

# Theory of Morphogenesis

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## Abstract

A model of morphogenesis is proposed based upon seven explicit postulates. The mathematical import and biological significance of the postulates are explored and discussed.

Théorie de la morphogénèse

Résumé : Un modèle de morphogénèse est proposé sur la base de sept postulats explicites. L'importance mathématique et la signification biologique de ces postulats sont explorées et discutées.

## Introduction/Background

Morphogenesis is the evolution of shape of an organism together with the differentiation of its parts. The discovery of differential gene expression, that is, the spatio-temporal distribution of gene expression patterns during morphogenesis together with its key regulators which are again given by gene expression is one of the main recent achievements in developmental biology, cf. [2] and references therein. Nevertheless, differential gene expression cannot explain the development of the precise geometry of an organism and its parts, cf. [4, 6].

The popular theory of morphogen gradients governing morphogenesis and accordingly differential gene expression, though correct for some special cases, still leaves more questions than answers [10]. For example, the mechanism of coordination of proper locations of specific morphogen production, the exact molecular pathways leading to morphogen gradient formation, the dependence of tissue formation and especially of their geometrical shapes on exact gradients, along with many other key points, must still be elucidated in order to accept this theory as the basis for pattern formation rather than a part of molecular instruments implementing more general laws. It is these more general laws which we shall postulate.

It is plausible to suggest the existence of a cell-surface molecular code which bears information about the geometrical pattern of an organism and thus coordinates the cascades of molecular events implementing pattern formation, e.g.,

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differential gene expression, directed protein traffic, growth of microtubules and others. Whatever the precise nature, this coding is epigenetic—literally, beyond genes—since diverse cell lineages with their diverse cell fates and morphogenetic evolutions nevertheless share the common genome of the organism itself.

Though the concrete signal transduction pathways connecting the morphogenetic coding information and expression of given sets of genes are not yet elucidated, we can suggest a set of postulates and possible approaches for discovering the correspondence between this code and its realization in the given geometry of an organism in space-time. This paper is a sequel to [5, 6] with the main new innovations being the inclusion of cell-to-cell communication, the emphasis on the role of cell potency, and the process of cell de-differentiation.

Our goal is to formalize the mechanisms and details of morphogenesis in order to uncover its underlying general laws, two significant manifestations being embryonic development and physiological response to various crises such as amputation, transplantation or biochemical intervention

## 1 Overview/Fundamental Hypotheses

Our proposed theory of morphogenesis is based on several fundamental hypotheses as follows:

- For each cell in an organism there is a cell-surface distribution of chemical substances called its (*epigenetic*) *spectrum* governing morphogenesis.
- There is transmission to a certain collection of neighbors from each cell of its own epigenetic spectrum called *cellular signaling*.
- Each cell comprising an organism performs one of several possible *cell events* at various times, namely, change of spectrum, change of position, change of shape including growth, mitotic division, and apoptosis (that is, programmed cell death).
- There is a collection of *universal rules* obeyed throughout Nature for a specific cell event for each cell at each instant depending upon its own epigenetic spectrum and the cellular signals it receives.
- For each zygote for each organism, there is an *optimal sequence* of cell events following the universal rules which describes the normal evolution of the embryo.
- If the optimal cell event is impossible due for instance to crisis or malfunction, then the cell response is to *de-differentiate* and return its spectrum to that of its ancestor cell.
- The strength of the signal transmitted by a cell is inversely proportional to its *potency*.

More explicitly and to fix ideas, we assume that the spectrum is comprised of a collection of oligosaccharide residues of glycoconjugates lying in the lipid bilayer cell-surface membrane of each cell. The concentration of these residues in different sectors of the cell can be described by a matrix with integer entries. Note that the spectrum of each cell could as well consist of other cell-surface molecules, in which case our general framework still applies *mutatis mutandi*.

The precise nature of signaling between cells likewise can remain unspecified. The detailed signal could be a direct mechanical interaction of cell-surface compounds or structures, or it could be molecular, such as ion exchange, ligand-receptor interactions or others. We assume that whatever its nature, the sent signal is itself determined by the spectrum of the sender cell which we may therefore take to be the signal itself. It is in the *interpretation* of that sent signal by the receiver cell that distinctions are made depending on precise details.

Each cell in fact receives a set of signals from a collection of its neighbors, and from these various signals determines an appropriate single new *target spectrum*. Under normal development after the cell event, the new spectrum agrees with the target spectrum, thus explicating a basic universal rule of morphogenesis. Other external attributes of the cell, such as its position within the embryo or its shape may also alter as the result of a cell event. If the target spectrum is unachievable by the cell in its current state, then the spectrum of the cell reverts to that of the previous cell event, a kind of backtracking which assumes a certain level of redundancy in the epigenetic spectrum.

The last postulate that signal strength varies inversely to the potency is the most difficult to explain here precisely because the concept of “potency” requires a number of further considerations. This final postulate is explained further in §4. Roughly, the potency of a cell is its ability to produce a diversity of different cell states during the optimal sequence of cell events, cf. §3. For instance, a zygote has maximal potency (totipotency), and a fully differentiated cell that admits no further mitotic divisions, such as a mammalian brain cell or eye lens cell, has minimal or no potency. All cells at early stages of embryonic development, which are called embryonic stem cells, enjoy so-called pluripotency, or in some cases even the totipotency of the zygote, which allows them easily to change their cell fates, while stem cells existing in tissues of adult organisms, or adult stem cells, enjoy bipotency, meaning that they can produce only two types of cells—themselves and the differentiated cells of the corresponding tissue.

We hope and expect that future laboratory experimental work will confirm or refine aspects of the model presented here. Furthermore, the model itself is currently being probed via computer implementation and experimentation.

## 2 Shapes

It is problematic to rigorously define the notion of *shape* or *form* in biology, cf. [9, 9.1.1]. We shall do so at two scales: the microscopic shape of a cell and the macroscopic shape of an organism.

To define the *shape of a cell* we proceed as follows, cf. [5, §2] for more details. We assume that the cell  $c$  contains a distinguished point  $O = O_c$  with respect to which it is star-convex, that is, the line segment  $\overline{OP}$  lies in  $c$  for any other point  $P$  in  $c$ . Though the example of a neuron cell shows this is not strictly true for all cells, we can accept that the few such counter-examples are not especially critical in determining shapes of organisms. Specifically, we take  $O$  to be the so-called microtubule organizing center or centrosome.

It follows that the shape in space of the cell membrane  $\mu(c)$  of  $c$  can be described by a positive real function  $\sigma = \sigma_c : S^2 \rightarrow \mathbb{R}_{>0}$  on the unit-radius two-dimensional sphere  $S^2$  centered at  $O$ , namely, if  $P_0 \in S^2$ , then the point  $P \in \mu(c)$  in the direction of  $P_0$  from  $O$  is uniquely defined by the equality  $\overrightarrow{OP} = \sigma(P_0) \overrightarrow{OP_0}$  of vectors. Thus as a subset of Euclidean space  $\mathbb{R}^3$ , the cell  $c$  in space is given by the convex set  $B_\sigma(O) = \{P \in \mathbb{R}^3 : \|\overrightarrow{OP}\| \leq \sigma(P_0)\} \subset \mathbb{R}^3$  containing  $O$ . To fix ideas, let us assume that  $\sigma_c \in L^2(S^2)$ , i.e.,  $\sigma_c$  is square integrable for each cell  $c$ .

Now turning to the *shape of an organism*  $\Omega$  regarded as the union of its constituent cells, one encounters the following difficulty ([9], *loc. cit.*): At an instant in time the organism  $\Omega$  is embedded as a closed subset in  $\mathbb{R}^3$ , and the coordinate axes can be chosen to coincide with the three embryonic axes (anterior-posterior, dorsal-ventral, left-right) of the organism determined already in the zygote. Insofar as the organism  $\Omega$  can move in space, it admits multiple manifestations as subsets, and it is not clear how to specify precisely when two such explicit manifestations of  $\Omega$  are equivalent.

We propose to proceed as follows. The shape of  $\Omega$  is determined by a finite and connected graph  $I(\Omega)$ , called the *graph of adjacency* whose vertices are given by the cells of  $\Omega$  with an edge between vertices  $c_1$  and  $c_2$  when the cells  $c_1$  and  $c_2$  touch one another; in fact,  $I(\Omega)$  is equipped with a natural metric assigning to the edge between  $c_1$  and  $c_2$  the distance between  $O_{c_1}$  and  $O_{c_2}$  in  $\mathbb{R}^3$ , in contrast to the simpler combinatorial length determined by the number of edges traversed. This metrized graph  $I(\Omega)$  can be isometrically embedded in  $\mathbb{R}^3$  in such a way that the vertex  $c$  is mapped to the distinguished point of the cell  $c$ , and  $\sigma_c$  determines the extent of the cell in space. Notice that the collection of functions  $\sigma_c$  are not arbitrary, e.g., because two cells cannot overlap. A crucial point is that we *do not fix the embedding* of  $I(\Omega)$  into  $\mathbb{R}^3$ . Two different closed subsets of  $\mathbb{R}^3$  have the same shape when they share the same data  $(I(\Omega), \sigma_c)$ .

We can now go further and give a notion of distance between two organisms  $\Omega_1$  and  $\Omega_2$ . Namely  $d(\Omega_1, \Omega_2)$  is the *Gromov-Hausdorff (GH) distance* (cf. [3]) between the metrized graphs  $I(\Omega_1)$  and  $I(\Omega_2)$ . There is also the related notion considered in [5] where one regards the organism  $\Omega = \cup_{c \in \Omega} B_{\sigma_c}(O_c) \subset \mathbb{R}^3$  as the union of its cells in space as a metric subspace of  $\mathbb{R}^3$  and again measures distances between organisms using GH distance; the distance based on the metrized graph of adjacency is more easily computable.

### 3 Cell State and Cell Event

As mentioned in §1 we postulate that the development of an organism is driven by cell-surface molecular codes called (epigenetic) spectra of its constituent cells. As a simplification to describe this code, we consider the set  $Mat$  of  $N$ -by-8 matrices  $A_c = (a_{ij})$  with natural integer entries  $a_{ij}$ , where  $N$  is the number of species of glycosidues we shall record for each cell, and the three coordinate planes decompose each cell surface into eight orthants within which we record the number  $a_{ij} \geq 0$  of each of the  $N$  species, for  $i = 1, \dots, N$  and  $j = 1, \dots, 8$ . As in [5] a more sophisticated approach would be to record the actual densities with further real-valued functions defined on sphere  $S^2$ , one such function for each species for each cell, rather than the discrete model with integral matrices considered here.

There are several data intrinsically associated with each cell  $c$ , namely,

- the epigenetic spectrum  $A_c \in Mat$ ,
- the shape function  $\sigma_c \in L^2(S^2)$ ,
- the coordinates of the distinguished point  $O_c \in \mathbb{R}^3$ ,
- the number  $t_c$  of cell divisions directly leading to  $c$  from the zygote called the *cell timer*,
- the number  $s_c$  of cell events occurring since the most recent cell division called the *cell stopwatch*,
- the relative age of the most recently inherited centrosome,  $\alpha_c = m$  for the older (mother) and  $\alpha_c = d$  for the younger (daughter) centrosome, called the *m/d invariant*.

These data are intrinsic in the sense that the cell might be removed from its organism yet preserving each of these attributes which could then be measured. Together these data comprise the *cell state*  $S_c = (A_c, \sigma_c, O_c, t_c, s_c, \alpha_c)$ , and we shall regard  $I_c = (A_c, t_c, s_c, \alpha_c)$  as the *internal state* and  $E_c = (\sigma_c, O_c)$  as the *external state* of the cell  $c$ . We should note that the first three pieces of data can be organized into a bundle over the configuration space of distinguished points in space with fiber given by shapes and spectra, cf. [5].

The biological and mathematical significance of  $A_c$ ,  $\sigma_c$  and  $O_c$  have already been discussed. In order to elucidate the two timers, let us first construct the *tree*  $T = T_\Omega$  of *cell events* whose vertices are in correspondence with the cell states  $S_c$  with an edge connecting vertices when they are related by a cell event. The zygote in its initial state forms the root of the tree  $T$  and has valence 2 corresponding to the fact that it divides from its current state at the outset of the construction; other 2-valent vertices arise from change of spectrum, position or shape, while 3-valent vertices correspond to division and 1-valent vertices to apoptosis. The tree  $T$  is metrized where the length of an edge is given by the temporal duration of the corresponding cell event.

The path in  $T$  from the zygote to the vertex of  $T$  labeled by cell state  $S_c$  passes through a certain number of 3-valent vertices, and this number is the value of the timer  $t_c$ . The biological determination of  $t_c$  can be approximated in terms of the length deficit of the so-called telomeric tail of the DNA contained in the cell  $c$ , which loses one telomere for each division, cf. [1], cf. [2]; strictly speaking, a single cell division might remove several telomeres from the tail due to oxidative effects, and indeed there are proteins called TERTs which serve to lengthen the telomeric tail, cf. [8]; let us nevertheless regard  $t_c$  as an intrinsic datum roughly determined by the telomeric tail length and given precisely by this and some other intrinsic cell data which can remain unspecified for now.

Analogously, the path in  $T$  from zygote to the vertex  $v$  has a last passage through a 3-valent vertex before arrival at  $v$ , and the number of 2-valent vertices it meets after visiting this 3-valent vertex, or in other words the number of changes of spectrum, shape or position that occur from the most recent division, gives the cell stopwatch  $s_c$  in terms of  $T$ . The biological determination of the stopwatch requires a short digression. All cells contain microtubules in particular supporting the cell surface, and as we have mentioned, have a specific microtubule organizing center which gives a distinguished point within each cell. In fact, microtubules are not static and cycle through a process of adding to the base (proximal) and removing from the tip (distal), and this cycle time in fact correlates with the cell cycle controlling mitosis. Thus, a notch on the microtubule moves up and away from the base towards the tip, and the distance of this notch from the base again gives an approximate biological interpretation to the intrinsic stopwatch  $s_c$ .

To explain the  $m/d$  invariant  $\alpha = \alpha_c$  let us note that the centrosome is duplicated during mitotic as well as meiotic cell division, cf. [1, 2]. The daughter cell inheriting the older centrosome has  $\alpha = m$ , and the other daughter cell has its  $\alpha = d$ . For diplosomes whose centrosome is comprised of two centrioles and which includes all animal cells, one centriole is older than the other and each duplicates to produce another pair of complete centrosomes each comprised of two centrioles; the daughter cell inheriting the oldest of the four constituent centrioles has  $\alpha = m$  the other having  $\alpha = d$ . The  $m/d$  invariant is indeed intrinsic in particular for diplosomes insofar as asymmetries between  $m$  and  $d$  centrioles go beyond simply age presenting notable differences in molecular composition, function and ultrastructure. The ovum and sperm in the diplosomic case each contain just one centriole, and the former is  $m$  in the zygote. Numerous experiments for diplosomes have shown that cell fate is tied to the  $m/d$  invariant cf. [7]. We again assume this  $m/d$  invariant is likewise intrinsic in general by these or other unspecified attributes.

Keeping track of the  $m/d$  invariant starting from the zygote, each cell  $c$  in an organism has a well-defined word of length  $t_c$  in the letters  $\{m, d\}$  called the  $m/d$  code which uniquely determines the phylogeny of its centrosome starting from the zygote. It is an interesting question whether the full  $m/d$  code is intrinsic or perhaps just a terminal segment of it of some fixed length definitely greater than or equal to one. It seems unlikely that cell events could depend upon more than the last few letters since otherwise presumably inevitable errors

in  $m/d$  code would be catastrophic for embryogenesis.

Fix some organism  $\Omega$  with zygote  $z$  and consider a cell state  $S_c$  labelling a vertex on the tree  $T$  of optimal cell events with its well-defined subtree  $T(S_c) \subseteq T = T(S_z)$  with this vertex as its root. Define the collection  $X(S_c)$  of all pairs  $(A_d, \alpha_d)$  occurring as data among vertices of  $T(S_c)$ . The ratio of the measure of  $X(S_c)$  to that of  $X(S_z)$  for some appropriate measure of the set of all pairs comprised of spectrum and  $m/d$  invariant is the (*normalized*) *potency* of the cell state  $S_c$ .

Potency is *not* an intrinsic attribute of the cell state in the sense discussed previously, and its definition requires *a priori* knowledge of the tree of optimal cell events. Despite much attention, we do not know a reasonable definition of intrinsic potency since one must specify under exactly which conditions a cell in its state is allowed to evolve: under *all possible conditions* being too broad and unmeasurable and under *specific laboratory conditions* being too specialized. Notice however that if the full  $m/d$  code were intrinsic, then potency for counting measure on  $Mat \times \{m, d\}$  could actually be determined in laboratory experiment without killing the organism: sample each type of internal cell state  $(A, \alpha)$  in the complete mature organism and compare with the histogram initial segments of  $m/d$  code.

## 4 Signaling and Cell Response

We have already in §1 explained that each cell  $c$  of an organism  $\Omega$  provides its signal to a collection of its neighbors, and the signal is given by its own spectrum. The set of cells of  $\Omega$  that receives this signal can be defined in various ways. For example, the signal might propagate uniformly in all directions or may have vectorial characteristic, it might decay with spatial distance from  $c$  or with combinatorial distance in the graph of adjacency from the vertex corresponding to  $c$ , the simplest possibility, or it might depend on the subsets of epigenetic spectra through which it is transmitted.

Each cell  $c$  of  $\Omega$  thus also receives a certain collection of signals from its neighbors, and these must be combined in some manner to produce the target spectrum also discussed in §1. There are again various possibilities ranging from a simple average over signals received possibly weighted by distance or other attributes again including perhaps the spectra through which it is transmitted, also possibly allowing for stochastic effects and depending inversely upon the potency of the sender according to our final postulate.

In the optimal situation, the spectrum of the cell after the cell event coincides with the target spectrum. In particular if the cell  $c$  in its state with spectrum  $A$  is provided with a target spectrum that agrees with  $A$ , this means that the optimal (coded) cell event for the cell  $c$  is confirmed by the signal. It is this “harmony” between cell current spectrum and the target spectrum determined by the received signal that communicates to the cell that it should “move” along the optimal tree of cell events. In this way, the shape of the organism through signaling can communicate stasis to its constituent cells at the conclusion of

morphogenetic processes.

More generally though, the target spectrum differs from the current spectrum and the cell state evolves. This evolution normally follows a pattern of differentiation, by which we mean that the cell states becomes more and more specialized, less capable of diverse evolution, thus with diminished potency.

Note that cell events depend on parameters. For instance the division of a cell requires the specification of a plane of division. Another aspect of cell events requires determining the rules for the resulting distribution of coding species (epigenetic spectrum) on a cell surface after a cell event. For example for the cell event of division, it is quite reasonable to postulate that half of the daughter cell-surface spectra are directly inherited unchanged from the mother spectrum while the remaining daughter spectra adjacent to the division plane are filled in by certain rules as yet to be determined.

We have thus far concentrated primarily on normal evolution of an embryo and finally briefly consider cell response under unusual circumstances. For example an amputated limb in the frog species *Xenopus* is capable of regeneration, and even small body fragments of the *Planaria* worm can generate an entire and complete organism, cf. [4]. Even human babies are capable of regenerating amputated fingertips during the first months following birth it turns out. The removal of the cell membrane from a plant cell produces a so-called callus of many undifferentiated cells capable of generating an entire and complete plant organism. Transplantation of limb fragments in non-standard orientations in *Drosophila*, *Axolotl* and other species can result in supernumerary limb regeneration as well as other bizarre outcomes. The literature abounds with experiments illustrating these remarkable phenomena, cf. [2, 4] and references therein.

In our model, the cell in its cell state is provided by signaling with a target spectrum and then responds with its optimal cell event under normal conditions, but if the conditions are not normal so the target spectrum is for some reason unachievable, then we posit that the cell has the only possible responses of stagnation (that is, no cell event), cell death (a form of apoptosis under these unusual conditions), or a de-differentiation (that is, the return to its previous epigenetic spectrum). In particular, the cell epigenetic spectrum can return to that of its mother in the case of cell division and may then de-differentiate further perhaps to its grandmother and beyond, or it may perhaps again divide. This cycle of devolution to ancestor and division accurately reflects the kind of de-differentiation and cell proliferation that typically precedes regeneration in experiments. It is the final postulate that signal strength varies inversely to potency that finally drives regeneration via the harmony discussed above.

We have articulated and discussed in this short paper seven explicit laws of morphogenesis which when taken together explain a plethora of phenomena in many hundreds of experiments in the literature.

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