Integrating Mechanical Control Theory into Models of Biological Development - Analytical Review

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Summary
This paper presents both a general review on developmental biomechanics and a concrete proposition for the computation of a symmetry breaking instability of a model of biological development in terms of self-organization theory. The necessary biological and physical facts taken from the literature are described and discussed in the context of a unified statement of the problems for mathematical modeling of pattern formation. This is then applied to planar cell polarization of the *Drosophila* wing. In this way the process is modeled by an elasto-polarization equation. In terms of this statement, the mechanical specificity (interaction with basal plate) of wing planar cell polarization is characterized. Some aspects of modeling somite formation as well as other developmental processes are also concerned.

1. Introduction

We start from the point of view that the epistemological principle of classical Laplacian determinism cannot explain developmental processes either in living or nonliving natural systems. One remarkable attempt to go beyond classical determinism in biology is Driesch's theory of vitalism (Oppenheimer, J.M. 1970), but we accept here, that only Self-Organization Theory (SOT) gives a broad enough conception for treating the problem of morphogenesis in living systems.

In the recent past a large number of theoretical models have been published in the field of developmental biology, which are classified in (Held,L.I.Jr. 1992). The models can be grouped into two rather distinct classes: biochemical and biomechanical ones. We can present this classification by the following scheme:

biochemical models: ***biochemistry*** $\longrightarrow$ ***morphogenesis*** $\longrightarrow$ ***biomechanics***
biomechanical models: ***biochemistry*** $\longrightarrow$ ***biomechanics*** $\longrightarrow$ ***morphogenesis***

Similar understanding can also be found in (Oster,G.F. et al. 1983; Oster,G.F. et al. 1985, Oster,G.F. et al. 1988). A basic assumption in the bio-chemical modeling is the existence of specific biochemical substances, which act as morphogens and signaling molecules. They react to other substances in accordance with corresponding rules of their kinetics and propagate (diffuse) in correspondence with physical laws. As a result they create chemical concentration patterns that precede morphological ones. The famous Turing theory (Turing, A. 1952), despite of having no direct parallels in biology, greatly affected the entire scientific community by increasing the interest in applying the theory of morphogenesis in a form of SOT to biological development. From Turing model of chemical morphogenesis we can turn directly to models of biological morphogenesis (development), firstly to chemo-kinetic models (represented most clearly by those of Gierer and Meinhardt (Meinhardt,1982) and then to those that include mechanical components (Belintsev et al, 1987). Mainly this approach is treated further in this review. However some more recent considerations are also taken into account in our approach.

To understand the self-organizational relationship between mechanical and informational components of these phenomena we need to test corresponding hypotheses in the framework of appropriate mathematical models. In (Oates,A. et all. 2009), the importance of mathematical modeling to integrate quantitative biological information into the morphomechanics of developmental processes is described. Certainly, in the modern literature the idea that gene expression informationally controls all processes is dominant (Caussinus et al., 2008). However there are many authors (Ingber, D. E. 2006, Keller, R. et all. 2008, Lecuit, T. & Lenne, P. F. 2007) recently developed the idea that mechanical forces can also govern cell behaviour in development.

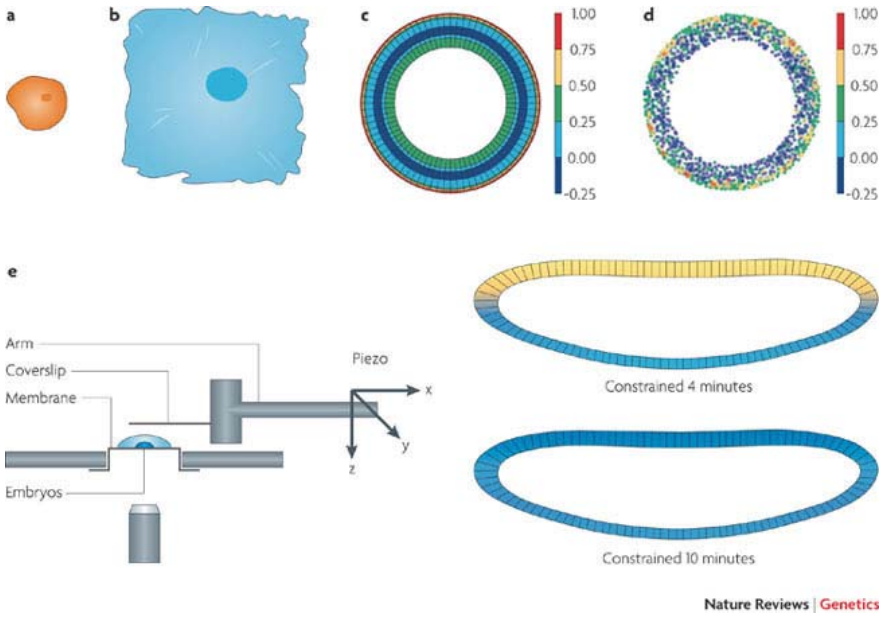

Figure 1. Mechanosensing in single cells (taken from Oates,A. Et al. 2009)

As it is seen in Figure 1, when some patterned substrates are used to control cell shape and their spread, two different developments are possible: (i) small and round cells enter apoptosis; (ii) spread-out cells proliferate and differentiate. More details on this point can be found in (Chen, C. S. et al. 1997, Singhvi, R. *et al*. 1994), where direct experimental evidence is provided that cell shape governs whether individual cells grow or die. So, the mechanical forces have in turn been shown to regulate gene expression and growth (Hamant et al., 2008).

Subsequently in this review, other examples for experimental evidence of a mechanical influence on development will be presented. In addition, here we take into account recently established biochemical conditions (Goldbeter,A. et al. 2007), in which mechanical effects can take place. By using these conditions, in author's recent work (Petrov, V. & Timmer, J. 2009) we propose a mechano-mathematical consideration of somite formation process with an emphasis on insights gained from exact qualitative modeling (in terms of geometrical theory of differential equations).

2. Mechano-Polarization Approach for Modelling Intercellular Elastic Interactions During Biological Development

The starting point of this topic is the *scaling invariance*, which corresponds to the observation, first made by Driesch in 1891, that any of the meridian blastomers of 2-cell or 4-cell sea urchin embryos are capable of forming a complete embryo, if cultured in isolation. One modern attempt to explain this point (scaling invariance) is the mechano-polarization model of intercellular interactions during morphogenesis (Belintsev et al, 1987). In that paper, the polarisation of embryonic cells is an active process accompanied by profound transformations of the cytoskeleton-associated intracellular structures. The experimental data are summarised in the form of following principal statements: (i) Individual cells of morpho-genetically active embryonic epithelia are mechanically active. They can deform themselves to acquire one of two discrete shapes iso-diametric or elongated normal to a layer surface, both being stable against small perturbations; (ii) The cell material of an epithelial tissue considered as a solid medium posseses passive elasticity in a purely mechanical sense; (iii) The mechanical activity of a given cell is modified by contact influences exerted by adjacent cells. The contact elastic interactions provide for the short-range co-operativity of cell polarisation; (iv) The elastic stresses in epithelium interfere with mechano-chemical activity. For instance, lateral transitions suppress cell polarisation normal to the cell sheet surface.

The mechano-polarization model, based on the above four statements is phenomenological, i.e. it does not assume any particular mechanism responsible for the active cell deformation. Instead, a scalar phenomenological parameter w is introduced as a function of space coordinate x and time t. By assumption, w equals zero if the mechano-chemical activity is switched off. Based on this general definition, in the simplest one-dimensional consideration, the observed features of the cell polarisation process may be rewritten as a kinetic equation:

$$\tau \frac{\partial w}{\partial t} = -\alpha w(w-1)(w-\beta) + D\frac{\partial^2 w(x,t)}{\partial x^2} - \kappa\sigma, \tag{2.1}$$

where τ is charactestic time of the process, σ is an *axial* elastic tension and κ is a coefficient of proportionality. When $\sigma > 0$, we say it is an axial *stretch* tension. For $\sigma < 0$ it is an axial *compression.* We use also the terms *positive* cross polarization when $w > 0$ and *negative* one for $w < 0$. D is a diffusion-like coefficient, α and β are parameters characterizing cell mechano-chemical activity.

The first term of the equation (2.1) presents the self-regulated character of the mechano-chemical activity; the second term - its transition between adjacent cells and the third term - its modulation by lateral stress in the sheet. The fact that one can observe two distinct classes of cell morphology allows one to assume the trigger-like control of the cell activity. One can simulate this by the *N*-like shape of the function

$$f(w) = -\alpha w(w-1)(w-\beta) \tag{2.2}$$

The graph of the function (2.2) is shown in fig.2.

The qualitative analysis made in (Petrov, V. & Timmer, J. 2009) shows that, if a traveling wave solution of (2.1) exists, then the sign of the wave velocity c is the same as the sign of the area under the curve $f(w)$ between the points 0 and 1. If this area is positive, then the traveling front propagates from the point 0 to 1, and the second point is called *dominant.* For sufficiently small values of β, the fixed point 1 is dominant. Thus in this case the polarization front propagates from the point 0 to 1. On the other hand, if β is close to 1, the fixed point 0 is dominant and the wave front propagates in the opposite direction (from 1 to 0). For some intermediate value of β, the situation would be neutral - neither right nor left direction of propagation is preferable. However, by appropriate slow dependence of β on w (through σ, which is proportional to w averaged), the neutral value of β could be made stable. Such a possibility is inherent to equation (2.1), where the tension σ can indeed be expressed to be proportional to w averaged. So, equation (2.1) is a closed integro-differential equation, which contains the principle property of a loss of the dominance of the state 0, changing dominance to 1, and vice versa. At the same time, state β is stabilized. In this way, (2.1) has the actual feature to tend towards a state of inhomogeneous distribution of cell polarity. This feature does not exist if the term of elastic tension in (2.1) is constant. Then the two states 0 and 1 are always stable and their destabilization is not possible, thus a stable inhomogeneous state is also not possible. By introducing the elasto-polarization dependence in (2.1), we actually involve a Turing bifurcation in the model.

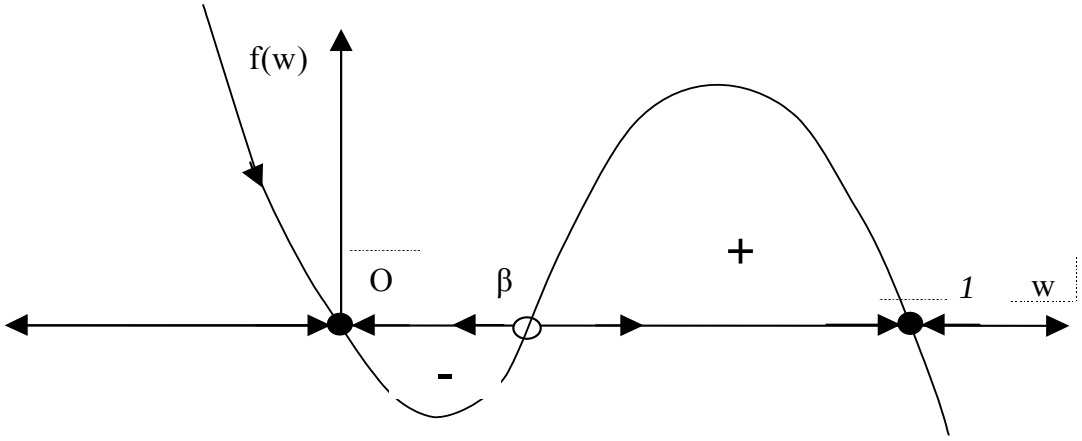

Figure 2. Graphical presentation of steady state points of (2.1) [Petrov,V.&Timmer,J. 2009]

One of the paradigmatic examples of biological development is the well-known process of somite formation in a bistability window of PreSomitic Mesoderm (PSM). Besides the universal sense of this example of biological development, the somite formation has also a vast underlying basis of biochemical, cytological and physiological investigations in the recent literature from the beginning of this millennium up to the essential study of Goldbeter,A. et al. 2007, and the last author's paper (Petrov,V.&Timmer,J. 2009) on the subject.

It is argued that somitogenesis is a *robust* process based upon the mechanical relationship between a long-range tension force stretching the axial mesoderm and short-range forces of pre-somite cell cohesion (Beloussov,L.V. 2001). In the dynamical theory context the terms *robust* and *structurally stable* have one and the same sense (Nikolov,S. et al. 2006). It is of interest therefore to analyze stability in terms both of Lyapunov's definition and structural one and to interpret it in the context of

somitogenesis robustness. Both polarized and non-polarized states should be considered as stable ones by analogy with the sharp developmental thresholds defined through bistability by antagonistic gradients of Retinoic Acid (RA) and Fibroblast Growth Factor (FGF) signaling (Goldbeter,A. et al. 2007).

In the model (2.1), cell polarization can be transmitted by contact from a given somite cell to its neighbor one. Moreover a mutual relationship between cross polarization and axial elastic tension of the mesoderm is introduced. So, we call this the elasto-polarization model. In our paper (Petrov,V. and Timmer,J. 2009) we show that, as a result of the interaction of elastic forces, spontaneous and contact polarization, a traveling front of somatic cell polarization propagates through the bistability window of the mesoderm layer. The mathematical scheme of the above described mechanism has been developed in the paper of Belintsev, B. N. et al. (1987).

In order to approach to the main problem of transforming temporal oscillations in PSM into spatial pattern formation, we can add a corresponding clock-wise oscillator of the type of the cell cycle to the wave front equation (2.1). As is shown in (Petrov,V., Pfeifer,M.&Timmer,J. 2006), the following negative feed-back oscillator for the key-regulator variable u and dynamical mass variable v of the cell cycle process can be defined:

$$\frac{du}{dt} = -n^2(u+p)^3 + m(u+p) - l/n - ks(v+q)/n, \tag{2.3}$$

$$\frac{dv}{dt} = \left(n(u+p) - s(v+q)\right)/Ts\ . \tag{2.4}$$

The other constant parameters n, p, q, k, s in (2.3) are defined in the mentioned author's paper (Petrov,V. et al. 2006). This oscillator is qualitatively identical to the FitzHugh-Nagumo model (FNm) (FitzHugh,1960,1961; Nagumo et al. 1962).

It is shown, that the model (2.3-4) is remarkable with the critical role of the parameter k, which determines the transition from bi-stability to self-oscillatory behavior of the oscillator (2.3-4). For sufficiently large values of the parameter T, the key-regulator variable u is fast with respect to the mass growth rate v. This means in Slow and Fast Variables Approximation (SFVA) described in authors paper (Petrov,V., Nikolova,E., Wolkenhauer,O. 2006) in a particular case of Quasi-Steady-State-Approximation (QSSA), we can separately consider on the one hand the key-regulator u, as a switch of the polarization process described by (2.1), and on the other hand to treat the higher steady state value of polarization w as a switch of the clock-wise oscillations.

This possibility could be realized in the form of self-organizational theory of somite formation to explain the mechanism of transforming temporal oscillations into spatial patterns. For this purpose we connect equations (2.1) and (2.3-4) by introducing relations $\beta = 1 - \beta_0 u$ and $k = k_0 w$. Certainly, such additional relationships (making the constant coefficients β and k variable ones) need corresponding argumentation. Our basic consideration is that somite formation correlates with cycles of cell-autonomous gene expression that spread from the tail bud to the rostral PreSomitic Mesoderm (PSM) border with a *periodicity equal* to that of the somitogenesis (Palmeirim et al. 1997).

3. Modeling Planar Cell Polarization of *Drosophila* Wing. Statement of One- and Two- Dimensional Problems

The previous considerations suggest that the model (2.1) seems to posses some advantages which are of interest for a possible extension of the approach to other

cases, and applying it to other processes of biological development. In the case of pattern formation of wing cell polarity, we have again to consider the following items: The first one is in introducing mechanical feedback between local and global elastic events, one of which is exemplified by a single cell's tendency to actively polarize itself, and the other one is presented by cell to cell transmission of polarization via elastic interactions. The second one is exemplified by the overall passive elastic tension of a cell layer that prevents the further active elasto-polarization of its cells. As a result, an initially homogeneous cell layer can be segregated, independently of scaling, into domains of highly polarized and flattened cells.

3.1. One-Dimensional Model of Planar Cell Polarization. Statement of the Problem.

The process of planar cell polarization (PCP) occurs as a result of mechanical interaction between cells of epithelium and the basal plate. The corresponding pattern of polarized and non-polarized cells predetermines the further formation of the scale. It is of interest to establish possible relationships between the regular or irregular distribution of patterns on the one hand and the intensity of tangential stresses between adherens junctions and lamina (extra-cellular matrix) on the other. For this purpose we firstly write the one-dimensional equation of PCP in the form

$$\tau \frac{\partial w}{\partial t} = -\alpha w(w-1)(w-\beta) + D\frac{\partial^2 w(x,t)}{\partial x^2} - \kappa\sigma, \tag{3.1.1}$$

Here the notations have the same sense already explained in section 2.

The force of interaction between one-dimensional epidermis and basal plate is proportional to $-qu$, where u is the scalar displacement and q is a coefficient of proportionality characterizing the elastic properties of the basal lamina. The equation of mechanical equilibrium of this "one-dimensional epidermis" is

$$-qu + \frac{\partial \sigma^a}{\partial x} + \frac{\partial \sigma^e}{\partial x} = 0 \tag{3.1.2}$$

By taking into account the additional elastic connections in equation (3.1.1), we introduce a new quantity expressed with the parameter $R_e^{\,2} = E/q$. The parameter R_e presents the characteristic length of exponential decay of elastic tensions in the layer when a local source of active force σ^a exists. After some transformations of (3.1.2) and its substitution in (3.1.1) we obtain a closed equation of cell polarization dynamics of a"one-dimensional epidermis" in the form

$$\tau \frac{\partial w}{\partial t} = f(w) + D\frac{\partial^2 w}{\partial x^2} - \frac{\kappa\varepsilon}{2R_e}\int \exp\left\{-\frac{\left|x-x'\right|}{R_e}\right\} w(x',t)\,dx \tag{3.1.3}$$

Here ε is a proportionality coefficient between the elastic tension σ and planar cell polarity w. If we accept an initial state with switched mechano-chemical activity (i.e. - without cell polarity)

$$w = 0, \qquad \sigma = 0,$$

The stationary problem $\frac{\partial w}{\partial t} = 0$ of the equation (3.1.2) will be evidently satisfied, but by parametrical variation of α, $R_e = \sqrt{\frac{E}{q}}$ and $R_p = \sqrt{D\tau}$ the equation can be approached to the bifurcation point of loss of asymptotic stability with the appearance

of new, spatially modulated regimes of behavior. (The parameter R_p appears in (3.1.3) after its transition to dimensionless form).

3.2. Two-Dimensional Modeling Planar Cell Polarization. Statement of the problem

It will be interesting to elaborate on the mechano-polarization model (2.1) by applying a two-dimensional generalization to the problem of modeling pattern formation of wing cell polarization with mechanical instability. Following (Belintsev,B.N. et al. 1987) we consider a mathematically idealized epithelial sheet in a form of flat monolayer of cells. Cells are considered as elastic units, attached to each other and to a basal plate made up of extra cellular matrix material, mainly chitinous structures, which is essential as a substrate reacting to planar polarization forces. In the basic equation (2.1), the variable w has a sense of temporarily limiting the degrees of freedom for all processes laying in the active cell deformation. The function governing PCP dynamics takes into account only the fact of relative stability of two steady states of the cell, which can be distinguished at a morphological level (for example – isotropic and polarized states). This fact is modeled by the specific type of relationship $f(w)$, shown graphically in fig.2.

Let $w = 0$ describe an isotropic cell. Any elongation normal to the cell sheet has a positive value of w, and the polarization of cells, stretched longitudinally in the sheet plane, have a negative value of w. The one-dimensional equation of cell polarization (2.1) in two-dimensions takes the form

$$\tau \frac{\partial w}{\partial t} = -\alpha w(w-1)(w-\beta) + D.\Delta w(x,y,t) - \kappa \sigma_{ii}^{e}, \tag{3.2.1}$$

where Δ means Laplacian operator $\Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}$ in the plane (x, y). The other symbols have the same sense as in equation (2.1), but considered in the plane. In the two-dimensional case only the trace σ_{ii}^{e} of stress tensor σ_{ik}^{e} (rank 2) is essential for the cell elongation normal or longitudinal to sheet. So, shear stresses are neglected. But we distinguish two parts of stress tensor components: passive purely elastic (σ_{ik}^{e}) and active (σ_{ik}^{a}), connected with the polarization process. The mechanical steady state (equilibrium) condition is presented by the equation

$$-qu_i + \frac{\partial \sigma_{ik}^{e}}{\partial x_k} + \frac{\partial \sigma_{ik}^{a}}{\partial x_k} = 0. \tag{3.2.2}$$

The term $-qu_i$ corresponds to the elastic external connections of the considered cell layer with the basal lamina (q is the coefficient of rigidity of the dermal layer). The corresponding force is proportional to the displacement u. As in the one-dimensional case, the active stress component is linearly related to the polarization w under additional assumption for isotropic stress of compression or dilation. Formally this active stress – polarization relationship is expressed in the form

$$\sigma_{ik}^{a} = \varepsilon \delta_{ik} w. \tag{3.2.3}$$

Here ε is a phenomenological coefficient of proportionality, and δ_{ik} is the Kronekker symbol expressing the plane isotropic character of the tensions produced by the apico-basally (i.e. normally to the sheet) elongated cells. In view of these considerations, it

follows that in equation (3.2.1), the member $k\sigma$ has a sense of trace of the tensor $\sigma_{ik} = \sigma^e_{ik} + \sigma^a_{ik}$.

Following (Landau,L.D.&Lifshitz,E.M. 1960) we firstly take into account the relation

(3.2.4) $$\sigma^e_{ii} = \frac{E}{1-m} div \vec{u}$$

Then we apply operator div to equation (3.2.2) and obtain the equation of elastic and active stress in the form

(3.2.5) $$-U + R_e^2 \Delta U + a\Delta w = 0,$$

where

(3.2.6) $$U = \kappa\sigma^e_{ii}, \qquad R_e^2 = \frac{E}{q(1-m^2)}, \qquad a = \frac{\varepsilon\kappa E}{q(1-m)}$$

E and m are Young modulus and Poisson ratio of the cellular sheet respectively.

The system of nonlinear partial differential equation (3.2.1) and linear partial differential equation (3.2.5) presents a closed system for the variables $w(x,y,t)$ and $U(x,y,t)$ defined in an idealized quadratic epidermal sheet. The latter is considered as a flat monolayer of cells that are elastic units, so w characterizes the morphological state of a single cell. In this sense $w = 0$ corresponds to an isotropic (non-polarized) state of a cell, while any cell elongated perpendicular to the cell sheet has $w > 0$. Vice versa, cells stretched longitudinally in the sheet plane have negative values of w.

The equations (3.2.1) and (3.2.5) can be analyzed mathematically as a system of two partial differential equations.

4. SOT-Based Computation of Symmetry Breaking Instability of One-Dimensional Model of Planar Cell Polarization.

The above described one- and two-dimensional models of PCP can be analyzed in the framework of more formal approaches of Self-Organization Theory (SOT). Such pure mathematical approach is presented in the paper (Sattinger,D.H. 1979) and later used to analyze more concrete formal models in (Belintsev,B.N. et al., 1981). The results, obtained in the mentioned papers can be directly applied to calculate **S**ymmetry Breaking Instability (SBI) of the elasto-polarization models formulated in the previous section. Let us first resume the essence of the general result obtained in (Sattinger,D.H. 1979):

Consider the equilibrium states of a dynamical system that have the form of equation

(4.1) $$(L - \mu B)u + F(\mu;u) = 0.$$

Here L and B are linear operators and F is a nonlinear one. The operators are defined in a Banach space. The parameter μ is real. Moreover we assume that $u = 0$ is always a steady state. In the paper (Sattinger,D.H. 1979), it is yet assumed that $\tilde{u}$ is a stable steady state (fixed point) if all eigenvalues of the linearized operator

(4.2) $$L - \mu B + F'_u(\mu;\tilde{u})$$

has positive real parts, and $\tilde{u}$ is unstable if at least one eigenvalue has negative real part. It is also assumed that $F'_\mu(\mu;0) = 0$ is valid. At the end it is supposed that for $0 \le \mu \le \mu_0$ the trivial solution is stable, and the instability occurs as parameter μ crosses some value μ_0 (bifurcation point).

Under the above conditions, which are rather general and include as particular cases the conditions of the models formulated in the previous section, the following can be proved: Besides the trivial solution, one of the following 3 different solutions also exists:

(a) two stable solutions of (4.1) for $\mu > \mu_0$ and no solutions for $\mu < \mu_0$;

(b) one stable solution for $\mu > \mu_0$ and one unstable solution for $\mu < \mu_0$;

(c) two unstable solutions for $\mu < \mu_0$ and no solutions for $\mu > \mu_0$;

Moreover, the non-trivial solutions tend to zero as μ tends to μ_0. The suitable conditions under which the physically interesting case (a) occurs, is treated in (Sattinger,D.H. 1979). These conditions in more concrete form are substantially considered in (Belintsev,B.N. et al., 1981) and applied to a formal model of a dissipative system with counteraction of local activation and an effective nonlocal inhibition. This system is a paradigmatic model of SOT, which is shown to be reducible to the equation

$$Tu_t = f(u) - \frac{1}{2R_h}\int \exp\left\{-\frac{|x-x'|}{R_h}\right\} u(x',t)dx' + R_u^2 u_{xx}, \tag{4.3}$$

Here derivatives are denoted by indices. It is seen that (4.3) differs from (3.1.3) by parameter and variable notations only. The two equations are mathematically identical and differ only by the biomechanical sense involved in (4.3). So the results obtained in (Belintsev,B.N. et al., 1981) can be directly recomputed in terms of the one-dimensional elasto-polarization model of placode formation formulated in section 3. More in detail this recalculation can be accomplished in the following way:

The initial state of (3.1.3) is $w = 0$ presenting trivial steady state solution of this equation as it is required from the Sattinger theory. Every parameter $\alpha, R_e, R_p, \eta = \varepsilon k\tau$ and their combinations play the role of the parameter μ in the above outlined Sattinger theory. The initial state can be replaced by variation of these parameters to the critical point $\mu = \mu_0$ of loss of stability as it is predicted by the theory. The presence of a Symmetry Breaking Instability (SBI) in the system described by (3.1.3) (or 4.3)) is due to the monotonic dependence of the time decrement ϖ for the harmonic disturbance modes proportional to $\exp[\varpi(\kappa)t + i\kappa x]$. To prove this we follow (Belintsev,B.N. et al., 1981) by first defining the dispersion equation, which in our case has the form

$$\tau\varpi(\kappa) = \alpha - R_p^2\kappa^2 + \frac{\eta {R_e}^2\kappa^2}{1 + R_e^2\kappa^2}; \qquad \eta = \varepsilon k\tau. \tag{4.4}$$

The condition of increasing decrement with the wave number is

$$\sqrt{\eta}\,R_e > R_p. \tag{4.5}$$

This means the characteristic length R_e of the elastic interaction must be sufficiently larger than the coherence length R_p of contact polarization.

In terms of the Sattinger theory, from (4.4) it follows that the threshold (bifurcation point) of the Turing instability is

$$\mu = \mu_0 = 0, \text{ where } \mu = \sqrt{\alpha + 2\eta} - \sqrt{\mu} - R_p / R_e. \tag{4.6}$$

The wave number of the corresponding bifurcation (critical) mode is

(4.7) $$\kappa_c = \sqrt{\eta}(R_p R_e) - \frac{1}{R_e{}^2}.$$

5. SOT-Based Computation of Symmetry Breaking Instability of Two-Dimensional Model of PCP.

The formulas (4.4-7) are also valid for the two-dimensional elasto-polarization model presented by the system of equations (3.2.1) and (3.2.5), formulated in section (3.2). Following (Belintsev et al. 1987), this system of equations can be reduced to the integro-differential equation

(5.1) $$\frac{\partial w}{\partial t} = -\frac{\alpha}{\tau} w(w-1)(w-\beta) + D.\Delta w(x,y,t) - \frac{a}{R_e}\int G_0\left(\frac{\vec{r}-\vec{r}'}{R_e}\right) w(\vec{r}',t)d\vec{r}'$$

Here G_0 is Green's function of the Laplace operator. For the further bifurcation analysis of (5.1), it is essential to underline that the integral operator in (5.1) has the same eigenvalue functions as the Laplacian. Moreover, the dispersion equation corresponding to the linearized equation (5.1) coincides with that of the one-dimensional model (4.4). And what is specific for the two-dimensionality of the considered case, in the bifurcation point of Turing instability only the absolute value of wave vector $\vec{k}$ is determined, but it has many directions.

Under these conditions, a more particular bifurcation analysis can be provided directly from the SOT of reaction-diffusion systems available in the literature (Nitzan,A. and Ortoleva,P. 1980.) This theory accentuates on the application of scaling and Ginzburg criteria to critical bifurcation analysis in non-equilibrium reacting systems. Mathematically the theory is described in general terms allowing to be used for solving the problem of SBI in the two dimensional elasto-polarization model, presented by (5.1). The constructive discovery of the paper is the reduction of vast set of nonequilibrium physical and chemical systems near their transition points to the form analogous to the time-dependent Ginzburg-Landau) equation of critical phenomena. The very reduction of a given arbitrary complicated system (for example – of type (5.1)) to a simple equation of Ginzburg-Landau type is accomplished in a general form by a separation of temporal and spatial scales near the transition point. This approach is called scaling method and can be applied for the reduction of (5.1) to TDGL near its critical point (in this case – Turing bifurcation point).

A remarkable trait of the Turing bifurcation in a planar case is the existence of a *degeneracy* of the wave-vector (the multiple analog of wave number in one-dimensional case) along its directions. It is known that only the absolute value of a wave vector for the normal perturbation mode $e^{i\vec{k}\vec{r}}$ is determined by Turing condition (Turing,A. 1952) while its orientation remains undefined. As it is shown in (Nitzan,A. and Ortoleva,P. 1980), when another kind of degeneracy, i.e. that of more than one wave numbers, is not superimposed, the solution of equation (5.1) near the critical point is presented in the following general form:

(5.2) $$w(\vec{r}) = \sum_{\theta} W_\theta e^{i\vec{k}_\theta\vec{r}}, \qquad \left|\vec{k}_\theta\right| = k_c$$

The selection of a discrete number of directions in the sum (7.2) is performed by the nonlinearity of function $f(w)$. It can be shown that the second-order term in the power series expansion of the function $f(w)$ selects the triads of plane waves

$e^{ik\rho}$, $(l = 1, 2, 3.)$, which wave vectors form an equilateral triangle (Nitzan,A. and Ortoleva,P. 1980):

$$\sum_{\theta=1}^{3} k_{\vartheta} = 0 \,. \tag{5.3}$$

So, the two-dimensional patterns of cell polarization must be presented by regular hexagonal lattice, originated by a subcritical bifurcation ($\mu < 0$), (Nitzan,A. and Ortoleva,P. 1980). This essential conclusion is valid also for (5.1), and it has a concrete biological concern to the recent experimental findings for pattern formation in imaginal discs of *Drosophila* (Aegerter-Wilmsen,T. et al. 2010; Aigouy, B et al. 2010; Classen,A. et al. 2005). In the paper of Aegerter-Wilmsen et al., it is experimentally shown that apical cell surfaces in discs metazoan epithelia of *Drosophila*, present polygons with different number of neighboring cells (including 6 too). By corresponding theoretical considerations there it is established that "only scenarios that include mechanical-stress dependent growth rates are in agreement with the experimental data". Our developmental model (5.1) principally confirms this conclusion with the result (5.3), showing the validity of hexagonal lattice. Other numbers of lattice polygons should correspond to modifications of (5.1) in a way compatible with a structural stability of the theoretical scheme proposed in (Nitzan,A. and Ortoleva,P. 1980). Such a type of structural stability for the developmental models is extensively discussed in the recent literature (Beloussov,L.V. 2001; Petrov,V.& Timmer,J. 2009). What is also of interest in this case is the role of planar cell polarity in the hexagonal packing of *Drosophila* wing epithelial cells, established in the paper of (Classen, A. et al. 2005). It is proposed there, that the specific "proteins polarize trafficking of Cadherin-containing exocyst vesicles during junction remodeling", which can be "a common mechanism for the action of planar cell polarity proteins in diverse systems". Such an assertion is in accordance with the principle introducing of a general polarization function $f(w)$ we made to determine the hexagonal pattern formation in the form (5.3). So, the obtained result (5.3) has a hopeful experimental and theoretical value to be used as a starting point in future investigations.

From (Nitzan,A. and Ortoleva,P. 1980) it follows that the subcritical character of such a polygonal pattern formation is a direct consequence of the orientation degeneracy of a Turing bifurcation point. If the latter is additionally degenerate with respect to wave number (this is the case if $R_p << R_e$, then the breaking of regularity occurs and low limitation appears for the polarization domain sizes (as well as for the distances between the domains). More detailed information about similar results can be found in (Meinhardt, H. 1982). In this book, the short-range mutual repression of two morphogenes is introduced, which is analogous to short-range polarization forces used in the Elasto-Polarization (EP) model in section 3.2. In this way two different scales of local activation and global inhibition are incorporated in the EP model.

6. On the Universality of Elasto-Polarization Model of Biological Morphogenesis

Equation (2.1) presented here is an universal mechanical model based on the assumption that the elasto-polarization processes are carried out in all epithelial layers with or without interaction by basal plate. In some cases such an interaction does not exist, but in others, the second layer is essential in the patterning mechanism as an elastic substrate which reacts to polarization forces. Then the universality of the Elasto-Polarization (EP) model is demonstrated in the possibility to simulate such

complex patterns as stripes and spots of epidermis modeled by well-known diffusion reaction models (Meinhardt, H. 1982). The applicability of EP both to somite and placode formation is based on the common existence of two feedback mechanisms that regulate all morphogenetic processes: The first positive one is that between single cell polarization activity and the contact transmission of polarization process. The second is negative between polarization and elastic forces. The EP model accounts for a number of embryonal patterns to which animal tissues are added in morphogenesis. The simulations of corresponding embryonal processes are reducible to the well-known model of (Meinhardt, H. 1982), which is of high order of universality too.

There is quite a large number of mathematical (both mechanical and biochemical) models distinguishing with high universality in reproducing embryogenetic experimental results with equal success. Which one of them is preferable for the experimentalists is a question of pragmatic convention between them. In this review we presented two illustrations of the descriptive power of the model for somite and placode formations. In addition, in (Belintsev,B.N. et al. 1987), some other examples demonstrating the universality of EP model are presented. They include: (a) Induction of budding in marine hydrozoa; (b) Analysis of regulations in sea-urchin embryos; (c) Morphogenesis of skin and cuticular structures; (d) Comparison with the well-known Odell's model (Odell,G.M. et al. 1981). The standpoint of Odell's model is the proposition that elastic properties of cells and the epithelial tissues they form are considered as a whole. The EP model also originates from this point of view and is similar to Odell's one in many respects. The only essential difference is that in EP model the elastic restoring forces have different signs. This means in Odell's model, these forces have a positive sign, i.e. they help cell contraction, while in the EP model, elastic forces restrict the deformation (polarization) process. Other mathematically developed mechanical models consider mesenchymal tissues (Oster, et al. 1983; Murray et al. 1988). In the model of (Nagorska et al. 1987), reaction diffusion and cell traction mechanisms are combined in a way that cell traction, acting in the epidermis depends on morphogen concentration, produced in the epidermis.

A common advantage of the EP model is the circumstance that it gives quantitative estimations of different scaling; a regular macro-state on the one hand and the parameters of the cell material available to experimental control, on the other hand. Among them, the relative lengths of polarization coherence R_p (polarization coherence length) and the elastic response of the embryonic material R_e (stress decay length) appear to be the most important. These two scales present local activation and global inhibition incorporated in the EP model by analogy with more general treatment of these notion in SOT (Belintsev,B.N. et al. 1981). Here, they are deduced from the other more elementary and in principle measurable parameters of the cell material. For example it can be shown that $R_p = \sqrt{D\tau}$, and $R_e = \sqrt{\frac{E}{q}}$, where D is a coefficient of polarization diffusion, τ is characteristic time of polarization, E is Young modulus, and q is coefficient of elastic connection between epithelial and layer and basal plate.

In accordance with the above cited SOTs of (Sattinger,D.H. 1979; Belintsev,B.N. et al. 1987; Nitzan,A. and Ortoleva,P. 1980), when the two scales are of one and the same order, the initially inhomogeneous state transform in a regular long range ordered patterns. In the two dimensional case these patterns take the form of hexagonal lattice as the adherens junctions of imaginal discs of *Drosophila* are to a

certain extent. If the scaling relationship $R_p << R_e$ is valid, the polarization process is self-restricted to a limited polarized domain as it is the case of somite formation.

In a more general context, mechanical laws were demonstrated to play an universal role in pattern formation and morphogenesis (Beloussov,L.V. 2008). The EP model is a theoretical example for the possibility to formulate robust macroscopic laws from which the observed shapes of concrete patterns (somites and placodes) could be derived.

There are many experimental examples showing the universal role of mechanical stresses in developmental processes. The following sequence of establishments could be cited in this connection: Stretching of *Xenopus* suprablastopore zone perpendicularly to the anterior-posterior axis of the embryo leads to the formation of the axis, which is perpendicular to the future head-tail orientation (Beloussov,L.V. et al. 1998, Beloussov,L.V. and Ermakov,A.S. 2001); Induction of involution movements within ventro-lateral parts of *Xenopus* early gastrula results in the formation of axial rudiments in abnormal locations (Beloussov,L.V. and Snetkova,E.V. 1994); Similar mechanical manipulations lead to inversion of dorso-ventral polarity in the loach embryo Cherdantsev,V.G. 2003). In both cases, axis formation was induced on the side opposite to that of growth; Artificial alteration of mechanical stresses in the *Drosophila* embryo leads to change of gene expression profile (Farge,E. 2003); Dorso-ventral and animal-vegetative mechanical stresses in *Xenopus* blastula cause embryonic axis formation (de Robertis,E.M. et al. 2000, de Robertis,E.M. and Kuroda,H. (2004)); It is established in (Opas,M. 1994; Ingber,D.E. 2003) that cells and their genomes are able to respond upon mechanical influence in a specific manner; Alteration of gene expression in response to flow-induced forces was shown for both cardiac and vascular endothelial cells (Garcia-Cardena,G. et al. 2001; Hove,J.R. et al. 2003); Cells are able to distinguish between different mechanical stimuli, for example between uni-axial and multi-axial forces (Hornberger,T.A. et al. 2005).

7. On the Specificity of Planar Cell Polarization in Terms of EP Model of *Drosophila* Wing Disc Development

As it was noted in section 5, from a universal point of view, the two-dimensional patterns of PCP in *Drosophila* wing development must be presented by a regular *hexagonal* lattice, originated by a subcritical bifurcation ($\mu < 0$), (Nitzan,A. and Ortoleva,P. 1980). However, many experimental observations show the cells of *Drosophila* imaginal wing disc have different polygon numbers with corresponding different number of neighbors for every cell (Aegerter-Wilmsen,T. et al. 2010; Gibson,M. et al. 2006). This means that the wing epithelial lattice is not exactly hexagonal, but polygonal. This partial discrepancy between the SOT of elasto-polarization and biological facts needs explanation. For this purpose, we need to take into account the fact of disc growth termination which should be derived as a consequence from the SOT model. Concerning this, we make the following considerations:

(i) We accept the model proposed in (Aegerter-Wilmsen,T. et al., 2007) that growth is regulated by mechanical stresses and define them originated from cell polarization in accordance with the elasto-polarization model discussed in the previous sections. Similarly to (Aegerter-Wilmsen,T. et al., 2007; Hufnagel, L. et al. 2007) we assume compression in the imaginal disc center as a cause of growth termination.

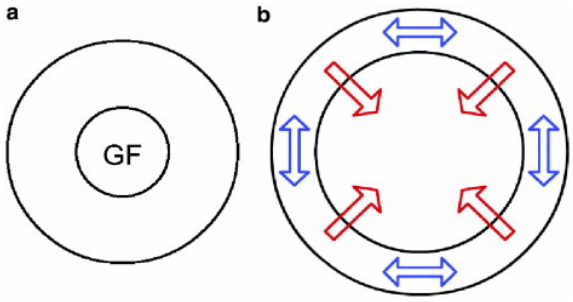

Figure 6. Principal scheme of growth termination model taken from (Aegerter-Wilmsen,T. et al., 2007).

The stretching in peripheral region induces compression in central zone (in analogy with the well-known Laplace formula). This type of radial distribution of stresses is analytically derived from the EP model considered here.

We accept the compression in the disc center as playing role of *mechanical control* of the growth. Moreover, we concretize this control, by analyzing an idealized mathematical statement of this model.

(ii) We consider a hexagonal lattice as self-organizationally predetermined by the PCP process at inter-cellular level and inducing a hexagonal form of the cells too. In addition, we accept the idea of (Aegerter-Wilmsen,T. et al. 2010) to explain the breaking of hexagonal cell forms by cell division and rearrangements. Moreover, we consider the center possibly inducing polygon distribution at inter-cellular level.

An idealized model of wing disc growth dynamics can be presented by the equation

$$\frac{dS}{dt} = \sigma S - \lambda P \,. \tag{7.1}$$

Here $S = \pi R^2$, $R = R(t)$ is the variable radius of the wing disc, $\sigma > 0$ is an effective coefficient presenting the rate constant of food consumption, under condition of unrestricted food supply through the disc area, $P = \frac{Th}{R}$ is the well-known Laplace formula for the central pressure P and peripheral tension T , λ is a phenomenological coefficient, h is the thickness of the peripheral ring of the disc. This is a highly simplified version of the model discussed in (Aegerter-Wilmsen,T. et al., 2007).

After replacing the mentioned formulas in (7.1) and accomplishing some transformations we obtain

$$\frac{dR}{dt} = \frac{\sigma}{2} R - \frac{\lambda T h}{2\pi R^2} \,. \tag{7.2}$$

This nonlinear differential equation (7.2) has a single steady state

$$R_s = \left(\frac{\lambda T h}{\pi \sigma} \right)^{\frac{1}{3}} . \tag{7.3}$$

It can be rigorously proved to be asymptotically stable. The steady state radius R_s we call the termination radius. It is seen that a sufficiently large value of the tension (stretching) T leads to large pressure P, terminating the growth (the increase of R),

which is a main assertion in the works (Aegerter-Wilmsen,T. et al., 2007; Hufnagel, L. et al. 2007).

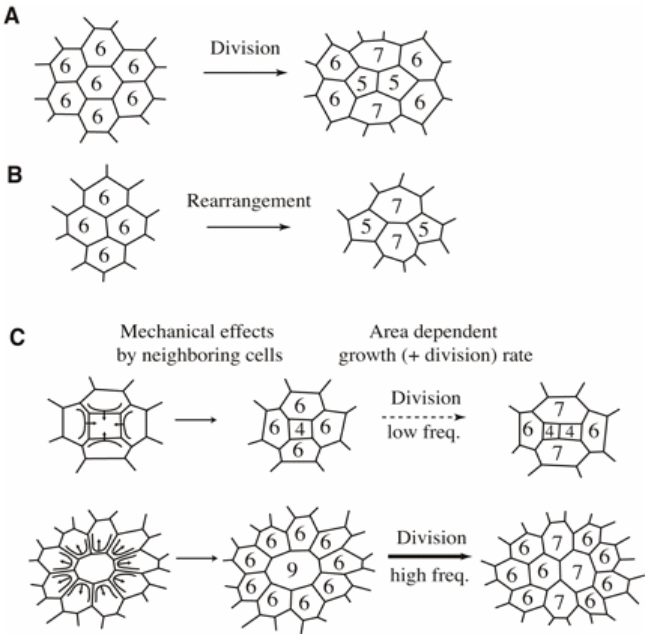

Figure 7. Schematic presentation of polygon distribution processes in epithelial layer, taken from from (Aegerter-Wilmsen,T. et al., 2010).

In terms of EP model, the nature of circumferencial tension T we use in equation (7.2) must be considered as a passive mechanical reaction to the active tension of polarization. So, we can consider the passive mechanical tension T is equal to the active one presented by the term $\frac{\varepsilon}{1-m}\left[\frac{1}{1+\varpi}(1-2m-\varpi)\overline{w}+w\right]$ in (7.4) below. Thus, the polarity w is a cause of the circumferencial tension discussed in (Aegerter-Wilmsen,T. et al., 2007; Hufnagel, L. et al. 2007) and equation (7.2) depends on the PCP model (7.4) below. We underline this circumstance by writing the governing equation of PCP (3.2.1) in a form appropriate for our case of wing disc epithelium as it is done in (Belintsev,B.N. and Savich,D. 1985):

$$(7.4)\qquad \tau\frac{\partial w}{\partial t}=-\alpha w(w-1)(w-\beta)+\frac{D}{r}\frac{\partial w}{\partial r}+D\frac{\partial^2 w}{\partial r^2}+\frac{k\varepsilon}{1-m}\left[\frac{1}{1+\varpi}(1-2m-\varpi)\overline{w}+w\right].$$

Here m is a coefficient of Poisson and $\varpi = qR(1+m)(1-2m)/E$. Moreover, $\overline{w}$ is the average polarization, and u is the displacement of the wing epithelium with respect to the basal lamina we mentioned in previous sections. On the other hand, the growth model (7.2) plays the role of movable boundary of (7.4). The experimental findings in (*Classen,A. et al. 2005*) allow us to consider the PCP variable w as a fast variable with respect to the slow variable R. That is why, the steady state value of w can be used in (7.2) as a parameter and the slow variable R can be used as a fixed boundary in (7.4). Thus every one of (7.2) and (7.4) can be analyzed separately by using the information for the behavior of the other one.

The results show that, the second term in equation (7.1) has a negative sign only in the case that the elasto-polarization w is positive in the center of the wing disc. This means the polarization process propagates from the center to the periphery.

Then the peripheral tension of the disc has a character of stretching one, as it is established in the paper of (Belintsev,B.N. and Savich,D. 1985). Therefore, this PCP model has a crucial role in defining the applicability of Laplace stretching-compression relationship to mechanical control of growth termination. This theoretical prediction of the inhomogeneous distribution of mechanical stresses in the wing disc is in accordance with experimental findings (Nienhaus,U. et al. 2009).

8. Discussion on the PCP Model of *Drosophila* Wing Pattern Formation

In order to model pattern formation in the *Drosophila* wing, we consider both a one-dimensional and a two-dimensional model of planar cell polarization in the wing epithelium, elastically connected with the basal plate (Sections 3 and 4). We accept this statement as a prospective one. Our understanding is that while the considerations in one dimension have more exhaustive general sense, the two dimensional statement allows more concrete insight into the process. Thus both approaches have their scientific values.

We consider in the wing discs, pattern formation develops as a result of the interactions of epithelial cells with a basal plate. They start to develop as epidermal thickenings (placodes) over the basal plate (extra cellular matrix). In the literature, similar processes are described as existing in various kinds of skin (Sengel,P. 1976). It is noted that these events determine the program of the prospective development.

The experimentally observed types of spatial patterns of *Drosophila* wing structures present regular lattices (for example – hexagonal distributions) of cells (Aigouy, B *et al. 2010; Classen,A. et al. 2005*) or irregular ones. Both regular and irregular structures can be predicted in terms of EP model analyzed in this review. The irregular case cannot be characterized as "chaotic" because of possible restriction underneath of distances between the cells (Meinhardt,H. 1982). In the frame work of PCP model the mechanical nature of the well-known "inhibition region" around every placode (Meinhardt,H. 1982) is revealed. It consists in the circumstance that in the vicinity of every placode the appearance of another one is inhibited by the existing tangential tensions (stresses).

The ratio between the two scales $R_p = \sqrt{D\tau}$ and $R_e = \sqrt{\frac{E}{q}}$ in the EP model determines the type of macroscopic order of placode distributions. When the characteristic length of polarization R_p, and the length of elastic interaction R_e (between epithelium and basal plate), are of one and the same order (scaling equality), then hexagonal lattice of placodes takes place. This circumstance is both a theoretical result in terms of PCP model and observed experimentally. But another theoretical and experimental fact is also the circumstance that if R_e is essentially larger than R_p then irregular distributions of placodes appear.

On the basis of the above accomplished analysis of PCP model we conclude that the leading role in forming hexagonal or irregular distributions of placodes in wing disc is due to the PCP activity of the epithelial cells. This can be achieved by the parameter q describing the *elastic interaction between epithelial and basal plate*. It is involved in the scale R_e (characteristic length of distant interactions) and is sufficiently large in case of regular patterns and small enough for irregular ones. So, the increase of parameter q leads to regularization of epithelial pattern formation. A second role of parameter q is that if it is large enough then the peripheral stress of the

layer is stretching one. In this way the central zone of the layer is under compression controlling the growth and terminating it.

The ratio $\frac{R_p^{\,2}}{R_e^{\,2}}$ is equal to $\frac{\tau q D}{E}$. When this ratio is near 1 we have regular pattern formation and mechanical control on the growth termination. For small ratio, the pattern formation is irregular and the termination is uncontrolled mechanically. It is a question of experimental convenience and possibilities to choose which ones of the parameters τ, q, D, E can be determined. However, at first glance, it seems reasonable to determine the scale R_p as approximately equal to the intermediate zone between compressed (central) and stretched (peripheral) regions of the wing disc. This zone is possibly measurable. Supposing that the ratio R_p / R_e is of order of 1, the parameters τ and E seem to be measurable, thus using the above formulas for the scales, D and q can be also evaluated.

9. Conclusions

PCP model of SOT predicts the following two basic events in *Drosophila* wing:

1. *Distribution of type* ***central compression – peripheral stretching*** *and mechanical control of the wing growth (Aegerter-Wilmsen,T. et al. 2010; Aegerter-Wilmsen,T. et al., 2007; Hufnagel, L. et al. 2007).*

2. *Transition from* ***irregular*** *to* ***regular*** *pattern formations (Classen,A. et al. 2005; Fahradifar,R. et al. 2007) under the following relationships:*

(i) *In case of* ***irregular*** *pattern formation, the inequality* $R_p / R_e << 1$ *is valid, and the coefficient of elastic interaction* q *between the epithelium and basal plate can be theoretically estimated by the relationship* $q << \frac{E}{R_p^{\,2}}$.

(ii) *For the* ***regular*** *pattern formation, the ratio* R_p / R_e *is of order of 1, and the coefficient of elastic interaction* q*, between the epithelium and basal plate, can be evaluated by the formula* $q = \frac{E}{R_p^{\,2}}$*, where* E *and* R_p *are experimentally measurable.*

In this way, PCP model of SOT suggests the pattern regularization is caused either by an increase of mechanical interaction between epithelium and basal plate, or by decrease of epithelium elastic modulus. How to verify this causal prediction is a question of future experimental investigations concerning the cell-molecular mechanisms of *Drosophila* wing mechanical properties.

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