BE 159: Signal Transduction and Mechanics in Morphogenesis

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7 Delta-Notch signaling

In the previous lecture, we investigated the large-scale effects of coupled genetic oscillators. In the homework, we will expore a simple model for how genetic oscillations may arise. Today, we will discuss a signaling pathway that enables genetic oscillations in one cell can be coupled to oscillations in its neighbor. The **Delta-Notch** signaling system is responsible for coupling cells in the presomitic mesoderm in developing zebrafish embryos. We will explore how coupling leads to synchronization of oscillation in the homework. Here, we will discuss some of the more generic features of the Delta-Notch system and introduce techniques for modeling it.

7.1 Molecular biology of the Delta-Notch signaling system

Delta-Notch signaling provides a mechanism for neighboring cells to communicate with each other. The molecular mechanism is shown in Fig. 10. Notch is a transmembrane protein that is the receptor for another transmembrane protein Delta. When a cell is expressing Notch and its neighbor is expressing Delta, Delta binds Notch, which results in a conformational change. This enables proteolytic cleavage of Notch, resulting in the Notch intracellular domain (Nicd) detaching from the membrane complex. Nicd acts as a transcription factor. It is a co-activator with Mastermind and a co-repressor with hairless, in addition to having other binding partners that control gene expression.

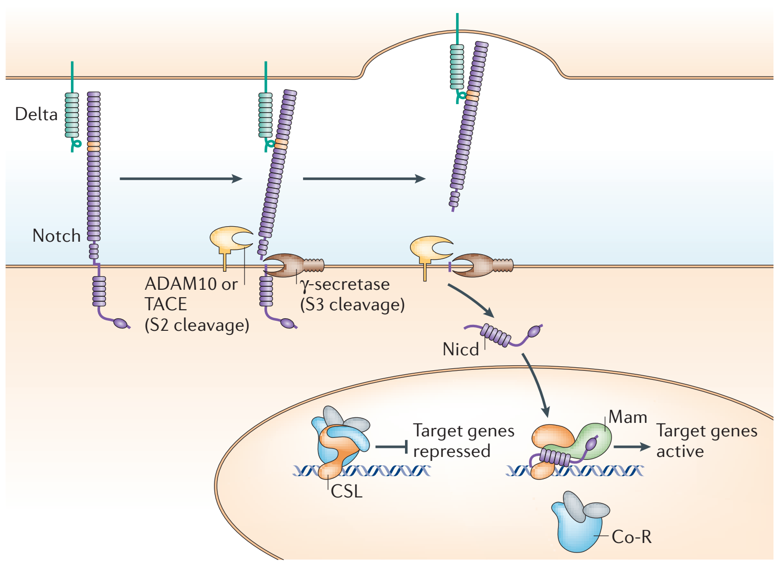

Figure 10: Sketch of the molecular details of Delta-Notch signaling. The insides of neighboring cells are shown in brown and the intercellular space is shown in light blue. Taken from Bray, *Nat. Rev. Mol. Cell Biol.*, **7**, 678–689, 2006.

Importantly, Nicd represses production of Delta. So, a cell that has a lot of cleaved Notch will stop producing Delta. Thus, a cell expressing a lot of Delta will suppress Delta expression in the neighboring cell by activating Notch in the neighbor. A schematic of this process is shown in Fig. 11.

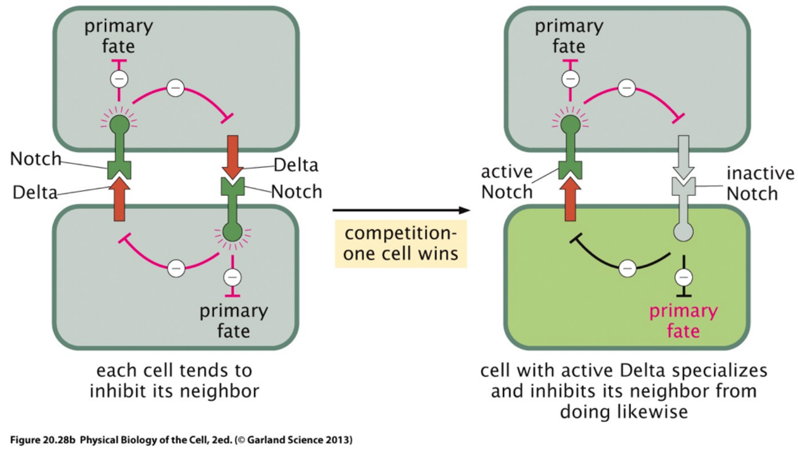

Figure 11: Schematic of nearest-neighbor cell differentiation by Delta-Notch. Delta expressed by the bottom cell activates Notch in the top cell. The activated Notch in the top cell suppresses Delta in the top cell. Because there is no Delta on the surface of the top cell, Notch is inactive in the bottom cell. Since Notch is inactive, Delta continues being expressed in the bottom cell.

So, the Delta-Notch system enables a cell to access a cell fate *and* instruct its neighbors *not* to access the same fate.

7.2 Mathematical analysis of the Delta-Notch system

We will develop a simple model to describe the dynamics of the Delta-Notch signaling between two nearest-neighbor cells. Let N_1 be the number of active Notch molecules in cell 1 and D_1 be the number of Delta molecules, with N_2 and D_2 similarly defined. We then write the dynamics as

$$\frac{\mathrm{d}N_1}{\mathrm{d}t} = F(D_2) - \gamma_N N_1 \tag{7.1}$$

$$\frac{\mathrm{d}D_1}{\mathrm{d}t} = G(N_1) - \gamma_D D_1 \tag{7.2}$$

$$\frac{\mathrm{d}N_2}{\mathrm{d}t} = F(D_1) - \gamma_N N_2 \tag{7.3}$$

$$\frac{\mathrm{d}D_2}{\mathrm{d}t} = G(N_2) - \gamma_D D_2. \tag{7.4}$$

We have defined γ_N and γ_D to be the respective autodegradation rates of Notch and Delta. The function $F(D)$ describes how the Delta level in a neighboring cell affects the Notch level. This function should be monotonically increasing, since more Delta implies more active Notch. The function $G(N)$ describes how the level of active Notch in a cell affects its Delta level. Since Notch represses Delta, this should be monotonically decreasing.

7.2.1 Nondimensionalization

As usual, we will nondimensionalize these dynamical equations. We define the following, with dimensionless quantities being either lowercase or marked with a tilde.

$$t = \tau \tilde{t} \tag{7.5}$$

$$G(N_2) = G_0 g(N_2/N_0) \tag{7.6}$$

$$F(D_2) = F_0 f(D_2/D_0) \tag{7.7}$$

$$N_1 = N_0 n_1 \tag{7.8}$$

$$D_1 = D_0 d_1, \tag{7.9}$$

with other variables similarly defined. After substitution and rearrangement, we get

$$\dot{n}_1 = \frac{F_0 \tau}{N_0} f(d_2) - \gamma_N \tau n_1 \tag{7.10}$$

$$\dot{d}_1 = \frac{G_0 \tau}{D_0} g(n_1) - \gamma_D \tau d_1 \tag{7.11}$$

$$\dot{n}_2 = \frac{F_0 \tau}{N_0} f(d_1) - \gamma_N \tau n_2 \tag{7.12}$$

$$\dot{d}_2 = \frac{G_0 \tau}{D_0} g(n_2) - \gamma_D \tau d_2, \tag{7.13}$$

where the over-dot indicates differentiation by $\tilde{t}$. Now, we choose $\tau = \gamma_N^{-1}$ and N_0 and D_0 such that $\lim_{d\to\infty} f(d) = 1$ and $g(n = 0) = 1$. We further choose $F_0 = N_0/\tau$ and $G_0 = D_0/\tau$. With these choices, we have

$$\dot{n}_1 = f(d_2) - n_1 \tag{7.14}$$

$$\dot{d}_1 = \nu\,(g(n_1) - d_1) \tag{7.15}$$

$$\dot{n}_2 = f(d_1) - n_2 \tag{7.16}$$

$$\dot{d}_2 = \nu\,(g(n_2) - d_2)\,, \tag{7.17}$$

where we are left with a single parameter, $\nu = \gamma_D/\gamma_N$, the ratio of the decay rates of Delta and Notch.

7.2.2 Homogeneous steady state

We are interested in knowing if these two neighboring cells can differentiate from each other. We therefore wish to find a homogeneous steady state, $n_1 = n_2 = n_0$ and $d_1 = d_2 = d_0$, and test its stability. If this homogeneous steady state is unstable, we expect the cells to be able to differentiate. If it is stable, they cannot spontaneously differentiate.

To find the steady state, we solve the system of equations with all time derivatives set to zero. I.e., we wish to solve

$$f(d_0) - n_0 = 0, \tag{7.18}$$

$$g(n_0) - d_0 = 0. \tag{7.19}$$

The first equation gives $n_0 = f(d_0)$, so the second equation tells us we must have $g(f(d_0)) = d_0$. We will write $g(f(x))$ as $gf(x)$, where $gf(x)$ is called the *composition* of the functions g and f. Now, $f(x)$ is a monotonically increasing function and $g(x)$ is a monotonically decreasing function, so $gf(x)$ is a monotonically decreasing function. So we have that $gf(d_0)$ is monotonically decreasing toward zero while the function $h(d_0) = d_0$ is monotonically increasing from zero. This means that these two functions cross exactly once, so there exists a *unique* homogeneous steady state.

7.2.3 Linear stability analysis

We saw linear stability analysis in section 2.5.3. We will perform the same type of analysis on the Delta-Notch dynamical system. Let n_0, d_0 be the homogeneous steady state. We take a small perturbation off of this steady state such that

$$n_1 = n_0 + \delta n_1 \tag{7.20}$$

$$d_1 = d_0 + \delta d_1 \tag{7.21}$$

$$n_2 = n_0 + \delta n_2 \tag{7.22}$$

$$d_1 = d_0 + \delta d_2, \tag{7.23}$$

where $\mathbf{u} \equiv (\delta n_1, \delta d_1, \delta n_2, \delta d_2)$ is a small perturbation about the homogeneous steady state. We expand the functions $f(d)$ and $g(n)$ to first order in the perturbation.

$$f(d_2) = f(d_0) + f'(d_0)\,\delta d_2 + \mathcal{O}\left((\delta d_2)^2\right), \tag{7.24}$$

$$g(n_1) = g(n_0) + g'(n_0)\,\delta n_1 + \mathcal{O}\left((\delta n_1)^2\right), \tag{7.25}$$

and so on. We define $f_0 = f'(d_0)$ and $g_0 = g'(n_0)$ for notational convenience. Then, to linear order in the perturbation, we have

$$\frac{\mathrm{d}}{\mathrm{d}t}\,\delta n_1 = f_0\,\delta d_2 - \delta n_1 \tag{7.26}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\,\delta n_1 = \nu\,(g_0\,\delta n_1 - \delta d_1) \tag{7.27}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\,\delta n_2 = f_0\,\delta d_1 - \delta n_2 \tag{7.28}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\,\delta d_2 = \nu\,(g_0\,\delta n_2 - \delta d_2)\,. \tag{7.29}$$

This can be written in matrix form as

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathbf{u} = \mathsf{A}\cdot\mathbf{u}, \tag{7.30}$$

with

$$\mathsf{A} = \begin{pmatrix} -1 & 0 & 0 & f_0 \\ \nu\,g_0 & 0 & -\nu & 0 \\ 0 & -1 & f_0 & 0 \\ 0 & \nu\,g_0 & 0 & -\nu \end{pmatrix}. \tag{7.31}$$

The sum of the eigenvalues of this matrix is given by the trace and the product of the eigenvalues is given by the determinant.

$$\operatorname{tr}\mathsf{A} = f_0 - \nu - 1 \tag{7.32}$$

$$\det\mathsf{A} = \nu^2\left(f_0^2 g_0^2 - 1\right). \tag{7.33}$$

So, a sufficient condition for having an eigenvalue with positive real part, and the homogeneous steady state thereby being unstable, is that $f_0 > \nu + 1$. We cannot say much more without having the functional forms of $f(x)$ and $g(x)$ and/or the numerical values of f_0 and g_0.

7.2.4 Linear stability in the $\nu \gg 1$ regime

To make more analytical progress, we consider the case where $\nu \gg 1$, which is to say that the Delta dynamics are much faster than the Notch dynamics. We note that the terms multiplying ν in equations (7.15) and (7.17) must be of order ν, since all of the variables have been scaled to unity. This means that $g(n_1) \approx d_1$ and $g(n_2) \approx d_2$. With this approximation, we can reduce the dynamical system to two equations.

$$\dot{n}_1 = fg(n_2) - n_1 \tag{7.34}$$

$$\dot{n}_1 = fg(n_1) - n_2. \tag{7.35}$$

We can again perform linear stability analysis, defining now

$$fg_0 \equiv \left.\frac{\mathrm{d}fg(n)}{\mathrm{d}n}\right|_{n=n_0}. \tag{7.36}$$

We get

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}\delta n_1 \\ \delta n_2\end{pmatrix} = \begin{pmatrix}-1 & fg_0 \\ fg_0 & -1\end{pmatrix}\cdot\begin{pmatrix}\delta n_1 \\ \delta n_2\end{pmatrix}. \tag{7.37}$$

The sum of the eigenvalues of this linear stability matrix is $\lambda_1 + \lambda_2 = -2$, implying that at least one of the eigenvalues has a positive real part. The product of the eigenvalues is given by the determinant, or $\lambda_1\lambda_2 = 1 - (fg_0)^2$. Since at least one of the eigenvalues has a negative real part, we must have $\lambda_1\lambda_2 < 0$ to have an instability. So, we must have $(fg_0)^2 > 1$, or $fg_0 < -1$, since $fg_0 < 0$. This tells us that the composite function $fg(x)$ must be steep.

7.2.5 Cooperativity in the $\nu \gg 1$ regime

We will model $f(x)$ and $g(x)$ as Hill functions to gain some more insights into the requirements for instability.

$$f(x) = \frac{x^{n_f}}{a + x^{n_f}}, \tag{7.38}$$

$$g(x) = \frac{b}{b + x^{n_g}}. \tag{7.39}$$

Then, we have

$$fg(x) = \frac{[b/(b+x^{n_g})]^{n_f}}{a + [b/(b+x^{n_g})]^{n_f}}. \tag{7.40}$$

We compute the differential of this function for $n_f = n_g = 1$.

$$\frac{\mathrm{d}fg}{\mathrm{d}x} = -\frac{ab}{(b + ab + ax)^2}. \tag{7.41}$$

Thus, we have that fg_0 can never have a magnitude greater than unity, since the denominator in this expression is equal to the numerator plus only posivite values. Therefore, if $n_f = n_g = 1$, we cannot have an instability. So, a requirement for instability of the Delta-Notch system in the limit where Delta dynamics are much faster than Notch dynamics is that we must have cooperativity, i.e., $n_f > 1$, $n_g > 1$, or both.