Early Neurodevelopment: Axial Differentiation, Brain Patterning, and Neurogenesis

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Introduction

The vastly complex, delicate nature of the nervous system calls for a highly effective development system. The development of the nervous system begins early in embryogenesis and is one of the last systems to be completed after birth. Deemed to be one of the most important steps in the evolutionary progression towards sophisticated life, the pathways regulating neurodevelopment are highly specialized and conserved. Embryonic neurodevelopment is an important starting point for the understanding of brain anatomy, function, and its neurobiology. The past few decades have brought about numerous technological advancements allowing for the study of the earliest stages of embryonic development further shedding light on the process that develops the most complex system known to man. This review aims to highlight major findings and provide a general understanding of neurodevelopment pertaining to crucial signaling pathways resulting in axial patterning and neurogenesis.

Notch Signaling Pathway

The Notch signaling pathway is a highly conserved signaling system critical to embryogenesis that is present in the majority of multicellular organisms. Notch has been demonstrated to influence a broad range of events throughout the entire life of a multicellular organism including somitogenesis, hematopoiesis, neurogenesis, stem cell renewal, stem cell differentiation, and late-life neurodegeneration.¹

Mammals possess four different transmembrane Notch receptors referred to as NOTCH1, NOTCH2, NOTCH3, and NOTCH4, to which Delta-like ligands -1 and -3 as well as Serrate-like ligands (Jagged-1 and -2) bind.² In *Drosophila melanogaster*, there exists one Notch receptor in addition to two ligands- Delta and Serrate. The Notch receptor is synthesized as a single protein and exists in a heterodimeric form grounded in the plasma membrane.¹ This pathway remains activated when Notch ligands from an adjacent cell interact with a receptor, triggering the cleavage and release of the Notch Intracellular Domain (NICD).³

The NCID translocates to the nucleus where knocks the N-CoR corepressor off of the CSL complex and recruits MAML and P300, allowing for the transcription of Hairy Enhancer of Splits 1 (HES1).⁴ HES1 is a basic-helix-loop-helix (bHLH) protein, which functions as dedicated transcriptional repressor by binding to target DNA regions. It is specifically known to inhibit expression of proneural genes.⁵



Figure 1. Adapted from Lasky and Wu (2005). Interaction between Delta-ligand from neighboring cells with the transmembrane Notch protein results in the cleavage of the Notch intracellular domain (NICD) by γ-secretase, resulting in translocation to the nucleus. NICD binds to CSL, knocking off CoR and recruiting CoA, along with MAML and P300 (not shown), in order to activate transcription of HES genes.

Notch Lateral and Inductive Signaling

Notch mediates its effect through two mechanisms classified as lateral inhibitory and inductive signaling.⁶ Lateral inhibition is responsible for controlling cellular differentiation by ensuring production of correct numbers of varying cell types from an equipotent population.⁷ Cells in an equipotent population initially express equal amounts of Delta ligand and Notch. The exposure of a cell to Delta ligand activates the Notch receptor, thus down-regulating Delta ligand expression and therefore blocking the Notch signaling pathway in the Delta-expressing cell.⁸ As a result, the Notch activated cell will only express receptor while in the Delta-expressing cell receptor expression will be down-regulated. Notch signaling will result in the maintenance of a uncommitted progenitor state while Delta expression will result in the commencement of neural differentiation.8



Figure 2, Adapted from Lasky and Wu (2005). Model of lateral inhibition: Two cells expressing equal amounts of ligand and receptor interact. The Notch pathway is activated in the cell on the left, resulting in increased receptor expression and down-regulation of Delta. Consequently, the cell on the right experiences a decrease in receptor expression and up-regulation of ligand production, creating two asymmetric cells that possess different

Inductive Notch signaling, on the other hand, involves two distinct cell types expressing either Notch or Delta ligand, resulting in Notch activation being possible only on the receptor-bearing cell. Consequently, cell fate determination occurs. Multiple possible interactions between neural stem cells expressing Notch and other cell types depending on anatomical location will result in varying pathways of differentiation. Exposure to committed neuroblasts which are expressing Delta will result in glial cell commitment in the presence of other extrinsic signals. Lack of exposure to Delta ligand will result in neurogenesis in the presence of strong proneural signals such as bone morphogenic protein 2 (BMP2).⁹ Interaction with Delta ligand will result in definite glial fate commitment even in the presence of strong proneurogenic signals. Relating this model to that of inhibitory signaling, the conjecture that Notch signaling is responsible for the dominating presence of glial cells in the adult brain remains well supported.



Figure 3. Adapted from Lasky and Wu (2005). Model of inductive signaling: Neural stem cells expressing Notch may come in contact with ligand-expressing neuroblasts, resulting in glial cell fate commitment even in the presence of strong proneural signals. In the absence of such an interaction, the neural stem cell continues towards neural development.

Notch-Dependent Embryonic Patterning in C. elegans

The Notch pathway is very simple yet diverse as it also plays an important role in early anteroposterior and transverse axis patterning. During fertilization by sperm, which occurs at the end opposite of the oocyte nucleus, a cytoplasmic flux results in the movement of the spermdeposited pronucleus and centrioles to one pole.¹⁰ It has been demonstrated that the location of the sperm-derived centrosomes will always determine the posterior pole of the zygote as possession of a mutant centrosome results in the failure of the establishment of zygotic polarity. The centrosomes will recruit a group of proteins present within the zygote known as PAR, furthering inducing cell polarity at the posterior end.¹¹ The unequal distribution of multiple cellular factors results in cellular asymmetry, allowing asymmetric cell division in the future.

The first zygotic division in *C. elegans* produces two cells called AB and P1. These two cells will then undergo patterned division in very distinct and deliberate manners.¹² The division of P1 occurs along the anterior/posterior axis, producing asymmetric daughter cells (EMS and P2) that express varying proteins. AB will divide symmetrically along the transverse axis in ABa and ABp. The AB descendants express GLP-1/Notch receptor, while only P2 expresses APX-1/Delta.¹² The GLP-1/Notch receptor components found in AB descendants and the APX-1/Delta components found in P1 descendants are maternally expressed.¹³ The displacement of ABp to the posterior of the 4-cell embryo puts it in contact with the APX-1/Delta-expressing P2 cell, therefore activating the Notch pathway in ABp, but not in ABa. The activated Notch signaling induces embryonic expression of the aforementioned ref-1 genes resulting in the birth of ABp granddaughters.

The 12-cell zygote still possesses maternally-expressed GLP-1/Notch in ABa descendants, but two new P1 descendants (MS and E) become the first signaling cells that produce embryonically-expressed signals controlled by the SKN-1 transcription factor.^{14,15} The MS cell is in contact with 2/4 of the ABa descendants causing GLP-1 activation, resulting in ref-1 gene expression within 25 minutes of ligand-receptor interaction.¹⁶ Identity of the MS signal-molecule is unknown, but it is thought to be related to APX-1 as studies have shown that in an embryo lacking MS, P2 is able to fill-in for MS signaling to a certain degree.¹⁵ ABp descendants are in contact with either MS or E in this stage and will continue expressing the receptor, but Notch activated ABp granddaughters will not respond to the the second Notch interaction unless the first interaction was inhibited by some means.¹⁷



Figure 4. Adapted from Hutter and Schnabel (1994). The division of AB and P1 cells along the transverse and anterior/posterior axis respectively. Cells outlined in red are expressing GLP-1/Notch while blue cells are expressing APX-1/Delta, The position of ABp in the 4-cell stage results in it being the only cell exposed to APX-1/Delta. Green cells represent Notch activation with light green representing early activation and dark green representing later activation.

The role of the first two Notch interactions is to determine which of The first two Notch interactions, which occur at the 4- and 12-cell

the AB cell descendants will differentiate into pharyngeal cells, a process controlled primarily by PHA-4.¹⁸ Pharyngeal tissue of *C. elegans* is primarily mesodermal and contains gland cells, muscle cells, and support cells, as well as a few neurons. In the earliest stages of embryogenesis, only ABa descendants (which are Notch-activated), will express PHA-4. Interruption of the second Notch interaction will prevent ABa descendants from producing pharyngeal cells, while prevention of the first interaction will prevent ABp refraction of the MS and E signals, resulting in ectopic expression of pharyngeal characteristics.¹⁴ stage, produce different results due to age-dependent expression of varying proteins. TBX-37 and TBX-38 are essential for AB descendants to produce pharyngeal cells, but the first Notch interaction induces a down-regulation of these proteins.¹⁹ The second Notch interaction then dictates which of the ABa descendants that are currently expressing TBX-37 and TBX-38 will differentiate into pharyngeal cells. Further Notch interactions in ABplaaa and ABarpap will contribute to the patterning of the left and right sides of the head.¹² While these two cells have very similar differentiation patterns, their surrounding cousins develop in distinct manners. The termination of surrounding cells does not influence the development of the right side of the head, while inhibiting patterning of the left side. The fourth Notch interaction causes AB descendant ABplpapp to give rise to the excretory system.²⁰

During gastrulation, the epiblast ingresses through the blastocoel towards the hypoblast through the primitive streak, forming the three layered gastrula. The gastrula is made up of an endoderm, mesoderm, and ectoderm (from deepest to most superficial). After gastrulation is complete, organogenesis may begin. Initiation of neurogenesis starts with notochord formation from the dorsal mesoderm.² The initial fate of the ectoderm is neural, but BMP4 and Wnt

Notch and *C. elegans* Cell Fate



Figure 5. Adapted from Neves and Priess (2005). Representation of the first four Notch interactions in the AB lineage. Certain descendants of ABa and ABp that express GLP-1 and LIN-12 will come in contact with ligand expressing cells (blue), resulting in Notch pathway activation. Black lines represent REF-1 expression 25 minutes after interaction.

Gastrulation and Neurogenesis

presence inhibits differentiation as these proteins are anti-neuralizing agents. Chordin, noggin, and follistatin (block BMP4) and FrzB, dickkopf, cerberus (block Wnt) are secreted by the organizer (dorsal mesoderm), inducing the neuralization of the ectoderm.²² Cells that receive FGF in addition to BMP4 inhibitory signals form the posterior neural plate. Anterior to posterior gradients of retinoic acid (RA) and FGF must also be present in order to cause mesodermal differentiation into the posterior neural plate.²³

Next, the cells of the neural plate fold inward on themselves to create the neural tube, a structure that runs along the anterior/posterior axis of the embryo and is the precursor to the brain and spinal cord. Sonic hedgehog (Shh) which is secreted by the notochord, patterns the ventral neural tube.²⁴



Figure 6. Adapted from (http://courses.biology.utah.edu/bastiani/3230/db%20lecture/lectures/b15neuro.html). The image on the left portrays the posterior/anterior gradients of RA and FGF, which are more present in the posterior, and Wnt/BMP-inhibitors noggin, follistatin, and chordin, which are more present in the anterior. On the right, BMP4 signaling, its inhibition, and anterior/posterior FGF/RA expression alongside BMP4 inhibition is portrayed in the ectoderm along with resultant patterning.

The neural crest, which is a band of migratory cells that appear at the border of the neural tube and ectoderm, begin to form during neurulation. These cells will later migrate and form the peripheral nervous system, bones, and teeth. Subsequently, another set of migratory cells form when mesodermal cells lateral to the notochord split into blocks called somites. which are responsible for organizing and segmenting the vertebrate's body.²⁵



Figure 7. Adapted from (http://cnx.org/content/col11496/1.6/). Visual representation of the formation of the neural tube as a result of neural plate convergence and associated structures. Ingression and convergence of the ectoderm will result in neural tube and neural crest formation, accompanied by the appearance of somites.

A mouse embryo at E7 will begin to produce signals that result in its differentiation into the major derivatives of the neural tube. The node serves as the primary organizing center and is the source of FGF and RA gradients that posteriorize the embryo.²⁶ It is also responsible for production of the aforementioned BMP and Wnt inhibitors (follistatin, noggin, chordin, Frzb, dickkopf). The anterior visceral endoderm (AVE) produces signals that result in the induction of brain development through dickkopf and cerberus expression.



(http://courses.biology.utah.edu/bastiani/3230/db%20lecture/lectures/b15neuro.html). Nodular expression of FGF, RA, BMP and Wnt inhibitors. Red arrows represent proneural signals.



Gastrulation and Neurogenesis Cont.

After the successful formation of the neural plate, FGF and RA will begin to posteriorize the neural axis through the expression of hox genes. The AVE will secrete cerberus and dickkopf that anteriorize the neural tube through the inhibition of Wnt and BMP, followed by subsequent TIc secretion, which also acts as an antagonist of Wnt.²⁷ This patterning results in the development of the telencephalon at the anterior most end, followed by the diencephalon (which together make up the forebrain), followed by the midbrain, hindbrain, and spinal cord.²⁵



BMP4 signaling

+FGF + RA

neural plate at E7 experiences Wnt, FGF, and RA expression from the posterior end, while cerberus and dickkopf are expressed from the anterior end of the AVE. Subsequent AVE produced TIc gradients at E8 result in neural tube segmentation into the telencephalon. diencephalon, midbrain, hindbrain, and spinal cord.



Figure 10. Adapted from

(http://courses.biology.utah.edu/bastiani/3230/db%20lecture/lectures/b15neuro.html). Major morphogenic gradients responsible for anterior-posterior and dorsal-ventral patterning in the mammalian brain are seen above. Shh is expressed at the ventral midline while BMP4 is expressed at the dorsal midline. These two proteins are crucial for the dorsal ventral patterning of the brain.

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