Optimizing the readout of morphogen gradients

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In multicellular organisms, the initial patterns of gene expression are regulated by spatial gradients of biochemical factors, known as morphogen gradients. Because of biochemical noise in the morphogen gradients there are associated spatial errors in the positions of target gene patterns. Using a simple single morphogen and/or single target gene model, we use propagation of error analysis to derive a condition on the amount of morphogen that needs to be produced in order to have precise spatial patterning of the target. We find that there is an optimal morphogen gradient profile that requires the least amount of morphogen to be produced. Experimental results for the Bicoid-Hunchback system in early *Drosophila* development are consistent with the predictions of this analysis. We also discuss our results in the context of recent work that analyzed this system using mutual information as an organizing principle, and show that minimizing the amount of morphogen produced also leads to a near optimal flow of information between input and target.

A developing organism generates its complex pattern of tissue specification by controlling the precise spatial patterning of its genes. Initially, there are so-called morphogen gradients, that correspond to spatially graded patterns of biochemical factors that regulate the expression of a set of target genes [1]. These target genes generate more complex patterns of spatial expression that in turn regulate further downstream genes, ultimately leading to patterns of expression that have the single cell precision required for many developing tissues [2]. Behind all of this spatial patterning are noisy chemical reactions that can lead to cell-to-cell variation in input and output expression [3,4]. This biochemical noise in turn leads to spatial uncertainty in the expression patterns. If the organism requires that patterns should be patterned to a specific precision then this will set bounds on what the biochemical noise can be.

A well-studied morphogen gradient is the embryonic expression pattern of the transcription factor bicoid in the developing fruit fly [5]. The bicoid profile decays along the anterior to posterior axis of the embryo and regulates the expression of several target genes, one of which is the transcription factor hunchback [6]. Unlike bicoid, the expression profile of hunchback shows a sharp transition half-way along the egg's length. It has been shown that this sharp transition can be generated through the action of bicoid alone [7]. There is now quantitative data for the expression levels and noise in the bicoid-hunchback system [8,9] thus providing the chance to analyze how the organism controls biochemical noise so as to achieve a certain level of spatial resolution. In setting this spatial precision and balancing its noise requirements the organism can choose among a host of morphogen gradient profiles and target gene responses. How does it choose? On setting the morphogen gradient, there has been prior work that focused on the physical mechanisms which could lead to robust profiles [10]. With respect to choosing the specific input-output response, recent work has suggested that the organism might be trying to optimize the flow of information from the morphogen to the target [11]. Other work has focused on the limits of precision imposed by the biochemical noise [12]. In this paper, using complementary analysis we will argue that in addition to the possibility of

maximizing information transfer, the organism may also be trying to choose the morphogen profile which minimizes the amount of cellular resources that are used to set up the gradient.

In many developing organisms there are initial morphogen gradients that decay along one spatial dimension. A given morphogen gradient, described by an average concentration profile, $c(x)$ along its primary axis, x, is then readout by target genes that typically show much sharper transitions and define spatial boundaries (see Fig. 1). For a single target that sets up a single spatial boundary, we define the transition to occur at a specific spatial location $x_{1/2}$ where its concentration is one-half of its maximal value. For simplicity we will assume that the initial morphogen gradient decays exponentially with position; which has been shown to be the case for the morphogen bicoid in the *Drosophila* embryo [8] and is the solution profile for diffusion from a localized source with uniform degradation [12]. Thus, the average concentration of the morphogen, $c(x)$, along its axis, x, can be written as

$$c(x) = F \exp(x_{1/2}/\lambda)\exp(-x/\lambda), \qquad (1)$$

where λ is a decay constant and F is the concentration at $x_{1/2}$. Due to the biochemical reactions that produce this gradient, there will also be associated fluctuations around the above average value. We will assume that the variance in the average concentration is Poissonian and goes as $\delta_c^2(x) = c(x)$, which matches well with the noise profile of the bicoid gradient [8]. Hence, the noise at $x_{1/2}$ in the morphogen gradient is $\delta_c(x_{1/2})/c(x_{1/2}) = 1/\sqrt{F}$, and thus F sets the biochemical noise level in the input at the target's spatial boundary.

Most target genes display Hill function characteristics as a function of their input's concentration (target response in Fig. 1). Thus, the average concentration of a target gene, $g(c)$, that has a transition at $x_{1/2}$ as a function of the input concentration, c, where $c(x_{1/2}) = F$, can be written as

$$g(c) = \frac{(c/F)^m}{1 + (c/F)^m}, \qquad (2)$$

where m is a measure of the cooperativity or specificity of the response. This target gene is measuring the concentration
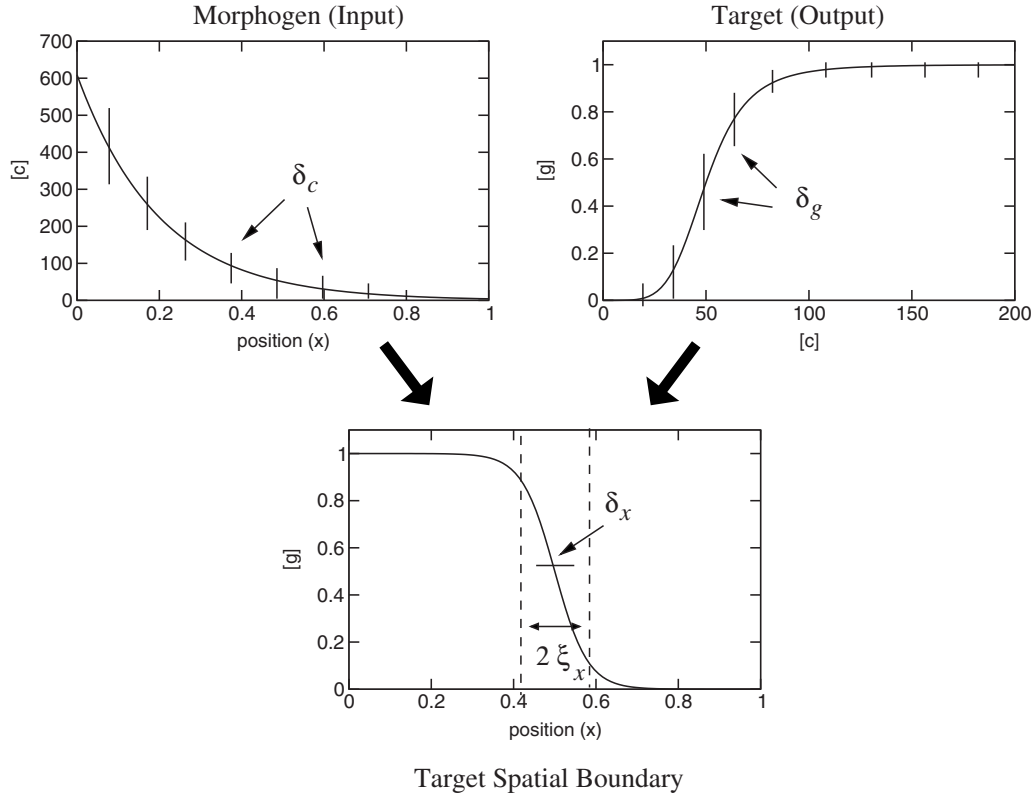
FIG. 1. Schematic of morphogen gradient readout by a target gene. The morphogen gradient, c decays along one of the spatial dimensions of the organism (left-hand top panel). The biochemical noise that exists in the generation of this gradient causes fluctuations in the input, δ_c. The morphogen gradient is readout by a target gene, g, that is assumed to have a Hill function type of response to the input (right-hand top panel). Due to the fluctuations in the input gradient, the target gene has output fluctuations δ_g. The target gene's response as a function of spatial position is shown in the bottom panel. It exhibits a transition from high to low expression at a position $x_{1/2}$, which happens to be 0.5 in the figure. The transition also has a characteristic spatial width given by $\xi_x = (1/2)|dg/dx|^{-1}$. Because of the fluctuations in the target gene's expression, there is also a corresponding error in position given by δ_x. In order for a precise resolution of the boundary, δ_x is bounded to be within ξ_x.

of the input morphogen. Transcription requires the diffusion of transcription factors to cognate binding sites in the promoter of the target, and hence measuring the input's concentration accurately is limited by diffusion and the amount of time spent measuring the signal [13]. Irrespective of these diffusion-limited measurement inaccuracies, there will also be associated fluctuations in the output signal that are in part due to the inherent biochemical fluctuations of the input signal. We now consider how this impacts the limits of precision on the spatial boundary of the target.

In order for proper development to proceed, the organism often requires the spatial boundary of the target gene to meet a specified amount of spatial precision. For instance, if the target gene is to be utilized to set a sharp boundary later in development, then the width of its transition must be narrow. Since $g(c)$ depends implicitly on the spatial location, x, we can define a transition width, ξ_x, that is a measure of how sharp the target gene's boundary is (see Fig. 1). This is given by

$$\xi_x = \frac{1}{2}\left|\frac{dg}{dx}\right|^{-1}_{x=x_{1/2}} = \frac{1}{2}\left|\frac{dg}{dc}\right|^{-1}_{c=F}\left|\frac{dc}{dx}\right|^{-1}_{x=x_{1/2}} = \frac{2\lambda}{m}. \quad (3)$$

Thus the transition width, ξ_x, depends on both the shape of the morphogen via λ and also on the cooperativity of the

target gene's response, through m. Increasing m leads to a sharper transition for a fixed λ, while increasing λ for fixed m causes the transition to be more broad. Or conversely, Eq. (3) states that there are different morphogen and target gene function combinations that can lead to the same transition width. If the organism is trying to have a fixed transition width, how does it choose between these different combinations?

In order to answer the above question, we must take into account that responding cells must be able to measure this transition accurately. More precisely, the accuracy of the spatial measurement requires that the spatial error associated with the cell-to-cell fluctuations in $g(c)$ should be less than the transition width, ξ_x. The spatial error at the boundary, δ_x, associated with the corresponding target gene's fluctuations, δ_g is given by

$$\delta_x = \left\{\left|\frac{dg}{dx}\right|^{-1}\delta_g\right\}_{x=x_{1/2}} = \left\{\left|\frac{dc}{dx}\right|^{-1}\delta_c(x)\right\}_{x=x_{1/2}} = \frac{\lambda}{\sqrt{F}}, \quad (4)$$

where we have assumed that the fluctuations in the output δ_g are determined solely by the input fluctuations via $\delta_g = |dg/dc|\delta_c$. Under this assumption the error in position is solely specified by the fluctuations in the input morphogen
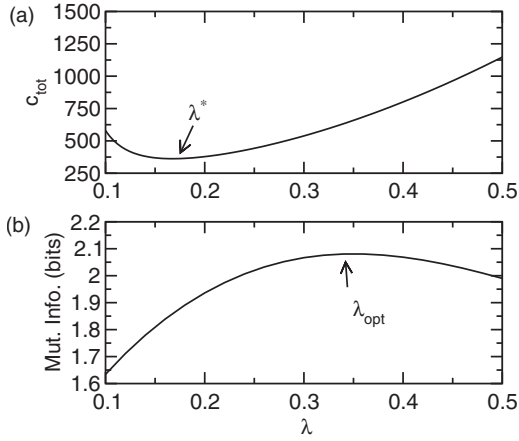
FIG. 2. (a) Plot of the amount of morphogen, c_{tot}, that would lead to an accurate spatial boundary as a function of decay length, λ. The cell width was taken to be $\Delta_x=0.016$ of the egg length, and the spatial boundary was set to $x_{1/2}=0.5$. For this value of $x_{1/2}$, the decay length which minimizes c_{tot} is at $\lambda^*=0.168$. (b) Mutual information as a function of decay length, λ, using parameters $F=100$, $x_{1/2}=0.5$, and $m=5$.

gradient. If the transition occurs over n cells of width Δx and we make the conservative assumption that noise fluctuations are shared equally over these cells, then the requirement that the spatial error fluctuations should be less than or equal to the transition width, gives

$$n\delta_x \leq \xi_x = n\Delta x. \qquad (5)$$

This leads to the simple requirement that $\delta_x \leq \Delta x$, which states that the spatial error associated with the morphogen's fluctuations is bounded by the cellular width. (This is too conservative a bound, and could be relaxed to the far more generous bound of $\delta_x \leq \xi_x$, but the following results do not depend on either choice.) The above relation sets a requirement that the morphogen concentration at the transition must be $F \geq (\lambda/\Delta x)^2$. Broader λ thus requires a larger concentration at the transition in order to keep the noise below the desired threshold. With F fixed by the above constraint, the spatial error is guaranteed to be less than the cellular dimensions at the transition boundary, and thus an accurate measurement of the transition by the target gene can be made.

By substituting the above value of F that bounds fluctuations into Eq. (1), we arrive at the steady-state concentration profile for $c(x)$ that will yield a precise output transition at $x_{1/2}$ for a given decay constant, λ. The total amount of morphogen at steady state is given by

$$c_{tot} = \int_0^1 dx \left(\frac{\lambda}{\Delta x}\right)^2 e^{x_{1/2}/\lambda} e^{-x/\lambda} = \frac{\lambda^3}{\Delta x^2} e^{(x_{1/2}-1)/\lambda}(e^{1/\lambda}-1).$$

$$(6)$$

Thus at steady state, the organism must make this amount of morphogen in order to allow the target gene to make an accurate measurement of the transition boundary. This function is plotted as a function of λ in Fig. 2(a), and is seen to go through a minimum. Assuming that the expenditure of cellular resources to maintain the morphogen gradient is pro-

portional to c_{tot}, then the organism could minimize these expenditures by choosing λ^* such that c_{tot} is minimized. Setting $\frac{dc_{tot}}{d\lambda}=0$ yields the following equation for λ^*:

$$\frac{1}{\lambda^*} = \ln\left(\frac{1}{3\lambda^*-x_{1/2}}+1\right). \qquad (7)$$

This equation has a unique solution for positive λ^*, with $\lambda^* > x_{1/2}/3$. Thus, for a given target gene transition boundary, $x_{1/2}$, there is an optimal decay length for the morphogen gradient that minimizes the amount of morphogen protein that the organism needs to produce. For a target gene with cooperativity, m, the corresponding spatial resolution of the transition is given by $\xi_x^*=2\lambda^*/m$. Or conversely, we can answer the question that was posed earlier, namely that if the organism wishes to have a specified transition width, ξ_x, then it can set the cooperativity of the target gene's response to be $m^*=2\lambda^*/\xi_x$, which will then minimize the amount of morphogen protein required. We also note that if evolutionary constraints make m difficult to alter such that ξ_x^* proves to be too broad a transition width, then λ could be decreased to obtain the desired ξ_x. The price for this decreased λ would be a greater total morphogen amount according to Eq. (6).

It is illustrative to explore the above results in the context of the developing *Drosophila* embryo where the bicoid morphogen gradient is readoff by the target gene hunchback. The transition boundary of Hunchback occurs at one-half the egg length that corresponds to $x_{1/2}=0.5$. The decay constant for the gradient that would minimize the amount of bicoid protein is $\lambda^*=0.168$ which is in good agreement with the measured decay constant of $\lambda_m=0.2$ [8]. The cooperativity of the hunchback response to bicoid has been measured to be $m=5$ [8]. With this value for m, the optimal transition width is found to be $\xi_x^*=0.07$, or 7% of the egg length, which is consistent with observed widths of the hunchback response prior to cellularization [7]. With this transition width the fluctuations in the input are bounded by $1/\sqrt{F_{max}}=0.09 < \delta_c/c < 1/\sqrt{F_{min}}=0.41$. The observed fluctuation at the transition point is $\delta_{c,m}/c=0.1$ that is within the above bounds. Thus, the measured experimental data are consistent with predictions derived from the assumption that the organism is trying to make as little morphogen as possible while keeping fluctuations bounded.

In addition to the above possibility of setting up morphogen gradients which minimize the expenditure of cellular resources, it has been recently proposed that the organism may also be trying to optimize the passage of information from the input morphogen to the output target [11]. This corresponds to maximizing the mutual information, $I(g,c)$ between input, c, and output, g, given by

$$I(g,c) = \int dc P(c) \int dg P(g|c) \log_2\left(\frac{P(g|c)}{P(g)}\right), \qquad (8)$$

where $P(c)$, $P(g)$ are the respective input-output distributions and $P(g|c)$ is the conditional probability between input and output that characterizes the target's response. Since a target gene's response, $P(g|c)$ is fixed by its promoter, it is argued that the organism might be trying to optimize the mutual information by adjusting the input morphogen distribution

$P(c)$. They calculated the mutual information from the experimental bicoid-hunchback distribution and compared it to the case where the bicoid input distribution was optimized so as to maximize the mutual information. The experimental mutual information was found to be $\approx 90\%$ of the optimal case and so it was argued that the bicoid gradient might be set by the organism to maximize the flow of information.

We have analyzed the possibility of the organism maximizing the bicoid-hunchback mutual information in the context of the model for the input and output responses given in Eqs. (1) and (2) along with the corresponding noise characteristics (see the Appendix for details). Using the parameters $F=100$, $x_{1/2}=0.5$, and $m=5$ that closely match the experimental morphogen profile, output response and noise fluctuations at the midpoint, we have calculated the mutual information $I(g,c)$ as a function of decay length λ [Fig. 2(b)]. It can be seen that the λ which maximizes the mutual information is $\lambda_{opt} \approx 0.35$. However the mutual information over the range from the experimentally measured $\lambda_m=0.2$ to $\lambda_{opt}=0.35$ does not change much, with the mutual information at λ_m being 93% of the optimal value. Based on these findings, it seems that the organism can fortuitously minimize the amount of morphogen produced while at the same time managing to be near optimal in the passage of information.

In summary, we have used propagation of error analysis to derive a condition on the amount of morphogen that needs to be produced [Eq. (6)] in order for a target gene to meet the desired spatial precision of its transition boundary. This condition predicts that there is an optimal morphogen decay length which minimizes the amount of required morphogen. The experimental data for the bicoid-hunchback system is at least consistent with the predictions from this analysis. In addition to this we also showed that the mutual information is rather insensitive to the decay length and that in minimizing the amount of morphogen produced it could also achieve a near optimal passage of information from input to target. It will be interesting to test this analysis on other morphogen gradient systems like the readout of the Dpp morphogen gradient in the developing wing-disk of *Drosophila* [14]. Currently quantitative data on the profiles and noise distributions of the morphogen and targets are lacking, and the system is complicated by other opposing input morphogen gradients. Only with the aquisition of such data will it become clearer as to how an organism chooses to set up its morphogen profiles and target responses, if it cares to choose at all.

APPENDIX

In order to calculate the mutual information, Eq. (8), from the model outlined in Eqs. (1) and (2), the distributions $P(c)$, $P(g|c)$, and $P(g)$ need to be evaluated. Equation (1) specifies the average concentration of the input morphogen, $c(x)$, as a function of position x, and we assume that the variance about this average is $\delta_c^2 = c(x)$. Assuming that the distribution $P(c|x)$ of c at a given x is Gaussian, then $P(c)$ is calculated by

$$P(c) = \int_{x=0}^{x=1} dx \frac{1}{\sqrt{2\pi\delta_c^2(x)}} \exp\left(\frac{-[c-c(x)]^2}{2\delta_c^2(x)}\right). \quad \text{(A1)}$$

With respect to the conditional probability distribution $P(g|c)$, we assume that it follows a Gaussian distribution centered about an average output, $g(c)$, with a variance $\delta_g^2(c)$ for a given input level, c. Thus, $P(g|c)$ is given by

$$P(g|c) = \frac{1}{\sqrt{2\pi\delta_g^2(c)}} \exp\left(\frac{-[g-g(c)]^2}{2\delta_g^2(c)}\right). \quad \text{(A2)}$$

Since the output g depends on the input c its variance is given by $\delta_g^2(c) = |dg(c)/dc|^2 \delta_c^2$ and using the average output function for $g(c)$ given by Eq. (2), the fluctuations in g are given by

$$\delta_g(c) = \frac{m(c/F)^m}{\sqrt{c}[1+(c/F)^m]^2}. \quad \text{(A3)}$$

Using the above two distributions, the probability distribution $P(g)$ can be computed via

$$P(g) = \int dc P(c) P(g|c). \quad \text{(A4)}$$

Thus if the parameters in the model, F, $x_{1/2}$, λ, and m are specified, the above two distributions can be evaluated and the mutual information, Eq. (8), can be calculated. In optimizing the mutual information, λ is adjusted until the maximal value of the mutual information is obtained.

[1] L. Wolpert, J. Theor. Biol. **25**, 1 (1969).

[2] For an excellent introduction to animal development and spatial patterning via morphogens, see S. B. Carroll, J. K. Grenier, and S. D. Weatherbee, *From DNA to Diversity* (Blackwell Science, New York, 2001).

[3] M. B. Elowitz, A. J. Levine, E. D. Siggia, and P. D. Swain, Science **297**, 1183 (2002).

[4] M. Thattai and A. van Oudenaarden, Proc. Natl. Acad. Sci. U.S.A. **98**, 8614 (2001).

[5] W. Driever and C. Nüsslein-Volhard, Cell **54**, 83 (1988); W. Driever and C. Nüsslein-Volhard, *ibid.* **54**, 95 (1988).

[6] W. Driever and C. Nüsslein-Volhard, Nature (London) **337**, 138 (1989).

[7] O. Crauk and N. Dostatni, Curr. Biol. **15**, 1888 (2005).

[8] T. Gregor, D. W. Tank, E. F. Wieschaus, and W. Bialek, Cell **130**, 153 (2007).

[9] B. Houchmandzadeh, E. Wieschaus, and S. Leibler, Nature (London) **415**, 798 (2007).

[10] A. Eldar, D. Rosin, B. Z. Shilo, and N. Barkai, Dev. Cell **5**, 635 (2003).

[11] G. Tkacik, C. G. Callan, and W. Bialek, e-print arXiv:/ 0705.0313.

[12] F. Tostevin, P. Rein ten Wolde, and M. Howard, PLOS Comput. Biol. **3**, e78 (2007).

[13] H. Berg and E. Purcell, Biophys. J. **20**, 193 (1977).

[14] For an excellent review of morphogen gradients in the developing wing of *Drosophila*, see T. Tabata and Y. Takei, Development **131**, 703 (2004).