Multiscale Modelling of Delta-Notch Pathways

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Notch signalling can be linked to drosophila hair growth on wings, or development of a chick's inner ear epithelium, it also occurs within eye development and now believed to cause tissue development in cardiac cells. It does this by assigning a fate to a particular cell where each cell adopts a certain cell fate, dependant on the communication through the Delta-Notch network. Notch operates by encoding a transmembrane protein which has a receptor. In the extracellular region the receptor contains 36 epidermal growth factor (EGF)-like repeats that control the interactions between Delta/Serrate/JAG ligands, though in this case the concentration is Delta ligands [1] [2].

Once bound, the signalling network is initialised by the Delta of the sending cell. The Notch receptor is then cleaved and relocates within the nucleus of the receiving cell. This can then alter the state of the cell to change its fate by repressing or activating certain gene expressions [3] as Figure 1 shows. In this case it will be assumed that Delta ligands will bind with Notch receptors and Notch receptors can also bind to Delta ligands.

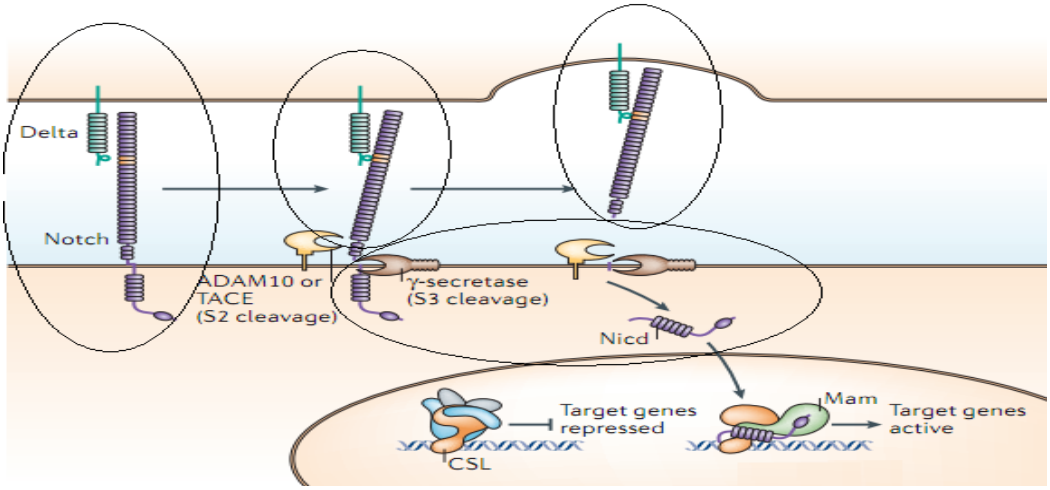

Figure 1- Step A. Delta and Notch bind together. Step B. S2 cleavage occurs in the extracellular domain. Step C. The Delta ligand and the Notch receptor become a product of endocytosis and are recycled or degraded. Step D. The NICD is cleaved in the intracellular domain due to γ-secretase, this process is known as S3 and S4 cleavage, though the diagram only shows the S3 cleave, as the S4 cleave is small. Step E. This shows the NICD moving into the nucleus, where it binds to CSL to activate target genes [4].

The cleaving process of NICD is first catalyzed by the ADAM-family of metalloproteases, this is known as S2 cleavage, it generates a substrate for S3/S4 cleavage. The second cleavage, S3/S4, is caused by γ-secretase, an enzyme complex. The S4 cleave removes any remnants of the receptor from the extracellular region and the S3 cleave releases the NICD in order for it to relocate within the nucleus. Once inside the nucleus, the NICD will bind and convert CSL from a transcriptional repressor to a transcriptional activator. Post cleavage, the remains of the Delta ligands and the Notch receptors become a product of endocytosis within the cell, enabling the protein to be recycled or degraded through the multivesicular-body pathway which is contrary to other models, which shows a dissociation between the receptor and ligand once bound. There is certain evidence to show that endocytosis can regulate the level of active Notch in the cell by negatively using Numb protein. At low levels, Notch is inhibited, but at high levels, it is thought that Notch regulates Numb. Though Numb regulates Notch, the effects of this process can be assumed to be negligible while modelling, due to minimal affects on Notch relative to the levels of lateral induction [4–6].

When the NICD enters the nucleus of a cardiac cell, lateral induction will occur. Lateral induction increases the production of Delta in the cell. The cell is then able to up-regulate the production of Delta, resulting in more Delta ligands, this process is shown in Figure 2. Certain work suggests that lateral induction may not only induce the Delta ligand, they state lateral induction "promotes ligand expression and activation of Notch on both cells" [7]. More recent work however, suggests that the increase in Notch is not caused specifically by the lateral induction process, but could be a by-product because of it, as it helps to "strengthens and maintains Notch activation". Therefore the model can assume that the lateral induction process induces both Delta Ligands and Notch receptors [8], [9].

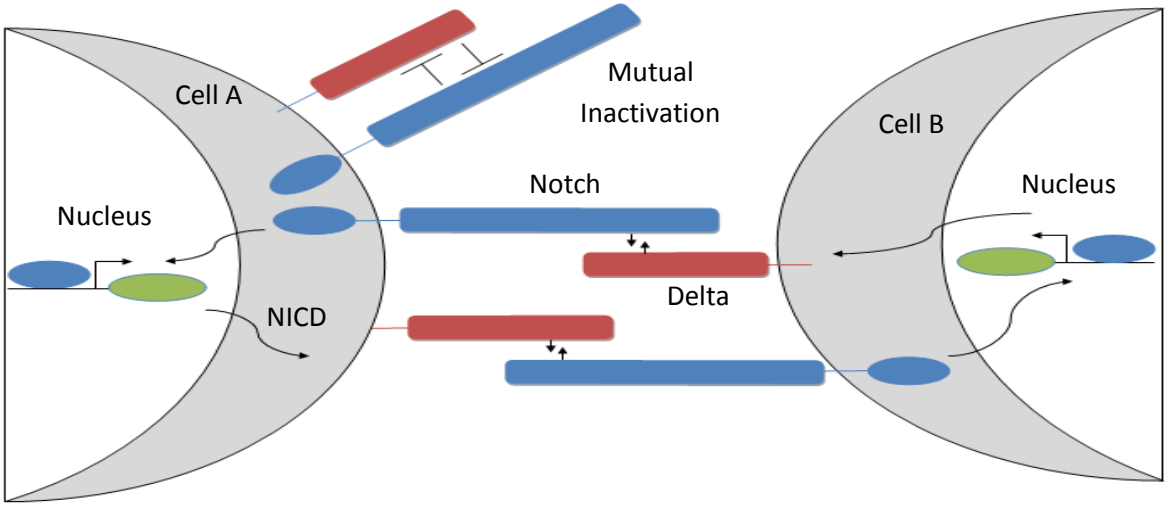

Figure 2 –A model of lateral induction. Lateral induction shows that after the bind of the Delta ligand of cell B and the Notch receptor of cell A, the NICD enters the nucleus of cell A, which not only alters gene expression, but induces the production of Delta, so it can bind to the Notch receptor of cell B and the process can repeat itself. This will lead to both cells sharing the same fate. Mutual inactivation also shown, will cause the cells to assume separate fates

Mutual inactivation, also known as cis-inhibition, is the process where the Notch receptor and Delta ligand in the same cell suppress each other [10]. In an individual cell, mutual inactivation results in a switch-like behaviour, where a large Delta/Notch ratio will result in "sending" and a large Notch/Delta ratio will cause the cell to "receive". This effect can be used to investigate pattern formation in Figure 2 and justify which cells send the signal to neighbouring cells to change fate and the boundaries this creates between cells, as in the model of the drosophila wing [11].

The cis-inhibition process renders the Notch receptors and Delta ligands involved useless, as it becomes a product of endocytosis and is then recycled as Delta and Notch or degraded into the nucleus. Mutual inactivation is a large factor that contributes to the non-linearity of the Delta-Notch signalling networks; therefore to model it mathematically, complex systems must be used. Mutual inactivation can occur in three ways. The first method is that the Notch receptor and Delta ligand can mutually inhibit each other. The second method shows that the Notch receptor can solely inhibit the Delta ligand. The final method is the Delta ligand inhibiting the Notch receptor. Though there are three separate types of inactivation, they all have the same effect and can therefore be modelled as the same inactivation [12].

Using the model produced by Owen et al [13], [14] the basic model of juxtacrine signalling can be adapted to produce the model in Figure 3. The original model was firstly adapted to include mutual inactivation. This would alter the results of bound Delta-Notch complexes per cell, dependant on the strength of the inactivation. The next step was to remove the dissociation rate, as the Delta Notch complex does not dissociate; instead it becomes a product of endocytosis and degrades internally, which is shown by the decay rate of the bound Delta-Notch complex. Due to the NICD cleaving once the Delta ligands and Notch receptor are bound, it can be assumed that all NICD relocates to the nucleus of the cell without losses. The process of cleavage of NICD can also remove the internalised ligand-receptor complex, as the NICD is the important factor for lateral induction.

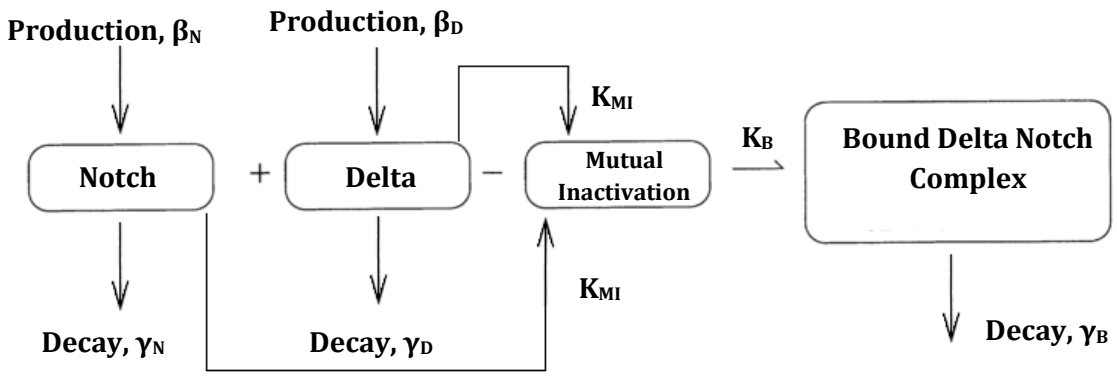

Figure 3 - A model of the Delta-Notch Pathway expressing mutual inactivation

The model was then put into a set of mathematical equations based on those produced by Owen et al [14] and mutual inactivation as described by Sprinzak et al [15] to produce the amount of free Delta, the amount of free Notch and the amount of bound Delta-Notch in time specific intervals over a certain number of cells using Delta as D(x,t), Notch as N(x, t) and the Bound Delta-Notch complex as B(x, t) to produce a multiscale model:

$$\frac{\delta D}{\delta t} = \beta_D - \gamma_D + P_D(B) - MI - K_B D < N >$$

$$\frac{\delta N}{\delta t} = \beta_N - \gamma_N + P_N(B) - MI - K_B N < D >$$

$$\frac{\delta B}{\delta t} = K_B D < N > + K_B N < D > - \gamma_B$$

The equations show β_D , β_N as the rate of production of both Delta and Notch. γ_D, γ_N and γ_B represent the decay rates of Delta, Notch and the bound Delta-Notch complex in a cell. $P_D(B)$ and $P_N(B)$ are functions that represent the ligand and receptor production via lateral induction. MI is the mutual inactivation of both Delta and Notch and as it requires the same number Delta and Notch, will affect both. K_B is the rate at which Delta and Notch bind between cells and these are relative to the amount of Delta in the cell and the average amount of Notch in immediate neighbouring cells represented as $< N >$. This may also work with Notch in the cell binding with the average amount of Delta in the neighbouring cell represented as $< D >$.

The equations and model can then be represented visually using Compucell3D™ to reproduce certain pattern formations without mutual inactivation in the same format as Owen et al [13]. By adding different strengths of mutual inactivation the results show that pattern formations vary and can deform at certain concentrations. This shows that there is a lack of pattern formation when mutual inactivation occurs. The deformation of patterns could provide a possible explanation for the lack of adhesion of certain regions within EMT, though further study would be required.

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