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Role of Pax6 in Specifying Dorso-Ventral Identities in th
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Multiple molecular mechanisms control the development of the simple neural plate into neural tube that ultimately gives rise to diverse neural and glial cells. Bmp and Wnt signals emanating from the roof plate and overlaying ectoderm initiate patterning of the dorsal neural tube (Lee and Jessell, 1999), while ventral spinal cord patterning is controlled by Shh that is secreted from the notochord and floor plate (Chiang et al., 1996; Ericson et al., 1996). Absence of notochord leading to loss of Shh signaling ventrally, leads to dorsalization of the whole neural tube (Yamada et al., 19991; Liem et al, 1995). The present study however suggests an additional mechanism, where dorsal fates are kept off in the ventral spinal cord (SC) due to presence of inhibitors of dorsal signals. Specifically my analysis shows that Lbx1 is usually kept off in the ventral SC due to the presence of the Wnt inhibitors, the secreted frizzled related proteins (sFRP's). Loss of Pax6 in *Pax6* $^{Sey/Sey}$ mutant's leads to loss of sFRP's in the ventral SC and results in activation of ectopic Lbx1 expression in cells normally fated to become V2 interneurons under the influence of Shh signaling. These results suggest that Pax6 and sFRP2 control ventral neuronal fates by restricting their response to Wnt signaling in the developing neural tube.

1. Original aims of the project & project successes.

1. Characterization of the molecular changes in the post- mitotic V2 interneurons due to ectopic Lbx1 up- regulation in *Pax6* $^{Sey/Sey}$ mutant mice.

The first specific aim of the proposed project was to characterize the molecular changes in the post-mitotic V2 interneurons due to ectopic Lbx1 up regulation in the Pax6 $^{Sey/Sey}$ mutant mice. This issue involved

    a). Determining the specific identity of ectopic Lbx1 cells.

    b). Determining which specific sub-populations of V2 interneurons are affected.

Both of these sub-aims have been accomplished as discussed in results section 2.

2. Characterization of the molecular changes in p2 progenitors leading to ectopic Lbx1 cells in V2 domain.

This aim had two specific sub-aims:

    a). Characterize the changes in progenitor factors that lead to ventral expansion of Lbx1.

    b). Determine the requirement of Mash1 in ventral Lbx1 up-regulation by generating *Pax6* $^{Sey/Sey}$; *Mash1* $^{-/-}$ double mutants.

a). The first part was to characterize the changes in the V2 progenitor domain of the *Pax6* $^{Sey/Sey}$ mutants that might suggest a possible mechanism of up- regulation of Lbx1 in this domain. We predicted that this might involve either loss of expression or ectopic gain of expression of a dorsal progenitor marker in this domain. ***An extensive array of testing has been carried out for this analysis*** based on expression patterns of genes that have already been identified (a "candidate-gene" approach). However, other unidentified genes might be involved in this up- regulation and these have not been included in the study. This part is addressed in **results section 1.**

b). As proposed in this aim in our grant proposal, Mash1 might be one candidate gene since it is expressed in the Lbx1 progenitors both dorsally and in the V2 domain. To specifically address the role of Mash1 we have obtained the *Mash1* $^{-/-}$ mutant mice and analyzed the generation of V2 interneurons in these mice. This analysis has provided us with some valuable data of the genetic pathways involved in generation of the two sub- populations of the V2 domain: V2a and V2b. **We have successfully generated Mash** $^{-/-}$**; Sey** $^{-/-}$ **double mutants** to determine whether ectopic Lbx1 observed in *Pax6* $^{Sey/Sey}$ mutants require Mash1 activity. This part of the work is discussed in **results section 3.**

3. In-ovo electroporations to determine the ability of dorsal and ventral progenitor factors t regulate Lbx1 expression in the spinal cord.

The final aim was to employ *in-ovo* electroporations to determine the ability of dorsal and ventra progenitor factors to regulate Lbx1 expression in the SC. Several candidates have been tested that hav led to a hypothesis elucidating the mechanism involved in the up- regulation of Lbx1 in the V2 domair The details of this specific aim are discussed **in results sections 4 and 5.**

Results.

1. Altered expression of sFRP's in *Pax6$^{Sey/Sey}$* mutant embryos.

Previous studies have shown that expression of the secreted Wnt antagonist *sFRP2* is down regulated in the ventral spinal cords of *Pax6$^{Sey/Sey}$* mutants (Kim et al., 2001), suggesting that Wnt signaling could be altered in these mice. To study this in more detail, we examined *sFRP* gene expression in *Pax6$^{Sey/Sey}$* mutant embryos. In wild-type (*WT*) mouse embryos, both *sFRP1* and *sFRP2* expression is confined to dividing ventral ventricular zone (VZ) and we determined that *sFRP1* is expressed in the p3, p2, p1 and p0 domains but only weakly if at all in the pMN domain at E10.5 (Fig. 1A,E), while *sFRP2* expression is seen in all ventral progenitor domains except the p3 domain (Fig. 1C,E). In *Pax6$^{Sey/Sey}$* mutant embryos, the expression of both *sFRP1* and *sFRP2* was altered in the thoracic spinal cord. The ventral domain of *sFRP1* was absent; while the more dorsal domain was reduced in size and occupied an area within the expanded "p0" domain that generates Evx1+ cells *Pax6$^{Sey/Sey}$* mutants (Fig. 1B,F). In contrast *sFRP2* expression was down regulated in the VZ in *Pax6$^{Sey/Sey}$* embryos, although weak expression was retained in the FP, a tissue that does not normally express Pax6 (Fig. 1D,F). Thus, loss of Pax6 results ir down regulation of both *sFRP1* and *sFRP2* in ventral progenitors.

2. A dorsal interneuron marker is up regulated ventrally in *Pax6$^{Sey/Sey}$* embryonic spinal cords.

Since sFRP proteins are negative regulators of the Wnt pathway, the loss of *sFRP1* and *sFRP2* expression from the ventral spinal cord of *Pax6* mutants might be expected to result in aberrant activation of Wnt signaling in this region. As Wnt signaling is required for the proliferation and specification of some dorsal cell types, we analyzed dorsal interneuron development in *Pax6* mutants using cell-type specific markers. Consistent with this possibility, we found that Lbx1, a homeodomain (HD)- containing transcription factor normally restricted to post-mitotic dI4-6 dorsal interneurons (Gross et al., 2002; Muller et al., 2002), was up regulated in a specific and reproducible location in the ventral spinal cord of mutant embryos (Fig. 2A). Ectopic Lbx1+ cells were distinct from ventrally migrating Lbx1+ cells generated in the dorsal SC in wild-type embryos at these stages since and many co-expressed Lhx3 or Mash1 that specifically mark cells in the ventral V2 progenitor domain (Figs 2Aii 3C). Ectopic Lbx1+ cells were intermingled with, but distinct from Chx10+ and Gata3+ cells that normally differentiate in the V2 domain (Fig. 2iii, iv). Very few if any ectopic Lbx1+ cells were found in the adjacent motoneuron (MN) or V0/V1 domains (Fig.2Av-vii). Furthermore, ectopic expression of Lmx1b (dI5), or Tlx1/3 (dI3 & dI5) which are normally co-expressed with Lbx1 in the dorsal spinal cord, was not seen in *Pax6$^{Sey/Sey}$* embryos, but some ectopic Lbx1 cells co-expressed Lim1/2, a LIM-HD containing factor found in both dorsal (dI2, dI4, dI6) and ventral (V0, V1 and V2) populations (Matise and Joyner, 1997). Thus, ectopic Lbx1 cells in the V2 domain in *Pax6$^{Sey/Sey}$* embryos bear some of the molecular characteristics of dI4 or dI6 interneurons.

3. A role for Mash1 in generating ectopic Lbx1 cells in the V2 domain of *Pax6$^{Sey/Sey}$* embryos.

One striking observation is that Mash1 is common to two of the three dorsal progenitor domains from which Lbx1 cells normally arise and to the V2 domain where ectopic Lbx1 cells appear in *Pax6$^{Sey/Sey}$*

mutants (Fig. 3B-D). It has been shown that Mash1 is required for the proper generation of V2 interneuron populations (Parras et al., 2002; Li et al., 2005); however, no ectopic Lbx1+ cells are seen in the ventral V2 domain in Mash1$^{-/-}$ mutants (Fig. 3G). To determine the potential role of Mash1 in the generation of ectopic Lbx1 in the V2 domain of $Pax6^{Sey/Sey}$ embryos, we generated $Pax6^{Sey/Sey}$; $Mash1^{-/-}$ double mutants. In these double mutant mice at both E10.5 and 11.5, ~60% fewer Lbx1+ cells were detected in the V2 domain, compared to single $Pax6^{Sey/Sey}$ mutants (Fig. 3E-H, O). These results suggest that expression of Mash1 is partly required for the generation of ectopic Lbx1 cells in the V2 domain in $Pax6^{Sey/Sey}$ embryos.

Next we examined the effect of ectopic Lbx1 expression in the V2 domain of $Pax6^{Sey/Sey}$ embryos on Chx10 expressing V2a and Gata2/3 expressing V2b neurons that are normally generated in this domain in WT embryos. At E10.5, both V2a and V2b interneurons were reduced in the $Pax6^{Sey/Sey}$ embryos; however, by E11.5 the V2a (Chx10+) population in the mutants recovered in $Pax6^{Sey/Sey}$ embryos to a level comparable to WT, while the reduction in V2b (Gata3+) interneurons persisted (Fig. 3M,N). We have recently shown that in Mash$^{-/-}$ mutants there is a selective reduction of V2b interneurons (Li et al., 2005). Therefore, Mash1 is required for the generation of V2b as well as most of the ectopic Lbx1+ cells in the V2 domain in $Pax6^{Sey/Sey}$ embryos.

4. Gsh1 and Mash1 synergize to induce Lbx1 interneuron cell fates.

It has recently been shown that overexpression of Mash1 using in ovo electroporation (EP) in chick embryos can induce dI3 interneurons (Fig. 4C,D). However, we found that EP of Mash1 repressed Lbx1 expression in the dl4-6 domains (Fig. 3B). Another closely related gene, Gsh1, is expressed in dI4 and dI5 progenitors, but is not required on its own for dorsal cell patterning and specification in mice (Fig. 4A), so its role is less clear. To address this issue, we transfected these factors into progenitor cells in chick embryo spinal cords. Interestingly, Gsh1 induced ectopic Lbx1+ cells in the ventral spinal cord (Fig. 4E; Fig. S1) and repressed Brn3a+/Isl1+ dl3 fates dorsally (Fig. 4F). However, co-EP of Gsh1 and Mash1 together induced even more widespread ectopic Lbx1 expression in ventral regions (Fig.4G; Fig. S1). In addition, ventral cell types were reduced in transfected embryos, indicating that Gsh1 and Mash1 had redirected their fates to a more dorsal (Lbx1+) phenotype. Taken together, these results are consistent with the idea that Gsh1 and Mash1 synergize to activate Lbx1 expression in the dorsal spinal cord, with Gsh1 functioning to modify Mash1 activity, and Mash1 potentiating the ability of Gsh1 to induce Lbx1+ cells (Fig. 4J).

5. sFRP2 blocks induction of Lbx1.

To examine the effect of sFRP2 on Lbx1 in the dorsal neural tube, we transfected sFRP2 into the developing chick neural SC. In embryos transfected with sFRP2, neuronal differentiation was broadly inhibited, resulting in a smaller neural tube on the transfected side (Fig. S2), consistent with the previously demonstrated role for Wnt signaling in providing mitogenic cues in the dorsal spinal cord (Megason and McMahon, 2002). In addition, most dorsal cell fates, including Lbx1 and Lmx1b, which mark dl4-6 and dI5 cells, respectively, were reduced or absent from the transfected side (Fig.6A, B). However, $sFRP2$ did not block Pax6 or Lhx3 expression in ventral progenitors (Fig. 6C,D), or the appearance of differentiated cell types that derive from ventral regions where $sFRP2$ is normally expressed, including MN (HB9+ or Isl1+), V1 (En1+), or V2 (Gata3+ or Chx10+) cells (Fig. 6E,F). These results suggest that dorsal neuron subtypes are disproportionately sensitive to the inhibition of Wnt signaling by sFRP2.

To further examine the role of sFRP's in generating ectopic Lbx1 interneurons, we made use of our transfection assay where Lbx1+ cells can be efficiently induced by co-transfection of $Gsh1$ and $Mash1$ (Fig. 4G). Transfection of a full-length mouse $sFRP2$ cDNA with $Gsh1$ and $Mash1$ completely blocked

the induction of ectopic Lbx1 cells (Fig. 5A,B; Fig. S1), while co-transfection of *sFRP1* reduced, but di not block, induction of Lbx1 (Fig. 5C; Fig. S1). Since sFRP's are secreted Wnt antagonists and can inhibit Lbx1 induction, this suggests requirement of Wnt signaling for generating Lbx1 by co-EP Gsh1 and Mash1. To test whether canonical Wnt signaling was required, we co-transfected three different factors which each selectively block canonical, but not non-canonical, Wnt signaling: an N-terminally deleted dominant-negative-Tcf4, dkk, or Axin (Peifer and Polakis, 2000; Megason and McMahon, 2002). Co-EP of each of these factors with Gsh1 and Mash1 failed to completely block the induction of Lbx1 like sFRP2 (Fig. 5D-F; Fig. S1). Because sFRP2 should be capable of blocking both canonical and non-canonical Wnt signaling extracellularly, these results raise the possibility that a non-canonical Wnt signal transduction pathway that is sensitive to inhibition by sFRP2 is involved in Lbx1 induction.

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II. Project challenges.

All the project goals have been largely addressed and have led to some very important conclusions, as addressed in the result section. One small challenge has been to equate the results observed in the two model systems employed: chick and the mouse.

III. Implications for future research.

Multiple molecular mechanisms control the development of the simple neural plate into neural tube that ultimately gives rise to diverse neural and glial cells. Bmp and Wnt signals emanating from the roof plate and overlaying ectoderm initiate patterning of the dorsal neural tube (Lee and Jessell, 1999), while ventral spinal cord patterning is controlled by Shh that is secreted from the notochord and floor plate (Ericson et al., 1996). Absence of notochord leading to loss of Shh signaling ventrally, leads to dorsalization of the whole neural tube (Yamada et al., 19991; Liem et al, 1995). The present study however suggests an additional mechanism, where dorsal fates are kept off in the ventral spinal cord (SC) due to presence of inhibitors of dorsal signals.

IV. Plans to continue this research.

The analysis of $Pax6^{Sey/Sey}$ mutants showed a loss of sFRP's in the ventral SC and led to the present hypothesis that sFRP's normally prevent ventral expansion of Lbx1 in the developing SC. However, multiple sFRP's are lost in the $Pax6^{Sey/Sey}$ mutants and it is difficult to infer whether all sFRP's contribute equally to this function, or one specific sFRP is enough. To address this question, we have started examining the individual sFRP1 & sFRP2 mutant mice. This has led to several very interesting observations about regulation of Wnt signalling that I plan to pursue further.

V. List of publications emerging from this research.

1. Foxn4 acts synergistically with Mash1 to specify subtype identity of V2 interneurons in the spinal cord. *Shengguo Li,*[1] **Kamana Misra**[1]*, Michael P. Matise*[2] *and Mengqing Xiang*[2]. **Proc Natl. Acad. Sci. U S A**. 2005 Jul 26; 102(30): 10688-93.
    [1] These authors contributed equally to this work, [2] Corresponding authors.

2. Pax6 controls ventral cell fates by regulating Wnt signaling in the developing spinal cord. **Kamana Misra**, *Eddie Kuang, Shike Li, and Michael P. Matise*. **Manuscript in preperation**.

Abstracts of Posters presented:

1. **Kamana Misra,** Eddie Kuang, Shike Li, Michael P Matise (2005). Pax6 controls cell fates in the developing spinal cord by regulating Wnt signaling. **Society for Developmental Biology 64**[th] **Annual meeting.**

2. Kamana Misra, Eddie Kuang, Shike Li, Michael P Matise (2005). Pax6 regulates neuronal fates by controlling the expression of Wnt antagonists in the developing spinal cord. **Society for Neuroscience 35**[th] **annual meeting.**
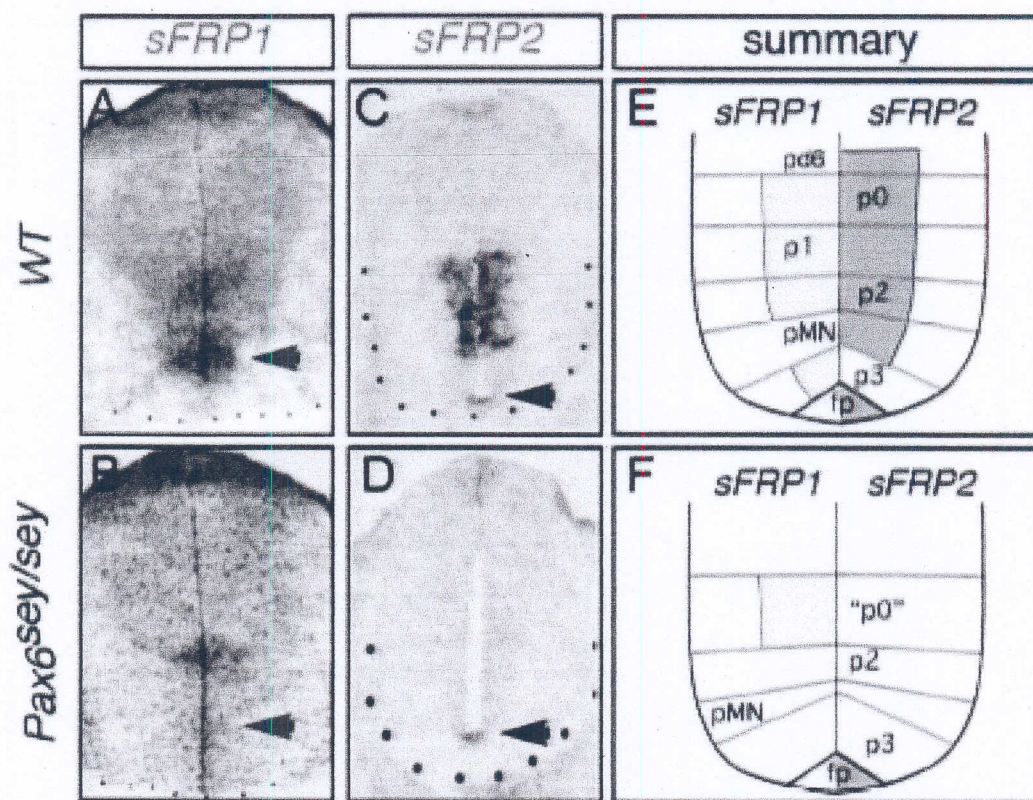
Figure 1. Down-regulation of *sFRP1* and *sFRP2* in the ventral spinal cord in *Pax6* [Sey/Sey] mutants.
(A,C) *sFRP1* and *sFRP2* are expressed in specific domains in the ventral VZ in the thoracic spinal cord
of *WT* embryos. *sFRP1* is expressed in the p3, p2, p1 and p0 domains, while *sFRP2* is expressed in the
FP, pMN, p2, p1, p0 and pdI6 progenitor domains (Q.L., M.P.M., unpublished). (B,D) In *Pax6* [Sey/Sey]
mutants, both *sFRP1* and *sFRP2* are down-regulated in the ventral spinal cord. *sFPR1* expression is lost
from the most ventral domain (red arrowheads), while *sFRP2* is down-regulated everywhere except in
the floorplate (arrowhead). (E,F) Summary of expression patterns of *sFRP1* and *sFRP2* in *WT* and *Pax6*
[Sey/Sey] mutant embryos. Transverse sections through the thoracic spinal cord at E11.5 shown in all
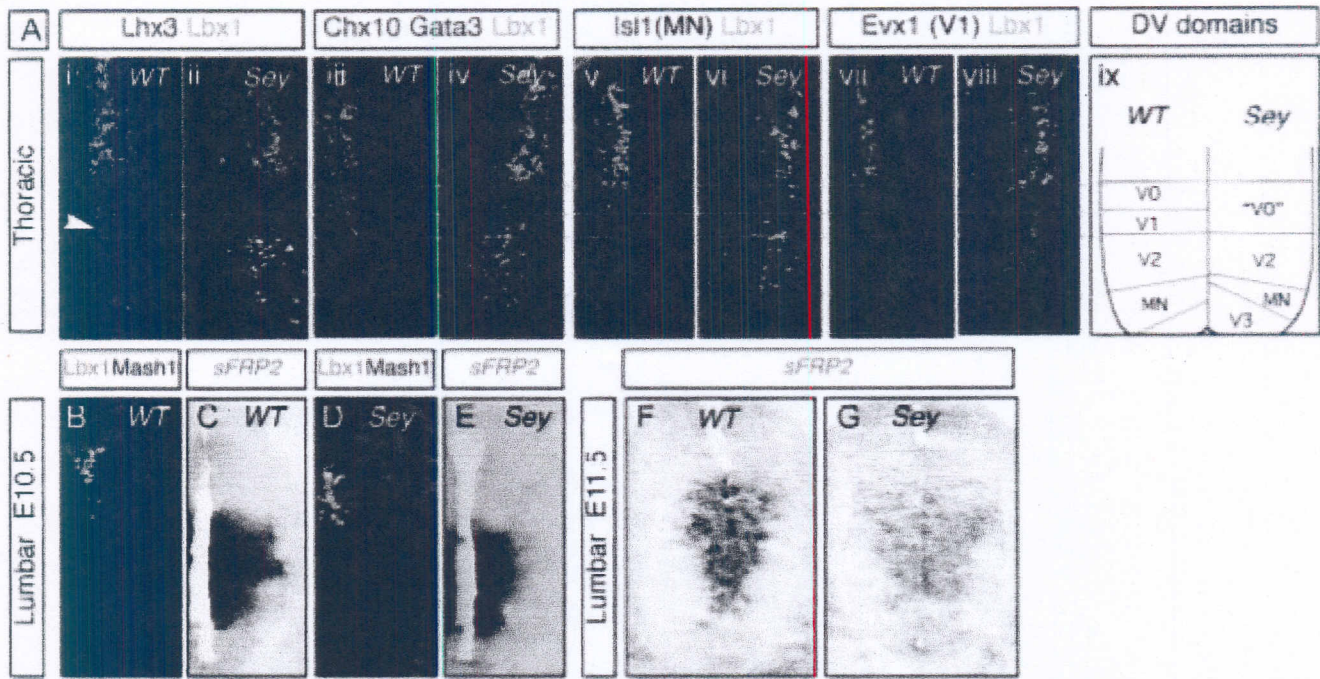figures.

Figure 2. Up-regulation of the dorsal interneuron marker Lbx1 in the ventral spinal cord of Pax6Sey/Sey mutants. (Ai-viii) The dorsal interneuron marker Lbx1 (dI4-6 cells) is up-regulated in the ventral V2 interneuron domain in $Pax6^{Sey/Sey}$ mutant embryos. Each pair of images shows left-right hemi-sections through *WT* (left panel) and $Pax6^{Sey/Sey}$ mutant (right panel) spinal cords, stained with the same markers (i, ii) Lbx1 expression arises within the dorsal aspect of the Lhx3 expression domain in *Pax6Sey/Sey* mutants, which demarcates MN and V2 progenitors. Arrowhead in (i) indicates ventrally migrating Lbx1 cells derived from the dorsal spinal cord. (iii, iv) Lbx1+ cells intermingle with, but are distinct from, Chx10+ and Gata3+ V2 interneurons in $Pax6^{Sey/Sey}$. (v-viii) Very few ectopic Lbx1 cells are found within the MN or V1/V0 generating domains in $Pax6^{Sey/Sey}$ embryos. Note that En1+ V1 interneurons are absent in these mutants at this stage and Evx1+ V0 interneurons extend ventrally into the V1 domain (Burrill et al., 1997; Ericson et al., 1997). (B-G) Appearance of ectopic Lbx1 cells in the ventral lumbar spinal cord correlates with loss of *sFRP2* expression in this region in $Pax6^{Sey/Sey}$ mutants. At E10.5, no ectopic Lbx1 cells are detected in lumbar regions of $Pax6^{Sey/Sey}$ mutants (D), and correspondingly *sFRP2* expression is still detected (E). Later, at E11.5 when ectopic Lbx1+ cells appear in this region, *sFRP2* expression is down-regulated (G). Transverse sections through the indicated regions of spinal cord at E10.5 (A-E) and E11.5 (F-G).
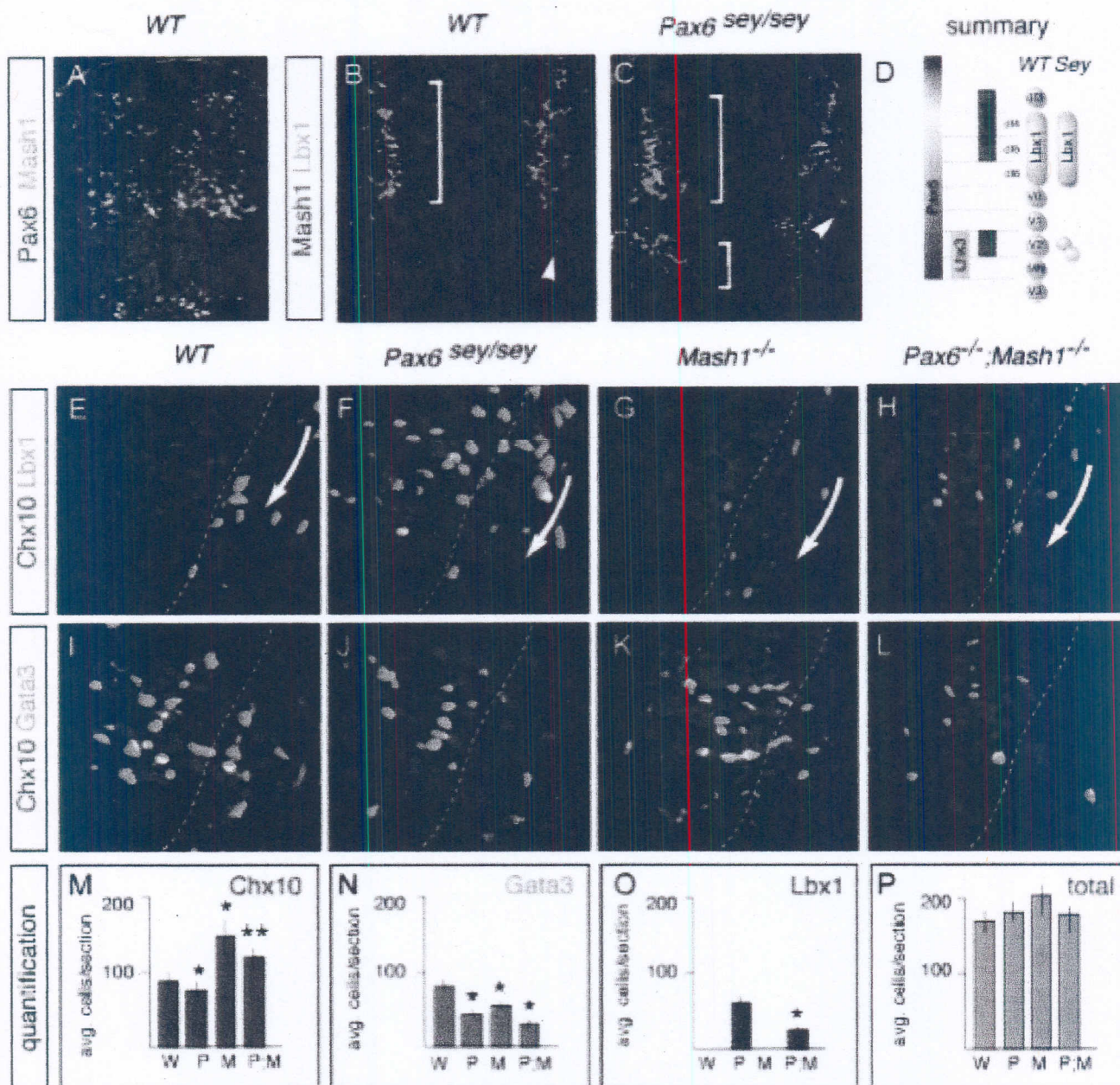
(A) Pax6 and Mash1 have overlapping expression patterns in the dI4, 5 as well as V2 progenitor domains in *WT* embryos. (B, C) Mash1 expression is common to Lbx1 progenitors in the dorsal spinal cord of WT embryos, as well as the V2 progenitor domain where ectopic Lbx1 appear in the **Pax6** $^{Sey/Sey}$ mutant embryos. Arrowhead in (B) indicates ventrally migrating Lbx1 cells derived from the dorsal spinal cord, large bracket shows normal Lbx1 domain in the dorsal spinal cord, smaller bracket in (C) indicates V2 domain. (D) Schematic summarizing Mash1 expression patterns and changes observed in Lbx1 expression in *Pax6Sey/Sey* mutants. (E-G) Thoracic hemi-sections of the spinal cord show no ectopic Lbx1 in the V2 domain of *WT* (E) and *Mash1-/-* mutant (G) embryos. The number of ectopic Lbx1 cells in the V2 domain is greatly reduced in **Pax6** $^{Sey/Sey}$; *Mash1-/-* double homozygotes (H) as

compared to *Pax6Sey/Sey* mutants (F,O). (I-P) Concomitant with the appearance of ectopic Lbx1 in the V2 domain, the number of Chx10+/Gata3+ V2 cells is reduced in **Pax6** $^{Sey/Sey}$ and **Pax6** $^{Sey/Sey}$; *Mash1* $^{-/-}$ double mutant embryos. (I-N) In *WT* embryos, similar numbers of Chx10 and Gata3 expressing interneurons are generated in the V2 domain, while in *Mash* $^{-/-}$ mutants (K, O) there is an increase in number of Chx10 cells at the expense of Gata3 cells (Li et al., 2005). (J,N) In **Pax6** $^{Sey/Sey}$ y mutants, both the Chx10 and Gata3 cells are reduced proportionately, while in **Pax6** $^{Sey/Sey}$; *Mash1* $^{-/-}$ double homozygotes the number of Chx10 cells falls between the numbers observed in *Mash* $^{-/-}$ embryos and **Pax6** $^{Sey/Sey}$ mutants (M,N). A compounding loss of Gata3 expressingV2b cells is observed in the double mutants. (M-P) Quantification of V2 interneurons in each genetic background, represented as the average number of stained cells per section ± s.e.m. (error bars) W=Wild-type, P= **Pax6** $^{Sey/Sey}$, M= *Mash1* $^{-/-}$, P;M=double mutants (P) Total number of cells within the V2 domain in each case (obtained by adding Chx10+/Gata3+/Lbx1+ cells). Transverse sections through the thoracic spinal cord at E10.5 (A-D) and E11.5 (E-P) are shown. Dashed line in E-L indicates approximate boundary between the VZ (to the left) and MZ (to the right). Arrows in E-H indicate stream of ventrally migrating Lbx1 cells derived from the dorsal spinal cord that migrate outside in the MZ. *=significant at p<0.001; **=significant at p<0.01,compared to *WT* (M-N) or *Pax6Sey/Sey* (O).
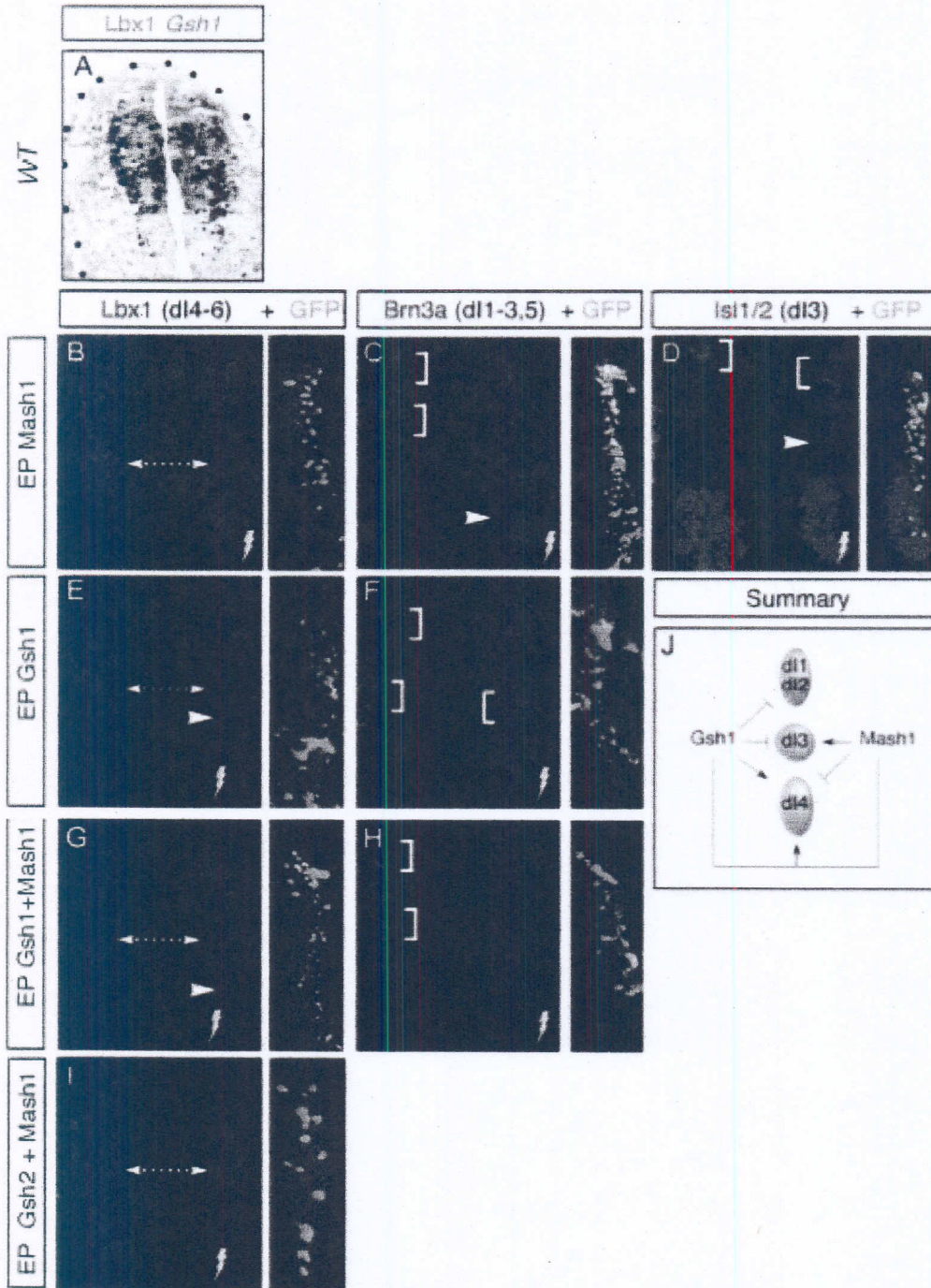
Figure 4. Efficient induction of ectopic Lbx1 by co-transfection of Gsh1 and Mash1.
(A) *Gsh1* mRNA (purple) is expressed in the dI4-6 progenitor region from where cells expressing Lbx1
HD protein (brown) originate. (B-D) Transfection of a mouse *Mash1* cDNA into chick embryos using in
ovo electroporation (EP). (B) Mash1 suppresses Lbx1 expression (blue asterisk). The double headed
dashed arrow indicates the ventral boundary of Lbx1 expression in the dorsal spinal cord. Panel at right
shows GFP expression to indicate extent of transfection. The right side in this and all subsequent figures
is the EP side. (C,D) Mash1 induces ectopic Brn3a (arrowhead in C), which is normally expressed in the

dI1-3 and dI5 domains, and Isl1/2 (arrowhead in D), which marks dI3 cells (brackets mark normal domains in each figure). (E,F) EP of *Gsh1* alone induces Lbx1 (arrowhead) in ventral cells, and represses dI1-3 interneuron fates (blue asterisk in F). (G,H) Co-EP of Mash1 with Gsh1 results in induction of ectopic Lbx1+ cells in the ventral spinal cord (arrowhead) at much higher efficiency than EP of Gsh1 alone (see also Fig. S1). In addition, Brn3a+ dI3/5 cell fates are repressed (blue asterisks). (I) Co-EP of Gsh2 with Mash1 suppresses Lbx1 expression, unlike EP of Gsh1 + Mash1 (compare I with G). (J) Summary of individual and combined effects of Gsh1 and Mash1 on dorsal neuronal fate induction. Transfected side is indicated by yellow bolt; transverse sections through thoracic spinal cord region shown in all figures.
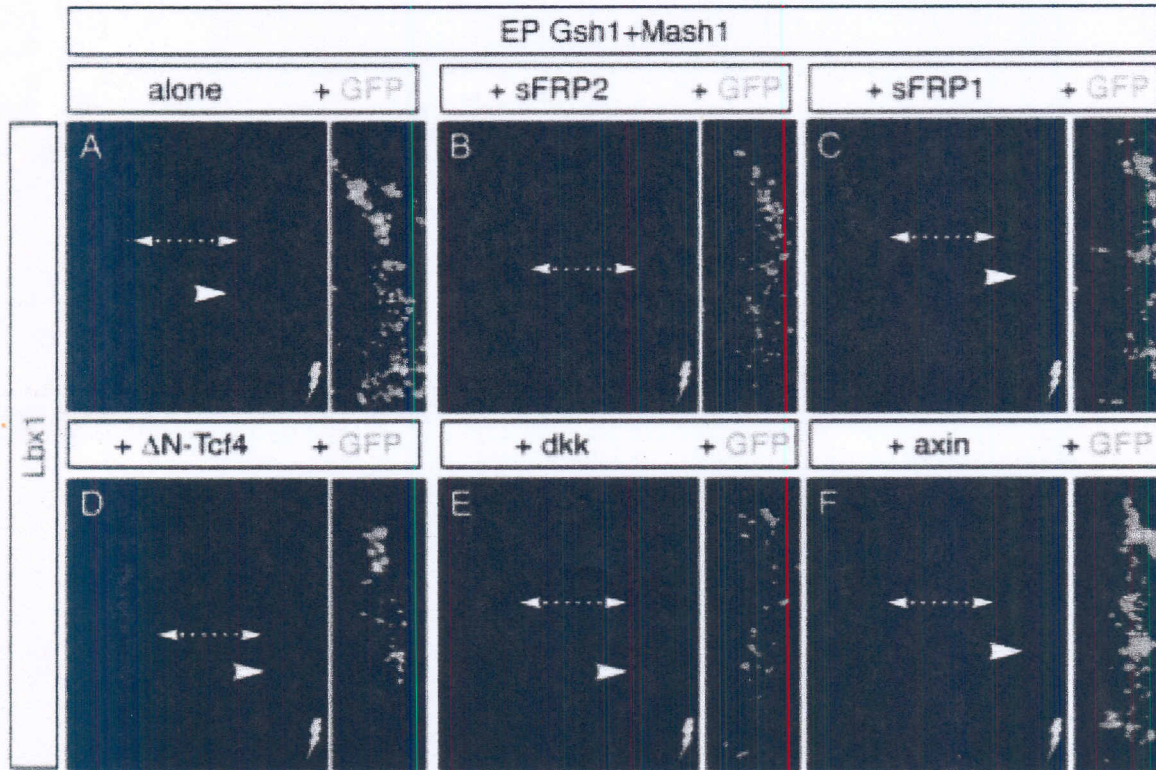
Figure 5. sFRP2 blocks the induction of Lbx1. (A,B) Overexpression of a full-length mouse *sFRP2* cDNA blocks the induction of ectopic Lbx1 induction in the ventral spinal cord by co-transfection of Gsh1+Mash1. (C) *sFRP1* does not antagonize the induction of ectopic Lbx1+ cells by Gsh1+Mash1 as efficiently as sFRP2. (D-F) Blockage of the canonical Wnt pathway signal transduction by co-electroporation of dominant-negative-*Tcf4* transcription factor (D), *dkk* (E) or Axin (F) fail to block Lbx1 induction by Gsh1+Mash.
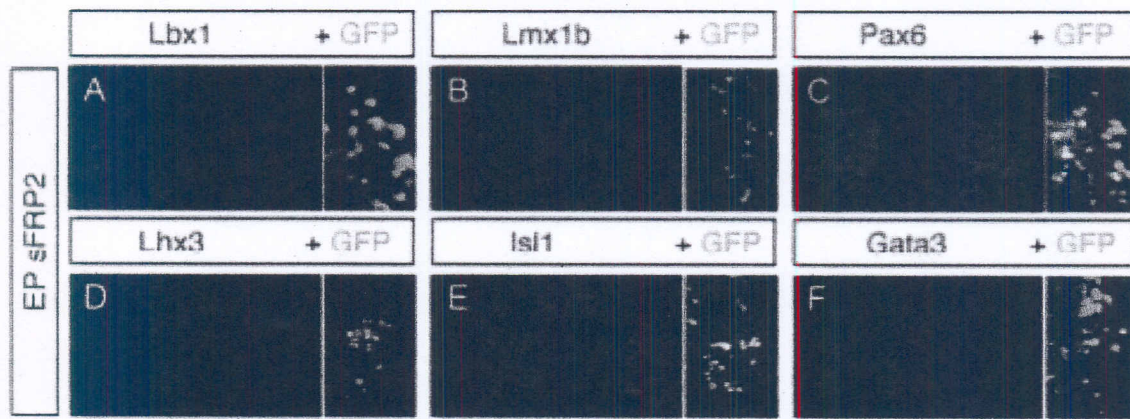

Figure 6. sFRP2 inhibits Lbx1 cell fate specification in the dorsal spinal cord. (A,B) Transfection of *sFRP2* into dorsal progenitors inhibits neurogenesis and blocks dorsal Lbx1+ and Lmx1b cell fates, but has no effect on Pax6 (C) or Lhx3 (D) expression in progenitors. (E, F) Ventral cell fates such as Isl1/2 and Gata3 are reduced but not blocked by EP of sFRP2 in ventral regions.
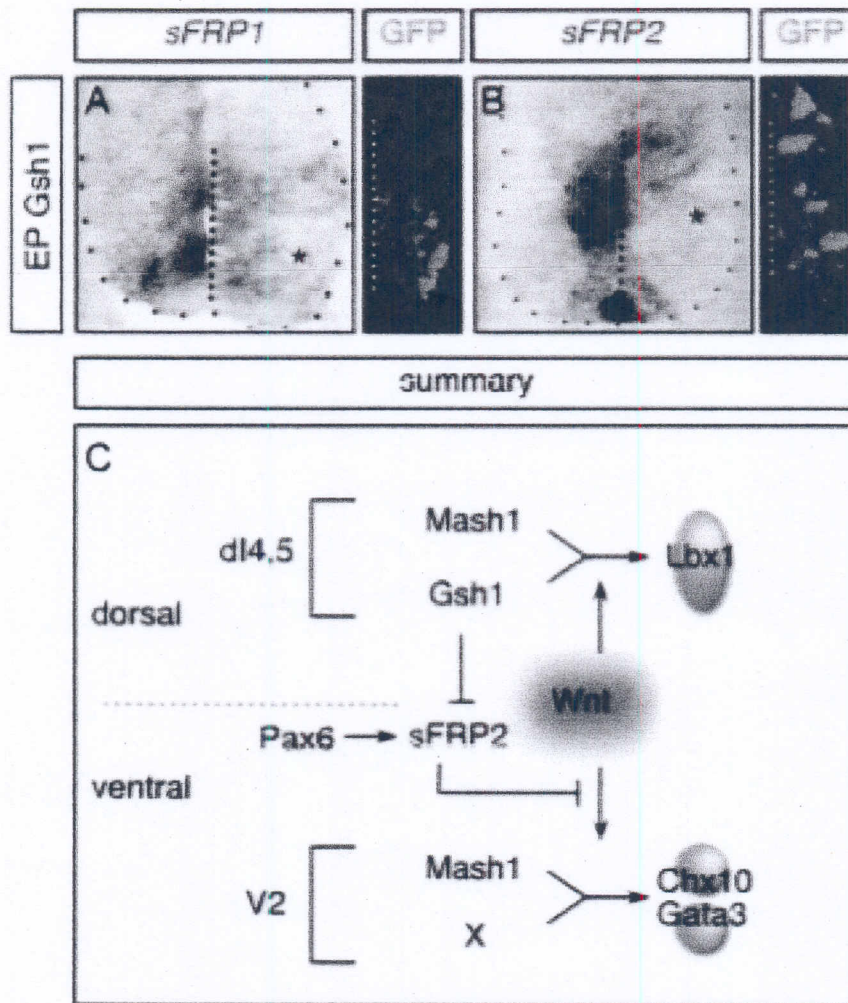
Figure 7. Transfection of *Gsh1* into ventral progenitors blocks *sFRP1* and *sFRP2* expression.
(A,B) EP of *Gsh1* represses both *sFRP1* and *sFRP2* expression in ventral progenitors. Panel at right shows GFP expression in near-adjacent sections to indicate extent of transfections. (C) Model for the regulation of Wnt signaling by Pax6 and sFRP2 in the spinal cord.