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Growth rate and shape as possible control mechanisms for the selection of mode development in optimal biological branching processes

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Abstract. Recently three branching modes were characterized during the formation of the lung in mice. These modes are highly stereotyped and correspond to domain formation, planar bifurcation and three dimensional branching respectively. At the same time it is proved that although genetic control mechanisms are presumably related to the selection of any of these modes, other external factors will most probably be involved in the branching process during development. In this paper we propose that the underlying controlling factors might be related to the rate at which the tubes that form the lung network grow. We present a mathematical model that allows us to formulate specific experimental predictions on these growth rates. Moreover we show that according to this formulation, there is an optimization criterion which governs the branching process during lung development, namely, efficient local space filling properties of the network. If there is no space limitation the branches are allowed to grow freely and faster, selecting one branching mode, namely, domain formation. As soon as volume constraints appear the growth rate decreases, triggering the selection of planar and orthogonal bifurcation.

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1 Introduction

The increasing understanding of the genetic regulatory control mechanisms during development has given the possibility of establishing detailed explanations of specific processes (for instance segmentation in drosophila or the ABC model in plants to mention two of the most celebrated ([2, 5]). There are, from our perspective, at least two more questions that are fundamental, which are related to morphogenesis. First, how external and contextual factors might interact with genetic regulatory networks in order to regulate developmental processes (see for instance [1]). Second, it is well known that many biological structures possess some (pseudo)optimality property. For instance, beehives have been extensively studied for the efficient use of material in order to partition a region in cells of equal size or transport properties of biological networks are also well known. Although the emergence of optimal structures can be understood, from a more global perspective, merely as the result of natural selection acting on biological systems, the detailed and local (both in space and time) control mechanisms that during development determine the (pseudo)optimality of a certain biological structure are not clear. In many cases, however, these questions are not independent and here we attempt to show in a very specific example how contextual features such as geometrical or size constraints as well as growth properties during development can robustly select structures that, on the other hand, are optimal or close to optimal according to some well defined criterion. One of the most important aspects we would like to emphasize is the fact that with the experimental information that is already available or that can be obtained, it is possible to formulate mathematical models that in turn can give quantitative or semiquantitative predictions to be tested experimentally. Not only that, they would provide insights into the mechanisms underlying some developmental processes. In fact, the suggestion that growth (or to be more precise, growth rate) as a contextual feature possibly affecting development (regeneration) goes back at least to D'Arcy Thompson. In his book *On growth and form* he describes that already Aristotle had noticed that lobsters had the capability of regenerating a claw that had been chopped off into a crusher or pinching one, depending on the speed of growth. It is the main aim of this paper to provide a mathematical model that accounts for the mode selection during the branching process of lung formation in mice.

In the remaining of the introduction we present our results in a nontechnical way. In section two we recall the experiments and results on the lung branching program obtained by Metzger et al. in [3]. In Sect. 3 we present a mathematical model and discuss the role that shape and growth might have as control mechanisms in these processes. In the same section we discuss how this is related to an optimization problem, namely, space filling. In section four we put our results in perspective and discuss some open problems and further research.

Summarizing the three different kinds of branching that take place during lung formation in mice, they correspond to domain bifurcation, planar bifurcation and orthogonal bifurcation. The authors, in their experimental work, show that these three modes are highly stereotyped. Moreover, according to them each mode of branching is controlled by a genetically encoded subroutine, a series of local patterning and morphogenetical operations, which are themselves controlled by a more global master routine. They show that this hierarchical and modular program is genetically tractable, and that it is suited to encoding and evolving the network of the lung. In their paper, a detailed model for the genetically coded subroutine is given. In the present paper we show that it is sufficient in order to recover the branching program of the network to postulate that the local realization of this global master underlying mechanism can be taken as growth rate of the branches and that, in turn, this provides a reasonable explanation for the optimal, in some sense, space filling properties

of the lungs. Assume that a branch during the first stages of lung formation, corresponding to domain branching, grows faster than during the following ones. This is consistent with the fact that, since during earlier stages there is no space limitation, a branch has no volume constraints and is free to grow. As soon as the bulk of the available space has been filled, some limitation arises and the rate of growth should diminish. We claim that this decrease triggers the appearance of the second growth mode, that is, planar bifurcation. Finally, when planar bifurcation becomes difficult, the growth rate should decrease even further, prompting orthogonal bifurcation. In order to make sense of this qualitative proposal, we use as a mathematical model, the so called activator-inhibitor Turing system proposed by Gierer and Meinhardt. More specifically, we use a reduction of this system called the shadow equation. In this approach, a morphogen, which in this case might very well be taken as a growth factor, is assumed to diffuse throughout the network and obey a nonlinear reaction-diffusion equation (see Sect. 3). When the growth rate is taken into account, the effective parameters of this equation are modified and we show using analytical, asymptotic and numerical results that the stereotyped branching behavior is recovered.

2 Biological preliminaries: the branching program during lung formation

Recent experiments in lung development have revealed in a very detailed way how the complex branching structure of the bronchial tree emerges. In these experiments, Metzger et al. [3] have traced the lineage of each branch of a full three-dimensional bronchial tree. They have realized that the whole structure emerges from only three different bifurcation modes, namely, *domain bifurcation*, where branches sprout in directions perpendicular to the growth direction of the corresponding mother branch, *planar bifurcations*, where the tip of a branch splits into two daughter branches all of them within the same plane, and, last, *orthogonal bifurcation*, which is like the planar bifurcation mode except that the daughter branches grow in orthogonal directions leaving the original plane.

Each of these bifurcation modes is associated to different aspects of the design of the bronchial tree. Domain bifurcation appears first and is used to generate the scaffold of lobe, planar bifurcation is then used to generate the thin edges of the lobes, whereas orthogonal bifurcation is used to create the lobe surface and fill the interior of the lobes.

Other relevant issues are both the timing and order in which these bifurcation modes appear. As for the former, Metzger et al. conclude that there is no global developmental program or clock controlling switching from one mode to the other with all of them being active at any particular time. The order in which they are used seems to obey a tighter regulation. Of all possible combinations, only three global sequences, which are described in detail in [3], are possible. These sequences are composed of transitions from one mode to another such that only the two following transitions are observed: from domain to orthogonal bifurcation, and from domain bifurcation to planar bifurcation back to domain bifurcation and then to orthogonal bifurcation.

Metzger et al. [3] state that the formation of the bronchial tree is completely controlled by a genetic program. Whilst we do not object to this point, and some aspects of the developmental process, such as the switch from one bifurcation mode to the other, are very likely to be exclusively controlled by the genetic program, we argue that some restrictions of physical origin also play a role in the morphogenetic process. We show that space restrictions, which impose limitations on the growth speed of the different branches, may be instrumental to understand some of the experimental observations reported in [3].

3 Growth and shape as control mechanisms

3.1 Model

The model we consider is an activator-inhibitor system in a growing domain. In order to simplify the analysis, and without loss of generality, we consider the reduction to the shadow equation instead of the full activator-inhibitor system:

$$u_t = d\Delta u - \lambda u + u^p.$$

3.1.1 Justification of model reduction

This model for fixed domains has in fact been extensively studied. We refer to [4] for a general overview and references therein for a more technical account. As a matter of fact, the qualitative features of the solutions that reflect the behavior of some pattern formation processes in some biological systems can be summarized as follows. For suitable values of the parameters involved, specifically if d is sufficiently small or if p is sufficiently close to a critical value, solutions tend to “condensate” in spikes, which in the context of biological pattern formation are identified with formation of spots. Another important property of these solutions is precisely the way these peaks arrange themselves. When only one concentration is present, and provided we prescribe zero flux boundary conditions, the center of the peak is located at the boundary, precisely at the point of maximal mean curvature.

When there are more peaks present, the behavior is more complicated, it can be said that there is a competition effect where, on the one hand, peaks repel themselves and tend to be as far as possible from each other, and on the other hand have the tendency of localizing at points with big curvature. For instance if the equation is considered on the sphere, where no boundary effect is present and all points are geometrically equivalent, the only effect that is important is that of repulsion and the solutions exhibit the property that the peaks arrange themselves in such a way that they are as far as possible from each other [6].

3.1.2 Model formulation

We have to adapt the general model to the specific case of the lung geometry and incorporate different growth rates in different directions (namely radial and longitudinal).

Our model formulation closely follows reference [7] where a general framework for activator-inhibitor systems on growing domains is presented. The corresponding shadow equation on a two dimensional growing domain reads:

$$u_t = \Delta_s u - u(\partial_t(\ln(h_1 h_2))) + f(u), \quad (1)$$

where h_1 and h_2 are the expansion velocities of the domain, $f(u) = -\lambda u + u^p$, and

$$\Delta_s = \frac{1}{h_1 h_2} \left(\left(\frac{h_2}{h_1} u_\xi \right)_\xi + \left(\frac{h_1}{h_2} u_\eta \right)_\eta \right) \quad (2)$$

is the Laplace-Beltrami operator.

In our particular case, the domain $\Omega(t)$ is a cylindrical shell, $\xi = z$, $\eta = \theta$, h_1 and h_2 , the expansion rates in the axial and radial direction, respectively, depend only on time. Therefore

$$\Delta_s = \frac{1}{h_1^2} u_{zz} + \frac{1}{h_2^2} u_{\theta\theta}. \quad (3)$$

There are a number of scenarios that we need to consider. For simplicity, we take $R \simeq \text{constant}$. Within this first scenario, there are two cases that we must consider. The first of these cases corresponds to $h_1(t) \gg R$. Equation (1) reads:

$$u_t = \frac{1}{R^2} u_{\theta\theta} - u \partial_t (\ln(h_1(t))) + f(u), \quad (4)$$

which implies that a possible symmetry breaking can occur only along the angular variable. This situation will be referred to as *slow growth*.

The second case corresponds to $h_1(t) \ll R$. In this case:

$$u_t = \frac{1}{h_1^2} u_{zz} - u \partial_t (\ln(h_1(t))) + f(u), \quad (5)$$

which implies that a possible symmetry breaking can occur only along the axial variable. This scenario will be described as *fast growth*.

4 Results

4.1 Linear stability analysis: fast growth

Let us consider the shadow equation for an activator-inhibitor reaction-diffusion system on a growing cylindrical shell with radius $R \simeq \text{constant}$:

$$u_t = \frac{1}{h_1^2} u_{zz} + \frac{1}{R^2} u_{\theta\theta} - u \partial_t (\ln(h_1(t))) - \lambda u + u^p. \quad (6)$$

In order to search for possible instabilities we perform a linearized stability analysis around the uniform non-trivial solution of Eq. (6) which is given by:

$$u_0 = (\mu + \lambda)^{1/(p-1)}. \quad (7)$$

We consider $u(z, \theta, t) = u_0 + b(z, \theta, t)$. Up to first order in the perturbation b , and assuming $h_1(t) = e^{\mu t}$ Eq. (6) reads:

$$b_t = \epsilon_h b_{zz} + \frac{1}{R^2} b_{\theta\theta} + (p-1)(\mu + \lambda)b, \quad (8)$$

where we define $\epsilon_h \equiv 1/h_1^2$. To proceed further, we assume separation of variables and write b as $b \sim e^{\nu t} Z(z) \Theta(\theta)$. Following the standard procedure Eq. (8) yields:

$$\begin{aligned} Z_{zz} &= \frac{\nu}{\epsilon_h} Z \\ \frac{1}{R^2} \Theta_{\theta\theta} &= (\nu - (p-1)(\mu + \lambda)) \Theta. \end{aligned} \quad (9)$$

In order for the corresponding mode to be unstable $\nu > 0$. This implies that the solution for $Z(z)$ is going to be a (real) exponential $Z(z) = e^{-Az}$ with $A = \pm \sqrt{\nu/\epsilon}$. Out of these two possible solutions, the boundary conditions select the one with $A > 0$. Since $p > 1$, the solution to the equation for $\Theta(\theta)$ is an oscillatory mode provided $\mu > (\nu/(p-1)) - \lambda$. In other words, given that the growth in the axial direction is fast enough, the only observable patterns are in the radial component.

No observable structures are possible along the axial direction. From the point of view of the theory of reaction-diffusion systems in growing domains, this observation makes sense, as one would expect the fast growth along the axial direction to suppress the formation of patterns in that direction. It is also consistent with the experimental observations of Metzger et al. [3], where they notice that domain bifurcation is the first mode to appear where it is arguable that no space restrictions are in place and thus the growth velocity is likely to be the larger.

Now we consider the other case, namely, when $R \ll h_1$. This in fact corresponds to the biological situation in which there is for the first time an important limitation of space. Therefore growth in the longitudinal direction decreases and for the purpose of analyzing the qualitative features of the solution, it is natural to consider a fixed domain as illustrated in Fig. 2. For this situation the results already mentioned in 3.1.1 due to the nonlinear effects of the reactive term become important. In other words, solutions with condensations are such that the peaks are located at points of maximal curvature. In Fig. 2 we present a simulation in which a solution with two peaks is computed. As can be seen, the location is consistent with the previous discussion. Moreover, it also corresponds to the experimental results in [3]. Although the simulation was performed on a planar domain, the same analytical result holds for a cylinder in three dimensions. In particular this also agrees with the fact that bifurcation is planar, since besides the maximal curvature effect, in 3d peaks arrange themselves as far as possible from each other. This is achieved if they are at antipodal points and so on the same plane.

4.2 Numerical results

1. Consistently with the above linear stability analysis, numerical simulations shown in Fig. 1 corresponding to the scenario where $h_1 \ll R$ and μ being large enough (i.e. fast growth along the axial direction), we obtain a periodic array of buds sprouting off the main branch whose layout abides by the rule of maximal separation between peaks in reaction-diffusion systems.
2. Furthermore, depending on the initial conditions, our model allows for different number of buds as well as different layouts, as shown in Fig. 1. We can have, for example, two or four peaks on a same plane orthogonal to the axial direction (Figs. 1(a) and (b)), always arranged according to the principle of maximal separation, or four peaks with arranged as in Fig. 1(c), namely, two sets of two buds on different planes with orthogonal sprouting directions.
3. Note that the above results fit perfectly with the *domain bifurcation* mode described in [3].
4. We also obtained consistent numerical evidence as can be seen in Fig. 2. We solved the equation in the situation in which growth along the axial direction is sufficiently slow so that it can be neglected, i.e. the domain can be taken as fixed. In this case it is observed that, due to the non-linear effects already mentioned, the buds select the points of maximal curvature. These results are in agreement with the *planar bifurcation* scenario reported by Metzger et al. [3].

5 Discussion

We have considered the influence of growth rate and space limitations on the possible branching modes and their timing during the developmental process. In particular, we have shown that under no space limitations, where (axial) growth is likely to be fast, domain bifurcations are robustly selected. If, on the contrary, space limitations

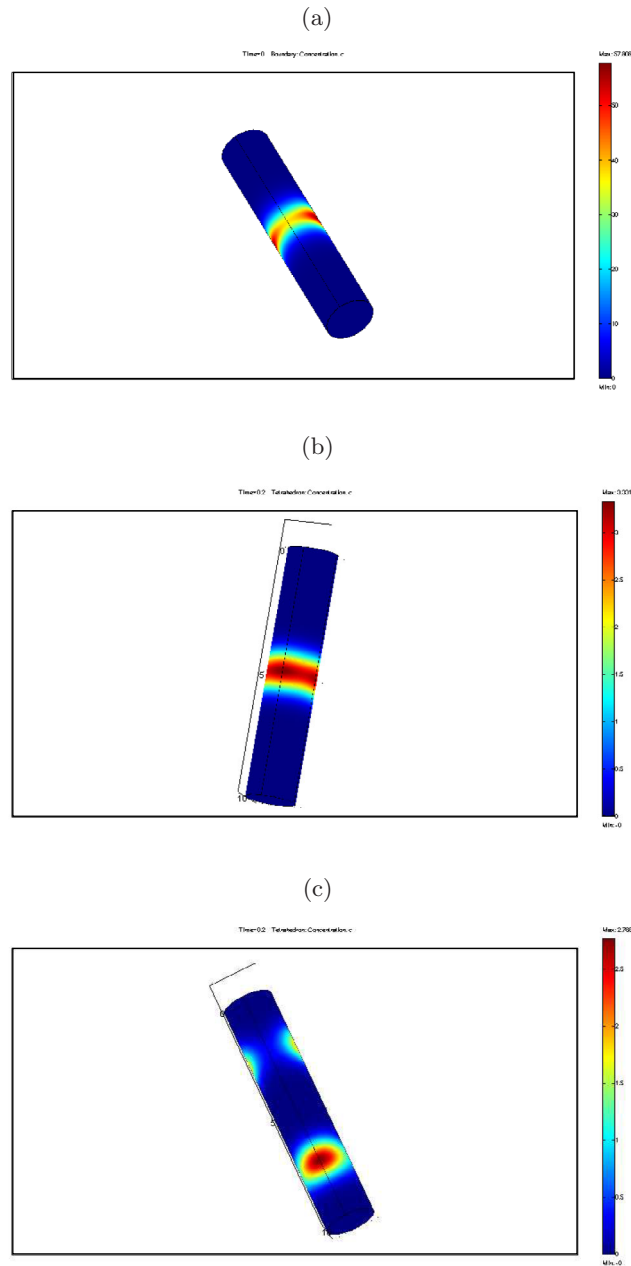


Fig. 1. Numerical simulations.

restrict the growth rate the selected bifurcation mode corresponds to planar bifurcations [3]. Using results from reaction-diffusion systems theory, we were able to give a rationale of the different modes of bifurcation observed in lung formation as well as the time ordering of their appearance.

A number of interesting issues arise from our results. For example, Metzger et al. [3] argue that the formation of the bronchial tree during lung development is genetically controlled. Although our results may seem to contradict such assertion,

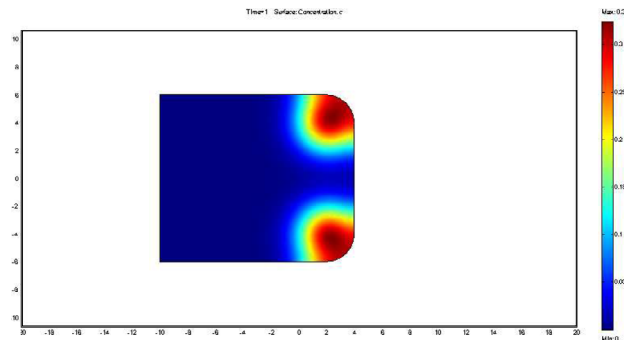


Fig. 2. Slow growth.

it is not so. They propose that a global clock controlling a global switch from one bifurcation mode to another does not exist, suggesting a local mechanism. Our proposal is that such local switch is partly controlled by space limitation which, in turn, controls the growth rate. Furthermore, our results are compatible with genetic control of the morphogenetic process. As pointed out in the Results section, the actual patterns exhibited by our model depend critically on the initial condition. We argue that this initial condition correspond to a genetically-controlled pre-pattern. Later development of this pre-pattern into the bifurcation modes observed occurs under the restrictions imposed by space and growth rate limitations on the corresponding reaction-diffusion system.

Although we have not directly addressed the orthogonal bifurcation mode in this paper, general results regarding reaction-diffusion systems allow us to draw some conclusions on its role. First, this mode is consistent with the principle that peaks in reaction-diffusion systems tend to be maximally separated. In fact, this property may explain several of the experimental observations of Metzger et al.. Orthogonal bifurcation is the most sensitive mode to space limitations in the sense that only by planar bifurcation, which is also a space-sensitive mode, the corresponding local planar region will eventually switch to domain bifurcation, as described by [3]. Since domain bifurcations need space available to occur, under space limitations these will robustly switch to orthogonal bifurcation. This may provide a rationale for both the sequencing observed in mode switching (see the discussion in the Biological Preliminaries section and [3]) and also for the fact that switching to the orthogonal mode is irreversible: It is the only mode that under space limitations can fill the space.

These results allow us to suggest several experiments in order to validate our conclusions. The most obvious of these experiments would be to physically limit the growth by imposing geometrical constraints. Under these conditions, we propose that space limitations would trigger the system to switch to either planar or orthogonal bifurcations. Conversely, if growth factors are injected and no space limitations are present the system should then switch to domain bifurcation. In general, a direct epigenetic relation between the genes considered by Metzger et al. and growth factors is strongly suggested by our conclusions and would provide further evidence in this direction.

Similar ideas can be applied to the development of the root in plants and it is the subject of current work.

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