

A mechanical perspective on vertebral segmentation



L. Truskinovsky^{a,*}, G. Vitale^{a,b}, T.H. Smit^c

^a LMS, CNRS-UMR 7649, École Polytechnique, Route de Saclay, 91128 Palaiseau, France

^b LiPhy, CNRS-UMR 5588, Université Joseph Fourier de Grenoble, 140 Avenue de la Physique - BP 87, 38402 Saint Martin d'Hères, France

^c Department of Orthopedic Surgery, VU University Medical Center, MOVE Research Institute, P.O. Box 7057, 1007MB Amsterdam, The Netherlands

ARTICLE INFO

Article history:

Received 11 March 2014

Accepted 2 May 2014

Available online 6 June 2014

Keywords:

Morphogenesis

Somitogenesis

Mechanical signaling

Multiple cracking

Gradient elasticity

ABSTRACT

Segmentation is a characteristic feature of the vertebrate body plan. The prevailing paradigm explaining its origin is the 'clock and wave-front' model, which assumes that the interaction of a molecular oscillator (clock) with a traveling gradient of morphogens (wave) pre-defines spatial periodicity. While many genes potentially responsible for these processes have been identified, the precise role of molecular oscillations and the mechanism leading to physical separation of the somites remain elusive. In this paper we argue that the periodicity along the embryonic body axis anticipating somitogenesis is controlled by mechanical rather than bio-chemical signaling. Using a prototypical model we show that regular patterning can result from a mechanical instability induced by differential strains developing between the segmenting mesoderm and the surrounding tissues. The main ingredients of the model are the assumptions that cell–cell adhesions soften when overstretched, and that there is an internal length scale defining the cohesive properties of the mesoderm. The proposed mechanism generates a robust number of segments without dependence on genetic oscillations.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Segmentation, the repetitive division of the body axis in modular units, is a ubiquitous motif in biology (Bhat & Newman, 2009; Cooke, 1988, Ten Tusscher, 2013). In vertebrates, segmentation is established early in embryogenesis by the formation of somites, blocks of tissue that bud off periodically from the anterior part of the pre-somitic mesoderm (PSM). Somites are transient structures that eventually give rise to a variety of tissues, including the spine, skeletal muscles, and the dorsal skin (Brent & Tabin, 2002; Christ, Huang, & Scaal, 2007). Unveiling the mechanism of somite formation is one of the major challenges in developmental biology (Bénazéraf & Pourquié, 2013; Dias, de Almeida, Belmonte, Glazier, & Stern, 2014; Herrgen et al., 2010; Hester, Belmonte, Gens, Clendenon, & Glazier, 2011; Pourquié, 1999, 2011; Stern & Vasilias, 1999).

The process by which the somites are formed can be viewed as a subdivision of an initially continuous cylinder into a row of separate blocks. Segmentation appears as a sequential self-slicing and an adequate theory of somite formation must provide an explanation for the physical process of cell clustering and cleavage. These mechanical phenomena are driven by internally generated active tractions. Externally driven processes of this type are ubiquitous in non-animate Nature with

* Corresponding author. Tel.: +33 1 69335808.

E-mail addresses: trusk@lms.polytechnique.fr (L. Truskinovsky), vitale.gui@gmail.com, guido.vitale@ujf-grenoble.fr (G. Vitale), th.smit@vumc.nl (T.H. Smit).

formation of cracks in drying mud and stress induced fracturing of coating films as some of the most well known examples (Hutchinson & Suo, 1992).

The prevailing paradigm for vertebrate segmentation, does not address the issue of cleavage and mechanical separation is viewed as of secondary importance. It is believed that the principal role is played by a cellular oscillator which interacts with a traveling wave of morphogens and in this way produces a periodic biochemical pattern (Baker & Schnell, 2009; Cooke & Zeeman, 1976; Meinhardt, 2008; Murray, Maini, & Baker, 2011; Rué & Garcia-Ojalvo, 2013). The underlying biochemical mechanism, known as the ‘clock and wave-front’ model (see Fig. 1), has been substantiated by the identification of both: genes that oscillate (Li, Fenger, Niehrs, & Pollet, 2003; Palmeirim, Henrique, Ish-Horowicz, & Pourquié, 1997; Schröter et al., 2012), and diffusion gradients of morphogens that propagate along the body axis (Dubrulle & Pourquié, 2002; Kicheva, Bollenbach, Wartlick, Jülicher, & Gonzalez-Gaitan, 2012). The long range synchronization issue for independent genetic oscillators has also been addressed and various components have been integrated into a comprehensive network model (Baker, Schnell, & Maini, 2008; Goldbeter & Pourquié, 2008; Hester et al., 2011). Even though the ‘clock and wave-front’ model does not specify how the finite blocks of cells undergo synchronized consolidation into somites, it is supported by the observations that mutations to some of the proposed genetic candidates alter the period of somitogenesis and affect the total number of somites in the body (Harima et al., 2013; Herrgen et al., 2010; Kim et al., 2011; Schröter et al., 2012).

The ambiguity, however, remains because it has not been yet possible to smoothly and predictably tune the period of the proposed pacemakers. More importantly, the ‘clock and wave-front’ mechanism appears to be incompatible with some experimental observations (Kondo, 2014). In particular, it does not explain why despite a nearly twofold fluctuation in the overall size of the presomitic mesoderm during embryonic development, a relatively constant number of somitomeres is found in tandem sequences: these observations suggest that without any changes in the temporal periodicity, the spatial scale of somites can be affected by the size of the PSM (Tam, Meier, & Jacobson, 1982). It is also alerting that the ‘clock and wave-front’ mechanism does not rely on the concomitancy of somitogenesis and the elongation of the body axis (Gomez et al., 2008).

In this paper we discuss an alternative *hypothesis* that somitogenesis is largely driven by the mechanical stresses induced by growth and active contraction (cf. Belousov, 2001). We developed a simple model showing how the emerging periodicity can result from mechanical self-organization. Our model suggests that the hypothetical segmentation clock invoked in the ‘clock and wave-front’ mechanism may have *spatial* rather than *temporal* nature.

The paper is organized as follows. In Section 2 we review the experimental evidence showing that mechanical signaling plays an important role during vertebrate segmentation. Various theoretical approaches to the mechanical modelling of morphogenetic instabilities are discussed in Section 3. Our mathematical model is formulated in Section 4, where we also review the related work in non-biological setting. The linear problem capturing the pre-patterning stage of the somitogenesis process is discussed in Section 5. Some remarks about the actual separation of somites are collected in Section 6. Finally, our conclusions and some future perspectives are presented in Section 7.

2. Mechanical signaling

A general limitation of the ‘clock and wave-front’ mechanism is that it focuses exclusively on genes and biochemical pathways, thereby neglecting the mechanical stresses in the growing embryo. It is well known, however, that cells can extract as much information from the mechanical cues as they do from diffusing factors (Mammoto & Ingber, 2010; Schwarz & Safran, 2013). It would be then rather natural for the embryo to employ mechanical forces as long-range communication means to guide morphogenesis and to trigger the appropriate response of the genome (Belousov, 2012; Davidson et al., 2010). This

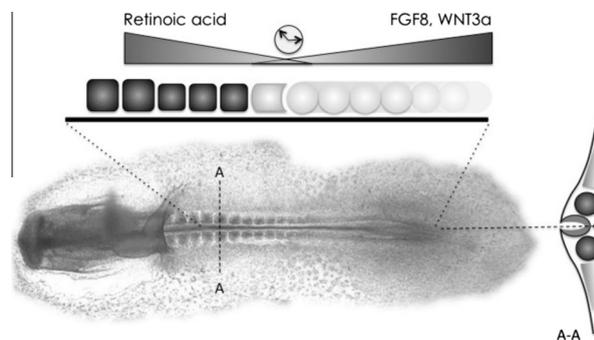


Fig. 1. Conventional picture of the vertebral segmentation in a chicken embryo. Bottom: Physical image of somites (black blocks) sequentially budding off from the PSM. The growing notochord is shown by the black line and by the dot in the cross section A-A. Within the non-differentiated PSM the incipient periodic pattern can be readily identified with somitomeres appearing as white blocks. Top: Schematics of the ‘clock and wave-front’ mechanism. Cells at the growing tail produce FGF8 and WNT3a signaling which keep them in a non-differentiated state. Retinoic acid, produced by the newly formed somites, facilitates differentiation into epithelial cells. This morphogenetic profile is traveling from head to tail with a constant speed while the genetic oscillation clock at the moving differentiation front sets the boundaries of the somites.

line of reasoning goes back to the pioneering work of A. Harris, who was the first to show convincingly that spatial morphogenetic patterns may be created by mechanical rather than chemical signaling (Harris, Stopak, & Wild, 1981; Harris, Stopak, & Warner, 1984; Stopak & Harris, 1982).

Harris and collaborators studied active contraction of fibroblasts suspended in a gel which was physically *restrained* by the attachment to a glass substrate. The overall contraction of the gel was therefore mechanically prevented and it was shown that the ensuing tensile instability gives rise to a regular geometric pattern. The authors concluded that tensile forces exerted by the fibroblasts caused stretching, tearing and eventual fragmentation of the initially homogeneous gel into a series of compacted clumps. Since the final segmented structure did not require any bio-chemical pre-patterning, Harris later argued in a series of papers (Harris, 1987, 1994, 1984, 2005) that mechanical instabilities can serve the morphological function which is conventionally attributed to diffusible morphogens (Meinhardt, 1982).

In other words, Harris conjectured that inhomogeneities induced by mechanical instabilities can provide cells with 'positional information' (Wolpert, 1971), thus allowing stress to play the role of the morphogen. In relation to somite segmentation, Harris suggested that a self-propagating mechanical instability may be responsible for the proliferation of the patterned domains and conjectured that somitogenesis is mechanically analogous to the aggregation of fibroblasts.

Similar ideas have been expressed by J. Bard who argued that chick somites form because pre-somitic cells exert mechanical forces on one another (Bard & Lauder, 1974; Bard, 1988). He showed that tractions lead to the aggregation of uniformly distributed cells only if the adhesion to the substratum is sufficiently strong which is analogous to the insistence of Harris on the importance of the deformational constraints. Bard emphasized that mechanical forces can propagate much faster and more robustly than diffusional gradients and that mechanical instabilities can yield the actual structures instead of just 'blueprints' capable of guiding the subsequent formation. In particular, he conjectured that the 'traction mechanism' can explain the ability of stirred mesoderm to produce normal somites (Menkes & Sandor, 1977) and the formation of multiple rows of somites in wide mesenchyme (Stern & Bellairs, 1984).

The ideas of Harris and Bard have been supported by the experimental observations that fibronectin is essential for somitogenesis (George, Georges-Labouesse, Patel-King, Rayburn, & Hynes, 1993), that cell adhesion molecules (CAMs) are formed well before somitogenesis (Duband et al., 1987) and that integrins responsible for the physical connection of the cells to the fibronectin matrix of the PSM are crucial for the segmentation process to actually take place (Dray et al., 2013; Girós, Grgur, Gossler, & Costell, 2011).

This mechanical perspective sheds a new light on molecular studies aimed at alteration of specific gene activities. For instance, the most natural target of such studies is Notch which is a critical component of the mouse somitogenesis because in its absence segmentation stops (Ferjentsik et al., 2009; Gibb, Maroto, & Dale, 2010). Notch signaling, however, also has a mechanical signature, more specifically, this gene can be linked to the adhesion forces between cells through the expression of Delta ligands (Ahimou, Mok, Bardot, & Wesley, 2004) and it plays an important role in cellular condensation (Fujimaki, Toyama, & Hozumi, 2006). One can then argue that mutations, believed to be affecting the 'clock' mechanism, may in fact be modifying the mechanical properties of the system by influencing the formation of cadherins and by affecting the adherence properties of the cells.

The experimental observations and the theoretical considerations presented above provide support for the hypothesis that long-range mechanical stresses play a decisive role in the generation of somitogenetic patterns. This role may be more important than previously believed because the alternative diffusional perspective can be criticized for not providing sufficiently robust mechanism (Wolpert, 2011). Moreover, it has been repeatedly mentioned that mechanics can regulate patterning with length scales exceeding those that can be generated by diffusion alone (Mansurov, Stein, & Belousov, 2012). An important argument is also that mechanical processes not only *pattern* and but also *shape* (Howard, Grill, & Bois, 2011).

The hypothesis about the essential role of mechanics in somitogenesis and in other similar developmental processes such as condensation of feather germs in birds, is in full agreement with the fact that the pattern formation in all these cases is an outcome of a constrained growth which subjects tissues to mechanical forces. As we have already mentioned, forces can be also generated by contracting cells (Harris et al., 1981) and can appear as a result of osmosis (Belousov, 2008). Since the ensuing mechanical interactions produce a feedback on cell behavior (Belousov, 2013) they may also affect the tasks conventionally attributed to bio-chemical morphogenes (Nelson et al., 2005; Pourquié, 2011).

As we argue in this paper, an important insight into the mechanical origin of somitogenesis arrives from the observation that segmentation has a precursor: as the somitogenesis front arrives, mesenchymal cells within the PSM appear to be already arranged inhomogeneously forming modulation clusters. These clusters are known either as somitomeres (Gossler & Tam, 2002; Meier, 1979, 1984) or as 'determined segments' (Bénazéraf & Pourquié, 2013; Hester et al., 2011). We show that such pre-aggregation can be explained by a destabilizing (positive) feedback of a purely mechanical origin. A particular advantage of the proposed mechanism is that the ensuing patterns are parametrically *robust* which makes the underlying mechanical signaling biologically relevant. We also discuss a possible scenario how the initial pre-patterning can evolve into the fully separation of somites.

3. Instabilities caused by stresses

Theoretical studies of the role of stresses in morphogenesis have been domineered by the "mechanical school" (Urduy, 2012) which replaced the chemical pre-pattern models (Cooke & Zeeman, 1976; Turing, 1952) by cell aggregation models

(Murray, Oster, & Harris, 1983; Murray, 2003; Odell, Oster, Alberch, & Burnside, 1981; Vaughan, Baker, Kay, & Maini, 2013). It pioneered the point of view that chemical patterning and morphogenesis form a *single* process. The proposed modeling methodology was based on the idea of a combined chemo-mechanical instability. More specifically, it implied stress induced chemotaxis where actively generated mechanical forces play the role of a chemo-attractor and chemical pattern is laid down simultaneously with the cell aggregation. The chemo-mechanical approach, however, was never directly applied to somitogenesis where advection–diffusion processes appear to be secondary to stress induced separation of cell aggregates.

More recently diffusion-free buckling due to different growth rates in mechanically coupled tissues has been actively explored as an even more radical mechanism of ‘morphogenesis without morphogenes’ (Osborn, 1993). It was realized that compressive instabilities play a crucial role in a broad range of morphogenetic phenomena from the folding of leaves to the wrinkling of guts (Liang & Mahadevan, 2009; Mirabet, Das, Boudaoud, & Hamant, 2011; Milani, Braybrook, & Boudaoud, 2013; Nakayama et al., 2012; Savin et al., 2011). In somitogenesis context, however, the buckling approach is hardly relevant because stresses in the growing PSM are mostly tensile.

Other types of mechanical signaling in developmental patterning have been considered as well (Wyczalkowski, Chen, Filas, Varner, & Taber, 2012). In particular, the morphogenetic role of surface forces and adhesive preferences has been repeatedly emphasized (Foty, Pflieger, Forgacs, & Steinberg, 1996; Steinberg, 1970; Thompson, 1963). The idea that surface tension plays an important role in the formation of somites was proposed rather early (Waddington & Deuchar, 1953), however, more recently it has been dismissed (Grima & Schnell, 2007). The formation of mesodermal somites was also linked with (columnar) polarization of cells in the axial mesoderm (Belintsev, Belousov, & Zaraisky, 1987) and in the proposed reaction–diffusion type model long-range mechanical interactions were modelled as an influence of the ‘whole’ upon the state of an individual cell. A related model of the mechanical feedback based on the ‘hyper-restoration’ hypothesis (Belousov, 2013) was proposed in Taber (2009).

In this paper we argue that somitogenesis is an outcome of a *strain localization* instability which, to our knowledge, has not been explored before in the morphogenetic context. Following the original insights of Harris, Bard and Belousov, we develop a diffusion-free model which is conceptually close to the buckling approach. However, while buckling originates from the *geometrical* nonlinearity of the elasticity equations and requires compressive loading (Grabovsky & Truskinovsky, 2007), we link the fragmentation of the PSM with the *physical* nonlinearity, which originates from material softening and reveals itself in tension (Rice, 1976).

Tensile stresses in tissues are ubiquitous (Bainier & Weaver, 2013; Kritikou, 2008) and the role of such stresses in the development of somites is directly supported by experiments showing that the explanted PSM can form molecularly-defined segments and that these segments progress further to become somites only when the surface ectoderm is left in place (Correia & Conlon, 2000; Palmeirim, Dubrulle, Henrique, Ish-Horowitz, & Pourquié, 1998). In another observation of this type cells from the PSM, placed ectopically on the area opaca, also formed somites, albeit without anterior and posterior polarization (Dias et al., 2014). Tension in these experiments is generated by direct contraction of PSM which was constrained by elastic coupling to the background. As in experiments of Harris and Bard, such coupling provided by the fibronectin (Rifes et al., 2007; Rifes & Thorsteinsdóttir, 2012) was found to be crucial for the segmentation to take place: it was shown that the pattern disappears when either fibronectin (Georges-Labouesse, George, Rayburn, & Hynes, 1996) or integrins (Dray et al., 2013) are lacking. The fundamental relation of segmentation to tension is also clear from the fact that somitogenesis terminates exactly when the elongation of the body axis stops (Bénazéraf & Pourquié, 2013).

So far, there have been no direct experimental evidence that PSM is of ‘softening nature’, which means that force diminishes with elongation, making the corresponding elastic modulus negative. However, softening behavior under tension has been established for other tissues of similar nature including embryonic epithelia (Wiebe & Brodland, 2005), aggregates of cancer cells (Gonzalez-Rodriguez et al., 2013) and collagen fiber-embedded gels (Gentleman et al., 2003). Measurements have also shown that intercellular attachment forces, which are mainly due to cadherins, start to diminish upon elongation beyond several micrometers (Benoit & Gaub, 2002; Puech, Poole, Knebel, & Muller, 2006). This behavior is compatible with the fundamental physics of molecular forces and with the behavior of capillary bridges. Most importantly, softening behavior has been observed in a physiological range for cellular deformations during embryonic growth (Wilson, Oster, & Keller, 1989).

It has been also noticed that (softening-induced) multicellular collective cleavage plays a key role in tissue separation and in segregation of a continuous tissue into different units (Duband et al., 1987). Some related experimental results showing the possibility of brittle failure for gels, including the protein ones, were reported in Leocmach, Perge, Divoux, and Manneville (2014), Liguore and Mora (2013) and Ronsin, Caroli, and Baumberger (2011). It is also appropriate to mention here the observations showing the increased strength of fibroblast traction in response to unloading associated with trauma or explantation. The positive feedback produced by such softening reveals an autocatalytic, anti-diffusive mechanism which serves to close the wound and bring torn tissue back together (Harris, 1987).

The most well-known manifestations of softening in solid mechanics are strain localization, loss of cohesion and material fracture. Periodic failure of reinforced concrete and multi-cracking associated with surface drying show that if the stretched material is sufficiently constrained, the strain localization zones appear in regular patterns (Sluys & DeBorst, 1996; Thouless, Li, Douville, & Takayama, 2011). The scale of these patterns, observed in a broad range of physical systems from cohesive granular materials (Alarcón et al., 2010) to crocodile skin (Milinkovitch et al., 2013), is determined by the cohesive properties

of the material and the geometry of the specimen (Bourdin, Marigo, Maurini, & Sicsic, 2014; Corson, Henry, & Adda-Bedia, 2010).

As in the case of stretching of reinforced concrete, the structure that undergoes segmentation during vertebrate morphogenesis (PSM) is elastically constrained by the surrounding tissues: neural tube, the lateral mesoderm, the notochord, the ectoderm and the endoderm, see Bellairs (1979) and Fig. 1. The tension leading to segmentation may be caused either by the elongating notochord (Adams, Keller, & Koehl, 1990; Grotmol et al., 2006) or by differential strains due to active condensation of the cells within the mesoderm (Bénazéraf et al., 2010). Yet another source of stress may be the embryonic straining due to area opaca, the ring of cells that stretch the embryo along the membrane of the yolk sack (New, 1959). In all these situations the elastic constraint is crucial and its loss inhibits the formation of somites (Dray et al., 2013; George et al., 1993; Girós et al., 2011).

4. The model

To explore the implied analogy between somitogenesis and periodic cracking we propose a toy model. Its general goal is to provide a *prototypical* description of the softening-induced periodic patterning in a mechanical system subjected to tensile stresses. Our more specific goal is to show that the anticipated breaking of translational symmetry can be induced by constrained growth in a setting appropriate for vertebrate segmentation.

In the interests of analytical transparency we model the PSM as a thin elastic rod undergoing longitudinal deformation, see the schematic setup shown in Fig. 2. We assume that the rod is attached (constrained) through linear shear (leaf) springs to a rigid foundation subjected to finite strain ϵ_0 .

Pre-straining characterized by the parameter ϵ_0 represents a distributed loading device mimicking differential growth. The springs in turn mimic interconnecting fibronectin matrix; their stiffness depends on the effective thickness of this matrix and we denote the corresponding length scale by λ_1 . This parameter characterizes the constraint which makes the separation of the PSM along a singular ‘crack’ energetically unfavorable. The *dimensionless* elastic energy density associated with such an ‘on-site’ interaction can be written as

$$f_1(u) = \frac{1}{2\lambda_1^2}(u - \epsilon_0 x)^2. \tag{1}$$

Here $u(x)$ is the longitudinal displacement and the loading appears as a set of distributed body forces.

To exhibit tensile instability, the material of the bar must be of the *softening* type, which in our setting means that beyond certain stretch, the axial force starts to diminish with elongation. Therefore we assume that the elastic energy density of the bar $f(\epsilon)$, where $\epsilon(x) = u'(x)$ is the longitudinal strain, is a convex function at small levels of stretching $\epsilon < \epsilon^*$ and that it

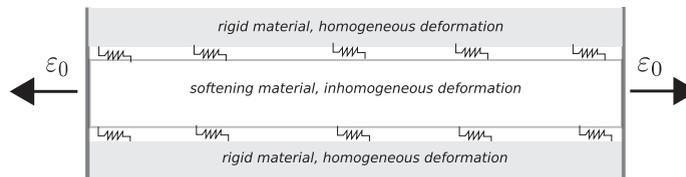


Fig. 2. A schematic illustration of the elements constituting the prototypical model. The layer in the middle, representing the PSM, is a nonlinear elastic bar which may deform inhomogeneously. The two layers above and below represent the homogeneously growing surrounding tissues. The shear springs, representing the interconnecting fibronectin, provide the linear elastic coupling. The arrows show the overall tension (pre-stress).

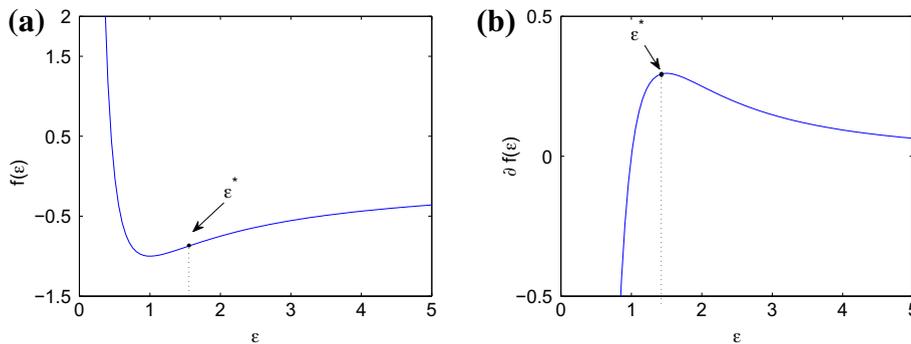


Fig. 3. (a) Elastic energy density represented by the concave-convex function $f(\epsilon) = \epsilon^{-2} - 2\epsilon^{-1}$; (b) The derivative of the energy $\partial f/\partial \epsilon$ representing axial force. The value of strain corresponding to the maximum axial force is ϵ^* .

becomes concave for $\varepsilon > \varepsilon^*$, see Fig. 3. The concave part represents the softening branch along which the cohesive properties progressively deteriorate.

Softening materials subjected to tensile loading are known to exhibit *infinite* localization of strain (shear banding, brittle fracture). To ensure that the resulting pattern has a finite scale one must add an energy term penalizing infinite localization. Such penalization necessarily brings with it an internal ‘coherence’ length which defines the scale where deformation can be considered as uniform.

In substances like hydrogels or wetted colloidal crystals the coherency length is determined by capillary interactions (Gallego-Gómez, Morales-Flórez, Blanco, de la Rosa-Fox, & López, 2012; Kawai, Nitta, & Nishinari, 2008). For growing tissues, the coherence length can be obtained from observations of stable surface-controlled cellular arrangements of a finite size (Dias et al., 2014; Stern & Bellairs, 1984). The value of coherence length may also depend on the internal architecture of the cell packing.

The simplest way to account for non-locality induced by the presence of a coherence length is through a gradient term in the energy density. Following the approach, originally due to van-der-Waals (Rowlinson & Widom, 1982), we assume that the *dimensionless* energy density of the bar has the form

$$f_2(\varepsilon, \varepsilon') = f(\varepsilon) + \frac{\lambda_2^2}{2} \varepsilon'^2, \quad (2)$$

where the internal parameter λ_2 has a dimension of length, the prime denotes spatial derivative and the potential $f(\varepsilon)$ is shown in Fig. 3. The simplifying assumption that the internal length λ_2 does not depend on ε is justifiable in view of our focus on the linear stability problem.

If we now add the energy densities $f_1(u)$ and $f_2(u', u'')$, we obtain the energy functional

$$E(u) = \int_{-l}^l \frac{\lambda_2^2}{2} u'^2 + f(u') + \frac{1}{2\lambda_1^2} (u - \varepsilon_0 x)^2 dx. \quad (3)$$

Here $2l$ is the length of the *pre-stressed* subdomain of the PSM. This region is expected to be smaller than the whole PSM, and parameter l may also depend on time.

To avoid a special treatment of the boundary layers we choose the boundary conditions to be compatible with the pre-strain and assume that (see Fig. 2)

$$u(-l) = -\varepsilon_0 l, \quad u(l) = \varepsilon_0 l. \quad (4)$$

To complement (4) we adopt the ‘natural’ (clamping) boundary conditions on the higher displacement gradients

$$u''(-l) = 0, \quad u''(l) = 0. \quad (5)$$

These conditions are obviously rather arbitrary given the complexity of the surface interaction (Charlotte & Truskinovsky, 2008) and their sole advantage is that they are the simplest possible.

To find the configuration where all mechanical forces are balanced, we need to solve the Euler–Lagrange equation $\delta E/\delta u = 0$, where $\delta E/\delta u$ is the variational derivative. We obtain

$$-\lambda_2^2 u'''' + \partial^2 f(u') u'' - \frac{1}{\lambda_1^2} (u - \varepsilon_0 x) = 0. \quad (6)$$

Here the notation $\partial^2 f$ is used as a shortcut for a second derivative. The experience with similar problems in the theory of phase transitions (Truskinovsky & Zanzotto, 1996) suggests that Eq. (6) may have a large number of nontrivial inhomogeneous solutions in addition to the trivial homogeneous solution with $u = \varepsilon_0 x$.

In order to decide which of these solutions are stable without specifying dynamics we make an assumption that the elastic energy functional (3) is (locally) minimized at each value of the prestress ε_0 . While the idea of the energy minimization has been previously used in the studies of morphogenesis (Steinberg, 2007), it has also encountered strong opposition because some of the forces involved in *active* functioning of living matter are not conservative (Harris, 1987). Without questioning the importance of these general objections, we stress that in our model the PSM and the surrounding tissues are treated as *passive* elastic materials. The *activity* in our model originates exclusively from the variation of the prestress ε_0 representing differential growth of the surrounding tissues to which the PSM is elastically attached.

Notice, that we are in a situation rather similar to the one encountered in the studies of a single adherent cell adapting its shape and orientation to the given background. It is broadly believed that such cells attempt to minimize the energy invested into straining of the environment and that they can actually reach the energy minimizing shape (Bischofs & Schwarz, 2003; Schwarz & Safran, 2013; Vianay et al., 2010). Despite the precarious nature of such a simplified interpretation of the behavior of the growing embryo, we adopt in this paper the same approach and associate the patterning of the PSM with the drive of the cell aggregates to minimize the elastic energy induced by differential growth.

To select among potentially numerous local minima of the energy (metastable states) different strategies can be proposed. Probably the least realistic assumption is that the energy is minimized globally at each value of the pre-strain ε_0 . It is more reasonable to assume that the dynamics is described by the simplest gradient flow $\dot{u} = -\delta E/\delta u$ and to focus on the limit $\nu \rightarrow 0$ reflecting the idea of a quasistatic driving (through ε_0). Since we are mostly interested in pre-patterning

and the exchange of stability between homogeneous and inhomogeneous states, the selection issue will not be pursued in full detail.

It is clear that the pre-patterning is initiated by the increase of the differential strain ε_0 beyond a certain threshold and the task is to express the critical value of ε_0 and the corresponding wavelength λ of the emerging pattern as functions of the non-dimensional parameters λ_1/l , λ_2/l . The quantitative solution of this problem depends on the precise form of the function $f(u')$, however, since we are interested in the qualitative behavior, we shall work with a softening potential of a generic form, see Fig. 3.

An important feature of our problem is that it involves *competing* interactions. Indeed, we observe that the term in the energy containing the function $f(u')$ is minimized at configurations with infinitely localized strain (Truskinovsky, 1996). Instead, the (quadratic in u) term describing elastic foundation favors homogeneous deformation. In other words, our material ‘prefers’ to break with a single fully developed crack, while the elastic constraint ‘drives’ the system towards the formation of an infinite number of infinitesimal cracks. The ‘gradient term’ in the energy (introducing cohesive length) smoothes the strain field without fully de-localizing strain gradients. To summarize, the formation of a pattern with a particular length scale (which depends on λ_1/l , λ_2/l) can be viewed as a resolution of a conflict between the tendencies toward *localization* and *spreading*.

The ensuing mathematical problem is conceptually similar to the problem of finding finite scale microstructures associated with martensitic phase transitions in constrained samples (Truskinovsky & Zanzotto, 1996; Vainchtein, Healey, Rosakis, & Truskinovsky, 1998; Vainchtein, Healey, & Rosakis, 1999). In this type of problems the parametric dependence of the energy minimizing configurations may be rather complex and dynamical studies have shown the existence of propagating fronts which separate growing segmented domains from metastable unsegmented domains (Belintsev et al., 1987; Ren & Truskinovsky, 2000; Vainchtein, 1999). It was also shown that by introducing *irreversibility* in the same framework one can generate complex hierarchical patterns resembling the ones observed in living Nature (Corson et al., 2010; Laguna, Bohn, & Jagla, 2008). Another relevant previous work concerns the patterns formed by irreversibly growing cracks in coating layers. The statistical features of these patterns have been thoroughly analyzed in the lattice setting, where discreteness played the same regularizing role as our gradient term (Handge, Leterrier, Rochat, Sokolov, & Blumen, 2000; Hornig, Sokolov, & Blumen, 1996; Meakin, 1986; Morgenstern, Sokolov, & Blumen, 1993). More recently, some hierarchical fracture patterns observed in experiment were reproduced numerically with an amazing precision by using the 3D phase field formulation with rate independent dissipation (Bourdin et al., 2014).

Our approach to pre-patterning during somitogenesis shares some common features with all this previous work. It is conceptually closer to the phase transition models modulo the replacement of a double-well Landau–Ginzburg type potential, by a convex-concave potential describing softening material. The unconstrained equilibria in the ensuing model exhibit only isolated cracks (Triantafyllidis & Aifantis, 1986; Triantafyllidis & Bardenhagen, 1993) and our goal is to account for the *constraint* responsible for multiple cracking. Another goal is to study in some detail the finite size effects.

5. Pre-patterning

It is easy to see that the trivial homogeneous state is locally stable for sufficiently small ε_0 and in this section we study the dependence of the critical value $\varepsilon_0 = \varepsilon_{crit}$, marking the loss of stability, on the parameters λ_1/l , λ_2/l . To this end we need to solve the linearized equation

$$-\lambda_2^2 v'''' + \partial^2 f(\varepsilon_0) v'' - \frac{1}{\lambda_1^2} v = 0 \quad (7)$$

with the boundary conditions

$$v''(-l) = v''(l) = v(-l) = v(l) = 0. \quad (8)$$

The linear boundary value problem (7) and (8) has nontrivial solutions exhibiting periodic modulations

$$v_n(x) = \sin(n\pi x)$$

if and only if the following condition is satisfied (cf. Vainchtein et al., 1999)

$$\left(\frac{\lambda_2}{l}\right)^2 (n\pi)^4 + \partial^2 f(\varepsilon_0) (n\pi)^2 - \left(\frac{l}{\lambda_1}\right)^2 = 0. \quad (9)$$

This equation can be solved explicitly and in Fig. 4 we show a (linearly interpolated) set of pairs $(\varepsilon_0, n = l/\lambda)$ satisfying Eq. (9) for a realistic choice of parameters λ_1/l , λ_2/l . The polygonal set presented in Fig. 4 indicates the domain where the homogeneous state is unstable.

Observe, that for the given value of the pre-strain ε_0 the number of potential pre-somites is bounded both from above and from below. The critical value of strain

$$\varepsilon_{crit} = \varepsilon_{crit}(\lambda_1/l, \lambda_2/l)$$

can be defined as the smallest solution of Eq. (9) with n restricted to be an integer. The integer n associated with ε_{crit} will be denoted by

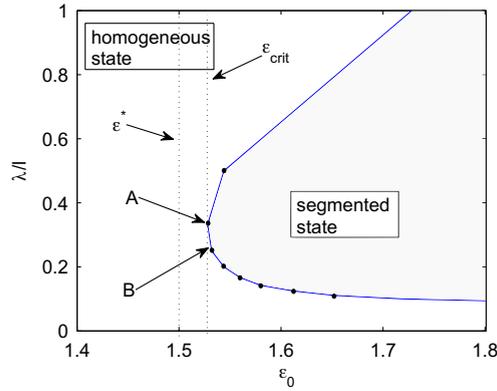


Fig. 4. Pairs $(\epsilon_0, 1/n)$ solving Eq. (9) at $\lambda_1/l = 1$, $\lambda_2/l = 10^{-2}$ for the potential $f(\epsilon) = \epsilon^{-2} - 2\epsilon^{-1}$. Point A corresponds to $\epsilon_{crit} = 1.5274$ and $n_{crit} = 3$. Point B describes the next bifurcation with $\epsilon_0 = 1.5308$ and $n = 4$.

$$n_{crit} = n_{crit}(\lambda_1/l, \lambda_2/l).$$

In Fig. 5 we illustrate the dependence of ϵ_{crit} on the parameters $\lambda_1/l, \lambda_2/l$. Notice that at fixed λ_1/l the curve $\epsilon_{crit}(\lambda_1/l)$ shown in Fig. 5 can be viewed as a stability boundary separating homogeneous and segmented states. One can see that for $\epsilon_0 < \epsilon^*$, i.e. outside of the domain of softening, the homogeneous configuration is always stable. The boundary of the stability domain is not smooth because of the corners where the number of the pre-somites n_{crit} , constituting the incipient pattern, changes abruptly. We observe that each pattern, characterized by a fixed number of pre-somites, persists over a finite range of pre-stresses.

It is interesting that inside these ‘robustness intervals’ the critical wavelength λ_{crit} , defining the size of the incipient somi-
tomeres, depends linearly on the length of the pre-stressed region of the PSM. Such dependence on l is compatible with the fundamental ‘size invariance’ principle stating that larger cell aggregates generate proportionally larger structures (Wolpert, 1969). In the somitogenesis context the ‘size invariance’ idea is also in agreement with the observations of that larger PSMs produce larger somites (Tam et al., 1982).

The dependence of the wave length of the incipient periodic pattern on l disappears for sufficiently large samples. In the ‘thermodynamic’ limit $\lambda_1/l \rightarrow 0, \lambda_2/l \rightarrow 0$ the number of somi-
tomeres increases indefinitely and the size of the robust parametric intervals tends to zero. To find the asymptotic formula for λ_{crit} in this limit we first rewrite Eq. (9) in the form

$$(n\pi)^2 = \frac{l}{\lambda_2} \left(-\partial^2 f(\epsilon_0) \pm \sqrt{\partial^2 f(\epsilon_0)^2 - 4 \left(\frac{\lambda_2}{\lambda_1} \right)^2} \right). \tag{10}$$

If we now neglect the fact that n is an integer, we obtain that the critical strain ϵ_{crit} is the smallest value of ϵ_0 making the expression under the square root equal to zero. Therefore in the limit $l \gg \sqrt{\lambda_1 \lambda_2}$ we obtain

$$\partial^2 f(\epsilon_{crit}) \sim -2 \frac{\lambda_2}{\lambda_1}. \tag{11}$$

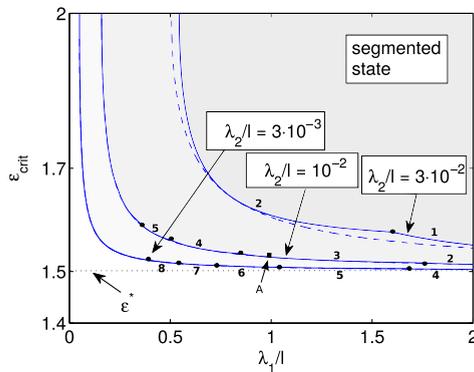


Fig. 5. Stability diagram in the space of parameters for $f(\epsilon) = \epsilon^{-2} - 2\epsilon^{-1}$. Shadowed regions correspond to the regimes where the homogeneous state is unstable. Small integer numbers show the values of n_{crit} . The dashed lines, that are almost indistinguishable from the solid lines except for $\lambda_2/l = 310^{-2}$, show the approximate solutions obtained from Eq. (11).

This asymptotics is illustrated in Fig. 5 by the dashed lines.

Finally, by substituting Eq. (11) back into Eq. (10) we obtain the Turing type asymptotic expression for the critical wavelength

$$\lambda_{crit} \sim \sqrt{\lambda_1 \lambda_2}. \tag{12}$$

To illustrate the asymptotics (12) we show in Fig. 6 the dependence of the function λ_{crit}/l on λ_1/l at different values of λ_2/l .

The ‘staircase’ structure of the function $n_{crit}(\lambda_1/l, \lambda_2/l)$ suggests that due to finite size effect the number of somitomeres in the pre-segmented PSM is a *robust* function of both the geometrical and the constitutive parameters. It reflects the ‘locking’ of the periodic patterns and demonstrates stability of the proposed morphogenetic mechanism. The parameter independence of the number of segments is, of course, an expected property of a system evolved through evolutionary selection process.

Notice also that the size of the individual somitomeres depends discontinuously on the geometrical parameters (thickness, length). Since in the process of the evolution of the embryo the geometry is changing with time, the parameters l and λ_1 are likely to vary as well. This gradual parameter drift may explain why the size of the somites changes from anterior to posterior (Tam et al., 1982). Given that the changes in the PSM geometry cannot affect the segmentation clock, such observations would be difficult to reconcile with the ‘clock and wave-front’ mechanism.

6. Developed somites

While the main focus of this paper is on the *pre-patterning* mechanism, it is instructive to illustrate the fact that an appropriate dynamic extension of the model allows one to trace the segmentation process all the way till the physical separation of individual somites.

One potential outcome of a dynamical model is presented in Fig. 7 where we show the strain profile associated with the *global minimum* of the energy (found numerically) and compare it with the incipient periodic profile at the same value of ϵ_0 (corresponding to the instability point A introduced in Fig. 4).

The localized peaks of the positive strain shown in Fig. 7 correspond to smoothed cracks while the spatially extended domains of negative strain correspond to aggregated somites. For determinacy, we selected parameters in such a way that the global minimizer of the energy contains only one (and a half) fully developed somite; the position of this somite is not symmetric due to our ‘natural’ boundary conditions. It is clear that by an appropriate change of parameters one can easily generate configurations with an arbitrary number of somites.

Observe next that the number of segments selected by the linear stability analysis survives in the nonlinear (post-buckling) regime. The unspecified dynamic process leading from the unstable pre-pattern to the globally stable configuration involves sharpening of the boundaries between the segments. The developing localization of strain is limited only by the magnitude of the cohesive length. The fact that the bifurcating pattern and the global minimizer share the same number of segments, is an indication that the proposed mechanism is robust not only in the pre-patterning stage but also in the regime of final separation.

To show that the global minimizer presented in Fig. 7 is not the only metastable configuration, we compare in Fig. 8 the equilibrium branch bifurcating at $\epsilon_0 = \epsilon_{crit}$ and delivering (after a turning point) the global minimum to the energy (branch I), with another equilibrium branch bifurcating at larger pre-stress $\epsilon_0 > \epsilon_{crit}$ (branch II). More specifically, we juxtapose the energies and the stresses for the patterns with $n = 3$ (bifurcating at point A with $\epsilon_{crit} = 1.5274$) and with $n = 4$ (bifurcating at point B with $\epsilon_{crit} = 1.5303$); see Fig. 9 for the definition of points A, B.

The blow up around the bifurcation points in Fig. 8 is shown in Fig. 9. As we see, in both cases the bifurcations are sub-critical and therefore the incipient patterns are unstable. The instability cannot be resolved quasi-statically and therefore a *dynamic* development is unavoidable. It will lead either to the global minimum of the energy (back to the branch I) or to one

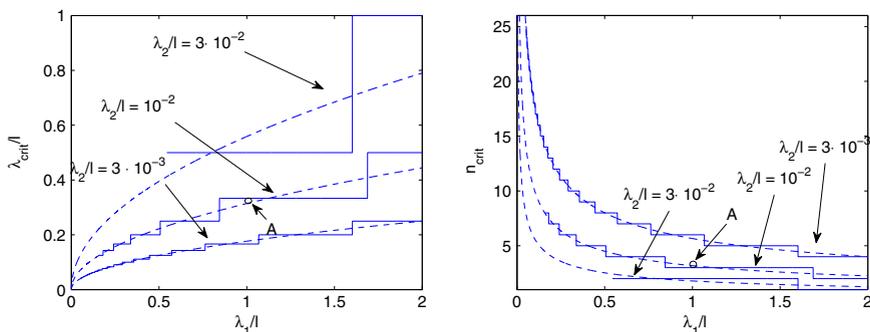


Fig. 6. The parametric dependence of the dimensionless critical wavelength λ_{crit} and the critical number of the segments n_{crit} . The dashed lines show the asymptotics given by Eq. (12). The elastic potential is $f(\epsilon) = \epsilon^{-2} - 2\epsilon^{-1}$.

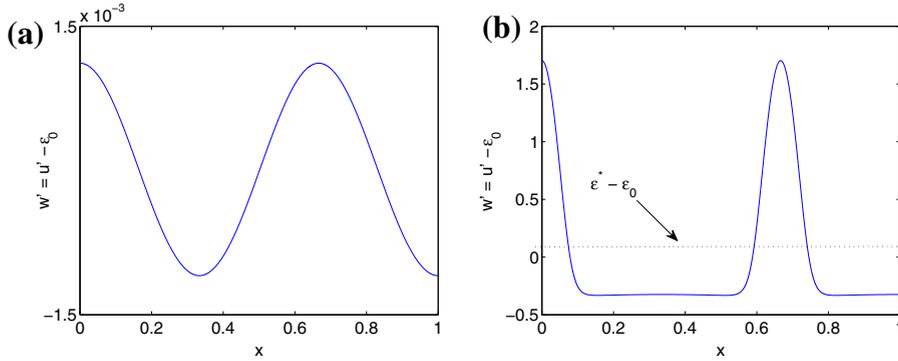


Fig. 7. Initial and fully developed strain configurations $w'(x) = u'(x) - \epsilon_0$ for $f(\epsilon) = \epsilon^{-2} - 2\epsilon^{-1}$ and $\lambda_1 = l$, $\lambda_2 = 10^{-2}l$. (a) The incipient strain profile close to the bifurcation point A from Fig. 6 corresponding to $\epsilon_{crit} = 1.5274$ and $n_{crit} = 3$; (b) The strain profile corresponding to the global minimum of the energy at the same parameters as in (a). In this figure we see one (and a half) fully developed somite.

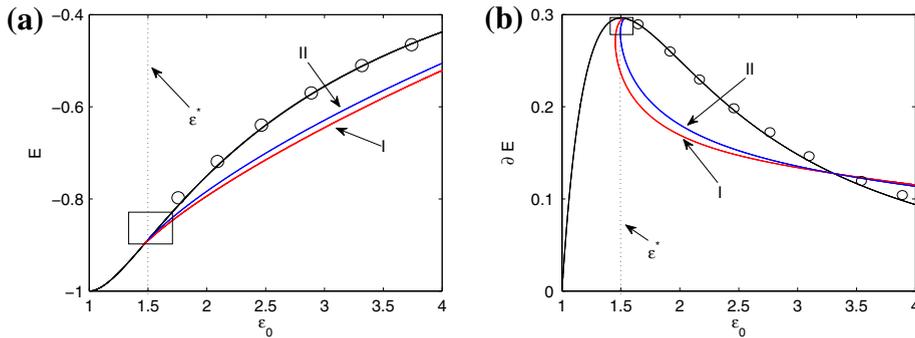


Fig. 8. Energy (a) and stresses (b) variation along the two equilibrium branches bifurcating from the trivial homogeneous branch. (a) Energies $E(\epsilon_0)$ of two equilibrium branches in the non-linear regime. Homogeneous branch is stable till point A; small circles indicate the region where homogeneous branch is unstable. The first nontrivial branch (I), which departs from the bifurcation point A (see Figs. 4–6) with the critical strain $\epsilon_{crit} = 1.5274$ and critical wave number $n_{crit} = 3$. After the turning point it describes the stable states (parameterized global minima of (3)). The second nontrivial branch (II) bifurcates from point B (see Fig. 9) with the critical strain $\epsilon_{crit} = 1.5303$ and critical wave number $n_{crit} = 4$. After the turning point it describes metastable states (parameterized local minima of (3)). Here we use $f(\epsilon) = \epsilon^{-2} - 2\epsilon^{-1}$ and $\lambda_1 = l$, $\lambda_2 = 10^{-2}l$. The total stress is computed as $\partial E / \partial \epsilon_0$.

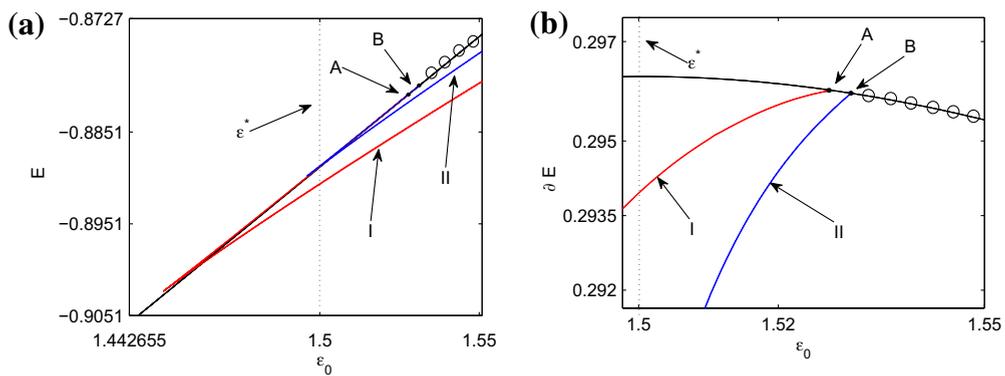


Fig. 9. Zoom into the rectangles indicated in Fig. 8.

of the local minima illustrated here by the branch II. In the former case the number of segments remains the same from the pre-patterning stage to the final separation stage, while in the latter case the number of segments necessarily changes.

The natural question whether the over-damped, gradient flow type dynamics allows the system to reach the global minimum of the energy remains outside the scope of this paper. In fact, one has to be careful in extending the purely mechanical model towards the stage where the somatic cleft is formed. It is clear that the ultimate separation of somites may involve secondary, non mechanical mechanisms (Henry, Hall, Burr Hille, Solnica-Krezel, & Cooper, 2000; Kulesa & Fraser, 2002;

Mosaliganti, Noche, Xiong, Swinburne, & Megason, 2012). While these mechanisms, implying chemical and biological adjustment (spatially-periodic expression of genes, periodically-spaced production of growth factors), are expected to be triggered by the *strain inhomogeneity* studied in this paper, our current understanding of their nature is incomplete. The purely mechanical model ignores a plethora of nonmechanical signaling events accompanying the initial instability and has to be complemented appropriately in order to be able to generate specific experimentally testable predictions.

7. Conclusions

Vertebral segmentation is a periodic process in space and time. It is generally assumed that periodicity is controlled by a molecular ‘clock’ which regulates temporally synchronized gene expression. Periodicity in space then emerges as a projection of the temporal periodicity performed by a steadily moving wave. In this paper we argue that the primary periodicity may be of *spatial* rather than *temporal* origin and that the required synchronization may be due to *mechanical* rather than *biochemical* signaling.

We utilize a known fact that mechanical instabilities can lead to the development of stress inhomogeneities in spatially distant material points and we interpret the observed segmentally-expressed genes as a readout of tissue mechanics. Our model suggests that the observed spatially inhomogeneous expression of genes is not necessarily the primary phenomenon. We show the possibility that it can be *induced* by a mechanical instability which is then a process *anticipating* the final segmentation phenomenon.

The goal of our oversimplified model was not to develop a comprehensive description of somitogenesis, but rather to demonstrate the very feasibility that a uniformly pre-stressed PSM can become globally periodic through a tension-induced instability. Our model is based on the assumptions that are well established in the literature, such as the important role of differential elongation during somitogenesis, the elastic constraint of the PSM due to the surrounding tissues and the strain softening nature of the cellular clusters. We have shown that the model readily generates spatial pre-patterning and that the underlying morphogenetic mechanism is robust.

The proposed mechanical perspective on the origin of somitogenesis is, however, incomplete because the temporal development through sequential formation of somites is not captured by the static model. Although there exist an experimental demonstration that somite segmentation can occur simultaneously instead of sequentially (Lipton & Jacobson, 1974), the normal development clearly requires a mechanism generating a propagating wave.

A conventional option would be to view the development of somites as an interaction of an *exterior* wave of morphogens with a mechanically induced spatially periodic pattern. An alternative possibility is that mechanics is also responsible for the propagation of the periodic pattern. It was previously shown in the phase transformation framework that if a model of this type is equipped with appropriate dynamics, it can generate propagating fronts that separate segmented and unsegmented configurations (Belintsev et al., 1987; Ren & Truskinovsky, 2000; Vainchtein, 1999); in the fracture setting segmentation fronts have been studied in Bourdin, Francfort, and Marigo (2009). If similar dynamic mechanism operates during somitogenesis the propagating front of the advancing mechanical instability can replace the front of diffusing morphogen. This would mean that not only the ‘clock’ but also the ‘wave’ in the ‘clock and wave-front’ mechanism has a mechanical origin.

To test these hypothesis and to fully understand the implications of our prototypical model, it is necessary to refine the simplified mechanical description by accounting for realistic geometry, adequate constitutive behavior and by incorporating dynamics. However, the qualitative predictions of the model can be tested already now. In particular, it would be of interest to study systematically to what extent the scale and the speed of the somitogenesis can be affected by the superimposed stretching or by the modification of the mechanical constraints. First experimental results of this type, showing the effect of the externally imposed thermal and mechanical loading on the outcome of a morphogenetic process, fully support the mechanical perspective (Kornikova, Troshina, Kremnyov, & Belousov, 2010; Primmitt, Norris, Carlson, Keynes, & Stern, 1989).

Acknowledgements

The authors thank M. Schmitz and B. Nelemans for insightful discussions. This work was initiated while LT and TS were visiting scholar at the Wyss Institute, Harvard University. TS and GV acknowledge the support from ZonMW-VICI Grant 918.11.635. GV also acknowledges the support from the GRE, École Polytechnique.

References

- Adams, D. S., Keller, R., & Koehl, M. A. (1990). The mechanics of notochord elongation, straightening and stiffening in the embryo of *Xenopus laevis*. *Development*, *110*, 115–130.
- Ahimou, F., Mok, L. P., Bardot, B., & Wesley, C. (2004). The adhesion force of Notch with Delta and the rate of Notch signaling. *Journal of Cell Biology*, *167*(6), 1217–1229.
- Alarcón, H., Ramos, O., Vanel, L., Vitztoz, F., Melo, F., & Gémard, J.-C. (2010). Softening induced instability of a stretched cohesive granular layer. *Physical Review Letters*, *105*(20), 1–4.
- Bainier, R., & Weaver, V. (2013). Cell biology. Strength under tension. *Science*, *341*(6149), 965–966.
- Baker, R. E., & Schnell, S. (2009). How can mathematics help us explore vertebrate segmentation? *HFSP Journal*, *3*, 1–5.
- Baker, R. E., Schnell, S., & Maini, P. K. (2008). Mathematical models for somite formation. *Current Topics in Developmental Biology*, *81*, 183–203.
- Bard, J. B. L. (1988). A traction-based mechanism for somitogenesis in the chick. *Roux's Archives of Developmental Biology*, *197*, 513–517.

- Bard, J., & Lauder, I. (1974). How well does Turing's theory of morphogenesis work? *Journal of Theoretical Biology*, 45(2), 501–531.
- Belintsev, B., Belousov, L., & Zaraksky, A. (1987). Model of pattern formation in epithelial morphogenesis. *Journal of Theoretical Biology*, 129(4), 369–394.
- Bellaïrs, R. (1979). The mechanism of somite segmentation in the chick embryo. *Journal of Embryology and Experimental Morphology*, 51, 227–243.
- Belousov, L. V. (2001). Somitogenesis in vertebrate embryos as a robust macromorphological process. In *NATO science series: Subseries I, life and behavioural sciences* (Vol. 329, pp. 97–106).
- Belousov, L. V. (2008). Mechanically based generative laws of morphogenesis. *Physical Biology*, 5(1), 015009.
- Belousov, L. V. (2012). Morphogenesis as a macroscopic self-organizing process. *Biosystems*, 109(3), 262–279.
- Belousov, L. V. (2013). Morphogenesis can be driven by properly parametrised mechanical feedback. *The European Physical Journal E*, 36(11), 1–16.
- Bénazéraf, B., Francois, P., Baker, R. E., Denans, N., Little, C. D., & Pourquié, O. (2010). A random cell motility gradient downstream of FGF controls elongation of an amniote embryo. *Nature*, 466(7303), 248–252.
- Bénazéraf, B., & Pourquié, O. (2013). Formation and segmentation of the vertebrate body axis. *Annual Review of Cell and Developmental Biology*, 29, 1–26.
- Benoit, M., & Gaub, H. E. (2002). Measuring cell adhesion forces with the atomic force microscope at the molecular level. *Cells Tissues Organs*, 172(3), 174–189.
- Bhat, R., & Newman, S. a. (2009). Snakes and ladders: The ups and downs of animal segmentation. *Journal of Biosciences*, 34(2), 163–166.
- Bischofs, I. B., & Schwarz, U. S. (2003). Cell organization in soft media due to active mechanosensing. *Proceedings of the National Academy of Sciences*, 16(100), 9274–9279.
- Bourdin, B., Francfort, G. A., & Marigo, J.-J. (2009). *Variational approach to fracture*. Springer.
- Bourdin, B., Marigo, J.-J., Maurini, C., & Sicsic, P. (2014). Morphogenesis and propagation of complex cracks induced by thermal shocks. *Physical Review Letters*, 112(1), 014301.
- Brent, A. E., & Tabin, C. J. (2002). Developmental regulation of somite derivatives: Muscle, cartilage and tendon. *Current Opinion in Genetics and Development*, 12, 548–557.
- Charlotte, M., & Truskinovsky, L. (2008). Towards multi-scale continuum elasticity theory. *Continuum Mechanics and Thermodynamics*, 20(3), 133–161.
- Christ, B., Huang, R., & Scaal, M. (2007). Amniote somite derivatives. *Developmental Dynamics*, 236, 2382–2396.
- Cooke, J. (1988). A note on segmentation and the scale of pattern formation in insects and in vertebrates. *Development*, 104(Supplement), 245–248.
- Cooke, J., & Zeeman, E. C. (1976). A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. *Journal of Theoretical Biology*, 58(2), 455–476.
- Correia, K. M., & Conlon, R. (2000). Surface ectoderm is necessary for the morphogenesis of somites. *Mechanisms of Development*, 91(1–2), 19–30.
- Corson, F., Henry, H., & Adda-Bedia, M. (2010). A model for hierarchical patterns under mechanical stresses. *Philosophical Magazine*, 90(1–4), 357–373.
- Davidson, L. a., Joshi, S. D., Kim, H. Y., von Dassow, M., Zhang, L., & Zhou, J. (2010). Emergent morphogenesis: Elastic mechanics of a self-deforming tissue. *Journal of Biomechanics*, 43(1), 63–70.
- Dias, A. S., de Almeida, I., Belmonte, J. M., Glazier, J. A., & Stern, C. D. (2014). Somites without a clock. *Science*, 343(6172), 791–795.
- Dray, N., Lawton, A., Nandi, A., Jülich, D., Emonet, T., & Holley, S. a. (2013). Cell-fibronectin interactions propel vertebrate trunk elongation via tissue mechanics. *Current Biology*, 23(14), 1335–1341.
- Duband, J. L., Dufour, S., Hatta, K., Takeichi, M., Edelman, G. M., & Thiery, J. P. (1987). Adhesion molecules during somitogenesis in the avian embryo. *The Journal of cell biology*, 104(5), 1361–1374.
- Dubrulle, J., & Pourquié, O. (2002). From head to tail: Links between the segmentation clock and antero–posterior patterning of the embryo. *Current Opinion in Genetics and Development*, 12, 519–523.
- Ferjentsik, Z., Hayashi, S., Dale, J. K., Bessho, Y., Herreman, A., De Strooper, B., et al (2009). Notch is a critical component of the mouse somitogenesis oscillator and is essential for the formation of the somites. *PLoS Genetics*, 5(9), e1000662.
- Foty, R., Pflieger, C., Forgacs, G., & Steinberg, M. (1996). Surface tensions of embryonic tissues predict their mutual envelopment behavior. *Development*, 122(5), 1611–1620.
- Fujimaki, R., Toyama, Y., & Hozumi, N. (2006). Involvement of Notch signaling in initiation of prechondrogenic condensation and nodule formation in limb bud micromass cultures. *Journal of Bone and Mineral Metabolism*, 24, 191–198.
- Gallego-Gómez, F., Morales-Flórez, V., Blanco, A., de la Rosa-Fox, N., & López, C. (2012). Water-dependent micromechanical and rheological properties of silica colloidal crystals studied by nanoindentation. *Nano Letters*, 12(9), 4920–4924.
- Gentleman, E., Lay, A. N., Dickerson, D. A., Nauman, E. A., Livesay, G. A., & Dee, K. C. (2003). Mechanical characterization of collagen fibers and scaffolds for tissue engineering. *Biomaterials*, 24(21), 3805–3813.
- George, E. L., Georges-Labouesse, E. N., Patel-King, R. S., Rayburn, H., & Hynes, R. O. (1993). Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. *Development*, 119(4), 1079–1091.
- Georges-Labouesse, E. N., George, E. L., Rayburn, H., & Hynes, R. O. (1996). Mesodermal development in mouse embryos mutant for fibronectin. *Developmental Dynamics*, 207(2), 145–156.
- Gibb, S., Maroto, M., & Dale, J. K. (2010). Trends in cell biology the segmentation clock mechanism moves up a notch. *Trends in Cell Biology*, 20, 593–600.
- Girós, A., Grgur, K., Gossler, A., & Costell, M. (2011). 51 integrin-mediated adhesion to fibronectin is required for axis elongation and somitogenesis in mice. *PLoS One*, 6(7), e22002.
- Goldbeter, A., & Pourquié, O. (2008). Modeling the segmentation clock as a network of coupled oscillations in the Notch, Wnt and FGF signaling pathways. *Journal of Theoretical Biology*, 252, 574–585.
- Gomez, C., Ozbudak, E. M., Wunderlich, J., Baumann, D., Lewis, J., & Pourquié, O. (2008). Control of segment number in vertebrate embryos. *Nature*, 454, 335–339.
- Gonzalez-Rodriguez, D., Bonnemay, L., Elgeti, J., Dufour, S., Cuvelier, D., & Brochard-Wyart, F. (2013). Detachment and fracture of cellular aggregates. *Soft Matter*, 9(7), 2282.
- Gossler, A., & Tam, P. P. (2002). Somitogenesis: Segmentation of the paraxial mesoderm and the delineation of tissue compartments. In J. Rossant & P. P. Tam (Eds.), *Mouse development* (pp. 127–149). San Diego: Academic Press.
- Grabovskiy, Y., & Truskinovsky, L. (2007). The flip side of buckling. *Continuum Mechanics and Thermodynamics*, 19(3–4), 211–243.
- Grima, R., & Schnell, S. (2007). Can tissue surface tension drive somite formation? *Developmental Biology*, 307(2), 248–257.
- Grotmol, S., Kryvi, H., Keynes, R., Krossoy, C., Nordvik, K., & Totland, G. K. (2006). Stepwise enforcement of the notochord and its intersection with the myoseptum: An evolutionary path leading to development of the vertebra? *Journal of Anatomy*, 209, 339–357.
- Handge, U., Leterrier, Y., Rochat, G., Sokolov, I., & Blumen, A. (2000). Two scaling domains in multiple cracking phenomena. *Physical Review E*, 62(6), 7807–7810.
- Harima, Y., Takashima, Y., Ueda, Y., Ohtsuka, T., Kageyama, R., & Gene, H. (2013). Report accelerating the tempo of the segmentation clock by reducing the number of introns in the *hes7* gene. *Cell Reports*, 3(1), 1–7.
- Harris, A. K. (1984). Cell traction and the generation of anatomical structure. In W. Jger & J. Murray (Eds.), *Modelling of patterns in space and time. Lecture notes in biomathematics* (Vol. 55, pp. 103–122). Springer.
- Harris, A. K. (1987). Cell motility and the problem of anatomical homeostasis. *Journal of Cell Science*, 8, 121–140.
- Harris, A. (1994). Multicellular mechanics in the creation of anatomical structures. In N. Akka (Ed.), *Biomechanics of active movement and division of cells. NATO ASI series* (Vol. 84, pp. 87–129). Springer.
- Harris, A. K. (2005). Direct physical formation of anatomical structures by cell traction forces. *The International Journal of Developmental Biology*, 23(50), 93–101.
- Harris, A. K., Stopak, D., & Warner, P. (1984). Generation of spatially periodic patterns by a mechanical instability: A mechanical alternative to the Turing model. *Journal of Embryology and Experimental Morphology*, 80, 1–20.

- Harris, A. K., Stopak, D., & Wild, P. (1981). Fibroblast traction as a mechanism for collagen morphogenesis. *Nature*, 290, 249–251.
- Henry, C. a., Hall, L. a., Burr Hille, M., Solnica-Krezel, L., & Cooper, M. S. (2000). Somites in zebrafish doubly mutant for knypek and trilobite form without internal mesenchymal cells or compaction. *Current Biology*, 10(17), 1063–1066.
- Herrgen, L., Ares, S., Morelli, L. G., Schröter, C., Jülicher, F., & Oates, A. C. (2010). Intercellular coupling regulates the period of the segmentation clock. *Current Biology*, 20(14), 1244–1253.
- Hester, S. D., Belmonte, J. M., Gens, J. S., Clendenen, S. G., & Glazier, J. a. (2011). A multi-cell, multi-scale model of vertebrate segmentation and somite formation. *PLoS Computational Biology*, 7(10), e1002155.
- Hornig, T., Sokolov, I. M., & Blumen, A. (1996). Patterns and scaling in surface fragmentation processes. *Physical Review E*, 54(4239).
- Howard, J., Grill, S., & Bois, J. (2011). Turing's next steps: The mechanochemical basis of morphogenesis. *Nature Reviews. Molecular Cell Biology*, 6(12), 392–398.
- Hutchinson, J. W., & Suo, Z. (1992). Mixed mode cracking in layered materials. *Advances in Applied Mechanics*, 29, 63–191.
- Kawai, S., Nitta, Y., & Nishinari, K. (2008). Model study for large deformation of physical polymeric gels. *The Journal of Chemical Physics*, 128(13), 134903.
- Kicheva, A., Bollenbach, T., Jagla, E. A., Wartlick, O., Jülicher, F., & Gonzalez-Gaitan, M. (2012). Investigating the principles of morphogen gradient formation: From tissues to cells. *Current Opinion in Genetics & Development*, 22(6), 527–532.
- Kim, W., Matsui, T., Yamao, M., Ishibashi, M., Tamada, K., Takumi, T., et al (2011). The period of the somite segmentation clock is sensitive to Notch activity. *Molecular Biology of the Cell*, 22(18), 3541–3549.
- Kondo, S. (2014). Self-organizing somites. *Science*, 343(6172), 736–737.
- Kornikova, E. S., Troshina, T., Kremnyov, S. V., & Belousov, L. V. (2010). Neuromesodermal patterns in artificially deformed embryonic explants: A role for mechanogeometry in tissue differentiation. *Developmental Dynamics*, 239(3), 885–896.
- Kritikou, E. (2008). Mechanotransduction: Under tension. *Nature Reviews Molecular Cell Biology*, 10(1), 3.
- Kulesa, P. M., & Fraser, S. E. (2002). Cell dynamics during somite boundary formation revealed by time-lapse analysis. *Science*, 298(5595), 991–995.
- Laguna, M. F., Bohn, S., & Jagla, E. A. (2008). The role of elastic stresses on leaf venation morphogenesis. *PLoS Computational Biology*, 4(4).
- Leocmach, M., Perge, C., Divoux, T., & Manneville, S. (2014). Creep and brittle failure of a protein gel under stress. arXiv:1401.8234 [cond-mat.soft].
- Liang, H., & Mahadevan, L. (2009). The shape of a long leaf. *Proceedings of the National Academy of Sciences U.S.A.*, 106, 22049–22054.
- Li, Y., Fenger, U., Niehrs, C., & Pollet, N. (2003). Cyclic expression of *esr9* gene in *Xenopus* presomitic mesoderm. *Differentiation*, 71, 83–89.
- Liguore, C., & Mora, S. (2013). Fractures in complex fluids: The case of transient networks. *Rheologica Acta*, 2(52), 91–114.
- Lipton, B. H., & Jacobson, A. G. (1974). Experimental analysis of the mechanisms of somite morphogenesis. *Developmental Biology*, 38(1), 91–103.
- Mammoto, T., & Ingber, D. E. (2010). Mechanical control of tissue and organ development. *Development*, 137, 1407–1420.
- Mansurov, A. N., Stein, A., & Belousov, L. (2012). A simple model for estimating the active reactions of embryonic tissues to a deforming mechanical force. *Biomechanics and Modeling in Mechanobiology*, 8(11), 1123–1136.
- Meakin, P. (1986). Fractal scaling in thin film condensation and material surfaces. *Critical Reviews in Solid State and Material Sciences*, 13, 143–189.
- Meier, S. (1979). Development of the chick embryo mesoblast. Formation of the embryonic axis and establishment of the metameric pattern. *Developmental Biology*, 73, 24–45.
- Meier, S. (1984). Somite formation and its relationship to metameric patterning of the mesoderm. *Cell Differentiation*, 14, 235–243.
- Meinhardt, H. (1982). *Models of biological pattern formation*. London: Academic Press.
- Meinhardt, H. (2008). Models of biological pattern formation: From elementary steps to the organization of embryonic axes. *Current Topics in Developmental Biology*, 81, 1–63.
- Menkes, B., & Sandor, S. (1977). Somitogenesis: Regulation potencies, sequence determination and primordial interactions. In D. Ede, J. R. Hinchcliffe, & M. Balls (Eds.), *Vertebrate limb and somite morphogenesis* (pp. 405–419). Cambridge University Press.
- Milani, P., Braybrook, S. A., & Boudaoud, A. (2013). Shrinking the hammer: Micromechanical approaches to morphogenesis. *Journal of Experimental Botany*, 64(15), 4651–4662.
- Milinkovitch, M., Manukyan, L., Debry, A., Di-Poi, N., Martin, S., Singh, D., et al (2013). Crocodile head scales are not developmental units but emerge from physical cracking. *Science*, 339(6115), 78–81.
- Mirabet, V., Das, P., Boudaoud, A., & Hamant, O. (2011). The role of mechanical forces in plant morphogenesis. *Annual Review of Plant Biology*, 62(1), 365–385.
- Morgenstern, O., Sokolov, I., & Blumen, A. (1993). Analysis of a one-dimensional fracture model. *Journal of Physics A: Mathematical and General*, 26(4521).
- Mosaliganti, K. R., Noche, R. R., Xiong, F., Swinburne, I. a., & Megason, S. G. (2012). ACME: Automated cell morphology extractor for comprehensive reconstruction of cell membranes. *PLoS Computational Biology*, 8(12), e1002780.
- Murray, J. D. (2003). Mechanical theory for generating pattern and form in development. In J. D. Murray (Ed.), *Mathematical biology* (Vol. 18, pp. 311–395). Springer.
- Murray, P. J., Maini, P. K., & Baker, R. E. (2011). The clock and wavefront model revisited. *Journal of Theoretical Biology*, 283(1), 227–238.
- Murray, J. D., Oster, G. F., & Harris, A. K. (1983). A mechanical model for mesenchymal morphogenesis. *Journal of Mathematical Biology*, 17, 125–129.
- Nakayama, N., Smith, R., Mandel, T., Robinson, S., Kimura, S., Boudaoud, A., et al (2012). Mechanical regulation of auxin-mediated growth. *Current Biology*, 22(16), 1468–1476.
- Nelson, C. M., Jean, R. P., Tan, J. L., Liu, W. F., Sniadecki, N. J., Spector, A. A., et al (2005). Emergent patterns of growth controlled by multicellular form and mechanics. *Proceedings of the National Academy of Sciences U.S.A.*, 102, 11594–11599.
- New, D. (1959). The adhesive properties and expansion of the chick blastoderm. *Journal of Embryology & Experimental Morphology*, 7(June), 146–164.
- Odell, G., Oster, G., Alberch, P., & Burnside, B. (1981). The mechanical basis of morphogenesis: Epithelial folding and invagination. *Developmental Biology*, 85(2), 446–462.
- Osborn, J. (1993). A model simulating tooth morphogenesis without morphogenes. *Journal of Theoretical Biology*, 165, 429–445.
- Palmeirim, I., Dubrulle, J., Henrique, D., Ish-Horowicz, D., & Pourquié, O. (1998). Uncoupling segmentation and somitogenesis in the chick presomitic mesoderm. *Developmental Genetics*, 23, 77–85.
- Palmeirim, I., Henrique, D., Ish-Horowicz, D., & Pourquié, O. (1997). Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. *Cell*, 91, 639–648.
- Pourquié, O. (1999). Segmentation of the paraxial mesoderm and vertebrate somitogenesis. *Current Topics in Developmental Biology*, 47, 81–105.
- Pourquié, O. (2011). Vertebrate segmentation: From cyclic gene networks to scoliosis. *Cell*, 145(5), 650–663.
- Primmitt, D., Norris, W., Carlson, G., Keynes, R., & Stern, C. (1989). Periodic segmental anomalies induced by heat shock in the chick embryo are associated with the cell cycle. *Development*, 105(1), 119–130.
- Puech, P.-H., Poole, K., Knebel, D., & Muller, D. J. (2006). A new technical approach to quantify cell-cell adhesion forces by AFM. *Ultramicroscopy*, 106(8–9), 637–644.
- Ren, X., & Truskinovsky, L. (2000). Finite scale microstructures in nonlocal elasticity. *Journal of Elasticity*, 59, 319–355.
- Rice, J. (1976). The Localization of Plastic Deformation. In W. Koiter (Ed.), *14th international congress on theoretical and applied mechanics* (pp. 207–220). Delft: North-Holland Publishing.
- Rifes, P., Carvalho, L., Lopes, C., Andrade, R. P., Rodrigues, G., Palmeirim, I., et al (2007). Redefining the role of ectoderm in somitogenesis: A player in the formation of the fibronectin matrix of presomitic mesoderm. *Development*, 134(17), 3155–3165.
- Rifes, P., & Thorsteinsdóttir, S. (2012). Extracellular matrix assembly and 3D organization during paraxial mesoderm development in the chick embryo. *Developmental Biology*, 368(2), 370–381.
- Ronsin, O., Caroli, C., & Baumberger, T. (2011). Microstructure stages during gelation of gelatin under shear. *The European Physical Journal E*, 6(34), 1–7.
- Rowlinson, J., & Widom, B. (1982). *Molecular theory of capillarity*. Dover.
- Rué, P., & Garcia-Ojalvo, J. (2013). Modeling gene expression in time and space. *Annual Review of Biophysics*, 42, 605–627.

- Savin, T., Kurpios, N. A., Shyer, A. E., Florescu, P., Liang, H., Mahadevan, L., et al (2011). On the growth and form of the gut. *Nature*, 476, 57–62.
- Schröter, C., Ares, S., Morelli, L. G., Isakova, A., Hens, K., Soroldoni, D., et al (2012). Topology and dynamics of the zebrafish segmentation clock core circuit. *PLoS Biology*, 10(7), e1001364.
- Schwarz, U. S., & Safran, S. A. (2013). Physics of adherent cells. *Reviews of Modern Physics*, 85(3), 1327–1381.
- Sluys, L. J., & DeBorst, R. (1996). Concrete – an analysis of crack width and spacing. *International Journal of Solids and Structures*, 33, 3257–3276.
- Steinberg, M. S. (1970). Does differential adhesion govern self-assembly processes in histogenesis? equilibrium configurations and the emergence of a hierarchy among populations of embryonic cells. *Journal of Experimental Zoology*, 4(173), 395–433.
- Steinberg, M. S. (2007). Differential adhesion in morphogenesis: A modern view. *Current Opinion in Genetics and Development*, 4(17), 281–286.
- Stern, C. D., & Bellairs, R. (1984). The roles of node regression and elongation of the area pellucida in the formation of somites in avian embryos. *Journal of Embryology and Experimental Morphology*, 81, 75–92.
- Stern, C. D., & Vasilias, D. (1999). Segmentation: A view from the border. *Current Topics in Developmental Biology*, 47, 107–129.
- Stopak, D., & Harris, A. K. (1982). Connective tissue morphogenesis by fibroblast traction. I. Tissue culture observations. *Developmental Biology*, 90, 383–398.
- Taber, L. A. (2009). Towards a unified theory for morphomechanics. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 367(1902), 3555–3583.
- Tam, P., Meier, S., & Jacobson, A. (1982). Differentiation of the metameric pattern in the embryonic axis of the mouse ii. somitomic organization of the presoditic mesoderm. *Differentiation*, 109–122(1–3), 183–224.
- Ten Tusscher, K. H. W. J. (2013). Mechanisms and constraints shaping the evolution of body plan segmentation. *The European Physical Journal E*, 36(5), 54.
- Thompson, D. (1963). *On growth and form*. Cambridge University Press.
- Thouless, M. D., Li, Z., Douville, N. J., & Takayama, S. (2011). Periodic cracking of films supported on compliant substrates. *Journal of the Mechanics and Physics of Solids*, 59(9), 1927–1937.
- Triantafyllidis, N., & Aifantis, E. C. (1986). A gradient approach to localization of deformation. i. Hyperelastic materials. *Journal of Elasticity*, 16(3), 225–237.
- Triantafyllidis, N., & Bardenhagen, S. (1993). On higher order gradient continuum theories in 1-d nonlinear elasticity. Derivation from and comparison to the corresponding discrete models. *Journal of Elasticity*, 33(3), 259–293.
- Truskinovsky, L. (1996). Fracture as a phase transition. In R. C. Batra, & M. F. Beatty (Eds.), *Contemporary research in the mechanics and mathematics of materials*. CIMNE (pp. 322–332).
- Truskinovsky, L., & Zanzotto, G. (1996). Ericksen's bar revisited: Energy wriggles. *Journal of the Mechanics and Physics of Solids*, 44(8), 1371–1408.
- Turing, A. (1952). The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London Series B*, 237, 37–72.
- Urdu, S. (2012). On the evolution of morphogenetic models: Mechano-chemical interactions and an integrated view of cell differentiation, growth, pattern formation and morphogenesis. *Biological Reviews of the Cambridge Philosophical Society*, 87(4), 786–803.
- Vainchtein, A. (1999). Dynamics of phase transitions and hysteresis in a viscoelastic Ericksen's bar on an elastic foundation. *Journal of Elasticity*, 57, 243–280.
- Vainchtein, A., Healey, T., & Rosakis, P. (1999). Bifurcation and metastability in a new one-dimensional model for martensitic phase transitions. *Computer Methods in Applied Mechanics and Engineering*, 170(3), 407–421.
- Vainchtein, A., Healey, T., Rosakis, P., & Truskinovsky, L. (1998). The role of the spinodal region in one-dimensional martensitic phase transitions. *Physica D: Nonlinear Phenomena*, 115(12), 29–48.
- Vaughan, B. L., Baker, R. E., Kay, D., & Maini, P. K. (2013). A modified Oster–Murray–Harris mechanical model of morphogenesis. *SIAM Journal on Applied Mathematics*, 73(6), 2124–2142.
- Vianay, B., Käfer, J., Planus, E., Block, M., Graner, F., & Guillou, H. (2010). Single cells spreading on a protein lattice adopt an energy minimizing shape. *Physical Review Letters*, 105(12), 128101.
- Waddington, C. H., & Deuchar, E. M. (1953). Studies on the mechanism of meristic segmentation: I. The dimensions of somites. *Journal of Embryology and Experimental Morphology*, 1(4), 349–356.
- Wiebe, C., & Brodland, G. W. (2005). Tensile properties of embryonic epithelia measured using a novel instrument. *Journal of Biomechanics*, 38(10), 2087–2094.
- Wilson, P. A., Oster, G., & Keller, R. (1989). Cell rearrangement and segmentation in *Xenopus*: Direct observation of cultured explants. *Development*, 105, 155–166.
- Wolpert, L. (1969). Positional information and the spatial pattern of cellular differentiation. *Journal of Theoretical Biology*, 1(25), 1–47.
- Wolpert, L. (1971). Positional information and pattern formation. *Current Topics in Developmental Biology*, 6(6), 183–224.
- Wolpert, L. (2011). Positional information and patterning revisited. *Current Topics in Developmental Biology*, 1(269), 359–365.
- Wyczalkowski, M. A., Chen, Z., Filas, B. A., Varner, V. D., & Taber, L. A. (2012). Computational models for mechanics of morphogenesis. *Birth Defects Research. Part C*, 96(2), 132–152.