Computational analysis of signal dynamics induced by ligands

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Abstract: During tissue development, cells differentiate creating a cellular pattern thanks to communication frameworks like the one studied, the *Notch signalling pathway*. In such mechanisms a cell interacts with the surrounding cells through proteins called *ligands*. With a numerical simulation program we manage to confirm the formation of cell patterns and doing a lineal stability analysis we find under which conditions the pattern can arise for a sustained signal activation. Furthermore, when implementing a transitory pulsating signal dynamics we find that a minimum interaction time is required if the cells are to differentiate.

I. INTRODUCTION

Cellular differentiation is key for a proper biological development of a living organism. To ensure this, cells need to communicate between each other [1]. One of such communication frameworks is the so-called *Notch/Delta signalling pathway* and it is present in a vast variety of species [2]. This pathway enables communication between neighbouring cells and usually drives an inhibitory interaction called lateral inhibition: a cell in a certain cellular state prevents its neighbouring cells from developing in the same way.

Several models for this communication framework have been proposed in the last decades [2, 3]. However, in order to reduce its biochemical complexity we simplify its description to the short-range interaction between the ligand (*Delta*) of a cell with the receptors (*Notch*) of the neighbouring cells as it is done in [3]. According to the mentioned model, a cell with high *Delta* activity may induce a high *Notch* activity in its neighbours. Likewise, in the intra-cellular domain, the high *Notch* expressed cell may reduce its own *Delta* activity. This feedback loop involves two adjacent cells that mutually inhibit each other. It could lead to the formation of a pattern where a high *Delta* - low *Notch* expressed cell is adjacent to low *Delta* - high *Notch* expressed cells.

In this report we implement the basic model proposed by Collier et al. [3] and discuss how a pattern arises. We pay special attention to the two cell system since the locality of this interaction enables us to gain valuable insight of this behavior from it. In fact, there are experimental scenarios where lateral inhibition occurs in isolated pairs of cells [4]. Furthermore, in their recent and experimental work [5], Elowitz group have shown that *Notch* signalling can be activated whether in a sustained or in a transitory way leading to different cell responses. In the last section of this report, we take into account their results including this new conception of transitory activation.

II. DEVELOPING SECTIONS

A. The model

We take the model proposed by Joanne Collier and colleagues in [3]. This model offers a mathematical framework for the *Notch / Delta* signalling pathway assuming a sustained activation (no explicit time dependence). In short, the equations ruling this communication mechanism are:

$$\dot{n_i} = f(\bar{d}_i) - n_i$$
$$\dot{d_i} = v[g(n_i) - d_i] \qquad (1)$$

where $\dot{}$ denotes d/dt. In order to reduce the number of parameters involved in the model we have taken the dimensionless equation system [3]. n_i, d_i stand for the *Notch* and *Delta* activity normalized with the constants N_0, D_0 (typical levels of *Notch* and *Delta*) respectively, so $n_i, d_i \in [0, 1]$. These two variables determine the cell state in any instant. f is a monotonically increasing function that describes how *Notch* production grows with the *Delta* level of the neighbouring cells; $\bar{d}_i$ is the average *Delta* activity of the first neighbours of the $i-$cell. g is a monotonically decreasing function describing the inhibition of *Delta* activity due to the of *Notch* level in the $i-$cell. Moreover, both, *Notch* and *Delta* production, are balanced by an exponential decay. Timescale has been adimensionalized to the characteristic decay time of *Notch*. Parameter $v = \rho/\mu$ gives the measure of the relative time-scales over which the levels of and *Delta* and *Notch* vary in terms of their respective decay rates: ρ, μ.

As the functions f and g we take a form that is vastly used in the literature and as proposed in [3]:

$$f(x) = \frac{x^k}{a + x^k} \; ; \; g(x) = \frac{1}{1 + bx^h} \qquad (2)$$

$\forall x \geq 0$ and with $a, b, h, k > 0$.

B. Particular case: The two cell system

It is interesting to introduce a two-cell finite system since it gives us sufficient information of the phenomenology. Under this condition the equation system reduces to four equations:

$$\dot{n_1} = f(d_2/2) - n_1 \,;\; \dot{d_1} = v[g(n_1) - d_1]$$
$$\dot{n_2} = f(d_1/2) - n_2 \,;\; \dot{d_2} = v[g(n_2) - d_2] \qquad (3)$$

In this system each cell has only a single neighbour and we consider they interact through half of their surface, so $\bar{d_1} = d_2/2$ and analogously $\bar{d_2} = d_1/2$.

Furthermore, this situation allows a visual representation of the phase plane of each characteristic cell-state variable d_i, n_i. The *Delta* phase-plane (d_2 vs d_1) will be of interest when the *Delta* variable has a relative slow dynamics compared to *Notch*. That means $v < 1$. Otherwise, if $v > 1$ we will be interested in the *Notch* phase-plane (n_2 vs n_1).

In the phase plane we can look for the equilibrium states by representing the *null-clines*. In order to introduce these null-clines we define them particularly in the *Delta* plane. In this case the null-cliens for each variable will be given by $\dot{d_1} = \dot{d_2} = 0$ leading us to: $d_1 = g(n_1) \,;\; d_2 = g(n_2)$. If we make a quasi-steady-state assumption that $\dot{n_1} = \dot{n_2} = 0$ the *Delta* null-clines are given by:

$$d_1 = g(f(d_2/2)) \,;\; d_2 = g(f(d_1/2))$$

Hence, the cut points of the null-clines in the phase-planes will be, by definition, the equilibrium states. Since f is a monotonically increasing function and g a monotonically decreasing one, there is exactly one homogeneous steady state ($d_1^{eq} = g(f(d_2^{eq}/2)) = d_2^{eq}$). Depending on the values of parameters $\{a, b, h, k\}$ one or more pair of additional cut points of the null-clines can be found. These points shall not appear on the bisectrice of the plane ($d_1 = d_2$) but in states such as $d_1^{eq} \neq d_2^{eq}$. We shall refer to these points as heterogeneous steady states.

We propose a set of values $\{a, b, h, k\}$ for a system with a single homogeneous steady state (4) and a set which results in three steady states (5). These sets are chosen because they offer a proper visual representation of the null-clines in both phase planes. Despite we define these parameters for a two cell system, they are used in all the numerical simulations involving the functions in (2)

$$a = 0.07 \,,\, b = 40 \,,\, k = 1 \,,\, h = 1 \qquad (4)$$
$$a = 0.07 \,,\, b = 40 \,,\, k = 2 \,,\, h = 3 \qquad (5)$$

C. Numerical simulations

In order to evaluate the model dynamics we integrate equations (1) in time taking parameters that ensure three equilibrium states for a pair of interacting cells (set (5)).
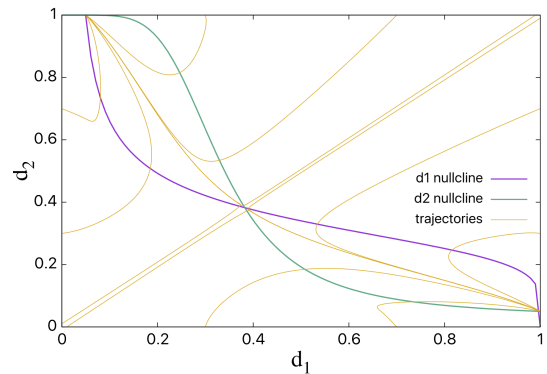

FIG. 1: Trajectories $d_2(d_1)$ (yellow lines) in *Delta* phase plane for a three steady states situation. The initial conditions are set in the edge of the figure for *Delta*: $d_1 = 1$, $d_2 = 1$, $d_1 = 0$ or $d_2 = 0$; and homogeneous for *Notch*. For the numerical integration of (1) we take the set (5) and $v = 0.1$. Green and purple lines show the *Delta* null-clines with the steady states in the cut points.

We use our own *python* program where we implemented an order four Runge-Kutta method.

Firstly, we investigate the two cell system for different initial conditions and for $v = 0.1$, slow *Delta* dynamics. In Figure 1, all simulated trajectories for $v = 0.1$ are represented in the *Delta* plane. It can be seen that every path dies in one of the two heterogeneous steady states, depending only on the initial condition. Separating the phase plane by the bisectrice ($d_2 = d_1$) we can see that the trajectories with an initial condition above the separatrix evolve to the heterogeneous steady state above the separatrix, and analogously for an initial condition below it. For the particular case of a trajectory with an initial condition near to an homogeneous state, initially it evolves towards the saddle-point (homogeneous steady state) following the bisectrice and then it shall escape to one of the two heterogeneous steady states depending on the initial perturbation. So, the phase plane has two symmetric parts separated by the line $d_2 = d_1$. We find that in any case the stationary stable state corresponds to a high *Delta* (low *Notch*) activity for one cell and a low *Delta* (high *Notch*) activity for the other. In order to see how a pattern arises, we study larger systems with periodic boundary conditions and set an initial condition close to an homogeneous state: $n_i \approx n_{i+1} \,;\; d_i \approx d_{i+1} \,\forall i$. Considering a one dimensional array of ten cells or a 10×10 2 dimensional cell system we have a quite visual representation of the stationary pattern. As shown in Figures 2 and 3, adjacent cells achieve opposite cell states creating an ordered periodic pattern of cell states. Therefore, the lateral inhibition communication amplifies the small differences between cells and drives patterning.

In order to analyse the effect of the dynamics we expand Collier's model including an order parameter for the one-dimensional array of N cells with periodic boundary
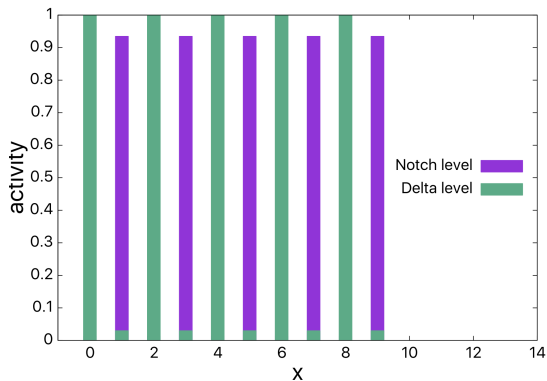
FIG. 2: Stationary activity profiles for *Notch* (purple) and *Delta* (green) in each cell position (x) for a one dimensional array of ten cells. We integrate equations (1) assuming periodic boundary conditions and an almost homogeneous initial condition for both *Notch* and *Delta*. As numerical parameters we use set (5) and $v = 1$.
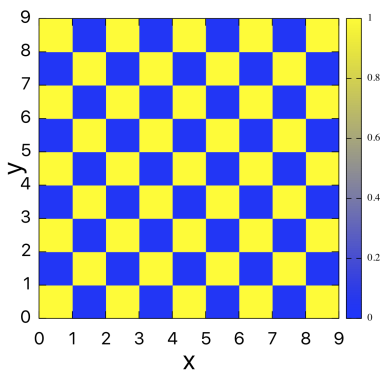

FIG. 3: Stationary *Delta* activity profile for a two dimensional squared cell grid. For representation purposes we can denote each cell as a square. We integrate equations (1) assuming periodic boundary conditions and an almost homogeneous initial condition for both *Notch* and *Delta*. As numerical parameters we use set (5) and $v = 1$. Yellow correspond to a high *Delta* activity whereas blue to a low one. (x, y) give the position of the cell in the grid.

conditions:

$$\phi_n(t) = \frac{1}{N} \sum_{i=1}^{N} [n_i(t) - n_{i+1}(t)]^2$$

$$\phi_d(t) = \frac{1}{N} \sum_{i=1}^{N} [d_i(t) - d_{i+1}(t)]^2. \qquad (6)$$

These magnitudes are zero when the system is in an homogeneous state. Otherwise they are positive saturating in a maximum value when the system reaches an heterogeneous steady state.

The evolution of the order parameter allows us to easily compare situations with different dynamics. In Figure
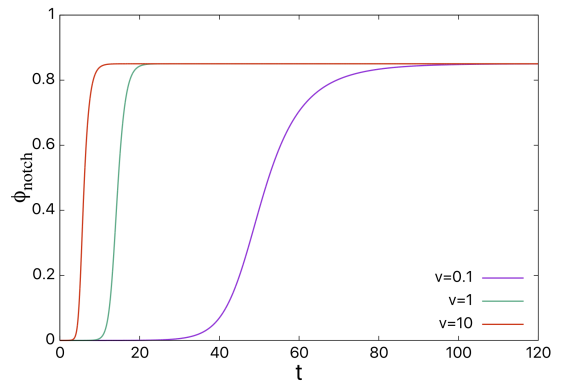

FIG. 4: Time evolution of the *Notch* order parameter, $\phi_n(t)$, for different values of v. A one dimension array of 10 cells with periodic boundary condition set initially in the homogeneous steady state has been considered. The integration has been computed using the parameter set (5).

4 we present the evolution of $\phi_n(t)$ for $v = 0.1, 1, 10$. As expected, it is seen that the smaller the v value is, the longer it takes to reach the heterogeneous steady state. Moreover, when taking the system initially in a scant perturbed homogeneous steady state, we can define a remaining time in this state, τ, as the time needed to reach a value of the order parameter greater than a given quantity δ: $\phi_n(t > \tau)$, $\phi_d(t > \tau) > \delta$. That is the time needed for the perturbation to expel the system from the homogeneous state. Time τ grows for slower *Delta* dynamics (small values of v).

D.   Linear stability analysis

In the previous section we have seen how a system initially in an homogeneous situation evolves towards an steady heterogeneous state, meaning the homogeneous equilibrium state is unstable. In order to find the conditions for a stable homogeneous state we do the linear stability analysis, as done in [3] and [6].

Consider a one-dimensional array of N cells with periodic boundary conditions. The evolution of this system is ruled by equation (1). We have already discussed the existence of a cut point of the null-clines on the bisectrice (an homogeneous steady state) which satisfies $x_0 = f(g(x_0))$, being $n_i^{eq} = x_0$, $d_i^{eq} = g(x_0)$ $\forall i$. We analyse the stability of the homogeneous steady state by studying the time evolution of a small perturbation in a system initially in this state. Since $|n_j - x_0|, |d_j - g(x_0)| << 1$ we can do a Taylor development of the equation system (1) around the homogeneous steady state:

$$\frac{d}{dt}[n_j - x_0] = \frac{\alpha}{2}\{d_{j-1} + d_{j+1} - 2g(x_0)\} - [n_j - x_0]$$

$$\frac{d}{dt}[d_j - g(x_0)] = v\{\beta(n_j - x_0) - [d_j - g(x_0)]\} \qquad (7)$$

where $\alpha = f'(g(x_0))$ and $\beta = g'(x_0)$. For definition

of $f(x)$ and $g(x)$, $\alpha > 0$, $\beta < 0$, $\forall x_0 \in \mathbb{R}$. Letting the perturbations be expressed as a superposition of modes in Fourier space:

$$n_j - x_0 = \sum_{s=1}^{N} \xi_s \exp\left(2\pi i \frac{sj}{N}\right)$$

$$d_j - g(x_0) = \sum_{s=1}^{N} \eta_s \exp\left(2\pi i \frac{sj}{N}\right) \qquad (8)$$

and replacing (8) in (7), the system can be expressed as:

$$\frac{d\xi_s}{dt} = \alpha \cos\left(\frac{2\pi s}{N}\right) \eta_s - \xi_s$$

$$\frac{d\eta_s}{dt} = v\left(\beta \xi_s - \eta_s\right) \qquad (9)$$

where we have accounted for the uniqueness of the Fourier series. When diagonalizing the Jacobian matrix (J) of the differential equation system (9), the solution for (ξ_s, η_s) can be written as the sum of the eigenstates of J times $e^{\lambda_s t}$, being λ_s the corresponding eigenvalue.

If we are to consider the homogeneous steady state unstable, at least one of the eigenvalues must have a real positive part so the perturbation indefinitely grows in time. If we do the diagonalization process we find:

$$\lambda_\pm^s = \frac{1}{2}\left\{-(1+v) \pm \sqrt{(1+v)^2 - 4v\left[1 - \alpha\beta \cos\left(\frac{2\pi s}{N}\right)\right]}\right\}.$$

Several cases can occur. If the discriminant is negative so $\lambda_\pm^s \in \mathbb{C}$, we have $\mathbb{R}e(\lambda_\pm^s) = -\frac{1+v}{2} < 0$, $\forall v > 0$. On the contrary, when the discriminant is positive, the eigenvalue is real and we need to study λ_+^s and λ_-^s separately. Since λ_-^s consists in the addition of two negative quantities, $\lambda_-^s < 0$ in any case.

The case of $\lambda_+^s \in \mathbb{R}$ is far more complex. The condition for $\lambda_+^s > 0$ is $1 - \alpha\beta \cos\left(\frac{2\pi s}{N}\right) < 0$. Since $\alpha\beta = f'(g(x_0)) \cdot g'(x_0) < 0$, necessarily $\cos\left(\frac{2\pi s}{N}\right) < 0$. However, it is not sufficient as the cosine is an enclosed function: $\cos\left(\frac{2\pi s}{N}\right) > -1$. Hence, a sufficient condition to ensure $\lambda_+^s > 0$ is $|\alpha\beta| > 1$, so the stability condition can be written as

$$|\alpha\beta| = |f'(g(x_0)) \cdot g'(x_0)| = |(fg)'(x_0)| < 1 \qquad (10)$$

Else, it exists one or more modes such as their eigenvalues are $\lambda_+^s > 0$. Equation 10 gives us an analytical condition for the stability of the homogeneous steady state. We can numerically check it for the sets of parameters $\{a, b, k, h\}$ presented in section II B for the functions $f(x)$, $g(x)$.

Set (5), used in all simulations in section II C, corresponds to a situation where the null-clines present three cut points, one in the bisectrice (homogeneous) and two in heterogeneous states (see Figure (1)). Moreover, according to our numerical results the homogeneous steady state is unstable. For $x_0 = 0.425$, $g(x_0) = 0.225$: $|\alpha \cdot \beta|_{set\,5} = 2.832 > 1$. On the other hand, when studying

the null-clines for set (4) a single cut point on the bisectrice is found. For $x_0 = 0.5375$ $g(x_0) = 0.175$ and the stability condition results in: $|\alpha \cdot \beta|_{set\,4} = 0.0921 < 1$. In both cases the analytical condition (10) for the stability fits with the numerical results.

Furthermore, we can interpret $\lambda_+(v)$ as a growing rate for the perturbations and define a characteristic time for the perturbation, $\tau(v)$. This time is given by the largest mode, s and is found for $s/N = 1/2$, meaning that the dominant mode results in a period two pattern. So, we define $\tau(v) = \lambda_{max}^{-1}(v)$.

$$\lambda_{max} = \lambda_+^{s=N/2} = -\frac{(1+v)}{2} + \frac{1}{2}\sqrt{(1-v)^2 - 4v\alpha\beta}$$

We can predict analytically the behaviour of this timescale as a function of v studying the limits for $v \to 0, \infty$ of $\lambda_{max}(v)$. In the first case, $v \to 0$, we find $\lim_{v \to 0} \lambda_{max}(v) = 0$. That is, when *Delta* has a much slower dynamics, the stability time, $\tau(v \to 0)$, its indefinitely large. Whereas for the opposite case $\lim_{v \to \infty} \lambda_{max}(v) = -(1 + \alpha\beta) > 0$, meaning that if *Notch* is the slow variable, there is a lower bound for the remaining time. Figure 4 shows how τ for $v = 0.1$ is far more larger than τ for $v = 1, 10$ which have comparable time-scales.

E.   Transitory activation

It has recently been observed that some ligands do not activate the *Notch* signalling in a sustained way, as discussed in the previous sections, but in a pulsating transitory way [5]. As a first approach, we try to model a transitory activation modifying the *Notch* production equation with a Heaviside function:

$$\frac{dn_i}{dt} = f(\bar{d}_i) \cdot \Theta(T - t)\Theta(t) - n_i \qquad (11)$$

In this new model, the signalling pathway is operative only for a time interval, given by T.

Numerically integrating the new equations for a system set at an initial homogeneous state we see that the signalling pathway needs a minimum performing time if it is to differentiate the cellular state (Figure 5-A). This time depends on the relative dynamics of the system (v). Assuming $v = 1$, our results give a minimum time around $T \sim 20$ to create a pattern. This time exceeds the experimental results given in [5]. They find for proteins (*Delta* and *Notch*) with life-times such as $\tau_p \sim 3 - 4\,h$, pulses of a duration of the order of $12\,h$.

As a first approach we identify the proteins life-times as their decay rates: ρ, μ (see section II A), and we work in a dimensionless unit system in which the time has been normalized to *Notch* decay rate μ. So, according to experimental results, if we consider that both proteins have the same life-time ($v = 1$), a real time of 12 h corresponds to a simulation time $T \sim 3 - 4$.
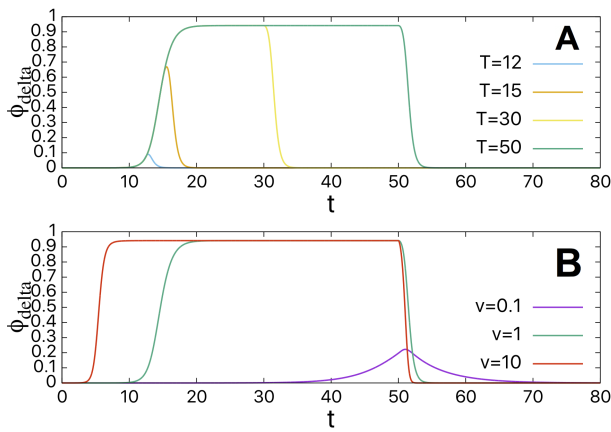
FIG. 5: Time evolution of the *Delta* order parameter, $\phi_d(t)$, in a transitory activation; using equation 11. **A:** $\phi_d(t)$ for different T values and assuming $v = 1$. **B:** $\phi_d(t)$ for different values of v and taking $T = 50$. In both cases a one dimensional array of 10 cells with periodic boundary conditions set initially in the homogeneous steady state has been considered. Parameters in (5) have been used.

Nevertheless, when simplifying the biological background of the process, the model has a lack of realism. Firstly, the estimation of the protein life-times as the exponential decay rates is not accurate. Furthermore, communication is considered to be immediate, when there is experimental evidence that the processes in the intracellular domain result in a time delay that might be important [7]. Despite these biological considerations, we have seen that larger v result in a smaller response times (Figure 5-B). So, our results may suggest that, with a quicker *Delta* dynamics, a time such as $T \sim 12\,h$ could be enough to induce cell differentiation.

III. CONCLUSIONS

Implementing and analysing Collier's model [3] we have seen how the feedback loop described by equations in (1) can lead to the cell differentiation creating a cellular pattern. That is, in the stationary state two cell states are possible and they are orderly distributed [3]. Moreover, analysing the stability of the equations system (1) around its homogeneous solution ($d_1^{eq} = d_2^{eq}$), we have found its stability condition (10), as it is done in [3]. For a given parameters $\{a, b, k, h\}$ values, if inequation (10) is satisfied, the homogeneous steady state is stable and no pattern arises. Otherwise, the system finds its stability in an heterogeneous state and the stationary state results in a pattern.

A more realistic model could be designed taking into account the cell morphology and contact surface [2]. Besides, this basic model can be expanded including a long range interaction via filopodia [2] or diffusive ligands [6], among others.

Current research on this topic focuses on the role of the activation dynamics in the signalling pathway [5]. We made a first approach to a transitory dynamics in equation (11). Our results suggest that the formation of a pattern does not only depend on the stability of the homogeneous state but on the time the communication is active (T) and the relative dynamics (v). Further analysis of Figure 5 shows how the pattern disappears after the pulse duration (T). Due to *Notch* exponential decay the new stationary state would correspond to a homogeneous system in which all cells have saturated *Delta* activity and null *Notch* activity. Experimentally, it has been observed that more than one pulse can occur repeatedly, but not necessarily periodically. One arising question would be whether cells dispose of mechanisms capable to keep the pattern once it has been formed and the signal has decayed or the pattern appears and disappears as the pulses occur.

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