Received: 19 October 2018

DOI: 10.1111/sapm.12258

WILEY STUDIES IN APPLIED MATHEMATICS

ORIGINAL ARTICLE

The necessity and benefits of multiple feedback for robust biological development

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Funding information
The research was supported in part by NIH Grants R01GM067247 and P50-GM076516 and by NSF Grant DMS-1129008. The R01 was awarded through the Joint NSF/NIGMS Initiative to support Research in the Area of Mathematical Biology. The author is also a member of the Center for Complex Biological Systems and the Center for Mathematical and Computational Biology at UC Irvine.

Abstract

Robust development of biological organisms is known to involve a variety of inhibitors whose specific roles in downregulating the undesirably excessive signaling activities are reasonably well understood. Empirical evidence points to existence of feedback mechanisms for upregulating inhibitory agents. At least two conventional feedback models have been found by model analysis to be ineffective or biologically inappropriate. In this paper, we explore more fully the recently formulated new type of regulatory feedback for robustness to examine all such feedback processes possible in a three-component basic extracellular model for ligand signaling. Acting alone, all processes will again be shown to be ineffective for promoting robustness. The principal finding of the present theoretical analysis is that a concurrent implementation of some combinations of two of these feedback processes actually renders the signaling gradient robust with respect to genetic and epi-genetic perturbations. Moreover, different multifeedback combinations achieve robustness in different ways. These findings provide a possible explanation for the concurrent presence of several such individually ineffective feedback processes in robust signaling gradients.

KEYWORDS

developmental biology, feedback, mathematical biology, robustness

1 | INTRODUCTION

Development of biological organisms through appropriate signaling morphogen (aka ligand) gradients[1–3] is mostly insensitive (robust) to genetic and/or epigenetic perturbations that lead to

abnormally enhanced or reduced expression of signaling ligand. Recent experimental evidence observed by Zhou in Lander's lab (see also Refs. 4 and 5) shows that synthesis rate of *decapentaplegic* (Dpp), a member of the *bone morphogen protein* (BMP) family responsible for the development of Drosophila wing imaginal disc, doubles when the ambient temperature is raised by 5.9°C. Such an increase in Dpp synthesis rate is expected to result in signaling gradients qualitatively different from that at the normal ambient temperature[6–9] (see also Section 2.2). Yet, little abnormality in the development of the wing imaginal disc (possibly size-normalized[4]) is observed under such a change in ambient temperature.[10–13] In effect, Dpp-mediated tissue patterning of the Drosophila wing is substantially robust to a significant increase in Dpp synthesis rate.

To be concrete, we focus herein on promotion of robustness with respect to *ectopic* signaling ligand expression of the enhancement (and not reduction) type. A variety of inhibitors for reducing such ectopic signaling activities are known to exist (see Refs. 14–33 for examples) and their roles in downregulating the undesirable ectopic activities have been investigated theoretically in Refs. 34–40 and references therein. However, just how a developing organism manages to upregulate inhibition in response to genetic and epigenetic perturbations remains largely not understood.

Adjustment for ectopic signaling activities is expected to require the presence of one or more feedback mechanisms to stimulate the needed level of inhibition. Existing empirical evidence to support this expectation can be found in Refs. 41–46 for examples. Specific feedback processes identified in the literature include:

- High levels of BMP-2 cause significant upregulation of Sox9 and Noggin expression.[44,45,47]
- 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.[43]

At the same time, computational and mathematical evidence also exists showing some possible feedback processes to be ineffective or biologically inappropriate. For example, numerical simulations performed in Ref. 36 show that a Hill function-type negative feedback on receptor synthesis rate does not by itself promote robustness. That conclusion was proved mathematically in Ref. 48 where some insight was gained on the reason for its ineffectiveness and strongly suggests that some form of nonlocal feedback may be responsible for promoting robustness instead. A self-enhanced ligand degradation-type feedback was suggested in Refs. 49 and 50 and found to be effective toward robustness. However, ligand degradation is receptor mediated in many developments. For such systems, self-enhanced degradation of (unbound) ligands would not be the appropriate feedback for robustness.

Downregulation of signaling activities is known to be accomplished in different ways. Whether it is through more inhibitors, more nonreceptors, or higher degradation rate of free or bound ligands, the net effect is a lower concentration of free ligand available for binding with signaling receptors. To initiate a nonlocal approach to feedback, a proof of concept effort was made in[51] to investigate a negative feedback induced by a signaling robustness index $R_b(t)$ to downregulate the ligand synthesis rate V_L. The feedback instrument $R_b(t)$, previously defined in[4,36,51] (see (26)-(29) below) is a root-mean-square measure of the deviation from the wild-type signaling gradient over the span of the gradient at time t. It was shown in[51] that robustness of development can be attained in the steady state with such a feedback. More importantly, the effect of the feedback on ligand synthesis rate succeeded in reducing the level of ectopicity of the signaling ligand gradient both in its magnitude and shape (unlike a Hill function-type feedback that distorts the signaling gradient shape and thereby works against robustness).

To the extent that feedback often does not reduce the ligand synthesis rate directly, we explore in this article the consequences of other possible feedback processes in the three-component basic extracellular model[7] and show that each by itself is ineffective in promoting signaling gradient robustness. Their

concurrent presence in developing biological organisms is then shown to result in possible benefits toward robust signaling associated with strategic combinations of these normally ineffective feedback processes.

2 | BASIC MODEL FOR MORPHOGEN GRADIENT FORMATION

2.1 | The mathematical model

In this paper, we focus on a typical one-dimensional extracellular space model first introduced and analyzed in Ref. 7 for *Dpp* gradient formation in the posterior compartment of a *Drosophila* wing imaginal disc (see Ref. 4 for a geometrical configuration of this disc model). The model was extended in Ref. 51 to investigate a new type of feedback for the robustness of such gradients. The model is generally applicable or helpful in gaining insight to other morphogen gradient systems with similar characteristics. The idealization of activities in the wing imaginal disc leading to this basic model has already been described.[6,7] Here, we only state the dimensionless form of the *initial-boundary value problem* (IBVP) for the model consisting of the three differential equations:

$$\frac{\partial a}{\partial t} = \frac{\partial^2 a}{\partial x^2} - h_0 ar + f_0 b - g_L a + v_L(x,t), \tag{1}$$

$$\frac{\partial b}{\partial t} = h_0 ar - (f_0 + g_0)b, \qquad \frac{\partial r}{\partial t} = v_R(x) - h_0 ar + f_0 b - g_R r, \tag{2}$$

for the dimensionless concentrations, a, r, and b, of free morphogen (eg, *Dpp* in the Drosophila wing imaginal disc), unoccupied (signaling) receptors (eg, *Thickveins* (*Tkv*) for *Dpp* in Drosophila wing disc), and bound (signaling) morphogen (eg, the Dpp-Tkv complexes), respectively, all normalized by the initial steady-state receptor concentration R_0 (at the onset of ligand synthesis at time $t = 0$). The one physical space variable X (the span in the distal direction from the edge of the ligand synthesis zone $X = 0$ in the case of the Drosophila wing imaginal disc) is normalized by its maximum span $X_{\max}$ so that $x = X/X_{\max}$ is the normalized spatial variable. The time variable T and the various rate constants $\{k_{\text{on}}R_0, k_{\text{off}}, k_L, k_{\text{deg}}, k_R\}$ for binding, dissociation, free ligand degradation, bound ligand degradation, and unoccupied receptor degradation as well as the (per receptor) ligand synthesis rate V_L/R_0 are all normalized by the same *time constant* $D/X_{\max}^2$,

$$\{t, h_0, f_0, g_L, g_0, g_R, v_L\} = \frac{X_{\max}^2}{D}\{T, k_{on}R_0, k_{off}, k_L, k_{\text{deg}}, k_R, V_L/R_0\}, \tag{3}$$

where D is the diffusion coefficient for the diffusive ligand concentration.

While the distribution of signaling receptors is generally not uniform throughout the extracellular space (with *Tkv* level higher in cells further away from the source of *Dpp* in the wing imaginal disc, for example, Ref. 23), we take the receptor synthesis rate $\bar{V}_R$ to be invariant in both time and space with

$$v_R(x) = \bar{v}_R = \frac{\bar{V}_R/R_0}{D/X_{\max}^2}, \tag{4}$$

to simplify analysis. As unoccupied receptors are seen to degrade at a rate proportional to its concentration with the degradation rate constant k_R, the receptor concentration $r(x,t)$ should be in a steady state for $t < 0$ determined by the second equation in (2) to be

$$\bar{v}_R = g_R r(x,0) = g_R, \tag{5}$$

since $r(x,0) = R(x,0)/R_{x,0} = 1$. It follows from (4) and (5) that

$$R_0 = \frac{\bar{V}_R}{k_R}. \tag{6}$$

For *Dpp* in the Drosophila wing imaginal disc synthesized only in a narrow production region $(-X_{\min}, 0)$ at a rate (usually) uniform in time, we set

$$v_L(x,t) = e\bar{v}_L H(-x) = \frac{e\bar{V}_L/R_0}{D/X_{\max}^2} H(-x) = \begin{cases} e\bar{v}_L & (-x_m \le x < 0). \\ 0 & (0 < x \le 1) \end{cases} \tag{7}$$

The constant e is an *enhancement (ectopicity) factor*; normally equal to 1 but may take on other values due to environmental changes.

The three differential equations are augmented by the following two (normalized) boundary conditions

$$x = -x_m : \frac{\partial a}{\partial x} = 0, \qquad x = 1 : a = 0, \tag{8}$$

all for $t > 0$. For the wing imaginal disc, the no flux condition at the compartment border is a consequence of assumed symmetry of the two (anterior and posterior) compartments of the wing disc. The kill end condition at the edge, $X = X_{\max}$, reflects the assumption of an absorbing end. Until morphogen being generated at $t = 0$, the biological system is in quiescence so that we have the (normalized) initial conditions

$$t = 0 : a = b = 0, \qquad r = 1, \tag{9}$$

keeping in mind $\bar{v}_R = g_R$ (see (5)).

The IBVP defined by (1), (2), (8), and (9) has been analyzed mathematically and computationally in Refs. 7 and 37 and elsewhere.

2.2 | Time-independent steady-state behavior

Given that both the ligand and receptor synthesis rates are time-independent, the diffusive IBVP of our model is known to tend to a unique time-independent steady state,

$$\{\bar{a}_e(x), \bar{b}_e(x), \bar{r}_e(x)\} = \lim_{t\to\infty} \{a(x,t), b(x,t), r(x,t)\}, \tag{10}$$

which is linearly stable with respect to a small perturbation from the steady state. The subscript "e" indicates the level of ectopicity with $e = 1$ corresponding to the wild-type (normal) gradient. This steady-state solution is determined by the following two-point BVP for $\bar{a}_e(x)$:

$$\bar{a}_e'' - \frac{g_0 \bar{a}_e}{\alpha_0 + \zeta_0 \bar{a}_e} - g_L \bar{a}_e + e\bar{v}_L H(-x) = 0, \tag{11}$$

$$\bar{a}_e'(-x_m) = 0, \qquad \bar{a}_e(1) = 0, \tag{12}$$

where

$$\alpha_0 = \frac{f_0 + g_0}{h_0}, \quad \zeta_0 = \frac{k_{\text{deg}}}{k_R} = \frac{g_0}{g_R}, \quad ()' = \frac{d()}{dx}. \tag{13}$$

The corresponding signaling morphogen concentration and unoccupied (signaling) receptor concentration are given in terms of $\bar{a}_e(x)$ by

$$\bar{b}_e(x) = \frac{\bar{a}_e(x)}{\alpha_0 + \zeta_0 \bar{a}_e(x)}, \qquad \bar{r}_e(x) = \frac{\alpha_0}{\alpha_0 + \zeta_0 \bar{a}_e(x)}, \tag{14}$$

keeping in mind (5). The reduction to (11)-(14) and the proof of well-posedness, monotonicity, and nonnegativity of the solution of the BVP (11)–(12) can be found in Ref. 7.

For completeness, we give here a proof of the following intuitively expected inequality:

Proposition 1. *$\bar{a}_e(x) \geq \bar{a}_1(x)$ for $e > 1$.*

Proof. The proof below for this proposition will serve as a prototype for related inequalities in subsequent development. The BVP for $\bar{a}_e(x)$ and $\bar{a}_1(x)$ may be combined to give the following relation for $\Delta(x) = \bar{a}_e(x) - \bar{a}_1(x)$:

$$\Delta'' - \frac{g_0 \Delta}{\left(\alpha_0 + \zeta_0 \bar{a}_1\right)\left(\alpha_0 + \zeta_0 \bar{a}_e\right)} - g_L \Delta + f(x) = 0, \qquad \Delta'(-x_m) = \Delta(1) = 0, \tag{15}$$

where

$$0 \leq f(x) = (e-1)\bar{v}_L H(-x) \leq (e-1)\bar{v}_L. \tag{16}$$

The existence, uniqueness, nonnegativity, and monotonicity of the solution $\Delta(x)$ of the linear BVP (15) can be proved by the method of upper-lower solution[52–54] used in Ref. 7. We mention here only that the lower and upper solutions, $\Delta_\ell(x)$ and $\Delta_u(x)$, needed in the proof may be taken to be $\Delta_\ell(x) = 0$ and

$$\Delta_u(x) = (e-1)\bar{v}_L \left\{ \left(x_m + \frac{1}{2} \right) - x_m x - \frac{1}{2} x^2 \right\} \geq 0, \tag{17}$$

respectively, for $-x_m \leq x \leq 1$. With $0 = \Delta_\ell(x) \leq \Delta(x) \leq \Delta_u(x)$, we have $\Delta(x) \geq 0$ as we wish to prove. ■

The corresponding inequality for the signaling gradients, $\bar{b}_1(x) \leq \bar{b}_e(x)$, is assured by the following corollary of Proposition 1:

Corollary 1. *$\bar{b}_1(x) \leq \bar{b}_e(x)$ $(-x_m \leq x \leq 1)$.*

Proof. The claim follows from

$$\bar{b}_e(x) - \bar{b}_1(x) = \frac{\alpha_0(\bar{a}_e - \bar{a}_1)}{(\alpha_0 + \zeta_0 \bar{a}_1)(\alpha_0 + \zeta_0 \bar{a}_e)},$$

because the right-hand side is nonnegative by Proposition 1 and the nonnegativity of $\bar{a}_e(x)$ and $\bar{a}_1(x)$. ■

2.3 | Low receptor occupancy

The nonlinear BVP (11)-(12) may be linearized if $\zeta_0 \bar{a}_e \ll \alpha_0$ to get an approximate set of solutions $\{\bar{A}_e(x), \bar{B}_e(x), \bar{R}_e(x)\}$ determined by

$$\bar{A}_e'' - \mu_L^2 \bar{A}_e + e\bar{v}_L H(-x) = 0, \qquad \mu_L^2 = \frac{g_0}{\alpha_0} + g_L \equiv \mu_0^2 + g_L, \tag{18}$$

$$\bar{A}_e'(-x_m) = 0, \qquad \bar{A}_e(1) = 0. \tag{19}$$

The exact solution of the linear BVP above has already been obtained in Ref. 7 and elsewhere. For subsequent applications, it is summarized below starting with

$$\bar{A}_e(x) = e\bar{A}_L(x),$$

where

$$\bar{A}_L(x) = \frac{\bar{v}_L}{\mu_L^2}\begin{cases} \{1 - \frac{\cosh(\mu_L)}{\cosh(\mu_L(1+x_m))}\cosh(\mu_L(x+x_m))\} & (-x_m \le x \le 0) \\ \frac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1+x_m))}\sinh(\mu_L(1-x)) & (0 \le x \le 1) \end{cases}. \tag{20}$$

The corresponding signaling morphogen and signaling receptor concentrations are

$$\bar{b}_e(x) \simeq \bar{B}_e(x) = e\bar{B}_L(x) = \frac{e\bar{A}_L(x)}{\alpha_0}, \qquad \bar{r}_e(x) \simeq 1. \tag{21}$$

For $\mu_L \gg 1$, the expression for $\bar{A}_e(x)$ is effectively a boundary layer adjacent to the edge of ligand synthesis zone, dropping sharply from $O(\bar{v}_L/2\mu_L^2)$ at $x = 0$ to $O(\bar{v}_L/2\mu_L^2)e^{-\mu_L x}$ for positive x. The gradient changes much more gradually if μ_L is $O(1)$ instead.

Definition 1. The morphogen gradient system is in a (steady) state of *low receptor occupancy* (LRO) if $\zeta_0 \bar{a}_e \ll \alpha_0$ and $\mu_L = O(1)$.

Remark 1. Layer gradients generally do not generate biologically realistic tissues or patterns. Biological developments of complex patterns are usually associated with LRO gradient systems. Considerable effort will be made to obtain useful information about such systems.

The following inequality is not as obvious as that of Proposition 1:

Proposition 2. $\bar{A}_1(x) \le \bar{A}_e(x) \le \bar{a}_e(x)$ $(-x_m \le x \le 1)$.

Proof. The proof of the first inequality is similar to Proposition 1. For the second, the BVP for $\bar{a}_e(x)$ and $\bar{A}_e(x)$ may be combined to give the following relation for $\Delta_A = \bar{a}_e(x) - \bar{A}_e(x) = \bar{a}_e(x) - e\bar{A}_L(x)$:

$$\Delta_A'' - \frac{g_0}{\alpha_0}\frac{\Delta_A}{\alpha_0 + \zeta_0\bar{a}_e} - g_L\Delta_A + f(x) = 0, \quad \Delta_A'(-x_m) = \Delta_A(1) = 0, \tag{22}$$

where

$$0 \le f(x) \le \frac{g_0\zeta_0\bar{a}_e\bar{A}_e}{\alpha_0\big(\alpha_0 + \zeta_0\bar{a}_e\big)} \le f_{\max}, \tag{23}$$

for some $f_{\max} > 0$. The existence, uniqueness, nonnegativity, and monotonicity of the solution $\Delta_A(x)$ of the linear BVP (22) can also be proved by the method of upper-lower solution[52–54] used in Ref. 7.

The required lower and upper solutions, $\Delta_\ell(x)$ and $\Delta_u(x)$, in the proof may be taken to be $\Delta_\ell(x) = 0$ and

$$\Delta_u(x) = f_{\max}\left\{\left(x_m + \frac{1}{2}\right) - x_m x - \frac{1}{2}x^2\right\} \geq 0, \tag{24}$$

respectively, for $-x_m \leq x \leq 1$. With $0 = \Delta_\ell(x) \leq \Delta_A(x) \leq \Delta_u(x)$, we have $\Delta_A(x) \geq 0$ and the second half of the inequalities is proved. ■

It also follows from the first inequality that

$$\bar{B}_e(x) = \frac{\bar{A}_e(x)}{\alpha_0} \geq \frac{\bar{A}_1(x)}{\alpha_0} = \bar{B}_1(x) \quad (-x_m \leq x \leq 1), \tag{25}$$

which is LRO version of Corollary1.

3 | ROBUSTNESS OF SIGNALING GRADIENT

3.1 | Perturbation due to enhanced morphogen synthesis

Normal development of wing imaginal disc and other biological entities may be altered by an enhanced morphogen synthesis rate stimulated by genetic or epigenetic changes. For example, *Dpp* synthesis rate in Drosophila imaginal disc doubles when the ambient temperature is increased by 5.9°C (see Ref. 4). At a state of LRO, a significant increase in morphogen synthesis rate is seen from (20) and (21) to increase proportionately the gradient magnitude and thereby alter the cell fate at each spatial location. Without the restriction of LRO, the steady-state signaling gradient has also been shown to increase with synthesis rate, though not necessarily proportionately (see Corollary 1). Yet, the actual development of biological organisms is 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 attain robustness of such biological developments. To investigate possible effective feedback, we introduced in Refs. 4, 36, 40, and 51 a robustness index $R_b(t)$ to measure deviations of signaling gradient after ligand synthesis rate enhancement from the wild type.

3.2 | Root-mean-square signaling differential

A possible global measure of signaling gradient robustness is the following *signal robustness index* R_b first introduced in[4,36]:

$$R_b(t) = \frac{1}{b_h(t) - b_\ell(t)}\sqrt{\frac{1}{x_\ell - x_h}\int_{x_h}^{x_\ell}[b_e(x,t) - b_1(x,t)]^2 dx}, \tag{26}$$

where $0 \leq b_\ell(t) < b_h(t) \leq b(-x_m, t)$ and $-x_m \leq x_h < x_\ell \leq 1$ with $\{b_\ell(t), b_h(t)\} = \{b_1(x_\ell, t), b_1(x_h, t)\}$. The quantities x_ℓ and x_h may be chosen away from the extremities to minimize the exaggerated effects of outliers. $R_b(t)$ is essentially the root mean square of the difference between the ectopic gradient $b_e(x,t)$ and the wild-type gradient $b_1(x,t)$.

For a system in steady state with

$$\bar{b}_1(x) = \lim_{t\to\infty} b_1(x,t), \quad \bar{b}_e(x) = \lim_{t\to\infty} b_e(x,t), \tag{27}$$

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

$$\bar{R}_b = \lim_{t\to\infty} R_b(t) = \frac{1}{b_h - b_\ell}\sqrt{\frac{1}{x_\ell - x_h}\int_{x_h}^{x_\ell}[\bar{b}_e(x) - \bar{b}_1(x)]^2 dx}. \tag{28}$$

To be concrete, we take in subsequent development $x_h = 0$ (because signaling is not particularly relevant in the interval of ligand synthesis) and $x_\ell = 1$. In that case, we have $\bar{b}_\ell = 0$. It would be natural to set $\bar{b}_h = \bar{b}_1(0)$. However, to avoid having to solve for $\bar{b}_1(0)$ numerically, we take $\bar{b}_h$ to be $\bar{B}_L(0)$ known explicitly from the LRO solution so that (28) simplifies to

$$\bar{R}_b = \frac{1}{\bar{B}_L(0))}\sqrt{\int_0^1 [\bar{b}_e(x) - \bar{b}_1(x)]^2 dx}. \tag{29}$$

Remark 2. The signal robustness index R_b is not the only global measure of the deviation of the ectopic signaling gradient from the original one prior to *Dpp* synthesis rate enhancement. Given an existing genetic program for individual cells, another relevant measure of robustness is the displacement of the same level of ligand-receptor complex concentration due to a change of morphogen synthesis rate. A more detailed discussion of this robustness measure and its application can be found in Ref. 4. In this paper, we focus on the signaling robust index defined (26), mainly in the form (29) for steady-state gradients.

3.3 | Exact and LRO solution

3.3.1 | LRO solution

For a morphogen system in a state of LRO (before and after ligand synthesis rate enhancement) so that $g_0 a/g_R \ll \alpha_0$, we have from Ref. 7 the following approximate steady-state solution for the signaling gradient of the (environmentally or genetically) perturbed system:

$$\bar{b}_e(x) \sim \frac{e\bar{v}_L}{\alpha_0\mu_L^2}\frac{\sinh(\mu_L x_m)\sinh(\mu_L(1-x))}{\cosh(\mu_L(1+x_m))} = e\bar{B}_L(x), \quad (0 \le x \le 1), \tag{30}$$

where $\mu_L^2 = g_L + g_0/\alpha_0$ with $\mu_L^2 \simeq h_0 + g_L$ whenever $f_0 \ll g_0$ (as it is for *Dpp* in Drosophila wing imaginal disc).

With $x_h = 0$, we have from (20) and (21)

$$\bar{b}_h = \bar{B}_L(0) = \frac{\bar{v}_L}{\alpha_0\mu_L^2}\frac{\sinh(\mu_L x_m)\sinh(\mu_L)}{\cosh(\mu_L(1+x_m))}\,. \tag{31}$$

(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 instead.) By taking $x_\ell = 1$ and $\bar{b}_\ell = \bar{b}_1(1) = \bar{B}_L(1) = 0$, the LRO approximation of $\bar{R}_b$ is given by

$$\begin{aligned}\bar{R}_b \simeq \rho_L &= \frac{e-1}{\sinh(\mu_L)}\sqrt{\int_0^1 [\sinh(\mu_L(1-x))]^2 dx} \\ &= \frac{e-1}{\sinh(\mu_L)}\sqrt{\frac{1}{2}\left(\frac{\sinh(2\mu_L)}{2\mu_L} - 1\right)} \equiv (e-1)\gamma(\mu_L).\end{aligned} \tag{32}$$

3.3.2 | Exact solution

Numerical solutions for the original nonlinear steady-state BVP and the corresponding robustness index $\bar{R}_b$ have been obtained for several sets of parameter values in Refs. 36 and 51 to assess the level of robustness of the corresponding *Dpp* gradients. (For the first example computed in Ref. 51, we have $\gamma(\mu_L) = 0.3938\ldots$ and $\bar{R}_b = 0.3939\ldots$ for $e = 2,\ h_0 = 10,\ g_0 = 0.2,\ f_0 = 0.001$, and $g_L = 0$.) They also serve to validate the appropriateness of the approximate solution (30) for signaling gradient and the approximation ρ_L in (32) for robustness index for systems in a state of LRO. Here, our main interest is in examining robustness-index-induced feedback for reducing ectopicity of enhanced signaling morphogen gradients (to below an acceptable threshold such as $\bar{R}_b \leq 0.2$) beyond the proof of concept investigation of Ref. 51. In the following sections, we examine the individual effects of each of the five such feedback processes that are possible in the basic three-component extracellular model. Our negative findings for these models lead to a study of concurrent applications of multiple feedback processes in the same gradient system.

4 | POSITIVE FEEDBACK ON BOUND LIGAND DEGRADATION

4.1 | Receptor-mediated degradation with positive nonlocal feedback

Signaling morphogens are known to internalize and degrade at a higher than normal rate when induced to do so by relevant molecular agents. For example, *Plasminogen activator inhibitor-1* (*PAI-1*) is known to induce receptor-mediated internalization and degradation of *urokinase* (uPA).[20] In extracellular space, uPA can activate surface bound plasminogen to produce surface bound plasmin. In the presence of PAI-1, uPA activity is inhibited and plasmin production interrupted, as the uPA-PAI-l complex is internalized and degraded. Hence, upregulating the inhibitor PAI-1 would downregulate the signaling uPA complex. Also, it is known that an upregulation of *transforming growth factor-beta* (TGF-β) would induce PAI-1 production via Smads.[41]

Another example is the ubiquitin-mediated internalization and degradation of the activated growth factor receptors leading to the downregulation of active receptor signaling. Inappropriate activity of growth factor receptors such as the *epidermal growth factor receptor* (EGFR) family is associated with the development of a wide variety of human cancers, including breast cancer.[25] Under normal condition, there appears to be a feedback mechanism to ensure adequate production of *ubiquitin* to prevent cancerous growth. With *Casitas B – lineage Lymphoma* (*Cbl*) proteins being critical regulators of receptor downregulation, mutant forms of *mouse Cbl* (*c-Cbl*) lead to inhibition of the downregulation of growth factor receptors and act as oncogenes for pathogenesis of cancer.

With the downregulation of signaling ligand-receptor complexes (aka bound or signaling morphogen gradient) ultimately accomplished through the increase of their degradation rate, we may, for simplicity, bypass the processes of upregulating regulator proteins (such as Cb1 proteins for ubiquitin and TGF-β for PA1) and consider a feedback on the bound morphogen degradation rate constant directly. Analogous to the new type of spatially uniform nonlocal feedback for achieving robustness first introduced in Ref. 51, we consider in this section the effect of a positive feedback on the normalized receptor-mediated degradation rate constant for signaling morphogen concentration of the form

$$g_c = g_0\left[1 + c\,R_b^n(t-\tau)\right], \tag{33}$$

where c and n are two parameters that regulate the strength and sensitivity, respectively, of the feedback process with $g_c = g_0$ in the absence of feedback ($c = 0$) or ectopicity ($R_b = 0$). The parameter τ allows the possibility of a time delay if the effect of feedback is not instantaneous. More will be said later on the appropriateness of the nonlocal feedback (33) and similar type of nonlocal feedback mechanisms investigated in the next few sections after we have worked out their consequences.

Before embarking on a study of the effects of (33), we observe that (i) ligand degradation is receptor-mediated solely for most morphogen system (including Dpp in the wing imaginal disc of Drosophila), and (ii) the normalized dissociation rate constant f_0 is usually much smaller (by at least an order of magnitude) than the normalized degradation rate constant g_0. Unless it is specifically stated otherwise, we take

$$g_L = f_0 = 0 \tag{34}$$

so that

$$\alpha_0 = \frac{g_0}{h_0}, \qquad \mu_L^2 = h_0 = \mu_0^2, \tag{35}$$

in the subsequent development (with $\zeta_0 = g_0/g_R$ unaffected by (34)).

4.2 | Steady state

It has been shown in Ref. 7 that a unique steady state exists for the extracellular model without feedback. It can be proved similarly that the same is true for the new model with feedback on the receptor-mediated degradation rate constant with the corresponding robustness index $R_b(t) \to \bar{R}_b$ (see (26)-(28)). Upon setting $\partial(\,)/\partial t = 0$, the governing differential equations and boundary conditions formally reduce again to (11) and (12) but now with g_0 replaced by

$$g_c = g_0\left[1 + c\bar{R}_b^n\right]. \tag{36}$$

The BVP for the steady-state solution, now denoted by $\{\bar{a}_c(x), \bar{b}_c(x), \bar{r}_c(x)\}$, depends on $\bar{R}_b$ (as well as the two parameters c and n). Given the approximation $g_0 + f_0 \simeq g_0$, the BVP for $\bar{a}_c(x)$ remains unchanged as given by (11)-(12) with the factor $1 + c\bar{R}_b^n$ canceled in (11) to get

$$\bar{a}_c'' - \frac{g_0\bar{a}_c}{\alpha_0 + \zeta_0\bar{a}_c} + e\bar{v}_L H(-x) = 0, \tag{37}$$

with

$$\bar{a}_c'(-x_m) = 0, \quad \bar{a}_c(1) = 0. \tag{38}$$

It follows that the free ligand concentration remains the same as that with no feedback so that

$$\bar{a}_c(x) = \bar{a}_e(x) \tag{39}$$

(and all the results in Ref. 7 for $\bar{a}_e(x)$ apply).

On the other hand, the bound morphogen and unbound (or free) receptor concentrations are given in terms of $\bar{a}_c(x)$ by

$$\bar{b}_c(x) = \frac{\bar{a}_c(x)}{\alpha_c + \zeta_c\bar{a}_c(x)} = \frac{\bar{b}_e(x)}{1 + cR_b^n}, \qquad \bar{r}_c(x) = \frac{\alpha_0}{\alpha_0 + \zeta_0\bar{a}_e(x)}, \tag{40}$$

where

$$\alpha_c = \frac{g_c}{h_0} = \frac{g_0}{h_0}\left(1 + cR_b^n\right), \qquad \zeta_c = \frac{g_c}{g_R} = \frac{g_0}{g_R}\left(1 + cR_b^n\right) \tag{41}$$

(as (34) is enforced in the present development).

Correspondingly, the expression for the robustness index now reads

$$\bar{R}_b = \frac{1}{\bar{B}_1(0)}\sqrt{\int_0^1 [\bar{b}_c(x) - \bar{b}_1(x)]^2 dx}, \tag{42}$$

with

$$\bar{B}_1(x) = \left[\bar{B}_L(x)\right]_{g_L = f_0 = 0}. \tag{43}$$

It is rather remarkable that, as a consequence of the very appropriate approximation $g_0 + f_0 \simeq g_0$, the free morphogen concentration for the new model is completely independent of robustness index $\bar{R}_b$ even when the degradation rate constant now depends on that index as given by (33). On the other hand, the signaling gradient concentration $\bar{b}_c(x)$ now depends on $\bar{R}_b$, rather simply through the multiplicative factor $(1 + cR_b^n)^{-1}$ as shown in (40). As such, expression (42) for determining $\bar{R}_b$ becomes a nonlinear equation for the unknown index (and not simply integrating a known integrand).

4.3 | Low receptor occupancy

Without solving (42) for the unknown robustness index $\bar{R}_b$, we know that it is positive (as long as $e > 1$). In that case, the expression for $\bar{b}_c(x)$ in (40) suggests that there would be a lower concentration of signaling ligand-receptor complexes than the corresponding concentration without feedback, $\bar{b}_c(x) < \bar{b}_e(x)$. In this subsection, we examine how this reduction changes the robustness index for a gradient system in a steady state of LRO (with assumptions (34) enforced).

In view of (39), the exact LRO solution is the same as that given in (20) with $g_L = f_0 = 0$ so that

$$\bar{a}_c(x) \simeq a_0(x) = e\bar{A}_1(x), \tag{44}$$

where

$$\bar{A}_1(x) = \frac{\bar{v}_L}{\mu_0^2}\begin{cases} 1 - \frac{\cosh(\mu_0)}{\cosh(\mu_0(1+x_m))}\cosh(\mu_0(x + x_m)) & (-x_m \le x \le 0) \\ \frac{\sinh(\mu_0 x_m)}{\cosh(\mu_0(1+x_m))}\sinh(\mu_0(1 - x)) & (0 \le x \le 1) \end{cases}, \tag{45}$$

and

$$\mu_0^2 = h_0 \tag{46}$$

(resulting from the assumptions made in (34)). Now $\bar{A}_1(x)$ is just $\bar{A}_L(x)$ as given by (20) with μ_L replaced by μ_0. Similar to $\bar{a}_c(x) = \bar{a}_e(x)$, $\bar{A}_1(x)$ is independent of the feedback on receptor-mediated degradation.

The corresponding signaling gradient in an LRO state with the same prescribed feedback is given by

$$\bar{b}_c(x) \simeq b_0(x; c\rho_c^n) = \frac{e\bar{A}_1(x)}{\alpha_c} = \frac{e\bar{B}_1(x)}{1 + c\rho_c^n} \equiv e\bar{B}_c(x), \tag{47}$$

where ρ_c denotes the LRO approximation for $\bar{R}_b$ with $[\rho_c]_{c=0} = [\rho_L]_{g_L=0}$ and

$$\bar{B}_1(x) = \frac{\bar{v}_L}{g_0} \begin{cases} 1 - \frac{\cosh(\mu_0)}{\cosh(\mu_0(1+x_m))} \cosh(\mu_0(x+x_m)) & (-x_m \le x \le 0) \\ \frac{\sinh(\mu_0 x_m)}{\cosh(\mu_0(1+x_m))} \sinh(\mu_0(1-x)) & (0 \le x \le 1) \end{cases}. \tag{48}$$

Even without an explicit solution of the approximate robustness index ρ_c, we see that

Proposition 3. *In a state of LRO with $c > 1$, the signaling morphogen gradient is reduced in magnitude by a positive feedback on receptor-mediated degradation.*

Remark 3. Note that the shape parameter μ_0 is not affected by the robustness index $\bar{R}_b \simeq \rho_c$ for our particular feedback. This is generally not the case for other feedback processes to be investigated herein.

For an explicit solution for ρ_c, we note the expression (42) for $\bar{R}_b$ is approximated by

$$\bar{R}_b \simeq \rho_c = \frac{1}{\bar{B}_1(0)} \sqrt{\int_0^1 [\bar{b}_c(x) - \bar{B}_1(x)]^2 dx} \tag{49}$$

$$= \frac{1}{\sinh(\mu_0)} \left(\frac{e}{1 + c\rho_c^n} - 1 \right) \sqrt{\int_0^1 [\sinh(\mu_0(1-x))]^2 dx},$$

when the gradient system is in a state of LRO (and the approximations $g_L = f_0 = 0$ are observed). After integration, the relation above simplifies to

$$\rho_c = \gamma_0 \left(\frac{e}{1 + c\rho_c^n} - 1 \right), \tag{50}$$

where

$$\gamma_0 \equiv \frac{1}{\sqrt{2}\sinh(\mu_0)} \sqrt{\frac{\sinh(2\mu_0)}{2\mu_0} - 1} = \gamma(\mu_0), \quad \mu_0^2 = h_0 \tag{51}$$

is as previously found in (32) with $g_L = 0$. However, unlike the case without feedback, the relation (50) now is a nonlinear equation for the (LRO) robustness index ρ_c. The solution of the equation depends on the yet unspecified parameters c and n.

With $c = 0$ (corresponding the case of no feedback), we have immediately from (50)

$$\left[\rho_c\right]_{c=0} = (e-1)\gamma_0.$$

For $e = 2$, we have

$$\left[\rho_c\right]_{c=0,e=2} = \gamma_0 = 0.394\ldots,$$

as previously computed for the first numerical example in Ref. 51 (for which $g_L = 0$ but $f_0 > 0$). The corresponding accurate numerical solution for the original nonlinear problem to be reported in Table 1 below gives $\bar{R}_b = 0.393\ldots$

For $0 < c < \infty$, the relation (51) may be written as

$$f_c(\rho_c) \equiv c\rho_c^{n+1} + c\gamma_0\rho_c^n + \rho_c - (e-1)\gamma_0 = 0. \tag{52}$$

TABLE 1 Comparison of LRO and exact solutions

$X_{\max} = 0.01$ cm, $X_{\min} = 0.001$ cm, $k_{on}R_0 = 0/01/\text{s}/\mu\text{M}$, $k_{deg} = 2\times10^{-4}/\text{s}$, $k_R = 0.001/\text{s}$, $k_{off} = 0$, $k_L = 0$, $D = 10^{-7}$ cm^2/s, $\bar{V}_L = 0.002\ \mu\text{M}/s$, $\bar{V}_R = 0.04\ \mu\text{M}/\text{s}$					
c	ρ_c	$\bar{R}_b$	$\bar{b}_1(0)$	$b_0(0; c\rho_c)$	$\bar{b}_2(0; c\bar{R}_b)$
0	0.394	0.393	0.0581	0.1162	0.1156
1	0.241	0.241	0.0581	0.0942	0.0932
2	0.183	0.183	0.0581	0.0856	0.0847
4	0.128	0.128	0.0581	0.0774	0.0766
8	0.082	0.082	0.0581	0.0706	0.0699

For $n = 1$, $f_c(\rho_c) = 0$ has one positive solution

$$\rho_c = \frac{1}{2c}\left[-(1 + c\gamma_0) + \sqrt{(1 + c\gamma_0)^2 + 4c(e-1)\gamma_0}\right] > 0. \tag{53}$$

Equation 52 is formally the same as the corresponding equation for an LRO gradient system with negative feedback on ligand synthesis rate investigated in Ref. 51 but with $g_L = f_0 = 0$. Subject to proper interpretation, the numerical results for LRO examples computed in Ref. 51 are also accurate approximations for the present LRO problem. For the same previously mentioned numerical example of Ref. 51 with $c = 1$ and $n = 1$, the expression (53) gives

$$\left[\bar{R}_b\right]_{c=1} \simeq \left[\rho_c\right]_{c=1} = 0.241\ldots, \tag{54}$$

which agrees with accurate numerical solution to three significant figures. In terms of the robustness index, the feedback mechanism (36) is also seen to reduce the ectopicity of the signaling gradient. Whether or not this value corresponds to an acceptable level of robustness for the gradient system considered, it is what our particular type of feedback (with $c = 1$ and $n = 1$) can attain. We may, of course, modify the feedback process such as increasing the value of c and/or n to (possibly) further reduce the ectopic signaling activities. It is straightforward to deduce from (52) the following useful results for systems in a steady state of LRO:

Proposition 4. *The LRO robustness index ρ_c decreases with increasing c but, for $\rho_c < 1$, increases with increasing n.*

Proof. Upon differentiating both sides of (52) with respect to c and n, respectively, we obtain

$$\frac{d\rho_c}{dc} < 0, \quad \left[\frac{d\rho_c}{dn}\right]_{\rho_c<1} < 0.$$

■

For $n \geq 1$, we have $f_c(0) = -(e-1)\gamma_0 < 0$ and, for $c + 1 \geq e\gamma_0/(1+\gamma_0)$,

$$f_c(1) = (c+1)(1+\gamma_0) - e\gamma_0 > 0,$$

so that there is a root ρ_c in the interval (0,1). With

$$\frac{df_c}{d\rho_c} = (n+1)c\rho_c^n + nc\gamma_0\rho_c^{n-1} + 1 > 0,$$

$f_c(\rho_c) = 0$ also has one and only one positive solution in $(0, \infty)$ that is < 1 if c is sufficiently large.

4.4 | The nonlinear problem

For morphogen gradient systems not in a state of LRO, we do not have a *useful* explicit solution for the gradients to enable us to see that the ectopic signaling ligand concentration $\bar{b}_c(x)$ should be sandwiched between $\bar{b}_e(x)$ and $\bar{b}_1(x)$. However, we know from Proposition 1 that $\bar{a}_1(x) \le (\bar{a}_c(x) =)\ \bar{a}_e(x)$. As a corollary, we have the following intuitively expected relations

Corollary 2. *The inequalities (i) $\bar{b}_1(x) \le \bar{b}_e(x)$, and (ii) $\bar{b}_c(x) \le \bar{b}_e(x)$ hold for ($-x_m \le x \le 1$)*

Proof. With

$$\begin{aligned}\bar{b}_e(x) - \bar{b}_1(x) &= \frac{\bar{a}_e(x)}{\alpha_0 + \zeta_0 \bar{a}_e(x)} - \frac{\bar{a}_1(x)}{\alpha_0 + \zeta_0 \bar{a}_1(x)} \\ &= \frac{\alpha_0 \{\bar{a}_e(x) - \bar{a}_1(x)\}}{\{\alpha_0 + \zeta_0 \bar{a}_e(x)\}\{\alpha_0 + \zeta_0 \bar{a}_1(x)\}} \ge 0,\end{aligned}$$

the first inequality follows from Proposition 1.

The second inequality follows from

$$\begin{aligned}\bar{b}_e(x) - \bar{b}_c(x) &= \frac{\bar{a}_e(x)}{\alpha_0 + \zeta_0 \bar{a}_e(x)} - \frac{\bar{a}_c(x)}{\alpha_c + \zeta_c \bar{a}_c(x)} \\ &= \frac{\bar{a}_e(x)}{\alpha_0 + \zeta_0 \bar{a}_e(x)} \left\{ 1 - \frac{1}{1 + c\bar{R}_b^n} \right\} \\ &= \frac{c\bar{R}_b^n}{1 + c\bar{R}_b^n} \bar{b}_e(x) \ge 0.\end{aligned}$$

■

As for the relative magnitude of $\bar{b}_c(x)$ and $\bar{b}_1(x)$, we see from the

$$\bar{b}_c(x) - \bar{b}_1(x) = \frac{1}{1 + c\bar{R}_b^n} \bar{b}_e(x) - \bar{b}_1(x)$$

that $\bar{b}_c(x) > \bar{b}_1(x)$ for $c\bar{R}_b^n$ sufficiently small but $\bar{b}_c(x) < \bar{b}_1(x)$ for $c\bar{R}_b^n$ sufficiently large.

For the LRO case, we saw from (50) that $\bar{R}_b \simeq \rho_c$ is a decreasing function of c. While it is satisfying that the feedback mechanism selected does reduce $\bar{b}_e(x)$ toward $\bar{b}_1(x)$, it may not reduce enough in steady state for the gradient system to be robust with respect to undesirable changes in ligand synthesis rate. Hence, we need to know more about $\bar{R}_b$ to determine how adequate is the chosen feedback for robustness.

4.5 | Robustness index with and without feedback

With our nonlocal positive feedback on receptor-mediated degradation, the steady-state signaling gradient $\bar{b}_e(x)$ in the definition of (29) of our nonlocal measure of robustness is replaced by the signaling gradient with feedback

$$\bar{b}_c(x) = \frac{\bar{b}_e(x)}{1 + c\bar{R}_b^n} \equiv \bar{b}_e\left(x; c\bar{R}_b^n\right) \tag{55}$$

as shown in (42). In adopting $\bar{b}_e(x; c\bar{R}_b^n)$ as an alternate notation for $\bar{b}_c(x)$, it is understood that the corresponding ectopic signaling gradient without feedback $\bar{b}_e(x)$ corresponds to $[\bar{b}_e(x; c\bar{R}_b^n)]_{c=0}$, ie,

$$\bar{b}_e(x) = \left[\bar{b}_e\left(x; c\bar{R}_b^n\right)\right]_{c=0} = \left[\bar{b}_e\left(x; c\bar{R}_b^n\right)\right]_{\bar{R}_b=0}.$$

The expression (42) for $\bar{R}_b$ may be written as

$$\bar{R}_b^2 = \Phi\left(c\bar{R}_b^n\right), \tag{56}$$

where

$$\Phi\left(c\bar{R}_b^n\right) = \frac{1}{\bar{B}_1^2(0)} \int_0^1 \left[\bar{b}_e\left(x; c\bar{R}_b^n\right) - \bar{b}_1(x)\right]^2 dx. \tag{57}$$

The relation (56) is now an equation to be solved for $\bar{R}_b$ (as a function of c and n). An explicit solution for $\bar{R}_b(c, n)$ is not likely or expected. We have, however, the following simple upper bound for $\bar{R}_b(c, n)$:

Lemma 1. $0 \le \bar{R}_b^2 \le [\Phi(c\bar{R}_b)]_{c=0} = \Phi(0)$.

Proof. For $e > 1$ and with $\bar{b}_e(x) \equiv \bar{b}_e(x; 0) = [\bar{b}_c(x)]_{c=0} \ge \bar{b}_c(x)$ by second inequality of Lemma 2, we have

$$\bar{R}_b^2 = \Phi\left(c\bar{R}_b^n\right) \le \Phi(0) = \frac{1}{\bar{B}_1^2(0)} \int_0^1 \left[\bar{b}_e(x) - \bar{b}_1(x)\right]^2 dx,$$

with the right-hand side corresponding the value of $\bar{R}_b$ without feedback found in (29) with $g_L = f_0 = 0$. The lemma follows with the right-hand side independent of $\bar{R}_b$ and had been calculated previously, ie,

$$\left[\bar{R}_b^2\right]_{c>0} \le \left[\bar{R}_b^2\right]_{c=0} = \Phi(0).$$

■

The upper bound $\Phi(0)$ is generally larger than the threshold for a robust system for $e = 2$ and larger (which was the reason for seeking some feedback mechanism for attaining robustness). For $c > 0$, the determination of the steady-state robustness index $\bar{R}_b$ requires the solution of (56) written as

$$f_R(\bar{R}_b) \equiv \bar{R}_b^2 - \Phi\left(c\bar{R}_b^n\right) = 0, \tag{58}$$

where

$$\Phi\left(c\bar{R}_b^n\right) = \int_0^1 \left[\frac{1}{1 + c\bar{R}_b^n} \frac{\bar{b}_e(x)}{\bar{B}_1(0)} - \frac{\bar{b}_1(x)}{\bar{B}_1(0)}\right]^2 dx.$$

Lemma 2. *The nonlinear equation $f_R(\bar{R}_b) = 0$ has exactly one root in (0,∞).*

Proof. Since

$$f_R(0) = -\Phi(0) = -\int_0^1 \left[\frac{\bar{b}_e(x)}{\bar{B}_1(0)} - \frac{\bar{b}_1(x)}{\bar{B}_1(0)}\right]^2 dx < 0,$$

and

$$\lim_{\bar{R}_b \to \infty} \left[f_R(\bar{R}_b) \right] = \infty,$$

$f_R(\bar{R}_b)$ has at least one root in $(0, \infty)$. Upon differentiating the relation (58) to get $df_R/d\bar{R}_b > 0$, we conclude that $f_R(\bar{R}_b) = 0$ has exactly one positive root. ■

Having established the existence and uniqueness of a positive robustness index for our problem, we still need to know the value of $\bar{R}_b$ to see if robustness is attained by the chosen feedback process. For that purpose, we rewrite the nonlinear equation (56) as

$$\bar{R}_b^2 = \frac{\beta_2}{\left(1 + c\bar{R}_b^n\right)^2} - \frac{2\beta_1}{1 + c\bar{R}_b^n} + \beta_0, \tag{59}$$

where

$$\beta_2 = \int_0^1 \left[\frac{\bar{b}_e(x)}{\bar{B}_1(0)} \right]^2 dx, \quad \beta_1 = \int_0^1 \frac{\bar{b}_e(x)\bar{b}_1(x)}{\bar{B}_1^2(0)} dx, \quad \beta_0 = \int_0^1 \left[\frac{\bar{b}_1(x)}{\bar{B}_1(0)} \right]^2 dx,$$

keeping in mind $\bar{a}_c(x) = \bar{a}_e(x)$ upon observing (34). The three quantities $\{\beta_0, \beta_1, \beta_2\}$ may be calculated once for all for a particular gradient system. The relation (59) is then a nonlinear equation for $\bar{R}_b$,

$$\left(\bar{R}_b^2 - \beta_0\right)\left(1 + c\bar{R}_b^n\right)^2 + 2\beta_1\left(1 + c\bar{R}_b^n\right) - \beta_2 = 0, \tag{60}$$

with c and n as parameters. Since the (LOR) robustness index increases with n for $n \geq 1$, the $n = 1$ case is of primary interest. For this case, (60) further simplifies to

$$\left(\bar{R}_b^2 - \beta_0\right)\left(1 + c\bar{R}_b\right)^2 + 2\beta_1\left(1 + c\bar{R}_b\right) - \beta_2 = 0. \tag{61}$$

The unique root of (61) (as well as (60) for a positive integer $n > 1$) may be obtained numerically by any available equation solver on mathematical software such as MatLab, Mathematica, and Maple.

To gain some insight to the steady-state robustness index $\bar{R}_b$, we compute its value for the system characterized by the parameter values, as shown in Table 1. This system meets the condition $\zeta_0\bar{a}_c = \zeta_0\bar{a}_e \ll \alpha_0$ 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 various quantities computed are shown in Table 1 for $n = 1$, $e = 2$, and several values of c.

For each value of c, we report in Table 1 the values of robustness index $\bar{R}_b$ and the corresponding LRO approximate solution ρ_c, the wild-type signaling gradients $\bar{b}_1(0)$, the exact ectopic signaling ligand-receptor concentration $\bar{b}_2(0; c\bar{R}_b)$, and the LRO ectopic signaling ligand-receptor concentration $\bar{b}_0(0; c\rho_c)$. Note that the row for $c = 0$ are values for the ectopic gradient without feedback. At the nominal basic strength/sensitivity level of $c = 1$ and $n = 1$, we have $\bar{b}_2(0; c\bar{R}_b) \simeq b_0(0; c\rho_c) < \bar{b}_2(0; 0)$ but still closer to the ectopic concentration without feedback than the wild-type concentration $\bar{b}_1(0)$. The corresponding robustness index $\bar{R}_b$ and its LRO approximation ρ_c are both > 0.2 (the rather conservative robustness criterion adopted in Ref. 36). Both are only slightly < 0.2 for $c = 2$. It is, of course, possible that $\bar{R}_b$ may be lowered by taking $c > 2$. However, as shown in Table 1, $\bar{R}_b$ is still well above 0.1 for $c = 4$. In the next few sections, we examine a few other nonlocal feedback mechanisms to see their effectiveness in promoting robustness at the basic strength/sensitivity level.

5 | NEGATIVE FEEDBACK ON RECEPTOR SYNTHESIS

5.1 | Receptor synthesis rate with negative robustness index induced feedback

Evidence exists that excessive Dpp signaling downregulates the receptor Tkv expression (and that the level of the receptor influences the effective range of the Dpp gradient). For example, the *inhibitory* (transcription factor) *Smads* (I-Smads) has been found to downregulate expression of type I receptors Thickveins and punt and functions as negative feedback regulators.[23] As another example, Hedgehog (HH) is known to downregulate expression of Tkv.[29] These Dpp signaling-induced inhibitory agents for Dpp receptors suggest another possible feedback mechanism for reducing the ectopicity of the perturbed gradients and rendering the morphogen system robust. Consistent with our approach to attain robustness through nonlocal feedback, we model the downregulation of receptor expression in the presence of excessive morphogen signaling by a negative feedback on the receptor synthesis rate. In terms of the three-component extracellular model (1)-(2) with a uniform receptor synthesis rate, this amounts to replacing in the second equation of (2) the (temporally and spatially) uniform synthesis rate $\bar{v}_R$ by the robustness dependent synthesis rate

$$v_c(t) = \frac{\bar{v}_R}{1 + cR_b^n(t-\tau)}, \tag{62}$$

where c and n are two parameters that regulate the strength of the feedback process and the parameter τ provides the effect of a time delay if it should be appropriate.

5.2 | Steady state

We are interested in the limiting behavior as $t \to \infty$ with $R_b(t) \to \bar{R}_b$ and all concentrations involved approaching their time-independent steady state denoted by $\{\bar{a}_v(x), \bar{b}_v(x), \bar{r}_v(x)\} \equiv \{\bar{a}_e(x; c\bar{R}_b^n), \bar{b}_e(x; c\bar{R}_b^n), \bar{r}_e(x; c\bar{R}_b^n)\}$. With $\partial(\ )/\partial t = 0$ (and $R_b(t) \to \bar{R}_b$), a similar calculation as in the previous cases reduces the governing differential equations and boundary conditions to

$$\bar{a}_v'' - \frac{g_0 \bar{a}_v}{\alpha_c + \zeta_c \bar{a}_v} + e\bar{v}_L H(-x) = 0, \tag{63}$$

$$\bar{a}_v'(-x_m) = 0, \quad \bar{a}_v(1) = 0, \tag{64}$$

where

$$\alpha_c = \alpha_0\left(1 + c\bar{R}_b^n\right), \quad \zeta_c = \zeta_0\left(1 + c\bar{R}_b^n\right), \tag{65}$$

with $[\bar{a}_v(x)]_{c=0} = \bar{a}_e(x)$ being the (ectopic) free ligand concentration without feedback. Unlike the model of the previous section, the steady-state free morphogen concentration $\bar{a}_v(x)$ depends on the unknown robustness index $\bar{R}_b$.

The signaling morphogen concentration and unoccupied receptor concentration are given in terms of $\bar{a}_v(x)$ by

$$\bar{b}_v(x) = \frac{\bar{a}_v}{\alpha_c + \zeta_c \bar{a}_v}, \qquad \bar{r}_v(x) = \frac{\alpha_0}{\alpha_c + \zeta_c \bar{a}_v}. \tag{66}$$

The corresponding robustness index $\bar{R}_b$ is now given by (42) with $\bar{b}_c(x)$ replaced by $\bar{b}_v(x) \equiv \bar{b}_e(x; c\bar{R}_b^n)$.

5.3 | Low receptor occupancy

For morphogen system in an LRO state prior to and after ligand synthesis enhancement so that

$$\zeta_0 \bar{a}_1(x) \ll \alpha_0 \quad \text{and} \quad \zeta_c \bar{a}_\upsilon(x) \ll \alpha_c, \tag{67}$$

we may obtain an accurate approximate solution $a_0(x; c\rho_\upsilon^n)$ for $\bar{a}_\upsilon(x)$ from the linearized BVP

$$a_0'' - \mu_c^2 a_0 + e\bar{v}_L H(-x) = 0, \tag{68}$$

$$a_0'\left(-x_m; c\rho_\upsilon^n\right) = 0, \quad a_0\left(1; c\rho_\upsilon^n\right) = 0, \tag{69}$$

with

$$\mu_c^2 = \frac{g_0}{\alpha_c} = \frac{h_0}{1 + c\rho_\upsilon^n} \leq h_0 = \mu_0^2, \tag{70}$$

where ρ_υ denotes the LRO approximation for $\bar{R}_b$ for the few feedback process.

The exact solution of the BVP (68)-(69) is

$$a_0\left(x; c\rho_\upsilon^n\right) = e\bar{A}_\upsilon(x),$$

with

$$\bar{A}_\upsilon(x) = \frac{\bar{v}_L}{\mu_c^2} \begin{cases} 1 - \frac{\cosh(\mu_c)}{\cosh(\mu_c(1+x_m))} \cosh(\mu_c(x + x_m)) & (-x_m \leq x \leq 0) \\ \frac{\sinh(\mu_c x_m)}{\cosh(\mu_c(1+x_m))} \sinh(\mu_c(1 - x)) & (0 \leq x \leq 1) \end{cases}. \tag{71}$$

In other words, $\bar{A}_\upsilon(x)$ is $\bar{A}_1(x)$ of (20) with μ_0^2 replaced by $\mu_c^2 = h_0/(1 + c\rho_\upsilon^n) = \mu_0^2/(1 + c\rho_\upsilon^n)$ (keeping in mind (34)). Hence, the negative feedback (62) results in, more steady-state free ligand molecules available in the extracellular space.

On the other hand, the LRO signaling gradient and free receptor concentration are given by

$$b_0\left(x; c\rho_\upsilon^n\right) = \frac{a_0\left(x; c\rho_\upsilon^n\right)}{\alpha_c} \equiv e\bar{B}_\upsilon(x), \quad r_0\left(x; c\rho_\upsilon^n\right) = \frac{1}{1 + c\rho_\upsilon^n},$$

respectively, with

$$\bar{B}_\upsilon(x) = \frac{\bar{v}_L}{g_0} \begin{cases} 1 - \frac{\cosh(\mu_c)}{\cosh(\mu_c(1+x_m))} \cosh(\mu_c(x + x_m)) & (-x_m \leq x \leq 0) \\ \frac{\sinh(\mu_c x_m)}{\cosh(\mu_c(1+x_m))} \sinh(\mu_c(1 - x)) & (0 \leq x \leq 1) \end{cases}. \tag{72}$$

The amplitude factor $e\bar{v}_L/g_0$ of the LRO approximation for the signaling morphogen concentration $\bar{b}_\upsilon(x)$ is unaffected by feedback on receptor synthesis rate constant in the form (62). The following proposition provides a more substantive characterization of the effect of the new feedback mechanism being examined:

Proposition 5. *For a gradient system in a state of LRO, the robustness-induced negative feedback on receptor synthesis rate principally (i) reduces the steady-state receptor concentration from its initial concentration, and (ii) changes the slope and convexity of the signaling morphogen gradient* $\bar{b}_\upsilon(x) \simeq b_0(x; c\rho_\upsilon^n) = e\bar{B}_\upsilon(x)$.

TABLE 2 The LRO approximation for robustness index (for the same parameter values as in Table 1)

c	0	1	2	4	8
ρ_v	0.394	0.357	0.328	0.287	0.235

Remark 4. With the end condition $A_v'(-x_m) = 0$, the amplitude of the signaling gradient (in a state of LRO) should nevertheless be affected somewhat with the change in slope and convexity. The numerical example in a later section confirms this effect to be secondary in an LRO approximation.

The LRO approximation ρ_v robustness index is now determined by

$$\bar{R}_b^2 \simeq \rho_v^2 = \int_0^1 \left[\frac{e\bar{B}_v(x)}{\bar{B}_1(0)} - \frac{\bar{B}_1(x)}{\bar{B}_1(0)}\right]^2 dx \tag{73}$$

$$= \int_0^1 \left[e_c \frac{\sinh(\mu_c(1-x))}{\sinh(\mu_0)} - \frac{\sinh(\mu_0(1-x))}{\sinh(\mu_0)}\right]^2 dx,$$

where

$$e_c = e\frac{\sinh(\mu_c x_m)\cosh(\mu_0(1+x_m))}{\sinh(\mu_0 x_m)\cosh(\mu_c(1+x_m)}, \quad \mu_c^2 = \frac{h_0}{1+c\rho_R^n}. \tag{74}$$

Unlike the explicit solution ρ_L given in (32) or the unique positive root ρ_c of a simple polynomial equation (50) for a positive feedback on the receptor-mediated degradation rate constant, the robustness index for a negative feedback on receptor synthesis rate for a gradient system in an LRO state is determined by a highly nonlinear equation for ρ_v:

$$f_c(\rho_v) = 0,$$

where

$$f_c(\rho_v) \equiv 2\sinh^2(\mu_0)\rho_v^2 - e_c^2\left(\frac{\sinh(2\mu_c)}{2\mu_c} - 1\right) - \left(\frac{\sinh(2\mu_0)}{2\mu_0} - 1\right) \tag{75}$$

$$+ 2e_c\left\{\frac{\sinh(\mu_c+\mu_0)}{\mu_c+\mu_0} - \frac{\sinh(\mu_c-\mu_0)}{\mu_c-\mu_0}\right\}.$$

For $n = 1$ of primary interest, we report in Table 2 values of ρ_v for the example in Table 1 for different $c \geq 0$. The results show $\rho_v > 0.2$ for $c \leq 8$, indicating that a robustness index induced negative feedback on the receptor synthesis rate is not at all effective toward a robust signaling gradient (at least when it is in an LRO state).

5.4 | The nonlinear problem

For $\zeta_0\bar{a}_1(x) = O(\alpha_0)$ and $\zeta_0\bar{a}_v(x; c\bar{R}_b^n) = O(\alpha_0)$, the ordinary differential equation (ODE) (63) admits no simplifications. However, the BVP (63)-(64) can be solved accurately by a number of numerical methods available on scientific computing software such as Mathematica, MatLab, and Maple. Numerical results have been obtained for the same example in Table 1 and reported in Table 3. The results generally confirm the finding for a system in a state of LRO for the particular example investigated.

It is of some interest to note that for the case $n = 1$ of primary interest, we have $[\bar{b}_v(0)]_{c=8} = 0.0731$ compared to a corresponding reduction of $[\bar{b}_c(0)]_{c=8} = 0.0699$ for the previous feedback

TABLE 3 Numerical solutions for exact robustness index ($n = 1$)

$X_{\max} = 0.01$ cm, $X_{\min} = 0.001$ cm, $k_{on}R_0 = 0/01/s/\mu M$, $k_{deg} = 2\times10^{-4}/s$, $k_R = 0.001/s$, $k_{off} = 0$, $k_L = 0$, $D = 10^{-7}$ cm^2/s, $\bar{V}_L = 0.002\ \mu$M/s, $\bar{V}_R = 0.04\ \mu$M/s					
c	ρ_v	$\bar{R}_b$	$\bar{b}_1(0)$	$b_0(0; c\rho_v)$	$\bar{b}_2(0; c\bar{R}_b)$
0	0.394	0.393	0.0581	0.1162	0.1156
1	0.357	0.355	0.0581	0.1040	0.1026
2	0.328	0.327	0.0581	0.0959	0.0944
4	0.287	0.285	0.0581	0.0857	0.0841
8	0.235	0.233	0.0581	0.0747	0.0731

mechanism (on receptor-mediated degradation of signaling gradient). The difference is $< 3\%$ of $\bar{b}_2(0)$. On the other hand, the difference between the two corresponding robustness indices (for $c = 8$ and $n = 1$),

$$\bar{R}_b\big(\bar{b}_v(x)\big) - \bar{R}_b\big(\bar{b}_c(x)\big) = 0.235 - 0.082 = 0.153,$$

is more than 38% of $\bar{R}_b(\bar{b}_2(x))$. The reason for this disproportionately large difference in $\bar{R}_b$ is seen from the graph for $[\bar{b}_v(x)]_{c=8} = [\bar{b}_2(x)]_{c_R=8,c_l=0}$ in Figure 1 in Section 9 below (principally for illustrating the benefits of the second multifeedback arrangement). There is a much more substantial and qualitative change in the shape of gradient $\bar{b}_v(x)$ relative to $\bar{b}_1(x)$ and $\bar{b}_2(x)$, particularly the change in its convexity, due to the change of the shape parameter from μ_0^2 of $\bar{b}_e(x)$ to μ_c^2 of $\bar{b}_v(x)$ at high feedback strength ($c = 8$). It is this substantial shape difference that leads to a larger difference between robustness index than the difference $\bar{b}_v(0) - \bar{b}_c(0)$ would suggest.

Together, these observations indicate an ineffectiveness of the present feedback process in promoting robust signaling gradient. Before we attempt to seek an explanation for the presence of this ineffective feedback in gradient systems, we continue our investigation of other feedback processes known to exist during gradient formation.

6 | POSITIVE NONLOCAL FEEDBACK ON FREE RECEPTOR DEGRADATION

6.1 | Free receptor degradation with positive nonlocal feedback

Members of the TGF-β superfamily of secreted signaling molecules regulate growth and cellular patterning during development and interact with specific type I and type II membrane receptors. Two members of the type I receptor family in Drosophila are encoded by the genes saxophone (sax) and thickveins (Tkv). Mutations that abolish sax or Tkv activity cause phenotypes similar to partial or complete loss of activity, respectively, of the TGF-β homolog Dpp.[26] Within the framework of the present three-component extracellular model, an increase in the rate of receptor mutation may be taken as an increase in the rate of free receptor degradation. A robustness index induced feedback on the free receptor degradation rate would replace the (wild-type) normalized degradation rate constant g_R by

$$g_R^{(c)} = g_R\big[1 + cR_b^n(t-\tau)\big],$$

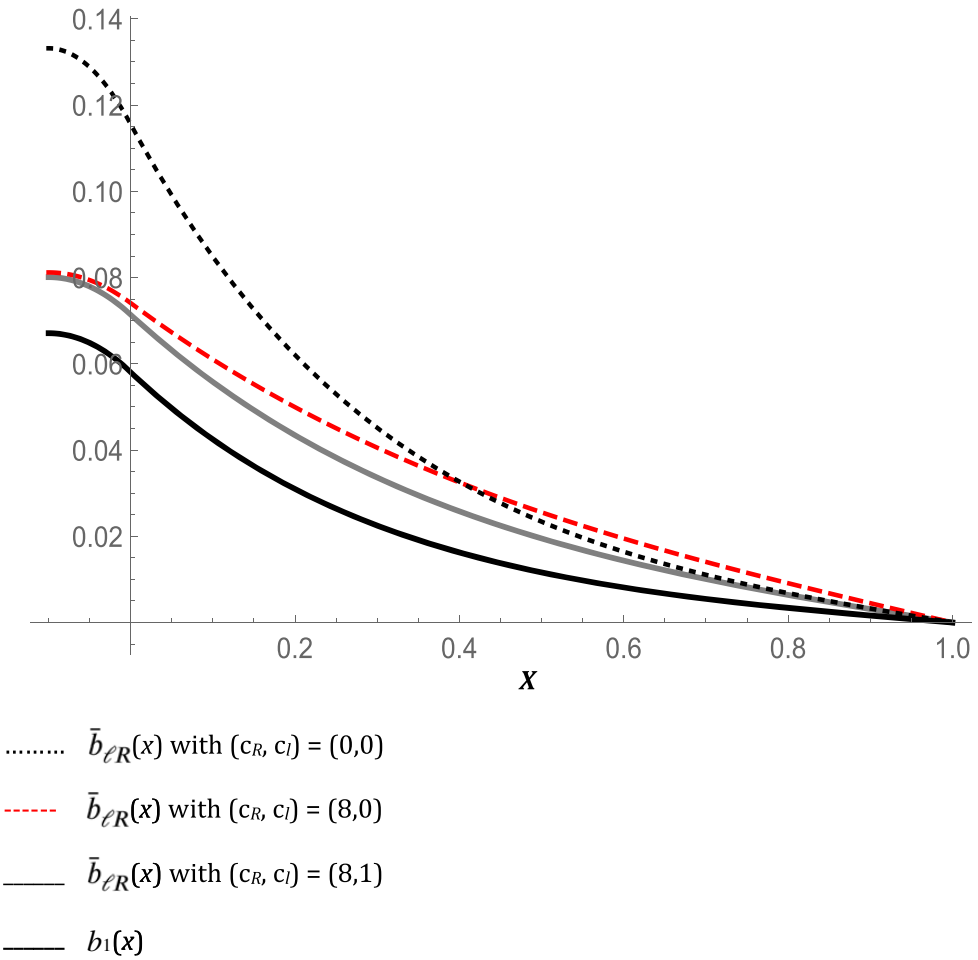

FIGURE 1 Signaling gradient robustness with concurrent feedback on free receptor degradation and free ligand degradation for $e = 2$

where c and n are two parameters that regulate the strength of the feedback process with $[g_R^{(c)}]_{c=0} = g_R$ in the absence of feedback. The parameter $\tau \geq 0$ provides the effect of a possible time delay.

6.2 | Steady state

Similar to the extracellular model without feedback, the present system also has a unique nonnegative signaling gradient with $R_b(t) \to \bar{R}_b$ so that

$$g_R^{(c)} \to g_R\left[1 + c\bar{R}_b^n\right]. \tag{76}$$

With (76) (and again with $g_L = f_0 = 0$), the steady-state solution of the problem, denoted by $\{\bar{a}_R(x), \bar{b}_R(x), \bar{r}_R(x)\}$, now depends on $\bar{R}_b$ (and the two parameters c and n). The relevant BVP for $\bar{a}_R(x)$ is

$$\bar{a}_R'' - \frac{g_0\bar{a}_R}{\alpha_c + \zeta_0\bar{a}_R} + e\bar{v}_L H(-x) = 0, \tag{77}$$

with

$$\alpha_c = \alpha_0\left(1 + c\bar{R}_b^n\right) = \frac{g_0}{h_0}\left(1 + c\bar{R}_b^n\right). \tag{78}$$

The free ligand concentration satisfies the same boundary condition previously given in (12) when there was no feedback:

$$\bar{a}_R'(-x_m) = 0, \quad \bar{a}_R(1) = 0. \tag{79}$$

The bound morphogen and unbound receptor concentrations are given in terms of $\bar{a}_R(x)$ by

$$\bar{b}_R(x) = \frac{\bar{a}_R(x)}{\alpha_c + \zeta_0 \bar{a}_R(x)}, \qquad \bar{r}_R(x) = \frac{\alpha_0}{\alpha_c + \zeta_0 \bar{a}_R(x)}. \tag{80}$$

Before moving on to examine the LRO approximation of this problem, we note that the only difference between the BVP for $\bar{a}_R(x)$ and that for $\bar{a}_v(x)$ of the previous section is ζ_c in (63) and (66) being replaced by ζ_0 in the ODE for $\bar{a}_R(x)$ and the expressions for $\bar{b}_R(x)$ and $\bar{r}_R(x)$.

6.3 | Low and high receptor occupancy

If the morphogen system is in an LRO state prior to and after ligand synthesis enhancement so that

$$\zeta_0 \bar{a}_1(x) \ll \alpha_0 \quad \text{and} \quad \zeta_0 \bar{a}_R(x) \ll \alpha_c, \tag{81}$$

$\bar{a}_R(x)$ may be approximated accurately by $a_0(x; c\rho_R^n)$ to be determined by linearized BVP

$$a_0'' - \mu_c^2 a_0 + e\bar{v}_L H(-x) = 0, \tag{82}$$

$$a_0'\left(-x_m; c\rho_R^n\right) = 0, \quad a_0\left(1; c\rho_R^n\right) = 0, \tag{83}$$

with

$$\mu_c^2 = \frac{g_0}{\alpha_c} = \frac{h_0}{1 + c\rho_R^n}. \tag{84}$$

Here, ρ_R denotes the LRO approximation for $\bar{R}_b$.

The BVP (82)-(83) is the same as the corresponding LRO problem (68)-(69) in the previous section for a negative feedback on receptor synthesis rate; so are the expressions for the signaling gradient and the unoccupied receptor concentration. Hence, the exact solution for $a_0(x; c\rho_R^n) = e\bar{A}_R(x)$ and the corresponding signaling gradient $b_0(x; c\rho_R^n) = e\bar{B}_R(x)$ are as given by (71) and (72) with $\rho_R = \rho_v$:

$$a_0\left(x; c\rho_R^n\right) = e\bar{A}_R(x) = e\bar{A}_v(x), \tag{85}$$

$$b_0\left(x; c\rho_R^n\right) = e\bar{B}_R(x) = \frac{e\bar{A}_R(x)}{\alpha_0\left(1 + c\rho_R^n\right)}. \tag{86}$$

In particular, $\bar{B}_R(x)$ is given by

$$\bar{B}_R(x) = \frac{\bar{v}_L}{g_0}\begin{cases} 1 - \frac{\cosh(\mu_c)}{\cosh(\mu_c(1+x_m))}\cosh(\mu_c(x+x_m)) & (-x_m \le x \le 0) \\ \frac{\sinh(\mu_c x_m)}{\cosh(\mu_c(1+x_m))}\sinh(\mu_c(1-x)), & (0 \le x \le 1), \end{cases} \tag{87}$$

where μ_c^2 is now given by (84).

TABLE 4 (for the same parameter values as in Table 1)

c	ρ_R	$\bar{R}_b$	$\bar{b}_1(0)$	$b_0(0; c\rho_R)$	$\bar{b}_2(0; c\bar{R}_b)$
0	0.394	0.393	0.0581	0.1162	0.1156
1	0.357	0.356	0.0581	0.1040	0.1030
2	0.328	0.328	0.0581	0.0959	0.0951
4	0.287	0.286	0.0581	0.0857	0.0850
8	0.235	0.234	0.0581	0.0747	0.0741

The robustness index ρ_R, being the same as ρ_v, is again the unique positive root of $f_c(\rho_R) = 0$ (see (75)) with the same numerical value as those given for ρ_v in Table 2 for $n = 1$. That ρ_R should vary with c (for a fixed n) is due again to the fact that the feedback on receptor synthesis does have an effect on the shape of the LRO signaling gradients $e\bar{B}_R(x)$.

6.4 | The nonlinear problem

For the original nonlinear BVP, we do not have a useful explicit solution for the gradients to enable us to see how much, if any, the ectopic signaling ligand concentration with $e > 1$ is reduced by feedback on free receptor degradation rate. To see whether or not the system with the new feedback is actually robust, we compute some typical robustness index values for our problem from the integral relation

$$\bar{R}_b^2 = \frac{1}{B_1^2(0)} \int_0^1 \left[\bar{b}_R(x) - \bar{b}_1(x)\right]^2 dx. \tag{88}$$

With $\bar{b}_R(x) = b_e(x; c\bar{R}_b^n)$ dependent on the robustness index, the relation (88) is a nonlinear equation for $\bar{R}_b$ once c and n are prescribed. Note that, unlike their LRO approximations, $\bar{b}_R(x)$ is not the same as $\bar{b}_v(x)$ because the ODE for $\bar{a}_R(x)$ is not the same as the ODE for $\bar{a}_v(x)$.

As can be seen from Table 4, the values for $\bar{R}_b$ as determined by (88) differ slightly from the corresponding values in Table 3. However, the differences are not particularly significant to warrant further discussion other than to note again that $\bar{R}_b > 0.2$ for $c \leq 8$. Hence, the positive feedback on free receptor degradation is also not at all effective in promoting robustness.

7 | NEGATIVE FEEDBACK ON LIGAND-RECEPTOR BINDING

It is known that there is a negative feedback circuit in which Dpp induces expression of its own antagonist, *Daughters against dpp* (DAd).[30,44] Overexpression of Dad blocks Dpp signaling activity (as seen from a lack dpp target gene *optomotor blind* [omb]). A similar observation has been made on the BMP antagonist Chordin.[28,32] Such inhibiting effects may be modeled by a negative feedback on the binding rate of the ligand with its signaling receptor (and thereby reducing the signaling activity). In the context of a robustness index induced nonlocal feedback, we take this negative feedback loop in the form

$$k_{on}^c = \frac{k_{on}}{1 + c\left[R_b(t-\tau)\right]^n}. \tag{89}$$

We investigate here the steady-state behavior of gradient system subject to this type of feedback. For that purpose, we replace the dimensionless wild-type binding rate constant h_0 by

$$h_c = \frac{h_0}{1 + c\bar{R}_b^n}, \tag{90}$$

where c and n are two parameters to be specified for specific application and h_c reduces to h_0 in the absence of feedback ($c = 0$) or ectopicity ($R_b = 0$).

The governing equations for the steady-state free ligand concentration $\bar{a}_h(x) = \bar{a}_e(x; c\bar{R}_b^n)$ of the gradient system with the new feedback remain as given by (11)-(13) but now the dimensionless binding rate constant h_0 is replaced by h_c so that

$$\bar{b}_h(x) = \frac{\bar{a}_h(x)}{\alpha_c + \zeta_0\bar{a}_h(x)}, \qquad \bar{r}_h(x) = \frac{\alpha_c}{\alpha_c + \zeta_0\bar{a}_h(x)}, \tag{91}$$

where

$$\alpha_c = \alpha_0\left(1 + c\bar{R}_b^n\right).$$

Upon using (91) to eliminate $\bar{b}_h$ and $\bar{r}_h$ from the remaining equation, we obtain

$$\bar{a}_h'' - \frac{g_0\bar{a}_h}{\alpha_c + \zeta_0\bar{a}_h} + e\bar{v}_L H(-x) = 0. \tag{92}$$

The boundary conditions for this second-order nonlinear ODE remain as given by (12).

The BVP for $\bar{a}_h(x)$ and the expressions for the corresponding signaling ligand concentration and unoccupied receptor concentration are identical to those for the problem with a positive feedback on the free receptor degradation rate. As such, the results from that section, particularly the results in Table 3, also apply to this problem, showing that the new feedback mechanism, acting alone, is also not effective in promoting robustness.

8 | POSITIVE FEEDBACK ON FREE LIGAND DEGRADATION

8.1 | Robustness-index-induced nonlocal positive feedback on free ligand degradation

At the basic level of strength and sensitivity of $(c, n) = (1, 1)$, the action of each of the four feedback mechanisms investigated so far does not lead to a robust signaling gradient when experiencing an enhanced ligand synthesis rate. Moreover, the last three do not reduce ectopicity to $e < 0.2$ even at higher feedback strength well above the $c = 1$. In this section, we examine one remaining possible feedback process for the same three-component model. Non(-signaling)-receptors such as heparan sulfate proteoglycans and other BMP family antagonists are known to upregulate when there is ectopic signaling and bind with ligand to reduce the ectopic ligand concentration.[24,31,44] As a first effort to investigate the effects of this feedback phenomenon on the robustness of the signaling gradient, we bypass the intermediate step of upregulating nonreceptors and their binding with free ligand units and simply model it as a positive feedback on a free ligand degradation rate in the form

$$k_L^{(c)} = ch_0R_b^n(t - \tau).$$

Though it is formally analogous to the self-enhanced ligand degradation of Refs. 49 and 50, the proposed feedback process fully acknowledges that ligand degradation is generally receptor-mediated in the absence of feedback (through $k_L^{(c)} = 0$ for either $c = 0$ (no feedback) or $R_b(t-\tau) = 0$ (no ectopicity)). (The actual downregulation of ligand concentration by nonreceptors will be treated in a separate publication.) In terms of the scaled parameters, the feedback in steady state takes the form

$$g_L^{(c)} = ch_0 \bar{R}_b^n. \tag{93}$$

Note that in contrast to previous feedback forms, we have taken the feedback strength parameter in units of the dimensionless binding rate constant h_0 (ch_0 instead of c). The appropriateness of the form (93) will be become clear when we examine the LOR solution of the problem.

8.2 | Time independent steady state with feedback

Similar to extracellular model system without feedback[7], the present modeled gradient system tends to a unique steady state $\{\bar{a}_\ell(x), \bar{b}_\ell(x), \bar{r}_\ell(x)\}$ with $R_b(t) \to \bar{R}_b$. For the steady-state solution. the governing partial differential equations and boundary conditions become

$$\bar{a}_\ell'' - h_0 \bar{a}_\ell \bar{r}_\ell - g_L^{(c)} \bar{a}_\ell + e\bar{v}_L H(-x) = 0, \tag{94}$$

$$h_0 \bar{a}_\ell \bar{r}_\ell - g_0 \bar{b}_\ell(x) = 0, \qquad (g_r + h_0 \bar{a}_\ell)\bar{r}_\ell = \bar{v}_R, \tag{95}$$

with

$$\bar{a}_\ell'(-x_m) = 0, \qquad \bar{a}_\ell(1) = 0, \tag{96}$$

where a prime again indicates differentiation with respect to x.

As in the case without feedback, we can solve (95) for $\bar{b}_\ell(x)$ and $\bar{r}_\ell(x)$ in terms of $\bar{a}_\ell(x)$ to get

$$\bar{b}_\ell(x) = \frac{\bar{a}_\ell(x)}{\alpha_0 + \zeta_0 \bar{a}_\ell(x)}, \qquad \bar{r}_\ell(x) = \frac{\alpha_0}{\alpha_0 + \zeta_0 \bar{a}_\ell(x)}, \tag{97}$$

with $\alpha_0 = g_0/h_0$ and $\zeta_0 = g_0/g_R$ as previously indicated (having taken $f_0 = 0$). The results are used to eliminate these two quantities from the only ODE (94) to get a BVP for $\bar{a}_\ell(x)$ alone:

$$\bar{a}_\ell'' - \frac{g_0 \bar{a}_\ell}{\alpha_0 + \zeta_0 \bar{a}_\ell} - g_L^{(c)} \bar{a}_\ell + e\bar{v}_L H(-x) = 0. \tag{98}$$

For nonnegative values of the parameters $g_0, g_L^{(c)}, g_R, h_0, \bar{v}_L$, and $\bar{v}_R$, there exists a unique, nonnegative solution $\bar{a}_\ell(x)$ of the BVP defined by (98) and (96). The corresponding concentrations $\bar{b}_\ell(x)$ and $\bar{r}_\ell(x)$ can then be calculated from (97).

8.3 | Low receptor occupancy

When the morphogen system is in a state of *LRO* prior to and after ligand synthesis enhancement so that:

$$\zeta_0 \bar{a}_1 \ll \alpha_0, \quad \zeta_0 \bar{a}_\ell \ll \alpha_0,$$

we may use as an approximate solution for $\bar{a}_\ell(x)$ the solution $a_0(x; ch_0\rho_\ell^n)$ of the linearized BVP

$$a_0'' = \mu_\ell^2 a_0 - e\bar{v}_L H(-x), \quad a_0'\left(-x_m; ch_0\rho_\ell^n\right) = a_0\left(1; ch_0\rho_\ell^n\right) = 0, \tag{99}$$

with

$$\mu_\ell^2 = g_L^{(c)} + \frac{g_0}{\alpha_0} = h_0\left(1 + c\rho_\ell^n\right) = \mu_0^2\left(1 + c\rho_\ell^n\right), \tag{100}$$

where ρ_ℓ is the corresponding approximate value for $\bar{R}_b$. The exact solution of (99) is given by

$$a_0\left(x; ch_0\rho_\ell^n\right) \equiv e\bar{A}_\ell(x),$$

where

$$\bar{A}_\ell(x) = \frac{\bar{v}_L}{\mu_\ell^2}\begin{cases} 1 - \frac{\cosh(\mu_\ell)}{\cosh(\mu_\ell(1+x_m))}\cosh(\mu_\ell(x+x_m)) & (-x_m \le x \le 0) \\ \frac{\sinh(\mu_\ell x_m)}{\cosh(\mu_\ell(1+x_m))}\sinh(\mu_\ell(1-x)) & (0 \le x \le 1) \end{cases}. \tag{101}$$

The LRO signaling gradient is then given by

$$b_0\left(x; ch_0\rho_\ell^n\right) = \frac{e\bar{A}_\ell(x)}{\alpha_0} \equiv e\bar{B}_\ell(x) \tag{102}$$

with

$$\bar{B}_\ell(x) = \frac{\bar{v}_L}{g_0\left(1 + c\rho_\ell^n\right)}\begin{cases} 1 - \frac{\cosh(\mu_\ell)}{\cosh(\mu_\ell(1+x_m))}\cosh(\mu_\ell(x+x_m)) & (-x_m \le x \le 0) \\ \frac{\sinh(\mu_\ell x_m)}{\cosh(\mu_\ell(1+x_m))}\sinh(\mu_\ell(1-x)) & (0 \le x \le 1). \end{cases} \tag{103}$$

Evidently, the positive feedback on free ligand degradation rate has the effect of reducing the ectopicity of signaling ligand concentration, at least when the system is in an LRO state. However, the reduction is negligibly small for moderate feedback strength (with $ch_0 = O(1)$ at most and $n \ge 1$) as h_0 is typically $O(10)$.

With $\bar{R}_b \simeq \rho_\ell$ typically < 1 in magnitude, we have $1 + c\rho_\ell^n \simeq 1$ for the feedback strength $ch_0 = O(1)$. In that case, the approximate robustness index ρ_ℓ is the unique root of

$$f_\ell(\rho_\ell) \equiv 2\sinh^2(\mu_0)\rho_\ell^2 - e_\ell^2\left(\frac{\sinh(2\mu_\ell)}{2\mu_\ell} - 1\right) - \left(\frac{\sinh(2\mu_0)}{2\mu_0} - 1\right) \tag{104}$$

$$+ 2e_\ell\left\{\frac{\sinh(\mu_\ell + \mu_0)}{\mu_\ell + \mu_0} - \frac{\sinh(\mu_\ell - \mu_0)}{\mu_\ell - \mu_0}\right\} = 0,$$

where

$$e_\ell = e\frac{\cosh(\mu_0(1+x_m))\sinh(\mu_\ell x_m)}{\cosh(\mu_\ell(1+x_m))\sinh(\mu_0 x_m)}. \tag{105}$$

While $f_\ell(x)$ is structurally the same as $f_c(x)$ of (75) with μ_c replaced by μ_ℓ, the unique zero of (104) for ρ_ℓ is not the same as ρ_v of (75) because the dependence of μ_ℓ on ρ_ℓ is different from the dependence of μ_c on ρ_v. The solution ρ_ℓ of (104) is also easily obtained using the approximate relation (104) by any of the root finders available on scientific computing software. With that solution, the corresponding

TABLE 5 (for the same parameter values as in Table 1 and $n = 1$)

c	ρ_ℓ	$\bar{R}_b$	$\bar{b}_1(0)$	$b_0(0; ch_0\rho_\ell^n)$	$\bar{b}_2(0; ch_0\bar{R}_b^n)$
0	0.394	0.393	0.0581	0.1162	0.1156
0.1	0.370	0.369	0.0581	0.1142	0.1130
0.2	0.349	0.349	0.0581	0.1120	0.1107
0.4	0.317	0.316	0.0581	0.1083	0.1071
1	0.254	0.253	0.0581	0.1011	0.1000
2	0.197	0.196	0.0581	0.0943	0.0933
2.5	0.179	0.178	0.0581	0.0920	0.0910

signaling gradient can be calculated from (102) to (103) as was done for a negative robustness-induced feedback on receptor synthesis rate in a previous section.

Before leaving the LRO solution of the problem, we note that with $\bar{R}_b \simeq \rho_\ell$ typically < 1 in magnitude, we need $c = O(1)$ (instead of $O(h_0^{-1})$) in order for $c\rho_\ell^n$ to have an effect on the signaling gradient. This observation suggests that we take the negative feedback on free ligand degradation in the form (93) with the feedback strength parameter in units of h_0 because it offers the option of taking $c = 1$ as the basic feedback strength level consistent with all other feedback processes considered previously.

8.4 | Numerical results

For $n = 1$, we report in Table 5 values of ρ_ℓ for the example in Table 1 for different $c \geq 0$. The results show $\rho_\ell > 0.2$ for $c \leq 1$, indicating that a robustness-index-induced positive feedback on the free ligand degradation rate is not effective in promoting a robust signaling gradient for this gradient system at a feedback strength level of $ch_0 \leq 10$. The robustness threshold of 0.2 is barely met by $c = 2$ ($ch_0 = 20$). Since the particular system considered is in a state of LRO, this approximate solution is expected to be adequate the problem.

The corresponding robustness index $\bar{R}_b$ and the signaling gradient $\bar{b}_\ell(x) = \bar{b}_2(x; ch_0\rho_\ell^n)$ of the original nonlinear BVP (98) and (96) obtained for the same system reported in Table 5 confirm this expectation. The values for $\bar{R}_b$ are consistent with the corresponding LOR approximation for the sample problem supporting the conclusion that the positive feedback on free ligand degradation rate is not effective in promoting robustness in the model system for the range of feedback strength $c < 2$ ($ch_0 < 20$).

Even with $b_0(0; ch_0\rho_\ell^n)$ approaching $\bar{b}_1(0)$ for larger values of c, the significant shape difference between $\bar{b}_1(x)$ and $b_0(0; ch_0\rho_\ell^n)$ associated with the change in the shape parameter from μ_0^2 to $\mu_\ell^2 = \mu_0^2(1 + c\rho_\ell^n)$ helps to diminish possible reduction of the high robustness index for higher values of c.

9 | MULTIPLE FEEDBACK

Having examined five different feedback mechanisms for promoting signaling gradient robustness herein (in addition to one other on ligand synthesis rate in Ref. 51), we have found that none by itself (at basic feedback strength and sensitivity $c = n = 1$) would ensure a robust signaling gradient ($\bar{R}_b < 0.2$). Only three would do so for $c \geq 2$ (with $ch_0 \geq 20$ in the case of a positive feedback on free ligand degradation), while the other three fail to do so for the very high feedback strength of $c = 8$ ($n = 1$). Even if we accept those effective for $c \geq 2$ as possible mechanisms for promoting robustness (ignoring possible changes in gradient shape), there is still the obvious question why some of the other four ineffective

mechanisms should be present concurrently in many biological developments. To answer this question and, more importantly, to show the necessity and benefits associated with these ineffective feedback mechanisms, we consider in this section two simple multifeedback models each involving a different combination of two concurrent feedback processes among those previously examined herein.

9.1 | Negative feedback on ligand synthesis rate and bound receptor degradation rate

9.1.1 | Steady-state behavior

In this first multifeedback gradient system, we examine the effects of concurrent applications of a negative feedback on ligand synthesis rate (as in Ref. 51) and a positive feedback on receptor-mediated ligand degradation rate (see (33) and (36)). The steady-state BVP for the free ligand concentration in response to the feedback

$$v_L = \frac{\bar{v}_L H(-x)}{1 + c_m \bar{R}_b}, \quad g_c = \frac{g_0}{1 + c_g \bar{R}_b}, \tag{106}$$

may again be reduced to a single ODE for the free ligand concentration $\bar{a}_{cm}(x) = \bar{a}_e(x; c_m, c_g)$:

$$\bar{a}''_{cm} - \frac{g_0 \bar{a}_{cm}}{\alpha_0 + \zeta_0 \bar{a}_{cm}} + \frac{e\bar{v}_L H(-x)}{1 + c_m \bar{R}_b} = 0, \tag{107}$$

and the same two boundary condition previously given in (12) when there was no feedback:

$$\bar{a}'_{cm}(-x_m) = 0, \quad \bar{a}_{cm}(1) = 0. \tag{108}$$

The signaling morphogen and free receptor concentrations are given in terms of $\bar{a}_{cm}(x)$ by

$$\left\{\bar{b}_{cm}(x), \bar{r}_{cm}(x)\right\} = \frac{1}{\alpha_0 + \zeta_0 \bar{a}_{cm}(x)} \left\{ \frac{\bar{a}_{cm}}{1 + c_g \bar{R}_b}, \alpha_0 \right\}. \tag{109}$$

9.1.2 | Low receptor occupancy

To gain some insight to the benefits of this multifeedback system, we first examine the system in a steady state of LRO. With

$$\zeta_0 \bar{a}_1(x) \ll \alpha_0 \quad \text{and} \quad \zeta_0 \bar{a}_{cm}(x) \ll \alpha_{cm},$$

$\bar{a}_{cm}(x)$ may be approximated accurately by $a_0(x; c_m, c_g)$ determined by the linearized BVP

$$a''_0 - h_0 a_0 + \frac{e\bar{v}_L H(-x)}{1 + c_m \rho_{cm}} = 0, \quad a'_0(-x_m; c_m, c_g) = a_0(1; c_m, c_g) = 0, \tag{110}$$

with ρ_{cm} being the LRO approximation for $\bar{R}_b$.

The exact solution of this BVP is

$$a_0(x; c_m, c_g) = \frac{e\bar{A}_1(x)}{1 + c_m \rho_{cm}}. \tag{111}$$

The corresponding signaling gradient in an LRO state with the same feedback is given by

$$\bar{b}_{cm}(x) \simeq b_0(x; c_m, c_g) = \frac{a_0(x; c_m, c_g)}{\alpha_0\big(1 + c_g\rho_{cm}\big)} \tag{112}$$

$$= \frac{e\bar{A}_1(x)}{\alpha_0\big(1 + c_m\rho_{cm}\big)\big(1 + c_g\rho_{cm}\big)} = \frac{e\bar{B}_1(x)}{\big(1 + c_m\rho_{cm}\big)\big(1 + c_g\rho_{cm}\big)}$$

with

$$\bar{B}_1(x) = \frac{\bar{\nu}_L}{g_0}\begin{cases} 1 - \frac{\cosh(\mu_0)}{\cosh(\mu_0(1+x_m))}\cosh(\mu_0(x + x_m)) \quad (-x_m \le x \le 0) \\ \frac{\sinh(\mu_0 x_m)}{\cosh(\mu_0(1+x_m))}\sinh(\mu_0(1 - x)) \quad (0 \le x \le 1). \end{cases} \tag{113}$$

For the LRO approximation ρ_{cm} of $\bar{R}_b$, we have the analogue of (49)

$$\bar{R}_b \simeq \rho_{cm} = \left(\frac{e}{\big(1 + c_m\rho_{cm}\big)\big(1 + c_g\rho_{cm}\big)} - 1\right)\sqrt{\int_0^1 \left[\frac{\bar{B}_1(x)}{\bar{B}_1(0)}\right]^2 dx}$$

so that

$$\rho_{cm} = \gamma_0\left\{\frac{e}{\big(1 + c_m\rho_{cm}\big)\big(1 + c_g\rho_{cm}\big)} - 1\right\}, \tag{114}$$

with γ_0 given by (51). Even without the actual solution ρ_{cm} of the nonlinear equation (114) above, it is clear that we have $\rho_{cm} < \rho_c$ (where ρ_c is given by (53)) as well as $\rho_{cm} < \rho_m$ (where ρ_m is the LRO approximation for the robustness index with $c_g = 0$ investigated in Ref. 51). For brevity, we will not compute the solution of (114) for specific sets of parameter values but only summarize these observations on ρ_{cm} in the following proposition:

Proposition 6. *The multifeedback gradient system subject to* (106) *is more robust than a system with only one constituent feedback acting alone. Moreover, we have*

$$\big[\rho_{cm}\big]_{c_m=c_g=c} < \big[\rho_{cm}\big]_{c_m=2c,c_g=0} = \big[\rho_{cm}\big]_{c_m=0,c_g=2c}. \tag{115}$$

Proof. The first claim follows from

$$\rho_{cm} < \gamma_0\left\{\frac{e}{1 + c_m\rho_{cm}} - 1\right\}, \quad \rho_{cm} < \gamma_0\left\{\frac{e}{1 + c_g\rho_{cm}} - 1\right\}.$$

The inequality (115) follows from

$$\big[\rho_{cm}\big]_{c_m=c_g=c} = \gamma_0\left\{\frac{e}{\big(1 + c\rho_{cm}\big)^2} - 1\right\} < \gamma_0\left\{\frac{e}{1 + 2c\rho_{cm}} - 1\right\}.$$

■

TABLE 6 (for the same parameter values as in Table 1 and $c_g = c_m = 2$ with $\bar{b}_1(0) = 0.05811$)

$c_g \backslash c_m$	**0**	**1**	**2**	
0	0.393	0.241	0.183	$\bar{R}_b$
	0.1156	0.0934	0.0849	$\bar{b}_{cm}(0)$
1	0.241	0.176	0.143	$\bar{R}_b$
	0.0932	0.0838	0.0789	$\bar{b}_{cm}(0)$
2	0.183	0.143	0.119	$\bar{R}_b$
	0.0847	0.0788	0.0755	$\bar{b}_{cm}(0)$

9.1.3 | Numerical solutions

To further confirm the benefits from the two concurrent feedback processes, we report in Table 6 accurate numerical solutions of the original nonlinear steady-state problem for the same set of parameter values as in Table 1. Not surprisingly, the results clearly demonstrate how robustness improves with increasing c_g or c_m. In particular, $\bar{R}_b$ decreases from 0.394 (well above the threshold of 0.2) in the absence of any feedback, ie, $(c_g, c_m) = (0, 0)$, to 0.183 (< 0.2) for $(c_g, c_m) = (2, 0)$ or (0,2). The magnitude of the signaling gradient $\bar{b}_{cm}(0)$ is certainly reduced by about $1/3$ of the $\bar{b}_e(0) - \bar{b}_{cm}(0)$ differential at the higher feedback sensitivity/strength $(c_g, c_m) = (2, 2)$.

More significantly, the combined effects of the two-feedback combination of this section at the *basic feedback strength level* of $c_m = 1$ and $c_g = 1$ not only lower the robustness index below the robustness threshold (not attained by either feedback alone at that strength), but also below the robustness index value for either individual feedback at the higher feedback strength of $\{c_m = 2, c_g = 0\}$ or $\{c_m = 0, c_g = 2\}$, confirming the second part of Proposition 6. Hence, the concurrent presence of the two feedback processes reduces the demand on the feedback strength required by either feedback process operating alone. Consequently, the two-feedback combination is preferred over either individual process at a higher feedback strength level.

9.2 | Positive feedback on free receptor and free ligand degradation

That the benefits accrued to the two-feedback combination of the previous subsection should be accumulative when executed concurrently is not a complete surprise because each alone is effective in promoting robustness at higher feedback strengths ($c \geq 2$). However, how may we justify the presence of feedback processes that are ineffective even at high feedback strengths? In this subsection, we consider a different combination of two of these ineffective feedback processes for an explanation. The new combination involves a positive feedback on both free receptor degradation and free ligand degradation. When implemented alone in an LRO state, each has been seen to induce a change in the shape parameter (from μ_0^2 to μ_R^2 and μ_ℓ^2, respectively) and thereby hamper its effectiveness in promoting robustness. Surprisingly, the concurrent activities of these two feedback processes will be seen to be capable of ensuring robust signaling at moderate feedback strengths, while each acting alone would fail to do so even at rather high levels of feedback strength.

9.2.1 | Steady-state behavior

To support the assertion above, it suffices to consider the concurrent activities of two steady-state feedback processes

$$g_R^{(c)} = g_R\left[1 + c_R \bar{R}_b\right], \quad g_L^{(c)} = c_\ell h_0 \bar{R}_b. \tag{116}$$

For such a multifeedback system, the governing steady-state equations may be reduced to a single ODE for the free ligand concentration $\bar{a}_{\ell R}(x) = \bar{a}_e(x; c_R, c_\ell)$:

$$\bar{a}''_{\ell R} - \frac{g_0 \bar{a}_{\ell R}}{\alpha_R + \zeta_0 \bar{a}_{\ell R}} - c_\ell h_0 \bar{R}_b \bar{a}_{\ell R} + e\bar{\nu}_L H(-x) = 0, \tag{117}$$

with

$$\alpha_R = \alpha_0\big(1 + c_R \bar{R}_b\big) = \frac{g_0}{h_0}\big(1 + c_R \bar{R}_b\big). \tag{118}$$

The free ligand concentration satisfies the same boundary condition previously given in (12) when there was no feedback:

$$\bar{a}'_{\ell R}(-x_m) = 0, \quad \bar{a}_{\ell R}(1) = 0. \tag{119}$$

The signaling morphogen and free receptor concentrations are given in terms of $\bar{a}_{\ell R}(x)$ by

$$\bar{b}_{\ell R}(x) = \frac{\bar{a}_{\ell R}(x)}{\alpha_R + \zeta_0 \bar{a}_{\ell R}(x)}, \qquad \bar{r}_{\ell R}(x) = \frac{\alpha_0}{\alpha_R + \zeta_0 \bar{a}_{\ell R}(x)}. \tag{120}$$

9.2.2 | Low receptor occupancy

To gain some insight on the advantage of this multifeedback system, we again consider the corresponding system in a steady state of LRO. With

$$\zeta_0 \bar{a}_1(x) \ll \alpha_0 \quad \text{and} \quad \zeta_0 \bar{a}_{\ell R}(x) \ll \alpha_{\ell R}, \tag{121}$$

$\bar{a}_{\ell R}(x)$ may be approximated accurately by $a_0(x; c_R, c_\ell)$ determined by the linearized ODE

$$a_0'' - \mu_{\ell R}^2 a_0 + e\bar{\nu}_L H(-x) = 0, \tag{122}$$

and the boundary conditions (119) with

$$\mu_{\ell R}^2 = \mu_0^2 \eta^2, \quad \eta^2 = \frac{1}{1 + c_R \rho_{\ell R}} + c_\ell \rho_{\ell R}. \tag{123}$$

Here, $\rho_{\ell R}$ denotes the LRO approximation for $\bar{R}_b$ for our multifeedback problem.

The exact solution of the BVP defined by (122) and the boundary conditions $a_0'(-x_m; c_R, c_\ell) = 0$ and $a_0(1; c_R, c_\ell) = 0$ is

$$a_0(x; c_R, c_\ell) = e\bar{A}_{\ell R}(x)$$

with

$$\bar{A}_{\ell R}(x) = \frac{\bar{\nu}_L}{\mu_{\ell R}^2} \begin{cases} 1 - \frac{\cosh(\mu_{\ell R})}{\cosh(\mu_{\ell R}(1+x_m))} \cosh(\mu_{\ell R}(x + x_m)) & (-x_m \le x \le 0) \\ \frac{\sinh(\mu_{\ell R} x_m)}{\cosh(\mu_{\ell R}(1+x_m))} \sinh(\mu_{\ell R}(1 - x)) & (0 \le x \le 1) \end{cases}. \tag{124}$$

In other words, $\bar{A}_{\ell R}(x)$ is $\bar{A}_1(x)$ of (45) with μ_0^2 replaced by $\mu_{\ell R}^2$ (see (123)). The corresponding LRO signaling gradient is given by

$$\bar{b}_{\ell R}(x) \simeq b_0(x; c_R, c_\ell) = \frac{a_0(x; c_R, c_\ell)}{\alpha_R} = \frac{e\bar{A}_{\ell R}(x)}{\alpha_R} \equiv e\bar{B}_{\ell R}(x). \tag{125}$$

With $\mu_{\ell R}^2 = \eta^2\mu_0^2$, the ratio for $\bar{b}_{\ell R}(x)/\bar{b}_1(x)$ in a state of LRO becomes

$$\frac{\bar{b}_{\ell R}(x)}{\bar{b}_1(x)} \simeq \frac{eB_{\ell R}(x)}{B_1(x)} = e_{\ell R}\frac{\sinh(\mu_{\ell R}(1-x))}{\sinh(\mu_0(1-x))}, \tag{126}$$

where

$$e_{\ell R} = \frac{e}{\eta^2\big(1 + c_R\rho_{\ell R}\big)}\frac{\sinh(\eta\mu_0 x_m)\cosh(\mu_0(1+x_m))}{\sinh(\mu_0 x_m)\cosh(\eta\mu_0(1+x_m)}, \tag{127}$$

With $\rho_{\ell R} \geq 0$, we have

$$\eta^2\big(1 + c_R\rho_{\ell R}\big) = 1 + c_\ell\rho_{\ell R}\big(1 + c_R\rho_{\ell R}\big) > 1,$$

and, given $\mu_0^2 = h_0 \gg 1$,

$$0 < \frac{\sinh(\eta\mu_0 x_m)\cosh(\mu_0(1+x_m))}{\sinh(\mu_0 x_m)\cosh(\eta\mu_0(1+x_m)} \sim e^{-\mu_0(\eta-1)},$$

$$\frac{\bar{b}_{\ell R}(0)}{\bar{b}_1(0)} \simeq \frac{eB_{\ell R}(0)}{B_1(0)} = e_{\ell R}\frac{\sinh(\mu_{\ell R})}{\sinh(\mu_0)} \sim \frac{e}{\eta^2\big(1 + c_R\rho_{\ell R}\big)} < e.$$

Also, with $\rho_{\ell R} > 0$, any $c_\ell > 0$ would bring $\mu_{\ell R}$ closer to μ_0 and thereby reduce gradient ectopicity by moving $\sinh(\mu_{\ell R}(1-x))$ closer to $\sinh(\mu_0(1-x))$. We summarize these observations in the following proposition:

Proposition 7. *The multifeedback* (116) *promotes robustness, not only by decreasing the signaling ligand concentration magnitude near the ligand source but also the shape deviation from the wild-type gradient (given $\mu_{\ell R} \simeq \mu_0$).*

9.2.3 | The LRO robustness index

For the LRO approximation $\rho_{\ell R}$ of $\bar{R}_b$, we have the analog of (73)

$$\bar{R}_b^2 \simeq \rho_{\ell R}^2 = \int_0^1 \left[\frac{e\bar{B}_{\ell R}(x)}{\bar{B}_1(0)} - \frac{\bar{B}_1(x)}{\bar{B}_1(0)}\right]^2 dx \tag{128}$$

$$= \int_0^1 \left[e_{\ell R}\frac{\sinh(\mu_{\ell R}(1-x))}{\sinh(\mu_0)} - \frac{\sinh(\mu_0(1-x))}{\sinh(\mu_0)}\right]^2 dx.$$

With (123) and (127), this relation becomes a nonlinear equation for $\rho_{\ell R}$:

$$f_c(\rho_{\ell R}) = 0, \tag{129}$$

TABLE 7 The LRO approximation $\rho_{\ell R}$ for robustness index (for the same parameter values as in Table 1 and $n = 1$)

$c_R \setminus c_\ell$	0	0.1	0.2	0.4	1	2
0	0.394	0.370	0.349	0.317	0.254	0.197
1	0.357	0.332	0.313	0.283	0.226	0.177
2	0.328	0.305	0.287	0.259	0.207	0.163
4	0.287	0.267	0.251	0.226	0.182	0.143
8	0.235	0.219	0.207	0.187	0.151	0.120

TABLE 8 The exact robustness index $\bar{R}_b$ and signal ligand concentration $\bar{b}_{\ell R}(0)$ (for the same parameter values as in Table 1 with $\bar{b}_1(0) = 0.0581$)

$c_R \setminus c_\ell$		0	0.1	0.2	0.4	1	2	2.5
0	$\bar{R}_b$	0.394	0.369	0.349	0.316	0.253	0.196	0.178
	$\bar{b}_{\ell R}(0)$	0.1156	0.1130	0.1107	0.1071	0.1000	0.0933	0.0910
1	$\bar{R}_b$	0.356	0.332	0.312	0.282	0.225	0.176	0.160
	$\bar{b}_{\ell R}(0)$	0.1030	0.1010	0.0994	0.0969	0.0920	0.0874	0.0858
2	$\bar{R}_b$	0.327	0.304	0.286	0.258	0.206	0.162	0.148
	$\bar{b}_{\ell R}(0)$	0.0950	0.0935	0.0922	0.0903	0.0866	0.0833	0.0821
4	$\bar{R}_b$	0.286	0.266	0.250	0.225	0.181	0.142	0.130
	$\bar{b}_{\ell R}(0)$	0.0850	0.0839	0.0831	0.0818	0.0796	0.0776	0.0769
8	$\bar{R}_b$	0.234	0.218	0.206	0.186	0.150	0.119	0.109
	$\bar{b}_{\ell R}(0)$	0.0741	0.0735	0.0731	0.0724	0.0714	0.0708	0.0706

where

$$f_c(\rho_{\ell R}) \equiv 2\sinh^2(\mu_0)\rho_{\ell R}^2 - e_{\ell R}^2\left(\frac{\sinh(2\mu_{\ell R})}{2\mu_{\ell R}} - 1\right) - \left(\frac{\sinh(2\mu_0)}{2\mu_0} - 1\right) \tag{130}$$
$$+2e_{\ell R}\left\{\frac{\sinh(\mu_{\ell R}+\mu_0)}{\mu_{\ell R}+\mu_0} - \frac{\sinh(\mu_{\ell R}-\mu_0)}{\mu_{\ell R}-\mu_0}\right\}.$$

For $c_\ell = 0$, the expression for $f_c(\rho_{\ell R})$ reduces to (75) after some appropriate changes of notations.

In Table 7, we report some results from the LRO solution to show how combinations of the two feedback processes of this section can ensure robustness, while they fail to do so individually even at higher levels of feedback strength keeping in mind the strength parameter being $c_\ell h_0$ as shown in (116).

9.2.4 | Numerical solutions

To further confirm the benefits from the particular combination of the two concurrent feedback processes (116), we report in Table 8 accurate numerical solutions of the original nonlinear steady-state problem for the same set of parameter values as in Table 7. The results clearly demonstrate again how closely is the LRO solution $\rho_{\ell R}$ approximating the exact robustness index $\bar{R}_b$. In addition, the results reported in Table 8 also show how robustness improves with increasing feedback strength parameter pair $(c_R,\ c_\ell)$. In particular, $\bar{R}_b$ decreases from 0.394 (well above the threshold of 0.2) with no feedback $(c_R, c_\ell) = (0, 0)$ to 0.162 (well below the threshold value) for $(c_R, c_\ell) = (2, 2)$.

Similar to Table 7, the exact results of Table 8 also show that a single constituent feedback is again ineffective for promoting robustness even operating at well above the basic level. For example, $(c_R, c_\ell) = (8, 0)$ would lead to a robustness index $\bar{R}_b = 0.234$, well above the threshold of 0.2. For $(0, c_\ell)$, $\bar{R}_b$ is still above the robustness threshold of 0.2 for $c_\ell < 2$. At (0,2) with $c_\ell h_0 = 20$, we have $\bar{R}_b = 0.196$, which is barely below the robustness threshold. However, by implementing the two feedback processes concurrently, we see from Table 8 that robustness, rather remarkably, can be attained with $(c_R, c_\ell) = (8, 0.4), (4, 1)$, and (1,2) and other combinations.

The magnitude of the signaling gradient $\bar{b}_{\ell R}(x)$ is certainly seen to tend to $\bar{b}_1(x)$ with higher feedback strength as shown by the changes of $\bar{b}_{\ell R}(0)$ in Table 8. The reduction is more substantial with c_R increasing from 0 to 8 compared to changes in $c_\ell h_0$ from 0 to 25. This is consistent with the impact of c_ℓ on the magnitude being proportional $(1 + c_\ell \bar{R}_b)^{-1}$ (and not $(1 + c_\ell h_0 \bar{R}_b)^{-1}$ when the system is in a state of LRO), while that of c_R is proportional to $(1 + c_R \bar{R}_b)^{-1}$. In contrast, the differential changes in robustness index are more comparable over the same ranges. Even more important is how the shape parameter $\mu^2_{\ell R}$ as defined in (123) is changed by the two types of feedback: one reduces its magnitude, while the other increases it resulting in a net effect of little change when both feedback processes operate at the same level of strength. Consequently, the shape of the ectopic signaling gradient modified by the two concurrent types of feedback remains more or less the same as the wild-type gradient shape.

Together, the combined effects of the two concurrent feedback process (116) have the distribution of $\bar{b}_{\ell R}(x)$ tending to $\bar{b}_1(x)$ in both magnitude and shape with increasing feedback strength, unlike the corresponding gradient with a single feedback process (of the last four types). The graph of $\bar{b}_{\ell R}(x) = [\bar{b}_2(x)]_{(c_R, c_l) = (8,1)}$ in Figure 1 further illustrates the impact of the additional second feedback associated with c_ℓ. The graph for $\bar{b}_{\ell R}(x)$ with $(c_R, c_\ell) = (8, 0)$ (which is the same as $\bar{b}_v(x)$ with $c = 8$) is not much further away from the wild type than that for $(c_R, c_\ell) = (8, 1)$. However, the slope and convexity of the former graph are much more distorted to result in a substantially higher robustness index.

10 | SUMMARY AND CONCLUDING REMARKS

10.1 | A new nonlocal feedback and gene regulation network

To provide a quantitative measure of the abnormality of biological signaling activities induced by genetic (such as mutation) or epigenetic (such as environmental) perturbations, a *robustness index* as defined in (26) was introduced in Refs. 4 and 36 to gauge the degree of ectopicity in the affected biological development. As seen from the definition, it is effectively the root mean square of the deviation of the ectopic signaling gradient from the wild type over the span of the signaling region normalized by some reference signaling activity level. While feedback has long been known from experimental observations and modeling results (see Ref. 55 and references cited earlier) to be a required instrument for reducing undesirable ectopic signaling and promoting robust development, theoretical analysis and numerical simulations have suggested that Hill's function-type local feedback induced by an ectopic signaling gradient is likely to be ineffective. It was pointed out in Refs. 48 and 51 that pointwise feedback mechanisms are functionally unrealistic and theoretically inappropriate for preserving magnitude and shape of wild-type signaling gradients. There are good reasons to think that appropriate nonlocal, spatially uniform feedback processes are more likely to be successful for that task. To this end, a new spatially uniform feedback mechanism was previously formulated in Refs. 51 and 56 based on the robustness index (26).

Though no explicit documentation is available to support nonlocal feedback, experience from our daily life suggests that it is certainly not unrealistic. At the cellular level, it could be induced by the

transported local feedback activities of gene regulatory networks. It is generally accepted that gene expression levels of its mRNA and proteins are governed by gene regulation networks, consisting of collections of molecular regulators. They interact with each other and with other substances in a cell as well as respond to external environment (epigenetic perturbations) to ensure survival. In multicellular organisms, a gene that is turned on in one cell may make a product that is transported away from the cell by diffusion (in extracellular space) and transcytosis/bucket brigade (through neighboring cells) and enter one or more other cells to turn on genes only when it is present above a certain threshold level. The impacted cells are thus induced into a new fate by response to local feedback of far away cells, and may even generate other morphogens that signal back to the original cell (see Ref. 57 and references therein). Hence, gene regulation activities (resulting from some feedback process) in one cell are likely to impact multiple cells in the multicellular organism in a coordinated nonlocal feedback. While the link from this coordinated gene regulatory networks generated nonlocal feedback to the several robustness-index-induced feedback mechanisms remains to be established, the actual impact of the transported gene-regulation-generated product may be tracked by modeling their spatial dynamics throughout the organism. Spatial dynamics models of interaction among cell regulatory networks at different locations are complicated. It seems prudent to consider the simpler (though theoretically more tenuous) robustness index induced feedback models in this paper for a first investigation of the necessity and benefits of the concurrent application of more than one such feedback processes. Knowing the payoff of multifeedback combinations, there would be more incentive to invest the time and effort on more realistic multiple feedback models based on multicellular regulatory network interaction.

10.2 | A proof of concept investigation

A proof of concept effort to establish the efficacy of the new robustness-index-induced feedback mechanism was undertaken in Refs. 51 and 56 to investigate the effects of a negative feedback that modifies the ligand synthesis rate in the form

$$v_L(x,t) = \frac{e\bar{v}_L}{1 + cR_b^n(t-\tau)} H(-x), \tag{131}$$

where c and n are two parameters characterizing the strength and sensitivity of the feedback, respectively, and τ measuring possible time delay of the effect of the feedback on the morphogen dynamics. The modified synthesis rate reduces to (7) in the absence of feedback, ie, when $cR_b^n = 0$. In that proof-of-concept effort, we bypassed the intermediate process of upregulating the inhibiting agent(s) (such as Dad[30]) and applied the negative feedback directly on the ligand synthesis rate as the feedback process ultimately downregulates expression of the relevant ligand. The results obtained in Refs. 51 and 56 are encouraging in that the new feedback process is substantially more effective in promoting robust signaling. In fact, robustness index of the ectopic signaling gradient below the acceptable robustness threshold of 0.2 was attained for moderate values of the feedback strength parameter ($c \geq 2$, $n = 1$).

10.3 | Multiple feedback mechanisms

In reality, feedback processes do not regulate ligand synthesis rate directly. Instead, they are known to upregulate some inhibiting agents that downregulate (free or bound) ligand expression. One such process is for Dpp concentration to induce expression of its own antagonist, Dad.[30] Also, not all feedback for robustness pertains to downregulating ligand expression. It is therefore necessary to continue our investigation of the new approach to feedback on other type of feedback processes for promoting

robustness. In this article, we have limited our effort to examining possible meaningful feedback processes for the three-component extracellular morphogen gradient model of Ref. 7. More specifically, we examined the effects of the following robustness-index-induced feedback processes on the model defined by the IBVP (1), (2), (8), and (9) known to exist during the formation of various signaling gradient systems:

- Positive feedback on the receptor-mediated degradation rate of the (signaling) ligand-receptor complexes.
- Negative feedback on the (signaling) receptor synthesis rate.
- Positive feedback on the free receptor degradation rate.
- Negative feedback on the ligand-receptor binding rate.
- Positive feedback on the free (unbound) ligand degradation rate.

(While the explicit action of nonsignaling receptor-type inhibitors such as heparan sulfate proteoglycans to reduce the concentration of free ligand concentration has been investigated in Refs. 34 and 36–38 40 and references therein, feedback on this type of inhibitors has not been included. Instead, their ultimate effect to downregulate the concentration of free ligand has been included in a negative feedback loop on ligand synthesis rate.) It was found that each of the five feedback processes is by itself ineffective in promoting robustness, at least at the basic feedback strength level of $(c, n) = (1, 1)$. Furthermore, the last four feedback processes listed above actually fail to promote robustness at rather high feedback strength of $c \leq 10$. Examinations of the signaling gradient distributions show that these feedback processes have the effect of modifying the gradient shape and thereby work against robustness. Why then do we find their concurrent presence in many signaling gradient developments when each of them does not promote robustness?

10.4 | Benefits of some concurrent feedback processes

For an answer, we examined the activities and effects of concurrent implementation of two different combinations of two feedback processes to see their impact on the corresponding signaling gradient that would be ectopic in the absence of feedback. In the process, we learned of at least two different ways how multifeedback promotes and enhances signaling gradient robustness.

The first combination consists of a negative feedback on ligand synthesis rate (see Ref. 51) and a positive feedback on receptor-mediated degradation rate (36). For this combination, the cumulative nature of the effects of constituent feedback processes is succinctly illustrated by

$$\frac{\bar{b}_{cm}(x)}{\bar{b}_1(x)} \simeq \frac{e\boldsymbol{B}_{cm}(x)}{\boldsymbol{B}_1(x)} = \frac{e}{\big(1 + c_m\rho_{cm}\big)\big(1 + c_g\rho_{cm}\big)},$$

in the case of an LRO gradient system. That the ratio is independent of spatial location reflects the fact that the shape parameter μ_0^2 is unaffected by the constituents of this multifeedback combination.

The second combination consists of a positive feedback on both the free receptor degradation rate and the free ligand degradation rate. Both feedback processes are ineffective in promoting robustness as each distorts the signaling gradient shape while reducing the ectopic gradient magnitude rather modestly. However, when implemented concurrently, the two individual shape changes work in opposite direction so that the net shape distortion becomes much less ectopic relative to the wild-type gradient (Proposition 7) as shown in Figure 1. Hence, this second multifeedback combination offers a different instrument for promoting signaling gradient robustness.

10.5 | Principal findings and suggested research

Other combinations of the feedback processes listed earlier in this section may or may not improve the performance of each of its constituents acting alone. That some (including the two described above) do constitutes the principal finding of our effort herein: a possible explanation for the concurrent presence and interaction of the many known feedback mechanisms that are theoretically ineffective in promoting robustness. More specifically, multifeedback mechanisms, when properly configured, serve to promote robust signaling in at least two ways: (1) reduction of the feedback strengths required of constituent feedback processes for attaining robustness, and (2) inducing complementary changes in the gradient shape to minimize distortion relative to the wild-type gradient. This second unanticipated role renders individual ineffective inhibitory activities useful instruments for promoting robustness. Together, the two effects provide a theoretical explanation for the concurrent presence of multiple feedback processes.

In our first effort to uncover possible reasons for the appearance of multiple feedback processes, we conveniently omitted or abbreviated the intermediate biological events of some of these processes such as how the feedback process generates the inhibiting agents and their transport to locations with less concentration of these agents. The spatial dynamics of these activities should help relate them to the spatially uniform effects of inhibition captured by robustness-index-induced feedback.

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How to cite this article: Wan FM. The necessity and benefits of multiple feedback for robust biological development. *Stud Appl Math*. 2019;1–39. https://doi.org/10.1111/sapm.12258