

Delta-Notch signaling and lateral inhibition in zebrafish spinal cord development

ABSTRACT

Our results are consistent with the idea that zebrafish Delta proteins, expressed by newly specified neurons, promote Notch activity in neighboring precursors. This signaling is required to maintain a proliferative precursor population and generate late-born neurons and glia. Thus, Delta-Notch signaling may diversify vertebrate neural cell fates by coordinating cell cycle control and cell specification.

INTRODUCTION

Background Specification of cells at different times and places is critical for generation of cellular diversity in the vertebrate nervous system. Distinct types of neurons and glia develop at characteristic times and places and molecular signals that promote formation of different cell types are regulated spatially and temporally. Thus, neural pattern formation requires coordination of signals that provide spatial and temporal information. Lateral inhibition, or lateral specification, is one process by which fine patterns of distinct cell types are generated. Among cells that have the potential to adopt the same fate, lateral inhibition specifies some cells for a primary or preferred fate and others for a secondary or alternative fate. Cell ablation experiments are used to identify lateral inhibition: when cells that would normally adopt a primary fate are removed, cells that would otherwise acquire a secondary fate replace them. For example, when neuroblasts of the grasshopper central nervous system were ablated, they were replaced by cells normally fated to give rise to epidermis. Similar demonstrations performed in other insects, leeches, nematodes, ascidians, and zebrafish neural crest indicate that lateral inhibition operates in diverse developmental contexts throughout metazoan development. The molecular mechanism of lateral inhibition is rooted in interaction of the ligand, Delta, and its receptor, Notch. Various lines of evidence indicate that Delta, expressed by specified cells, activates Notch in neighboring cells directing them into alternative developmental pathways. The genetic demonstration that Delta-Notch signaling regulates insect neuroblast formation has been performed using *Drosophila melanogaster*: embryos lacking Delta or Notch function develop excess neural cells at the expense of epidermis. In the nervous system of vertebrate embryos, Notch gene expression generally is associated with proliferative zones of undifferentiated cells. Scattered cells within proliferative zones express Delta, although, in chick, cells in S-phase of the cell cycle do not express Delta1 suggesting that expression is limited to newly post-mitotic cells. In frog and zebrafish neural plate, Delta gene expression appears to precede expression of early neuronal markers. However, whether this indicates that proliferative cells of anamniote nervous systems express Delta genes is still an open question. Newly-specified neurons may use Delta to signal to unspecified neighboring precursor cells via Notch. Such signaling could diversify neural cell fate by maintaining a pool of precursor cells that respond to other instructive signals or by promoting alternative developmental pathways. Gain-of-function experiments provide evidence for both ideas. For example, overexpression of a constitutively active form of Notch (Nac) in frog embryos led to an increase in the amount of neural tissue, even when cell division was blocked. It was suggested that this excess neural tissue arose from specification of excess uncommitted precursor cells to a neural fate once levels of the overexpressed Notch protein fell. Frog retinal cells forced to express Nac maintained a neuroepithelial morphology rather than

undergoing differentiation, which also suggests that Notch activation maintains cells in an undifferentiated state. Similarly, overexpression of Delta proteins, which presumably activate endogenous Notch signaling, prevented retinal cell differentiation in frog and chick and formation of the earliest-born neurons in frog and zebrafish. Each of these observations is consistent with the notion that Notch activity maintains cells in a proliferative, unspecified state. More recently, several reports have raised the possibility that Notch activity promotes specification of certain cell types, particularly glial cells. Forced expression of Nac promoted formation of cells expressing markers of Müller glia in rat and zebrafish retinæ. As Müller glia are among the last retinal cell types born, these results are consistent with the idea that Notch activity delays differentiation. However, Notch activity did not promote expression of markers of other late-born cell types in rat and appeared to inhibit, rather than promote, cell proliferation in zebrafish. Additionally, Nac expression in the mouse forebrain promoted formation of radial glia, the earliest specified cell type in forebrain and either permitted or promoted gliogenesis in the peripheral nervous system. By contrast, disruption of Notch signaling results in formation of excess early-born neurons. Antisense Notch RNA treatment or expression of a dominant negative form of Notch in chick resulted in excess ganglion cells, one of the first retinal cell types to differentiate. Similarly, expression of dominant negative Delta proteins caused formation of excess early-born neurons in the retina of frog and chick and in the neural plate of frog and zebrafish, concomitant with decreased cell proliferation and reduced numbers of late-born neurons. Mouse embryos homozygous for a targeted mutation of Notch1 or RBP-J κ , which encodes a downstream component of the Notch signaling pathway, had elevated levels of expression of neuronal markers, indicating formation of excess neurons. Excess neurons also developed in mouse embryos homozygous for targeted mutation of HES1, a presumptive downstream effector of Notch signaling. Recently, a mutant allele of the zebrafish deltaA (dIA) gene was identified and shown to cause formation of excess Rohon-Beard sensory neurons and decreased trunk neural crest progenitor cells and excess hair cells and decreased support cells in the ear. To investigate further the role of Notch-mediated cell-cell signaling in vertebrate neural development we have tested neural pattern formation in zebrafish embryos. We show by ablating single, identified primary motor neurons that these cells normally inhibit nearby cells from adopting the primary motor neuron fate. Our experiments lead us to conclude that in the absence of lateral inhibition the time during which neighboring cells can replace a missing cell is transient. This suggests that the temporal and spatial cues that specify neural cell fate are tightly regulated. We also show that in dIA mutant embryos neural precursor cells prematurely cease dividing and differentiate as early-born neurons with identities appropriate for their positions within the spinal cord and that mutant embryos have reduced numbers of late-born neurons and glia. These observations support the idea that Delta-Notch mediated lateral inhibition coordinates transition of proliferative neural precursors into specified neurons and glia of the spinal cord.

CONCLUSION

Conclusions In zebrafish, at least three notch genes are expressed by proliferative neural precursors and at least three delta genes are expressed by subsets of proliferative precursors and post-mitotic neurons. Thus, precursors fated to exit the cell cycle and differentiate may express elevated levels of delta genes, causing them to signal to neighboring notch-expressing precursors. Signaling between neighboring cells appears to regulate primary motor neuron specification: when we removed primary

motor neurons soon after birth they were replaced, presumably by neighboring precursors. Genetic disruption of Delta-Notch signaling causes precursors to prematurely terminate cell division and differentiate as early-born neurons, with identities appropriate to their cell body positions, at the expense of later born neurons and glia. We propose that in the zebrafish central nervous system cell-cell interactions, mediated by Delta-Notch signaling, help coordinate transition of proliferative precursors to specified neurons and glia.