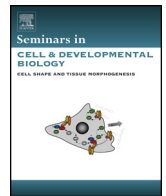




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Review

Computational and mathematical methods for morphogenetic gradient analysis, boundary formation and axonal targeting

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ABSTRACT

Morphogenesis and axonal targeting are key processes during development that depend on complex interactions at molecular, cellular and tissue level. Mathematical modeling is essential to bridge this multi-scale gap in order to understand how the emergence of large structures is controlled at molecular level by interactions between various signaling pathways. We summarize mathematical modeling and computational methods for time evolution and precision of morphogenetic gradient formation. We discuss tissue patterning and the formation of borders between regions labeled by different morphogens. Finally, we review models and algorithms that reveal the interplay between morphogenetic gradients and patterned activity for axonal pathfinding and the generation of the retinotopic map in the visual system.

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1. Introduction

Physical modeling, mathematical analysis and simulations are currently converging to better understand basic biological questions such as morphogenetic gradients genesis and axonal targeting. Morphogenesis depends on the formation of gradients that are necessary to pattern a tissue. For example, the French flag metaphor by L. Wolpert [1] proposed that cells choose to belong to a given territory depending on their location in a morphogenetic gradient. How do cells sense the local concentration and the direction of the gradient? How do they convert this information into a cell fate using differentiation pathways? What defines the precision of the boundary formation between territories?

The formation of a morphogenetic gradient depends on a source where proteins are synthesized, a transport mechanism (e.g. diffusion [2] or active transport) and a sink or degradation process [3]. Whereas monotonically increasing or decreasing gradients can be generated by a single morphogen, two or more interacting morphogens give rise to a large variety of concentration profiles [3]. Moreover, multiple interacting morphogens increase the sensibility to initial conditions and amplify fluctuations. These properties have already been reported by Turing [4], and haven been subsequently extended and generalized by Meinhardt and collaborators [5–8].

In the first part, we review computational methods related to the formation and read out of morphogenetic gradients, and the precision and stability of boundaries generated between morphogenetic regions. Gradients are well studied in a syncytium where the absence of membranes between nuclei permits the use of diffusion theory [9]. However, this is not the case in organized tissue, where all cells participate and at the same time “sense” the local concentration. We further review methods to analyze how a cell perceives an ambient concentration based on the steady flux of morphogens to binding sites [10]. Transforming a bound number of molecules at steady state into a cell fate may involve many molecular steps to guarantee multiple controls, such as regulatory loops to amplify a small signal.

The formation and read-out of protein gradients is not only essential for morphogenesis and tissue patterning, but also plays a fundamental role for axonal pathfinding and the formation of neuronal networks in the brain. During neuronal development, axons migrate from the region where they are born to specific target regions, thereby generating long range connections leading to brain wiring and functional maps between two brain regions [11,12]. A topographic or continuous map is generated when neighboring neurons in the area of origin connect to neighboring neurons in the target area. The formation of such a continuous map depends crucially on the existence of guidance proteins that are distributed in gradients in both areas. Gradients are necessary to label and survey the areas, and to guide axons to their correct target site. One of the best studied topographic map is the retinotopic map of the visual system that connects the retina to the visual centers in the brain (Fig. 3A). During the development of the visual system, retinal ganglion cells (RGCs) connect to the midbrain where they form synaptic connections with neurons in the superior colliculus (SC) in mammals and optic tectum (OT) in fish, amphibians

and chicks. In mammals, RGC additionally project to the lateral geniculate nucleus (LGN) of the thalamus. When RGCs have reached the tectum, they form ordered connections with tectal neurons thereby generating a retina–tectum topographic map, which is important to preserve the visual information projected onto the retina. Similar to morphogenesis, guidance molecule gradients in the retina and the SC or OT play a crucial role in the map formation process. What are the requirements on the gradients to establish a precise map? How many gradients are needed? It is found that gradients alone are not sufficient to generate a precise map, and additional patterned activity generates correlations and interactions between axons and target neurons that refines the map. In the last section we review theoretical approaches to describe the formation of the retinotopic map. The effective models strongly rely on pre-established gradients, and it remains challenging to understand how the formation, read-out, precision and interaction between morphogenic gradients and activity affect the properties of the final map.

2. Morphogenetic gradients

2.1. A minimal model for gradient formation

The minimal physical requirement for morphogenetic gradient formation is morphogen synthesis, transport and degradation. In one dimensional homogenized ensemble, in the absence of unpenetrated membranes, such as a syncytium (i.e. fly embryos or muscle fibers), the morphogen concentration $c(x, t)$ at position x and time t is modeled by

$$\frac{\partial c(x, t)}{\partial t} = D \frac{\partial^2 c(x, t)}{\partial x^2} - \frac{\partial}{\partial x} [c(x, t) b_0(x)] - k(x) c(x, t), \quad (1)$$

where $k(x)$ represents degradation and $b_0(x)$ active transport, for example induced by cilia [13]. When transport is only due to diffusion we have $b_0(x) = 0$. Degradation can occur due to an enzyme, receptor-mediated endocytosis [14], or dispersion of morphogens into the tissue. Depending on the boundary conditions, various types of gradient shapes can be obtained from Eq. (1) [3,15]. Steep gradients allow patterning of a small territory. Shallow gradients can be used to pattern large regions where the concentration remains almost constant and decays steeply towards the end of region due to additional nonlinear mechanisms (see [16]). With constant degradation rate $k(x) = k$ and $b_0 = 0$, the steady state solution of Eq. (1) decays exponentially

$$c(x) = c_0 e^{-\lambda x}, \quad (2)$$

where $\lambda = \sqrt{k/D}$ and c_0 is the imposed concentration at $x = 0$. For cells imbedded in a living tissue, the formation of a morphogenetic gradient requires additional mechanisms [1,4,5,17–19] for receptor–ligand interactions.

In multicellular systems, secreted morphogens and cell surface receptors [20] have been modeled by coupling Eq. (1) to other reaction–diffusion equations [21]. To better understand early patterning, we proposed [22,23] that during gradient formation, morphogens that are transcription factors are internalized

by cells, inducing their own irreversible synthesis and thereby “infecting” or labeling cells. In this process cells become labeled, cell 1 infects cell 2, cell 2 infects cell 3, etc, thereby generating a group of cells that defines a morphogenetic domain (Fig. 1A). In addition, feedback loops ensure that the labeling is permanent [24].

A conceptual difficulty in this labeling process of initially identical cells is the capability to generate a gradient: how morphogens expressed in the infectivity process described above can produce a graded expression? After induction, the morphogen production should converge to the same steady state in all cell, as they are identical. To resolve this difficulty, additional mechanisms are needed, such as a reduction in morphogen efficiency, or transport of morphogens, or degradation. These additional effects create a decreasing concentration and/or production of morphogens [23]. In an asymmetric propagation model (propagating from the left to the right in one dimensional domain)[23], an intracellular endocytosed morphogen A (which can also be a secondary messenger pathway due to binding of receptors) needs to be distinguished from an external morphogens A and the model formulation along a finite number of cells for $k \geq 1$ is

$$\frac{dA_k}{dt} = \beta \frac{\tilde{K}A_k^*}{1 + \tilde{K}A_k^*} - \gamma_1 A_k \text{ and } \frac{dA_k^*}{dt} = \varphi A_{k-1} - \gamma_2 A_k^*, \quad (3)$$

where γ_1, γ_2 are the parameters for degradation, φ is the flux from the left, and $\beta, \tilde{K}$ are the Michealis–Menten production rate, finally, introducing the decay factor

$$\alpha = \frac{\beta \tilde{K} \varphi}{\gamma_1 \gamma_2}, \quad (4)$$

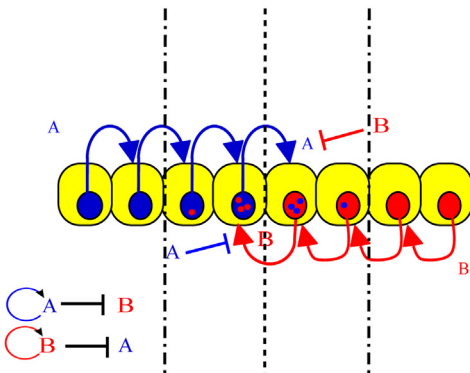
the steady state concentration of morphogens decays either exponentially or algebraically [23]

$$A_k = B_1 \frac{(1 - \alpha)\alpha^k}{B_2(1 - \alpha) + 1 - \alpha^k}, \text{ for } \alpha \neq 1 \quad (5)$$

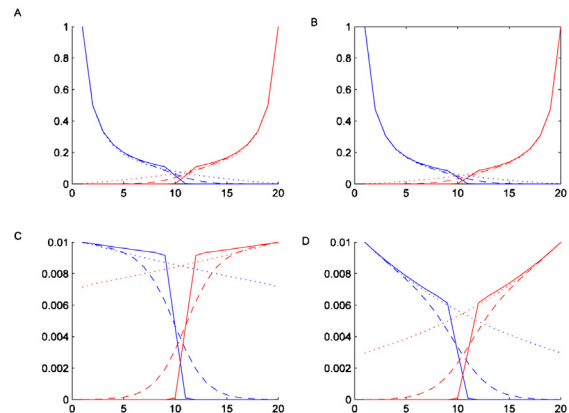
$$A_k = B_1 \frac{1}{B_3 + k}, \text{ for } \alpha = 1. \quad (6)$$

Long range gradients can be obtained when $\alpha > 1$ [23]: the rich variety of solutions of reaction–diffusion equations allows generating very different types of gradients [4], for example, short or long range, stable or unstable. What are the role of short versus long range gradient? It might be possible that initial patterning requires steep gradients across few cells only, while, a secondary patterning generated when cells are already organized, requires longer gradients.

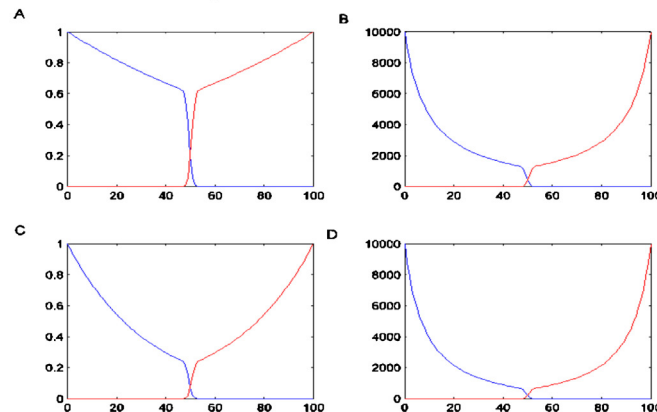
A Morphogenetics Border between two opposite gradients



B Modeling boundary formation



C Boundary formation with dimers



D Gradient smoothing during cell division

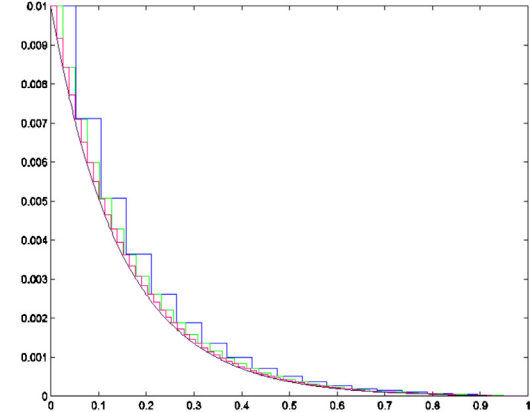


Fig. 1. Boundary between morphogenetic gradients. (A) Schematic representation of two opposite gradients. The boundary is formed at the intersection where morphogen A and B inhibit each other. (B) Modeling boundary formation using Eqs. (11) and (12): In A and C (magnification): $\alpha = 1$, while in B and D (magnification) $\alpha = 0.95$. Parameter β was attributed three values, $\beta = 0$ (dotted lines) $\beta = 0.01$ (dash continuous lines) and $\beta = 0.1$ (continuous lines). A and C (resp. B and D) differ by the initial concentration X_0 . Sharper gradients are formed for the largest value of β [23]. (C) Effect of the dimer formation on gradient position: sharper gradients are formed. (D) Cell division leads to gradient smoothing. After each division, the local cell concentration results from averaging with the ones of the neighbors [16].

2.2. Cooperativity between morphogens and gradient shape

We now give an example of how cooperativity leads to a sharp gradient. When two copies of morphogen A^* can form a dimer or two promoter sites are driven by a cooperativity mechanism (closely located at the same transcriptional regulator) or the production of morphogen A becomes efficient when two A^* are bound to the promoter site, the morphogen equation for a unidirectional flux (see Eq. (3)) can be extended by adding the cooperativity in the Michaelis–Menten term:

$$\frac{dA_k}{dt} = \beta \frac{\tilde{K}A_k^* + \tilde{K}_2A_k^{*2}}{1 + \tilde{K}A_k^* + \tilde{K}_2A_k^{*2}} - \gamma_1 A_k, \quad \frac{dA_k^*}{dt} = \varphi A_{k-1} - \gamma_2 A_k^*. \quad (7)$$

As a result, morphogen cooperativity leads to very steep gradients and sharp boundaries [16,25], as shown in Fig. 1C) and allows generating different types of gradients (steep or shallow). Direct cooperativity has also been proposed to enlarge gradients [14,26], so that regions of different sizes can be labeled. Eq. (3) can also be used to model the coupling between mRNA and protein production, where the protein is a morphogen that activates the gene. The production rate is proportional to the probability of finding the protein on the gene with a single site, see also [25] for a mRNA-protein example.

2.3. Effect of on-exponential gradients

For a nonlinear degradation rate $k(x) = k_1(x)c(x, t)^{n-1}$, $n > 1$ of morphogens, steady state gradients decay algebraically, more rapidly close to the source, much slower far away. The case $n=2$ was considered initially in [14]. The generic profile, solution of Eq. (1) is

$$c(x) = \left(\frac{A_n}{(a+x)^2} \right)^{1/(n-1)}, \quad (8)$$

where a and A_n are constants. This long tail decay generates robust morphogen gradients that can account for long range adaptation in tissue size [14,26], see also [27,28]. Another scenario to generate long range gradient was proposed in [29] based on two interacting ligands: a morphogen and a diffusible molecule, called “the expander”, the role of which is to expand the morphogen gradient, either by facilitating diffusion or inhibiting its degradation. In this model, morphogens repress expander production or secretion [14,26].

2.4. Maintaining a gradient during cell division

It is not clear how the morphogen concentration is changed or maintained during cell divisions. Are daughter cells producing the same amount of morphogens as mother cells? When the number of cells is doubled, we expect the gradient to get smoother. This effect is modeled using the bidirectional fluxes model at each cell [16]

$$\frac{dA_k}{dt} = \beta \frac{\tilde{K}A_k^*}{1 + \tilde{K}A_k^*} - \gamma_1 A_k, \quad \frac{dA_k^*}{dt} = \frac{\varphi}{2}(A_{k-1} + A_{k+1}) - \gamma_2 A_k^*. \quad (9)$$

The redistribution of morphogen during cell division is computed from Eq. (9). At steady state [16], the morphogenetic gradient concentration is smoothed out and following each iteration, the width of the cell is divided by two (no growth of the tissue), while the total number is doubled. The steady state gradient becomes smooth as predicted by classical diffusion–reaction equation [15]. An example of iteration procedure starting with 10 cells is illustrated in Fig. 1D. Gaining in accuracy during cell division has also been reported recently for a different mechanism in the *Drosophila* wing imaginal disc, that correlates with the temporal increase in

Dpp signaling. Recent models consider the case where the length L can vary [30] in *Drosophila*, but to explore the difference between the tissue growth and gradient time scale, new computations are needed. In particular, when the number of cells double, does their size double at the same time? In vertebrates, where all cells participate to gradient formation, the effect of cell division is associated with tissue growth, that should also be modeled. Finally, the precise molecular mechanisms that a cell is using to sample the concentration coming from a bidirectional flux remains unclear. It might be conceivable that local averaging is a mechanism employed by the cell to sample the local concentration.

2.5. Time-dependent gradient formation

Has a morphogenetic gradient the time to reach steady state (with less than a few percent), before cells start to differentiate? Non-stationary gradients can influence cell fate. For example, the duration of exposure of Shh is essential to label cells into motor neurons, longer exposure leads to a different fate (see review [31]). Once a gradient is generated, during its deterministic evolution to steady state, it can also determine the cell fate. This is the case for Shh patterning of the dorso-ventral neural tube in the chick (a review dedicated to time-integration is [31]). Very few models today integrate time-dependent cell fate generation and examine the consequences of transient time-dependent gradients on cell fate and differentiation. In reaction–diffusion models, the time-dependent gradient is studied by solving numerically the reaction–diffusion equations [4,5,15,21] (see also [32]). Recently, to analyze the time-dependent profile, an explicit one-dimensional computation has been performed with a morphogen degradation rate that is a power law of the concentration [28]. The result showed that the concentration can be expressed as $c(x, t) = C_s(x)\phi(x/\sqrt{Dt})$, where $\xi = x/\sqrt{Dt}$ and $\phi(\xi)$ is a hypergeometric confluent function, solution of

$$\xi^2 \phi_{\xi\xi} + \left(\frac{\xi^3}{2} - \frac{4\xi}{n-1} \right) \phi_{\xi} + \frac{2(n+1)}{(n-1)^2} \phi(1 - \phi^{n-1}) = 0. \quad (10)$$

For $n=2$ (cooperativity of degradation with two morphogens) we have the approximation $\phi(\xi) = 4000 + \xi^9/4000 + 5\xi^6 e^{\xi^2/4}$. Thus, time-dependent profiles are obtained from the steady-state one ϕ [28]. Similar to classical front-propagation [33], in this model of local interaction for degradation, gradient formation shows a front of propagation. This model opens the possibility to get new experimental characterization time-dependent gradient.

3. Boundary between morphogenetic gradients

During brain development, genetically labeled areas define functional domains [34]. To delimit such areas, a boundary has to be generated between them. Morphogens with auto-activating and reciprocal-inhibiting activities propagating in opposite directions and competing for cell labeling lead to boundary formation (Fig. 1) [35–38]. Keeping a stable position seems crucial especially during brain development [39,40], but physical rules that define the boundary between two regions are still missing. We review models that quantify the precision of the boundary and how it varies with respect to parameters, such as morphogen concentration fluctuations or initial gradient concentration.

3.1. Boundary induced by successive morphogen gradients

In flies, the successive formation of gradients can generate sharp boundary. Indeed, the sharp Hunchback (hb) boundary in the *Drosophila* embryo is formed after a broad Bicoid (bcd) morphogen gradient. Once the bcd gradient is generated [41], hb transcription

is activated when several bcd and hb are bound at the same time to the promoter site [42–44]. This nonlinear cooperativity effects creates a bistability regime, which is at the origin of the sharp hb gradient [42,45] (see also the discussion below). Thus, the hb profile is generated with a sharp transition region along the dorso-ventral axis.

3.2. Boundary formation using opposing morphogen gradients

The boundary between two regions can also be generated at the intersection of two morphogen gradients A and B, where the two morphogens inhibit each others. The ensemble of cells labeled by A then belongs to the A-territory, and cannot anymore be labelled as a B-cell. For example, during brain development, the competition between Otx2 and Gbx2 morphogens is responsible for the position of the Mid/Hindbrain (MHB) (see Fig. 2C) [37,46,47]. To account for this boundary formation, it is necessary to use self-activating and reciprocal inhibiting properties of A and B (Fig. 1A) [23]: a cell at the boundary between two regions receives two different morphogens, but will express preferentially one of them. Taking a reaction–diffusion equation for the propagation of identical morphogens A and B, to model the boundary formation, additional terms in the equation are needed to model the interactions between the two factors. This interaction is modeled at two places: first, morphogen B can bind to the production site of A, leading to a competition and thus reducing the production of A; second, a non exclusive possibility is that morphogen A directly binds B, leading to the formation of an inactive A*B* dimer or a degradation. The fraction of A destroyed by B is proportional to the number of A and B that is $[A][B]$. For example, based on Eq. (3), the interaction is modeled by (see notations of the previous paragraph):

$$\frac{dA_k}{dt} = K_A \frac{\tilde{K}A_k^*}{1 + \tilde{K}B_k^* + \tilde{K}A_k^*} - (\gamma + \gamma_t)A_k, \quad \frac{dA_k^*}{dt} = \varphi A_{k-1} - \gamma A_k^* - \beta A_k^* B_k^* \quad (11)$$

and

$$\frac{dB_k}{dt} = K_B \frac{\tilde{K}B_k^*}{1 + \tilde{K}B_k^* + \tilde{K}A_k^*} - (\gamma + \gamma_t)B_k, \quad \frac{dB_k^*}{dt} = \varphi B_{k+1} - \gamma B_k^* - \beta A_k^* B_k^*, \quad (12)$$

where β is the dimer degradation rate. Similar equations can be used for a bidirectional flux leading to classical reaction–diffusion equations (9). Fig. 1B shows boundaries between two morphogen regions. The boundaries become much sharper when morphogens form dimers (Fig. 1C). Other modeling studies [48,49] focused on variations in the boundary position based on exponential decays.

3.3. Shift in the boundary induced by gradient fluctuation

Random perturbations of a gradient can affect the precise location of the boundary between two morphogenetic regions. These fluctuations can be generated by various parameters such as the injection rate of morphogens (for example, Bicoid proteins and its co-repressor [50]), the degradation rate, the inherent change in the geometry (tortuosity) where morphogens diffuse between cells, the number of receptors on each cell or any other quantity involved in internalization or synthesis of new morphogens. Although there are many experimental reports [49,51] about the role of noise in shifting the position of the boundary, few studies have modeled the shift due to noise. Two quantities have been computed: one is the change of the concentration amplitude across the gradient domain, and the second is the variance of the boundary position. Computing the variance with respect to the random perturbations reduces

the exploration of the parameter space and allows identifying key regimes.

To compute the variance of the boundary position, a model for random fluctuations is needed. A random flux of morphogens is given by $\varphi(x) = \varphi(1 + \varepsilon\sigma(x))$, where $\sigma(x)$ is a random field of mean zero. The variance is $E(\sigma(x)\sigma(y)) = \xi^2 e^{-\lambda|x-y|}$. For a fixed steady state variance ξ , the steady state probability density function p is a centered Gaussian of variance ξ , with $Pr(\sigma \in (x, x+dx)) = 1/\sqrt{2\pi\xi} e^{-(x/2\xi)^2} dx$ [16]. When the random field σ is added on two inhibitory and opposed morphogenetic gradients u (resp. w), it generates local fluctuations in the morphogen flux and in the concentration of morphogen A (resp. B) in an ensemble of M cells. The boundary is located at a position $M/2 + \tilde{b}_\sigma$, where $\tilde{b}_\sigma$ is the shift due to the cumulative effect of the fluctuations (Fig. 2A). For two symmetric unperturbed gradients, the boundary is where the concentration of A equals the concentration of B:

$$u\left(\frac{M}{2} + b_\sigma\right) - w\left(\frac{M}{2} + b_\sigma\right) = 0. \quad (13)$$

The shift $\tilde{b}_\sigma$ can be computed by expanding the concentration u (and w) as a function of ε near the point $M/2$: $u = u_0 + \varepsilon u_1 + \dots$, where u_0 is the solution in the absence of fluctuations. Expressions for u_1 as a function of u_0 is solved in [16]. At first order in ε , $\{u'_0(M/2) - w'_0(M/2)\}b_\sigma + \varepsilon\{u_1(M/2) - w_1(M/2)\} = 0 + o(\varepsilon)$ and thus $\langle |b_\sigma|^2 \rangle = \varepsilon^2 \langle |u_1(M/2) - w_1(M/2)|^2 \rangle / (u'_0(M/2) - w'_0(M/2))^2$. For $\alpha < 1$, the mean of the shift is zero, while the variance is

$$\langle |\tilde{b}_\sigma|^2 \rangle \sim \xi^2 \varepsilon^2 \frac{1}{\lambda^2 \ln^2 \alpha} \left(\frac{M\lambda}{2} - \frac{3}{2} \right) + O(\varepsilon^3), \quad (14)$$

which consists of three terms: (1) the variance of the fluctuation and its spatial correlation, (2) the gradient decay ($\log \alpha$), and (3) the size of the region (total number of cells). 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), $\alpha = 0.95$, the variance is $\langle |\tilde{b}_\sigma|^2 \rangle \approx 1$, (about one cell out of 100).

To conclude, the shift in the boundary between morphogenetic regions is a general and inherent feature due to noise. Thus, the boundary position varies between individuals. The standard deviation depends on the square root of the number of labeled cells and the exponential decay of the gradient. Thus, fluctuations in the production rate can alter the boundary position [14]. Randomness can also serve to sharpen the boundary. Indeed, recently, a coarse-grained model for hindbrain patterning involving cross inhibition and auto-activation of the retinoic acid (RA) interacting with two transcription factors Krox20, Hoxb1a [24,52], predicted striped pattern of gene expression where fluctuations in RA concentration alone can induce a rough boundary, whereas additional noise in hoxb1a/krox20 expression sharpens the boundary.

3.4. Boundary shift induced by changing the level of gene expression

The position of the boundary between two morphogenetic regions labeled by two opposed gradient (reciprocally inhibitory) can shift significantly by changing the strength of the initial inducer (Fig. 2C). For different initial concentrations of inducers X_A and X_B , a shift in the boundary position is generated between the two morphogen regions A and B. When $X_A \neq X_B$ (all other characteristics are the same), the shift ΔL of the boundary can be computed as

$$\Delta L = \frac{1}{2} \left| \frac{1}{X_A} - \frac{1}{X_B} \right| \text{ for } \alpha = 1 \quad (15)$$

$$\Delta L = -\frac{1}{2} \frac{\log(X_B/X_A(\alpha^{-1} - 1))}{\log \alpha} \text{ for } \alpha < 1, \quad (16)$$

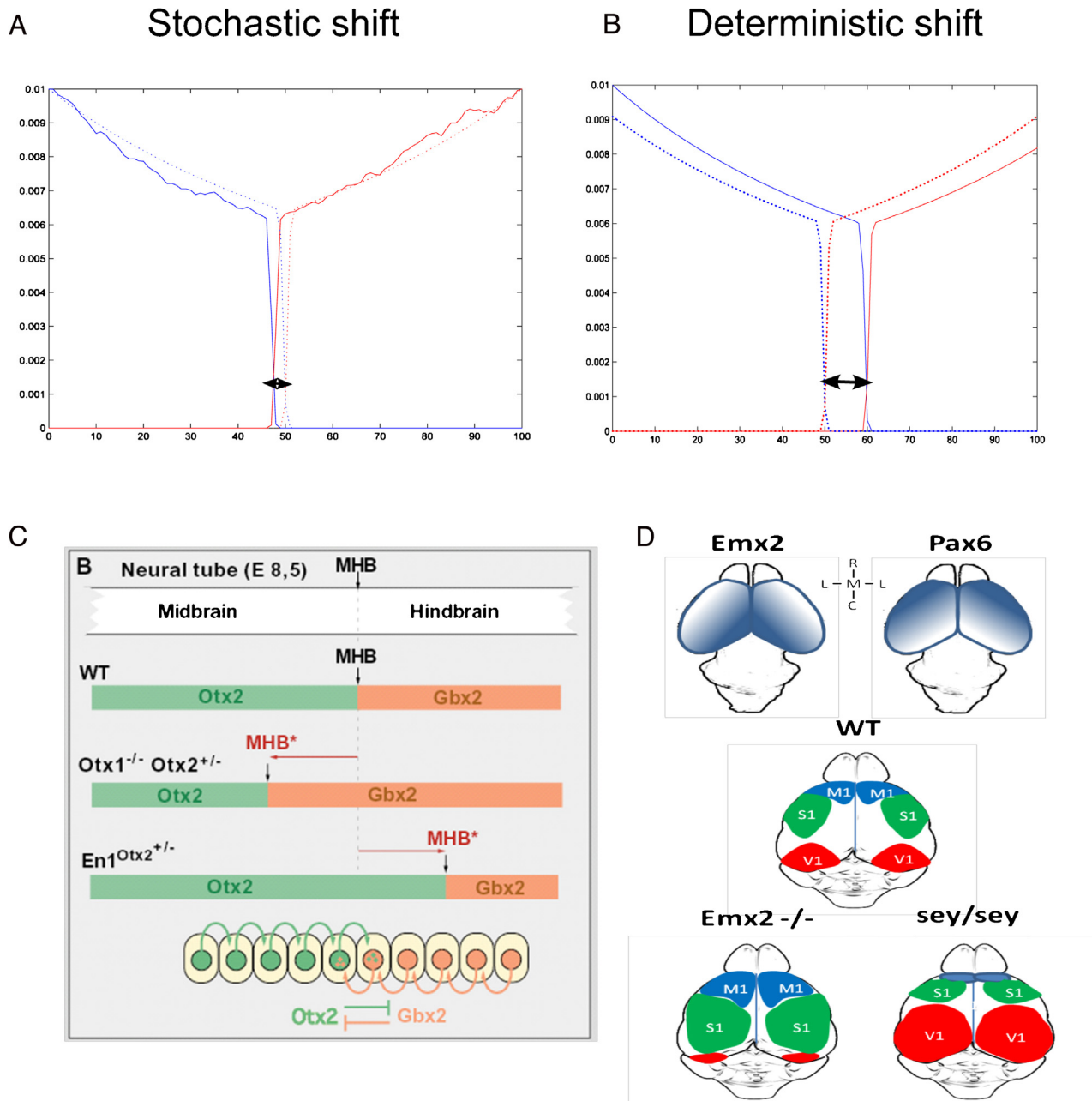


Fig. 2. Shift of the boundary between two morphogenetic regions. (A) A small random fluctuation in the flux leads to a fluctuation in the position of the boundary between two morphogenetic regions. For the simulation, 100 cells are used, with the parameters $\beta = 1$, $\varepsilon = 0.01$. The dotted lines correspond to deterministic simulation where the fluctuation term is set to zero. The continuous line shows a realization, obtained by using the amplitude $\varepsilon = 0.01$ in Eq. (16). The shift of boundary position is only of 2–3 cells. (B) Following an asymmetry between the initial concentration of A versus B, the boundary is shifted. The simulation uses $\alpha = 0.95$. A shift of 10 cells (10%) is obtained for a 20% difference in initial concentration. (C) Schematic representation of the shift in the Midbrain position, resulting from the asymmetric initial concentration of Otx2/Gbx2 [22]. (D) Illustration of the shift between morphogenetic regions in the neocortex [34]. (C) Drawing from Stettler and Moya, this issue).

where α is the gradient exponential decay rate (see Eq. (4)) [23]. The shift does not depend on the size of the morphogenetic region, but only on the difference in the initial concentrations and the decay factor α (see Fig. 2B). For an algebraic decaying gradient $\alpha = 1$ and a total of 100 cells, a twofold difference in concentration generates a shift of 25 cells. An explicit formula for the position of the midpoint was also developed for interacting auto-inhibiting morphogens [48], assuming exponential gradients. In contrast, Eq. (15) links the boundary shift to the difference in initial concentrations. Deterministic shift has been examined by modifying the

gene expression of Otx2 and Gbx2 modifying the mid/hindbrain (MHB) position (see Fig. 2C): a disequilibrium in favor of Otx2 or Gbx2 pushes the border caudally or rostrally, respectively [37]. A second example is provided by Pax6 and Emx2. Indeed, the border between the primary sensory and visual areas is regulated by the respective levels of the two proteins [34,35,46] (see Fig. 2D). An expression for the shift in the boundary position can be used to predict behavioral changes: for mice, in which Otx2 was given an advantage over Gbx2, have a higher number of DA neurons and show an hyper-locomotor phenotype [39,40,53].

4. How a cell measures the external concentration of morphogens

The ability of the cell to read-out the local characteristics of a gradient concentration is a critical step to determine the cell fate, to pattern a morphogenetic region, to generate borders between territories, for axonal guidance and synapse formation. Yet, how this read-out is implemented at a molecular level remains unclear. Several steps can be discerned: the binding of morphogen to receptors is the first step of this process. The read-out should depend ultimately on the number of promoter sites occupied by of morphogens in the genome, to generated transcription. It is however not clear how the number of promoter sites can play the role of analogue–digital converter where the input is the external gradient of morphogens, the output gives the cell identity.

In addition, changing the relative position of receptors by clustering or de-clustering on the cell surface can change the diffusion flux and thus modulate the interaction between morphogens and receptors, that can thus modulate the read-out of a gradient [54,55]. However, this modulation still does not explain how cells reads a gradient. To understand how the morphogen flux is converted into cell fate, downstream signaling should be considered. The fluctuation in the number of diffusers at an absorbing or reflecting sphere allows estimating the ratio $\delta c/c$, [56], which measures the sensitivity of detection.

4.1. Extracting positional information from a gradient

While the first step of sensing a gradient is the binding of morphogens (ligands) to receptors located on the surface of a cell, a second step requires analyzing the rate at which ligand binds receptors. Competition with degradation introduces a new time scale in this measure and the notion of time spent before degradation becomes a relevant quantity [57]. In that case, the survival concentration of free c_m or bound c_b receptors can be used to compute two local time scales characterizing the formation of the steady-state gradient concentration profile of diffusing morphogen molecules and the morphogen–receptor interaction. These time-scales are estimated analytically in one dimension [58] by solving the reaction–diffusion equations

$$\frac{\partial c_m}{\partial t} = D \frac{\partial^2 c_m}{\partial x^2} - (k_{deg} + k_{on})c_m + k_{off}c_b. \quad (17)$$

$$\frac{\partial c_b}{\partial t} = k_{on}c_m - (k_{deg} + k_{on})c_b. \quad (18)$$

The local time at position x prior to degradation is

$$\tau_m(x) = \int_0^\infty \left(1 - \frac{c_m(x, t)}{c_m(x, \infty)}\right) dt, \quad (19)$$

where $c_m(x, \infty)$ is the steady state concentration. Using the characteristic length $\lambda_e = \sqrt{D/k_{deg}^e} = \sqrt{D(k_{off} + k_e)/k_{deg}k_{off} + k_{deg}k_e + k_{on}k_e}$ the two time scales [27] are (see also [58] in higher dimensions)

$$\tau_b(x) = \tau_m(x) + \frac{1}{k_e k_{off}}, \quad \tau_m(x) = \frac{1}{2k_{deg}^e} \left(1 + \frac{x}{\lambda_e}\right) \left[1 + \frac{k_{on}k_{off}}{(k_{on} + k_e)^2}\right]. \quad (20)$$

These two time scales allow estimating the interaction between cells and morphogenetic gradients thereby providing a better understanding of the first steps in reading a morphogenetic gradient.

4.1.1. Extracting positional information from a gradient requires to memorize binding events

While the first step of sensing a gradient direction involves binding of ligands to receptors, it is unclear how the direction is evaluated. Possibly receptors in one side are more frequently activated than the other side. Assuming that this is the case, then in the next step, a second messenger pathway should preserve this directionality. For example, this pathway cannot be carried out by diffusible molecules that would immediately lose the memory of the initial position. Thus some precise mechanisms should keep track of the historical directional binding events. One possibility is to break locally the symmetry, for example by a fast redistribution of morphogen spatial indicators, which can be surface receptors or internal second messengers. Such strategy is used in neurite turning assay, induced by GABA neurotransmitters: receptors accumulate in the direction of the gradient, following a nonlinear mechanism involving actin dynamics [59]. Similar molecular mechanisms are expected to be found in searching axons, where historical relay are implemented in molecular terms in order to communicate the direction of a gradient at a subcellular level. Changing direction can be used as a local read-out of the spatial gradient [3,15,24].

4.1.2. Converting a morphogenetic gradient into gene activation

Although the initial step of reading a gradient have not been elucidated, the ultimate goal is to differentiate according to the specific location. For a cell embedded in a steady state gradient, the number of occupied transcription sites in the nucleus should depend on the morphogen flux and thus on the cell position. In the nucleus, several mechanisms are controlling the number of bound TFs, such as the degradation rate, the rate of attachment, possible competitive ligands, the diffusion coefficient and the search time for promoter sites [60–66]. In the absence of any positive or negative feedback look, the probability $\mathbb{P}r_k(x|n_s)$ that k sites are occupied when there are n_s possible binding sites at a position x is [45] for $1 \leq k \leq n_s$,

$$\mathbb{P}r_k(x|n_s) = \sum_{n_f=k}^{\infty} \frac{\alpha^{n_f}(x)}{n_f!} e^{-\alpha(x)} \frac{1/\beta^k k! \prod_{j=0}^{k-1} (n_f - j)(n_s - j)}{1 + \sum_{l=1}^{\min(n_f, n_s)} 1/\beta^l l! \prod_{j=0}^{l-1} (n_f - j)(n_s - j)}, \quad (21)$$

where $\alpha(x) = \lambda(x)/K$ is the ratio of the Poissonian arrival rate $\lambda(x)$ of morphogens to the cell nucleus (which depends on the location x and is proportional to the steady state concentration $c(x)$, $\lambda(x) = \Lambda c(x)$) to the degradation rate K . The parameter $\beta = \bar{T}_s/\bar{T}_b$ is the ratio of morphogen search of the bound time with the promoter site. The probability $\mathbb{P}r_k(x|n_s)$ gives an estimate for the number of promoter sited activated by morphogens.

Interestingly, the presence of two binding sites is sufficient to generate a genetic switch, which can be analyzed by the probability $\mathbb{P}r_k(x|n_s)$: at steady state, the production is balanced by degradation, thus for a morphogen efficacy production rate r , the steady state production is $\lambda = r \mathbb{P}r_k(x|n_s)$, where x is the cell position and $K > 0$. Bistability appears when this equation has two stable solutions (and one unstable in between).

For example, in the fly embryo, the gene Hunchback (hb) is transcribed at a rate r when there are two hb or at least one Bicoid (bcd) bound to the promoter sites. Thus the steady state production of hb is $\lambda = r(1 - Pr)$, where Pr is the probability that hb is not transcribed and is given by

$$Pr = \mathbb{P}_{0,b}(1 - \mathbb{P}_{2,h}). \quad (22)$$

where $\mathbb{P}_{0,b}$ is the probability that no bcd are bound to the promoter and $1 - \mathbb{P}_{2,h}$ the probability that there are no two hb bound. The probability is connected to the gradient concentration by

$$\mathbb{P}_{0,b}(x) = e^{-\alpha_b(x)} \left(1 + \frac{\beta_b(x)\alpha_b(x)}{6}\right) \quad (23)$$

and the probability $\mathbb{P}_{2,h}$ with $k = n_s = 2$, is

$$1 - \mathbb{P}_{2,h} = e^{-\alpha_h(x)} + \alpha_h(x)e^{-\alpha_h(x)}. \quad (24)$$

The profile of the hb gradient $\alpha_h(x)$ can be deduced from $\alpha_b(x)$ [45]. For some value of $\alpha_b(x)$, a bistability occurs and cells located right to a point x_c switch from a low to a high response to a smooth input signal. Thus for an auto-regulated hb, there is a sharp boundary of hb in the embryo and this boundary does not require any other mechanism, such as a repressor. To conclude, the conversion of a morphogenetic gradient [25], into gene activation depends on their local interaction. It involves computing the probability that k -sites of a specific gene are activated while other sites are not bound. The combinatoric can be expressed in terms of the external concentration. However when few morphogens are involved, to convert efficiently a few number of morphogens into a full expression, feedback loop mechanisms are needed and serve to amplify the small initial signal [52,67].

To conclude, there are many steps where fluctuations affect gene expression, starting from a noisy morphogen gradient with local morphogen concentration fluctuations, variations in the number of promoter activated, and in the entire regulatory mechanisms that can involved feedback loops. Although the variance of fluctuations should be additive in linear systems, the presence of multiple feedback loops leads to nonlinear dynamics and thus to various nonintuitive responses to a noisy input signal. Each different architecture of a feedback circuit leads to different response that should be studied separately [68].

5. Axonal targeting and retinotopic map formation

In this section we discuss computational approaches to understand retinotopic map formation in the tectum (to simplify the notation, we refer to SC and OT as tectum). We do not consider the axonal migration process from the retina to the tectum, see for example [69]). After RGC axons reach the tectum, a complex process starts that lasts for several days and eventually leads to the formation of a topographic map between the retina and the tectum (Fig. 3C). This process depends strongly on guidance molecules that are distributed in gradients in the tectum and the retina, trans and cis interactions between axons and tectal neurons, and correlated activity in the retina (see also [47,70–74]). A general view is that activity independent mechanisms based on guidance molecules establish an initial map that is further refined by activity dependent processes.

In his chemoaffinity hypothesis (formulated in 1963) Sperry first realized that protein gradients in the retina and the tectum can encode for positional information and guide axons [75]. Similar to Turing whose work on morphogenetic gradients pioneered the field of morphogenesis, Sperry's proposition pioneered the mathematical modeling of retinotopic map formation. Around 30 years later, Sperry's hypothesis has been validated by the identification of two transmembrane families of guidance proteins that are distributed in gradients in the retina and the tectum [76–78]: EphA and EphB receptors together with their ephrin-A and ephrin-B ligands. Eph receptors and ephrin ligands are co-expressed by RGC and tectal neurons and play a fundamental role in retinotopic map formation [79–83]. The EphA/ephrin-A family is necessary to map the nasal–temporal (N–T) axis of the retina onto the anterior–posterior (A–P) axis of the tectum, while EphB/ephrin-B family serves for the mapping of the dorsal–ventral (D–V) axis onto the medial–lateral (M–L) axis, thereby generating an orthogonal frame of coordinates (Fig. 3A). Trans-interactions between Eph receptors and ephrin ligands between two cells gives rise to bidirectional signaling in which a response is generated in both cells, and not only in cell expressing the receptor (Fig. 3B). The classical forward signaling refers to

Eph receptors whereas reverse signaling to ephrin ligands signaling into their host cell. Ephrin-A reverse signaling additionally requires p75-neurotrophin receptors [84]). Because ligands and receptors are co-expressed by the same cell, cis-interactions also affect map formation [85,86]. Thus, the Eph/ephrin signaling system provides a rich basis for the formation of a topographic map.

Sperry assumed that RGC axons stop when they encounter a critical protein level depending on their anchoring position in the retina (Fig. 4A). However, experiments in which the retina and/or tectum had been surgically manipulated revealed that even under such distorted conditions a correctly ordered map is formed (for review see [74]). This suggested that the target position cannot be encoded by pre-defined levels of guidance proteins. To obtain a flexible mapping process, additional axon–axon interactions and competition between axons have been introduced, later on confirmed by genetic manipulations of the expression levels of EphA receptors and ephrin-A ligands [87–89]. In mouse knock-in experiments, Brown et al. [87] generated two populations of RGC neurons with different EphA receptor levels that were randomly mixed in the retina. In the tectum, these two intermingled populations became partitioned and generated two separate maps. This suggested that map formation depends on axonal competition for tectal space and on relative but not absolute levels of EphA receptors. Finally, map formation depends on correlated electrical activity in the retina [90–95].

5.1. Computational models of retinotopic map formation

Modeling retinotopic map formation started in the 1970s at a time when many of the molecular details were still unknown (for modeling reviews see [96–99]). However, already these early phenomenological models introduced many key concepts governing map formation, such as guidance molecules distributed in gradients, axon–tectal interactions, axon–axon interactions, axonal competition, axonal branching and modulation of synaptic strength. Experimental discoveries during the past 20 years brought the molecular ground for many of those concepts. However, because map formation is a multi-component process that occurs over several days and depends on interactions between neurons (Fig. 3C), it remains challenging to quantitatively understand map formation from first physical principles. We now give an overview over computational models and present main ideas.

5.1.1. Models independent of tectal gradients

To obtain a flexible mapping, von der Malsburg and Willshaw conceived an algorithm that does not depend on pre-established protein gradients in the tectum [100]. In their “marker induction model” markers are distributed in gradients only in the retina, and the tectum is initially devoid of markers. RGC axons that innervate the tectum tend to induce their marker level into tectal neurons via feedback mechanisms based on the formation and modulation of synaptic connections. The optimal target is the tectal neuron that eventually has the same marker level. Although it is known today that preestablished gradients exist in the tectum, this work introduced many important concepts, such as axonal competition, axon–axon interactions, axonal branching and modulation of synaptic strength.

5.1.2. Models based on interchange

Starting from a random position in a lattice tectum, an ordered map is generated by implementing algorithms where neighboring axons are compared and interchanged depending on their retinal position. The arrow model of Hope [101] is a sorting algorithm that does not need tectal gradients of guidance proteins, however, global directional information is necessary (anterior vs posterior and medial vs lateral). Beside interchange, the model also allows to

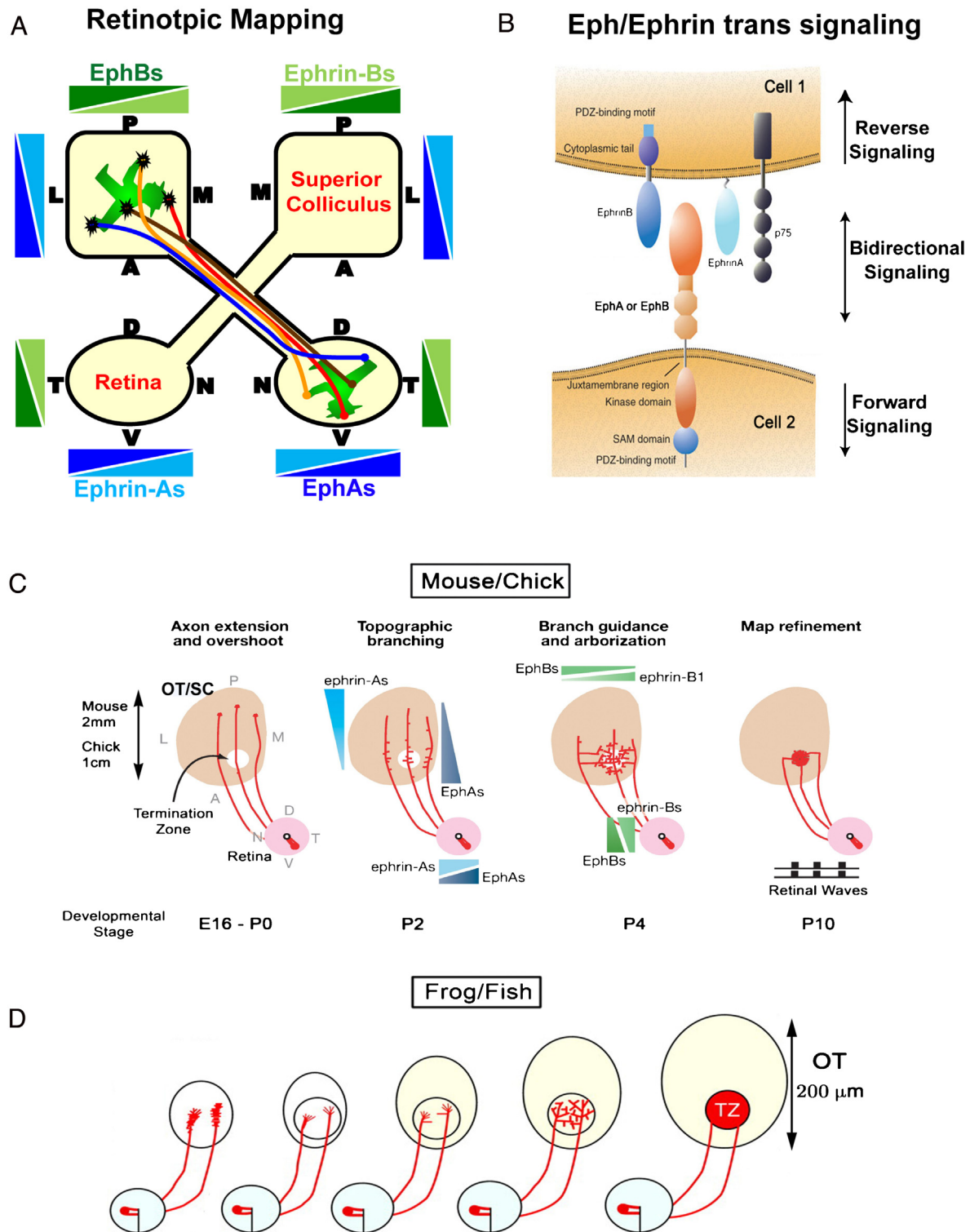


Fig. 3. Properties of the topographic projection from the retina to the SC/OT. (A) The mapping of the N–T (D–V) axis onto the A–P (M–L) axis is controlled by the family of EphA/ephrinA (EphB/ephrinB) receptor/ligand proteins that are expressed in gradients in the retina and SC/OT. RGC from regions with high EphA project to regions with low ephrin-A (repulsive interaction), whereas it is opposite for EphB/ephrinB (attractive interaction). Additional guidance proteins like Ryk, Wnt, Semaphorins, Plexins, Neuropilins or Engrailed are not depicted. (B) Schematic that illustrate the concept of bidirectional signaling between two cells (trans-interactions). Cis-interactions (receptor/ligand interactions on the same cell) are not depicted. EphrinA reverse signaling additionally requires the p75 neurotrophin receptor. (C) Development of the retinotopic map in mouse and chick via overshooting and interstitial branching. (D) In fish and amphibians the RGC target more directly their correct TZ (Panel (A) is adapted from [97], (B) from [81], (C) and (D) from [47,71].).

switch to free neighboring tectal sites in order to reproduce map expansion experiments. A stochastic version of the Hope model was introduced by Koulakov and Tsigankov to explain map formation along the tectal A–P axis [102]. The model is based on graded

distribution of EphA receptors in the retina and ephrinA ligands in the tectum, and EphA/ephrin-A forward signaling that is repulsive [88]. The exchange probability is reduced compared to the default value 0.5 if two neighboring axons are correctly ordered (the axon

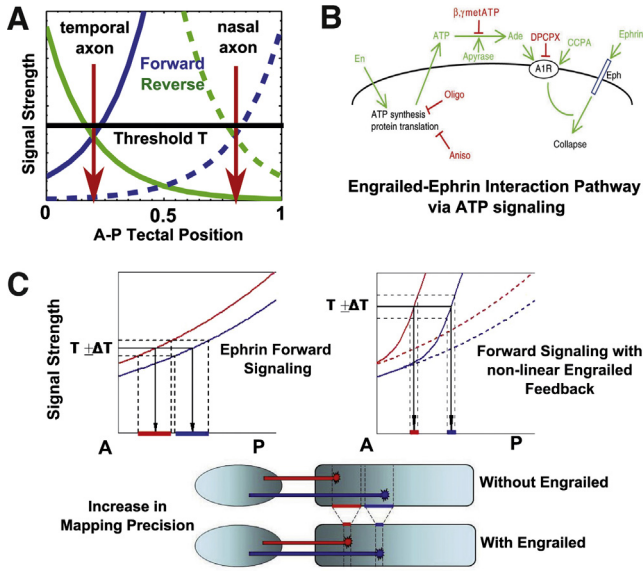


Fig. 4. Eph/ephrin signaling. (A) Ephrin forward S_{fwd} and reverse signal S_{rev} in a RGC axon generated by interactions with tectal cells. With exponential gradients in the retina and tectum and a signal $S \sim [R] \times [L]$, where $[L]$ and $[R]$ are ligand and receptor concentrations, we obtain: $S_{fwd} \sim e^{\alpha_{ret}x_0 + \beta_{tec}y}$ and $S_{rev} \sim e^{-\alpha_{tec}y - \beta_{ret}x_0}$, where x_0 is the retinal and y the tectal position. Assuming that axons have a tendency to move and stop if S_{fwd} overcomes a threshold value $T \sim e^{\kappa_{appa}}$, the stop positions generate a linear map between the retina and tectum, see Eq. (25) (with S_{rev} one has to assume that axons tend to stop but are pushed forward by the reverse signal). The combined signal $S_{fwd} + S_{rev}$ generates a parabolic potential around the TZ that can be applied to restrict branching. (B) The signaling pathway of Engrailed affecting growth cone collapse: Green, Engrailed pathway for growth cone collapse, and red, inhibition of growth cone collapse. (C) Non-linear engrailed feedback generates a signal strength that increases steeply in the region around the TZ, thereby reducing the variance in the stop position (see [126] for more information). (Panel (B) and (C) are adapted from [126]).

with lower receptor level occupies the site with higher ligand density), if not the probability is increased. The algorithm reproduces the experimental map duplication results of Brown et al. [87].

5.1.3. Models based on directed motion

The servomechanism model of Honda [103] is based on the graded distribution of receptors $R(x)$ in the retina and ligands $L(y)$ in the tectum. Their interaction generates a signal $S(x, y) = R(x)L(y)$. Axons carry a receptor level $R(x_0)$ depending on their origin x_0 in the retina, and they stop at tectal position y_0 where $S_0 = R(x_0)L(y_0)$. In general, axons are moving into the direction that minimizes the quantity $|S(x_0, y) - S_0|$. At each step, axons explore neighboring tectal positions by computing $S(x_0, y)$, and then move with a certain probability towards the new position y' that minimizes $|S(x_0, y') - S_0|$. The model does not include interactions between axons. As a novel feature, Honda used the model to reproduce stripe assay experiments. The model was generalized by adding a tendency of axons to avoid crowded sites, which represents axonal competition for target space [104].

To illustrate the scenario conceived by Honda, we give a simple example based on ephrinA forward signaling and experimental results suggesting that the response of RGC to ephrinA is concentration-dependent and switches from promotion to repulsion [105,106]. With exponential gradients, $S(x_0, y) = R_{max}e^{\alpha(x_0-1)}L_{max}e^{-\beta y}$ (scaled coordinates $0 \leq x_0, y \leq 1$), and using $S_0 = R_{max}L_{max}e^{-\kappa}$, the stop positions generates a linear map as observed experimentally (see also Fig. 4A)

$$y(x_0) = \frac{\kappa}{\beta} + \frac{\alpha}{\beta}(x_0 - 1). \quad (25)$$

Based on the servomechanism model, Gebhardt, Bastmeyer and Weth [107] conceived an algorithm where ephrin bidirectional

signaling is used to map the N–T retinal axis onto the A–P tectal axis. The effect of correlated retinal activity is neglected. Axonal movement is derived from an axon-specific guidance potential $G_i(\tilde{y}_i)$ (axon i) that depends on the interaction of the axon with neighboring axons and tectal neurons. The interactions lead to forward and reverse signaling with trans- and cis-contributions. The potential is chosen to be the logarithm of the ratio between forward and reverse signaling,

$$P_i(\tilde{y}_i) = \ln \left(\frac{S_{i,fwd}(\tilde{y}_i)}{S_{i,rev}(\tilde{y}_i)} \right). \quad (26)$$

The probability that an axon moves to a neighboring site (movement to an occupied site was permitted, e.g. no competition for target space was implemented) is computed from the potential. An axon reached its TZ and stops if $P_i(\tilde{y}_i) = 0$, e.g. when forward and reverse signaling are balanced. The model is able to reproduce novel stripe assay experiments and a variety of other results including map duplication, map compression, mismatch and polarity reversal experiments.

Based on ideas from earlier models [101,108], Simpson and Goodhill proposed an algorithm to update the tectal position $\tilde{y}_i$ of an axonal branch i (each axon has eight branches), $\mathbf{y}_{i,t+1} = \mathbf{y}_{i,t} + v\tilde{\mathbf{e}}_{i,t}$ [109]. The movement direction is determined by chemoaffinity, competition and axon–axon interactions,

$$\tilde{\mathbf{e}}_{i,t} = \alpha_{chem}\tilde{\mathbf{e}}_{i,chem} + \alpha_{comp}\tilde{\mathbf{e}}_{i,comp} + \alpha_{int}\tilde{\mathbf{e}}_{i,int}. \quad (27)$$

Neural activity is neglected. The chemoaffinity term $\tilde{\mathbf{e}}_{i,chem}$ moves branches towards their predefined TZ. The competition part $\tilde{\mathbf{e}}_{i,comp}$ moves the branch from high to low branch density regions. The interaction part $\tilde{\mathbf{e}}_{i,int}$ is motivated by repulsive ephrin-A forward signaling that is not derived from receptor–ligand interactions, but is based on the comparison of EphA receptor levels (see also [110]). It has the tendency to disperse nearby axons with very different EphA receptor levels. The framework reproduces a large range of surgical and genetic manipulations experiments.

5.1.4. Models based on minimization of guidance potentials

Gierer first assumed that guidance proteins distributed in gradients in the retina and the tectum generate an axon specific parabolic potential $P(x_0, y)$ (retinal position x_0 and the tectal position y) [111,112]. The potential minimum corresponds to the TZ, and the potential gradient determines the direction of the movement. To illustrate Gierer's approach, we construct an axon specific parabolic potential based on ephrin forward and reverse signaling (see Fig. 4A)

$$P(x_0, y) = S_{fwd}(x_0, y) + S_{rev}(x_0, y) = R_{r,max}e^{\alpha_r(x_0-1)}L_{t,max}e^{-\beta_t y} + R_{t,max}e^{\alpha_t(y-1)}L_{r,max}e^{-\beta_r x_0} \quad (28)$$

The minimum $y_0(x_0)$ of $P(x_0, y)$ depends linearly on x_0 and induces a linear mapping.

Koulakov and Tsigankov conceived a map formation algorithm based on the minimization of an overall effective energy functional E which is the sum of chemoaffinity and activity dependent contributions from all axons i in the tectum ($\tilde{y}_i$ is the tectal position of axon i) [113]:

$$E = E_{chem} + E_{act} = \sum_i (\alpha R_A(i)L_A(\tilde{y}_i) - \beta \alpha R_B(i)L_B(\tilde{y}_i)) - \frac{\gamma}{2} \sum_{i,j} C_{i,j}U(\tilde{y}_i - \tilde{y}_j). \quad (29)$$

The chemoaffinity potential E_{chem} depends on ephrin forward signaling that is repulsive for ephrin-A and attractive for ephrin-B. Cis-interactions are introduced by assuming that axonal receptors are masked by axonal ligands and tectal ligands by tectal receptors. The activity potential E_{act} considers the effect of correlated activity using a pair-wise attraction between axons depending on their distance in the retina and tectum. Starting from a random initial distribution in the tectum, the final configuration is found through an iteration procedure that aims to minimize E . At each step, two randomly chosen axons (not necessarily neighbors) become interchanged with probability $p = (1 + e^{\Delta E})^{-1}$, where ΔE is the energy difference before and after the exchange. Because E_{chem} depends only on forward signaling, the lowest energy is achieved when all axons are accumulated at the border of the tectum. To avoid accumulation and to induce a homogenous distribution, it is introduced that a tectal position can only be occupied by a single axon, motivated by axonal competition. The model reproduces the map in ephrin-A knockout mice [88,89]. A modified version is later on introduced to reproduce the map duplication results from Brown et al. [114].

Triplett et al. [115] used simulations based on Eq. (29) to discuss their experimental results for Math5 mutant mice that have 5–10% of the normal amounts of RGCs. Axons in these mutants do not homogeneously fill the tectum and are enriched in the anterior part. This is explained by assuming a reduced axon–axon competition in the mutant. From this it is concluded that competition is a driving force for retinotopic mapping. However, this contrasts earlier results by Gosse et al. [116] suggesting that competition is not required for targeting, but merely serves to restrict arbor size and shape. To test the role of competition and to discuss the discrepancy with [116], simulations are performed with an additional competition term E_{comp} in Eq. (29).

5.1.5. Models based on interstitial branching

In frogs and fish, RGC axons that enter the tectum stop around their correct TZ where they form synaptic connections that are refined by activity (Fig. 3D). In contrast, in mice or chicks, axons first substantially overshoot their TZ along the A–P axis, then form interstitial branches along the axon shaft preferentially around the correct TZ. Finally, overshoot and ectopic branches are eliminated and the connection is refined [106,117] (Fig. 3C). Yates et al. [118] conceived a stochastic algorithm for map formation along the A–P axis in chicks based on interstitial branching. Initially, axons span the whole AP axis. Ephrin-A forward and reverse signaling between axons and tectal cells restricts the branching probability to a region around the TZ (see Fig. 4A). Axon–axon interactions mediated by ephrin signaling further restrict the branching probability. It is found that a model based on ephrin signaling alone cannot reproduce the mapping precision observed in vivo, and a non-linear feedback mechanism is introduced that refines the mapping. The model does not include correlated retinal activity or synaptic properties, and it reproduces the maps in ephrin-A deficient mice [88,89] and in transgenic EphA knockin mice [87].

Grimbert and Cang [119] conceived a phenomenological model for retinotopic mapping in mice that proceeds through two consecutive stages (similar to [120]): an initial arborization and a subsequent maturation phase. The first part results in an immature map where branch positions are determined using a stochastic model based on branching probabilities that are restricted around the TZ by forward and reverse ephrin signaling. The immature map is then refined in the second dynamic phase that models the movement of branches using a partial differential equation that has two contributions: an attractive interaction between arbors, motivated by Hebbian plasticity induced by retinal waves, and a dispersive term that accounts for competition for tectal resources. Grimberty

and Cang find that the dynamic phase can strongly remodel the initial chemoaffinity map, thereby challenging the common view that retinotopic maps are merely refined by activity-dependent processes. The model is validated by reproducing the transgenic EphA knockin mice results [87] and EphA7-KO results that not have been modeled previously [121].

5.1.6. Modeling the time evolution of the map formation

Previous models implemented algorithms to iteratively generate a map, but no time evolution has been simulated. Godfrey, Eglen and Swindale conceived a complex branching model based on rate equations to simulate the map evolution during 1 week maturation period in mice and chicks [120]. Moreover, time dependent correlated activity derived from retinal wave simulations are used. The model takes into account the effect of ephrinA and ephrinB signaling, trophic factors, correlated activity, axon branching and growth, neuronal and synaptic properties. The map formation occurs in two stages: the first is determined by chemoaffinity-mediated axon growth and branching, whereas the second is dominated by activity-mediated refinement via trophic feedback on axonal and synaptic growth. The model predicts that gradient tracking abilities of growth cones are not required due to spatial integration via arborization, and spike time dependent plasticity is not relevant for map refinement. The model is used to discuss many details of the map formation process, however, simulated maps are not compared with observations. A drawback is that the model complexity strongly limits its applicability and reproducibility (simulation runtime is around 1–6 days). Nevertheless, this approach is remarkable because it is a first attempt to simulate a time dependent map formation.

5.2. Open questions and future directions

By analyzing retinotopic map formation models [107,109,114,115,119,120] one realizes that we are still missing an answer to the question how the process proceeds over time at molecular level. Models are still largely phenomenological and focus on effective algorithms to reproduce final maps. There is consensus about general principles that contribute to map formation, like bidirectional signaling, axon–axon interactions, axon competition or correlated activity. However, because equations are postulated rather than derived from analysis of underlying processes, several theoretical frameworks exist. Parameters are model specific and fitted to reproduce final maps, which makes it difficult to compare values across frameworks. Furthermore, models are mostly validated using a fraction of available experimental data, and it remains unclear how they would perform in other conditions.

It is currently difficult to differentiate between various approaches. For example, there is controversy regarding the impact of the various map formation mechanisms, e.g. correlated affinity versus chemoaffinity (e.g. [115,119] versus [107,109]). Due to complexity, the map formation cannot be understood by proposing more complex systems of phenomenological equations based on qualitative arguments. Instead, detailed models have to be constructed from the analysis of underlying biochemical interactions. This will provide a common basis for the derivation of more coarse-grained models that will eventually lead to a unified framework.

5.2.1. Cooperative interaction between several guidance proteins

There are still many open questions regarding the details of the map formation process. For example, because Eph/ephrin proteins are expressed by axons and tectal neurons, how does an axon distinguish between an interaction with a tectal cell compared to a competitive axon? What exactly is axon competition? What is the

role of different type of Eph/ephrin proteins? Trans interactions of Eph receptors with ephrin ligands are part of a large signaling network controlling actin cytoskeleton dynamics and a variety of other cellular responses [79,122–124]. Are several ephrin types needed to regulate various biological processes involved in the map formation, and/or are they important for the mapping precision? Besides the Eph/ephrin system, there are other guidance proteins like Ryk, Wnt, Semaphorins, Plexins, Neuropilins or Engrailed that are involved in axon guidance [70,76,124,125]. Is there synergetic interaction between several guidance molecules to increase the mapping precision (Fig. 4C)? For example, engrailed homeoproteins are expressed in gradients in the tectum where that affect transcription and translation, regulate the expression of ephrinA5, increase the sensitivity of growth cones to ephrin activity and participate in axon guidance [126–128]. Because homeoproteins can easily shuttle between cells, they can mediate axon–tectum and axon–axon interactions [129]. Recently a mechanism has been proposed that illustrates how synergetic interactions between engrailed and ephrinA5 (Fig. 4B) can increase the precision of the map formation (Fig. 4C) [126].

5.2.2. Growth cone dynamics and signaling along axon shaft

In amphibians and fish the map formation is considered to rely on growth cone guidance (Fig. 3D). Here it would be important to include models for growth cone dynamics into the map formation process [130]. In contrast, in vertebrates, growth cone dynamics might be less important for the mapping precision because map generation proceeds through arborization and branching along the axons shaft (Fig. 3C). In this case signal integration has to occur all along the axon and is not restricted to the growth cone, [73,120]. How is signaling along the axon shaft spatially integrated? What is the role of the growth cone in vertebrates? Recent experimental data suggest that branching might also be an important mechanism for map formation in zebrafish [131].

5.2.3. Correlated activity and cAMP signaling

Map refinement is assumed to occur through correlated activity that affects synaptic properties. However, Eph/ephrin signaling also influences synapse formation and plasticity [82]. Furthermore, experiments show that correlated activity and chemoaffinity both affect the cAMP signaling, a fact that is so far ignored by models [132–134]. Engrailed mediated signaling via ATP secretion leads to the activation adenylate cyclase thereby also affecting the cAMP concentration [126]. What is the role of cAMP signaling? Does it control basic cellular behavior which therefore makes it a target for other signaling pathways?

5.2.4. Cooperative effort between experiment and theory is needed

To unravel the complexity of the map formation process an integrative effort between modeling and experiment is needed. It will be necessary to develop models that predict details of the time dependent map formation process. Experiments are needed that do not focus on the final map, but study details of the formation process. Innovative in-vitro experiments with strict controlled conditions are determinant, such as the novel stripe assay experiments [107]. Studying map formation in artificial tecta would provide minimal condition that could lead to new models of axonal guidance in such artificial environments. To conclude, although general principles that govern retinotopic map formation are known, we are still far from understanding how these principles emerge at a fundamental molecular and cellular level and how they exactly govern the map formation process.

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