ROBUSTNESS OF MORPHOGEN GRADIENTS WITH "BUCKET BRIGADE" TRANSPORT THROUGH MEMBRANE-ASSOCIATED NON-RECEPTORS

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ABSTRACT. Robust multiple-fate morphogen gradients are essential for embryo development. Here, we analyze mathematically a model of morphogen gradient (such as Dpp in Drosophila wing imaginal disc) formation in the presence of non-receptors with both diffusion of free morphogens and the movement of morphogens bound to non-receptors. Under the assumption of rapid degradation of unbound morphogen, we introduce a method of functional boundary value problem and prove the existence, uniqueness and linear stability of a biologically acceptable steady-state solution. Next, we investigate the robustness of this steady-state solution with respect to significant changes in the morphogen synthesis rate. We prove that the model is able to produce robust biological morphogen gradients when production and degradation rates of morphogens are large enough and non-receptors are abundant. Our results provide mathematical and biological insight to a mechanism of achieving stable robust long distance morphogen gradients. Key elements of this mechanism are rapid turnover of morphogen to non-receptors of neighoring cells resulting in significant degradation and transport of non-receptor-morphogen complexes, the latter moving downstream through a "bucket brigade" process.

1. **Introduction.** At some stages of embryonic development, signaling protein molecules known as *Morphogens* (aka *ligands*) are synthesized at a localized site, some of them disperse from their production site, bind to cell receptors along the way, and result in different receptor occupancies at different cell locations. The spatial concentration gradient of morphogen-receptor complexes (aka *signaling gradients*) induces spatially graded differences in cell signaling. The differential cell signaling in turn gives rise to different gene expressions from which follow different stable cell fates and visual tissue patterns and organs during development.

In general, it is important for a developing biological organism to form appropriate morphogen gradients that appear at a proper time and proper place and are robust with respect to perturbations in system architecture, environmental changes, or signaling noise. As conflicting biochemical processes and strategies may be required to attain precision and robustness, the delineation of how both characteristics can be achieved in biological development remains a challenge in systems biology [19]. Different mechanisms and processes involved in the formation of different morphogen gradients are either known or have been proposed [2, 3, 7, 8, 9, 13, 14, 15, 16, 29, 30]. One process that remains indispensible is the transport of morphogens away from their localized source.

A number of models have been proposed for morphogen transport; most are based on diffusion of morphogen molecules and their interactions with signalling and non-signaling extracellular molecules [5, 7, 11, 12, 13, 16, 17, 18]. It has been argued that diffusion alone may not be a reliable mechanism as the resulting gradients are sensitive to substantial changes in system parameters, leading to a signaling gradient that is no longer biologically useful [19, 21, 24, 31]. Many additional mechanisms such as transcytosis, dynamin-mediated endocytosis, feedback control and regulations by membrane-associated non-receptors have been suggested for achieving robustness [4, 5, 7, 15, 23, 24].

Regulations by non-signaling receptor (or non-receptor for short) such as heparan sulfate proteoglycans (HSPG) in morphogen movement are observed in many experiments [1, 2, 3, 6, 9, 10], and the effects of the presence of non-receptors on the existence and characteristics of the steady-state signaling morphogen gradients have been studied in [20, 22, 23, 24]. From these studies, the desired robustness with respected to substantial perturbations of morphogen synthesis rate is seen to be achievable through two different mechanisms involving regulations of non-receptors:

Mechanism 1: Substantial (reversible) binding of *slowly turned over* morphogen molecules with membrane-bound non-receptors with the resulting non-signaling (morphogen) complexes degrading at a sufficiently rapid rate [24].

Mechanism 2: Fast binding of *rapidly turned over* free morphogen molecules with non-receptors so that the non-signaling complexes move downstream through a "bucket brigade" process [23].

Mathematical analysis of the effectiveness of these two robustness mechanisms have been carried out in [23, 24] based on reaction-diffusion models with only one diffusion term in either free or non-signaling bound-morphogens. These one diffusion models however are biologically incomplete because it is possible to have both types of transport. Models with two diffusions have been developed and studied in [22, 26, 32] with the numerical simulations carried out in [22] suggesting that Mechanism 2 continues to ensure robustness if the free morphogen degradation is large enough.

In this paper, we analyze mathematically a model of morphogen gradient formation with both diffusion of free morphogens and the movement of non-signaling

morphogen complexes through a "bucket brigade" process to establish a theory capable of predicting previous results from numerical simulations [22]. The model here (corresponding to the Dpp gradient in the wing imaginal disc) is unrelated to, and conceptually different from those of [26, 32] where the non-receptors are freely diffusing (Sog) molecules. We prove for our model the existence, uniqueness, and stability of the (quasi-)steady-state signaling gradient and thereby validating Mechanism 2 for tissue pattern formation. We also prove robustness of the steady state signaling gradient with respect to a significant increase in ligand synthesis rate under the assumption of low receptor and non-receptor occupancy and high free morphogen degradation rate compared with its diffusion rate.

2. Formulations.

2.1. **Model equations.** We refer the mathematical model in [22, 23], which was based on the formation of morphogen gradient in *Drosophila* wing imaginal discs regulated by the glypican members of heparan sulfate proteoglycans [3]. The model involves concentrations of free ligands [L], receptors [R], ligand-receptor complexes [LR], non-receptors [N], and ligand-non-receptor complexes [LN]. Distributions of various morphogen concentrations in the wing imaginal disc are assumed to be sufficiently uniform in two directions (except possibly for boundary layers) to change only along the antero-posterior axis. The anterior and posterior compartments are taken to be sufficiently symmetric so that we can focus on the posterior compartment that spans the range $-d_0 \leq X \leq X_{\max}$ with a narrow region of ligand synthesis at $-d_0 \leq X \leq 0$. Both [L] and [LN] are allowed to diffuse; other main reactions include binding and unbinding of ligand to receptors and non-receptors, degradation of ligands, receptors and the ligand-receptor complexes. The total concentration of non-receptor binding sites is abundant and assumed to be a constant N_0. Therefore, the resulting reaction-diffusion equations is (see [23] for an expanded discussion of the D_{LN} term):

$$\frac{\partial[\mathrm{L}]}{\partial T} = D_{\mathrm{L}}\frac{\partial^2[\mathrm{L}]}{\partial X^2} - k_{\mathrm{on}}[\mathrm{L}]\,[\mathrm{R}] + k_{\mathrm{off}}[\mathrm{LR}] \tag{1}$$
$$- j_{\mathrm{on}}[\mathrm{L}]\,(N_0 - [\mathrm{LN}]) + j_{\mathrm{off}}[\mathrm{LN}] - \delta_{\mathrm{L}}[\mathrm{L}] + V(X)$$

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

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

$$\frac{\partial[\mathrm{LN}]}{\partial T} = D_{\mathrm{LN}}\frac{\partial^2[\mathrm{LN}]}{\partial X^2} + j_{\mathrm{on}}[\mathrm{L}]\,(N_0 - [\mathrm{LN}]) - j_{\mathrm{off}}[\mathrm{LN}], \tag{4}$$

where $X \in (-d_0, X_{\max})$. The production rate of ligands is given in terms of the *Heaviside unit step function* $H(z)$:

$$V(X) = v_0 H(-X), \qquad H(z) \equiv \left\{ \begin{array}{ll} 0 & (z < 0) \\ 1 & (0 < z) \end{array} \right. \tag{5}$$

where v_0 is a constant ligand synthesis rate in the production region $-d_0 \leq X < 0$. The boundary of the region is specified by $X = X_{\max}$. The receptor synthesis rate is assumed to depend only on [LR] and not on X and T explicitly.

Assumed symmetry at the border $X = -d_0$ requires the no flux conditions:

$$\frac{\partial[\mathrm{L}]}{\partial X} = 0, \quad \frac{\partial[\mathrm{LN}]}{\partial X} = 0 \quad \text{at} \quad X = -d_0. \tag{6}$$

The edge $X = X_{\max}$ is taken to be a sink for all diffusive elements so that

$$[L] = [LN] = 0 \quad \text{at} \quad X = X_{\max}. \tag{7}$$

With $V(X)$ discontinuous at $X = 0$, we stipulate the continuity of $[L]$, $[LN]$ and the partial derivatives $\partial[L]/\partial X$, $\partial[LN]/\partial X$ at $X = 0$, consistent with the PDE (1) requiring $\partial^2[L]/\partial X^2$ to have only a simple jump discontinuity at $X = 0$.

Before the onset of ligand production ($V(X) = 0$), there is no ligand concentration of any kind so that

$$[L] = [LR] = [LN] = 0, \quad (-d_0 < X < X_{\max}, \quad T \le 0). \tag{8}$$

The receptors are expected to be in a steady-state prior to the onset of ligand production, so that

$$\frac{\partial[R]}{\partial T} = 0.$$

This implies

$$T = 0 : \quad [R] = \omega_R(0)/\delta_R \equiv R_0 \tag{9}$$

where R_0 is the concentration of unbound receptors at the steady-state prior to the onset of ligand production.

The conditions (1)-(9) define an initial-boundary value problem (IBVP) for morphogen gradient formation. The model in [23] corresponding to the special case with $D_L = 0$. With $D_L \ne 0$ herein, the corresponding mathematical problem becomes more difficult to analyze and requires a new approach.

Similar to [23], we introduce the following non-dimensional quantities:

$$l = \frac{[L]}{j_{\text{off}} N_0/\delta_L}, \quad r = \frac{[R]}{R_0}, \quad u = \frac{[LR]}{R_0 k_{\text{on}} j_{\text{off}} N_0/(\delta_L \delta_{LR})}, \quad w = \frac{[LN]}{N_0}, \tag{10}$$

$$t = \delta_L T, \quad x = \frac{X + d_0}{X_{\max} + d_0}, \quad d = \frac{d_0}{(X_{\max} + d_0)}, \quad k(u) = \frac{\omega_R([LR])}{\delta_R R_0}, \tag{11}$$

$$\varepsilon = \frac{j_{\text{on}} N_0}{\delta_L}, \quad \lambda^2 = \frac{j_{\text{off}}(X_{\max} + d_0)^2}{D_{LN}}, \quad \delta_r = \frac{\delta_R}{\delta_L}, \quad \delta_u = \frac{\delta_{LR}}{\delta_L}, \tag{12}$$

$$\alpha = \frac{k_{\text{off}}}{\delta_{LR}}, \quad \gamma = \frac{k_{\text{on}} R_0}{j_{\text{on}} N_0}, \quad \mu = \frac{k_{\text{on}} j_{\text{off}} N_0}{\delta_R \delta_L}, \quad \eta = \frac{v_0 j_{\text{on}}}{\delta_L j_{\text{off}}}, \tag{13}$$

$$\theta_l = \frac{D_L}{(X_{\max} + d_0)^2 \delta_L}, \quad \theta_w = \frac{D_{LN}}{(X_{\max} + d_0)^2 \delta_L}. \tag{14}$$

Here $k(u)$ is the normalized receptor synthesis rate satisfying $k(0) = 1$. The normalized ligand production rate is given by

$$v(x) = (\eta/\varepsilon) H(d - x).$$

Here η is the (dimensionless) effective production rate which is important for our discussion below.

Using the above non-dimensional variables, equations (1)-(4) become

$$\frac{\partial l}{\partial t} = \theta_l \frac{\partial^2 l}{\partial x^2} - (l - w) - \varepsilon \left(l(1 - w) - \gamma(\alpha u - lr)\right) + v(x) \tag{15}$$

$$\frac{\partial r}{\partial t} = \delta_r \left(\mu(\alpha u - lr) + (k(u) - r)\right) \tag{16}$$

$$\frac{\partial u}{\partial t} = -\delta_u((\alpha + 1)u - lr) \tag{17}$$

$$\frac{\partial w}{\partial t} = \theta_w \left(\frac{\partial^2 w}{\partial x^2} - \lambda^2(w - \varepsilon l(1 - w))\right), \tag{18}$$

with initial conditions

$$l = u = w = 0, \quad r = 1, \quad (t = 0, 0 \le x \le 1) \tag{19}$$

and boundary conditions

$$\left.\frac{\partial l}{\partial x}\right|_{x=0} = \left.\frac{\partial w}{\partial x}\right|_{x=0} = l|_{x=1} = w|_{x=1} = 0, \quad (t \ge 0). \tag{20}$$

2.2. **Steady-state behavior.** In cell signaling and pattern formation, we are interested in the (quasi-)steady-state signaling gradient long after the onset of ligand production. The steady-state gradients satisfy (15)-(18) with all time partial derivatives set to zero. With $\bar{l}(x), \bar{r}(x), \bar{u}(x), \bar{w}(x)$ denoting the steady-state solution for $l(x,t), r(x,t), u(x,t)$ and $w(x,t)$ of (15)-(20), respectively, we get the following two ODE for $\bar{l}$ and $\bar{w}$

$$\begin{aligned}
\theta_l \frac{\partial^2 \bar{l}}{\partial x^2} - (\bar{l} - \bar{w}) - \varepsilon(\bar{l}(1 - \bar{w}) - \gamma(\alpha\bar{u} - \bar{l}\bar{r})) + v(x) &= 0 \\
\frac{\partial^2 \bar{w}}{\partial x^2} - \lambda^2(\bar{w} - \varepsilon\bar{l}(1 - \bar{w})) &= 0
\end{aligned} \tag{21}$$

where the functions $\bar{u}$ and $\bar{r}$ depend on $\bar{l}$ through

$$\bar{l} = (\alpha + 1)\bar{u}/\bar{r}, \quad \bar{r} = k(\bar{u}) - \mu\bar{u}. \tag{22}$$

The boundary conditions for (21) are

$$\bar{w}'(0) = \bar{l}'(0) = \bar{w}(1) = \bar{l}(1) = 0. \tag{23}$$

Hereafter a prime, $'$, indicates differentiation with respect to x.

We are only interested in solutions of (21)-(23) that are biologically realistic (or "biological gradient" for brevity). The concentration of the corresponding bound morphogen-non-receptor gradient is restricted by the total number of binding sites of non-receptors, i.e., $0 \le [LN] \le N_0$. Also, the (realistic) biological gradients must be non-negative. In terms of non-dimensional variables, biological gradients should satisfy

$$0 \le \bar{w}(x) \le 1, \quad \bar{l}(x) \ge 0, \quad \bar{r}(x) \ge 0, \quad \bar{u}(x) \ge 0, \quad (0 \le x \le 1). \tag{24}$$

We note that it is possible to have $\bar{l}(x) > 1$ when x is small because of a high ligand synthesis rate. This motivates the following definition for a biologically acceptable gradient.

Definition 2.1. The four functions $\{\bar{w}(x), \bar{l}(x), \bar{r}(x), \bar{u}(x)\}$ are said to be *biologically acceptable gradients* if the functions satisfy equations (22) and inequalities (24) for any $0 \le x \le 1$ and if $\bar{w}(x)$ and $\bar{l}(x)$ satisfy the BVP (21)-(23).

We always assume a non-positive feedback of receptor production, which is characterized by the function $k(u)$ as follows.

Definition 2.2. The function $k(u)$ is said to be a non-positive feedback if

$$k(0) = 1, k'(u) \le 0, k(u) > 0, \quad \forall u \ge 0.$$

In the following discussion, we extend the domain of $k(u)$ to all real number by setting $k(u) = k(0)$ for all $u < 0$.

In the results section below, we study the biologically acceptable gradients of (21)-(23). In [23], we investigated the well-posedness of the BVP for the special case of $\theta_l = 0$ through the methods of upper and lower solutions and nonlinear eigenvalue problem previously developed [25, 27]. However, when $\theta_l \ne 0$, these methods are not applicable directly. To establish well-posedness, we introduce new

methods based on functional differential equations which are also different from those employed in [26, 32] for problems with two diffusion elements.

3. Results.

3.1. **Existence and uniqueness of the steady-state solution.** This section proves the the following theorem on the existence and uniqueness of the steady-state solution.

Theorem 3.1. *Consider the BVP* (21)*-*(23)*, if $k(u)$ is a non-positive feedback, and*

$$\varepsilon(\varepsilon + 2) < 1/(\eta + 1), \tag{25}$$

there exists a unique combination of biologically acceptable gradients $\{\bar{w}, \bar{l}, \bar{u}, \bar{r}\}$.

Before proving Theorem 3.1, we observe the following gradient properties:

Lemma 3.2. *Suppose $k(u)$ is a non-positive feedback and $\mu > 0$. Then for any $\bar{l} > 0$, we have*

$$\frac{\partial \bar{u}}{\partial \bar{l}} > 0, \quad \frac{\partial \bar{r}}{\partial \bar{l}} < 0. \tag{26}$$

for the unique positive solution $\bar{u} = \bar{u}(\bar{l}), \bar{r} = \bar{r}(\bar{l})$ of (22)

Proof. All conclusions in this lemma are direct consequences of (22). □

From Lemma 3.2, the BVP (21)-(23) can be rewritten in the following form:

$$\begin{cases} \theta_l \bar{l}'' - (\bar{l} - \bar{w}) - \varepsilon \bar{l}(1 - \bar{w}) - \varepsilon \gamma \bar{u}(\bar{l}) + v(x) = 0 \\ \bar{w}'' - \lambda^2(\bar{w} - \varepsilon \bar{l}(1 - \bar{w})) = 0 \\ \bar{l}'(0) = \bar{l}(1) = 0, \\ \bar{w}'(0) = \bar{w}(1) = 0. \end{cases} \tag{27}$$

To prove Theorem 3.1, we only need to show that the BVP (27) has a unique solution satisfying $0 < \bar{w}(x) < 1$ and $\bar{l}(x) > 0$ for $0 \le x \le 1$. This is done by treating two particular cases of the uncoupled form of (27):

1. For any $\bar{w}(x)$ with $0 \le \bar{w}(x) \le 1$, prove that the BVP

$$\begin{cases} \theta_l \bar{l}'' - (\bar{l} - \bar{w}) - \varepsilon \bar{l}(1 - \bar{w}) - \varepsilon \gamma \bar{u}(\bar{l}) + v(x) = 0 \\ \bar{l}'(0) = \bar{l}(1) = 0, \end{cases} \tag{28}$$

   has a unique solution $\bar{l}(x) > 0$. In particular, the solution is written as

$$\bar{l}(x) = \bar{l}_0(x) + \mathcal{L}_{\bar{w}}(x) \tag{29}$$

   where $\bar{l}_0(x)$ is the solution of (28) when $\bar{w} \equiv 0$, and $\mathcal{L}_{\bar{w}}(x)$, as defined by (29), is a functional of $\bar{w}$ satisfying

$$0 \le \mathcal{L}_{\bar{w}}(x) \le (\eta + 1), \quad (0 \le x \le 1). \tag{30}$$

2. Upon rewriting the BVP

$$\begin{cases} \bar{w}'' - \lambda^2(\bar{w} - \varepsilon \bar{l}(1 - \bar{w})) = 0 \\ \bar{w}'(0) = \bar{w}(1) = 0. \end{cases} \tag{31}$$

   as a *functional boundary value problem*

$$\begin{cases} \bar{w}'' - \lambda^2(\bar{w} - \varepsilon(\bar{l}_0(x) + \mathcal{L}_{\bar{w}}(x))(1 - \bar{w})) = 0 \\ \bar{w}'(0) = \bar{w}(1) = 0. \end{cases}, \tag{32}$$

   prove that (32) has a unique solution.

The proofs of these two propositions above are given below in Lemmas 3.3-3.5. For the proofs, we hereafter denote $H^0(I, \mathbb{R})$ the space of continuous real-valued functions defined on $I = [0, 1]$, with the L^∞ norm

$$\|a\| = \sup_{0 \le x \le 1} |a(x)|$$

for any $a \in H^0(I, \mathbb{R})$. Then $(H^0(I, \mathbb{R}), \|\cdot\|)$ is a Banach space. The Banach spaces $H^0(I, I)$ and $H^0(I, \mathbb{C})$ are defined similarly.

Lemma 3.3. *For any $\bar{w} \in H^0(I, I)$, the equation*

$$\begin{cases} \theta_l \bar{l}'' - (\bar{l} - \bar{w}) - \varepsilon \bar{l}(1 - \bar{w}) - \varepsilon \gamma \bar{u}(\bar{l}) + v(x) = 0 \\ \bar{l}'(0) = \bar{l}(1) = 0, \end{cases} \tag{33}$$

has a unique positive solution. Furthermore, the solution satisfies

$$0 \le \bar{l}(x) \le \eta/\varepsilon + 1, \qquad (0 \le x \le 1). \tag{34}$$

Let $\bar{l}_0(x)$ be the solution of (33) *with $\bar{w}(x) \equiv 0$, then*

$$0 < \bar{l}_0(x) < \eta/\varepsilon \tag{35}$$

and

$$0 \le \bar{l}(x) - \bar{l}_0(x) \le \eta + 1, \quad (0 \le x \le 1). \tag{36}$$

Proof. For any $\bar{w} \in H^0(I, I)$, it is easy to see that $\bar{l}_U(x) \equiv \eta/\varepsilon + 1$ and $\bar{l}_L(x) \equiv 0$ are respectively upper and lower solutions of (33). From Lemma A.1 in the Appendix, there exits a unique solution of the BVP (33) such that $0 \le \bar{l}(x) \le \eta/\varepsilon + 1$. Similarly, we have $0 < \bar{l}_0(x) < \eta/\varepsilon$.

Let $\bar{l}_1(x) = \bar{l}(x) - \bar{l}_0(x)$, then $\bar{l}_1(x)$ satisfies

$$\begin{cases} \theta_l \bar{l}_1'' - \bar{l}_1 - \varepsilon (\bar{l}_1(1 - \bar{w}) + \gamma(\bar{u}(\bar{l}_0(x) + \bar{l}_1) - \bar{u}(\bar{l}_0(x)))) \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad + (1 + \varepsilon l_0(x))\bar{w} = 0 \\ \bar{l}_1'(0) = \bar{l}_1(1) = 0, \end{cases} \tag{37}$$

Again, Lemma A.1 yields a unique solution of (37) that satisfies

$$0 \le \bar{l}_1(x) \le \eta + 1, \quad (0 \le x \le 1)$$

and the Lemma is proved. $\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\square$

From Lemma 3.3, we define an operator $\mathcal{L} : H^0(I, I) \mapsto H^0(I, \mathbb{R})$ such that

$$\bar{l}(x) = \bar{l}_0(x) + \mathcal{L}_{\bar{w}}(x), \tag{38}$$

with $\bar{l}(x)$ and $\bar{l}_0(x)$ as in Lemma 3.3, then for any $\bar{w} \in H^0(I, I)$

$$0 \le \mathcal{L}_{\bar{w}}(x) \le \eta + 1, \quad (0 \le x \le 1). \tag{39}$$

Lemma 3.4. *Let $\mathcal{L}_w$ as defined in* (38)*, then for any $\bar{w}_1, \bar{w}_2 \in H^0(I, I)$,*

$$\|\mathcal{L}_{\bar{w}_1} - \mathcal{L}_{\bar{w}_2}\| \le (1 + \varepsilon)(1 + \eta)\|\bar{w}_1 - \bar{w}_2\|. \tag{40}$$

Proof. Write $\varphi(x) = \mathcal{L}_{\bar{w}_1}(x) - \mathcal{L}_{\bar{w}_2}(x)$, and

$$q(x) = 1 - \bar{w}_1(x) + \gamma \frac{\bar{u}(\bar{l}_0(x) + \mathcal{L}_{\bar{w}_1}(x)) - \bar{u}(\bar{l}_0(x) + \mathcal{L}_{\bar{w}_2}(x))}{\mathcal{L}_{\bar{w}_1}(x) - \mathcal{L}_{\bar{w}_2}(x)},$$

then $\varphi(x)$ satisfies

$$\begin{cases} \theta_l \varphi'' - (1 + \varepsilon q(x))\varphi + (1 + \varepsilon \bar{l}_0(x) + \varepsilon \mathcal{L}_{\bar{w}_2}(x))(\bar{w}_1 - \bar{w}_2) = 0 \\ \varphi'(0) = \varphi(1) = 0. \end{cases} \tag{41}$$

Since $\partial\bar{u}/\partial\bar{l} > 0$, we have $q(x) > 0$. Now, applying Lemma A.3, we obtain

$$\|\varphi\| < \|1 + \varepsilon\bar{l}_0(x) + \varepsilon\mathcal{L}_{\bar{w}_2}\|\|\bar{w}_1 - \bar{w}_2)\| < (1 + \varepsilon)(1 + \eta)\|\bar{w}_1 - \bar{w}_2\|,$$

and the Lemma is proved. □

Lemma 3.5. *Consider the functional differential equation*

$$\begin{cases} \bar{w}'' - \lambda^2(\bar{w} - \varepsilon(\bar{l}_0(x) + \mathcal{L}_{\bar{w}}(x))(1 - \bar{w})) = 0 \\ \bar{w}'(0) = \bar{w}(1) = 0. \end{cases} \tag{42}$$

where $\mathcal{L}_{\bar{w}}$ is defined as previous so that

$$0 \leq \mathcal{L}_{\bar{w}}(x) \leq \eta + 1, \quad (0 \leq x \leq 1) \tag{43}$$

and

$$\|\mathcal{L}_{\bar{w}_1} - \mathcal{L}_{\bar{w}_2}\| \leq (1 + \varepsilon)(1 + \eta)\|\bar{w}_1 - \bar{w}_2\|. \tag{44}$$

If $\varepsilon > 0$ satisfies

$$\varepsilon(\varepsilon + 2)(\eta + 1) < 1, \tag{45}$$

then (42) *has a unique solution in $H^0(I, I)$.*

Proof. Let $\bar{w}_0(x)$ to be the solution of

$$\begin{cases} \bar{w}_0'' - \lambda^2(\bar{w}_0 - \varepsilon\bar{l}_0(x)(1 - \bar{w}_0)) = 0 \\ \bar{w}_0'(0) = \bar{w}_0(1) = 0. \end{cases} \tag{46}$$

Lemma A.1 yields that (46) has a unique solution and $0 \leq \bar{w}_0(x) \leq 1, (0 \leq x \leq 1)$. Let $\bar{w}_1(x) = \bar{w}(x) - \bar{w}_0(x)$, then $\bar{w}_1$ satisfies

$$\begin{cases} \bar{w}_1'' - \lambda^2(1 + \varepsilon\bar{l}_0(x))\bar{w}_1 + \varepsilon\lambda^2\mathcal{L}_{\bar{w}}(x)(1 - \bar{w}(x)) = 0 \\ \bar{w}_1'(0) = \bar{w}_1(1) = 0. \end{cases} \tag{47}$$

From Lemma A.3, there is a Green function $G(x, s)$ that is positive for any $0 \leq x, s \leq 1$, and (47) is equivalent to the following functional integral equation

$$\bar{w}_1 = \varepsilon\lambda^2 \int_0^1 G(x, s)\mathcal{L}_{\bar{w}}(s)(1 - \bar{w}(s))ds. \tag{48}$$

Thus, the boundary value problem (42) is equivalent to the following functional integral equation

$$\bar{w} = \mathcal{T}_{\bar{w}} \tag{49}$$

where

$$\mathcal{T}_{\bar{w}}(x) = \bar{w}_0(x) + \varepsilon\lambda^2 \int_0^1 G(x, s)\mathcal{L}_{\bar{w}}(s)(1 - \bar{w}(s))ds.$$

Hence, it is sufficient to show that the operator $\mathcal{T}$ maps $H^0(I, I)$ into itself and is a contraction map.

First, it is easy to see $\mathcal{T}_{\bar{w}} \in H^0(I, \mathbb{R})$ and $\mathcal{T}_{\bar{w}}(x) \geq 0$ ($0 \leq x \leq 1$) for any $\bar{w} \in H^0(I, I)$. Second, we have $G(x, s) > 0$ from Lemma A.3. Note (43), therefore

$$\mathcal{T}_{\bar{w}}(x) \leq \bar{w}_0(x) + \varepsilon\lambda^2(\eta + 1) \int_0^1 G(x, s)ds. \tag{50}$$

The right hand side of (50), denoted as $\bar{w}_U(x)$, satisfies the equation

$$\begin{cases} \bar{w}_U'' - \lambda^2(\bar{w}_U - \varepsilon\bar{l}_0(x)(1 - \bar{w}_U)) + \varepsilon\lambda^2(\eta + 1) = 0 \\ \bar{w}_U'(0) = \bar{w}_U(1) = 0, \end{cases} \tag{51}$$

which yields

$$\bar{w}_U(x) = \bar{w}_0(x) + \varepsilon(\eta + 1)(1 - \bar{w}_0(x)) < 1$$

by (45). It follows that $\mathcal{T}_{\bar{w}}(x) \leq 1, (0 \leq x \leq 1)$ and hence $\mathcal{T}_{\bar{w}}$ maps $H^0(I, I)$ into itself.

Next, we show that $\mathcal{T}$ is a contraction map. Let $\bar{w}_1, \bar{w}_2 \in H^0(I, I)$, then

$$
\begin{aligned}
\|\mathcal{T}_{\bar{w}_1} - \mathcal{T}_{\bar{w}_2}\| &= \varepsilon\lambda^2 \|\mathcal{L}_{\bar{w}_1}(1 - \bar{w}_1) - \mathcal{L}_{\bar{w}_2}(1 - \bar{w}_2)\| \left\| \int_0^1 G(x, s) ds \right\| \\
&\leq \varepsilon\lambda^2 \left(\frac{\|\mathcal{L}_{\bar{w}_1} - \mathcal{L}_{\bar{w}_2}\|}{\|\bar{w}_1 - \bar{w}_2\|} + \|\mathcal{L}_{\bar{w}_2}\| \right) \|\bar{w}_1 - \bar{w}_2\| \left\| \int_0^1 G(x, s) ds \right\| \\
&\leq \varepsilon\lambda^2(\varepsilon + 2)(1 + \eta) \cdot \frac{1}{\lambda^2} \cdot \|\bar{w}_1 - \bar{w}_2\| \\
&< \|\bar{w}_1 - \bar{w}_2\|
\end{aligned}
$$

and therefore $\mathcal{T}$ is a contraction (since $\| \int_0^1 G(x, s) ds \| \leq \frac{1}{\lambda^2}$ according to Lemma A.3).

As the map $\mathcal{T}$ is a contraction map from $H^0(I, I)$ into itself, it has a unique fixed point, which is the solution of (42). The Lemma is proved. $\qquad\square$

3.2. Linear stability of the steady-state gradient.

For the biological (quasi-) steady state behavior to be relevant for tissue patterning, it should be stable. We show here that this is in fact the case, at least by a linear stability analysis. For linear stability, we consider a small perturbation from the steady-state in the form

$$
\begin{aligned}
l(x, t) &= \bar{l}(x) + e^{-\xi t}\tilde{l}(x), \quad r(x, t) = \bar{r}(x) + e^{-\xi t}\tilde{r}(x) \\
u(x, t) &= \bar{u}(x) + e^{-\xi t}\tilde{u}(x), \quad w(x, t) = \bar{w}(x) + e^{-\xi t}\tilde{w}(x)
\end{aligned} \tag{52}
$$

where $\bar{l}, \bar{r}, \bar{u}, \bar{w}$ are the steady-state solutions and where the time-independent portion of the perturbations, $\tilde{l}, \tilde{r}, \tilde{u}$ and $\tilde{w}$, are negligibly small. After linearlization, we have the following eigenvalue problem

$$
\begin{aligned}
-\xi\tilde{l} &= \theta_l \tilde{l}'' - (\tilde{l} - \tilde{w}) - \varepsilon((1 - \bar{w})\tilde{l} - \bar{l}\tilde{w}) + \varepsilon\gamma(\alpha\tilde{u} - \bar{r}\tilde{l} - \bar{l}\tilde{r}) \tag{53} \\
-\xi\tilde{r} &= \delta_r(\mu(\alpha\tilde{u} - \bar{r}\tilde{l} - \bar{l}\tilde{r}) + (k'(\bar{u})\tilde{u} - \tilde{r})) \tag{54} \\
-\xi\tilde{u} &= -\delta_u((\alpha + 1)\tilde{u} - \bar{r}\tilde{l} - \bar{l}\tilde{r}) \tag{55} \\
-\xi\tilde{w} &= \theta_w(\tilde{w}'' - \lambda^2(\tilde{w} - \varepsilon((1 - \bar{w})\tilde{l} - \bar{l}\tilde{w}))) \tag{56}
\end{aligned}
$$

with boundary conditions

$$
\tilde{l}'(0) = \tilde{l}(1) = \tilde{w}'(0) = \tilde{w}(1) = 0.
$$

We solve (54)-(55) for $\tilde{r}, \tilde{u}$ to get

$$
\begin{aligned}
\tilde{r} &= \frac{\mu(\xi/\delta_u - 1) + k'(\bar{u})}{1 - \xi/\delta_r}\tilde{u} \\
\tilde{u} &= \frac{\bar{r}}{\alpha + (1 - \xi/\delta_u) + \dfrac{\mu(1 - \xi/\delta_u) - k'(\bar{u})}{1 - \xi/\delta_r}\bar{l}}\tilde{l}.
\end{aligned}
$$

Hence, the perturbations $\tilde{l}, \tilde{w}$ satisfy the following nonlinear eigenvalue problem

$$
\begin{cases}
\theta_l \tilde{l}'' - A_\xi(x)\tilde{l} + (1 + \varepsilon\bar{l})\tilde{w} = 0, \\
\theta_w \tilde{w}'' - B_\xi(x)\tilde{w} + \varepsilon\lambda^2(1 - \bar{w})\tilde{l} = 0, \\
\tilde{l}'(0) = \tilde{l}(1) = \tilde{w}'(0) = \tilde{w}(1) = 0,
\end{cases} \tag{57}
$$

where

$$A_\xi(x) = 1 - \xi + \varepsilon(1 - \bar{w}) + \cfrac{\varepsilon\gamma(1 - \xi/\delta_u)\bar{r}}{\alpha + (1 - \xi/\delta_u) + \cfrac{\mu(1 - \xi/\delta_u) - k'(\bar{u})}{1 - \xi/\delta_r}\bar{l}}$$

$$B_\xi(x) = \lambda^2(1 + \varepsilon\bar{l}) - \xi.$$

The following theorem establishes the local stability of the steady-state solution.

Theorem 3.6. *If $k(u)$ is a non-positive feedback, and*

$$\varepsilon(1 + \varepsilon + \eta) < 1, \tag{58}$$

then all eigenvalues of the nonlinear eigenvalue problem (57) *have positive real parts and therefore the biologically acceptable gradient combination $\{\bar{w}(x), \bar{u}(x), \bar{l}(x), \bar{r}(x)\}$ is asymptotically stable.*

Proof. We assume $\Re(\xi) \leq 0$ and prove a contradiction by showing that for any ξ such that $\Re(\xi) \leq 0$, the BVP problem (57) has only a zero solution.

First, consider the equation

$$\theta_l \tilde{l}'' - A_\xi(x)\tilde{l} + (1 + \varepsilon\bar{l})\tilde{w} = 0, \ \tilde{l}'(0) = \tilde{l}(1) = 0. \tag{59}$$

When $\Re(\xi) \leq 0$, we have

$$\Re(A_\xi(x)) \geq 1 - \Re(\xi) > 0.$$

Thus, Lemma A.2 implies that for any $\tilde{w} \in H^0(I, \mathbb{C})$, (59) has a unique solution, which is given by an operator $\mathcal{G}_\xi$ below:

$$\tilde{l}(x) := \mathcal{G}_\xi(\tilde{w}) = \int_0^1 G(x, s)(1 + \varepsilon\bar{l}(s))\tilde{w}(s)ds.$$

Here $G(x, s)$ is the corresponding Green function and satisfies

$$\|\int_0^1 G(x, s)\| \leq \frac{1}{1 - \Re(\xi)} \tag{60}$$

from Lemma A.3. The operator $\mathcal{G}_\xi$ maps $\tilde{w} \in H^0(I, \mathbb{C})$ to the unique solution $\tilde{l}(x)$ of (59). Moreover, $\mathcal{G}_\xi$ is linear, and from Lemma A.2 and (34), (60),

$$\|\mathcal{G}_\xi(\tilde{w})\| \leq \frac{1 + \varepsilon + \eta}{1 - \Re(\xi)}\|\tilde{w}\|. \tag{61}$$

Now, the eigenvalue problem (57) reduces to a functional eigenvalue equation

$$\theta_w \tilde{w}'' - B_\xi(x)\tilde{w} + \varepsilon\lambda^2(1 - \bar{w})\mathcal{G}_\xi(\tilde{w}) = 0, \ \ \tilde{w}'(0) = \tilde{w}(1) = 0. \tag{62}$$

It is easy to see $\Re(B_\xi(x)) > \lambda^2 - \Re(\xi) > 0$. Thus Lemma A.3 yields a Green function $K_\xi(x, s)$ such that (62) is equivalent to the following functional integral equation

$$\tilde{w}(x) = \int_0^1 K_\xi(x, s)\varepsilon\lambda^2(1 - \bar{w})\mathcal{G}_\xi(\tilde{w})(s)ds := \mathcal{F}_\xi(\tilde{w}). \tag{63}$$

Here $\mathcal{F}_\xi$ is an operator maps $H^0(I, \mathbb{C})$ into itself. From Lemma A.3, we have

$$\|\int_0^1 K_\xi(x, s)ds\| \leq \frac{1}{\inf_{0 < x < 1}\Re(B_\xi(x))} \leq \frac{1}{\lambda^2 - \Re(\xi)}.$$

For any $w_1, w_2 \in H^0(I, \mathbb{C})$,

$$
\begin{aligned}
\|\mathcal{F}_\xi(w_1) - \mathcal{F}_\xi(w_2)\| &\leq \varepsilon\lambda^2\|\int_0^1 K_\xi(x,s)ds\|\|\mathcal{G}_\xi(w_1) - \mathcal{G}_\xi(w_2)\| \\
&\leq \varepsilon\lambda^2 \cdot \frac{1}{\lambda^2 - \Re(\xi)}\|\mathcal{G}_\xi(w_1 - w_2)\| \\
&\leq \varepsilon\lambda^2 \cdot \frac{1}{\lambda^2 - \Re(\xi)} \cdot \frac{1 + \varepsilon + \eta}{1 - \Re(\xi)}\|w_1 - w_2\| \\
&< \varepsilon(1 + \varepsilon + \eta)\|w_1 - w_2\|.
\end{aligned}
$$

Thus, (58) implies $\|\mathcal{F}_\xi(w_1) - \mathcal{F}_\xi(w_2)\| < \|w_1 - w_2\|$. Hence, the operator $\mathcal{F}_\xi$ is a contraction map and (62) has only the zero solution.

We have proved that if $\Re(\xi) \leq 0$, (57) has only the zero solution. Therefore, all eigenvalues of problem (57) must have positive real part and the Theorem is proved. $\square$

3.3. **Robustness of the steady-state solution.** We have established the existence, uniqueness and stability of the biologically acceptable gradients. In this section, we consider the robustness of the corresponding signaling gradient when the ligand production rate is changed.

We focus here on the dependence of the signaling gradient on the ligand production rate characterized by the dimensionless constant η. To this end, denote the dimensionless signaling concentration by $\bar{u}(x; \eta)$. As in [21, 22, 23, 24], we characterize the robustness of the signaling gradient with respect to changes in ligand production rate by a *robustness index* $R(\eta, \eta')$, defined as the relative change of the signaling gradient when the parameter η is changed to η'

$$
R(\eta, \eta') = \frac{1}{\Delta\eta/\eta} \frac{1}{1 - d} \int_d^1 \frac{\Delta\bar{u}(x)}{\bar{u}(x; \eta)} dx \tag{64}
$$

where

$$
\Delta\eta = |\eta - \eta'|, \quad \Delta\bar{u}(x) = |\bar{u}(x; \eta) - \bar{u}(x; \eta')|.
$$

Evidently, the smaller the value of R, the more robust is the signaling gradient. We adopt $R < 0.2$ for acceptable robustness as in [24].

In the definition (64), the explicit solution $\bar{u}(x; \eta)$ is not known, and therefore not easy to calculate the robustness index. Here, we first perform analysis for the extreme case of $\varepsilon \ll 1$ and $\theta_l \ll 1$ to find a sufficient condition to have good robustness, and then examine more general cases by numerical simulations.

3.3.1. *Approximation solution when $\varepsilon \ll 1$ and $\theta_l \ll 1$.* First, we obtain an approximate solution when $\varepsilon \ll 1$ and $\theta_l \ll 1$ by the method of asymptotic expansions.

For $0 < \varepsilon \ll 1$, a regular perturbation solution in ε of $\bar{w}(x; \varepsilon, \theta_l)$ and $\bar{l}(x; \varepsilon, \theta_l)$ in (27) is appropriate. For the leading term solution, denoted by $\bar{w}_0(x; \theta_l)$ and $\bar{l}_0(x; \theta_l)$, the boundary value problem (22) decouples to result in two linear problems:

$$
\begin{aligned}
&\bar{w}_0'' - \lambda^2(\bar{w}_0 - \eta H(d - x)(1 - \bar{w}_0)) = 0, \\
&\bar{w}_0'(0) = \bar{w}_0(1) = 0
\end{aligned} \tag{65}
$$

and

$$
\theta_l \bar{l}_0'' - (\bar{l}_0 - \bar{w}_0) + v(x) = 0, \qquad \bar{l}_0'(0) = \bar{l}_0(1) = 0. \tag{66}
$$

Note that the boundary value problem (66) is a direct consequence of the relevant equations in (27) for the leading term solution, while the problem (65) is obtained

by making use of the differential equation in (66) to eliminate $\bar{l}_0(x; \theta_l)$ from the leading term equation

$$\bar{w}_0'' - \lambda^2(\bar{w}_0 - \varepsilon \bar{l}_0(1 - \bar{w}_0)) = 0,$$

keeping in mind that $v(x)$ (and consequently also $(\bar{l}_0 - \bar{w}_0)$) is $O(\eta/\varepsilon)$ in the region $[0, d]$.

The exact solution of the linear boundary value problem (65) is

$$\bar{w}_0(x; \theta_l) = \begin{cases} \dfrac{\eta}{1+\eta} - a \cosh(\lambda\sqrt{1+\eta}x), & (0 \le x \le d) \\ \dfrac{\bar{w}_d \sinh(\lambda(1-x))}{\sinh(\lambda(1-d))}, & (d \le x \le 1) \end{cases} \tag{67}$$

where

$$\bar{w}_d = \frac{\eta}{1+\eta}\left[1 + \frac{1}{\sqrt{1+\eta}}\coth(\lambda(1-d))\coth(\lambda d\sqrt{1+\eta})\right]^{-1}, \tag{68}$$

$$a = \frac{\coth(\lambda(1-d))}{\sqrt{1+\eta}\sinh(\lambda\sqrt{1+\eta}d)}\bar{w}_d. \tag{69}$$

Since the order of the differential equation (65) for $\bar{w}_0$ is the same as that of (27), the solution (67)–(69) is in fact the leading term approximation (in ε) for $\bar{w}(x; \varepsilon, \theta_l)$ (without any supplementary boundary layer component, at least not to this order of approximation).

Having $\bar{w}_0(x; \theta_l)$, the boundary value problem (66) for $\bar{l}_0(x; \theta_l)$ becomes

$$\theta_l \bar{l}_0'' - \bar{l}_0 = -\bar{w}_0 - \frac{\eta}{\varepsilon}H(d-x), \qquad \bar{l}_0'(0) = \bar{l}_0(1) = 0.$$

This simple linear problem may be solved by the method of variation of parameters. With $0 < \theta_l \ll 1$, we omit terms of order θ_l (and ε) to get the approximate solution

$$\bar{l}_0(x; \theta_l) = \begin{cases} \dfrac{\eta}{\varepsilon} + \dfrac{\eta}{1+\eta} - a\cosh(\lambda\sqrt{1+\eta}x) & (0 < x \le d) \\ \qquad - \dfrac{\eta}{\varepsilon}\dfrac{\cosh\frac{1-d}{\sqrt{\theta_l}}}{\cosh\frac{1}{\sqrt{\theta_l}}}\cosh(x/\sqrt{\theta_l}) & \\ \dfrac{\bar{w}_d\sinh(\lambda(1-x))}{\sinh(\lambda(1-d))} + \dfrac{\eta}{\varepsilon}\dfrac{\sinh\frac{d}{\sqrt{\theta_l}}}{\cosh\frac{1}{\sqrt{\theta_l}}}\sinh((1-x)/\sqrt{\theta_l}) & (d \le x < 1). \end{cases} \tag{70}$$

Correspondingly, the leading term solution for the signaling gradient is given implicitly by

$$\bar{u}(x; \varepsilon, \theta_l) = \bar{u}_0(x; \theta_l)[1 + O(\varepsilon)] = \frac{k(\bar{u}_0(x; \theta_l))\bar{l}_0(x; \theta_l)}{(\alpha+1) + \mu\bar{l}_0(x; \theta_l)}[1 + O(\varepsilon)] \tag{71}$$

and the receptor gradient is given by

$$\bar{r}(x; \varepsilon, \theta_l) = \bar{r}_0(x; \theta_l)[1 + O(\varepsilon)] = \{k(\bar{u}_0(x; \theta_l)) - \mu\bar{u}_0(x; \theta_l)\}[1 + O(\varepsilon)]. \tag{72}$$

3.3.2. *Robustness with $\varepsilon \ll 1$ and $\theta_l \ll 1$.* Now, we apply the above approximation to study the robustness when $\varepsilon \ll 1$ and $\theta_l \ll 1$. In this case, the robustness index R is approximated by

$$R_0(\eta, \eta') = \frac{1}{\Delta\eta/\eta}\frac{1}{1-d}\int_d^1 \frac{\Delta\bar{u}_0(x)}{\bar{u}_0(x; \eta)}dx. \tag{73}$$

The following Theorem established an upper bound of the robustness index R_0 when the ligand production rate is increased ($\eta' > \eta$).

Theorem 3.7. *Assume that $\eta' > \eta$ and $k(u)$ is a non-positive feedback, the robustness defined by* (73) *satisfies*

$$R_0(\eta, \eta') < \frac{A(\eta)}{\sqrt{1+\eta}} \left(1 + \frac{\coth(\lambda d\sqrt{1+\eta})}{\sqrt{1+\eta}} \right) + \frac{B(\eta)}{\sqrt{\eta}}, \quad (\eta' > \eta), \qquad (74)$$

where $A(\eta), B(\eta)$ are bounded for all $\eta > 0$. In particular, we have $R_0(\eta, \eta') = O(\eta^{-1/2})$ $(\eta' > \eta)$ when η is sufficiently large.

Proof. First, we have

$$\frac{\Delta \bar{u}_0(x)}{\bar{u}_0(x;\eta)} = \left| \frac{\partial \bar{u}_0(x;\eta)}{\partial \eta} \right|_{\eta=\tilde{\eta}(x)} \cdot \frac{\Delta \eta}{\bar{u}_0(x;\eta)} \right|$$

for some $\eta \leq \tilde{\eta}(x) \leq \eta'$ and therewith

$$R_0(\eta, \eta') < \frac{\eta}{1-d} \int_d^1 \max_{\eta \leq \tilde{\eta}(x) \leq \eta'} \left| \frac{\partial \bar{u}_0(x;\eta)}{\partial \eta} \right|_{\eta=\tilde{\eta}(x)} \cdot \frac{1}{\bar{u}_0(x;\eta)} \right| dx. \qquad (75)$$

It is readily shown from (70) that

$$\frac{\partial \bar{l}_0}{\partial \eta} > 0,$$

and from (71), we obtain

$$0 < \frac{\partial \bar{u}_0}{\partial \eta} = \frac{\frac{(\alpha+1)k(\bar{u}_0)}{[(\alpha+1)+\mu \bar{l}_0]^2}}{1 - \frac{k'(\bar{u}_0)\bar{l}_0}{(\alpha+1)+\mu \bar{l}_0}} \frac{\partial \bar{l}_0}{\partial \eta} < \frac{1}{1+\Phi_L \bar{l}_0} \frac{\bar{u}_0}{\bar{l}_0} \frac{\partial \bar{l}_0}{\partial \eta}, \quad \left(\Phi_L = \frac{\mu}{1+\alpha} \right)$$

which implies

$$\frac{\partial}{\partial \eta} \left(\frac{\bar{u}_0}{\bar{l}_0} \right) < 0.$$

Thus, we have

$$0 < \left. \frac{\partial \bar{u}_0(x;\eta)}{\partial \eta} \right|_{\eta=\tilde{\eta}(x)} < \left. \left(\frac{\bar{u}_0}{\bar{l}_0} \frac{1}{1+\Phi_L \bar{l}_0} \frac{\partial \bar{l}_0}{\partial \eta} \right) \right|_{\eta=\tilde{\eta}(x)}$$

$$< \frac{\bar{u}_0(x;\eta)}{\bar{l}_0(x;\eta)} \frac{1}{1+\Phi_L \bar{l}_0(x;\eta)} \left. \left(\frac{\partial \bar{l}_0}{\partial \eta} \right) \right|_{\eta=\tilde{\eta}(x)}. \qquad (76)$$

Now, from (75), (76) and (70), we have

$$R_0(\eta, \eta') < \frac{\eta}{1-d} \int_d^1 \frac{1}{\bar{l}_0(x;\eta)} \frac{1}{1+\Phi_L \bar{l}_0(x;\eta)} \left. \left(\frac{\partial \bar{l}_0}{\partial \eta} \right) \right|_{\eta=\tilde{\eta}(x)} dx$$

$$< \frac{\eta}{\bar{w}_d} \max_{\tilde{\eta}>\eta} \left. \frac{\partial \bar{w}_d}{\partial \eta} \right|_{\eta=\tilde{\eta}} + \frac{B(\eta)}{\sqrt{\eta}}$$

where

$$B(\eta) = \frac{\eta^{1/2}}{1-d} \frac{\eta}{\varepsilon} \frac{\sinh(d/\sqrt{\theta_l})}{\cosh(1/\sqrt{\theta_l})} \int_d^1 \frac{\sinh((1-x)/\sqrt{\theta_l})}{\bar{l}_0(x;\eta)(1+\Phi_L \bar{l}_0(x;\eta))} dx.$$

It is straightforward to obtain from (68)

$$
\begin{aligned}
\frac{\partial \bar{w}_d}{\partial \eta}\bigg|_{\eta=\tilde{\eta}} &= \frac{1}{(1+\tilde{\eta})^{3/2}} \frac{1}{\coth(d\lambda\sqrt{1+\tilde{\eta}})\coth(\lambda(1-d)) + \sqrt{1+\tilde{\eta}}} \\
&+ \frac{\tilde{\eta}}{2(1+\tilde{\eta})^{3/2}} \frac{\left[\coth(d\lambda\sqrt{1+\tilde{\eta}}) + d\lambda\sqrt{1+\tilde{\eta}}\mathrm{csch}^2(d\lambda\sqrt{1+\tilde{\eta}})\right]\coth(\lambda(1-d))}{\left[\coth(d\lambda\sqrt{1+\tilde{\eta}})\coth(\lambda(1-d)) + \sqrt{1+\tilde{\eta}}\right]^2} \\
&< \frac{A(\eta)}{(1+\eta)^{3/2}}
\end{aligned}
$$

where

$$
\begin{aligned}
A(\eta) &= \max_{\tilde{\eta}>\eta} \left\{ \frac{1}{\coth(d\lambda\sqrt{1+\tilde{\eta}}) + \sqrt{1+\tilde{\eta}}} \right. \\
&\left. + \frac{\tilde{\eta}\left[\coth(d\lambda\sqrt{1+\tilde{\eta}}) + d\lambda\sqrt{1+\tilde{\eta}}\mathrm{csch}^2(d\lambda\sqrt{1+\tilde{\eta}})\right]}{2\left[\coth(d\lambda\sqrt{1+\tilde{\eta}}) + \sqrt{1+\tilde{\eta}}\right]^2} \right\} \\
&< 1 + \coth(d\lambda\sqrt{1+\eta}) < 1 + \coth(d\lambda).
\end{aligned}
$$

Thus,

$$
\begin{aligned}
\frac{\eta}{\bar{w}_d} \max_{\tilde{\eta}>\eta} \frac{\partial \bar{w}_d}{\partial \eta}\bigg|_{\eta=\tilde{\eta}} &< \frac{\eta}{\bar{w}_d} \frac{A(\eta)}{(1+\eta)^{3/2}} \\
&\leq \frac{A(\eta)}{\sqrt{1+\eta}} \left(1 + \frac{\coth(\lambda d\sqrt{1+\eta})}{\sqrt{1+\eta}}\right).
\end{aligned}
$$

Next, we show that $B(\eta) < \infty$ for all $\eta > 0$. First, we see from (68) that there exist $\eta_1 > 0$, such that $\bar{w}_d > \frac{1}{2}$ when $\eta > \eta_1$. Let

$$
w_1(x) = \frac{\sinh(\lambda(1-x))}{2\sinh(\lambda(1-d))}, \quad h(x) = \frac{1}{\varepsilon} \frac{\sinh(d/\sqrt{\theta_l})}{\cosh(1/\sqrt{\theta_l})} \sinh((1-x)/\sqrt{\theta_l}),
$$

then $\bar{l}_0(x;\eta) > w_1(x) + \eta h(x)$ for any $\eta > \eta_1$, and therefore

$$
\begin{aligned}
B(\eta) &< \frac{\eta^{1/2}}{1-d} \int_d^1 \frac{\eta h(x)}{(w_1(x) + \eta h(x))(1 + \Phi_{\mathrm{L}}(w_1(x) + \eta h(x)))} dx \\
&< \frac{\eta^{1/2}}{\Phi_{\mathrm{L}}(1-d)} \int_d^1 \frac{\eta h(x)}{(w_1(x) + \eta h(x))^2} dx := B_1(\eta).
\end{aligned}
$$

Thus, we only need to show that $B_1(\eta) < \infty$ for all $\eta > 0$.

It is straightforward to obtain from $B_1(\eta)$ above

$$
B_1'(\eta) = \frac{\sqrt{\eta}}{\Phi_{\mathrm{L}}(1-d)} \int_d^1 \frac{h(x)(3w_1(x) - \eta h(x))}{(w_1(x) + \eta h(x))^3} dx
$$

which is negative when η is large enough. Therefore, we conclude that $B_1(\eta) < \infty$. The Theorem has been proved. □

From Theorem 3.7, the system is robust provide that the ligand synthesis rate is high. This theoretical result is confirmed by numerical simulations in Fig.1a (see [22] for simulations of a two-dimensional model).

3.3.3. *Robustness for general cases.* From the above analysis, when both ε and θ_l are small and η is large, the system has good robustness. For general cases when either ε or θ is not small, analytic calculation of the robustness R is difficult. We perform numerical simulation with results given at Fig. 1b, which shows a contour plot of the threshold η as a function of (ε, θ_l) so that $R = 0.2$. From Fig. 1b, the threshold η increases quickly with ε and θ_l. Thus, higher ligand synthesis rate is required to achieve good robustness for larger values of ε and θ_l.
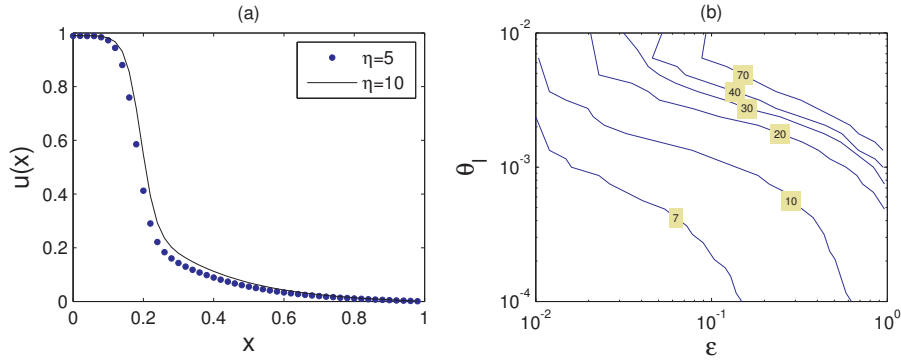
FIGURE 1. Direct numerical simulations for robustness of morphogen gradient. (a) Profiles of $u(x)$ at the steady-state for two morphogen production rates. Parameters used are referred to [23]: $d = 0.06$, $\lambda = 5.0$, $\gamma = 0.8$, $\mu = 0.6$, $\alpha = 0.1$, $\varepsilon = 0.01$, $\theta_l = 4.0 \times 10^{-4}$, and $\eta = 5$ (circle dots) or $\eta = 10$ (solid line). The feedback function is $k(u) = \frac{1}{1+(u/1.2)^2}$. (b) Contour plot of η (values shown on the lines) as a function of (ε, θ_l) so that the robustness $R = 0.2$.

4. **Discussion.** In this paper, we study a mathematical model for signaling morphogen gradient formation in the presence of cell membrane-associated non-receptors and a non-positive feedback for signaling receptor production (such as Tkv for Dpp in *Drosophila* wing imaginal disc). We proved that when

$$\varepsilon(\eta + 1) < 1 \tag{77}$$

the system has a unique steady-state solution, and the solution is linearly stable for all realistic range of parameter values. The steady state BVP is therefore well-posed. Furthermore, if

$$\varepsilon \ll 1, \quad \theta_l \ll 1, \quad \eta \gg 1, \tag{78}$$

the gradient is shown analytically to be robust with respect to changes in ligand production rate. Numerical simulations confirm this conclusion and show similar robustness for the general case.

Biological significances of the conditions (77) and (78) can be understood in the following way by considering the concentration of extracellular ligands in the production region. In that region, when the ligand production rate is very high and the concentration of total non-receptors (N_0) is large compare to that of receptors (R_0), the effect of receptors is negligible because most ligands bind to non-receptors. We further neglect the diffusion because ligands are synthesis uniformly in this region. Therefore, the system includes only ligand production, degradation, and reversible binding with non-receptors:

$$\begin{cases} \dfrac{d[\text{L}]}{dT} & = \ v_0 - \delta_\text{L}[\text{L}] - j_\text{on}[\text{L}][\text{N}] + j_\text{off}[\text{LN}] \\ \dfrac{d[\text{LN}]}{dT} & = \ j_\text{on}[\text{L}][\text{N}] - j_\text{off}[\text{LN}] \end{cases} \tag{79}$$

where $[\text{N}] + [\text{LN}] = N_0$.

From equation (79), free ligands are removed by either binding to non-receptors or degradation. The ratio of flux between these two ways of removing free ligands is given by

$$(j_{\text{on}}[\text{L}][\text{N}])/(\delta_{\text{L}}[\text{L}]),$$

which is of the order of ε when non-receptors are in low saturation. Thus ε measures the ability of ligand non-receptors binding relative to ligand degradation.

From the steady-state version of (79), we get $j_{\text{on}}[\text{L}]/j_{\text{off}} = [\text{LN}]/[\text{N}]$ from the second equation and, with it, $[\text{L}] = v_0/\delta_{\text{L}}$ from the first. Upon using the latter to eliminate $[\text{L}]$ from the former, we get $[\text{LN}]/[\text{N}] = (j_{\text{on}}/j_{\text{off}})(v_0/\delta_{\text{L}})$. With the definition of η from (13), we have

$$\eta = [\text{LN}]/[\text{N}]$$

showing that η is the ratio of bound to free non-receptor concentration in the ligand production region.

The condition (77) is satisfied when ε is small enough, i.e., most of the free ligands are removed by degradation. A sufficiently large δ_{L} also yields a small value of θ_l. The condition $\eta \gg 1$ is satisfied when the ligand synthesis rate is large (so that the ligand concentration is high at the production region) and non-receptors are in a state of high occupancy. Thus, the conditions (77) and (78) are satisfied with rapid synthesis and degradation of ligands.

From these theoretical results, we infer the following process for the formation of stable signaling biological gradients that are robust with respect to significant changes in ligand synthesis rate. A high ligand synthesis rate saturates the receptors and much of the non-receptors in the ligand production region where the signaling does not contribute to development and tissue patterning. Though excess ligand molecules are mostly removed by degradation with some transported downstream by normal diffusion (to bind with receptor and contribute to signaling gradient), those bound to non-receptors move along the "bridge" of cell bound non-receptors. These ligands then disassociate from the non-receptors, and are removed either by degradation or by binding with receptors to form signaling complex to regulate the expression of downstream genes. In this way, a signaling gradient is determined mainly by the amount of non-receptors available and is robust with respect to a perturbation in the ligand production rate as long as sufficient non-receptors are available for the task of bucket brigade transport. These observations are consistent with what has been observed in the case of Dpp gradient in *Drosophila* wing imaginal disc [3, 28], in which cell membrane bound non-receptors are important in the formation of morphogen gradient and tissue development.

Appendix A. Preliminary lemmas.

Lemma A.1. *Consider the boundary value problem*

$$\begin{cases} y'' - f(x,y) = 0 \\ y'(0) = y(1) = 0. \end{cases} \tag{80}$$

If

$$\frac{f(x,y_1) - f(x,y_2)}{y_1 - y_2} > 0 \tag{81}$$

for any $0 \le x \le 1$ and $y_1 \ne y_2$, and if there exists $b_1 \le 0 \le b_2$ such that

$$f(x,b_1) \le 0 \le f(x,b_2) \quad (0 \le x \le 1),$$

then (80) *has a unique solution* $y(x)$, *and the solution satisfies*

$$b_1 \leq y(x) \leq b_2, \quad (0 \leq x \leq 1). \tag{82}$$

Proof. It is easy to verify that $y_L(x) \equiv b_1$ and $y_U(x) \equiv b_2$ are lower and upper solutions of (80), respectively. Thus (80) has at least one solution that satisfies (82) according to the method of upper and lower solution [27].

Assume that there are two solutions $y_1(x)$ and $y_2(x)$. Let

$$q(x) = \frac{f(x, y_1(x)) - f(x, y_2(x))}{y_1(x) - y_2(x)},$$

then $q(x) \geq 0$. Let $\varphi(x) = y_1(x) - y_2(x)$, then $\varphi(x)$ satisfies

$$\varphi''(x) - q(x)\varphi(x) = 0, \quad \varphi'(0) = \varphi(1) = 0,$$

which implies

$$\int_0^1 (\varphi'(x))^2 dx + \int_0^1 q(x)\varphi^2(x) dx = 0.$$

Thus, we have $\varphi(x) \equiv 0$, and the uniqueness is proved. $\qquad \square$

Lemma A.2. *Let* $a(x) \in H^0(I, \mathbb{C})$. *If* $\Re(a(x)) > 0$, *the boundary value problem*

$$y'' - a(x)y = 0, \quad y'(0) = y(1) = 0 \tag{83}$$

has only the zero solution.

Proof. Assume that there is a nonzero solution $y(x)$. Multiply (83) by $y^*(x)$ and integral over the solution domain, we have

$$\int_0^1 |y'(x)|^2 dx + \int_0^1 \Re(a(x))|y(x)|^2 dx = 0,$$

which implies $y(x) \equiv 0$. Thus, (83) has only the zero solution. $\qquad \square$

Lemma A.3. *If* $a(x) \in H^0(I, \mathbb{C})$ *and* $\Re(a(x)) > 0$, *the boundary value problem*

$$y'' - a(x)y + f(x) = 0, y'(0) = y(1) = 0 \tag{84}$$

has a unique solution given in terms of a Green's function $G(x, s)$ *by*

$$y(x) = \int_0^1 G(x, s)f(s)ds. \tag{85}$$

Furthermore, the Green's function satisfies

$$\left\| \int_0^1 G(x, s)ds \right\| \leq \frac{1}{\inf_{0 \leq x \leq 1} \Re(a(x))}. \tag{86}$$

If $a(x) \in H^0(I, \mathbb{R})$, *then* $G(x, s) > 0$ *for all* $(x, s) \in [0, 1] \times [0, 1]$.

Proof. Let $\phi_1(x), \phi_2(x)$ be the solutions of the linear homogeneous equation

$$y'' - a(x)y = 0 \tag{87}$$

satisfying the boundary conditions

$$-\phi_1'(0) = \phi_2(1) = 1, \quad \phi_1(1) = \phi_2'(0) = 0.$$

Then the Green function is given by

$$G(x, s) = \begin{cases} \dfrac{\phi_1(x)\phi_2(s)}{\phi_1(s)\phi_2'(s) - \phi_1'(s)\phi_2(s)}, & (0 \leq s \leq x \leq 1) \\ \dfrac{\phi_1(s)\phi_2(x)}{\phi_1(s)\phi_2'(s) - \phi_1'(s)\phi_2(s)}, & (0 \leq x \leq s \leq 1), \end{cases}$$

where the Wronskian is nonzero with $\phi_1(s)\phi_2'(s) - \phi_1'(s)\phi_2(s) = \phi_2(0) \neq 0$. It is easy to verify that (85) defines a solution of (84).

The uniqueness follows from Lemma A.2.

The function $\int_0^1 G(x,s)ds$ satisfies (84) with $f(x) = 1$. Let

$$\int_0^1 G(x,s)ds = r(x)e^{i\varphi(x)}, \quad r(x), \varphi(x) \in H^0(I, \mathbb{R}).$$

It is a lengthy but straightforward calculation to show that $r(x)$ and $\varphi(x)$ satisfy two coupled differential equations one of which is

$$r'' - (\Re(a(x)) + \varphi'^2(x))r - \cos\varphi(x) = 0, \quad r'(0) = r(1) = 0. \tag{88}$$

Since $\varphi'^2(x) > 0$ and $|\cos\varphi(x)| \leq 1$, Lemma A.1 yields

$$\left\| \int_0^1 G(x,s)ds \right\| = \|r(x)\| \leq \frac{1}{\inf_{0 \leq x \leq 1} \Re(a(x))},$$

and (86) is proved.

When $a(x) \in H^0(I, \mathbb{R})$, the relevant maximum principle requires that both $\phi_1(x)$ and $\phi_2(x)$ are positive with $\phi_1'(x) \leq 0 \leq \phi_2'(x)$, and thus $G(x,s) > 0$. $\square$

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