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A New Approach to Feedback for Robust Signaling Gradients

By T. Kushner, A. Simonyan, and F. Y. M. Wan

The patterning of many developing tissues is orchestrated by gradients of morphogens through a variety of elaborate regulatory interactions. Such interactions are thought to make gradients robust, that is, resistant to changes induced by genetic or environmental perturbations; but just how this might be done is a major unanswered question. Recently extensive numerical simulations suggest that robustness of signaling gradients cannot be attained by negative feedback (of the Hill's function type) on signaling receptors but can be achieved through binding with nonsignaling receptors (or nonreceptors for short) such as heparan sulfate proteoglycans with the resulting complexes degrading after endocytosis. These were followed by a number of analytical and numerical studies in support of the aforementioned observations. However, evidence of feedback regulating signaling gradients has been reported in literature. The present paper undertakes a different approach to the role of feedback in robust signaling gradients. The overall goal of the project is to investigate the effectiveness of feedback mechanisms on ligand synthesis, receptor synthesis, nonreceptor synthesis, and other regulatory processes in the morphogen gradient system. As a first step, we embark herein a proof-of-concept examination of a new spatially uniform feedback process that is distinctly different from the conventional spatially nonuniform Hill function approach.

1. Introduction

In the early stage of biological development, cells receive positional information, usually from spatially distributed, signaling-receptor bound *morphogens*, to

Address for correspondence: Frederic Y. M. Wan, Department of Mathematics, Center for Mathematical and Computational Biology and Center for Complex Biological Systems, University of California at Irvine, Irvine, CA 92697-3875; e-mail:fwan@uci.edu

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adopt different fates resulting in tissue patterning. Morphogens (aka *ligands*) such as Decapentaplegic (*Dpp)* in a Drosophila wing imaginal disc are secreted signaling molecules synthesized (often at a localized source) and transported downstream (e.g., by active or passive diffusion) for binding with signaling receptors (such as Thickvein [*Tkv*] for *Dpp*) to form signaling spatial gradients. Graded differences in receptor occupancy at different locations underlie the signaling differences that ultimately lead cells down different paths of development [1–3].

An important requirement for signaling morphogen gradients is to produce patterns that are not easily altered by genetic or epigenetic (such as environmental) fluctuations. The insensitivity of a system's output to variations in input or system parameters is often termed *robustness* . How this requirement is met has been the subject of a number of recent studies [4–11]. Understanding how robustness is attained is important not only to shed light on the reliability of developing systems, but also to help explain the ubiquitous presence of elaborate regulatory schemes in morphogen systems.

Formation of concentration gradients of signaling morphogen–receptor complexes (signaling gradients for short) is expected to be affected by other known ligand activities including binding with molecular entities (such as heparan sulfate proteoglycans) other than signaling receptors. Such nonsignaling entities are called *nonreceptors* because they bind with morphogens but the resulting bound morphogen complexes do not signal. As such, the presence of nonreceptors reduces the amount of morphogens available for binding with signaling receptors and thereby inhibits or downregulates cell signaling. Effects of nonreceptors have been examined briefly in [12] where we extend a simplest wing disc morphogen model of [13, 14] to include the possibility of morphogens binding with a certain kind of cell-surface nonreceptor to investigate their inhibiting effects on the formation and properties of steady-state signaling gradients.

Available experimental results carried out by S. Zhou in A.D. Lander's lab (see also [15]) show that Dpp synthesis rate doubles when the ambient temperature is increased by 6°C. With such an increase in Dpp synthesis rate, the simple models developed in [13, 14, 16, 17] would predict signaling gradients qualitatively different from that at the lower ambient temperature. Yet, little abnormality in the development of the wing imaginal disc is observed under such a change of ambient temperature (see also [10, 16]). In effect, ligand-mediated patterning of the Drosophila wing is substantially robust to a significant increase in synthesis rate. On the other hand, modification of models to include a feedback loop in which receptor synthesis rate is downregulated by an increase in signaling morphogen concentration was found not to lead to robustness [18, 19, 11]. This suggests different types of feedback mechanisms are probably at work. Two novel strategies for achieving robustness were identified in [19]; both involve cell surface nonreceptors mediating a large

portion of overall morphogen degradation. That nonreceptors provide a vehicle to robust signaling in the presence of an enhanced ligand synthesis rate was shown computationally for a portion of the 10^6 biologically realistic sets of parameter values in a six-dimensional parameter space [19]. Subsequent investigations, for example [18, 11, 20], validated the two major findings of [19]: (i) A Hill function-type negative feedback on receptor synthesis rate alone does not lead to robustness, and (ii) robustness may be achieved by a sufficient level of nonreceptor-mediated degradation and enhanced by the same feedback process.

Empirical evidence exists showing that nonreceptors constitute a possible mechanism for downregulating morphogen signaling. Introduction of *noggin* (*NOG*) leads to the blocking of endogenous *BMP* signaling [21–23] and the presence of *BMP* inhibitor *chordin* (*Chd*) antagonizes BMP signaling by blocking binding to their receptors [24, 25] are but only two examples. Other known inhibitors include *follistatin* (*FST*, which binds *BMP*-7 and *BMP*-2 reversibly) [26–28] (see also [29]) and *Short Gastrulation* (*Sog*, which binds directly with *Dpp, see* [30] and references therein). However, there are also inhibitors of morphogen signaling that achieve the same outcome by different biological processes from those of nonreceptors. Among these are *wingless* (*Wg*, which downregulates *Dpp* receptor frizzle 2 (*Fz*2) expression) [31, 32], *Daughter against Dpp* (*Dad* , which downregulates the *Dpp* target gene optomotor-blind, *Omb*) [33], and PAI-1 (which induces receptor-mediated internalization and degradation of urokinase [34]). In all cases, robustness with respect to an upregulated signaling gradient resulting from environmental or genetic perturbations requires additional expression of one or more inhibiting agents above their normal concentration to be stimulated by signal enhancement. This suggests the existence of some kind of feedback process to induce robustness.

Feedback has long been seen as a mechanism for responding to an enhanced signaling gradient and stimulating upregulation of inhibitors of morphogen signaling to achieve robustness (see [5, 35–37] for examples). Specific feedback loops identified in the literature include:

- BMP-2 causes significant upregulation of Sox9 and the BMP antagonist Noggin expression [22, 35].
- High levels of Wingless signaling induce Notum expression and Notum modifies the heparan sulfate proteoglycans Dally-like and Dally that contribute to shaping Wingless gradient [38].

It is important to note that the negative results of [18, 19, 11] are not inconsistent with the findings above. They merely suggest that negative feedback of the Hill function type on signaling receptors is inappropriate for achieving a robust signaling gradient for the low receptor occupancy (LRO) type of morphogen systems investigated (while high receptor occupancy does not induce distinctive patterns as seen in nature).

In this paper, we initiate a different approach to the role of feedback in ensuring robust signaling gradients. The overall goal of the project is to investigate the effectiveness of feedback mechanisms other than a negative feedback, especially that of the Hill's function type, on receptor and a positive feedback on nonreceptor synthesis rate. As a first step, we embark herein a proof-of-concept examination of a new spatially uniform feedback process that is distinctly different from the conventional spatially nonuniform Hill function approach.

2. A model of drosophila wing imaginal disc

In this paper, we focus on *Dpp* gradients in the extracellular space of the posterior compartment of a Drosophila wing imaginal disc. It has been shown in [17] that the inclusion of transcytosis leads only to a re-interpretation of the system parameters in the steady state results. At the same time, we may simplify the morphogen activities in the wing imaginal disc as a one-dimensional reaction–diffusion problem in which morphogen is introduced at the rate V_L locally adjacent (and symmetric with respect) to the border, $X = -X_m$, between the *anterior* and *posterior* compartment of the disc, and absorbed at the other end, $X = X_{\max}$, the edge of the posterior compartment. The biological development is taken to be uniform in the direction along the compartment border (except possibly for a layer phenomenon at each end of the compartment) to reflect the fact that the ligand synthesis rate is taken to be uniform in that direction. Extensions to a two-dimensional model to allow for nonuniform activities in more than one spatial directions have been carried out in [16,10,39]. Although formulated as a model for *Dpp* in Drosophila wing imaginal disc to make use of the known biology of that system, the developments in this paper, with some modifications, also apply to other morphogen systems.

2.1. An extracellular model

Let $[L(X, T)]$ be the concentration of a diffusing ligand (such as *Dpp*) at time T and distance X toward wing disc edge normal to the compartment boundary with the localized source spanning $-X_m < X < 0$. As in [13], we take the diffusion of the ligand to be governed by $\partial[L]/\partial T = D\partial^2[L]/\partial X^2$, D being the constant diffusion coefficient. We add to this reversible binding and degradation of ligands and receptors as well as degradation of ligand–receptor complexes with the binding rate $k_{on}[L][R]$, dissociation rate $k_{off}[LR]$, degradation rate $k_{\deg}[LR]$ for the bound ligands along with the degradation rates for the free ligands and receptors $k_L[L]$ and $k_R[R]$, respectively. In these expressions, $[R]$ is the concentration of signaling receptors (e.g., *Tkv* for *Dpp*) synthesized at the spatially distributed rate of $V_R(X, T)$, and $[LR]$ is the concentration

of ligand–receptor (*Dpp–Tkv*) complexes. The parameters k_{on}, k_R, k_L, k_{deg}, and k_{off} are the various binding, degradation, and dissociation rate constants, which may not be known (or constant) due to possible feedback phenomena. Except for k_{on}, all the other rate constants are in units of $1/\sec$. While the "binding rate constant" k_{on} is in units of $1/\sec/\text{mole}$.

There is no endocytosis prior to degradation in this formulation. The omission of receptor internalization results in no loss of generality for the purpose of this investigation; we have already established in [17] that the boundary value problem (BVP) governing the steady-state behavior of a more general system with transcytosis can be reduced to the same BVP for our simpler system.

For a proof-of-concept investigation, we focus in this paper on direct feedback on the ligand synthesis rate but also discuss others such as feedback on free and bound ligand degradation rates. Indirect feedback such as that on nonreceptors is being investigated in [40]. In this way, we are led to the following nonlinear reaction–diffusion model governing the evolution of the three unknown concentrations $[L]$, $[R]$, and $[LR]$, which generally vary in space and time:

$$\frac{\partial [L]}{\partial T} = D\frac{\partial^2 [L]}{\partial X^2} - k_{on}[L][R] + k_{off}[LR] - k_L[L] + V_L, \tag{1}$$

$$\frac{\partial [LR]}{\partial T} = k_{on}[L][R] - (k_{off} + k_{\text{deg}})[LR], \tag{2}$$

$$\frac{\partial [R]}{\partial T} = -k_{on}[L][R] + k_{off}[LR] - k_R[R] + V_R, \tag{3}$$

where $V_L(X, T)$ is the localized morphogen synthesis rate (centered at and) spanning symmetrically with respect to the border $X = -X_{\min}$ between the two wing disc compartments. Below is a typical form of such synthesis rate relevant to our investigation:

$$V_L(X, T) = \bar{V}_L H(-X) = \begin{cases} \bar{V}_L & (-X_m < X < 0) \\ 0 & (0 < X < X_{\max}). \end{cases} \tag{4}$$

The receptor synthesis rate is typically taken to be uniform in space and time with $V_R(X, T) = \bar{V}_R > 0$ for $-X_{\min} < X < X_{\max}$ and all $T > 0$. Except for the inclusion of the term $-k_L[L]$ in (1), the system (1)–(4) is the same as the one investigated in [14]. The free ligand degradation term is included here to allow for a feedback process possibly appropriate for the Hedgehog (Hh) morphogen [41] and other similarly behaved gradient systems.

With the early stage of the anterior compartment and posterior compartment developing more or less similarly, we consider here only the ligand activities in the posterior compartment for which we have the following idealized boundary

conditions:

$$X = -X_{\min}: \qquad \frac{\partial [L]}{\partial X} = 0, \qquad X = X_{\max}: \qquad [L] = 0, \tag{5}$$

for all $T > 0$, where the no-flux condition at the compartment border is a consequence of symmetry, and the kill end condition at the distal edge, $X = X_{\max}$, of the compartment reflects the assumption of an absorbing edge (which we will occasionally take to be infinitely far away to avoid making such an assumption).

Until morphogens being generated starting at $T = 0$, ligand activities are expected to be in quiescence so that we have as initial conditions

$$T = 0: \qquad [L] = [LR] = 0, \quad [R] = R_0, \tag{6}$$

for $-X_m \le X \le X_{\max}$. For the case of a uniform receptor synthesis rate, we have from (2)

$$R_0 = \frac{\bar{V}_R}{k_R}. \tag{7}$$

by steady-state consideration prior to the onset of morphogen synthesis. With $k_L = 0$. the initial-boundary value problem (IBVP for short) defined by (1)–(6) corresponds to the model treated in [14].

2.2. Dimensionless form

To reduce the number of parameters in the problem, we introduce the normalized quantities

$$t = \frac{D}{X_0^2} T, \qquad x = \frac{X}{X_0}, \qquad \ell_M = \frac{X_{\max}}{X_0}, \quad x_m = \frac{X_{\min}}{X_0}, \tag{8}$$

$$\{a, b, r\} = \frac{1}{R_0}\{[L], [LR], [R]\}, \tag{9}$$

$$\{f_0, g_0, h_0, g_R, g_L\} = \frac{X_0^2}{D}\{k_{off}, k_{deg}, k_{on} R_0, k_R, k_L\}, \tag{10}$$

$$\{v_L(x,t), v_R(x,t)\} = \frac{X_0^2}{D}\left\{\frac{V_L}{R_0}, \frac{V_R}{R_0}\right\}, \qquad \{\bar{v}_L, \bar{v}_R\} = \frac{X_0^2}{D}\left\{\frac{\bar{V}_L}{R_0}, \frac{\bar{V}_R}{R_0}\right\}, \tag{11}$$

where X_0 is some typical scale length, taken to be $X_{\max}$ for the finite domain case so that $\ell_M = X_{\max}/X_0 = 1$. With these normalized quantities, we rewrite the IBVP for the three unknowns $[L]$, $[LR]$, and $[R]$ in the following

normalized form:

$$\frac{\partial a}{\partial t} = \frac{\partial^2 a}{\partial x^2} - h_0 ar + f_0 b - g_L a + v_L(x,t), \tag{12}$$

$$\frac{\partial b}{\partial t} = h_0 ar - (f_0 + g_0)b, \qquad \frac{\partial r}{\partial t} = v_R(x,t) - h_0 ar + f_0 b - g_R r, \tag{13}$$

with the boundary conditions

$$x = -x_m : \quad \frac{\partial a}{\partial x} = 0, \quad x = \ell_M : \quad a = 0, \tag{14}$$

all for $t > 0$, and the initial conditions

$$t = 0 : \quad a = b = 0,\ r = 1. \tag{15}$$

In (12) and (13), we have kept the normalized ligand and signaling receptor synthesis rates general to allow for inclusion of feedback in later sections.

The IBVP defined by (12)–(15) and its modified forms have been analyzed as mathematical models for ligand activities and tissue pattern formation.

2.3. Time-independent steady-state behavior

2.3.1. Reduction to a well-posed BVP for $\bar{a}(x)$. Given that both the ligand and receptor synthesis rates are time independent, it can be shown [14] that the extracellular model system has a unique steady state given by

$$\{\bar{a}(x), \bar{b}(x), \bar{r}(x)\} = \lim_{t\to\infty} \{a(x,t), b(x,t), r(x,t)\}, \tag{16}$$

which is linearly stable with respect to a small perturbation. It was shown in [14] that the three governing equations may be reduced to a well-posed two-point BVP for $\bar{a}(x)$:

$$\bar{a}'' - \frac{g_0 \bar{a}}{\alpha_0 + \zeta_0 \bar{a}} - g_L \bar{a} + \bar{v}_L H(-x) = 0, \tag{17}$$

$$\bar{a}'(-x_m) = 0, \qquad \bar{a}(\ell_M) = 0, \tag{18}$$

with

$$\bar{b}(x) = \frac{\bar{a}(x)}{\alpha_0 + \zeta_0 \bar{a}(x)}, \qquad \bar{r}(x) = \frac{\alpha_0}{\alpha_0 + \zeta_0 \bar{a}(x)}, \tag{19}$$

where

$$\alpha_0 = \frac{f_0 + g_0}{h_0}, \qquad \zeta_0 = \frac{k_{\deg}}{k_R}. \tag{20}$$

For a finite domain, X_0 would normally be $X_{\max}$ so that $\ell_M = 1$.

2.3.2. Low receptor occupancy. The morphogen system is said to be in a state of LRO if

$$\zeta_0 a = k_{\text{deg}} a / k_R \ll \alpha_0. \tag{21}$$

For such a system, we may neglect terms involving $\zeta_{0,} a$ in (17)–(19) to get an approximate set of solutions $\{a_0(x), b_0(x), r_0(x)\}$ determined by

$$a_0'' - \mu_L^2 a_0 + \bar{\nu}_L H(-x) = 0, \quad \mu_L^2 = \frac{g_0}{\alpha_0} + g_L \tag{22}$$

$$a_0'(-x_m) = 0, \qquad a_0(\ell_M) = 0, \tag{23}$$

with

$$b_0(x) = \frac{a_0(x)}{\alpha_0}, \qquad r_0(x) = 1. \tag{24}$$

We limit our discussion to a finite positive $X_{\max}$ so that the exact solution for $\bar{a}_0(x)$ is

$$a_0(x) = \begin{cases} \dfrac{\bar{\nu}_L}{\mu_L^2}\left\{1 - \dfrac{\cosh(\mu_L)}{\cosh(\mu_L(1+x_m))}\cosh(\mu_L(x+x_m))\right\} & (-x_m \le x \le 0) \\ \dfrac{\bar{\nu}_L}{\mu_L^2}\dfrac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1+x_m))}\sinh(\mu_L(1-x)) & (0 \le x \le 1) \end{cases}, \tag{25}$$

with

$$\bar{b}(x) \simeq \frac{a_0(x)}{\alpha_0}, \qquad \bar{r}(x) \simeq 1. \tag{26}$$

3. Robustness of signaling gradient

In this paper, we make use of the extracellular model summarized in the preceding section to investigate the effectiveness of feedback processes for achieving robust signaling gradients with respect to a significant change in the morphogen synthesis rate. We do this in a broader context than the conventional Hill function approach. In particular, the results for gradient systems in a state of LRO will be useful in later developments for at least two reasons. Biological gradients that are differentiating tend to be suitably convex, which is typically achieved through a state of LRO. The mathematical model for systems in an LRO state may be linearized to yield explicit solutions for the relevant BVP and IBVP and thereby offering clearer insight to the system behavior. In the subsections below, we recall certain aspects of robustness of signaling

gradients with respect to significant changes in the morphogen synthesis rate first formulated in [19] and further developed in [10, 11, 20, 42]. While other measures of robustness have also been considered and analyzed (see [43]), the main purpose of our summary below is to introduce a global measure of robustness to provide a key ingredient for a new approach to effective feedback mechanisms for achieving stable biological developments.

3.1. Perturbation due to enhanced morphogen synthesis

Normal development of wing imaginal disc and other biological organisms may be altered by an enhanced morphogen synthesis rate stimulated by genetic or epigenetic changes. As mentioned earlier, *Dpp* synthesis rate in Drosophila imaginal disc doubles when the ambient temperature is increased by 6°C (shown by S. Zhou while in A.D. Lander's Lab, see also [15]). At a state of lower receptor occupancy, a significant increase in morphogen synthesis rate has been shown to increase the steady-state signaling gradient magnitude proportionately and change the slope and convexity of the gradient as well. As such, the cell fate at each spatial location would be altered [10, 14, 42]. Without the restriction of LRO, the steady-state signaling gradient has also been shown to be an increasing function of synthesis rate, though not necessarily proportionately [10, 14].

Even if the difference between the normal and enhanced signaling gradients is small at a particular location x as it would be for a system in a state of high receptor occupancy (except for a narrow region near the edge of wing disc), the pattern developed would still be significantly different because the cell type that was at $\bar{x}$ is now at some distance away at $\tilde{x}$. Yet, the development of biological organisms are generally not particularly sensitive to a significant change in the ambient temperature that leads to significant signaling morphogen synthesis rate change. Some kind of feedback control process must be at work to minimize the effects of such changes on the biological developments. First attempts in finding such feedback control mechanisms focused on a Hill function type negative feedback on receptor synthesis rate. It was found by numerical simulations [19] that such a feedback process does not lead to robustness. That conclusion was proved mathematically in [18] where some insight was gained on the reason for the ineffectiveness of such feedback. Briefly, the effect of a Hill function-type negative feedback on receptor synthesis rate tends to reduce the convexity of the gradient leading to significant qualitative difference in the convexity between normal and enhanced signaling gradients even if the difference in their magnitude along much of the spatial span may have improved by the feedback.

It was suggested in [18] that a different kind of feedback process would be more appropriate for safeguarding against such unwanted enhanced signaling gradient. Two robustness indices have been introduced in [10, 19, 42] to provide

global measures of the deviation from normal signaling gradient after synthesis rate enhancement. We recall one of these in the next section to be used in our proof-of-concept development of a new approach to feedback for robustness.

3.2. Root-mean-square signaling differential

Let $b(x,t)$ be the normalized signaling morphogen concentration $[LR]/R_0$ for a normal (wild-type) ligand synthesis rate $V_L(X,T) = \bar{V}_L H(-X)$ (or $v_L(x,t) = \bar{v}_L H(-x)$ after normalization). Let $b_e(x,t)$ be same quantity for an enhanced (ectopic) synthesis rate $e\bar{V}_L H(-X)$ (or $e\bar{v}_L H(-x)$ after normalization) for some amplification factor e. A rather natural global measure of signaling gradient robustness is the following *signal robustness index* R_b corresponding to the root-mean-square of the deviation between $b_e(x,t)$ and $b(x,t)$:

$$R_b(t) = \frac{1}{b_h - b_\ell}\sqrt{\frac{1}{x_\ell - x_h}\int_{x_h}^{x_\ell}[b_e(x,t) - b(x,t)]^2 dx}, \tag{27}$$

where $0 \le b_\ell(t) < b_h(t) \le b(-x_m, t)$ and $-x_m \le x_h < x_\ell \le \ell_M = 1$. The quantities x_ℓ, x_h, b_ℓ, and b_h may be chosen away from the extremities to minimize the exaggerated effects of outliers.

For a system in steady state with

$$\bar{b}(x) = \lim_{t\to\infty} b(x,t), \qquad \tilde{b}(x) = \lim_{t\to\infty} b_e(x,t), \tag{28}$$

the robustness index $R_b(t)$ tends to a constant $\bar{R}_b$:

$$\bar{R}_b = \lim_{t\to\infty} R_b(t) = \frac{1}{b_h - b_\ell}\sqrt{\frac{1}{x_\ell - x_h}\int_{x_h}^{x_\ell}[\tilde{b}(x) - \bar{b}(x)]^2 dx}. \tag{29}$$

In subsequent developments, we set $x_h = 0$ in part because signaling is irrelevant in the interval of ligand synthesis. We also take $b_\ell = b(1,t) = 0$ for simplicity. For the case of LRO, we take b_h to be the explicit (approximate) steady-state value for $\bar{b}(0)$ known from (25) and (26) to be

$$b_h = \frac{\bar{v}_L}{\alpha_0 \mu_L^2}\frac{\sinh(\mu_L x_m)\sinh(\mu_L \ell_M)}{\cosh(\mu_L(\ell_M + x_m))} \sim \bar{b}(0). \tag{30}$$

For the case of high receptor occupancy (which is usually not biologically useful), it would be more appropriate to take $b_h = g_r/g_0$ corresponding to receptor saturation.

The signal robustness index $R_b(t)$ is not the only measure of the deviation of the modified signaling gradient from the one prior to morphogen synthesis rate enhancement. Given an existing genetic program for individual cells, a more relevant measure of robustness may be the displacement of the same level of morphogen–receptor complex concentration due to an ectopic morphogen

synthesis rate. Such a robustness index, denoted by $R_x(t)$, was first introduced in [19] and investigated in [42] and references cited therein. The present proof-of-concept study limits itself only to working with $R_b(t)$ and $\bar{R}_b$ and leaves the discussion on $R_x(t)$ and $\bar{R}_x$ to a separate investigation [40].

3.3. Approximate solution for LRO

For a morphogen system in a state of low occupancy so that $g_0 a/g_R \ll \alpha_0$, we have from [14] (and with $\rho = 1$) the following approximate steady-state solutions for the signaling gradients of the normal (wild type) and (environmentally or genetically) perturbed system:

$$\tilde{b}(x) \sim e\bar{b}(x) = \frac{e\bar{v}_L}{\alpha_0 \mu_L^2} \frac{\sinh(\mu_L x_m)\sinh(\mu_L(1-x))}{\cosh(\mu_L(1+x_m))}, \quad (0 \le x \le \ell_M), \quad (31)$$

where $\mu_L^2 = g_L + g_0/\alpha_0$ with $\mu_L^2 \simeq h_0 + g_L$ whenever $f_0 \ll g_0$ (assuming that the perturbed system is also in a state of LRO). In the absence of any feedback, the parameter e is the amplification factor of the ligand synthesis rate. In particular, for $e = 2$, $x_\ell = 1$, $x_h = 0$, we have

$$\begin{aligned} \bar{R}_b &\sim \frac{1}{\sinh(\mu_L)} \sqrt{\int_0^1 [\sinh(\mu_L(1-x))]^2 dx} \\ &= \frac{1}{\sinh(\mu_L)} \sqrt{\frac{1}{2}\left(\frac{\sinh(2\mu_L)}{2\mu_L} - 1\right)}. \end{aligned} \quad (32)$$

For a gradient system with $g_0 = 0.2$, $f_0 = 0.001$, $g_r = 1$, $h_0 = 10$, $\ell_M = 1$, $x_m = 0.1$, and $\bar{v}_L = 0.05$ (together with $\bar{V}_L = 0.002$ μM, $\bar{V}_R = 0.04$ μM, $D = 10^{-7}\text{cm}^2/\text{sec}$, $X_{\max} = 0.01$ cm) corresponding to $\beta = 0.25$ in table 2 of [14], the steady state is in low (receptor) occupancy. For this case, the approximate solution for $\bar{R}_b$ given by (32) is $0.3938\ldots$, while accurate numerical solutions of the BVP for $\bar{a}(x)$ gives $0.3939\ldots$ for a percentage error of less than 0.01%.

If ligand synthesis rate is increased 20 times to $\bar{V}_L = 0.04$ μM, the accurate numerical solution for $\bar{R}_b$ is found to be $0.37486\ldots$ The percentage error of the LRO approximate is still less than 1%. These comparisons serve to validate the numerical simulation code developed for exact numerical solutions of our model.

Our main interest however is in the use of $\bar{R}_b$, or more generally $R_b(t)$, in an appropriate feedback mechanism for attaining robustness of signaling morphogen gradients. To the extent that some enhanced ligand systems may only be *near* LRO (and still sufficiently differentiating), the use of the approximate signaling robustness index based on the approximate solution (31)

may not be sufficiently accurate. For these cases, it is necessary to obtain numerical solutions for $\bar{a}(x)$ and $\tilde{a}_e(x)$ and the corresponding value for $\bar{R}_b$.

4. Feedback on ligand synthesis rate

4.1. A nonlocal feedback with delay

Downregulation of signaling activities are known to be accomplished in different ways. Whether it is through more nonreceptors or higher degradation rate of free or bound ligands, the net effect is equivalent to a lower concentration of free ligand available for binding with signaling receptors. To initiate our new approach to feedback, we consider in this first effort the effect of a negative feedback stimulated by a higher than normal signaling ligand concentration to be simply a reduction of the ligand synthesis rate V_L. To implement this approach, we take the normalized synthesis rate $v_L(x, t)$ to include a negative feedback factor using the signaling robustness index $R_b(t)$ as an instrument for downregulating the synthesis rate:

$$v_L(x, t) = \kappa(t; \tau)\bar{v}_L H(-x) \equiv \frac{e\bar{v}_L H(-x)}{1 + c\,[R_b(t - \tau)]^n}, \tag{33}$$

where the *amplification factor* e is as previously defined in Section 3.2 and where c and n are two parameters to be chosen for appropriate feedback strength similar to those for a Hill's function. Two features of the feedback process in (33) should be noted. First, with $c = n = 1$, the feedback mechanism reduces the synthesis rate by a fraction that depends on the average deviation over an appropriate spatial span (e.g., the distal span of the posterior compartment of the wing imaginal disc of the Drosophila). Second, the feedback may not be instantaneous as a delay of τ unit of dimensionless time is allowed for the feedback to become effective.

With $\tau > 0$ (and $v_R(x, t) = \bar{v}_R$ uniformly throughout the entire distal-proximal span of the wing imaginal disc), the IBVP for the three normalized concentration may be computed as we would for the problem without feedback except that the ligand synthesis rate change with time. In particular, for the period $[0, \tau]$, the problem is identical to the one without feedback. For the interval $[k\tau, (k + 1)\tau]$ and with $t = k\tau + \eta$, the synthesis rate is modified to

$$v_L(x, t) = \frac{e\bar{v}_L H(-x)}{1 + c\,[R_b((k - 1)\tau + \eta)]^n} \qquad (0 < \eta < \tau) \tag{34}$$

with all concentrations continuous at the junctions between the time intervals. This solution process is being implemented and the results analyzed in [40].

4.2. Time-independent steady-state with feedback

It has been shown in [14] that the extracellular model system without feedback has a unique steady state that is linearly stable with respect to small perturbations from the steady state. We show here that the same is true for our model with feedback on the ligand synthesis rate. Suppose $\{a(x,t),\ b(x,t), r(x,t)\}$ of (12)–(15) tend to the time-independent states $\{\tilde{a}(x), \tilde{b}(x), \tilde{r}(x)\}$ and therewith $R_b(t) \to \bar{R}_b$ (see (27) and (29)). In that case, we have $v_L(x,t)$ of (34) tends to $\bar{\kappa}(\bar{R}_b)\bar{v}_L H(-x)$, where

$$\bar{\kappa}(\bar{R}_b) = \lim_{t\to\infty} \kappa(t;\tau) = \frac{e}{1 + c\left(\bar{R}_b\right)^n}. \tag{35}$$

Note that we have used $\bar{\kappa}(\bar{R}_b)$ for $\kappa(t;\tau)$ in the steady-state case because the *amplitude factor* $\kappa(t;\tau)$ is no longer time dependent and is only a function of $\bar{R}_b$ (and of course of $e, n,$ and c in both cases).

For the steady-state solution $\{\tilde{a}(x), \tilde{b}(x), \tilde{r}(x)\}$, we have $\partial(\ )/\partial t = 0$ so that the governing partial differential equations and boundary conditions become

$$\tilde{a}'' - h_0\tilde{a}\tilde{r} + f_0\tilde{b} - g_L\tilde{a} + \bar{\kappa}(\bar{R}_b)\bar{v}_L H(-x) = 0, \tag{36}$$

$$h_0\tilde{a}\tilde{r} - (f_0 + g_0)\tilde{b} = 0, \qquad (g_r + h_0\tilde{a})\tilde{r} - f_0\tilde{b} = \bar{v}_R, \tag{37}$$

with

$$\tilde{a}'(-x_m) = 0, \qquad \tilde{a}(1) = 0, \tag{38}$$

where a prime indicates differentiation with respect to x, that is, $(\ )' = d(\ )/dx$.

As in the case without feedback, we can solve (37) for $\tilde{b}$ and $\tilde{r}$ in terms of $\tilde{a}$ (analogous to (19) and (20)):

$$\tilde{b}(x) = \frac{\tilde{a}(x)}{\alpha_0 + \zeta_0\tilde{a}(x)}, \qquad \tilde{r}(x) = \frac{\alpha_0}{\alpha_0 + \zeta_0\tilde{a}(x)} \tag{39}$$

with

$$\alpha_0 = \frac{f_0 + g_0}{h_0}, \qquad \zeta_0 = \frac{k_{\text{deg}}}{k_R}. \tag{40}$$

and use the results to eliminate these two quantities from the only ordinary differential equations (ODE) (36) to get a BVP for $\tilde{a}$ alone:

$$\tilde{a}'' - \frac{g_0\tilde{a}}{\alpha_0 + \zeta_0\tilde{a}} - g_L\tilde{a} + \bar{\kappa}(\bar{R}_b)\bar{v}_L H(-x) = 0, \tag{41}$$

$$\tilde{a}'(-x_m) = 0, \qquad \tilde{a}(1) = 0, \tag{42}$$

where $\bar{\kappa}(\bar{R}_b)$ is given by (35).

The following theorem, similar to the one in [14], ensures the BVP for the steady-state concentration $\bar{a}(x)$ above is well-posed, nonnegative, and monotone decreasing:

THEOREM 2. *For positive values of the parameters* g_0, f_0, h_0, $\bar{v}_L$, *and* $\bar{v}_R$, *there exists a unique, nonnegative solution* $\bar{a}(x)$ *of the BVPs* (41) *and* (42). *The corresponding concentrations* $\bar{b}(x)$ *and* $\bar{r}(x)$ *can then be calculated from* (39).

Proof: The existence proof is similar to that in [14] for the case without feedback. It suffices to produce an upper solution and a lower solution for the problem to apply the known monotone method of [44] (see also [45,46]).

Evidently, $a_\ell(x) \equiv 0$ is a *lower* solution because

$$-[a_\ell]'' + \frac{g_0 a_\ell}{\alpha_0 + \zeta_0 a_\ell} + g_L a_\ell - \frac{e\bar{v}_L}{1 + c\left(\bar{R}_b\right)^n} H(-x)$$
$$= -\frac{e\bar{v}_L}{1 + c\left(\bar{R}_b\right)^n} H(-x) \le 0 \qquad (-x_m < x < 1),$$

with

$$a_\ell'(-x_m) = 0, \qquad a_\ell(1) = 0.$$

For an *upper* solution, we note that

$$\tilde{a}'' + e\bar{v}_L \ge \tilde{a}'' - \frac{g_0 \tilde{a}}{\alpha_0 + \zeta_0 \tilde{a}} - g_L \tilde{a} + \frac{e\bar{v}_L}{1 + c\left(\bar{R}_b\right)^n} H(-x) = 0.$$

The exact solution for $\tilde{a}'' + e\bar{v}_L = 0$ is

$$a_u(x) = e\bar{v}_L \left\{ \left(x_m + \frac{1}{2} \right) - x_m x - \frac{1}{2} x^2 \right\}$$

with $a_u'(-x_m) = 0$ and $a_u(1) = 0$. From $(i)\ a_u(-x_m) = \frac{\bar{v}_L}{2}(1 + x_m)^2 > 0$, $(ii)\ a_u'(x) = -\bar{v}_L(x + x_m) < 0$ for $x > -x_m$, and $(iii)\ a_u(1) = 0$, we have

$$a_u(x) > 0 \qquad (-x_m \le x < 1).$$

It follows that

$$
\begin{aligned}
&-[a_u]'' + \frac{g_0 a_u}{\alpha_0 + \zeta_0 a_u} + g_L a_u - e\bar{v}_L H(-x) \\
&\quad = e\bar{v}_L + \frac{g_0 a_u}{\alpha_0 + \zeta_0 a_u} + g_L a_u - e\bar{v}_L H(-x) > e\bar{v}_L - e\bar{v}_L H(-x) \geq 0
\end{aligned}
$$

for $-x_m < x < 1$ so that $a_u(x)$ is an upper solution for the BVP for $\bar{a}(x)$. The monotone method assures us that there exists a solution $\tilde{a}(x)$ of the BVPs (41) and (42) with

$$0 = a_\ell(x) \leq \tilde{a}(x) \leq a_u(x).$$

Because $a_u(x)$ is already known to be positive for $-x_m \leq x < 1$, $\tilde{a}(x)$ must be nonnegative in the whole solution domain.

To prove uniqueness, let $a^{(1)}(x)$ and $a^{(2)}(x)$ be two (nonnegative) solutions and $a(x) = a^{(1)}(x) - a^{(2)}(x)$. Then as a consequence of the differential equation (41) for $a^{(1)}(x)$ and $a^{(2)}(x)$, the difference $a(x)$ satisfies the following differential equation:

$$-a'' + \frac{g_0 \zeta_0 \alpha_0 a}{(\alpha_0 + \zeta_0 a^{(1)})(\alpha_0 + \zeta_0 a^{(2)})} + g_L a = 0.$$

Form

$$\int_{-x_m}^{1} \left[-a'' + \frac{g_0 \zeta_0 \alpha_0 a}{(\alpha_0 + \zeta_0 a^{(1)})(\alpha_0 + \zeta_0 a^{(2)})} + g_L a \right] a dx = 0,$$

and integrate by parts. Upon observing continuity of $\tilde{a}(x)$ and $\tilde{a}'(x)$, and application of the boundary conditions in (42), the relation above may be transformed into

$$\int_{-x_m}^{1} [a'(x)]^2 dx + \int_{-x_m}^{1} \left\{ \frac{g_0 \zeta_0 \alpha_0 [a(x)]^2}{(\alpha_0 + \zeta_0 a^{(1)}(x))(\alpha_0 + \zeta_0 a^{(2)}(x))} + g_L [a(x)]^2 \right\} dx = 0.$$

Both integrands are nonnegative and not identically zero; therefore we must have $a(x) \equiv 0$ and uniqueness is proved. ■

Stability of the steady-state solution with respect to small perturbations in the presence of feedback is more complicated to analyze and will be omitted because it is not needed in subsequent developments.

4.3. Monotonicity

As for the model analyzed in [14], free morphogen concentration $\tilde{a}(x)$ and the corresponding signaling morphogen gradient $\tilde{b}(x)$ can be shown to be (positive and) monotone decreasing in the open interval $(-x_m, 1)$. First we rule out the possibility of any extremum in that interval.

PROPOSITION 2. *Under the same hypotheses as those in Theorem 2, the nonnegative steady-state concentration* $\tilde{a}(x)$ *does not attain a maximum or minimum in* $(0, 1)$ *and hence is monotone decreasing in that interval.*

Proof: First, it is easy to see that the nonnegative $\tilde{a}(x)$ does not have an interior maximum in the interval $0 < x < 1$. If it should have a local maximum at some interior point x_0, then we must have ($\tilde{a}'(x_0) = 0$ and) $\tilde{a}''(x_0) \leq 0$. However, because $\tilde{a}(x) \geq 0$ and $v_L(x) = 0$ in $x > 0$, we have

$$\tilde{a}'' = \frac{g_0 \tilde{a}}{\alpha_0 + \varsigma_0 \tilde{a}} + g_L \tilde{a} \geq 0.$$

It follows that we must have $\tilde{a}''(x_0) = 0$ and therewith $\tilde{a}(x_0) = 0$. Because x_0 is a maximum point, we must have $\tilde{a}(x) = 0$ in $0 < x < 1$. The continuity requirements imply $\tilde{a}(0) = \tilde{a}'(0) = 0$. However, it is impossible for any nontrivial solution of the ODE (41) to satisfy both of these conditions unless $\tilde{a}(x) = 0$ for all x in $[-x_m, 0]$ as well. Such a free morphogen concentration does not satisfy (41) in the interval $(-x_m, 0)$ where the normalized *Dpp* synthesis rate is a positive constant $\bar{v}_L$. Hence $\tilde{a}(x)$ does not have a maximum in $(-x_m, \infty)$.

Also, $\tilde{a}(x)$ does not have a positive interior minimum. If it should have one at x_0 (with $\tilde{a}(x_0) > 0$), then it must have an interior maximum at some $x_1 > x_0$ in order for $\tilde{a}(x)$ to decrease from $\tilde{a}(x_1) > 0$ to $\tilde{a}(1) = 0$. However, this contradicts the fact that $\tilde{a}(x)$ does not have an interior maximum. There is still the possibility of a local interior minimum $\tilde{a}(x_0) = 0$. With $\tilde{a}'(x_0) = 0$ at the local minimum, we have $\tilde{a}(x) \equiv 0$, which does not satisfy the ODE (41) in the interval $(-x_m, 0)$.

Altogether, the solution $\tilde{a}(x)$ of the BVP must be (nonnegative and) monotone decreasing from $\tilde{a}(-x_m) > 0$ to $\tilde{a}(\ell_M) = 0$. ■

We can actually prove that the relevant morphogen concentrations are positive for $x < 1$, which we will need in subsequent development.

COROLLARY 4. *Under the hypotheses of Theorem 2, the concentrations* $\tilde{a}(x)$, $\tilde{b}(x)$, *and* $\tilde{r}(x)$ *do not vanish in* $(-x_m, 1)$.

Proof: Suppose $\tilde{a}$ vanishes at x_0 in $(-x_m, 1)$ and hence attains a local minimum there (because $\tilde{a}(x)$ is nonnegative). However, this contradicts Proposition 2, which asserts that $\tilde{a}(x)$ does not have an interior minimum. That the remaining quantities do not vanish follows from (39) and (40). ■

4.4. Low receptor occupancy

If the morphogen system is in a state of *LRO* prior to and after ligand synthesis enhancement so that (21) is met generally by the present feedback model (including the special case where $c = 0$ and $e = 1$ so that $\tilde{a}(x; \bar{R}_b)$ reduces to

$\bar{a}(x)$), we may use the linearized model

$$a_0'' = \mu_L^2 a_0 - \bar{\kappa}(\bar{R}_b)\bar{v}_L H(-x), \tag{43}$$

$$a_0'(-x_m) = 0, \quad a_0(1) = 0 \tag{44}$$

with

$$\mu_L^2 = g_L + \frac{g_0}{\alpha_0} \tag{45}$$

for an approximate solution of our problem. The exact solution of (43) and (44), denoted by $a_0(x; \bar{R}_b)$ for its dependence on $\bar{R}_b$, is expected to be an accurate approximation of the exact solution $\tilde{a}(x; \bar{R}_b)$. It reduces to the (approximate) wild-type ligand concentration when $c = 0$ and $e = 1$.

For a finite positive $X_{\max}$, the exact solution for $a_0(x)$ is

$$a_0(x) = \begin{cases} \dfrac{\bar{\kappa}\bar{v}_L}{\mu_L^2}\left\{1 - \dfrac{\cosh(\mu_L \ell_m)}{\cosh(\mu_L(1+x_m))}\cosh(\mu_L(x+x_m))\right\} & (-x_m \le x \le 0) \\ \dfrac{\bar{\kappa}\bar{v}_L}{\mu_L^2}\dfrac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1+x_m))}\sinh(\mu_L(1-x)) & (0 \le x \le 1) \end{cases}, \tag{46}$$

with

$$\tilde{b}(x) \simeq \frac{a_0(x)}{\alpha_0}, \qquad \alpha_0\tilde{b}(0) \simeq \tilde{a}(0) \simeq a_0(0) = \frac{\bar{\kappa}\bar{v}_L}{\mu_L^2}\frac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1+x_m))}\sinh(\mu_L). \tag{47}$$

For $\mu_L \gg 1$, the expression for $a_0(x)$ in the signaling range of $0 \le x < 1$ is asymptotically

$$a_0(x) \sim \frac{\bar{\kappa}\bar{v}_L}{\mu_L^2}e^{-\mu_L x} \qquad (0 \le x < 1),$$

so that the gradient is effectively a boundary layer adjacent to $x = 0$, steep near $x = 0$, and dropping sharply to near zero away from $x = 0$.

The discussion above leads to the following observation:

PROPOSITION 4. *Even if a morphogen system is in a steady state of LRO (so that the condition* (21) *is satisfied), its signaling gradient may not be a biologically meaningful gradient for the intended tissue patterning if the condition* $\mu_L = O(1)$ *is not met.*

5. Numerical algorithms for steady-state solutions

5.1. A single pass solution scheme

The presence of the factor $\bar{R}_b$ in the ODE for $\tilde{a}$ makes the solution of the BVP (41) and (42) much less straightforward. As $\bar{R}_b$ encapsulates the unknown concentrations of normal and enhanced signaling ligand–receptor complexes, it depends on the solutions of two BVPs over the entire span of the solution domain through the integrated condition (29). To the extent that there are reliable software for solving BVP in ODE, we may make use of these tools by reconfiguring the integro-differential equation problem for $\tilde{a}$ to a BVP for a system of ODEs.

For this purpose, we let $\bar{a}(x)$ and $\tilde{a}(x)$ be the unknown free (unbound) ligand concentration for a wild-type ligand synthesis rate $\bar{\nu}_L H(-x)$ and an ectopic synthesis rate $\bar{\kappa}\bar{\nu}_L H(-x)$, respectively, with the amplification factor $\bar{\kappa}$ to be specified (as previously done in the discussion of the robustness index $\bar{R}_b$). The wild-type concentration $\bar{a}(x)$ is determined by the BVP (41)–(42) with $e = 1$ and $c = 0$ so that

$$\bar{a}'' - \frac{g_0\bar{a}}{\alpha_0 + \zeta_0\bar{a}} - g_L\bar{a} + \bar{\nu}_L H(-x) = 0, \tag{48}$$

$$\bar{a}'(-x_m) = 0, \qquad \bar{a}(1) = 0. \tag{49}$$

Correspondingly, $\tilde{a}$ is determined by the BVP (41) and (42) and the integral condition (29) with $x_\ell = 1$, $x_h = 0$, and $b_\ell = 0$ so that

$$\bar{R}_b = \frac{1}{b_h}\sqrt{\int_0^1 [\tilde{b}(x;\bar{R}_b) - \bar{b}(x)]^2 dx}, \tag{50}$$

where

$$\tilde{b}(x;\bar{R}_b) = \frac{\tilde{a}(x;\bar{R}_b)}{\alpha_0 + \zeta_0\tilde{a}(x;\bar{R}_b)}, \qquad \bar{b}(x) = \frac{\bar{a}(x)}{\alpha_0 + \zeta_0\bar{a}(x)}. \tag{51}$$

As indicated previously, we take b_h to be given by (30) for systems of LRO (and $b_h = k_R/k_{\text{deg}}$ for less likely systems of high receptor occupancy).

For a single pass algorithm for the solution of our problem where the nonlinear relation (29) involves the unknown $\tilde{a}(x;\bar{\kappa}(R_b))$, we introduce two new functions to replace the integral relation (29). The first is the function $R_2(x)$ defined by

$$R_2' = \frac{1}{b_h^2}[\tilde{b}(x) - \bar{b}(x)]^2 = \frac{1}{b_h^2}\left(\frac{\tilde{a}(x)}{\alpha_0 + \zeta_0\tilde{a}(x)} - \frac{\bar{a}(x)}{\alpha_0 + \zeta_0\bar{a}(x)}\right)^2 H(x), \tag{52}$$

and the initial condition

$$R_2(-x_m) = 0. \tag{53}$$

With the Heaviside function $H(x)$ on the right hand side of (52), we may stipulate $R_2(x)$ to be continuous $x = 0$.

The second new function is $R_b(x)$ defined by

$$R_b' = 0 \tag{54}$$

specifying that it does not change with location and is therefore some (unknown) constant $\bar{R}_b$, that is, $R_b(x) = \bar{R}_b$. The two new functions are related by the integral condition (50) taken in the form

$$R_2(1) = \bar{R}_b^2. \tag{55}$$

In terms of the two new functions, we may rewrite (without altering the content of the ODE (36)) the BVP for $\tilde{a}$ as

$$\tilde{a}'' - \frac{g_0\tilde{a}}{\alpha_0 + \zeta_0\tilde{a}} - g_L\tilde{a} + \bar{\kappa}(R_b)\bar{v}_L H(-x) = 0, \tag{56}$$

$$\tilde{a}'(-x_m) = 0, \qquad \tilde{a}(1) = 0, \tag{57}$$

with

$$\bar{\kappa}(R_b) = \frac{2}{1 + cR_b}, \tag{58}$$

where we have taken $e = 2$ and $n = 1$ to be concrete (with c still to be specified). In this form, R_b is treated as a function of position $R_b(x)$.

Note that (56), (52) and (54) are three coupled ODE for the three unknowns $\tilde{a}(x)$, $R_2(x)$ and $R_b(x)$ to be solved simultaneously. It is a fourth order system with four auxiliary conditions given in (57), (53) and (55) with the latter taken in the form

$$R_2(1) = [R_b(1)]^2 . \tag{59}$$

Together, the BVP for the fourth-order system defined by (56), (52), (54), (57), (53), and (59) enables us to avoid having the global parameter $\bar{R}_b$ as an unknown to be determined by an integral on the yet unknown solutions of the two principal ODEs over the entire solution domain.

Adding to this the BVP defined by (48) and (49), we have a sixth order system for the four unknowns $\bar{a}(x)$, $\tilde{a}(x)$, $R_b(x)$, and $R_2(x)$. Such a BVP can be solved by computing software generally available on *MatLab*, *Mathematica*, and *Maple*. It should be noted however that a single pass solution algorithm for this problem requires the software to have the capability of handling a vanishing Jacobian in the linearization of the nonlinear BVP by some form of Newton's method.

5.2. An iterative algorithm

It is possible to avoid computing with vanishing Jacobians. Given the dependence of $\tilde{a}(x)$ (and hence $\tilde{b}(x)$) on $\bar{R}_b$, the relation (50) may be written abstractly as

$$\bar{R}_b = C(\bar{R}_b), \tag{60}$$

where

$$C(\bar{R}_b) = \frac{1}{b_h}\sqrt{\int_0^1 [\tilde{b}(x;\bar{R}_b) - \bar{b}(x)]^2 dx}. \tag{61}$$

Observe that $0 \le C(0) < 1$ and, for $0 \le c < 1$,

$$0 \le C(\bar{R}_b) < 1, \tag{62}$$

given $[\tilde{b}(x;\bar{R}_b) - \bar{b}(x)]/b_h < 1$ for $x > 0$.

A typical iterative solution scheme would start with some initial estimate $\bar{R}_0$ and calculate successive iterates $\bar{R}_k$ by the simple iteration

$$\bar{R}_{k+1} = C(\bar{R}_k) = \frac{1}{b_h}\sqrt{\int_0^1 \left[\tilde{b}(x;\bar{R}_k) - \bar{b}(x)\right]^2 dx}, \quad k = 0, 1, 2, 3, \ldots., \tag{63}$$

where $\tilde{b}(x;\bar{R}_k)$ is determined from the solution $\tilde{a}(x;\bar{R}_k)$ of the BVP (56)–(58) with $\bar{R}_b = \bar{R}_k$ in

$$\bar{\kappa}(\bar{R}_b) = \frac{2}{1 + c\bar{R}_k}. \tag{64}$$

The sequence $\{\bar{R}_k\}$ is guaranteed to converge to $\bar{R}_b$ if $C(\cdot)$ should be a contraction map, that is, if

$$\left|\frac{dC}{d\bar{R}_b}\right| < 1. \tag{65}$$

Rather than attempting to establish the contracting property of $C(\bar{R}_b)$, we show first that $\tilde{a}(x;\bar{R}_b)$ and $\tilde{b}(x;\bar{R}_b)$ are both decreasing functions of $\bar{R}_b$. The nonpositivity of the marginal change of $\tilde{b}(x;\bar{R}_b)$ with $\bar{R}_b$ is then used in

$$\frac{dC}{d\bar{R}_b} = \frac{1}{b_h^2 C}\int_0^1 [\tilde{b}(x;\bar{R}_b) - \bar{b}(x)]\frac{\partial\tilde{b}(x;\bar{R}_b)}{\partial\bar{R}_b}dx \tag{66}$$

to analyze the convergence of the iterative process (63).

Upon differentiating all relations in the BVP for $\tilde{a}(x;\bar{R}_b)$ partially with respect to $\bar{R}_b$, we obtain

$$-w'' + \left(\frac{\alpha_0}{\alpha_0 + \zeta_0\tilde{a}} + g_L\right)w - \frac{2\bar{v}_L}{\left(1 + \bar{R}_b\right)^2}H(-x) = 0, \tag{67}$$

$$w'(-x_m;\bar{R}_b)=0, \qquad w(1;\bar{R}_b)=0, \tag{68}$$

where

$$w(x;\bar{R}_b)=-\frac{\partial\tilde{a}(x;\bar{R}_b)}{\partial\bar{R}_b}.$$

Clearly, $w_\ell(x;\bar{R}_b)\equiv 0$ is a lower solution of the BVP for $u(x;\bar{R}_b)$ given

$$\begin{aligned}&-w_\ell''+\frac{\alpha_0(1+g_L)+g_L\zeta_0\tilde{a}}{\alpha_0+\zeta_0\tilde{a}}w_\ell-\frac{2\bar{v}_L}{\left(1+\bar{R}_b\right)^2}H(-x)\\&\quad=-\frac{2\bar{v}_L}{\left(1+\bar{R}_b\right)^2}H(-x)\le 0 \qquad (0\le x\le 1).\end{aligned}$$

As an upper solution, we have

$$w_u(x;\bar{R}_b)=\frac{2\bar{v}_L}{\left(1+\bar{R}_b\right)^2}\left\{\left(x_m+\frac{1}{2}\right)-x_m x-\frac{1}{2}x^2\right\}$$

with

$$-w_u''-\frac{2\bar{v}_L}{\left(1+\bar{R}_b\right)^2}=0.$$

Note that

$$\begin{aligned}&-w_u''+\left(\frac{\alpha_0}{\alpha_0+\zeta_0\tilde{a}}+g_L\right)w_u-\frac{2\bar{v}_L}{\left(1+\bar{R}_b\right)^2}H(-x)\\&\quad\ge -w_u''-\frac{2\bar{v}_L}{\left(1+\bar{R}_b\right)^2}H(-x)=\frac{2\bar{v}_L}{\left(1+\bar{R}_b\right)^2}[1-H(-x)]\ge 0,\end{aligned}$$

and

$$w_u'(-x_m;\bar{R}_b)=0, \qquad w_u(1;\bar{R}_b)=0.$$

The monotone method of [44] implies that $w(x;\bar{R}_b)$ exists, is unique, and nonnegative so that

$$-w_u(x;\bar{R}_b)\le\frac{\partial\tilde{a}(x;\bar{R}_b)}{\partial\bar{R}_b}\le 0.$$

This leads to the following proposition on the nonpositivity of the marginal value $\partial\tilde{b}(x;\bar{R}_b)/\partial\bar{R}_b$:

PROPOSITION 5. $\partial\tilde{b}(x;\bar{R}_b)/\partial\bar{R}_b\le 0.$

Table 1
Numerical Solutions by the Iterative Algorithm
$X_{\max} = 0.01$ cm, $X_{\min} = 0.001$ cm, $k_{on}R_0 = 0.01$ sec/μM,
$k_{deg} = 2 \times 10^{-4}$/ sec, $k_R = 0.001$/ sec, $k_{off} = 10^{-6}$/ sec, $k_L = 0$,
$D = 10^{-7}$ cm^2/ sec, $\bar{V}_L = 0.002$ μM/ sec, $\bar{V}_R = 0.04$ μM/ sec

c	$\bar{R}_k$	$\bar{R}_{k+1}$	$\bar{b}(0)$	$\tilde{b}(0;\bar{R}_k)$	$\tilde{b}(0;\bar{R}_{k+1})$	$\tilde{b}(0;0)$
1	0.24190	0.24051	0.05798	0.09327	0.09306	0.11533
2	0.18177	0.18296	0.05798	0.08451	0.08469	0.11533
4	0.11114	0.11163	0.05798	0.07422	0.07431	0.11533

Proof: Upon differentiating the expression for $\tilde{b}(x;\bar{R}_b)$ in (51) partially with respect to $\bar{R}_b$, we obtain

$$\frac{\partial \tilde{b}(x;\bar{R}_b)}{\partial \bar{R}_b} = \frac{\alpha_0}{(\alpha_0 + \zeta_0 \tilde{a})^2} \frac{\partial \tilde{a}(x;\bar{R}_b)}{\partial \bar{R}_b} \leq 0. \qquad \blacksquare$$

Together with (62) and $\tilde{b}(x;\bar{R}_b) > 0$, Proposition 5 implies $dC/d\bar{R}_b \leq 0$ as long as $[\tilde{b}(x;\bar{R}_b) - \bar{b}(x)] \geq 0$ (which is the case at the start of the iterative scheme). However, this does not make $\{\bar{R}_k\}$ a nonincreasing sequence (though bounded below by 0). If $\bar{R}_{k+1} < \bar{R}_k$, we would have $\tilde{a}(x;\bar{R}_{k+1}) > \tilde{a}(x;\bar{R}_k)$ and therewith $\bar{R}_{k+2} > \bar{R}_{k+1}$ (consistent with $dC/d\bar{R}_b \leq 0$). As such, we have a nonnegative sequence $\{\bar{R}_k\}$ alternately increasing and decreasing with successive iterations bounded below (by 0) and above (by $\bar{R}_b(c=0)$) offering the prospect of convergence. As we see from an illustrative example in the next section, the iterative scheme converges rapidly for $c = 1$ but the steady-state value found is unstable for $c \gg 1$.

5.3. An illustrative example

To gain some insight to the iterative algorithm for the steady-state value $\bar{R}_b$ of the robustness index, we apply it to the system characterized by the parameter values shown in Table 1. This system meets the condition (21) for a state of LRO and is further confirmed to be so by comparison of the exact numerical solution with that of the linearized model. The steady-state robustness index $\bar{R}_b$ is found after less than 10 iterations with less than 0.2% discrepancy between the 8th and 9th iterations as shown on the line for $c = 1$ in Table 1.

The quick convergence of the scheme for the particular example is gratifying. However, the biological implication is not as satisfying. Taking the average of the two iterates for $\bar{R}_k$ shown in the table gives a rather accurate numerical solution of $\bar{R}_b \simeq 0.24121\ldots$ for the steady-state robustness index. This is

above the acceptable threshold of $\bar{R}_b \leq 0.2$ set (arbitrarily) in [19] for robustness. While there is some flexibility in setting the reference value b_h and reinterpreting the new definition, a more serious issue is the magnitude and shape of the resulting steady-state signaling gradient.

Comparing the values of $\bar{b}(0)$ and $\tilde{b}(0;0)$ with the accurate numerical solution $\tilde{b}(0;\bar{R}_k)$ associated with the final acceptable iterate $\bar{R}_k$ shows that the solution with feedback is reduced but still closer to the corresponding enhanced concentration $\tilde{b}(0;0)$ than the concentration for the wild-type system. The same is true for the entire signaling region of the solution domain. Two questions suggest themselves: Is this the best we can do by the new feedback mechanism in the form (33) (and consequently robustness cannot be attained by such a mechanism)? If so, are there modifications that would lead to robustness? We examine possible answers to these questions in the following sections.

5.3.1. Wild-type and perturbed systems at LRO. For our example, the model system is in a state of LRO before and after ligand synthesis enhancement. In that case, accurate approximate solutions for $\tilde{a}(x)$ and $\bar{a}(x)$ can be obtained as was done previously for the model without feedback. Briefly, the ODE for $\tilde{a}(x)$ is linearized to give a linear equation for the approximate solution $a_0(x)$:

$$a_0'' - \mu_L^2 a_0 + \bar{\kappa}(\bar{r}_b)\bar{v}_L H(-x) = 0,$$
$$a_0'(-x_m) = 0, \qquad a_0(1) = 0,$$

where μ_L^2 is as given by (45) and where $\bar{R}_b$ in the expression (64) for $\bar{\kappa}$ is now replaced by the corresponding approximate expression $\bar{r}_b$ using the LRO approximate solution $b_0(x;\bar{r}_b)$ for $\tilde{b}(x;\bar{R}_b)$. The exact solution for this problem is given by (46) with

$$\alpha_0\tilde{b}(x;\bar{R}_b) \simeq a_0(x;\bar{r}_b) = \frac{\bar{\kappa}(\bar{r}_b)\bar{v}_L}{\mu_L^2}\frac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1+x_m))}\sinh(\mu_L(1-x)), \quad (69)$$

$$\alpha_0\bar{b}(x) \simeq [a_0(x)]_{\bar{\kappa}=1} = \frac{\bar{v}_L}{\mu_L^2}\frac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1+x_m))}\sinh(\mu_L(1-x)), \quad (70)$$

for the signaling region $0 \leq x \leq 1$.

5.3.2. $\bar{R}_b$ at LRO. The expressions (69) and (70) for the enhanced and wild-type normalized signaling morphogen gradients, $\tilde{b}(x;\bar{R}_b)$ and $\bar{b}(x)$, in the range relevant for cell signaling are to be used, respectively, in the expression (50) as was done in (32) to obtain for an LRO system

$$\bar{R}_b \simeq \bar{r}_b(c) = \gamma(\mu_L)\left[\frac{2}{1+c\bar{r}_b} - 1\right] \quad (71)$$

with

$$\gamma(\mu_L) = \frac{1}{\sqrt{2}\sinh(\mu_L)}\sqrt{\left(\frac{\sinh(2\mu_L)}{2\mu_L} - 1\right)}. \tag{72}$$

For $c = 0$ (corresponding the case of no feedback), we have immediately

$$\left[\bar{R}_b\right]_{c=0} \simeq \bar{r}_b(0) = \gamma(\mu_L),$$

which is $0.3938\ldots..$ for our example (as already reported in the discussion following (32)) while accurate numerical solution by the iterative algorithm of the previous section gives $\bar{R}_b = 0.3939\ldots$

For $0 < c < \infty$, the relation (71) may be written as the quadratic equation

$$c\bar{r}_b^2 + (1 + c\gamma)\bar{r}_b - \gamma = 0 \tag{73}$$

for $\bar{r}_b$ with one positive solution

$$\bar{R}_b \simeq \bar{r}_b = \frac{1}{2c}\left[-(1 + c\gamma) + \sqrt{(1 + c\gamma)^2 + 4\gamma}\right] > 0. \tag{74}$$

For the problem specified by the parameter values in Table 1 and $c = 1$, such a feedback process gives

$$\left[\bar{R}_b\right]_{c=1} \simeq \bar{r}_b(1) = 0.24108\ldots, \tag{75}$$

which is nearly identical to the average $0.24160\ldots$ of the 8th and 9th iterates found earlier for $\bar{R}_b(c = 1)$. As such, $\bar{R}_b(c = 1) \simeq 0.24121\ldots$ (together with a corrected signaling gradient that is closer to the perturbed gradient than the unperturbed one) is the best the feedback (33) with $c = 1$ can attain.

5.3.3. Modification for a more effective feedback process. To improve on the feedback mechanism toward robustness of signaling gradients, we note that a larger value of c in the expression (33) would reduce the enhanced synthesis rate to result in a lower concentration level of $\tilde{a}(x; \bar{R}_b)$ and $\tilde{b}(x; \bar{R}_b)$. This in turn should lead to a smaller robustness index $\bar{R}_b(c)$ as we would like to have. This expectation is easily proved for systems in a state of LRO by differentiating the relation (73) with respect to c to get for $c > 0$:

$$\frac{d\bar{r}_b}{dc} = -\frac{\gamma - \bar{r}_b}{c(1 + c\gamma + 2c\bar{r}_b)} < 0,$$

given $\bar{r}_b > 0$ for $c > 0$. The same result can be established for gradient systems of more general receptor occupancy:

PROPOSITION 6. $d\bar{R}_b/dc < 0$ *for* $c > 0$.

Proof: We first prove

$$\partial\tilde{a}(x; \bar{R}_b(c))/\partial c < 0, \qquad \partial\tilde{b}(x; \bar{R}_b(c))/\partial c < 0,$$

using the approach for proving

$$\partial \tilde{b}(x; \bar{R}_b)/\partial \bar{R}_b < 0,$$

when c was set equal to 1. The proposition follows from

$$\frac{d\bar{R}_b}{dc} = \frac{1}{b_h^2 \bar{R}_b(c)} \int_0^1 [\tilde{b}(x; \bar{R}_b(c)) - \bar{b}(x)] \frac{\partial \tilde{b}(x; \bar{R}_b(c))}{\partial c} dx,$$

given $[\tilde{b}(x; \bar{R}_b(c)) - \bar{b}(x)] > 0$ for $0 < c < \infty$. ■

From (74), we have

$$\lim_{c \to \infty} \bar{R}_b(c) = 0.$$

Thus by choosing c sufficiently large, we should be able to reduce the robustness index and scale down $\tilde{b}(x; \bar{R}_b(c))$ to be close to $\bar{b}(x)$. The results for such an effort for $c = 2$ and $c = 4$ are reported in the last two rows of Table 1. For $c = 4$, not only is $\bar{R}_b$ ($\simeq 0.111385\ldots$) well below the robustness threshold of 0.2, the feedback-adjusted normalized signaling ligand concentration ($\simeq 0.074265\ldots$) at $x = 0$ is now much closer to the wild-type concentration ($\simeq 0.05798\ldots$) than the enhanced concentration ($\simeq 0.11533\ldots$) without feedback. In fact, the feedback adjusted gradient $\tilde{b}(x; \bar{R}_b(c))$ (not shown here) is also closer to $\bar{b}(x)$ than $\tilde{b}(x; 0)$ for all $x > 0$.

Note that a large c value also has the effect of changing $\bar{R}_b(c)$, and therewith $\tilde{b}(x; \bar{R}_b(c))$, more drastically from iteration to iteration. The larger swings would make the iterative scheme more erratic. Experiments on specific examples show a rather slow convergence for a straightforward application of the iterative algorithm (63). Some judicious modifications of the algorithm have led to faster convergence to the limiting value for $\bar{R}_b$.

5.3.4. A Hill function-type modification. For comparison, we consider here a different kind of feedback process on the synthesis rate for the steady-state behavior of the form

$$\hat{\kappa} = \frac{2}{1 + [\phi(x)]^2}, \qquad \phi = \frac{1}{b_h} \left[\tilde{b}(x; 0) - \bar{b}(x)\right]. \tag{76}$$

This Hill function-type feedback is spatially nonuniform and provides a crude model for a delay feedback with effects quickly reaching a steady state. The steady-state solution of the morphogen system with such a feedback correction gives $\hat{R}_b = 0.2050885\ldots$ and $\hat{b}(0) \simeq 0.0879065\ldots$.

While the results are slightly better than those by the spatially uniform feedback (58), the two corresponding signaling gradients $\tilde{b}(x; \bar{R}_k)$ and $\hat{b}(x)$ are not significantly different. More importantly, the comparison would favor the spatially uniform feedback if the enhanced synthesis rate should induce a receptor saturated state. For then, the signaling gradient resulting from (76)

would be more concave and biologically less differentiating for the purpose of differential cell fates similar to the results found in (18). Further comparison between spatially uniform and nonuniform feedback processes should be more properly investigated.

5.4. *Delay time long compared to time to steady state*

In this section, we consider the extreme case of a feedback delay time τ being substantially longer than the time t_∞ for the ligand system to reach its steady-state behavior, for example, $\tau = 2t_\infty$. (For this purpose, we take $g_0 t_\infty = 10$ with $\lambda_0 \gtrsim g_0$ being the smallest decay rate constant for a transient to decrease exponentially in time as shown in the discussion of linear stability in [14].) For this case, the various concentrations would already be in a time-independent steady state at $(k-1)\tau \ll t \leq k\tau \equiv t_k$ for $k = 1, 2, 3, \ldots$. with $\tilde{a}^{(k)}(x; \bar{R}_b^{(k)})$ determined by (41) and (42) for an appropriate time-independent synthesis rate amplitude factor $\bar{\kappa}$ adjusted for the effect of feedback determined by the solution of the corresponding BVP for the previous interval as described below.

With $\bar{R}_b^{(0)} = 0$ (because feedback has not become effective for $0 \leq t \leq \tau$), the steady-state solution $\tilde{a}^{(1)}(x)$ appropriate for t near τ (say $0 \ll t \leq \tau$) is found by solving the BVPs (41) and (42) with

$$\bar{\kappa} = \bar{\kappa}^{(1)} = \frac{2}{1 + c\bar{R}_b^{(0)}}. \tag{77}$$

The solution can be computed by solving (41)–(42) with (78) and the corresponding $\tilde{b}^{(1)}(x; \bar{R}_b^{(0)})$ can be obtained by the relation (39) to be used in

$$\bar{R}_b^{(1)} = \frac{1}{b_h}\sqrt{\int_0^1 [\tilde{b}^{(1)}(x; \bar{R}_b^{(0)}) - \bar{b}(x)]^2 dx}, \tag{78}$$

for the determination of the unknown steady-state robustness index $\bar{R}_b^{(1)}$ for the interval $0 \ll t \leq \tau$.

Near the end of the interval $[\tau, 2\tau]$, the steady-state solution $\tilde{a}^{(2)}(x)$ appropriate for $\tau \ll t \leq 2\tau$ is again determined by (41) and (42) but now with

$$\bar{\kappa} = \bar{\kappa}^{(2)} = \frac{2}{\left(1 + c\bar{R}_b^{(1)}\right)\left(1 + c\bar{R}_b^{(0)}\right)}.$$

With $\tilde{a}(x; \bar{\kappa}^{(1)}) = \tilde{a}^{(1)}(x)$ having reached its steady state for t near τ, say $0 \ll t \leq \tau$, the effect of feedback is felt permanently during the next time period $\tau \ll t \leq 2\tau$ through a reduction of the synthesis rate so that the actual

synthesis rate should be

$$v_L(x,t) = \frac{2}{\left(1 + c\bar{R}_b^{(1)}\right)} \bar{v}_L H(-x) \equiv \bar{\kappa}^{(1)} \bar{v}_L H(-x), \tag{79}$$

until there is a further change at the start of the next time interval $[2\tau, 3\tau]$. The effect of the feedback in the next period would be to further reduce this modified synthesis rate.

The solution for $\tilde{a}(x;\bar{\kappa}^{(2)}) = \tilde{a}^{(2)}(x)$ enables us to compute $\tilde{b}(x;\bar{\kappa}^{(2)}) = \tilde{b}^{(2)}(x)$ and therewith

$$\bar{R}_b^{(2)} = \frac{1}{b_h} \sqrt{\int_0^1 \left[\tilde{b}^{(2)}(x) - \bar{b}(x)\right]^2 dx}. \tag{80}$$

With $\bar{R}_b^{(1)}$ already known from (78) for the previous interval, (80) determines $\bar{R}_b^{(2)}$ to be used for adjusting the ligand synthesis rate in the next interval $[2\tau, 3\tau]$.

In general, near the end of the interval $[(k-1)\tau, k\tau]$, the steady-state solution $\tilde{a}^{(k)}(x)$ appropriate for $(k-1)\tau \ll t \leq k\tau$ is again determined by (41) and (42) but with

$$\bar{\kappa} \sim \bar{\kappa}^{(k)} = \frac{2}{\left(1 + c\bar{R}_b^{(1)}\right)\left(1 + c\bar{R}_b^{(2)}\right) \cdots \left(1 + c\bar{R}_b^{(k-1)}\right)}. \tag{81}$$

and with

$$\bar{R}_b^{(k)} = \frac{1}{b_h} \sqrt{\int_0^1 \left[\tilde{b}^{(k)}(x) - \bar{b}(x)\right]^2 dx}. \tag{82}$$

The quantity $\bar{R}_b^{(k)}$ is the (steady-state) robustness index for $(k-1)\tau \ll t \leq k\tau$.

5.4.1. LRO approximation. For systems at LRO, the steady-state solution for successive time intervals $(k-1)\tau \ll t \leq k\tau$, $k = 1, 2, 3 \ldots$ can be written down explicitly. This will be carried out for $c = 1$ later.

For $k = 1$, we have $\bar{R}_b^{(1)} \sim [\bar{r}_b]_{c=1}$ and correspondingly $\bar{\kappa}^{(1)} \sim \bar{\kappa}_1$ for $0 \ll t \leq \tau$, where $\bar{r}_b(c)$ is as given by

$$\bar{R}_b^{(1)} \sim \bar{r}_b^{(1)} = \gamma(\bar{\kappa}^{(1)} - 1) = \gamma,$$

since $\bar{r}_b^{(0)} = \bar{R}_b^{(0)} = 0$ and $\bar{\kappa}^{(1)} = \bar{\kappa}_1 = 2$.

Similar calculations lead to the following results for $(k-1)\tau \ll t \leq k\tau$ for general k:

$$\bar{R}_b^{(k)} \sim \bar{r}_b^{(k)} = (\bar{\kappa}^{(k)} - 1)\gamma$$

$$\tilde{b}^{(k)}(x) \sim b_0(x; r_b^{(k)}) = b_0^{(k)}(x) = \frac{\bar{\kappa}_k \bar{v}_L}{\alpha_0 \mu_L^2} \frac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1 + x_m))} \sinh(\mu_L(1 - x))$$

for $0 \le x \le 1$, where

$$\bar{\kappa}^{(k)} \simeq \bar{\kappa}_k = \frac{2}{\left(1+\bar{r}_b^{(1)}\right)\cdots\left(1+\bar{r}_b^{(k-1)}\right)}.$$

With $\bar{\kappa}^{(1)} = \bar{\kappa}_1 = 2$, it is straightforward to prove by induction the following proposition

PROPOSITION 7. $\bar{\kappa}_{k+1} = \dfrac{2}{2-(1-\gamma)^k}, \quad \bar{r}_b^{(k+1)} = \dfrac{\gamma(1-\gamma)^k}{2-(1-\gamma)^k}$

It follows that $\bar{\kappa}_k \to 1$ and $\bar{r}_b^{(k)} \to 0$ as $k \to \infty$ so that $b_0^{(k)}(x)$ tends to $\bar{b}(x)$ for sufficiently large k.

5.4.2. Numerical results. Accurate numerical results for $\bar{R}_b^{(k)}$ and $\tilde{b}^{(k)}(x)$ obtained by solving (41)–(42) numerically without the LRO approximation generally show that $\bar{r}_b^{(k)}$ and $b_0^{(k)}(x)$ are accurate approximations of the corresponding $\bar{R}_b^{(k)}$ and $\tilde{b}_0^{(k)}$. As such, robustness generally is achieved eventually (e.g., for $t \ge 3\tau$ in the case of the illustrative example of Table 1). Biologically, cell differentiation would have been completed after $k\tau$ for a relatively small k. Even so, numerical solutions computed suggest that feedback with a long delay is more likely to lead to robust signaling gradients if the time to cell differentiation is considerably longer than the delay time.

6. Other new feedback mechanisms

Though the conventional Hill's function-type negative feedback on receptor synthesis rate proves to be ineffective against signaling gradient distortion [19, 18, 11], the results of previous sections suggest that some feedback mechanisms may still be effective. Below are some additional possible spatially uniform feedback controls on a number of known regulatory processes that may promote robust signaling. The corresponding spatially nonuniform feedback will be discussed elsewhere.

6.1. Positive feedback on ligand degradation

It has been observed that introduction of polypeptide *noggin* (encoded by the *NOG gene*) binds and inactivates members of the transforming growth factor-beta (TGF-beta) superfamily signaling proteins, such as *bone morphogenetic proteins* (BMPs). At the same time, an ectopic concentration of BMP causes significant upregulation of Sox9 and Noggin expression [21–23]. By-passing the processes of ligand upregulating noggin expression, we could model, as it was done earlier in this paper, this reduction of available BMP molecules very crudely by a negative feedback on ligand synthesis rate. In that

case, the results of the previous sections apply. A somewhat more biologically realistic feedback process would be a positive feedback on the degradation rate constant k_L as the reduction of ligand pertain to its concentration (and not its synthesis rate). For such a model, we would keep the ligand synthesis rate for both the wild-type and perturbed system unaffected by the robustness index, but now with a positive feedback on the (free) ligand degradation rate. Such a positive feedback control may be taken in the form:

$$k_L = \frac{\bar{k}_l R_b(t-\tau)}{1 + R_b(t-\tau)}$$

with $k_L = 0$ when $\bar{R}_b = 0$ given that ligand degradation is normally receptor-mediated. The effect of such a feedback loop is being investigated.

6.2. Negative feedback on the signaling complex binding rate

It is also known that overexpression of Dad (*Daughters against dpp*) blocks Dpp signaling activity (as seen from a lack of dpp target gene *optomotor blind* [omb]) and there is a negative feedback circuit in which Dpp induces expression of its own antagonist, Dad [33; 35]. (A similar observation has been made on the BMP antagonist Chordin 24; 25.) One important signaling activity that affects signaling gradient is the binding rate of the ligand with its signaling receptor. For a possible model of this feedback loop, we may take

$$k_{on} = \frac{\bar{k}_{on}}{1 + cR_b(t-\tau)},$$

again with both wild-type and ectopic synthesis rate unaffected by the robustness index. Some interesting outcome from an investigation of such a negative feedback mechanism will be reported separately.

6.3. Positive feedback on the nonreceptor synthesis rate

Certain nonreceptors molecules such as Dally (*division abnormally delayed*) [47], FST (*follistatin* ([26–29], Sog (*short gastrulation*) [48; 30], and various *heparan sulfate proteoglycans* [49] bind members of the BMP family and prevent their interactions with signaling receptors, thereby inhibiting its signaling. In fact, the previously mentioned BMP antagonists noggin and chordin [23; 35] may be considered playing a similar role in BMP signaling. As such, they may be seen as siphoning off the relevant morphogen to reduce its concentration and activity in the extracellular space and are members of the so-called “nonreceptors,” effective agents for reducing ectopic signaling morphgen concentrations [19]. At the same time, the expression of these nonreceptors is often stimulated and upregulated by BMP (see [22] for example). This may be accomplished by a positive feedback on the nonreceptor

synthesis rate $V_N(X, T)$ (see [42]) in the form

$$V_N(X, T) = \bar{V}_N \left[1 + \frac{c R_b(t - \tau)}{1 + R_b(t - \tau)} \right] \equiv \kappa_N \bar{V}_N. \tag{83}$$

Spatially uniform and nonuniform feedback mechanisms are being investigated [40].

6.4. Other known signaling inhibiting processes

Other possible feedback controls may come from modeling the following experimental observations:

- Dpp represses the synthesis of its own receptor Tkv, which in turn enhances Dpp destruction [50].
- Wingless (Wg) represses its signaling receptor DFz2 but Dpp signaling mediated by DFz2 leads to stabilization of Wg rather than degradation [31].
- Positive feedback on receptor-mediated degradation rate [34].
- Dlp (Dally-like) has opposite effects at high and low levels of Wingless. Dlp promotes low-level Wingless activity but reduces high-level Wingless activity [51].

In reality, robust signaling gradients in the presence of genetic and epigenetic changes are likely to be the consequences of a combination of different feedback activities including those mentioned above. However, appropriate feedback processes may well depend on the existing ligand and signaling receptor concentration. This is illustrated by how the effects of Dlp on Wg activity depend of the level of Wg concentration [51]. Another example is the different outcomes from an enhanced nonreceptor concentration observed in [50] and [31] (see also [12]).

7. Concluding remarks

Robustness with respect to an ectopic signaling gradient resulting from genetic or epigenetic perturbations requires one or more signaling-inhibiting agents to be stimulated (by the enhanced signaling morphogen concentration) and upregulated above their normal level. This means the existence of some kind of feedback process in order to promote robustness. Feedback has long been seen as a mechanism for maintaining stable developments and specific feedback loops have been identified in the morphogen literature such as [5, 35–38], and elsewhere. Though the conventional Hill function-type negative feedback on receptor synthesis rate proves to be ineffective for this purpose [19; 18; 11], we have shown in this paper that a spatially uniform feedback process based on a spanwise average of excess signaling can play such a role. With the two algorithms developed for the solution of specific integro-differential equation

system for such a feedback mechanism, the results obtained confirm that at least one such feedback mechanism can be effective for ensuring robustness and suggest that many other effective feedback mechanisms are also possible and should be investigated.

Among the possible agents for achieving robustness that appear biologically realistic, nonreceptors appear to be ubiquitous for downregulating signaling. Such down-regulation has already been observed and investigated theoretically in [11, 20]. Research on feedback processes for up-regulating nonreceptors should be a high priority item. At the same time, other mechanisms for down-regulating ectopic signaling are also known to exist. As such, feedback controls other than nonreceptor-based process also require our attention. The matter is further complicated by the fact that there are more than one feedback processes for modeling the effect of each of these inhibiting agents and that the effects of a particular mechanism may vary depending on existing conditions. All these observations suggest that feedback as a mean for promoting and attaining robustness of biological developments constitutes a rich area for theoretical and empirical research. Some additional findings from our investigation of this highly complex phenomenon will be reported in [40] and elsewhere.

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St. Olaf College
University of California at Irvine
University of California at Irvine

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