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ORIGINAL ARTICLE

Membrane-Associated Non-Receptors and Morphogen Gradients

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Abstract A previously investigated basic model (System B) for the study of signaling morphogen gradient formation that allows for reversible binding of morphogens (aka ligands) with signaling receptors, degradation of bound morphogens and diffusion of unbound morphogens is extended to include the effects of membrane-bound non-signaling molecules (or non-receptors for short) such as proteoglycans that bind reversibly with the same morphogens and degrade them. Our main goal is to delineate the effects of the presence of non-receptors on the existence and properties of the steady-state concentration gradient of signaling ligand–receptor complexes. Stability of the steady-state morphogen gradients is established and the time to reach steady-state behavior after the onset of morphogen production will be analyzed. The theoretical findings offer explanations for observations reported in several previous experiments on Drosophila wing imaginal discs.

Keywords Morphogens · Morphogen gradients · Non-receptors · Proteoglygans · Tissue patterning · Stability

1. Introduction

Morphogens (aka *ligands*) are molecules that activate signal-transducing mechanisms to generate cellular responses when they bind to (signaling) cell receptors. Concentration gradients of morphogen–receptor complexes are known to be responsible for patterning biological tissues during development. For a number of morphogen families, it is well established that concentration gradients are formed by the transport of morphogens from a localized site of production (see Kerszberg and Wolpert, 1998; Entchev et al., 2000; Strigini and Cohen, 2000; Teleman and

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Cohen, 2000 and other references in Lander et al., 2002). This can be seen from an in vivo visualization of the gradient of the morphogen decapentaplegic (Dpp) in a Drosophila fruit fly wing imaginal disc shown in Fig. 1 of Entchev et al. (2000). Figure 2 of the same reference shows the evolution of the bound morphogen gradient in the wing imaginal disc, which ultimately specifies the locations of wing vein structures on the anterior and posterior portions of the adult wing (Gurdon and Bourillot, 2001). Recent mathematical modeling and quantitative analysis in Lander et al. (2002) and Lou et al. (2004) show that diffusion as a mechanism for morphogen transport and formation of steady-state morphogen concentration gradients is consistent with available experimental observations (Entchev et al., 2000; Teleman and Cohen, 2000). Specific results include the establishment of the existence of various steady-state morphogen concentration gradients and the characterization of the shape of these gradients.

Formation of concentration gradients of different morphogen–receptor complexes is expected to be affected by other known ligand activities including binding with molecular entities other than signaling receptors. Such non-signaling entities will be called *non-receptors* since they bind with morphogens similar to receptors but the resulting complexes do not signal. From this perspective, the presence of non-receptors reduces the amount of morphogen available for binding with receptors and thereby inhibits cell signaling. In this paper, we extend the basic model *System B* of Lander et al. (2002) to include the possibility of morphogens binding with nondiffusive non-receptors such as *cell surface proteoglycans*. Such a model will be used to investigate the inhibiting effects of non-receptors on the formation and properties of steady-state morphogen gradients and the related transient decay time. Similar to System B, the more general model considered herein is an extracellular model in that it does not explicitly represent endocytosis (and cell surface molecules are treated as if they degrade directly); it also takes the concentration of receptors and non-receptors to remain unchanged in time (which can hold whenever synthesis and degradation of receptors and non-receptors are not affected by morphogen binding or signaling). Investigation of more general models with fewer simplifications (see Lander et al., 2002, 2006 and references therein) have led to the same mathematical boundary value problem (BVP) for the determination of the steady-state gradients up to a different interpretation of the biological parameters. Hence, we can and will work with extracellular models whenever possible to simplify our analysis and presentation.

Morphogens are known to bind both nondiffusible and diffusible non-receptors. Regarding nondiffusible non-receptors, a variety of molecules, especially heparan sulfate proteoglycans (HSPG), such as syndecans and glypicans, are known to be important in Drosophila imaginal discs, influencing gradients formed by morphogens such as Dpp, Wingless (Wg), and Hedgehog (Hh) (Lin, 2004). A brief treatment of the effects of nondiffusive non-receptors can be found in Eldar et al. (2003), in which it was concluded that the steady-state shapes of morphogen gradients are necessarily independent of non-receptors. However, in that study the ability of non-receptors to mediate morphogen degradation was neglected (reflecting the relatively common misconception that most HSPG are static components of the extracellular matrix, not rapidly turning-over cell surface molecules).

In the present investigation of nondiffusible non-receptors, we explicitly account for non-receptor-mediated morphogen degradation and characterize its dynamic and steady-state effects on gradient formation. Existence, uniqueness, monotonicity and linear stability of a steady-state gradient of signaling morphogen–receptors are proved. Theoretical results obtained for steady-state gradients herein will provide an explanation in Section 5 of the opposite effects from overexpression of different receptors in Drosophila wing imaginal discs observed experimentally in Cadigan et al. (1998) and Lecuit and Cohen (1998). Morphogen binding with diffusible non-receptors has also been studied. Findings include a possible mechanism responsible for some unexpectedly sharp enhancement of Dpp–receptor gradient at the dorsal midline prior to reaching a substantially lower and less accentuated steady-state concentration (Eldar et al., 2002; Kao et al., 2003; Lou et al., 2005; Mizutani et al., 2005; Shimmi et al., 2005). The findings strongly suggest that the binding of Dpp with the diffusible non-receptor Sog is responsible for some unexpectedly sharp enhancement of Dpp–receptor gradient at the dorsal midline of the biological organism prior to reaching a substantially lower and less accentuated steady-state concentration.

Mathematical models of the binding of morphogens to signaling receptors have been shown to produce biologically realistic morphogen gradients for tissue patterning. The gradients from these models have also been shown to be asymptotically stable with respect to small perturbations. The (usually sharp) lower bound of of the decay rate is found to depend only on the degradation rate constant, offering a way to measure the latter (Mizutani et al., 2005). However, such gradients are not robust to substantial changes in the various parameters, including the kinds of changes that would be induced by environmental fluctuations (e.g. varying temperature). For some morphogen gradient systems, introduction of typical feedback control mechanisms surprisingly does not lead to robustness while the presence of cell-membrane-bound non-receptors that compete for the relevant morphogens alone does so in a significant region of the system parameter space (Nie et al., 2004; Lander et al., 2005c; Lei et al., 2005). It is therefore important to acquire some understanding of, and insight to, the effects of nondiffusive non-receptors to morphogen gradient formation as we do herein.

2. A one-dimensional formulation

In this paper, we focus on the extracellular space of the posterior compartment of a Drosophila wing imaginal disc so that our results can be compared with the those for some of the end source models considered in Lander et al. (2002, 2005a) and Lou et al. (2004) and applied to explain the experimental observations of Cadigan et al. (1998) and Lecuit and Cohen (1998). In formulating a relevant mathematical model for the problem of interest, we simplified the development of the wing imaginal disc to a one-dimensional reaction–diffusion problem in which morphogen is introduced at the rate ν at one end, the border between the *anterior* and *posterior* compartment of the disc, and absorbed at the other end, the edge of the posterior compartment. Based on what we have learned from Lander

et al. (2003) and Vergas (2006), extensions to two- and three-dimensional models should be straightforward.

Let $[L(X, T)]$ be the concentration of a diffusing ligand, such as Dpp or Wg, at time T and distance X from the source end $X = 0$, with $X_{\max}$ being the distance to the edge of the compartment. As in Lander et al. (2002), we take the diffusion of the ligand to be governed by Fick's second law, $\partial[L]/\partial T = D\partial^2[L]/\partial X^2$, D being the diffusion coefficient. We add to this relation the formation and dissociation of morphogen–receptor complexes at the binding rate $-K_{\text{on}}[L](R_0 - [LR])$ and dissociation rate $K_{\text{off}}[LR]$. Here $[LR]$ is the concentration of morphogen–receptor complexes which degrade at the degradation rate $K_{\text{deg}}[LR]$. In these expressions, K_{on}, K_{off} and K_{deg} are the binding rate constant, dissociation rate constant, and degradation rate constant, respectively. The parameter R_0 is the total fixed concentration of receptors available. Though there is no explicit account for the synthesis, internalization (through endocytosis) and degradation of receptor in this formulation, designated as System B in Lander et al. (2002) and Lou et al. (2004), a fixed receptor concentration R_0 corresponds to a receptor synthesis rate matching its degradation rate with internalization implicit in receptor-mediated degradation. The omission of an explicit account of receptor renewal and internalization results in no loss of generality for the purpose of analysis; we have already established in Lou et al. (2004) and Lander et al. (2006) that the BVP governing the steady-state behavior of the more general Systems R and C which include receptor renewal and internalization can be reduced the corresponding BVP for the simpler System B. With appropriate interpretation of the biological parameters involved, the results from the simpler model system also apply to more complex systems with receptor (and non-receptors) renewal and internalization and without the restriction on the receptor synthesis rate being equal to the degradation rate.

To investigate the effects of a fixed concentration N_0 of a proteoglycan type non diffusive non-receptors, we augment System B by a set of similar activities for the non-receptor sites resulting in a concentration of morphogen–non-receptor complexes $[LN(X, T)]$ with J_{on}, J_{off}, and J_{deg} being the corresponding binding rate constant, dissociation rate constant, and degradation rate constant, respectively. For the present study, we work with the analogue of System B without an explicit accounting of non-receptor renewal and internalization. Similar to System B, the stipulation of a fixed non-receptor concentration N_0 corresponds to a non-receptor synthesis rate matching its degradation rate.

For the extension of System B above, we have the following nonlinear reaction–diffusion system governing the evolution of the concentrations $[L]$, $[LR]$, and $[LN]$:

$$\frac{\partial[L]}{\partial T} = D\frac{\partial^2[L]}{\partial X^2} - K_{\text{on}}[L](R_0 - [LR]) + K_{\text{off}}[LR] - J_{\text{on}}[L](N_0 - [LN]) + J_{\text{off}}[LN] \quad (0 < X < X_{\max},\ T > 0), \tag{1}$$

$$\frac{\partial[LR]}{\partial T} = K_{\text{on}}[L](R_0 - [LR]) - (K_{\text{off}} + K_{\text{deg}})[LR] \quad (0 \le X \le X_{\max},\ T > 0), \tag{2}$$

and

$$\frac{\partial[LN]}{\partial T} = J_{\text{on}}[L](N_0 - [LN]) - (J_{\text{off}} + J_{\text{deg}})[LN] \quad (0 \le X \le X_{\max},\ T > 0). \tag{3}$$

With a morphogen synthesis rate $\nu(T)$ at the localized source $X = 0$ between the two wing disc compartments, we have the following idealized end condition at the source end:

$$X = 0: \quad \frac{\partial[L]}{\partial T} = \sigma D \frac{\partial[L]}{\partial X} - \{K_{\text{on}}[L](\rho^2 R_0 - [LR]) - K_{\text{off}}[LR]\} - \{J_{\text{on}}[L](\rho_z^2 N_0 - [LN]) - J_{\text{off}}[LN]\} + \nu, \tag{4}$$

for all $T > 0$, where, for an aggregated source model (System A of Lander et al., 2005a), $1/\sigma = X_{\min}$ is the width of the ligand synthesis zone. The parameters $\rho^2 R_0$ and $\rho_z^2 N_0$ are respectively the uniform receptor and non-receptor concentration densities in the narrow receptor production region between the two compartments. The edge of the posterior compartment of the wing disc at the other end, $X = X_{\max}$, is taken to be absorbing; hence, we have

$$X = X_{\max}: \quad [L] = 0 \quad (T > 0). \tag{5}$$

Until morphogens being generated at $T = 0$, the biological system was in quiescence so that we have the homogeneous initial conditions

$$T = 0: \quad [L] = [LR] = [LN] = 0, \quad (0 \le X \le X_{\max}). \tag{6}$$

The initial-boundary value problem (IBVP) defined by (1)–(6) will be designated as *System N* henceforth. In the absence of non-receptor sites (so that $N_0 = J_{\text{on}} = J_{\text{deg}} = J_{\text{off}} = 0$), the limiting case of $\sigma = 0\,(X_{\min} = \infty)$ reduces to System B upon setting $\rho^2 = 1$.

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

$$t = \frac{D}{X_0^2}T, \quad x = \frac{X}{X_0}, \quad a = \frac{[L]}{R_0}, \quad b = \frac{[LR]}{R_0}, \quad c = \frac{[LN]}{N_0}, \quad Z = \frac{N_0}{R_0}, \tag{7}$$

$$f_0 = \frac{X_0^2}{D}K_{\text{off}}, \quad g_0 = \frac{X_0^2}{D}K_{\text{deg}}, \quad h_0 = \frac{X_0^2}{D}K_{\text{on}}R_0, \quad \nu_0 = \frac{X_0^2}{DR_0}\nu, \tag{8}$$

$$f_1 = \frac{X_0^2}{D}J_{\text{off}}, \quad g_1 = \frac{X_0^2}{D}J_{\text{deg}}, \quad h_1 = \frac{X_0^2}{D}J_{\text{on}}N_0, \quad \bar{h}_1 = \frac{h_1}{Z}, \tag{9}$$

where X_0 is some typical scale length which will be taken to be $X_{\max}$ unless specifically indicated otherwise. With these normalized quantities, we rewrite the IBVP

for $[L]$, $[LR]$, and $[LN]$ in the following normalized form

$$\frac{\partial a}{\partial t} = \frac{\partial^2 a}{\partial x^2} - h_0 a(1-b) + f_0 b - \bar{h}_1 Z a(1-c) + f_1 Z c \quad (0 < x < 1) \tag{10}$$

$$\frac{\partial b}{\partial t} = h_0 a(1-b) - (f_0 + g_0) b \quad (0 \le x \le 1) \tag{11}$$

$$\frac{\partial c}{\partial t} = \bar{h}_1 a(1-c) - (f_1 + g_1) c \quad (0 \le x \le 1) \tag{12}$$

with the boundary conditions

$$x = 0: \quad \frac{\partial a}{\partial t} = \sigma_0 \frac{\partial a}{\partial x} - [h_0 a(\rho^2 - b) - f_0 b] - \left[\bar{h}_1 Z a\left(\rho_z^2 - c\right) - f_1 Z c\right] + \nu_0 \tag{13}$$

$$x = 1: \quad a = 0 \tag{14}$$

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

$$t = 0: a = b = c = 0 \quad (0 \le x \le 1). \tag{15}$$

In (13), the parameter σ_0 will be taken to be $X_{\max}/X_{\min}$ herein to correspond to an aggregated source model (Lander et al., 2005a,b).

The IBVP defined by (10)–(15) constitutes a new mathematical model for morphogen activities in the presence of nondiffusive non-receptors such as cell membrane-bound Syndecans and Glypicans (e.g., Dally and Dlp) in Drosophila (Jackson et al., 1997; Bellin et al., 2003; Fujise et al., 2003; Lin, 2004). It will be used to study the effects of such non-receptor sites on the amplitude, steepness and convexity of the various steady-state morphogen concentration gradients, and the decay rate of transient behavior. For this purpose, we limit ourselves hereafter to the case $\rho^2 = \rho_z^2 = 1$ to simplify the discussion.

3. Time-independent state (σ_0=0)

We denote by $\bar{a}(x)$, $\bar{b}(x)$, and $\bar{c}(x)$ the time-independent steady-state solution for $a(x,t)$ and $b(x,t)$ and $c(x,t)$ of (10)–(15), respectively. For this steady-state solution, we have $\partial\bar{a}/\partial t = \partial\bar{b}/\partial t = \partial\bar{c}/\partial t = 0$ so that the governing partial differential equations and boundary conditions become

$$\bar{a}'' - h_0\bar{a}(1-\bar{b}) + f_0\bar{b} - \bar{h}_1 Z\bar{a}(1-\bar{c}) + f_1 Z\bar{c} = 0 \quad (0 < x < 1) \tag{16}$$

$$h_0\bar{a}(1-\bar{b}) - (f_0 + g_0)\bar{b} = 0, \quad (0 \le x \le 1)$$

$$\bar{h}_1\bar{a}(1-\bar{c}) - (f_1 + g_1)\bar{c} = 0 \quad (0 \le x \le 1) \tag{17}$$

with

$$\sigma_0\bar{a}'(0) - \{h_0\bar{a}(0)[1-\bar{b}(0)] - f_0\bar{b}(0)\} - Z\{\bar{h}_1\bar{a}(0)[1-\bar{c}(0)] - f_1\bar{c}(0)\} + \nu_0 = 0, \tag{18}$$

$$\bar{a}(1) = 1, \tag{19}$$

where a prime indicates differentiation with respect to x, i.e., $(\ )' = \mathrm{d}(\ )/\mathrm{d}x$.

We can use (17), written as

$$\bar{b}(x) = \frac{\bar{a}(x)}{\bar{a}(x)+\alpha_0}, \qquad \alpha_0 = \frac{f_0+g_0}{h_0} = \frac{K_{\mathrm{off}}+K_{\mathrm{deg}}}{K_{\mathrm{on}}R_0}, \tag{20}$$

$$\bar{c}(x) = \frac{\bar{a}(x)}{\bar{a}(x)+\bar{\alpha}_1}, \qquad \bar{\alpha}_1 = \frac{f_1+g_1}{\bar{h}_1} = \frac{J_{\mathrm{off}}+J_{\mathrm{deg}}}{J_{\mathrm{on}}R_0}, \tag{21}$$

to eliminate $\bar{b}$ and $\bar{c}$ from all other relevant equations to obtain a BVP for $\bar{a}$ alone:

$$\bar{a}'' = \frac{g_0\bar{a}}{\bar{a}+\alpha_0} + \frac{Zg_1\bar{a}}{\bar{a}+\bar{\alpha}_1}, \tag{22}$$

$$-\sigma_0\bar{a}'(0) + \frac{g_0\bar{a}(0)}{\bar{a}(0)+\alpha_0} + \frac{Zg_1\bar{a}(0)}{\bar{a}(0)+\bar{\alpha}_1} = \nu_0, \qquad \bar{a}(1) = 0. \tag{23}$$

We consider here first the special case $\sigma_0 = 0$, designated as *System NB*. For this case, the boundary condition at $x = 0$ becomes a quadratic equation for $\bar{a}(0)$:

$$[g_0 + Zg_1 - \nu_0]\bar{a}^2(0) + [g_0\bar{\alpha}_1 + Zg_1\alpha_0 - \nu_0(\alpha_0+\bar{\alpha}_1)]\bar{a}(0) - \nu_0\alpha_0\bar{\alpha}_1 = 0. \tag{24}$$

This can be solved to obtain

$$\bar{a}(0) = \frac{-\kappa \pm \sqrt{\kappa^2 + 4\nu_0\alpha_0\bar{\alpha}_1(g_0+Zg_1-\nu_0)}}{2(g_0+Zg_1-\nu_0)} \tag{25}$$

with

$$\kappa = g_0\bar{\alpha}_1 + Zg_1\alpha_0 - \nu_0(\alpha_0+\bar{\alpha}_1). \tag{26}$$

The free ligand concentration at the source end for System NB $\bar{a}(0)$ is completely specified as in System B investigated in Lander et al. (2002, 2003) and Lou et al. (2004). For System B, it was required that g_0 be greater than ν_0 for the existence of a steady state. For any prescribed set of values for $g_0, \bar{\alpha}_1, g_1, \alpha_0$, and ν_0, the expression (25) for $\bar{a}(0)$ enables us to prove the following necessary condition for the existence of a steady state for System NB:

Lemma 1. *$g_0 + Zg_1 > \nu_0$ is a necessary condition for the existence of nonnegative steady-state concentration gradients.*

Proof: Re-write the expression (26) for κ as

$$\kappa = (g_0 + Zg_1 - \nu_0)(\bar{\alpha}_1 + \alpha_0) - (Zg_1\bar{\alpha}_1 + g_0\alpha_0).$$

It follows that $\kappa < 0$ and both roots for $\bar{a}(0)$ would be negative if $g_0 + Zg_1 < \nu_0$.

With $g_0 + Zg_1 > \nu_0$, we must take the positive square root in (25) to get

$$\bar{a}(0) = \frac{-\kappa + \sqrt{\kappa^2 + 4\nu_0\alpha_0\bar{\alpha}_1(g_0 + Zg_1 - \nu_0)}}{2(g_0 + Zg_1 - \nu_0)} \equiv \bar{a}_0 > 0 \tag{27}$$

to avoid a negative $\bar{a}_0$ and ensure a meaningful steady-state concentration of morphogens at the source point.

In the absence of non-receptors, the necessary condition $g_0 + Zg_1 > \nu_0$ reduces to the known condition $g_0 > \nu_0$ found in Lander et al. (2002) and Lou et al. (2004). For the simpler case of $Z = 0$, the various normalized steady-state morphogen concentrations depend on the following three parameters: the dimensionless *effective on rate* ψ, the ligand s*ynthesis-to-degradation ratio* β, and the ligand–receptor *loss–gain ratio* α_0:

$$\begin{aligned} \psi &\equiv \mu^2 = \frac{g_0}{\alpha_0} = \frac{K_{\text{deg}}}{K_{\text{deg}} + K_{\text{off}}} \frac{x_{\max}^2}{D} K_{\text{on}} R_0, \\ \beta &\equiv \frac{\nu_0}{g_0} = \frac{\nu}{K_{\text{deg}} R_0}, \qquad \alpha_0 = \frac{K_{\text{deg}} + K_{\text{off}}}{K_{\text{on}} R_0}. \end{aligned} \tag{28}$$

The *synthesis–degradation ratio* β is a measure of the relative strength of the morphogen production rate and the morphogens degradation rate. The dimensionless *effective on rate* $\psi = h_0 g_0/(g_0 + f_0)$ characterizes essentially the relative strength of the effective morphogen–receptor binding rate and morphogen diffusion rate (see (28)). The loss–gain ratio of morphogen–receptor complexes α_0 is a measure of the effective replacement of lost ligand–receptor complexes through new binding. It is rather remarkable that for the limiting case of $\sigma_0 = 0$, the existence of the steady-state concentration gradients $\bar{a}$ and $\bar{b}$ in the absence of non-receptors depends on the value of only one of these three parameters, namely β.

In the presence of a concentration of membrane-bound non-receptors, Lemma 1 shows that the parameter relevant to the existence of steady-state gradients corresponding to β is

$$\beta_a(Z) \equiv \frac{\nu_0}{g_0 + Zg_1} = \frac{\nu}{K_{\text{deg}} R_0 + J_{\text{deg}} N_0} \tag{29}$$

with $\beta_a(0) = \beta$. Analogous to the $Z = 0$ case, we expect $\beta_a(Z) \equiv \nu_0/(g_0 + Zg_1) < 1$ to be a sufficient condition for the existence of steady-state morphogen gradients as well. It is straightforward to prove the following existence, uniqueness, and monotonicity theorem by the method previously indicated in Lou et al. (2004) (see also Lander et al., 2003).

Theorem 1. *For $g_0 + Zg_1 > \nu_0$ so that $\beta_a(Z) < 1$, there exist a unique triplet of non negative (time-independent) steady-state gradients $\bar{a}$, $\bar{b}$, and $\bar{c}$, all strictly decreasing in* $[0, 1]$.

Proof: (sketched) With $a_u(x) = \bar{a}_0$ and $a_\ell(x) = 0$ being an upper and a lower solution of the BVP for $\bar{a}(x)$, respectively, a theorem of Sattinger (1972) assures us that $\bar{a}(x)$ exists with $0 \le \bar{a}(x) \le \bar{a}_0$.

Uniqueness is proved by integrating the BVP for the difference $a(x)$ between two possible solutions $\bar{a}_1$ and $\bar{a}_2$ to obtain

$$\int_0^1 (a')^2 \, dx + \int_0^1 \left\{ \frac{g_0 \alpha_0 a^2}{(\bar{a}_1 + \alpha_0)(\bar{a}_2 + \alpha_0)} + \frac{Z g_1 \bar{\alpha}_1 a^2}{(\bar{a}_1 + \bar{\alpha}_1)(\bar{a}_2 + \bar{\alpha}_1)} \right\} dx = 0.$$

Since the integrands are all non negative, we must have $a(x) = 0$.

For monotonicity, suppose that $\bar{a}(x)$ has a maximum at x_0; then $\bar{a}''(x_0) \le 0$. But the ODE for $\bar{a}(x)$ requires $\bar{a}''(x_0) \ge 0$. Hence, we must have $\bar{a}''(x_0) = 0$ and therewith $\bar{a}(x_0) = 0$ by the ODE. But $0 \le \bar{a}(x) \le \bar{a}(x_0) = 0$ requires $\bar{a}(x) = 0$ which contradicts $\bar{a}(0) = \bar{a}_0 > 0$. That $\bar{a}(x)$ does not admit an interior minimum can also be proved similarly.

We see from the condition $\beta_a \equiv \nu_0/(g_0 + Zg_1) < 1$ that the presence of nondiffusive non-receptors allows steady-state concentration gradients for a larger normalized morphogen synthesis rate ν_0. Hence, non-receptors help the formation of steady-state gradients in that the normalized production rate of the morphogens no longer needs to be smaller than their normalized degradation rate, provided there is a sufficiently large concentration of non-receptors. On the other hand, for a fixed concentration of non-receptors, there will always be a threshold ligand synthesis rate for the existence of a steady state. It is significant that the restriction on the synthesis rate (for the existence of steady-state behavior) in the ad hoc point source model Systems B is not removed by the introduction of nondiffusive non-receptors to that model.

4. Time-independent state ($\sigma_0 \neq 0$)

For the more typical case of $\sigma_0 > 0$, the end condition at $x = 0$ no longer specifies the end value $\bar{a}(0)$. Correspondingly, there is no longer any requirement for $g_0 + Zg_1$ to be greater than ν_0. In fact, we have the following theorem which ensure the existence of a unique nonnegative monotone decreasing steady-state concentration $\bar{a}(x)$ with no restriction on the ligand synthesis rate:

Theorem 2. *For positive values of the parameters $\sigma_0, g_0, \alpha_0, g_1, \bar{\alpha}_1$, and ν_0, there exists a nonnegative regular solution $\bar{a}(x)$ of the BVP (22) and (23). (The corresponding concentrations $\bar{b}(x)$ and $\bar{c}(x)$ can then be calculated from (20) and (21), respectively.)*

The proof this theorem is similar to that for $Z = 0$ case in Lander et al. (2005a) and will not be given here. Instead we will obtain an approximate steady-state solution for low morphogen synthesis rates.

For sufficiently low values of ν_0, we expect $h_0\bar{a}(x) \ll g_0 + f_0$ and $\bar{h}_1\bar{a}(x) \ll g_1 + f_1$. For such cases, henceforth designated as the *Low Ligand Synthesis Rate* (LLSR) cases, a first approximation $\bar{a}_0(x)$ of $\bar{a}(x)$ may be obtained by neglecting the $\bar{a}(x)$ terms in the denominators of (22) and (23) to get

$$[\bar{a}_0]'' = \mu_s^2\bar{a}_0, \qquad -\sigma_0\bar{a}_0'(0) + \mu_s^2\bar{a}_0(0) - \nu_0 = 0, \quad \bar{a}_0(1) = 0 \tag{30}$$

with

$$\mu^2 = \psi = \frac{g_0}{\alpha_0}, \qquad \mu_z^2 = \psi_z = \frac{g_1}{\bar{\alpha}_1}Z, \qquad \mu_s^2 = \mu^2 + \mu_z^2. \tag{31}$$

The exact solution of this linear BVP is

$$\begin{aligned}\bar{a}_0(x) &= \frac{\nu_0}{\Delta_0(\mu_s, \sigma_0)}\sinh(\mu_s(1-x)) \\ &\equiv \frac{\nu_0\sinh(\mu_s(1-x))}{\mu_s^2\sinh(\mu_s) + \sigma_0\mu_s\cosh(\mu_s)} \sim \alpha_0\bar{b}(x)\end{aligned} \tag{32}$$

with

$$\alpha_0\bar{b}(0) \sim \bar{a}(0) \sim \bar{a}_0(0) = \frac{\nu_0}{\mu_s^2 + \sigma_0\mu_s\coth(\mu_s)} \equiv a_0. \tag{33}$$

Note that the corresponding solution for the special case $\sigma_0 = 0$ (System NB) is

$$\bar{a}_0(x) = \frac{\nu_0\sinh(\mu_s(1-x))}{\mu_s^2\sinh(\mu_s)}, \qquad \bar{a}_0(0) = \frac{\nu_0}{\mu_s^2}. \tag{34}$$

With (32), we now see that if the ad hoc point source model of System NB is to be an adequate approximation of System N, it is necessary to have the binding rate sufficiently high so that $\sigma_0\coth(\mu_s)/\mu_s \ll 1$.

More important information on the effects of a concentration of nondiffusive non-receptors can be obtained from (32). Since $\mu_s^2 = \mu^2 + \mu_z^2 \geq \mu^2$, the introduction of nondiffusive non-receptors has the following effects on the amplitude, slope and convexity of the *normalized* morphogen concentrations gradient $\bar{a}(x)$ and $\bar{b}(x)$.

Theorem 3. *For sufficiently low morphogen synthesis rates (so that (32) is an adequate approximation of $\bar{a}(x)$), the presence of nondiffusive non-receptors generally lowers the normalized concentration level of both $\bar{a}(x)$ and $\bar{b}(x)$ at each point of the solution domain, reduces the steepness of the negative slope, and increases the convexity of the concentrations.*

Proof: The conclusions are consequences of computing $\partial[\bar{a}_0(x)]/\partial Z$, $-\partial[\partial\bar{a}_0(x)/\partial x]/\partial Z$, and $-\partial[\partial^2\bar{a}_0(x)/\partial x^2]/\partial Z$ and showing that they are all negative. But even with the explicit solution (32), the task is not straightforward. Given $\partial[\ ]/\partial Z = \{\partial[\ ]/\partial\mu_s\}\{g_1/(2\mu_s\bar{\alpha}_1)\}$, we consider

$$\frac{\partial\bar{a}_0}{\partial\mu_s} = \frac{\nu_0}{[\Delta_0]^2}\{\mu_s^2\sinh(\mu_s x) + \sigma_0\mu_s\cosh(\mu_s x) - \phi(x)\}$$

where

$$\phi(x) = \Delta_0 x\cosh(\mu_s(1-x)) + [2\mu_s\sinh(\mu_s) + \sigma_0\cosh(\mu_s)]\sinh(\mu_s(1-x)$$
$$\frac{\partial\phi}{\partial x} = -\mu_s^2\sinh(\mu_s)\cosh(\mu_s(1-x)) - \Delta_0(\mu_s x)\sinh(\mu_s(1-x)).$$

Since $\partial\phi/\partial x < 0$, we have

$$\frac{\partial\bar{a}_0}{\partial\mu_s} < \frac{\nu_0}{[\Delta_0]^2}\{\mu_s^2\sinh(\mu_s x) + \sigma_0\mu_s\cosh(\mu_s x) - \phi(1)\} \le 0$$

for $0 \le x \le 1$. This show that $\bar{a}_0$ is a decreasing function of Z. Next, we have

$$\frac{\partial}{\partial\mu_s}\left[\frac{\partial\bar{a}_0}{\partial x}\right] = \frac{\nu_0}{[\Delta_0]^2}\{\mu_s^3\cosh(\mu_s x) + \sigma_0\mu_s^2\sinh(\mu_s x)$$
$$+\mu_s^2\sinh(\mu_s)\cosh(\mu_s(1-x)) + \Delta_0(\mu_s x)\sinh(\mu_s(1-x)\}$$

which is positive for $0 \le x \le 1$; hence the slope $\mathrm{d}\bar{a}_0/\mathrm{d}x$ becomes less negative with increasing Z. Finally, the expression

$$\frac{\partial}{\partial\mu_s}\left[\frac{\partial^2\bar{a}_0}{\partial x^2}\right] = \frac{\nu_0}{[\Delta_0]^2}\{\mu_s^4\sinh(\mu_s x) + \sigma_0\mu_s^3\cosh(\mu_s x)$$
$$+\sigma_0\mu_s^2\cosh(\mu_s)\sinh(\mu_s(1-x)) - \mu_s\Delta_0(\mu_s x)\cosh(\mu_s(1-x))\}$$

which is nonnegative for $0 \le x \le 1$ and strictly positive for $0 \le x < 1$.

As we shall see in the next section, we can prove similar results for more general morphogen synthesis rates for which linearization is inappropriate.

Remark 1. It is important to observe that the presence of non-receptor sites does not automatically affect the free and bound morphogen gradients. In the

absence of non-receptor-mediated ligand degradation so that $g_1 = 0$, we have $\mu_z^2 = Zg_1/\bar{\alpha}_1 = 0$ (even when $Z \neq 0$) and $\mu_s^2 = \mu^2$. In that case, both $\bar{a}_0(x)$ and $\bar{b}_0(x)$ reduce to the corresponding expression without non-receptors in Lou et al. (2004) and Lander et al. (2005a). As such, our results provide an analytical confirmation of the same observation in Eldar et al. (2003) based on numerical simulations. At the same time, they extend the work of Eldar et al. (2003) to the more general case which allows for non-receptor-mediated ligand degradation.

While non-receptors always reduce the negative slope of gradients, many biologists are more concerned about "slope relative to concentration" (or "relative slope" for brevity), $s(x; Z) \equiv \bar{a}'(x)/\bar{a}(x)$; how does $s(x; Z)$ change with the introduction of non-receptors? For the *LLSR* case, it is a straightforward calculation to show that $\partial s/\partial Z < 0$ so that we have the following result.

Theorem 4. *Whereas a higher concentration of nondiffusive non-receptors always reduces negative slopes of gradients for the LLSR case, it always increases the relative slopes $\bar{a}'(x)/\bar{a}(x)$ and $\bar{b}'(x)/\bar{b}(x)$, making them steeper downward.*

How does the conclusion above change if the ligand synthesis rate is higher (so that we cannot linearize the BVP for the steady-state solution)? We will attempt to answer this question in the next section (but postpone a discussion of a more general model with receptor and non-receptor renewal to a future report). Another task that requires attention is to see what the results for normalized quantities obtained above tell us about experimentally observable quantities (generally the unnormalized quantities such as $[L]$, $[LR]$ and $[LN]$) since the rescaling of these quantities involves the parameter R_0. We will also do this in the next section.

5. Effects of non-receptor-mediated degradation on steady-state gradients

For the general case of an unrestricted synthesis rate ν_0, we will obtain the dependence of amplitude and shape of the free morphogen gradient on the amount of non-receptors introduced by first showing that $\bar{a}(x)$ is a decreasing function of Z. Let $y(x) = \partial\bar{a}/\partial Z$ and differentiate all the relations in (22) and (23) with respect to Z to get

$$y'' = \left[\frac{\alpha_0 g_0}{(\bar{a}+\alpha_0)^2} + \frac{Z\bar{\alpha}_1 g_1}{(\bar{a}+\bar{\alpha}_1)^2}\right] y + \frac{g_1\bar{a}}{(\bar{a}+\bar{\alpha}_1)}, \qquad y(1) = 0, \tag{35}$$

$$B[y(0)] \equiv -\sigma_0 y'(0) + \frac{\alpha_0 g_0 y(0)}{[\bar{a}(0)+\alpha_0]^2} + \frac{Z\bar{\alpha}_1 g_1 y(0)}{[\bar{a}(0)+\bar{\alpha}_1]^2} + \frac{g_1\bar{a}(0)}{[\bar{a}(0)+\bar{\alpha}_1]} = 0. \tag{36}$$

We can establish the following existence and uniqueness theorem for this linear BVP.

Theorem 5. *For any set of positive values of the parameters $\sigma_0, g_0, \alpha_0, \bar{\alpha}_1$ and ν_0, there exists a unique nonpositive monotone increasing solution $y(x)$ of the BVP (35) and (36).*

Proof: Because of the form of the boundary condition (36), the monotonicity theorem of Sattinger (1972) is not directly applicable. Consider the auxiliary BVP defined by (35) with $y(1) = 0$ and $y(0) = y_0$ for any y_0 in $[-y_z, 0]$ where

$$y_z = \frac{\bar{a}(0)\,[\bar{a}(0) + \bar{\alpha}_1]}{Z\bar{\alpha}_1} > 0.$$

Now, $y_u(x) = 0$ is an upper solution for this auxiliary problem. With

$$\frac{Z\bar{\alpha}_1 g_1 y_z}{[\bar{a}(x) + \bar{\alpha}_1]^2} = \frac{g_1\bar{a}(0)\,[\bar{a}(0) + \bar{\alpha}_1]}{[\bar{a}(x) + \bar{\alpha}_1]^2} \geq \frac{g_1\bar{a}(0)}{[\bar{a}(x) + \bar{\alpha}_1]} \geq \frac{g_1\bar{a}(x)}{[\bar{a}(x) + \bar{\alpha}_1]},$$

$y_\ell(x) = -y_z$ is a lower solution of the same auxiliary BVP. Hence, a unique, nonpositive, monotone increasing solution $y(x)$ of auxiliary BVP exists for any y_0 (in the chosen range) with $-y_z \leq y(x) \leq 0$. Let $s(y_0)$ be the slope $y'(0)$ of the *auxiliary* BVP for the particular y_0; then we have from (36) $s(0) = 0$ and $s(y_0) > 0$ (since the solution of the auxiliary BVP is monotone increasing for y_0 in $[-y_z, 0]$. Correspondingly, we have

$$B[y(0) = 0] = \frac{g_1\bar{a}(0)}{[\bar{a}(0) + \bar{\alpha}_1]} > 0,$$

$$B[y(0) = -y_z] \leq -\sigma_0 s(-y_z) - \frac{\alpha_0 g_0 y_z}{[\bar{a}(0) + \alpha_0]^2} < 0.$$

Continuous dependence of the solution $y(x)$ on the parameter y_0 implies that there is an intermediate value $\bar{y}_0$, $-y_z < \bar{y}_0 < 0$, for which $B[\bar{y}_0] = 0$. This proves the existence part of the lemma. Uniqueness and monotonicity are proved by arguments similar to that for System A in Lander et al. (2005a).

The following useful theorem extends the explicit results for the case of low morphogen synthesis rates on the amplitude and shape of the concentration gradients.

Theorem 6. *The presence of nondiffusive non-receptors generally lowers the amplitude and reduces the downward slope of the concentration gradients $\bar{a}(x)$ and $\bar{b}(x)$ for all $x < 1$ (rendering it less steep) and generally increases the convexity of $\bar{a}(x)$ in a region adjacent to the morphogen source.*

Proof: We have from Theorem 5 that $y(x) = \partial\bar{a}/\partial Z$ is negative in $[0, 1)$. It follows that $\bar{a}(x)$ is a decreasing function of Z so that the presence of the non-receptors generally lowers the magnitude of $\bar{a}(x)$ throughout the wing disc interior. The effects of non-receptors on the amplitude of $\bar{b}(x)$ follows from the relation

$$\frac{\partial\bar{b}}{\partial Z} = \frac{\alpha_0}{[\bar{a}(x) + \alpha_0]^2}\frac{\partial\bar{a}}{\partial Z}.$$

We also have from the same theorem that $y(x)$ is monotone increasing so that

$$\acute{y}(x) = \frac{\partial}{\partial x}\left(\frac{\partial \bar{a}}{\partial Z}\right) = \frac{\partial}{\partial Z}(\acute{\bar{a}}) > 0.$$

In other words, the negative slope $\acute{\bar{a}}(x)$ is an increasing function of Z and hence becomes less steep downward with the introduction of nondiffusive non-receptors. At the sink end, we have $y(1) = 0$ and therewith

$$y''(1) = \frac{g_1 \bar{a}(1)}{[\bar{a}(1) + \bar{\alpha}_1]^2} = 0.$$

At the source end, we have

$$y''(0) = \left\{\frac{\alpha_0 g_0}{[\bar{a}(0) + \alpha_0]^2} + \frac{Z\bar{\alpha}_1 g_1}{[\bar{a}(0) + \bar{\alpha}_1]^2}\right\} y(0) + \frac{g_1 \bar{a}(0)}{\bar{a}(0) + \bar{\alpha}_1} = \sigma_0 \acute{y}(0) > 0. \quad (37)$$

With $y''(x; Z) = \partial[a''(x; Z)]/\partial Z$ being continuous, the positive $a''(x)$ increases with Z at least in some neighborhood of $x = 0$ so that $\bar{a}(x)$ becomes more convex there.

Note that even without an explicit solution outside the LLSR range, we manage to deduce the same effect of the introduction of nondiffusive non-receptors on the magnitude and slope of the concentration gradients as in the LLSR range for the entire (posterior) compartment. In the case of the convexity of the concentration gradients, we succeeded only in deducing from (37) that more non-receptor sites only makes the gradients more convex in a region adjacent to the source end (while these same gradients remain flat at the sink end with or without non-receptors). Without an explicit solution or additional information, the change of relative slope with non-receptor concentration, $\partial s/\partial Z$, is not known to be of one sign.

The effects of the addition of non-receptor sites obtained in this and the last section help to explain why overexpression of morphogen receptors can have different (and sometimes opposite) effects on different morphogen systems. When *Dpp* receptors *thick veins* (*tkv)* in wing imaginal discs are overexpressed in vivo (Lecuit and Cohen, 1998), they produce effects significantly different from (and nearly opposite to) those of overexpressing the Wingless (Wg) receptor *Drosophila frizzled* 2 (*Dfz2*) Cadigan et al. (1998). More specifically, we have from (Lecuit and Cohen, 1998) that "high levels of *tkv* generally sequester ligand and limit its movement across the wing disc." The corresponding ligand–receptor gradient become steeper and more convex, tending to a boundary layer, while the amplitude remains pretty much unchanged. On the other hand, we have from Cadigan et al. (1998) that "high levels of a Wg receptor, *Drosophila frizzled* 2 (*Dfz2*), stabilize Wg, allowing it to reach cells far from its site of synthesis." To reconcile these different effects, we write the expressions (33) and (31) in terms of the biological parameters to

obtain

$$\mu_s^2 = \mu^2 + \mu_z^2 = \frac{k_{\text{on}} R_0}{D/X_{\text{max}}^2} + \frac{j_{\text{on}} N_0}{D/X_{\text{max}}^2}, \tag{38}$$

$$[LR]_{x=0} = R_0 \bar{b}(0) = \frac{R_0 \nu_0/\alpha_0}{\mu_s^2 + \sigma_0 \mu_s \coth(\mu_s)} \approx \frac{\nu/k_{\text{deg}}}{1 + \Gamma N_0/R_0}, \tag{39}$$

with

$$k_{\text{on}} = \frac{K_{\text{on}} K_{\text{deg}}}{K_{\text{deg}} + K_{\text{off}}}, \quad j_{\text{on}} = \frac{J_{\text{on}} J_{\text{deg}}}{J_{\text{deg}} + J_{\text{off}}}, \quad \Gamma = \frac{j_{\text{on}}}{k_{\text{on}}}. \tag{40}$$

The approximation in the expression (39) for $[LR]$ at the source end is appropriate for sufficiently large μ_s. For the *Dpp* receptor *thick veins* with $\mu^2 \gg \mu_z^2$ (Lecuit and Cohen, 1998), overexpression of the receptor further increases μ^2 and thereby μ_s^2, resulting in steeper and a more convex $[LR]$ gradient as observed experimentally in Lecuit and Cohen (1998). For the Wg receptor *Dfz2* with $\mu^2 \ll \mu_z^2$ (Cadigan et al., 1998), overexpressing the receptor (resulting in a larger value of R_0 and μ^2) does not change μ_s^2 significantly but does increase the amplitude of $[LR]$ throughout the wing disc (through a reduction of the denominator of the expression for $[LR]_{x=0}$). The concentration $[LR]$ is higher at each location from the source end to the edge of the wing imaginal disc and hence reach more receptors at different locations downstream than does the wild type at the corresponding location as observed experimentally in Cadigan et al. (1998). We know from experimental evidence summarized in the recent review of Lin (2004) that at least one class of membrane bound non-receptor—the cell surface heparan sulfate proteoglycan (including syndecans and glypicans)—clearly plays a major role in both Wg and Dpp gradients, as well as in the gradients formed by other morphogens. The mathematical results obtained above therefore offer an explanation on how the effects of such non-receptors could account for important experimental observations in these systems, including seemingly inconsistent ones such as those in Cadigan et al. (1998) and Lecuit and Cohen (1998).

6. Linear stability for the time-independent steady states

Now that the existence of a unique set of time-independent steady-state morphogen concentration gradients has been established, we want to know if the gradients are stable. For a linear stability analysis, we consider a small perturbation from the steady state in the form

$$\begin{aligned} a(x,t) &= \bar{a}(x) + \mathrm{e}^{-\lambda t}\hat{a}(x), \quad b(x,t) = \bar{b}(x) + e^{-\lambda t}\hat{b}(x), \\ c(x,t) &= \bar{c}(x) + \mathrm{e}^{-\lambda t}\hat{c}(x) \end{aligned} \tag{41}$$

where $\bar{a}$, $\bar{b}$ and $\bar{c}$ are the steady-state solutions and where the time-independent portion of the perturbations, $\hat{a}$, $\hat{b}$ and $\hat{c}$, are negligibly small compared to the

corresponding steady-state solution. After linearization, we have the following eigenvalue problem for $\hat{a}$ and $\hat{b}$:

$$-\lambda\hat{b} = h_0(1-\bar{b})\hat{a} - (f_0 + g_0 + h_0\bar{a})\hat{b} \tag{42}$$

$$-\lambda\hat{c} = \bar{h}_1(1-\bar{c})\hat{a} - (f_1 + g_1 + \bar{h}_1\bar{a})\hat{c} \tag{43}$$

and

$$-\lambda\hat{a} = \hat{a}'' - h_0(1-\bar{b})\hat{a} + (f_0 + h_0\bar{a})\hat{b} - \bar{h}_1 Z(1-\bar{c})\hat{a} + Z(f_1 + \bar{h}_1\bar{a})\hat{c} \tag{44}$$

with

$$-\lambda\hat{a}(0) = \sigma_0\hat{a}'(0) - \{h_0[1-\bar{b}(0)] + \bar{h}_1 Z[1-\bar{c}(0)]\}\hat{a}(0) + [f_0 + h_0\bar{a}(0)]\hat{b}(0) + Z[f_1 + \bar{h}_1\bar{a}(0)]\hat{c}(0), \tag{45}$$

$$\hat{a}(1) = 0. \tag{46}$$

The above system can be reduced to an eigenvalue problem for $\hat{a}$ alone. We begin by solving (42) for $\hat{b}$ in terms of $\hat{a}$, making use of (20) to get:

$$\hat{b} = -\frac{h_0[1-\bar{b}(x)]}{\lambda - [h_0\bar{a}(x) + g_0 + f_0]}\hat{a} \tag{47}$$

$$= -\frac{h_0(g_0+f_0)}{[h_0\bar{a} + f_0 + g_0][\lambda - (h_0\bar{a} + g_0 + f_0)]}\hat{a}.$$

Similarly, we can obtain the following expression for $\hat{c}$ in terms of $\hat{a}$:

$$\hat{c} = -\frac{\bar{h}_1[1-\bar{c}(x)]}{\lambda - [\bar{h}_1\bar{a}(x) + g_1 + f_1]}\hat{a} \tag{48}$$

$$= -\frac{\bar{h}_1(g_1+f_1)}{[\bar{h}_1\bar{a} + f_1 + g_1][\lambda - (\bar{h}_1\bar{a} + g_1 + f_1)]}\hat{a}.$$

Next, we use (47) and (48) to eliminate $\hat{b}$ and $\hat{c}$ from (44) and (45) to get

$$\hat{a}'' + [\lambda - q_z(x,\lambda)]\hat{a} = 0 \tag{49}$$

$$\sigma_0\hat{a}'(0) + [\lambda - q_z(0;\lambda)]\hat{a}(0) = 0, \quad \hat{a}(1) = 0 \tag{50}$$

where

$$q_z(x,\lambda) = \frac{h_0(1-\bar{b})(g_0-\lambda)}{g_0 + f_0 + h_0\bar{a} - \lambda} + \frac{\bar{h}_1 Z(1-\bar{c})(g_1-\lambda)}{g_1 + f_1 + \bar{h}_1\bar{a} - \lambda}$$

$$= \frac{(f_0 + g_0)(g_0 - \lambda)}{(\alpha_0 + \bar{a})[\lambda_{c0}(x) - \lambda]} + \frac{Z(f_1 + g_1)(g_1 - \lambda)}{(\bar{\alpha}_1 + \bar{a})[\lambda_{c1}(x) - \lambda]}, \tag{51}$$

with

$$\lambda_{c0}(x) = g_0 + f_0 + h_0\bar{a}(x), \qquad \lambda_{c1}(x) = g_1 + f_1 + \bar{h}_1\bar{a}(x). \tag{52}$$

Note that $\lambda_{ck}(x) > 0$ are positive.

The eigenvalue problem (49) – (50) enables us to establish the following stability theorem for the unique steady-state solution $\bar{a}(x)$.

Theorem 7. *All eigenvalues of the nonlinear eigenvalue problem (49) and (50) are positive and the steady-state concentrations $\bar{a}(x)$, $\bar{b}(x)$ and $\bar{c}(x)$ are asymptotically stable by a linear stability analysis.*

The theorem is proved by modifying the argument used in Lander et al. (2005a) and will not be given here.

7. The smallest eigenvalue for the LLSR case

While knowing the eigenvalues being positive is sufficient to ensure the linear stability of the steady-state morphogen concentration gradients, it is important to determine the dependence of the eigenvalues on the biological parameters to gain more insight to the effects of non-receptors on the decay rate of transients. In particular, the magnitude of the smallest eigenvalue would give us some idea of how quickly the system returns to the steady state after small perturbations. As we do a great deal of computing for the time evolution of the concentration of both free and bound morphogens from their initial conditions, the rate of decay obtained will also provide an estimate of the time it takes to reach steady state. More importantly, there is currently only some rough estimates on the ranges of various rate constants for different biological systems. Knowing how the decay rate depends on the biological parameters will suggest ways for experimentalists to measure them more adequately. We will illustrate the benefits of knowing this dependence by working out some explicit results for the smallest eigenvalue in the LLSR case.

The *smallest eigenvalue* of (49) and (50), denoted by λ_s, generally can only be found by numerical methods. Accurate numerical solutions for the nonlinear eigenvalue problem is possible but tedious. For a sufficiently *low ligand synthesis rate* so that terms involving $h_0\bar{a}(x)$ and $\bar{h}_1\bar{a}(x)$ in $q_z(x;\lambda)$ may be neglected (see Section 4), a first approximation (or leading term perturbation solution) $\{\lambda^{(0)}, \hat{a}^{(0)}(x)\}$ of the exact solution of (49) and (50) is determined by the simpler eigenvalue problem:

$$[\hat{a}^{(0)}]'' + \eta^2\hat{a}^{(0)} = 0, \tag{53}$$

$$\{\sigma_0[\hat{a}^{(0)}]' - \eta^2[\hat{a}^{(0)}]\}_{x=0} = 0, \quad \hat{a}_0(1) = 0 \tag{54}$$

where

$$\eta^2 = \lambda^{(0)} - \frac{h_0(\lambda^{(0)} - g_0)}{\lambda^{(0)} - g_0 - f_0} - \frac{\bar{h}_1 Z(\lambda^{(0)} - g_1)}{\lambda^{(0)} - (f_1 + g_1)}. \tag{55}$$

The solution of (53) and (54) is given by $\hat{a}^{(0)}(x) = c_0 \sin(\eta(1-x))$ for an arbitrary constant c_0, with $\lambda^{(0)}$ being a solution of (55) where η is to be found from

$$\sigma_0 \eta = \eta^2 \tan(\eta). \tag{56}$$

The solutions of this equation depends only on σ_0 and no other parameter, particularly not on Z. Though $\eta = 0$ satisfies (56), it is not a solution of the eigenvalue problem (53) and (54) except for the limiting case $\sigma_0 = 0$, since there is not a corresponding nontrivial eigenfunction. (The $\sigma_0 = 0$ case was analyzed in Lou et al. (2004) where $\lambda_s^{(0)}$ was shown to be the smallest root of (55) with $\eta^2 = 0$.)

To find the smallest eigenvalue $\lambda_s^{(0)}$ for (53) and (54) for a prescribed $\sigma_0 > 0$, it would appear that we should solve (55) for each of the countably infinite number of solutions $\{\pm\eta_k\}$, $k = 1, 2, 3, \ldots$, of (56) (with η_k anti-symmetric about the origin, $(k-1)\pi < \eta_k < (k - \frac{1}{2})\pi$ and each increasing with σ_0) and then compare magnitude of the infinite number of smallest roots for different k. However, by differentiating both sides of the relation (55) with respect to η^2, we have immediately the following lemma on $\eta^2(\lambda^{(0)})$.

Lemma 2. *η^2 is a monotone increasing function of $\lambda^{(0)}$ and, conversely, $\lambda^{(0)}$ is a monotone increasing function of η^2.*

It follows from the lemma above that the smallest eigenvalue $\lambda_s^{(0)}$ of (53) and (54) is the smallest of the three roots of

$$\eta^2\big(\lambda^{(0)}\big) \equiv \lambda^{(0)} - \frac{h_0(\lambda^{(0)} - g_0)}{\lambda^{(0)} - g_0 - f_0} - \frac{\bar{h}_1 Z(\lambda^{(0)} - g_1)}{\lambda^{(0)} - f_1 - g_1} = \eta_1^2 < \left(\frac{\pi}{2}\right)^2. \tag{57}$$

Moreover, given $\sigma_0 \gg 1$ for Drosophila wing discs (see Lander et al., 2005b), the smallest eigenvalue $\lambda_s^{(0)}$ corresponds to $\eta = \eta_1 \lesssim \pi/2$ with $\eta_1 \to \pi/2$ from below as $\sigma_0 \to \infty$. We summarize these observations in the following theorem most relevant for *Dpp* in a Drosophila wing disc.

Theorem 8. *For $\sigma_0 > 0$, the smallest eigenvalue $\lambda_s^{(0)}$ of (53) and (54) is the smallest of the three roots of $\eta^2(\lambda^{(0)}) = \eta_1^2$ where η_1^2 is the smallest positive solution of (56) which depends only on σ_0 and tends to $(\pi/2)^2$ monotonically as $\sigma_0 \to \infty$.*

The above result made it unnecessary to solve a countably infinite number of cubic equations in order to find $\lambda_s^{(0)}$. However, the solutions of (57) depends on $h_0, g_0, f_0, \bar{h}_1, g_1, f_0$, and Z in a rather complicated way. It is of considerable interest to see that some of the critical features of $\lambda_s^{(0)}$ and the corresponding decay

rate of transients depend very simply on only a few of these parameters. We will illustrate this briefly by obtaining an upper bound and a lower bound for $\lambda_s^{(0)}$ below and show how they help us explain some experimental observations in Bellin et al. (2003) on the effect of non-receptors on the time to steady-state gradients.

Let $g_\ell \equiv \min\{g_0, g_1\}$ and $g_L \equiv \max\{g_0, g_1\}$. With $\eta^2(\lambda^{(0)} = 0) < 0$ and $\eta^2 \uparrow \infty$ as $\lambda^{(0)} \uparrow g_\ell + f_\ell$, we have from Lemma 2 the following theoretical useful upper bound for $\lambda_s^{(0)}$.

Lemma 3. $0 < \lambda_s^{(0)} < g_\ell + f_\ell$.

For the biologically realistic range of $g_\ell \leq \eta_1^2 \leq (\pi/2)^2$ (with $0 < g_\ell \ll 1$ for Dpp in Drosophila wing disc), we have $\eta^2(\lambda^{(0)} = g_\ell) \leq g_\ell \leq \eta_1^2$; from which follows $g_\ell \leq \lambda_s^{(0)}$ giving a lower bound for $\lambda_s^{(0)}$. We summarize the useful observations above in the following theorem.

Theorem 9. *At low morphogen synthesis rates and* $\min\{g_0, g_1\} \leq \eta_1^2 \leq (\pi/2)^2$, *we have* $\lambda_s \sim \lambda_s^{(0)}$ *with*

$$\min\{g_0, g_1\} < \lambda_s^{(0)} < \min\{g_0 + f_0, g_1 + f_1\}.$$

For $g_L > g_\ell + f_\ell$, we have also $\eta^2(\lambda^{(0)} = g_L) < g_L$. However, g_L is not a sharper lower bound for $\lambda_s^{(0)}$ since the lesser quantity $g_\ell + f_\ell$ is already an upper bound. Note that the upper and lower bound obtained above do not depend on $h_0, \bar{h}_1, \sigma_0$ and Z. It follows that the half life of transients must be shorter than the greater of $\ln(2)/K_{\text{deg}}$ and $\ln(2)/J_{\text{deg}}$ but must be longer than the greater of $\ln(2)/(K_{\text{deg}} + K_{\text{off}})$ and $\ln(2)/(J_{\text{deg}} + J_{\text{off}})$. For Drosophila, the off rate constants are typically much smaller than the corresponding degradation rate constants. Hence, these upper and lower bounds sharply delimit the time to a steady signaling gradient. With some extensive analysis, the same conclusion can be extended beyond the *LLSR* range and thereby offer a way of estimating the degradation rate constants (Mizutani et al., 2005).

When $g_1 \gg g_0$ ($J_{\text{deg}} \gg K_{\text{deg}}$), the presence of non-receptors is seen from Theorem 9 to have insignificant effect on the rate of transient decay. However, for $g_0 \gg g_1$ ($K_{\text{deg}} \gg J_{\text{deg}}$), the addition of non-receptors would slow down the decay rate significantly. Hence, only a lengthening (and not shortening) of the time to a steady-state gradient is associated with the presence of non-receptors. It is interesting that in real biological gradient systems, Dpp in a Drosophila early embryo that is known to operate very fast (with gradient formation time of about 30 min) happens to lack nondiffusive non-receptors (Bellin et al., 2003; Mizutani et al., 2005), whereas the Dpp gradient in the Drosophila wing disc that has a much slower dynamics (with a time to gradient of several hours) operates in the presence of a substantial level of HSPG (Teleman and Cohen, 2000; Fujise et al., 2003). With $K_{\text{deg}} \gg J_{\text{deg}}$ generally for Dpp in Drosophila, our mathematical results predict that a very fast gradient should lack (or have a low concentration of) nondiffusive non-receptors whereas the slower dynamics should operates in the

presence of a substantial level of these non-receptors. Both predictions are consistent with the little evidence we have on gradient dynamics of Drosophila cited previously.

8. Concluding remarks

In this paper, we initiated the first analytical study (not just numerical simulations such as Eldar et al., 2003) of the effects of membrane-bound non-receptor-mediated degradation on morphogen gradient formation and cell signaling. Considerable experimental evidence, recently reviewed in Lin (2004), shows that non-receptors have an important role in the formation and robustness of morphogen gradients and therefore deserve greater theoretical scrutiny. At the most basic level, non-receptors compete with receptors for binding with, and degradation of available morphogens; their presence is expected to reduce the steady-state concentration of signaling morphogen–receptor complexes throughout the wing disc interior. Analysis of the basic model, System N, for *Dpp* in a wing imaginal disc with non-receptors, formulated and analyzed herein, confirms this expected consequence. Results obtained also show that the introduction of non-receptors generally increases the convexity of the steady-state concentration gradients, at least in a region adjacent to the source end. For *LLSR* cases where the governing ODE and boundary conditions for the steady concentration of free morphogens $\bar{a}(x)$ can be linearized, asymptotic results show also that the addition of non-receptor sites reduces the negative slope of the gradients (making it less steep) and increases their convexity throughout the wing disc interior. However, it has the opposite effects of further steepening the relative slopes $\bar{a}'(x)/\bar{a}(x)$ and $\bar{b}'(x)/\bar{b}(x)$ which are of more interest to biologists.

The structure of the BVP governing the steady-state gradients and the explicit asymptotic solution for low synthesis rates show that the effects of a fixed concentration of non-receptor sites are generally negligible when the effective binding rate of non-receptors, g_1/α_1, is sufficiently small compared to that of the receptors, g_0/α_0, but is significant when g_1/α_1 is relatively large. This result provides an explanation for why in vivo receptor overexpression can have opposite effects in different morphogen systems. It can now be explained why overexpression of the *Dpp* receptor *tkv* in wing discs (Lecuit and Cohen, 1998) could result in nearly opposite observed effects from an overexpression of the Wg receptor *Dfz2* (Cadigan et al., 1998).

The results obtained in this paper on steady-state morphogen gradients in the presence of a single class of membrane-bound non-receptor can be extended to several species of such non-receptors. For two non-receptor species with the corresponding normalized degradation rates g_k, normalized effective loss–gain ratio $\bar{\alpha}_k$, and the ratio of non-receptor-to-receptor concentration ratios Z_k, the boundary value problem for the steady-state free *Dpp* concentration may be reduced to

$$\bar{a}'' = \frac{g_0\bar{a}}{\bar{a}+\alpha_0} + \frac{Z_1 g_1 \bar{a}}{\bar{a}+\bar{\alpha}_1} + \frac{Z_2 g_2 \bar{a}}{\bar{a}+\bar{\alpha}_2}, \tag{58}$$

with

$$-\sigma_0\bar{a}'(0)+\frac{g_0\bar{a}(0)}{\bar{a}(0)+\alpha_0}+\frac{Z_1g_1\bar{a}(0)}{\bar{a}(0)+\bar{\alpha}_1}+\frac{Z_2g_2\bar{a}(0)}{\bar{a}(0)+\bar{\alpha}_2}=\nu_0,\quad \bar{a}(1)=0. \tag{59}$$

More realistic two- and three-dimensional models that would allow for diffusion in the ventral-dorsal direction and the apical-basal direction can also be developed and analyzed as we did in Lander et al. (2003) and Vergas (2006). We will not pursue a discussion of these extensions since there are other more fundamental issues (e.g., stability) that need to be addressed.

As we may expect from our previous work on morphogen gradients (Lou et al., 2004; Lander et al., 2005a), steady-state morphogen gradients with non-receptors can also be shown to be asymptotically stable with respect to small perturbations. A more interesting theoretical finding on gradient dynamics pertains to the effects of non-receptors on the time to steady-state behavior. Introduction of nondiffusive non-receptors generally does not speed up and often substantially slows down the time to gradient formation for our model, offering the presence of HSPG non-receptors as a likely cause for the slower dynamics of Dpp gradient in Drosophila wing disc (Teleman and Cohen, 2000; Fujise et al., 2003) and, less directly, their absence for the fast dynamics of Dpp gradient in the early Drosophila embryo (Bellin et al., 2003; Mizutani et al., 2005). When applicable, the simple relation between decay rate of transients and degradation rate constants of receptors and nondiffusive non-receptors offers a way of estimating the latter as done in Mizutani et al. (2005).

As pointed out in Section 1 of this paper, the main thrust for our interest in non-receptors is their possible role in the robustness of morphogen gradients during development to changes in system parameters including those induced by environmental fluctuations. We know from Lander et al. (2005c); Lei et al. (2005) that non-receptors constitute a significant mechanism for robustness. It is therefore important to acquire sufficient theoretical understanding of their effects on gradient formation which we have attempted to do in this paper.

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