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REVIEW

Mathematical modelling in developmental biology

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Abstract

In recent decades, molecular and cellular biology has benefited from numerous fascinating developments in experimental technique, generating an overwhelming amount of data on various biological objects and processes. This, in turn, has led biologists to look for appropriate tools to facilitate systematic analysis of data. Thus, the need for mathematical techniques, which can be used to aid the classification and understanding of this ever-growing body of experimental data, is more profound now than ever before. Mathematical modelling is becoming increasingly integrated into biological studies in general and into developmental biology particularly. This review outlines some achievements of mathematics as applied to developmental biology and demonstrates the mathematical formulation of basic principles driving morphogenesis. We begin by describing a mathematical formalism used to analyse the formation and scaling of morphogen gradients. Then we address a problem of interplay between the dynamics of morphogen gradients and movement of cells, referring to mathematical models of gastrulation in the chick embryo. In the last section, we give an overview of various mathematical models used in the study of the developmental cycle of *Dictyostelium discoideum*, which is probably the best example of successful mathematical modelling in developmental biology.

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Introduction

One can state that mathematical modelling in developmental biology started with the publication of the book 'On growth and form' by D'Arcy Thomson (Thompson 1917). In this book, mathematics was used in a very descriptive way for the analysis of various biological shapes observed in nature. This, to a certain extent, was dictated by the descriptive nature of biology at that time. However, the use of mathematical methods in other branches of biology, such as population dynamics and demography, had started much earlier (the main contributors here are Thomas Malthus in the 18th century, Pierre Verhulst and Benjamin Gompertz in the 19th century) and had become far more than just descriptive by the early 20th century. The book 'Elements of physical biology' by Alfred Lotka (Lotka 1925), where mathematical language is used to describe various aspects of population dynamics and bioenergetics, is considered by many scientists as the first book on mathematical biology: the use of mathematics in biology demonstrated in this book was, at least to some extent, similar to its use in physics.

Developmental biology was rapidly advancing in the late 19th and early 20th century. A number of scientists had started experimenting with embryos and this caused old paradigms, like 'vitality' and 'entelechy', to be left behind, while experimentally observable processes and objects had attracted attention. Studies of developmental and regenerative processes in lower organisms led to the introduction of an important concept of a physiological gradient to explain the difference in physiological processes occurring in different locations in an organism (Child 1911). Later on, experiments on embryos led to the discovery of an important morphological object, termed an 'organising centre' (Spemann 1927), which orchestrates the behaviour of cells during gastrulation in the embryo. Gastrulation, in turn, was defined as the stage in embryonic development when the formation of first tissues and setting of the preliminary body plan of the future organism take place. The way the organising centre acts and directs the behaviour of the developing embryo appeared to be different between species (Wolpert *et al.* 2002), although it is always associated with the movement of the organising centre and its impact on the movement and differentiation of other cells comprising the embryo.

One of the main challenges in developmental biology since the discovery of the organising centre is to explore the mechanisms of cellular differentiation. Many efforts have been made to check whether or not the cellular differentiation takes place according to the developmental programme each cell acquires from its maternal cell, run by internal cellular clock. However, on the basis of a vast amount of experimental observations, it was concluded that embryonic cells differentiate according to their location with respect to positional information

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provided by a so-called 'morphogen gradient'. The term 'morphogen gradient' refers to a substance that is present and non-uniformly distributed in the embryo. Often the differentiated cells start to migrate resulting in the transportation of the positional information across the embryo. Despite the accumulation of an overwhelming amount of data on cell movement patterns in developing tissues of different geometry (Beloussov 1998), little is known about the mechanisms governing the migration and rearrangement of cells in embryonic tissue. One of the commonly discussed mechanisms is chemotaxis when the migration of a cell is driven by the gradient of a substance, which is, in this case, called a chemotactic agent (Dormann *et al*. 2002, Yang *et al*. 2002). Other known mechanisms such as apical constriction (Odell *et al*. 1981, Dawes-Hoang *et al*. 2005) or cellular intercalation (Warga & Kimmel 1990) result in the deformation of embryonic tissue and thus lead to the rearrangement of cells with a net effect similar to migration of cells.

No mathematical formalism has been developed so far to describe tissue dynamics due to apical constriction or cellular intercalation. The contrary is true for chemotaxis, which has been studied mathematically by many authors especially in the context chemotactic signalling and motion of unicellular organisms (Keller & Segel 1971, Keller *et al*. 2003) including the aggregating population of *Dictyostelium discoideum* amoebae. Studies of the developmental cycle in *D. discoideum* probably represent the best examples of the use of mathematical modelling in developmental biology. Here, we will give an overview of models and modelling results from these studies.

Modelling morphogen gradients

The classical illustration of how a morphogen can provide positional information is given by the French Flag model suggested by Lewis Wolpert (Wolpert 1969). This model demonstrates how a simple, linear concentration profile of a morphogen (Fig. 1A) can define domains of cellular determination in an otherwise homogeneous tissue. The linear concentration profiles can form naturally in various settings. The simplest case is when the production and degradation of the morphogen take place outside the tissue on its opposing sides and the morphogen passively diffuses along the tissue, from the side where it is produced to the side where it is degraded. Mathematically, the stationary concentration profile of the morphogen in this system should obey the so-called Laplace's equation with Dirichlet boundary conditions, which for a tissue represented by a one-dimensional domain of length L, is given by the following mathematical formulation:

$$D\frac{\mathrm{d}^2u}{\mathrm{d}x^2}=0;\quad u(0)=u_1;\quad u(L)=u_2 \qquad (1)$$

where u represents the concentration of the morphogen, D represents the diffusion coefficient and u_1 and u_2 represent the buffered (fixed) concentrations of morphogen on the boundaries of the tissue, $x=0$ and $x=L$. The advantage of a linear profile, the solution of equation (1), is that it scales with the size of the tissue. This means that if, for example, we double the size of the tissue, then the sizes of all domains of cellular determination (as defined by the threshold concentration values T_1 and T_2, see Fig. 1A) will also double.

However, the experimental observations do not always confirm the linear shape of a morphogen gradient. Most commonly, measurements point to an exponential shape, as in the case of the transcriptional factor Bicoid in the early embryo of the fruit fly *Drosophila melanogaster* (Driever & Nüsslein-Volhard 1988, Gregor *et al*. 2007). Formation of the exponential profile can be shown mathematically under the assumption that the morphogen not only diffuses but also degrades inside the domain. The concentration of morphogen can be buffered (fixed) on the boundaries of the tissue (similar to the above case expressed in equation (1)). Alternatively, we can assume that the tissue is isolated (no flow on the boundaries) and the production of the morphogen takes place in a restricted area inside the domain (Fig. 1B). These assumptions are perfectly reasonable for many studied cases. For example, the maternal Bicoid mRNA in *D. melanogaster* embryo is localised in a small region on its apical side;

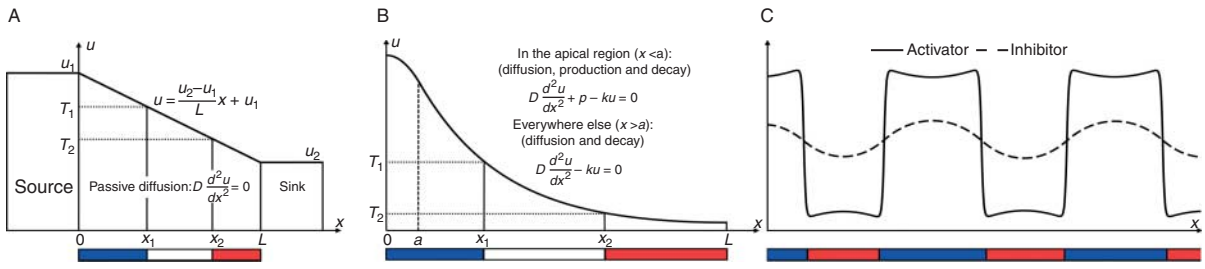

Figure 1 Modelling morphogen gradient. (A) Linear gradient in the French Flag model forms due to passive diffusion of the morphogen (equation (1)). (B) Exponential gradient forms when the diffusion is combined with decay (equation (2)). (C) A periodic (Turing) pattern forms due to non-linear interactions between the involved morphogens (equation (3)). (A and B) T_1 and T_2 are the threshold concentrations of the morphogen, defining the borders of the cell determination domains. (C) The sharp profile of the activator makes the locations of cell determination domains insensitive to the exact value of the threshold concentrations.

the Bicoid protein produced in this region diffusively spreads and decays along the entire embryo. A stationary concentration profile of the morphogen in this system will satisfy the following equation:

$$D\frac{\mathrm{d}^2u}{\mathrm{d}x^2}+p(x)-ku=0;\quad \frac{\mathrm{d}u}{\mathrm{d}x}=0\quad \text{for}\quad x=0,L;$$
$$p(x)=\begin{cases} p & \text{for}\quad 0\le x\le a \\ 0 & \text{for}\quad a<x\le L \end{cases} \tag{2}$$

where the term *p(x)* defines the production of a protein in the apical (left-side) region of size *a* and the term -*ku* is its degradation. The main problem with the exponential (or nearly exponential as in Fig. 1B) profile, as described by the solution of equation (2), is that it does not scale. For example, if, in the case shown in Fig. 1B, we increase the size of the domain from *L* to *2L*, the sizes of blue and white sub-domains will not change while the red sub-domain will increase to cover all added *L* units of length. This property of the solution of equation (2) contradicts the observation that the exponential gradient of Bicoid in *D. melanogaster* embryo scales with the size of the embryo with very high precision (Gregor *et al.* 2008).

The models represented by equations (1) and (2) fall into the class of linear models (that is, the variable *u* and its derivative appear only in their first power). Since the introduction of a classical predator–prey model (Lotka 1925), many non-linear models have been developed and used for studies in mathematical biology. Contrary to linear models, which have a limited range of possible solutions, non-linear models can be used to reproduce virtually any kind of known dynamics in concentration fields of morphogens. This is especially true if more than one morphogen is considered and the model is represented by a set of reaction–diffusion equations. The dynamics of two interacting morphogens can be described by the following reaction–diffusion equations:

$$\begin{cases} \dfrac{\partial u}{\partial t}=\dfrac{\partial^2u}{\partial x^2}+f(u,v); & \dfrac{\partial u}{\partial x}=0\ \text{ for }\ x=0,L \\ \dfrac{\partial v}{\partial t}=D\dfrac{\partial^2v}{\partial x^2}+\varepsilon g(u,v); & \dfrac{\partial v}{\partial x}=0\quad \text{for}\ x=0,L \end{cases} \tag{3}$$

These equations state that the rates of change of morphogen concentrations are defined by two processes, diffusion (first term on the right-hand side) and reaction (second term) with the latter accounting for the production and decay of morphogens. In the classical FitzHugh-Nagumo (FHN) model (Nagumo *et al.* 1962), the kinetics in the first equation has cubic non-linearity $f(u,v)=k_uu(u-a)(u-1)-v$, and the kinetics in the second equation are linear $g(u,v)=k_vu-v+b$ and the second variable (*v*) does not diffuse, $D=0$. The FHN model was originally designed as a generic model for signal propagation along a nerve fibre. Its modification, which takes into account the diffusion of second variable ($D\neq0$), also serves as a generic model for morphogenetic pattern formation (Vasiev 2004). The morphogen, with concentration *u*, is called the activator as it promotes its own production and the production of the second morphogen, the inhibitor. The inhibitor promotes the degradation of the activator. Parameters *D* and ε define the rates of diffusion and kinetics of the inhibitor respectively compared with the rates of activator. Model (3) has a rich set of solutions, including stationary patterns and travelling waves that can be obtained by varying the values of parameters *D* and ε (Vasiev 2004).

One of the best-known examples of the phenomena observed in non-linear systems is the so-called diffusion-driven instability (or 'Turing instability') (Turing 1952), which provides a possible mechanism of morphogen gradient formation from an initially uniform state. According to this mechanism, a spatially periodic pattern can spontaneously arise under certain conditions due to the intrinsic noise in the system. Figure 1C shows a periodic stationary pattern that has emerged in system (3) from nearly homogeneous (noisy) initial conditions. This pattern, which could represent a morphogen gradient, only occurs when the inhibitor diffuses quickly and has slow kinetics (i.e. the product $D\varepsilon$ is above a certain threshold (Vasiev 2004)). The later condition is often stated only in terms of the diffusion coefficient *D*, which should be sufficiently large, $D>1/\varepsilon$, meaning that the diffusion of the inhibitor must be considerably larger than that of the activator. This difference in diffusion rates was phrased as 'long-range inhibitor and local activator' in classical works of Gierer and Meinhardt where Turing instability was brought forward to explain various biological patterning processes including the regeneration of fresh-water polyp hydra (Meinhardt 2008, 2009). Recent experimental studies of pigmentation patterns of fish (Yamaguchi *et al.* 2007) have directly confirmed that these patterns occur due to Turing instability. However, the Turing patterns fail the scaling test: the distance between spikes (Fig. 1C) in the model is only defined by the values of two parameters: diffusion, *D*, and kinetics rate, ε, of the inhibitor. This means that the number of spikes should increase with an increase in the size of the medium.

In models represented by equations (1), (2) and (3), formation of the gradient is conditioned by diffusion of the morphogen. A number of mathematical studies have addressed the formation of gradients in the absence of morphogen diffusion, for example due to proliferation (Ibanes *et al.* 2006, Chisholm *et al.* 2010) or migration (Harrison *et al.* 2011) of cells. Non-diffusive patterning mechanisms can be provided by direct contacts between cells, of which the classical example is Delta-Notch signalling associated with the binding of non-diffusive Delta to the Notch receptor of neighbouring cells (Collier *et al.* 1996). Cell determination patterns due to contact signalling can be studied using the cellular automaton models, represented by sets of rules defining

the interactions and states of cells or using so-called hybrid models combining cellular automata with differential equation (Ghosh & Tomlin 2004).

Robustness and scaling of morphogen gradient

The shape of a developing biological structure often demonstrates remarkable robustness with respect to various changes in its developmental conditions. Scaling is a particular case of robustness and there are many instances recorded (including classical experiments of Hans Driesch (Sander 1992)) where perfectly capable organisms emerge from embryos of different sizes. Robustness and scaling of biological patterns increasingly attract the attention of mathematical biologists (Umulis 2009, de Lachapelle & Bergmann 2010).

Possible mechanisms ensuring the robustness of dorso-ventral patterning to changes in production rates of involved proteins (BMP and Sog) in the *D. melanogaster* embryo have been revealed using mathematical modelling (Eldar *et al*. 2002). In the model, it was assumed that BMP is produced on the dorsal side of the embryo, Sog is produced on the ventral side, Sog inhibits BMP, and BMP and Sog interact to form the BMP–Sog complex. It was shown that if the BMP–Sog complex is highly diffusive so that the diffusion and degradation of the BMP–Sog complex considerably enhances the dorso-ventral transportation of the BMP (the term used by authors is 'shuttling'), then the BMP gradient is robust with respect to the changes in its kinetics. A modification of this model (Ben-Zvi *et al*. 2008) with two additional equations describing the dynamics of BMP ligand Admp, which also forms a highly mobile complex with the BMP inhibitor, was used to demonstrate that a shuttling mechanism can also explain scaling of BMP gradient in the *Xenopus* embryo.

It is a well-established fact that the Bicoid gradient in *D. melanogaster* embryo is exponential and it scales with the size of the embryo (Gregor *et al*. 2008). Formation of the Bicoid gradient has been addressed in a number of modelling studies (Grimm *et al*. 2010). Its exponential profile can easily be modelled but the real challenge is to explain its scaling. It is known that Bicoid molecules are partly located in the syncytium (freely diffuse) and are partly bound by nuclei. A mathematical model combing this observation (so-called 'nuclear-trapping' model), with the assumption that Bicoid is predominantly degraded in nuclei rather than in syncytium, was designed to reproduce the Bicoid gradient scaling (Umulis 2009). However, there are two difficulties associated with this mechanism of scaling. First, the total amount of Bicoid strongly depends on the density of nuclei, thus after each nuclear division (every 10 min), the concentration of Bicoid should significantly reduce. This contradicts to the experimental observations indicating that the Bicoid gradient is stable over cell cycles 10–14 (Gregor *et al*. 2007). Another argument is more philosophical: it is very unlikely that the degradation in nuclei is stronger than in syncytium, simply because there is no 'degradation machinery' associated with the nucleus.

Scaling of morphogen gradients can result from the non-linear interactions of involved morphogens. For example, one can imagine that the scaling of the Bicoid gradient is possible because Bicoid and Caudal, which are expressed in the opposite sides of embryo, mutually affect their diffusion and/or degradation rates in some particular way. Possible non-linear interactions between morphogens resulting in gradient scaling have recently been addressed in a framework of the expansion–repression model (Ben-Zvi & Barkai 2010). In this model, one morphogen ('repressor') reduces the production rate of another morphogen ('expander'), which, in turn, reduces the degradation rate of the repressor. This model is indeed capable of reproducing the scaling of a morphogen, although the biological interpretation of particular kinetics used in the model is neither simple nor intuitive.

One of the mechanisms of scaling (which is also behind the expansion–repression model (Ben-Zvi & Barkai 2010)) can be illustrated in a simple setting. Assume that the production rate of a certain morphogen is constant and it degrades uniformly in the medium. Also assume that the morphogen diffuses quickly so that its level is roughly the same over the entire medium. If v denotes the concentration of the morphogen and it degrades with rate $k_v v$ at each 'point', then the total degradation rate over the medium of size L is $k_v vL$. If the total production rate over the entire medium is p (and constant), then balance is achieved if $p = k_v vL$, indicating that the concentration, v, is inversely proportional to the size of the medium $v = p/k_v L$. Furthermore, assume that there is another morphogen whose degradation is affected by the first morphogen so that its concentration u is given by the equation:

$$D\frac{\mathrm{d}^2u}{\mathrm{d}x^2} - k_u v^2 u = 0; \quad u(0) = u_0; \quad u(L) = 0. \tag{4}$$

Generally (if v is constant), the solution of equation (4) is given by a superposition of two exponents. However, if the medium is sufficiently large, the concentration profile $u(x)$ can be approximated by a single exponent:

$$\begin{aligned} u(x) &= u_o \exp\left(-\sqrt{\frac{k_u v^2}{D}}x\right) = u_o \exp\left(-\sqrt{\frac{k_u}{D}}vx\right) \\ &= u_o \exp\left(-\sqrt{\frac{k_u}{D}}\frac{p}{k_v L}x\right) \\ &= u_o \exp\left(-\sqrt{\frac{k_u}{D}}\frac{p}{k_v}\xi\right) \end{aligned} \tag{5}$$

which appears to be a function of relative position, $\xi = x/L$, rather than actual position, x, and therefore scales with the size of the medium.

All the above models of morphogen gradient formation treat involved processes as continuous in space and in time. However, the scaling of biological structures could be due to their discrete nature, that is, the scaling could be conditioned by the interactions between entities, such as cells or nuclei. Thus, it may be more informative to model the system as spatially discrete. In other words, if we measure the size of the embryo by the number of nuclei along its length, rather than in millimetres, then embryos of different physical length are equal if they contain the same number of nuclei (i.e. at the same stage of development). The discrete approach provided by hybrid models (continuous in time and discrete in space) have been used by many authors (Jaeger 2009). For example, the gene circuit model that clearly postulates that the embryo consists of nuclear divisions, each represented by a single nucleus and surrounding syncytium, has been used to model gap domain formation (Jaeger *et al*. 2004). In this model, the pattern of gap gene expression scales with the number of nuclei rather than with the physical size of the embryo and thus reflects the segmentation scaling.

Modelling cell movement

In the previous sections, we have considered mathematical models for stationary morphogen gradients. However, morphogen gradients are typically dynamic, and in many cases, their dynamics are conditioned by the movement of cells (Fig. 2A). Gastrulation in the chick embryo is a good example for which detailed data on the dynamics of gene expression patterns and cell movement is available (Bachvarova 1999, Stern 2004, Chuai & Weijer 2009*a*). Extensive cell motion observed during gastrulation in the epiblast can be considered as having three distinct parts: i) cell motion along the embryo midline, associated with progression and regression of the primitive streak; ii) lateral movement of cells on both sides of the midline, which is vortex-shaped at early gastrulation and forms lateral flows toward the midline at later stages and iii) transformation of cells approaching the midline from epithelial cells to the mesenchyme forming a sink in the epiblast along its midline and giving rise to outward lateral flows formed by mesenchyme cells. As the movement involves different cell groups that express different genes, the forming morphogenetic patterns are dynamic and change following the relocation of domains of transcription. Furthermore, the movement of cells can, in turn, be affected by morphogen concentrations, for example if the movement is chemotactic and morphogens act as chemotactic agents. These possibilities have recently been explored in studies combining mathematical modelling and experiments (Vasiev *et al*. 2010, Harrison *et al*. 2011).

Two different interpretations of cell movement data during gastrulation in the chick embryo have been reported: one pointing to the cellular intercalation mechanism (Voiculescu *et al*. 2007) and the other to the chemotactic mechanism (Yang *et al*. 2002). We feel that the general reasoning would be in favour of the chemotactic mechanism. This mechanism can explain the formation of a compact group of moving cells (i.e. follicle cells in the *D. melanogaster* embryo or Hensen's node in the chick embryo), while formation of such groups due to cellular interaction is problematic, if not impossible. However, there are many other cases, such as movement of cells in the zebrafish dorsal mesoderm or during the germ band elongation in *D. melanogaster* embryo (Montell 2008), that are better explained by a cellular interaction mechanism.

The interplay between the dynamics of morphogen gradients and chemotactic cell movement has recently been addressed in mathematical studies (Vasiev *et al*. 2010, Harrison *et al*. 2011). The simplest scenario to consider is when the cells forming the moving group transcribe a gene that is not transcribed in the surrounding tissue. Then this group of cells (which will be referred as the domain of transcription, DoT) can be represented mathematically by a segment of line (of length a), which is also moving (Fig. 2B). If u represents the concentration of transcribed mRNA, then we can presume that $u=1$ inside the DoT and $u=0$ outside. Furthermore, one can assume that the protein, which is associated with the transcribed gene and produced inside the DoT, can diffuse into surrounding tissue and degrade. The concentration profile, $v(x)$, of the protein is stationary from the perspective of the moving DoT (i.e. in the moving frame of reference) and satisfies the following equation:

$$D\frac{d^2v}{dx^2}-s\frac{dv}{dx}+k_1u-k_2v=0;\quad v(\pm\infty)=0$$
$$\text{where}\quad u=\begin{cases}1 & \text{for}\quad 0\le x\le a\\ 0 & \text{for}\quad x<0\quad\text{and}\quad x>a\end{cases} \tag{6}$$

where the first term describes diffusion of the protein, the second term takes into account the movement of DoT, the third and fourth terms explain production and decay of the protein respectively. The solution of equation (6) is plotted in Fig. 2B; it produces an asymmetric gradient, with its maximum concentration shifted to the back of the moving DoT. This concentration profile can explain the movement of the DoT provided that the cells forming the DoT are chemotactically repelled by the protein. Indeed, the concentration of protein is lower on the front of DoT than on its back (the difference is denoted by Δv in Fig. 2B), and if the protein acts as a chemorepellent on cells comprising the DoT, then the net repulsion could keep the DoT moving to the right. In fact, each cell would experience a different chemotactic signal corresponding to the local gradient of chemotactic agent, which could even force cells to move in opposite directions. However, if we assume that the

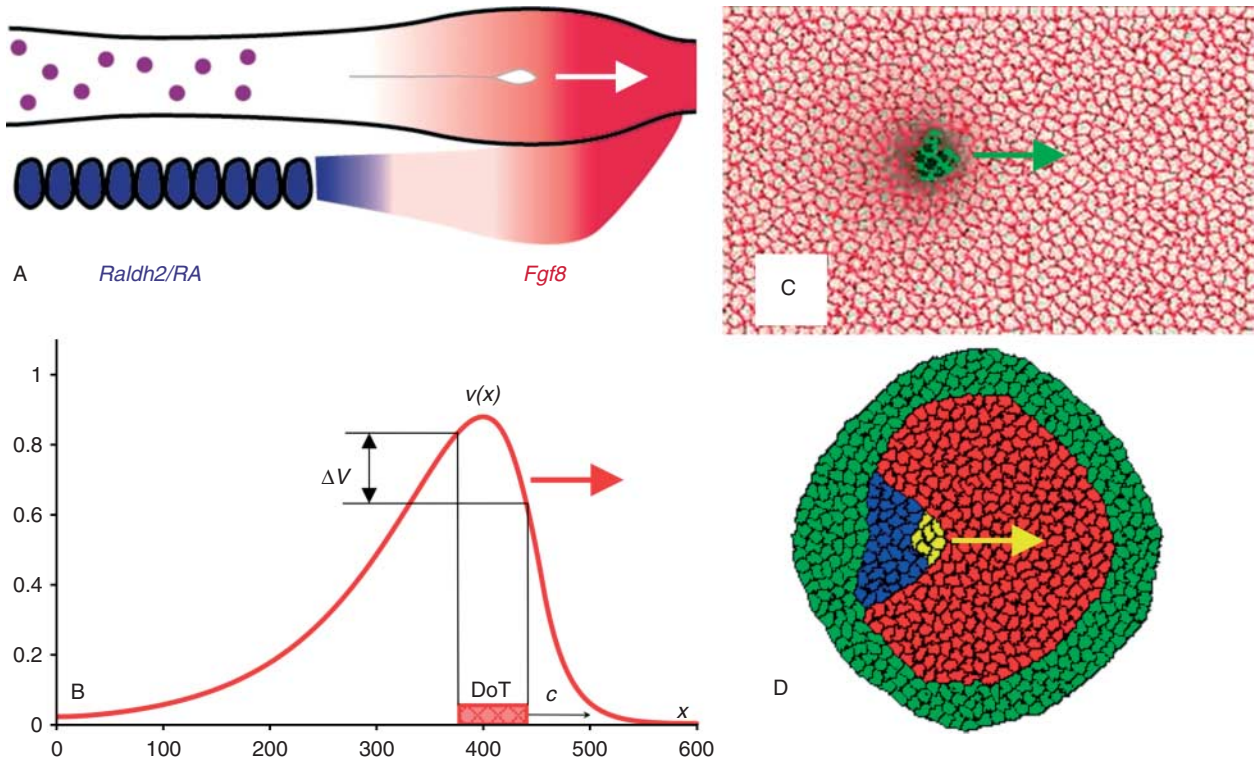

Figure 2 Dynamics of morphogen gradient and movement of cells. (A) Fibroblast growth factor (FGF8, red) and retinoic acid (RA, blue) are expressed in neighbouring domains with the border moving in an anterior-to-posterior direction (Diez del Corral *et al.* 2003). The border follows the stem zone, which moves during the primitive streak regression in the chick embryo. (B) Illustration of the solution to equation (6). The domain of transcription (DoT) is represented by the red bar. The graph of the propagating morphogen gradient (solution of equation (6)) is shown by the solid red line. The difference, Δv, between the morphogen concentration on the front and back of the moving DoT indicates that this gradient can provide the chemotactic signal causing the DoTs migration. (C) Moving DoT in the cellular Potts model. The DoT (group of green transparent cells) moves in the tissue (red transparent cells) and is repelled by the chemotactic agent (concentration shown by grey shadows) produced in the DoT. (D) Simulation of the primitive streak extension in the chick embryo (Vasiev *et al.* 2010). Yellow cells form streak tip, blue – Koller's sickle, red – *area pellucida* and green – *area opaca*. Primitive streak extension is associated with posterior-to-anterior movement of streak tip cells (left-to-right in panel). Four possible chemotactic mechanisms can explain this motion (see text).

cells forming DoT are bound by strong adhesive forces and act as a coherent group, then the net velocity of the DoT would be defined only by the difference in concentration of the chemotactic agent on its boundaries. This hypothesis was confirmed using cellular Potts model (Harrison *et al.* 2011), which has become a widely adopted model for the simulation of biological tissues (Merks & Glazier 2005). The snapshot of simulated tissue in Fig. 2C shows the group of cells representing the DoT (shown in green) and placed in a tissue comprising another type of cell (shown in red). The DoT moves (to the right) as cells forming the DoT are repelled by the protein produced by themselves. This demonstrates an important chemotactic scenario associated with the ability of a coherent group of cells to move due to self-repulsion.

Analysis of chemotactic scenarios that can explain formation of the primitive streak in chick embryo (Vasiev *et al.* 2010) has indicated that they always came in pairs. That is, if a certain movement pattern can be explained by chemotaxis, then it will have at least two explanations. For example, we just saw that the DoT can move if the cells comprising the DoT produce a self-repellent. The counterpart explanation is the following: the DoT will show the same movement pattern if the cells comprising the surrounding tissue produce a protein that acts as chemoattractant to the cells forming the DoT. Furthermore, movement patterns can be explained even by more than two chemotactic mechanisms if more than two cell types are involved in the scenario. This statement can be illustrated by the simulations of primitive streak extension (Vasiev *et al.* 2010). Figure 2D gives a snapshot from one of these simulations with green cells comprising the *area opaca*, red cells – *area pellucida*, blue cells – Koller's sickle and yellow cells – cells forming the streak tip (which is as a fraction of Koller's sickle transforming later into the Hensen's node). Primitive streak extension is associated with the posterior-to-anterior movement of streak tip cells (yellow) that are followed by the cells comprising Koller's sickle (blue cells). It was assumed that the movement of yellow cells is active (chemotactic) while the movement of blue cells (which passively follow the yellow cells) is conditioned by strong adhesive ties

between the yellow and blue cells. Movement of the yellow cells can be explained by their interactions with the red cells: that is, either the red cells produce an attractant for the yellow cells or (the counterpart explanation) the yellow cells produce a repellent for the red cells. Two more explanations are associated with the interaction of yellow and blue cells: either yellow cells produce an attractant for blue cells or blue cells produce a repellent for yellow cells. All four mechanisms have been confirmed to reproduce the primitive streak extension in numerical simulations. In order to discriminate these mechanisms, more sophisticated scenarios including the interaction between two primitive streaks have been simulated (Vasiev *et al*. 2010). Analysis of the patterns formed by interacting primitive streaks, which form under alternative assumptions, has led to the conclusion that the mechanism involving the repulsion of streak tip cells (yellow) by cells forming Koller's sickle (blue) fits much better with experimental observations.

Modelling *D. discoideum* development

In the previous section, we have addressed hypothetical mechanisms of movement of cells during gastrulation in the chick embryo. Despite the fact that expression patterns of a number of genes associated with morphological structures like Koller's sickle or primitive streak tip are known (Bachvarova 1999) and even the candidate chemotactic agents have been identified (Chuai & Weijer 2009*b*), we have no solid experimental evidence confirming any of those mechanisms. However, there is a biological process, namely the aggregation of *D. discoideum* amoebae, which involves chemotactic movement of cells and which has been studied in far more detail (Cohen 1971, Parent & Devreotes 1999, Dormann *et al*. 2002). *D. discoideum* amoebae cooperate and show striking social behaviour when they are deprived of food. Starving cells communicate by means of a chemical signal to synchronise their otherwise random and unorganised motion.

D. discoideum is a relatively simple organism for which the signalling molecule (cAMP), signalling machinery and cellular chemotactic response to cAMP are well studied. It is known that cells communicate by means of travelling waves of cAMP. A number of models have been suggested to describe these waves (Martiel & Goldbeter 1987, Tang & Othmer 1995) falling into the class of models given by equation (3). The aggregation of chemotactically moving cells is commonly described by the Keller–Segel equation (Keller & Segel 1971):

$$\frac{\partial \rho}{\partial t} = D\Delta\rho - \nabla(\rho \mathbf{V}) \qquad (7)$$

which states how the rate of change of the density of cells, ρ, is defined by the random motion of cells (the first term in the right-hand side) and the chemotactic motion (second term). The velocity of chemotactic motion, $\boldsymbol{V}$, is proportional to the gradient of chemotactic agent, u, $\boldsymbol{V} = \chi \nabla u$.

A particular feature of aggregating *D. discoideum* cells is that they form streams (Fig. 3A). The mechanism of stream formation had puzzled researches for several decades and was uncovered by means of mathematical modelling. A cellular automaton model (Vasieva *et al*. 1994) gave the simplest answer to this question. In this model, it was postulated that aggregating cells could be in three states: resting, excited or refractory. Resting cells get excited when they receive a signal from a locally signalling cell and also respond by moving (a fixed distance) towards it. Excited cells send the signal to all cells in their vicinity, defined as a circle of a predefined radius. Excited cells stay excited for a few time steps and then become refractory. Refractory cells do not react to any signal and turn into resting cells after a predefined number of time steps have elapsed. Initially, cells are randomly placed in the model medium and one cell is periodically stimulated (excited). This cell passes the excitation to the neighbouring cells, which pass it further on, causing the excitation to propagate. In this model, it is possible to identify paths of excitation from the (externally) stimulated cell to all other cells in the medium. The collection of all such paths forms a branching pattern and cellular streams develop along its branches. Furthermore, the model has demonstrated

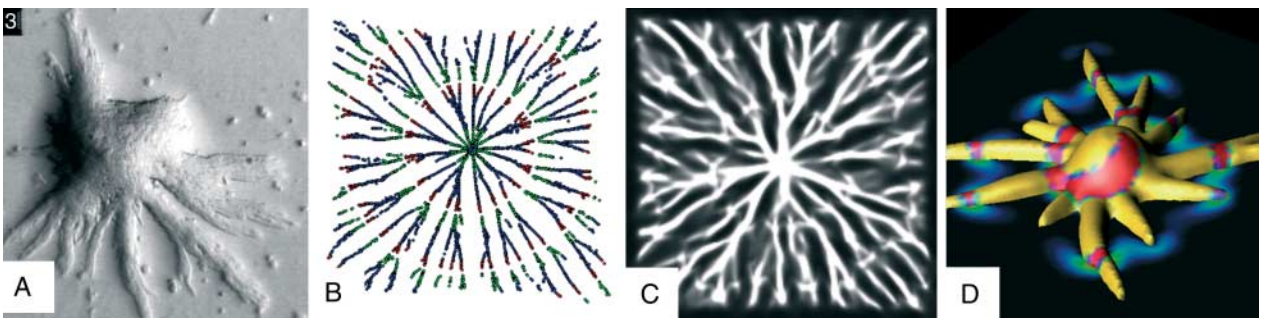

Figure 3 Streaming patterns formed in an aggregating *Dictyostelium discoideum* population. (A) Experiment data (roughly 10^5 cells gather together to form a mound). (B, C, and D) Results generated by a cellular automaton model (Vasieva *et al*. 1994), a reaction–diffusion model (Vasiev *et al*. 1994) and a hydrodynamic model (Vasiev *et al*. 1997) respectively.

that the irregularities in the initial distribution of cells can cause breaks in the propagating waves of excitation leading to the formation of rotating spiral-shaped waves of excitation, associated with vortex-shaped cell movements and curved cellular streams (Vasieva *et al*. 1994).

A more formal mathematical explanation of streaming was given using a model that combines amended equation (3), describing cAMP waves, and equation (7), describing the dynamics of cell density (Vasiev *et al*. 1994). It was shown that the aggregation streams form only when the velocity of cAMP waves increases with the density of cells. This condition amplifies the (initial) heterogeneity in the distribution of cells over the aggregating field, that is, dense cell spots get denser after propagation of each successive excitation wave. In addition, these spots elongate while moving towards the aggregation centre or the source of excitation waves. The movement, deformation and collisions of initially denser spots cause the formation of merging streams in the aggregation field. Simulations have confirmed that there should be some initial heterogeneity in the cellular density, for example due to random fluctuations, for the streaming patterns to form. The experimental observations on the dependence of the speed of cAMP waves on density of cells was controversial at the time (Hashimoto *et al*. 1975), but this theoretical result has helped in design of experiments that have confirmed that indeed the cAMP waves move faster as cell density increases (vanOss *et al*. 1996).

The hydrodynamic model, which was later developed to study three-dimensional processes associated with further steps of *D. discoideum* development, has also reproduced the formation of cellular streams as they merge into a three-dimensional mound (Fig. 3D). This model has been used to analyse cellular flows in the *D. discoideum* mound and slug. In particular, the model could reproduce, by means of computer simulations, the circular motion of cells in the mound and in the tip of the slug, as conditioned by the chemotactic signal delivered by a rotating scroll wave of cAMP. This model has also revealed the mechanisms of cell sorting in the mound (Vasiev & Weijer 1999) and explained patterns formed by cellular flows in the migrating slug (Bretschneider *et al*. 1999). In experimental conditions, cells in the mound differentiate into prestalk and prespore cells in a salt-and-pepper manner. However, these two cell types sort out so that prestalk cells collect at the tip of the mound. Model simulations have shown that two critical differences between prespore and prestalk cells allow sorting to occur in this particular way. First, prestalk cells move faster than prespore cells and this allows the prespore cells to accumulate along the vertical midline of the hemispherical mound. The chemotactic signal delivered by the scroll wave has a component pointing to the midline of the mound, both prestalk and prespore cells tend to move to the midline but faster prestalk cells win the competition. As prestalk cells collect along the midline, they are pushed up by other cells that are also forced to move to the centre of the mound. Thus, the prestalk cells start to collect on the top of the hemispherical mound and now the second difference between prestalk and prespore cells starts to play a role. Prestalk cells produce more cAMP than prespore cells and the heterogeneity in the mound, due to initial cell sorting, causes the scroll wave to twist so that its rotation is now combined with downward propagation. As a consequence, all cells are forced to move up but again as the prestalk cells move faster they accumulate at the top of the mound completing the sorting process (Vasiev & Weijer 1999).

Conclusions

In this review, we have focused on a few illustrative examples of mathematical models used for the analysis of morphogen gradients and cellular motion. Many other models and modelling approaches that have played a significant role in understanding developmental processes have been left out of this review. For instance, mathematical modelling is an important component in studies of mechanical forces that are associated with migration of cells and tissue deformation during morphogenesis (Beloussov 1998). Typical examples of such studies are given by a theoretical analysis of the hyper-restoration hypothesis (Taber 2008) and computational modelling of mechanical feedback in deformed tissue (Ramasubramanian & Taber 2008) both highlighting the role of stress perturbations in developing tissue during morphogenesis.

All our examples of mathematical models for gradient formation addressed the case of a stationary gradient. However, there are experimental studies that indicate that it can take a considerable amount of time for the morphogen gradients to stabilise and positional information must therefore be provided by a dynamic gradient (Drocco *et al*. 2011). Mathematical models addressing properties of pre-steady-state morphogen gradients (including their scaling) have started to appear (Bergmann *et al*. 2007) and we can expect significant progress in this area in the near future. There are many indications that positional information is provided by (or interpreted according to) a signal that is integrated over time, rather than by its present value (Dessaud *et al*. 2010). This important observation has not been explored by means of mathematical modelling so far.

Our examples of mathematical modelling of cellular motion were limited to the case of chemotaxis. The models designed for the analysis of gastrulation in chick embryo have allowed us to check and confirm that several chemotactic mechanisms can equally explain movement patterns in the epiblast. These models also suggested how the mechanisms can be discriminated experimentally. However, we cannot exclude the possibility that the rearrangement of cells in tissues

could be conditioned by anisotropy in adhesive properties of cells along their surface, that is, by various cellular intercalation mechanisms. The explanations based on these mechanisms are always given on a semi-intuitive level (Meinhardt 2006) and so far there have been no serious attempts to model them mathematically.

Mathematical modelling in developmental biology is one of the fundamental and harmonic examples of systemic knowledge integration. It helps in discovery of biophysical mechanisms of development and allows a rigorous check of hypotheses concerning these mechanisms as they emerge from experimental observations. Mathematical modelling also helps to generate new hypotheses and design new experiments. Ideally, there would be iterative relationships between experimental observations and mathematical modelling allowing for their cross-validation so that our understanding of biological development goes beyond collection of experimental data on one side and idealistic ungrounded theoretical models on the other. Mathematical modelling in developmental biology is gradually evolving from exploring possible mechanisms of processes causing the break in symmetry and scaling, to more narrow quantitative descriptions of the processes that can be validated experimentally. It is evident that mathematical modelling plays an important role and brings significant insights into developmental biology. It is also evident that modelling will play even more crucial role in biological studies in the future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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