

# Can morphogen activity be enhanced by its inhibitors?

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## Abstract

Biological experiments show that the DPP inhibitor SOG actually increases DPP activity along the mid-line of the *Drosophila* embryo. This counterintuitive behavior is investigated via analysis and numerical simulations of two ligand-receptor models, one with and one without SOG. It is shown that these simple models provide a mathematical explanation for the effect of SOG: DPP ligand is stored and transported as bound DPP-SOG complex, then released near the mid-line to create the observed peaked morphogen activity.

**Keywords:** Morphogen gradient; Ligand-receptor; DPP; SOG

## 1. Introduction

Morphogens are secreted signaling molecules that organize a field of surrounding cells into patterns. They form a gradient of concentration and determine the fate of responding cells according to the different concentrations of the morphogen perceived by the cells [1]. Some of the most interesting and best-analyzed examples are the morphogen-mediated events that occur during *Drosophila* development. In the fly embryo, high levels of the BMP-like (Bone Morphogenetic Protein) ligand DPP (Decapentaplegic) are responsible for specifying the dorsal-most cell fates. Curiously, establishing this concentration of DPP activity requires the activity of several secreted proteins including a secreted factor SOG (short gastrulation) that inhibits DPP.

Mutation of SOG results in a decrease of DPP activity [2]. Although there are other participants in this system, such as the co-inhibitor TSG, extracellular protease TLD and a second ligand SCW, in this paper we only address the effect of SOG, an apparent inhibitor of DPP, which can lead to a local elevation of DPP signaling.

We use a ligand-receptor model [3] to describe the DPP-SOG system, in which DPP is produced uniformly over the dorsal half of the embryo and SOG is produced in the ventral half of the embryo. DPP activity is measured

by the concentration of DPP-receptor complexes. Then the questions at hand are: (a) does this simple model allow a higher concentration of the bound DPP around the mid-line of the dorsal region with the presence of SOG (a DPP inhibitor) than without SOG? (b) if so, under what conditions does this happen? and (c) is it robust?

In Section 2, we examine a system with only DPP and the receptor. In Section 3, we add SOG to the system. In Section 4, we study the effect of SOG. In Section 5, we present some discussions and conclusions.

## 2. DPP-only

Consider a ligand-receptor model [3] on a ring of cells (a cross section of the *Drosophila* embryo) where ligand is produced in the top half only. Reversible binding, diffusion and degradation of the bound ligand occur. The equations governing this system are

$$\frac{\partial [L]}{\partial T} = D_L \frac{\partial^2 [L]}{\partial X^2} - K_{on}[L](R_t - [LR]) + K_{off}[LR] + V_L \quad (1)$$

$$\frac{\partial [LR]}{\partial T} = K_{on}[L](R_t - [LR]) - (K_{off} + K_{deg})[LR] \quad (2)$$

where  $[L]$  and  $[LR]$  are concentrations of free ligand and receptor-bound ligand,  $D_L$  is the diffusion constant,  $V_L$  is ligand production rate, and  $K_{on}$ ,  $K_{off}$ ,  $K_{deg}$  are association, disassociation and degradation rates, respectively. Initial

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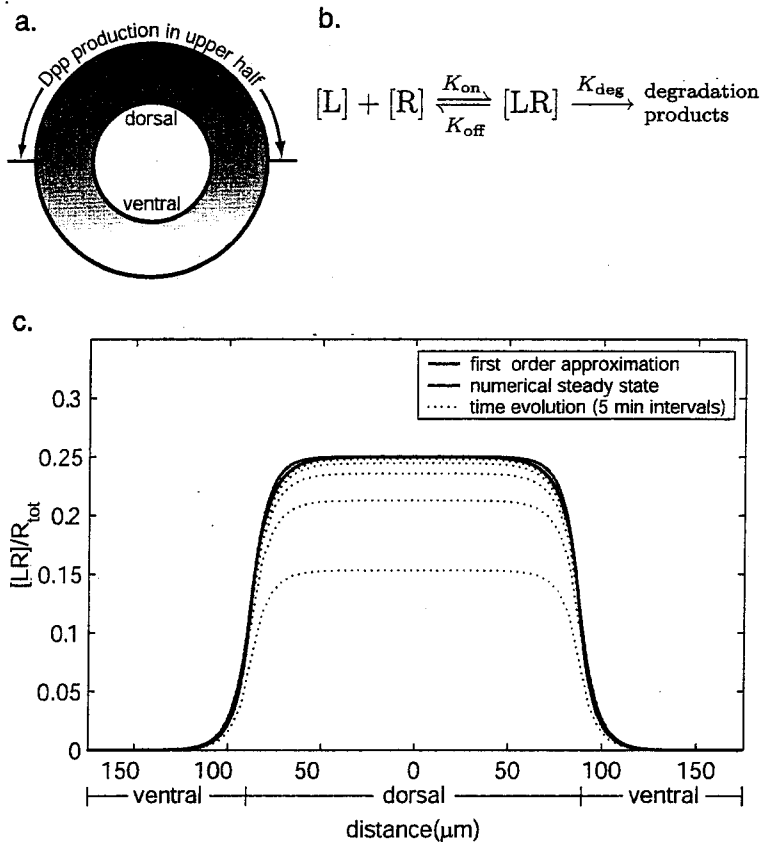


Fig. 1. DPP-only system. (a) Cross section of *Drosophila* embryo and region of DPP production. (b) Reactions and rate constants for this system. We allow diffusion of the free DPP ligand, reversible binding, and degradation of bound DPP. (c) Numerical and perturbation solutions for steady state and time evolution of the system. (Parameters:  $X_{\max} = 0.0175$  cm,  $D_L = 2 \times 10^7$  cm<sup>2</sup> s<sup>-1</sup>,  $V_L/R_t = 8 \times 10^4$  s<sup>-1</sup>,  $K_{\text{on}}R_t = 0.4$  s<sup>-1</sup>,  $K_{\text{off}} = 4 \times 10^{-6}$  s<sup>-1</sup>,  $K_{\text{deg}} = 3.2 \times 10^{-3}$  s<sup>-1</sup>.)

concentrations are zero ( $[\cdot] = 0$  at  $T = 0$ ) and boundary conditions are periodic with the ring cut artificially at the ventral mid-line. The properties of the steady-state solutions of this system can be studied similarly as those in [3,4].

Numerical steady-state solutions for [LR] were computed using the COLNEW package [5] and full, time-dependent solutions were computed with a standard finite difference method. Steady-state, time-dependent, and perturbation solutions for a typical case are shown in Fig. 1. It can be seen from Fig. 1 that the "gradient" resulting from this morphogen system is very flat in the center of both dorsal and ventral regions, with the only substantial change in [LR] occurring at the border junctions of the dorsal and ventral regions.

### 3. Effects of SOG

We now consider the reaction-diffusion system with inhibitor SOG that binds to the original ligand, but not

to the receptor. In this model the degradation of the bound complex [LS] involves the destruction of the inhibitor SOG, [S], while the original ligand [L] is released intact [6,7]. As before, ligand [L] is produced in the upper half, and the new factor [S] is produced in the lower half (Fig. 2). This is the simplified *Drosophila* system discussed in the introduction.

The corresponding differential equations are:

$$\frac{\partial [L]}{\partial T} = D_L \frac{\partial^2 [L]}{\partial X^2} - K_{\text{on}}[L](R_t - [LR]) - J_{\text{on}}[L][S] + K_{\text{off}}[LR] + (J_{\text{off}} + J_{\text{deg}})[LS] + V_L \quad (3)$$

$$\frac{\partial [LR]}{\partial T} = K_{\text{on}}[L](R_t - [LR]) - (K_{\text{off}} + K_{\text{deg}})[LR] \quad (4)$$

$$\frac{\partial [LS]}{\partial T} = D_{LS} \frac{\partial^2 [LS]}{\partial X^2} + J_{\text{on}}[L][S] - (J_{\text{off}} + J_{\text{deg}})[LS] \quad (5)$$

$$\frac{\partial [S]}{\partial T} = D_S \frac{\partial^2 [S]}{\partial X^2} - J_{\text{on}}[L][S] + J_{\text{off}}[LS] + V_S \quad (6)$$

Again, all concentrations start at zero ( $[\cdot] = 0$  at  $T = 0$ ) and the boundary conditions are periodic.

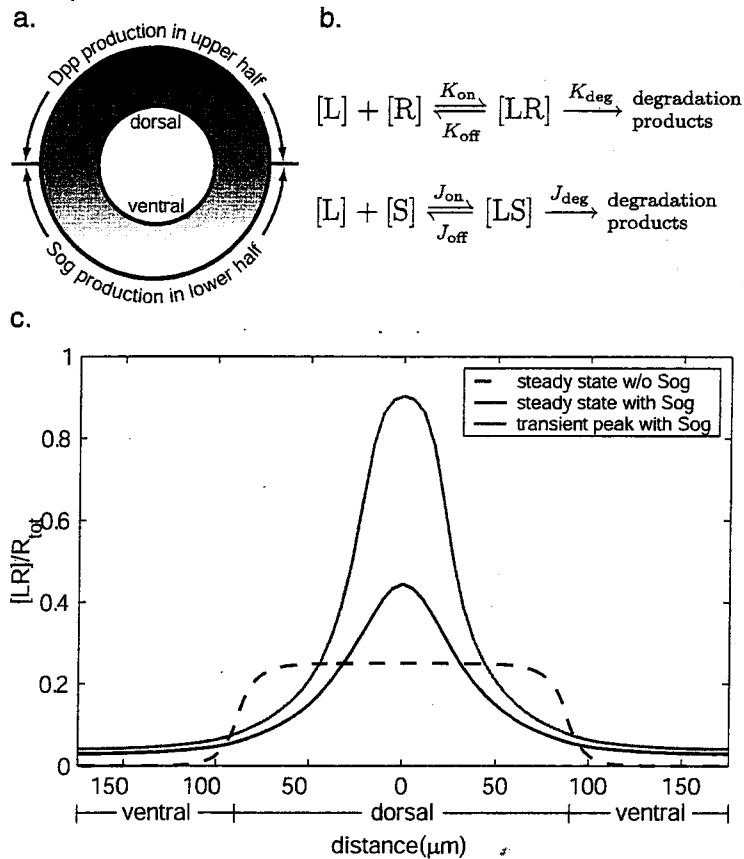


Fig. 2. The system with the addition of SOG. (a) Cross section of *Drosophila* embryo and regions of DPP and SOG production. (b) Reactions and rate constants. In addition to the reactions in Fig. 1b, we add diffusible SOG with reversible DPP–SOG binding and degradation of bound SOG. (c) Steady-state and transient gradients of bound DPP, compared to the system without SOG. (Parameters:  $D_{LS} = 2 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ ,  $V_S/R_t = 6 \times 10^{-2} \text{ s}^{-1}$ ,  $J_{on}R_t = 10 \text{ s}^{-1}$ ,  $J_{off} = 1 \times 10^{-5} \text{ s}^{-1}$ ,  $J_{deg} = 6.0 \times 10^{-2} \text{ s}^{-1}$ . Other parameters as in Fig. 1.)

From a simple analysis of the system, it can be shown that the balance between DPP production and degradation at steady-state requires  $\int K_{deg}[LR]dx = \int V_L(x)dx$ . Hence the average concentration of receptor-bound DPP is  $V_L/2K_{deg}$ , independent of the presence of SOG – the inhibitor’s effect on bound DPP in steady-state is a spatial redistribution, not an increase or decrease in quantity. A comparison of steady-state between DPP-only and DPP-with-SOG systems is shown in Fig. 2c.

Unlike the previous system though, DPP-with-SOG has considerably more complicated dynamics. In particular, for a certain (fairly broad) range of reaction rates and other parameters, the bound ligand concentration forms a transient peak that is higher than its steady-state (see Fig. 3). More interestingly, the transient peak with SOG is significantly higher than that of the system without SOG and some of the steady-state peaks with SOG are also higher than peaks in the system without SOG (Fig. 2c). This will be discussed in detail in the next section.

#### 4. Peak formation

When SOG is destroyed in the cleavage (or degradation) of the DPP–SOG complex, it is possible for the two-ligand system to give rise to a peaked gradient in [LR], with higher concentrations than for the single-ligand system without SOG (Figs. 2 and 3). This counterintuitive result occurs both as a transient effect and, to a lesser extent, in steady-state.

##### 4.1. How does SOG raise the peak?

For both transient and steady-states, the peaked shape of the gradient is due to the transport of DPP by SOG through diffusion of DPP–SOG. The transient peak differs from the steady-state peak in that it is sharper and has peak concentrations of [LR] that are often *higher* by a factor of two or more (Fig. 3). Here is an outline of the dynamics of this system.

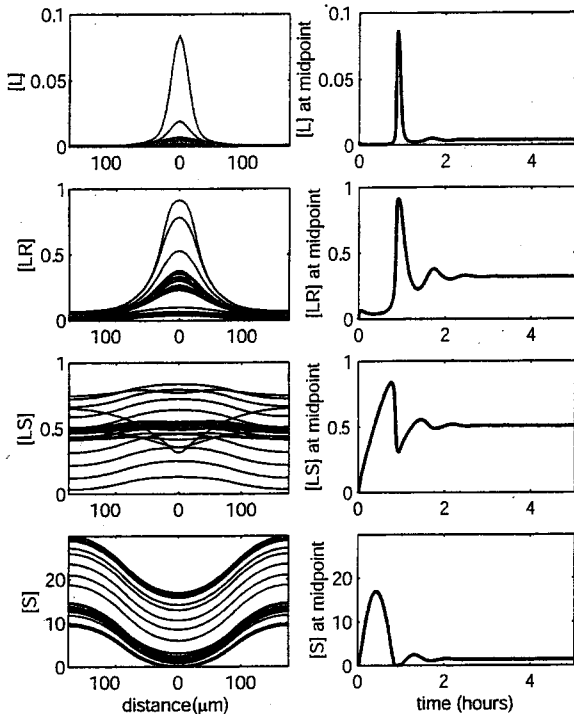


Fig. 3. Typical time evolution of the system with SOG. Note that initially nearly all DPP produced is taken up as DPP-SOG. Since only SOG is destroyed in the degradation of this complex, a stockpile of DOG is built up, leading to a substantial (although transient) DPP-receptor gradient when SOG runs out. Likewise, even in steady state, the spatial inhomogeneity in SOG production leads to DPP build-up in the center of the dorsal half. (Parameters:  $J_{on}R_t = 6 \text{ s}^{-1}$ . Other parameters as in Fig. 2.)

- (1) If  $J_{on} \gg K_{on}$ , free DPP will preferentially bind to SOG rather than to receptors. [L] and [LR] remain small while [LS] increases, particularly in the ventral due to the availability of SOG.
- (2) As [LS] has accumulated in the ventral, it starts to diffuse to the dorsal. As a result, [LS] increases in the dorsal.
- (3) Because the SOG is continuously produced in the ventral, an excess of SOG allows rapid re-forming of DPP-SOG as long as DPP is available (due to either the degradation of SOG-DPP or diffusion of DPP from the dorsal).
- (4) Since the degradation of [LS] destroys SOG but releases DPP intact in the dorsal region, eventually there is not enough SOG for DPP to reform SOG-DPP. Instead, the slower reaction  $[L] + [R] \rightarrow [LR]$  takes over. Consequently, a sharp gradient  $cLR$  around the mid-line of the dorsal.
- (5) Given parameters that are considered to be biologically feasible, this process typically occurs on the time-scale of one hour.

## 4.2. Robustness

The DPP-SOG system is robust with regard to changes in production and reaction rates. Some plots of peak heights corresponding to various sets of parameters are shown in Fig. 4. Keeping constants related to DPP unchanged, we find that increasing  $J_{deg}$  increases the consumption rate of SOG and therefore decreases the time to transient peak, but also decreases the height of the peak since less DPP-SOG is stockpiled. Likewise, increasing  $V_S$  allows more DPP-SOG to be created, which increases the time to a transient peak but also increases its height. Finally, the formation rate of DPP-SOG,  $J_{on}$ , governs the amount of interaction between DPP and SOG. A larger  $J_{on}$  increases the effect of SOG, raising the peak. This occurs almost linearly for the steady-state peak (Fig. 4a,c) but *nonlinearly* for the transient peak (Fig. 4b,d). Another effect of  $J_{on}$ , not visible in Fig. 4, is an increased "ringing" after the maximum transient peak. This is seen in the time plots of Fig. 3.

## 4.3. Requirements for peak formation

Based on the above observations of the mechanisms involved and the effects of parameter changes, it appears that the following conditions are necessary for the formation of a peaked gradient:

- (1)  $J_{on} \gg K_{on}$  and  $V_S \gg V_L$ . The formation of [LS] must dominate  $[L] + [R]$  binding. This is important for both steady-state and transient peaks to be higher than peaks in the system without SOG.
- (2)  $J_{deg}$  should be small, but not zero. A small  $J_{deg}$  allows large amounts of [LS] to accumulate, which is essential for a tall transient peak.

## 5. Conclusion

We have presented here two ligand-receptor models for morphogen activity in *Drosophila*, first with DPP alone, then with the addition of its inhibitor SOG. Our analysis and numerical simulations suggest that the presence of SOG allows for the formation of a peaked gradient and in fact an increase of morphogen activity on the mid-line of the *Drosophila* embryo, both at steady-state and with a large transient peak. In particular, the height of the steady-state [LR] peak is nearly *linearly* increasing in  $J_{on}$ , and uniform in  $J_{deg}$  and the transient peak is (*nonlinearly*) increasing in  $J_{on}$ .

Although our assumption of degradation of DPP-SOG into free DPP may be a mathematical simplification of the biological reality [6,7], preliminary simulations of a more complex model show that involving TSG (Twisted gastrulation), SCW (Screw) and TLD (Tolloid), for example, does not change the fundamental features of this system. A detailed discussion will be presented in a separate paper.

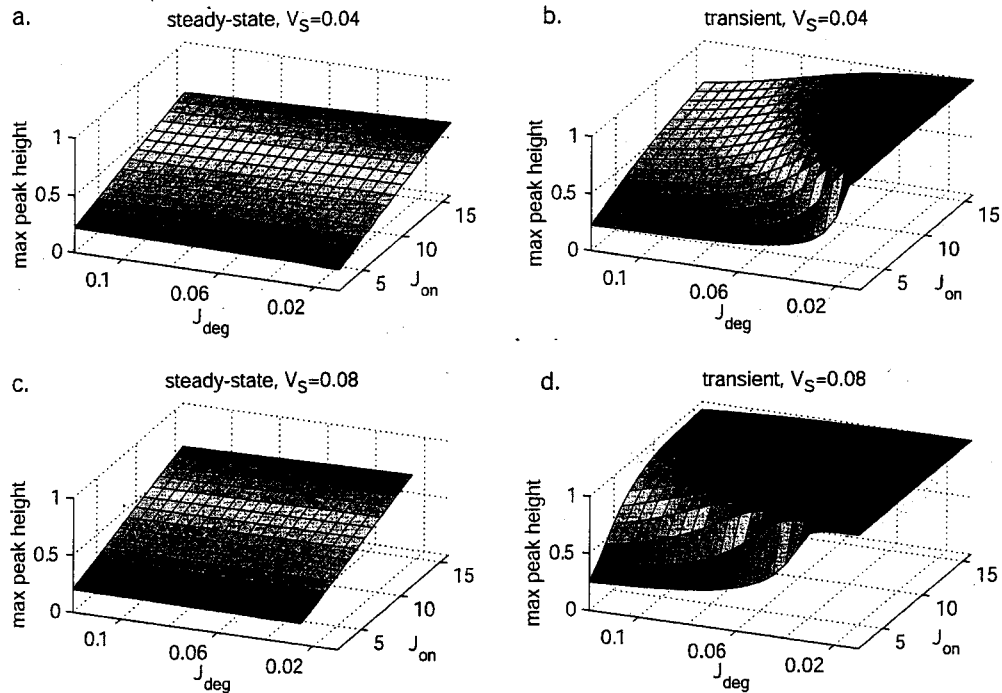


Fig. 4. The height of steady-state and transient peaks as a function of  $J_{on}$  and  $J_{deg}$ . (The corresponding DPP-only system has a steady-state peak of 0.25, as in the previous figures.) It can be seen that in the parameter ranges examined, the steady-state peak height is nearly linear in  $J_{on}$  and unaffected by changes in  $J_{deg}$  and  $V_S$ , while the transient peak height increases nonlinearly with  $J_{on}$  and seems to be stretched in  $J_{deg}$  by the increase in  $V_S$ .

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