

MORPHOGEN GRADIENT WITH EXPANSION-REPRESSION MECHANISM: STEADY-STATE AND ROBUSTNESS STUDIES

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ABSTRACT. Robust morphogen gradient formation is important for embryo development. Patterns of developmental tissue are encoded by the morphogen gradient that drives the process of cell differentiation in response to different morphogen levels. Experiments have shown that tissue patterning is robust with respect to morphogen overexpression. However, the mechanisms for this robust patterning remain unclear. The expansion-repression mechanism, which was proposed for achieving scaling of patterning with organ size, is a type of self-enhanced clearance through a non-local feedback regulation and may contribute to the robustness with respect to morphogen overexpression. In this paper, we study the role of the expansion-repression mechanism in morphogen gradient robustness through a two-equation model with general forms of feedback functions. We prove the existence of steady-state solutions, and, through model reduction and simplification, show that the expansion-repression mechanism is able to improve the robustness against changes in the morphogen production rate. However, this improvement is restricted by the biological requirement of multi-fate long-range morphogen gradient.

1. Introduction. Embryonic patterning of a developing organism is typically governed by signaling molecules known as morphogens. Morphogens diffuse away from a local production site and bind with membrane receptors to form a morphogen gradient in a patterning region. Patterns of developmental tissue are encoded by the morphogen gradient that drives the process of cell differentiation in response to different morphogen levels [26, 27]. The *Drosophila melanogaster* wing imaginal disc is a popular model system for studying morphogen-mediated patterning. In this model system, several morphogens are involved: Decapentaplegic (Dpp) which functions for the anterior-posterior patterning [1, 16, 25], Wingless (Wg) which controls the dorsal-ventral patterning [7, 28], and Hedgehog (Hh) which regulates the central part of the disc [24].

Many mathematical models have been proposed to study the formation of morphogen gradients. In [12, 13, 17, 18, 19], different models were proposed, and the existence, uniqueness and local stability of steady-state solutions were studied. These theoretical studies have shown how morphogen gradients depend on biological parameters and provided a stepping stone for studying morphogen gradient robustness. In [20], experiments have shown that Dpp overexpression induces

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no detectable effect on the anterior-posterior patterning of the *Drosophila* wing disc. Different strategies have been proposed to explain such robustness, including self-enhanced clearance (SEC) [8], multi-gradient system [11] and pre-steady-state decoding [5]. With SEC, morphogen signaling upregulates morphogen receptor synthesis so that the degradation of morphogens by binding to their receptors is enhanced by their own signaling. Through this strategy, the robustness is improved by reducing the sensitivity to morphogen synthesis and suspending overexpressed morphogens in the signaling region.

Aside from the robustness, scaling of patterning with organ size is another important topic in morphogen studies. Ben-Zvi and Barkai [2, 4] predicted that scaling may be achieved through the expansion-repression mechanism. They showed that a newly identified component, Pentagone (Pent), which is repressed by Dpp signaling and expands Dpp gradient, acts as an expander in expansion-repression motif [3, 10]. In [2], a generic two-equation model of the expansion-repression mechanism in which morphogen signaling upregulates morphogen degradation through diffusive expanders was introduced. This mechanism can be considered as a type of SEC through a non-local feedback regulation. This point of view motivates a study on how the expansion-repression mechanism contributes to the robustness of morphogen patterning. In this paper, we consider a two-equation model of a morphogen-expander system with general forms of feedback functions. Through quantifying the effect of morphogen overexpression, we demonstrate how the expansion-repression mechanism improves the robustness of morphogen system.

This paper is organized as follows. In Section 2, we introduce a two-equation model of morphogen-expander system with general forms of feedback functions. In Section 3, we prove the existence of steady-state solutions. In Section 4, we perform model reduction with the assumption that expanders diffuse rapidly and degrade slowly [3, 4]. In Section 5, we define a robustness measure for quantifying the effect of morphogen overexpression and, through this measure, we show how the expansion-repression mechanism improves the robustness of a steady-state solution against changes in the morphogen production rate and the limitation of this improvement is also discussed in this section. Finally, discussion is given in Section 6.

2. Mathematical Model. We consider a morphogen model in which morphogens, denoted by M , are produced in a local region and diffuse out to a patterning region. The gradient formed by morphogens governs the patterning of a tissue through establishing the boundaries between cell fates at particular thresholds of morphogen concentration. One such example is the anterior-posterior patterning of the *Drosophila* wing disc driven by Dpp [25].

In the model, diffusive molecule expanders, denoted by E , are produced everywhere while the production rate is downregulated by morphogen level. Also expanders reduce the degradation rate of morphogens to extend the signaling distance. Figure 1 shows the schematic diagram of the morphogen-expander system which was proposed in [2].

The dynamics of the concentrations of M and E are modeled as follows:

$$\frac{\partial[M]}{\partial t} = D_M \Delta[M] - \beta_M([E])[M] + V_M(x) \quad (1)$$

$$\frac{\partial[E]}{\partial t} = D_E \Delta[E] - \beta_E[E] + V_E([M]), \quad (2)$$

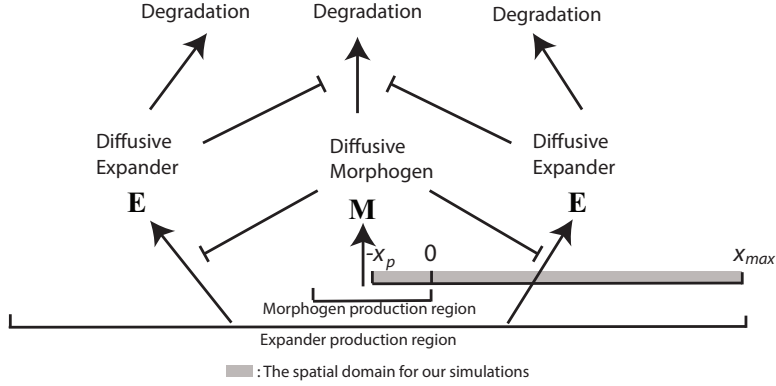


FIGURE 1. Schematic diagram of the morphogen-expander system which was proposed in [2].

where $\beta_M([E])$ and $V_E([M])$ are positive functions which are strictly decreasing with respect to $[E]$ and $[M]$, respectively. In this paper, for simplification, we assume that β_M and V_E are smooth functions.

As in [15, 18, 19], we simplify the problem by representing the patterned tissue as an one-dimensional domain, as shown in Figure 1. The midpoint of the morphogen production region is denoted by $x = -x_p$; the boundary between the production region and the patterning region is $x = 0$; and the edge of the patterning region is $x = x_{max}$. According to these settings, we define

$$V_M(x) = \begin{cases} v_M & \text{if } x \in (-x_p, 0); \\ 0 & \text{if } x \in [0, x_{max}), \end{cases} \quad (3)$$

where v_M is the morphogen production rate in the production region. We assume that morphogen and expander gradients are symmetric with respect to the center $x = -x_p$ and the boundary condition at $x = x_{max}$ is reflective [3, 4]. Accordingly we define the boundary conditions as follows:

$$\frac{\partial[M]}{\partial x}(t, -x_p) = 0, \quad \frac{\partial[M]}{\partial x}(t, x_{max}) = 0; \quad (4)$$

$$\frac{\partial[E]}{\partial x}(t, -x_p) = 0, \quad \frac{\partial[E]}{\partial x}(t, x_{max}) = 0. \quad (5)$$

3. Existence of Steady-State Solution. The formation of morphogen gradients typically requires only a few hours, which is relatively short compared with the time scale of cell growth, hence, we are only interested in steady-state morphogen gradients in this study. In this section, we consider the steady-state equations for the model (1)-(2). Let a and b be the steady-state solutions of $[M]$ and $[E]$, respectively. By setting the left hand side of (1)-(2) to be zero, we obtain a steady-state system:

$$0 = D_M \frac{d^2 a}{dx^2} - \beta_M(b)a + V_M(x), \quad (6)$$

$$0 = D_E \frac{d^2 b}{dx^2} - \beta_E b + V_E(a), \quad (7)$$

with

$$\frac{da}{dx}(-x_p) = 0, \quad \frac{da}{dx}(x_{max}) = 0; \quad (8)$$

$$\frac{db}{dx}(-x_p) = 0, \quad \frac{db}{dx}(x_{max}) = 0. \quad (9)$$

Theorem 3.1. *There exists a non-negative steady-state solution (a, b) for (6)-(9).*

Before proving Theorem 3.1, we state the following lemma which will be used in the proof of Theorem 3.1:

Lemma 3.2. *For any continuous non-negative functions \bar{a} and \bar{b} such that*

$$0 \leq \sup_{-x_p \leq x \leq x_{max}} \bar{a} \leq \frac{v_M}{\beta_M(V_E(0)/\beta_E)}, \quad (10)$$

$$0 \leq \sup_{-x_p \leq x \leq x_{max}} \bar{b} \leq \frac{V_E(0)}{\beta_E}, \quad (11)$$

there is a unique pair of continuous non-negative functions (a, b) satisfying

$$0 = D_M \frac{d^2 a}{dx^2} - \beta_M(0)a + (\beta_M(0) - \beta_M(\bar{b}))\bar{a} + V_M(x), \quad (12)$$

$$0 = D_E \frac{d^2 b}{dx^2} - \beta_E b + V_E(\bar{a}), \quad (13)$$

with

$$\frac{da}{dx}(-x_p) = 0, \quad \frac{da}{dx}(x_{max}) = 0; \quad (14)$$

$$\frac{db}{dx}(-x_p) = 0, \quad \frac{db}{dx}(x_{max}) = 0. \quad (15)$$

Moreover, the functions a and b satisfy

$$0 \leq \sup_{-x_p \leq x \leq x_{max}} a \leq \frac{v_M}{\beta_M(V_E(0)/\beta_E)},$$

$$0 \leq \sup_{-x_p \leq x \leq x_{max}} b \leq \frac{V_E(0)}{\beta_E}.$$

The proof is based on the monotone method by Sattinger established in [23], which was widely used in studying other morphogen systems [14, 15, 12, 18, 19].

Proof. Using the assumption (10)-(11) and the definitions of V_E and β_M , we have

$$\begin{aligned} 0 \leq (\beta_M(0) - \beta_M(\bar{b}))\bar{a} + V_M(x) &\leq (\beta_M(0) - \beta_M(V_E(0)/\beta_E)) \frac{v_M}{\beta_M(V_E(0)/\beta_E)} + v_M, \\ &\leq \frac{v_M \beta_M(0)}{\beta_M(V_E(0)/\beta_E)}, \end{aligned}$$

and

$$0 \leq V_E(\bar{a}) \leq V_E(0).$$

It is easy to verify that $a_l = 0$ is a lower solution and $a_u = \frac{v_M}{\beta_M(V_E(0)/\beta_E)}$ is an upper solution for (12) with the boundary condition (14); $b_l = 0$ is a lower solution and $b_u = \frac{V_E(0)}{\beta_E}$ is an upper solution for (13) with the boundary condition (15). The

monotone method by Sattinger established in [23] shows that there are solutions a and b of (12)-(15) satisfying

$$\begin{aligned} 0 = a_l \leq a(x) \leq a_u &= \frac{v_M}{\beta_M(V_E(0)/\beta_E)}, \\ 0 = b_l \leq b(x) \leq b_u &= \frac{V_E(0)}{\beta_E}. \end{aligned}$$

To prove the uniqueness, it is as same as proving the uniqueness of the solution c of the following equation:

$$0 = D \frac{d^2 c}{dx^2} - \beta c + f(x), \quad (16)$$

with no-flux boundaries, and the constants β and D are positive constants.

We suppose that $c_1(x)$ and $c_2(x)$ satisfy (16) and set $c_3(x) = c_1(x) - c_2(x)$, which satisfies

$$0 = D \frac{d^2 c_3}{dx^2} - \beta c_3,$$

then we have

$$0 = D \int_{-x_p}^{x_{max}} \frac{d^2 c_3}{dx^2} c_3 dx - \int_{-x_p}^{x_{max}} \beta c_3^2 dx.$$

Using integration by parts and the continuity of the solutions, we obtain

$$0 = -D \int_{-x_p}^{x_{max}} \left(\frac{dc_3}{dx} \right)^2 dx - \int_{-x_p}^{x_{max}} \beta c_3^2 dx,$$

therefore it implies that c_3 is a zero function and $c_1 = c_2$. \square

Now we start the proof of Theorem 3.1.

Proof. (Theorem 3.1) First we set Ω , a subspace of the Banach space $[C[-x_p, x_{max}]]^2$, with the L^∞ norm and define

$$\begin{aligned} \Omega = \{(a, b) \in [C[-x_p, x_{max}]]^2 \mid 0 \leq \sup_{-x_p \leq x \leq x_{max}} |a| \leq \frac{v_M}{\beta_M(V_E(0)/\beta_E)} \\ \text{and } 0 \leq \sup_{-x_p \leq x \leq x_{max}} |b| \leq \frac{V_E(0)}{\beta_E}\}, \end{aligned}$$

which is a closed and convex set in the Banach space $[C[-x_p, x_{max}]]^2$.

From Lemma 3.2, we define an operator $T : \Omega \rightarrow \Omega$ such that

$$T(\bar{a}, \bar{b}) = (a, b),$$

with (\bar{a}, \bar{b}) and (a, b) as in Lemma 3.2.

By the standard regularity theory, we see that T is a continuous mapping. If $T(\Omega)$ is a relatively compact subset of $[C[-x_p, x_{max}]]^2$, we can apply the Schauder fixed point theorem [9] to prove that there exists a pair (a, b) such that

$$T(a, b) = (a, b)$$

and the proof of Theorem 3.1 is completed.

Now let us complete the proof by showing that $T(\Omega)$ is a relatively compact subset of $[C[-x_p, x_{max}]]^2$. Using the Arzela-Ascoli theorem [22], $T(\Omega)$ is a relatively compact subset if and only if the elements in $T(\Omega)$ are uniformly bounded and equicontinuous on $[-x_p, x_{max}]$. By Lemma 3.2, we see that the elements are

uniformly bounded by $\text{Max} \left\{ \frac{v_M}{\beta_M(V_E(0)/\beta_E)}, \frac{V_E(0)}{\beta_E} \right\}$. According to the definition of T and the Schauder estimate of elliptic equation [9], it is easy to show that $T(\Omega)$ is a bounded subset of $[C^1[-x_p, x_{max}]]^2$ so we prove that the elements in $T(\Omega)$ are equicontinuous on $[-x_p, x_{max}]$. Overall, we have finished the proof of Theorem 3.1. \square

4. Model Reduction. To reveal the features of the expansion-repression mechanism, we simplified the system (6)-(9) with the assumption that expanders diffuse rapidly and degrade slowly [3, 4]. Under this assumption, we can approximate that the concentration of the steady-state expander is uniform across the domain, and thus, after replacing (a, b) by (a_s, b_s) , the system (6)-(9) becomes

$$0 = D_M \frac{d^2 a_s}{dx^2} - \beta_M(b_s) a_s + V_M(x) \quad (17)$$

with

$$b_s = \frac{1}{\beta_E} \frac{\int_{-x_p}^{x_{max}} V_E(a_s(y)) dy}{x_{max} + x_p}, \quad (18)$$

and the boundary conditions

$$\frac{da_s}{dx}(-x_p) = 0 \text{ and } \frac{da_s}{dx}(x_{max}) = 0. \quad (19)$$

In this simplified system, the effective degradation rate of morphogen, $\beta_M(b_s)$, increases with the morphogen concentration a_s , and thus the expansion-repression mechanism can be considered as a type of self-enhanced clearance through a non-local feedback regulation. In the next section, a numerical simulation shows that this simplification gives a good approximation for the steady-state solutions of the original system (6)-(9).

Theorem 4.1. *There exists a unique non-negative solution for (17)-(19).*

Proof. First we define a function $\theta(x; k, v)$ which is the unique positive solution of the equation:

$$0 = D_M \frac{\partial^2 \theta}{\partial x^2} - k\theta + V(x) \text{ for } x \in (-x_p, x_{max}) \quad (20)$$

where

$$V(x) = \begin{cases} v & \text{if } x \in (-x_p, 0); \\ 0 & \text{if } x \in [0, x_{max}), \end{cases} \quad (21)$$

and the boundary conditions

$$\frac{\partial \theta}{\partial x}(-x_p) = 0 \text{ and } \frac{\partial \theta}{\partial x}(x_{max}) = 0. \quad (22)$$

Define a function $f(k)$ as

$$f(k) = k - \beta_M \left(\frac{1}{\beta_E} \frac{\int_{-x_p}^{x_{max}} V_E(\theta(y; k, v)) dy}{x_{max} + x_p} \right) \text{ for } k > 0.$$

It is easy to prove that $f(k_1) = 0$ if and only if $a_s(x) = \theta(x; k_1, v_m)$ is a solution of (17)-(19).

From (20)-(22), we obtain $\theta(x; k, v)$ in terms of v, k and D_M as:

$$\theta(x; k, v) = \begin{cases} \frac{v}{k} \left(1 - \frac{e^{2x_{max}/\lambda} - 1}{2(e^{2(x_{max}+x_p)/\lambda} - 1)} (e^{-x/\lambda} + e^{(x+2x_p)/\lambda}) \right) & \text{for } -x_p \leq x < 0 \\ \frac{v}{k} \frac{e^{2x_p/\lambda} - 1}{2(e^{2(x_{max}+x_p)/\lambda} - 1)} (e^{-(x+2x_{max})/\lambda} + e^{x/\lambda}) & \text{for } 0 \leq x \leq x_{max} \end{cases} \quad (23)$$

where $\lambda = \sqrt{D_M/k}$. It is easy to see that $\theta(x; k, v)$ decreases with respect to k . Furthermore, since V_E and β_M are strictly decreasing functions, the function $f(k)$ is continuous and strictly increases with respect to k . Moreover, since $f(0) < 0$ and $f(2\beta_M(0)) > 0$, there exists a unique positive k_1 so that $f(k_1) = 0$, and thus $a_s(x) = \theta(x; k_1, v_m)$ is a unique positive solution of (17)-(19). \square

5. Robustness to Morphogen Synthesis. In this section, we consider the robustness of a steady-state morphogen gradient with respect to changes in the morphogen production rate. In order to achieve this, we define the robustness $R(a)$ as an average of the relative change of a when the parameter v_M is increased to \bar{v}_M [17, 19]:

$$R(a) = \frac{1}{\Delta v_M / v_M} \frac{1}{x_{max}} \int_0^{x_{max}} \frac{|\Delta a(x)|}{a(x)} dx$$

with

$$\Delta a(x) = \bar{a}(x) - a(x) \text{ and } \Delta v_M = \bar{v}_M - v_M > 0,$$

where $a(x)$ and $\bar{a}(x)$ are the steady-state morphogen gradients for morphogen production rates v_M and \bar{v}_M , respectively. As in [17, 19], smaller value of $R(a)$ means better robustness.

In this section, we first consider the simplified system (17)-(19). For this simplified system, we approximate $R(a_s)$ for sufficiently small change of morphogen production ($\Delta v_M \ll 1$) and then analyze how the expansion-repression mechanism improves the robustness of morphogen gradient. Next, we apply numerical simulations to verify the results for the following two cases with larger value of v_M : the simplified system (17)-(19) and the two-equation system (6)-(9).

5.1. Approximation for sufficiently small Δv_M . Define the sensitivity coefficient of a_s to v_M [21],

$$S(x) = \frac{v_M}{a_s(x; v_M)} \frac{\partial a_s(x; v_M)}{\partial v_M}, \quad (24)$$

then, when $\Delta v_M \ll 1$, the robustness can be approximated by the average of the sensitivity coefficient over $[0, x_{max}]$:

$$R(a_s) \approx \frac{1}{x_{max}} \int_0^{x_{max}} |S(x)| dx. \quad (25)$$

In the following theorem, we approximate $R(a_s)$ in terms of the parameters in (17)-(19) and the functions β_M and V_E .

Theorem 5.1. *For $\Delta v_M \ll 1$, the robustness $R(a_s)$ for the simplified system (17)-(19) can be approximated as (25) with*

$$S(x) = 1 + \frac{\theta_1(x; \beta_M, v_M)}{a(x; v_M)} \frac{\frac{\beta'_M}{\beta_E(x_{max}+x_p)} \int_{-x_p}^{x_{max}} V'_E a(y; v_M) dy}{1 - \frac{\beta'_M}{\beta_E(x_{max}+x_p)} \int_{-x_p}^{x_{max}} V'_E \theta_1(y; \beta_M, v_M) dy},$$

where θ is defined in (23) and $\theta_1 = \frac{\partial \theta(x; y_1, y_2)}{\partial y_1}$, and β'_M and V'_E are the derivatives of β_M and V_E at $b_s = \frac{1}{\beta_E} \frac{\int_{-x_p}^{x_{max}} V_E(a(y; v_M)) dy}{x_{max} + x_p}$ and $a_s = a_s(y; v_M)$, respectively.

Proof. First, we define

$$\bar{\beta}_M(v_M) = \beta_M \left(\frac{1}{\beta_E} \frac{\int_{-x_p}^{x_{max}} V_E(a_s(y; v_M)) dy}{x_{max} + x_p} \right), \quad (26)$$

and therefore we obtain

$$a_s(x; v_M) = \theta(x; \bar{\beta}_M(v_M), v_M)$$

where θ is defined in (20)-(22). Moreover, the derivative of a_s with respect to v_M is given by

$$\frac{\partial a_s(x; v_M)}{\partial v_M} = \theta_2 + \theta_1 \frac{d\bar{\beta}_M}{dv_M}, \quad (27)$$

where $\theta_i = \frac{\partial \theta(x; y_1, y_2)}{\partial y_i}$ for $i = 1, 2$. From (26) and (27), we have

$$\begin{aligned} \frac{d\bar{\beta}_M}{dv_M} &= \frac{\beta'_M}{\beta_E(x_{max} + x_p)} \left(\int_{-x_p}^{x_{max}} V'_E \theta_2(y; \beta_M, v_M) dy \right. \\ &\quad \left. + \frac{d\bar{\beta}_M}{dv_M} \int_{-x_p}^{x_{max}} V'_E \theta_1(y; \beta_M, v_M) dy \right) \end{aligned}$$

and thus

$$\frac{d\bar{\beta}_M}{dv_M} = \frac{\frac{\beta'_M}{\beta_E(x_{max} + x_p)} \int_{-x_p}^{x_{max}} V'_E \theta_2(y; \beta_M, v_M) dy}{1 - \frac{\beta'_M}{\beta_E(x_{max} + x_p)} \int_{-x_p}^{x_{max}} V'_E \theta_1(y; \beta_M, v_M) dy}. \quad (28)$$

From the definition of the function θ , it is easy to verify that

$$\theta_2(y; \beta_M, v_M) = a_s(y; v_M)/v_M,$$

then combining with (24), (27) and (28), we finally obtain

$$S(x) = 1 + \frac{\theta_1(x; \beta_M, v_M)}{a_s(x; v_M)} \frac{\frac{\beta'_M}{\beta_E(x_{max} + x_p)} \int_{-x_p}^{x_{max}} V'_E a_s(y; v_M) dy}{1 - \frac{\beta'_M}{\beta_E(x_{max} + x_p)} \int_{-x_p}^{x_{max}} V'_E \theta_1(y; \beta_M, v_M) dy}.$$

□

From Theorem 5.1, we write the approximated $R(a_s)$ as

$$R(a_s) \approx \frac{1}{x_{max}} \int_0^{x_{max}} \left| 1 + \frac{\theta_1(x; \beta_M, v_M)}{a_s(x; v_M)} C \right| dx, \quad (29)$$

where C is a positive constant depending on all the parameters and the functions V_E and β_M .

A value $\lambda = \sqrt{D_M/\beta_M(b_s)}$, known as the length scale [13], captures the distance that morphogens are able to reach before they are degraded by certain ratio. In terms of λ , x_p , x_{max} , v_M and β_M , the steady-state solution $a_s(x; v_M)$ can be expressed as $\theta(x; \beta_M, v_M)$ defined in (23). Moreover, the partial derivative $\theta_1(x; \beta_M, v_M) = \frac{\partial \theta(x; \beta_M, v_M)}{\partial \beta_M}$ for $x > 0$ can be expressed as

$$a_s(x; v_M) \frac{\hat{\lambda}}{\widehat{D}_M} \left(-\hat{\lambda} + \frac{\widehat{x}_p e^{2\widehat{x}_p/\hat{\lambda}}}{(e^{2\widehat{x}_p/\hat{\lambda}} - 1)} - \frac{(1 + \widehat{x}_p) e^{2(1+\widehat{x}_p)/\hat{\lambda}}}{(e^{2(1+\widehat{x}_p)/\hat{\lambda}} - 1)} + \frac{(-\widehat{x} + 2) e^{(-\widehat{x}+2)/\hat{\lambda}} + \widehat{x} e^{\widehat{x}/\hat{\lambda}}}{2(e^{(-\widehat{x}+2)/\hat{\lambda}} + e^{\widehat{x}/\hat{\lambda}})} \right)$$

where $\widehat{D}_M = D_M/x_{max}^2$, $\widehat{x}_p = x_p/x_{max}$, $\hat{\lambda} = \lambda/x_{max}$ and $\widehat{x} = x/x_{max}$. So if we fix the values of \widehat{D}_M and \widehat{x}_p , the approximated $R(a_s)$ in (29) is only determined by the values of $\hat{\lambda}$ and C .

In Figure 2, we set $D_M/x_{max}^2 = 10^{-4} sec^{-1}$ and $x_p = 0.1$, as in [3, 13], and plot the approximated $R(a_s)$ in (29) as a function of C with different values of λ/x_{max} in a biologically possible range [0.1, 0.3], suggested by [13].

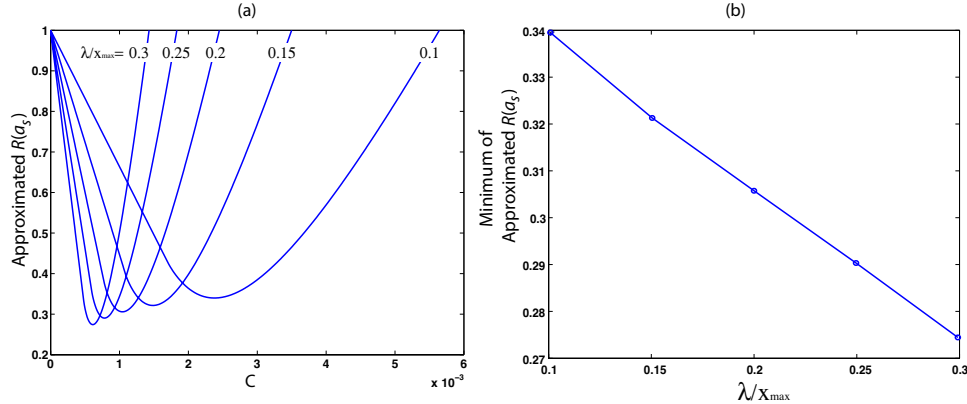


FIGURE 2. The approximated $R(a_s)$ defined in (29). (a) The approximated $R(a_s)$ as a function of C with different values of λ/x_{max} . (b) The minimum of the approximated $R(a_s)$ with different values of λ/x_{max} .

Figure 2(a) demonstrates how the approximated $R(a_s)$ depends on C . When V_E and β_M are constants (without the expansion-repression mechanism), the constant C equals to zero, and hence $R(a_s) = 1$. In the case with the expansion-repression mechanism so that V_E and β_M are decreasing functions, we have $C > 0$. Since $\theta_1(x; \beta_M, v_M)$ is negative ($\theta(x; k, v)$ decreases with respect to k), Figure 2(a) shows that $R(a_s)$ decreases with respect to C when C is small. This fact suggests that the expansion-repression mechanism can improve the robustness.

Figure 2(b) shows that the minimum of the approximated $R(a_s)$ decreases with respect to λ/x_{max} . But we know that there is a lower bound of the relative length scale λ/x_{max} for achieving multi-fate long-range morphogen gradient [13], for example, the length scale is around $20\mu m$ for the Dpp gradient of the *Drosophila* wing disc with $x_p = 10\mu m$ and $x_{max} = 100\mu m$ [6]. Overall, the results in Figure 2 suggest that although the expansion-repression mechanism can improve the robustness against changes of the morphogen production rate, the restriction on achieving multi-fate long-range morphogen gradient induces the limitation that the robustness $R(a_s)$ is always larger than 0.2 which was adopted for an upper bound for an acceptable robustness value [18, 19].

5.2. Numerical simulations. In the previous subsection, we showed that, for the simplified system (17)-(19) with a sufficiently small v_M , the approximated $R(a_s)$ has been improved but this effect of improvement is restricted by the biological requirement of multi-fate long-range morphogen gradient.

Here we first verify this result for the simplified system (17)-(19) with relatively large $\Delta v_M = v_M/2$ corresponding to 50% change of the morphogen production rate. We consider the sensitivity coefficient and the robustness with fixed $\lambda =$

$\sqrt{D_M/\beta_M(b)} = 20\mu m$ and define

$$V_E(z) = \frac{v_E}{1 + (z/K_a)^4} \text{ and } \beta_M(z) = \frac{\beta_{max}}{1 + (z/K_b)^n}. \quad (30)$$

which were used in [3]. The parameters v_E and β_{max} are the maximum values of V_E and β_E , respectively; the parameters K_a and K_b are the half-saturation constants of the feedback functions.

As in [3, 13], we set $v_M = 8 \times 10^{-2}\mu M$, $v_E = 10^{-3}\mu M$, $\beta_E = 10^{-4}$, $x_p = 10\mu m$, $x_{max} = 100\mu m$, $K_a = 0.2a(-x_p) = 2.5183\mu M$, $K_b = b = 6.5472\mu M$, $D_M = 1\mu m^2 sec^{-1}$ and $\beta_{max} = 2/20^2 sec^{-1}$ so that $\lambda = \sqrt{D_M/\beta_M(b)} = 20\mu m$. The morphogen and expander gradients for these settings are shown in Figure 3(c). In Figures 3(a,b,d,e), the sensitivity coefficient and $R(a_s)$ are plotted with different n varied from 0 to 8. We note that n is the Hill function coefficient in (30), which describes the cooperativity of the feedback response, and in our case, larger n implies larger value of β'_M at the steady-state solution. For $n = 0$ (the case without feedback), β_M is a constant function and the robustness is equal to one. The term β'_M increases along with n and thus the robustness decreases from 1 to around 0.24 (see Figures 3(b,e)).

Now we consider the original system (6)-(9) with $\Delta v_M = v_M/2$. We set $D_E = 10D_M = 10\mu m^2 sec^{-1}$, as in [3], and other parameters are set the same as which we used above. Figure 3(f) displays the steady-state solutions for the two-equation system (6)-(9), which are consistent with the steady-state solutions for the simplified system (17)-(19), shown in Figure 3(c). Also, Figures 3(b,e,h) support that $R(a_s)$ gives a good approximation for $R(a)$ and the robustness has a lower bound which is larger than 0.2.

6. Discussion. In this paper, we have proved the existence of steady-state solutions of the two-equation morphogen-expander system (6)-(9) with general forms of feedback functions. With the assumption that expanders diffuse rapidly and degrade slowly, a model reduction was used to simplify the two-equation system into the one-equation non-local system (17)-(19). For the simplified model, we showed that the expansion-repression mechanism improves the robustness with respect to changes in the morphogen production rate and discussed the limitation of this improvement. Our analytic and numerical results supported that $R(a)$, the robustness measure used in [17, 19], is always larger than 0.2 which is an upper bound for an acceptable robust system.

Although a limitation exists for the expansion-repression mechanism, a potential modification can be proposed according to some recent experimental and mathematical results. Experiments presented in [3] support that Pent, as an expander, expands the Dpp gradient through an interaction with Dally, a heparin sulfate proteoglycan, which acts as a non-signaling receptor in the morphogen system. Mathematical studies suggest that the presence of non-signaling receptors allows the morphogen gradient to have better robustness against changes of the morphogen production rate [18, 19]. Non-signaling receptors, coupled with expanders (as depicted in Figure 4), may provide a solution to the problem of achieving better robustness while remaining biologically feasible, and our studies give an insight for this future study.

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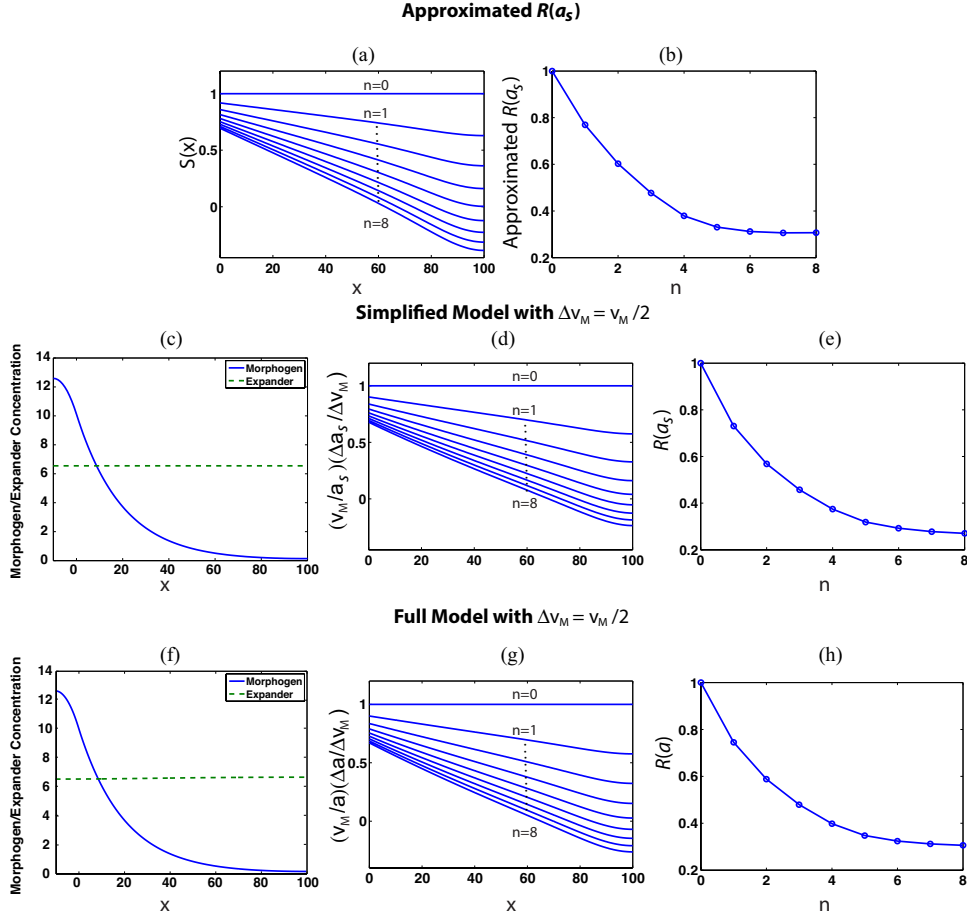


FIGURE 3. Sensitivities and robustness measures with different values of n in (30). (a) Sensitivity coefficient $S(x)$. (b) Approximated $R(a_s)$ as a function of n . (c) Steady-state solution for the simplified system (17)-(19). (d) The simplified system (17)-(19): $\frac{v_M}{a_s(x)} \frac{\Delta a_s(x)}{\Delta v_M}$ with $\Delta v_M = v_M/2$. (e) The simplified system (17)-(19): $R(a_s)$ as a function of n with $\Delta v_M = v_M/2$. (f) Steady-state solution for the two-equation system (6)-(9). (g) The two-equation system (6)-(9): $\frac{v_M}{a(x)} \frac{\Delta a(x)}{\Delta v_M}$ with $\Delta v_M = v_M/2$. (h) The two-equation system (6)-(9): $R(a)$ as a function of n with $\Delta v_M = v_M/2$.

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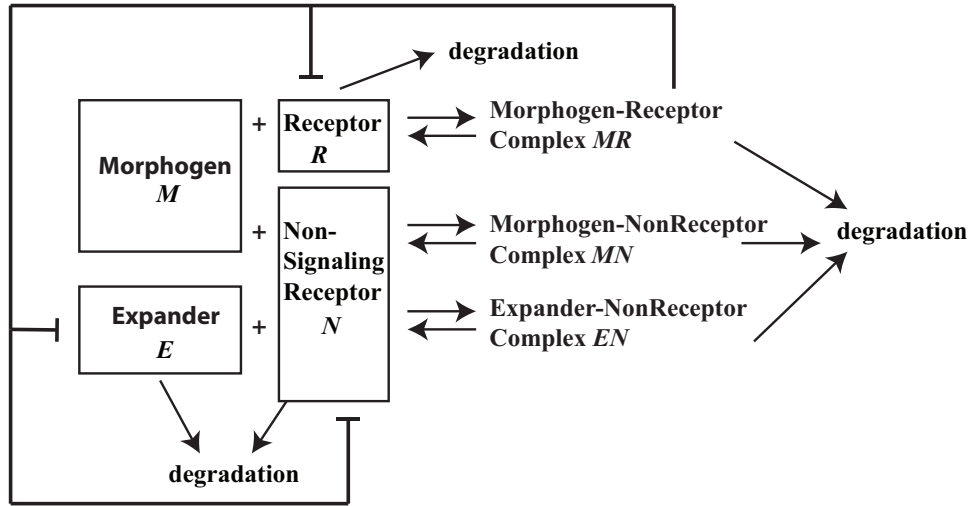


FIGURE 4. Schematic diagram of morphogen model involving non-signaling receptors, as well as expanders. In this system, free morphogens, denoted by M bind with non-diffusive receptors to form a gradient of morphogen-receptor complexes, denoted by MR , that governs the patterning of a tissue. Other than signaling receptors, morphogens also bind with other non-signaling receptors, denoted by N , to form a gradient of morphogen-nonreceptor complex, which is denoted by MN . Diffusive expanders, denoted by E , reduce the number of morphogen-nonreceptor complex through binding with non-signaling receptors to form a complex EN . From this point of view, E expands a morphogen gradient by increasing the concentration of free diffusive morphogens. Also, morphogen-receptor complexes inhibit the production rates of receptors, non-signaling receptors and expanders.

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