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Review

Mechanics and self-organization in tissue development

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ABSTRACT

Self-organization is an all-important feature of living systems that provides the means to achieve specialization and functionality at distinct spatio-temporal scales. Herein, we review this concept by addressing the packing organization of cells, the sorting/compartmentalization phenomenon of cell populations, and the propagation of organizing cues at the tissue level through traveling waves. We elaborate on how different theoretical models and tools from Topology, Physics, and Dynamical Systems have improved the understanding of self-organization by shedding light on the role played by mechanics as a driver of morphogenesis. Altogether, by providing a historical perspective, we show how ideas and hypotheses in the field have been revisited, developed, and/or rejected and what are the open questions that need to be tackled by future research.

1. Introduction

Self-organization is, somehow, a slippery concept. A standard definition reads as follows: *"self-organization is a process in which pattern at the global level of a system emerges solely from numerous interactions among lower-level component of the system"* [1]. While this definition sounds convincing at first, it also raises a number of questions: what do we mean by pattern and, further, how do we quantify it?, what do we mean by interactions? On the other hand, we would all agree that, for example, human development is truly an example of a self-organizing process: starting from a single cell —the zygote— that grows and divide repeatedly, an organism with highly specialized functions and organizational traits emerges (instead of an amorphous mass of cells). H. Haken provided in his seminal book *Synergetics* another example, herein adapted, from the field of social behavior that hints at the characteristics that define self-organization [2]. A group of workers in a factory, driven by the orders of a boss, produce a car. In the absence of the boss, the workers might as well achieve enough level of mutual understanding (*i.e.*, they could self-organize) and end up producing the same car (or even an improved version of it!). Thus, features of self-organization systems are, first, the existence of a functional, global, "ordered" output (*i.e.*, the car and not just some junk); second, the lack of external drivers orchestrating the process (*i.e.*, the boss); third, the need of interactions between individual, lower-level, components (*i.e.*, the communication that leads to the mutual understanding of factory workers); and, fourth, those interactions are local and independent of the final output (*i.e.*, workers do not use megaphones to coordinate their efforts that, otherwise, are focused on creating and assembling parts of the car rather than on the final model). This set of features is acknowledged in most definitions of self-organization in chemical, physical, and biological systems [1,2]. In addition to this, another interesting quality of self-organization is that covers different spatio-temporal scales. Namely, the aforementioned lower-level components can be lipids forming a raft, cells shaping a tissue, or flocking birds.

Herein we review some self-organization mechanisms in developmental processes at different scales where mechanics has been proven to play a key role. To include all the mechanisms that fit within that framework is clearly out of the scope of this manuscript. We made a judgment call aimed at using examples where elements from different fields — Geometry, Topology, Physics, and Dynamical Systems theory— have been shown to be successful to quantitatively describe the self-organizing mechanisms. In particular, we discuss the packing organization of cells, the cell sorting phenomenon, and the propagation of active waves in tissues. As for the former, cells in epithelia, the skeletal muscle, the adipose tissue, or even the central nervous system of animals are tightly packed [3–9]. In that context, a relevant question is do the

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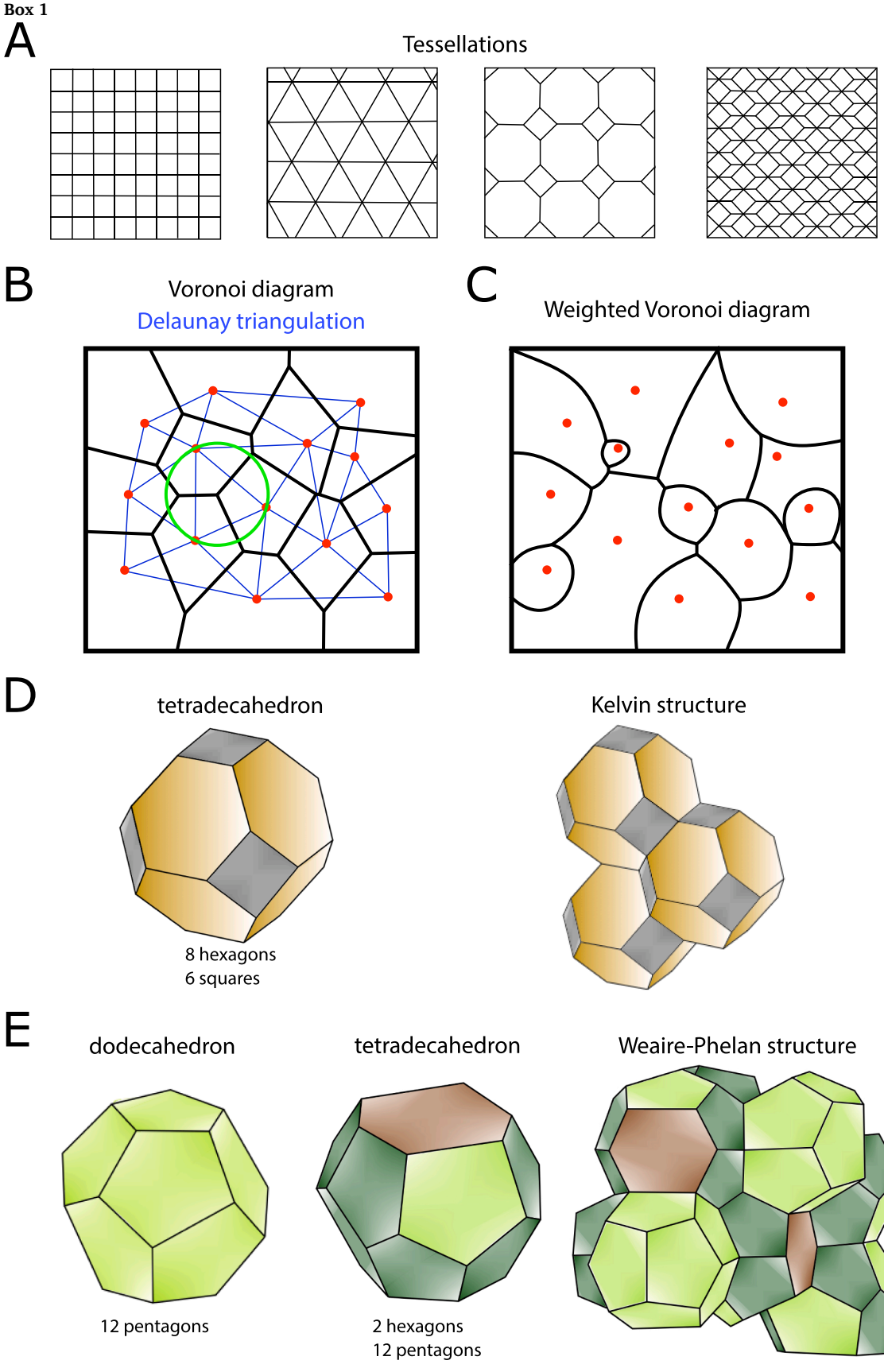
Box 1


A
Tessellations
B
Voronoi diagram
Delaunay triangulation
C
Weighted Voronoi diagram
D
tetradecahedron
8 hexagons
6 squares
Kelvin structure
E
dodecahedron
12 pentagons
tetradecahedron
2 hexagons
12 pentagons
Weaire-Phelan structure

Topological concepts. A: A **tessellation** is formed by geometrical entities that tile the space without enabling gaps or overlapping. These elements are arranged as mosaics. The box shows that a tessellation can be formed by the same type of polygons (*e.g.* triangles or squares) or by different types of geometrical figures. **B:** The **Voronoi diagram** (black lines) is a particular type of tessellation. It is obtained through a simple mathematical rule: given a set of seeds (red dots) the Voronoi tessellation delimits the regions of space closer to any seed. Those regions have convex polygonal shapes (or convex polyhedral shapes in a 3D space) and are called Voronoi cells. The dual (mathematical equivalent through a transformation) of the Voronoi diagram is the so-called **Delaunay triangulation** (blue lines), where seeds are connected to nearest neighbors and lead to a triangulation diagram. The nearest neighbors connectivity condition then satisfies the Delaunay condition: the circumscribed circumferences that link three seeds (green) cannot contain any seeds inside (otherwise that seed would be a nearest neighbor) and circumcenters are located at the vertexes shared by three Voronoi cells. **C:** There are more general forms to generate a Voronoi diagram where seeds could be assigned with different "weights" in terms of a distance function: a **Weighted Voronoi diagram**. In that case, the usual Euclidean distance is modified depending on the seed's weight: the larger the weight of a seed, the easier a faraway coordinate belongs to its emerging Voronoi cell. Thus, the Voronoi cells associated to seeds with larger weights are larger in size. This type of Voronoi diagrams display Voronoi cells with concavities. **D:** For years, researchers have devised methods to optimize the compartmentalization of space. One of the most famous examples is Kelvin's problem, introduced in 1887 by William Thomson (Lord Kelvin) in his classical article *"On the Division of Space with Minimum Partitional Area"* [38]. There, he raised the question of the optimal way to partition a space by using cells with equal volume and with the minimal surface area. He proposed the **Kelvin structure**, a tiling of **tetradecahedra** with 6 squared sides and 8 hexagonal sides. **E:** As of today, the more optimal solution to Kelvin's problem was found in 1993 by Denis Weaire and Robert Phelan [40]. They improved Kelvin's solution by using a 3D tiling structure with a surface area 0.3% smaller than that used in Kelvin structure: the **Weaire-Phelan structure** is composed by two different cells with equal volume, a **tetradecahedron** with 2 hexagons and 12 pentagons, and a pentagonal **dodecahedron**.

packing and connectivity properties of cells qualify as a self-organizing process? In order to address this question, one option is to find an adequate modeling framework able to capture some important characteristics. For example, nervous tissues organize as a circuit, where neurons synaptically connect different regions, and this feature makes feasible the usage of network modeling [10,11]. This approach, as well as additional evidence, have then indicated the existence of self-organizing features [12]. However, in the case of tissues where cells pack together and organize resembling tessellations (a way to fill a bi-dimensional (2D) or three-dimensional (3D) space with compact elements, Box 1) the network approach disregards the biophysical constraints that shape the cell packing and hence determine their organizational properties. In these cases, the application of models that account for the mechanics driving tissue packing are necessary to understand the formation and homeostasis of complex multi-cellular structures. Here we focus on how the marriage of geometrical, topological, and biophysical concepts has been proved fruitful to describe and model the organizational traits of tissues such as epithelia [6].

Another level of self-organization refers to the formation of diverse cell and tissue types during development. Intuitively, this process relies on sorting mechanisms able to segregate distinct tissue types. While cellular signaling has been shown to be essential in this process, the mechanical properties of cells have been shown to be equally relevant [13]. Townes and Holtfreter reported one the first experimental observations of embryonic cell sorting: the spontaneous aggregation of gastrulating amphibian embryos mixtures into their distinct germ layers *in vitro* [14]. This observation was associated with a differential "affinity" where cells with distinct fates, or identities, displayed different levels of "attraction" to each other. This conceptual model provided a hand-waving explanation of the cell sorting phenomenon and was also able to account for the formation of separating boundaries between different cell populations and the lack of cell intermingling along those boundaries [15,16]. However, the precise underlying molecular and/or cellular mechanisms that define and characterize "affinity" and "attraction" remained unclear at that time. Since then, several mechanisms have been proposed to explain the self-organization process of cell sorting and boundary formation. Here we will review proposed theories based on molecular and cell mechanical properties, and shed light on the experimental and computational works that support, and also question, these mechanisms.

In the aforementioned examples we skipped the details about the communication mechanisms that orchestrate the interactions between the individual components. In the case of the topological/packing organization of cells or the segregation of cellular populations those interactions are mainly *short-range*. That is, the collective behavior emerges from neighboring cell interactions, *e.g.*, cellular adhesion. Indeed, a number of developmental problems display short-range communication mechanisms that elicit the *local* coordination of cells. A remarkable example is the ubiquitous Delta-Notch (ligand-receptor) system responsible of the lateral inhibition, the lateral induction phenomena, and other self-organized patterning phenomena [17–19]. In particular, Delta-Notch lateral inhibition is a conserved mechanism of patterned cell fate specification among organisms that amplifies differences in ligand/receptor expression and results in Delta (ligand) expressing cells to inhibit ligand expression, and the activation of Notch (receptor) pathway, in neighboring cells [20]. Notably, a recent study has shown that other pathways are also able to produce a lateral inhibition response using mechanical regulation [21]. Thus, during zebrafish oogenesis the prospective micropyle precursor cell (MPC) accumulates the transcriptional coactivator TAZ and grows faster than neighboring cells. Those cells become mechanically compressed and, as a consequence, lose nuclear TAZ and the MPC fate specification. However, the successful development of organisms require additional communication mechanisms that enable cells to share organizational information at a broader level. Thus, tissue patterning rely on cell-cell protrusive interactions and, more generally, on diffusive signals, that go beyond nearest-neighbors interactions [18,22]. However, pure diffusive transport of signaling molecules, while efficient at the intracellular spatio-temporal scales, it is highly inefficient at the intercellular (*i.e.*, tissue-level or full embryo) scales: *e.g.* it takes $\mathscr{O}(10^0)s$ for a typical protein to diffuse $\mathscr{O}(10^1)\mu m$ (cell size), but $\mathscr{O}(10^4)s$, *i.e.* hours, to diffuse $\mathscr{O}(10^3)\mu m$ (embryo size) [23]. Moreover, diffusion is, by definition, an homogenizing process, and the observed spatio-temporal organization of the embryo during development demands inhomogeneous, yet coordinated, responses. The answer to this conundrum relies on reaction-diffusion mechanisms [24,25]. Generically, a reaction-diffusion mechanism corresponds to a spatio-temporal dynamics where *local* physicochemical reactions are *long-ranged*, spatially coupled, through a transport mechanism [26] (Box 3). We notice that a number of distinct morphogenetic mechanisms fall within this definition of a reaction-diffusion system [27]. One example is Turing patterning that has been reviewed in a number of studies [24,25,27–29], and for which recent research has shown that mechanical effects can play a key role [30,31]. Herein, instead, we illustrate the reaction-diffusion mechanism of self-organization by focusing on the formation of excitable traveling waves and the effect of mechanochemical feedbacks [32].

Altogether, we cover different examples about the self-organization phenomenon during development where mechanics has been proved relevant. On the one hand, our review contributes to the increasing interest on the role played by mechanical signals in development; on the

other hand, we aim at providing basic understanding about some of the theoretical foundations used to describe quantitatively these developmental problems.

2. Tissue packing and tessellations

2.1. Cellular packing as a self-organization trait

Packing optimization of objects is a self-organization feature commonly found in nature that leads to different space tessellation patterns in 2D and 3D (Box 1). A famous example from the inorganic realm are the basaltic columns formed by fast cooling of volcanic lava that tessellate as columnar prisms such as those of the Giant's Causeway in Northern Ireland (Fig. 1A). Bubbles in foams also tessellate (Fig. 1B) and acquire, preferentially, a tetradecahedral shape [37]. The tetradecahedron (a.k.a. tetrakaidecahedron, generically a polyhedron with fourteen faces) was postulated by Lord Kelvin [38] as the optimum way to fill a 3D space with a tessellation of elements having the same volume and minimal surface area (Box 1). This type of organization has been observed in other cases of tessellating structures such as those obtained in compression experiments of lead shot balls [39]. Kelvin's conjecture (Box 1) was further improved by the so-called Weaire-Phelan structure (Box 1) that increases the 3D packing optimization [40]. This structure is formed by two types of cell shapes and was computationally postulated and, interestingly, found latter in some crystal structures, such as those of clathrates [41]. As of today, the Weaire-Phelan structure has not been found in any living material. Also, we point out that there is no proof that the Weaire-Phelan structure is in fact a global packing optimum but a counter-example of Kelvin's conjecture.

In the context of living matter, muscular, bone, adipose tissues, some cell types of the nervous system, and especially epithelia, are also organized and packed following space tessellations (Fig. 1C–E). Cells in epithelial monolayers are closely joined together through intercellular connections ("tight junctions") and the basal membrane that operates as a connective tissue [4,6]. Tissue packing is then revealed as a layer of cells that, for example, cover the surfaces of the organs or form glands (Fig. 1C-D). Skeletal muscles are packed arranged by fascicles that are separated by a fine connective tissue, the endomysium. As a result, muscle fibers appear as long multinucleated cells that are grouped in bundles [4,7]. Fat (*i.e.*, adipose) cells organize by forming closely packed bulbous spheres surrounded by strands of a supportive connective tissue [5,8]. All these tissues, including the brain parenchyma, that is tiled by a network of contacting astrocytes, are arranged as cellular tessellations [9]. Interestingly, in the case of the trabecular bone, the observed tessellation corresponds to the pores of the tissue (Fig. 1E) [42,43].

Cellular packing organization is indeed a key factor for regulating cellular communication, growth, or the structural support and the material properties of tissues. As for the driving force underlying cell packing, the hypothesis that living matter satisfies the same basic principle that inorganic materials has been proved successful. Thus, mono-disperse foam bubbles tessellate and self-organize by minimizing their surface-tension energy (Box 2) [44] that in turn leads, as mentioned above, to their tetradecahedral shape. Indeed, the tetradecahedron is the cell polyhedral shape that predominates on multilayer animal tissues such as fat and epidermis [45,46]. Moreover, surface-tension minimization has been shown to drive the organization pattern of many other systems such as the grouping of cells during the cleavage phase in early embryo development [47], the ommatidia configuration of the developing retina [48], the scutoidal cellular shape (Fig. 1C-D) [49], and, as shown below, in the cell sorting process.

2.2. The topological organization of cells

Independently of the physical principles underlying the packing configuration of cells, the mathematical characterization of the topology of cell contacts has been also revealed as a powerful tool to shed light on the self-organizing properties of tissues. In particular, monolayer epithelia (*i.e.*, the tissues that coat many organs and constitute most of the embryonic structures and glands [50]) have been studied in detail from such a viewpoint. Thus, the cellular organization on the apical surface of these tissues can be characterized as tessellations of planar polygonal shapes. From that perspective, the application of the so-called Euler principle implies, and experiments confirm, that cells have six neighbors on average [6]. Notably, while there is an unlimited number of different polygonal distributions (*i.e.*, set of frequencies of cells that belong to a given polygonal class) that satisfy such a property, the one found in surfaces of metazoan epithelia seems to be unique and highly conserved [51]. Gibson *et al.*, showed that this self-organization trait emerges from the cellular proliferation/division process [51]. We notice that while proliferation and division are active cellular processes, this research revealed that the emergence of a self-organized tessellation can be derived from a probabilistic model where these processes occur instantaneously and are passive (in the sense that growth/division happen regardless of the behavior of the cellular environment and not as an active physiological response). Moreover, other "laws" can be derived from the topological analysis of epithelial surfaces and reveal correlations between cell properties, such as the cell area, and their polygonal class [6].

Further insight has been obtained by applying concepts from the field of computational geometry to study and characterize epithelia. A clear example are the analyses based on Voronoi diagrams (or their dual form, the Delaunay triangulations, Box 1). Strikingly, if the centroids of epithelial cells arranged in their characteristic polygonal pattern are used to build a Voronoi tessellation, the outline of cell junctions fits, almost perfectly, with such a pattern [52]. The Voronoi methodology has been widely used to quantify the connectivity features of tissue packing in normal and pathological conditions in epithelial, muscle, bone, adipose and neural tissues [4,5,8,9,42,43,52–55]. Moreover, Voronoi models allow to analyze and simulate packed tissues with an efficient computational workload when compared with other popular simulation models [56].

Recent progress has shown that the organization, geometry, and tissue connectivity studies of epithelia using Voronoi concepts can be linked to biophysical analyses. Some examples include, *i*) the study of the tissue fluidity [57,58], *ii*) the analysis of the limits of epithelial self-organization driven by physical constraints [4], and *iii*) the discovery of novel 3D cellular shapes, *i.e.* the *scutoid* (Fig. 1C-D), and its consequences [49,59]. In a different context, the Voronoi approach has also been used as a design tool for tissue engineering and for the development of bioimplants (Fig. 1E-F). In that regard, Voronoi models have helped to match the trabecular morphometry (density, volume, surface area…) and the mechanical (Young's modulus) and permeability properties of bones. The resulting artificial implants can be then printed using biocompatible and bioresorbable materials that favor osteoconduction (formation of new bone cells that slowly replace the scaffold material) due to the high degree of biostructural-mimicking characteristics provided by Voronoi models [42,43]. As a final comment about the usage of the Voronoi approach, it is worth mentioning a methodological variation that has led to some interesting results in the context of cellular mechanics: the so-called weighted Voronoi diagrams ([60], Box 1) where the cellular centroids used to estimate a tessellation are "weighted" depending on different factors. This method, originally used to investigate polycrystal growing [61,62], has been applied to study the cellular mechanics in tumor growth or during lymphoid follicle morphogenesis [63].

2.3. Combining topology, geometry and physics: the vertex model

According to the previous comments, reliable tissue modeling frameworks must be able to link the emergence of the topological organization to the biomechanical properties of packed cells and tissues and to their geometrical contraints. Then, the biomechanics needs to

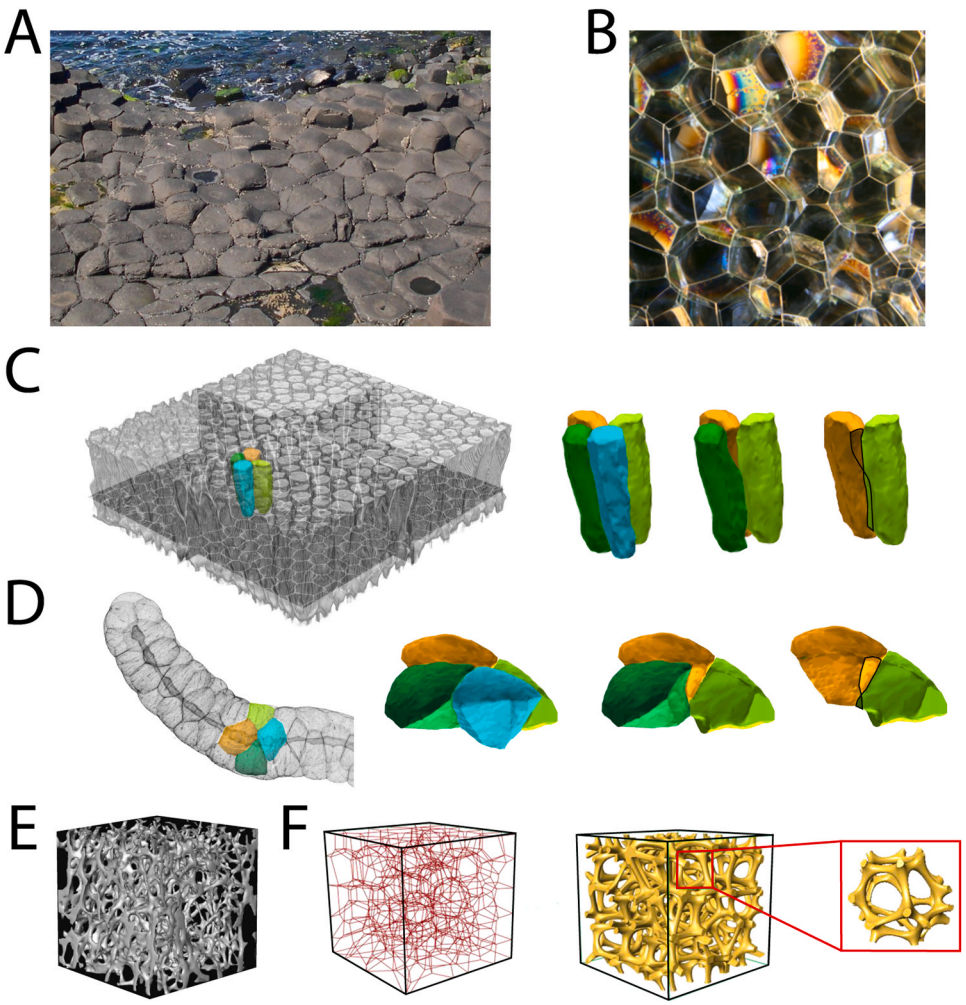


Fig. 1. Self-organization and 3D packing: from inert to living matter. A: Giant's Causeway (Clocha´n an Aifir), Northern Ireland (adapted from [33]). **B:** Foam bubbles organized as a 3D tessellation (adapted from [34]). **C:** (Left) Monolayer epithelium of *Drosophila* embryo at the end of the cellularization process. A four-cell motif has been highlighted. (Right) Zooming in the four-cell motif reveals scutoidal shapes (*i.e.*, neighbor exchanges along the apico-basal axis): the orange and blue cells contact on the top surface but not on the bottom one. The opposite occurs with the dark/light green cells. **D:** (Left) Full projection of a *Drosophila* salivary gland at third larval instar revealing a similar packing configuration, *i.e.*, a scutoidal four-cell motif. Same color code as in panel **C. E:** 3D reconstruction of coronal section of the proximal metaphysis of a 3 years old child (adapted from [35]). **F:** Design of a porous scaffold mimicking the trabecular bone architecture: 3D Voronoi cellular configuration (Left) and the resulting porous scaffold obtained from simulations (Right) (adapted from [36]).

mimic the "adhesiveness" that keep the cells connected and, in addition, must allow for the observed tissue plasticity due to cell shape changes, cellular movements, topological tissue rearrangements, and collective cell migration [64,65]. In that regard, epithelial cells, unlike for example foam bubbles, are active/reactive entities equipped with a biological machinery that transduce signals into mechanical responses (and *vice versa*). The cytoskeleton, adhesion molecules, cellular membranes, and other biological elements ultimately render the physical properties to tissues, define their contractility, elasticity, and surface tension features (Box 2), that in turn determine key biological functions such as proliferation, migration, apoptosis, or tissue repair. Some efforts to provide such a bridge across scales have focused on either developing theoretical frameworks or characterizing the continuum viscoelastic behavior of tissues [66–68].

In this context, the vertex model is one of the most popular simulation methods of epithelial cell behavior (Box 2). The model initially considered properties such as the cellular adhesion, the contractility, and elasticity effects in 2D planar epithelial layers [69,70]. Subsequent implementations have included biological events such as proliferation, migration, or mechanobiology effects [31,64,65,71], active tension fluctuations and tissue voids [72], active friction/stress [73], as well as pathological conditions (*e.g.*, tumorigenesis) [74,75]. Remarkably, the application of the vertex model in combination with experiments has been able to establish, quantitatively, the cell biomechanical parameters that lead to the observed self-organization in epithelia [76,77]. Further, from planar 2D simulations, the vertex model has evolved to what are known as "2D and 1/2" (a.k.a. 2.5D) models to study epithelial surfaces in a curved space in order to investigate epithelial folding, invagination/evagination processes, or tubulogenesis [65,78–80]. Finally, some implementations of the model have been able to simulate 3D epithelial monolayers thus considering cellular volume effects [6]. Recent developments of the vertex model in a 3D context include accounting for the recently reported epithelial packing events (on-transient apico-basal intercalations) that have been shown to minimize the tissue energy (the aforementioned *scutoids*) (Figure 1C-D) [81–83]. An open question remains in regards of the usability of vertex models to investigate the morphogenesis of multilayer epithelia, where cellular packing can differ considerably with respect to monolayers and the aforementioned tetradecahedral cellular shapes can play a key role [84]. Recent progress in that direction refers to the study of the self-organization process that leads to the segregation of cellular populations and well-defined boundaries [85], *i.e.*, cell sorting.

3. Cell sorting

3.1. Boundaries as a signature of self-organization during development

In the late sixties and early seventies of the 20th century clonal/mosaic techniques made possible to track cells and their progeny. Interestingly, these analyses revealed the existence of "barriers" within cellular populations that cells could not cross and that delimit distinct cellular compartments that ultimately map into different parts of primordia [86,87] (Fig. 2A). Subsequent studies using cellular lineage tracking have shown that compartmentalization is a conserved

self-organization strategy during development [16]. Compartment boundaries are usually linked to signaling centers –organizers– that, serve as axes of a coordinate system, pattern cellular populations, and provide positional information to cells [88] (Fig. 2B). In this context, different studies have revealed the pathways and mechanisms that are responsible for boundary establishment and their maintenance when they are challenged, for example, by cell division events [15,89]. Thus, it has shown that both short- and long-ranged communication mechanisms are required for boundary establishment [87,90,91] and that the subsequent downstream regulation of actin, myosin, and cadherins expression, *i.e.* the cell mechanical properties, underlies their robust maintenance [16,71,89,92]. Boundary formation and maintenance is then a process that beautifully illustrates the existence of self-organization traits in development that rely on mechanical cues and it can be considered as a trademark of a broader self-organizing mechanism: cell sorting.

3.2. Cellular affinities: the differential adhesion hypothesis and beyond

Generally speaking, the formation of distinct functional organs relies on the proper segregation of cell populations into different "groups" as well as on the formation, and the active maintenance, of spatial boundaries that prevent different cell types from mixing as mentioned above. In order to understand this process, the physical properties of cells have been long studied and, together with the experimental observation of an spontaneous separation of mixed cells with different fates, led to the principle that cells display different "affinities" between them [14]. To explain these results, M.S. Steinberg proposed the "Differential Adhesion Hypothesis" (DAH). Thus, by drawing parallels with the behavior observed in immiscible liquids, Steinberg hypothesized that cells aggregates could behave similarly due to the distinct mechanical properties of cells (Fig. 2C-D) [93–98]. According to Steinberg's theory, "adhesion" was defined as the minimum amount of work that is required to separate cells. The adhesion strength could vary from one cell type to another and, consequently, the movement of cells was just a transient dynamics toward a final, segregated, configuration that minimizes the free energy of the system [99]. The DAH has been widely accepted for understanding the mechanism of cell sorting and has been considered as the primary driving force of cell segregation in many experimental and computational studies [93,100–105].

Harris criticized the DAH model and raised questions about Steinberg's analogy by identifying fundamental differences between cells and liquids [106]. In the same study, he presented a number of alternative theories including the "Differential Surface Contraction Hypothesis" (DSCH). Based on this mechanism, the cells are supposed to have different degrees of "surface contraction" due to the acto-myosin cortical network: surface contractility is minimal for homotypic cell contacts, higher for contacts between different cell types, and reaches its highest value when cells are in contact with the medium (*e.g.*, the extracellular matrix in *in vivo* experiments). However, the DSCH theory has been also criticized for not being able to explain some of the actual liquid-like behavior of tissues [107,108]. Later, the balance of surface contractility and adhesion activity was taken into account by means of the "Differential Interfacial Tension Hypothesis" (DITH). This model was first initiated by Brodland and Chen using computational work to improve the flaws of the DAH approach [109] and then supported by an analytical framework that showed that the DITH theory captures the self-rearrangement of cells and tissues comprehensively [110]. Based on this theory, cell sorting process is controlled by mechanical tension which depends on both adhesion and cortical activity (Box 2). Some of the evidence that support the DITH model comes from the aforementioned studies about boundaries in the wing imaginal disc of *Drosophila*. Thus, it has been shown that cells at the interface of different cellular populations are enriched with contractile acto-myosin cables that increase tension and confer mechanical stability [16,71,92]. Moreover, the experimental studies about the germ-layer organization in Zebrafish by Krieg *et al.* showed that the differential adhesion is not enough to explain the cell sorting, and that cortical activity plays an important role [111]. Further, the interplay between contractility and adhesion terms was also studied theoretically by Manning *et al.*, where they provided a minimal model to estimate the ratio between the adhesion and cortical forces that determine, effectively, the tissue surface tension that in turns drives cell sorting [112]. Nonetheless, it is important to notice that when it comes to molecular effectors, a distinction between adhesion and contractility can be problematic: mechanical tension results from adhesion and contractile forces that feedback each other and, consequently, to separate their individual contributions is a convoluted problem [16]. Finally, a number of studies suggest that cortical activity plays the most important role for controlling the cell-cell contact area and that adhesion plays a supporting, indirect, role [113–117]. However, the DITH theory has been also recently questioned. Thus, Yanagida and coworkers, by investigating the segregation of the primitive endoderm from the epiblast have suggested that differences in mechanical properties are not enough to explain phase separation and that surface fluctuations, *i.e.* noise, drive cell sorting as it occurs in colloidal mixtures [118].

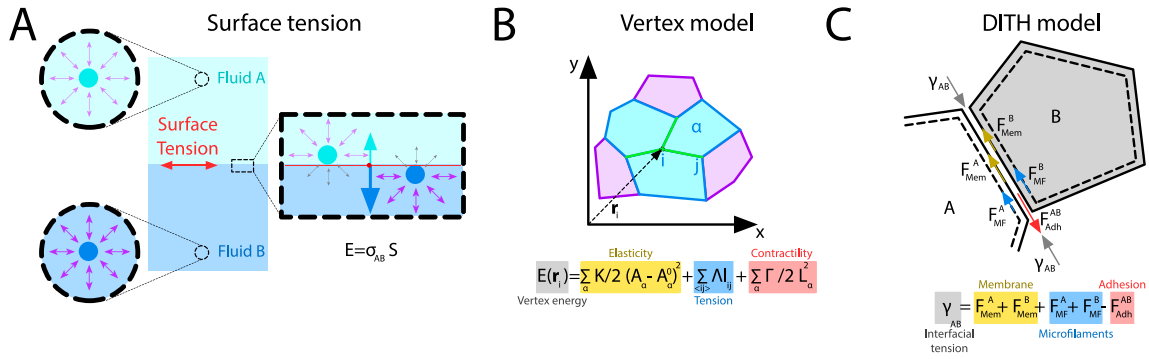
Recent contributions have also explored the problem of cell segregation in a 3D context. On the one hand, it has been shown that in small multi-cellular aggregates, with sizes similar to those seen in early development, sorting can occur only if there are relatively large differences in interfacial tension, very large differences in adhesion, or both, between different cell types [119]. This could actually explain, the need of additional mechanisms as mentioned above [118]. On the other hand, previous studies had showed, by testing different tissues, that the cellular tension is remarkably constant when it is assumed that its value is independent of cell shape [120]. However, Sahu *et al.* have concluded that the interfacial tension largely affects the cellular geometry which they claimed could be useful to detect tumor invasiveness [85]. Additionally, there has been inspiring work in the *Caenorhabditis elegans* model showing how packing, cell sorting, and mechanics are linked. Thus, embryo patterning is achieved by cell sorting and linked with cell fate specification (a mechanism known as "cell focusing") [121,122]. Then, cell identity changes promote the positional rerouting of cells following a cell sorting mechanism that relies on mechanical properties that ultimately affects cell packing and body shape [123–125]. Altogether, the mechanical drivers and mechanisms underlying the self-organization phenomena *via* cell sorting have shed light on morphogenetic processes but are still under debate more than sixty years after the seminal work of Townes and Holtfreter. As shown below, a similarly long time span has last since the original discovery of another self-organizing mechanism until its recent application in the context of developmental mechanobiology: excitable traveling waves.

4. Excitable traveling waves

4.1. Self-organization and dissipative structures

The existence of self-organization mechanisms driven by excitable traveling waves can be traced back to the early fifties of the 20th century due to the work of B.P. Belousov [126]. Belousov was trying to produce an inorganic version of the Krebs cycle and, to his surprised, he observed the spontaneous generation of chemical oscillations [127,128]. Unfortunately, Belousov's work was rejected by several journals and the results were even claimed to be impossible from a thermodynamics perspective [129]. The (false) argument used against Belusov reads as follows. The entropic arrow of time states that the total entropy must always increase, $\dot{S}_T > 0$, and given the conditions of the experiments (constant pressure and temperature) this implies that the so-called free energy must always decrease in any spontaneous process. However, the phenomenon reported by Belousov indicated an oscillatory behavior of the free energy and, consequently, it was deemed to be impossible. It

Box 2



Mechanical concepts. A: Surface tension arises from unbalanced molecular forces in immiscible fluids. Away from the interface between fluids *A* and *B*, cohesive forces (purple arrows) are isotropic. As a result, the resulting force acting upon molecules is, in average, null. However, at the interface, cohesive forces become unbalanced by adhesive forces (small grey arrows). In this example, cohesive forces dominate over adhesion forces in both fluids and inward (with respect to each fluid) forces, *i.e.*, pressure terms, develop that aim at reducing the interface area. At equilibrium these effective pressure terms are compensated by the internal pressure of the fluids. Also, acting forces at the interface imply the existence of a tension (force) that resist deformation: surface tension. The so-called Young-Laplace formula relates the geometry of the interface with the acting pressure gradient and the surface tension, σ_{AB}, between fluids *A* and *B* [6]. The surface tension then accounts for the force per unit of length acting at the interface and defines the surface energy as $E = \sigma_{AB}S$, where *S* is the interfacial area. Energy minimization requires minimization of *S*. **B:** The **vertex model** prescribes an energy to each of the vertexes that define the geometry of cells and has been typically used to model epithelia. In this example, the energy of vertex *i* located at position $\mathbf{r}_i$ combines the elastic energy of all cells, α, sharing vertex *i* (*i.e.*, blue cells), the (line) tension energy of all edges sharing that same vertex (*i.e.*, green segments), and the energy due to the cortex activity of cells α. The elastic energy consider cells as 2D elastic objects ("springs") with area A_α that aims at approaching the target area A_α^0 (*K* being proportional to the Young modulus). The line tension energy is the 1D equivalent of the surface energy, where Λ is the tension between cells and l_{ij} is the length of the edge connecting vertexes *i* and *j*. Finally, the contractility energy mimics the effect of the acto-myosin contractile ring and aims at minimizing the cellular perimeter, L_α (where Γ is the energy per unit of area of the ring). **C:** The **DITH** model relates the effective **interfacial tension** between cells as a result of the combination of other acting forces. Thus, in the absence of other force terms, the membrane (Mem) and microfilament (MF) forces acting of both cells, *A* and *B*, together with the adhesion (Adh) force determine the interfacial tension, γ_{AB}. Adhesion has a negative sign since it contributes to increase the interfacial edge between cells.

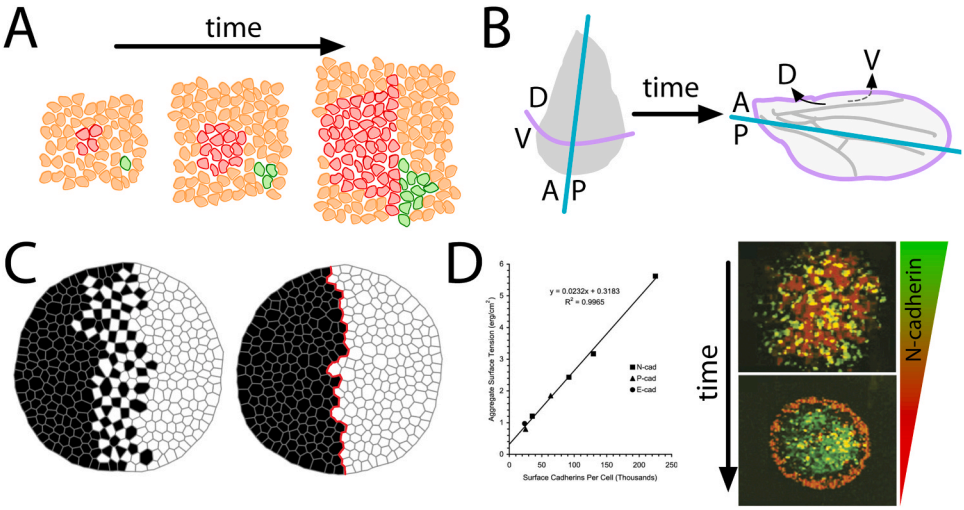


Fig. 2. Cell sorting: boundaries and tension. **A:** During tissue growth, as cells proliferate, lineage boundaries indicate the existence of mechanical barriers that help to segregate cell populations. **B:** Segregated cell populations of primordia (Left) lead to distinct parts of tissues/organs in the adult organism (Right). In this cartoon the *Drosophila* third instar larva wing imaginal disc (Left) is compartmentalized in the dorsal (D), ventral (V), anterior (A), and posterior (P) cellular regions that determine the corresponding regions of the prospective, adult, wing blade (Right). **C:** Numerical simulations (vertex model approach) support the differential adhesion hypothesis and show that line tension (adhesion) can drive either cell intermingling (Left) or cellular segregation (Right) [31]. **D:** The differential adhesion hypothesis (DAH) pictures cell aggregates as liquid-like mixtures where mixing or phase separation is driven by surface tension. (Left) Experiments reveal a linear relation between cadherins concentration and the value of the surface tension of cell aggregates. (Right) Mixtures of cells with distinct cadherin expression levels segregate as time progresses and, as expected by the DAH theory, the cell population with lower surface tension engulfes that with higher levels of cadherins, *i.e.* higher surface tension (adapted from [93]).

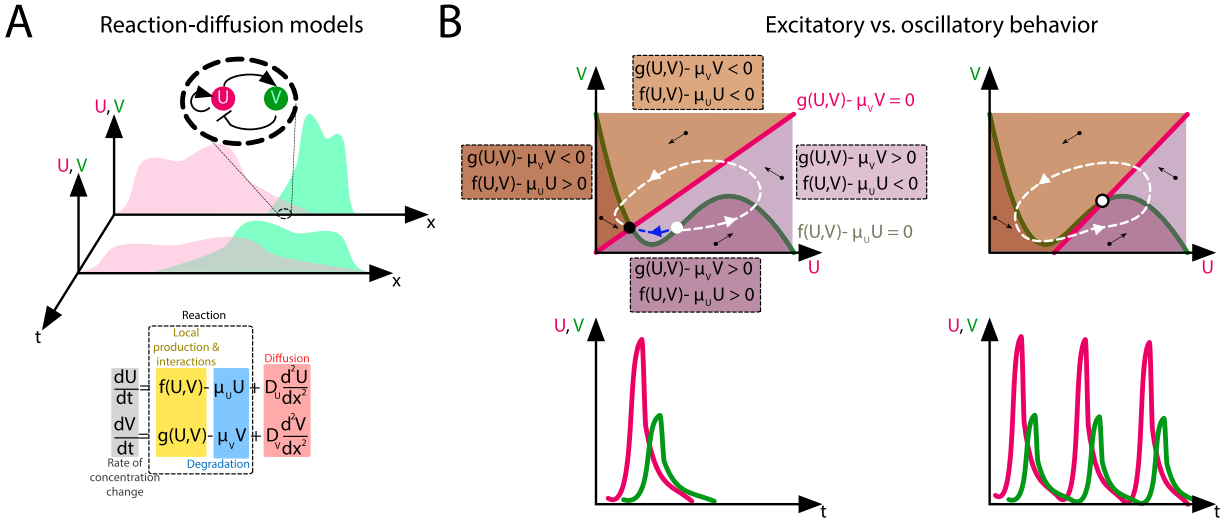
took the efforts of A. Zhabotinsky and co-workers to show that Belousov's results were veracious. Importantly, Zhabotinsky revealed that the system displayed not just a temporal oscillatory behavior, but also spatial self-organization through the formation of traveling waves [127, 128] (Fig. 3A). These discoveries eventually led to a growing interest on the so-called Belousov-Zhabotinsky (BZ) reaction. In particular it captured the attention of biologists such as A. Winfree and in the issue of the 11th of February of 1972 the cover of *Science* featured a photo of the traveling wave patterns of the BZ reaction [130] (Fig. 3A). And yet, what about the thermodynamic arguments used to critizise Belousov's work?

The answer to the apparent dilemma relies on the studies of I. Prigogine and co-workers on systems far from equilibrium. This latter concept refers to systems that are driven away from their equilibrium state due to the input/exchange of energy (and/or matter) with the surrounding environment. This is obviously a framework more adequate to describe life than classical thermodynamics, hence its importance. Prigogine showed that in these systems, the energy dissipated to the environment could lead to self-organization phenomena: a negative local entropy production, $\dot{S}_\Omega < 0$, that produces order, spontaneously, in the system could be compensated by the entropy production in the surrounding environment, $\dot{S}_E > 0$, such that thermodynamics was not contradicted: $\dot{S}_T = \dot{S}_\Omega + \dot{S}_E > 0$ [131,132]. By his works on self-organization in dissipative structures and non-equilibrium thermodynamics Prigogine was awarded in 1977 with the Nobel prize in Chemistry. Crucially, he laid the foundation for understanding why self-organization processes are even possible during morphogenesis from an energetics standpoint. Unfortunately, Belousov never witnessed the success of his pioneering work: it was only after his death that the scientific community began to recognize the utter importance of his studies [126]. We stress that the specific chemistry of the BZ reaction has not been found, to the best of our knowledge, in any biological system. However, BZ-like spiral patterning has been found repeatedly in living systems, including the aggregation pattern of *Dictyostelium*, the Calcium waves formed in the frog oocyte, the activity of pacemaker cells and, more recently, it has been suggested that spontaneous brain activity follows a BZ-like pattern [127,133]. In fact, as we revealed below, excitable traveling waves are ubiquitous in biology from intracellular processes to ecology [128].

4.2. *Traveling waves regulation in development: from ionic transport to mechanical inputs*

The reaction-diffusion theory provides a framework to understand the origin and propagation of excitable traveling waves (Box 3) but does not hint at the communication mechanism driving this self-organization phenomenon. In the particular context of development, recent progress has been achieved in the understanding of how excitable waves regulated by ionic transport control morphogenetic signals. For example, K^+ channels display excitable dynamics and regulate the release of Dpp in the fly wing primordium thereby controlling the temporal pattern of this important morphogen [137,138]. Ca^{2+} ions also regulate the activity of voltage-gated channels implicated on developmental processes driven by the excitable dynamics of cells. Thus, Huang and co-workers have recently showed that in *Drosophila* the Dpp signaling was compromised when Ca^{2+} channels were inhibited [139]. Additional evidence along those lines comes from studies about the role of Ca^{2+} signaling for regulating Rab5 (a conserved GTPase that regulates endocytosis and acts as a tumor-suppressor protein in the imaginal epithelium of *Drosophila* [140]) [141] or for modulating the bone morphogenetic protein (BMP)

Box 3



Dynamical Systems concepts. A: The **reaction-diffusion** formalism models the behavior of species that depend on space and time. In this example, species U and V depend on a single spatial coordinate, x, and on time, t. As time progresses, the concentration profile changes due to reaction and diffusivity: reaction terms account for the *local* interactions among species and their degradation, and diffusion terms account for their transport. **B: Excitatory *vs.* oscillatory behavior**: a primer on dynamical systems. Given species U and V, the reaction terms dictate the local increase or decrease. *e.g.*, if $f(U,V) - \mu_U U > 0$ then $dU/dt > 0$, *i.e.*, the concentration of U grows with time. By the same token, the condition $f(U,V) - \mu_U U = 0$ determines the set of values of U and V that do not contribute to the change of the concentration of U, the so-called nullcline. In these examples the nullclines for U and V are plotted in the phase-space $U - V$ (thick lines). The stationary states are determined by the points where nullclines cross (black circles). Nullclines compartmentalize the phase-space into regions with distinct behaviors in terms of the growth of U and V and indicate how the system evolves in those regions as schematically indicated by the arrows. *e.g.* in the top region of the phase-space plots both species, U and V, decrease their concentration with time. (Left) In this example, if the system is at the (stable) stationary state and their concentration are changed by a perturbation, two possible behaviors are possible. On the one hand, if the perturbation is small (to the left of the white circle), then systems returns rapidly to the steady state (blue trajectory). On the other hand, if the perturbation is large (to the right of the white circle), the dynamics to go back to the steady state requires a long excursion through the phase-space (white trajectory). As shown at the bottom, these long excursions appear a function of time as peaks of activity and the system is referred to as being "excitable": if a perturbation is large enough, it becomes transiently amplified. (Right) In this case the steady state (open circle) is unstable and trajectories always escape "away" from it. Due to the structure of the phase-space these trajectories are orbits around the steady state. As a function of time (bottom) the behavior of U and V is oscillatory. When combined with diffusivity effects, excitatory or oscillatory dynamics can lead to spatial **traveling waves**.

signaling in *Xenopus* [142]. Ionic excitatory waves have been shown to play also a role during tissue differentiation. In this context, Belgacem and Borodinsky have shown the existence of an interplay between the morphogenetic signal Shh and the dynamics of Ca^{2+} [143]. In addition, Calcium waves have been shown to interact with Shh pathway coordinating mesenchymal cell movements during development [144]. In particular, Li and co-workers recently revealed that Shh-responsive mesenchymal cells display synchronized Ca^{2+} oscillations during feather bud elongation in chicken. Remarkably, this study suggests a role for excitatory waves in the regulation of the cellular adhesion and communication machinery thus hinting to the existence of ion-based mechanochemical interactions. The latter has been also indirectly suggested in wound-healing assays where it has been recently shown that actomyosin cables display high levels of reactive oxygen species that in turn depend on Ca^{2+} activity waves [145].

Excitatory dynamics and traveling waves also appear in development independently of ion-based responses. A recent example is the reported proneural wave in the *Drosophila* optic lobe [146]. Namely, during the development of the *Drosophila* brain, a traveling wave of proneural gene expression initiates neurogenesis and drives the transition of neuroepithelial cells into neuroblasts. This wave is driven by an excitable reaction-diffusion system due to the diffusive transport of the epidermal growth factor receptor (EGFR). Notably, numerous examples of an excitatory dynamics that results in the formation of traveling waves come from studies about the collective cell migration during wound healing where mechanical responses are key. Thus, Serra-Picamal and colleagues showed that traveling mechanical waves associated with the cellular migration velocity and the propagation of the strain rate appear in epithelial monolayers [134,147] (Fig. 3B). Interestingly, this study suggests that these mechanical waves depend on a stiffening-fluidization dynamics of cells and might trigger mechanotransduction pathways during wound healing, morphogenesis, and the collective cellular invasion in cancer. In that direction, recent results indicate that force transmission at cell-cell junctions and cell polarity are crucial for driving a collective dynamics in epithelia and that this phenomenon is orchestrated by the mechanotransduction of Yes-associated protein (YAP) activity [148] (see also [149]). Nonetheless, while the existence of traveling mechanical waves during cell migration has been proved in different studies, there is still an active debate on the field about the more appropriate formalism to describe, and consequently finally understand, the origin of this propagating excitations [150]. Further insight has been obtained in a system that has captured the attention of researchers during the last decade: the ERK pathway.

4.3. *The ERK pathway: mechanochemical excitation waves during development*

The ERK (extracellular signal regulated kinase) pathway, aka ERK/MAPK (mitogen activated protein kinase) pathway, transduces an extracellular signal (growth factors levels, such as EGF), into phosphorylation levels of ERK (Fig. 3C). The phosphorylation of ERK turns on its kinase activity and phosphorylates a number of downstream targets (transcription factors) that regulate key cellular processes such as proliferation, differentiation, and the response to cellular stress. Malfunctioning of the ERK pathway plays an all-important role during tumorigenesis [151].

The ERK pathway displays a rich dynamical behavior that includes ultra-sensitivity, bistability, and oscillations [152]. More importantly, during the last decade it has revealed as a pivotal pathway to understand mechanochemical feedbacks. Thus, it has been shown that ERK is

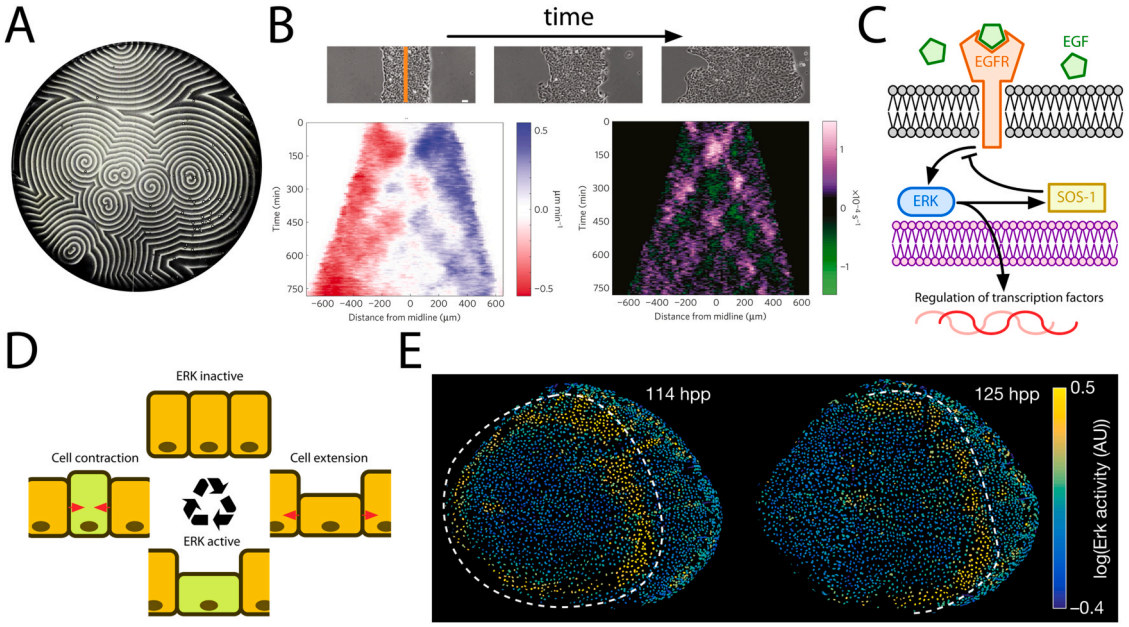


Fig. 3. Traveling waves: from chemical systems to mechabiology. A: The so-called BZ reaction was the first observation of traveling waves in a chemical system. The pattern arises from an excitatory dynamics phenomenon that propagates through the media (adapted from [130]). **B:** (Top) Time-lapse of a epithelial monolayer expansion experiment. The orange line in the first frame indicates the tissue midline. (Bottom) Kinematic and mechanical waves develop as the tissue migrates as revealed by the expansion velocity quantification (Left) and the rate of cellular deformation, *i.e.* the strain rate (Right) (adapted from [134]). **C:** Schematic representation of the main signaling features of the ERK pathway. Upon binding with external signals (*e.g.*, EGF), the membrane receptor triggers a number of biochemical and phosphorilation steps that end into the activation of ERK. The cytoplasmatic activity of ERK includes the activation of the repressor of the pathway (SOS-1). The nuclear activity of ERK includes the regulation of transcription factors that control multiple processes such as the cellular mechanical activity. **D:** Mechanochemical feedback of ERK activity: cell extension triggers ERK activation that in turn drives cell contraction. As a result fronts of mechanochemical activity develop in the form of traveling waves that instruct, among other processes, cell polarization and tissue expansion [135]. E(a) The pattern arises from an excitatory dynamics phenomenon that propagates through the media (adapted from [130]). (b) (Bottom) Kinematic and mechanical waves develop as the tissue migrates as revealed by the expansion velocity quantification (Left) and the rate of cellular deformation, i.e. the strain rate (Right) (adapted from [134]). (c) **E**: ERK activity during osteoblast regeneration: ERK activity propagates as excitable waves and control regeneration speed (adapted from [136]).

activated upon mechanical stress (*e.g.* tissue stretching triggers ERK activation) and, complementary, that ERK activation induces cellular mechanical activity (*e.g.*, ERK activity drives cellular contraction) [135, 153–156] (Fig. 3C). ERK has been shown to be an instructive signal to drive collective cell migration during wound healing and tissue expansion [135,136,153,156], it is a driver of morphogenetic shape changes such as invagination [157], regulates tissue homeostasis and stress responses [158], it is involved in the differentiation and maintenance of embryonic stem cells [159,160], and it coordinates cells during tissue growth and regeneration [136].

Importantly, these developmental functionalities at the collective cell level are achieved by mechanochemical waves. In the case of tissues with free edges (*e.g.* wound healing), the directionality of the waves is correlated with that of the expansion of the tissue (the tissue moving in the opposite direction of the ERK waves) [135]. Intriguingly, the opposite directionality has been recently reported during tissue regeneration [136]. Interestingly, in the case of confluent epithelial sheets where no expansion of the tissue is possible, the directionality of ERK waves is random [154]. Different mathematical models have been proposed to understand the generation of ERK waves and the interplay between signaling and mechanics. Hino *et al.* coupled phenomenologically the growth of ERK activity and cell area strain and the rate of change of cellular length (contraction) to ERK activity [135]. As for mechanics, the polarity force associated with tissue expansion is driven by ERK activity levels through cell contractility levels. Another noteworthy study is that of Boocock and coworkers that aims at deriving a mechanobiology theory of ERK wave propagation from first principles and supported by experiments [154]. They also analyze different effects and alternative options in order to understand ERK biomechanical activity under different situations. These two modeling attempts do not rely on diffusive signals and ERK signaling is considered to be intracellular. Thus, intercellular coupling is driven by mechanical interactions. In particular, Boocock and coworkers analyzed theoretically the possible effects of effective ERK diffusive signals and concluded that this"would have only very weak effects on the instability and pattern formation" [154]. Moreover, the proposed intracellular signaling of dynamics of ERK is not excitable. However, recent studies have proposed a more detailed modeling approach aimed at describing ERK signaling mechanisms that rely on an activator-inhibitor dynamics (backed up by experimental work) where an effective diffusive transport mechanism is required [136]. When coupled with a model of the mechanical mechanism acting upon ERK signaling, this model leads to excitatory traveling waves that direct and coordinate the spatio-temporal growth of osteoblasts and instruct localized tissue growth [136] (Fig. 3D). To challenge their model and reveal the underlying nature of ERK waves, *i.e.* phase *versus* traveling, they performed laser ablation and observed that ERK waves follows indeed an excitable dynamics and do not overcome obstacles. In agreement with other studies the study revealed that ERK activation relies on a feedback between growth and mechanical stress.

5. Discussion

Body structures display heterogeneous shapes and organizational traits depending on the nature of their cellular components. We find multilayer or monolayer packed tissues, flatten or curved surfaces tiled by cells, glandular tissues with cylindrical or spherical-like shapes, mixtures of different specialized cells types in compact or soft structures, or complicated synaptic networks of nervous cells in confined spaces. However, within all this diversity we also find unifying principles. In this review we have shown some examples where tissue organization, at different levels, can be described under the common umbrella of self-organization. The latter is an all-important component of the developmental plan by linking cellular packing, fate, communication, and functionality with mechanics. In this context, epithelia constitute the paradigm of tissues that are amenable to be described by different theoretical approaches from Topology to Dynamical Systems.

Here we have shown that, in spite of organ specialization, biological function, or even the 3D spatial organization, the particular characteristics of epithelial cells make feasible their analysis as living-matter tessellations. This is partly due to the geometrical "simplicity" of epithelial cells that allows for reductionist approaches in terms of their shapes: polygons, prisms, and, more recently, scutoids. In this regard, the scutoidal shape illustrates that the packing topology relies on the geometrical constraints of tissues. Namely, the optimal cellular packing of epithelial monolayers depends on the anisotropy of the local curvature, *i.e.*, on the level of deformation with respect to a planar geometry. Moreover, besides the topological and geometrical analyses, the biophysical features underlying tissue organization has been well characterized in monolayer epithelia with the help of simulation tools, such as the vertex model, that link cellular packing an mechanical properties. Current challenges of the field include the possibility of using similar tessellation and biophysical tools to understand self-organization in multilayer tissues [161]. In that regard, the solution could come from the implementation of Voronoi approaches to simulate the globular-like shapes of these cells (similar to complex polyhedra such as the tetradecahedrum). In that case, by following a similar methodological pipeline that in 2D, the Voronoi approach could be used to extract geometrical and connectivity information from which inferring tissue mechanics. As a matter of fact, we have reported here on the success of applying 3D Voronoi tessellations to develop bone bioimplants [42,43] and there are promising possibilities to adapt this theoretical approach to other cellular structures [162]. For example, it could be used to study a prominent feature of astrocyte tissue organization that tile the entire brain parenchyma conforming a 3D tessellation [163–165].

As reviewed here, tissue organization implies not just the ability to pack cells but also the possibility of segregate different cellular populations. The self-organizing compartmentalization properties of tissues are also driven by mechanical interactions that are transduced by feedbacks between the activity of biomolecules (*e.g.*, cadherins) and the material-like characteristics of cells. In that regard, while lot of progress has been achieved to understand the process of cell sorting, more work is still required to elucidate the details and the consequences of the aforementioned feedbacks. On that matter, one of the main challenges is to *quantitatively* determine the role played by different biomolecules and the mechanosignaling properties of their pathways. Also, while differences in cellular mechanical properties is the more widely supported mechanism of cell sorting, there are still open questions about its applicability *in vivo*. For example, the majority of controlled experiments performed to understand the cell sorting mechanism have been carried out *in vitro* by mixing together cells with distinct fates; something that does not happen *in vivo* often [13]. Moreover, the proposed theoretical mechanisms usually rely on large differences in adhesion and/or contractility; something that may not be supported in some morphogenetic processes [166]. Boundary formation/maintenance seems to be the more promising *in vivo* problem to shed light on these questions [92, 166–171]. Another important point to consider is, as in the case of cell packing, how the 3D conformation of cells affects the proposed mechanisms of cell sorting. Again, developing 3D computational models would be of great help to test hypotheses in that regard. For example, Revell *et al.* have recently developed a 3D local force based simulation to study the process of cell sorting and evaluate the relative weight of contractility *vs.* adhesion forces [119].

Finally, as for the mechanism of self-organization through traveling waves, we have shown that the reaction-diffusion formalism can explain how local excitability can be propagated at the tissue level scale by means of either diffusive signal or mechanical coupling among cells. These waves can ultimately coordinate tissue growth and other biological responses and in some cases, such as the ERK pathway, involve mechanochemical feedbacks [32]. Interestingly, it has been shown that ERK activity relies on Ca^{2+} signaling that in turn depends on the cellular stretching level that stimulates cell proliferation [172]. This fact makes

possible to connect some of the different signaling mechanisms reviewed in the context of excitable dynamics and, additionally, raises intriguing questions. In particular, cellular growth implies the existence of another time scale (the cell cycle duration) that, at least in some of the reported cases *e.g.*, [136], is of the same order of magnitude that the refractory period of the propagating traveling waves, and also induces protein dilution effects. How these effects compete and/or collaborate, to regulate the mechanochemical activity of ERK waves is an open question. Further, one of the challenges of the field is to understand how ERK waves induces complex morphogenetic activity, for example, epithelia invagination [157]. Finally, the propagation of traveling mechanochemical waves in 3D cellular environments awaits to be explored and characterized. As beautifully summarized by Hannezo and Heisenberg, *"how such mechanochemical pulses and waves of signaling pathway activation translate into cellular responses has only began to be elucidated"* [32].

Altogether, we, as many others, envision that the understanding of self-organization in developmental systems will open incredible opportunities to engineer both inorganic and organic materials in the future [173,174]. To that end, quantitative approaches from different fields able to integrate and characterize the structure, the mechanics, and the dynamical behavior at different biological spatio-temporal scales are clearly needed. Herein, we have reviewed some of these phenomena and theoretical frameworks and, undoubtedly, the forthcoming years will bring to light new and promising progress in the field.

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