Embracing Laws for Developmental Successions

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Abstract: - A model permitting to derive each next embryonic shape from a preceded one is suggested. It implies that each embryonic shape is associated with a definite pattern of mechanical stresses having a tendency to be hyperrestored (restored with an overshoot) after any deviation from the initial value. We describe several morphomechanical feedbacks deduced from the model and apply them to gastrulation of amphibian embryos and to other morphogenetic processes, including the main types of Metazoa development. We relate this model to Van der Pol non-linear equations and discuss the role of genetic and epigenetic parameters in modifying hyper-restoration responses.

Key-Words: Self-organization - Morphogenesis – Mechanical stresses – Gastrulation – Shape models.

1 Introduction

Suggest that we observe a prolonged chain of events which repeats, may be with some deviations, for a lot of times, and we want to know why this happens. A human mind developed two alternative approaches for resolving such problems. By first of them, this chain should be split into a number of "cause-effect" links, each one of them being governed by a specific factor, unique for this very link and being free for having something in common with other links. Within the framework of this approach, in order to apprehend such a chain one should discover and enumerate, one after one, all of these specific factors. A visual allegory of this approach might be a route of an old-fashioned postman, delivering letters to their destinations. It is quite obvious, that the postman's trajectory consists of a number of mutually independent steps, which are determined by the letters' addresses alone and which do not have, in general, anything in common with each other.

Another approach invites us to search for a common dynamic law, invariable for an entire given succession, or even for an extended family of such successions. This approach in no way excludes the exploration of "specific causes", but strives to formulate all of them, so to say, in the language of this law. It is widely known, that this is a dominated methodology of the physical sciences, making them indisputable leaders of the human's knowledge. And, if remembering Maxwell's [1] definition of physics ("Physical Science is that department of knowledge which relates to the order of nature, or, in other words, to the regular succession of events"), a person, unknown with the modern situation in developmental biology, will firmly believe that such a law-centered approach can be definitely applied to this science as well.

Actually however, the situation is far from being so simple. The classical physics was never dealing with so prolonged, tortuous and complicated chains as those exemplified by space-temporal successions of developmental processes. Nobody can be sure a priori, that such chains can be embraced by any single law. Moreover, until the emergence of a self-organization theory (SOT) [2], a self-complication of spatial organization, observed almost during each next step of development, looked from a physical point of view as something incomprehensible.

On the other hand, a total, unbound domination of a "specific factors" approach led to grave contradictions and dead-locks in developmental biology. First and the main one of them came from a discovery of embryonic regulations, that is, embryos' capacity to develop into normally structured organisms out of single egg parts, fused eggs, and totally intermixed embryonic cells. These results showed unambiguously, that any "specific causes", whatever they are, cannot have any pre-established definite localizations. Another set of contradictions, unexpectedly arisen in the modern molecular cell biology is a far-going universality and hence non-specificity of developmentally important genes and signaling pathways: same or closely homologous genes and pathways participate in development of quite different embryonic rudiments, and vice versa: homologous rudiments of different species can be controlled by largely non-homologous sets of genes [3].

Therefore, even a comprehensive knowledge of the molecular level events taking place at a given developmental stage do not permits us to predict, what morphological structures will at any stage emerge.

Meanwhile, these shortcomings can be successfully overcome within the SOT framework. The matter is, that for the case of complex systems SOT denies classical deterministic notion of one-to-one (and hence "specific") cause-effect relations, replacing it by the principle of parametrically regulated non-linear feedbacks. The parameters' space-time scales are in an order or more greater than those of the system's variables, which behavior we are interested in. Therefore, the parameters *ex definitio* are unspecific. The role of parameters is very important: there are their values which determine the mode of a system's behavior (say, monotonous or oscillatory) and even decide whether the given system exists (have stable solutions) or do not exist at all (is unstable). However, taken *per se*, out of the context of the feedbacks which they control, the parameters are completely "blind". The information about the parameters values without knowing anything about the feedbacks' structure gives us nothing. The reverse is not true: knowing the structure of the feedbacks alone we'll become informed about a range of the system's possibilities, even if the real mode of the system's behavior will remain uncertain.

So far as genetic factors are equivalent and temporally constant in all somatic cells of a given species (that is, largely smoothed throughout both space and time), they perfectly fit the notion of parameters. Among the latter, they occupy the upper level (due to the greatest characteristic times and space dimensions) leaving several lower levels to epigenetic parameters, which occupy more restricted space-time domains, controlling within the latter gene expression and signaling pathways.

Another fundamental property of self-organizing systems, having a deep similarity with developing embryos, is their ability to establish de novo macroscopic order within large populations of microscopic entities lacking within themselves any signs of such an order. A famous law derived by Driesh from his discovery of embryonic regulations ("A fate of a part is a function of its position within a whole") can be applied to these inanimate systems to no less extent than to embryos. For example, in so called Benard "cells" (macroscopic dissipative structures supported by intense convection) the "fates" of individual molecules (i.e. whether they are involved into an upward or downward stream or are not involved into any stream at all) depend upon their macroscopic positions [2]. Within such a context, a notion of holistic determination, having earlier a vitalistic flavor, should be regarded as a universal property of all the far from equilibrium non-linear systems, both inanimate and animate. At the same time, it is a function of parameters, because by changing the parameter values quite different kinds of macroscopic order (say, different wave-lengths of periodic structures) or no order at all can be obtained.

2 Problem Formulation

Taking the above said into consideration, we may formulate our task as an attempt to reveal universal and robust enough non-linear feedbacks, able to derive each next developmental stage (regarded as a macroscopic entity) from a preceded one and opened at the same time for modulations by genetic and epigenetic factors. Up to now, two types of non-linear models have been elaborated for interpreting pattern formation in developing organisms. In the first one [4], the feedbacks were interpreted in terms of cross-catalysis, cross-inhibition and diffusion of specific substances. The second one, without neglecting a chemical component, emphasized the role of mechanical stresses, born by cells' motile activity, in establishing the morphogenetically important long-range feedbacks [5, 6]. As regarding our task, the second type of the models looks most promising, because its connections with the problem of shapes formation are much more direct and at the same time less specific than in the case of chemo-diffusion models.

3 Problem Solution

3.1. Mechanical Stresses (MS) in Cells and Developing Embryos.

The developing animal embryos of all the species studied in this respect were found to be mechanically stressed, the tension and compression MS being properly balanced [7-10]. By several estimations [11], the average stresses in embryonic cells lie within several dozens N/m^2 (=Pa) range while Young modulus can exceed hundred Pa. Worth mentioning however, these average values are concentrated in very narrow cell-cell contact zones, where they may reach $5x10^3$ Pa. This is enough to deform molecules of extracellular matrix and cell adhesion complexes. In embryos substantial MS occur since blastula stage, when they are generated by osmotically driven turgor pressure within blastocoel [11]. At the later stages precisely directed tensile and pressure MS are mostly created by cooperative

cell movements driven by actin-based mechanisms. At least in Vertebrate embryos, tensile MS are arranged according to definite 3D patterns, which remain topologically invariable during rather prolonged periods of development (blastulation, gastrulation, neurulation etc), but are drastically changed in between [7, 10]. Average MS values within embryonic tissues are within several N/m^2 range. Assuming however that cell-cell contacts to which the tensions are applied occupy no more than 1% of the total cell-cell surfaces, the real tensions may be in two orders greater [10, 12]. Relaxations or reorientation of normal MS patterns at the early stages lead to grave anomalies in subsequent development [10, 10].

3.2. Stress-mediated Feedbacks

There is a plenty of metabolic and structural networks and hence feedbacks in the living cells [13, 14], Many of them involve stress-generating molecular machines, most of which were shown to be mechanosensitive [12, 15]. Thus, a possibility to create feedback loops between the *passive* stresses, produced by outside forces and the *active* ones, generated by the machines themselves, is opened. Several kinds of such feedbacks have been postulated and partly approved: induction of active tissue contraction by its passive stretching [16]; inhibition of cells aggregation by cell-generated stretching of elastic substrate [5]; induction of contact cell polarization by relaxation of tangential tensions [6]; induction of exocytosis by membrane stretching and endocytosis by its relaxation [17]; stimulation of cell divisions by tension [18]. We suggest the rule which includes these reactions as particular cases [10, 19]:

- Whenever a change is produced in the amount of local stress applied to a cell or local region of tissue (regardless of whether this change in force comes from a neighboring part of the embryo or has been exerted by an experimenter), the cells or tissue will respond by actively generating forces directed towards the restoration of the initial stress value, but as a rule overshooting it (Fig. 1A$_1$, B$_1$).

According to this "hyper-restoration" (HR) hypothesis, if a sample is stretched by an external force, it tends to diminish actively the stretching even up to the point of generating the internal pressure stresses. In the case of free edges and/or small resistance to its shrinkage it will do the same by contraction, as postulated in [16].

Meanwhile, if the edges are firmly fixed (which is usual for embryonic tissues) the only way for reaching the same mechanical result at the tissue level would be cells rearrangement (a so-called cell-cell intercalation), Fig. 1A$_2$. Correspondingly, if a sample is relaxed or, moreover, compressed, it will tend to "hyper-restore" its initial tension either by active cells spreading (in the case of free edges), or (if the edges are fixed) via tangential contraction (Fig.1B$_2$) and/or by immigration of some cells.

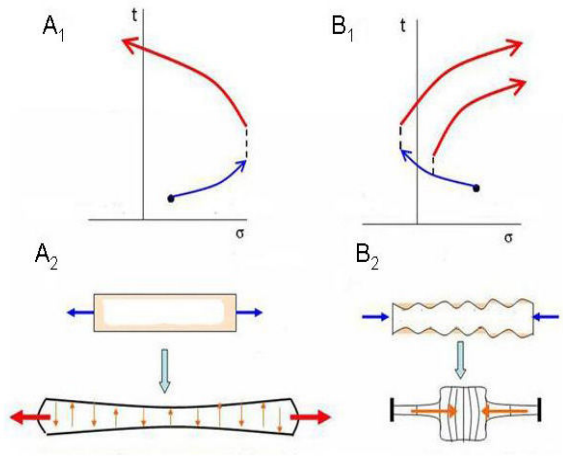

Fig. 1. Schemes (A$_1$, B$_1$) and illustrations (A$_2$, B$_2$) of the active HR responses (red) to the shifts of mechanical stress values (blue). In A$_1$, B$_1$ stresses σ are plotted along horizontal axes (stretching to the right, relaxation/compression to the left), and time (t) along vertical axes. A$_2$: intercalation response to a stretching of a sample with fixed edges. B$_2$: tangential contraction/perpendicular elongation of a cell group within a relaxed/compressed sample.

Similar reactions are taking place at the level of single cells and cell-cell contacts. For example, a moderate cell's stretching leads to increase, while reduction of tensions results in decrease of cell-cell contact areas [20]. So far as tensile stresses applied to cell contacts are inversely proportional to the latter's areas, the both reactions are obviously directed oppositely to stress shifts caused by external forces.

Importantly, these reactions have themselves an intrinsic tendency to be coupled into the second order feedbacks. For example, if a part α of a sample actively contracts, the adjacent part β will be passively stretched (Fig.2A), responding to this by cell intercalation (Fig.2B). The intercalation-mediated β extension will, in its turn compress α, inducing its further contraction. We denote this loop as a contraction-extension (CE-) feedback. A particular case of CE-feedback should take place within an epithelial layer of non-zero curvature, passively bent by an external force (Fig 3A). In this case the convex surface of a layer will be passively stretched, responding to this by active extension, mediated probably by the radial cells insertion.

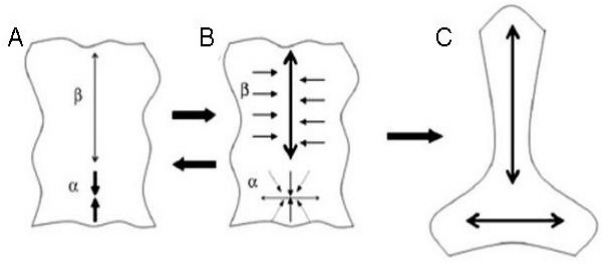

Fig. 2. A scheme of contraction-extension (CE-) feedback. States A and B can numerously exchange each other. A final state may look like C.

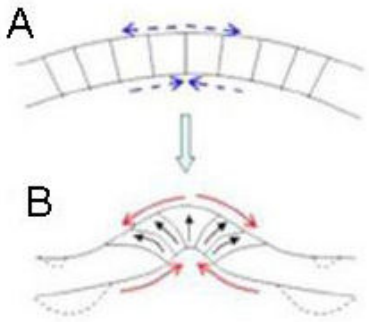

Fig. 3. Active reinforcement of the passively initiated bending due to the action of curvature-increasing (CI) feedback. Dashed blue arrows denote external stresses while red arrows the active responses. Black curved arrows: expected cell migration.

At the same time, the concave side will be passively compressed, responding by active contraction. As a result, the passively imposed bending will be actively reinforced (Fig. 3B). We call this reaction a curvature-increasing (CI-) feedback loop.

Lastly, suggest that two or more cells or entire cell layers (α and β) contact each other by their extensible surfaces. Now, if α unit actively extends the β unit will be firstly passively stretched and then actively extended, stretching now α unit, etc. In this way the both units mutually enhance each other extension along their common contact surface. This is an extension-extension (EE-) feedback.

3.3. Experimental and Model Evidences of HR reactions

3.3.1. Experimental evidences of stretch-promoted active extension

A fluorescently labeled piece of a supra-blastoporal area of an early gastrula *Xenopus* embryo (Fig. 4A) has been stretched perpendicularly to its normal elongation axis up to a state shown in Fig. 4B. Within next several hours a labeled area spread throughout the entire sample (Fig. 4C) indicating active cell rearrangements in the direction of the imposed stretching. Similarly, the pieces of ventral ectoderm stretched in arbitrary direction took various dumb-bell shapes indicating generation of internal pressure oriented along stretch direction (Fig. 4D-F) [19].

3.3.2. Experimental evidences of relaxation-promoted tangential cell contraction and cell immigration

Relaxation of circumferential tensions in the ectoderm of early gastrula *Xenopus* embryos leads to the apical contraction of a large fraction of ectodermal cells and to the corresponding differentiation of initially homogeneous cell net (Fig. 5, cf A and B) [21].

3.3.3. Active increase of the imposed curvature

A sandwich of a ventral ectoderm from *Xenopus* early gastrula has been folded by inserting it into a vertical slit made into an agarose bed (Fig. 6A). Within next three hours the opening has been gradually closed and in 3 h transformed into a narrow slit (Fig. 6 B, C). In other words, a spontaneous increase of the imposed curvature took place [19].

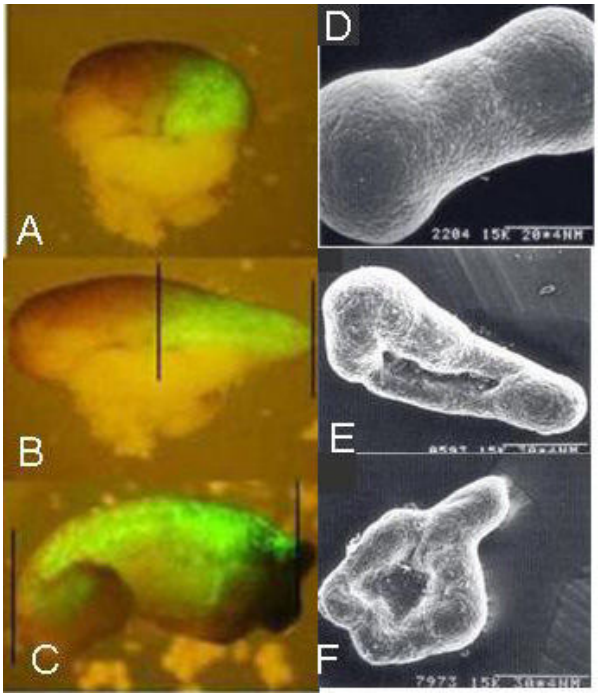

Fig.4. Examples of active stretch-generated extension associated with cells rearrangements. A-C: transversal extension of partly labeled sandwiches of a suprablastoporal area. D-B: dumb-bell shapes produced by the pieces of ventral ectoderm stretched in arbitrary direction [16].

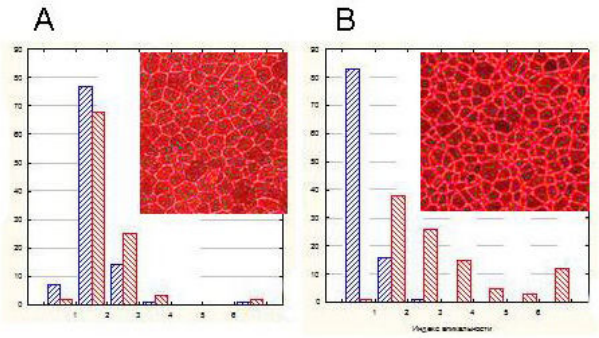

Fig.5. Histograms of apical indexes, A_i (apical/radial diameters ratios) in ventral ectodermal cells in few minutes (A) and 2h (B) after relaxation (red boxes) as compared with A_i of same stage intact embryos. Vertical axis: percent of cells. Insets: planar views of ectodermal fragments from relaxed embryos.

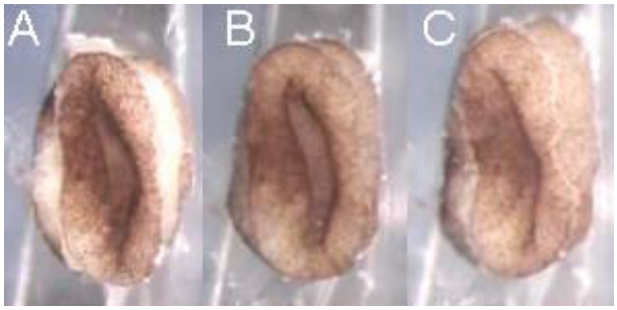

Fig.6. Spontaneous curvature increase of the artificially bent ectodermal sandwich. A: just after bending, B and C: 1 h and 3 h later, correspondingly.

3.3.4. Modeling of CE and CI feedbacks

Morphogenetic consequences of contraction-extension (CE) feedback have been modeled [22] under different values of the parameters controlling:

(1) threshold value of a tensile stress triggering the active extension response. We denote this parameter as a global threshold, GT, and take $0<GT<1$.

(2) amount of the active extension response.

GT parameter turned out to be crucial for determining the resulted patterns. Namely, under high GT values an initially homogeneous cell layer became segregated into single domains of tangentially contracted (columnar) and extended (flattened) cells (Fig. 7A). Under progressive GT diminishment, the length of the columnar domain was at first reduced (Fig. 7B) and then split into an increased number of smaller domains, imitating a segmentation process (Fig. 7C, D). Under even lower GT values the arisen patterns became non-stationary and transformed into perpetually running waves of cell contraction-extension. By varying the parameter (2), different segmentation patterns could be reproduced.

By modeling curvature-increasing (CI) feedback, we had into mind, first of all, the morphogenesis of lower invertebrates, hydroid polyps, which consists of a series of quite regular and species-specific osmotically driven pressure impulses (growth pulsations, GP) deforming an elastic outer skeleton (perisarc). Correspondingly, we imitated different biologically realistic GP patterns, introduced the elasticity parameter W ($0<W<1$) and the parameter related to a bending rigidity of a cell layer [16]. Under a wide enough range of the parameters values, we were able to generate the shapes of hydroid colonies rather similar to the real ones (Fig. 8B, C) from simple initial shapes imitating hydroids stems (Fig. 8A). At the same time, the shapes generated under non-periodic regime (Fig. 8D) as well as under extreme W values (Fig. 8E, F) were far from being realistic. In particular, under high W values smooth extended cusps (Fig. 8E) while under low W values closely interwoven bundles (Fig. 8F) were produced.

Same kind of modeling with the same set of parameters could be used for generating biologically realistic shapes of a definite radial symmetry order out of circular shapes. In all these cases a small local deviation from a precise circular shape was enough for triggering an entire series of folds whose radii and wave-lengths were determined by the parameters values. While some sets of parameters produced stationary shapes with a precise radial symmetry, others generated non-stationary dissymmetric shapes [19].

The main results of the modeling based upon the presumptions of CE and EE feedbacks have been confirmed elsewhere [23].

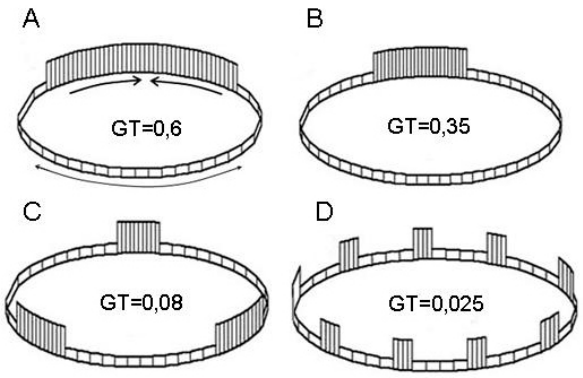

Fig. 7. Generation of one-domain and multi-domain (metameric) patterns out of a homogeneous precisely circular cell layer under different values of GT parameter (shown by figures) [22].

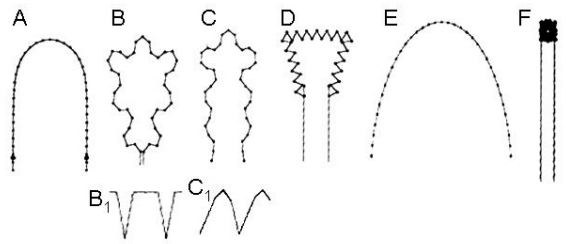

Fig. 8. Various shapes produced out of a shape A under different periodicity of pressure impulses and different values of W parameter. B, C: realistic shapes generated under periodic regimes B_1 and C_1 correspondingly. D: a shape generated under constant pressure. E, F: figures generated under high and low W values correspondingly. From [19], modified.

3.4. Gastrulation in amphibian embryos, regarded as a chain of morphomechanical feedbacks.

Now we come to our main task which is in representing a real complicated morphogenetic process as a succession of the above described morphomechanical feedbacks, each previous one triggering the next. For doing this, we took a well-studied morphogenetic process playing a central role in development, namely gastrulation [11]. In amphibian embryos the entire gastrulation process can be divided into several steps: (1) radial cell intercalation (RCI) in the blastocoel roof, actively extending this part of embryo; (2) formation of apically contracted bottle-shaped cells in the dorsal part of the marginal zone; (3) convergent (ventro-dorsal) cell intercalation (CCI) in the suprablastoporal area; (4) invagination of the bottle-cells area; (5) coordinated antero-posterior extension of pre- and postinvoluted parts of the suprablastoporal area. Our interpretation, based upon HR hypothesis, is in following.

(1) RCI is regarded as HR response of the blastocoel roof to its stretching by the increased turgor pressure in the blastocoel (Fig.9a, b, α). Indeed, RCI is completely hampered by the decrease of turgor pressure [11].

(2) Formation of bottle-shaped cells in the marginal zone is interpreted as HR reaction to the relaxation/slight compression of this zone by the actively extended blastocoel roof (Fig.9b, β). This is confirmed by relaxation-induced apical cell contraction (Fig. 5).

(3) As a next step, a powerful CE feedback is

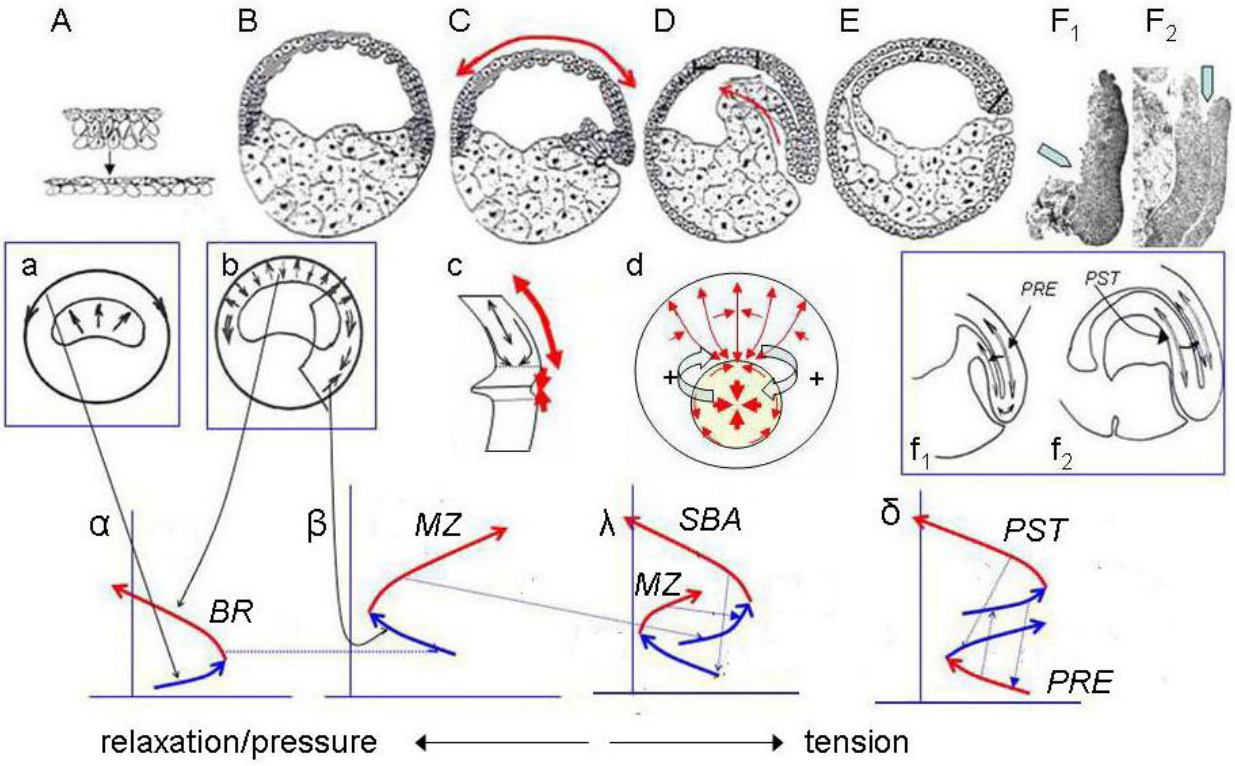

Fig. 9. Successive steps of *Xenopus* gastrulation (upper row) interpreted as a chain of morphomechanical feedbacks. Middle row frames are the maps of cell movements and the associated mechanical stresses. Lower row frames α - δ are the diagrams of mutually coupled feedbacks (active stresses are denoted by red and passive stresses by blue). BR: blastocoel roof; MZ: marginal zone; PRE: preinvoluted zone; PST: postinvoluted zone; SBA: suprablastoporal area. In α - γ frames these legends accompany active stresses of the given embryonic areas. From [11], modified.

established between the marginal zone (MZ) and the suprablastoporal area (SBA) (Fig. 9C, D, γ): apically contracted MZ cells stretch SBA, triggering active CCI-mediated SBA extension; the latter event further relaxes/compresses MZ, triggering further contraction in this area, and vice versa. This interpretation is supported by a number of data, including immediate canceling of gastrulation movements by relaxation of tensions within SBA [10, 11].

(4) Invagination of a blastoporal area (Fig. 9C, c) is regarded as a consequence of the curvature-increase feedback. The initial MZ curvature has been imposed by its RCI-mediated compression.

(5) Further involution is regarded as a consequence of elongation-elongation (EE) feedback between pre- and postinvoluted cell layers, *PRE* and *PST* correspondingly (Fig. 9 f_1, f_2, σ). At first the active role is played by PRE (identical to SBA) which stretches PST. The latter's stretching is confirmed by its immediate contraction after being detached from PRE (Fig. 9F1). At the later stage, meanwhile, the detached PST extends rather than contracts, indicating the rise of intercalation-mediated internal pressure.

4. Discussion

The above presented reconstruction of amphibians' gastrulation supports the idea that prolonged enough successions of developmental events can indeed be embraced by a common morphomechanical rule (HR model), acting onto a macroscopic level. The main points for further discussion are:

- Is this model robust enough to be extrapolated to different types of development?
- Can it be applied to the processes of cyto-differentiation, rather than to a morphogenesis in its strict sense?
- What might be the way for an adequate mathematical elaboration of HR model?

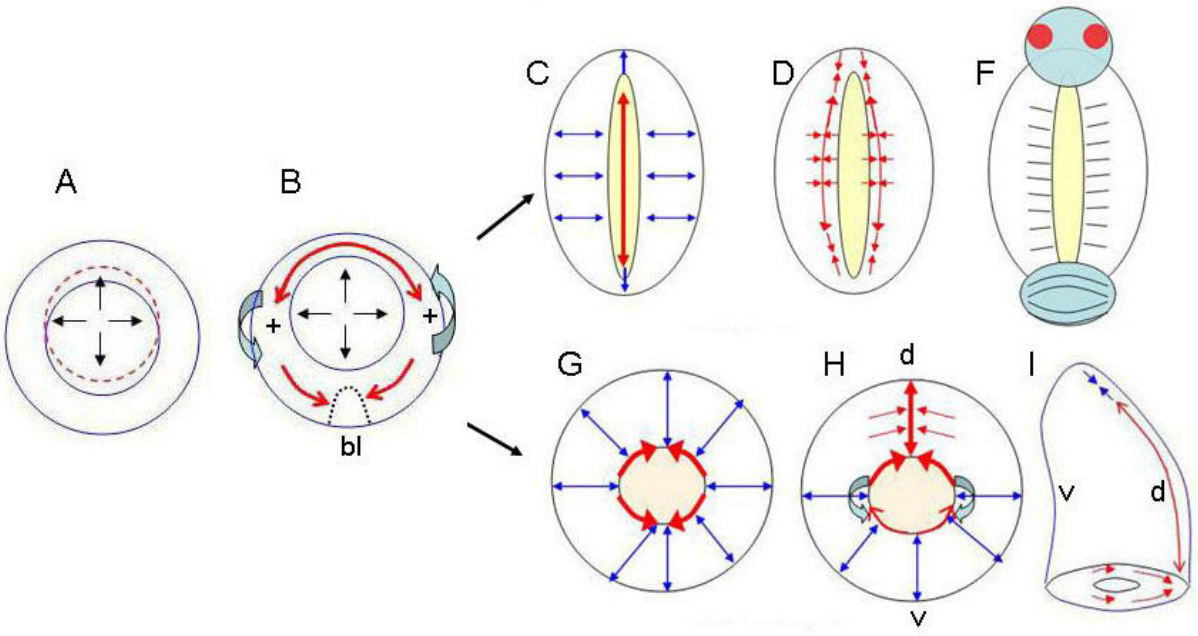

Fig. 10. Two main types of Metazoan gastrulation interpreted within the framework of HR model. A: a slight eccentric shift of a turgor pressurized cavity (dashed contour) at the blastula stage; B: a resulted inequality of mechanical stresses in the opposite areas of blastula wall triggers a contraction-extension (CE) positive feedback (blue curved arrows). bl: future site of a blastopore. C-F: development of Protostomia with a slit-like blastopore. Note the formation of the actively extending areas laterally to blastopore and compression zones on its poles (D). The latter are transformed into head and proctodeum regions (F). G-I: development of Deuterostomia with the actively contracted circular blastopore. At stage H a second order CE feedback is established along the blastopore circumference (cf Fig. 9d). d: dorsal, v: ventral side. Straight blue arrows indicate passive MS and red arrows indicate active MS.

4.1. "Generalized gastrulation and formation of the main body plans.

As follows from HR model, a spherically symmetric embryonic shape (a blastula) with precisely centered pressurized cavity inside should be unstable: if a cavity undergoes even a small eccentric shift, the tensile stresses in the thinner part of a blastula wall will exceed those in the remaining part. Accordingly, a CE-feedback should be established between the both parts: the thinner part will be actively extended due to cell intercalation, while the resting part should respond to its relaxation/compression either by immigration of some cells, or by invaginations of different geometries, or by a combination of the both processes (Fig. 10A, B). As a result of such instability, we inevitably get a transition of a blastula-like shape into any one of several gastrulae types known for Metazoans. Among those, the domination of immigration response requires a low cell-cell adhesion, while epithelial invaginations indicate stronger cell-cell connections.

Two main geometric types of epithelial invaginations are known in Metazoa: slit-like (typical for Protostomia) and round/circular blastopores (dominating in Deuterostomia, including lower Vertebrates). A slit-like invagination (a straight fold) is geometrically the smoothest one, easily achieved by a proliferating pressure of the surrounding cells, without requiring any accurately arranged and finely regulated contractions of the blastoporal cells. The second type meanwhile is not so smooth and requires just such contractions. The morphomechanical consequences of different types of gastrulation largely deviate from each other. Gastrulation going on via cells immigration does not create any substantial tensile fields around. A slit-like blastopore produced by proliferating cells pressure generates a tangential pressure on its opposite poles and some tensions oriented perpendicularly to a slit (Fig. 10 C-F). Meanwhile, a circular actively contracted blastopore becomes a focus of a powerful tensile field which may spread throughout an entire gastrula surface (Fig. 10G). Even more important is that in this case a second order CE feedback is established along the entire blastopore periphery, segregating this latter into the domains of tangentially contracted and extended cells (Fig. 10H; cf Fig. 7A, B; Fig. 10d, λ). Due to this feedback, a dorso-ventral polarity in the early Deuterostomia embryos is established.

4.2.Coupling morphomechanics with cell differentiation.

Several recent findings, e.g. [24] indicate that at least some of developmentally important genes are mechanosensitive, and hence depend upon morphogenesis-generated stresses. Recently we traced the expression patterns of neural and mesodermal genes in the artificially bent double explants of a suprablastoporal tissue of gastrula stage amphibian embryos [25]. Firstly, we confirmed (see Fig. 6 and the corresponding comments), that the imposed curvature of explants has been actively increased; second, and most important was that in a great majority of cases the areas of the neural genes expression were located on the concave (compressed) while those of mesodermal genes on the convex (stretched) sides of explants. Remarkably, in spherical explants, where mechanical stresses were evenly distributed, the location of the both kinds of rudiments was quite variable and even chaotic. One may conclude that the chemical inductive agents concentrated in the suprablastoporal areas are themselves not enough for setting up precise spatial patterns of the induced rudiments; this job is made by mechano-geometry, either provided by normal morphogenetic movements, or artificially imposed.

4.3. Van der Pol equations as an adequate model for HR responses.

A well known family of Van der Pol equations describes a set of non-linear oscillatory processes, either with or without stable nodules, and includes two variables: a slow variable x and a fast variable y. We take that version of Van der Pol equations which provides two stable nodules:

$$dx/dt = y - Kx - B \qquad (K > 0)$$
$$\varepsilon\, dy/dt = -(Cy^3 + Ay + x) - D \qquad (A < 0)$$

A phase portrait of this equations system with two stable nodules P and Q (situated at the intersections of the both zero isoclines) is given in Fig. 11. .
We plot the mechanical stress values along the horizontal axis and represent the perturbations which are caused by external forces and directed away from a closest nodule by short blue arrows. At the same time the shifts along the vertical axis display the relatively fast switches of mechanochemical parameter(s) responsible just for HR responses, that is, either for a rise of internal compression stresses after external stretching or

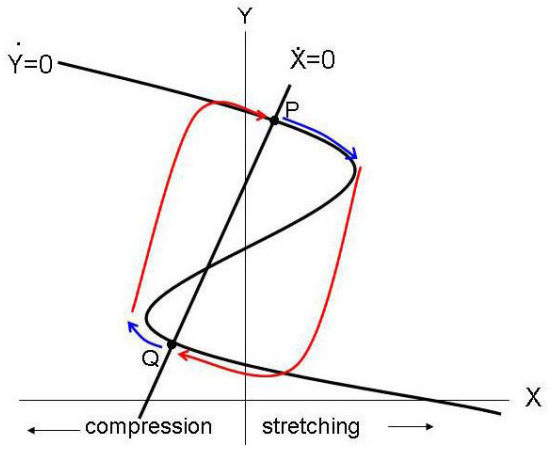

Fig. 11. Phase portrait of Van der Pol equations with two stable nodules P and Q.

for rise of tensile stresses after external relaxation/compression. These responses are shown by curved red arrows. One can see that due to inclination of *x*-zero isocline from lower left to upper right (the opposite inclination direction should make the both nodules unstable and hence the entire system non-existing) the horizontal shifts during active responses will always exceed the external perturbations, which is just what we call a hyper-restoration.

It is also clear, that the changes in the parameters values should largely modify HR responses, bringing thus far-going morphological consequences. For example, a shift of a nodule away or towards the closest bending point of *y*-zero isocline (due to changes in *K* and/or *B* parameters values) is identical to the changes in the values of the threshold parameter which we defined earlier as GT (see 3.3.4) and which was shown to affect profoundly the resulted morphology (Fig. 7). On the other hand, since the model's parameters keep constancy both in space and time, they well may be attributed either to genetic and/or slow and smoothed epigenetic factors. Being "blind" out of the model's context, they acquire within such a context quite a definite meaning.

Worth mentioning, Van der Pol equations reproduce just a single HR response, rather than a consecutive series of such responses which comprise, by our suggestion, a real morphogenesis. Correspondingly, the equations themselves are not directly related to morphogenesis. In order to establish this relation, we must introduce in our construction one more set of events, namely embryonic shapes, which may be at the beginning quite simple (see Figs 9, 10). Each shape serves as initial (border) conditions for the next round of shape changes, governed by the same HR mechanics. Hence, by a suggested view, a succession of embryonic shapes should be regarded as a series of discrete reflections X_{n+1}, X_{n+2} … $[X_{n+1} = f(X_n)]$, rather than, say, consecutive values of a continuous function. In biological terms, the suggested concept fits a strong statement made several decades ago by two independent thinkers [26, 27] about the possibility to derive each next embryonic shape from the previous one ("form out of form"). HR hypothesis suggest a biophysical mechanism permitting to make such a deriving.

It may be also of interest to compare HR model with Le Chatelier principle, which claims that any physico-chemical system shifted from thermo-dynamic equilibrium tends to return towards its initial state. HR responses are started just from such reactions, but continue them by performing overshoots, which is never the case for the classical Le Chatelier responses. In the other words, HR reactions prolong Le Chatelier trajectories (in the phase space) in the out of equilibrium direction. Hence, HR reactions can be considered as natural extensions of Le Chatelier responses for the systems characterized by a constant inflow of energy.

5. Conclusions

Modern biology achieved a great progress in revealing numerous regulatory feedbacks and networks acting on a molecular - supramolecular level [13, 14]. Much less attention has been paid however to the emergence of some of these feedbacks on a macroscopic (supracellular) level [28]. Meanwhile, without such an emergence (inherent in self-organizing systems) no large scale dynamic order so typical for the living beings could be established. The formation of coherent macroscopic fields of mechanical stresses based upon the activity of multiple miniature molecular – supramolecular devices is an important example of such an emergence.

The above presented HR model should be regarded as an attempt to formulate the macroscopic laws for mechanodependent morphogenetic processes. It is still a matter of discussion whether this model can be applied not only to embryonic morphogenesis proper but also to several categories of morphogenetically related processes with different characteristic times [29]. Extreme examples of such processes are postembryonic growth and wound healing [30]. In any case however, taking into consideration an increased role of cell and tissue mechanics for bioengineering applications [31, 32] one can hope that HR model may be also of use in biotechnology.

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