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Notch signalling regulates the contribution of progenitor cells from the chick Hensen's node to the floor plate and notochord

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SUMMARY

Hensen's node of the chick embryo contains multipotent self-renewing progenitor cells that can contribute to either the floor plate or the notochord. Floor plate cells are a population of epithelial cells that lie at the ventral midline of the developing neural tube, whereas the notochord is a rod of axial mesoderm that lies directly beneath the floor plate. These two tissues serve as a source of a potent signalling morphogen, sonic hedgehog (Shh), which patterns the dorsoventral axis of the neural tube. We show, through both gain- and loss-of-function approaches, that Notch signalling promotes the contribution of chick axial progenitor cells to the floor plate and inhibits contribution to the notochord. Thus, we propose that Notch regulates the allocation of appropriate numbers of progenitor cells from Hensen's node of the chick embryo to the notochord and the floor plate.

KEY WORDS: Notch, Shh, Embryo, Chick, Notochord, Floor plate

INTRODUCTION

The tissues that lie at the vertebrate embryonic midline are an important source of signals that pattern surrounding tissues during development. In particular, the floor plate, a population of prospective radial glia-like cells that lie at the ventral midline of the developing neural tube (Kingsbury, 1930), is a source of signals involved in patterning the dorsoventral axis of this tissue (Appel, 2000; Colamarino and Tessier-Lavigne, 1995; Jessell and Dodd, 1990; Placzek and Briscoe, 2005; Strahle et al., 2004; Tanabe and Jessell, 1996).

There is a wealth of data focussed on how floor plate develops. The axial mesoderm of the notochord that lies directly beneath the floor plate can induce ectopic floor plate differentiation (Dodd et al., 1998; Placzek, 1995; Placzek et al., 1990; Smith and Schoenwolf, 1989; van Straaten et al., 1985; van Straaten et al., 1988; Yamada et al., 1991). Moreover, notochord removal from caudal regions of the chick embryo results in the absence of the floor plate (Placzek et al., 1990; Van Straaten and Drukker, 1987; Van Straaten and Hekking, 1991; Yamada et al., 1991; Placzek et al., 2000). The signal mediating this induction is sonic hedgehog (Shh) (Echelard et al., 1993; Krauss et al., 1993; Marti et al., 1995; Roelink et al., 1994; Ericson et al., 1996; Marti et al., 1995; Roelink et al., 1995). Mice homozygous null for *Shh* lack a floor plate (Chiang et al., 1996). Thus, Shh appears necessary and sufficient to induce floor plate differentiation (Tanabe and Jessell, 1996).

However, there is some controversy as to whether floor plate induction by the notochord is the means by which floor plate development is initiated in the embryo. Careful fate map studies suggest that the floor plate and notochord share a common progenitor cell pool in the organiser (the blastopore lip of amphibians, the embryonic shield of fish, Hensen's node in mammals and birds) (Amacher et al., 2002; Catala et al., 1996; Catala et al., 1995; Latimer et al., 2002; Melby et al., 1996; Selleck and Stern, 1991; Selleck and Stern, 1992; Shih and Fraser, 1995; Spemann and Mangold, 2001; Wilson and Beddington, 1996). It has been suggested that caudal notochord removal can also ablate the precursors of the floor plate (Catala et al., 1996; Le Douarin and Halpern, 2000; Teillet et al., 1998). Thus, it is necessary to reconcile reports showing a requirement for the notochord (and Shh) for floor plate development, with reports showing the two tissues share common progenitors.

The floor plate arises via slightly different means in distinct vertebrate species. Although Shh appears necessary and sufficient for differentiation of all floor plate cells in mouse (Chiang et al., 1996), this is only true of the lateral floor plate in zebrafish (Chen et al., 2001; Etheridge et al., 2001; Karlstrom et al., 2003; Neumann et al., 1999; Schauerte et al., 1998; Varga et al., 2001). Development of the medial floor plate in zebrafish is dependent on Nodal signalling (Hatta et al., 1991; Muller and Basler, 2000; Odenthal et al., 2000; Rebagliati et al., 1998; Sampath et al., 1998; Schauerte et al., 1998). These two populations express different markers; in fish and mice, both the medial and lateral floor plate express Foxa2, whereas only the medial floor plate expresses Shh. In chick, however, the medial and lateral floor plate initially express both markers but Foxa2 becomes downregulated in the lateral floor plate, while Shh continues to be expressed by both populations (Placzek and Briscoe, 2005; Strahle et al., 2004). Moreover, in chick, Shh is necessary and sufficient for differentiation of floor plate cells in the trunk/tail; however, a combination of Nodal and Shh is required for floor plate development at the anterior end of the embryo (Patten et al., 2003).

Studies in zebrafish and *Xenopus* suggest that Notch signalling might regulate midline cell fate specification in axial progenitors located in the organiser (Appel et al., 1999; Latimer and Appel, 2006; Latimer et al., 2002; Latimer et al., 2005; Lopez et al., 2003; Lopez et al., 2005). These midline structures are namely the notochord, floor plate and hypochord (a structure found solely in anamniotes, which lies between the notochord and the dorsal aorta). It has been proposed that Notch has two distinct roles in midline tissue development in fish: first, to specify a subset of midline

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precursors in the shield to develop as the hypochord at the expense of the notochord, and second, to promote proliferation of floor plate cells (Latimer and Appel, 2006). Notch has been shown to play a role in the maintenance of floor plate identity at much later stages in the chick neural tube (le Roux et al., 2003); however, a role for this pathway in chick has not yet been addressed in terms of cell fate choice in the node. Notch has been implicated in many instances of binary cell fate decisions in vertebrate and invertebrate embryos, where it also acts to maintain stem cell characteristics (reviewed by Hansson et al., 2004). Whether Notch is required simply to maintain pools of undifferentiated progenitor cells, or whether it also plays instructive roles in promoting specific cell fates, has been the subject of debate (Del Barrio et al., 2007; Gaiano and Fishell, 2002; Liu et al., 2006; Park and Appel, 2003; Rocha et al., 2009; Yeo and Chitnis, 2007). In this study, we dissect the role of Notch signalling in cell fate choice in the chick organizer, Hensen's node.

MATERIALS AND METHODS

Chick embryo culture

White Leghorn *Gallus gallus* eggs (Henry Stewart & Co., Lincolnshire and Winter Farm, Royston) or GFP-expressing chick embryos [Roslin Institute, Midlothian (McGrew et al., 2004)] were incubated at 38.5°C in a humidified incubator to yield embryos staged according to Hamburger and Hamilton (HH) (Hamburger and Hamilton, 1992). GFP-embryos were set up in early chick (EC) culture from HH1-4 and wild-type embryos were set up in EC culture at HH4 (Chapman et al., 2001). EC plates were supplemented with 100 μM γ-secretase inhibitor IX (DAPT; Calbiochem) dissolved in dimethylsulphoxide (DMSO; Sigma) or DMSO alone.

Grafting technique

Grafting of the medial sector of Hensen's node

The medial sector of Hensen's node was isolated from GFP donors and grafted to a homotopic site using the same criteria as described by Selleck and Stern (Selleck and Stern, 1992) or a heterotopic site as described by Storey (Storey et al., 1992) in a non-GFP host (Figs 1-3). For host stage, see each figure. Hosts were incubated overnight. Embryos were sectioned along the length of the embryo and analyzed for GFP-cell contribution to the floor plate (see below).

In situ hybridization and immunocytochemistry

Standard methods for wholemount in situ hybridization (ISH) were used (Henrique et al., 1995). Antibody protocols have been described for Foxa2 and 3B9 (Developmental Studies Hybridoma Bank) and anti-phospho-histone-H3 antibody (Upstate). Fluorescent signal was analyzed using a compound microscope (Leica DM5000 B). Images were recorded using Openlab 4.0.3.

Apoptosis assay

Cryosectioned neuroectoderm explants were processed using the In Situ Cell Death Detection Kit, Fluorescein (Roche). The number of apoptotic cells per unit area was calculated using Volocity5 (×64) software. Twenty one sections from three DAPT-treated explants and 15 sections from three DMSO-treated explants were analyzed.

Phospho-histone-H3 assay

The number of fluorescent cells per unit area was calculated using Volocity5 (×64) software. Ten sections from two DAPT-treated explants and ten sections from two DMSO-treated explants were analyzed.

In ovo electroporation

Hensen's node

The pCIG-NICD or pCIG-dnRBPjκ constructs (Dale et al., 2003) or an empty vector were introduced to Hensen's node at HH4 using standard in ovo electroporation.

Statistics

Results were analyzed using the parametric 1-way Analysis of Variance (ANOVA) test and the non-parametric Kruskal-Wallis 1-way ANOVA on ranks test.

Cell counts and measurements

Grafting of the medial sector of Hensen's node

Serial transverse sections were taken along the entire length of each embryo in order to assess GFP cell contribution to the floor plate along the anterior-posterior (A/P) axis. Thus, each and every section along the embryo from the tip of the head right back to Hensen's node was analyzed for GFP cells in the floor plate. In each embryo, the A/P level at which the first GFP-expressing cell was found in the floor plate was recorded. No GFP cells at all were present in the floor plate throughout the majority of the axis in embryos receiving a DAPT-treated node graft. GFP-cell contribution to the floor plate in these embryos was limited to the most caudal region. The percentage length of the A/P axis containing GFP-cell contribution in the floor plate was then calculated in control versus treated embryos. This analysis is presented in a box plot showing the percentage of the entire length of the axial midline (head to node) that contained GFP cells in the floor plate in embryos receiving treated or untreated node grafts.

Hensen's node electroporation

A count of GFP cells in the floor plate and notochord was performed in the region from the start of the presomitic mesoderm to Hensen's node. The floor plate domain was limited to the Foxa2-expressing domain using Volocity5 (×64) software. Ten pCIG, 4 pCIG-dnRBPjK and 7 pCIG-NICD electroporated embryos were analyzed.

Measuring notochord size

The circumference of the notochord was measured in transverse cryosections from embryos hybridised for *Hairy2* using ImageJ software. The statistical test was performed on measurements along the entire A/P axis (Kruskal-Wallis 1-way ANOVA; df=1; H=63.831; P<0.001) and in the region between the headfold and the first somite (Kruskal-Wallis 1-way ANOVA; df=1; H=37.672; P<0.001).

RESULTS

Notch inhibition in Hensen's node progenitors prevents these cells from populating the floor plate

We performed a detailed mRNA expression analysis of the Notch target gene *Hairy2* in Hensen's node and the axial tissues of the notochord and floor plate along the embryonic axis of the chick embryo at HH5-8. It is expressed in Hensen's node as well as in the axial tissues themselves as they leave the node (see Fig. S1A-E in the supplementary material; data not shown), coincident with initiation of floor plate characteristics in these cells, as judged by expression of the floor plate marker *Foxa2* (see Fig. S1F-J in the supplementary material). As axial progenitor cells exit the node and extend along the axis, expression of *Hairy2* is higher in the floor plate than in the notochord (see Fig. S1C,D in the supplementary material). Thus, *Hairy2* is expressed at the right time and place to play a role in chick floor plate development.

To address whether Notch plays a role in influencing the cell fate choice that occurs in Hensen's node of the chick, we used a pharmacological approach to inhibit Notch signalling using the γ-secretase inhibitor DAPT (Dale et al., 2003; Morohashi et al., 2006). We cultured GFP-expressing donor embryos from HH1-4 in the presence or absence of DAPT. We grafted the medial sector of Hensen's node from these donors to an equivalent site in non-GFP HH4 host embryos, which were then cultured overnight (15-18 hours) in the absence of DAPT (Fig. 1A). The embryos were then sectioned and analyzed for contribution of GFP cells to the floor plate and notochord along the A/P embryonic axis. Control grafted embryos displayed contribution of GFP cells to the axial mesoderm of the entire A/P axis (including prechordal plate mesoderm, head process notochord and notochord of the trunk) and some labelled cells were also present in the node, indicative

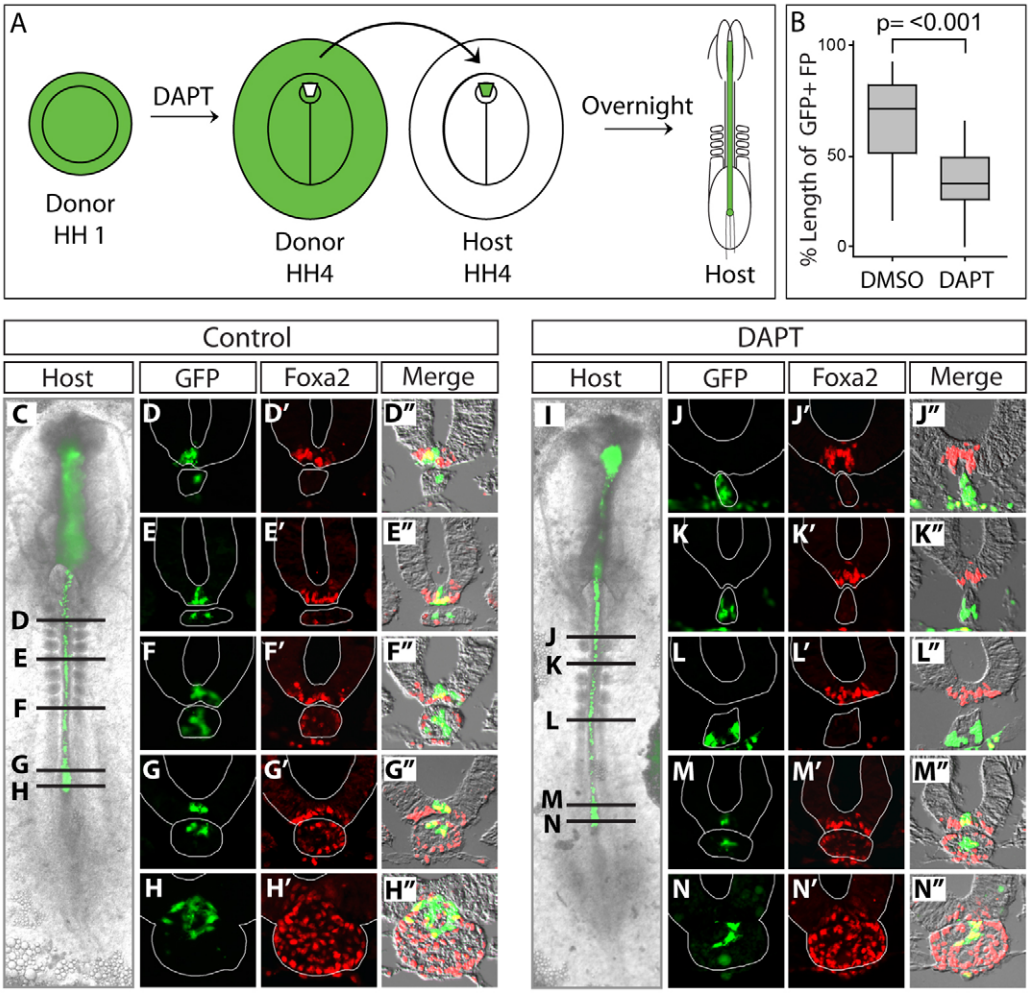

Fig. 1. Notch inhibition prevents progenitor cells from populating the chick floor plate. (**A**) Schematic of the assay: GFP-expressing donor cultured HH1-4 in DMSO or DAPT. The medial sector of donor Hensen's node is grafted to a homochronic site in a non-GFP HH4 non-treated host and cultured overnight. (**B**) Boxplot showing that Notch inhibition significantly reduces the contribution of progenitors to the floor plate (FP) along the embryonic axis. (**C**,**I**) Host embryos after culture. (**D-H**) Transverse sections of C showing GFP cells contributing to the FP for most of the axis and notochord over the entire axis. (**D′-H′**) Same sections as D-H showing Foxa2. (**D″-H″**) Overlay of D-H with D′-H′. (**J-N**) Sections of I showing GFP cells contributing to the notochord over the entire axis; FP contribution is absent throughout the majority of the axis with FP contribution limited to the most caudal end of the embryo. (**J′-N′**) Same sections as J-N showing Foxa2. (**J″-N″**) Overlay of J-N with J′-N′.

of this being a self-renewing population of progenitor cells (n=17; Fig. 1C-H). The floor plate in the anterior region of the body axis is not derived from Hensen's node but instead arises from region 'a' just anterior to the node (Patten et al., 2003). For this reason, we were not surprised by the absence of any contribution of either control or inhibitor-treated GFP cells to the ventral neural tube in the head of grafted embryos. Control grafted embryos displayed contribution of GFP cells to the floor plate from the level of the first somite all the way back to Hensen's node, which corresponds to published data (Patten et al., 2003). By contrast, GFP-expressing progenitor cells that had been exposed to DAPT were strikingly absent from the floor plate for most of the axis (apart from some sections in the caudal embryo; see Fig. S2 in the supplementary material). However, DAPT-treated progenitor cells contributed to the axial mesoderm along the entire length of the embryonic axis. Thus, there is proportionately more contribution of Notch-inhibited progenitors to the notochord than to the floor plate (n=16; Fig. 1I-N). Consequently, Notch inhibition also caused a highly significant reduction in the proportion of progenitor cells that populated the floor plate along the axis as compared with controls (1-way ANOVA df=1, F=14.225, P<0.001; comparison of the percentage length of embryos that contained control or DAPT-treated GFP cells in the floor plate) (Fig. 1B; see also Fig. S2 in the supplementary material). *Foxa2* analysis revealed that a GFP-negative floor plate population is nevertheless present throughout the axis in embryos carrying a DAPT-treated graft (Fig. 1J′-N′). These *Foxa2* cells might be derived from untreated host progenitors and/or from neuroepithelial cells that would not normally become the floor plate, which became exposed to inductive cues from the underlying notochord.

The TUNEL assay did not reveal any significant difference in apoptosis in DAPT-treated versus control explants (controls n=3, DAPT n=3; 1-way ANOVA df=1, F=1.235, P=0.274) (data not shown). However, DAPT explants were smaller than controls which is probably owing to the significant reduction in the mitotic index (reduced number of phospho-histone-H3-labelled cells) following DAPT treatment (controls n=2, DAPT n=2; Kruskal-Wallis 1-way ANOVA df=1, H=14.286, P<0.001) (data not shown), as expected in the absence of Notch activity.

Some of the grafted embryos did show limited contribution from DAPT-treated progenitors to the floor plate in the caudal embryo just anterior to the node (n=13/16; Fig. 1M), whereas others had no floor plate contribution (n=3/16; data not shown). To investigate whether late contribution to the floor plate was owing to insufficient DAPT in the medial sector, we modified the experiment by exposing the hosts to DAPT after grafting (Fig. 2A). Under these conditions, we again observed GFP cells in axial mesoderm but not the floor plate for most of the axis. This result was significantly different from controls (1-way ANOVA df=1; F=5.31; P=0.05; controls n=4, DAPT n=6 embryos; Fig. 2B-L). However, as before, we observed contribution of DAPT-treated GFP cells to the caudal floor plate just

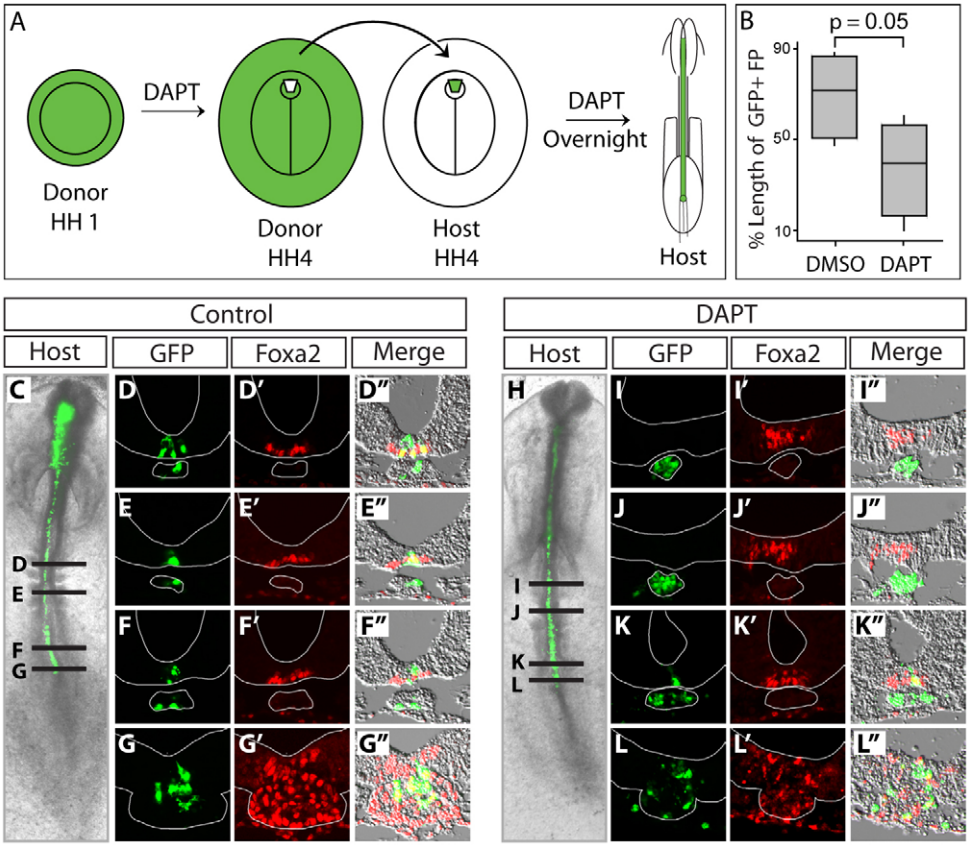

Fig. 2. Notch inhibition throughout the host prevents progenitor cells from populating the floor plate. (**A**) Schematic of the assay: same as Fig. 1 except that the host was cultured overnight in DMSO or DAPT. (**B**) Boxplot showing that Notch inhibition significantly reduces the contribution of progenitor cells to FP. (**C**,**H**) Host embryos after culture. The DAPT-treated host (H) displays severe somite defects. (**D-G**) Transverse sections of C showing GFP cells contributing to the FP and notochord. (**D′-G′**) Same as D-G showing Foxa2. (**D″-G″**) Overlay of D-G with D′-G′. (**I-L**) Sections of H showing GFP cells contributing to the notochord over the entire axis; FP contribution is absent throughout the majority of the axis with FP contribution limited to the most caudal end of the embryo. (**I′-L′**) Same as I-L showing Foxa2. (**I″-L″**) Overlay of I-L with I′-L′.

anterior to the node (Fig. 2K). Possible explanations for this late contribution to the floor plate in both experiments, where either just the donor or both donor and host were treated with DAPT, might be that the decision of cells to populate either the floor plate or the notochord may not be limited to progenitors residing in the node, but might also occur in a region just anterior to the node, consistent with studies showing shared expression of notochord and floor plate markers in this region (Placzek et al., 2000). Alternatively, Notch might not influence cell fate choice in axial progenitors along the whole A/P axis or, conversely, DAPT might only be effective for a specific period of time.

To distinguish between these possibilities, we cultured HH4 embryos overnight (15-18 hours) in the presence of DAPT. As expected, this led to a loss/severe downregulation of *Hairy2* in the floor plate over most of the axis (see Fig. S1P-T in the supplementary material), although some expression was still present just anterior to Hensen's node (n=2/7; see Fig. S1S,T in the supplementary material), in a comparable domain to where inhibitor-treated progenitors start to contribute to the floor plate in grafted embryos. By contrast, embryos transferred onto fresh DAPT-treated plates midway through the experiment showed a complete loss/severe downregulation of *Hairy2* in the floor plate over the entire embryonic axis (n=6; see Fig. S1U-Y in the supplementary material). These findings suggest that DAPT activity decreases below threshold during the culture, therefore liberating axial progenitors from Notch inhibition towards the end of the culture period. It is noteworthy that these embryos also displayed somite defects (see Fig. S1P,U in the supplementary material), as expected, as Notch is required for somite formation (Bessho et al., 2003; Dale et al., 2003; Ferjentsik et al., 2009; Holley et al., 2002; Rida et al., 2004).

When we repeated these experiments and analysed expression of the floor plate markers Foxa2 and *Netrin1* in embryos cultured overnight in the presence of DAPT, as well as in embryos transferred to a fresh DAPT plate midway through the culture period (n=8 and n=15, respectively; Fig. 2I′-L′, Fig. 5, data not shown), we found that in both conditions both markers were still strongly expressed along the entire axis. Taken together, these data suggest that in the absence of Notch, Hensen's node-derived progenitors primarily populate the notochord at the expense of floor plate. Nevertheless, a floor plate develops in these embryos, possibly via inductive signals derived from the underlying notochord.

Notch inhibition does not prevent node cells from becoming notochord

The midline sector of the node contains notochord precursors that are predominantly committed to a notochord fate (Selleck and Stern, 1992; Storey et al., 1995). Our data indicate that Notch inhibition does not appear to affect the ability of these cells to become notochord (Fig. 1I-N″, Fig. 2H-L″). To test this idea further, we repeated the graft experiment but this time the tissue was placed in a challenging environment (where notochord does not usually develop), namely the area opaca at the border between embryonic and extraembryonic tissue, at the level of Hensen's node of a non-GFP HH3+ embryo and then cultured for 24-30 hours (Fig. 3A). Under these conditions, the grafted tissue undergoes convergent extension movements as it would in the normal environment of the midline of the embryo (Dias and Schoenwolf, 1990; Gallera, 1971; Waddington and Schmidt, 1933). Transverse sections of control explants showed expression of the notochord marker 3B9/*Not*I and the floor plate marker Foxa2 (n=8; Fig. 3B-G′). However, as in the homotopic grafts, we found that DAPT-treated explants showed no

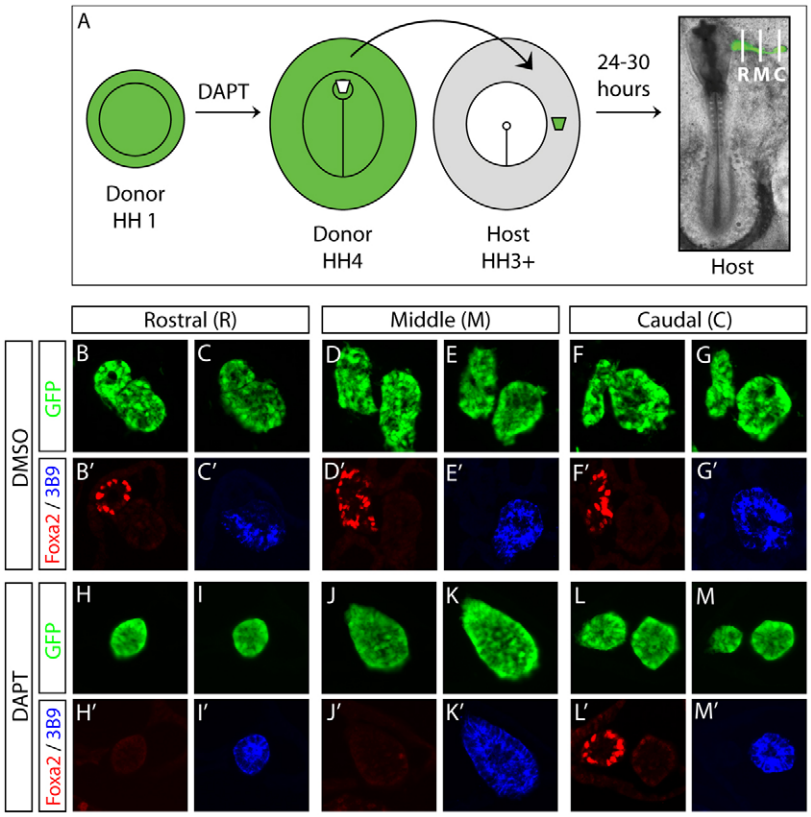

Fig. 3. Notch inhibition does not prevent progenitor cells from becoming notochord. (**A**) Schematic of the assay: same as Fig. 1 except the graft made at the boundary between the area opaca and area pelucida at the level of Hensen's node in a non-GFP-expressing HH3$^+$ host and cultured overnight. Serial sections were analyzed using Foxa2 or 3B9 antibodies. (**B-G**) Transverse sections of the control. (**B′-G′**) Same as in B-G showing Foxa2 or 3B9 expression. (**H-M**) Sections of DAPT-treated sample. (**H′-K′**) Same as in H-K showing lack of Foxa2 and presence of 3B9. (**L′**,**M′**) At the caudal end of these explants some GFP cells are Foxa2 positive.

Foxa2 throughout most of the explant but they did show 3B9/*Not*I along the full extent of the explant (n=3; Fig. 3H-M′). Thus, our data suggests that Notch inhibition prevents axial progenitors from contributing to the floor plate but this treatment does not appear to affect their ability to become notochord. It is noteworthy that, as *Hairy2* is downregulated in notochord following DAPT treatment (see Fig. S1U-Y in the supplementary material), we cannot rule out the possibility that, despite maintaining 3B9, notochord character might be affected under these conditions, although it has yet to be shown if *Hairy2* is a requisite feature of notochord functionality.

Inhibition of Notch signalling promotes contribution of chick axial progenitor cells to the notochord

To verify our observations with DAPT, we used a second approach to inhibit Notch in Hensen's node. A construct encoding both GFP and a dominant-negative version of the RBPjκ transcription factor (dnRBPjκ) was electroporated in ovo into Hensen's node of HH4 embryos (Fig. 4A). dnRBPjκ can still bind the intracellular portion of the Notch receptor (NICD) but is no longer able to bind DNA, and thus sequesters NICD and inhibits target gene transcription (Chung et al., 1994). Electroporation of the empty vector led to GFP cells in both the floor plate and notochord in roughly equal proportions along the A/P axis (of total GFP cells counted, an average of 47% in the floor plate and 53% in notochord, n=44 embryos; Fig. 4B-E′). Strikingly, in dnRBPjκ electroporated embryos, contribution of the electroporated pool of cells to notochord was very significantly greater than to the floor plate, as compared with controls (of total GFP-cells counted, an average of 33% in the floor plate and 67% in notochord; Kruskal-Wallis 1-way ANOVA df=1, H=11.283, P<0.001, n=19 embryos electroporated; Fig. 4F-I′). We cannot rule out the possibility that some Notch-inhibited cells undergo apoptosis or contribute to another structure(s). However, we see no significant difference of apoptosis in Notch-inhibited and untreated samples (data not shown). Thus, both means of Notch inhibition, exposure to DAPT and dnRBPJκ electroporation, led to a significantly reduced contribution of progenitors to the floor plate and a proportional increase in the contribution of these progenitors to notochord.

Notch activation in Hensen's node progenitors promotes contribution of these cells to the floor plate and inhibits them from populating the notochord

We next performed the converse experiment of constitutively activating the Notch pathway in Hensen's node by in ovo electroporation of a construct encoding both GFP and NICD. NICD activates the signal transduction pathway independently of ligand activation (Schroeter et al., 1998). Strikingly, half the embryos electroporated with NICD showed no contribution of GFP cells to notochord (n=7/15; Fig. 4J-M′), and in those embryos that did show contribution to both the floor plate and notochord, contribution to notochord was very significantly less than in controls (an average of 82.5% of cells were found in the floor plate and 17.5% in notochord; Kruskal-Wallis 1-way ANOVA df=1, H=112.265, P=<0.001, n=15 embryos electroporated; see Fig. S3 in the supplementary material). In summary, our data strongly suggest that Notch biases axial progenitors to contribute to the floor plate at the expense of the notochord.

Loss of Notch signalling leads to formation of an enlarged notochord

At first glance, our data appear to present a paradox in that Notch appears to be required for progenitor cells in Hensen's node to populate the floor plate (Figs 1-4), yet we also show that in the prolonged absence of Notch signalling a floor plate develops

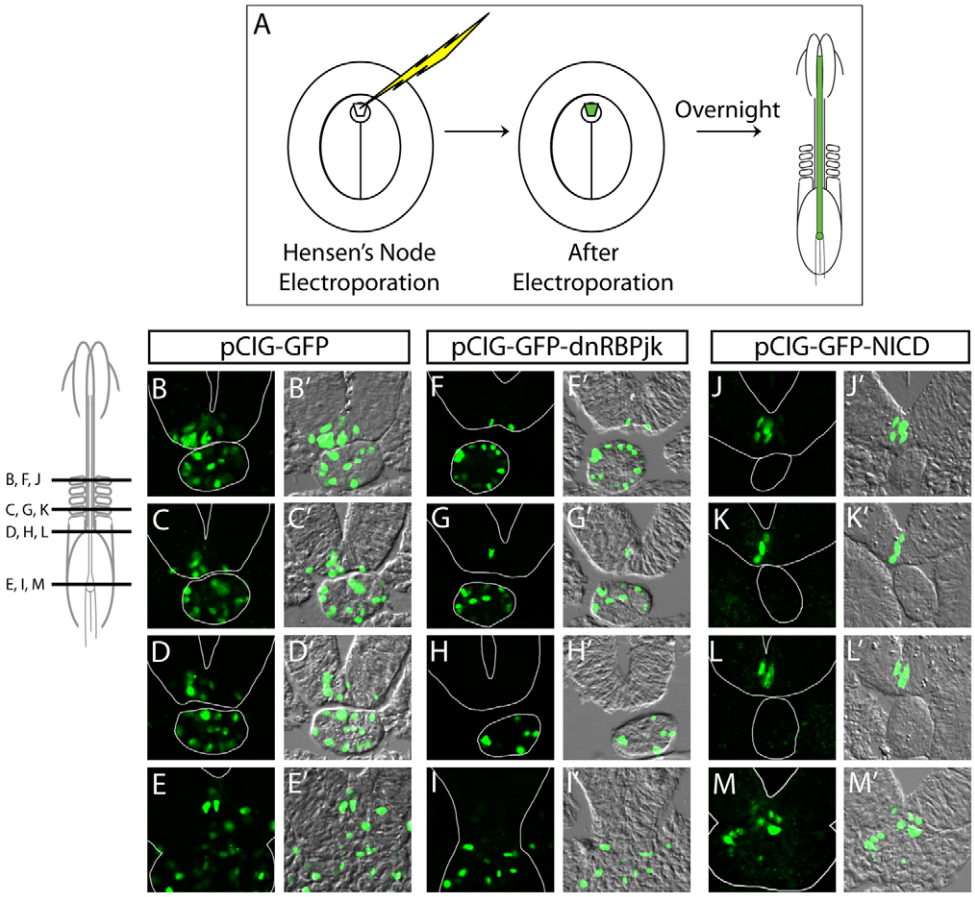

Fig. 4. Notch activation in Hensen's node progenitors promotes the contribution of these cells to the floor plate and inhibits them from populating the notochord. (**A**) Schematic of the assay. (**B-E′**) Serial transverse sections of embryo electroporated with pCIG-GFP showing contribution of electroporated cells to FP and the notochord. (**F-I′**) Sections of embryo electroporated with pCIG-dnRBPjκ showing a minor contribution to the FP and predominant contribution to notochord. (**J-M′**) Sections of embryo electroporated with pCIG-NICD showing exclusive contribution to the FP.

(Figs 2 and 5). However, the notochord, which can induce floor plate differentiation in adjacent neural tissue, is present in DAPT-treated embryos, and careful measurement revealed that, in fact, the notochord is significantly bigger in these treated embryos (n=7) compared with controls (n=3). (Kruskal-Wallis 1-way ANOVA df=1, H=63.831, P<0.001; Fig. 5A-B′; see also Fig. S1 in the supplementary material.) As we have shown that under conditions of Notch inhibition Hensen's node progenitor cells continue to contribute robustly to the notochord, it is consistent that under these conditions more progenitor cells would contribute to notochord than to the floor plate, thereby leading to the formation of a bigger notochord. We therefore suggest that the presence of a notochord in DAPT-treated embryos facilitates the induction of a floor plate in neural tissue that did not derive from Hensen's node and would therefore not normally be fated to become floor plate tissue.

DISCUSSION

In this study, we have investigated the implication of Notch signalling in the development of the axial tissues of the notochord and floor plate that derive from Hensen's node in the chick embryo. We found that Notch plays a crucial role within the progenitor cells that give rise to these two tissues, promoting them to contribute to the floor plate and inhibiting their contribution to the notochord.

It was previously reported that Notch acts within cells of the zebrafish organizer/shield to regulate allocation of appropriate numbers of cells to the notochord, floor plate and hypochord. The authors propose that Notch has two distinct roles: first, to specify trunk hypochord at the expense of notochord and second, to promote proliferation but not specification of floor plate cells (Latimer and Appel, 2006; Latimer et al., 2002; Latimer et al., 2005). Our data argue that Notch plays a more prominent role in floor plate development within the organizer in chick, as in the absence of Notch activity there is no contribution of progenitor cells to the floor plate within the region of the embryo where the inhibition took place. Moreover, in embryos devoid of Notch signalling, the notochord is significantly enlarged, which implies that the notochord population expands at the expense of the floor plate. Taken together with our gain-of-function data, Notch therefore appears to be required in Hensen's node to regulate progenitor cell contribution to the floor plate and the notochord.

The mechanism by which Notch acts to promote the floor plate at the expense of the notochord remains to be determined. This role might be permissive such that Notch inhibits induction of notochord markers rather than actively promoting the floor plate. Alternatively, Notch might play an instructive role directly via induction of floor plate markers. In this case, it is probable that Notch would exclusively specify the floor plate of medial character as Hensen's node contains progenitors for only this subset, whereas the lateral floor plate in chick arises from neuralized ectoderm (Charrier et al., 2002).

Our data show that in the absence of Notch a floor plate still forms. This might be owing to induction of floor plate characteristics from the larger notochord that forms under these conditions. Alternatively, a small proportion of node precursor cells might continue to adopt the floor plate fate despite the absence of Notch signalling. Indeed, recent findings by Lowell et al. suggested that in the context of mES cells, Notch acts not as a primary inducer, but as an amplifier such that NICD has no effect on its own on the stability

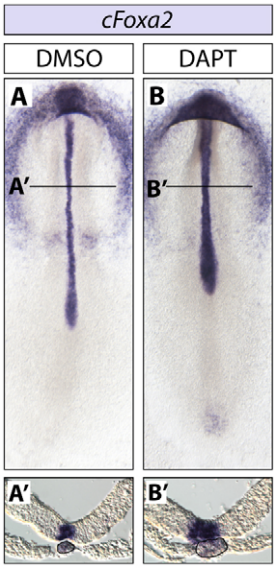

Fig. 5. Following Notch inhibition in a whole chick embryo, a larger notochord develops. (**A-B′**) *Foxa2* in embryos cultured overnight in DAPT or DMSO and their corresponding sections. *Foxa2* in the floor plate (A,A′) is not affected in a DAPT-treated embryo (B,B′). DAPT-treated embryos have a larger notochord.

of the stem cell state, nor on the acquisition of neural cell fate, but it increases the effectiveness of Fgf in mediating this transition (Lowell et al., 2006). It is possible we have identified a similar role for Notch in Hensen's node.

Alternatively, Notch activity might promote epithelial versus mesodermal characteristics within a subpopulation of precursor cells, which then acquires floor plate characteristics after becoming exposed to notochord-derived inductive cues once it exits Hensen's node. Further characterization of the cell behaviours following Notch activation within Hensen's node would provide more insight into which of these possibilities might hold true.

The node and streak comprise precursors of a great many cell derivatives. Aside from classical embryological studies, very little is known about the transcriptional profile or the mechanisms implicated in the adoption of a specific cell fate within these progenitor cells. An important future goal will be to investigate the biochemical basis of different signalling mechanisms implicated in the acquisition of specific cell fates within these different progenitor cell populations. The Notch pathway plays a key role in many binary cell fate decisions. Thus, Notch might influence binary cell fate choice of many tissues other than the axial tissues and this has potential implications for the developing embryo as a whole.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

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