

Jagged–Delta asymmetry in Notch signaling can give rise to a Sender/Receiver hybrid phenotype

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Notch signaling pathway mediates cell-fate determination during embryonic development, wound healing, and tumorigenesis. This pathway is activated when the ligand Delta or the ligand Jagged of one cell interacts with the Notch receptor of its neighboring cell, releasing the Notch Intracellular Domain (NICD) that activates many downstream target genes. NICD affects ligand production asymmetrically—it represses Delta, but activates Jagged. Although the dynamical role of Notch–Jagged signaling remains elusive, it is widely recognized that Notch–Delta signaling behaves as an intercellular toggle switch, giving rise to two distinct fates that neighboring cells adopt—Sender (high ligand, low receptor) and Receiver (low ligand, high receptor). Here, we devise a specific theoretical framework that incorporates both Delta and Jagged in Notch signaling circuit to explore the functional role of Jagged in cell-fate determination. We find that the asymmetric effect of NICD renders the circuit to behave as a three-way switch, giving rise to an additional state—a hybrid Sender/Receiver (medium ligand, medium receptor). This phenotype allows neighboring cells to both send and receive signals, thereby attaining similar fates. We also show that due to the asymmetric effect of the glycosyltransferase Fringe, different outcomes are generated depending on which ligand is dominant: Delta-mediated signaling drives neighboring cells to have an opposite fate; Jagged-mediated signaling drives the cell to maintain a similar fate to that of its neighbor. We elucidate the role of Jagged in cell-fate determination and discuss its possible implications in understanding tumor–stroma cross-talk, which frequently entails Notch–Jagged communication.

Notch signaling | Jagged | Fringe | cell signaling | developmental biology

Notch signaling pathway is an evolutionarily conserved mechanism that plays a crucial role in controlling cell-fate differentiation during embryonic development (1, 2). This pathway is often aberrantly activated in many cancers and controls the proliferation and survival of cancer cells, as well as their malignant progression (3). The signaling pathway consists of the Notch transmembrane receptor and its ligands Delta and/or Jagged. The interaction between the receptor and the ligand of the same cell (*cis*-interaction) leads to the degradation of both proteins, therefore not generating a signal. The interaction between the receptor of one cell with the ligand of a neighboring cell (*trans*-interaction) leads to the release of the Notch Intracellular Domain (NICD) signal into the cytoplasm. The NICD then enters the nucleus where it associates with the CSL transcription factor complex, resulting in subsequent activation of downstream target genes (1, 2) (Fig. 1).

Notch signaling through Jagged and that through Delta have different dynamics because of two elements of asymmetry in the signaling circuit. First, NICD inhibits Delta through its downstream effector Hes1 (4), but activates Jagged both directly (5) and indirectly through miR-200 (see discussion in *SI Text, section S1*). These modes of regulation effectively create an intercellular double-negative feedback loop between Notch and Delta (6), but an intercellular double-positive feedback loop between Notch and Jagged (5) (Fig. 1). Consequently, Notch–Delta signaling

between two cells behaves as a two-way switch: one cell has [high Delta (ligand), low Notch (receptor)] expression on its surface, whereas the other cell has [high Notch (receptor), low Delta (ligand)] on its surface. According to common terminology, the first cell behaves as a Sender (S) and the second one as a Receiver (R). In other words, the Notch–Delta signaling standalone causes the two neighboring cells to acquire opposite fates. This mechanism, known as lateral inhibition, is implicated, for example, in control of neurogenesis in *Drosophila* and vertebrates (7), and in salt-and-pepper patterns observed during wing vein formation (6). On the other hand, for standalone Notch–Jagged signaling between two cells, Notch and Jagged levels in both cells go hand in hand (high Notch, high Jagged). Therefore, both cells can act as both Receiver (R) and Sender (S)—or the two cells acquire similar fates. This mechanism, known as lateral induction, is implicated, for example, in mammalian inner-ear development (8, 9), control of epidermal stem cell clusters (10), as well as inner cardiac development (11). Therefore, Delta and Jagged affect the collective cell-fate decisions in a group of cells quite differently.

The second asymmetry between signaling through the ligands Delta and Jagged arises due to posttranslational modifications of Notch that modulate the binding of Notch to Delta and to Jagged. Fringe, a glycosyltransferase, can decrease the affinity of Notch to bind to Jagged, but increase the affinity of Notch to bind to Delta (12). Consequently, Fringe creates two distinct Notch populations on the cell surface: one that has comparable binding affinity to both Jagged and Delta, and one that strongly prefers

Significance

Notch signaling pathway plays crucial roles in cell-fate determination during embryonic development and cancer progression. According to the current paradigm, the Notch–Delta signaling leads to complementary cell-fate selection between two neighboring cells where one acts as Sender or Receiver. However, this picture is not complete because an additional ligand, Jagged, is involved in the Notch signaling. We devise a specific theoretical framework to decipher the functional role of Jagged. We find that the asymmetry between the modulations of Delta and Jagged leads to the existence of the previously unexplored possibility of a Sender–Receiver phenotype enabling two interacting cells to share a similar fate. This realization can provide important clues regarding embryonic development, wound healing, and how to target tumor–stroma signaling.

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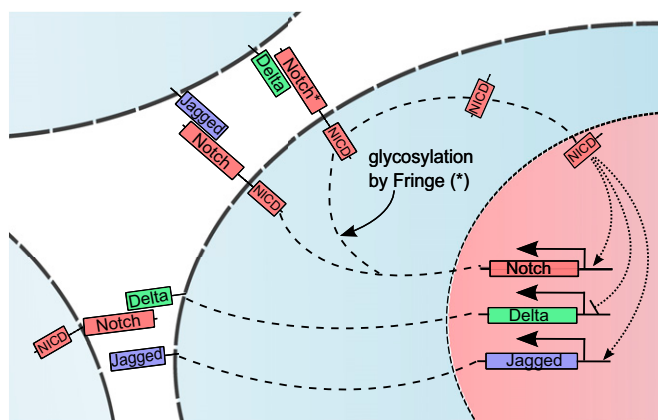


Fig. 1. Overview of the intracellular and intercellular Notch signaling pathway. Notch, the transmembrane receptor of one cell, binds to Delta or Jagged, the transmembrane ligands belonging to the neighboring cell. This *trans*-interaction cleaves the Notch receptor to release NICD. NICD migrates to the nucleus and modulates the transcription of many genes. This modulation indirectly leads to the transcriptional activation of Notch and Jagged and inhibition of Delta. Interaction between Notch receptor and ligands (Delta or Jagged) of the same cell (*cis*-interaction) leads to the degradation of both the receptor and the ligand. Glycosylation of Notch by Fringe (*) modifies Notch to have higher affinity for binding to Delta and lower affinity for binding to Jagged.

binding to Delta. The effects of these two elements of asymmetry in Notch signaling remain elusive and call for clarification of their corresponding role in cell-fate determination mediated by Notch signaling.

Many experimental and theoretical research efforts have been directed toward understanding the Notch–Delta-dependent cell-fate determination (6, 13–17). In contrast, the role of Notch–Jagged signaling has gained limited research attention despite the recognized role of Jagged in tumorigenesis. For example, overexpression of Jagged has been associated with poor prognosis, at least in breast cancer and prostate cancer (18), thus highlighting the importance of understanding its role in Notch signaling. Other recent studies have shown that Notch signaling can be activated by soluble forms of the ligands Jagged and Delta (19–21). The soluble Jagged and Delta have different effects on tumor progression—soluble Delta inhibits tumor growth (22, 23), whereas soluble Jagged strongly aggravates the malignant progression of cancer. More specifically, Jagged plays an important role in inducing epithelial to mesenchymal transition (EMT) as well as promoting cells to acquire cancer stem cell (CSC) properties (20). Notably, Notch–Jagged signaling also plays a crucial role in angiogenesis (24), cancer metastasis (25), and rapid development of cancer chemotherapy and radiation therapy resistance (26).

Here, we have devised a tractable mathematical framework to evaluate the role of Jagged in cell-fate determination mediated by Notch signaling. We show that the Jagged–Delta asymmetry in Notch signaling can give rise to a Sender–Receiver (S/R) hybrid state, thus rendering the Notch signaling to operate as a three-way switch so that two interacting cells can acquire one of the three states—Sender (S), Receiver (R), and hybrid Sender/Receiver (S/R). More specifically, we demonstrate how including Jagged in the Notch–Delta signaling opens up and maintains a previously unidentified state in which the cells can both send and receive signals—suggesting that Jagged-mediated signaling allows interacting cells to acquire similar fates.

Results

The Theoretical Framework. To explore the effects of Jagged in cell-fate determination, we generalized earlier theoretical frame-

work devised by Sprinzak et al. (14) by incorporation of Jagged in addition to Delta, and the asymmetric transcription regulation of the ligands by NICD—a transcriptional activator of Jagged and transcriptional repressor of Delta. First, we investigated the model dynamics in the case when Jagged and Delta have similar binding affinity of Notch. Second, we analyzed a further extension of the model in which the asymmetric effect Fringe is included: Fringe increases the Notch–Delta binding affinity and decreases the Notch–Jagged binding affinity.

More specifically, within the framework proposed by Sprinzak et al. (14), Notch receptor (N) belonging to one cell can interact with both the ligands of the same cell (D or J)—known as *cis*-interaction, or with those of the neighboring cell—Jagged or Delta (D_{ext} or J_{ext})—known as *trans*-interaction. The *cis*-interaction, also referred to as *cis*-inhibition, causes the degradation of both the interacting proteins. On the other hand, the *trans*-interaction, also referred to as *trans*-activation, leads to the cleavage of Notch receptor, which releases NICD (represented as I in the model). Within the framework presented here, in addition to introducing Jagged as an additional element in the signaling circuit, we also include the feedback effects of NICD that indirectly activates Notch and Jagged and represses Delta, thereby creating an asymmetry between Notch–Delta and Notch–Jagged interactions (Fig. 2). The deterministic equations for the dynamics of Notch (N), Delta (D), Jagged (J), and NICD (I) are given by

$$\frac{dN}{dt} = N_0 H^{S+}(I) - k_C N(D+J) - k_T N(D_{ext} + J_{ext}) - \gamma N, \quad [1]$$

$$\frac{dD}{dt} = D_0 H^{S-}(I) - k_C D N - k_T D N_{ext} - \gamma D, \quad [2]$$

$$\frac{dJ}{dt} = J_0 H^{S+}(I) - k_C J N - k_T J N_{ext} - \gamma J, \quad [3]$$

$$\frac{dI}{dt} = k_T N(D_{ext} + J_{ext}) - \gamma I, \quad [4]$$

where γ represents the degradation rate of all three transmembrane proteins Notch, Jagged, and Delta, and γ_I the degradation rate of NICD. k_C and k_T are the strengths of *cis*-inhibition and *trans*-activation, respectively; and N_0 , D_0 , and J_0 are the production rates of Notch, Delta, and Jagged, respectively. N_{ext} , D_{ext} , and J_{ext} represent the amount of protein available for binding, which can be on the membrane surface of neighboring cells or in a soluble form. Experimental evidence suggests that membrane-bound ligands can

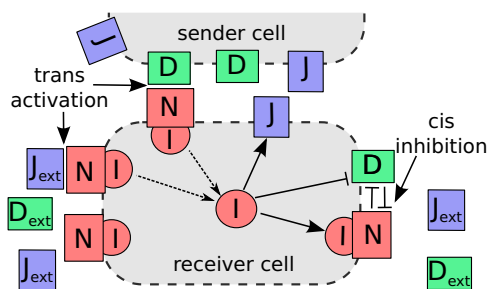


Fig. 2. Schematic illustration of Notch signaling circuit. NICD (I) is released when the receptor (N) of the receiver cell interacts with the ligand of the sender cell (D or J) or with external ligands in a soluble form (D_{ext} or J_{ext})—so-called *trans*-activation. The released signal activates the expression of N and J , and inhibits the expression of D . The *cis*-inhibition occurs between the receptor and ligand in the same cell and leads to the degradation of both proteins.

generate a stronger signal compared with their soluble forms (27). However, the distinction between these two forms of ligand—membrane-bound and soluble—is not addressed in the following analysis but can be easily incorporated in model by considering their different *trans*-activation rates (Eqs. S41–S44 in *SI Text*, section S6). We consider shifted Hill functions (28) to represent the effect of NICD (I) on the production rates of the proteins. Shifted Hill functions are defined as $H^S(I, \lambda) = H^-(I) + \lambda H^+(I)$ or in simpler notation: $H^{S^+}(I)$ if $\lambda > 1$ and $H^{S^-}(I)$ if $\lambda < 1$, where the weight factor λ represents the fold change in production rate, therefore, for activation, $\lambda > 1$; for repression, $\lambda < 1$; and for no effect, $\lambda = 1$ ($\lambda_N, \lambda_I > 1$, and $\lambda_D < 1$ in our model). Note that shifted Hill functions have a constitutive production term. For the case of two interacting cells, the variables N_{ext} , D_{ext} , and J_{ext} should be replaced by N , D , J of the neighboring cell (Eqs. S22–S25 in *SI Text*, section S3). A detailed discussion of the parameter values can be found in *SI Text*, section S1, and Table S1. The model shows a good robustness with respect to changes in parameter values as discussed in *SI Text*, section S5, and Figs. S1 and S2. Temporal dynamics and stochastic simulations are included in *SI Text*, section S7, and Fig. S3. All of the codes were developed in Python using the PyDSTool (29).

Notch–Delta Circuit: A Two-Cell Toggle Switch. We proceed to analyze the standalone dynamics of the Notch–Delta signaling by analyzing the reduced model given by Eqs. (5)–(7) below (a reduced version of the model described above):

$$\frac{dN}{dt} = N_0 H^{S^+}(I) - k_C N D - k_T N D_{ext} - \gamma N, \quad [5]$$

$$\frac{dD}{dt} = D_0 H^{S^-}(I) - k_C D N - k_T D N_{ext} - \gamma D, \quad [6]$$

$$\frac{dI}{dt} = k_T N D_{ext} - \gamma I. \quad [7]$$

We analyze two cases of this model: (i) single cell driven by a cell with fixed value of external Delta (D_{ext}) and fixed value of external Notch (N_{ext}); (ii) two interacting cells where (D_{ext}) and (N_{ext}) represent the D and N of the neighboring cell. For case (i), the Notch–Delta circuit is bistable with two possible states: (a) (high Notch, low Delta)—the cell is a Receiver (R) of the ligand D , and (b) (low Notch, high Delta)—the cell is a Sender (S) of the ligand D (Fig. 3A). For case (ii), the ligands D of both cells activate the receptors of the other cell, and the two-cell circuit presents two possible states: the first cell as Receiver and the second cell as Sender (R; S) and vice versa, i.e. (S; R) (Fig. 3B and Fig. S4D). The model for this case of two cells interacting through Notch–Delta is detailed in *SI Text*, section S3, Eqs. S19–S21.

Bifurcation and Phase Diagram. In Fig. 3C, we present a bifurcation diagram when the external Delta (D_{ext}) acts as a control parameter—the range of existence of the different Notch–Delta states of a single cell as function of (D_{ext}). We see that for small D_{ext} , the cell behaves as a Sender (S); and for large D_{ext} , the cell behaves as a Receiver (R). We further see the existence of bistability for intermediate levels of D_{ext} : the cell can either be a Sender (S) or a Receiver (R) (Fig. 3C and Fig. S5A). Next, in Fig. 3D, we present the phase diagram (two-parameter bifurcation diagram) for a single cell driven by two control parameters, the external Notch (N_{ext}) and the external Delta (D_{ext}). Doing so reveals the existence of three distinct phases: (i) monostable Sender {S} phase, (ii) monostable Receiver {R} phase, and (iii) a bistable phase {S,R}, where cells can either be Receiver or Sender. The results indicate that the Notch–Delta circuit behaves as an intercellular mutually inhibitory bistable (two-way) toggle-like switch.

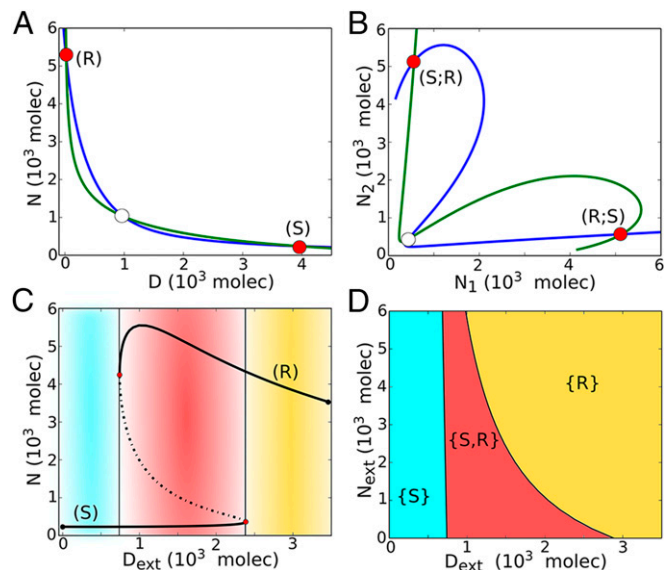


Fig. 3. Dynamical system characteristics of the Notch–Delta circuit. (A) Nullclines for the case of one cell interacting with fixed values of external proteins ($N_{ext} = 500$, $D_{ext} = 1,500$). The blue nullcline is for condition $dN/dt = 0$ and $dl/dt = 0$, and the green nullcline is for condition $dD/dt = 0$ and $dl/dt = 0$ (Eqs. 5–7). Unfilled circles represent unstable steady states, whereas red filled circles represent the two stable states—the Sender (S) and the Receiver (R). (B) Nullclines for the case of two cells interacting with each other through Notch–Delta. The blue nullcline is for condition of all ODEs being set to zero except for dN_2/dt and the green nullcline is for condition of all ODEs being set to zero except for dN_1/dt (Eqs. S19–S21). Unfilled circle represents unstable steady states, and the red filled circles represent the two stable states—(S; R) and (R; S). (C) Bifurcation, for the one-cell case, of Notch protein levels on the membrane as a function of the number of external Delta (D_{ext}) for fixed $N_{ext} = 500$ molecules. Starting in the Sender (S) state, i.e., (low Notch, high Delta) (blue region) and increasing the external Delta (D_{ext}) at some threshold the cell undergoes a transition to the Receiver state, i.e., (high Notch, low Delta) (yellow region). The reverse transition occurs at a different number of D_{ext} proteins that leads to a region of coexistence of both states—Sender and Receiver (red region). Solid curves represent stable steady states, whereas dotted curves represent unstable steady states. (D) Phenotype diagram as a function of external Notch (N_{ext}) and external Delta (D_{ext}) for one-cell model. The monostable phase {S} corresponds to the Sender state (low Notch, high Delta) and monostable phase {R} corresponds to the Receiver state (high Notch, low Delta). The bistable phase {S,R} corresponds to a region of coexistence of both states—Sender and Receiver.

As such, this switch drives two Notch–Delta interacting neighboring cells to adopt opposite fates: one cell as a Sender and the other as a Receiver or vice versa. This result is consistent with the mutual inhibition mechanism commonly associated with Delta-mediated Notch signaling (30), also referred to as lateral inhibition. For this reason, the Notch–Delta signaling is critical for generating checkboard-like patterns as well as sharp boundaries of wing vein formation in the *Drosophila* wing disc (13), and also in the differentiation of sensory cells (31).

The Ligands’ Asymmetric Transcription Regulation by NICD. In this section, we study the effect of the ligands’ asymmetric transcription regulation by NICD—inhibition of Delta and activation of Jagged. We consider the Notch–Jagged *cis*-inhibition and *trans*-activation rate to be the same as the Notch–Delta *cis*-inhibition and *trans*-activation rate. The effect of the post-translational modifications of Notch by Fringe is considered in the next section. We found that the ligands’ asymmetric transcription regulation enables the existence of a new Sender/Receiver (S/R) hybrid state in addition to the Sender (S) and

Receiver (R) states, thus turning the Notch–Delta–Jagged pathway to act as a three-way switch (Fig. 4A and Fig. S4A).

The S/R hybrid state has intermediate levels of both the receptor and the ligands, therefore allowing for bidirectional signaling. In the case of two interacting cells, in this hybrid state, indicated by (S/R; S/R), the two cells have similar intermediate levels of the ligands in contrast with the additional two opposite states [(S; R) and (R; S)] (Fig. 4B and Fig. S4E and F). In other words, whereas the Notch–Delta signaling enables only opposite fates, (S; R) and (R; S), the Notch–Delta–Jagged signaling enables two similar interacting cells to have similar fates of being in a hybrid state (S/R; S/R). Such similar fate adoption, also known as lateral induction, is a signature of Notch–Jagged signaling. For example, during inner-ear development, lateral induction through Jagged1 in specific regions of the developing otocyst (auditory vesicle) enables the propagation and maintenance of prosensory character in some cells (32). Also, during cardiac development, lateral induction specifies the cells that undergo EMT to form endocardial cushion and heart valves (11). Besides, lateral induction has been implicated in vertebrate so-

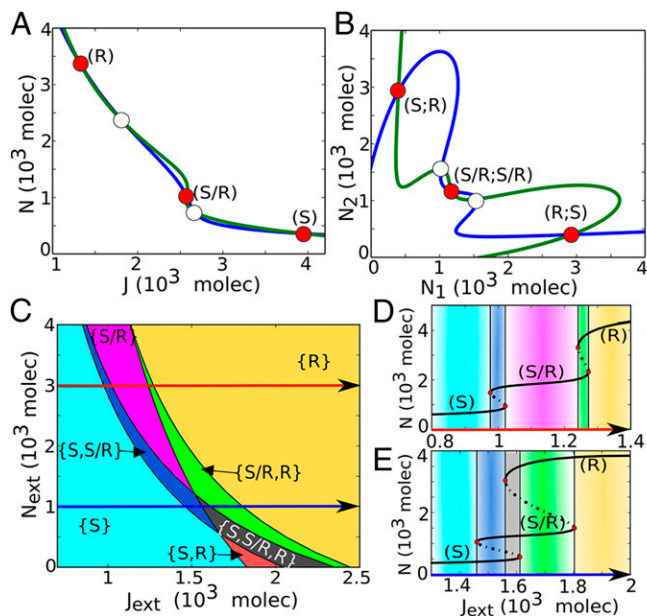


Fig. 4. Dynamical system characteristics of the Notch–Delta–Jagged circuit. (A) Nullclines for the case of one cell interacting with fixed values of external proteins ($N_{ext} = 500$, $D_{ext} = 0$, $J_{ext} = 1,750$). The blue nullcline is for condition $dN/dt = 0$, $dD/dt = 0$ and $dI/dt = 0$, and the green nullcline is for condition $dD/dt = 0$, $dJ/dt = 0$ and $dI/dt = 0$ (Eqs. 1–4). The red filled circles represent the three stable steady states—Sender (S), Receiver (R), and hybrid Sender/Receiver (S/R). Unfilled circles represent unstable steady states. (B) Nullclines for the case of two cells interacting with each other through Notch–Delta–Jagged. The blue nullcline is for condition of all ODEs being set to zero except for dN_1/dt and the green nullcline is for condition of all ODEs being set to zero except for dN_2/dt (Eqs. S22–S25). (C) Phenotype diagram when the one-cell Notch–Delta–Jagged circuit is driven by both the external Notch (N_{ext}) and external Jagged (J_{ext}), for ($D_{ext} = 0$). Each phase, denoted by a different color, corresponds to a different combination of coexisting phases. Same phenotype diagram is obtained when driven by N_{ext} and D_{ext} , for $J_{ext} = 0$, once Notch is considered to have the same binding affinity as J_{ext} and D_{ext} . (D) Bifurcation of Notch protein levels on the membrane when driven by external Jagged for fixed levels of $N_{ext} = 3,000$ proteins. This curve shows the existence of the monostable (S/R) phase (pink region) for a large range of external ligands. (E) Same as panel D for $N_{ext} = 1,000$ proteins. In this case, the hybrid S/R state coexists with other states, i.e., seen only in bistable (blue and green regions) and tristable phases (gray region).

mite boundary formation (33) as well as in wing margin development (6, 13).

Phase Diagram. In Fig. 4C we present the phase diagram (two-parameter bifurcation diagram) for a single cell driven by two control parameters: the external Notch (N_{ext}) and the external Jagged (J_{ext}). When the two ligands are included with asymmetric transcription regulation by NICD, the phase diagram comprises three monostable phases: {S}, {R}, and {S/R}; three phases of coexistence of two phenotypes {S,R}, {S,S/R}, and {S/R,R}; and also a tristable phase showing the coexistence of all three possible states {S,S/R,R} (Fig. 4C). At larger N_{ext} values, we see the hybrid S/R state can exist, for some range of J_{ext} , by itself, i.e., in the monostable {S/R} phase (Fig. 4D and Fig. S5B). However, at smaller N_{ext} values, the hybrid state always coexists with other states in a bistable phase {S, S/R} and {S/R, R}, or in a tristable phase {S, S/R, R} (Fig. 4E and Fig. S5C).

The Effect of Fringe-Mediated Asymmetric Notch–Ligand Binding. Glycosylation of Notch by Fringe creates additional asymmetry between Delta and Jagged by modulating the binding affinity of the two ligands to Notch; the glycosylated Notch has a higher affinity to bind to Delta, but lower affinity to bind to Jagged (34, 35). To incorporate this mechanism within our framework, we considered two distinct subpopulations of Notch—the one modified by Fringe, and the other unmodified. Because NICD, which is represented in the model by (I), activates Fringe (36), we have taken the fraction of glycosylated Notch (denoting the effect of Fringe on Notch) to increase with (I). This glycosylated Notch has different strengths of *cis*-inhibition and *trans*-activation for Delta and for Jagged (37) (see derivation of the model in *SI Text*, section S2). Thus, while representing effective Notch (sum of glycosylated and unglycosylated Notch), we consider the strengths of *cis*-inhibition and *trans*-activation of Notch for Delta and for Jagged to depend on (I). The resulting model for one cell is given by

$$\frac{dN}{dt} = N_0 H^{S^+}(I) - N(k_{C_D} D + k_{T_D} D_{ext} + k_{C_J} J + k_{T_J} J_{ext}) - \gamma N, \quad [8]$$

$$\frac{dD}{dt} = D_0 H^{S^-}(I) - k_{C_D} N D - k_{T_D} D N_{ext} - \gamma D, \quad [9]$$

$$\frac{dJ}{dt} = J_0 H^{S^+}(I) - k_{C_J} N J - k_{T_J} J N_{ext} - \gamma J, \quad [10]$$

$$\frac{dI}{dt} = N[k_{T_D} D_{ext} + k_{T_J} J_{ext}] - \gamma I, \quad [11]$$

where k_{C_D} , k_{C_J} , k_{T_D} , and k_{T_J} are now functions of the signal NICD given by $k(I) = k[1 + aH^+(I)] = kH^S(I, \lambda^F)$, where $\lambda^F = 1 + a$. The shifted Hill function $H^S(I, \lambda^F)$ represents the increase of the Fringe effect on the binding asymmetry with the increase of (I) and the parameter λ^F represents the increase ($\lambda^F > 1$), decrease ($\lambda^F < 1$) of both *trans*-activation and *cis*-inhibition rate due to glycosylation. Experimental evidence suggests that $\lambda_D^F > 1$ and $\lambda_J^F < 1$ (34, 35), representing the increase of the binding affinity between Notch and Delta, and the decrease of that between Notch and Jagged.

When $\lambda_D^F = \lambda_J^F = 1$, the model is the same as considered earlier without any effect of Fringe (Eqs. 1–4). In this case, the Notch–ligand binding has equal affinity for external Jagged and external Delta, as reflected in the symmetry of the phenotype diagram (two-parameter phase diagram) for external Jagged and external Delta (Fig. 5A, Center). The bifurcation diagram for a cell driven by external Delta (D_{ext}) and by external Jagged (J_{ext}) presents the same behavior—a large range of the intermediate state (S/R) in

a monostable phase (Fig. 5A). However, when the effect of Fringe is incorporated (e.g., $\lambda_D^F=3$ and $\lambda_J^F=0.3$), the circuit behaves differently. The range of the existence of the four phases containing the hybrid S/R state [the phases {S/R}, {S/R, R}, {S, S/R}, and {S, S/R, R}] increases with the level of external Jagged (Fig. 5B, Center, and Fig. S6). When Notch signaling is mainly mediated by Jagged (high J_{ext}), both the forward and backward transitions between (S) and (R) states require a transitions into and from the hybrid S/R state as an intermediary step (Fig. 5B, Right and Fig. S5D). Conversely, when the Notch signaling is mainly mediated by Delta (high D_{ext}), the forward and backward transitions between (S) and (R) do not go through the hybrid state (Fig. 5B, Left and Fig. S5E). When the effect of Fringe is considered to be too strong ($\lambda_D^F=5$ and $\lambda_J^F=0.2$), the circuit is mostly bistable and therefore the response of the circuit becomes similar to the case of standalone Notch–Delta signaling (Fig. 5C).

The results above suggest that signaling through Jagged has an important role in maintaining the hybrid Sender/Receiver (S/R) state, and that Jagged makes it much more likely that transition from Sender (S) to Receiver (R) and vice versa happens through the hybrid (S/R) state.

The Effect of Delta–Jagged Asymmetry on the Cell–Cell Fate Modulation. Notch signaling in mammals is mediated through four types of Notch (Notch 1–4) and three types of Fringe (lunatic, manic, and radical Fringe) (38). Experimental evidence suggests that

most Fringe proteins act with different types of Notch, possibly leading to different forms of glycosylated Notch, thereby expanding the repertoire of responses that the Notch signaling system can mediate (34, 35). Within our framework, different modulations of Notch by Fringe can be represented by different values of the parameters λ_D^F and λ_J^F , which represent either the increase ($\lambda^F > 1$) or decrease ($\lambda^F < 1$) of the *cis*-inhibition and *trans*-activation rates. Most experimental evidence suggests that Fringe increases the signaling mediated by Delta and decreases the signaling mediated by Jagged, resulting in $\lambda_D^F > 1$ and $\lambda_J^F < 1$ (34, 35). The phenotype diagram when the circuit is driven by different values of λ_D^F and λ_J^F presents the response of the circuit for different combinations of Fringe modulations (Fig. 6).

Because Fringe is activated by NICD (36), its effect is dominant in cells with high number of Notch molecules [Receiver (R) state] that cleave to form NICD. Therefore, to analyze the effect of Fringe on Notch–Delta–Jagged signaling, we choose the external signal to the cell to be composed mainly of ligands (J_{ext} , D_{ext}) and low values of N_{ext} ; i.e., the external signal can be considered equivalent to a Sender (S) cell. Two such different combinations are chosen: (high D_{ext} , low J_{ext}) and (low D_{ext} , high J_{ext}) (Fig. 6). In the case of (high D_{ext} , low J_{ext}) and at $\lambda_D^F > 2$ and $\lambda_J^F < 1$, i.e., when the external signal is mostly Delta, and Fringe increases the affinity of Notch for Delta, and decreases that for Jagged, the cell is mostly in monostable phase of the Receiver

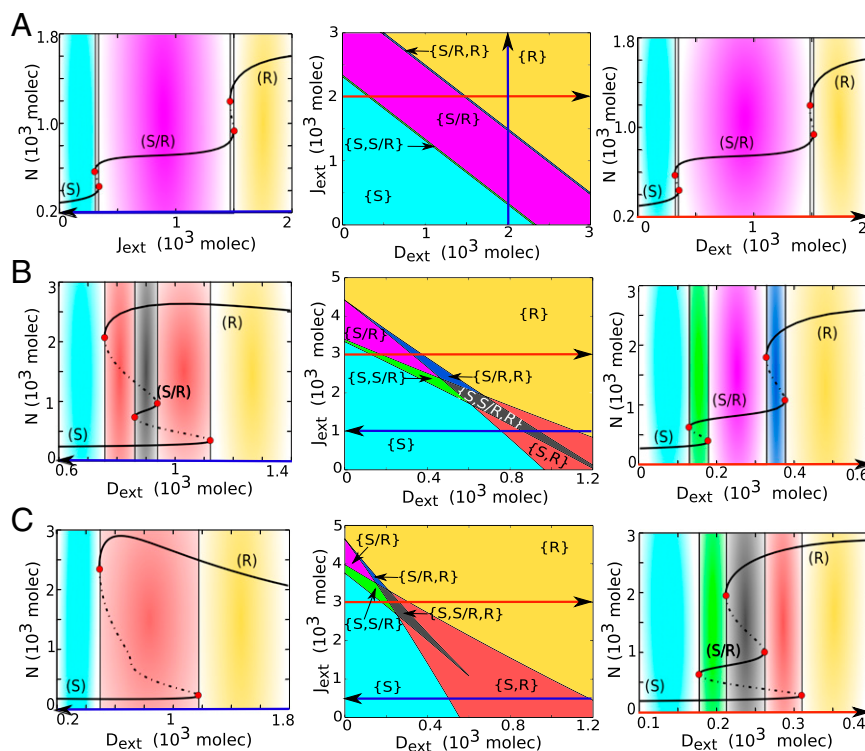


Fig. 5. Phenotype diagram and bifurcation curves for the one-cell Notch–Delta–Jagged–Fringe circuit. The phenotype diagram shows the different possible phases when the circuit is driven by variable levels of both external Jagged and external Delta. (A) Phenotype diagram (Center) for $\lambda_D^F = \lambda_J^F = 1$ (no Fringe effect). In this case, the circuit response to external Jagged and external Delta is symmetric. Bifurcation curve of Notch protein levels with respect to varying external Jagged values (Left) for fixed $D_{ext} = 2,000$ and $N_{ext} = 500$ molecules and (Right) bifurcation curve with respect to varying external Delta values for fixed $J_{ext} = 2,000$ and $N_{ext} = 500$ molecules. (B) Phenotype diagram (Center) for $\lambda_D^F = 3$ and $\lambda_J^F = 0.3$ (intermediate effect of Fringe). Bifurcation curves of Notch protein levels in response to varying D_{ext} for $J_{ext} = 1,000$ and $N_{ext} = 500$ molecules (Left), i.e., Notch signaling mainly mediated through Delta and for fixed $J_{ext} = 3,000$ and $N_{ext} = 500$ molecules (Right), i.e., Notch signaling mainly mediated through Jagged. (C) Phenotype diagram (Center) for $\lambda_D^F = 5.0$ and $\lambda_J^F = 0.2$ (very strong effect of Fringe). Bifurcation curves of Notch protein levels in response to D_{ext} for fixed $J_{ext} = 500$ and $N_{ext} = 500$ molecules (Left), in which the hybrid S/R state no longer exists, and the circuit behaves like a bistable toggle switch similar to the circuit considering Notch–Delta only, and for fixed $J_{ext} = 3,000$ and $N_{ext} = 500$ molecules (Right), in which the hybrid S/R state can be observed to coexist with other states (green and gray regions). Phenotype diagrams for $N_{ext} = 1,000$ are presented in Fig. S7.

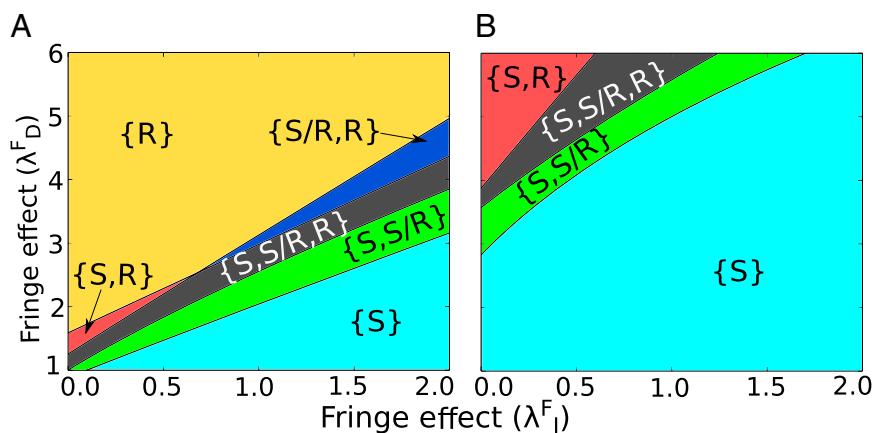


Fig. 6. Phenotype diagram of the Notch–Delta–Jagged–Fringe model when the circuit is driven by different values of the Fringe modulation for Notch–Delta interaction λ_D^F and Notch–Jagged interaction λ_J^F . In all curves the external signal represents cells in the Sender (S) state—low concentration of Notch ($N_{ext} = 500$ molecules) and high concentration of ligands. Each figure represents a different combination of the number of external ligands (in number of proteins available to binding). (A) $D_{ext} = 1,500$ and $J_{ext} = 500$ molecules. (B) $D_{ext} = 500$ and $J_{ext} = 1,800$ molecules.

(R) state, or, in other words, the cell attains the opposite fate as that of a cell representing the external signals (Fig. 6A). However, when the external signal is mainly Jagged, i.e., in (low D_{ext} , high J_{ext}), at smaller values of λ_D^F ($\lambda_D^F < 4$) and λ_J^F ($\lambda_J^F < 1$), i.e., the effect of Fringe is not very pronounced; the cell is mostly in the monostable phase of the Sender (S) state (Fig. 6B). Therefore, the cell attains a fate similar to the cell represented by the external signal. These results suggest that while signaling through the Notch–Delta circuit, the two cells attain opposite fates; however, when signaling through the Notch–Jagged circuit, the two cells attain similar fates, thereby suggesting that Jagged helps neighboring cells to maintain similar fates.

Ligand Production Rates Control Tissue Level Patterning. As mentioned earlier, Notch–Delta interactions lead to lateral inhibition—neighboring cells adopt alternate fates—Sender (S) and Receiver (R) (13, 14, 39, 40). Notch–Jagged interactions lead to lateral induction (41, 42)—neighboring cells adopt similar fates—both of them can simultaneously send and receive the signal—Sender/Receiver (S/R) state. Although these mechanisms have been well-studied individually, the tissue level patterns that might emerge when both mechanisms act simultaneously have not been explored.

To address this issue, we simulate a one-dimensional layer of cells interacting via Notch pathway, for different values of D_0 and J_0 —Delta and Jagged production rates, respectively. Our results show that when the relative production rate of Delta is increased, the so-called “salt-and-pepper” pattern (or alternate fate pattern) at a tissue level begins to emerge (Fig. 7A and C and Fig. S8). On the other hand, when the relative production rate of Jagged is increased, the salt-and-pepper pattern is disrupted, and the cells begin to adopt similar fates where they can both send and receive the signal (Fig. 7B and D and Fig. S8).

Our results are consistent with the patterns observed experimentally when both ligands are produced at different rates. For example, during hypoxia-mediated angiogenesis, increase in the concentration of vascular endothelial growth factor increases the production of Delta, thereby causing some cells to adopt a tip fate—those with (high Delta, low Notch). Consequently, the remaining cells adopt a stalk fate—(low Delta, high Notch). It may be noted that in this physiological context, the cells do not necessarily adopt a canonical salt-and-pepper pattern, rather two tip cells might be separated by a few stalk cells, the number of which is determined by Jagged (43). As another example, the

inflammatory factors such as TNF- α can lead to increased Jagged production and decreased Delta production (43), therefore driving the cells to a similar fate—hybrid Sender/Receiver (S/R)—that can promote bidirectional communication between tumor and stroma, a context where inflammation often plays a key role.

Discussion

Notch pathway plays crucial roles during embryonic development (1, 2) and also during tumor progression and metastasis (25). Whereas Notch–Delta signaling has been extensively studied both theoretically and experimentally (6, 13–17) and is well understood, the role of Jagged in the Notch–Delta–Jagged signaling is still elusive. Here, we introduced a specially designed theoretical framework to study Notch signaling through both Delta and Jagged which incorporates the effect of the asymmetries between these two key ligands. An earlier attempt included both Delta and Jagged in Notch signaling system (9). Although it explains some of their own experimental results, it does not include some fundamental features of Notch signaling, such as *cis*-inhibition between Notch and Delta (14), *cis*-inhibition between Notch and Jagged (37), and the effect of glycosyltransferase Fringe that causes an asymmetry between Notch–Delta and Notch–Jagged signaling (34, 35). Further, their model also does not discriminate between the transmembrane receptor Notch and the internalized signal NICD.

Our results confirmed that Notch–Delta alone allows only two states: Sender (S) or Receiver (R), which is consistent with previous studies (6, 13, 14, 17). Our key findings are that due to the Delta–Jagged asymmetry, the Notch signaling through both Delta and Jagged gives rise to a hybrid Sender/Receiver (S/R) state in addition to Sender and Receiver states. Sender (S) cells have high levels of ligands (Delta and Jagged) and low levels of receptor (Notch) on their surface, and Receiver (R) cells have high levels of receptor and low levels of ligands on their surface. The hybrid S/R cells have intermediate levels of both the receptor and ligands, therefore allowing them to both send and receive signals. Alternate arrangements of Sender and Receiver cells have been observed in checkerboard-like or salt-and-pepper pattern formation (30, 31). However, direct measurements of both Notch and ligands are needed in cells to identify the hybrid S/R cells.

The two-cell model explores the canonical signaling between two cells. In our one-cell model, we take the level of the external ligands Jagged and Delta (J_{ext} , D_{ext}) as control parameters that

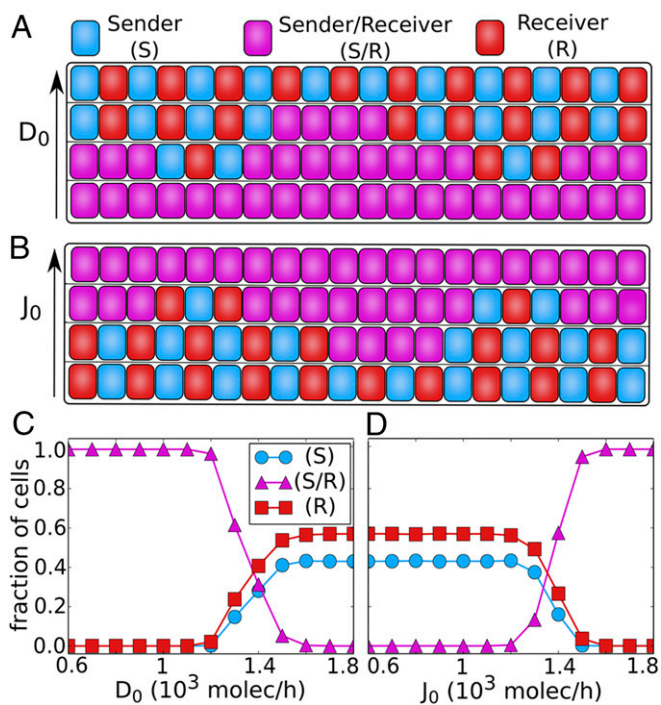


Fig. 7. Patterning at the tissue level. (A and B) Representation of a 1D layer of 20 interacting cells. (A) The increase of the production of Delta (D_0) leads to the formation of alternate patterns in which neighbor cells alternate between Sender and Receiver. (B) The increase of the production of Jagged leads all of the cells to the hybrid (S/R) state, therefore losing the alternate (S) and (R) pattern. (C and D) Average of the fraction of cells in (S), (S/R), or (R) state as a function of ligand production. The averages were taken over 100 simulations of a 1D layer of 100 interacting cells with periodic boundary condition. (C) Fraction of cells in (S), (S/R), or (R) state as a function of the production of Delta. (D) Fraction of cells in (S), (S/R), or (R) state as a function of the production of Jagged. The states of the cells are defined according to the amount of signaling (l): Sender ($l < 100$), Sender/Receiver ($100 < l < 300$), and Receiver ($l > 300$). For this figure, we used $k_T = 2.5e^{-5}$.

represent fixed levels of Delta and Jagged on a neighboring cell. In the case of Notch–Delta–Jagged between two neighboring cells, (J_{ext} , D_{ext}) for each cell represent the values of Delta and Jagged on the neighboring one.

We note that (J_{ext} , D_{ext}) can also represent external soluble ligands. However, currently the mechanism of receptor activation by soluble ligands is not clear and is still debated. Some studies suggest that mechanical pulling force is essential to activate proteolysis and the release of the signal (NICD) (44), and because soluble forms of the ligands tend to lack this pulling force, they are expected to inhibit signaling (45). Conversely, other studies indicate that other mechanisms such as ligand multimerization (46) can furnish sufficient mechanical leverage for receptor activation (12). Notwithstanding the incomplete understanding of how soluble ligands activate Notch, they play crucial roles in various contexts such as de novo generation of regulatory T cells (19), differentiation of adipocyte progenitor (47), hematopoietic progenitor (48), and neural crest stem cells (49).

Soluble Jagged1 has been specifically implicated in mediating long-distance communication between tumor cells and stromal cells. Jagged1 can be secreted by endothelial cells that can activate Notch signaling in cancer cells, inducing them to gain migratory and invasive characteristics by undergoing partial or complete EMT (50). Jagged1 can also induce the expression of NF- κ B (51), which can increase the population of CSCs and

further increase the secretion of Jagged1 (52), suggesting a wave-like mechanism in the tumor microenvironment to increase the production and maintenance of therapy-resistant CSCs. Future theoretical studies of these circuits hold promise for appreciating the key role of soluble Jagged1 in mediating two interlinked and clinically insuperable facets of cancer—metastasis (as a result of cells undergoing EMT) and tumor relapse (as a result of expanded CSC pool).

Not only soluble Jagged1, but also transmembrane Jagged1 mediates tumor progression in several ways, and has been proposed to be a therapeutic target (53). Notch–Jagged signaling plays a crucial role in the metastasis of breast cancer cells to bone, where prostate cancer cells expressing Jagged1 communicate with Notch-expressing osteoclasts to “home” in the bone (25). Also, overexpression of Jagged1 on cancer cells can trigger Notch activation in neighboring endothelial cells (which can possibly secrete more soluble Jagged1), promoting sprouting tumor angiogenesis (54) and thereby tumor growth. Consistent with their protumor roles, high levels of Notch and Jagged1 in cancer cells often correlate with poor patient survival (53).

We show that the hybrid S/R state is enabled only after including Jagged in the model. We expect that this hybrid state plays an absolutely critical role in mediating communication between these cells helps them to maintain that otherwise metastable hybrid epithelial/mesenchymal (E/M) phenotype. As has been recently observed in clusters of circulating tumor cells (CTCs), these hybrid E/M cells mediate tumor aggression and invasion (58), and can have more metastatic potential than the CTCs moving individually (59, 60). Future theoretical studies should investigate the coupling of EMT and Notch–Delta–Jagged signaling to explore this hypothesis.

Although our model provides a fresh theoretical framework to investigate the effect of both Delta and Jagged in the Notch signaling system, it is based on a well-mixed ordinary differential equation (ODE) approximation, ignoring most spatial effects that can be useful, for example, to understand the formation of sharply defined bands of Notch signaling that occur in *Drosophila* wing vein system (6). Other limitations of our model include: no distinction between soluble and membrane-bound ligands, no time delay between production of Fringe and its action on Notch, and grouping the different members of the family of Notch, Delta, Jagged, and Fringe into one variable. This grouping restricts understanding the context-specific function of different family members, for example, lunatic Fringe vs. radical Fringe (37).

To conclude, we present, to our knowledge, the first step toward including the role of Jagged in cell-fate determination. Jagged-mediated signaling indicates an evolutionary need to implicate different repertoires of responses in cell–cell communication, and has been shown to be critical in mammalian embryonic development as well as tumor progression. A better understanding of Notch–Delta–Jagged signaling, which is affected by various signals in the tumor microenvironment (43), can provide valuable clues how to target cancer survival by interfering with the tumor–stroma cross-talk.

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