How Active Mechanics and Regulatory Biochemistry Combine to Form Patterns in Development

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Abstract

The development of organisms starting from their zygotic state involves a tight integration of the myriad biochemical signaling interactions with the mechanical forces that eventually pattern and shape the resulting embryo. In the past decade, it has become increasingly evident that several important developmental processes involve mechanical forces in an essential manner. In this review, we highlight the multifaceted role of mechanics in pattern formation, from protein and cell sorting to the generation of tissue shape. We then review the ways in which the active cellular cytoskeleton self-organizes to form dynamic patterns. Finally, we focus on mechanochemical feedback, where signaling proteins can establish patterns via coupling to the activity of the cytoskeleton. Throughout the review, we focus on the generic physical principles of the establishment of active mechanochemical patterns and point toward future directions in studying how the principles of mechanics and chemistry combine to drive morphogenetic pattern formation.

Contents

1. INTRODUCTION

The molecular revolution of biology shapes the way we think about life today and puts DNA in the spotlight of biological research. Since its onset in the middle of the twentieth century, tremendous scientific effort has provided us with a wealth of knowledge on the molecular nature of genes and their control networks, all of which revolves around the questions of when and how many proteins are produced in the cell. DNA encodes the information about the conditions under which a protein is produced but generally does not encode the spatial location of the protein inside the cell. It is thus fascinating to see that life shows very complex spatiotemporal organization on all scales, which cannot directly be explained from a gene-centered view. How this spatial organization emerges is one of the less understood aspects of biology, but it is clear that a large-scale systems approach grounded in physical principles is required.

Probably the most dramatic process in the genesis of complex spatial organization in biology is embryonic development. Consequently, embryogenesis has attracted researchers from a broad range of disciplines who aim to understand how biological systems robustly generate patterns, structure, and three-dimensional (3D) shapes. Embryogenesis starts from a single fertilized cell, which is the origin of the higher organism (35, 121, 144). Two remarkable processes start soon after fertilization. On the one hand, pattern-formation mechanisms generate a biochemically encoded coordinate system for the embryo, while active mechanical deformations generate the final shape of the embryo, a process called morphogenesis (**Figure 1**). Traditionally, pattern formation and

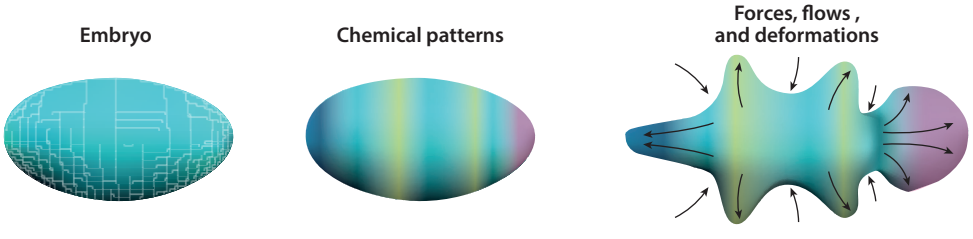

Figure 1

During embryonic development, the newly forming organism increases spatial complexity through a succession of pattern formation and morphogenetic shape changes. Frequently, the underlying forces are coordinated by patterns in biochemical signaling proteins.

morphogenesis have been studied separately. Pattern formation was studied in the context of diffusive signaling proteins, called morphogens, which encode biochemical information in spatially inhomogeneous concentration profiles [predicted in 1952 by Alan Turing (134) and experimentally discovered by Wolfgang Driever and Christiane Nüsslein-Volhard in 1988 (26)]. As an overarching theme of pattern formation, these mechanisms produce a nonuniform concentration profile with a length scale that is set by the combination of their diffusion constants and reaction rates. On the other hand, morphogenesis—the generation of the 3D shape of the embryo—has traditionally been studied without a direct connection to the molecular constituents of the cells and tissues (124, 132).

The impact of active transport and forces on pattern formation has been highlighted in earlier studies by influential scientists like D'Arcy Thompson, Alan Turing, and Lewis Wolpert (40, 132, 134, 143). It nevertheless took quite some time to uncover the molecular basis of force generation, with the key discoveries of the dynamic nature of the cytoskeleton, and to isolate and characterize molecular motors like kinesin, dynein, and myosin (46, 60, 75). It is only in the past decade that a quantitative understanding of the autonomous force generation of the cytoskeleton and the deformation it induces at the cellular and tissue scale has emerged (43, 44, 66, 68, 80, 109). Our emphasis in this review is on the physical principles involved in developmental pattern formation (28, 89, 90, 95). Numerous studies now investigate the impact of forces in the process of pattern formation, emphasizing the idea that pattern formation and morphogenesis are inseparably connected and that the emergence of patterns and shape is often founded on mechanochemical principles.

In this review, we focus on the role of mechanics and chemistry in pattern formation. The patterns discussed can be of a wide variety of natures, ranging from the more familiar non-homogeneous protein concentrations to patterns in shape, such as the emergence of a certain tissue morphology. In Section 2, we discuss several examples in which patterning processes are driven by mechanical forces but the mechanochemical origin of these forces is not accounted for. We then move on to systems where mechanochemical generation and regulation of forces are an integral part of the patterning process (Section 3). We end this review with a look at future questions and challenges in this field. At the outset, we would like to emphasize that this is not an exhaustive review of all the studies in developmental pattern formation combining chemistry and mechanics. Rather, we highlight a few examples where the regulation of physical forces by biochemical interactions leads to interesting examples of pattern formation in development.

2. MECHANICAL PATTERNING

Many of the patterns that surround us have their origin in mechanical forces, where they are driven, for example, by fluid instabilities on various length scales. We highlight some examples of how mechanics can generate a broad range of biological patterns, from inhomogeneous distributions of proteins and cells to distinct tissue topologies.

2.1. Interfacial Tension

We are well familiar with the spontaneous pattern that emerges in an emulsion of oil and vinegar after thorough mixing. The substantial difference in the surface tension of these two liquids results in a demixing process over time. Interestingly, such processes also happen in cell and developmental biology. We discuss two patterns—the sorting of cytoplasmic proteins into membraneless compartments and the sorting of cells into different germ layers—that are driven by different interfacial energies amongst their components. These examples share a similar patterning principle

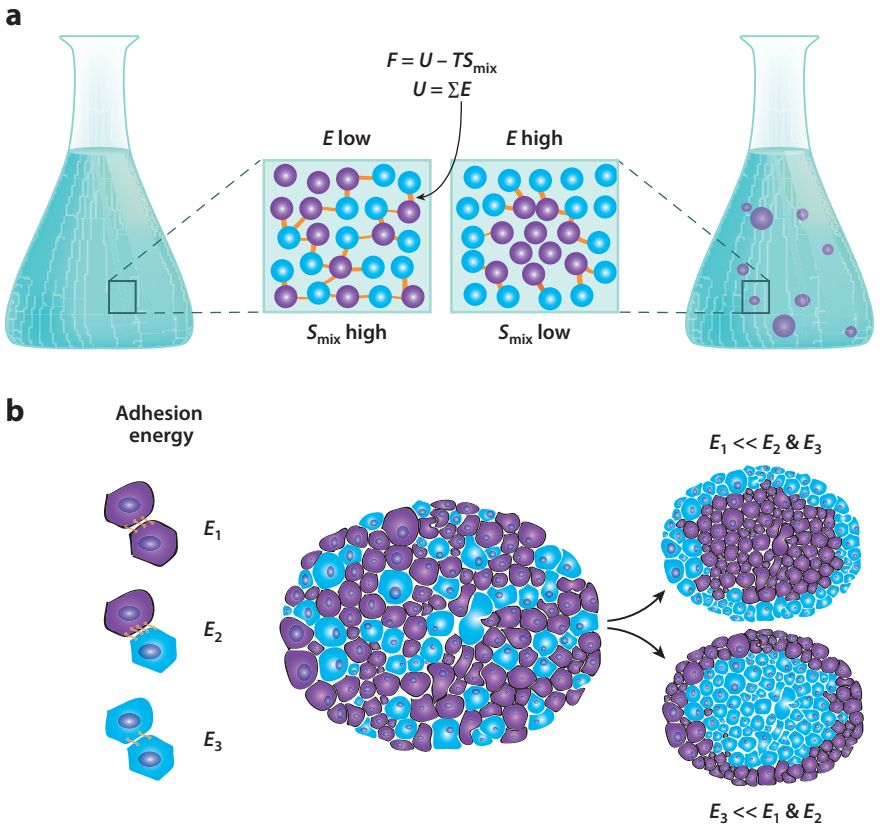

Figure 2

Interfacial tension as a mechanism to create spatial structure. (*a*) The liquid–liquid demixing transition is determined by the interfacial energy E between two particle species. Reducing the interfacial surface area decreases the internal energy U but increases the mixing entropy S_{mix}. Spontaneous demixing occurs if the free energy F in the demixed state is lower than in the mixed state. (*b*) Cells use adhesion proteins to hold on to their neighboring cells. The adhesion energy is the work that has to be invested to separate two cells, which is negative in sign for cells that hold together. The differential adhesion hypothesis (126) posits that cells that have a lower absolute value of their adhesion energy form a shell around those cells that have a higher absolute value of their adhesion energy.

and are thus discussed together in this section, even though the origins of the interfacial energy are very different.

Recently, the mechanism of forming membraneless compartments inside the cell cytoplasm has received significant attention. There are now very strong indications that different compartments, including stress granules (17), centrosomes (72), P granules (15), nucleoli (12), and Cajal bodies (33), share a similar mechanism of formation: a liquid–liquid demixing transition (50). In such a demixed state, the effective contact area between two different substances is greatly reduced, causing a decrease in the interfacial energy cost. If this drop in interfacial energy surpasses the entropic cost of the more ordered state, then demixing can happen spontaneously (**Figure 2*a***). Mechanics, in the form of surface tension differences, thus controls whether the system demixes or remains in a mixed state. Normal emulsions undergo Oswald ripening, where all liquid droplets

coalesce into one, but this is generally not observed inside the cell. It is thus plausible that additional biochemical regulatory networks exist to control the number of drops as well as their size (147).

Interestingly, similar mixing–demixing processes have also been observed to pattern tissues. Townes & Holtfreter (133) showed that an in vitro mixture of cells from different germ layers can spontaneously demix. Even though the underlying mechanism was not clear at that time, the similarity to liquid–liquid demixing processes resulted in the differential adhesion hypothesis (126). In essence, this hypothesis considers each cell in a tissue as a mobile unit that has an adhesive interaction with its neighboring cells. A mixture of two different types of cells with substantially different adhesive strengths will thus demix (**Figure 2*b***). The differential adhesion hypothesis was experimentally validated in aggregates of cells in which the number of adhesion proteins (in this case different classes of cadherins) was precisely controlled (29). It was first shown that the number of cadherins linearly correlated with the surface tension of the aggregate. Additionally, this study showed that a mixture of cells with different expression levels of cadherins segregates such that cells with lower adhesive strength form a shell around the sphere of cells with higher adhesive strength. This mechanism can have a broad impact on developmental processes. For example, in the developing zebrafish embryo, it has been shown that cells of the ectoderm and the mesendoderm have different tissue surface tensions (116). This results in a demixing process where a mixture of these cells self-organizes such that the ectodermal cells are positioned internally, whereas cells of the mesendoderm are situated peripherally. This organization can be inverted when the surface tension difference is inverted by genetically targeting adhesion proteins. A modification of this mechanism was recently uncovered in the organization of the human mammary and prostate glands (19). Two cell types (luminal cells and myoepithelial cells) have different interaction energies with the extracellular matrix. A mixture of these cells can self-organize into a core-shell structure. The establishment of such a pattern depends on the difference in interaction energies of the two cell types with the surrounding extracellular matrix. This interaction energy then determines which cell type is positioned at the periphery and which is positioned in the interior. We highlight here that the differential adhesion hypothesis is still a topic of active research, with several questions on the magnitudes of tissue surface tensions remaining unanswered (4).

Cell adhesion proteins are just one way to modulate the interfacial tension between cells. The contractile state of the actomyosin cytoskeleton can also induce such a demixing; this has recently been studied in mammalian development (73). Shortly after fertilization, cells of the mouse embryo sort such that some remain at the surface of the embryo, to later develop into the placenta, while others internalize and develop into the final organism. This process depends on the contractile state of the cells, with the strongly contracting cells being sorted into the interior.

In summary, these systems can switch between a mixed and a demixed heterogeneous state by modulating the interfacial tension, and this bistable switching has been shown to act on cellular and tissue scales.

2.2. Buckling Instabilities and Differential Growth

Several organs develop folds in the process of embryogenesis. For example, the human intestine creates folds and loops to compact its approximate length of 6 m into the body. Mechanical buckling instabilities are being considered as a mechanism for the patterning of a broad range of organs, like the intestine, the respiratory system, and the brain (88, 129, 138).

A buckling instability results when it is energetically favorable for an elongating thin rod to become bent rather than being compressed. Investigations of the looping morphogenesis of the chick gut showed that differential growth of two connected tissues, the intestinal tube and the

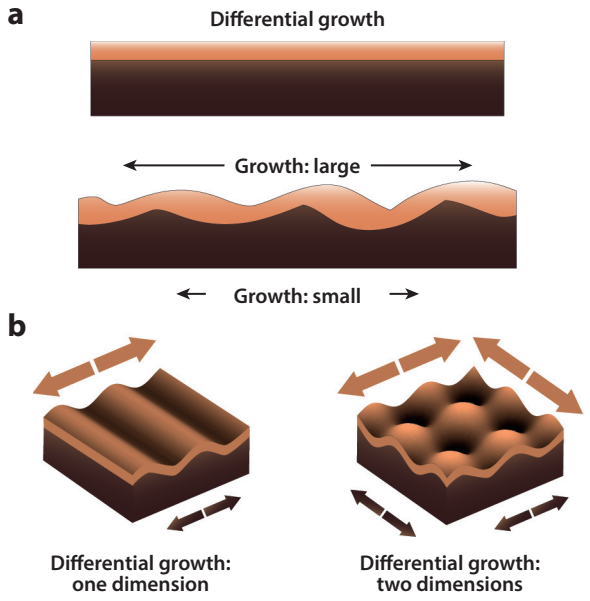

Figure 3

Differential growth as a mechanism to generate folded tissue topology. (*a*) Morphological undulations can occur when two tissues are connected but the top tissue grows faster than the bottom tissue. (*b*) Ridge-like or hill-like structures can result when the growth is isotropic or anisotropic, respectively.

mesentery, is responsible for the emergence of intestinal folds (**Figure 3**). These folds relax as the two tissues are separated (114). The spacing of these loops depends only on mechanical parameters, further emphasizing the mechanical nature of this patterning process. Another buckling event shapes the internal topology of the intestine. Pronounced finger-shaped protrusions, known as villi, increase the surface area of the inside of the intestine. The innermost tissue, a thin epithelial layer, is, however, initially flat. A sequence of three buckling events was found to be responsible for forming these villi in the gut of the chick (119). Key to this process is the expansion of the inner tissue and the constriction mediated by the surrounding smooth muscle layer, resulting in compressive stresses. The development of the respiratory system also shows indications of patterning via buckling instabilities. The developing lung starts with a Y-shaped topology, and numerous branching events then create the final shape of this organ. It was generally thought that branching is a result of biochemical signaling. However, it was recently demonstrated that branches in the growing epithelial tissue, which are constricted by a surrounding gel, occur in the absence of specific signaling and thus show characteristics of a mechanical buckling event (136). This study further demonstrated that the length scale of this buckling pattern is given by the tissue growth rate. Buckling instabilities have also long been implicated to be essential in the morphogenesis of another organ, namely the brain (106). Indeed, the complex folding of the brain can, in part, be of a mechanical origin. This idea was supported by a combination of numerical studies and 3D-printed gels that grow by absorbing a solvent. The constrained growth was mimicked by coating the 3D-printed gel with a thin film of a restrictive substance. Folding structures strikingly reminiscent of mammalian brains were observed when this object grew in size; these structures originated in mechanical buckling instabilities (131).

To conclude, buckling instabilities have the ability to translate the differential growth rates of two connected tissues or restricted growth into complex shapes with emergent length scales that depend on tissue mechanics.

3. MECHANOCHEMICAL PATTERNING

One of the fascinating properties of cells and tissues is their ability to autonomously generate forces. These force-generating processes are fundamental to morphogenesis and are generally controlled by regulatory proteins. Consequently, a complete picture of morphogenetic events must not only look at the mechanical shape changes but also incorporate the ways in which the concentration of these regulatory proteins is affected by shape changes in the morphology of the embryo. As such, a deep understanding of morphogenesis requires us to account for the mechanochemical nature of pattern formation.

In this section, we first briefly discuss examples of mechanisms to generate nonuniform patterns in biochemical regulators (in the absence of forces). We then turn our attention to active materials and their ability to autonomously generate mechanical stresses. Finally, we discuss systems in which active materials and regulators are coupled, with an emphasis on their ability to form patterns.

3.1. Biochemical Patterning

The framework of reaction–diffusion systems has been used very successfully to describe how biochemical patterns can emerge (16, 57, 61, 84, 107). This framework considers a set of n biochemical species (signaling proteins like morphogens) that diffuse in the ambient space and interact chemically. Mathematically, the concentration fields of the signaling proteins $c_i(\mathbf{x}, t)$ with $i = 1, \ldots n$ at spatial position $\mathbf{x}$ and time t evolve according to the reaction–diffusion equation

$$\partial_t c_i = D_i \nabla^2 c_i + R_i(c_1, \ldots, c_n), \quad i = 1, \ldots n, \qquad 1.$$

where D_i are the diffusion constants and R_i represent chemical reactions that affect morphogen i. These reactions can result in production or degradation of the species i at position $\mathbf{x}$ and time t, such that $R_i(c_1, \ldots, c_n)$ encodes the system-specific interaction network between the chemical species. The coupling between chemical interactions and diffusive transport, as encoded in the above equation, can lead to the emergence of patterns. The two archetypal paradigms within this framework, which have been very useful in developmental biology, are the positional information model (143) and the Turing pattern model (134).

In the positional information model of biological pattern formation (143), the concentration field of a morphogen secreted in a particular group of cells (source cells) serves to lay down a coordinate system for the embryo. The morphogen molecules diffuse from the region of the source cells and are absorbed by the target cells surrounding this region. These target cells regulate their gene expression patterns in response to the concentration levels of morphogen present at their location. This differential gene expression can generate patterns in cellular fate that lead to the emergence of organized structures in the embryo. For example, this morphogen gradient model has been quantitatively established in the patterning of the *Drosophila* wing disc (56).

Alan Turing showed in his seminal paper (134) that a system of two morphogens can spontaneously exhibit spatially periodic patterns if the slow-diffusing species (the activator) autocatalyzes its own production and also stimulates the production of the fast-diffusing species (the inhibitor). When this criterion is satisfied, the homogeneous state of a reaction–diffusion system becomes unstable in the presence of infinitesimally small perturbations, which then grow and spontaneously generate periodic patterns. Extensive studies have used the Turing mechanism to explain patterns

in diverse biological contexts (61, 84). For example, recent experiments have demonstrated that the specification of digits in mammalian limbs is governed by a Turing mechanism (102, 118).

3.2. Mechanically Active Materials

Mechanical forces in cells and tissues are typically generated by the cytoskeleton, which is a meshwork of filamentous proteins (such as actin and microtubules) and molecular motors (such as myosin and kinesin) (46, 60). The ATP-consuming activity of molecular motors leads to their self-propelled motion along the filamentous networks (51). The concerted action of motors can generate mechanical stresses on long length scales, reaching up to the cellular and tissue scales. Such forces are well known to be involved in cell division (39, 117), cell migration (1, 142), and embryogenesis (7, 109). This intrinsic force-generating mechanism in the cellular cytoskeleton drives it away from thermal equilibrium and puts it into a new class of materials called active matter (52, 76, 97, 101) (**Figure 4*a***).

The actomyosin cortex just underneath the cell membrane, a thin cytoskeleton meshwork, can be described as an active fluid on developmental timescales. Continuous turnover (exchange with the cytoplasm) of the proteins constituting the actomyosin cortex makes it flow like a viscous active

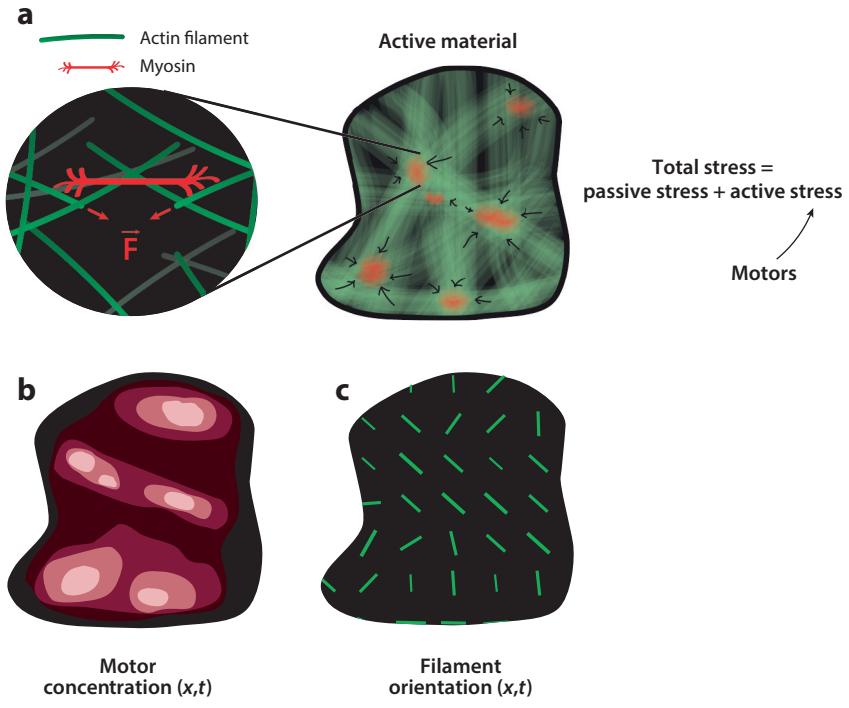

Figure 4

The actomyosin cytoskeleton as an active material. (*a*) The most important constituents of the actomyosin cytoskeleton are actin filaments and myosin motor proteins, which cross-link actin and generate mechanical forces by tugging filaments with respect to each other. This mechanical activity results in an additional active stress component in a mesoscopic element of the thin film actomyosin cortex. An active matter description of the actomyosin cortex considers (*b*) the motor density and (*c*) the filament orientation to determine the active stress distribution, both as a function of space (x) and of time (t).

fluid on timescales that are long compared to the turnover time. The molecular motor activity of myosin generates contractile stresses. In a hydrodynamic thin-film description, the total stress σ in a surface element of the actomyosin cortex is given by (11, 52, 77, 110)

$$\sigma = \sigma_p + \sigma_a. \quad 2.$$

The passive stress σ_p arises from the usual hydrodynamic description of a fluid or a solid (63, 64) dependent on the relevant timescales. The active stress σ_a results from motor activity and is regulated by the concentrations of molecular motors (**Figure 4*b***) and by the orientational order parameters of the actin filaments (**Figure 4*c***) (52, 76). It was recently shown that σ_a can also have an active chiral component (86). At the physiological scale of the actomyosin cytoskeleton, inertial forces do not play a significant role, and as such, dynamical evolution is governed only by a balance of forces acting on a fluid element. This force balance can be written as

$$\nabla \cdot \sigma = \mathbf{F}_{ext}, \quad 3.$$

where $\mathbf{F}_{ext}$ is any external force acting on the thin film layer. At the simplest level of description, $\mathbf{F}_{ext}$ can be modeled as a drag force of the form $\mathbf{F}_{ext} = \gamma \mathbf{v}$, where γ is a friction coefficient of the thin layer with its surroundings. If one assumes that σ_a is regulated by the concentration of myosin motors alone, one can solve for the hydrodynamic velocity field $\mathbf{v}$ in the case of a liquid (or the elastic displacement field $\mathbf{u}$ in the case of an elastic material) from the force–balance equation (Equation 3). This theoretical framework has been experimentally shown to quantitatively recapitulate the flow fields of the actomyosin cortex on viscous timescales (77, 86).

3.3. Patterns in the Cytoskeleton

A mechanically active system can show spontaneous emergence of patterns in concentration and orientation fields of cytoskeletal components. Such patterns are distinct from classical patterns observed in reaction–diffusion systems because forces and flows play an essential role in their generation. In this section, we highlight some theoretical concepts of this pattern formation process. Additionally, we mention experimental studies on reconstituted cytoskeletal systems.

The transport equations for the concentration fields of any molecule embedded in the cytoskeleton, e.g., myosin itself, now involve an additional advection term resulting from either flow or deformation of the underlying material. Specifically, we find that (11)

$$\partial_t c_i = -\nabla \cdot (\mathbf{v} c_i) + D_i \nabla^2 c_i + R_i(c_1, \ldots, c_n), \quad i = 1, \ldots n, \quad 4.$$

where the velocity $\mathbf{v}$ is obtained by solving the force–balance equation (Equation 3). In a simple approach, ignoring the orientational degrees of freedom, the active stress σ_a can be a function of the concentration fields, c_i, of all the species or of only one or a few active species (e.g., myosin motors), whereas the remaining passive species do not generate any active stress [e.g., signaling molecules like RhoA (120)]. However, these passive molecules will also be advected by the velocity field and can additionally have chemical interactions with the active species. We first consider the case in which there are no passive species present and discuss pattern formation in this scenario. The cellular cytoskeleton and the concomitant patterns observed in motors and filaments provide a prime example of this situation.

We first discuss the simplest case of just one species, which is also the regulator of the active stress, e.g., myosin motors. Even in the absence of the reaction terms $R(c)$, the transport equation above (Equation 4) for c, coupled with Equation 3, can display nontrivial patterns when the active stress σ_a is upregulated by the concentration field c (11). A physical way to understand this mechanochemical instability leading to patterns is the following. The concentration

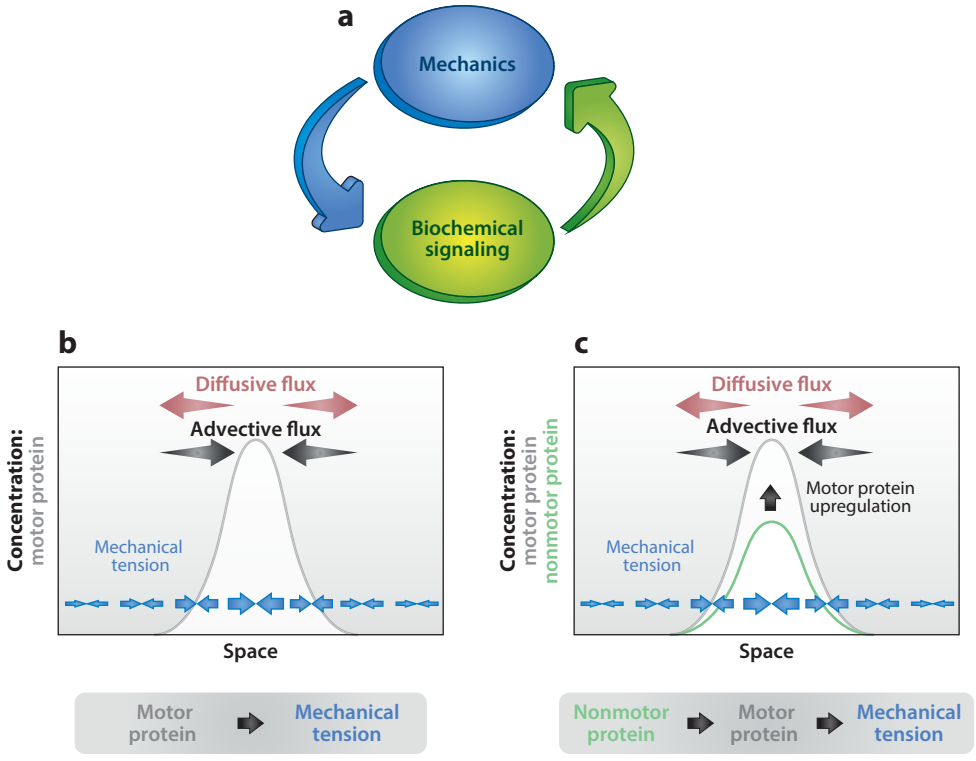

Figure 5

Pattern formation via an interplay between cell mechanics and biochemical signaling. (*a*) Positive feedback occurs among cell mechanics, transporting signaling molecules, and regulation of the cytoskeleton. (*b*) Such feedback can induce advective fluxes into regions of high motor density, which can compensate for the diffusive tendency to flatten inhomogeneous motor concentration profiles. (*c*) Nonmotor proteins can also use mechanochemical feedback to generate steady-state patterns by upregulating motor protein concentrations.

field c evolves in time under the effects of both advection and diffusion. Diffusion leads to suppression of fluctuations in the concentration field, whereas advection leads to clumping (which is a result of active stress upregulation). This competition between diffusive smoothing and contractile clumping balances at some point, which is where the steady-state patterns appear (11) (**Figure 5*b***). Specifically, the control parameter that drives the pattern-forming instability is the ratio of the strength of the active flows to that of passive diffusion—the Péclet number. Notably, this instability is essentially mechanical in nature, as there are no local chemical reactions that can generate any other instabilities à la reaction–diffusion systems.

Kumar et al. (62) studied a particularly interesting case of this kind of mechanochemical instability leading to pulsatile patterns in an active thin film. Consider two species A and I, both of which regulate active stress $\sigma_a = \sigma_a(c_A, c_I)$. A linear stability analysis shows the existence of Turing-like criteria for the emergence of spontaneous oscillatory patterns (62). Specifically, it was found that oscillatory patterns spontaneously emerge when (*a*) the fast-diffusing species upregulates and the slow-diffusing species downregulates the active stress or (*b*) the active stress upregulator turns over faster compared to the active stress downregulator. The key physical idea for the emergence of these pulsatile patterns is the coupling between differential regulation of active stress and the

differential relaxation of the stress-regulating species, either by different diffusivities or by different turnover rates.

In the cases discussed above, we have considered the regulation of the interfacial tension (active stress) in the cytoskeleton by the concentration of motor molecules alone. However, the cytoskeleton is really made of filamentous proteins, which are extended polymeric objects. In the case of actin and microtubules, these filaments are asymmetric (the filament ends are structurally different). As such, the orientation degrees of freedom (either polar or nematic) can appear in the active stress.

In such situations, one should note that the orientational degrees themselves change subject to the forces acting on them. In other words, there would be equations of motion for the polar or nematic order parameters even at thermal equilibrium (22). In the presence of motor activity, additional terms appear in these equations of motion (52). This coupling between filament density and orientation and motor activity leads to the possibility of the appearance of patterns in the orientation of the filaments as well.

These motor-driven patterning processes have been demonstrated in many reconstituted assays. These studies were pioneered in in vitro systems of kinesin and microtubules, which exhibit complex orientational patterns, like asters, vortices, and networks of interconnected poles (87, 128). Recently, several studies reconstituted a membrane-associated actin network, resembling the in vivo actomyosin cortex, and observed that spontaneous patterns occur when myosin and ATP are added to the system (2, 5, 58, 59, 69, 122, 123, 137). Over time, clusters of myosin, so-called myosin foci, form. Additionally, the actin network exhibits orientational ordered patterns similar to those in the microtubule systems, depending on the relative amounts of the ingredients. This interplay between actin filament orientation and collective myosin motor activity has been investigated in detail using micropatterning of actin nucleators (104).

Steric interactions between filamentous cytoskeleton components like actin and microtubules can also result in orientational order. So-called surface-gliding assays anchor molecular motors on a glass slide and add filaments on top of them. Using actin as the filament and a nonprocessive myosin as the anchor, it was shown that orientational order emerges when the actin density is above a critical threshold (115). Structures like clusters, swirls, and interconnected bands emerge, which can be long-lived and span the whole system. A similar assay made of microtubules and dynein studied by Sumino et al. (127) showed the existence of large-scale vortices of microtubules (with a diameter significantly larger than the microtubule length), which were shown to stem from steric interactions during filament collisions. Such reconstituted systems have also been used to study topological defects in orientation fields (23, 53). Furthermore, pattern formation in cytoskeletal systems has been studied with the bacterial FtsZ protein, which forms membrane-associated cytokinetic rings (71). This intrinsically curved protein shows treadmilling and is thus motile (78). Purified FtsZ and FtsA (required for membrane binding of FtsZ) proteins have the ability to autonomously self-organize into ring-like structures (71). Such vortex formation in curved active polymers was theoretically investigated by Denk et al. (24), who found that the formation of vortices is dependent on the filament density. Recently, pattern formation in the cytoskeleton has been studied in in vivo systems as well. The *Caenorhabditis elegans* zygote shows a period of 5 minutes with strong flows of the actomyosin cortex from the posterior to the anterior end. These flows induce a region of strong mechanical compression in the cortex. This compression leads to nematic order in the orientation of actin filaments perpendicular to the flow direction and creates a ring-like actin structure reminiscent of cytokinetic rings (105, 110). At a higher length scale, cells in the trachea (tubes in the airway of the *Drosophila* embryo) are mechanically stabilized by supracellular actin rings that arise from a mechanical instability (41). These supracellular structures are reminiscent of the large-scale actin cable seen during *Drosophila* dorsal closure (6).

In summary, the in vivo cytoskeleton or in vitro reconstituted systems of cytoskeletal components act as active force-generating materials and have an intrinsic pattern-forming potential. Mechanical instabilities can generate regions of high motor concentration, e.g., myosin foci, and also long-ranged polar and nematic order in the spatial orientation of the cytoskeleton filaments, such as asters, vortices, etc., in bacterial FtsZ, actin, and microtubules.

3.4. Patterns in Regulators of Active Materials

Pattern formation in active materials, as discussed in the previous section, only concerns those cytoskeleton proteins that are directly involved in generating mechanical stresses. Even though distinct structures in the cytoskeleton, for example, actin cables, are important during embryogenesis, the majority of proteins that need to establish distinct spatiotemporal patterns during development are nonmotor signaling proteins. This class of proteins drives cell differentiation and triggers tissue-specific transcriptional programs. Can proteins that do not themselves generate mechanical force use the active nature of the cytoskeleton to form patterns?

The key in answering this question is realizing that the cytoskeletal deformations and flows have a strong impact on the spatiotemporal distribution of nonmotor signaling proteins. Motile cells like fish keratocytes, for example, have a very dynamic cytoskeleton, with actin retrograde flow at the leading edge and myosin-induced contractions at the rear end. These cytoskeletal flows induce large-scale cytoplasmic flows and thus lead to the transport of cytoplasmic proteins (55). In other systems, like the neural axon, the *C. elegans* zygote, the mouse oocyte, and the *Drosophila* oocyte, it has been suggested that flows of the cytoskeleton induce large-scale transport of cytoplasmic proteins (34, 85, 92, 146). In summary, the flows and deformations produced by the cytoskeleton can transport proteins that do not themselves generate mechanical stresses. Chemical interactions between these signaling molecules and the force-generating motor molecules can lead to positive feedback, amplifying the original pattern-forming instabilities (**Figure 5*a*,*c***) (11, 25, 36, 48, 49, 62, 79, 112, 125). This mechanochemical feedback has a widespread potential for pattern formation in morphogenesis. We discuss a few systems in which mechanochemical feedback has been studied or that are interesting candidates for such a mechanism next.

Budding yeast is a system in which the interplay between chemical patterning and cytoskeleton-mediated transport has received significant attention. Budding yeast generally divides asymmetrically by forming a small bud, which then grows and separates from the mother cell during cytokinesis. The location of the initial bud is encoded by a patch of membrane-associated Cdc42 (10, 42). This Cdc42 patch is stable in time, despite diffusive losses. Two mechanisms have been identified to maintain this nonhomogeneous concentration of Cdc42. On the one hand, a biochemical positive feedback loop stimulates the recruitment of Cdc42 to regions of its high concentration (13, 18, 140). On the other hand, it has been shown that actin-mediated transport is important for the formation of Cdc42 patches (139, 140). These actin cables form in response to Cdc42 (20, 98) such that budding yeast exhibits features of mechanochemical feedback. The precise role of this actin-mediated transport is still debated (65, 113), although recent results suggest that it promotes robustness of the Cdc42 patch against stochastic fluctuations (30).

The activity of the cytoskeleton is also crucial in the establishment of cell polarity. For example, fish keratocytes can utilize their actomyosin cortex to establish a polarized state. These cells are stationary when unpolarized and motile when polarized. This polarization event does not require external cues. Rather, fluctuations of the contractile state of the actomyosin cortex can establish a polarized state (145). This link between cell polarization and fluctuations of the actomyosin cortex has also been established in zebrafish early embryonic progenitor cells, which when polarized show amoeboid cell motility (108). Interestingly, the persistence time of the polarized state universally

depends on the strength of the actin flow for a broad range of motile cell types (74). Thus, in these systems, a stronger mechanochemical feedback leads directly to a longer persistence of the patterned, polarized state of the cell.

Fibroblasts can also show features of mechanochemical pattern formation on a larger scale. These cells are durotactic such that they move toward regions of higher substrate stiffness (70). Fibroblasts additionally excrete extracellular matrix (ECM), which in turn results in an increase of the substrate stiffness (141). This system may possibly exhibit mechanochemical feedback and the concomitant patterns in fibroblast and ECM distributions, as a future study might reveal.

Mechanochemical feedback as a patterning mechanism has been implicated in the organization of lipid-anchored proteins, so-called glycosylphosphatidylinisotol (GPI)-anchored proteins (38). These proteins are organized in nanoscale clusters, and the mechanism of this clustering depends critically on the interaction between the GPI-anchored proteins and the dynamic actomyosin cytoskeleton. Another system that is studied for its mechanochemical pattern-forming potential is the slime mold *Physarum polycephalum*. This cell possesses a strikingly active cytoskeleton, which is implicated in intercellular vein formation and peristaltic fluid transport. Complex spatiotemporal thickness oscillations were observed in protoplasmic droplets [cell fragments of *P. polycephalum* (130)], which have inspired mechanochemical models of these dynamic patterns (99, 100).

The effect of the actomyosin flows on the polarity proteins has been well-studied in the emergence of cell polarity in the *C. elegans* zygote (37, 83). This cell polarizes by establishing two distinct membrane domains with proteins of either the anterior or the posterior partitioning-defective (PAR) protein complexes, thus establishing the anteroposterior axis. Importantly, cell polarity establishment happens concomitantly with the presence of actomyosin flows. It was recently shown that these flows are indeed sufficient to trigger the transition of the unpolarized zygote to the polarized state, both states being stable against thermal fluctuations (37). It has been demonstrated that the PAR proteins themselves also differentially regulate the actomyosin cortex and its contractile state (83). It will be interesting to see if the mechanism of pattern formation in this system is indeed mechanochemical in nature.

Cortical actomyosin flows and patterns have also been implicated in the establishment of the left–right (LR) axis of the *C. elegans* embryo at the six-cell stage. The hydrodynamic flows seen in the actomyosin cortex at the one-cell stage have a strong chiral component (86). These flows and the concomitant actomyosin distribution can be understood as left–right asymmetric patterns arising in an active chiral fluid (31, 32). The same chiral flows are seen at the six-cell stage just before a symmetry-breaking mechanism distinguishes the left and right axes. Notably, in this case, the mechanochemical patterns couple to the geometrical shape of the embryo in establishing the LR axis.

On the tissue scale, the coupling between mechanics and biochemical signaling has also been implicated in the establishment of complex morphogenetic patterns. For example, the forces generated by cell intercalation, which are regulated by actomyosin expression levels, tow epidermal tissue to close the mammalian eyelid (45). Examples of one particular pattern, the so-called myosin foci, frequently occur when the tension in the actomyosin cytoskeleton is high. These are transient structures with high myosin concentration that are pulsatile in nature. The mechanism of their formation has been investigated in *Drosophila* and *C. elegans*. The pulsatile myosin foci in *Drosophila* emerge as a self-organized pattern through the interactions between myosin-induced flows and myosin regulation through the RhoA-ROCK pathway (81). In *C. elegans*, however, it has been found that pulsatile myosin foci are the result of both advective flows and an external RhoA-mediated oscillator, which controls the contractile instability in the actomyosin cortex (91). Planar cell polarity (PCP) is another example of a system where the interplay between mechanics and

biochemical signaling establishes developmental patterns. PCP patterns in the *Drosophila* wing disc have been shown to be influenced by the flows created in the underlying epithelial tissue (3, 27). PCP pathways can themselves regulate mechanics; for example, the Fat/Dachsous/Four-jointed PCP pathway has been shown to regulate myosin (called Dachs in this case), and thereby tissue tension (14). Thus, the interplay between mechanics and signaling can go both ways, a hallmark of feedback in mechanochemicaln. Notably, at the tissue level, the concentration fields of signaling molecules can span several cell lengths, and as such, consideration of molecules that maintain tissue integrity, e.g., cadherins, and their couplings with signaling molecules and motors can be important in mechanochemical pattern formation (67).

4. OUTLOOK

The development of an organism from a zygote into a fully functional 3D creature is a process in which chemical patterns of molecules are strongly correlated with the embryonic shape. The emergence of patterns in shape space, i.e., morphogenesis, is controlled by the information encoded in the concentration fields of morphogens. A change in the shape of the embryo affects the concentration patterns of these morphogens, which in turn change the shape of the embryo itself. This strong coupling between morphogen patterns and patterns in shape space is the hallmark of self-organized pattern (and shape) formation in developing embryos (8). But how do the chemical concentration fields of morphogens affect the shape of a cell or embryo? The influence of morphogen concentration fields on surface geometry has been considered (21, 84), but because classical morphogenetic patterning mechanisms do not involve mechanical forces per se, one has to invoke ad hoc couplings between morphogens and the stresses needed for geometrical deformations.

As we have remarked above, force generation in cells and tissues is typically controlled by the cytoskeletal processes. In previous sections, we discussed how biochemical coupling between nonmotor signaling molecules, i.e., morphogens and force-generating molecular motors, can result in mechanochemical feedback, which can generate many of the important developmental patterns. The main difference between classical pattern-formation mechanisms, as studied in reaction–diffusion systems, and active mechanochemical patterns, as discussed in this review, is that forces and deformations and/or flows are essential ingredients in the latter. The key point to note is that the very same stresses involved in these active mechanochemical patterns can also deform the shape of the underlying embryo. As such, active mechanochemical patterns could be the natural and consistent way to couple chemical patterning with shape deformations (9, 81, 94, 103, 129, 135). We believe that future studies will take this approach to fruition and unify the two superficially distinct aspects of pattern formation and morphogenesis in developing organisms.

The mechanochemical patterns discussed in this review combine biochemical reaction networks with the physical principles of mechanical forces that can result in deformations and/or flows. The patterns in morphogen concentration fields, hydrodynamic flow fields, filament orientation fields, and the shape of the embryo are dynamic entities. Quantitative developmental biology experiments are beginning to capture these complex spatiotemporal dynamics in increasing detail (54, 93). It is the inseparable convolution of mechanics and biochemical signaling that poses the greatest challenge for understanding mechanochemical patterns. As such, it becomes imperative that experimental studies are combined with mathematical and physical theories of morphogenetic pattern formation to gain a deeper insight into developmental biology (47, 96).

To summarize, we began with developmental pattern formation processes where mechanical principles played a key role. We explored the role of interfacial tension and differential adhesion in sorting proteins and cells. We next discussed buckling instabilities that result from differential

growth in connected tissue layers. Active materials and their pattern-forming instabilities were discussed next, with an emphasis on the cellular cytoskeleton. In conclusion, our discussion of the feedback between mechanics and biochemical signaling shows that this is a generic feature of morphogenesis and can have a widespread impact on developmental pattern formation. A systematic and quantitative exploration of this feedback is just beginning and will open up new avenues in understanding developmental self-organization.

DISCLOSURE STATEMENT

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