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Mechanobiology and morphogenesis in living matter: a survey

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Mechanobiology and morphogenesis in living matter: a survey

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Abstract Morphogenesis in living tissues is the paramount example of a time- and space-dependent orchestration of living matter where shape and order emerge from undifferentiated initial conditions. The genes encode the protein expression that eventually drives the emergence of the phenotype, while energy supply and cell-to-cell communication mechanisms are necessary to such a process. The overall control of the system likely exploits the laws of chemistry and physics through robust and universal processes. Even if the identification of the communication mechanisms is a question of fundamental nature, a long-standing investigation settled in the realm of chemical factors only (also known as *morphogens*) faces a number of apparently unsolvable questions. In this paper, we investigate at what extent mechanical forces, alone or through their biological feedbacks, can direct some basic aspects of morphogenesis in development biology. In this branch of *mechanobiology*, we discuss the typical rheological regimes of soft living matter and the related forces, providing a survey on how local mechanical feedbacks can control global size or even gene expression. We finally highlight the pivotal role of nonlinear mechanics to explain the emergence of complex shapes in living matter.

Keywords Morphogenesis · Developmental biology · Mechanobiology · Elasticity

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1 Introduction

Imagine that you trace by an unbiased eye in time-lapse regime the development of any embryo. First of all, you will notice a plethora of extensive deformations and mutual shifts of its constituent parts, starting from large groups of cells up to so-called supramolecular structures, all of them together covering roughly the $10^{-3} - 10^{-6}$ m dimensional range. So far as any motion and deformation require mechanical forces, it is but natural to ascribe all these processes to the realm of mechanics and to conclude hence that the latter should lie in a very heart of embryology. However along centuries such a conclusion has been shared only by few outstanding scientists, such as His [51] and Thompson [88], although they were qualified as marginal by the mainstream community. The dominating view was that although the developmental importance of mechanical events can not be rejected in any case, these observations were no more than *epiphenomenae* (blind end results) of more deeply hidden controlling agents. At lower scales, they have nothing to do with mechanics but should be treated instead in terms of classical chemistry, such as a finely regulated production and diffusion of specific macromolecules.

This approach, even if promoted a number of important discoveries (mostly centered around the action of the so called embryonic inductors), has led to almost disregard several fundamental aspects of development. To these belongs, first of all, the entire problem of shapes formation (morphogenesis) including its holistic control,

vividly expressed by a miraculous capacity of eggs and embryos to produce normally structured organisms of different dimensions from their isolated parts or randomly mixed cells (so called “embryonic scaling”). Even most refined self-organization models, if based exclusively on such chemical notions as synthesis, inhibition and diffusion of some specific substances [70], cannot interpret these properties without additional *ad hoc* introduced assumptions. It is the proper time to ask whether the mechanics can be here of any help by suggesting some new feedback contours able to control morphogenesis.

The first section of this work is a critical review of recent discoveries in developmental biology: the importance of physical loads in the emergence of a macroscopic shape is highlighted from local mechano-transduction mechanisms at the cellular level. In the second section, we illustrate the conceptual difficulties that characterize the identification of the mechanisms of size control in embryogenesis. Even in simple system models, a mechanical feedback on the reaction-diffusion dynamics of morphogens seems to cooperate to provide a local control of global size and some arguments even support the hypothesis that stress itself conveys information on size. Finally, we review the theoretical bases of the mechanical modelling of morphogenesis and mechano-biology, highlighting not only the pivotal role of nonlinear mechanics for proving novel biological insights but also the many original advances in mechanical modeling triggered by biological questions.

2 The role of mechanics in developmental biology

2.1 Mechanical control of morphogenesis

The first unambiguous experimental demonstration of a mechanical control of morphogenesis-like events was given by Harris et al. [49]. The authors seeded fibroblasts onto a silicon substrate, whose compliance allowed the crawling cells to provoke a deformation along distances much exceeding the individual cells diameters. Under these conditions the cells, instead of spreading homogeneously along the entire substrate (as they did when cultivated onto a rigid surface) created a lattice consisting of regularly arranged clusters connected by thin files of extensively elongated cells. That means that cells were self-organized into definite patterns with new (not existing beforehand) characteristic linear dimensions of inter-cluster distances. To describe mathematically these results, the authors borrowed from chemokinetic models the idea of short-range activation and long-range inhibition but decoupled it from a chemical con-

text. Rather, they linked the short-range activation with increase of cell-cell contact areas and long-range inhibition with cells-generated tensions stretching the substrate and preventing thus further enlargement of cell clusters. As a result, non-observable scalar values have been replaced by directly observed tensorial ones (deformations of cells and tensions of a substrate). This opened the way for modeling realistic three-dimensional shapes instead of their hypothetical chemical counterparts. For applying the same approach to entire embryos, the first thing to explore was whether their tissues were mechanically stressed and if yes, whether the stresses were of developmental importance. This work was started on amphibian embryos by Moscow University group (transformed later in the lab of Developmental Biophysics) in the seventies [12]. The stresses were detected by the emergence of rapid ($t < 1s$) tissue deformations (Fig. 1A, B) after strictly localized incisions performed under close to 0° C temperatures. This procedure avoids the development of an active cellular response after a certain lag period, keeping only quasi-elastic relaxations to highlight the presence of pre-existing stresses.

As a result, stage-specific tensile patterns have been revealed consisting of three main elements: surface areas stretched in regular directions and as a rule in the gradient fashion; files of tensed cells transpiercing embryonic bodies; tension nodules which join these two components [11]. The patterns remained topologically invariable during rather prolonged time periods, drastically changing in between. The changes were associated with morphogenetic movements which produced tissue deformations remarkably similar to the rapid post-incision ones, although if proceeding several time orders slower. Therefore, one may conclude that normal morphogenesis is going on in a much retarded rate along a relaxation pathway. Based upon these observations, a model of epithelial morphogenesis has been proposed [10] which, similar to most other models implied short-range activation and long-range inhibition. However, the activation was identified with a relay-like enlargement of cell-cell contact areas while the inhibition with the increase of tangential tensions caused just by this relay if the layers edges were fixed. The unique advantage of this model was in representing the above mentioned phenomenon of embryonic scaling without any additional assumptions.

2.2 Mechanical feedbacks and size effects

The next step was focused on exploring whether and in what way the relaxation and reorientation of the main tensions affects development. A simple method

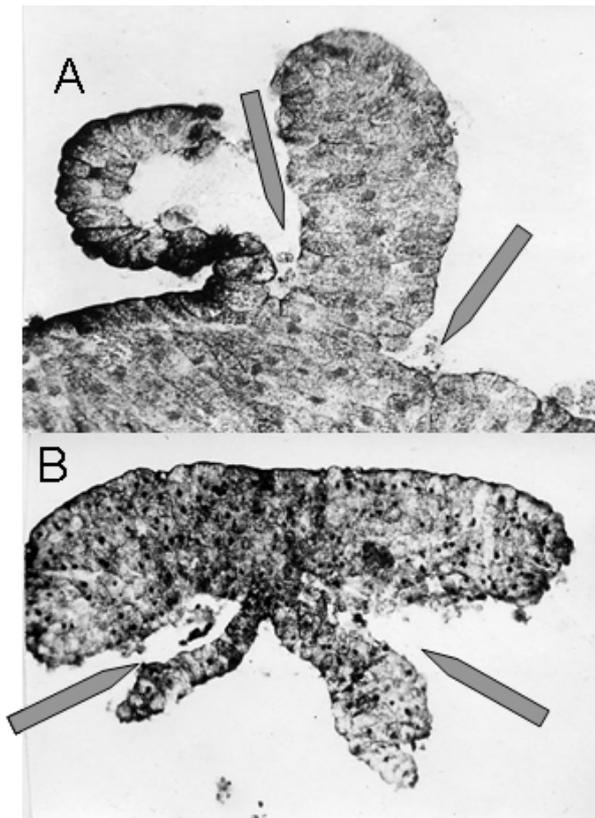


Fig. 1 Two examples of rapid ($t < 1s$) deformations following tissue incisions (wedges) of *Rana temporaria* tissues from late gastrula embryos. A: separated tissue of the outer (dark) and inner layers of neuroectoderm undergo same bending as intact tissues during neurulation. B: bending of incised parts of gastrocoel roof closely imitates normal morphogenetic movements during notochord formation. From [14, 16].

for relaxing tangential tensions on the embryonic surface was to insert a wedge of homologous tissue in the vegetal hemisphere of a blastula or early gastrula stage embryo (Fig.2A,D, pointers; see for details [13]). When performed during the blastula stage, such operation led to formation of abnormal protuberances (Fig.2B) and to a complete distortion of further development (Fig. 2C). If made on the early gastrula stage, similar relaxation led to abnormal extension and multiplication of the axial organs (Fig. 2E). Even if nothing more than a small region corresponding to the dorsal blastoporal lip was relaxed (outlined by bracket, Fig. 2F) the axial organs arrangement was completely disordered (Fig. 2G) and, most surprisingly, the entire shape of embryonic body largely deviated from amphibian phenotype towards that typical for Amniota embryos (Fig.2, cf F and H) [56]. This makes it plausible that, besides being a source of diffusible inductors, the dorsal blastoporal lip plays also a role of a main tensions nodule, controlling the entire body morphology.

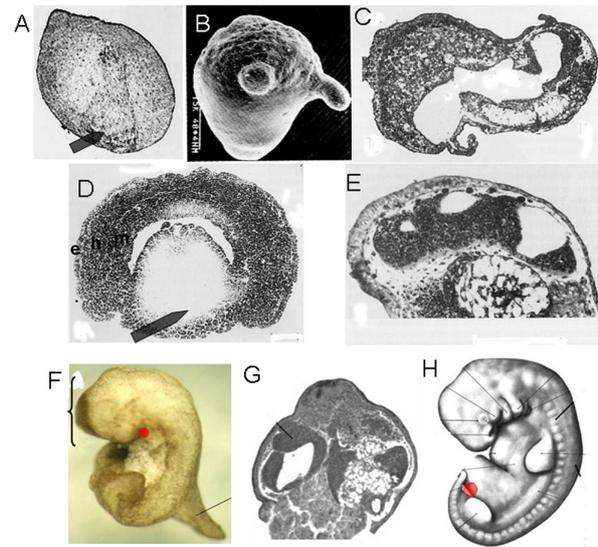


Fig. 2 Loss of morphological order and formation of abnormal phenotype as a result of experimental relaxation of tensile stresses on the surface of *Xenopus laevis* embryos. For detailed comments see text. A, B: wedge of a ventral tissue (pointers) inserted in a blastula stage embryo for relaxing surrounding tissues, 1h after operation. B, C, E: operated embryos, 48h after insertion. Note abnormal protuberances in B and a complete loss of order in C, E. F and G correspondingly: completely abnormal phenotype and chaotic arrangement of axial organs after a local relaxation of tensions on the dorsal lip surface of early gastrula *Xenopus* embryos (relaxed area is marked by brackets in F). H: text-book image of so-called pharyngula embryo similar to F but absent in normally developing amphibians and specific for other taxonomic group [13,56].

On the other hand, stretching by an external force induces or at least reorients a universal type of morphogenetic cell movements, so called “convergence–extension”, which elongates embryonic body by shifting its cells in convergent directions towards the elongation axis. A body of amphibian embryos consists of two parts so called suprablastoporal area (SBA) which displays autonomous convergence–extension elongating body in antero-posterior direction, and the ventral area (VA), normally incapable to these movements. It has been observed that within VA the convergence–extension movements could be initiated *de novo* by arbitrary oriented external force (Fig. 3B-D, cf A) and within SBA they could be reoriented perpendicularly to the normal ones (Fig. 3E-G) [15,90]. In the both cases most of explants took dumb-bell shapes (Fig.3B, D, G) and revealed extensive cell redistributions, pointing to active convergence–extension in the direction of applied force. This means that after the application of an external stretching force to a sample of embryonic tissue, its cells become rearranged in such a way that the sample becomes actively

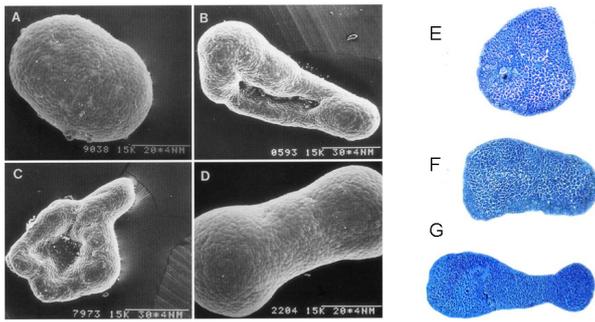


Fig. 3 Active response to external stretching in different explants of *Xenopus* embryonic tissues. A-D: ventral area, E-G: suprablastoporal area (SBA). A is non-stretched explant. B-D: explants stretched by outside applied forces produce various bulges, indicating generation of the active pressure forces which coincide with direction of stretch. E: a piece of SBA tissue before stretching; F: same piece immediately after the end of transverse stretching (which was oriented perpendicularly to normal elongation direction); G: same tissue piece after 3h incubation in a stretched state. Again, a dumb-bell shape indicates the action of a newly generated pressure force. From [15], and courtesy of A. Mansurov.

elongated in stretch direction, producing a certain pressure just in the direction of a previous stretching.

Another way to redistribute tensions was to bend forcibly tissue pieces, stretching thus their convex surfaces and compressing the concave ones. After stopping deformations, progressive deepening of just slightly outlined invaginations could be observed on the concave sides (Fig. 4 cf A, B and C, D; see for details [57, 59]). Common to all the above described reactions was the tendency not only to restore stress values perturbed during experimental interventions but to do it with certain overshoots. Indeed, as shown by morphometric measurements, the relaxation of tensions or slight compression triggered cells tangential contractions and endocytotic engulfment of cell membrane to the amount enough for *hyper-restoring* the initial tensions [56, 57, 59, 11]. Similarly, as shown in Fig. 3, the external stretching, by inducing cells convergence-extension, *hyper-restores* the initial state of a moderate tension by producing internal pressure, and thus reversing the sign of stresses. Examples of such reactions are numerous (reviewed in [11]). Within the tissues in which cells are close to immobile, the main tool for hyper-relaxing previously imposed tensions seem to be their mitotic divisions predominantly oriented along the axes of cell stretching [48].

Similar reactions, seemingly directed towards active hyper-restoration of the initial stress patterns, are taking place not only in time but also in space. Thus, in many cases tensile patterns are arranged within embryonic tissues in a gradient-like fashion. In order to keep the mechanical equilibrium, the cross-sections of

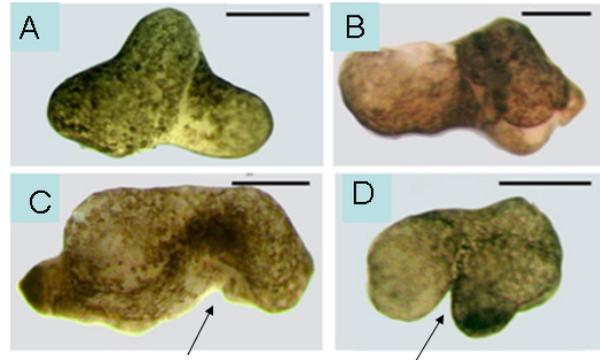


Fig. 4 The active increase of slightly imposed curvature in double explants of *Xenopus* SBA tissue. A, B: two explants 30min after preparation. C, D: same explants 4h later. The arrow points to the much increased invagination. Each vertical column displays same explants separated by several hours time interval. Bars = 100 μm .

the tensed cell files at the different levels of the gradient should be reversely proportional to this levels stresses: thus, cell files should be narrowed in the uphill direction of the gradient [23, 11, 42]. In these cases the cells or the particles of egg surface are always moving uphill the gradient [15], tending to smooth it out and even to reverse. A spectacular example is a behavior of a gastrula stage *Xenopus* embryo sucked by one of its ends into a pipette of a narrower diameter. For keeping mechanical equilibrium, tensile stresses in the sucked part should increase, creating thus a stress gradient going downhill to non-sucked part of embryo. Under these conditions, in spite of dropping down the sucking force up to zero (and even negative) value, the embryo continued to insert actively into a pipette up to complete penetration smoothing thus out the gradient [66]. Another example of the active responses to geometric constraints is a behavior of internally pressurized embryonic rudiments of a toroid or semi-toroid shapes (a circular blastoporal lip of many embryos is an instructive example). As known from mechanics, the meridional (circular) tensions on the surfaces of such bodies are twice as much as the equatorial ones. By a hyper-restoration principle, there are the first ones to be at first relaxed and then transformed into pressure stresses, molding from a torus a cucumber-like body elongated parallel to torus axis. This is just what is taking place during normal development of *Cnidarian* embryos [58] and in the isolated lips of amphibian blastopores [11].

2.3 Mechano-sensitivity of gene expression

These results show that, instead of passively responding to mechanical forces, embryonic tissues actively modulate both the forces' magnitude and their preferred di-

rections, in a way that would be impossible to achieve without affecting the molecular level events. Another group of evidences favoring this scenario was a discovery of mechano-sensitive regulatory pathways involved in genes expression. This line of investigations started from [43], reporting that a brief localized pressure applied to the stomodeum area of the mutant *Drosophila* embryos (unable to develop this rudiment), was sufficient to remove the developmental block by activating twist gene and possibly some others. The activation was achieved by stimulating a regulatory protein, β -catenin, otherwise stored in a subcortical depot, to be transported into cell nuclei. Next, Desprat et al. [34] showed that the deformation (indentation) of the mesodermal rudiment in *Drosophila* embryos lacking snail gene was enough to restore the normal mesoderm-forming capacities in these defective embryos. Addressing to the representatives of quite other phylum, zebrafish embryos, Brunet et al. [19] showed that if the expression of a gene *ntl*, a homolog of an early mesodermal marker brachyuri, was blocked by the arrest of epiboly movements, it could be still renewed if stretching the arrested cells by magnetic force. A key link stimulated by exogeneous mechanical forces appeared to be the same in all these events: this was phosphorylation of β -catenin by a tyrosine residue. As a result, the interactions between β -catenin and E-cadherines of cell-cell junctions were repressed, permitting thus β -catenin release from junctions to cell nuclei where it activates mesodermal genes. The role of tissue deformations in regulating genes expression was shown also in the above mentioned experiments by bending double explants (sandwiches) of SBA areas [57]. In these experiments a pan-neural gene, *Sox3*, was expressed in the concave (mostly compressed) area while muscle actin genes of the on the opposite stretched side (Fig.5, D,E). These patterns were in sharp contrast to the polar distribution of genes expression sites in straight explants (Fig.5A) and their random distribution in spherical ones (Fig. 5B, C).

Other promising trend was initiated by a discovery of mechanically prestressed state of cell nuclei [68, 22]. It is suggested that the effective shape of cell nuclei is based upon the balance of external forces exerted by microfilaments and microtubules and intrinsic forces caused by entropic nature of DNA polymers. That permits to regard the cell nucleus as a mechanosensor. The nucleus stiffness is the smallest and fluidity of lamin scaffold the greatest in non-differentiated cells as compared with those involved in differentiation. By specifying this suggestion, Chalut et al. [22] distinguishes a naïve, or totipotent state of stem cells from the primed, or pre-differentiation one. It is the latter period which

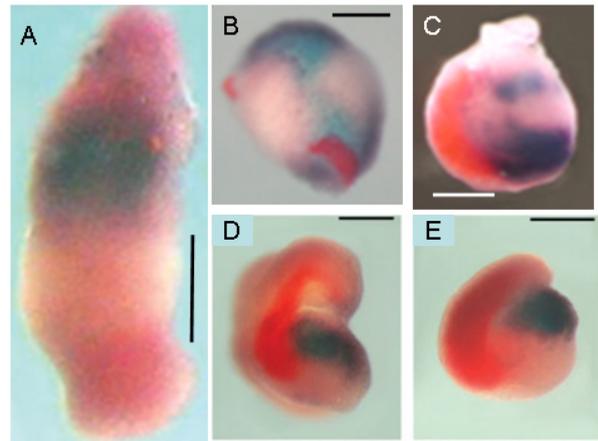


Fig. 5 Expression patterns of muscle actin gene (red) and panneural marker Sox3 (blue) in straight (A), spherical (B, C) and bent (D, E) explants. Bars = 100 μ m. For explanations see text.

corresponds to the maximally decondensed state of chromatin and the greatest softness of cell nuclei. Accordingly, the genome is maximally opened to the action of mechanical forces just in this state.

3 The puzzle of size control: lessons from simple systems

A specific area of morphogenesis where mechanics is expected to play a role is size control. In the section above we have learned that the tensional pattern can provide cells with mutual positional information, so that complex structures can be produced (like in vasculogenesis). However also a global control on the size of organs exists. In embryo development, cells stop proliferating at homeostasis, a target state that can account, at a macro level, for the shape and size of an organ. However, while control of mitosis is local, the spatial dimension of a tissue is a global information. How do single cells get aware of that at the same time? Which is their communication mechanism? While morphogen factors are demonstrated to play a key role in morphogenesis, and in particular for shape emergence, the possibility that they can produce a global control on size is controversial.

A fundamental contribution to the theory of morphogens is due to Lewis Wolpert [96], who proposed the "French flag model". The central element of this model is the spatial distribution of the concentration of specific substances in the tissue: it is detected by the cells which, according to specific thresholds, trigger the transcription of distinct sets of genes. According to this theory, there is a direct correlation between the input (the concentration level) and the output (the response

of the tissue): each threshold corresponds to the border of an expression territory. The first morphogen, the *bicoid*, was discovered in 1988 by Christiane Nüsslein–Vohard; after that, many others have been identified. It has been later demonstrated that cells do not only detect the magnitude, the intensity of the concentration of a morphogen, but they can also measure its local direction of variation; the gradient can provide instructions for polarization, migration, extension of filopodia and generation of complex structures [89]. Moreover, recent biological discoveries have also pointed out that physical and structural features inform cells about their proliferation ability through mechanical regulation of few known mediators, e.g. YAP and TAZ, of Hippo signaling and organ growth [64]. Their activity is indeed confined to cells subjected to mechanical cues, such as either local stretching/bending or a change of stiffness of the surrounding extracellular matrix [6].

To recast the idea of morphogenetic control in a formal way, we start considering a one-dimensional spatial domain $[0, L(t)]$ where a morphogen diffuses and degrades. We illustrate elementary arguments that could be specialized to a specific morphogenetic system [2]; as we are here mainly interested in methodological questions, we just capture the main ideas. The morphogen source is located in $x = 0$ where it is produced at a constant rate $\alpha > 0$, while it cannot flow at the right boundary $L(t)$. The qualitative description above rewrites in the following differential model, where $c(x, t)$ represents the morphogen concentration and $d(x, t)$ is a conjectured dilutant species. Without loss of generality, we take $d(x, t = 0) = 1$, so that:

$$-D \frac{\partial^2 c}{\partial x^2} = -kdc, \quad (1)$$

$$\frac{\partial c}{\partial x} \Big|_{x=0} = -\alpha, \quad \frac{\partial c}{\partial x} \Big|_{x=L} = 0 \quad (2)$$

$$(3)$$

The general solution of (1) is

$$c = c_0 \exp(-x/\lambda) + c_1 \exp(x/\lambda).$$

where $\lambda(t) = \sqrt{D/(d(t)k)}$. Enforcing the boundary conditions gives

$$c(x, t) = \alpha \lambda \frac{\cosh((x-L)/\lambda)}{\sinh(L/\lambda)} \quad (4)$$

The power and the limits of the theory of morphogens stems from analyzing the solution (4) in the standard case $d = 1$: the concentration field $c(x, t)$ decays with a characteristic decay length λ , independent on the domain size. The concentration of morphogen is therefore a measure of "how many λ 's" the boundary is far away and it can in principle provide the position for the

boundary of one or more regions of fixed size. However, the physical value of λ usually ranges few hundreds of microns, and for domain lengths $L \gg \lambda_0$,

$$c(x, t) \simeq \alpha \lambda \exp\left(\frac{-x}{\lambda}\right), \quad (5)$$

the morphogen concentration exponentially decays far from the source and becomes rapidly inappreciable. The theoretical difficulty could be overcome conjecturing the existence of an undegradable unidentified species $d(t)$ that simply dilutes with length

$$L^2 d = L_0^2 \quad (6)$$

but such a speculation is not yet supported by experiments. It follows that the concentration of morphogens can explain patterning in soft tissues of few λ 's, but its application to size control exhibits conceptual difficulties for large organs.

An alternative approach to explain the size control during growth via a limited proliferation is based on mechanobiology [53]. Cells produce forces and then displacement and flow thanks to two inner mechanisms: polymerization/depolymerization of the actin cytoskeleton (the main structural component of a cell) and the contraction of the actin network produced by the myosin motors. In mechanobiology it is therefore customary to distinguish between passive and active stress¹. The passive stress in the living matter is produced by traction at the boundary of the body and possibly residual stress due to geometrical incompatibility (see next section) when no internal forces are generated at a cellular level. A distinctive feature of mechanobiology is that the pure passive behavior can be in principle measured by standard techniques (nanoindentation, biaxial tests) after inhibition of the myosin activity and actin polymerization, while the active stress can never be measured *per se*. From an energetic point of view one could comment that passive stress can be dissipative or not, while the active one is always fully dissipative. The active stress can be modulated by the cells to control polarization, migration, patterning and duplication; it can depend on the stiffness of the environment and on the externally applied load, possibly interacting with the one the active matter produces [63]. Eventually, stress is a natural candidate to convey information on the size of the domain.

The first step to elucidate the possible role of mechanics in size control should be a clear statement of the rheology ("the constitutive laws") of the living matter. Despite its appealing simplicity, the idea that cellular

¹ Not to be confused with the distinction between active and reactive forces in classical mechanics

aggregates can be mechanically described as simple fluids (inviscid or viscous) with interface tension has been disregarded in recent years in the light of a number of experiments that elucidate the origin of the cell–cell stress in assemblies [75]. The force per unit surface in a cellular aggregate is not a simple hydrostatic pressure, it can be spatially inhomogeneous and it is produced by the interplay between cell–cell adhesion and their cortical actomyosin cables that contract according to specific directions. The perimetral anisotropic contraction is known to promote cell migration by intercalation mechanism. We are here interested in understanding if the active stress produced by the cells might also convey information about the domain size.

If the active stress is a key ingredient of the theory, it seems natural to add the active contribution to the passive behavior of the cell aggregate, seen as an inert material. This decoupling is in principle possible inhibiting the myosin activity in the cells and then performing standard stress-strain measures. However technical difficulties (cell aggregates are very soft) and the very complex rheological behavior of cells does not easily allow to draw a conclusion. Mechanically active cells in a monolayer exhibit a rheology that is more similar to a fluid rather than a solid; while the active stress generates cell flow, the tensional state is not trivially a pressure. Laser ablation techniques show that the stress is non spherical in the material, even at equilibrium [75]. Most likely, cell aggregates behave like a Bingham-type material: their tensional state depends on the strain above some tensional threshold, while they start flowing above such a yield stress [3, 93].

The simplest setting where mechanics might be tested as a possible long-range signal for size control is the case of a pointwise (singular) source. Here a difficulty analogous to the one illustrated for morphogenetic sources is encountered: in a three dimensional setting a pointwise force decays with the radial distance r like $1/r^2$ (take, for instance, the Kelvin solution in linear elasticity). This asymptotic behavior poorly supports the conjecture that the stress can convey information on size for two reasons: it vanishes fast (quadratically) with the distance and it involves no intrinsic length scale. Therefore the role of mechanics to disentangle puzzle of size control [94] should be investigated in more complex settings: when there is a cooperation with morphogens or in truly three dimensional problems, where the stress field *depends* on the size of the domain.

We formalize this idea with two elementary examples of finite elasticity reappraised in a “convey information” perspective; a much richer three-dimensional realm will be discussed in the next section. Consider first a bar of length $L(t)$, homogeneously loaded in one

direction only. The first evidence is that a simple load-stress mechanical theory is useless for the sake of size control: since the growth time scale is very long with respect to the relaxation times of the cellular material, viscosity plays no role, the tensional state in the body depends on the strain only and it is equal to the load (if any) on the boundary, whatever its rheological properties are. In other words, a homogeneous tensional field can not convey any information. The scenario does not dramatically change if a homogeneous active stress is introduced in the force balance: the tension will take a different value, but it remains size-independent.

A positional information based on the local strain can be instead obtained even for homogeneous materials when more complex geometries and loads are assumed. As a representative example, consider the classical Rivlin problem of a soft cylinder subject to a combined (homogeneous) finite axial stretch and torsion [73]. Assume that the deformation depends on the material cylindrical coordinates (R, Θ, Z) as follows

$$r = R/\sqrt{\lambda_z}, \quad \theta = \Theta + \gamma\lambda_z Z, \quad z = \lambda_z Z, \quad (7)$$

where γ is the torsion angle per unit length and λ_z is the uniform stretching ratio in the axial direction. The deformed configuration can be realized by application of a torque and an axial force only on the cylinder with unloaded lateral surface, whatever are the material constitutive properties, under the incompressibility constraint. The left Cauchy-Green deformation tensor \mathbf{C} takes the form

$$\mathbf{C} = \begin{pmatrix} \frac{1}{\lambda_z} & 0 & 0 \\ 0 & \frac{1}{\lambda_z} & r\gamma\sqrt{\lambda_z} \\ 0 & r\gamma\sqrt{\lambda_z} & \lambda_z^2(1 + r^2\gamma^2) \end{pmatrix} \quad (8)$$

We see that the strain (and then the stress) depend on the spatial radial coordinate r . It follows that, in principle, a local measure of a mechanical quantity (the strain) can provide positional information. In this case the C_{zr} component of the deformation is proportional to the radial position, but no information about the axial coordinate is provided. This kind of arguments could be extensively applied in embryology.

The role of mechanics, so obscure and promising in size control, is nevertheless much more established in morphogenesis, where in several systems it has been proved that the emergence of instabilities is the physical mechanism that nature uses to shape organs, as illustrated in the section below.

4 A continuum mechanics perspective for mechanobiology and morphoelasticity

The need to account for the peculiar characteristics of living matter versus inert materials has pushed towards both the refinement of classical mechanical models and the development of novel theoretical and numerical frameworks, such as [7, 50, 61]. Much interest in continuum mechanics has focused in modelling how soft living matter can either change its microstructure (remodeling) or vary its mass (growth) [83, 39].

One of the most active research branches in this field has coalesced around modeling living matter as an open chemo-thermo-mechanical system, where the chemical energy transported at the cellular level is adaptively spent for material rearrangements. This active behaviour, that has developed through evolution, displays two important features: on one hand it ensures a continuous dynamical balance with the environment, on the other hand it triggers morphological transitions. In the following, we critically review the continuum mechanics approaches that deal with modeling the mechanical cues of morphogenesis and shape emergence for materials, with a focus on systems that exhibit an elastic mechanical behavior.

4.1 Mechano-biological models coupling mechanical stress and growth

The main challenge of mechano-biological modeling is to build the simplest realistic description of the macroscopic effects triggered by a biochemical cascade of the inter-related developmental events from the molecular to the tissue scale, provoked by either genetic and epigenetic causes [32].

A reductionist viewpoint often relies on the seminal principle of *homeostasis*: all life phenomena result from the mutual balance between the inner the surrounding media. At the core of modern physiology, such a dynamic equilibrium is postulated to regulate living organisms despite the structural complexity of the building blocks [18], thus relying on a continuous feedback between mechanical and biochemical cues, occurring at the cellular and sub-cellular scale and provoking effect up to the macroscale.

Mechano-biological modeling complements classical continuous approaches to account for the two fundamental processes during tissue development, namely growth and remodeling. Both phenomena occur simultaneously in developmental biology, create tensional states that, in the time scale of interest, are even independent on the applied surface loads, thus making morphogenesis

a highly nonlinear process [62]. Two main theoretical frameworks have been developed to account for the material rearrangements of living matter. The first one focuses on the residual strains: the mechanical description settles on the introduction of a stress-free, natural state of the material. The starting point of the second approach is the pattern of residual stresses, i.e. the stress in the body in its unloaded configuration. Both methods are briefly illustrated in the following. For the sake of simplicity, here we look at living matter, which is always a multi-component material, as a single component one. This approach is not very restrictive: from a theoretical point of view, all the fundamental questions on morphodynamics are already encoded in the simpler one-component setting, although the mixture theory can be more appropriate when specifying the biomechanical system (see [4, 54]). In same vein, we neglect to account for surface growth processes [45, 82] as well as softening phenomena observed during cell proliferation [41, 74].

Initial strain method

The initial strain method is rooted in the plasticity theory [60] and can be formally stated as follows: given an hyperelastic material with strain energy $\bar{W}(\mathbf{F})$, the behaviour of the evolving (growing, remodelling) material is dictated by the strain energy per unit mass $\bar{W}(\mathbf{F}\mathbf{F}_g^{-1})$, where \mathbf{F}_g^{-1} has to be constitutively provided. Growth is therefore a distortion: the energy of the unloaded body in the reference configuration is $W(\mathbf{F}_g^{-1})$, and the minimum (typically unique) is locally transposed in \mathbf{F}_g . The intuitive idea is that, at each time, it exists a *relaxed state* in which the material is locally stress-free in any point [76], and the pull back to such a (local) state is provided by the tangent mapping \mathbf{F}_g^{-1} . We remark that the idea of an intermediate, relaxed placement is purely local: it is not a reachable global configuration in the Euclidean space, but a collection of (possibly infinite) stress-free states, one for each material point and global compatibility or integrity is not ensured. The virtual state is identified by the *growth tensor* $\mathbf{F}_g = \mathbf{F}_g(\mathbf{X}; t)$, whose inverse therefore gives the initial strain in every material point \mathbf{X} at time t , as shown in Figure 6. In practice, \mathbf{F}_g represents the stretching of tangent vectors due to volumetric growth processes in the interior of the body, whilst its inverse gives a linear transplant from the reference crystal to the tangent of the underformed configuration [38, 79]. As pointed out by Skalak [80, 81], in general \mathbf{F}_g cannot be expressed as the gradient of a vector field, thus growth is said “incompatible”. A major mechanical consequence is that an internal state of stress persists in the undeformed

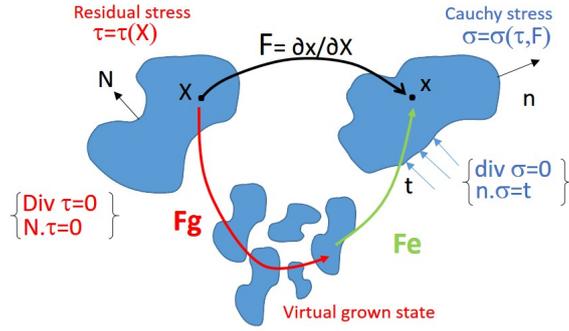


Fig. 6 Multiplicative decomposition for the initial strain model.

configuration even after the removal of either all external tractions or geometrical constraints. Since a great difference exists between the time-scales directing either growth or remodelling (days) and the viscoelastic response (seconds), even in viscoelastic materials the effects can be separated, thus such an internal state of stress represents the *residual stresses* due to the incompatible growth.

Since \mathbf{F} is the tensor gradient of deformation acting from the underformed to the deformed configuration, the tangent map from the virtual stress-free state to the deformed configuration is given by a multiplicative decomposition as:

$$\mathbf{F}_e = \mathbf{F} \mathbf{F}_g^{-1}. \quad (9)$$

As the strain energy per unit mass is supposed to depend on \mathbf{F}_e only, it is often called *elastic deformation tensor*. The strain energy function per unit reference volume $W = W(\mathbf{X}, t)$ for the material with evolving natural state [38] is

$$W = \rho \det(\mathbf{F}) \bar{W}(\mathbf{F}_e, c_\beta, t) \quad (10)$$

where ρ is the mass density and \bar{W} is here generalized to encompass the dependence on both the elastic deformation and on the local concentration c_β of biochemical species. Using classical thermo-mechanical arguments for Eqs.(9,10), hyperelastic constitutive relations can be derived [31]:

$$\boldsymbol{\sigma} = \rho \mathbf{F}_e \frac{\partial \bar{W}}{\partial \mathbf{F}_e}; \quad \mathbf{S} = \rho \det(\mathbf{F}) \mathbf{F}_g^{-1} \frac{\partial \bar{W}}{\partial \mathbf{F}_e} \quad (11)$$

where $\boldsymbol{\sigma}$ and \mathbf{S} are the elastic Cauchy and the transpose of the first Piola-Kirchhoff stress tensors, respectively. As living matter is mostly composed by water, the incompressibility constraint $\det(\mathbf{F}_e) = 1$ usually applies, and the evolution of the mass density over time obeys to:

$$\frac{\dot{\rho}_0}{\rho_0} = \frac{(\det \dot{\mathbf{F}}_g)}{\det \mathbf{F}_g} = \text{tr}(\dot{\mathbf{F}}_g \mathbf{F}_g^{-1}) \quad (12)$$

where $\rho_0 = \rho \det \mathbf{F}$ is the mass density per unit reference volume.

The definition of stress-dependent evolution laws for \mathbf{F}_g is thus crucial to model the mechano-biological effects during morphogenesis. A simple phenomenological law coupling stress and growth have been proposed by Taber [85]: living matter has a target *homeostatic* stress to be reached via remodelling actions. The theory has much in common with the hyper-restoration hypothesis discussed in the first section, although the two ideas were independently elaborated. Without resorting to thermo-dynamics arguments, this approach was originally introduced to recover the residual stress in arteries and has then been successful in a number of other of biomechanical systems [84]. We only mention here that, intriguingly enough, a neoplastic tissue could be conversely then defined, from a biomechanical point of view, as living matter that has lost the ability to self-regulate its duplication rate on the basis of the correct targeting of the homeostatic stress.

Growth and remodelling are dissipative processes and the exploitation of thermodynamic inequalities can be effective to provide admissible evolution laws for the growth tensor. Two approaches can be adopted, in strong analogy with earlier investigations in plasticity theory [69], and both identify a specific stress tensor driving the evolution of the natural state.

A first possible starting point is to adopt the formalism of the principle of virtual powers for growth too. In standard mechanics the power of internal forces can be at most fully stored in the material as increase of free energy; in full analogy, when remodelling occurs the same inequality applies with a new term, representing the power of *accretive forces* that induce growth [37]. If the Piola-Kirchhoff stress is tested versus $\dot{\mathbf{F}}$, the accretive stress is tested versus $\dot{\mathbf{F}}_g$. Exploiting this generalized principle of virtual powers, it naturally arises a role for the Eshelby stress \mathbf{E} , the energy conjugate of the growth tensor:

$$\mathbf{E} = \bar{W} \mathbf{I} - \mathbf{F}_e^T \frac{\partial \bar{W}}{\partial \mathbf{F}_e} \quad (13)$$

where \mathbf{I} is the identity matrix. Ambrosi and Guana [1] showed that a simple thermo-dynamically admissible mechano-biological growth law is:

$$K \dot{\mathbf{F}}_g = -(\mathbf{E} - \mathbf{E}_0) \mathbf{F}_g \quad (14)$$

where K is a positive-definite symmetric matrix, and \mathbf{E}_0 is a target homeostatic stress, representing a constant internal accretive stress, possibly dependent also on biochemical cues. A growth model of this type drives the model to the homeostatic mechanical equilibrium \mathbf{E}_0 , without the hyper-restoration dynamics discussed

in the previous section.

An alternative approach considers the thermo-mechanical balance laws of open systems accounting for the mass production inside the material [38], possibly occurring both as a volumetric production and as a non-convective mass flux [25]. In this case, it is found that the driving force of growth is the material Mandel stress $\mathbf{M} = \mathbf{S}\mathbf{F}$, and a thermo-dynamically consistent evolution law for growth and remodelling reads:

$$\dot{\mathbf{F}}_g = f^+(c_\beta, T)(\mathbf{M} - g(c_\beta, T)\mathbf{F}_g) \quad (15)$$

where $f^+(c_i, T)$, $g^+(c_i, T)$ are positive definite scalar functions, whose expressions may represent a specific dependence on the temperature T (e.g. Arrhenius-based relations) to model the chemical reactions occurring during the morphogenetic processes. In particular, $g(c_i, T)$ describes the amount of energy supplied by biochemical sources, and $f^+(c_i, T)$ the fraction that can be transformed in growth. This theoretical framework has also been extended to account for growth processes localized close to a boundary or an interface, thus providing a unified theory for modeling volumetric and surface growth processes [27].

Summarizing, Eqs.(14,15) both describe thermo-dynamical consistent laws for coupling stress-dependent evolution laws during morphogenesis. When a domain and boundary conditions are specified, they rewrite as systems of nonlinear partial differential equations that can be addressed in terms of rigorous methods [46].

An important example: the cardiac looping

The cardiac looping is an example of paramount importance where mechanics interacts with morphogenesis. During the Hamburger–Hamilton embryonic stage, the initially straight primitive heart tube bends and then transforms in a looped tube, thus generating the primordial structure of the four-chambers pump. The early stage of such a process is usually called c-looping; even the simple bending of the heart tube, is still very debated in its fundamental dynamics [78]. According to an early hypothesis, the heart tube bends because its elongation during growth is constrained at the boundary; this explanation has been abandoned after the observation that even an isolated, unbounded heart tube bends in culture [20, 65]. Two main conjectures are then debated to explain the heart tube bending: differential growth and differential active stress. Differential growth may be due to hyperplasia (more cells) or hypertrophy (cell enlargement). Differential tension may be due to the active generation of stress by the cells themselves, exploiting their actomyosin machinery. In both cases the correct mathematical formulation of the problem must be based on the observation that the placement

at rest of the tube is not the straight one, but it is distorted by the differential growth (or tension): the elastic system places itself in the bent configuration such that the elastic energy is minimal when compared with the *distorted* reference configuration.

Taber and coworkers [78] faced the questions of cardiac looping on the basis of experiments and numerical simulations. Isolated hearts in a lab have been treated with blebbistatin and cytochalasin, to inhibit myosin activity and actin polymerization, respectively. Numerical simulations employ the theory illustrated in the previous section, as specified in a cylindrical geometry: the growth tensor takes the diagonal form

$$\mathbf{F}_g = \text{diag}(F_{rr}, F_{\theta\theta}, F_{zz}) \quad (16)$$

and is not homogeneous: it depends on the circumferential coordinate θ . The specific form of \mathbf{F}_g depends on the investigated mechanism: growth (hypertrophy vs. hyperplasia) or active stress. Intriguingly enough, from a theoretical viewpoint all of these biomechanical behaviors can be framed in the very general theory of distortions discussed in the section above [5]. On the basis of the orchestrated combination of experiments, theory and numerical simulation (in cylinder as well in real geometry) the authors reach the conclusion that differential myocardial growth is the primary bending mechanism [78]. It remains however to be understood how the inhomogeneous growth pattern forms: an explanation could be provided by the elastic instability mechanisms.

Initial stress method

The initial strain method has been extensively used in the biomechanical literature in the last decades, however it has a drawback: a direct experimental measure of the growth tensor \mathbf{F}_g is impossible, because the residual stress should be locally released in every point. Thus, the initial strain method is often used in conjunction with simplifying assumptions of spherical or diagonal growth tensor, otherwise non-destructive data inversion techniques should be developed to obtain the form of \mathbf{F}_g .

Since one can only access the pre-stressed, undeformed configuration of the material, Guillou and Ogden [47] proposed to extend the multiplicative decomposition approach replacing \mathbf{F}_g with a residual deformation tensor \mathbf{F}_r , mapping the material evolution of the undeformed state. This approach does not involve unrealizable configurations, but calls for a functional dependence of \bar{W} also on \mathbf{F}_r .

A more straightforward approach has been recently proposed in [30]. The starting point is to assume that the strain energy functions explicitly depends on both

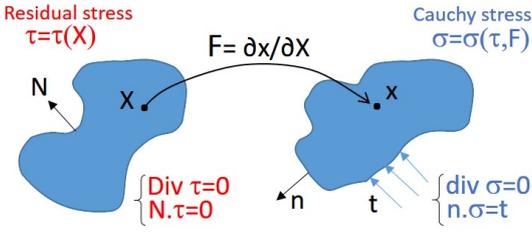


Fig. 7 Residually-stressed and deformed configurations for the initial stress model.

the pre-existing residual stresses and on the elastic deformation from the residually-stressed configurations. If the body occupies a volume Ω_0 with an unloaded boundary $\partial\Omega_0$ and outward unit normal \mathbf{N} in the reference configuration, its *residual stress tensor* $\boldsymbol{\tau}$ obeys the balance laws:

$$\begin{aligned} \text{Div } \boldsymbol{\tau} &= \mathbf{0}, & \boldsymbol{\tau} &= \boldsymbol{\tau}^T & \text{in } \Omega, \\ \mathbf{N} \cdot \boldsymbol{\tau} &= \mathbf{0} & & & \text{on } \partial\Omega, \end{aligned} \quad (17)$$

where Div is the divergence in material coordinates and $W = W(\mathbf{F}, \boldsymbol{\tau})$, as shown in Figure 7. If the W is an isotropic functional it can be written as a function of ten independent invariants [77]: the six principal invariants $I_{\alpha k}$, with $\alpha = (C, \tau)$ and $k = (1, 2, 3)$, of $\mathbf{C} = \mathbf{F}^T \mathbf{F}$ and $\boldsymbol{\tau}$, plus the four mixed invariants:

$$\begin{aligned} J_1 &= \text{tr}(\boldsymbol{\tau} \mathbf{C}), & J_2 &= \text{tr}(\boldsymbol{\tau} \mathbf{C}^2), \\ J_3 &= \text{tr}(\boldsymbol{\tau}^2 \mathbf{C}), & J_4 &= \text{tr}(\boldsymbol{\tau}^2 \mathbf{C}^2), \end{aligned} \quad (18)$$

where tr indicates the trace operator. From standard thermo-mechanical considerations, the symmetric Cauchy stress tensor $\boldsymbol{\sigma} = \boldsymbol{\sigma}(\mathbf{F}, \boldsymbol{\tau})$ in the deformed configuration reads:

$$\boldsymbol{\sigma} = \mathbf{F} \left(\sum_{k=1}^3 \frac{\partial W}{\partial I_{Ck}} \frac{\partial I_{Ck}}{\partial \mathbf{F}} + \sum_{m=1}^4 \frac{\partial W}{\partial J_m} \frac{\partial J_m}{\partial \mathbf{F}} \right). \quad (19)$$

The functional dependence of W on the residual stresses can be restricted exploiting compatibility and objectivity requirements. In fact, the free energy must be constant (say, zero) for the unstressed and undeformed body, so that $W(\mathbf{I}, \mathbf{0}) = 0$. Then, the enforcement of the *compatibility* of the residual stress, i.e. $\boldsymbol{\sigma}(\mathbf{I}, \boldsymbol{\tau}) = \boldsymbol{\tau}$, provides three scalar equations. Finally, *objectivity* imposes that

$$\boldsymbol{\tau} = \mathbf{F}^{-1} \frac{\partial W}{\partial \mathbf{F}}(\mathbf{F}^{-1}, \boldsymbol{\sigma}), \quad (20)$$

representing nine scalar equations on the derivatives of the free energy with respect to the invariants. These requirements impose restrictions in the form of W . For example, if a simple neo-Hookean response is expected

when $\boldsymbol{\tau}$ vanishes, the corresponding residually-stressed energy takes the following functional form:

$$\Psi = \frac{1}{2} [\lambda(I_{\tau_j}) I_{B1} + J_1 - 3\mu] \quad (21)$$

where μ is the shear modulus, and $\lambda = \lambda(I_{\tau_j})$ is the real positive root of $\lambda^3 + \lambda^2 I_{\tau_1} + \lambda I_{\tau_2} + I_{\tau_3} - \mu^3 = 0$. More complex natural behaviors can also be modeled, even if the functional dependence greatly complicates, possibly involving implicit relations between the invariants [55].

An example: optimal residual stresses in arteries

Initial strain and initial stress approaches can be compared using a simple system model, a residually stressed artery. Residual stresses arise during development and possibly evolves further at mature stages: they are believed to be the mechanical adaptation of arteries to homogenize the transmural stress gradient generated by the blood pressure [24].

Such tension are typically investigated by a radial cut of a tissue sample: the ring opens up, thus revealing an incompatible state. Circumferential layers are in different tensional states, the inner ones being even compressive [84]. The initial strain model has been employed to test the hypothesis of uniform initial strain formulated in [86]. It is assumed that arteries remodel until the elastic deformation tensor \mathbf{F}_e is homogeneous [35], being:

$$\mathbf{F}_e = \text{diag} \left(1/\sqrt{\lambda_\theta \lambda_z}, \sqrt{\lambda_\theta/\lambda_z}, \lambda_z \right) \quad (22)$$

where $\lambda_\theta = 2\pi/(2\pi - \alpha)$, λ_z are the initial circumferential and longitudinal strain, respectively, and α is the opening angle. The theoretical predictions of such an initial strain approach are found well within the physiological pressure range [36], and the residual stress produces the expected reduction of the transmural gradient of hoop stress caused by the physiological pressure.

Even if (a constant) initial strain approach provides one possible solution, this gives only an approximation of the underlying distribution of residual stresses in the tissue. Alternatively, the initial stress model can be used to look for the residual stress distribution that minimizes the transmural stress gradient. In particular, it is possible to detect the undeformed, residually-stressed configuration by removing the internal pressure. Thus, one can immediately guess a functional expression of $\boldsymbol{\tau}$ that automatically satisfies Eq.(17), for example introducing an Airy stress function. Then, it is possible to derive the underlying residual stress distribution by applying a simple principle of functional adaptation for homeostatic conditions. In the case of arteries, it leads to identify the expression of $\boldsymbol{\tau}$ which

minimizes the stress gradient when the artery is subjected to a physiological pressure. This case is discussed in [30], proving an optimal solution for the resulting Cauchy stresses. In particular, it is shown that the initial strain method overestimates the maximum stress inside the material.

In conclusion, although the initial strain model has a simpler functional expression for the strain energy function, the initial stress model might have advantages when the initial strain can not be determined experimentally. Relevant examples are the non-destructive determination of residual stresses, that can be inferred, for example, by nonlinear inverse-analysis from experimental measurements on wave propagation in the statically unloaded, residually stressed material.

4.2 Mechanical pre-patterning and morpho-elasticity

The focus of the section above was on the smooth interplay between growth and stress. However, as far as the remodelling process generates residual stresses, the tensional state can reach a limiting threshold such that an elastic instability arises, driving a morphological transition at the macroscopic scale that can be considered as a *mechanical pre-patterning*. *Morpho-elasticity* investigates the emergence of shape as a mechanical transition in growing living matter, merging together nonlinear elastic theories with perturbation techniques.

The starting point is the identification of the deformation gradient $\mathbf{F}_0 = \text{Grad } \mathbf{x}_0$ corresponding to the equilibrium solution of the elastic-growth problem. Such a finitely deformed configuration is associated with some scalar parameter: the eigenvalues of \mathbf{F}_g , for the initial strain model, or the components of $\boldsymbol{\tau}$, for the initial stress model. Such scalar variables will be later considered as *order parameters* for the onset of an elastic bifurcation. The linear stability of the equilibrium configuration is performed applying an infinitesimal deformation $\delta\mathbf{u}$ in the form:

$$\delta\mathbf{u} = u_j(\mathbf{x}_0)\mathbf{e}_j \quad (23)$$

where the scalar components u_j correspond to the direction of the canonical basis vectors \mathbf{e}_j ($j = 1, 2, 3$). At first order, the perturbation determines a variation on the geometric deformation tensor,

$$\delta\mathbf{F} = \text{grad}(\delta\mathbf{u})\mathbf{F}_0 \quad (24)$$

where grad is the gradient operator in the current configuration. If kinematic constraints apply, they must be

satisfied by the perturbation too. For example, if the incompressibility applies, it requires

$$\text{div}(\delta\mathbf{u}) = 0 \quad (25)$$

Accordingly, the boundary elastic problem can be rewritten by performing a series expansion around the basic solution. The balance of linear momentum has the following incremental form

$$\text{div}(\delta\bar{\mathbf{S}}) = 0 \quad (26)$$

The increment $\delta\bar{\mathbf{S}}$ of the push-forward of the Piola-Kirchhoff stress tensor in Eq.(26) has the following constitutive relation:

$$\delta\bar{\mathbf{S}} = \mathcal{A}_0^1 \text{grad}(\delta\mathbf{u}) + p_0 \text{grad}(\delta\mathbf{u}) - \delta p \mathbf{I} \quad (27)$$

where $p_0, \delta p$ are the Lagrange multiplier for the basic incompressibility constraint and its incremental counterpart, respectively, and:

$$\mathcal{A}_{hklj} = F_{h\gamma} F_{l\beta} \frac{\partial^2 W}{\partial F_{k\gamma} \partial F_{j\beta}} \quad (28)$$

are the component of the fourth-order tensor \mathcal{A} calculated in \mathbf{F}_0 , also known as *the fourth-order tensor of instantaneous elastic moduli* [72], where the sum over dummy subscripts applies. The incremental boundary value problem is finally complemented by the boundary conditions, which are typically expressed as a Dirichlet condition on a part $\partial\mathcal{B}_0$ of the boundary and a Neumann condition on the complementary portion $\partial\mathcal{B}_\sigma$:

$$\delta\mathbf{u} = \hat{\mathbf{u}} \quad \text{on } \partial\mathcal{B}_0, \quad \delta\bar{\mathbf{S}} = \hat{\mathbf{t}} \quad \text{on } \partial\mathcal{B}_\sigma. \quad (29)$$

where $\hat{\mathbf{u}}$ and $\hat{\mathbf{t}}$ are imposed incremental displacement and traction loads, respectively.

The solution of the (linear) incremental boundary value problem expressed by Eqs.(25,26,29) yields a dispersion relation, giving the values of the order parameter as a function of a spatial wavenumber of the perturbation. Since considerations of locally stability prove that the corresponding perturbed configurations are neutrally stable, it is possible to identify the minimum value of the order parameter, as well as the corresponding wavenumber, determining the onset of an elastic bifurcation.

In initial strain models the order parameter is often an eigenvalue of the growth tensor. Morphoelastic approaches have been successful to determine the morphological transitions in several system models, where residual stresses arise as a consequence of either spatial constraint or differential growth. Practical examples are the homogeneous, isotropic growth of an elastic layer [17] or of an annular tissue [8, 71] under a rigid external wall, and bi-layered tissues with different (homogeneous) swelling rates [26, 9]. Morphoelasticity has

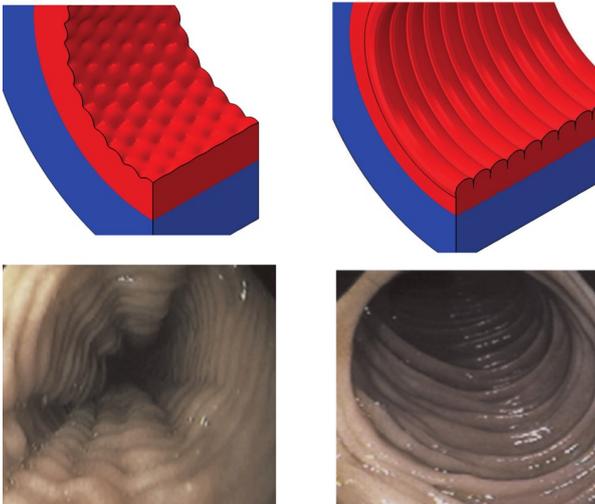


Fig. 8 Top: Numerical simulations of the differential growth of the gastrointestinal mucosal layer (blue) over a soft mesoderm (blue); courtesy of Valentina Balbi (University of Manchester). The two snapshots are compared with the mucosal patterns observed endoscopically on the feline oesophagus (down, left) and on the jejunum (down, right); adapted with permission from [95].

been successful to predict the onset of mechanical pre-patterns in developing vertebral segmentation [91], tumours [27,33], airways [40], brain gyrification [87]. An illustrative modeling example concerning intestinal embryogenesis is shown in Figure 8 [28].

In an initial stress setting the mathematical framework is analogue, but the pre-stress components naturally appear as order parameter. The main advantage of this method is that the perturbation is superposed over an underformed, pre-stressed configuration, with great simplifications for both the theoretical derivations and for the development of robust simulation tools. In particular, in plane strain elasticity a simple bifurcation parameter is a material constant of the Airy stress function for the pre-stress. This choice allows to investigate the effects of a different functional expression of the residual stresses on the onset of the bifurcations [29]. In both approaches, the incremental equations simply detect the onset of the bifurcation and the neutrally stable mode, but nothing can be inferred about the evolution of the bifurcated solution far from the threshold. One step toward a full nonlinear analysis can be performed either by higher order expansion in the incremental expansions [44] or performing a second variation of the energy functional [21]. Alternatively, finite element simulations can be performed, with specific continuation algorithms to capture the bifurcated path in the nonlinear regime. It is finally worth mentioning that linear stability techniques fail to determine the onset

of fully nonlinear instabilities, like crack nucleation or crease formation [52].

5 Conclusions

Morpho-mechanics has rapidly developed in the last decades. In the biological community there is a general agreement that mechanics plays a role in a very large number of morphogenetic systems. At the same time, important theoretical advancements have been established and the theory seems to be mature enough to be applied to a variety of new biomechanical questions of fundamental nature.

Notwithstanding the advancements, open questions are everywhere in morpho-mechanics. Take for example the notion of tension in cellular monolayers. From a structural point of view, this is a mesoscopic system, where the size of the elementary “periodic structure” (the cell) is not much smaller than the size of the overall sheet. The contractility of the cortex of the cells can generate a very directional pre-stress; however the representation such an active tension in terms of classical strain energies, provided by homogenization arguments might be questionable, because the length scales ratio between cells ($20\mu\text{m}$) and organ (1 mm) is not very small.

The same argument applies to a possible generalization of the notion of surface tension: it can be applied to biological tissues only allegorically, as characterizing tendency to internalization of particles (cells) which are in many orders greater than molecules. However such an improper usage has sometimes proven to be useful. Another example is a rarely mentioned physical force, the surface pressure, the inverse of the surface tension, that accounts for the tendency to externalize different scale particles and plays in morphogenesis a role greater than in inert matter.

Finally, a rational classification of stress-generated forces is closely associated with the poorly explored space-temporal structure of tissue stresses. What are their characteristic dimensions and times? Do they generate smooth or sharp gradients? Do they oscillate and, in positive case, what are their space-time domains of coherence? In any case, we should be prepared to accept that the space/time derivatives of stress will be of much greater biological importance than their absolute values.

An effective elaboration of these and other open problems calls for a novel approach, matching the rigour of exact sciences with an adequate, non hyper-simplified presentation of the biological behaviour.

Compliance with Ethical Standards

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