Robust Pattern in Development II

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Life cycle of the fruit fly (Drosophila melanogaster)
Gastrulation of the fruit fly embryo

- After cellulization of the syncytium, gastrulation starts, resulting in a cylinder layer structure, in which both anterior-posterior and ventral-dorsal axes are defined.

- Three distinct cell types are arranged on the periphery of the cylinder in three regions, namely, DR (dorsal), NE (neuroectoderm) and VE (ventral).
Sub-patterning of the DR region

During the subsequent development, DR is further sub-divided into sub-regions.

An interaction network of three protein molecules determines the fate of the DR cells.

1. The morphogen Scw (M): produced by all cell types in the embryo, its concentration gradient specifies different cell fates.

2. The inhibitor protein Sog (I): produced by NE cells, it inhibits the action of M;

3. The protease Tld (P): produced by DR cells, it degrades I.

![Interaction network diagram]
Some facts about the interaction network

- I inhibits M by forming a complex, MI;
- P cleaves I in the complex MI, thereby releasing M;
- M is degraded very slowly;
- I, P and M diffuse in the perivitelline fluid, a thin fluid layer outside of the cells.

![Diagram of the interaction network]

- P
- I
- M

![Diagram of the perivitelline fluid]

- DR
- NE
- VE
- Perivitelline fluid
The activity of M is robust to levels of M, I and P

- The free form of M has a high concentration in the middle of DR, and a low concentration at the two ends. This distribution is important for the sub-patterning of DR.

- The regulatory effects of P and I on M lead to a very robust distribution of M across the DR region. How is this achieved?

- Let’s first consider a simple mechanism.

![Diagram showing the distribution of M, P, and I across DR with high concentration in the middle and low at the ends.](image)
The simple mechanism for the distribution of M

- The high concentration of M in the middle of DR can be explained by the following facts:

1. The inhibitor I is produced at the boundaries between DR and NE, i.e., at $x = -1$ and $x = 1$.

2. As I is degraded by P that is evenly distributed, its concentration decays into the dorsal region.

3. Free morphogen, thus has the highest concentration at the center of the region, at $x = 0$.
The simple mechanism for the distribution of M is not robust

- Reduced production of P, I and M greatly affects the concentration profile of M, thus this simplest mechanism cannot explain the robustness of M’s activity in DR sub-patterning.
A mechanism for the robustness of M activity in DR sub-patterning

- Since M is not appreciably degraded, so the self-enhanced degradation is not likely the mechanism underlying the robustness of M in DR sub-patterning.

- Let’s consider the general equations that govern the behavior of I, P and M.

Assume that free I is degraded by P at a rate $\alpha_I$, and I binds M to form a stable complex $[IM]=C$, at a rate $k$, then,

$$\frac{\partial I}{\partial t} = D_I \frac{\partial^2 I}{\partial x^2} - kIM - \alpha_I PI.$$ 

Assume $[IM]$ is degraded by P at a rate $\alpha_C$, then,

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} + kIM - \alpha_C PC.$$
A mechanism for the robustness of M activity in DR sub-patterning

- Since M binds I at rate k, and is released when C is degraded by P, therefore,

\[
\frac{\partial M}{\partial t} = D_M \frac{\partial^2 M}{\partial x^2} - kIM + \alpha_C PC.
\]

- However, it is very difficulty to solve these equations analytically, so Eldar and Barkai studied them numerically.

Given a set of parameter values \((D_I, D_P, D_M, \alpha_I, \alpha_C, \text{ and } k)\), they computed the profiles of P, I and M.

The robustness of each set of values were evaluated by twofold change in the production rate of P, I, and M.

They found that most combination of the values of these parameters gave no robust solution.
A mechanism for the robustness of M activity in DR sub-patterning

However, a small set of parameter combinations did give robust activity of M.

These parameter set all belong to the same limiting class, in which some parameters are much smaller than the others.

Specifically, they found that:

1. Free M moves much slower than the bound form of M, C:
   \[ D_C >> D_M = 0. \]

2. Free I is not degraded by P, and only I in the complex C is degraded by P:
   \[ \alpha_C >> \alpha_I = 0. \]
If we assume $\alpha_I = 0$ and $D_M = 0$, then the original set of dynamics equations can be easily solved analytically at steady states.

\begin{align*}
1) \quad & \frac{\partial I}{\partial t} = D_I \frac{\partial^2 I}{\partial x^2} - kIM = 0, \\
2) \quad & \frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} + kIM - \alpha_C PC = 0, \\
3) \quad & \frac{\partial M}{\partial t} = -kIM + \alpha_C PC = 0.
\end{align*}

Add the two sides of 2) and 3), we have,

\[ D_C \frac{\partial^2 C}{\partial x^2} = 0. \]
A mechanism for the robustness of M activity in DR sub-patterning

- The general solution of \( D_C \frac{\partial^2 C}{\partial x^2} = 0 \) is \( C(x) = ax + C_0 \).

Since there are two symmetrical sources of the production of I, the concentration of C is actually spatially uniform, i.e.,

\[ C(x) = C_0. \]
A mechanism for the robustness of M activity in DR sub-patterning

- Substitute $C(x)=C_0$ into 3): $-kIM + \alpha_C PC = 0$, we have,

$$kIM = \alpha_C PC_0, \quad I = \frac{\alpha_C PC_0}{k} \cdot \frac{1}{M}.$$ 

- Substitute this expression for $I$ in 1): \[
\frac{\partial I}{\partial t} = D_I \frac{\partial^2 I}{\partial x^2} - kIM = 0,
\]

we have,

$$D_I \frac{\partial^2}{\partial x^2} \left( \frac{\alpha_C PC_0}{k} \cdot \frac{1}{M} \right) - \alpha_C PC_0 = 0,$$

$$\frac{\alpha_C PC_0 D_I}{k} \frac{\partial^2}{\partial x^2} \left( \frac{1}{M} \right) = \alpha_C PC_0,$$

$$\frac{\partial^2 M^{-1}}{\partial x^2} = \frac{k}{D_I}.$$
A mechanism for the robustness of M activity in DR sub-patterning

\[ \frac{\partial^2 M^{-1}}{\partial x^2} = \frac{k}{D_I}, \]

\[ \frac{\partial}{\partial x} \left( \frac{\partial M^{-1}}{\partial x} \right) = \frac{k}{D_I}, \]

\[ \frac{\partial M^{-1}}{\partial x} = \frac{k}{D_I} x + C_1, \]

\[ M^{-1} = \frac{k}{2D_I} x^2 + C_1 x + C_2, \]

\[ M^{-1} = \frac{k}{2D_I} \left( x^2 + \frac{2D_I C_1}{k} x + \frac{2D_I C_2}{k} \right), \]

\[ M = \frac{2D_I / k}{x^2 + \frac{2D_I C_1}{k} x + \frac{2D_I C_2}{k}}. \]

Let's find \( C_1 \) and \( C_2 \) under the boundary conditions:

\[ x=0, \quad M(0) = M_{tol}, \quad \text{and} \]

\[ \left. \frac{\partial M}{\partial x} \right|_{x=0} = 0. \]
A mechanism for the robustness of M activity in DR sub-patterning

From $\frac{\partial M^{-1}}{\partial x} = \frac{k}{D_I} x + C_1$, we have,

$$- \frac{1}{M^2} \frac{\partial M}{\partial x} = \frac{k}{D_I} x + C_1,$$

$$- \frac{1}{M_{\text{tol}}} \cdot 0 = \frac{k}{D_I} \cdot 0 + C_1,$$

$$C_1 = 0.$$

Therefore, $M = \frac{2D_I/k}{x^2 + \frac{2D_I}{k} C_1 x + \frac{2D_I}{k} C_2} = \frac{2D_I/k}{x^2 + \frac{2D_I}{M_{\text{tot}} k}} = \frac{2D_I/k}{x^2 + \frac{2D_I}{M_{\text{tot}} k}}$

$$= \frac{A}{x^2 + \varepsilon^2}, \text{ where } A = 2D_I/k, \text{ and } \varepsilon = \sqrt{A/M_{\text{tot}}}. \quad (\text{Equation 1})$$
A mechanism for the robustness of M activity in DR sub-patterning

- When $M_{tot}$ is significantly high, then,

$$M \approx \frac{A}{x^2}.$$ 

- In addition, at positions far away from the middle line, $x > > \varepsilon$,

$$M \approx \frac{A}{x^2}.$$ 

- Therefore, the profile of M become a power law, and is not dependent on the concentration of P, I or M.

- Thus, M is robust to the change in these parameters.
The molecular mechanism of the robustness of M activity in DR sub-patterning

- This mechanism of robustness is achieved through the shuttling of M by I.
- M cannot move until it is bound by I: $D_C >> D_M = 0$.
- Since I is high at the boundary, so the complex C=MI is pushed into DR.
- P does not work on free I: $\alpha_I = 0$.
- Once I in MI is degraded, M is released and deposited in DR. Thus M is accumulated high in the middle of DR.
Summary of the mechanisms of the robustness morphogen function

- We have discussed two distinct mechanisms of robustness of morphogen function: self-enhance degradation and regulated molecule shuttling and degradation.

- Surprisingly, they all result in a power law of concentration along the developmental axis:

\[ M \approx \frac{A}{x^2}. \]

- These modeling works can help identify correct molecular mechanisms of biological functions, e.g. the fact that \( M \) cannot move until it binds to \( I \) was experimentally discovered after Eldar and Barkai’s modeling work.