To achieve this, cells would need to measure the magnitude and duration of different mechanical stimuli. Here, it is proposed they could perform this task by using their mechanically activated ion channels, which directly transduce mechanical stimuli into changes in V_{mem}.

Under this framework, bioelectrics is not an "instructive driver of morphogenesis"[62], but a mechanical mechanism. As stressed by [43], a main feature of a code is arbitrariness: the response triggered by a signal is not a physical consequence, but something arbitrary. Borrowing an example from [43], exposure to a high temperature will lead to cell death as the physical consequence of protein denaturation, i.e., heating destroys the three-dimensional configuration that makes proteins functional. A specific value of V_{mem} could also lead to cell death, but this response would not be physically connected. Cells *interpret* this signal as a cue to trigger programmed cell death. The same signal could evolve to trigger, for example, cell proliferation, i.e., the link between the signal and the response is an evolutionary convention, and thus, a code.

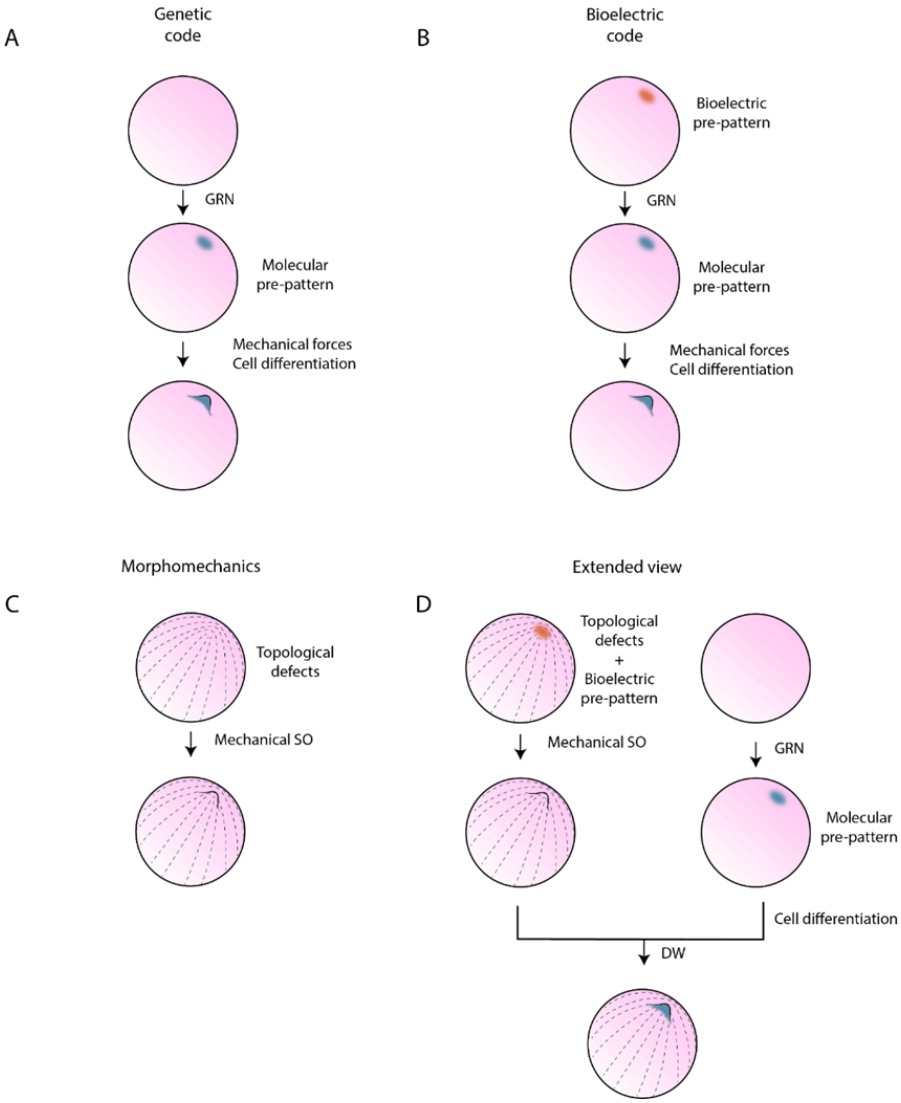

Figure 6. Schematic representation of different views of embryogenesis. The hypothetical formation of a bud in an epithelial layer is depicted for their comparison (see text for references). **(A)** In the gene-centric view, a gene regulatory network generates a molecular pre-pattern. This pre-pattern regulates a cell behavior capable of forming a bud (e.g., proliferation). It will also regulate cell differentiation by switching "on/off" specific genes. Mechanical forces are involved in the formation of the bud – e.g., proliferating cells will displace each other, and this would generate a pushing force capable of forming a bud –, however, they are downstream of gene regulatory networks, which are the drivers of morphogenesis. **(B)** According to the bioelectric code, electrical pre-patterns precede and control gene regulatory networks. After the activation of a molecular pre-pattern, the bud is generated as in the previous case. Changes in membrane voltage *instruct* cells which response they should perform. **(C)** In morphomechanics, living matter is conceived as an active medium capable of *mechanically* self-organizing into forms without the need for a pre-pattern. Topological defects would be *spontaneously* generated in living tissues. These defects are foci of high mechanical stress capable of leading tissue morphogenesis. Molecular pre-patterns (not depicted) would harness this self-shaping potential. For example, the total charge of defects on a sphere is +2, however, several combinations of defects can accomplish this requirement. Molecular pre-patterns could set the conditions necessary to generate a specific configuration in a reproducible way, as living systems require. **(D)** Morphomechanics is based on the idea that cells are capable of measuring the magnitude and duration of different mechanical stimuli. In the present work, it is suggested that cells could perform this task by using bioelectrical signals. These signals will be generated downstream of mechanical forces by means of mechanically activated ion channels. Cells could use this information for selecting among different responses (hyper-restoration, growth response, and stretch activation) and behaviors (proliferation, apoptosis, migration, elongation, etc.). Under this framework, bioelectric pre-patterns are not a code, but part of the mechanical mechanism of morphogenesis, i.e., they inform cells about their membrane

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mechanics. Morphomechanics is focused on morphogenesis, and therefore, is compatible with the idea that cell differentiation is driven by gene regulatory networks. Here it is proposed that differentiation waves could be the mechanism that couples these two processes. Differentiation waves are mechanical waves that propagate throughout an epithelium. Triggered by a mechanical stimulus, they would be initiated at topological defects. They can be contractile or extensile. Cells located at different regions of the embryo will undergo different combinations of these waves, which could guide their differentiation (GRN: gene regulatory network; SO: self-organization; DW: differentiation waves).

In morphomechanics, a change in V_{mem} is not arbitrary, but *physically* connected to a change in mechanical stress. Alterations in V_{mem} inform cells about their membrane mechanics. Bioelectric signals are part of the physical mechanism underlying morphogenesis. However, the cell response to these changes could be arbitrary, an evolved rule. Here, it is important to stress that, according to Beloussov[12][48], the hyper-restoration response is not specifically biological (i.e., arbitrary), but it can be understood as the extension of the Le Chatelier principle for active matter (i.e., at far from equilibrium conditions).

Embryonic tissues would not remain in a liquid crystal state for the whole process of embryogenesis. They can undergo unjamming-jamming transitions that will confer to them a solid-like state[63]. This transition gives rise to a new set of patterning processes, e.g., differential strain. Differential strain is a phenomenon first described in the inanimate realm[64] that has proven helpful for understanding embryonic development. It occurs when two solid materials are physically connected, and one of them extends or shrinks with respect to the other. This creates regularly spaced foci of high mechanical strain that lead to morphological changes. When an elastic material expands faster than a rigid underground, it compresses itself, which leads to geometric buckling phenomena like the wrinkling gut[65], the folded brain cortex[66], and the scoliotic spine[67]. When it shrinks, the rigid underground is overstretched, which leads to the formation of regularly spaced cracks. Cracking has been proposed to explain the fragmentation of the crocodile skin and the paraxial mesoderm into scales[68] and somites[69][70], respectively.

Finally, the extensile activity that allows out-of-plane morphological changes, cell-cell attachments that propagate mechanical and bioelectrical waves, the “fixed edges” condition necessary for a hyper-restoration response, the cell state splitter that could couple morphogenesis and cell differentiation—all of these are features of an epithelium. To better understand embryogenesis, it would be necessary to elucidate what is the best way to conceive an embryonic tissue, i.e., the relationships between the epithelium, mesenchyme, and extracellular matrix.

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