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Active stress as a local regulator of global size in morphogenesis

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ABSTRACT

While a general consensus exists that the morphogenesis of living organisms has its roots in genetically encoded information, there is a big debate about the physical mechanisms that actually mediate its control. In embryo development, cells stop proliferating at homeostasis, a target state in terms of physical conditions that can represent, for instance, the shape and size of an organ. However, while control of mitosis is *local*, the spatial dimension of a tissue is a *global* information. How do single cells get aware of that at the same time? Which is their communication mechanism? While morphogen factors are demonstrated to play a key role in morphogenesis, and in particular for shape emergence, they seem unable to produce a global control on size by themselves and, conversely, many recent experiments suggest that active mechanics plays a role. Here we focus on a paradigmatic larval structure: the imaginal disc that will become the wing of the fruit fly. By a formalization of theoretical conjectures in terms of simple mathematical models, we show that inhomogeneous stress, likely dictated by morphogenetic patterns, is an admissible mechanism to convey locally the global information of organ size.

1. Introduction

In life science, *morphogenesis* is the ensemble of biological processes that lead to the emergence of an organism shape. The orchestrated process of differentiation and duplication yields organs that have a precise shape and size. An important role in this phase is held by molecules that were first hypothesized by Alan Turing. In his seminal work [1] he described how the concentration of chemical substances in a tissue evolves in time because of reaction and diffusion processes. The density patterns, dictated by instability, can be conjectured to drive the system shaping. Even if he did not precisely identify such molecules, Turing called them *morphogens* to convey the idea of shape generation.

A fundamental contribution to the theory of morphogens is due to Lewis Wolpert [2], who proposed the "French flag model". The central element of this model is the spatial distribution of the concentration of specific substances in the tissue: it is detected by the cells which, according to specific thresholds, trigger the transcription of distinct sets of genes. According to this theory, there is a direct correlation between the input (the concentration level) and the output (the response of the tissue): each threshold corresponds to the border of an expression territory.

Since 1970s many efforts have been made to understand how morphogens influence growth [5–9] and how cells can sense and respond to their concentration changes [10]. These studies allowed to deeper understand morphogen properties, so Turing's idea was retained, but extended: not only morphogens are responsible of pattern formation, they also specify mutual cell position and then influence organism growth [11]. The first morphogen, the *bicoid*, was discovered in 1988 by Christiane Nüsslein-Volhard; after that, many others have been identified: *Decapentaplegic* (*Dpp*), *Hedgehog*, *Wingless*, *Epidermal growth factor*, *Fibroblast growth factor* and *Retinoic acid*, to cite a few.

While the theoretical argument that the concentration of a morphogen can drive growth is fascinating and successful in some cases, such an appealing explanation fails in many morphogenetic processes or, at least, it is to be corroborated by other physical mechanisms. The most popular model organisms in morphogenetic studies is probably the *Drosophila melanogaster*, also known as fruit fly. The importance of many morphogens like DPP, wingless, hedgehog and others in shaping is well assessed, because in many cases it has been observed that their absence inhibits a correct evolution of the organ. However, it is still a matter of debate *how* morphogens control growth in the drosophila fly and, in particular, how cells "know" when the final size has been achieved.

We concentrate our attention on the Drosophila wing *imaginal disc*, the structure of the larva from which the adult insect wing originates. Three different regions can be identified along the proximal–

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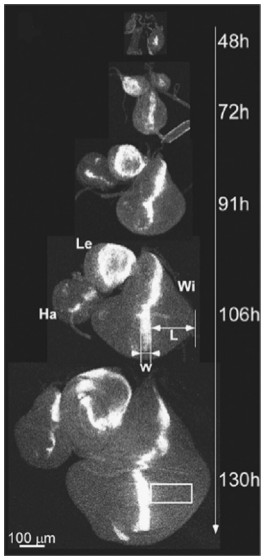

Fig. 1. Images of Dpp gradients inside wing (Wi), leg (Le), and haltere (Ha) discs at different developmental times. The parameters w and L represent the source and target widths, respectively; the scale bar is 100 μm. Reprinted with rights from [16].

distal axis of the disc: the *notum* (proximal), the *hinge* (central) and the *pouch* (distal). The notum will give rise to the torax, the pouch to the wing and the hinge to the flexible region between the two. The growth of the disc lasts about 150 h and during this morphogenetic stage the initial number of cells duplicates about 10 times, passing from 50 to 50 000 and eventually covering an area of about 1 mm^2, as depicted in Fig. 1. The wing disc is a sheet of epithelial cells, a very thin structure that can be effectively represented by a two-dimensional approximation.

One of the most studied morphogens is the Dpp, a bone morphogenetic protein that has a key role in the development of the fruit fly, since it has been proved that the genetic suppression of its production inhibits the morphogenesis [12–14]. It spreads from a central stripe of the disc that divides it into two almost equal parts: the anterior and the posterior one. This morphogen has been widely analyzed: thanks to techniques like fluorescence recovery after photobleaching, its production rate, effective diffusion coefficient and degradation rate [15] are precisely determined. The following experimental facts are well assessed.

- The spatial dynamics of Dpp is fast with respect to the growth of the disc: diffusion and decay of Dpp can be assumed to be always in mutual equilibrium so that the mass convection due to the material displacement caused by the cell duplication process does not distort the morphogen concentration field $c(x,t)$.
- In the early development the cell duplication rate is homogeneous in space, but changes during time: it is higher in the initial phase than in the final one. The disc area $S(t)$ evolves in time according to a Gompertzian law:

  $$\log\left(\dot{S}/S\right) = b_1 - b_2 t,$$

  with b_1 and b_2 positive constants. At a later stage, typically after 72 h, the mitosis is oriented along the proximal–distal axis [27,28].

In spite of the big amount of experimental data available, the research about Dpp spreading dynamics is still very active: recent studies show that Dpp profile scales in space with the disc length and the morphogen concentration adapts to disc size [16–18]. Such an experimental behavior reported by Wartlick and coworkers [17] is explained by the authors themselves assuming that an expander of constant mass dilutes during to cell division: if the degradation rate depends linearly on it, the morphogen concentration scales as the length of the domain (expander–dilution mechanism). An alternative hypothesis which might provide scaling of the morphogen is the expansion–repression mechanism proposed by Ben-Zvi and Barkai [19]: when the disc grows, the cells at the periphery of the imaginal disc experience morphogen levels below a given threshold, and they might produce an expander that diffuses in the tissue and reduces morphogen degradation.

Notwithstanding, a full explanation of the determination of the size of an organ in terms of pure reactive–diffusing agents remains elusive. One of the motivations is that even though chemical signaling scales with length, it has a very small concentration at the boundary of the disc. This remark suggested a different approach to Hufnagel and coworkers [18]; they argue that mechanical stress might compensate the decay of morphogen concentration in the periphery of the disc, so that a combination of morphogen diffusion and mechanics might be the key regulator of disc growth. This explanation is in agreement with a number of recent reports on the emerging role of mechanics in morphogenesis [20]. Mechanical stress is known to play a role in tissue development [21], sometimes in conjunction or superposition with chemical signaling [23]. In particular, evaluation of the stress in the wing imaginal disc by photoelasticity has been the subject of recent experimental works [24,49]. Their main results are that the stress is inhomogeneous, larger in the center than in the periphery, it is compressive and grows with the size of the disc.

One of the aims of this paper is to address whether a continuum mechanics model can reproduce a stress pattern qualitatively similar to the reported one. Moreover, we are interested in investigating whether a purely mechanical setting can be provided a signaling mechanism for size regulation during growth. While the complex interaction of morphogenetic factors will not be directly addressed, a possible output of concentration pattern will be used as a datum to modulate the activation of the mechanical stress.

The tensional state in a tissue can be due to external loads, residual stress and, most notably in the present context, we argue that it is actively produced by the cells, thanks their own actomyosin network. The stress is therefore a long range field, natural candidate for intercellular communication, and, as a matter of fact, cells are well known to modulate their motility and reproduction rate on the basis of their own tensional state [26]. Here, we formalize different conjectures about possible morphogenetic mechanisms in mathematical equations and analyze them in terms of physical admissibility. Our standpoint is that a physical field is an admissible mechanism of local transduction of global information if, under homogeneous growth, it depends in a specific functional form on the domain size and on its relative position in the organ only. Under this hypothesis, we show that a non-homogeneous stress field, possibly modulated by morphogen concentration, can be advocated as admissible mechanism of global information transmission.

This paper is organized as follows. First, we revisit, in a mathematically formal way, the most common physical mechanisms assumed in the literature for the role of the Dpp in the imaginal disc morphogenesis. We reformulate different conjectures in terms of mathematical models to clarify the weak and strong points of apparently opposite theories. Then, we propose a model of disc development where the cell duplication is driven by the spatial variation of mechanical tension, produced by cell contraction and spatially modulated by morphogen concentration. We argue that tissue growth is driven by an inhomogeneous mechanical stress: as its gradient drops below a threshold, the disc does not increase beyond a critical size. As discussed in the final remarks, the proposed mechanism does not suffer of