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We analyze here a variant version of the biophysical model proposed in Holcman et al. (Formation of morphogenetic gradients and boundaries during early development, submitted), where now morphogens can form dimers. As a result, gradients are smoother and borders are much sharper. Because random perturbations of a gradient can affect the precise location of the boundary between two morphogenetic regions, we also analyze these fluctuations and in particular, we obtain an analytic expression for the variance of the boundary location as a function of the variance of the random perturbations. This formula can be used to study the noise intrinsic effect on the boundary position between morphogenetic regions, which can be at the origin of interindividual variations.
Morphogenetic Gradients and the Stability of Boundaries Between Neighboring Morphogenetic Regions

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Keywords ???

1. Introduction

Positional information is a key feature in cellular development and actually precedes cell specialization. It is thus a fascinating problem to understand how positional information is spread across a cell ensemble. The notion that a messenger, called a morphogen, is generated and can propagate across cells was formulated early on by Turing (1952). The

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discussion shows that various types of concentration profiles can be produced across many cells. Cellular communication and thus spatio-temporal organization relies partly on morphogens transport across cells. Moreover, morphogenetic gradients define uniquely each cell position, which preludes their specific differentiation. We are interested here in revisiting the question of how a morphogenetic gradient can be generated and we would like to propose a complementary scenario to Holcman et al. (submitted) and Gierer and Meinhardt (1972).

For several decades, many groups have investigated both experimentally and theoretically, the origin of a molecular gradient (Turing, 1952; Wolpert, 1996; Gierer and Meinhardt, 1972; Meinhardt, 1983a; Lander et al., 2002; Houchmandzadeh et al., 2002). In 1952, Turing presented a model based on a system of reaction-diffusion equations, leading to various instable morphogen patterns (Turing, 1952). While the fundamental role of a molecular gradient in the context of cell differentiation was proposed in Wolpert (1969, 1996), a refined mathematical analysis using a combination of short range diffusive activator and long range inhibitor was first developed in Gierer and Meinhardt (1972). When the morphogen source is spatially distributed, mathematical analysis has shown that various concentration profiles can be generated, going from a decreasing gradient to oscillating morphogen distributions. Cells can be considered as relay sources and can generate long range gradients (Gierer and Meinhardt, 1972). The precise molecular mechanism associated with this process is still unclear, however, it has been demonstrated recently that gradient of transcription factors are at the basis of brain areas (Simeone, 2000). A summary of different morphogenetic gradients, generated by reaction-diffusion equations is presented in Lander et al. (2002), with applications to patterning in the drosophilae. Their model includes the scenario of a propagating activator that has to bind to cell receptors in order to activate the morphogen synthesis inside cells. Finally, when the number of receptors at the cell surface can be regulated by activator molecules, depending on the cell internal dynamical state, long gradient profiles or traveling waves can be generated (Monk, 1998).

Generating a morphogenetic gradient in a cell ensemble cannot be dissociated from cellular labeling which leads to specific differentiation. Cellular labeling can be understood as the cell’s ability to interpret the concentration level which results in cell specialization, as described in Wolpert (1996), Gierer and Meinhardt (1972), Meinhardt (1983a). For that purpose, Wolpert (1996) introduced the notion of the French flag, where in the developing embryo, according to the morphogen concentration, a cell will belong to a unique morphogenetic area. A monotonic gradient ascribes a unique positional information and in the nervous system, for example, this information can be responsible for the formation of boundaries in the developing neuroepithelium and is used for axonal guidance (Brunet et al., 2005). These facts show that a morphogenetic gradient can have several functions.

We have recently proposed in Holcman et al. (submitted), a biophysical scenario of morphogenetic gradients genesis, based on the assumption that morphogens act as transcription factors. In that model, morphogens propagate and generate at the same time their own replication in each cell. Each cell participates and is a relay in the morphogenetic gradient profile. We view the morphogen underlying dynamics as an autocatalytic process in which the morphogen is a transcription factor. This property relies on the messenger protein concept and it has been shown that distinct transcription factors, primarily homeoproteins, can traffic from one cell to the next one (Prochiantz and Joliot, 2003). In fact, homeoproteins have all the properties that are required to play the role of morphogens.
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and to be at the origin of border formation. Indeed, as shown in Bishop et al. (2003), Schwarz et al. (2000), Simeone (2000), Toresson et al. (2000), Yun et al. (2001), Stenman et al. (2003), Brunet et al. (2005), Howard and ten Wolde (2005), Wolpert (1996), McHale et al. (2006), Houchmandzadeh et al. (2002), they pass from cell to cell, but in addition, they very often activate their own transcription and participate in border formation, since homeoproteins on either side of the borders are reciprocal inhibitors.

Our previous model (Holcman et al., submitted) succeeded to generate morphogenetic gradients of various shapes in a one dimension. In contrast with previous existing models, it is interesting to note that in our model, morphogenetic gradients were generated only by two types of molecules: an activator $A$ and a modified version of it, denoted $A^\ast$. In that scenario, generating a gradient relies on the autocatalytic property of $A$. But when cells are all initially identical, after activation and in the absence of any inhibitory regulation, the morphogen concentration should be identical in all cells, leading to a flat steady state profile. To overcome this difficulty and introduce diversity in the gradient profile genesis, based on experimental data realized in Prochiantz' lab, we postulated a scenario in which a morphogen $A$, sees its transcription efficiency decreased after entering into a cell. This scenario has received some biophysical evidences based on the observations that morphogens were detected bound to some lipids at the cell membrane. It is remarkable that under this assumption, we obtained (Holcman et al., submitted) various types of long and short range gradients. This scenario has the advantage to be parsimonious in the sense that it uses really only one morphogen and a modified version of it.

We analyze here a variant version of the biophysical model proposed in Holcman et al. (submitted), where now morphogens can form dimers. First, because delimiting a specific area is a fundamental task in signaling and regionalization, we will study the formation of accurate boundaries between two regions obtained by inhibitory morphogens under the dimer formation assumption. As a result, we show that gradients are smoother and borders are much sharper. Our numerical simulations can be related to previous results, obtained for the Drosophilae (Howard and ten Wolde, 2005). Second, because random perturbations of a gradient can affect the precise location of the boundary between two morphogenetic regions, we further analyze the role of these fluctuations, and in particular, we obtain an analytic expression for the variance of the boundary location as a function of the variance of the random perturbations. This formula can be used to study the noise effect on the position of the boundary between morphogenetic regions. During early development of the Drosophilae, the stability of the boundary with respect to the random noise has been examined both experimentally (Houchmandzadeh et al., 2002) and numerically (Howard and ten Wolde, 2005). In Howard and ten Wolde (2005), the source of variability is the injection rate of both the Bicoid protein and its co-repressor. It is interesting that both studies reported some pretty robust stability with random perturbations of the hunchback gene boundary. However, to our knowledge, no analytical results were reported, relating the boundary position with the size of the fluctuations. We obtain here a precise estimate of the variance of the boundary location when the morphogen flux contains a fluctuation term. The main result of this part is the expression of the variance given by formula (45). The present analysis can be generalized to other types of morphogenetic gradients, which can be obtained by both a diffusion source and a relay activation system. We finally discuss a general formula derived in Holcman et al. (submitted), relating the shift in the boundary between two morphogenetic regions and the difference in the two promoter initial concentrations. Similar formulas were given in Houchmandzadeh et al.
(2005) and McHale et al. (2006), but for exponential gradients only, generated by a competition between diffusion and inhibition. Our formula is general and can be used, for example, to analyze quantitatively the shift observed in the midbrain dopaminergic neurons, obtained in mutant mice following the experimental work of Brodski et al. (2003).

2. Generating a morphogenetic gradient

2.1. General equations

Let us review briefly the essence of the biophysical model, developed in (Holcman et al., submitted): We assumed that a time 0 specified by the cell internal clock, an initial factor \( X_0 \) controlling the production of morphogen \( A \) is activated. Molecule \( A \) labels this very first cell as a \( A \)-cell “for ever.” The first \( A \)-cell synthesizes \( A \) at a given rate inside the nucleus by an autocatalytic mechanism. Only a small fraction of \( A \) is later packed into vesicles to be delivered at the cell surface. Outside the first \( A \)-cell, \( A \) diffuses and only a small fraction \( \phi \) will reach the neighboring cell membrane. Various mechanisms are possible to explain how \( A \) will label the new cells. First, it has to be internalized, and this step may involve either an endocytosis pathway or, as we suggested, when \( A \) is a homeoprotein, it can penetrate into the membrane of the neighboring cells (Prochiantz and Joliot, 2003). While penetrating the cell membrane, we further assumed that the replicating power of \( A \) is altered, due to the irreversible binding of lipid molecules. The modified \( A \) morphogen is denoted \( A^* \). Possibly \( A \) can also be internalized by binding to specific receptors located on the cell membrane.

Moreover, we also assumed in (Holcman et al., submitted) that the \( A \)-production in neighboring cells is mostly controlled by \( A^* \), which originally comes from \( A \) molecules of a previous cell. Using these considerations, we were able to derive a set of discreet equations in two cases: (1) when the morphogen flux is unidirectional, and (2) when it is bidirectional. In the continuum limit, the second set of equations can be compared to the phenomenological equations used in Turing (1952), Eldar and Barkai (2005), Lander et al. (2002). We will now recall the equations and the main parameters. We denote by

1. \( \tilde{K} \) the equilibrium constant of \( A^* \) with its DNA site.
2. \( K_A \) the synthesis rate of \( A \) per active DNA site, controlled by \( A^* \).
3. \( \gamma \) the hydrolysis rate of \( A \) within the cell.
4. \( \gamma_t \) the proportion of \( A \) molecules packed in vesicles, to fuse with the cell membrane.
5. \( \phi \) the fraction of \( A \) entering a cell and experiencing a conformational change (e.g. through the possible attachment of a lipid).

Due to possible loss of \( A \) molecules between two consecutive cells, we impose the general condition that \( \phi \leq \gamma \). In case (1), the propagation of \( A \) follows (see Holcman et al., submitted) the hyperbolic equations

\[
\begin{align*}
\frac{\partial A}{\partial t} &= K_A \frac{N \tilde{K} A^*}{1 + \tilde{K} A^*} - (\gamma + \gamma_t) A, \\
\frac{\partial A^*}{\partial t} &= \tilde{\varphi} \frac{\partial A}{\partial x} + \varphi A - \gamma A^*,
\end{align*}
\]
while in case (2), we have shown (Holcman et al., submitted) that $A$ is described by a
degenerate parabolic system of equations given by

$$
\frac{\partial A}{\partial t} = K_A \frac{N \tilde{K} A^*}{1 + K A^*} - (\gamma + \gamma_t)A,
$$

$$
\frac{\partial A^*}{\partial t} = \tilde{\phi} \frac{\partial^2 A}{\partial x^2} + \varphi A - \gamma A^*,
$$

(2)

where $\tilde{\phi} = \varphi (\Delta x)^2$. $\Delta x$ represents the size of a cell. It is interesting to note the system
of Eqs. (2) belongs to the general class of reaction–diffusion equations and should be
compared to those summarized in Eldar and Barkai (2005), Lander et al. (2002). If we
define the parameter $\alpha$ by

$$
\alpha = \frac{\tilde{K} K_A \varphi}{\gamma (\gamma + \gamma_t)},
$$

(3)

it represents the decay rate of the morphogenetic gradient obtained by solving (1) or (2)
at steady state. In the discreet version, these equations can be solved explicitly and for
system (1),

$$
A_0 = \frac{K_A X_A}{(\gamma + \gamma_t)}
$$

(4)

and for $k \geq 1$,

$$
A_k = \gamma \frac{1}{\tilde{K} \varphi} \frac{1}{\gamma (1 - \alpha) \alpha^k} + 1 - \alpha^k \quad \text{when } \alpha \neq 1,
$$

$$
A_k = \gamma \frac{1}{\tilde{K} \varphi} \frac{1}{\gamma (1 - \alpha) \alpha^k} + k = \gamma \frac{1}{\tilde{K} \varphi} \frac{1}{X_A (-1 + k)} \quad \text{when } \alpha = 1.
$$

(5)

When $\alpha < 1$, the gradient decays almost exponentially (see Holcman et al., submitted),
while for $\alpha = 1$, we have algebraic decays, and for $\alpha > 1$, long-range gradient can be
formed, since the solution converges after few cells to a constant.

### 2.2. Chemical reactions for dimer formation

The basis of dimer formation assumes that the production of $A$ is really efficient when
only two $A^*$ are bound to the promoter site according to the chemical reaction

$$
\text{DNA} + A^* \rightleftharpoons \text{DNA} \cdot A^*,
$$

$$
A^* + \text{DNA} \cdot A^* \rightleftharpoons A^* \cdot \text{DNA} \cdot A^*.
$$

(6)

When two complexes $\text{DNA} \cdot A^*$ and $A^* \cdot \text{DNA} \cdot A^*$ are synthesized, we assume that the
production of $A$ by $A^* \cdot \text{DNA} \cdot A^*$ is much more efficient than its production by one
$\text{DNA} \cdot A^*$ only. The production of $A$ by the second Eq. (6) is summarized by

$$
A^* \cdot \text{DNA} \cdot A^* \rightarrow A + A^* \cdot \text{DNA} \cdot A^*.
$$

(7)

We propose now to derive the general dynamical equations for the dimer case.
2.3. Morphogenetic gradient equations with dimers

When $A$ is synthesized either by DNA · $A^*$ or $A^* · DNA · A^*$, to derive the morphogen equations, we follow the steps presented in (Holcman et al., submitted). The main difference here is due to the synthesis rate of $A$, which is given in steady state by the Michaelis–Menten approximation. This approximation allows us to estimate the fraction $F_1$ of $A^* · DNA$ and $F_2$ of $A^* · DNA · A^*$ produced. These fractions are respectively given by

\[ F_1 = \frac{D \tilde{K} A^*}{1 + \tilde{K} A^* + \tilde{K} f_2 A^*}, \]
\[ F_2 = \frac{D \tilde{K} f_2 A^*}{1 + \tilde{K} A^* + \tilde{K} f_2 A^*}. \]

The total amount of $A$ molecules produced per unit of time depends on the synthesis rates $F_1$ and $F_2$ and is given by

\[ N = \frac{K_A \tilde{K} A^* + K_{A^2} \tilde{K} A^*}{1 + \tilde{K} A^*}, \]

where $K_{A^2}$ is the synthesis rate of $A$ by $A^* · DNA · A^*$. For the case of a unidirectional flux, Eq. (1) becomes

\[ \frac{\partial A}{\partial t} = \frac{K_A \tilde{K} A^* + K_{A^2} \tilde{K} A^*}{1 + \tilde{K} A^*} - (\gamma + \gamma_t)A, \]
\[ \frac{\partial A^*}{\partial t} = \frac{\partial A}{\partial x} + \varphi A - \gamma A^*. \]

(9)

and in the bidirectional case, we obtain a degenerate parabolic system of equations:

\[ \frac{\partial A}{\partial t} = \frac{K_A \tilde{K} A^* + K_{A^2} \tilde{K} A^*}{1 + \tilde{K} A^*} - (\gamma + \gamma_t)A, \]
\[ \frac{\partial A^*}{\partial t} = \frac{\varphi \partial^2 A}{2 \partial x^2} + \varphi A - \gamma A^*. \]

(10)

The steady state gradients generated by Eqs. (10) are shown in Fig. 1. The parameters are given in Table A.1, see also Holcman et al. (submitted). Using various set of parameters, we obtain different morphogenetic gradients (on each figure of panel 1, the lowest curve corresponds to the monomer case). We conclude that dimer formation does not change the nature of the shape, but can have nonlinear effects on the gradient profiles.

2.4. Formation of a boundary between two morphogenetic areas

In Holcman et al. (submitted), we examined the boundary formation between two neighboring morphogenetic regions. We wish now to study the effect of dimers formation on the boundary stiffness.
Morphogenetic Gradients and the Stability of Boundaries

Fig. 1 Steady state gradient generated by dimers. The solutions are obtained by solving numerically Eqs. (10). In each sub-figure, the lowest curve is the solution of the system of equation where \( K_2 = 0 \) (monomer). \( x \)-axis is the normalized cell ensemble and \( y \)-axis is the normalized concentration. The values of the remaining parameters are given in Table A.1.

To investigate this question, we suppose that dimers of the same morphogens can be synthesized, while we ignore the direct interaction between two different morphogens \( A^* \) and \( B^* \): that is \( A^* \cdot DNA \cdot B^* \) cannot synthesize any compounds. We further assume that \( A \) and \( B \) have similar biochemical properties. In the discreet case, in a dimensional cell ensemble for a unidirectional flux, the propagation of the two morphogens are described by the following system of equations: for \( k = 1, \ldots, N \),

\[
\begin{align*}
\frac{dA_k}{dt} &= \frac{K_A \tilde{K} A_k^* + K_{A2} \tilde{K}_2 A_k^*}{1 + \tilde{K} A_k^* + \tilde{K}_2 A_k^* + \tilde{K} B_k^* + \tilde{K}_2 B_k^*} - (\gamma + \gamma_t) A_k, \\
\frac{dA_k^*}{dt} &= \varphi A_{k-1} - \gamma A_k^*, \\
\frac{dB_k}{dt} &= \frac{K_B \tilde{K} B_k^* + K_{B2} \tilde{K}_2 B_k^*}{1 + \tilde{K} A_k^* + \tilde{K}_2 A_k^* + \tilde{K} B_k^* + \tilde{K}_2 B_k^*} - (\gamma + \gamma_t) B_k, \\
\frac{dB_k^*}{dt} &= \varphi B_{k+1} - \gamma B_k^*.
\end{align*}
\]

Results of numerical simulations associated with the system of Eqs. (11) are presented in Fig. 2. It shows that the precise localization of the boundary in an ensemble of 100 cells (the parameters of the simulations are reported in the legend of the figure). It is interesting to note that the nonlinearity induced by the dimers formation leads to a sharp boundary.
Fig. 2 Boundary formation with dimers. \( \gamma = \phi = 10^{-4}, \gamma_t = 0.05 \ast \gamma, K = 10^5, K_2 = 1, K_A = 1.0510^{-9} \). (A): \( X_A = X_B = 10^2, K_{A_2} = 10.395 \). (B): \( X_A = X_B = 10^5, K_{A_2} = 10.395 \). (C): \( X_A = X_B = 10^5, K_{A_2} = 10.185 \). (D): \( X_A = X_B = 10^9, K_{A_2} = 10.185 \). The curves are obtained by solving the system of Eqs. (11). X-axis is the number of cells and Y-axis is the normalized concentration. The values of the remaining parameters are given in Table A.1.

In comparison, when no dimers are formed, sharp boundaries can only appear when two different morphogens directly inhibit one another (see Holcman et al., submitted for the details). When a bidirectional flux of morphogens is generated between neighboring cells, Eqs. (11) are replaced by the following set of equations for \( k = 1, \ldots, N \),

\[
\frac{dA_k}{dt} = \frac{K_A \ddot{K}_A A_k^* + K_{A_2} \ddot{K}_2 A_k^{*2}}{1 + \ddot{K}_A A_k^* + \ddot{K}_2 A_k^{*2}} - (\gamma + \gamma_t) A_k, \\
\frac{dA_k^*}{dt} = \frac{\phi}{2} (A_{k-1} + A_k) - \gamma A_k^*, \\
\frac{dB_k}{dt} = \frac{K_B \ddot{K}_B B_k^* + K_{B_2} \ddot{K}_2 B_k^{*2}}{1 + \ddot{K}_B B_k^* + \ddot{K}_2 B_k^{*2}} - (\gamma + \gamma_t) B_k, \\
\frac{dB_k^*}{dt} = \frac{\phi}{2} (B_{k+1} + B_k) - \gamma B_k^*.
\]

The results of the numerical simulations are presented in Fig. 3: The boundary between the two morphogenetic regions is much sharper compared to the one obtained with a unidirectional flux (Fig. 2). Both concentrations decay very quickly near the boundary and this finding is similar to the one obtained in Fig. 1 of Howard and ten Wolde (2005),
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Fig. 3  Boundary between two morphogenetic gradients obtained with dimer formation and bidirectional fluxes. The dashed lines represent the gradient when a morphogen is alone. The boundary conditions are fixed to \( X \) at one extreme part and absorbing at the other part. The continuous line represents the morphogen gradient generated by Eqs. (12). \( \gamma = \phi = 10^{-4}, \gamma_t = 0.05, K = 10^5, K_2 = 1, K_A = 1.0510^{-9}. \) (A): \( X_A = X_B = 10^5, K_A2 = 10.5026. \) (B): \( X_A = X_B = 10^8, K_A2 = 10.5026. \) (C): \( X_A = X_B = 10^5, K_2 = 10.5. \) (D): \( X_A = X_B = 10^8, K_A2 = 10.5. \) The graphs correspond to the steady solution of Eqs. (12).

where the authors used a new model involving Bicoid to generate a morphogenetic gradient concentration in the drosophilae. Their sharp concentration profiles seem to be a consequence of the nonlinearity interactions of a small gene network. It is remarkable that our model, modified with the additional possibility that morphogen dimers can be formed, generates gradient in an ensemble of cells, as if membranes were ignored, and thus, this result is comparable to the morphogen gradient generated in a drosophilae syncytium. We conclude that in the absence of any direct chemical interactions between morphogens \( A \) and \( B, \) in both cases of unidirectional and bidirectional fluxes, dimers formation induces boundaries much sharper compared to the ones induced by monomers alone.

3. Small noise perturbation and gradient stability

In this section, we shall study the stability to small perturbations of the boundary between two morphogenetic gradients. We postulate that the main source of variability comes from the morphogen flux \( \phi \) across cells. Because it represents the fraction of \( A \) molecules that are finally entering a cell in the form of \( A^* \), the flux is the most likely fluctuating parameter. Fluctuations may come into two places: First, the number of \( A \) molecules may fluctuate due to the diffusion from a cell to the neighboring one, and second, there may be some variability in the efficacy of penetration the cell membrane.
We model these fluctuations as a small stochastic component added to the constant flux term. To examine the stability of the gradient concentration, we analyze Eq. (1) both in the discreet and the continuum case. We recall that in the discreet case, Eq. (1) is given by

\[
\frac{dA_k}{dt} = KA \tilde{K} \frac{A_k^*}{1 + KA_k^*} - (\gamma + \gamma_t)A_k,
\]

\[
\frac{dA_k^*}{dt} = \varphi A_{k-1} - \gamma A_k^*.
\] (13)

We shall first estimate the variance of the gradient concentration as a function of the flux amplitude. The flux entering cell \(k\) has the general form

\[
\varphi_k = \varphi(1 + \varepsilon \sigma_k),
\] (14)

where \(\sigma_k\) are independent identical random variables, with zero mean and variance \(\sigma\) \((E(\sigma_k \sigma_q) = \sigma \delta_{q,k}\), where \(\delta_{q,k} = 1\) only if \(q = k\)). We assume that \(\varepsilon \ll 1\). In the approximation \(\tilde{K}A^* \ll 1\) in systems (13) and (1), we now provide at steady state, an estimate of the concentration \(A_k\).

3.1. Random flux perturbation in the initial gradient formation

At steady state, when \(\tilde{K}A^* \ll 1\), using the explicit induction relation between the morphogen concentration between cell \(k\) and \(k + 1\) in system (13) (see Holcman et al., submitted), we have

\[
A_k = \frac{KA \varphi(1 + \varepsilon \sigma_k)}{\gamma(\gamma + \gamma_t)}A_{k-1}
\]

\[
= \alpha A_{k-1}(1 + \varepsilon \sigma_k).
\]

When \(\varepsilon\) is small, the solution is given by

\[
A_k = \alpha^k A_0 \prod_{n=1}^{k} (1 + \varepsilon \sigma_n) \approx A_0 \alpha^k \left(1 + \varepsilon \sum_{n=1}^{k} \sigma_n\right).
\]

We denote by \(\tilde{A}_k\), the steady state solution in the absence of any random fluctuations \((\varepsilon = 0)\), \(\tilde{A}_k = \alpha^k A_0\) and the variance of \(A_k\) is given by

\[
\langle |A_k - \tilde{A}_k|^2 \rangle = k\alpha^{2k}\varepsilon^2\sigma.
\] (15)

The total fluctuation \(FG\) of the gradient in a cell ensemble is defined by

\[
FG^2 = \sum_0^N \langle |A_k - \tilde{A}_k|^2 \rangle = \varepsilon^2 \sigma \sum_0^N k\alpha^{2k}.
\] (16)

Thus, in the limit of a large number of cells, we obtain the approximation

\[
FG \approx \varepsilon \sqrt{\sigma} \frac{\alpha}{1 - \alpha^2}.
\] (17)
This expression is valid only for $\alpha \ll 1$. In that case, we conclude that the variance of the gradient profile is linear in $\alpha$. The case $\alpha > 1$ is treated in the Appendix.

3.2. A random flux perturbation changes the position of the boundary between areas

To estimate the effect of the flux random fluctuations on the morphogenetic profile (2), we consider the continuum steady state regime where the flux is approximated by a random process $\sigma$:

$$\varphi(x) = \varphi(1 + \varepsilon \sigma(x)), \quad (18)$$

where $\sigma(x)$ is a random field of variance 0, such that

$$\mathbb{E}(\sigma(x)\sigma(y)) = \xi^2 e^{-\lambda|x-y|}. \quad (19)$$

$\sigma(x)$ is the solution of an Ornstein–Uhlenbeck process

$$\dot{\sigma} = -\lambda \sigma + \beta \dot{w}, \quad (20)$$

where $\lambda > 0$ is a constant. The steady state variance is given by $\xi^2 = \frac{\beta^2}{2\lambda}$ and the steady state probability density function (pdf) $p$ is a centered Gaussian of variance $\xi$,

$$p(\sigma \in (x, x + dx)) = \frac{1}{\sqrt{2\pi \xi}} e^{-\frac{(x^2}{2\xi^2}} dx. \quad (21)$$

3.2.1. The steady state profile with fluctuations

In that approximation, $1 \gg \tilde{K}A^*$ and in the steady state continuum limit, we propose to estimate the total variance in the $A$-morphogenetic profile. Equation (2) becomes

$$0 = \tilde{\varphi} \frac{\partial^2 A}{\partial x^2} + \varphi A - \gamma \frac{(\gamma + \gamma_t) A}{NKK_A} \quad (22)$$

and can be written as

$$0 = \frac{(\Delta x)^2}{2}(1 + \varepsilon \sigma(x)) \frac{\partial^2 A}{\partial x^2} + (1 + \varepsilon \sigma(x)) A - \frac{1}{\alpha} A. \quad (23)$$

Using an asymptotic expansion of the solution in power of $\varepsilon$, we are looking for a solution of the form

$$A(x) = A_0(x) + \varepsilon \tilde{A} + o(\varepsilon), \quad (24)$$

where $A_0$ is the first term. $\tilde{A}$ is solution of equation

$$\tilde{A}'' = 2 \frac{\alpha^{-1} - 1}{(\Delta x)^2} \tilde{A} - 2\alpha^{-1} \frac{\sigma(x)}{(\Delta x)^2} A_0, \quad \tilde{A}(0) = \tilde{A}(L) = 0.$$
where \( L \) is the size of the cell ensemble. The solution is given by

\[
\tilde{A}(x) = \sinh(\beta L - \beta x) \int_0^x \frac{2 \sinh(\beta x_1) \sigma(x_1)}{\beta \alpha \sinh(\beta L) (\Delta x)^2} A_0(x_1) \, dx_1 \\
+ \sinh(\beta x) \int_x^L \frac{2 \sinh(\beta L - \beta x_1) \sigma(x_1)}{\beta \alpha \sinh(\beta L) (\Delta x)^2} A_0(x_1) \, dx_1,
\]

(25)

where

\[
A_0(x) = a_0 \cosh(\omega x) + a_1 \sinh(\omega x).
\]

(26)

When \( \alpha < 1 \),

\[
\omega^2 = \frac{2}{(\Delta x)^2} \left( -1 + \frac{1}{\alpha} \right),
\]

(27)

and \( a_0, a_1 \) depend on the boundary conditions and \( \beta = \frac{\sqrt{2\alpha - 1} - 2}{\Delta x} \). We define the total variance as in the discrete case by

\[
TVF^2 = \int_0^L \langle |A(x) - A_0(x)|^2 \rangle \, dx.
\]

(28)

Thus

\[
TVF^2 \approx \varepsilon^2 \int_0^L \langle |\tilde{A}(x)|^2 \rangle \, dx + o(\varepsilon^2).
\]

(29)

Using the explicit expression (25), we get

\[
TVF^2 = \varepsilon^2 \int_0^L \langle |\tilde{A}(x)|^2 \rangle \, dx + o(\varepsilon^2)
\]

\[
= \frac{4 \varepsilon^2 \xi^2}{(\Delta x)^4 \beta^2 \alpha^2 \sinh^2(\beta L)}
\]

\[
\times \left\{ \int_0^L \sinh^2(\beta(L - x)) \int_0^x \int_0^x \sinh(\beta x_2) \sinh(\beta x_1) E(\sigma(x_1)\sigma(x_2)) \right. \\
\times A_0(x_1) A_0(x_2) \, dx_1 \, dx_2 \, dx \\
+ \int_0^L \sinh^2(\beta x) \int_x^L \int_x^L \sinh(\beta(L - x_1)) \sinh(\beta(L - x_2)) A_0(x_1) A_0(x_2) \right. \\
\times E(\sigma(x_1)\sigma(x_2)) \, dx_2 \, dx_1 \, dx \right\}.
\]

If we denote \( a = A(0) \), when \( \beta \) is small and \( \alpha \) is close to 1, then some tedious computations lead to the following asymptotic expansion for the total variance fluctuation as a
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function of the noise parameters:

\[
TVF^2 = \frac{\epsilon^2 \xi^2}{(\Delta x)^4 a^2} a^2 \left\{ 160L^{-2} \lambda^{-9} - 72L^{-1} \lambda^{-8} + 4L^{-1} \lambda^{-7} + 16L \lambda^{-6} / 3 - 2L^2 \lambda^{-5}
+ L^3 \lambda^{-4} / 3 - L^4 \lambda^{-3} / 90 - L^5 \lambda^{-2} / 105 + L^6 \lambda^{-1} / 315 \right\}
- a^2 e^{\lambda L} (160L^{-2} \lambda^{-9} + 88L^{-1} \lambda^{-8} + 12L^{-7}).
\]

Thus, the leading order term is

\[
TVF^2 = \frac{\epsilon^2 \xi^2}{315(\Delta x)^4 a^2} a^2 L^6 \lambda^{-1}.
\]

4. From how much a random flux shifts the position of the boundary?

They are various ways to shift the position of the boundary between two morphogenetic regions: one possibility, as described in (Holcman et al., submitted), is obtained for a different initial inductor concentration of morphogen A and B. Another possibility results from the cumulative effect of small random perturbations across cells. We study this case for a random flux component \( \phi \), described by Eq. (18). We will derive here an analytical expression of the shift of the boundary. Because the random field \( \sigma \) has zero mean, the average position \( b_\sigma \) of the boundary is equal to the mean, but the variance \( V(b_\sigma) \) should depend on the variance of the flux. Under the assumption \( \alpha < 1 \), we propose to relate the variance \( V(b_\sigma) \) of the boundary position to the noise variance \( \sigma \). The general case is left open.

We start from the explicit recurrent relation Eq. (13) derived at steady state, and we will extend this solution to the continuum limit. Because Eq. (13) is hyperbolic, only one boundary condition is necessary to determine the entire solution. This boundary condition is given by the initial transcription factor concentration.

To determine the position of the boundary, we use the criteria that for two morphogens A, B propagating in opposite directions, when they meet at a point denoted by \( b_\sigma \), they produce a sharp boundary between the morphogenetic regions (see Holcman et al., submitted). This is also the case when dimers are involved, but we only consider here the case of monomers and assume that the boundary is sharp enough (the amplitude of the derivative at the boundary is high enough), so that the concentration of morphogens decreases very quickly in a neighborhood of the point \( b_\sigma \). This remark allows us to use the explicit expression of solution (13). In the rest of the paper, we consider that morphogen A is initiated at position 0-th cell, and propagates to the right while morphogen B is initiated at the \( M \)-th cell and propagates to the left.

4.1. Deriving a continuum equation for a morphogen concentration

In the absence of any fluctuations, the position of the boundary \( b_\sigma \) is defined as the point where the concentrations A and B are equal. Thus, the position of the boundary can only be defined when A and B are continuous functions. We start by extending the discreet
sequences $A_k$ and $B_k$ to the continuum limit. The explicit recurrent relation for morphogen $A$ concentration is, for $k \geq 1$

$$A_k = \frac{\gamma}{K \varphi} \frac{(1 - \alpha) \alpha^k}{\gamma(1 - \alpha) + 1 - \alpha^k}, \quad \text{when } \alpha \neq 1. \quad (30)$$

Outside the boundary layer near the boundary $b_\sigma$, the two morphogens $A$ and $B$ are not mixed, so the concentration of $A$ is given by formula (30) and for $k \geq 1$,

$$A_k = \frac{\gamma}{K \varphi} \frac{(1 - \alpha) \alpha^k}{C - \alpha^k}. \quad (31)$$

If we define the continuous function $u$ by

$$u(x) = \frac{\alpha^x}{C - \alpha^x}, \quad (32)$$

then

$$A_k = \frac{\gamma}{K \varphi} (1 - \alpha) u(k). \quad (33)$$

Similarly we can define the normalized concentration $w$ of $B$ by

$$w(x) = \frac{\alpha^{M-x}}{C - \alpha^{M-x}}. \quad (34)$$

By deriving with respect to $x$ and using the identity

$$C = \alpha^k (u^{-1} + 1),$$

we get

$$0 = \frac{\partial}{\partial x} (\alpha^x (u^{-1} + 1)), \quad (35)$$

thus

$$u' = \ln (u + u^2).$$

This equation is used to include fluctuations. Indeed, when the fluctuation term in the flux $(\sigma_k)$ does not depend on $k$, we obtain using (18), the following expression for $A_k$:

$$A_k = \left( \frac{\gamma}{K \varphi (1 + \epsilon \sigma)} \right) \frac{(1 - \alpha (1 + \epsilon \sigma)) \alpha^k (1 + \epsilon \sigma)^k}{C + \frac{\epsilon \sigma}{1 - \alpha (1 + \epsilon \sigma)} - \alpha^k (1 + \epsilon \sigma)^k}. \quad (36)$$

To expand this solution as a function of the parameter $\epsilon$, we use Eq. (35) to obtain at order $O(\epsilon^2)$ the relation

$$0 = \frac{\partial}{\partial k} \alpha^k \left( \left( 1 + \left( k - \frac{1}{1 - \alpha} \right) \epsilon \sigma \right) u^{-1} + 1 + k \epsilon \sigma \right) + O(\epsilon^2),$$
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that is

\[ u' = \ln \alpha (u^2 + u) + \varepsilon \sigma \left( \frac{\ln \alpha}{1 - \alpha} u^2 + u^2 + u \right) + O(\varepsilon^2). \] (36)

Although this expression was obtained for a \( k \)-independent \( \sigma \) random field, up to a first order approximation, we will use it for a general noise, which might be \( k \) dependent. We leave open the derivation of the general perturbation formula starting from the explicit expression of \( A_k \) and the properties of the random field.

4.2. Asymptotic solution of Eq. (36)

Equation (36) describes at first order, the effect of perturbation on a morphogenetic gradient. We search for an asymptotic solution of Eq. (36) by considering a regular expansion of the form

\[ u = u_0 + \varepsilon u_1 + \cdots, \] (37)

where \( u_0 \) is the solution in the absence of fluctuations. Using expression (32) for \( u_0 \) and isolating terms of order \( O(\varepsilon) \), we obtain the following equation for \( u_1 \):

\[ u_1' = \ln \alpha (u_1 + 2u_0 u_1) + \sigma \left( \frac{\ln \alpha}{1 - \alpha} u^2_0 + u^2_0 + u_0 \right) \] (38)

which can be rewritten as

\[ \frac{d}{dx} (u_1 u_0^{-2} \alpha^x) = \sigma C + \frac{\sigma \alpha k \ln \alpha}{1 - \alpha}, \] (39)

where \( C \) is the constant of the unperturbed profile \( u_0 \). After integrating Eq. (39) with a zero boundary condition (\( u_1(0) = 0 \)), we obtain

\[ u_1(k) = u_0^2 \alpha^{-k} \left( C \int_0^k \sigma(l) dl + \frac{\ln \alpha}{1 - \alpha} \int_0^k \sigma(l) l^k \right) \] (40)

4.3. Estimation of the variance of the boundary fluctuation \( b_\sigma \)

When the random field \( \sigma \) satisfies condition (19), \( u \) (resp. \( w \)) represents the concentration of morphogen \( A \) (resp. \( B \)), then the boundary between the morphogens is located at a position \( M/2 + \tilde{b}_\sigma \), where \( \tilde{b}_\sigma \) converges to zero when \( \sigma \) goes to zero. To estimate \( \tilde{b}_\sigma \), we start by defining the boundary condition as the set of points where the concentration of \( A \) equals the concentration of \( B \):

\[ u(M/2 + \tilde{b}_\sigma) - w(M/2 + \tilde{b}_\sigma) = 0. \] (41)

To obtain an expression for \( \tilde{b}_\sigma \), we use on one hand, the expansion of \( u \) and \( w \) as a function of \( \varepsilon \), and on the second hand, the Taylor expansion of \( u \) and \( w \) near the point \( M/2 \). At first order in \( \varepsilon \), we get

\[ \{u'_0(M/2) - w'_0(M/2)\} \tilde{b}_\sigma + \varepsilon \{u_1(M/2) - w_1(M/2)\} = 0 + o(\varepsilon), \] (42)
where \( u = u_0 + \varepsilon u_1 \) and \( w = w_0 + \varepsilon w_1 \), and thus

\[
\langle |b_\sigma|^2 \rangle = \varepsilon^2 \frac{(|u_1(M/2) - w_1(M/2)|^2)}{(u_0'(M/2) - w_0'(M/2))^2}.
\]  

(43)

Under the assumption that \( \alpha < 1 \), the function \( u_0 - w_0 \) is strictly decreasing and thus the derivative at the point \( M/2 \) is not zero. Using Eq. (40), we now provide an estimation for the quantity \( \langle |u_1(M/2) - v_1(M/2)|^2 \rangle \). The expectation with respect to the noise term of the \( u_1 \) variance at a point \( R \) is given by

\[
\frac{1}{2} E \left( \int_0^R \sigma(x) dx \right)^2 = \int_0^R e^{-\lambda x} \int_x^R \alpha y dy dx = \xi^2 \left( \frac{R}{\lambda} + \frac{e^{-\lambda R} - 1}{\lambda^2} \right).
\]

Second,

\[
E \left( \int_0^R \int_0^x \sigma(x) \sigma(y) \alpha y dy dx \right) = \int_0^R e^{-\lambda x} \int_0^{R-x} \alpha y dy dx
\]

\[
= \xi^2 \frac{1}{\ln \alpha} \int_0^R e^{-\lambda x} (\alpha^{R-x} - 1) dx
\]

\[
= \xi^2 \frac{\alpha^R - e^{-\lambda R}}{\ln \alpha + \ln \alpha} + \frac{e^{-\lambda R} - 1}{\lambda \ln \alpha}.
\]

Third,

\[
E \left( \int_0^R \int_x^R \sigma(x) \sigma(y) \alpha^x dy dx \right) = \int_0^R e^{-\lambda x} \int_0^R \alpha^x dy dx
\]

\[
= \frac{1}{\ln \alpha} \int_0^R e^{-\lambda x} (\alpha^R - \alpha^x) dx
\]

\[
= \frac{\alpha^R (1 - e^{-\lambda R})}{\lambda \ln \alpha} + \frac{\alpha^R e^{-\lambda R} - 1}{\ln \alpha (\lambda - \ln \alpha)}.
\]

And fourth,

\[
\frac{1}{2} E \left( \int_0^R \sigma(x) \alpha^x dx \right)^2 = \int_0^R e^{-\lambda x} \int_x^R \alpha^{2y-x} dy dx
\]
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\[ e^2 \frac{1}{2 \ln \alpha} \int_0^R e^{-\lambda x} (\alpha^2 R - \alpha^x) dx \]

\[ = \frac{\alpha^2 R - \alpha R e^{-\lambda R}}{2 \ln \alpha (\ln \alpha + \lambda)} + \frac{1 - \alpha R e^{-\lambda R}}{2 \ln \alpha (\ln \alpha - \lambda)}. \]

4.3.1. Variance of \( b_\sigma \)

We estimate the variance (43) as follows

\[ E \left( u_1 \left( \frac{M}{2} \right) - u_1 \left( \frac{M}{2} \right) \right)^2 = 2E \left( u_1 \left( \frac{M}{2} \right) \right)^2 - 2E \left( u_1 \left( \frac{M}{2} \right) w_1 \left( \frac{M}{2} \right) \right). \]

Only the second term in the r.h.s. needs to be evaluated. Using the expression of \( w_1 \),

\[ w_1(k) = v_0^2 \alpha^{-M+k} \left( C \int_k^M \sigma(l) dl + \frac{\ln \alpha}{1 - \alpha} \int_k^M \sigma(l) \alpha^l dl \right) \]

we get

\[ E \left( u_1 \left( \frac{M}{2} \right) w_1 \left( \frac{M}{2} \right) \right) = C^2 E \left( \int_0^M \sigma(x) dx \int_0^M \sigma(y) dy \right) \]

\[ + 2 \frac{C \ln \alpha}{1 - \alpha} E \left( \int_0^M \int_0^M \sigma(x) \sigma(y) \alpha^{M-y} dy dx \right) \]

\[ + \frac{\ln^2 \alpha}{(1 - \alpha)^2} E \left( \int_0^M \sigma(x) \alpha^x dx \int_0^M \sigma(y) \alpha^{M-y} dy \right). \]

We estimate each term separately. First,

\[ E \left( \int_0^M \sigma(x) dx \int_0^M \sigma(y) dy \right) = \int_0^M \int_0^M \exp \left( -\frac{\lambda}{2} (y - x) \right) dy dx \]

\[ = \frac{1}{\lambda^2} \left( \exp \left( -\frac{\lambda M}{2} \right) - 1 \right)^2. \]

Second,

\[ E \left( \int_0^M \int_0^M \sigma(x) \sigma(y) \alpha^{M-y} dy dx \right) = \int_0^M e^{\lambda x} dx \int_0^M e^{-\lambda y} \alpha^{M-y} dy \]

\[ = \frac{(e^{\frac{\lambda M}{2}} - 1) (e^{\frac{\lambda M}{2}} - \alpha^\frac{M}{2})}{\lambda (\ln \alpha + \lambda)}. \]

And finally,

\[ E \left( \int_0^M \sigma(x) \alpha^x dx \int_0^M \sigma(y) \alpha^{M-y} dy \right) = -\frac{(e^{\frac{\lambda M}{2}} \alpha^\frac{M}{2} - 1) (e^{-\lambda M} - e^{\frac{\lambda M}{2}} \alpha^\frac{M}{2})}{(\ln \alpha + \lambda)^2}. \]
We can now plug the results of the previous integrals into the definition of the variance (43). Using the symmetry of $A$ and $B$, at the middle point, we have

$$(u'_0(M/2) - w'_0(M/2))^2 = 4u'_0(M/2)^2$$

and the derivative of $u_0$ is given by the formula (35),

$$u'_0(M/2) = \ln \alpha u_0^2(M/2) C \alpha^{-M/2}.$$ 

Finally, using the expression of the variance (43), gathering the expressions above, we get an estimation of the variance of $b_\sigma$ up to order $O(\varepsilon^3)$:

$$\langle |\tilde{b}_\sigma|^2 \rangle = \xi_\varepsilon^2 \varepsilon^2 \left\{ \frac{1}{\lambda^2 \ln \alpha} \left( \frac{M \lambda}{2} + 2e^{-\lambda M/2} - \frac{3}{2} - \frac{1}{2}e^{-\lambda M} \right) \right.$$ 

$$+ \frac{1}{C \ln \alpha (1 - \alpha)} \left( \frac{\alpha M/2 - e^{-\lambda M/2}}{\ln \alpha(\lambda + \ln \alpha)} + \frac{(\alpha M/2 - 1)(1 - e^{-\lambda M/2})}{\lambda \ln \alpha} \right)$$ 

$$+ \frac{\alpha M/2 e^{-\lambda M/2} - 1}{\ln \alpha(\lambda - \ln \alpha)} - \frac{(\alpha M/2 - e^{-\lambda M/2})(1 - e^{-\lambda M/2})}{\lambda(\ln \alpha + \lambda)} + \frac{1}{C^2(1 - \alpha)^2}$$ 

$$\times \left( \frac{\alpha M - aM^2e^{-\lambda M/2}}{2\ln(\alpha(\ln \alpha + \lambda))} + \frac{1 - aM^2e^{-\lambda M/2}}{2\ln(\alpha(\alpha - \lambda))} - \frac{(e^{-\lambda M} - \alpha M/2)^2}{2(\ln \alpha + \lambda)^2} \right) \left\} \right.$$ 

$$+ O(\varepsilon^3).$$

The leading order term in the expansion is for $\alpha < 1$,

$$\langle |\tilde{b}_\sigma|^2 \rangle \sim \beta^2 \varepsilon^2 \frac{1}{2\lambda^3 \ln \alpha} \left( \frac{M \lambda}{2} - \frac{3}{2} \right) + O(\varepsilon^3).$$

(45)

We can now estimate the shift due to noise. For an ensemble of $M = 100$ cells, with a noise amplitude $\varepsilon = 0.01$ (1% fluctuation per cell), $\beta^2/2 = \lambda = 1$ (the correlation length is of the order of one cell), $\alpha = 0.95$, we found that the variance is given by

$$\langle |\tilde{b}_\sigma|^2 \rangle \approx 1$$

(46)

that is the variance is about one cell.

### 4.4. Shift in the boundary position induced by a different activation factor concentration

Changing the initial concentrations of the inducers $X_A$ or $X_B$ may result in a shift of the boundary between the two morphogen regions (Holcman et al., submitted). Numerical simulations of the shift in the boundary position were given (Holcman et al., submitted). When both morphogens have identical characteristic parameters, we recall the analytical formula for the shift. When the shift $\Delta L$ is small, in $B$ direction and for $\alpha < 1$, the value of $X_B$ is given by

$$X_B \approx \frac{(\alpha^{-1} - 1)\alpha^2 \Delta L}{X_A^{-1}(\alpha^{-1} - 1) + 1 - \alpha^{-2} \Delta L}.$$ 

(47)
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Equivalently,

\[ 2\Delta L = |X_A^{-1} - X_B^{-1}| \quad \text{if} \quad \alpha = 1 \]
\[ = \frac{1}{\ln \alpha} \ln \left( \frac{X_B (\alpha^{-1} - 1)}{(\alpha^{-1} - 1) + X_B} \right) \quad \text{if} \quad \alpha < 1. \]

(48)

We refer to Holcman et al. (submitted) for the derivation of the formula. It is interesting to see that for \( \alpha = 1, \) \( X_B = 2X_A = 2 \) (doubling the concentration) then the shift is

\[ \Delta L = \frac{1}{4}. \]

(49)

that is for a total of 100 cells, such difference in the inductor concentration is the origin of a shift of 25 cells. Expression (48) generalizes expression obtained in Houchmandzadeh et al. (2005) for a symmetric steady state gradient induced by diffusion with a killing source.

5. Discussion and conclusion

Based on the finding that transcription factors, such as engrailed can travel from cell to cell (Prochiantz and Joliot, 2003), we have developed mathematical models where they can generate various morphogenetic gradients. The physiological significance of such effect is not clear yet, but in a recent study (Brunet et al., 2005), it was shown that homeoproteins are signaling molecules with morphological consequences in retinal axone guidance. Moreover, in a recent study (unpublished Prochiantz’s data), it has been found that in vivo, Pax6 transfer is necessary for the extension of the eye anlagen. There are various ways to test the present model. First, there are already some experiments that support it: They consist in modifying the levels of expression of homeoproteins that participate in border formation (Brodski et al., 2003). The difference in the expression level resulted in a border shift. This result was repeated with several homeoprotein pairs (see Bishop et al., 2003; Simeone, 2000). The most popular cases are Otx2/Gbx2 and the mid-hindbrain border as well as Emx2/Pax6 and the primary visual/somato-sensory cortices. A nice way to test the model would be to mutate the homeoproteins in the regions necessary for intercellular transfer. Unfortunately, these regions are in the homeodomain. This precludes that they can be mutated without affecting cell-autonomous transcriptional activity. Thus, an alternative strategy has to be found, which consists in blocking the homeoprotein outside of the cells by expressing single-chain antibodies in vivo. Prochiantz’s lab has applied this strategy to Pax6 and shows that blocking Pax6 passage does not modify the early definition of the eye anlagen (stage 3–6 somites in the Zebrafish), but blocks its homeogenetic extension, leading to a small eye phenotype (unpublished data).

We have also studied in this article, the stability of the boundary between morphogenetic regions, when the morphogen flux is driven by random fluctuations. This model is based on the assumption that morphogens are a transcription factor, which can enter the cell membrane (Prochiantz and Joliot, 2003) and label exclusively the cell when they induce their own production. This scenario is far from being restrictive and does not only model the propagation of homeoproteins, it also accounts for any molecular transport
through a cell surface. Moreover, we have seen that dimers formation resulted in sharp gradients compared to monomers. Thus, we conclude that a simple direct inhibition between two different morphogens does not generate sharp boundaries, while cooperative does. The present analysis confirms the numerical results obtained with a cooperativity model for the stability of hunchback boundary in the early morphogenetic patterning of the drosophilae (Howard and ten Wolde, 2005). The main difference between our model and their model is that no membrane needs to be crossed in the case of the drosophilae, while it is a fundamental obstacle of signaling in our case, and in general.

The scenario we have presented here and in Holcman et al. (submitted), can be compared to the traditional ones, based on reaction–diffusion equations (Lander et al., 2002; Eldar and Barkai, 2005), in which the mode of morphogen propagation requires membrane receptor activation, vesicular internalization or transcytosis (Vincent and Dubois, 2002). The present scenario derived from biophysical considerations on one hand can be interpreted as parsimonious and minimal, where diffusion alone is not enough to explain gradient, because it requires that each cell, once labeled becomes a source and participates to the gradient generation. On the other hand, the assumption that morphogens are homeoproteins allows us to explain the internalization without using transcytosis, which is usually characterized as time consuming. We have also obtained here an explicit asymptotic expression for the variance of the boundary as a function of the flux variance. Because fluctuations are inherent to the flux, the boundary between two morphogenetic gradients cannot be predicted with an arbitrary accuracy, rather it is given with an error described by formula (45). While formula (45) includes relay, it generalizes the formulas derived in McHale et al. (2006) and Houchmandzadeh et al. (2005), obtained for non-interacting gradients and based on diffusion and hydrolysis only. In early development, fluctuations of the boundary should be kept as small as possible to avoid labeling mistakes, which may be later on amplified by cellular divisions. It is not clear what are the molecular mechanisms controlling the level of fluctuations in a cell ensemble. Recent experiments about the anteroposterior axis of the Drosophila embryo (Houchmandzadeh et al., 2002) have revealed a large variability of the maternal morphogen Bicoid gradient expression, although this variability was observed to be decreased at the level of hunchback (hb) gene expression.

Finally, a shift in the border position between two morphogenetic gradient, as described by formula (48) can result in drastic functional and behavior consequences: In mutant mice, by shifting the boundary between the morphogen Otx2 and GBx2, the midbrain dopaminergic neuronal population expands and is enlarged. In addition, those mutants show a higher locomotor activity which persists in the adult stage (Brodski et al., 2003). At early stages, mistakes in boundary formation can lead to abnormal cortical maps and be responsible for developmental malformation which is at the basis of neuropsychiatric and neurological diseases.

Appendix Variance $\alpha > 1$ in the discreet case

We estimate in the appendix, the total fluctuation $FG^2$ defined by expression (16) when $\alpha > 1$. The flux $\varphi$ is given for $k = 1, \ldots, N$ by $\varphi_k = \varphi_0(1 + \varepsilon \sigma_k)$, where $\sigma_k$ are defined
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Table A.1 Table of parameters. We used normalized values. The variables $K_A$, $\varphi$, $\gamma$ and $\gamma_t$ are associated with synthesis rate and two values of the order of several hours. $\alpha$ represents the decay rate of the gradient

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>1</td>
</tr>
<tr>
<td>$K$</td>
<td>1</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>$10^{-4}$ s$^{-1}$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>$10^{-4}$ s$^{-1}$</td>
</tr>
<tr>
<td>$\gamma_t$</td>
<td>$5 \times 10^{-6}$ s$^{-1}$</td>
</tr>
<tr>
<td>$K_A$</td>
<td>$1.05 \times 10^{-4}$</td>
</tr>
<tr>
<td>$X_A$</td>
<td>$1/2$</td>
</tr>
</tbody>
</table>

in Section 3. The recurrent relation gives that

$$A_{k+1} = \frac{\alpha A_k (1 + \varepsilon \sigma_k)}{1 + \tilde{K} \varphi(0) \gamma A_k (1 + \varepsilon \sigma_k)} \quad (A.1)$$

where $\alpha = \tilde{K} K_A \varphi(0) / (\gamma + \gamma_t)$. Because the convergence to the steady state is exponentially fast, after few cells, the concentration of $A$ will be close to steady state and

$$A_s = (\alpha - 1) \frac{\gamma}{\tilde{K} \varphi(0)} \quad (A.2)$$

Then

$$A_{k+1} - A_s = \frac{A_k (1 + \varepsilon \sigma_k) - A_s}{1 + (\alpha - 1) \frac{A_k}{A_s} (1 + \varepsilon \sigma_k)} \quad (A.3)$$

or equivalently,

$$A_{k+1} - A_s = (A_s \varepsilon \sigma_k + A_k - A_s) \alpha^{-1} + O(\varepsilon^2) \quad (A.4)$$

Using that

$$\langle |A_{k+1} - A_s|^2 \rangle = A_s^2 \varepsilon^2 E(\sigma_k^2) + \langle |A_k - A_s|^2 \rangle \alpha^{-2} \quad (A.5)$$

in the steady state limit, we obtain

$$V(A) = \langle |A_\infty - A_s|^2 \rangle = \frac{A_s^2 \varepsilon^2 E(\sigma^2)}{\alpha^2 - 1} \quad (A.6)$$

Defining the total variance fluctuation by

$$TVF^2(N) = \sum_{k=1}^{N} \langle |A_k - A_s|^2 \rangle \quad (A.7)$$
we obtain the leading order term

\[ TVF^2(N) = \frac{A^2 \varepsilon^2 \sigma N}{\alpha^2 - 1}, \]

(A.8)

where \( E(\sigma^2) = \sigma \).

References


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