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Molecules and Cells

Proliferating Neural Progenitors in the Developing CNS of Zebrafish Require Jagged2 and Jagged1b

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In the central nervous system (CNS), giving rise to the diversity and the complexity of neurons is the spatial and temporal differentiation of neural stem cells and/or neural precursors. Here, we investigated the role of Jagged-mediated Notch signaling in the maintenance and differentiation of progenitor cells during late neurogenesis by analyzing the expression patterns of zebrafish *jagged* homologues, and by injecting their morpholinos. Expression of both *jagged2* and *jagged1b* mRNA in the CNS suggested that they might be involved in control of differentiating neural progenitors in which they are involved later in development. In Jagged2 and Jagged1b knock-down embryos, the overall rate of cell division dramatically decreased, and the ectopic VeMe neurons were generated. The results suggest that Jagged-Notch signaling plays a critical role in the maintenance of proliferating neural precursors, and that the generation of late-born neurons, especially VeMe neurons, is regulated by the interplay between Jagged2 and Jagged1b.

INTRODUCTION

Cellular diversity within the central nervous system (CNS) ensures the coordinated outputs of signals against various stimuli. Neurons are defined both physiologically and anatomically, and many of the identified neurons have been assigned their unique function. Molecular studies show that distinct subtypes of neurons are located topologically along the dorsoventral axis of the spinal cord and are generated from different progenitors (Briscoe et al., 2001). The complexity of this organization raises the question of how neuronal subtype identity and distribution patterns are regulated through the association of intrinsic factors with extrinsic signals. Although cellular signaling pathways such as FGF, BMP, Shh, and Notch is believed to play a central role in neurogenesis (Artavanis-Tsakonas et al., 1999; Briscoe and Ericson, 2001), relationship between ligands and receptors is still remained undefined in zebrafish.

During neurogenesis in both vertebrates and invertebrates, activation of Notch inhibits neuronal differentiation (Artavanis-Tsakonas et al., 1999; Chitnis et al., 1995; Fortini et al., 1993; Struhl et al., 1993), and suppresses the development oligodendrocytes from their precursors during gliogenesis (Park et al., 2003; Wang et al., 1998). In the CNS, Notch receptors and their ligands are expressed in the proliferative zones of undifferentiated cells (Appel et al., 2001; Lindsell et al., 1996). Previous studies have highlighted the role of early Delta-Notch signaling in mediating lateral inhibition in vertebrate neurogenesis, where Notch signaling within neurogenic domains prevents cells with neuronal potential from becoming early differentiating primary motor neurons (Appel et al., 2001; Itoh et al., 2003; Park et al., 2003). This allows them to adopt secondary motor neuron and OPC fates during the later stages of development. Although the Notch ligand, Jagged, functions by inhibiting the differentiation of OPC during gliogenesis, and zebrafish Jagged2 maintains proliferating neural progenitors (Lindsell et al., 1995; Wang et al., 1998; Yeo and Chitnis, 2007), the role of other Jagged homologues in zebrafish neurogenesis *in vivo* is largely unknown.

In the zebrafish embryo, distinct neurons such as primary and secondary motor neurons, GABAergic Kolmer-Agduhr (KA) cells, ventral medial neurons (VeMe), and Rohon-Beard sensory neurons (RB), emerge in a distinct spatial and temporal order from neural precursors (Bernhardt et al., 1992; Westerfield et al., 1986). Jagged2-mediated Notch activation is required for the generation of late-born neurons, such as motor neurons and GABAergic neurons throughout development (Yeo and Chitnis, 2007). The study by Yeo and Chitnis attempted to answer several key questions regarding the role played by Notch during neurogenesis. First, which Notch ligand specifies the development of late-born neurons, including motor neuron and ventral neurons? Second, which molecules regulate the transcription and/or post-translation modification of Notch ligands for the specification of late-born neurons? Third, does Notch activation directly, or indirectly, limit the number of neural precursors? The results demonstrated a critical role for Jagged2-mediated Notch signaling in the maintenance of proliferating neural precursors

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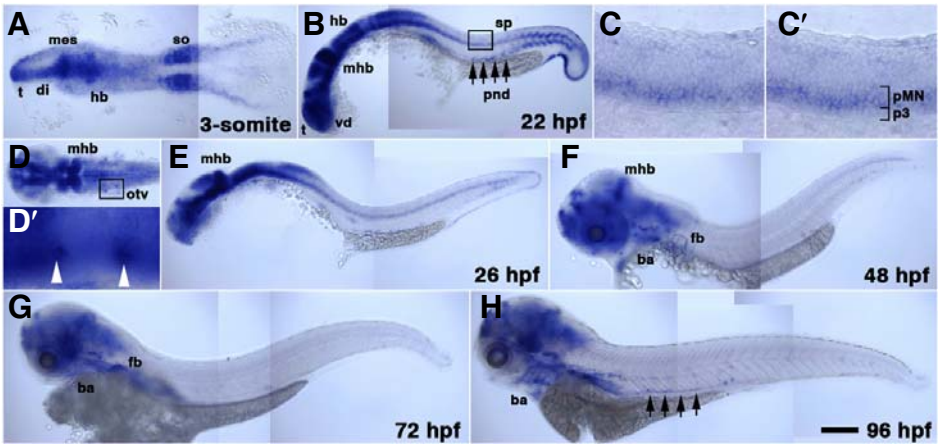

Fig. 1. Expression patterns of zebrafish *jagged2* mRNA. Anterior to the left. Dorsal (A, D) and lateral (B-C′, D′-H) views. At the 3-somite stage, *jagged2* is expressed in the telencephalon (t), the diencephalon (di), the mesencephalon (mes), the hindbrain and the somites (A). At 22-hpf, *jagged2*-expressing cells are detected in the ventral diencephalon (vd), the mid-and hindbrain boundary (mhb), the hindbrain, the pronephric duct (pnd, arrows) and cells in the pMN domain of the spinal cord (B). High magnification views of the boxed area of the spinal cord in B (C). A different focal plane of (C) is shown in (C′). At 26-hpf, *jagged2* transcripts are detected in the forebrain, the mhb, the ear and the pMN domain of the spinal cord (D, E). High magnification views of the boxed area of the ear in D (D'). The transcripts of *jagged2* are expressed in the fin bud (fb), the mhb and the branchial arches (ba) at 48-hpf (F), 72-hpf (G) and 96-hpf (H). Arrows indicate the pronephric duct (H). Scale bar: 100 μm (A, B, and E-H).

within a discrete compartment of the neural tube during vertebrate development (Yeo and Chitnis, 2007). However, this still provides insufficient answer to the second question, since proliferating neural progenitors are still present in Jagged2 knockdown embryos. In this study, we investigated the role of Jagged-mediated Notch signaling in the maintenance and differentiation of progenitor cells during late neurogenesis by analyzing the expression of zebrafish *jagged* homologues, and by injecting their morpholinos. Our results suggest that Jagged-mediated Notch signaling plays a critical role in the maintenance of proliferating neural precursors, and that the generation of late-born neurons is regulated by the interplay between Jagged2 and Jagged1b.

MATERIALS AND METHODS

Fish lines

Zebrafish were maintained as described by Yeo et al. (2007). The lines used in this study were: AB* wild-type, *Tg[her4-dRFP]* (Yeo et al., 2007), *Tg[ngn1-EGFP]* (Zecchin et al., 2005), and *Tg[olig2:egfp]* (Park et al., 2004).

Morpholino injections

2.5-4 ng of spliced *jagged1b-MO* (5′-AAA TCA AGA CTC ACC GTC GTC CGC A-3′; Gene Tools), *jagged1b mismatched control-MO* (5′-AAA TCt AGA CTC tgC GTC GTC CGg A-3′; Gene Tools), and *jagged2-MO* (Yeo and Chitnis, 2007) were injected into two-cell-stage zebrafish embryos as previously described (Lee et al., 2010; Yeo et al., 2007).

Whole-mount *in situ* hybridization

Whole-mount *in situ* hybridization was performed as previously described (Yeo and Chitnis, 2007). Antisense riboprobes were transcribed from cDNA for zebrafish *jagged1b*, *jagged2*. Photos were taken with a differential interference contrast microscope (Axioplan2; Carl Zeiss).

Immunohistochemistry

Immunohistochemistry was performed as previously described (Yeo and Chitnis, 2007). Primary antibodies used were anti-phospho-histone H3 (Upstate). Secondary antibodies used were Alexa Fluor 568-conjugated goat anti-rabbit IgG (Molecular Probes). Photos were taken with a confocal laser scanning microscope (LSM 510; Carl Zeiss).

RESULTS

Expression patterns of the zebrafish *jagged2* genes

We first analyzed the expression of four *delta* and three *jagged* genes in zebrafish embryos. In both *deltaA* mutant and *deltaA*/*deltaD* double mutant embryos, the number of late-born neurons and glia was reduced to levels similar to those seen in the *mib* mutant (Appel et al., 2001; data not shown). In zebrafish *beamter* (*deltaC*) mutant, the number of KA" cells slightly, but not significantly, reduced (Julich et al., 2005; Shaw et al., 2006; data not shown). As a first step toward understanding the role played by Jagged-mediated Notch signaling during neurogenesis, we compared the expression patterns of *jagged1a*, *jagged1b* and *jagged2* genes during development. The expression of *jagged1a* was restricted to cells within a relatively dorsal compartment of the spinal cord (Zecchin et al., 2005 and data not shown). The transcripts of *jagged2* were detected in the telencephalon, diencephalon, mesencephalon, hindbrain, and the somites at the 3-somite stage (Fig. 1A). At 22 hpf, *jagged2*-expressing cells were detected in the ventral diencephalon, the mid-and hindbrain boundary (MHB), the hindbrain, the pronephric duct, and in cells within the pMN domain of the spinal cord (Fig. 1B). In the spinal cord, *jagged2* transcripts were undetectable within the motor neuron domain up to 12 hpf, but were detected at 22 hpf (Figs. 1B, 1C, and 1C′; pMN domain). This domain is just dorsal to the most ventral part of the spinal cord in which KA" cells and VeMe cells differentiate (p3 domain, Fig. 1B, Yeo and Chitnis, 2007). At 26 hpf, *jagged2* transcripts were detected in the forebrain, the MHB, the ear, and the pMN domain of the spinal cord (Figs. 1D and 1E). These transcripts were also expressed in the fin bud, the MHB and the branchial arches at 48 hpf, 72 hpf, and 96 hpf (Figs. 1F-1H). Transcripts of *jagged2* were persistently detected in the spinal cord throughout development. These data imply the pleiotropic roles of Jagged2-mediated Notch signaling during vertebrate development, suggesting that the function of Jagged2 might play a role in differentiation of branchial cells and nephric cells as well as in maintenance of neural progenitors of the CNS (Yeo and Chitnis, 2007).

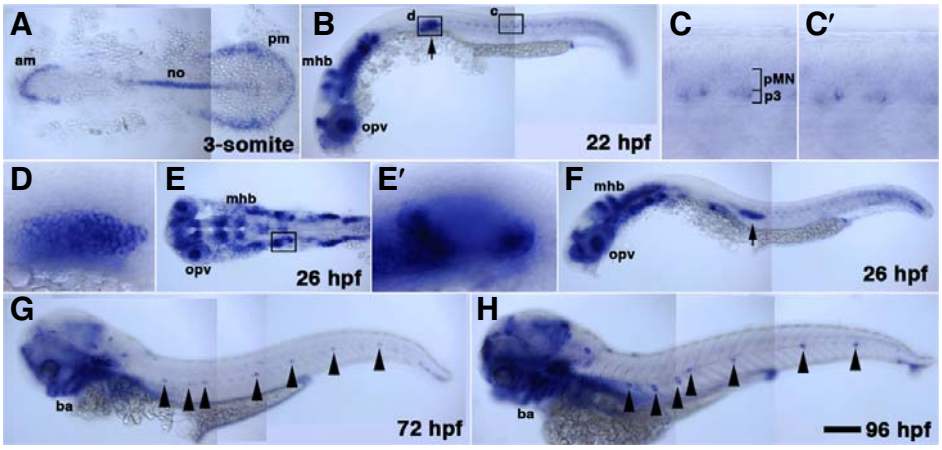

Fig. 2. Expression patterns of zebrafish *jagged1b* mRNA. Anterior to the left. Dorsal (A, E) and lateral (B-D, E′-H) views. At the 3-somite stage, *jagged1b* is expressed in the notochord, the anterior margin of neural plate (am) and the pronephros primordia (pm) (A). At 22-hpf, *jagged1b*-expressing cells are detected in the optic vesicles (opv), the mid-and hindbrain boundary (mhb), the lateral primordia (arrow) and some cells in the p3 and pMN domain of the spinal cord (B). High magnification views of the boxed area of the spinal cord in B (C). A different focal plane of (C) is shown in (C′). High magnification views of the boxed area of the lateral primordia in B (D). At 26-hpf, *jagged1b* transcripts are detected in the eye (opv), the mhb, the lateral primordia and the ear (E, F). High magnification views of the boxed area of the ear in E (E′). The transcripts of *jagged1b* are expressed in the posterior lateral line including seven neuromasts with functional hair cells on one side of the trunk and in the branchial arches at 72-hpf (G) and 96-hpf (H). Scale bar: 100 μm (A, B, E, and F-H).

Expression patterns of zebrafish the *jagged1b* genes

At the 3-somite stage, *jagged1b* was expressed in the notochord, the anterior margin of the neural plate and the pronephros primordia (Fig. 2A). In contrast to the relatively uniform expression of *jagged2*, *jagged1b* was expressed at high levels in a subset of ventral spinal cord cells and in the lateral primordial at 22 hpf (Figs. 2B-2D). It was also expressed broadly at very low levels in more dorsal parts of the cord (Figs. 2C and 2C′; Yeo and Chitnis, 2007). At 26 hpf, *jagged1b* transcripts were detected in the eye, the MHB, the lateral primordial and the ear (Figs. 2E and 2F), and in the posterior lateral line at 72 hpf and 96 hpf, including in seven neuromasts with functional hair cells on one side of the trunk and in the branchial arches (Figs. 2G and 2H). Transcripts of *jagged1b* were also persistently detected in the spinal cord throughout development. The observation that the expression of both *jagged2* and *jagged1b* mRNA starts before the initiation of neurogenesis suggests that they may be involved in the control of early embryogenesis different way from their involvement in the control of differentiating neural progenitors later in development. These data may also imply a pleiotropic role for Jagged-mediated Notch signaling during vertebrate development, suggesting that the reciprocal expression of *jagged2* and *jagged1b* might be involved in the maintenance of neural progenitors within the CNS.

Jagged2 and Jagged1b are required to maintain a population of neural progenitors

As a next step toward understanding the role of Jagged-mediated Notch signaling, we attempted a loss-of-function study using morpholinos (MO) directed against the *jagged1b* and *jagged2* genes expressed in the spinal cords of *Tg[ngn1-EGFP];Tg[her4-dRFP]* double transgenic zebrafish. Both *jagged2-MO (J2-MO)* and *jagged1b-MO (J1b-MO)*, designed to interfere with gene splicing, led to the generation of alternative transcripts that were expected to be non-functional (Yeo and Chitnis, 2007). Analysis of *Tg[her4-fluorescent reporter]* transgenic zebrafish showed that, while both the expression of proneural genes and the activation of Notch are critical for endogenous *her4* expression, the expression of the reporter gene is primarily regulated by Notch activity alone, and can be detected in proliferating neural precursors (Yeo et al., 2007). Zebrafish *neurogenin1* (*ngn1*) is expressed in the proneural domain (Kim et al., 1997). Expression analysis of *Tg[ngn1-EGFP]* showed that fluorescent proteins were detected not only in the telencephalon and diencephalon, but also in some spinal cord neurons, including ventral medial (VeMe) neurons (Blader et al., 2004, Supplementary Fig. S1). Fluorescent confocal images showed that, while EGFP-positive Rohon-Beard sensory neurons (RB) and circumferential descending neurons (CiD) developed normally at 24 hpf, a slight decrease in the number of dRFP-positive neural precursors was observed at 24 hpf in the *J2-* and *J1b-MO* injected embryos (Figs. 3A-3A″, and 3B-3B″). At 48 hpf, we observed not only an increase in the number of EGFP-positive VeMe neurons, but also a dramatic decrease in dRFP-positive neural precursors; the embryos injected with *J2-MO* or *J1b-MO* alone did not produce any obvious change (Figs. 3C-3C″, 3D-3D″ and data not shown). This suggests that the combined function of Jagged2 and Jagged1b is required for the differentiation of VeMe neurons at 48 hpf, consistent with the idea that Jagged-Notch signaling plays a role in the maintenance of neural precursors in a discrete compartment of the neural tube during the vertebrate development (Yeo and Chitnis, 2007).

Jagged2 and Jagged1b are required to maintain a population of proliferating cells

Previous study shows that the number of proliferating cells significantly decreased both in pMN domain and p3 domain, while overall rate of cell division in the CNS decreased slightly but insignificantly following knock-down of Jagged2 (Yeo and Chitnis, 2007). This study confirm the idea that Jagged2-mediated Notch signaling is required to maintain a population of proliferating cells in both the Olig2-positive pMN domain and in the most ventral p3 domain, and that other Jagged homologue is required for spatio-temporal differentiation of neural precursors in the CNS.

To determine whether the number of dividing precursors reduce following knock-down of Jagged2 and Jagged1b, embryos were labeled at 48 hpf with an antibody that recognizes phosphorylated histone H3 on Ser10, an established marker mitosis (Fig. 4). During mitosis, histone H3 ser10 is globally phosphorylated, which is required for chromosome condensation and segregation, while in G_0/G_1 transition, a small fraction of transient H3 ser10 phosphorylation at specific loci leads to

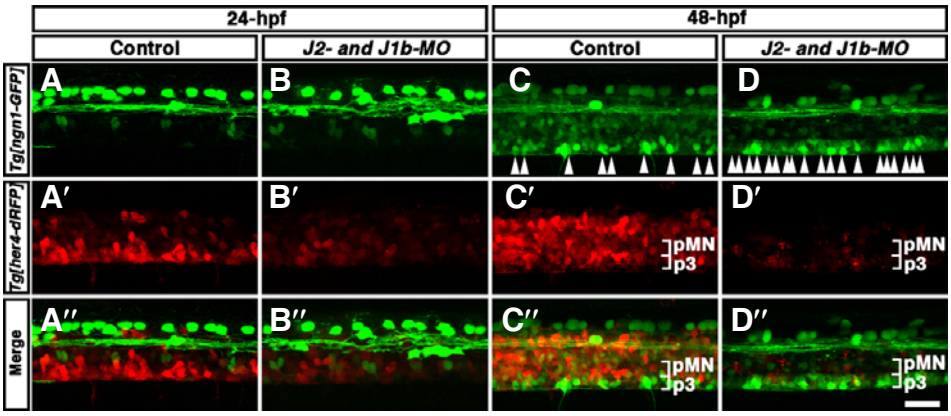

Fig. 3. Inhibition of Jagged-mediated Notch signaling causes a reduction in the number of the *her4*-positive cells in the spinal cord. Lateral views and anterior to the left are shown. Confocal images identify EGFP (A-D, green) and dRFP (A′-D′, red) in the *Con-MO* (A-A″, C-C″), and the *J2-* and *J1b-MO* injected *Tg[ngn1-EGFP]; Tg[her4-dRFP]* double transgenic embryos (B-B″, D-D″) at 24-hpf (A, B) and at 48-hpf (C, D). Upper brackets indicate the pMN domain, and lower brackets indicate the ventral region of spinal cord (p3 domain). White arrowheads indicate the EGFP-positive ventral neurons in the *Tg[ngn1-EGFP]* embryos. Scale bar: 50 μm.

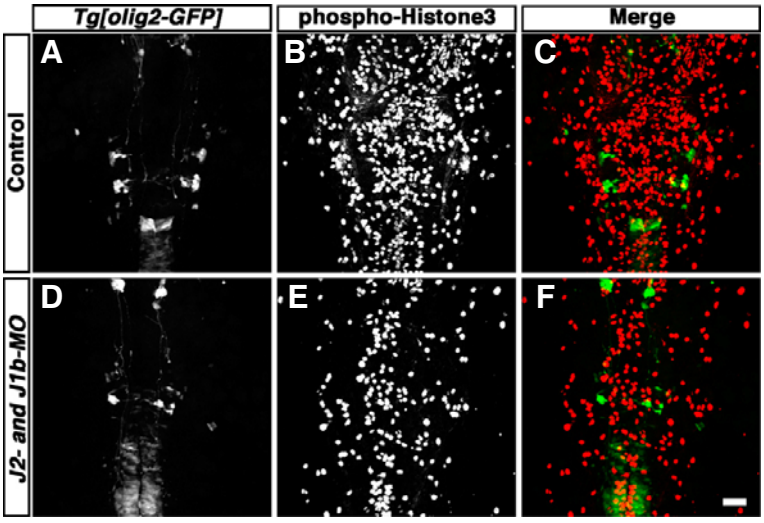

Fig. 4. Inhibition of Jagged-mediated Notch signaling causes a reduction in the number of the proliferative neural precursors in the brain. Dorsal views and anterior to the top are shown. Confocal images identify PH3-labeled nuclei (B and E, red) and EGFP (A and D, green) in the *Con-MO* (A-C), and the *J2-* and *J1b-MO* injected *Tg[olig2-EGFP]* embryos at 26-hpf (D-F). Scale bar: 50 μm.

chromatin relaxation (Davie, 2003). The overall rate of cell division decreased dramatically following knock-down of Jagged2 and Jagged1b, while the number of EGFP-positive cells in *Tg[olig2-EGF]* transgenic fish remained unchanged (Figs. 4E and 4F). These observations confirm the idea that Jagged-mediated Notch signaling is required to maintain a population of proliferating cells in the CNS.

DISCUSSION

The generation of late-born neurons including motor neurons, GABAergic neurons, and VeMe neurons, differs temporally from primary neurogenesis. There is a requirement for regulatory mechanisms that maintain the number of neural progenitor cells and temporally regulate the number of neurons by preventing uncommitted precursors from acquiring a neuronal fate, or by the spatial or temporal removal of suppression signals. Previous study showed that Jagged2 knockdown resulted in an increase in motor neurons and KA" cells, a subset of GABAergic KA cells that differentiate form the most ventral p3 progenitors at 17 hpf (Yeo and Chitnis, 2007). This study suggests Jagged2 and Jagged1b knockdown allow neural progenitors that are still dividing after 28 hpf to aberrantly differentiate as VeMe neurons, and they allow some neural progenitors to prematurely differentiate as neurons. How do the combined Jagged2 and Jagged1b expressed in the CNS influence the fate of neural progenitors? The simplest possibility is that Jagged2 and/or Jagged1b expressed in the CNS directly activates Notch in adjacent neural progenitors after 28 hpf. Notch activation at this stage prevents differentiation of the spinal progenitors, keeps them in a proliferative state and allows them to differentiate and adopt alternate fates later in development. However, a Notch target gene has not yet identified which is expressed in the CNS that effectively or selectively reports Notch activation by Jagged2 and Jagged1b. Expression of a Notch target gene *her4*, for example, whose expression we have used to monitor Notch activity (Yeo et al., 2007), is expressed at high levels in the CNS at the stages we examined and there are significant changes in its expression after knockdown of Jagged2 and Jagged1b. In the presence of such a reporter or experiments demonstrating a direct link between Jagged2/Jagged1b expression and Notch activation in the CNS, it provide formally possible that Notch activation by Jagged2 in the pMN and by Jagged1b in p3 domain indirectly influences fate in the more ventral p3 domain by inducing expression of a factor in the pMN domain that then influences fate in the more ventral p3 domain. Although the pMN and p3 domains are described as distinct progenitor domains, they represent adjacent cell populations between which direct interactions mediated by Jagged2 or by Jagged1b remain plausible, and additional se-

creted factors need not be invoked to account for effects of Jagged2 or Jagged1b on cell fate in a ventral domain.

Previous neurosphere assays using neural stem cells derived from *Notch1-/-*, *RBP-Jk-/-*, and *PS1+/-* mice showed that Notch pathway molecules are essential for the maintenance of neural stem cells (Hitoshi et al., 2002). The assays showed that Delta-mediated Notch signaling affects the outcome of neural stem cell differentiation both qualitatively and quantitatively (Grandbarbe et al., 2003). Although the conservation of signaling molecules strongly suggests the preserved function of the Notch-signaling pathway during evolution and development, individual subtypes of Notch, and their ligands, Jagged2, Jagged1b or Delta, contribute in unique ways to the building of the overall neural architecture. Although structural conservation strongly suggests a preserved function for Jagged homologues throughout evolution, the roles and the spatial-temporal expression patterns of the individual subtypes may have shuffled among themselves. Our results imply that the combined functions of the Jagged2- and Jagged1b-mediated Notch signaling pathways are indispensable for the *in vivo* maintenance of a dividing neural precursor population during later development and the surrounding microenvironment, perhaps to maintain a stem cell 'niche', during the course of neural development. Furthermore, Jagged2- and Jagged1b-mediated Notch signaling may help to diversify vertebrate neural cell fates.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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REFERENCES

Appel, B., Givan, L.A., and Eisen, J.S. (2001). Delta-Notch signaling and lateral inhibition in zebrafish spinal cord development. BMC Dev. Biol. *1*, 13.

Artavanis-Tsakonas, S., Rand, M.D., and Lake, R.J. (1999). Notch signaling: cell fate control and signal integration in development. Science *284*, 770-776.

Bernhardt, R.R., Patel, C.K., Wilson, S.W., and Kuwada, J.Y. (1992). Axonal trajectories and distribution of GABAergic spinal neurons in wildtype and mutant zebrafish lacking floor plate cells. J. Comp. Neurol. *326*, 263-272.

Blader, P., Lam, C.S., Rastegar, S., Scardigli, R., Nicod, J.C., Simplicio, N., Plessy, C., Fischer, N., Schuurmans, C., Guillemot, F., and Strähle, U. (2004). Conserved and acquired features of neurogenin1 regulation. Development *131*, 5627-5637.

Briscoe, J., and Ericson, J. (2001). Specification of neuronal fates in the ventral neural tube. Curr. Opin. Neurobiol. *11*, 43-49.

Chitnis, A., Henrique, D., Lewis, J., Ish-Horowicz, D., and Kintner, C. (1995). Primary neurogenesis in Xenopus embryos regulated by a homologue of the Drosophila neurogenic gene Delta. Nature *375*, 761-766.

Davie, J.R. (2003). MSK1 and MSK2 mediate mitogen- and stress-induced phosphorylation of histone H3: a controversy resolved. Sci. STKE. *2003*, PE33.

Fortini, M.E., Rebay, I., Caron, L.A., and Artavanis-Tsakonas, S. (1993). An activated Notch receptor blocks cell-fate commitment in the developing Drosophila eye. Nature *365*, 555-557.

Grandbarbe, L., Bouissac, J., Rand, M., Hrabe de Angelis, M., Artavanis-Tsakonas, S., and Mohier, E. (2003). Delta-Notch signaling controls the generation of neurons/glia from neural stem cells in a stepwise process. Development *130*, 1391-1402.

Hitoshi, S., Alexson, T., Tropepe, V., Donoviel, D., Elia, A.J., Nye, J.S., Conlon, R.A., Mak, T.W., Bernstein, A., and van der Kooy, D. (2002) Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. Genes Dev. *16*, 846-858.

Itoh, M., Kim, C.H., Palardy, G., Oda, T., Jiang, Y.J., Maust, D., Yeo, S.Y., Lorick, K., Wright, G.J., Ariza-McNaughton, L., et al. (2003). Mind bomb is a ubiquitin ligase that is essential for efficient activation of Notch signaling by Delta. Dev. Cell *4*, 67-82.

Julich, D., Hwee Lim, C., Round, J., Nicolaije, C., Schroeder, J., Davies, A., Geisler, R., Lewis, J., Jiang, Y.J., and Holley, S.A. (2005). beamter/deltaC and the role of Notch ligands in the zebrafish somite segmentation, hindbrain neurogenesis and hypochord differentiation. Dev. Biol. *286*, 391-404.

Kim, C.H., Bae, Y.K., Yamanaka, Y., Yamashita, S., Shimizu, T., Fujii, R., Park, H.C., Yeo, S.Y., Huh, T.L., Hibi, M., et al. (1997). Overexpression of neurogenin induces ectopic expression of HuC in zebrafish. Neurosci. Lett. *239*, 113-116.

Lindsell, C.E., Shawber, C.J., Boulter, J., and Weinmaster, G. (1995). Jagged: a mammalian ligand that activates Notch1. Cell *80*, 909-917.

Lindsell, C.E., Boulter, J., diSibio, G., Gossler, A., and Weinmaster, G. (1996). Expression patterns of Jagged, Delta1, Notch1, Notch2, and Notch3 genes identify ligand-receptor pairs that may function in neural development. Mol. Cell. Neurosci. *8*, 14-27.

Lee, H.O., Choe, H., Seo, K., Lee, H., Lee, J., and Kim, J. (2010). Fgfbp1 is essential for the cellular survival during zebrafish embryogenesis. Mol. Cells *29*, 501-507.

Park, H.C., and Appel, B. (2003). Delta-Notch signaling regulates oligodendrocyte specification. Development *130*, 3747-3755.

Park, H.C., Shin, J., and Appel, B. (2004). Spatial and temporal regulation of ventral spinal cord precursor specification by Hedgehog signaling. Development *131*, 5959-5969.

Shaw, K.M., Castranova, D.A., Pham, V.N., Kamei, M., Kidd, K.R., Lo, B.D., Torres-Vasquez, J., Ruby, A., and Weinstein, B.M. (2006). Fused-somites-like mutants exhibit defects in trunk vessel patterning. Dev. Dyn. *235*, 1753-1760.

Struhl, G., Fitzgerald, K., and Greenwald, I. (1993). Intrinsic activity of the Lin-12 and Notch intracellular domains *in vivo*. Cell *74*, 331-345.

Wang, S., Sdrulla, A.D., diSibio, G., Bush, G., Nofziger, D., Hicks, C., Weinmaster, G., and Barres, B.A. (1998). Notch receptor activation inhibits oligodendrocyte differentiation. Neuron *21*, 63-75.

Westerfield, M., McMurray, J.V., and Eisen, J.S. (1986). Identified motoneurons and their innervation of axial muscles in the zebrafish. J. Neurosci. *6*, 2267-2277.

Yeo, S.Y., and Chitnis, A.B. (2007). Jagged-mediated Notch signaling maintains proliferating neural progenitors and regulates cell diversity in the ventral spinal cord. Proc. Natl. Acad. Sci. USA *104*, 5913-5918.

Yeo, S.Y., Kim, M., Kim, H.S., Huh, T.L., and Chitnis, A.B. (2007). Fluorescent protein expression driven by her4 regulatory elements reveals the spatiotemporal pattern of Notch signaling in the nervous system of zebrafish embryos. Dev. Biol. *301*, 555-r67.

Zecchin, E., Conigliaro, A., Tiso, N., Argenton, F., and Bortolussi, M. (2005). Expression analysis of jagged genes in zebrafish embryos. Dev. Dyn. *233*, 638-645.