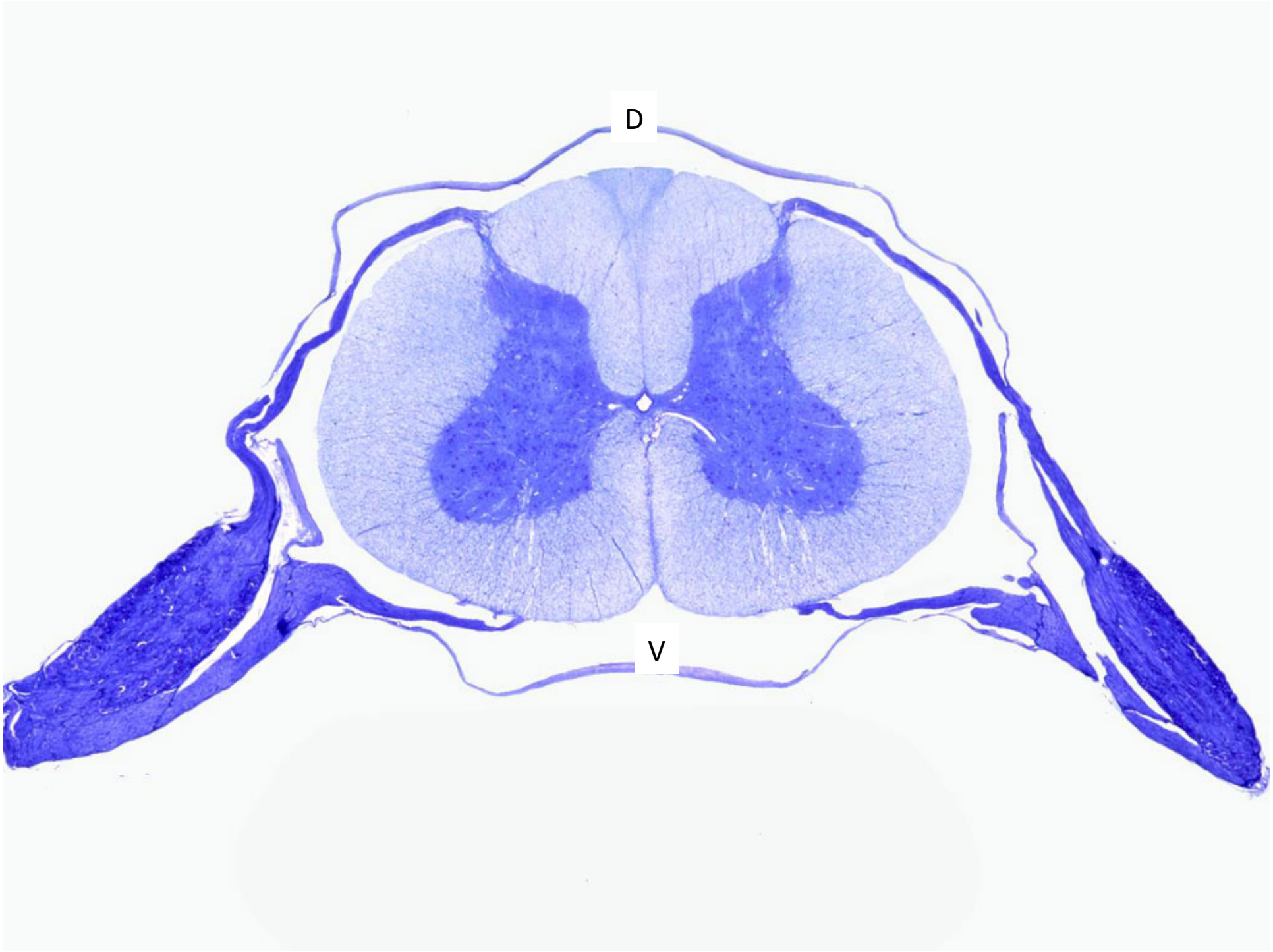
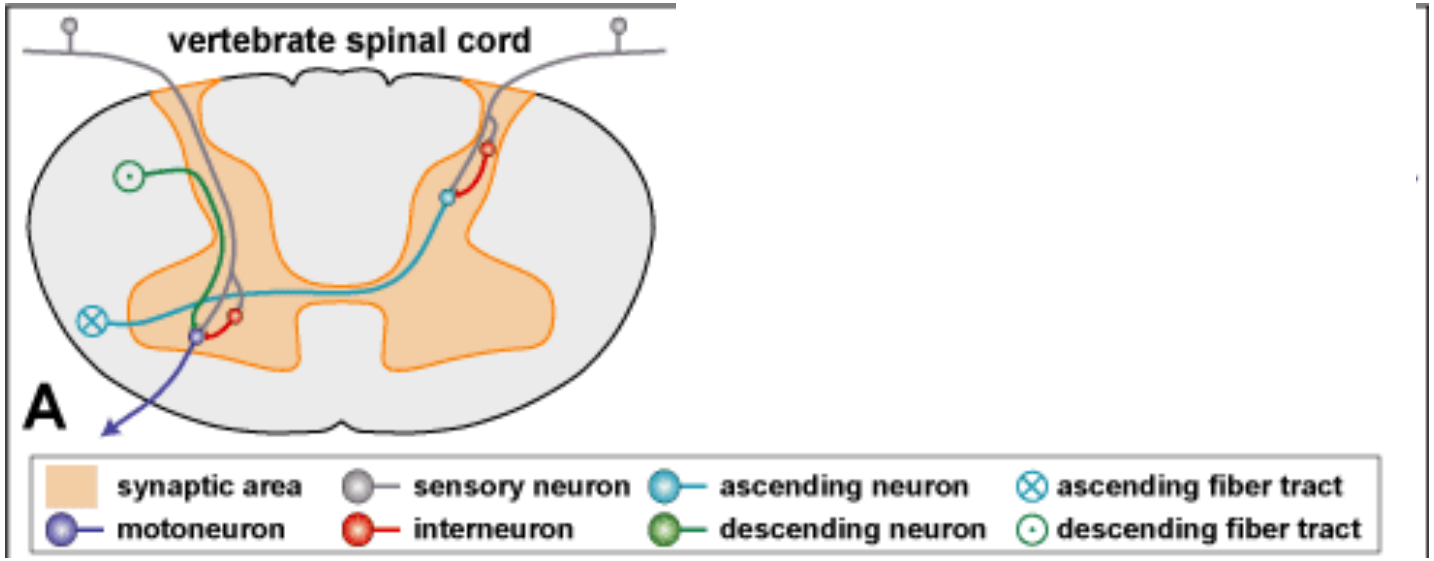
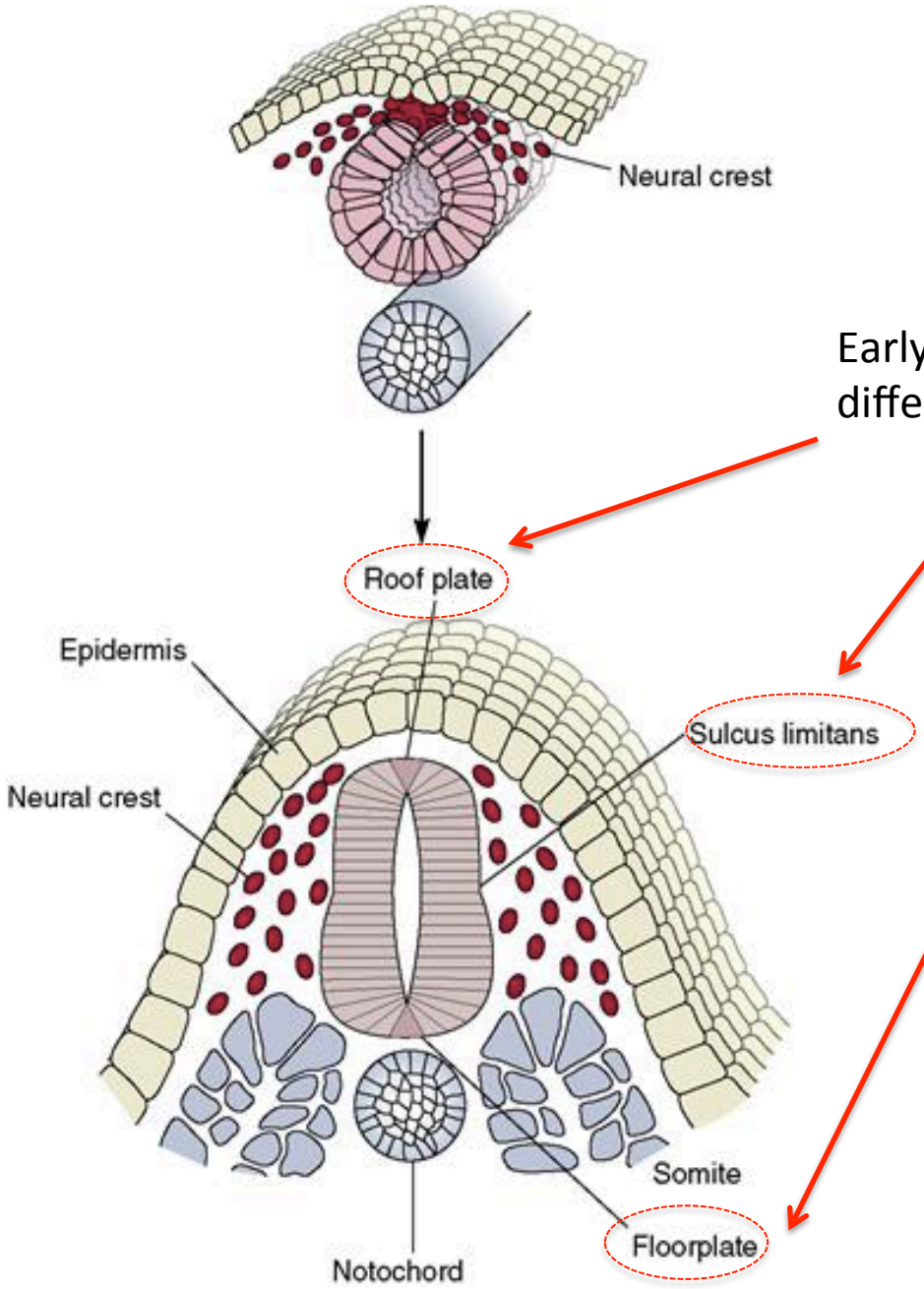

D
V



Different neuronal types are located at highly specific position

How does dorsal-ventral difference in the spinal cord organization emerge during development?

Dorsal –ventral polarity in the neural tube

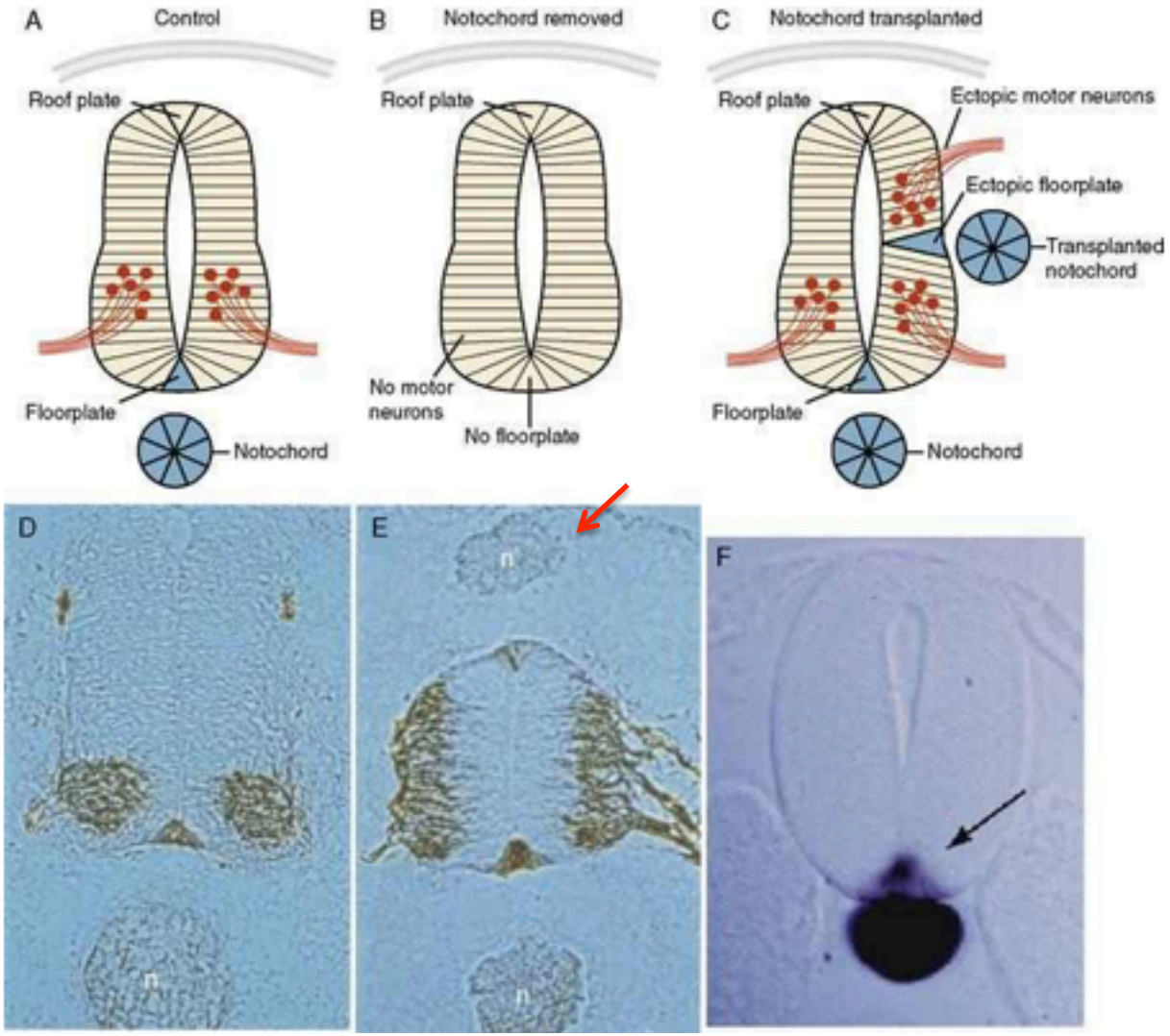


Early morphological sign that the neural tube is differentiating along the dorsal- ventral axis

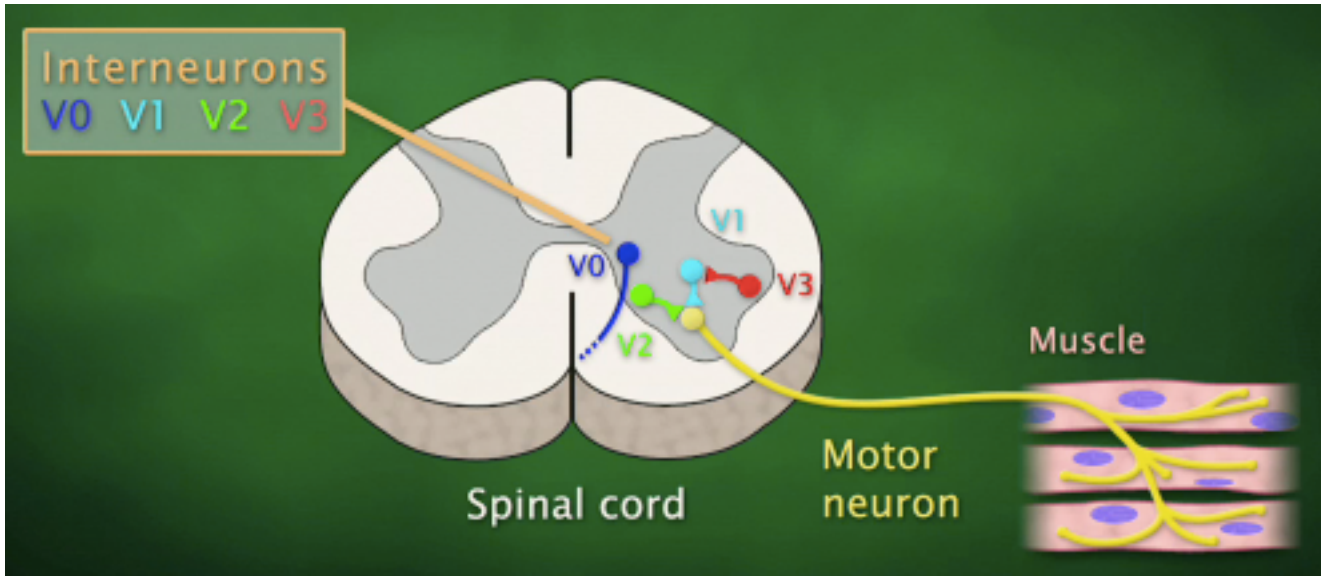
Dorsal-ventral polarity largely derives from interaction of the neural tube with the notochord

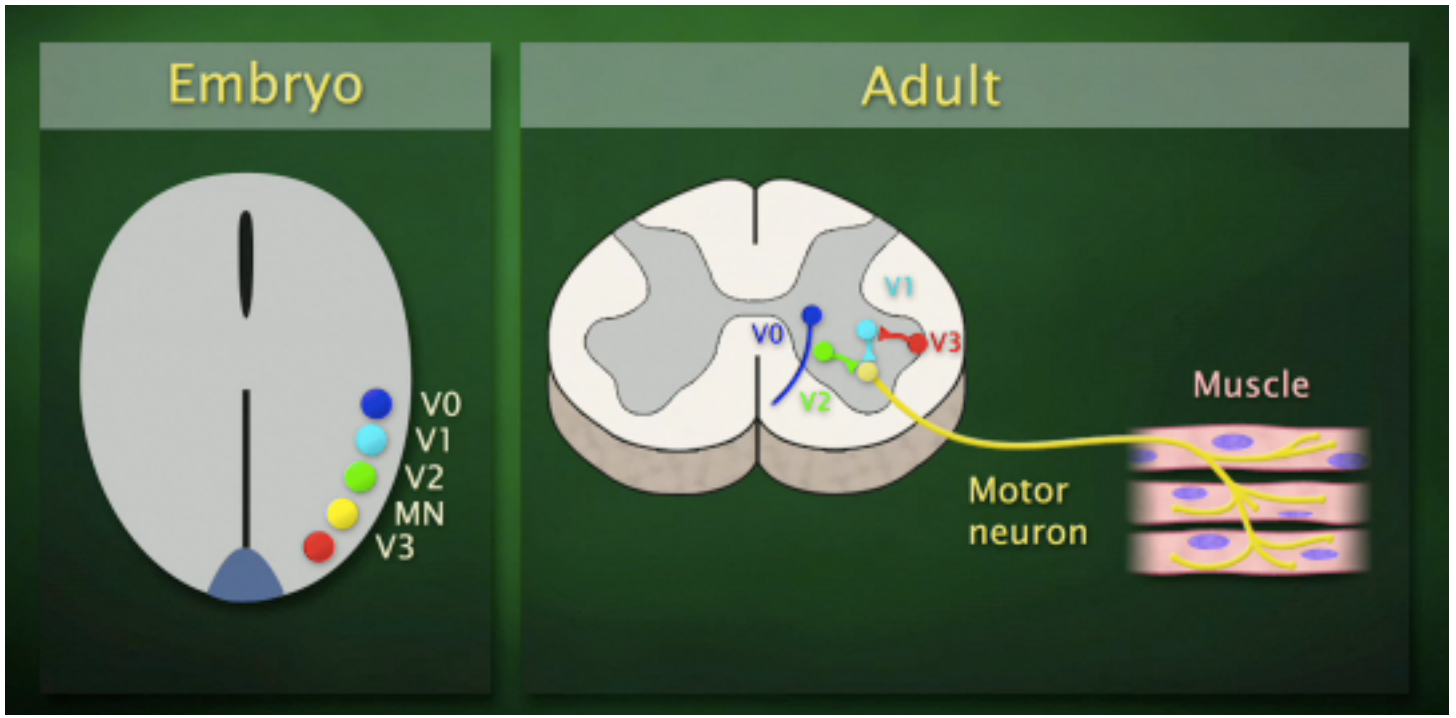
The notochord plays a prominent role for the development of the dorsal- ventral axis of the spinal cord

Holtfreter, 1934

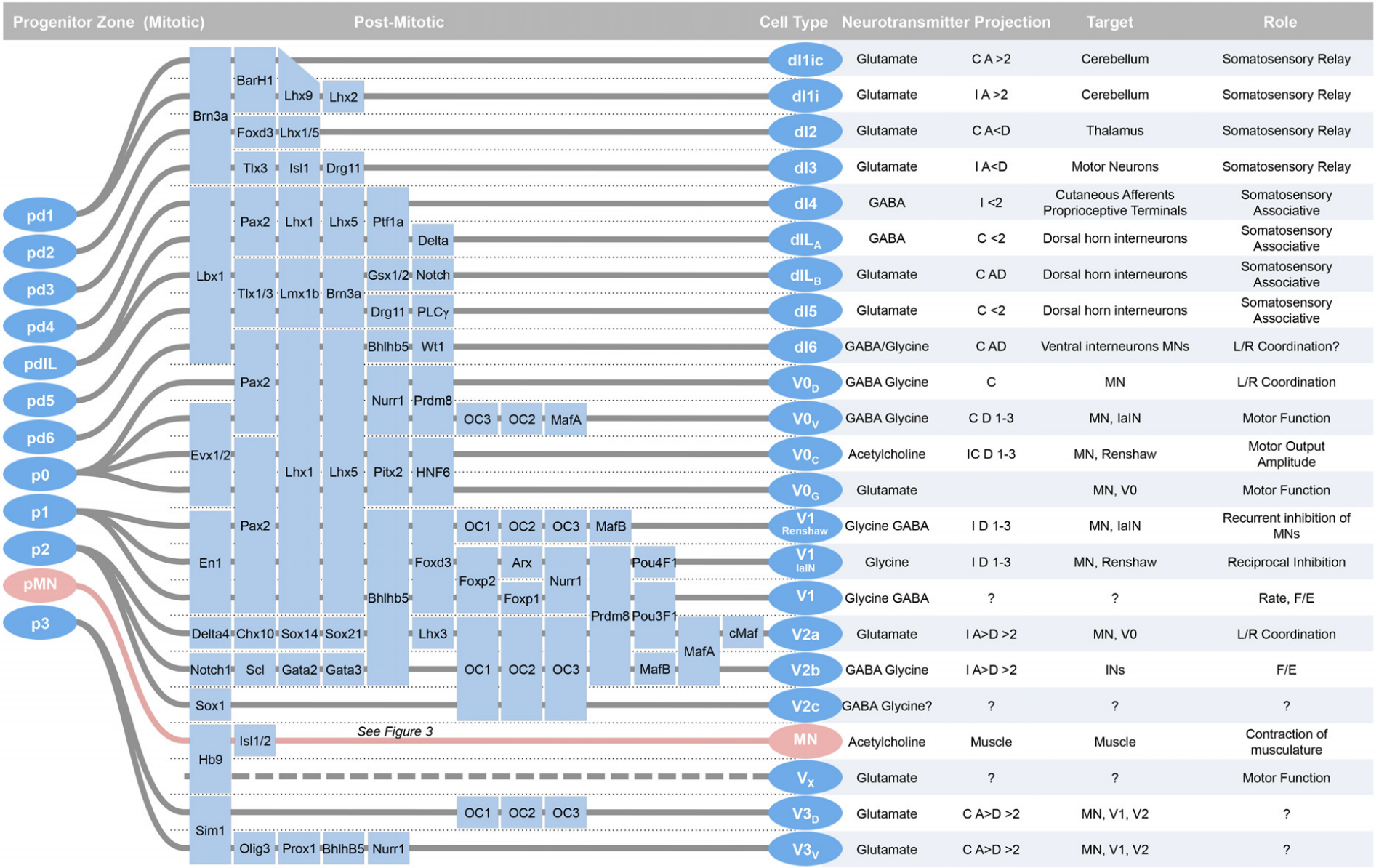


Placzek et al., 1991





Modified from T. Jessell



Over the last week of development, **23 classes of neurons can be defined by transcription factor expression.**

Lu et al., 2015

Projection Code

I: Ipsilateral
C: Contralateral
A: Ascending
D: Descending
1: Number of Segments

Dorsal–ventral patterning of the vertebrate developing spinal cord

11* distinct domains of neural progenitors with dorso-ventral regional identity

---

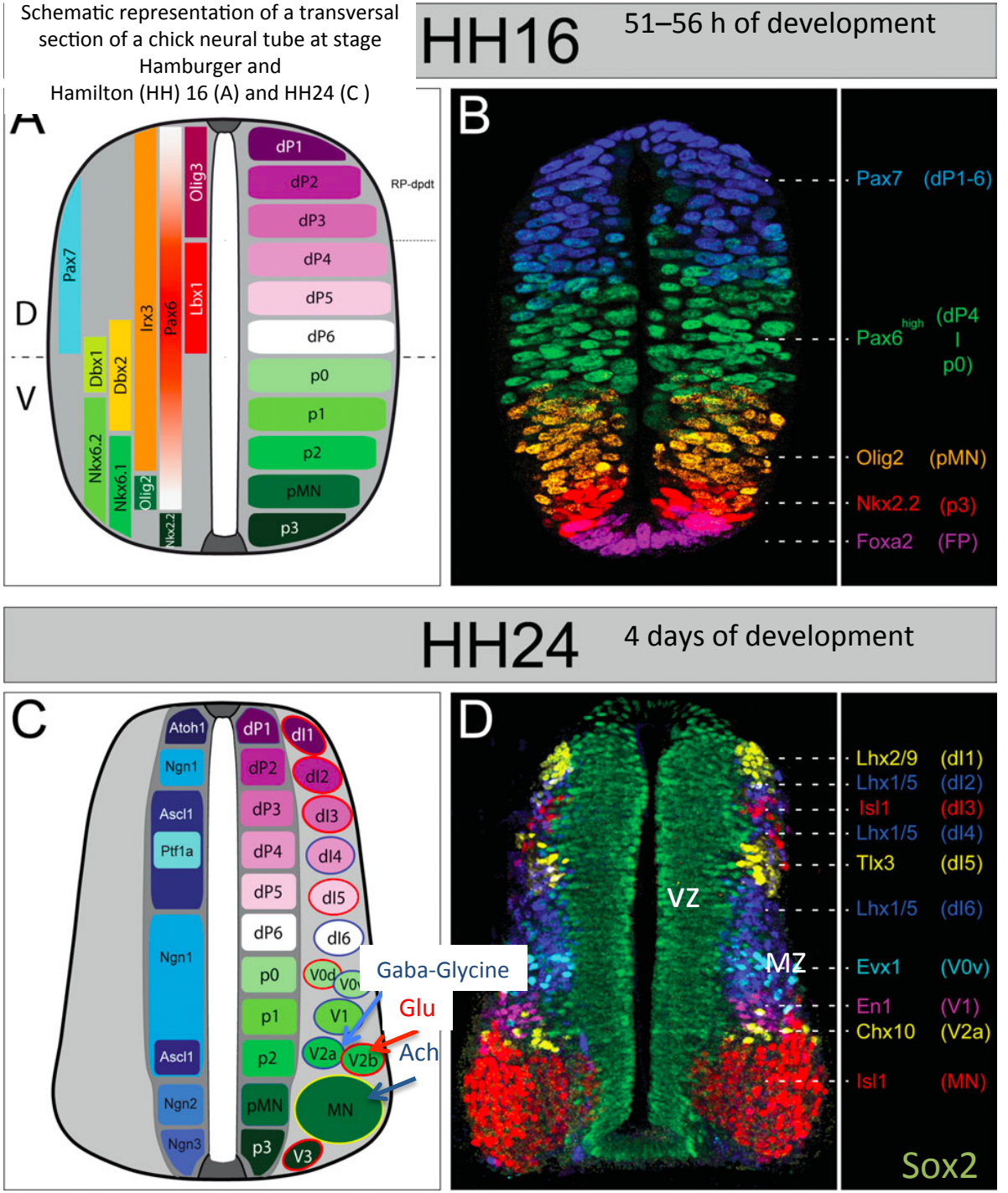
1) bHLH & homeodomain TFs define the progenitor domains

2) Additional TFs (mainly LIM-HD family e.g. Lhx1 and Isl1) are expressed in sub-groups in these domains refining cell fates into 23 different classes of neurons

*or 13 (considering also late born pdILA and pdILB)

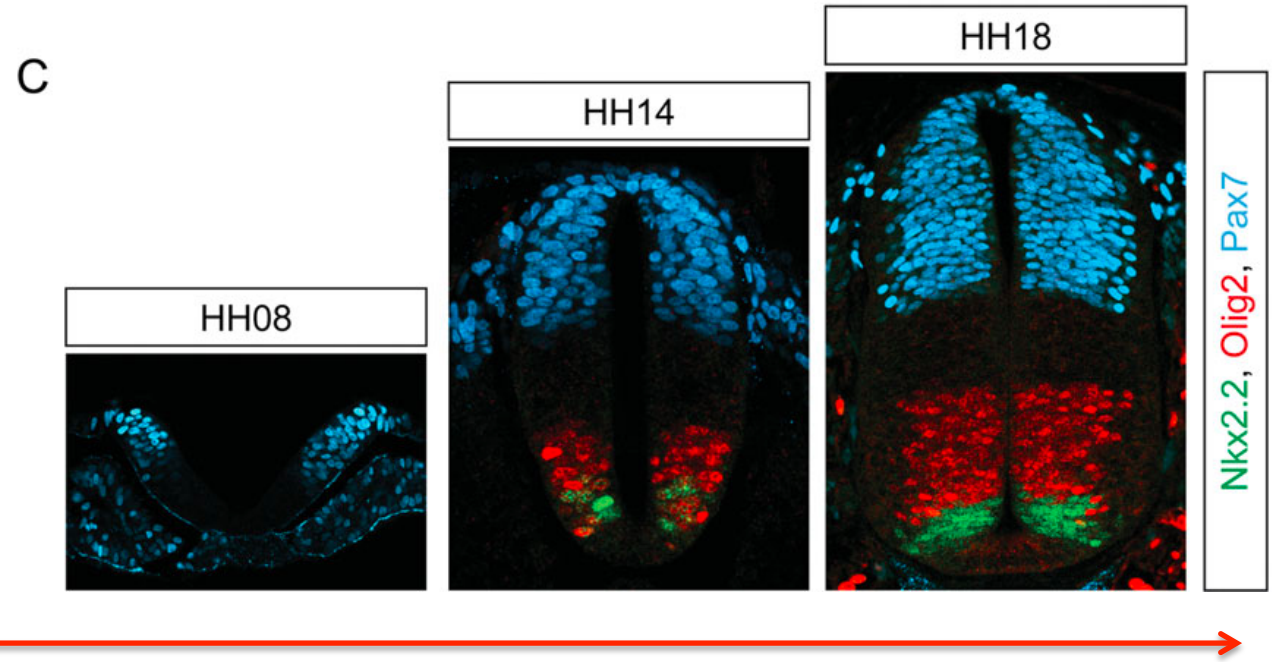
Le Dréau *Dev Neurobiol 2012*

Schematic representation of a transversal section of a chick neural tube at stage Hamburger and Hamilton (HH) 16 (A) and HH24 (C)



Photographic reconstruction immunostaining

3 critical genes that define the D-V pattern are: Pax7, Olig2 and Nkx2.2



Progressive emergence of ventral neural progenitor domains

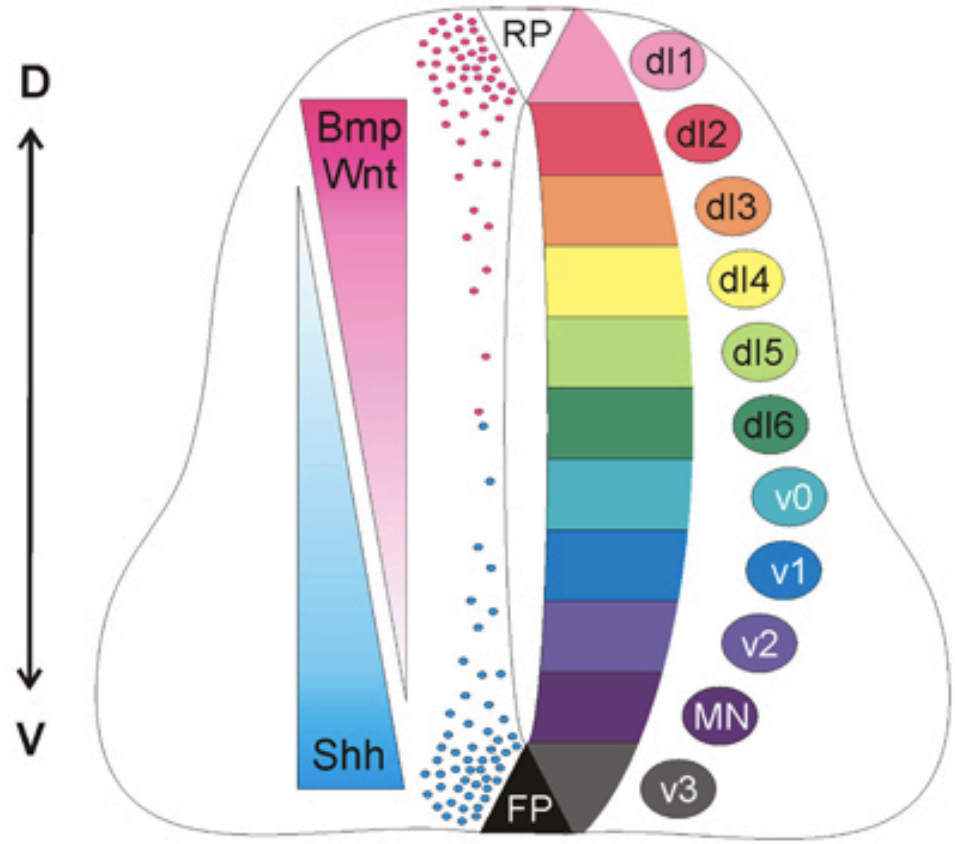
The main actors in spinal cord DV pattern formation

2 opposite signaling centers:

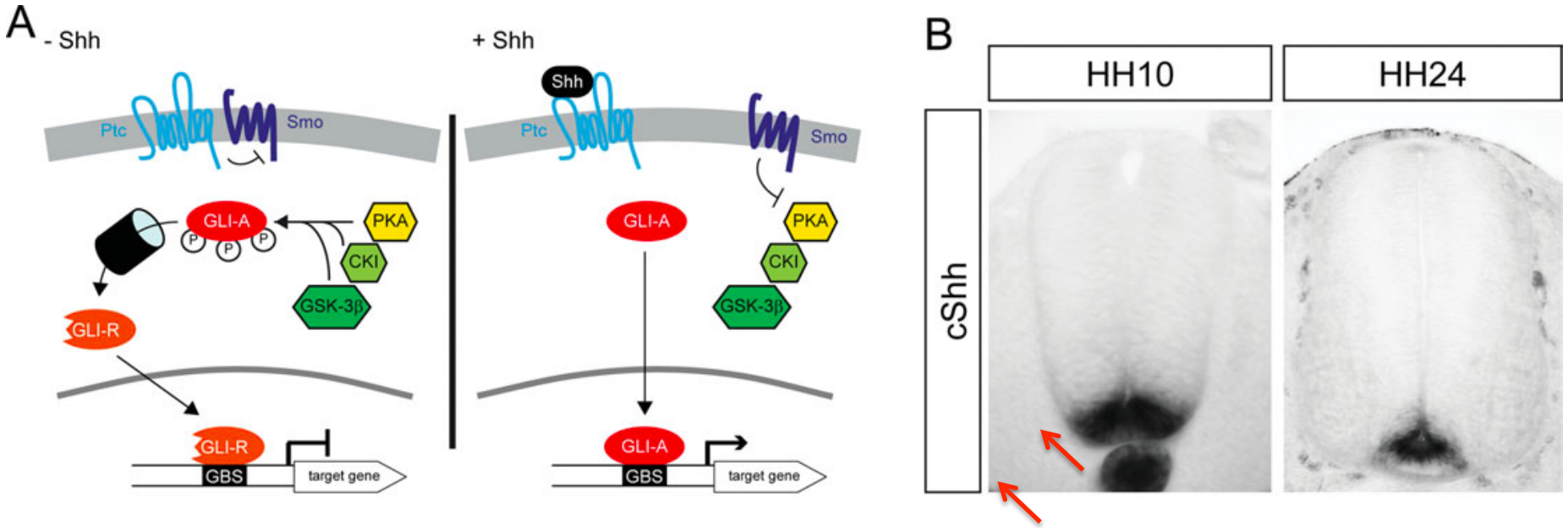
- the roof plate
- the ventral floor plate

Three main signals:

-Shh
-BMPs
-Wnt



Graded **shh signaling** controls the identity of ventral progenitors



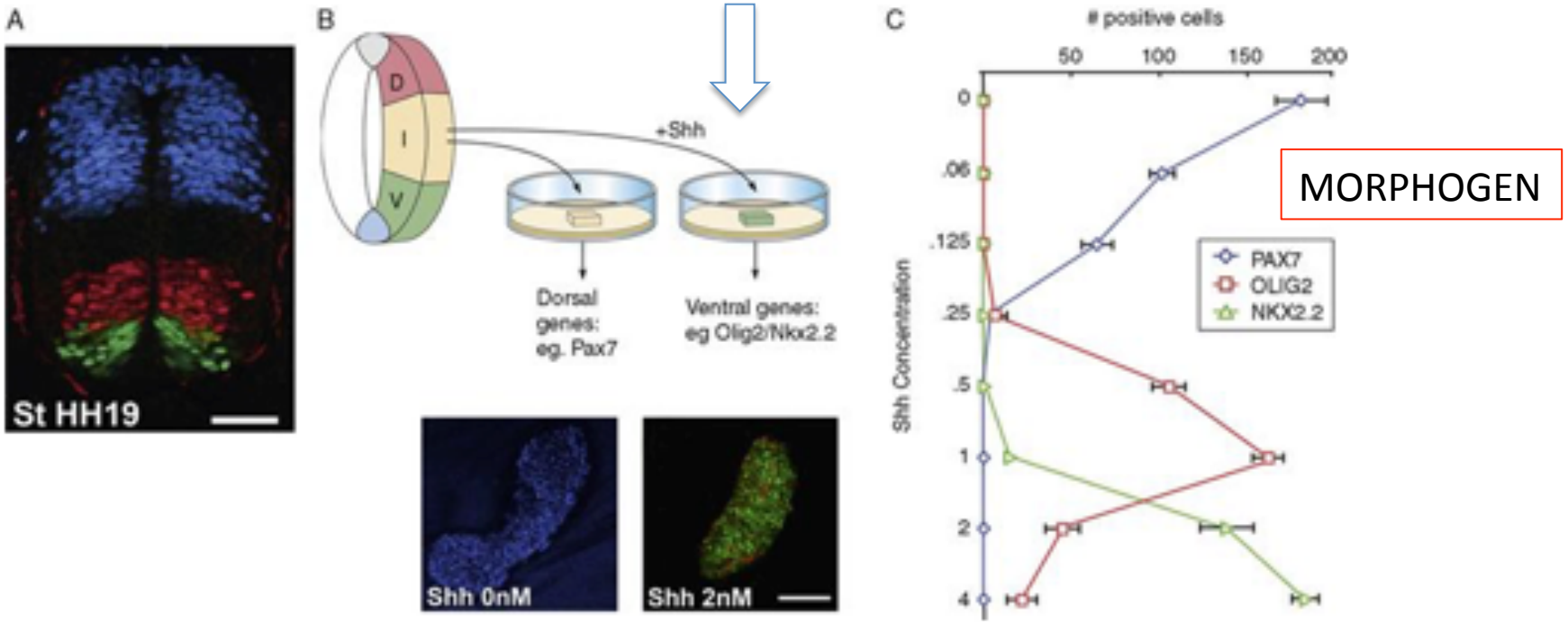
- Shh is expressed in the nothocord at the time when the V-D axis of the neural tube is being specified – soon after shh expression occurs also in the floorplate

- The nothocord and floor plate are the two main signaling centers responsible for ventralizing the neural tube.

Shh is sufficient to ventralize the neural tube during development

In vitro experiments:

-co-culture (neural tube + notochord)
-co-culture (neural tube + cells expressing shh)
- Neural tube + recombinant shh



MORPHOGEN

More ventral identities require higher levels and longer periods of Shh signaling

LOF

Anti-Shh antibodies block differentiation of the floorplate and motor neurons when added to neural tube explants

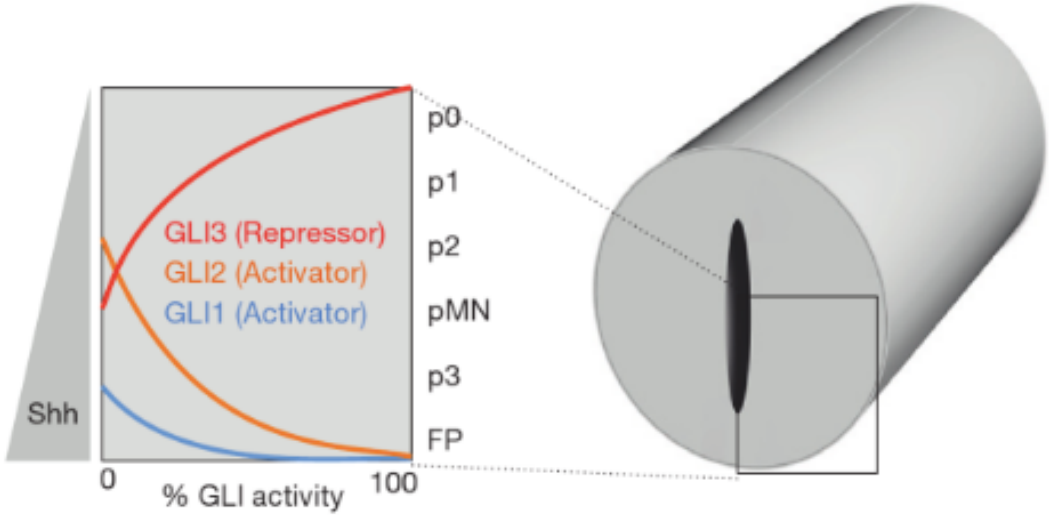
Targeted deletion of Shh in mice results in failure of the development of the ventral cell types in the spinal cord

Shh is released by Notochord and floor plate

GOF and LOF demonstrate Shh is neccessary and sufficient to induce ventral neural fate

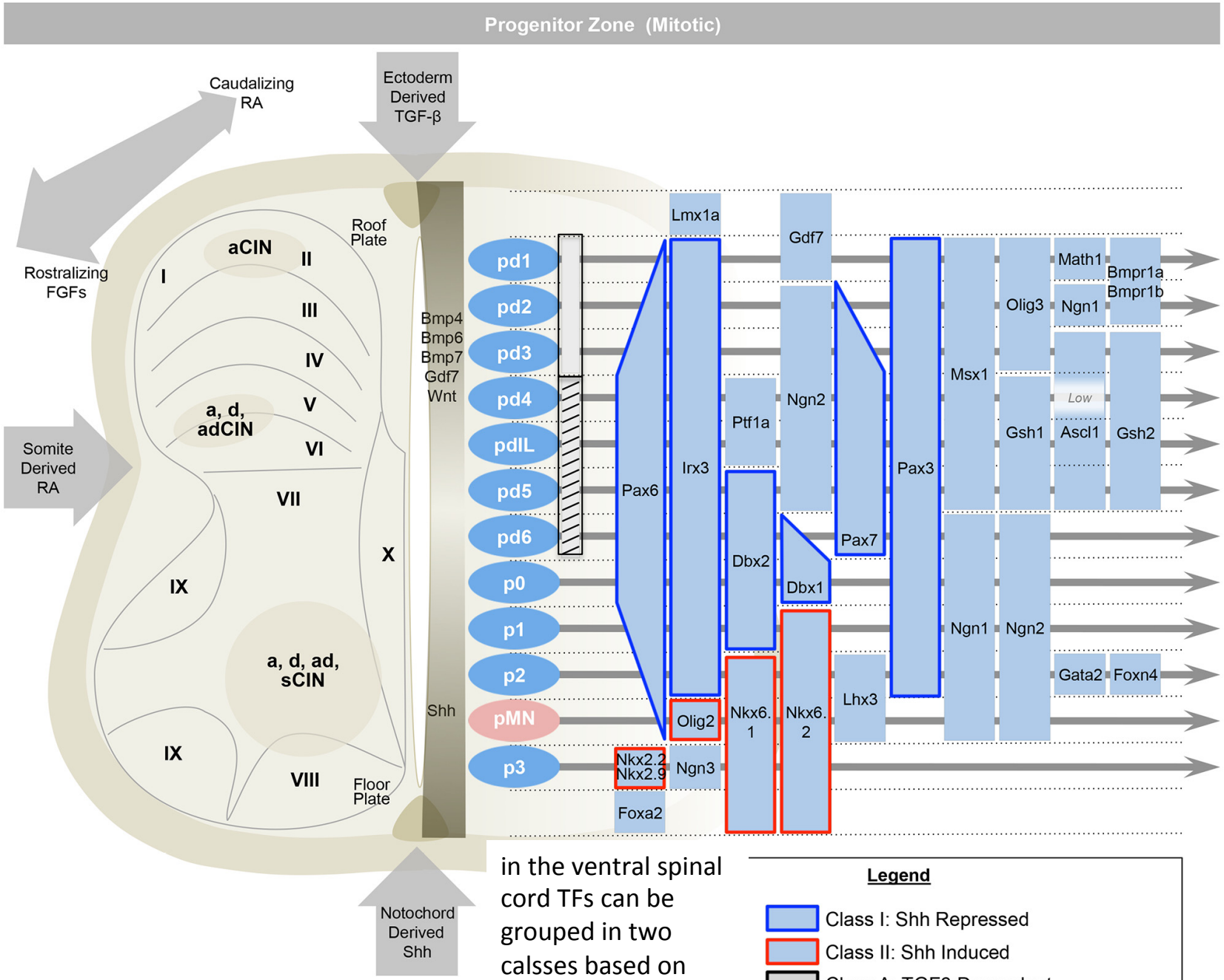
Shh function in a concentration-dependent way, as a gradient morphogen,
regulating the expression of patterning determinants in the ventral neural tube

Activation of the Shh pathway is transduced into **regulated levels and duration of Gli activity**



Progenitor cells adopt ventral identities

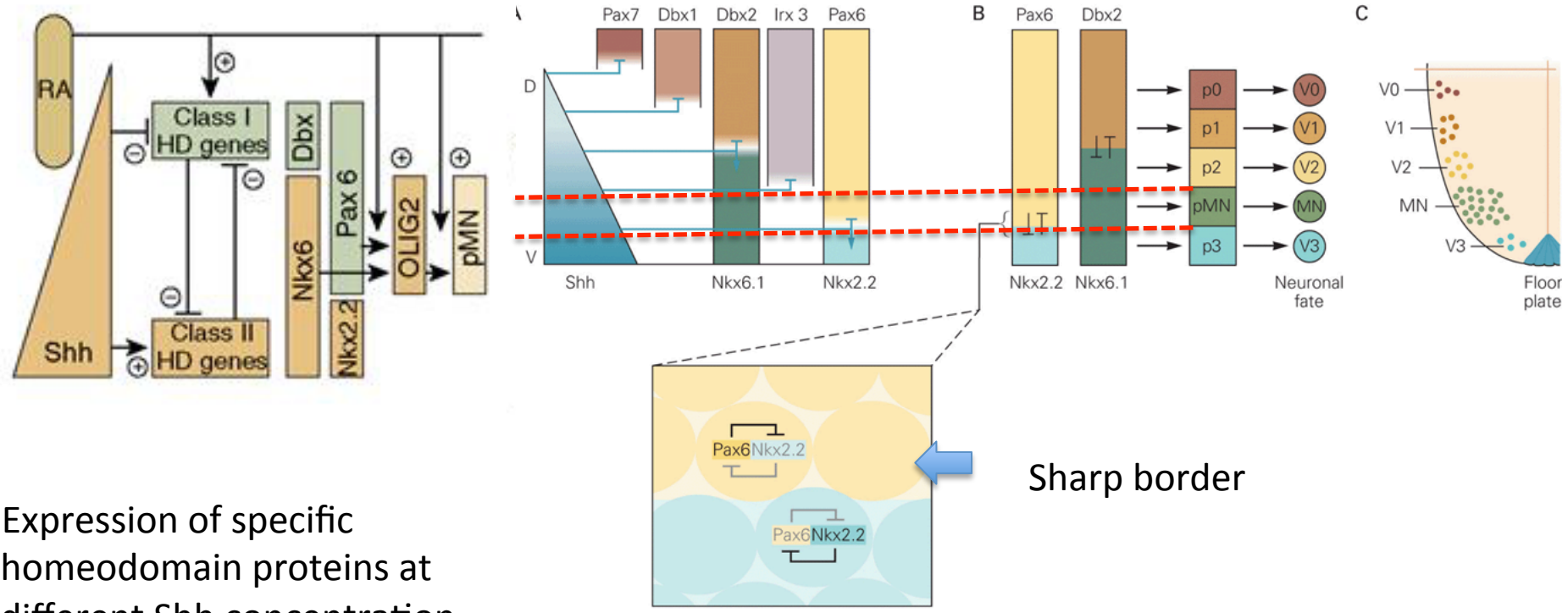
Subtype progenitor identities in the ventral spinal cord are established sequentially:
more ventral identities require higher levels and longer periods of Shh signaling



in the ventral spinal cord TFs can be grouped in two calsses based on response to Shh

Lu et al., 2015

How do cells at different D-V levels interpret their exposure to different levels of Shh?



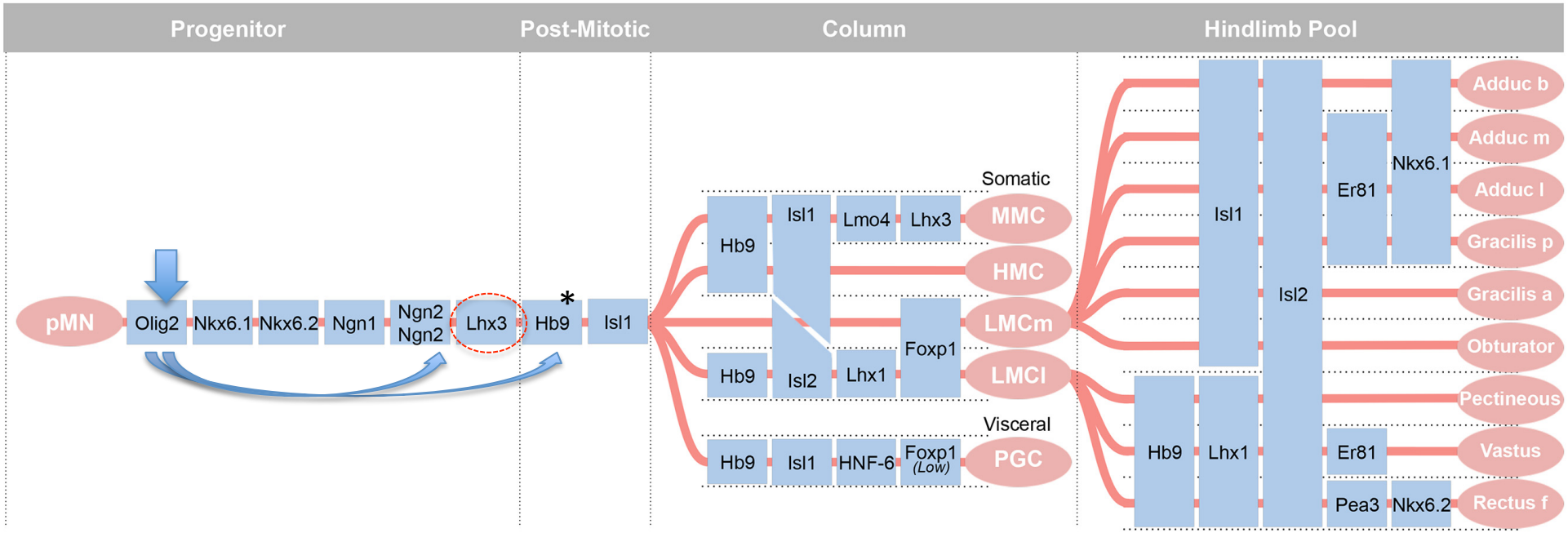
Sharp border

- Expression of specific homeodomain proteins at different Shh concentration
- Cross-repression between the two classes of genes sharpens the boundary

Figure 52-9 A sonic hedgehog signaling gradient controls neuronal identity and pattern in the ventral spinal cord.

A. A ventral-to-dorsal (**V–D**) gradient of sonic hedgehog (Shh) signaling establishes dorsoventral domains of homeodomain protein expression in progenitor cells within the ventral half of the neural tube. Graded Shh signaling generates a corresponding gradient of Gli transcription factor activity (not shown). At different concentrations the extracellular Shh and intracellular Gli gradients specify different neuronal classes. At each concentration a different homeodomain transcription factor (Pax7, Dbx1, Dbx2, Irx3, or Pax6) is repressed, with Pax7 the most sensitive and Pax6 the least sensitive to repression. Other homeodomain

Focus on motor neurons



Lu et al., 2015

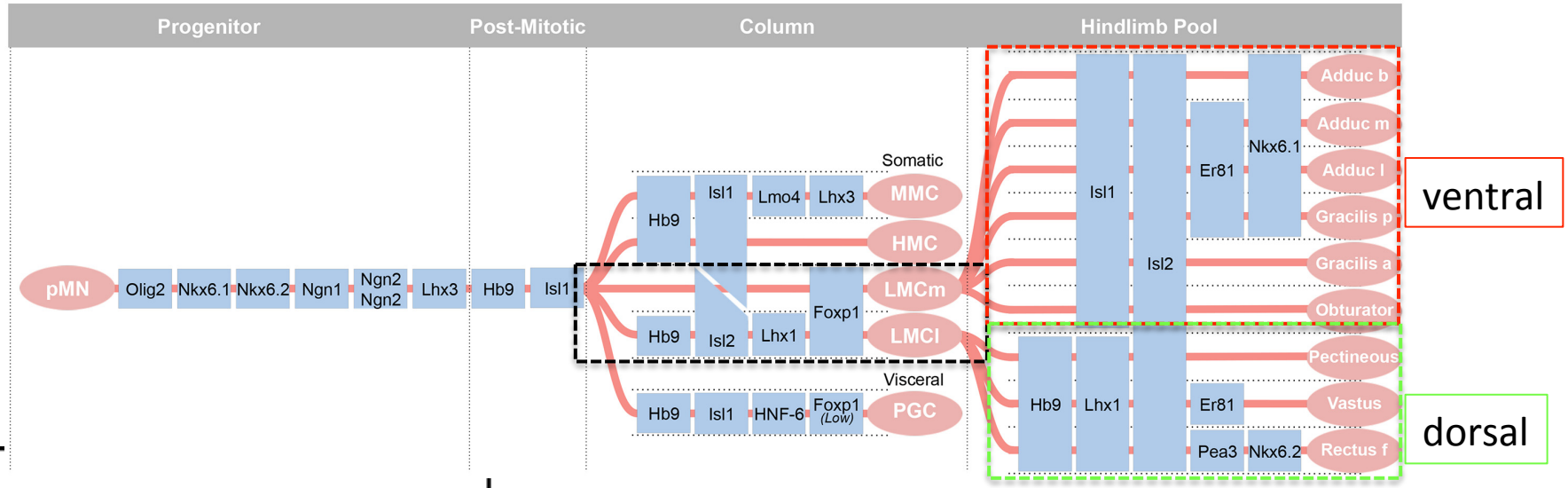
**Hb9 can induce motor neurons if ectopically expressed*

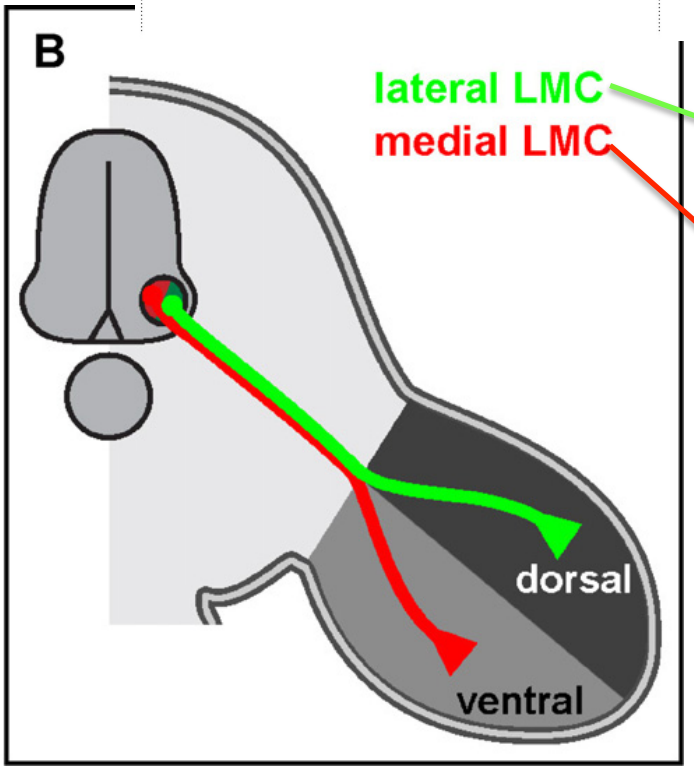
The pMN cell domain gives rise to the most diverse class of spinal cord neurons

Motor neurons are organized in motor column and motor pools→ **Genetic code**

MMC= medial motor column → innervates dorsal musculature
HMC= hypaxial motor column → innervates ventral musculature
LMC= lateral motor column → innervates muscle of the limb (LMCl-dorsal portion - LMCm ventral portion)
PGC= preganglionic motor neurons → autonomic nervous system

Focus on the lateral motor columns





Lim1 (Lhx1)

Isl1

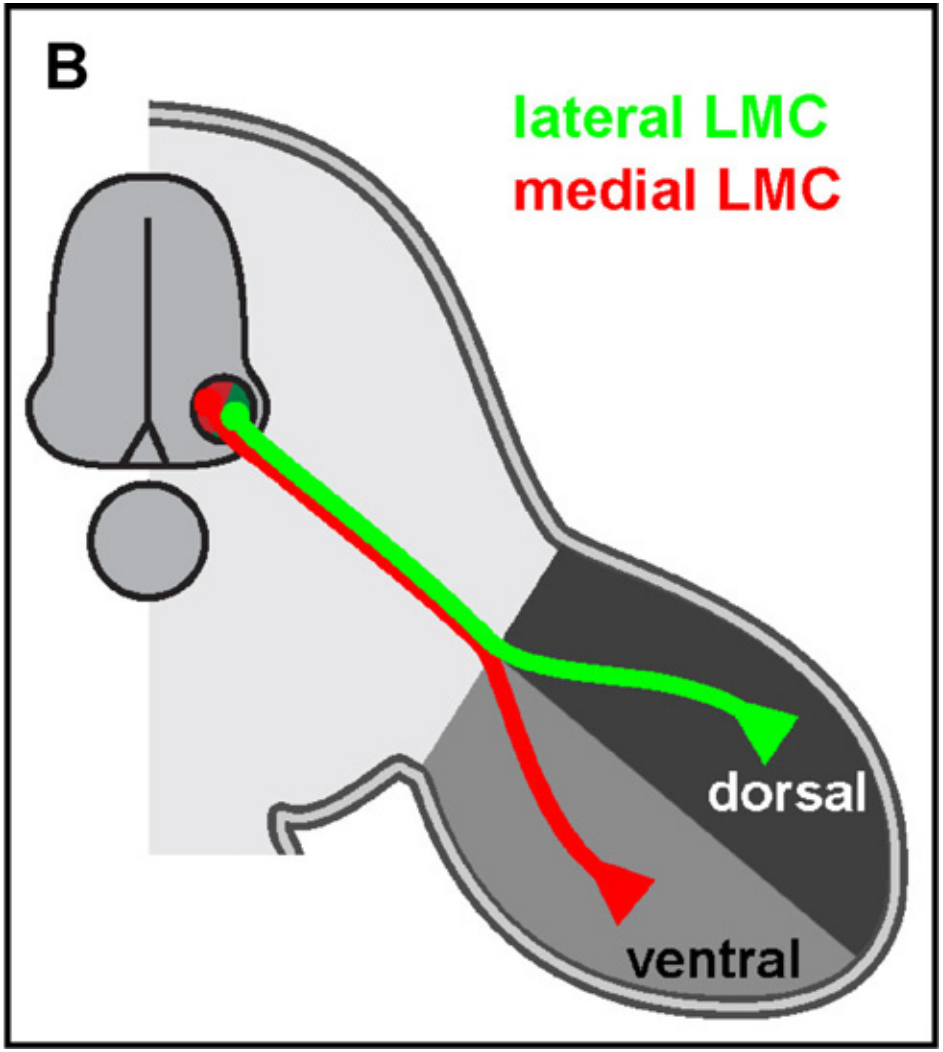
Kao et al., 2012

Formation of specific axon pathways by developing motor nerves in the vertebrate limb

At the cellular level, axons of the lateral and medial divisions of lateral motor column (LMC) reach the base of the limb and invariantly select a dorsal or a ventral limb trajectory

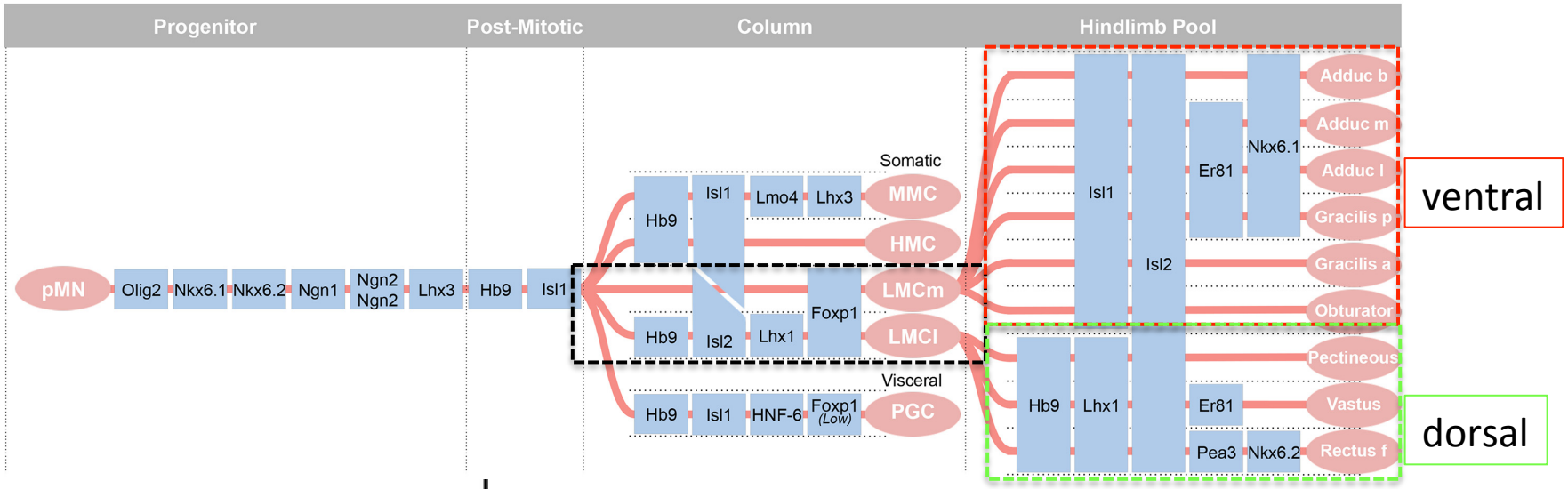
→trajectory selection is made at the level of the LMC growth cones

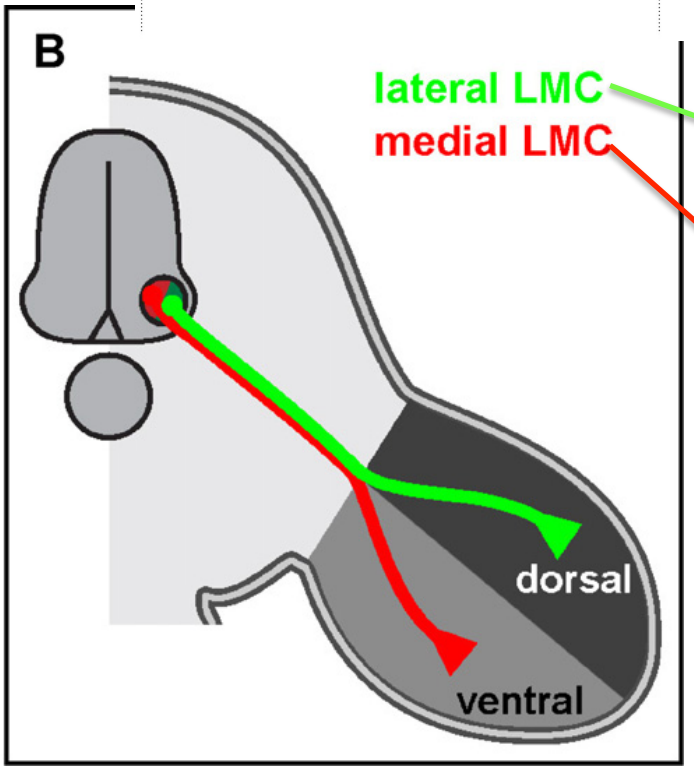
Contact-dependent Eph/ephrin-mediated guidance of axons



Kao et al., 2012

Focus on the lateral motor columns





lateral LMC

medial LMC

Lim1 (Lhx1) → EphA4 expression

Isl1 → EphB1 expression

Trascriptional regulation of Eph/ephrin expression

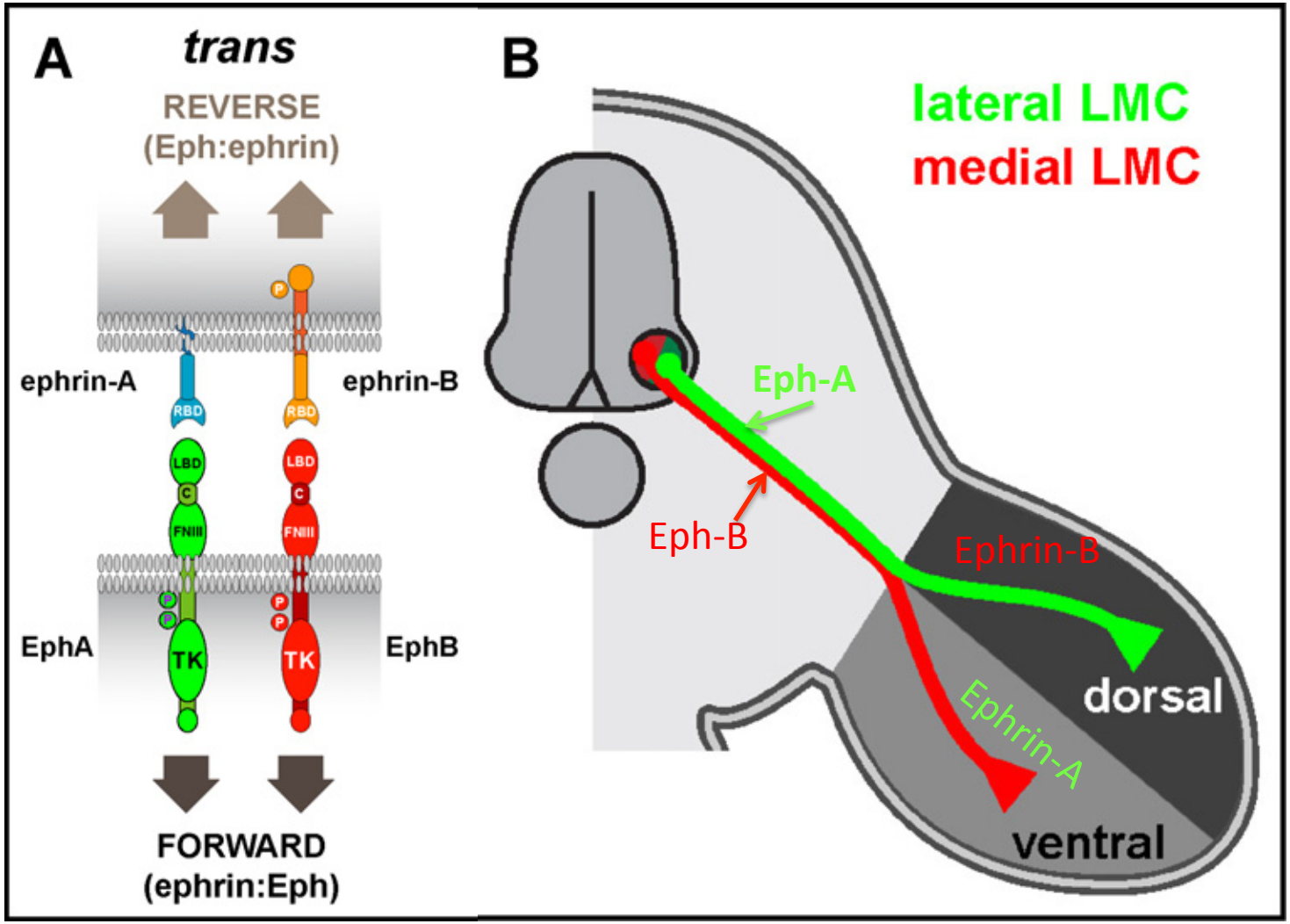
Precise control of Eph/ephrin expression in motor neurons and their targets

The role of Eph and ephrin signaling in axon guidance: axon pathfinding through repulsion

''Forward'' ephrin:Eph signaling leads to growth cone collapse and turning away from the source of ephrin

<u>Molecular symmetry</u> in the dorso-ventral guidance of the two sets of LMC axons:

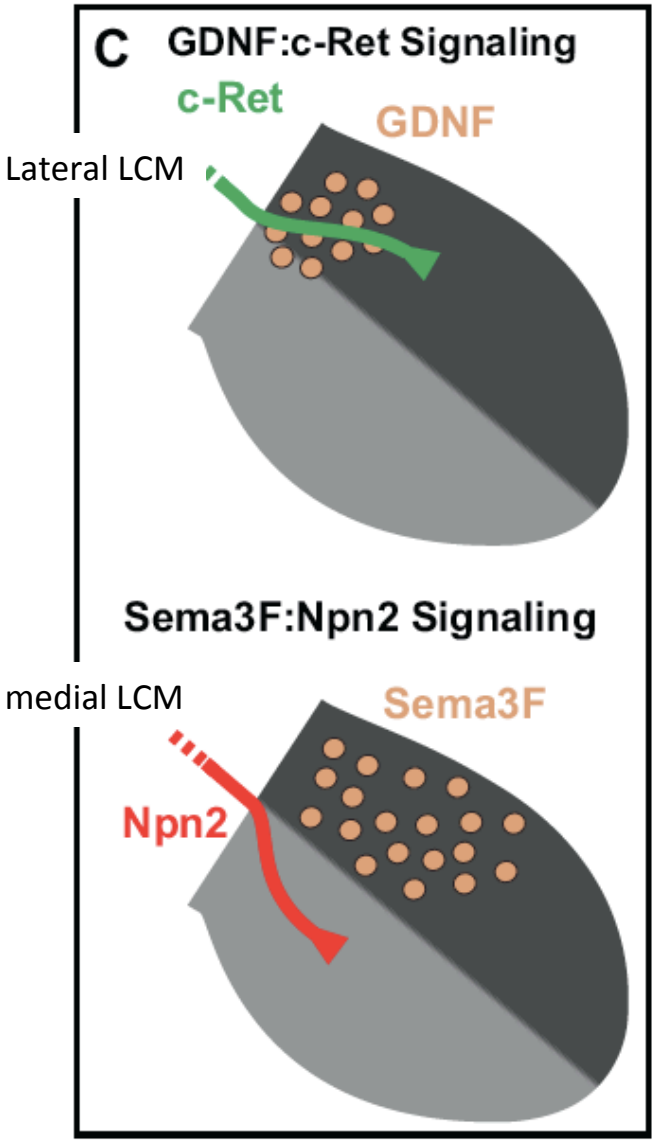
- EphA signaling directs lateral LMC axons dorsally
- EphB signaling directs medial LMC axons ventrally



Kaoa et al., 2012

Exp → Rotation of chick limb buds along D/V axis → switch of innervation

Other motor axon guidance cues act increasing the robustness and fidelity of the limb trajectory choice

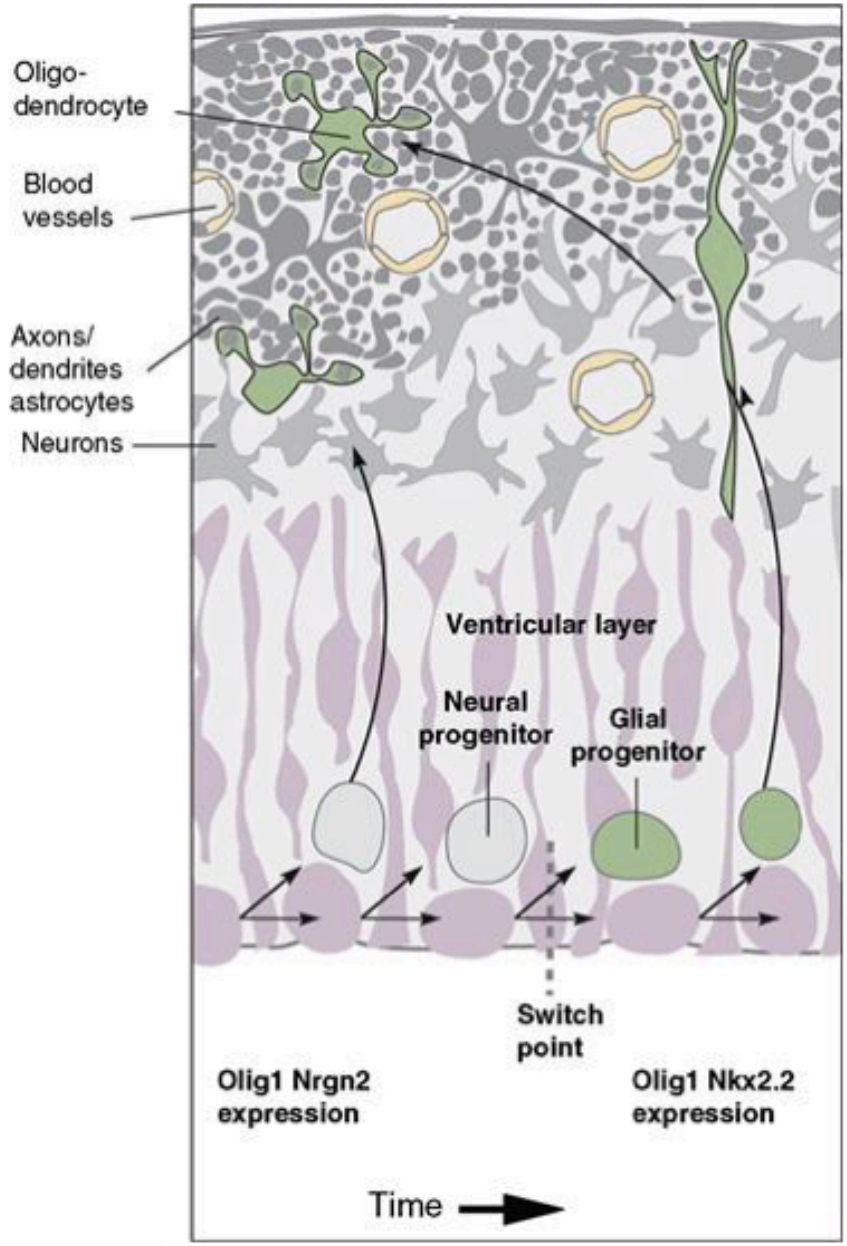


A subset of lateral LMC axons express c-Ret, receptor for the glial cell line-derived neurotrophic factor (GDNF) expressed by dorsal limb mesenchymal cells

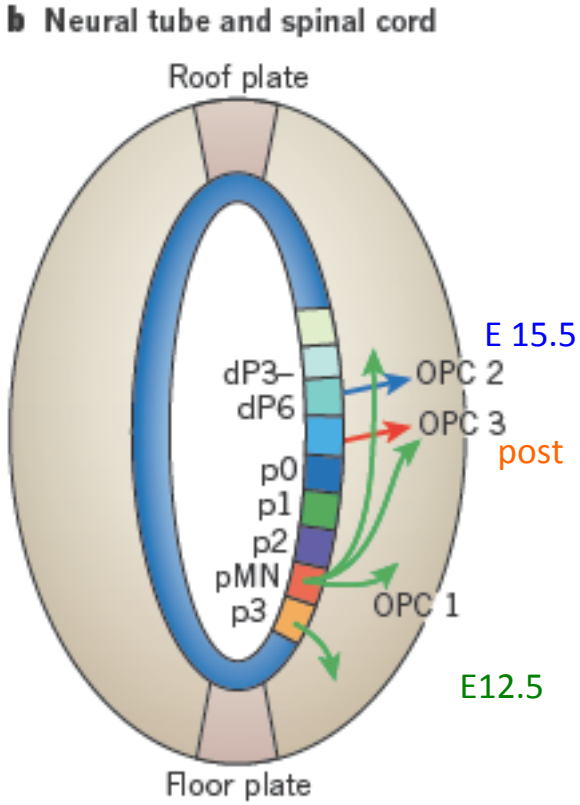
→ Synergy with ephrinA:EphA signalling

A subset of medial LMC neurons express Npn-2, a Semaphorin 3F receptor

pMN domain → originates oligodendrocytes



Sanes et al.,



Ngn2 downregulation is a determinant of the neuron–glial-cell switch

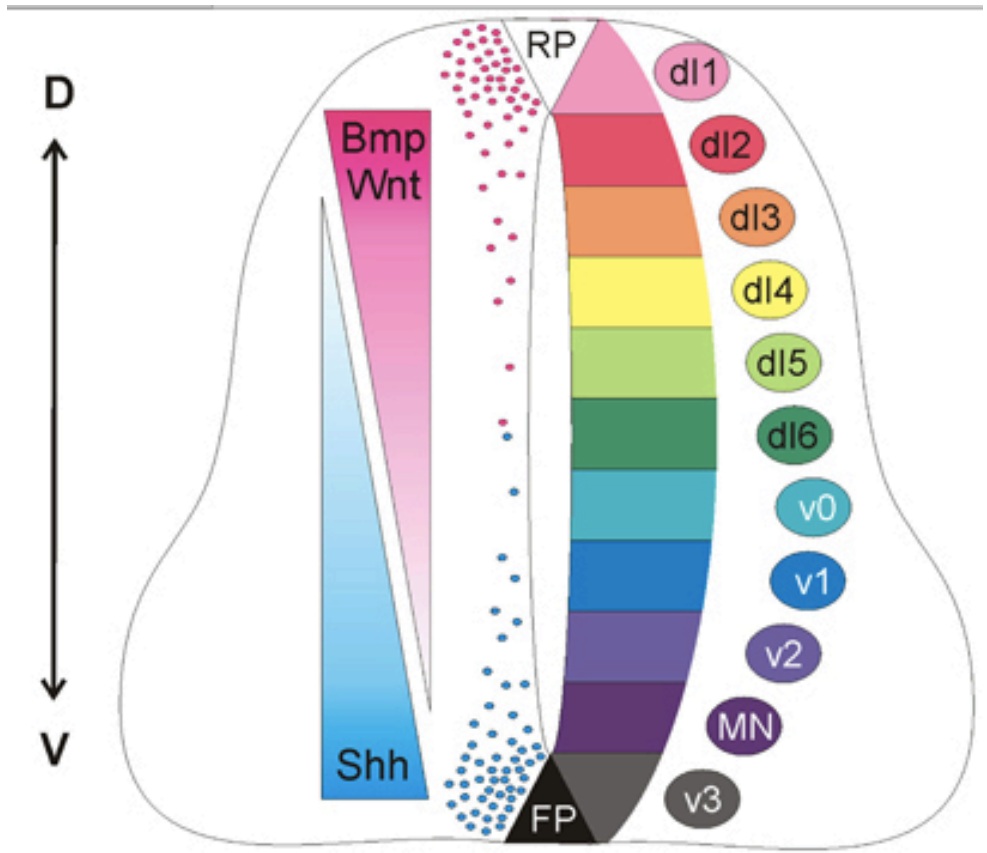
+

Pro-glial cell TFs (Sox9 and NFI)

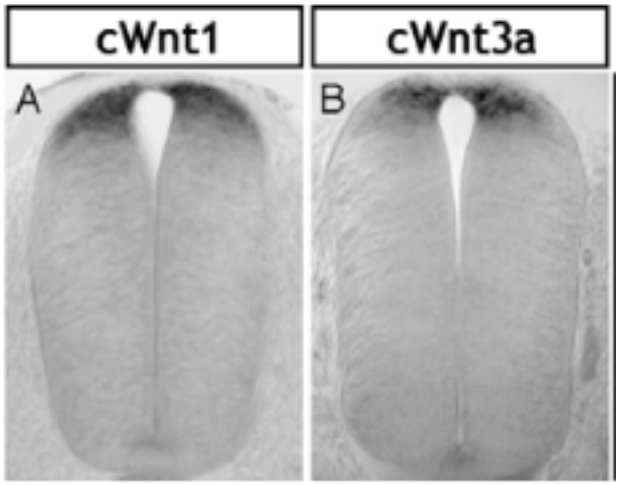
→ *A molecular switch allows progenitor cells to generate different cell types at different developmental stages*

The main actors in spinal cord DV pattern formation

→ The role of the RP in patterning the dorsal neural tube:
Explant assays + in vivo genetic ablation of the RP=loss of dI1-3 (RP-dependent)



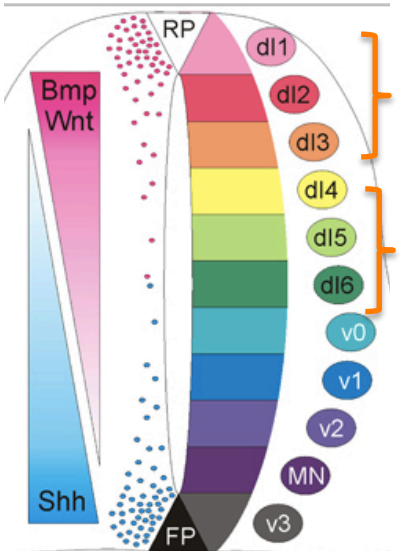
The role of the **Roof plate** in patterning the dorsal neural tube: **I) The effect of Wnt signalling**



HH stage 18 chick embryos

Several members of the Wnt family (including Wnt1 and Wnt3a) are expressed by the RP (both in chick and mouse)

Wnt proteins:
1) Mitogenic signals for neural cells
2) Neural cell fate specification



Severe reduction

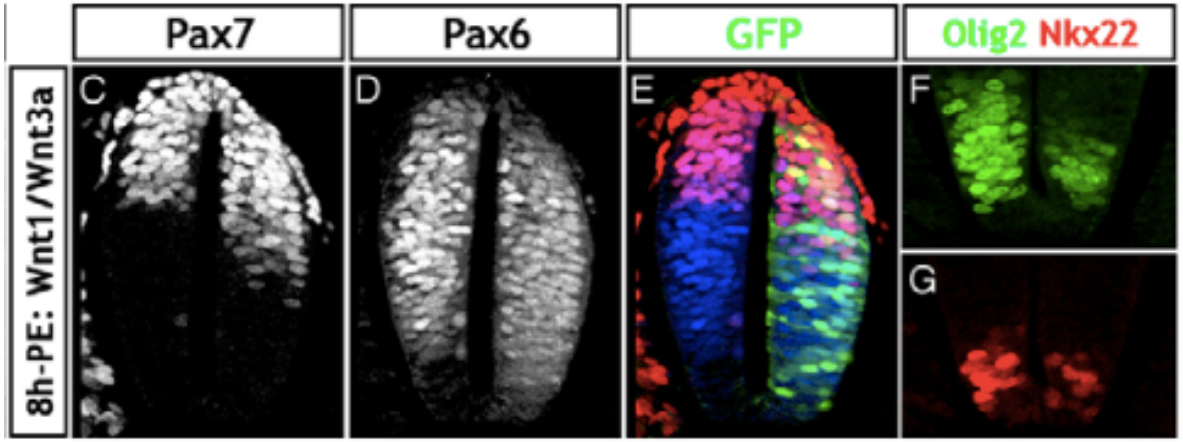
Mouse Double KO WNt1/3a

Expansion

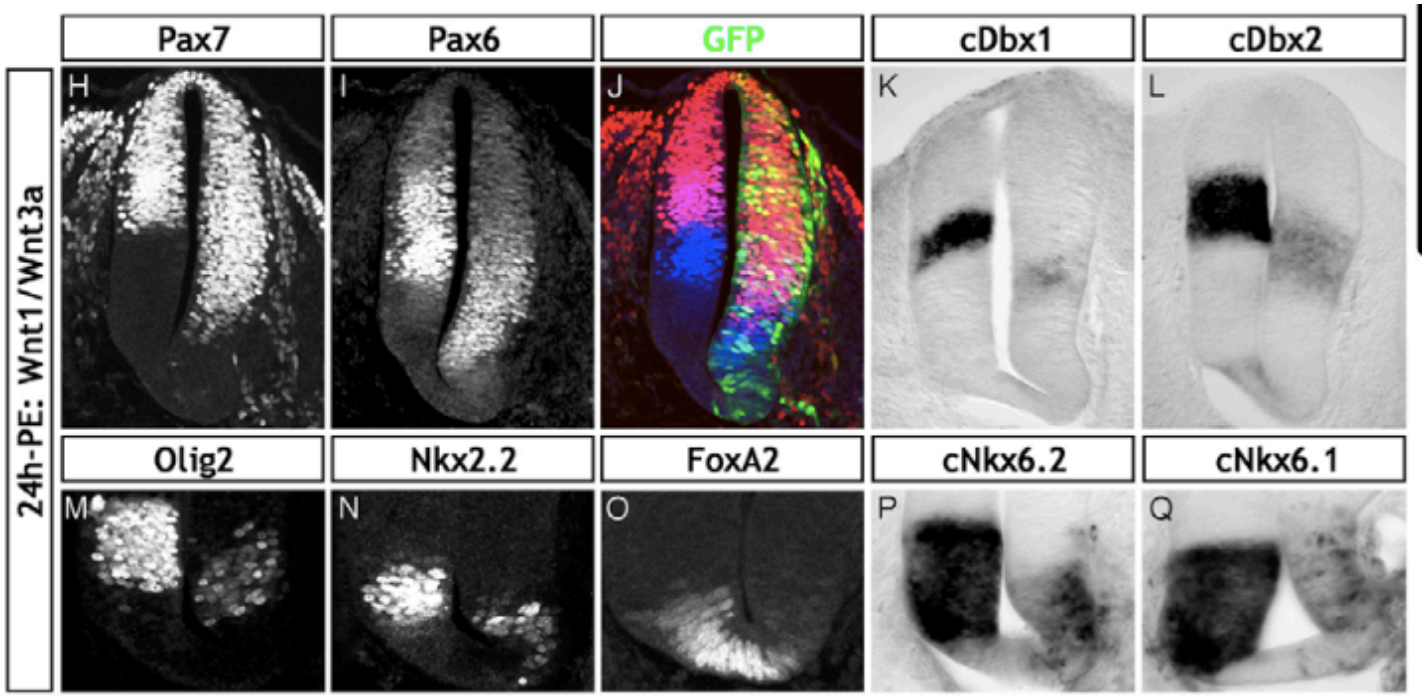
(Muroyama et al., 2002)

Experiments in chick embryos

Wnt1/Wnt3a misexpression along the DV axis



In ovo electroporation of plasmids encoding Wnt1/3a and GFP (green) at early neural developmental stages (HH11/12)

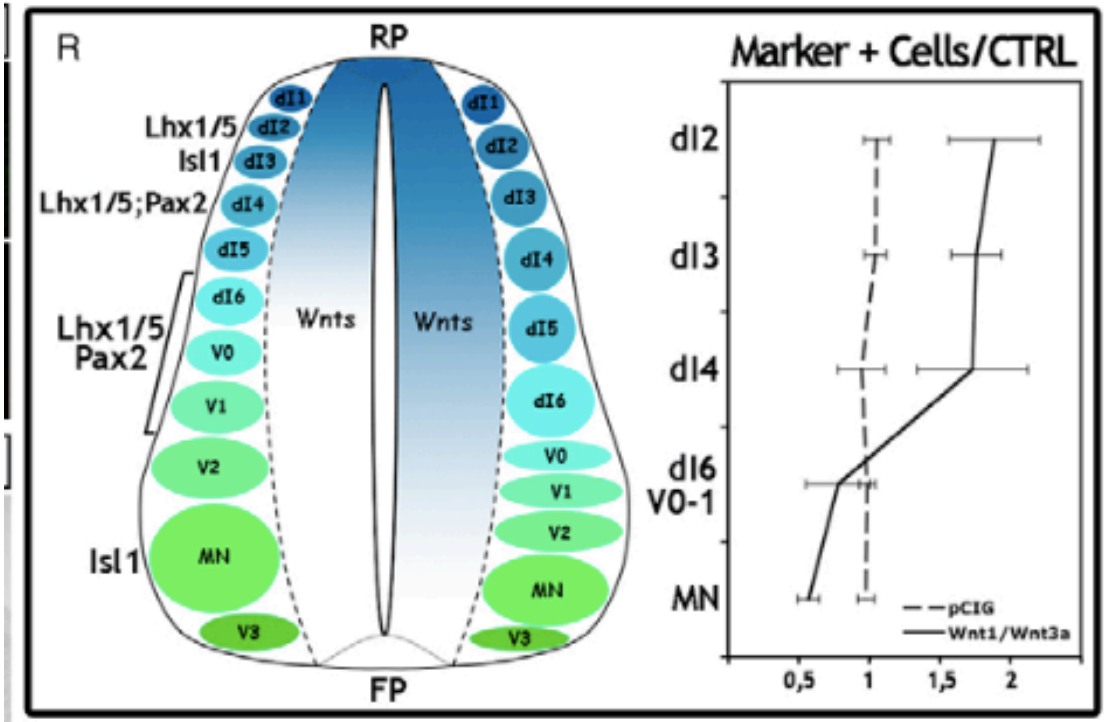


Wnt1/3a causes **overgrowth** of the electroporated side at 24h

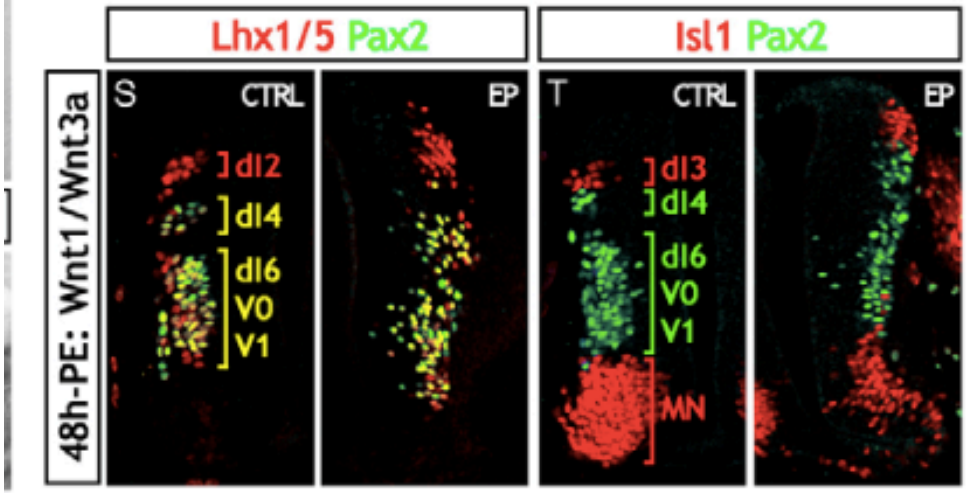
floorplate

Alvarez-Medina et al., 2008

Wnt1/Wnt3a misexpression along the DV axis



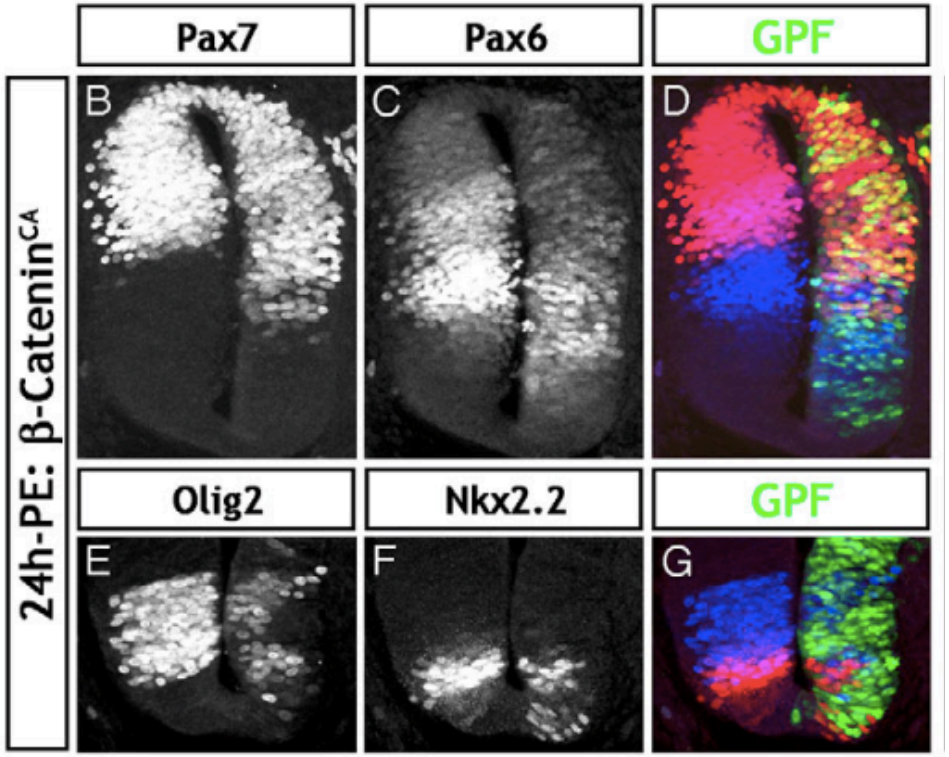
At 48 hr - Wnt1/3a causes phenotype changes on differentiated neurons



Overexpression of Wnt1/Wnt3a increased dorsal IN number (dI2, dI3 and dI4), at the expenses of the intermediate and the ventral IN subtypes dI6-V0/1 and MNs

Alvarez-Medina et al., 2008

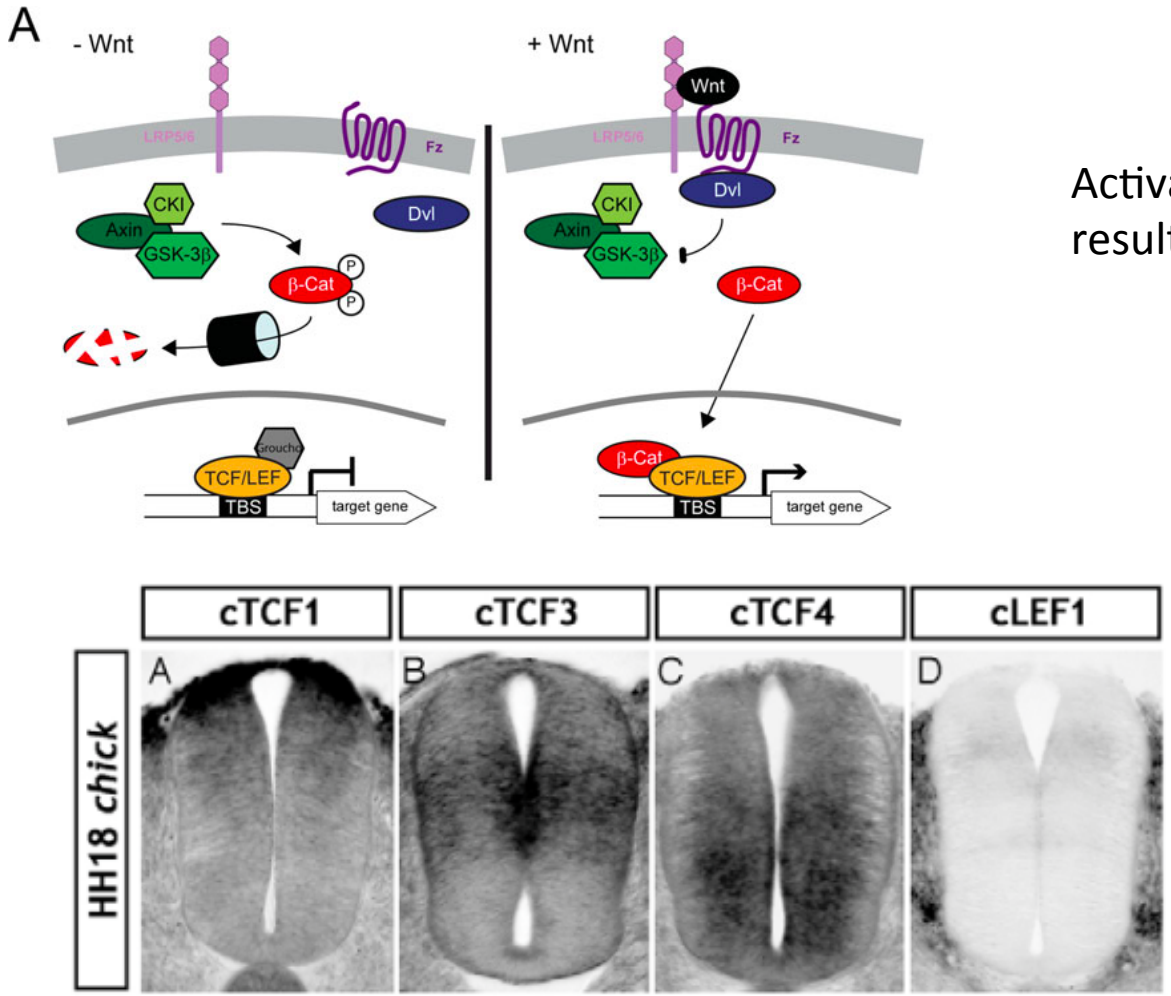
Wnt patterning activity is mediated by the canonical pathway



Electroporation of a stabilized form of beta-catenin that is resistant to targeted proteolysis (acts as a dominant active protein)

Induces cell-autonomous ectopic activation of dorsal genes such Pax7 or Pax6, together with a strong and cell-autonomous repression of ventral genes such as Olig2 or Nkx2.2

Alvarez-Medina et al., 2008

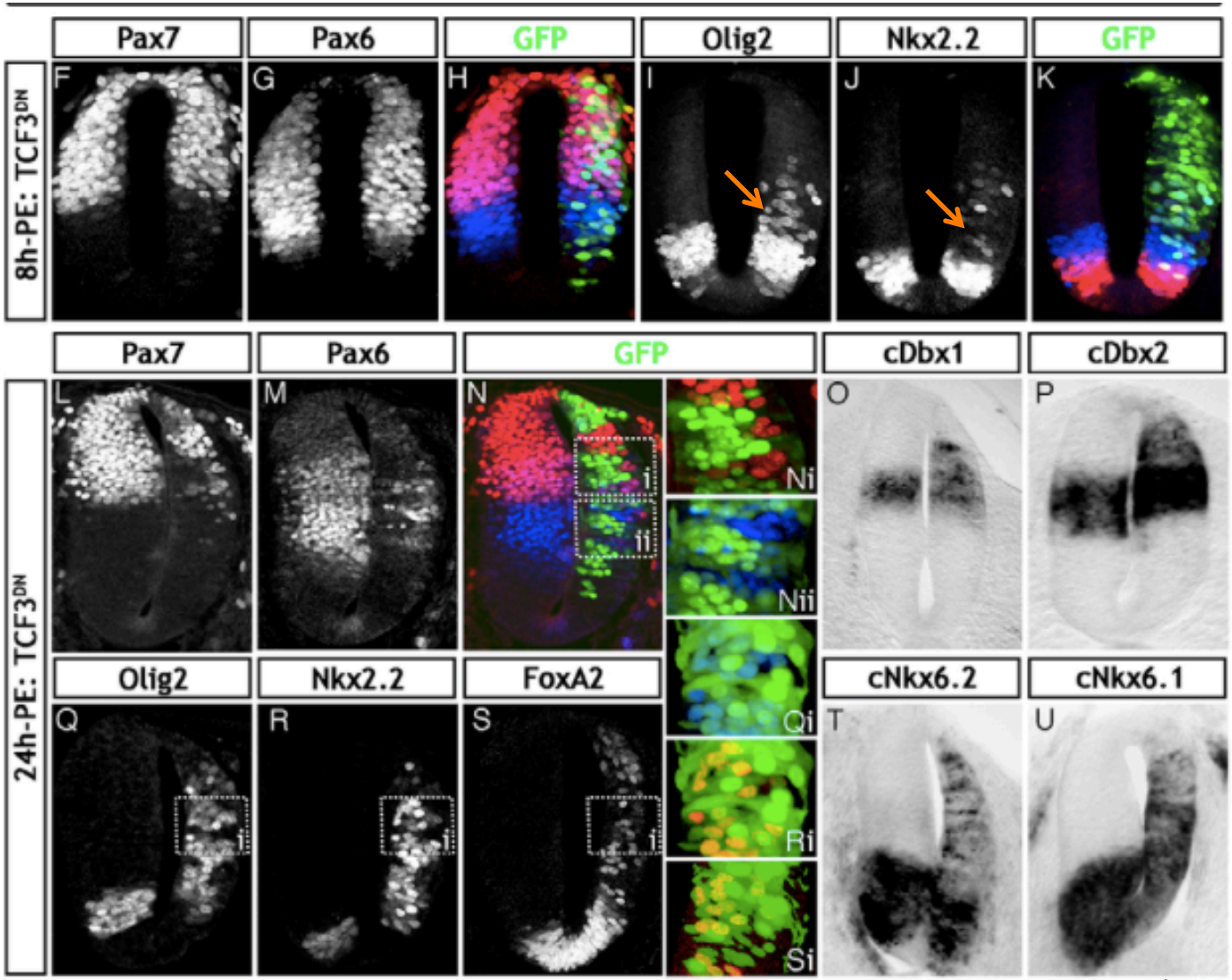


Activation of the canonical Wnt pathway results in Tcf target gene activation

Members of the Tcf/Lef family of HMG-box transcription factors are differentially expressed in the developing spinal cord: Tcf1, Tcf3 and Tcf4 encompass the entire DV axis of the neural tube

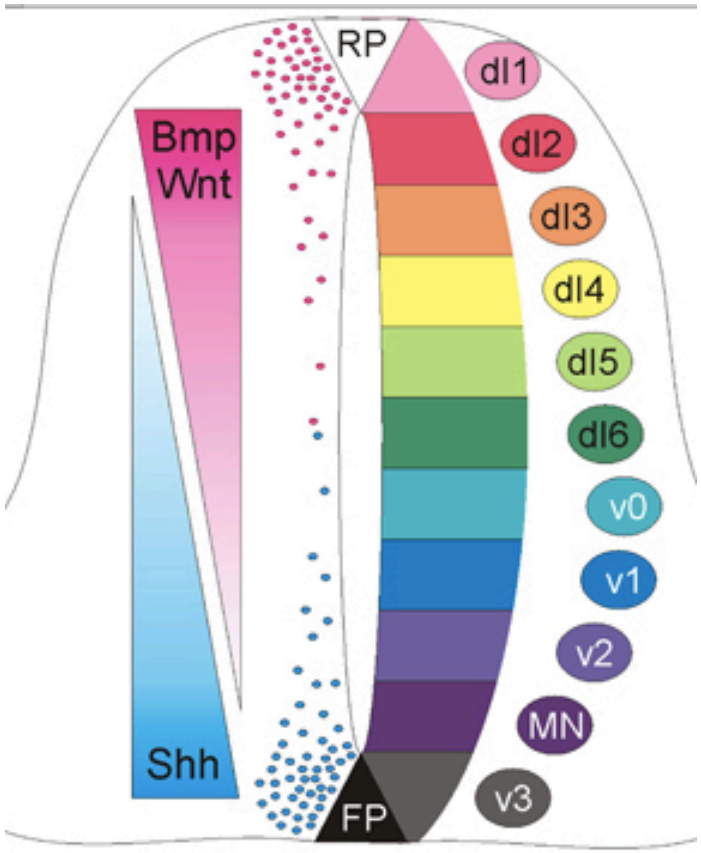
Alvarez-Medina et al., 2008

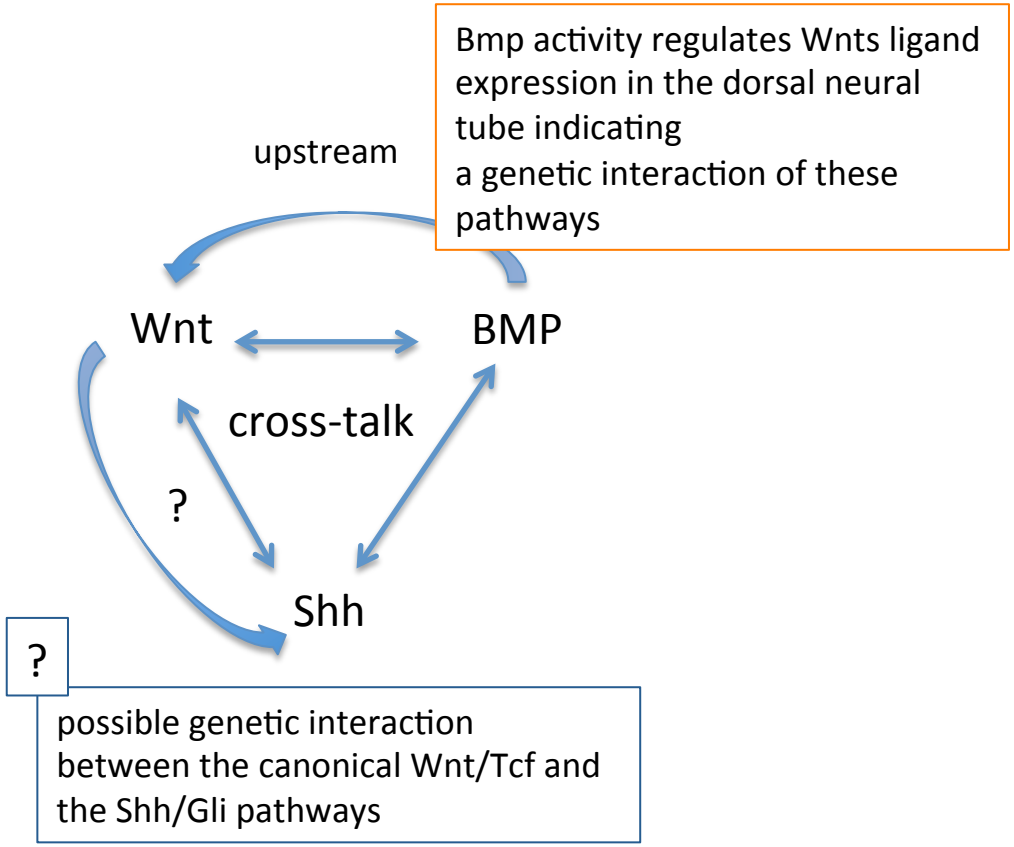
Electroporation of dominant-negative (DN) forms of Tcf1, **Tcf3** and Tcf4 lacking the beta-catenin-interacting domain, thus acting as **constitutive repressors of Wnt target genes**



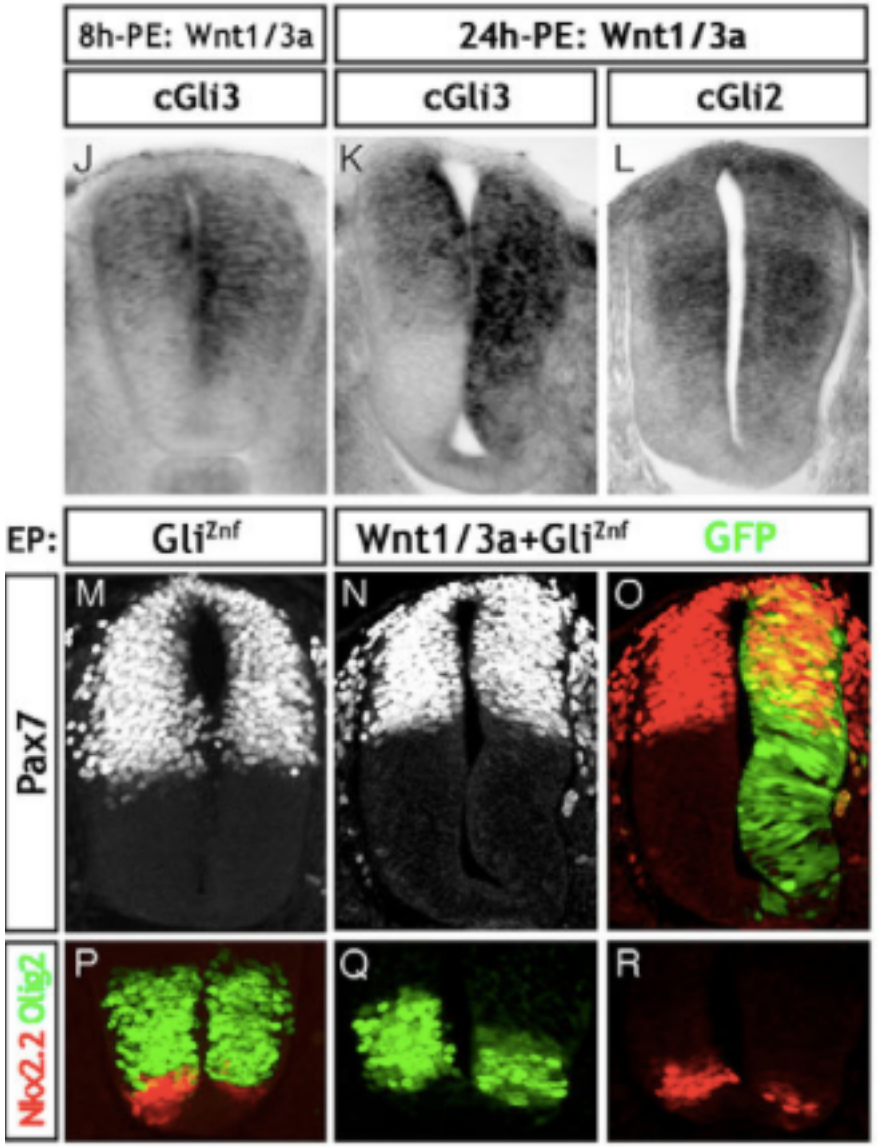
positive Wnt activity is required for the restriction of Olig2 and Nkx2.2 expression to their respective pMN and p3 progenitor domains

Alvarez-Medina et al., 2008


RP
dI1
dI2
dI3
dI4
dI5
dI6
v0
v1
v2
MN
v3
FP
Bmp
Wnt
Shh


Bmp activity regulates Wnts ligand expression in the dorsal neural tube indicating
a genetic interaction of these pathways
upstream
Wnt
BMP
cross-talk
?
Shh
?
possible genetic interaction between the canonical Wnt/Tcf and the Shh/Gli pathways

Does Wnt activity regulate Gli expression?



Ectopic Wnt1/3a expression causes a rapid (8h PE) and maintained (24 hours PE) ventral activation of Gli3, without affecting Gli2 expression

a deleted form of Gli3 protein that contained only the DNA binding zinc-finger-domain (GliZnF-no trascriptional activity) abolished Wnt-induced ventral expansion of Pax7.

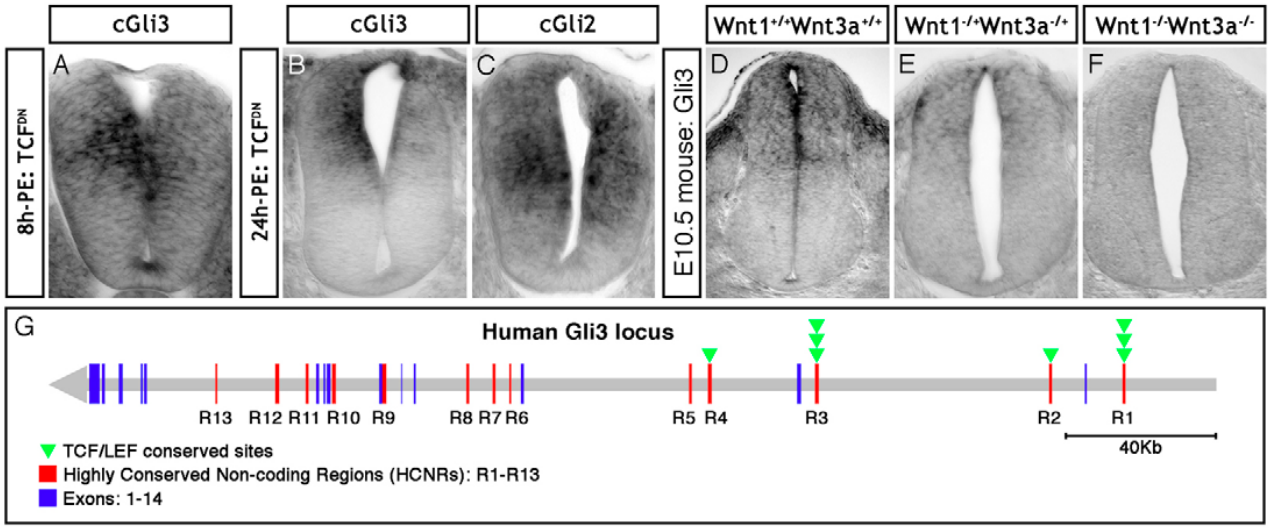
Twenty-four hours PE, GliZnF alone reduces Nkx2.2 (red) expression.

Co-electroporation of Wnt1/3a and GliZnF results in a partial rescue of Olig2 (green) and Nkx2.2 (red) expression

→the induction of Gli3 may explain the inhibitory effect of Wnt on the ventral programme

Alvarez-Medina et al., 2008

Wnt signalling controls Gli3 expression in the dorsal spinal cord



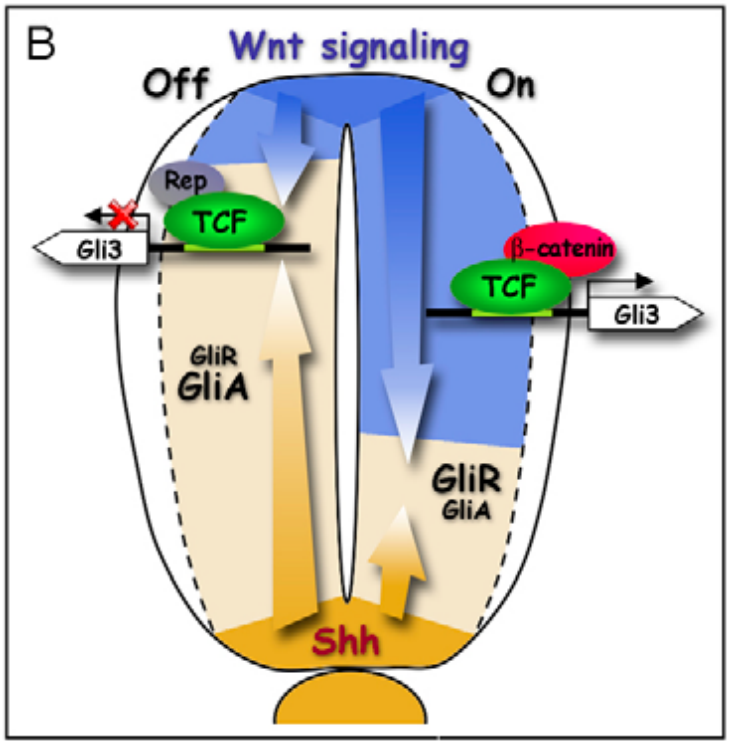
Schematic representation of the human *GLI3* locus.
Conserved coding sequences are depicted in blue and conserved non-coding sequences in pink. Grey arrow indicates the length of the *GLI3* gene and the direction of transcription. **Tcf-binding sites are depicted in green**

several Tcf binding consensus sequences have been identified within highly conserved non-coding regions in the human Gli3 locus

Gli3 is a direct target of Wnt/beta catenin

Alvarez-Medina et al., 2008

Wnt signaling antagonizes Shh activity



Proposed model:

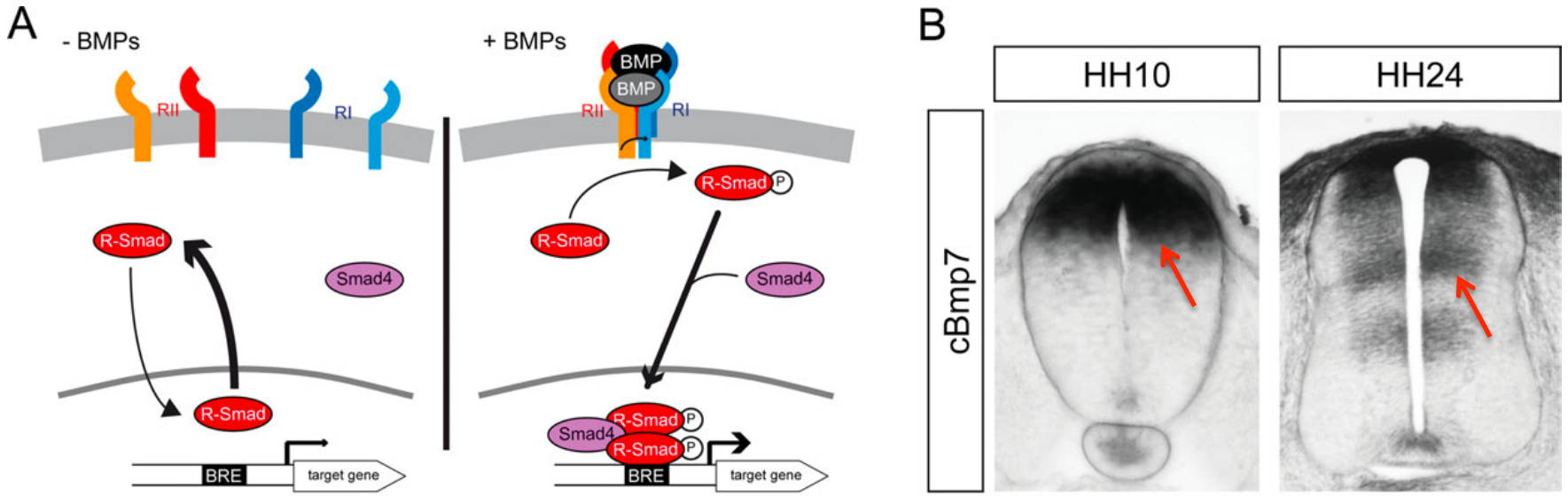
Wnt/Tcf signalling from the dorsal NT regulates the expression of the main inhibitor of the Shh/Gli pathway, Gli3.

In turn, Gli3, acting mainly as a transcriptional repressor, restricts the graded Shh/Gli ventral activity.

The balance between Shh and Wnt is critical to pattern the spinal cord along its DV axis

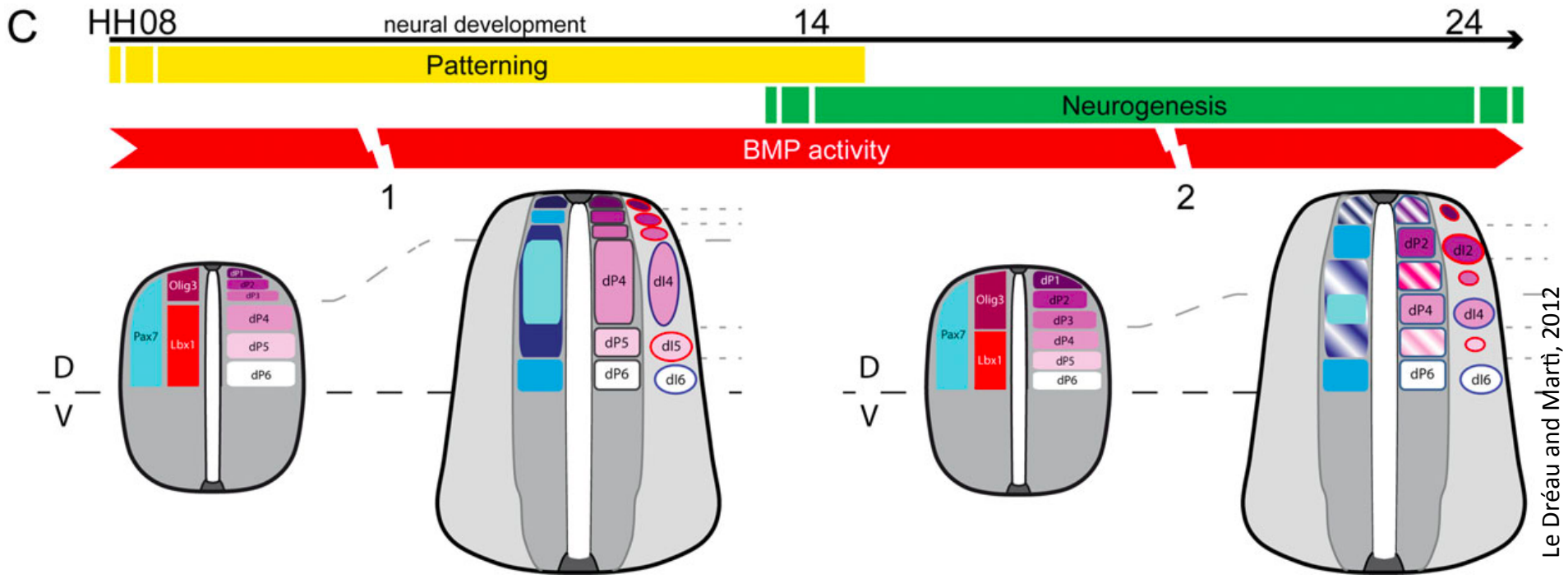
Alvarez-Medina et al., 2008

The role of the **Roof plate** in patterning the dorsal neural tube: I**I) The effect of BMP**



Expression of BMPs is highly dynamic and complex during neural tube development.

- Before neural tube closure BMP2 and 4 are expressed by the epidermal ectoderm while BMP4 and 5 are expressed by neural folds
- **After neural tube closure, several BMPs (BMP4, BMP5, and BMP7) are expressed in the RP.**
- At the time of primary neurogenesis of dINs BMP7 expression extend throughout the dorsal half of the neural tube.



A loss or a reduction in BMP signaling at early stages result in:

- decrease in the territory occupied by the three Olig3+ (purple) dorsalmost progenitor domains (dP1–3) compensated by an expansion of the Lbx1+ (red) ventral-most dorsal progenitor domains (dP4–6)
- the corresponding dIN populations generated by these progenitor domains will be generated in decreased (dI1–3) or increased (dI4–6) numbers

A reduction in the BMP activity occurring later during development:

- affects the generation of the dorsal interneurons populations without any obvious changes in patterning
- However, reduces expression of proneural basic helix–loop–helix factors Atoh1 and Ascl1, and the generation of the dI1, dI3, and dI5 populations.
- expression of Ngn1 (dP2 and dP6) and Ptf1a (dP4) in the progenitor domains is barely affected by reduced BMP activity and the corresponding dIN populations generate normally.

Different BMPs (e.g**. BMP4** and BMP7*) exert different roles in patterning & neurogenesis… (non redundant function) **not required for dorsal patterning but for neurogenesis of discrete neural identities*

The different functional areas of the CNS derive from regionally distinct subdivision of the neural tube during development

Take home message about neural patterning:

1) **Inductive interactions** guide the early pattern of cell differentiation in the neural tube

2) A **small number** of inducing factors:

- control programs of TF expression in target cells
- guide the extensive diversification of cell types

3) Despite differences in the organization of the CNS of vertebrates and invertebrates the signaling molecules responsable for differentiation and patterning of the nervous system are **highly conserved throughout animal evolution**