P H Y S I C A L   R E V I E W   L E T T E R S

Morphogen Gradient from a Noisy Source

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(Received 16 November 2004; published 24 February 2005)

We investigate the effect of time-dependent noise on the shape of a morphogen gradient in a developing embryo. Perturbation theory is used to calculate the deviations from deterministic behavior in a simple reaction-diffusion model of robust gradient formation, and the results are confirmed by numerical simulation. It is shown that such deviations can disrupt robustness for sufficiently high noise levels, and the implications of these findings for more complex models of gradient-shaping pathways are discussed.

Successful embryonic development requires that different regions within the embryo receive distinct sets of developmental instructions. These instructions are often relayed by a morphogen, a signaling molecule that induces different cell fates at different concentrations. By establishing concentration gradients of various morphogens during early stages of development, the embryo provides each cell with the positional information it needs for the proper implementation of a body plan (Fig. 1) [1].

A morphogen gradient must reliably demarcate precise and accurate boundaries between groups of cells despite substantial genetic and environmental variability. Past studies have argued that this task might be accomplished in at least two different ways. First, it has been proposed that the downstream pathway that responds to a morphogen may have built-in mechanisms for interpreting sharply defined positional information from a gradient that varies quite substantially from embryo to embryo [2]. Second, the shape of a gradient might itself be "robust" to certain kinds of interference. Eldar *et al.* have presented evidence that the shape of the bone morphogenic protein (BMP) gradient may not be altered significantly by several types of mutations in the pathway responsible for establishing the gradient [3]. Furthermore, their theoretical investigations of dynamical models for several gradient-shaping pathways have uncovered steady-state solutions that are effectively independent of certain kinetic parameters and initial or boundary conditions [3,4]. Thus, there is some indication that a pathway's architecture could ensure that the shape of the resulting gradient did not depend on the rates of synthesis or the concentrations of some or all components of the pathway.

The theoretical studies of robustness mentioned above rely on deterministic reaction-diffusion equations that treat the influxes of the components of a gradient-shaping pathway as constant, time-independent quantities. However, a growing number of experimental studies have demonstrated that gene expression at the cellular level is an inherently "noisy" process, during which the rates of synthesis of various gene products fluctuate substantially

about their average values over time [5–8]. In the presence of such noise, a gradient would be constantly driven away from its steady-state shape, and might even adopt a different shape, on average. Thus, the question of whether deterministic models can adequately capture the dynamics of morphogens that are, in reality, produced and secreted stochastically is an open one whose answer may bear profoundly on our understanding of how a viable final product is assured during the process of embryonic development.

This Letter introduces the novel dimension of time-dependent noise to the study of morphogen gradients. A perturbative treatment of a simple reaction-diffusion model for gradient formation is used to calculate both the noise-averaged shift of the system away from its robust deterministic steady state and the system's fluctuations about this average. These analytical results are subsequently confirmed by a numerical simulation. Comparing our findings
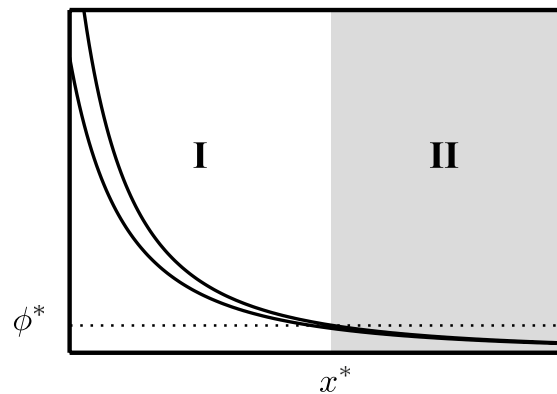

FIG. 1. Morphogen concentration gradients $\phi_0(x)$ are plotted from (2) for two different values of the production rate η_0. Where ϕ_0 is greater than the threshold concentration ϕ^*, a cell fate of type I is induced (unshaded). In contrast, $\phi_0 < \phi^*$ induces a cell fate of type II (shaded). The process by which the gradient is shaped is said to be robust to η_0 because both curves demarcate essentially the same location x^* for the boundary between cell types.

to the steady-state solution of the reaction-diffusion model, we find that deterministic robustness can, indeed, be compromised by noise, but only when the intensity of noise is sufficiently high. Finally, we argue that models of robust gradient formation that incorporate a more realistic number of components should be even more susceptible to the effects of noise than the simple model investigated here.

We begin by considering a single morphogen of local concentration $\phi(x, t)$ that is produced at the origin at a constant rate η_0 and diffuses out to infinity along the positive x axis with Fick's constant D. The morphogen accelerates its own rate of degradation such that it is removed from the system at a local rate of $f\phi(x, t)^2$, which gives the equation of motion

$$\partial_t \phi = D\partial_x^2 \phi - f\phi^2 + \eta_0 \delta(x). \quad (1)$$

Experimental evidence suggests that this mechanism of self-enhanced ligand degradation is a qualitatively correct description of how the wingless gradient is established in fruit flies [4].

When the gradient reaches a steady-state shape ϕ_0, it will divide space into two regions: one where the concentration of morphogen is higher than some threshold value ϕ^* and a cell fate of type I is induced, and one where the concentration is lower and a cell fate of type II is induced (Fig. 1). The boundary between the two regions lies at the coordinate x^* that satisfies $\phi_0(x^*) = \phi^*$.

The steady-state solution that satisfies $\partial_t \phi_0 = 0$ is given by

$$\phi_0(x) = \frac{6D}{f} \frac{1}{(x + \epsilon)^2} = \frac{6D}{f}\left[\frac{1}{x^2} - \frac{2}{x^3}\epsilon + \mathcal{O}(\epsilon^2)\right], \quad (2)$$

where $\epsilon = (12D^2/f\eta_0)^{1/3}$. The boundary coordinate is therefore given by $x^* = \sqrt{6D/f\phi^*} - \epsilon \equiv x^*(0) - \epsilon$. If $\epsilon \ll x^*(\epsilon)$, then $d\log x^*/d\log\epsilon$ will be very small, meaning that relatively large percentage changes in ϵ will introduce only minor percentage shifts in x^*. Thus, as long as $\epsilon \ll x^*(0)$, the location of a cell-type boundary specified as in Fig. 1 will be robust to the concerted shifts in η_0 that might result from genetic or environmental variation within a population of embryos [4].

The preceding analysis operated under the assumption that the rate at which the morphogen is synthesized and secreted is constant over time in a single embryo. We now relax this assumption in order to study the consequences of introducing time-dependent noise into the system. The first step in doing this is to define new quantities $\phi(x, t) = \phi_0(x) + \delta\phi(x, t)$ and $\eta(t) = \eta_0 + \delta\eta(t)$. A rescaling of space, time, noise, and concentration in the interest of consolidating free parameters results in the new equation of motion

$$\partial_t \delta\phi = \partial_x^2 \delta\phi - \frac{12}{(x + \epsilon)^2}\delta\phi - \delta\phi^2 + \delta\eta\delta(x). \quad (3)$$

We have computed the mean shift $\langle\delta\phi(x)\rangle$ and variance $\Delta \equiv \langle\delta\phi(x)^2\rangle - \langle\delta\phi(x)\rangle^2$. [Here, $\langle\cdots\rangle$ denotes averaging over all realizations of the noise $\delta\eta(t)$.] Since dimensional analysis requires that these two quantities depend on $\epsilon(\eta_0)$, they are a means to gauge the impact that noise has on the robustness of the gradient to changes in η_0. The noise itself is taken to satisfy $\langle\delta\eta(t)\rangle = 0$ and $\langle\delta\eta(t)\delta\eta(t')\rangle = 2\gamma\delta(t - t')$. A power-counting argument shows that the effects of more realistic noise correlations should not alter the behavior for large x.

The quantities of interest can be calculated using perturbation theory. The first step is to construct a Green's propagator, which is composed of the eigenfunctions $\hat{\psi}_\lambda$ of the linear operator $-\partial_x^2 + 12/(x + \epsilon)^2$ in the right-hand side of (3):

$$G(x_1, x_2; \omega) = \int_0^\infty d\lambda \frac{\hat{\psi}_\lambda(x_1)\hat{\psi}_\lambda(x_2)}{-i\omega + \lambda}. \quad (4)$$

The eigenfunctions may be approximated as $\hat{\psi}_\lambda(x) = \sqrt{(x + \epsilon)/2}J_{7/2}[\sqrt{\lambda}(x + \epsilon)]$. While terms proportional to Bessel functions of the second kind are needed for exactness when $\epsilon \neq 0$, their contribution should be small for the soft modes of the system that contribute most strongly to averages over the noise, particularly in the case of interest where ϵ is small compared with the size of the system.

The iterative solution to (3) obtained by repeated substitution for the nonlinear term can be expressed as a sum over tree Feynman diagrams rooted at x, each edge corresponding to G, each node to the nonlinear interaction, and the end of each branch corresponding to an insertion of the noise. Averaging over the noise then has the effect of pairing these ends in all possible ways. The nth-order contribution to Δ is represented in the inner box of Fig. 2. The two lines that converge on x' must originate at vertices located at points x_1 and x_2, which can be assumed to be less than x' for reasons that are explained below. Integration over ω and the propagator eigenvalue
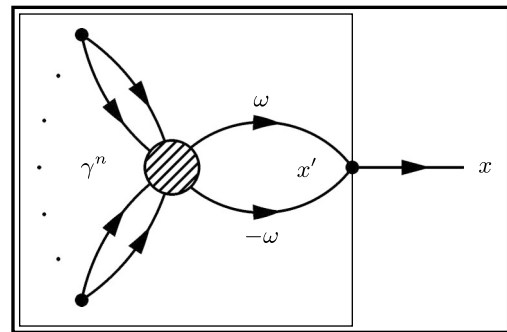

FIG. 2. The Feynman diagram for a general, nth-order process, with time increasing from left to right. n distinct fluctuation events at the origin propagate through space and interact with each other to produce a positive contribution to Δ at x' (inner box). The self-interaction of this variance summed over all points x' then induces a nonzero $\langle\delta\phi\rangle$ at x (outer box).

for $G(x_1, x')$ reveals that the nth-order diagram contains the integral

$$\ldots \frac{1}{4} \int d\lambda (x' + \epsilon)\sqrt{(x_1 + \epsilon)(x_2 + \epsilon)} J_{7/2}(\sqrt{\lambda}[x_1 + \epsilon]) J_{7/2}(\sqrt{\lambda}[x' + \epsilon]) I_{7/2}(\sqrt{\lambda}[x_2 + \epsilon]) K_{7/2}(\sqrt{\lambda}[x' + \epsilon]). \quad (5)$$

Assuming that $x' + \epsilon \gg x_i + \epsilon$ for all points x_i that temporally precede x' in Fig. 2, the heavy damping from $K_{7/2}$ permits the substitution $J_{7/2}(\sqrt{\lambda}[x_1 + \epsilon]) I_{7/2}(\sqrt{\lambda}[x_2 + \epsilon]) \sim \lambda^{7/2}$. The subsequent integral over λ in (5) comes out the same regardless of the order in perturbation theory. Thus, we obtain $\Delta \sim 1/(x')^8$.

The above result was based on the assumption that x' is always much larger than the spatial coordinates of vertices located at earlier time coordinates. Obviously, when one integrates over all possible vertex positions, one will be forced to consider cases where $x_1 \gg x'$. However, free fluctuations constantly relax towards $\delta\phi = 0$ as they propagate through space, and their effects are clearly the strongest if they interact nonlinearly prior to undergoing most of this relaxation. Thus, by assuming that all interactions contributing to a diagram occur before propagation over any significant distance takes place, we ignore processes that would have contributed only weakly to the final answer.

With Δ in hand, we now proceed to the outer box of Fig. 2 and calculate the average shift in $\delta\phi$ using

$$\langle \delta\phi(x) \rangle = \int dx' \Delta(x') G(x, x'; \omega = 0). \quad (6)$$

Since

$$G(x > x'; 0) = \frac{\sqrt{xx'}}{7}\left(\frac{x'}{x}\right)^{7/2}, \quad (7)$$

the leading term in (6) becomes $\langle \delta\phi(x) \rangle \sim 1/x^3$. It should be noted, however, that the cubic power law is not a result of the specific form of the fluctuations Δ as long as they fall off sufficiently rapidly with x'. Rather, the observed decay would be brought in as part of any computation of (6) because of the cubic spatial dependence of $G(x > x'; 0)$. Thus, it seems likely that little was sacrificed in generality by assuming a white spectrum for the noise.

The system defined in (1) and (3) was simulated in order to test whether $\langle \delta\phi \rangle$ and Δ would exhibit the predicted power law behaviors. As Fig. 3 illustrates, the concurrence of the data with the analytical calculations is essentially exact, except near $x = 8.8$, where a boundary condition requiring $\delta\phi$ to vanish artificially skews the curve towards zero.

We are now in the position to assess whether the gradient remains robust to changes in the average production rate $\langle \eta \rangle = \eta_0$ in the presence of white noise of strength γ. Recall that the condition for robustness to η_0 in the steady-state case was $\epsilon \ll x^*(0)$. Now examining the *noise-averaged* shape of the gradient, we find that

$$\langle \phi(x) \rangle \simeq \frac{6D/f}{(x + \epsilon)^2} - \frac{A}{x^3}, \quad (8)$$

where $A(\eta_0, \gamma)$ is an unknown, positive amplitude that

increases in a monotonic, unbounded fashion with γ from the value of zero at $\gamma = 0$. Comparing to (2), it is apparent the impact of the noise at leading order is that it drives up the effective value of ϵ. This means that, although our criterion for robustness may be met in the absence of noise, there must be a $\gamma^*(\eta_0)$ for which $\gamma > \gamma^*$ implies that $2\epsilon(\eta_0) + A(\gamma, \eta^0) > x^*(0)$. Thus, the robustness of the system to changes in η_0 is reduced in the presence of noise, and can be eliminated altogether if the noise level is sufficiently high.

It is also interesting to consider the effects that the fluctuations might themselves have on the process of development. If the embryo had an infinite amount of time to sample the fluctuating level of morphogen at each point in space, it could "measure" and respond to the gradient $\langle \phi \rangle$ with perfect precision. Instead, positional information must be recovered from a morphogen gradient over the course of some finite period T dictated by the timetable of embryonic growth. A calculation similar to the one carried out in (5) reveals that the variance measured at x during this period will be less than Δ and asymptotically proportional to $T^{-1}x^{-6}$. (The slower x^{-6} decay of fluctuations results from the fact that points more distant from the origin receive information about the effective value of ϵ more slowly.) Thus, the precision of cell-type boundaries must be limited approximately by $\Delta x^* \sim (x^*)^{-3}/\langle \phi'(x^*) \rangle$. This uncertainty in the boundary position should grow monotonically with γ and depend only very weakly on x^*. Thus, regardless of where the boundary lies on average, a sufficiently high noise strength could push the precision in x^* below tolerable levels.
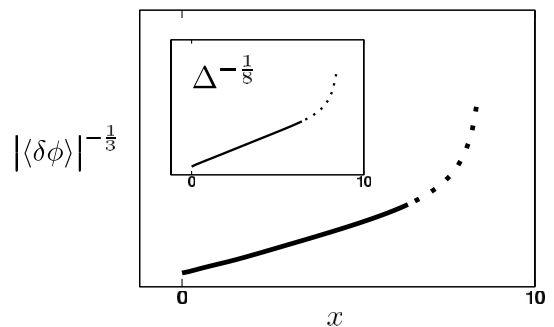

FIG. 3. The equation of motion (3) was solved numerically for $\Delta t = 0.001$, $\Delta x = 0.2$, $f = 5$, $\gamma = 0.2$, and $D = 1$ on $0 < x < 8.8$. Boundary conditions were imposed such that $\partial_x \delta\phi|_{x=0} = \delta\phi(8.8) = 0$. Here, we plot the inverse cube root of the magnitude of the average shift in $\delta\phi$, along with the inverse eighth root of the fluctuation of the shift about its average value (inset). Regression analysis of the solid lines gave linear correlation coefficients >0.998; the dotted lines represent regions where the finite-size effect due to the boundary condition at $x = 8.8$ becomes noticeable.

The above discussion focused on a reduced model of morphogen gradient formation that involves only one chemical species. In fact, even the simplest realizations of self-enhanced ligand degradation *in vivo* involve intermediary receptors and proteases that enable the morphogen to regulate its own rate of degradation [4]. However, there are at least two reasons to believe that this additional sophistication should only magnify the effects we have observed in the reduced model. First, this study has focused on a system in which the only source of noise was localized at the single point in space at which the damping of fluctuations was strongest. In contrast, more realistic models may contain multiple sources of noise that extend across the entire system. Second, in more complex pathways, it is possible for the concentration of one chemical species to control the relaxation time of fluctuations in a second species. Thus, a pathway that is robust to the concentration of a component in the deterministic steady state may exhibit fluctuations and an induced shift away from the steady state that are not robust to this concentration. For example, we have recently shown that under reasonable assumptions, the gradient shape established by the BMP pathway studied in [3] should exhibit increased variability when the ambient concentration of the short gastrulation protein is reduced. This predicted variability is anecdotally confirmed by experiments carried out in [9], but the issue still demands a more quantitative investigation.

This study is, to our knowledge, the first to elucidate the impact that time-varying stochasticity in gene expression can have on the shaping of morphogen gradients. Using both analytical and numerical techniques, we have shown that noise can interfere with the putatively robust specification of positional information by inducing a nonrobust mean shift in the gradient away from its steady-state shape, and can also cause fluctuations in the position of a cell fate boundary. There is, furthermore, good reason to believe that these effects should be more pronounced in models more realistic than the simplified system considered here.

However, while it has been made clear in this work that noise has potential significance, it remains a question for experimenters whether fluctuations are large enough that they can play a detectable role in the formation of morphogen gradients. Yet even in the cases where noise turns out to have little effect, there still remains the intriguing possibility that specific mechanisms are responsible for attenuating fluctuations in order to prevent them from interfering substantially with the action of morphogens. In either event, our findings should provide motivation for an exciting new line of experimental inquiry that may contribute to a richer understanding of the factors that play a decisive role during embryonic development.

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