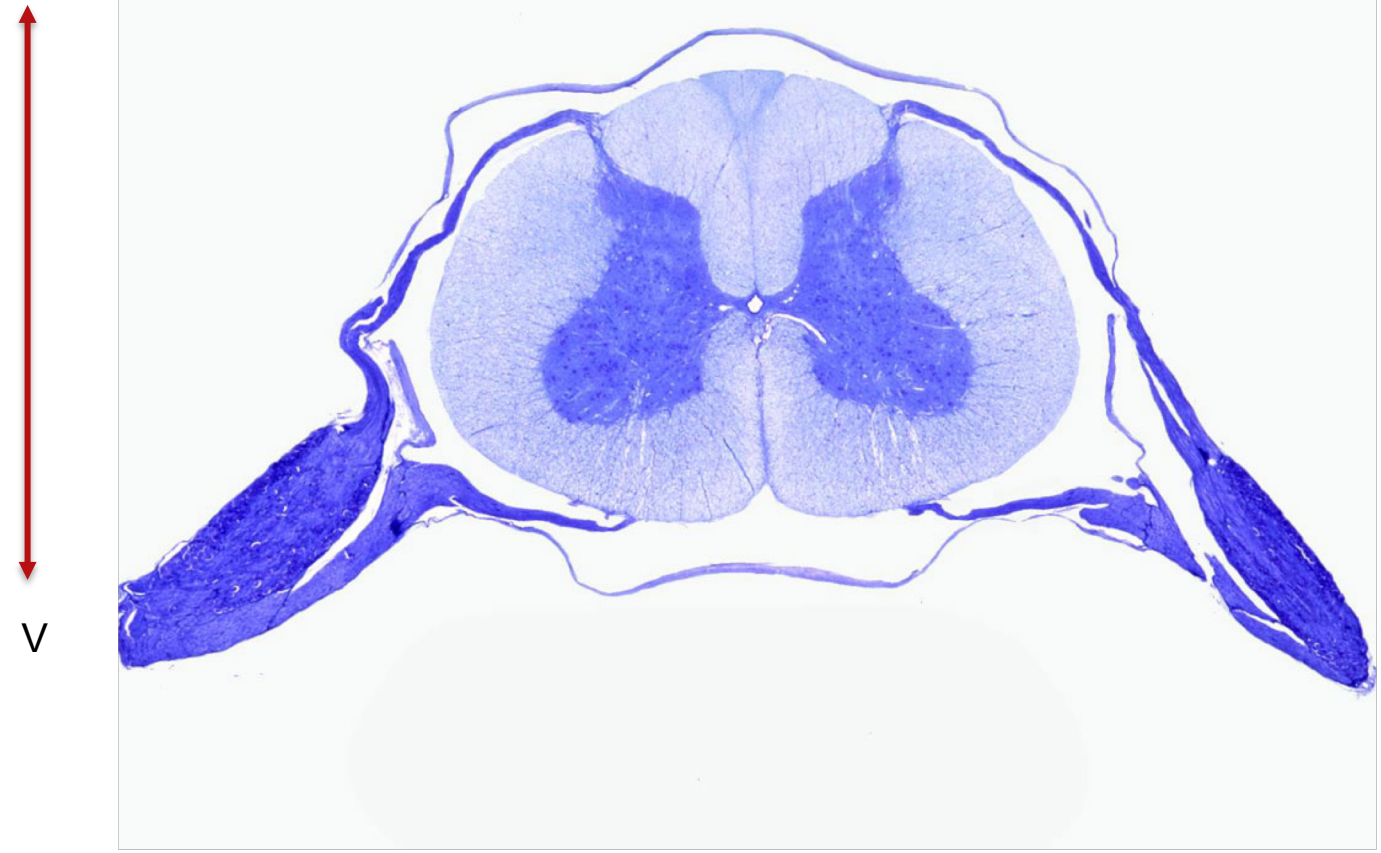
Dorso-ventral patterning

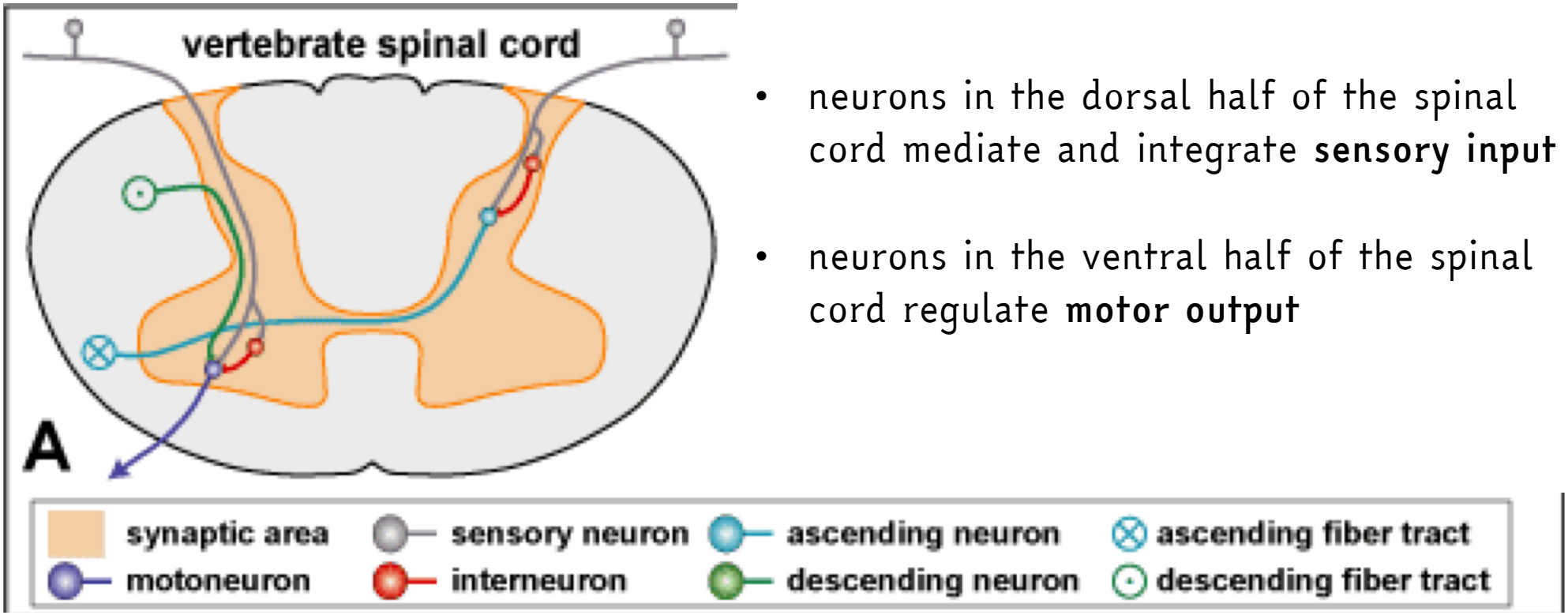
The spinal cord

D



V

The cells that process sensory and motor functions in the spinal cord are organized along the dorsal-ventral axis

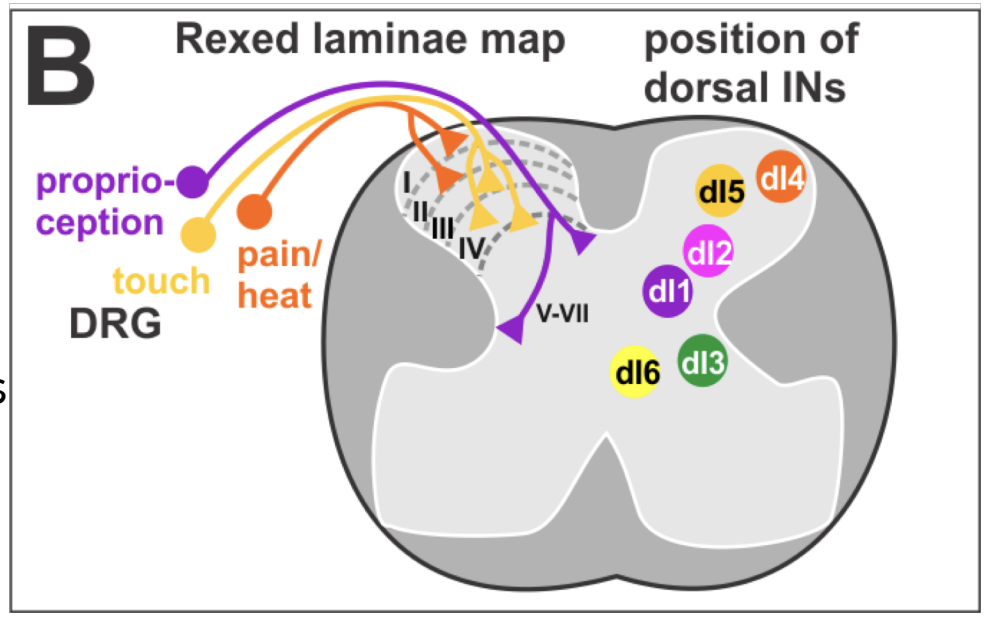


- neurons in the dorsal half of the spinal cord mediate and integrate **sensory input**
- neurons in the ventral half of the spinal cord regulate **motor output**

Different neuronal subtypes are located at highly specific position

The adult **dorsal spinal cord** is organized with a laminar architecture comprising seven distinct layers

Afferent sensory information reaches specific layers where it is mediated by different subtypes of **dorsal interneurons**

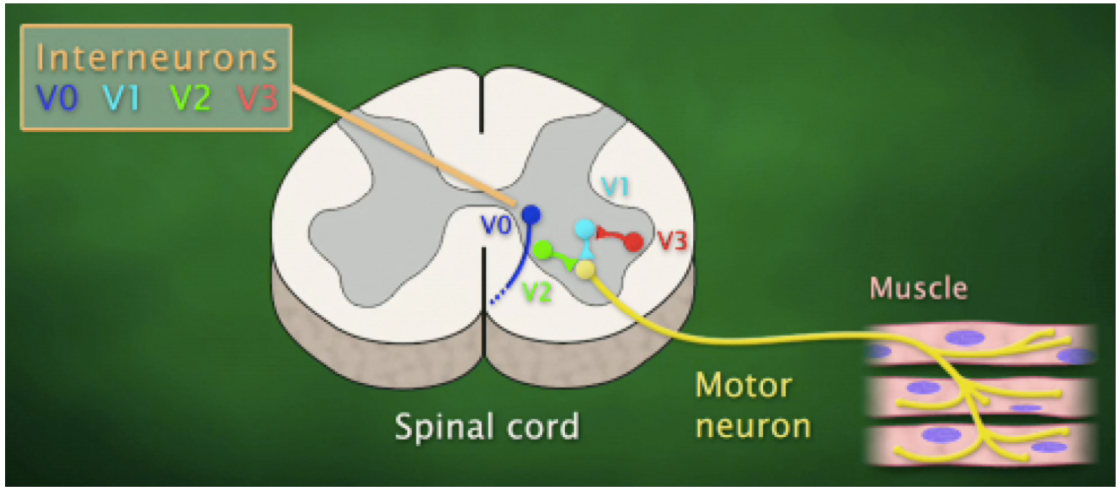


Andrews et al., 2019 Current topics in developmental biology

Different neuronal subtypes are located at highly specific position

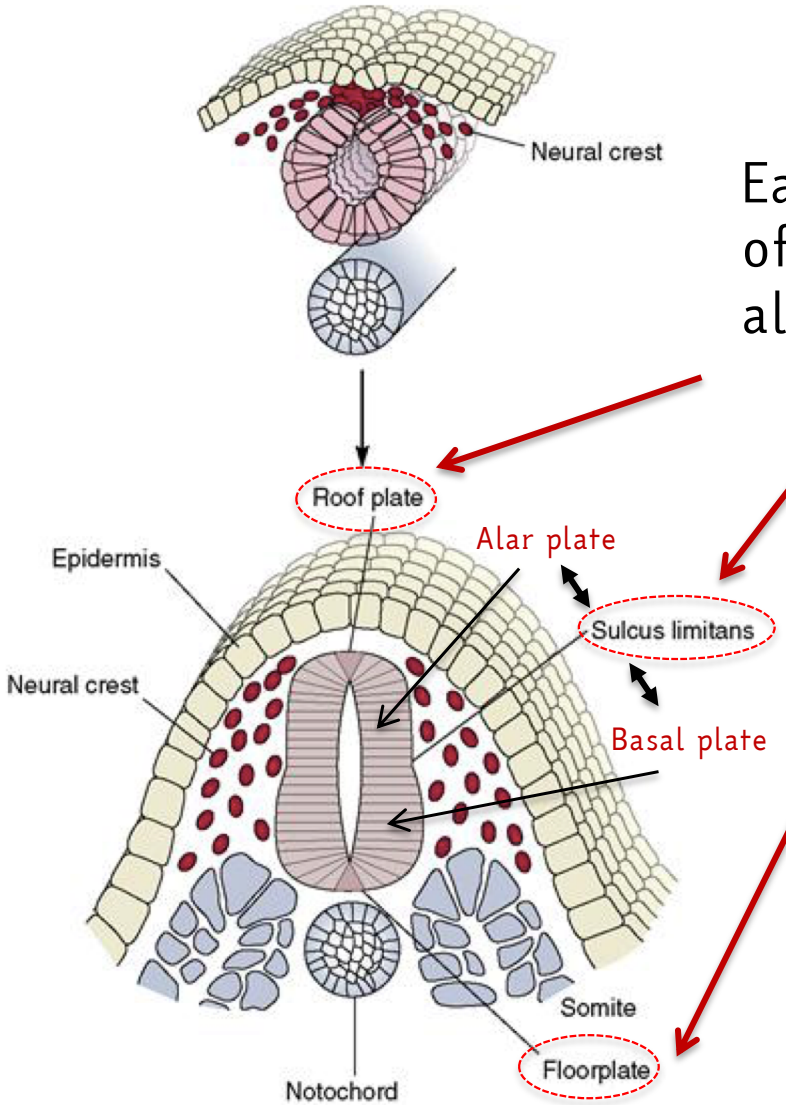
Motor circuits are comprised of:

- **motor neurons** (MNs), whose axons exit the spinal cord through the ventral root to synapse onto specific muscles;
- **ventral interneurons** (INs), which modulate the activity of MNs



Modified from T. Jessell

Early morphological signs
of neural tube differentiation
along the dorsal- ventral axis



The main actors in spinal cord D-V pattern formation

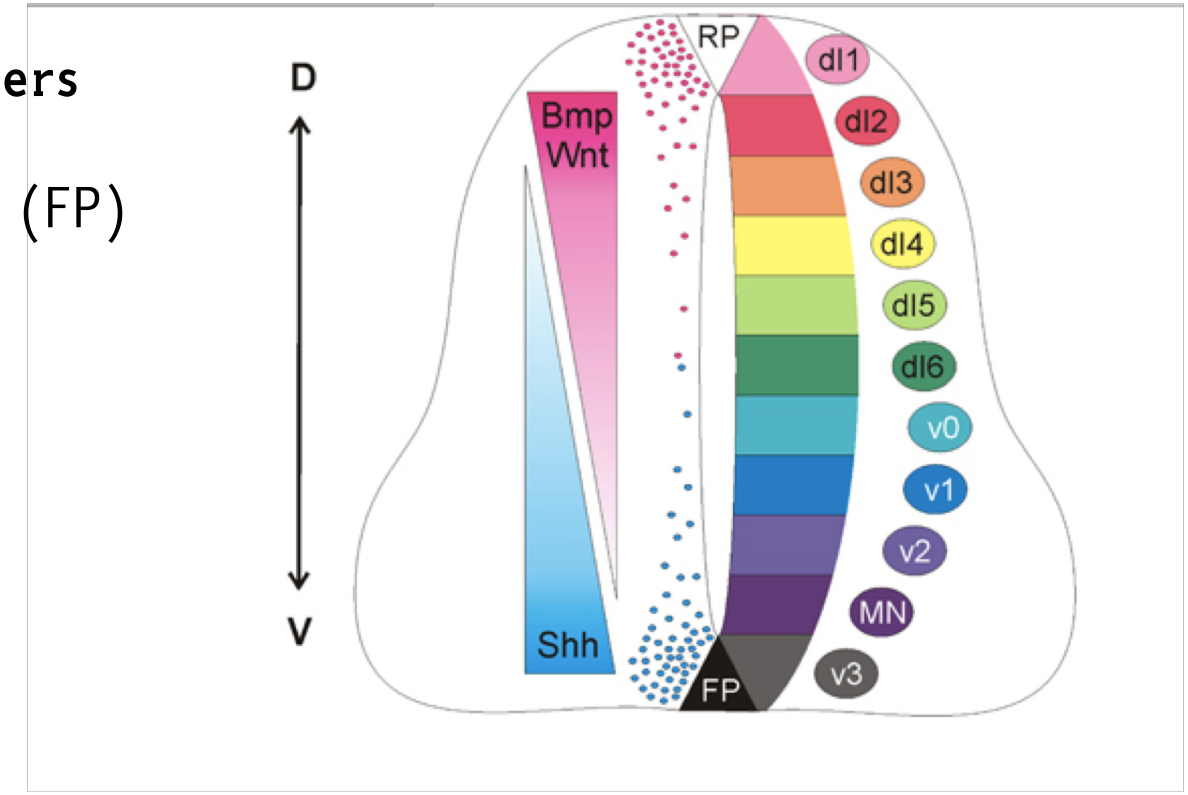
2 opposite signaling centers

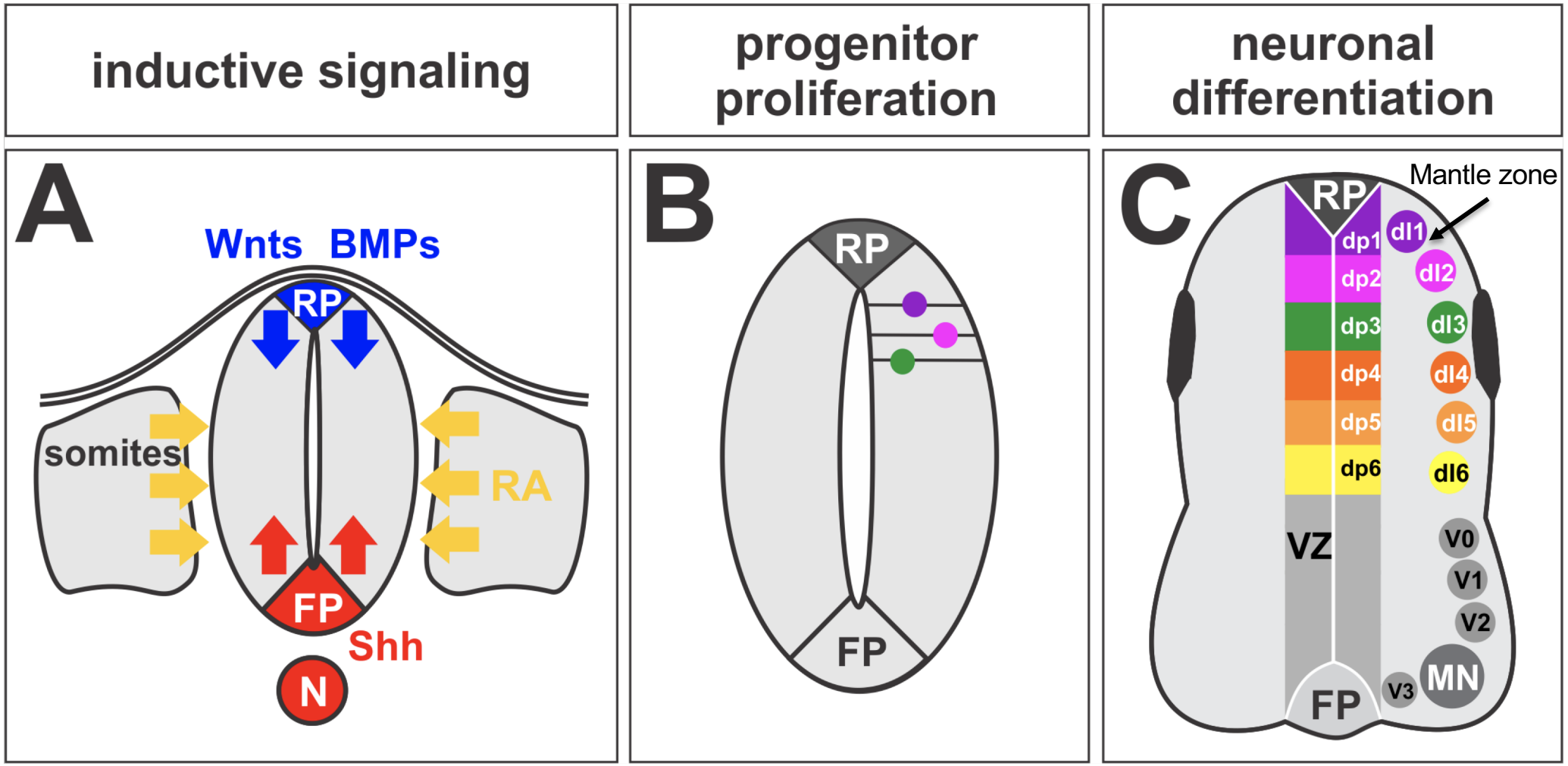
a. the roof plate (RP)

b. the ventral floor plate (FP)

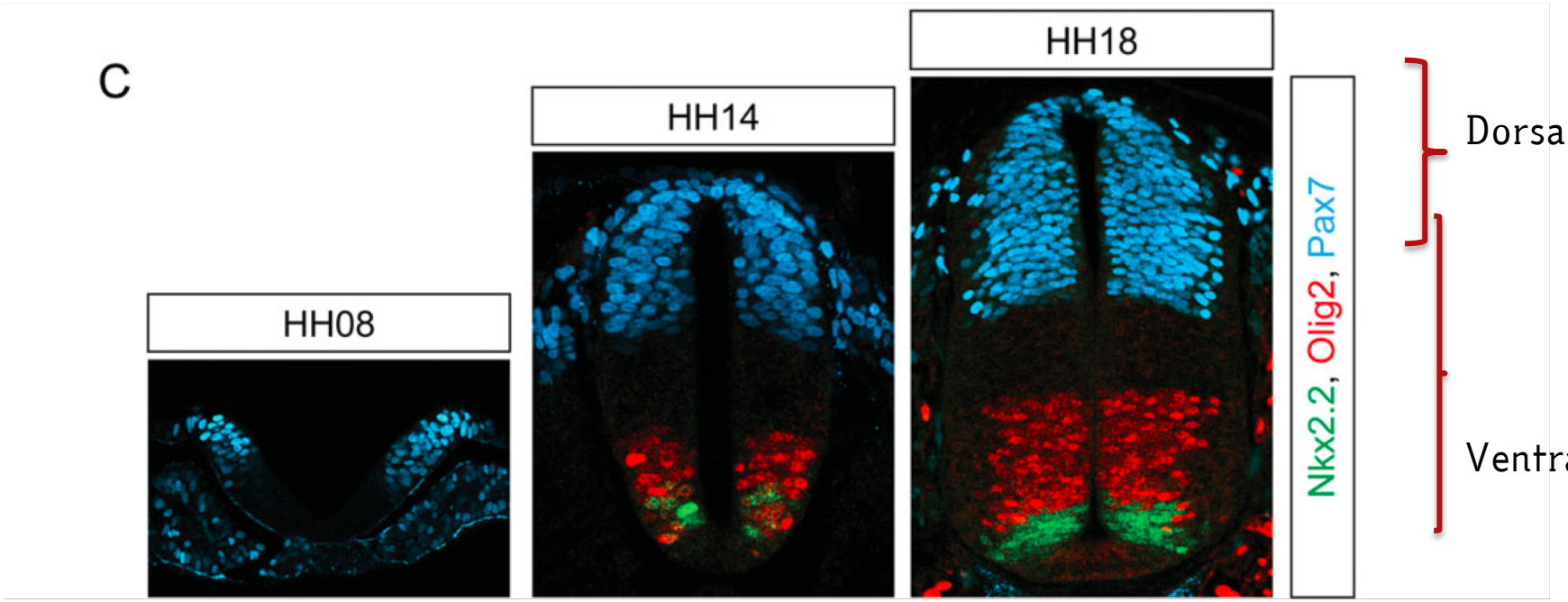
Three main signals

- BMPs
- Wnt
- Shh




inductive signaling
progenitor proliferation
neuronal differentiation
A
Wnts BMPs
RP
somites
RA
FP
Shh
N
B
RP
FP
C
Mantle zone
RP
dp1
dp2
dp3
dp4
dp5
dp6
dI1
dI2
dI3
dI4
dI5
dI6
VZ
V0
V1
V2
V3
MN
FP

Progressive emergence of ventral neural progenitor domains

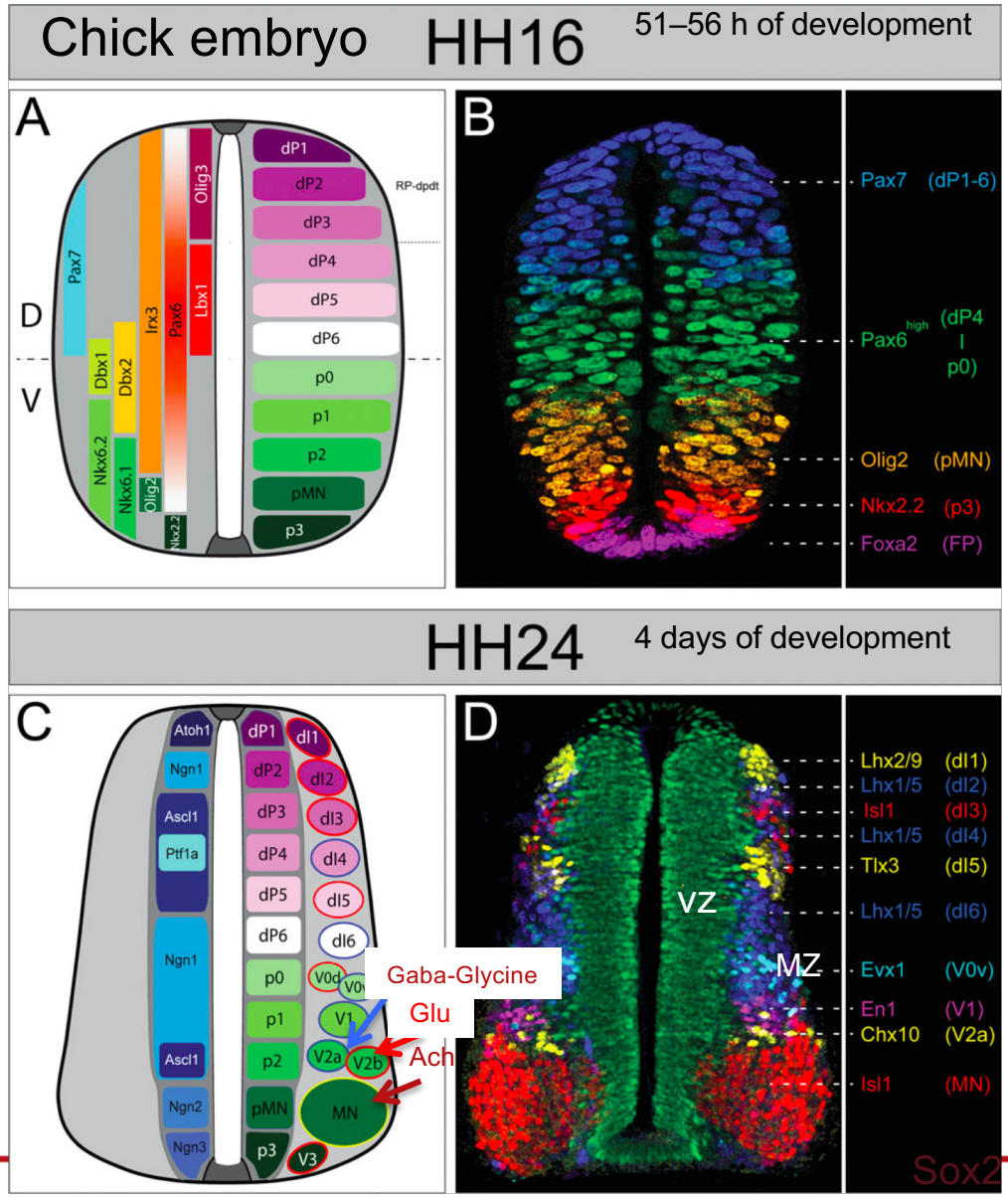


(chick embryo)

The **11 distinct domains** of neural progenitors with dorso-ventral regional identity are defined by aTFs code

→ Proneural bHLH TFs define the progenitor domains

→ 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**



Le Dréau *Dev Neurobiol 2012*

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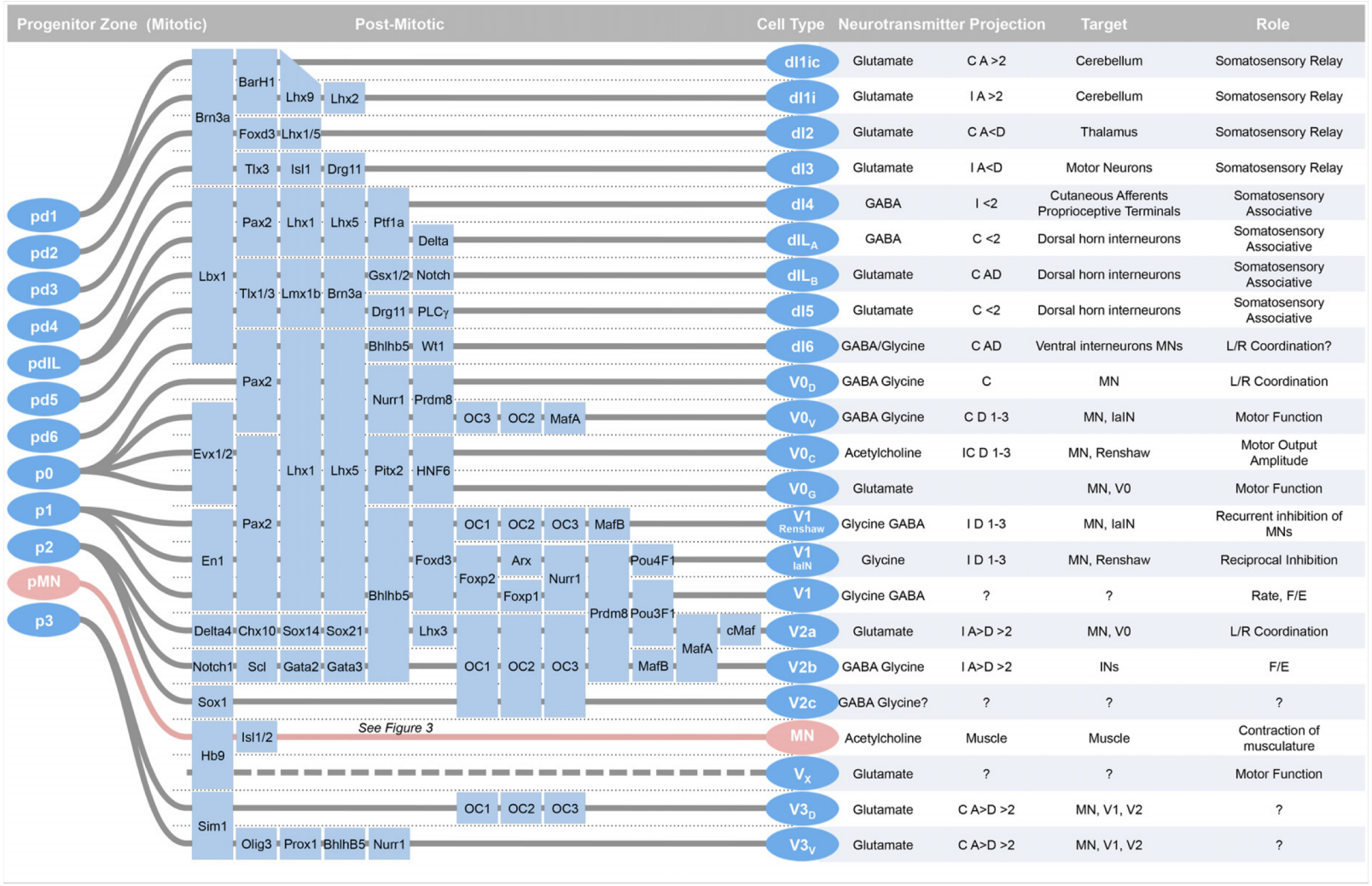

23 classes of neurons can be defined by transcription factor expression

Lu et al., 2015

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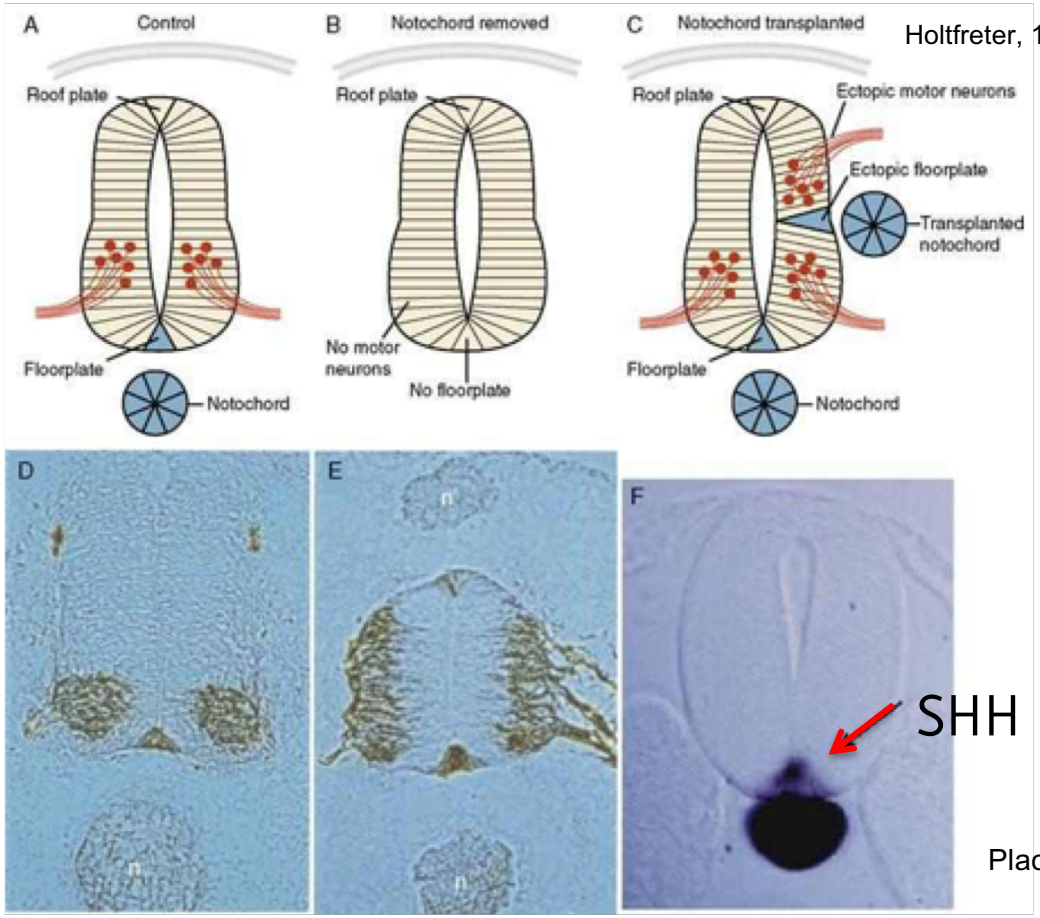
Specification of ventral fate

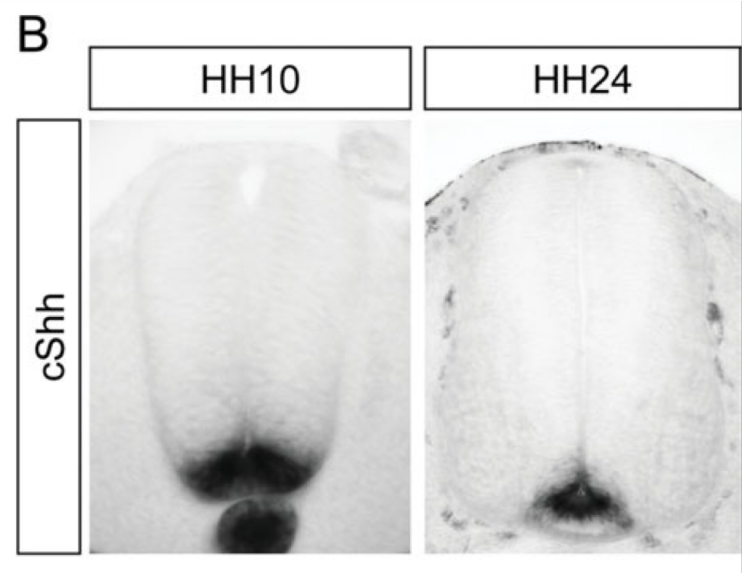


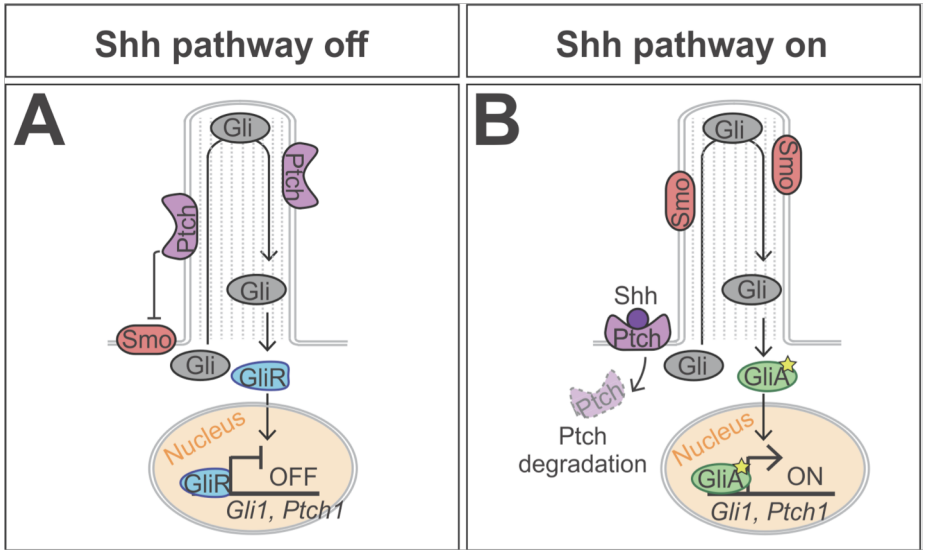
Holtfreter, 1934

Placzek et al., 1991

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

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



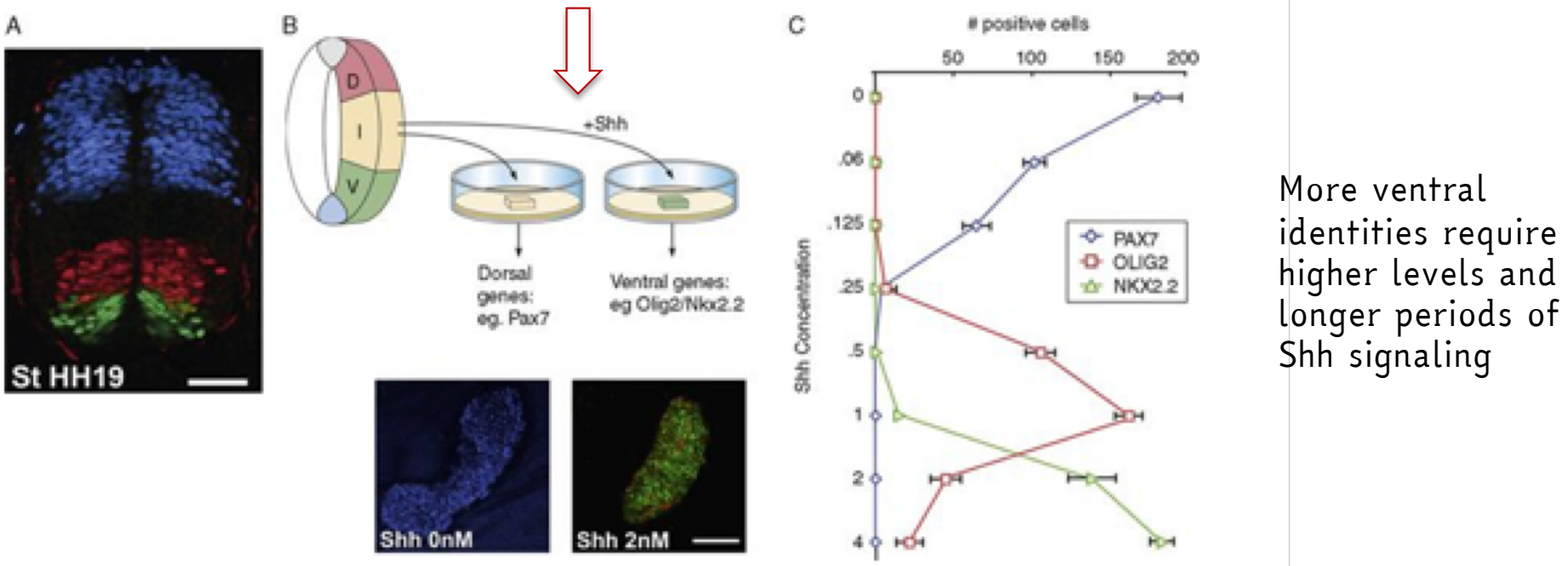


The Shh signal transduction pathway converges on Gli proteins

- Shh is expressed in the **nothocord** at the time when the D-V 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 acts as a canonical morphogen in ventral patterning

In vitro experiments:
co-culture (neural tube + notochord)
co-culture (neural tube + cells expressing shh)
neural tube + recombinant shh



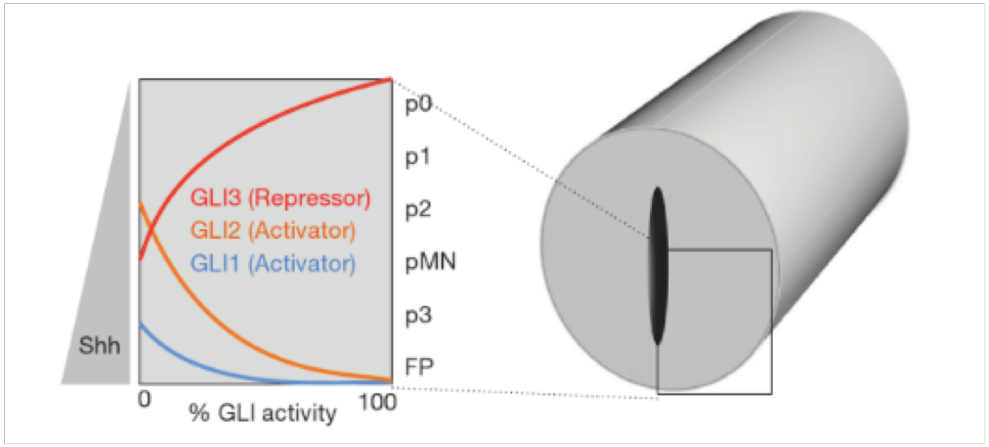
More ventral identities require higher levels and longer periods of Shh signaling

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

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

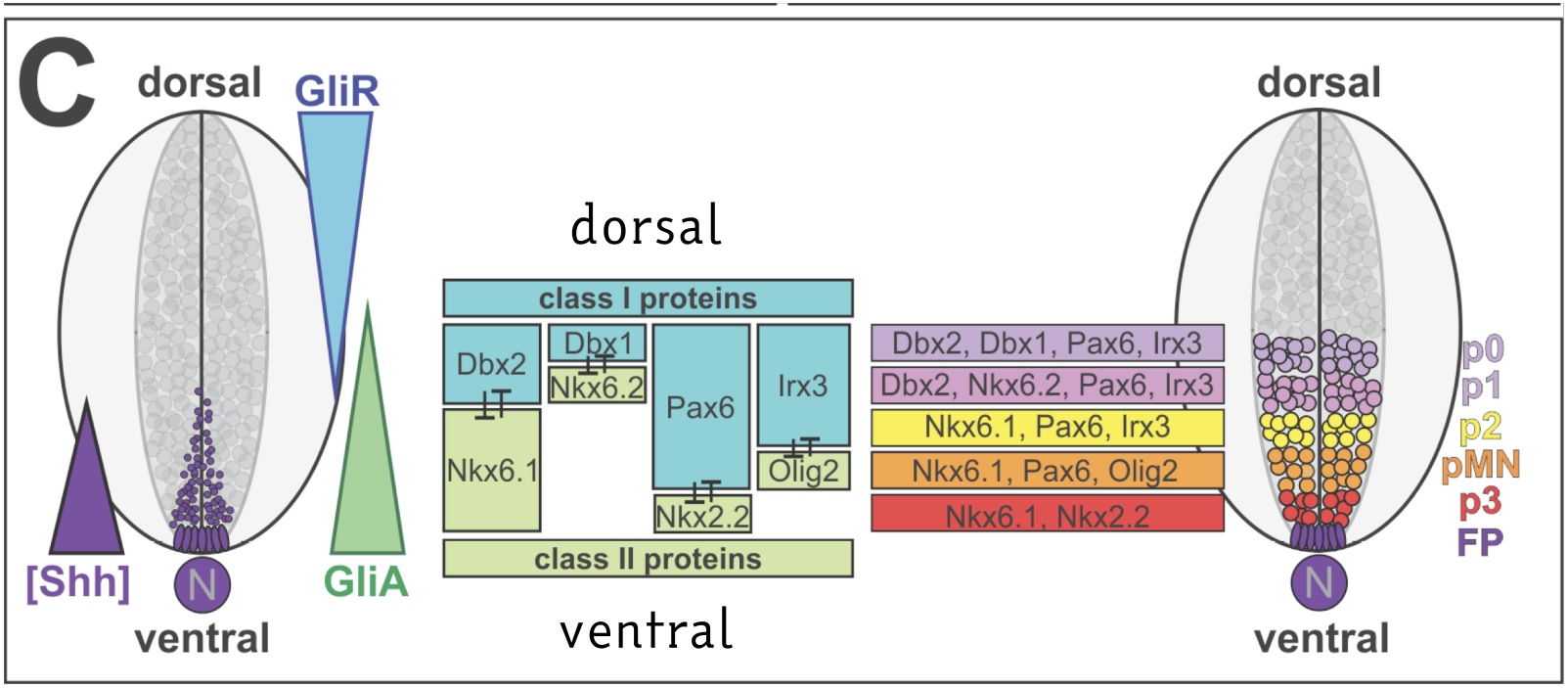


Vertebrates have three Gli homologs:
Gli1 = trascriptional activator (A)
Gli2 and **Gli3** = bifunctional (activators A or repressors R)
Gli2 mostly A – Gli3 mostly R

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

The Shh gradient is interpreted by a code of Gli transcription factors

The balance between GliA and GliR, regulates the expression of transcription factors that will define the progenitor identity of the cells

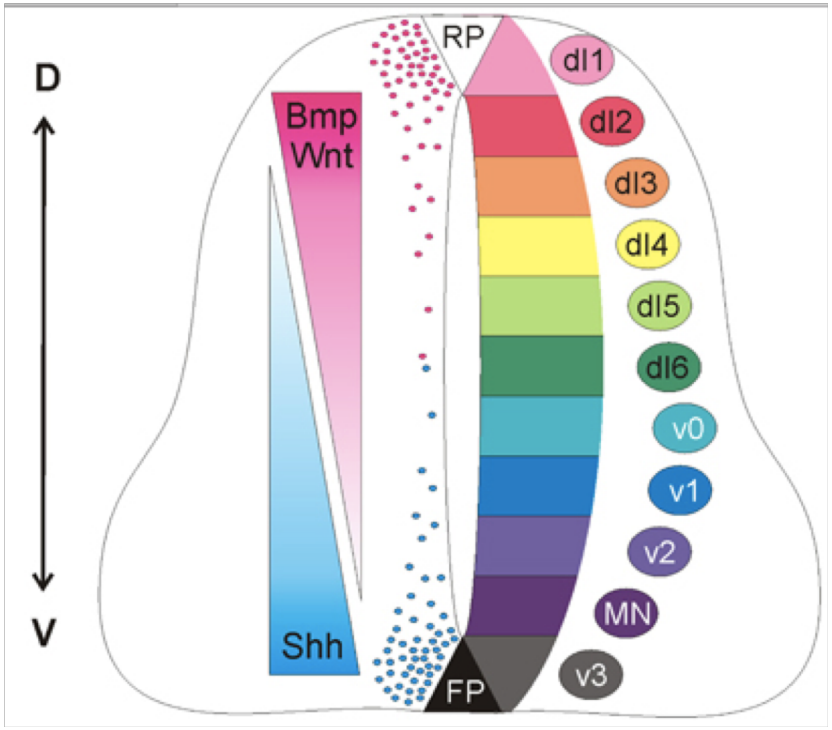


The D-V limits of transcription factor expression are a consequence of Shh signaling activity

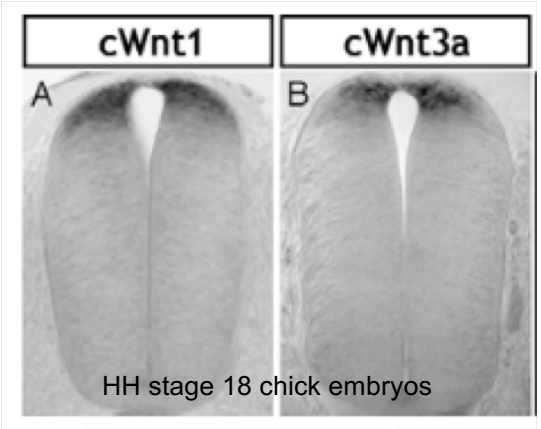
The boundaries between progenitor domains are established by cross repressive interactions between complementary class I/II protein pairs

The specification of dorsal patterning in the dorsal spinal cord

Multiple members of the **Wnt** and **BMP** families are critical for dorsal patterning



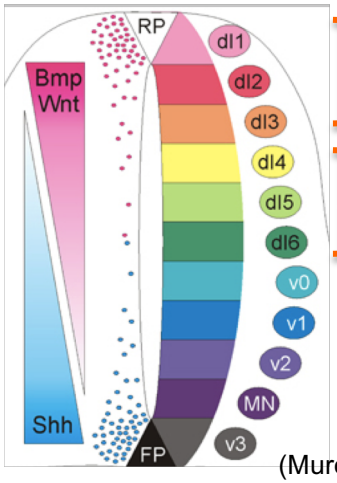
Wnt signaling controls dorsal progenitor proliferation



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

Wnt proteins:

- Mitogenic signals for neural cells
- Neural cell fate specification



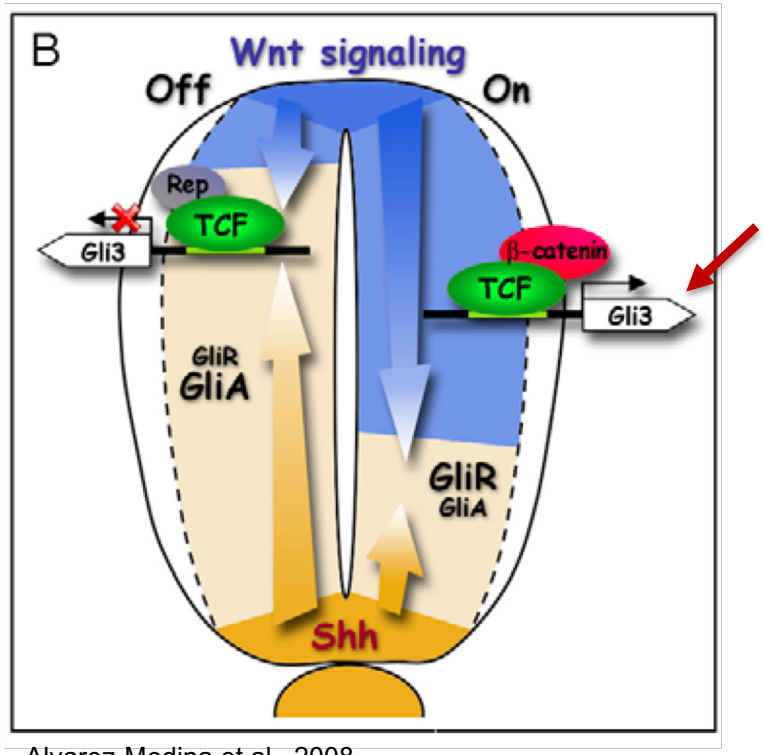
Severe reduction

Expansion

Mouse Double KO Wnt1;3a

(Muroyama et al., 2002)

Interaction beten Wnt signaling and Shh activity



Alvarez-Medina et al., 2008

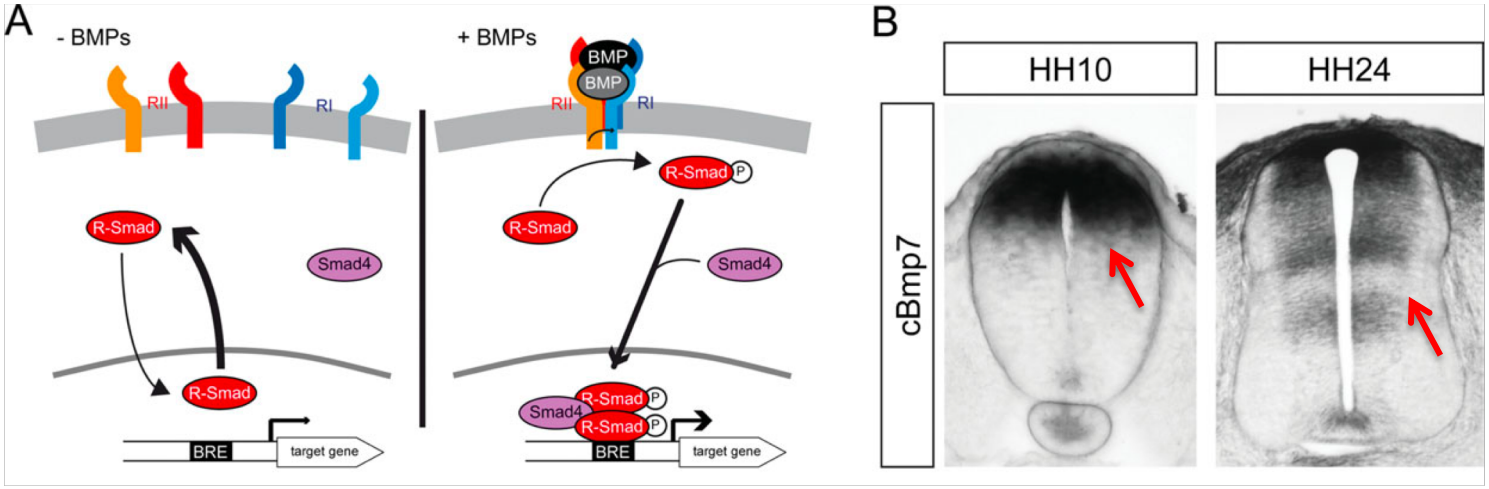
<u>Proposed model:</u>

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

The role **of BMP** in patterning the dorsal neural



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

- BMPs in the non-neural ectoderm promote the formation of the RP at the dorsal midline of the spinal cord
- RP itself expresses a range of BMPs in nested domains (rodents BMP6,7,GDF7 – chick BMP4,5,7,9)
- At later stages, the expression of the BMPs extends more broadly into the dorsal spinal cord

→ BMP signaling is critical *in vivo* for the formation of the dI1, dI2 and dI3 classes of sensory neurons

BMPs have distinct roles directing dorsal spinal fates

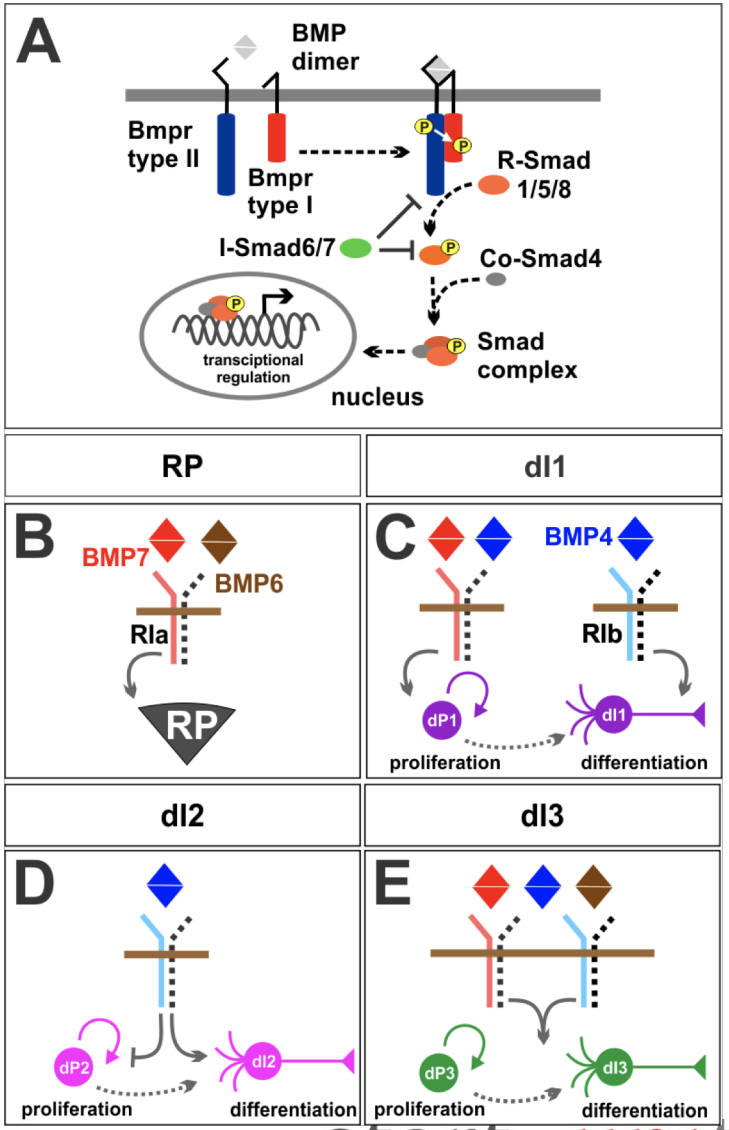
B - BMP6 (mouse) and BMP7 (chicken) are the most effective at directing RP identity through the BmprIa receptor (mouse)

C- Both BMP4 and BMP7 can promote dP1 patterning through BmprIa or BmprIb (chicken), but only BMP4 directs progenitors to differentiate as dI1s through BmprIb (mouse and chicken)

D - BMP4 specifically directs dP2s to differentiate in dI2 in chicken

E - All BMPs tested in both species, including BMP4, BMP5, BMP6 and BMP7, can act though either BmprIa or BmprIb to promote modest levels of dP3 proliferation and their differentiation into dI3s

No BMP was identified that direct the dI4-dI6 fates, rather BMP signaling tends to suppress the ventral-dorsal fates



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