

Pax6 is expressed in subsets of V0 and V2 interneurons in the ventral spinal cord in mice



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ABSTRACT

The embryonic spinal cord in mice is organized into eleven progenitor domains. Cells in each domain first produce neurons and then switch to specifying glia. Five of these domains known as p3, pMN, p2, p1 and p0 are located in the ventral spinal cord and each expresses a unique code of transcription factors (TFs) that define the molecular profile of progenitor cells. This code is largely responsible for determining the subtype specification of neurons generated from each domain. Pax6 codes for a homeobox-containing TF that plays a central role in defining the molecular boundaries between the two ventral-most domains, p3 and pMN. Using fate mapping and gene expression studies we show that PAX6, in addition to each patterning function, is expressed in a group of late born interneurons that derive from the p2 and p0 domains. The p2-derived neurons represent a subset of late born V2b interneurons and their specification depends on Notch signaling. The V0 neurons represent V0v ventral neurons expressing Pax2. Our data demonstrate that interneuron diversity in the ventral spinal cord is more complex than originally appreciated and point to the existence of additional mechanisms that determine interneuron diversity, particularly in the p2 domain.

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During embryo development the posterior neural plate gives rise to the developing neural tube which eventually forms the spinal cord. The neural plate is initially made up of a single layer of neuroepithelial progenitor (NEPs) cells with uniform molecular properties. NEPs in the neural tube, located in the ventricular zone (VZ), start to express different combinations of transcription factors (TFs) and become organized into eleven progenitor domains along the dorso–ventral axis. The VZ of the embryonic ventral spinal cord (vSC) is organized into five progenitor domains (ventral-p3-pMN-p2-p1-p0-dorsal), specified through the graded activity of Sonic Hedgehog released from the notochord and floor plate (Ericson et al., 1997a). Sonic Hedgehog either induces or represses the expression of homeobox and basic helix-loop-helix TFs in all domains of the vSC, creating a unique expression pattern of TFs in each domain (Jessell, 2000). The dorsal spinal cord (dSC) is organized into six progenitor domains. Members of the bone morphogenetic protein (BMP) family, produced from dorsal midline structures, create a gradient of BMP-dependent activity that is

responsible for patterning dorsal progenitor domains, along with other signaling molecules (Zhuang and Sockanathan, 2006). The TFs expressed in each progenitor domain are primarily responsible for specifying the neural and glial output of each domain (Briscoe et al., 2000; Muhr et al., 2001; Hochstim et al., 2008).

While neural patterning is the overarching mechanism responsible for establishing cellular diversity in the spinal cord, additional mechanisms operate in each domain that further enhance cellular diversity. For example, the p2 domain generates at least three different types of interneurons (INs), named V2a, V2b and V2c. Specification of V2a and V2b IN fates is determined in post-mitotic progenitors through the action of Notch/Delta signaling, both in mice (Del Barrio et al., 2007; Peng et al., 2007) and zebrafish (Battista et al., 2008; Kimura et al., 2008). The mechanism underlying the specification of V2c INs is yet to be determined (Panayi et al., 2010). Downstream of Notch, the action of the LIM-only protein (LMO4) either favors or inhibits transcriptional complexes in subsets of post-mitotic progenitors and consolidates the V2a and V2b IN fates (Joshi et al., 2009).

The p0 domain is known to express both Dbx1 and Dbx2, whereas the p1 domain is marked by the sole expression of Dbx2 (Pierani et al., 1999). Loss of Dbx1 leads to loss of V0 INs leading to expansion of the p1 domain and overproduction of V1 INs

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(Pierani et al., 2001). V0 INs are further subdivided into inhibitory ventral V0_v and excitatory dorsal V0_d INs (Lundfald et al., 2007). The mechanism underlying this sub-type specification is not yet known.

Pax6 codes for a homeodomain-containing TF and plays a crucial role in maintaining the identity of the pMN domain (Briscoe et al., 2000; Genethliou et al., 2009). While the role of PAX6 during patterning of the vSC has been studied extensively, little is known about its role in post-mitotic neurons. In this study we used expression analysis, fate mapping and genetic studies to show that Pax6 is expressed in a subset of late-born INs derived from the p2 and p0 domains of the vSC. The p2-derived INs are generated at least two days later than the stage when other V2 INs are specified and their specification still depends on Notch signaling. Our data suggest that the mechanisms guiding IN subtype specification in the p2 domain is more complicated than originally appreciated.

1. Results

1.1. Pax6 is expressed in ventral interneurons derived from the p0 and p2 domains

Pax6 is a transcription factor that is first expressed by most neural progenitors of the spinal cord and is required for correct neural patterning during neurogenesis (Ericson et al., 1997b; Briscoe et al., 2000) and gliogenesis (Hochstim et al., 2008; Genethliou et al., 2009). In addition to its expression in the VZ throughout spinal cord development, at around e12.0–e12.5 Pax6 expression becomes clearly detectable in a small group of ventrally migrating cells (Fig. 1A–C). We first confirmed that these cells expressed the pan-neuronal marker NeuN (and are thus neurons) and none of the motor neuron markers (data not shown). We conclude that these cells are most likely interneurons (INs) as no glial cells are produced at this stage (Hochstim et al., 2008).

To determine the precise developmental window during which these INs are born we used *in vivo* BrdU incorporation. We injected pregnant mice at consecutive intervals with BrdU solution at e10.5, e11.5 and e12.5 and harvested the embryos at e14.5 (Fig. 1D–F). This analysis showed that Pax6⁺ INs are mostly specified at e11.5 (Fig. 1G). We cannot exclude the possibility that some cells are born at e10.0 or even some at e12.0 since BrdU take up might be partial.

We then applied fate mapping to determine the progenitor domains where the progenitors producing these cells. Based on their final resting position, it seemed likely that the Pax6⁺ INs originated from ventral progenitor domains. We used genetic fate mapping using three Cre-expressing transgenic mice which, all together, mark all five ventral progenitor domains. These were Olig2-iCre [labeling p3-, pMN- and very few p2-derived cells (Chen et al., 2011)], Foxn4-iCre (labeling p2-derived neurons) and Dbx1-iCre (labeling dorsall6-, dl5-, p0- and p1-derived cells). Males from each line were crossed to Rosa26-stop-YFP females and embryos or adult progeny were analyzed at the appropriate stages.

Analysis of embryonic cords derived from a Dbx1-iCre × Rosa26-stop-YFP cross showed that approximately 50% of the Pax6⁺ INs derive from Dbx1 lineage suggesting origin from progenitors residing in the dl5–p1 region (Fig. 2A–C and J). Furthermore, about 25%, of Pax6⁺ INs originated from Foxn4⁺ progenitors suggesting origin from the p2 domain (Fig. 2D–F and J). The lineage relationship between Foxn4-expressing progenitors continued to hold true over a range of developmental stages and through to adulthood (Suppl. Fig. 1). Finally, analysis of the progeny of the Olig2⁺ lineage in Olig2-iCre × Rosa26-stop-YFP embryos failed to establish a lineage relationship between the p3/pMN/ventral-p2 domain and Pax6⁺ INs (Fig. 2G–I). These data demonstrate that

two distinct groups of Pax6⁺ INs originate from the p2 and dl5–p1 domains, respectively. We cannot exclude the possibility that some Pax6⁺ INs originate from other progenitor domains or that incomplete Cre recombination in the domains studied did not allow us to fate-map all cells.

Both the fate mapping studies and the birth-dating experiments are in line with previous studies showing that around e12.5 vSC progenitors stop producing neurons and switch to gliogenesis (Hochstim et al., 2008; Genethliou et al., 2009).

1.2. Most Pax6⁺ interneurons represent subsets of V2b and V0 interneurons

Having established the domains where Pax6⁺ INs originate from, we analyzed the expression of specific interneuron markers relative to Pax6. We first focused on interneurons derived from the p2 domain. These are divided into three groups V2a, V2b and V2c INs, expressing Chx10 (V2a), Gata3 (V2b) and Sox1 (V2c). We have previously shown that Pax6 INs are distinct from V2c INs (Panayi et al., 2010) and thus tested if they express Chx10 or Gata3. Our data showed that none of the Pax6⁺ INs express Chx10 (Fig. 3A–C) while at least some express Gata3 only when they settle in the ventral cord and not when they migrate out of the VZ (Fig. 3D–F).

Given that Gata3 is not expressed by any other IN type of the vSC, we conclude that at least some of the p2-derived Pax6⁺ INs represent a subclass of late-born V2b INs. We cannot exclude the possibility that some Pax6⁺ INs derive from the p2 domain and express only Pax6. The genetic tools available to us cannot test this possibility.

Our fate mapping data suggested that the majority of Pax6⁺ neurons derive from a region encompassing four domains (p1, p0, dl6 and dl5) which express Dbx1. The p1 and p0 domains generate V1 and V0 INs, respectively. We thus focus on markers expressed by these neurons using antibodies that label V0 and V1 INs. We used Evx1, a marker for V0 INs, Pax2 a marker for ventral V0 INs (V0_v) and FoxP2 a marker for V1 INs (Pierani et al., 1999; Lanuza et al., 2004; Morikawa et al., 2009).

Having established that some Pax6⁺ INs express Pax2 (and are therefore V0_v INs) and some express Gata3 (and represent a subset of V2b INs) we used triple staining using Pax6/Pax2/Gata3 to find out if Pax6⁺/Pax2⁺ and represent the total number of Pax6⁺ neurons (Fig. 4). We found that, in addition to Pax6/Pax2⁺ and Pax6⁺/Gata3⁺ INs, there were also only Pax6-expressing INs (Fig. 4 arrow heads). Pax6⁺ INs were also negative for Prdm8, known to mark V1 and some V2 INs (Komai et al., 2009) and HNF6 which labels V1 INs (Stamps and Krishnan, 2004) (data not shown).

Finally, analysis of neurotransmitter expression showed that Pax6⁺ INs were heterogeneous in that some were glutamatergic (excitatory) while others were glycinergic (inhibitory) (Suppl. Fig. 2). Antibody incompatibility did not allow us to determine the IN subtype relative to neurotransmitter expression but most likely the glycinergic Pax6⁺ INs represented the subset of V2b INs (Lundfald et al., 2007).

Together, these data suggest that Pax6⁺ INs fall into three categories. The first group represents a subset of V0_v neurons, the second group forms a subset of V2b neurons and the third is a group of Pax6⁺ INs whose origin cannot be defined using available genetic tools. Nevertheless, they are most likely ventrally derived neurons.

1.3. Pax6⁺/Gata3⁺ INs specification is controlled by Notch signaling

Previous work has shown that Notch signaling is responsible for the allocation of neuronal subtype specification in V2 INs operating in post-mitotic progenitors (Yang et al., 2006; Peng et al., 2007). More specifically, loss of Notch signaling leads to overproduction

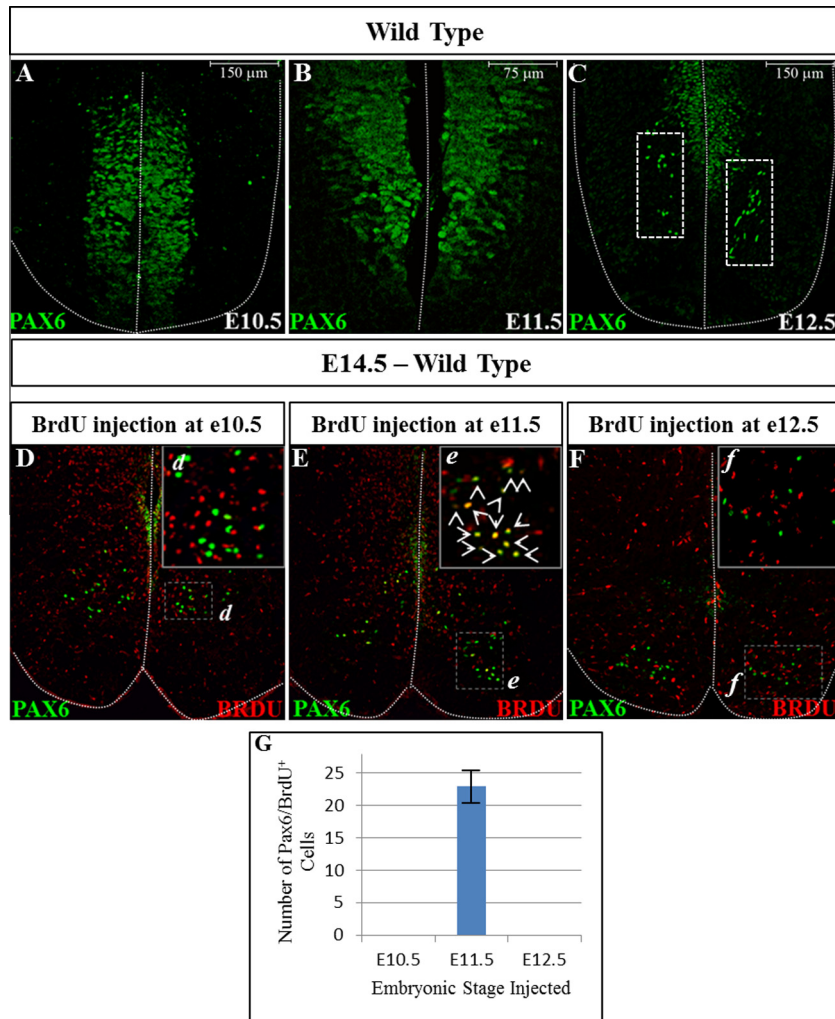


Fig. 1. PAX6 is expressed in ventral interneurons born at a narrow developmental window. Embryonic spinal cord sections at the denoted stages were immune-stained with anti-PAX6 antibody. Panels A and B depict the expression of PAX6 along the ventricular zone. Panel C shows PAX6⁺ neurons (dotted square) migrating ventrally. Panels D–F represent spinal cord sections from e14.5 embryos derived from pregnant mothers injected with BrdU at the denoted stage. Panel G shows the number of cells co-stained with anti-BrdU and anti-PAX6 antibodies. Note that only at stage e11.5 injections result in co-staining of PAX6 with anti-BrdU antibody.

of V2a INs and complete loss of V2b INs. These studies have been carried out at e10.5 embryos using Presenilin 1 (PSEN1) and FoxN4-deficient embryos (Li et al., 2005; Yang et al., 2006; Del Barrio et al., 2007; Peng et al., 2007). Given that Pax6 is expressed in a subset of V2b INs, we asked if the production of these cells was affected in PSEN1-mutant embryos. Analysis of spinal cord sections from mutant and wild type embryos showed that an equivalent number of Pax6⁺/Pax2⁺ neurons were still specified, despite a noticeable decrease in the total number of Pax6⁺ neurons (not shown). This is most likely due to an overall decrease of neurogenesis caused by diminished Notch signaling. However, the specification of Pax6⁺/Gata3⁺ V2b INs was totally abolished. First, we noted that in PSEN^{-/-} embryos there was some production of Gata3⁺ cells while at e10.5 these V2b cells are totally misspecified and convert to V2a cells expressing Chx10 (Peng et al., 2007). Importantly the very few Gata3⁺ cells produced in the mutants co-expressed Chx10 indicating that these cells have a mixed V2a/V2b phenotype. Similarly we failed to observe Pax6⁺/Gata3⁺ and instead we noted very few (1–2 cells per section) Pax6⁺/Chx10⁺ INs.

These data suggest that markedly diminished Notch signaling affected the phenotype of Pax6⁺/Gata3⁺ cells and caused a fate switch to Pax6⁺/Chx10⁺ cells in V2b INs. Interestingly some Gata3⁺/Chx10⁺ cells were also observed in mutant cords which most likely

represent V2b INs with partial V2b-to-V2a fate switch (Fig. 5). These results indicate that late born Gata3⁺/Pax6⁺ neurons, like the early born Gata3⁺/Pax6⁻ cells, are specified in a Notch-dependent manner, while the production of Pax6⁺/Pax2⁺ neurons was essentially unaffected.

2. Discussion

In this study we describe the expression of Pax6 in differentiated neurons in the vSC. Although this expression was noted a long time ago (Ericson et al., 1997b), no study has since described the subtype identity of these cells. We show that Pax6⁺ neurons form a heterogeneous population of INs which originates from at least two domains, the p0 domain constituting a subset of the V0V INs, and the p2 domain forming a subset of the V2b INs. This diverse population of Pax6⁺ cells was shown to include both inhibitory and excitatory neurons.

Neurogenesis in the p2 domain and subtype specification has been studied quite extensively. Here we show that p2-derived INs that express Pax6 also expresses Gata3. Unlike the other three sub-types of INs generated in this domain (V2a–c), the Pax6⁺ INs are born at least two days later. It is noteworthy that when these

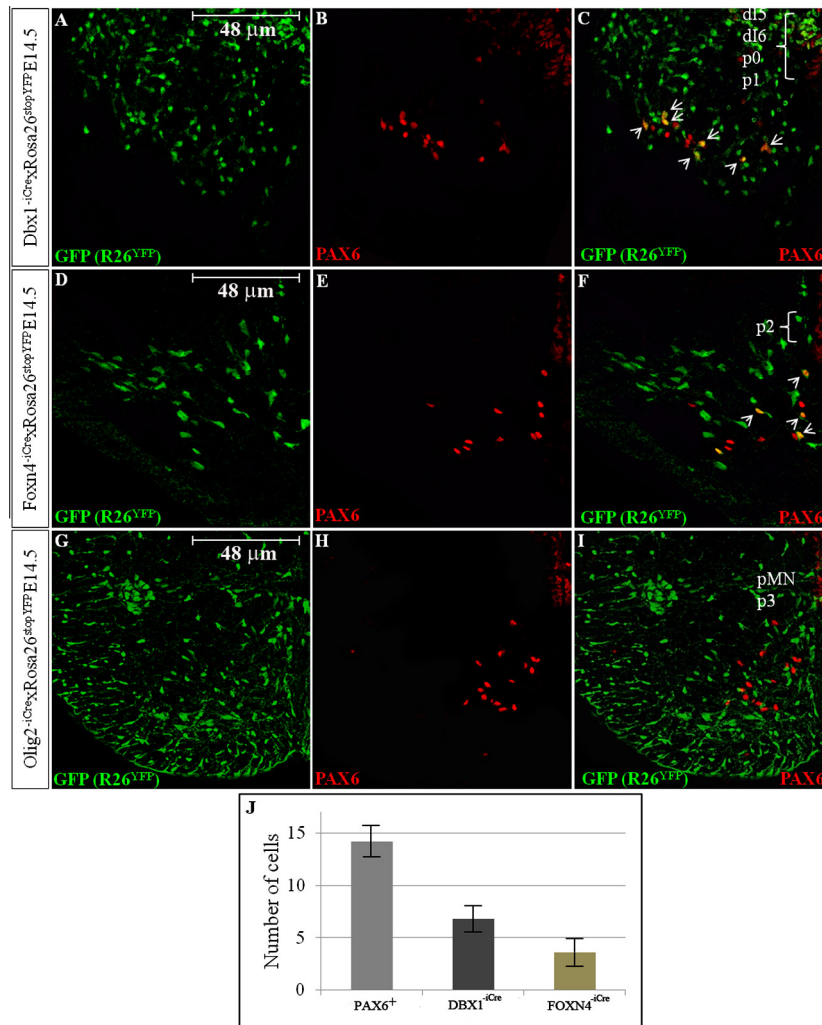


Fig. 2. Lineage analysis of the PAX6⁺ neurons. Spinal cord sections from e14.5 embryos from the denoted crosses were analyzed for PAX6 and YFP (detected with anti-GFP antibody) expression. Arrows in panel C indicate neurons from the Dbx1 lineage that co-express PAX6. Arrows in panel F show neurons from the Foxn4 lineage that also express PAX6 (p2-derived). Panel I, indicates that no Pax6⁺ neurons originate from the Olig2⁺ lineage. Panel J illustrates the total number of Pax6⁺ neurons and the number of Pax6⁺ neurons deriving from Dbx1 and Foxn4 lineages. Note that the total number of Pax6⁺ is more than the sum of the two subpopulations of neurons. This could be due to incomplete or delayed Cre-recombination or that some Pax6⁺ neurons derive from other domain(s).

cells migrate out of the ventricular zone they do not express Gata3. However, as they settle in their ventral position they switched on Gata3. This sequential expression of Pax6 followed by Gata3 clearly points to a different mechanism that drives the expression of Gata3 in these INs compared to other Gata3⁺ INs which acquire Gata3 expression on the borders of the VZ through a mechanism controlled by Notch signaling. What switches on Gata3 in these cells remains to be determined.

Previous studies have shown that the Notch pathway determines the specification of V2 INs (Del Barrio et al., 2007; Peng et al., 2007). Specifically loss of Notch at e9.5 converts all V2b INs to V2a INs. Our previous work has shown that a new group of INs, which we named V2c INs, when specified express Gata3 but its expression is repressed by SOX1 and these INs acquire a distinct fate (Panayi et al., 2010). Our present data suggest that Pax6⁺ INs first begin to express Pax6 and subsequently acquire Gata3 expression. In the absence of Notch they maintain Pax6 expression, lose Gata3 and switch on Chx10, a marker for V2a INs. Whatever the underlying mechanism operating to initially specify Pax6⁺ INs is, our data reveal that Notch signaling is responsible to direct expression of Gata3. Therefore, Notch signaling may not only func-

tion to specify neuronal fates in progenitors just exiting the cell cycle but may also do so in fully differentiated neuronal fates settling in the marginal zone. We noted that in the PSEN mutants some Gata3 expression was maintained, unlike early stages (Peng et al., 2007), but Gata3⁺ cells converted to Gata3⁺/Chx10⁺ cells. These cells are likely to be bona fide V2b INs generated from residual Notch signaling activity conferred by Presenilin II which is also expressed in the vSC.

3. Materials and methods

3.1. Transgenic mice

The following established mouse lines were used: Rosa26-stop-YFP (Srinivas et al., 2001), Foxn4-iCre and GATA3-eGFP (Panayi et al., 2010), Dbx1-iCre (Fogarty et al., 2005) and Olig2-iCre (Kessaris et al., 2006). All animals were kept in the normal 12 h light–12 h dark cycle and where allowed free access to water and food. All animal procedures were performed in accordance with a license issued by the Chief of Veterinary Services of the Republic

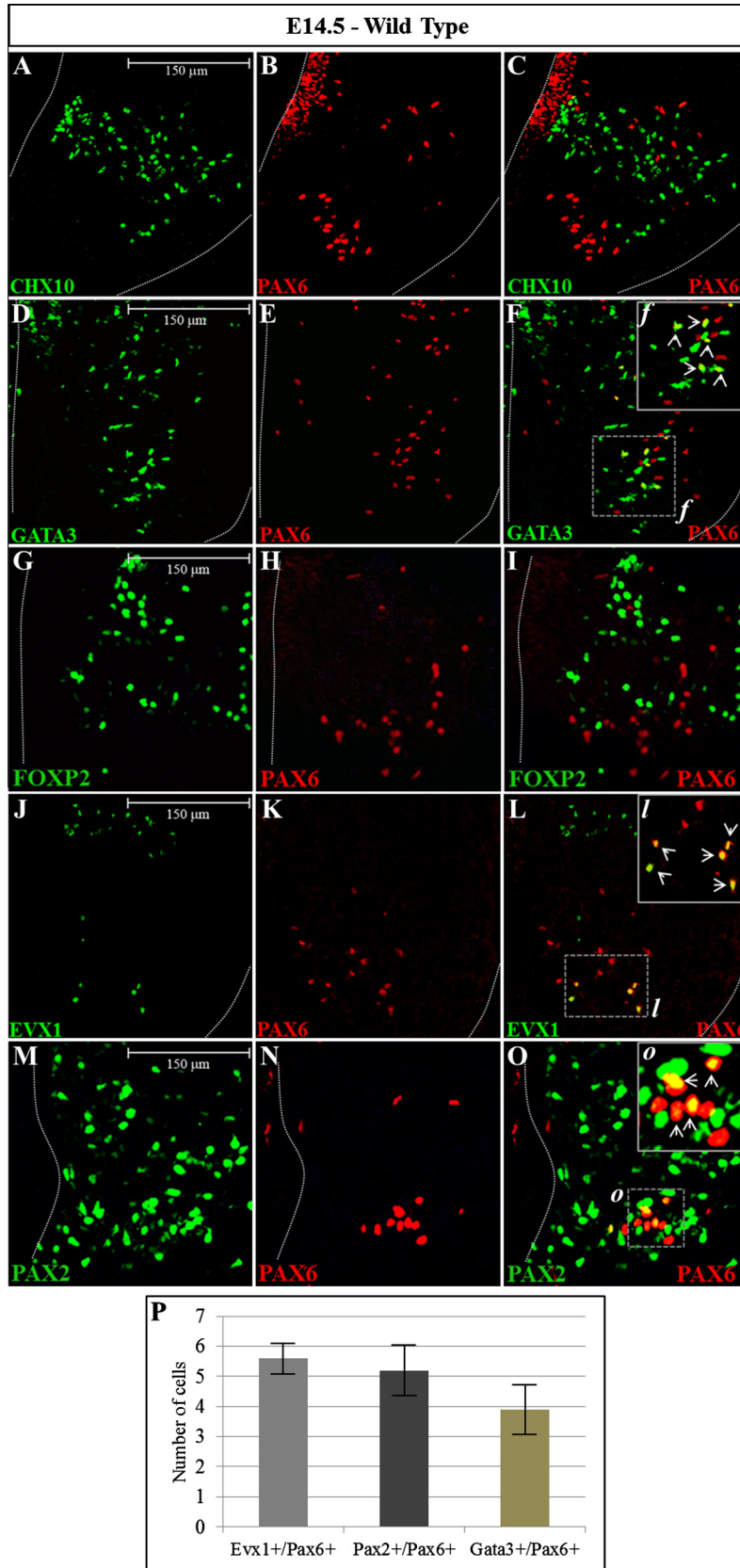


Fig. 3. Marker analysis of Pax6⁺ neurons in embryonic spinal cords. Spinal cord sections from e14.5 embryos were stained with anti-PAX6 antibody and the antibodies denoted in the panels representing V2a interneurons (CHX10), V2b interneurons (GATA3), V1 interneurons (FOXP2), V0 interneurons (EVX1) and V0v interneurons (PAX2). Arrow heads represent cells co-expressing PAX6 and the indicated marker. Panel P shows quantification of each population. Panel P represents a graph showing the number of cells expressing PAX6 and the respective marker. Note that the number of Evx1⁺/Pax6⁺ (V0) and Pax2⁺/Pax6⁺ (V0v) neurons is very similar indicating that the possibly all V0 Pax6⁺ INs are most likely V0v INs.

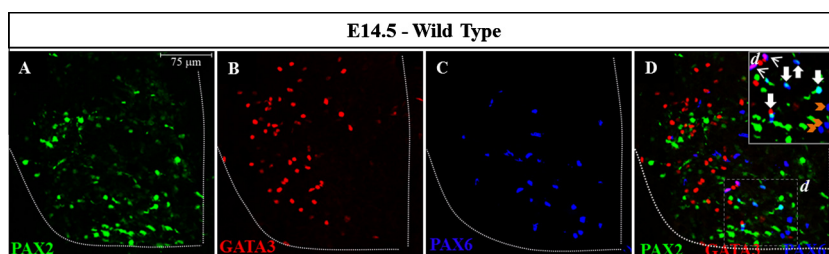


Fig. 4. Pax6⁺ neurons are not all V0v and V2b interneurons. Spinal cord sections from e14.5 embryos were triple immune-stained using anti-PAX2, anti-GATA3 and anti-PAX6 antibodies (panels A–D) indicating the three populations of PAX6⁺ neurons in the ventral spinal cord. Inset in panel D shows an enlarged view of the dotted area. Note that there are blue Pax6⁺ cells (indicated by orange chevrons) that do not express either PAX2 or GATA3. PAX2⁺/PAX6⁺ cells are indicated by white arrows and GATA3⁺/PAX6⁺ cells by arrow heads.

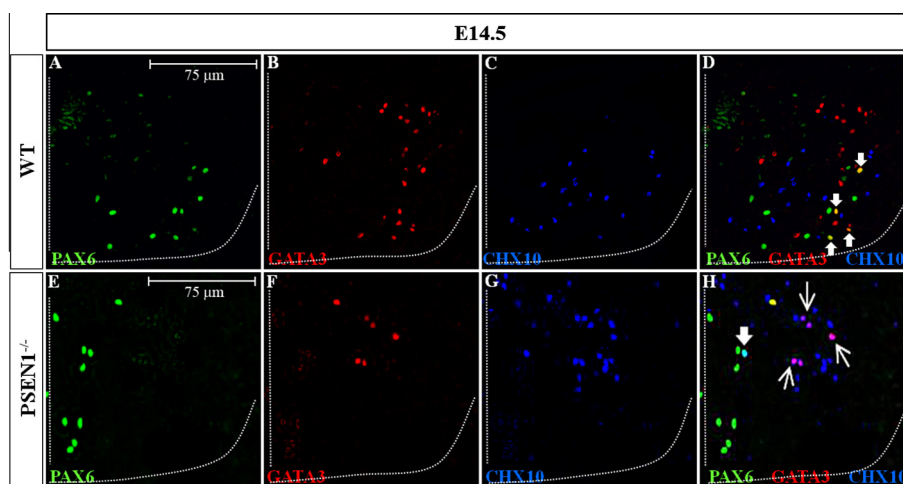


Fig. 5. The specification of PAX6⁺/GATA3⁺ cells depends on Notch signaling Spinal cord sections from E14.5 wild type (panels A–D) and PSEN1^{-/-} (panels E–H) embryos were immune-stained with anti-PAX6, anti-GATA3 and anti-CHX10 antibodies. Note that in wild type embryos there are only PAX6⁺/GATA3⁺ and PAX6⁺/GATA3⁻ cells (Panel D). On the contrary in mutant embryos (Panel H) we noted mostly PAX6⁺/CHX10⁺ (arrows in H) and occasionally GATA3⁺/CHX10⁺ (bold arrow in H) but never PAX6⁺/GATA3⁺ cells indicating a fate switch of PAX6⁺/GATA3⁺ cells to a PAX6⁺/CHX10⁺ phenotype.

of Cyprus, according to National Law. All animal involving experiments were carried out in accordance to the 86/609/EEC Directive. When required, embryo genotyping was done by PCR analysis.

3.2. Immunohistochemistry

Immunohistochemistry was performed using embryos fixed in MEMFA or 4% Paraformaldehyde (PFA) in phosphate-buffered saline (PBS) for a period of 15–35 min, depending on the age of the embryo and the antibodies used. This was followed by extensive washing in PBS and cryoprotection in 15–30% (w/v) sucrose prepared in PBS. Sections (10–12 μm) were cut on a cryostat. Antibody detection was performed using immunofluorescence according to standard procedures. The following primary antibodies were used: anti-BRDU (1/200 Abcam); anti-Chx10 (1/500 Santa Cruz Biotechnology); anti-EVX1 (1/50 Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA); anti-FoxP2 (1/1,000 Abcam); anti-GATA3 (1/100 gift from Frank Grosveld, Erasmus Medical Centre, Department of Cell Biology, Rotterdam, The Netherlands); anti-GFP (1/4000 Invitrogen); anti-GFP (1/2000 Nacalai USA); anti-Glutamate (1/10,000 Immunosolution); anti-Glycine (1/5000 Immunosolution); anti-HNF6 (1/5000 Laboratory of Neural Differentiation/LPAD Unit); anti-Pax2 (1/1000 Millipore Bioscience Research Reagents); anti-Pax6 (1/2000 Chemicon and 1/50 Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA); anti-PRDM8 (1/200 gift from Pr. Yoichi Shinkai). Images were captured on a TCSL confocal microscope (Leica, Germany).

3.3. Cell counting and statistical analysis

Serial sections from equivalently-staged embryos at forelimb level were used for cell counting. For each stage and genotype three embryos were used to obtain 10 sections for counting, totaling 30 sections. The quantitative results were analyzed by two-tailed distribution, homoscedastic Student's T-Test. The graphed results are shown as means ± SEM (Standard error of the mean).

3.4. BrdU labeling

Staged pregnant mice were injected intra peritoneally with BrdU (Sigma) at 50 μg/kg of body weight once two hours for a total of six injections. The injected female was euthanized at the stage required by the experimental design and the embryos were collected and processed for detection of BrdU labeling using immunohistochemical techniques as has been aforementioned.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.gep.2013.06.004>.

References

- Batista, M.F., Jacobstein, J., Lewis, K.E., 2008. Zebrafish V2 cells develop into excitatory CiD and Notch signalling dependent inhibitory VeLD interneurons. *Dev. Biol.* 322, 263–275.
- Briscoe, J., Pierani, A., Jessell, T.M., Ericson, J., 2000. A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* 101, 435–445.
- Chen, J.A., Huang, Y.P., Mazzoni, E.O., Tan, G.C., Zavadil, J., Wichterle, H., 2011. *Mir-17-3p* controls spinal neural progenitor patterning by regulating *Olig2/Irx3* cross-repressive loop. *Neuron* 69, 721–735.
- Del Barrio, M.G., Taveira-Marques, R., Muroyama, Y., Yuk, D.I., Li, S., Wines-Samuelson, M., Shen, J., Smith, H.K., Xiang, M., Rowitch, D., Richardson, W.D., 2007. A regulatory network involving *Foxn4*, *Mash1* and *delta-like 4/Notch1* generates V2a and V2b spinal interneurons from a common progenitor pool. *Development* 134, 3427–3436.
- Ericson, J., Briscoe, J., Rashbass, P., van Heyningen, V., Jessell, T.M., 1997a. Graded sonic hedgehog signaling and the specification of cell fate in the ventral neural tube. *Cold Spring Harb. Symp. Quant. Biol.* 62, 451–466.
- Ericson, J., Rashbass, P., Schedl, A., Brenner-Morton, S., Kawakami, A., van Heyningen, V., Jessell, T.M., Briscoe, J., 1997b. *Pax6* controls progenitor cell identity and neuronal fate in response to graded *Shh* signaling. *Cell* 90, 169–180.
- Fogarty, M., Richardson, W.D., Kessar, N., 2005. A subset of oligodendrocytes generated from radial glia in the dorsal spinal cord. *Development* 132, 1951–1959.
- Genethliou, N., Panayiotou, E., Panayi, H., Orford, M., Mean, R., Lapatitis, G., Malas, S., 2009. Spatially distinct functions of *PAX6* and *NKX2.2* during gliogenesis in the ventral spinal cord. *Biochem. Biophys. Res. Commun.* 382, 69–73.
- Hochstim, C., Deneen, B., Lukaszewicz, A., Zhou, Q., Anderson, D.J., 2008. Identification of positionally distinct astrocyte subtypes whose identities are specified by a homeodomain code. *Cell* 133, 510–522.
- Jessell, T.M., 2000. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat. Rev. Genet.* 1, 20–29.
- Joshi, K., Lee, S., Lee, B., Lee, J.W., Lee, S.K., 2009. *LMO4* controls the balance between excitatory and inhibitory spinal V2 interneurons. *Neuron* 61, 839–851.
- Kessar, N., Fogarty, M., Iannarelli, P., Grist, M., Wegner, M., Richardson, W.D., 2006. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nat. Neurosci.* 9, 173–179.
- Kimura, Y., Satou, C., Higashijima, S., 2008. V2a and V2b neurons are generated by the final divisions of pair-producing progenitors in the zebrafish spinal cord. *Development* 135, 3001–3005.
- Komai, T., Iwanari, H., Mochizuki, Y., Hamakubo, T., Shinkai, Y., 2009. Expression of the mouse PR domain protein *Prdm8* in the developing central nervous system. *Gene Expr. Patterns* 9, 503–514.
- Lanuza, G.M., Gosgnach, S., Pierani, A., Jessell, T.M., Goulding, M., 2004. Genetic identification of spinal interneurons that coordinate left–right locomotor activity necessary for walking movements. *Neuron* 42, 375–386.
- Li, S., Misra, K., Matise, M.P., Xiang, M., 2005. *Foxn4* acts synergistically with *Mash1* to specify subtype identity of V2 interneurons in the spinal cord. *Proc. Natl. Acad. Sci. USA* 102, 10688–10693.
- Lundfald, L., Restrepo, C.E., Butt, S.J., Peng, C.Y., Droho, S., Endo, T., Zeilhofer, H.U., Sharma, K., Kiehn, O., 2007. Phenotype of V2-derived interneurons and their relationship to the axon guidance molecule *EphA4* in the developing mouse spinal cord. *Eur. J. Neurosci.* 26, 2989–3002.
- Morikawa, Y., Hisaoka, T., Senba, E., 2009. Characterization of *Foxp2*-expressing cells in the developing spinal cord. *Neuroscience* 162, 1150–1162.
- Muhr, J., Andersson, E., Persson, M., Jessell, T.M., Ericson, J., 2001. Groucho-mediated transcriptional repression establishes progenitor cell pattern and neuronal fate in the ventral neural tube. *Cell* 104, 861–873.
- Panayi, H., Panayiotou, E., Orford, M., Genethliou, N., Mean, R., Lapatitis, G., Li, S., Xiang, M., Kessar, N., Richardson, W.D., Malas, S., 2010. *Sox1* is required for the specification of a novel p2-derived interneuron subtype in the mouse ventral spinal cord. *J. Neurosci.* 30, 12274–12280.
- Peng, C.Y., Yajima, H., Burns, C.E., Zon, L.I., Sisodia, S.S., Pfaff, S.L., Sharma, K., 2007. Notch and MAML signaling drives *Scf*-dependent interneuron diversity in the spinal cord. *Neuron* 53, 813–827.
- Pierani, A., Brenner-Morton, S., Chiang, C., Jessell, T.M., 1999. A sonic hedgehog-independent, retinoid-activated pathway of neurogenesis in the ventral spinal cord. *Cell* 97, 903–915.
- Pierani, A., Moran-Rivard, L., Sunshine, M.J., Littman, D.R., Goulding, M., Jessell, T.M., 2001. Control of interneuron fate in the developing spinal cord by the progenitor homeodomain protein *Dbx1*. *Neuron* 29, 367–384.
- Srinivas, S., Watanabe, T., Lin, C.S., Williams, C.M., Tanabe, Y., Jessell, T.M., Costantini, F., 2001. Cre reporter strains produced by targeted insertion of *EYFP* and *ECFP* into the *ROSA26* locus. *BMC Dev. Biol.* 1, 4.
- Stamps 3rd, A.E., Krishnan, V.V., 2004. Perceived enclosure of space, angle above observer, and distance to boundary. *Percept. Mot. Skills* 99, 1187–1192.
- Yang, X., Tomita, T., Wines-Samuelson, M., Beglopoulos, V., Tansey, M.G., Kopan, R., Shen, J., 2006. *Notch1* signaling influences v2 interneuron and motor neuron development in the spinal cord. *Dev. Neurosci.* 28, 102–117.
- Zhuang, B., Sockanathan, S., 2006. Dorsal–ventral patterning: a view from the top. *Curr. Opin. Neurobiol.* 16, 20–24.