# AP3162D: Lecture 5 - Basic modelling frameworks for developmental biology and cell-fate decisions - Part II

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In this lecture, we will discuss how the different parts of an embryo scale proportionally with the embryo's size. Robust scaling ensures that the right body parts are formed at the right locations, proportional to the size of the organism. We will discuss one particular mechanism that can achieve robust scaling, called "expansion-repression feedback". We will study this mechanism through reaction-diffusion equations and "numerical screening". Reaction-diffusion equations describe morphogens and their regulators diffusing, degrading, and possibly reacting with other molecules in a field of cells/nuclei of an embryo. Numerical screening is a widely used method for computationally screening hundreds of thousands of parameters in an equation, such as the reaction-diffusion equation, and find the sets of parameter values that yield the desired biological behaviours. In this lecture, we will discuss how to numerically screen large numbers of parameters in the reaction-diffusion equations to determine which parameter values enable robust scaling of developing embryos.

## I. BIOLOGICAL PHENOMENON: SCALING OF BODY PARTS IN DEVELOPING EMBRYOS

You and I have different heights and body sizes. Yet your and my arms are above our torsos and below our heads. Your and my legs are below our torsos. Given that you and I can be of vastly different heights, the my arms' lengths can largely deviate from those of your arms. But the length of my arms compared to the length of my height, in fact, would not be much different from those of yours. This also holds true for others animals and in fact, is crucial for them to properly function given that two animals from the same species will generally have different sizes, based on how they grew up and their genetic makeup. These facts, that the proportion of one body part relative to another body part in an organism is the conserved is called **scaling**. In this lecture, we will discuss an example of scaling in developing frog embryos and learn about numerical screening, a computational technique that one uses to screen for regulatory circuits that enable scaling in embryos (but it is also useful in other contexts).

## II. MECHANISM FOR ACHIEVING SCALING IN EMBRYOS: EXPANSION-REPRESSION FEEDBACK

In this section, we study a mathematical model proposed by Danny Ben-Zvi and Naama Barkai that achieves scaling in developing embryos ("Scaling of morphogen gradients by an expansion-repression integral feedback control" *PNAS* (2010)). The model relies on a mechanism that the authors called **expansion-repression feedback**.

### A. Conventional reaction-diffusion of one morphogen cannot achieve robust scaling

Before delving into the expansion-repression feedback that Ben-Zvi and Barkai proposed (and later tested in frog embryos), let us consider a simpler picture: A single morphogen, *Morph*, diffusing and degrading in a field of cells. For simplicity, we will consider diffusion along a line (one-dimensional diffusion) with the cells of the embryo sitting on the line of length $L$, one next to the other. Suppose that at the **proximal end** ($x = 0$), there is a source of *Morph* that produces *Morph* at some rate $\rho$. At the **distal end** ($x = L$), we can impose at least two types of boundary conditions:

- **Dirichlet condition**: We specify $M(L, t)$ for each time $t$.
- **Neumann condition**: We specify the **outward normal derivative**, $\partial M/\partial n$ at $x = L$ for each time $t$. The outward normal derivative points away from the domain of the reaction-diffusion equation. Thus, at $x = L$, $\partial M/\partial n = \partial M/\partial x$ whereas at $x = 0$, $\partial M/\partial n = -\partial M/\partial x$

The reaction-diffusion equation for describing the concentration $M$ of *Morph* on the line is

$$\frac{\partial M}{\partial t} = D_M \frac{\partial^2 M}{\partial x^2} - G(M) \tag{1}$$

where $D_M$ is the diffusion coefficient of *Morph*, and $G(M)$ is a function of $M$ and is a degradation rate of *Morph* that is either zero or a positive number. A simple example of $G$ that we have been using in the previous lectures is a **first-order degradation**: $G(M) = \alpha M$. Let us now solve Eq. 1 for a special case.

**Special case: Steady-state solution of Eq. 1 when there is no degradation**: Here we set $G = 0$ (no degradation) and $\partial M/\partial t = 0$ (steady-state). Then we have the following **Laplace's equation**:

$$0 = \frac{d^2 M_s}{dx^2} \tag{2}$$

Evidently, the steady-state solution $M_s$ does not depend on the diffusion coefficient $D_M$. To obtain $M_s$, we must specify the boundary conditions at $x = 0$ and $x = L$. Let us suppose that the source at the proximal end ($x = 0$) secretes and "takes away" *Morph* such that $M(x = 0, t)$ is a constant value $M_0$ at all times (thus $M_s(0) = M_0$). Let us also assume that at the distal end ($x = L$), we have a perfect sink (i.e., $M(x = L, t) = 0$ at all times). Thus $M_s(L) = 0$. With these Dirichlet conditions, the solution of Eq. 2 is

$$M_s(x) = M_0\left(1 - \frac{x}{L}\right) \tag{3}$$

We can check that Eq. 3 is indeed a solution of Eq. 2 by noting that (1) its second-derivative with respect to $x$ yields zero for $0 < x < L$, and that (2) $M_s(0) = M_0$ and $M_s(L) = 0$ (i.e., satisfies both boundary conditions). So we know that Eq. 3 is indeed a solution. But how do we know that it is the *only* solution of Eq. 2? The answer is that we have indeed found the *only* solution of the Laplace equation with the specified boundary conditions due to the following two theorems, one whose proof depends on the other:

**Theorem 1 (No local extrema)**: Given a boundary condition for domain $D$ (which can have any number of dimensions), a solution to the Laplace's equation on $D$ that is not a constant function cannot have any local extrema *inside* $D$. Thus the solution's maximum and minimum values must lie on the boundary of $D$.
*Proof*: We will use another fact about Laplace's equation without proof here - A solution $M$ to a Laplace equation $\nabla^2 M = 0$ has the property that its value $M(\vec{r}_0)$ at point $\vec{r}_0$ inside the domain $D$ is the average of all the values of $M$ on the surface of any sphere whose center is at $\vec{r}_0$ and fits inside $D$. In 1-, 2-, and 3-dimensions, this means

$$M(x_0) = \frac{M(x_0 - a) + M(x_0 + a)}{2} \qquad \text{(solution in 1-dimension: } x_0 - a \text{ and } x_0 + a \text{ are points in interval } D) \tag{4a}$$

$$M(\vec{r}_0) = \frac{1}{2\pi R}\int_{\text{circle}} M(r) \qquad \text{(solution in 2-dimensions: circle of radius } R \text{ inside } D) \tag{4b}$$

$$M(\vec{r}_0) = \frac{1}{4\pi R^2}\int_{\text{sphere}} M(r) \qquad \text{(solution in 3-dimensions: sphere of radius } R \text{ inside } D) \tag{4c}$$

The theorem then immediately follows. To see this, assume, for contradiction, that $M$ is not a constant function that has a local maximum at some point $\vec{r}_0$ inside $D$. Then by definition of a local maximum, we can find a small enough sphere that fits inside $D$ and whose center is at $\vec{r}_0$ such that $M$ is smaller than $M(\vec{r}_0)$ everywhere on the sphere's surface. Then the average of all values of $M$ on the sphere's surface must be smaller than $M_{\vec{r}_0}$, contradicting that $M_{\vec{r}_0}$ must, in fact, be the average. Thus, by contradiction, $M$ cannot have a local maximum inside $D$. By using the same logic, it follows that $M$ also cannot have any local minima inside $D$. Thus theorem 1 holds.

**Theorem 2 (Uniqueness theorem)**: Given a Dirichlet boundary condition for domain $D$ (which can have any number of dimensions), a solution to the Laplace's equation on $D$ is unique. Thus we can call it *the* solution.
*Proof*: We use theorem 1 to prove this. Suppose, for contradiction, that we have two distinct solutions, $M_1$ and $M_2$ in the same domain $D$ and with the same Dirichlet boundary conditions. Then since the Laplace's equation is a linear equation, we must have $\nabla^2(M_1 - M_2) = \nabla^2 M_1 - \nabla^2 M_2 = 0$. Thus $M_1 - M_2$ is a solution inside the domain $D$ as well. On the boundary of $D$, $M_1 - M_2$ is zero since $M_1$ and $M_2$ take the same values there. By theorem 1, we must then have $M_1 - M_2 = 0$ everywhere inside $D$ since, for otherwise, $M_1 - M_2$ would have a local extremum inside $D$. Thus $M_1 = M_2$ in the end. Thus once we find a solution $M_1$, we have found all the solution there is for the Laplace's equation on $D$ with a fixed Dirichlet boundary condition. Thus theorem 2 holds.

Long story short, theorem 2 tells us that Eq. 3 is the only solution to Eq. 2. Let us now return to the one-dimensional diffusion of *Morph* between $x = 0$ and $x = L$. The steady-state solution $M_s$, in fact, allows for **scaling**. That is, if we have two embryos - one has length $L$ and the other with length $\beta L$ - and $M_L$ and $M_{\beta L}$ are the steady-state concentrations of the two embryos respectively, then

$$M_L(x_1) = M_0\left(1 - \frac{x}{L}\right) \tag{5a}$$

$$M_{\beta L}(x_2) = M_0\left(1 - \frac{x}{\beta L}\right) \tag{5b}$$

where $x_1$ is the position within the embryo of length $L$ and $x_2$ is the position within the embryo of length $\beta L$. Note that if we scale both $x_1$ and $L$ in Eq. 5a by $\beta$, then we obtain

$$1 - \frac{\beta x_1}{\beta L} = 1 - \frac{x_2}{\beta L} \tag{6}$$

Thus we can write $M_L$ and $M_{\beta L}$ as

$$M_L(x_1) = M_0(1 - y) \tag{7a}$$

$$M_{\beta L}(x_2) = M_0(1 - y) \tag{7b}$$

where $y$ is the position divided by the embryo's length. Mathematically, this is what it means to have scaling. Biologically, scaling means that the morphogen concentration within an embryo is determined by the distance from the proximal end relative to the embryo's total length and not by the absolute distance from the proximal end. But this scheme is not robust as it strongly depends on $M_0$. Any slight changes to $M_0$ could lead to vastly different concentration profiles. Moreover, we assumed that *Morph* does not degrade, which is not true in real embryos. Thus this mechanism for scaling cannot provide robust scaling in real embryos.

### B. Expansion-repression feedback

We now describe a mechanism that can robustly generate scaling in embryos. As in the previous section, we consider a diffusing morphogen called *Morph*. Being a morphogen, its concentration $M$ is sensed by cells or nuclei inside the embryo and determines which type of cells and where they are generated inside the embryo. Let us assume that a localized source secretes *Morph* and that *Morph* then diffuses along one-dimension as before. Let $D_M$ be *Morph*'s diffusion coefficient and $\alpha_M$ be $M$'s degradation rate. Then the reaction-diffusion equation for $M$ is again given by

$$\frac{\partial M}{\partial t} = D_M \frac{\partial^2 M}{\partial x^2} - \alpha_M M \tag{8}$$

where $D_M$ is the diffusion coefficient of *Morph* and $\alpha_M$ is proportional to the degradation rate, $\alpha_M M$ (i.e., we have set $G(M)$ in Eq. 1 to be $\alpha_M M$). Crucial to the authors' model is that $D_M$ and $\alpha_M$ are *not* constants, as we will see shortly. We impose the following boundary condition at the proximal end: there is a constant flux $\eta_M$ of $M$ at the source located at $x = 0$. At the distal end ($x = L$), we can assume a number of different boundary conditions. The authors, in fact, show that their main conclusions are unaffected whether we set a reflective boundary condition (i.e., $\partial M/\partial x = 0$) or absorbing boundary condition (i.e., $M(L) = 0$) at $x = L$. Moreover, the authors consider a second diffusing molecule, called the **expander** *Exp*, whose concentration either directly or indirectly affects $D_M$ and the degradation rate through $\alpha_M$. Thus $D_M$ and $\alpha_M$ are functions of $M$ and $E$:

$$D_M = D_M(E, M) \tag{9a}$$

$$\alpha_M = \alpha_M(E, M) \tag{9b}$$

While we do not specfy the functional forms of $D_M$ and $\alpha_M$, we impose the following two conditions:

- As the concentration $E$ increases, $D_M$ increases. Thus the expander directly/indirectly **facilitates diffusion** of *Morph*.

- As $E$ increases, $\alpha_M$ decreases. Thus the expander directly/indirectly **inhibits degradation** of $Morph$.

We also assume that $Morph$ represses the production of $Exp$, which we characterize through a sigmoidal production-rate function as a function of the concentration $M$. Then the following coupled reaction-diffusion equations describe the $Morph$ and $Exp$ concentrations throughout the embryo:

$$\frac{\partial M}{\partial t} = D_M \frac{\partial^2 M}{\partial x^2} - \alpha_M M \tag{10a}$$

$$\frac{\partial E}{\partial t} = D_E \frac{\partial^2 E}{\partial x^2} - \alpha_E E + \beta_E \frac{T_{rep}^h}{T_{rep}^h + M^h} \tag{10b}$$

Here, $T_{rep}$ is the repression threshold, $h$ is a Hill coefficient, $D_E$ is diffusion constant for $E$, and $\alpha_E$ is the degradation rate for $E$. We assume a reflective boundary condition for $M$ and $E$. Eqs. 10a and 10b define the **expansion-repression feedback** system. We next analyze these equations, first using analytical estimates and next with a "numerical screening" method.

### 1. *Analytical approximation*

Analytical estimates before numerical simulations almost always provide mechanistic insights into the equations that the numerical simulations fail to yield. We can analytically approximate solutions to Eqs. 10a and 10b before numerically solving them. For a complex equations with many parameters like these, it often helps to rescale the variables, $x$ and $t$, which means that we measure the length and time relative to other parameters that have dimensions of length and time respectively (thus $x$ and $t$ would become dimensionless). Practically, we can do this by dividing Eq. 10a by $\alpha_M$, which has units of 1/time, to get

$$\frac{1}{\alpha_M}\frac{\partial M}{\partial t} = \frac{D_M}{\alpha_M}\frac{\partial^2 M}{\partial x^2} - M \tag{11a}$$

$$\implies \tau \frac{\partial M}{\partial t} = \lambda^2 \frac{\partial^2 M}{\partial x^2} - M \tag{11b}$$

where $\tau = 1/\alpha_M$ is a **characteristic time** and $\lambda = \sqrt{D_M/\alpha_M}$ is a **characteristic length**. We can similarly rescale Eq. 10b but that is not yet necessary. In the analyses below, we will assume that

$$D_M = D_M(E) \qquad \text{(Independent of } M\text{)} \tag{12a}$$

$$\alpha_M = \alpha_M(E) \qquad \text{(Independent of } M\text{)} \tag{12b}$$

$$\tag{12c}$$

Let us now consider what the steady-state solution $M_s(x)$ of Eq. 11b would look like, without actually solving the equation. Having identified $\lambda$ and $\tau$ as the relevant dimensions enable us to determine the main properties of $M_s(x)$. First, we note that, in steady-state (i.e., $\partial M_s/\partial t = 0$), Eq. 11b becomes

$$0 = \frac{\partial^2 M_s}{\partial y^2} - M_s \tag{13}$$

where $y = \frac{x}{\lambda_s}$ and $\lambda_s$ is the steady-state value of the function $\lambda(M, E)$ (remember, $D_M$ and $\alpha_M$ are functions of $E$ and possibly of $M$). . Thus,

$$M_s(x) = f(y) \tag{14}$$

where $f$ is some function. Furthermore, by imposing that the **flux** at $x = 0$ - this is $-D_M \partial M_s/\partial x|_{x=0}$ by Fick's law for diffusion - is $\eta_M$, we have

$$\eta_M = -D_M \left.\frac{\partial M_s}{\partial x}\right|_{x=0} \tag{15a}$$

$$\implies \frac{\lambda_s \eta_M}{D_M} = -\left.\frac{\partial M_s}{\partial y}\right|_{y=0} \tag{15b}$$

Eq. 15b expresses the boundary condition at $x = 0$ in terms of the rescaled position variable $y$. At the other boundary ($x = L$), we impose a Dirichlet condition:

$$M_s(L) = \epsilon \tag{16}$$

where $\epsilon$ is some constant. Eq. 14 and the two boundary conditions (Eqs. 15b and 16) completely specify the functional form of $M_s(x)$. We see that $M_s(x)$ is not only a function of $y$ (Eq. 14), it also depends on $\lambda\eta_M/D_M$, according to Eq. 15b. Thus we can write

$$M_s(x) = M_\lambda\left(\frac{x}{\lambda_s}; \frac{\lambda_s\eta_M}{D_M(E)}\right) \tag{17}$$

where $M_\lambda$ is some function that depends on the functional form of $\lambda$. Note that $M_s(x)$ indeed depends on the functional form of $\lambda$ because different functional forms of $\lambda$ can produce the same steady-state value $\lambda_s$. We can rewrite Eq. 17 in a simpler way by introducing a new function $\rho_s(E)$:

$$\rho_s(E) = \frac{\lambda_s\eta_M}{D_M(E)} \tag{18}$$

Then Eq. 17 becomes

$$M_s(x) = M_\lambda\left(\frac{x}{\lambda_s}; \rho_s(E)\right) \tag{19}$$

So far, we have not yet demonstrated if the proposed mechanism, the so-called "expansion-repression feedback", allows for scaling. To address this issue, we need to determine whether $M_s(x)$ admits scaling as a function of the proximal-distal length $L$. Our starting point is to note that increasing $E$ causes an increase in $D_M$ and a decrease in $\alpha_M$ - we imposed these conditions on the model. Thus, the steady-state value of $Morph$'s diffusion length $\lambda_s = \sqrt{D_M/\alpha_M}$ increases as $E$ increases. This means that $Morph$ will travel further away from the proximal tip ($x = 0$), and thus we say that the morphogen region **expands** in the field. But as the concentration $M$ of the morphogen increases at a given location, the production rate of the $Exp$ will decrease since $Morph$ represses production of $Exp$ - This is where the term **repression** in the "expansion-repression" mechanism arises. The repressed production of $Exp$, in turn, would decrease the $\lambda_M$ and thus $Morph$ field will contract. At this point, without doing calculations, we can envision at least two possibilities: (1) oscillatory behaviour in which the whole cycle of expansion-contraction of the morphogen field repeats over and over, or (2) a steady-state value of $E$ is reached. But we know from Eq. 19 that there is a steady-state of $M$ and thus scenario (2) is what we will get.

To analytically extract the behaviour of $M_s(x)$, Let us assume that $Exp$ diffuses very fast (i.e., $D_E$ is large) and degrades very slowly (i.e., $\alpha_E$ is nearly zero). The very large value of $D_E$ means that $E$ is essential uniform throughout the embryo at all times because any inhomogeneous distribution of $E$ along the embryo quickly homogenizes by the fast diffusion. Moreover, since the degradation of $Exp$ is very slow, if there is a production of $Exp$ anywhere in the embryo, the $Exp$ will quickly spread to the rest of the embryo and the nearly uniform $E$ will continue to increase over time until the production of $Exp$ is shut down everywhere. Physically, we know that $M$ will have the lowest value at the distal tip ($x = L$) since the source of $Morph$ is at the proximal tip ($x = 0$). There, $Morph$ must also repress the production of $Exp$ in order for $E$ and $M$ to simultaneously attain steady-state profiles. This means that at $x = L$, we must have $M_s$ roughly equal to or higher than the repression threshold $T_{rep}$. In other words,

$$M_\lambda\left(\frac{L}{\lambda_s}; \rho_s\right) \approx T_{rep} \tag{20}$$

Now, suppose that $M_\lambda$ is an invertible function - that is, it has an inverse function $M_\lambda^{-1}$. Then inverting Eq. 20, we have

$$\frac{L}{\lambda_s} = M_\lambda^{-1}(T_{rep}; \rho_s) \tag{21a}$$

$$\implies \lambda_s = \frac{L}{\alpha_0} \tag{21b}$$

where $\alpha_0 = M_\lambda^{-1}(T_{rep}; \rho_s)$. Thus we can, at least formally, find the steady-state diffusion length $\lambda_s$ in terms of $T_rep$, embryo's length $L$, and the steady-state flux $\rho_s$ at the proximal end. It is interesting to note here that the steady-state diffusion length depends on the embryo's length, unlike in the case of simple diffusion of the Morphogen without the expander that we considered in the previous section. Plugging Eq. 21b into Eq. 19, we obtain

$$M_s(x) = M_\lambda\left(\frac{x}{L}; \rho_s\right) \tag{22}$$

Here, we see that the steady-state concentration $M_s$ of the morphogen scales with the embryo's length $L$ since it is a function of $x/L$, rather than a function of the absolute value of the position $x$. But the scaling may not be robust - $M_s$ is also a function of the normalized steady-state flux $\rho_s$ of the morphogen at the proximal end. Thus fluctuations in $\rho$ may ruin the scaling that the $x/L$ term achieves.

As an example, suppose that the diffusing $Morph$ degrades via a first-order kinetics (Eq. 11b). Then, as you will show in problem set 2, we have

$$M_L(x/L) = \frac{\eta_M L}{D_M \mu} exp\left( - \mu \frac{x}{L}\right) \tag{23}$$

where $\mu = ln(\rho_s) - ln(T_{rep})$. Eq. 23 tells us that the scaling is partially ruined by the lone factor of $L$ outside the exponential. If the degradation rate of $M$ is quadratic (i.e., $G(M) = \alpha_M M/M_T$ in Eq. 1, where $M_T$ is a constant), then you will show in problem set 2 that

$$M_s(x) = \frac{6 D_M M_T}{\alpha_M (x+\epsilon)^2} \tag{24}$$

where $\epsilon = \sqrt[3]{\frac{12 D_M^2}{\alpha_M \eta_M}}$. In the limit of $x \gg \epsilon$ (i.e., for positions sufficiently far from the proximal end), Eq. 24 becomes

$$M_L(x/L) \approx \frac{T_{rep}}{(x/L)^2} \tag{25}$$

According to Eq. 25, a quadratic degradation rate of $Morph$ yields a nearly perfect scaling - the steady-state concentration of morphogen depends only on the relative position $x/L$, and no factors of $L$ and $x$ appear by themselves Eq. 25. But with the quadratic degradation of $Morph$, the scaling is still not perfect. In fact, when $x$ is comparable to $\epsilon$ (i.e., near the proximal end where $Morph$ is produced), there is no scaling. This agrees with experiments on embryos in which one observes no or poor scaling near the source.

## III. NUMERICAL SCREENING

We have so far used gross approximations (e.g., very fast diffusion and very slow degradation of $Exp$). But we would now like to know whether the scaling still holds for less drastic values of the parameters, and if so, precisely for which values for each parameter. In the previous section, we obtained the functional forms of the equations that describe the diffusion and degradations of morphogen and expander. Our job now is to iteratively assign different numbers to each parameter in the equation and check, for each set of assigned values, if system yields robust scaling. This procedure, called "**numerical screening**", is often used in systems biology model. The moral here is that two equations that have the same functional forms and same variables can yield qualitatively distinct behaviours if we assign different numbers to the same parameters. The set of equations that the authors numerically screened is

$$\frac{\partial M}{\partial t} = D_M \frac{\partial^2 M}{\partial x^2} - (1+E)^{p_1}\alpha_1 M - (1+E)^{p_1}\alpha_2 M^2 \tag{26a}$$

$$\frac{\partial E}{\partial t} = D_E \frac{\partial^2 E}{\partial x^2} - \alpha_E E + \beta_E \frac{(M/T_{p_2})^{hp_2}}{1+(M/T_{p_2})^h} \tag{26b}$$

We use the following boundary conditions

$$-D_M \left.\frac{\partial M}{\partial x}\right|_{x=0} = \eta \qquad \text{(Neumann condition: Constant flux of } Morph \text{ at the proximal end)} \tag{27a}$$

$$D_M \left.\frac{\partial M}{\partial x}\right|_{x=L} = 0 \qquad \text{(Neumann condition (Reflective condition): No flux of } Morph \text{ at the distal end)} \tag{27b}$$

$$D_E \left.\frac{\partial M}{\partial x}\right|_{x=0} = 0 \qquad \text{(Neumann condition (Reflective condition): No flux at the distal end)} \tag{27c}$$

$$D_E \left.\frac{\partial M}{\partial x}\right|_{x=L} = 0 \qquad \text{(Neumann condition (Reflective condition): No flux at the distal end)} \tag{27d}$$

$$\tag{27e}$$

In Eqs. 26a and 26b, we assign different numbers to the following constants and check if a particular set of values yield a robust scaling:

- $D_M$: Diffusion coefficient for $Morph$.
- $D_E$: Diffusion coefficient for $Exp$.
- $\alpha_1$: linear degradation term for $Morph$.
- $\alpha_2$: quadratic degradation term for $Morph$.
- $\alpha_E$: linear degradation term for $Exp$.
- $\eta$: Flux of $Morph$ at the proximal end ($x = 0$).
- $\beta_E$: maximum production rate of $Exp$.
- $p_1$: This is either -1 ($Exp$ **increases** degradation of $Morph$) or +1 ($Exp$ **decreases** degradation of $Morph$).
- $p_2$: This is either 0 ($Morph$ represses $Exp$) or +1 ($Morph$ induces $Exp$).
- $T_{p_2}$: $T_0$ is repression threshold for $Exp$ and $T_1$ is induction threshold for $Exp$.
- $h$: Hill coefficient.

**Procedure for numerical screening of Eqs. 26a and 26b**: Ben-zvi and Barkai assigned approximately 400,000 different sets of numbers to the above parameters and, for each of these parameters, used MATLAB to numerically solve Eqs. 26a and 26b. MATLAB has built-in PDE solvers. "Numerically solving" means using one of these solvers, the authors obtained graphs of $M$ and $E$ versus $x/L$. They then checked this graph for (1) normal length ($L$=100$\mu$ m) and (2) double length ($L$=200$\mu$ m). If there was a strong overlap between the two graphs (both functions of $x/L$), then they concluded that the particular set of parameters yielded scaling. But having scaling is not sufficient if the values of $M$ and $E$ are biologically non-sensical. To make biological sense, the authors tested if the solutions $M$ and $E$ in steady-state obeyed all of the following conditions:

**Conditions for $M$ to be a biologically valid concentration-profile**:

1. $M$ must be maximum at $x = 0$ (where the source is) and minimum at $x = L$ (distal end) with its maximum being larger than 10 times its minimum.
2. Concentration at $x = 0.5L$ or $x = 0.75L$ must be larger than $10^{-6}\mu$ M.
3. The ratio between the maximum of $M$ and the value of $M$ at $x = 0, 25L$ is larger than 1 and less than 100 (i.e., rule out extremely sharp concentration profiles).
4. $M$ and $E$ both reach their steady-state. To overcome numerical fluctuations in MATLAB's solver, we first pick two time points - $t_{1/2}$ and $2t_{1/2}$ - where $2t_{1/2}$ is the total time that we impose on simulating Eqs. 26a and 26b. We then measure the average values of $M$ and $E$ during the time interval $[t_{1/2}, 2t_{1/2}]$ at any of the following five positions: $x = 0$, $x = 0.25L$, $x = 0.5L$, $x = 0.75L$, and $L$. If $M$'s and $E$'s averages deviate less than 1% from their values at time $2t_{1/2}$ at the same position (i.e., one of the five values of $x$ that we picked), then we say that the system has reached a steady-state.

Using the four criteria above, the authors found that only 7% of the 400,000 parameter sets were biologically valid. The authors then scored each of the 7% of the parameter sets based on how well they scaled with $L$.

To quantify how well the two graphs overlapped, the authors assigned a score to each parameter set using the following scoring metric:

**Scoring metric**:

1. For $L$=100 $\mu$m: Find the steady-state value of $M$ at three positions: $y_1 = x_1/L = 0.25$, $y_2 = x_2/L = 0.5$, and $y_3 = x_3/L = 0.75$. Let these three values be $M_1$, $M_2$, and $M_3$.
2. For $L$=200 $\mu$m: Find positions $y_1^* = z_1/L$, $y_2^* = z_2/L$, and $y_3^* = z_3/L$ where the steady-state value of $M$ is $M_1$, $M_2$, and $M_3$.
3. Compute $\sigma = \frac{1}{3}\sum_{i=1}^{3} |\delta_i|$, where $\delta_i = y_i - y_i^*$

Note that $\sigma = 0$ would be a **perfect scaling** whereas no scaling at all would mean $\sigma = 0.25$. Furthermore, note that if one of the three values - $M_1$, $M_2$, and $M_3$ - was not reached when the embryo doubled in length, then scaling clearly does not occur. The authors then selected parameter sets with $\sigma < 0.1$ and then concluded that these were the ones that enabled robust scaling.