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Internalization and end flux in morphogen gradient formation

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Dedicated to Professor Roderick Wong on the occasion of his 60th birthday

Abstract

Two simple reaction–diffusion systems of partial differential equations and auxiliary conditions governing the activities of diffusible ligands such as Dpp in anterior–posterior axis of *Drosophila* wing imaginal discs were previously formulated and investigated by numerical simulations in [Developmental Cell 2 (2002) 785–796]. System B focuses on diffusion, reversible binding with receptors and ligand-mediated degradation for a fixed receptor concentration uniform in time and space. System C extended this basic but meaningful model to allow for endocytosis, exocytosis and receptor synthesis and degradation. The present paper provides a mathematical underpinning for the computational studies of these two systems and some insight gained from our analysis. We will see for instance that the two boundary value problems governing the steady state for the two systems are identical in form. This result will enable us to avoid dealing with internalization explicitly when we investigate other complex morphogen activities such as the effects of (1) feedback and (2) diffusible and non-diffusible molecules competing for ligands and receptors to inhibit cell signaling and pattern formation. The principal contribution of the present work pertains to the extension of System C to allow for a ligand flux at the source end. The more general model has many significant consequences including the removal of a limitation of previous models on ligand synthesis rate for the existence of steady state behavior. Linear stability of the corresponding steady state behavior is established. While the actual decay rate of transients is less accessible in this new model, it is possible to obtain tight upper and lower bounds for the decay rate in terms of the (effective) degradation rate of the receptors and that of the ligand-receptor complexes.
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1. Introduction

Morphogens (ligands) are molecular substances that bind to cell surface receptors and other molecules. The gradients of different morphogen-receptor concentrations are known to be responsible for cell signaling and patterning of biological tissues during the developmental phase of the biological host. For a number of morphogen families (including Dpp in the wing imaginal disc of Drosophila fruit flies), it is well established that the concentration gradients are formed by morphogens that are transported from a localized production site and bind to cell surface receptors downstream (see references cited in [7]). Recently, the mechanism of morphogen transport has been re-examined by both theoreticians and experimentalists, resulting in considerable uncertainty regarding the role of diffusion in transporting morphogens, and other mechanisms being suggested as replacements (e.g., [3,4,6,19] and references in [7]). The observations against diffusive transport were summarized and addressed in [7] by results from numerical simulations of two mathematical models, designated as Systems B and C, in the form of a system of partial differential equations and auxiliary conditions (defining an *initial-boundary value problem*, or IBVP for short) on ligand and receptor concentrations. The results in [7] show that *diffusive models* of morphogen transport can account for much of the data obtained on biological systems including those that have been used to argue against diffusive transport. When observations and data are correctly interpreted, they not only fail to rule out diffusive transport, they favor it.

The present paper provides the mathematical underpinning for the case of diffusive transport of morphogens made in [7] including establishing the existence of a unique set of asymptotically stable morphogen concentration gradients if the ligand synthesis rate is less than the receptor-mediated degradation rate of the ligand-receptor complexes. A remarkable outcome of our analysis of System C shows that the governing *boundary value problem* (BVP) for the steady state behavior of this more complete model (that allows for receptor internalization and renewal) may be reduced to the corresponding BVP for System B (without receptor internalization and renewal) with the amplitude parameter β and the shape parameter ψ in the latter replaced by the corresponding amplitude and shape parameters β_ω and ψ_ω (to be defined in Sections 3 and 4 of this paper), respectively. As such, the steady state morphogen activities in both intra- and extracellular space of *Drosophila* wing discs are adequately represented by System B if we suitably interpret the degradation and binding rates. It follows that all mathematical developments for the steady state problem of System B apply effectively verbatim to that of System C, and we will take advantage of the implication of this mathematical equivalence in a substantive way in Section 3 of this paper. In addition, the mathematical equivalence of the two systems will enable us to avoid dealing with internalization explicitly when we investigate other complex morphogen activities such as the effects of feedback and of competing (non-signaling) molecules such as diffusible Sog and Tsg (which bind with available receptors and thereby reduce ligand-receptor concentration) and non-diffusible proteoglycans (which bind with ligand to form non-signaling ligand-nonreceptor complexes and thereby, again, reduce ligand-receptor concentration).

The principal contribution of the present paper however is to investigate a fundamental significant extension of System C to allow for a ligand flux at the source end. This simple extension leads to some very significant results including the removal of the unrealistic limitation on ligand synthesis rate for the existence of steady state behavior. Linear stability of the corresponding steady state behavior is established. While the actual decay rate of transients is less accessible in this extended model, it is possible to obtain useful upper and lower bounds for the decay rate in terms of the (effective) degradation rate of the receptors and that of the ligand-receptor complexes.

In reporting the results mentioned above, we will focus on one-dimensional models. As shown in [10], similar results for higher dimensional models have been obtained by similar developments with some straightforward technical modifications.

2. A one-dimensional formulation with endocytosis and receptor synthesis

In the one-dimensional model of the morphogen activities along the anterior–posterior axis of a *Drosophila* wing imaginal disc with receptor internalization and renewal first formulated in [7], ligand molecules (Dpp in our case) are introduced into the extracellular space at a rate V_L at the end, $X = 0$, the border between the *anterior* and *posterior* compartment of the disc. The morphogens diffuse in extracellular space downstream toward the disc edge $X = X_{\max}$ according to Fick's second law. Along the way, some ligand molecules associate themselves with cell surface bound receptors at the binding rate $K_{\text{on}} L(X, T) R_{\text{out}}(X, T)$, where $L(X, T)$ and $R_{\text{out}}(X, T)$ are, respectively, ligand and (extracellular space) receptor concentration at time T and location X. The resulting ligand-receptor complexes of concentration $[LR(X, T)]_{\text{out}}$ are bound to a cell surface membrane just as the receptors. These complexes in turn *dissociate* at the rate $K_{\text{off}}[LR(X, T)]_{\text{out}}$. Altogether, we have the partial differential equation (1) below governing the rate of change of $L(X, T)$, with K_{on} and K_{off} known as the binding rate constant and dissociation rate constant, respectively. The ligand-receptor complexes are subject to endocytosis, exocytosis, and degradation modeled by Eqs. (2) and (3) below with

- *in* and *out* rate constant K_{in} and K_{out} for entering and exiting cell interior resulting in a concentration of ligand-receptor complexes $[LR(X, T)]_{\text{in}}$ in the cell interior, and
- *degradation* rate constant K_{deg} for the intracellular ligand-receptor complexes.

There is also the time evolution of the receptor concentrations in both the intracellular and extra-cellular space due to the formation, dissociation and degradation of morphogen-receptor complexes, degradation of receptors not bound to a morphogen, synthesis of new receptors and the movement of receptors in and out of the cell surface. These activities are captured by Eqs. (4) and (5) where K'_{in} and K'_{out} are the *in* and *out* rate constants for the receptors and V_R and K'_{deg} are the synthesis rate and the degradation rate constant for receptors. Altogether, we have the following five equation model designated as System C in [7]:

$$\frac{\partial L}{\partial T} = D_L \frac{\partial^2 L}{\partial X^2} - K_{\text{on}} LR_{\text{out}} + K_{\text{off}}[LR]_{\text{out}}, \tag{1}$$

$$\frac{\partial [LR]_{\text{out}}}{\partial T} = K_{\text{on}} LR_{\text{out}} - (K_{\text{off}} + K_{\text{in}})[LR]_{\text{out}} + K_{\text{out}}[LR]_{\text{in}}, \tag{2}$$

$$\frac{\partial [LR]_{\text{in}}}{\partial T} = K_{\text{in}}[LR]_{\text{out}} - (K_{\text{deg}} + K_{\text{out}})[LR]_{\text{in}}, \tag{3}$$

$$\frac{\partial R_{\text{out}}}{\partial T} = -K_{\text{on}} LR_{\text{out}} + K_{\text{off}}[LR]_{\text{out}} - K'_{\text{in}} R_{\text{out}} + K'_{\text{out}} R_{\text{in}}, \tag{4}$$

and

$$\frac{\partial R_{\text{in}}}{\partial T} = V_R(X, T, [LR]_{\text{in}}, R_{\text{in}}) - K'_{\text{deg}} R_{\text{in}} + K'_{\text{in}} R_{\text{out}} - K'_{\text{out}} R_{\text{in}} \tag{5}$$

with $(0 \leqslant X \leqslant X_{\max}, T > 0)$ as the domain of all the equations except the first equation which holds only away from the end points (so that $0 < X < X_{\max}, T > 0$). In (1), D_L is the *diffusion coefficient* for the morphogen in the extracellular space while K'_{in}, K'_{out}, and K'_{deg} for the receptor rate constants in (4) and (5) correspond to k_p, k_q and k_g, respectively, in [7]. For the possibility of steady state gradients, we will limit ourselves here to the case of a prescribed $V_R = V_R(X)$. To simplify analysis, we will focus in some parts of this paper on a spatially uniform receptor synthesis rate $V_R(X) = \bar{V}_R$.

The system of differential equations above is augmented by suitable boundary and initial conditions. Here, we extend System C of [7] by allowing for a ligand flux at the source end so that we have:

$$X = 0: \quad \frac{\partial L}{\partial T} = V_L - K_{\text{on}} L R_{\text{out}} + K_{\text{off}} [LR]_{\text{out}} + \sigma D_L \frac{\partial L}{\partial X}, \tag{6}$$

where V_L is synthesis rate of morphogen of the end source at $X = 0$ and σ is a *flux coefficient* to be specified. In this study, the ligand synthesis rate is taken to be uniform rate $\bar{V}_L$. The other end point is assumed to be a morphogen sink so that we have

$$X = X_{\max}: \quad L = 0. \tag{7}$$

Both (6) and (7) hold for all $T > 0$. Until the onset of morphogens synthesis at $T = 0$, there were only unoccupied receptors in both the intracellular and extra-cellular space so that

$$T = 0: \quad L = [LR]_{\text{out}} = [LR]_{\text{in}} = 0, \quad R_{\text{out}} = \bar{R}_{\text{out}}(X), \quad R_{\text{in}} = \bar{R}_{\text{in}}(X) \tag{8}$$

for $0 \leqslant X \leqslant X_{\max}$ where $\bar{R}_{\text{out}}(X)$ and $\bar{R}_{\text{in}}(X)$ are the steady state intra- and extra-cellular receptor concentration, respectively. Systems (1)–(8) constitutes an initial-boundary value problem (IBVP) for the five unknown concentrations L, $[LR]_{\text{out}}$, $[LR]_{\text{in}}$, R_{out}, and R_{in}.

To reduce the number of parameters of the problem, let

$$t = \frac{D_L}{X_{\max}^2} T, \quad x = \frac{X}{X_{\max}}, \quad V_R = \bar{V}_R w(x), \tag{9}$$

$$\bar{R}_0 = \frac{K'_{\text{out}} \bar{V}_R}{K'_{\text{deg}} K'_{\text{in}}}, \quad \{v_0, \omega_0\} = \frac{X_{\max}^2}{D_L \bar{R}_0} \{\bar{V}_L, \bar{V}_R\}, \quad \sigma_0 = \sigma X_{\max}, \tag{10}$$

$$\{L, [LR]_{\text{out}}, [LR]_{\text{in}}, R_{\text{out}}, R_{\text{in}}\} = \bar{R}_0 \{a, b, c, d, e\}, \tag{11}$$

$$\{f_0, g_0, j_0, k_0\} = \frac{X_{\max}^2}{D_L} \{K_{\text{off}}, K_{\text{deg}}, K_{\text{in}}, K_{\text{out}}\}, \tag{12}$$

$$\{g_1, j_1, k_1, h_0\} = \frac{X_{\max}^2}{D_L} \{K'_{\text{deg}}, K'_{\text{in}}, K'_{\text{out}}, K_{\text{on}} \bar{R}_0\}, \tag{13}$$

with $\bar{V}_R = V_R(X = 0)$ so that $w(0) = 1$. For the reference receptor concentration $\bar{R}_0$, we take it to be the steady state concentration $\bar{R}_{\text{out}}$ in the absence of morphogens. For our uniform receptor synthesis rate

$V_R(X,T)=\bar{V}_R$, we have $R_{\text{in}}(X,0)=\bar{V}_R/K'_{\text{deg}}$ and $\bar{R}_{\text{out}}(X)=R_{\text{out}}(X,0)=(K'_{\text{out}}\bar{V}_R)/(K'_{\text{deg}}K'_{\text{in}})=\bar{R}_0$. The initial-boundary value problem (IBVP) for the various concentrations can now be re-written in the following normalized form:

$$\frac{\partial a}{\partial t}=\frac{\partial^2 a}{\partial x^2}-h_0ad+f_0b, \tag{14}$$

$$\frac{\partial b}{\partial t}=h_0ad-(f_0+j_0)b+k_0c,\quad \frac{\partial c}{\partial t}=j_0b-(k_0+g_0)c, \tag{15}$$

$$\frac{\partial d}{\partial t}=-h_0ad+f_0b-j_1d+k_1e,\quad \frac{\partial e}{\partial t}=\frac{j_1g_1}{k_1}w-(k_1+g_1)e+j_1d \tag{16}$$

all for $t>0$, $0\leqslant x\leqslant 1$, except for the first which holds for the open interval $0<x<1$. Correspondingly, the boundary conditions become

$$x=0:\quad \frac{\partial a}{\partial t}=v_0-h_0ad+f_0b+\sigma_0\frac{\partial a}{\partial x}\quad (t>0), \tag{17}$$

$$x=1:\quad a=0\quad (t>0) \tag{18}$$

and the initial conditions

$$t=0: a=b=c=0,\quad d=1,\quad e=j_1/k_1\quad (0\leqslant x\leqslant 1). \tag{19}$$

For a prescribed set of rate constants and synthesis rates, numerical solutions for the IBVP above can be obtained by a number of conventional numerical methods. In this paper, we will be concerned mainly with the mathematical underpinning for such numerical solutions and qualitative insight gained from our analysis of the problem.

3. Time-independent solution ($\sigma_0=0$)

The first task for the five-component system is to establish the existence, uniqueness and monotonicity of a steady state morphogen-receptor concentration. More specifically, we consider the case of a non-negative, time-independent receptor synthesis rate, $V_R=\bar{V}_Rw(x)\geqslant 0$, and a time independent positive morphogen production rate $\bar{V}_L>0$ and investigate the condition(s) under which steady state concentration gradients can be sustained and how the shape of these gradients depends on the biological parameters. We first summarize briefly in this section the known results for $\sigma_0=0$ (System C) reported in [12]. The remaining sections of this paper will be concerned with the new and more interesting case of $\sigma_0>0$.

3.1. Reduction to a boundary value problem for one unknown

We denote by $\bar{a}(x)$, $\bar{b}(x)$, etc. the time-independent steady state solution for $a(x,t)$, $b(x,t)$, etc., of (14)–(19), respectively. For a time independent solution, we have $\partial(\ )/\partial t=0$ so that the governing

equations reduce to one second order *ordinary differential equation* (ODE) and four algebraic equations. We can solve the latter for $\bar{b}(x)$, $\bar{c}(x)$, $\bar{d}(x)$, and $\bar{e}(x)$ in terms of $\bar{a}(x)$ to get

$$\bar{b}(x) = \frac{\alpha_b(x)\bar{a}(x)}{\bar{a}(x)+\alpha_\omega}, \quad \bar{c}(x) = \frac{j_0\bar{b}(x)}{k_0+g_0} = \frac{\alpha_c(x)\bar{a}(x)}{\bar{a}(x)+\alpha_\omega}, \tag{20}$$

$$\bar{d}(x) = \frac{(j_0g_0+f_0g_0+f_0k_0)\bar{b}(x)}{(k_0+g_0)h_0\bar{a}(x)} = \frac{\alpha_d(x)}{\bar{a}(x)+\alpha_\omega}, \tag{21}$$

$$\begin{aligned}\bar{e}(x) &= \frac{1}{k_1+g_1}\left\{\frac{j_1g_1}{k_1}\,w(x) + \frac{j_1(j_0g_0+f_0g_0+f_0k_0)}{(k_0+g_0)h_0\bar{a}(x)}\,\bar{b}(x)\right\} \\ &= \frac{j_1g_1w(x)}{k_1(k_1+g_1)}\left\{1+\frac{\alpha_e}{\bar{a}(x)+\alpha_\omega}\right\},\end{aligned} \tag{22}$$

with

$$\alpha_\omega = \frac{j_1g_1(k_0f_0+f_0g_0+j_0g_0)}{j_0g_0h_0(k_1+g_1)}, \quad \alpha_b(x) = \frac{j_1g_1(k_0+g_0)w(x)}{j_0g_0(k_1+g_1)}, \tag{23}$$

$$\alpha_c(x) = \frac{j_1g_1w(x)}{g_0(k_1+g_1)}, \quad \alpha_d(x) = \alpha_\omega w(x), \quad \alpha_e = \frac{k_1}{g_1}\,\alpha_\omega. \tag{24}$$

Expressions (20)–(22) can be used to express the ODE from (14) in terms of $\bar{a}(x)$ alone:

$$\frac{\mathrm{d}^2\bar{a}}{\mathrm{d}x^2} = \frac{g_\omega w(x)\bar{a}}{\bar{a}+\alpha_\omega} = \frac{\psi_\omega w(x)\bar{a}}{1+\bar{a}/\alpha_\omega}, \tag{25}$$

where

$$g_\omega \equiv g_0\alpha_c(0) = \frac{j_1g_1}{k_1+g_1}, \quad \psi_\omega = \frac{g_\omega}{\alpha_\omega} = \frac{j_0g_0h_0}{j_0g_0+f_0g_0+k_0f_0}, \tag{26}$$

since $w(0)=1$. Similarly, the boundary conditions can also be expressed in terms of $\bar{a}(x)$. For $\sigma_0=0$, we may write them in the form

$$\bar{a}(0) \equiv \bar{a}_0 = \frac{\alpha_\omega v_0}{g_\omega - v_0} = \bar{\beta}_\omega\alpha_\omega, \quad \bar{a}(1)=0, \tag{27}$$

where

$$\bar{\beta}_\omega = \frac{\beta_\omega}{1-\beta_\omega}, \quad \beta_\omega = \frac{v_0}{g_\omega} = \frac{\bar{V}_L}{\bar{R}_0K'_{\mathrm{deg,obs}}}, \quad K'_{\mathrm{deg,obs}} = \frac{K'_{\mathrm{in}}}{K'_{\mathrm{deg}}+K'_{\mathrm{out}}}K'_{\mathrm{deg}}. \tag{28}$$

Note that $K'_{\mathrm{deg,obs}}$ is the receptor counterpart of observed degradation rate constant $K_{\mathrm{deg,obs}} = K_{\mathrm{in}}K_{\mathrm{deg}}/(K_{\mathrm{deg}}+K_{\mathrm{out}})$ for ligand-receptor complexes first introduced in [7].

The BVP for $\bar{a}(x)$ above is identical in form to the corresponding result for the much simpler System B (without endocytosis, exocytosis or receptor renewal) obtained in [7,12]. This observation is sufficiently useful to be summarized as a theorem below:

Theorem 1. *For a uniform receptor synthesis rate so that* $w(x)=1$, *the boundary value problem* (25) *and* (27) *for* $\bar{a}$ *is identical in form to the corresponding BVP for System B*; *only the parameters of the BVP*

are modified (*with* β_ω *and* ψ_ω *taking the place of the amplitude parameter* β *and the shape parameter* $\psi = \mu^2$).

3.2. Existence, uniqueness and monotonicity

If $g_\omega < v_0$, the expression for $\bar{a}(0)$ in (27) implies $\bar{a}(0) < 0$ which is not acceptable. If $g_\omega = v_0$, then the end condition $(g_\omega - v_0)\bar{a}(0) = \alpha_\omega v_0$ would require $\alpha_\omega v_0$ to vanish which is also unacceptable for a positive morphogen production rate. Hence, we must have $g_\omega > v_0$, i.e., $\beta_\omega < 1$, to have a steady state solution. The requirement is analogous to a corresponding requirement ($\beta = v_0/g_0 < 1$) for System B observed in [7,12]. The following existence theorem is proved by the monotonicity method of Amman [1] and Sattinger [17,18] with $a_u(x) = \bar{a}_0$ and $a_\ell(x) = 0$ as the upper and lower solution, respectively [10]:

Theorem 2. *For* $g_\omega > v_0$ *so that* $\beta_\omega < 1$, *there exist a unique non-negative* (*time-independent*) *steady state solution for the boundary value problem defined by* (25) *and* (27), *given* $w(x) \geqslant 0$.

The proof of uniqueness is similar to that for the more general case of $\sigma_0 > 0$ in Theorem 6. On the monotonicity of $\bar{a}(x)$ however, we need $w(x) > 0$ in order for the corresponding proof in Theorem 7 below to apply to the following theorem:

Theorem 3. *For* $g_\omega > v_0$ *and a* (*normalized*) *receptor synthesis rate* $w(x) > 0$, *the unique non-negative* (*time-independent*) *steady state solution for the boundary value problem* (25) *and* (27) *is strictly decreasing in* [0, 1].

4. Time independent steady state solution ($\sigma_0 > 0$)

The limitation imposed by the necessary condition of Theorem 2 on the ligand synthesis rate for the existence of a steady state behavior is not biologically realistic. The restriction may well be caused by setting $\sigma_0 = 0$ which was in part necessitated by the lack of experimental data on the parameter σ_0. The resulting unexpected restriction suggests that we should investigate also the case $\sigma_0 > 0$ to see if the limitation persists. The results obtained for $\sigma_0 > 0$ constitute the principal contribution of the present paper.

Since the differential equations are unchanged for $\sigma_0 > 0$, the same reduction in Section 3.1 for the steady state solution in that case gives again the same second order ODE for $\bar{a}(x)$. *To simplify our presentation, we consider only the case of a spatially uniform receptor synthesis rate so that* $w(x) = 1$ and

$$\bar{a}'' = \frac{g_\omega \bar{a}}{\bar{a} + \alpha_\omega} = \frac{\psi_\omega \alpha_\omega \bar{a}}{\alpha_\omega + \bar{a}}, \tag{29}$$

where $()' = d()/\mathrm{d}x$. Though the absorbing end condition at $x = 1$ remains unchanged, the previous Dirichlet condition at $x = 0$ is now changed to a inhomogeneous "mixed (or leaky)" condition so that we

have

$$\sigma_0 \bar{a}'(0) - \frac{g_\omega \bar{a}(0)}{\bar{a}(0) + \alpha_\omega} + v_0 = 0, \quad \bar{a}(1) = 0, \tag{30}$$

where g_ω, ψ_ω and α_ω have been defined in terms of the various rate constants in (26) and (28).

4.1. Existence theory

Because of the form of the first boundary condition in (30), the monotone method of Amann and Sattinger is not directly applicable for proving existence of a steady state solution. In this section, we develop an existence proof using the monotone method as a starting point to obtain the following result:

Theorem 4. *For positive values of the parameters* σ_0, g_ω, α_ω, *and* v_0, *there exists a regular solution* $\bar{a}(x) > 0$ *of the BVP* (29) *and* (30) *for all* x *in* $[0, 1)$.

Proof. For any $a_0 > 0$, Theorem 2 assures us that the BVP defined by (29) and the Dirichlet conditions $\bar{a}(0) = a_0$ and $\bar{a}(1) = 0$ has a unique, monotone decreasing positive (analytic) solution in $0 < x < 1$. Let $s(a_0)$ be the resulting $\bar{a}'(0)$; then $s(a_0)$ is negative for positive a_0 and $s(0) = 0$ since the corresponding $\bar{a}(x)$ necessarily vanishes throughout $[0, 1]$. Let $B[a_0] \equiv \sigma_0 s(a_0) + v_0 - g_\omega a_0/(\alpha_\omega + a_0)$. Evidently, we have $B[0] > 0$. In the range $\bar{\beta}_\omega = \beta_\omega/(1 - \beta_\omega) > 0$, then we can complete the proof simply by noting $B[\alpha_\omega \bar{\beta}_\omega] = \sigma_0 s(a_0) < 0$. Since $\bar{a}(x)$ and $\bar{a}'(x)$ depend continuously on a_0, we have by the intermediate value theorem that there is a value $\bar{a}_0$ for which $B[\bar{a}_0] = 0$. The solution of the Dirichlet BVP with $a_0 = \bar{a}_0$ is then a solution of the BVP (29)–(30).

The proof for the complementary range $\bar{\beta}_\omega \leqslant 0$ is slightly more complicated. Let $y(x; a_0) \equiv \partial \bar{a}/\partial a_0$; it follows from the BVP for $\bar{a}(x; a_0)$ that $y(x; a_0)$ is the solution of the BVP:

$$y' = \frac{g_\omega \alpha_\omega y}{(\alpha_\omega + \bar{a})^2}, \quad y(0) = 1, \quad y(1) = 0.$$

Evidently, $y_u(x) = 1$ and $y_\ell(x) = 0$ are, respectively, an upper and lower solution of the problem above. Hence by the monotonicity method of Amann and Sattinger, there is a unique, nonnegative, and monotone decreasing solution $y(x; a_0)$ for this problem with $y'(x; a_0) < 0$. In particular, we have $y'(0; a_0) = \partial[a'(0; a_0)]/\partial a_0 < 0$. Hence, $B[a_0]$ is a decreasing function of a_0. Given $B[0] > 0$, there exists some $\bar{a}_0 > 0$ for which $B[\bar{a}_0] = 0$. Again, the solution of the Dirichlet BVP with $a_0 = \bar{a}_0$ is a solution of the BVP (29)–(30). □

Remark 5. Note that the existence proof stipulates no limitation on the ligand synthesis rate relative to the receptor-mediated degradation rate (or any other limitation for that matter)! It seems reasonable to ask whether the model with $\sigma_0 = 0$ can adequately characterize the actual morphogen gradients even if $\beta_\omega < 1$. We will return to this question in the next subsection.

Theorem 6. *The non-negative solution of Theorem* 4 *is unique.*

Proof. Suppose there are two solutions $\bar{a}_1(x)$ and $\bar{a}_2(x)$. Then $a(x) = \bar{a}_1 - \bar{a}_2$ satisfies the ODE

$$a'' = \frac{g_\omega \bar{a}}{(\bar{a}_1 + \alpha_\omega)(\bar{a}_2 + \alpha_\omega)} \tag{31}$$

(since $w(x) = 1$) and the end condition $a(1) = 0$. Because $\sigma_0 > 0$, the other boundary condition at $x = 0$ is now replaced by

$$\sigma_0 a'(0) = \frac{g_\omega \alpha_\omega a(0)}{(\bar{a}_1 + \alpha_\omega)(\bar{a}_2 + \alpha_\omega)}.$$

Upon multiplying the ODE by $a(x)$ and integrate by parts we get after applying the end conditions

$$\frac{g_\omega \alpha_\omega a^2(0)}{\sigma_0(\bar{a}_1 + \alpha_\omega)(\bar{a}_2 + \alpha_\omega)} + \int_0^1 (a')^2 \, \mathrm{d}x + \int_0^1 \frac{g_\omega \alpha_\omega a^2}{(\bar{a}_1 + \alpha_\omega)(\bar{a}_2 + \alpha_\omega)} \, \mathrm{d}x = 0. \tag{32}$$

Condition (32) requires $a(x) \equiv 0$ and hence uniqueness. □

Theorem 7. *The non-negative solution of Theorem* 4 *is a monotone decreasing function.*

Proof. Suppose there is a local maximum of $\bar{a}$ at an interior point x_0 with $\bar{a}''(x_0) \leqslant 0$. At the same time, we have from (25)

$$\bar{a}''(x_0) = \frac{j_1 g_1 w(x_0) \bar{a}(x_0)}{(k_1 + g_1)[\bar{a}(x_0) + \alpha_\omega]} \geqslant 0 \tag{33}$$

because morphogen concentration has already been shown to be nonnegative and we have $w(x_0) > 0$. Together they require $\bar{a}''(x_0) = 0$ and therewith $\bar{a}(x_0) = 0$ by (33) given $w(x) = 1$ (though the proof goes through as long as $w(x) > 0$). Since $\bar{a}(x)$ is non-negative and $\bar{a}(x_0)$ is a maximum, we must have $\bar{a}(x) \equiv 0$ which violates the requirement that $\bar{a}(0) = \bar{a}_0 > 0$ (for $v_0 > 0$).

The ODE (25) requires $\bar{a}(x)$ to be continuous and smooth. It follows that the steady state concentration $\bar{a}(x)$ also cannot have a local minimum $\bar{a}(x_0) = 0$ at an interior point x_0. Otherwise, we would have $\bar{a}(x) = 0$ for $x \geqslant x_0$ and, by the continuity of $\bar{a}(x)$ and $\bar{a}'(x)$, $\bar{a}(x) \equiv 0$ for $0 \leqslant x \leqslant x_0$ as well. Hence, $\bar{a}(x)$ must be monotone, and, given the boundary conditions at the two ends, it must be monotone decreasing. □

4.2. Approximate solution for a low ligand synthesis rate

For a sufficiently small v_0, we expect that $\bar{a}(x_0) \ll \alpha_\omega$ and a good first approximation $a_0(x)$ of the exact solution is determined by the linear BVP

$$a_0'' - \mu_\omega^2 a_0 = 0, \quad \mu_\omega^2 = \frac{g_\omega}{\alpha_\omega} = \psi_\omega$$

subject to the boundary conditions

$$\sigma_0 a_0'(0) - \mu_\omega^2 a_0(0) + v_0 = 0, \quad a_0(1) = 0.$$

It is straightforward to obtain the exact solution of this linear BVP:

Theorem 8. *For sufficiently small v_0, a first approximation solution for $\bar{a}(x)$ is given by*

$$a_0(x) = \frac{v_0}{\mu_\omega^2} \frac{\sinh(\mu_\omega(1-x))}{\sinh(\mu_\omega)[1 + \frac{\sigma_0}{\mu_\omega}\coth(\mu_\omega)]} = \frac{\beta_\omega \alpha_\omega \sinh(\mu_\omega(1-x))}{\sinh(\mu_\omega)[1 + \frac{\sigma_0}{\mu_\omega}\coth(\mu)]}, \tag{34}$$

where $\beta_\omega = v_0/g_\omega$.

Remark 9. For relatively high binding rate, the parameter $\mu_\omega^2 = g_\omega/\alpha_\omega$ is generally large compared to 1. Hence, if σ_0 is O(1) or smaller, the contribution from the flux term is negligible. This observation provided the basis for the omission of the flux term in Systems B, C and R in [7,12]. The omission is attractive as it leads to simpler theoretical and computational treatments of the problem. However, it is possible to deduce from a model that allow for a finite region of morphogen production that the flux coefficient σ_0 is approximately $X_{\max}/X_{\min} = 1/x_m$ for a sufficiently small $X_{\min}$ (which is typically for a Drosophila wing disc) [8]. Unless μ_ω is sufficiently large so that $\sigma_0/\mu_\omega = (\mu_\omega x_m)^{-1}$ is negligibly small, the contribution of the flux term generally may not be negligible. We summarize the observation in the following corollary:

Corollary 10. *If $\beta_\omega = v_0/g_\omega \ll 1$ so that the approximate solution* (34) *is applicable, the limiting case of System C is an adequate characterization of the distributed source model* (*as well as the aggregated source model*) *of* [8] *provided $\sigma_0 \ll \mu_\omega$.*

As a measure of steepness and convexity of the gradient, we let x_h be the mid level location of the ligand-reception concentrations. With $\bar{b}(x)$ and $\bar{b}(x) + \bar{c}(x)$ both proportional to $\bar{a}(x)$, x_h is specified by $\bar{a}(x_h) = \frac{1}{2}\bar{a}(0)$ and we have from the expressions (34)

$$\sinh(\mu_\omega(1 - x_h)) = \frac{1}{2}\sinh(\mu_\omega), \quad x_h = 1 - \frac{1}{\mu_\omega}\sinh^{-1}\left(\frac{1}{2}\sinh\mu_\omega\right). \tag{35}$$

Corollary 11. *At low morphogen synthesis rate, the location of mid level ligand-receptor concentrations, x_h, is given by* (35) *to a first approximation. It does not depend on the morphogen or receptor synthesis rate and moves toward the origin as $\mu_\omega \to \infty$.*

For $X_{\max}$ large (say, relative to the production zone width), it is not difficult to show that the unnormalized mid level location X_h is simplified considerably to $\ln(2)/\kappa$ where $\kappa = \mu_\omega X_{\max}$ is independent of $X_{\max}$.

Another application of the approximate solution (34) to determine indirectly the effects of a diffusive non-receptor such as Sog on the gradient shape can be found in [14]. A direct determination of the same effects has been found in [5,13,15] to be much more difficult (by an order of magnitude at least).

4.3. Approximate solution for high Dpp synthesis rates

With all biological parameters other than v_0 fixed, it is expected that the maximum steady state free ligand concentration would increase with v_0 (as was the case in the approximate solution found in the last subsection). We let $\bar{a}(x) = v_0 A(x)$ and write the BVP for $\bar{a}(x)$ in terms of $A(x)$:

$$A'' - \frac{1}{\beta_\omega}\frac{A}{\varepsilon + A} = 0, \quad \varepsilon = \frac{\alpha_\omega}{v_0} = \frac{1}{\beta_\omega \mu_\omega^2}, \quad A(1) = 0, \quad \sigma_0 A'(0) - \frac{1}{\beta_\omega}\frac{A(0)}{\varepsilon + A(0)} + 1 = 0.$$

For a sufficiently high Dpp synthesis rate $\bar{V}_L$ so that $0 < \varepsilon \ll 1/\beta_\omega (= g_\omega/v_0) \ll 1$, we may seek a perturbation solution of $A(x)$ in $1/\beta_\omega$ with its leading term determined by

$$A_0'' = 0, \quad \sigma_0 A_0'(0) + 1 = 0, \tag{36}$$

or

$$A_0(x) = c_0 - \frac{x}{\sigma_0} \quad (0 \leqslant x < 1). \tag{37}$$

Correspondingly, we have for $w(x) = 1$

$$\{\bar{b}(x), \bar{b}(x) + \bar{c}(x)\} = \frac{(\gamma, \gamma')\bar{a}(x)}{\alpha_\omega + \bar{a}(x)} \sim \frac{(\gamma, \gamma')v_0 A_0(x)}{\alpha_\omega + v_0 A_0(x)}, \tag{38}$$

where

$$\gamma = \frac{K'_{\text{deg,obs}}}{K_{\text{deg,obs}}}, \quad \gamma' = \frac{K'_{\text{deg,obs}}}{K_{\text{deg,eff}}}. \tag{39}$$

For $v_0/\sigma_0 \gg \alpha_\omega$, the shape of $\bar{b}(x)$ and $\bar{b}(x) + \bar{c}(x)$ is sigmoidal, decreasing sharply from $O(\gamma, \gamma')$ to nearly 0 over a narrow interval centered at some location x_h.

One of the important result of interest is the location of x_h in terms of the biological rate parameters of the problem. For that and other results pertaining the case of high ligand synthesis rate, we need to find the constant of integration c_0 to complete the solution of the BVP. Evidently, it is not appropriate to apply the second end condition $A_0(1) = 0$ to determine c_0 since $A_0(x)$ is not large compared to ε near $x = 0$. (In fact, it is smaller than ε for x sufficiently close to the end $x = 1$ to warrant a layer analysis.) However, for the purpose of locating the sharp front of the $\bar{b}(x)$ gradient, it can be verified that allowing the use of (37) for the entire domain and applying the end condition on this solution gives the same first approximation for c_0. This approach would give $c_0 = 1/\sigma_0$ and therewith

$$A_0(x) = \frac{1}{\sigma_0}(1 - x), \quad (0 \leqslant x \leqslant 1). \tag{40}$$

Theorem 12. *For $\beta_\omega \gg \sigma_0/\mu_\omega^2$, a first approximation solution for the concentration gradients $\bar{a}(x)$, $\bar{b}(x)$ and $\bar{c}(x)$ is given by* (40) *and* (38).

As a condition for determining the location of the sharp gradient front of the receptor bound morphogen concentrations, we let $x_h = X_h/X_{\max}$ where the ligand-receptor concentrations are exactly half of its level at the origin. Given (38), this implies

$$\frac{(1 - x_h)}{\sigma_0\alpha_\omega + v_0(1 - x_h)} = \frac{1}{2}\frac{1}{\sigma_0\alpha_\omega + v_0}. \tag{41}$$

Corollary 13. *For $\beta_\omega \gg \sigma_0/\mu_\omega^2$, the location of the sharp gradient front of the receptor bound ligand complexes characterized by the location of mid level* (*or half peak*) *concentration is given to a first approximation by*

$$x_h = 1 - \frac{\sigma_0\alpha_\omega}{v_0 + 2\sigma_0\alpha_\omega} \simeq 1 - \frac{\sigma_0\alpha_\omega}{v_0} = 1 - \frac{\sigma_0}{\beta_\omega\mu_\omega^2} = 1 - \frac{K'_{\text{deg,obs}}}{K_{\text{on}}\bar{V}_L}.$$

Remark 14. Unlike the low ligand synthesis rate case, the mid level location now depends on the magnitude of the synthesis rate with $x_h \to 1$, i.e., $X_h \to X_{\max}$, as $\bar{V}_L \to \infty$, as it should be for this case. The biological implications of these results are discussed in [14].

5. Linear stability for the time-independent steady states

5.1. Perturbation from steady state

Now that the existence of time-independent steady state concentration gradients have been established, we want to know if they are stable. For a linear stability analysis, we consider

$$a(x,t) = \bar{a}(x) + \mathrm{e}^{-\lambda t}\hat{a}(x), \quad b(x,t) = \bar{b}(x) + \mathrm{e}^{-\lambda t}\hat{b}(x), \quad \text{etc.} \tag{42}$$

where $\bar{a}$, $\bar{b}$, etc., are the steady state concentrations and where the time independent portion of the perturbations, $\hat{a}$, $\hat{b}$, etc., are negligibly small compared to the corresponding steady state concentration. After linearization, we have the following eigenvalue problem for the perturbations, $\hat{a}$, $\hat{b}$, etc.:

$$-\lambda\hat{e} = -(k_1 + g_1)\hat{e} + j_1\hat{d}, \tag{43}$$

$$-\lambda\hat{d} = -h_0(\bar{d}\hat{a} + \bar{a}\hat{d}) + f_0\hat{b} - j_1\hat{d} + k_1\hat{e}, \tag{44}$$

$$-\lambda\hat{c} = j_0\hat{b} - (k_0 + g_0)\hat{c}, \tag{45}$$

$$-\lambda\hat{b} = h_0(\bar{a}\hat{d} + \bar{d}\hat{a}) - (f_0 + j_0)\hat{b} + k_0\hat{c} \tag{46}$$

and

$$-\lambda\hat{a} = \hat{a}'' - h_0(\bar{d}\hat{a} + \bar{a}\hat{d}) + f_0\hat{b} \tag{47}$$

with

$$-\lambda\hat{a}(0) = -h_0[\bar{d}(0)\hat{a}(0) + \bar{a}(0)\hat{d}(0)] + f_0\hat{b}(0) + \sigma_0\hat{a}'(0), \quad \hat{a}(1) = 0, \tag{48}$$

where a prime indicates differentiation with respect to x.

The above system can be reduced to an *eigenvalue problem* for $\hat{a}$ alone. We begin by solving the four relations (43)–(46) to get $\hat{b}$, $\hat{c}$, $\hat{d}$, and $\hat{e}$ in terms of $\hat{a}$ to obtain

$$\delta\hat{e} = -j_1 h_0\bar{d}(x)\,[\lambda^2 - \lambda(g_0 + j_0 + k_0) + j_0 g_0]\hat{a}, \tag{49}$$

$$\delta\hat{d} = h_0\bar{d}(x)(\lambda - g_1 - k_1)[\lambda^2 - \lambda(g_0 + j_0 + k_0) + j_0 g_0]\hat{a}, \tag{50}$$

$$\delta\hat{c} = j_0 h_0\bar{d}(x)[\lambda^2 - \lambda(g_1 + j_1 + k_1) + j_1 g_1]\hat{a}, \tag{51}$$

$$\delta\hat{b} = -h_0\bar{d}(x)\,(\lambda - g_0 - k_0)[\lambda^2 - \lambda(g_1 + j_1 + k_1) + j_1 g_1]\hat{a}, \tag{52}$$

where

$$\delta(x;\lambda) = \Delta_{21}(\lambda)\Delta_{20}(\lambda) - f_0\Delta_{21}(\lambda)\Delta_{10}(\lambda) - h_0\bar{a}(x)\Delta_{20}(\lambda)\Delta_{11}(\lambda) \tag{53}$$

with

$$\Delta_{2m}(\lambda) = \lambda^2 - (g_m + j_m + k_m)\lambda + j_m g_m, \quad (m = 0, 1), \tag{54}$$

$$\Delta_{1m}(\lambda) = \lambda - (g_m + k_m), \quad (m = 0, 1). \tag{55}$$

The three relations (45)–(47) can be combined to give $-\lambda\hat{a} = \hat{a}'' + \lambda\hat{b} + (\lambda - g_0)\hat{c}$. The relations (52) and (51) are now used to express $\hat{b}$ and $\hat{c}$ in the ODE above in terms of $\hat{a}$ to get

$$\hat{a}'' + \{\lambda - q(x;\lambda)\}\,\hat{a} = 0, \quad q(x;\lambda) = h_0\bar{d}(x)\,\frac{\varphi_0(\lambda)}{\delta(x;\lambda)}, \tag{56}$$

$$\varphi_0(\lambda) = \Delta_{20}(\lambda)\Delta_{21}(\lambda). \tag{57}$$

Similarly, we can also combine the three relations (45), (46) and (48) to write the boundary condition at the source end as $-\lambda\hat{a}(0) = \lambda\hat{b}(0) + (\lambda - g_0)\hat{c}(0) + \sigma_0\hat{a}'(0)$. We then use (52) and (51) to eliminate $\hat{b}$ and $\hat{c}$ so that the two boundary conditions in (48) are now in terms of $\hat{a}$ alone:

$$\sigma_0\hat{a}'(0) + \kappa(\lambda)\hat{a}(0) = 0, \quad \hat{a}(1) = 0 \tag{58}$$

with

$$\kappa(\lambda) = \lambda - q(0;\lambda) = \lambda - \frac{h_0\alpha_\omega}{\alpha_\omega + \bar{a}(0)}\,\frac{\varphi_0(\lambda)}{\delta(0;\lambda)}. \tag{59}$$

The special case of the nonlinear eigenvalue problem above with $\sigma_0 = 0$ has already been analyzed in [12]. We will limit our attention here to the more general and mathematically different case of $\sigma_0 > 0$.

5.2. Positive eigenvalues

In this subsection, we show that the eigenvalues of the ODE (56) and the homogeneous boundary conditions (58) must be positive. First, we prove that the eigenvalues must be real:

Lemma 15. *All the eigenvalues of* (56) *and* (58) *are real.*

Proof. Suppose λ is a complex eigenvalue and $a_\lambda(x)$ an associated nontrivial eigenfunction, then λ^* is also an eigenvalue with eigenfunction $a_\lambda^*(x)$ where $(\ )^*$ is the complex conjugate of $(\ )$. Integration by parts and applications of the boundary conditions in (58) give the bilinear relation

$$\int_0^1 [(a_\lambda^*)a_\lambda'' - (a_\lambda^*)''a_\lambda]\,\mathrm{d}x = \frac{|a_\lambda(0)|^2}{\sigma_0}[(\lambda - \lambda^*) - \{q(0;\lambda) - q(0;\lambda^*)\}], \tag{60}$$

which requires

$$\begin{aligned} 0 = &\int_0^1 \{(\lambda - \lambda^*) - [q(x;\lambda) - q(x;\lambda^*)]\}(a_\lambda^* a_\lambda)\,\mathrm{d}x \\ &+ \frac{|a_\lambda(0)|^2}{\sigma_0}\{(\lambda - \lambda^*) - [q(0;\lambda) - q(0;\lambda^*)]\}. \end{aligned} \tag{61}$$

It is straightforward to verify

$$q(x;\lambda) - q(x;\lambda^*) = -(\lambda - \lambda^*)\,\frac{h_0\alpha_\omega}{\alpha_\omega + \bar{a}(x)}\,\frac{G(x;\mathrm{Re}(\lambda),|\lambda|^2)}{|\delta(x,\lambda)|^2} \equiv -(\lambda - \lambda^*)\Phi(x;\lambda)$$

with

$$G(x; \mathrm{Re}(\lambda), |\lambda|^2) = f_0|\Delta_{21}|^2\{[\mathrm{Im}(\lambda)]^2 + [\mathrm{Re}(\lambda) - (g_0 + k_0)]^2 + j_0k_0\} \\ + h_0\bar{a}(x)|\Delta_{20}|^2\{[\mathrm{Im}(\lambda)]^2 + [\mathrm{Re}(\lambda) - (g_1 + k_1)]^2 + j_1k_1\} \tag{62}$$

being a real quantitative for any λ. In that case, condition (61) becomes

$$-(\lambda - \lambda^*)\left\{\int_0^1 a_\lambda a_\lambda^*[1 + \Phi(x; \lambda)]\,\mathrm{d}x + \frac{1}{\sigma_0}[1 + \Phi(0; \lambda)]|a_\lambda(0)|^2\right\} = 0,$$

where $\Phi(x; \lambda)$ is positive. Since the integral is positive for any nontrivial function $a_\lambda(x; \lambda)$, we must have $\lambda - \lambda^* = 0$. Hence, λ does not have an imaginary part. □

Theorem 16. *All eigenvalues of the nonlinear eigenvalue problem* (56)–(58) *are positive and the steady state concentration* $\bar{a}(x)$ *is asymptotically stable by a linear stability analysis* (*and so are the* $\bar{b}(x)$, $\bar{c}(x)$, $\bar{d}(x)$, *and* $\bar{e}(x)$).

Proof. Suppose $\lambda \leqslant 0$. Let $\hat{a}_\lambda(x)$ be a nontrivial eigenfunction of the homogeneous BVP (56)–(58) for this non-positive eigenvalue. Multiply (56) by $\hat{a}_\lambda$ and integrate over the solution domain to get

$$\int_0^1 \{\hat{a}_\lambda\hat{a}_\lambda'' - q(x; \lambda)(\hat{a}_\lambda)^2\}\,\mathrm{d}x = -\lambda\int_0^1 (\hat{a}_\lambda)^2\,\mathrm{d}x.$$

After integration by parts and applications of the homogeneous boundary conditions (58), we obtain

$$\lambda\int_0^1 (\hat{a}_\lambda)^2\,\mathrm{d}x = \int_0^1 (\hat{a}_\lambda')^2\,\mathrm{d}x + \int_0^1 q(x; \lambda)(\hat{a}_\lambda)^2\,\mathrm{d}x - \frac{1}{\sigma_0}(\hat{a}_\lambda(0))^2[\lambda - q(0; \lambda)]. \tag{63}$$

Suppose λ is not positive so that $\lambda = -|\lambda| \leqslant 0$, we have for $m = 0, 1$

$$\Delta_{2m}(-|\lambda|) = |\lambda|^2 + (g_m + j_m + k_m)|\lambda| + j_m g_m > 0, \tag{64}$$

$$\Delta_{1m}(-|\lambda|) = -[|\lambda| + (g_m + k_m)] < 0, \tag{65}$$

$$\delta(x; -|\lambda|) = \Delta_{21}(-|\lambda|)\Delta_{20}(-|\lambda|) - f_0\Delta_{10}(-|\lambda|)\Delta_{21}(-|\lambda|) \\ - h_0\bar{a}(x)\Delta_{11}(-|\lambda|)\Delta_{20}(-|\lambda|) > 0, \tag{66}$$

$$q(x; -|\lambda|) = \frac{h_0\alpha_\omega}{\alpha_\omega + \bar{a}(x)}\,\frac{\Delta_{21}(-|\lambda|)\Delta_{20}(-|\lambda|)}{\delta(x; -|\lambda|)} > 0, \tag{67}$$

$$-|\lambda| - q(0; -|\lambda|) = -\left\{|\lambda| + \frac{h_0\alpha_\omega}{\alpha_\omega + \bar{a}(0)}\,\frac{\Delta_{21}(-|\lambda|)\Delta_{20}(-|\lambda|)}{\delta(x; -|\lambda|)}\right\} < 0. \tag{68}$$

For any nontrivial solution of the eigenvalue problem under the assumption $\lambda \leqslant 0$, the right-hand side of (63) is positive while the left-hand side is non-positive. The contradiction means that the eigenvalues of (56)–(58) must be positive. □

6. The decay rate of the transients

While knowing the eigenvalues being positive is sufficient to ensure linear stability of the steady state morphogen concentration gradients, it is also important to know the dependence of the eigenvalues on the biological parameters to gain more insight to the time needed to get to steady state. In particular, the magnitude of the smallest eigenvalue would give us the approximate decay rate of the transient behavior of the system (such as how quickly the system returns to the steady state after a small perturbation). As we compute the time evolution of free and bound morphogen concentrations from their initial conditions, the same decay rate also gives us an estimate of the time it takes for the system to reach steady state starting from its initial configuration. This estimate will offer a reality check for the mathematical model. The system would not be an appropriate representation of the Drosophila wing disc development if it should take too long (or too short) a time to reach steady state. In this section, we will obtain some upper and lower bounds for the smallest eigenvalue of (56)–(58) to give some quantitative estimate and qualitative insight to the decay rate in terms of the biological parameters of the problem.

6.1. Approximate decay rate

For sufficiently low ligand synthesis rate, we expect free ligand concentration to be low so that $\hat{a}(x) \ll \alpha_\omega$. In that case, we have as a good first approximation solution $\{a_0(x), \lambda^{(0)}\}$ of the eigen-pair determined by the ODE and boundary conditions (56)–(59) with $\bar{a}(x)$ terms omitted compared to α_ω. This results in the following simpler eigenvalue problem:

$$a_0'' + [\lambda^{(0)} - q_0(\lambda^{(0)})]a_0 = 0 \quad (0 < x < 1), \tag{69}$$

$$\sigma_0 a_0'(0) + [\lambda^{(0)} - q_0(\lambda^{(0)})]a_0(0) = 0, \quad a_0(1) = 0, \tag{70}$$

with

$$q_0(\lambda^{(0)}) = \frac{\Delta_{20}(\lambda^{(0)})\Delta_{21}(\lambda^{(0)})}{\Delta_{21}(\lambda^{(0)})[\Delta_{20}(\lambda^{(0)}) - f_0\Delta_{10}(\lambda^{(0)})]}. \tag{71}$$

The exact solution for the eigenvalue problem (69)–(70) is

$$a_0(x) = c_0 \sin(\eta(1-x)), \quad \eta^2 = \lambda^{(0)} - q_0(\lambda^{(0)}) = \kappa(\lambda^{(0)}) \tag{72}$$

and $\lambda^{(0)}$ is a root of

$$\sigma_0 = \eta \tan(\eta) \tag{73}$$

(given that $\eta = 0$ is not an admissible solution since it leads to a trivial solution for $a_0(x)$). We will be interested in the smallest positive solution η_1 of (73). Observe that η_1 (as well as any other solution of (73)) depends on σ_0 only and no other parameters of the problem with $\eta_1 \to \pi/2$ from below as $\sigma_0 \to \infty$.

By Lemma 17 in the next section, we know that $\lambda^{(0)}$ is an increasing function of η^2 (and conversely η^2 is an increasing function of $\lambda^{(0)}$). Hence, the slowest decay rate of the transients is given (approximately) by the smallest positive $\lambda^{(0)}$, denoted by $\lambda_s^{(0)}$, that satisfies (73) with $\eta(\lambda^{(0)}) = \eta_1$ (see (72)). In other

words, $\lambda_s^{(0)}$ is the smallest root of

$$\lambda^{(0)} - \frac{\Delta_{20}(\lambda^{(0)})\Delta_{21}(\lambda^{(0)})}{\Delta_{21}(\lambda^{(0)})[\Delta_{20}(\lambda^{(0)}) - f_0\Delta_{10}(\lambda^{(0)})]} = \eta_1^2, \tag{74}$$

where η_1 is the smallest root of (73) with $\eta_1 \leqslant \pi/2$. For a prescribed value of σ_0, we find η_1^2 from (73) and then solve (74) for the smallest root. The latter amounts to finding the smallest root of a fifth degree polynomial. Both have been done numerically and found to be in excellent agreement with the time needed for the solution of the original IBVP to evolve to steady state obtained by integrating the IBVP numerically as described in [7].

6.2. *Bounds for the smallest eigenvalue λ_s*

Suppose λ_s is the smallest eigenvalue of the eigenvalue problem (56) and (58). When ligand synthesis rate is not low, it is still possible (but tedious) to obtain an accurate approximate solution for λ_s by numerical methods. In this subsection, we will obtain upper and lower bounds for λ_s to gain some insight on how the decay rate of transients depends on morphogen activity parameters. Let

$$\kappa_s = \lambda_s - \frac{h_0\alpha_\omega}{\alpha_\omega + \bar{a}(0)} \frac{\Delta_{21}(\lambda_s)\Delta_{20}(\lambda_s)}{\delta(0;\lambda_s)} \equiv \kappa(\lambda_s), \tag{75}$$

where $\kappa(\lambda)$ is given by (59). (Unlike the solution process for $\lambda_s^{(0)}$ where we determine η_1^2 separately from (73), we do not know κ_s here and therefore cannot solve (75) for λ_s.) The function $\kappa(\lambda)$ has four (generally simple) poles which are the four positive roots of

$$\delta(0;\lambda) = \Delta_{21}(\lambda)\Delta_{20}(\lambda) - f_0\Delta_{21}(\lambda)\Delta_{11}(\lambda) - h_0\bar{a}(0)\Delta_{20}(\lambda)\Delta_{10}(\lambda) = 0.$$

Let λ_c be the smaller of the four poles. It is straightforward to prove the following two key lemmas:

Lemma 17. *$\kappa(\lambda)$ as given by* (59) *is a monotone increasing function of λ in $0 \leqslant \lambda < \lambda_c$ where λ_c is the smallest root of $\delta(0;\lambda)$, i.e., the smallest pole of $\kappa(\lambda)$.*

Proof. We compute $\mathrm{d}\kappa/\mathrm{d}\lambda$ to obtain

$$\frac{\mathrm{d}\kappa}{\mathrm{d}\lambda} = 1 + \frac{h_0\alpha_\omega}{\alpha_\omega + \bar{a}(0)} \frac{Z(\lambda)}{[\delta(0;\lambda)]^2} \tag{76}$$

with

$$\begin{aligned} Z(\lambda) = {} & f_0[\Delta_{21}(\lambda)]^2[(\lambda - g_0 - k_0)^2 + j_0k_0] \\ & + h_0\bar{a}(0)[\Delta_{20}(\lambda)]^2[(\lambda - g_1 - k_1)^2 + j_1k_1] > 0 \end{aligned} \tag{77}$$

showing that $\mathrm{d}\kappa/\mathrm{d}\lambda$ is positive. □

Lemma 18. $\kappa(\lambda) > 0$.

Proof. By the first mean value theorem for integrals of [16], there exists some $\xi = \xi(\lambda)$ in (0,1) for which (63) can be written as

$$\kappa_\xi(\lambda) \equiv \lambda - q(\xi;\lambda) = \int_0^1 (\hat{a}'_\lambda)^2\,\mathrm{d}x - \frac{1}{\sigma_0}(\hat{a}_\lambda(0))^2\kappa(\lambda), \tag{78}$$

where we have normalized the eigenfunction so that the integral of $(\hat{a}'_\lambda)^2$ over [0,1] is unity. Upon writing (78) as

$$\kappa_\xi(\lambda) + \frac{1}{\sigma_0}(\hat{a}_\lambda(0))^2\kappa(\lambda) = \int_0^1 (\hat{a}'_\lambda)^2\,\mathrm{d}x, \tag{79}$$

and observing $q(\xi;\lambda) \geqslant q(0;\lambda)$ so that

$$\kappa_\xi(\lambda) \leqslant \kappa(\lambda) = \kappa_0(\lambda), \tag{80}$$

$\kappa(\lambda)$ must be positive for the left-hand side of (79) to be positive. □

The following lemma helps to narrow down the range of λ_s:

Lemma 19. $0 < \lambda_s < \lambda_c$.

Proof. Since $\kappa(0) < 0$ and $\kappa(\lambda) \uparrow \infty$ as $\lambda \uparrow \lambda_c$, there is a unique value of λ in $(0, \lambda_c)$ for which $\kappa(\lambda) = \kappa_s$ for any $\kappa_s > 0$. Hence, we have $0 < \lambda_s < \lambda_c$ for the smallest eigenvalue λ_s since we know that $\kappa(\lambda_s)$ must be positive. □

Lemma 19 above settles the existence and uniqueness of a positive λ_s. With the help of Lemma 17, we can obtain a better lower bound for λ_s. Let λ_{20} and λ_{21} be the smaller of the two roots of $\Delta_{20}(\lambda) = 0$ and $\Delta_{21}(\lambda) = 0$, respectively, with

$$\begin{Bmatrix} \Lambda_{2m} \\ \lambda_{2m} \end{Bmatrix} = \frac{1}{2}\left\{\theta_m \pm \sqrt{\theta_m^2 - 4j_m g_m}\right\}, \quad \theta_m = j_m + g_m + k_m \tag{81}$$

for $m = 0$ and 1. For the wing disc problem, we have $\{j_m, g_m, k_m\} < 1$ so that

$$\begin{Bmatrix} \lambda_{20} \\ \lambda_{21} \end{Bmatrix} \approx \begin{Bmatrix} j_0 g_0/\theta_0 \\ j_0 g_0/\theta_0 \end{Bmatrix} = \begin{Bmatrix} K_{\mathrm{deg,eff}} \\ K'_{\mathrm{deg,eff}} \end{Bmatrix} \equiv \begin{Bmatrix} g_{0,\mathrm{eff}} \\ g_{1,\mathrm{eff}} \end{Bmatrix} \tag{82}$$

with

$$K_{\mathrm{deg,eff}} = \frac{K_{\mathrm{deg}}}{1 + (K_{\mathrm{out}} + K_{\mathrm{deg}})/K_{\mathrm{in}}} = \frac{K_{\mathrm{in}}}{K_{\mathrm{in}} + K_{\mathrm{out}} + K_{\mathrm{deg}}}\,K_{\mathrm{deg}} \tag{83}$$

and $K'_{\mathrm{deg,eff}}$ similarly defined. We have the following lower bound on the decay rate for the case $\min\{\lambda_{20}, \lambda_{21}\} < \kappa_s$ most relevant to the Dpp gradients in the Drosophila wing disc:

Theorem 20. *If* $\min\{\lambda_{20}, \lambda_{21}\} < \kappa_s$, *we have* $\lambda_s > \min\{\lambda_{20}, \lambda_{21}\}$ *and hence* $\min\{\lambda_{20}, \lambda_{21}\} < \lambda_s < \lambda_c$.

Proof. The lower bound on λ_s is a direct consequence of Lemma 17 given $\kappa(0) < 0$ and $0 < \kappa(\lambda_{2k}) = \lambda_{2k} < \kappa_s$, $k = 0$ or 1. □

Remark 21. It should be noted that λ_{20} and λ_{21} depend only on the normalized in, out and degradation rate constants of receptors and ligand receptor complexes (and thus on the diffusion coefficient D_L and $X_{\max}$ as well). It follows that the lower bound for the slowest possible decay rate does not depend on the synthesis rate of either ligands or receptors. Furthermore, λ_{20} and λ_{21} are identical to the approximate λ_s obtained for System C ($\sigma_0 = 0$) in [12] for the same parameter range. It appears then that the decay rate of transients is not significantly affected by the dimensionless flux rate coefficient σ_0.

In the complementary range ($\kappa(0) < 0 <$) $\kappa_s < \min\{\lambda_{20}, \lambda_{21}\}$, we have $\kappa(\kappa_s) < \kappa_s$ and $\kappa(\lambda_{2m}) = \lambda_{2m} > \kappa_s$ which gives the following consequence of Lemmas 17 and 19:

Theorem 22. *For the range* $\kappa_s < \min\{\lambda_{20}, \lambda_{21}\}$, *we have* $\lambda_\ell \equiv \kappa_s < \lambda_s < \min\{\lambda_c, \lambda_{20}, \lambda_{21}\}$.

7. Conclusion

In this paper, we formulated a new model for the essential morphogen activities along the anterior–posterior axis of the wing imaginal disc of Drosophilas. The model allows for diffusion, reversible binding with receptors, internalization, receptor mediated degradation, and receptor renewal. As such it contains System C of [7] as a special case when we do not permit ligand flux at the source end. Section 3 provided the mathematical underpinning for the computational studies of System C in [7]. One remarkable outcome for the steady state problem is that the relevant BVP has the same mathematical form as that for the simpler System B (without internalization or receptor renewal) with the *degradation rate constant*, $K_{\deg}$, and *effective binding rate constant*, $K^*_{\text{on}} = \psi D_L / X^2_{\max}$, of the latter being replaced by the corresponding *observed* rate constants, $K_{\deg,\text{obs}}$ (see note following (28)) and $K^*_{\text{on,obs}} = \psi_\omega D_L / X^2_{\max}$. In Section 3.1, we substantiated this result first reported in [7]. It follows that the proof for the existence of a unique steady state of the free ligand concentration for System B in [10,12] may be used verbatim to prove a similar result for System C (sketched in Subsection 3.2). The same observation also allows us to avoid dealing with endo- and exocytosis explicitly as separate biological processes when we extend the model to investigate additional morphogen activities such as the effects of inhibitors and feedback mechanisms.

The principal contribution of the present paper is on the extended model allowing for ligand flux at the source end. The simple change from $\sigma_0 = 0$ to $\sigma_0 > 0$ in the end condition at $x = 0$ not only necessitated a different proof of existence (as the theorem of Sattinger in [17] does not apply directly) but also led to some fundamentally different characterization of the morphogen activities. For instance, there is no longer any limitation on the ligand synthesis rate for the existence of steady state behavior. A perturbation analysis for low morphogen synthesis rates enabled us to delineate the limitation of System C for modeling the morphogen activities of interest. Approximate analytic solutions for both low and high ligand synthesis rates have been applied to offer insight to issues of interest to the community of developmental biologists [9,14].

Linear stability of the steady state behavior was established for the extended system. While the decay rate of the transients (given by the smallest eigenvalue λ_s from the stability analysis) for System C was reduced to finding the smallest root of a fifth degree polynomial [12], the problem is much less tractable for

$\sigma_0 > 0$. However, useful upper and lower bounds were obtained for λ_s of the extended (aggregated source) model and the values for the lower bound for different parameter ranges were found to be the same as the approximate decay rate of System C obtained in [12]. The simple expression for the lower bound shows that the (slowest) decay rate does not depend on the synthesis rate of ligands or receptors. Furthermore, by comparing the lower bound to that for System C, it appears that the decay rate of transients is not significantly affected by the dimensionless flux rate coefficient σ_0.

Two- and three-dimensional versions of Systems B and C that allow for diffusion in the ventral-dorsal direction and the apical-basal direction of the Dpp activities in wing discs have been formulated and investigated by both analytical and computational methods [10]. Similar higher dimensional studies can be carried out for the aggregated source model treated herein. However, the important issues pertaining to steady state behavior and decay rate of transients (and the related time to steady state) have already been successfully addressed by results of our one-dimensional model. In addition, the analytical results on the gradient shapes have already found applications in actual biological issues and phenomena including (1) an explanation (see [9]) of the opposite effects resulting from over-expression of different receptors in Drosophila wing imaginal discs observed experimentally in [11,2], (2) an indirect determination of the effects of a diffusive non-receptor such as Sog on the gradient shape (see [13,14]).

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