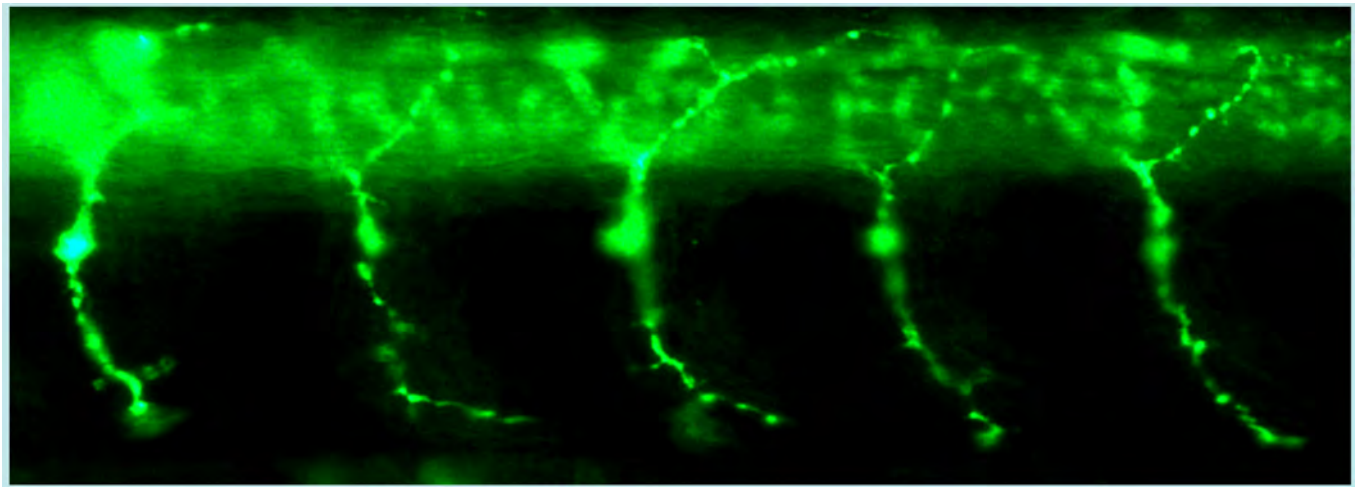
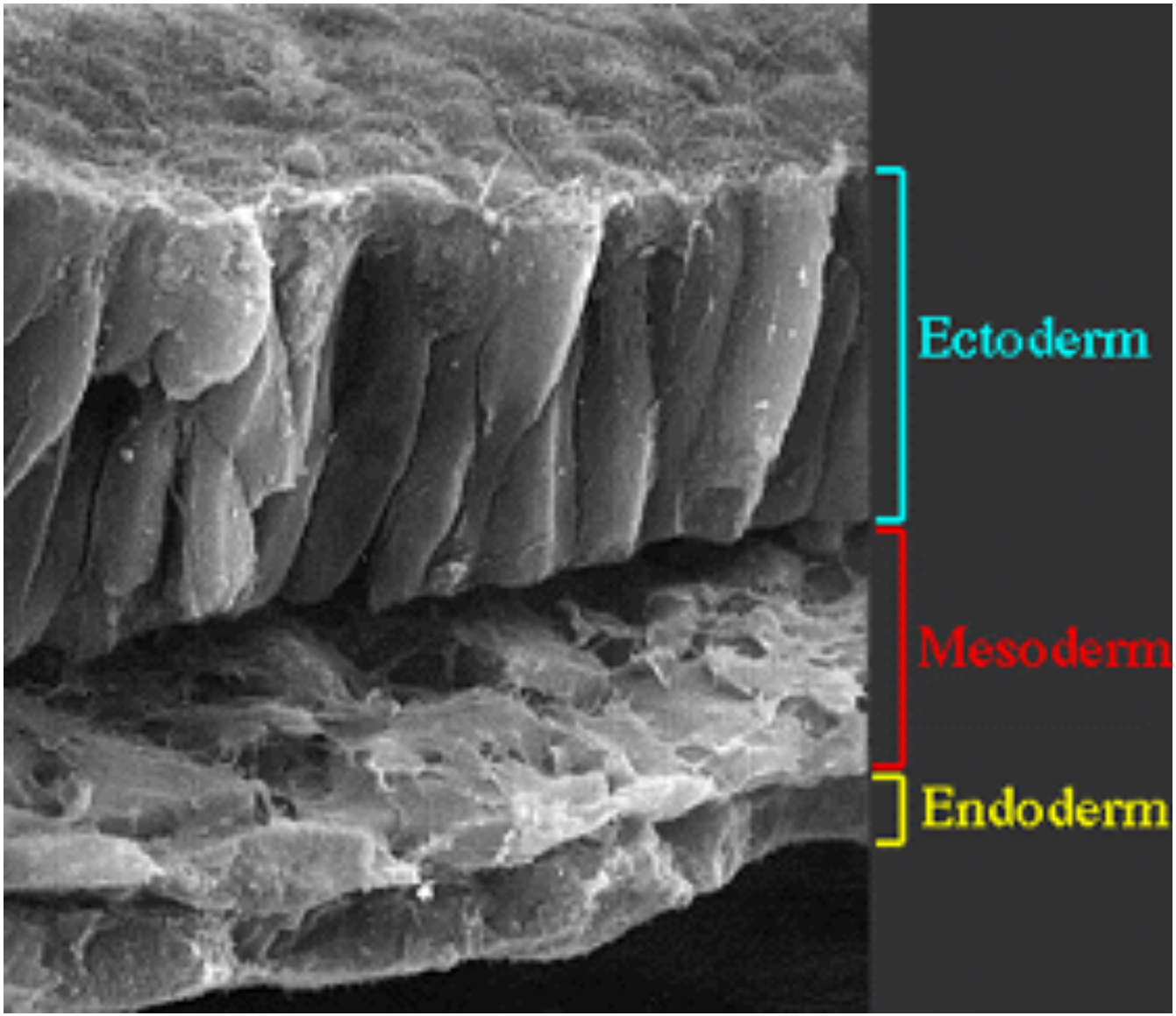
Motor and sensory neuron development

MVIMG 747
Neuromuscular Biology
April 12, 2012

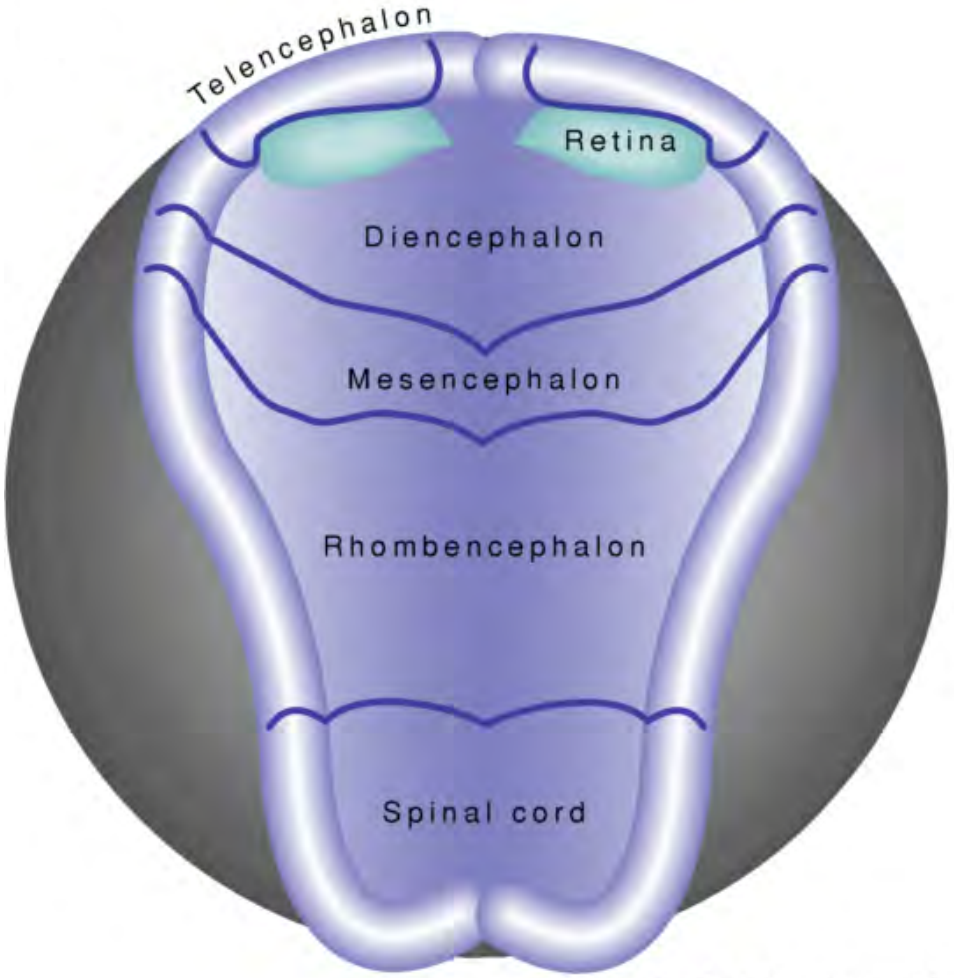


After gastrulation there are three germ layers



Neurulation

The neural plate stage in vertebrates

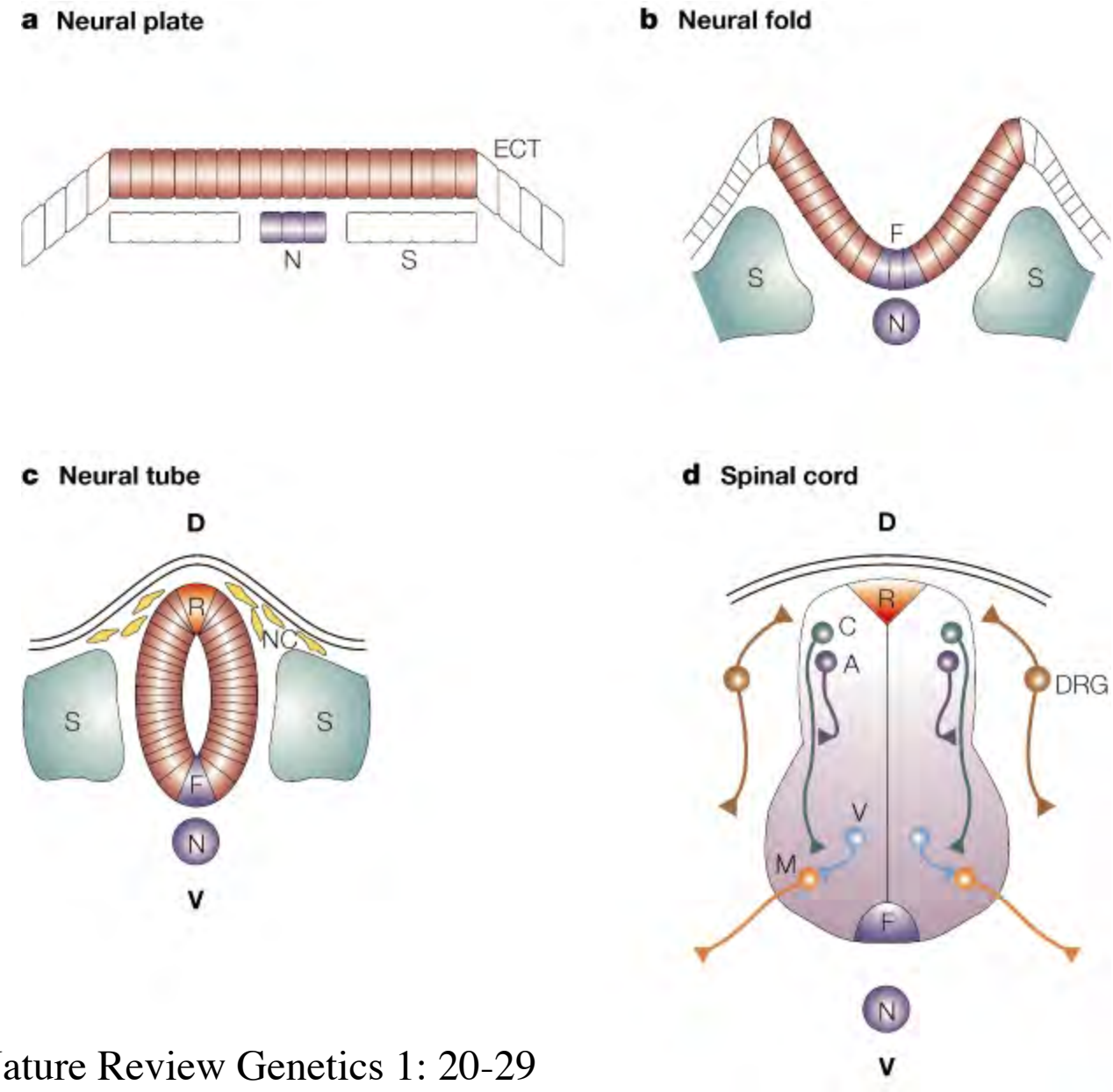


Anterior

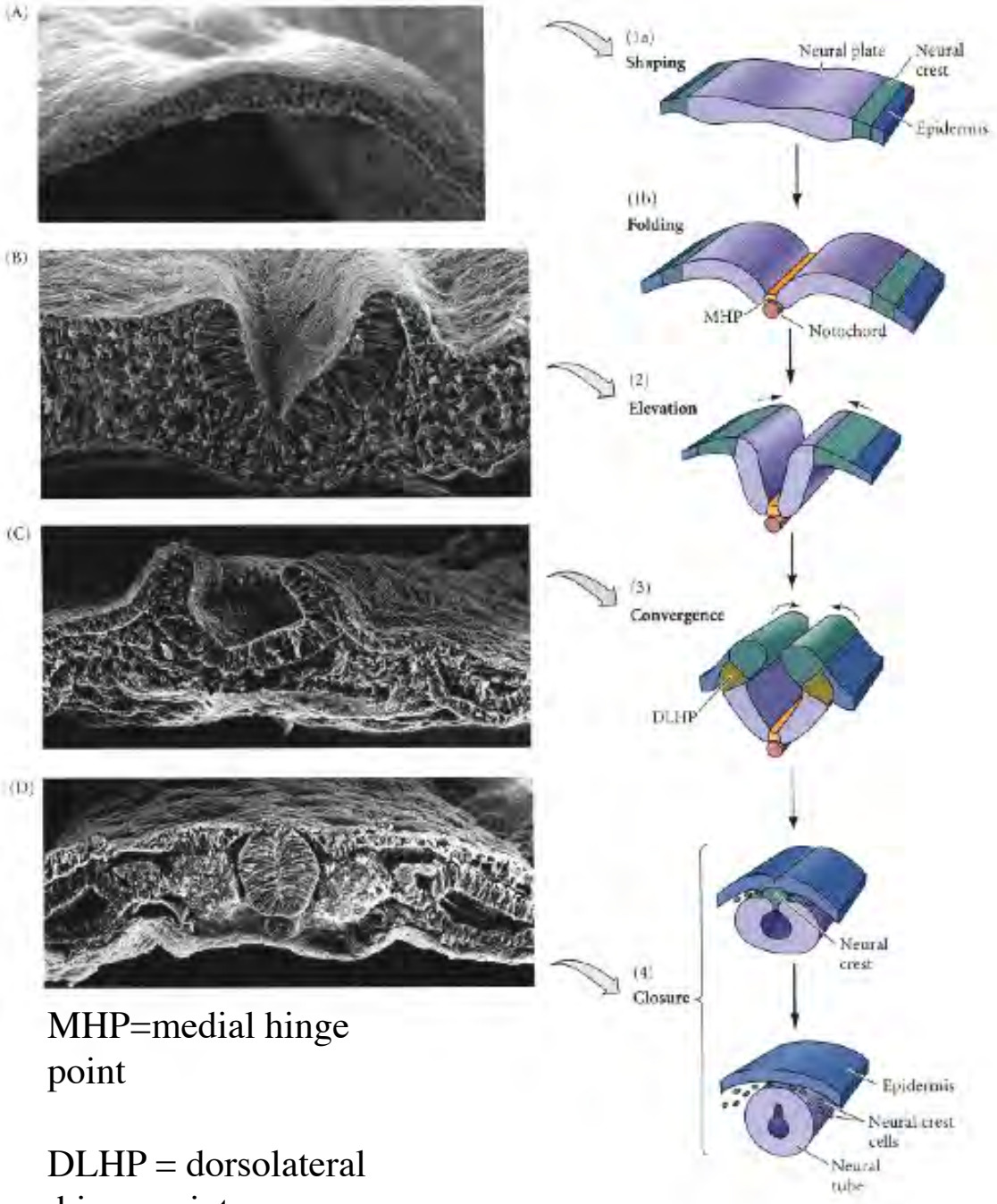
Posterior

Dorsal view

Primary neurulation:



Jessell (2000) Nature Review Genetics 1: 20-29



MHP=medial hinge point

DLHP = dorsolateral hinge point

Cells of the neural plate are the elongated cells in the dorsal region of the ectoderm.

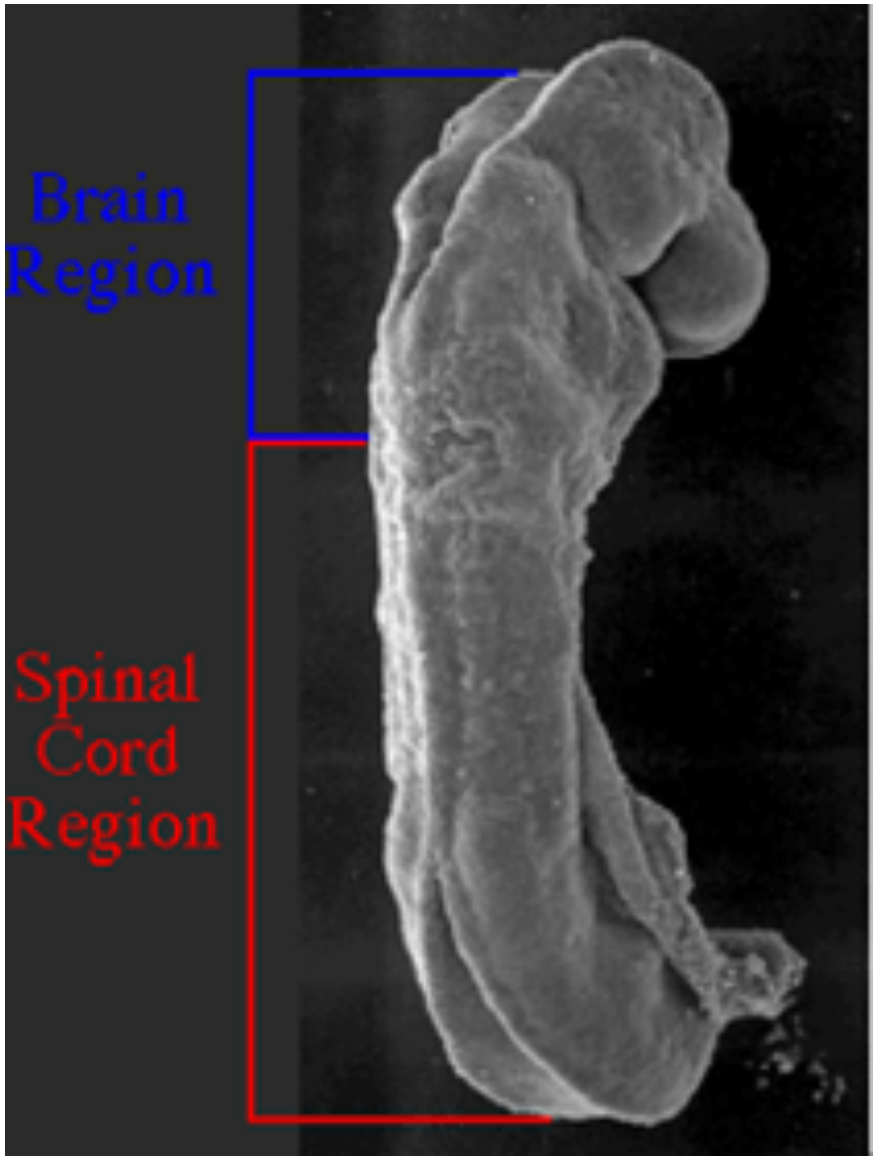
Folding begins as the MHP cells anchor to the notochord and change their shape.

The epidermis moves towards the midine

Convergence of the neural folds occurs as the DLHP become wedge shaped and the epidermal cells push towards the center

Neural folds are brought into contact with one another. Neural crest links the neural tube with the epidermis.

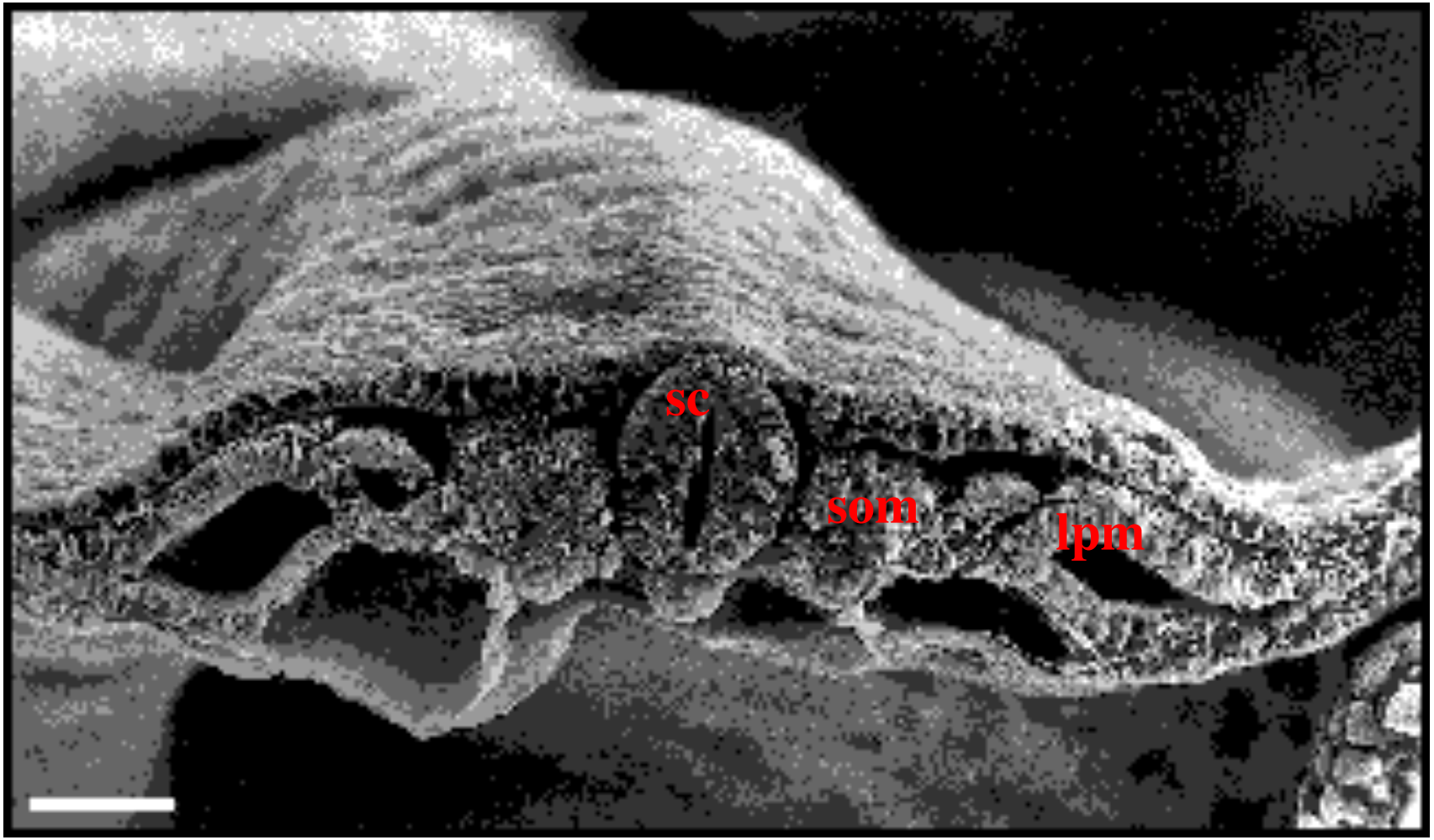
Neural crest disperses



The neural folds fuse forming the portion of the neural tube that will be:
Brain
Spinal cord

Spinal Cord

Scanning EM of chick embryo showing spinal cord



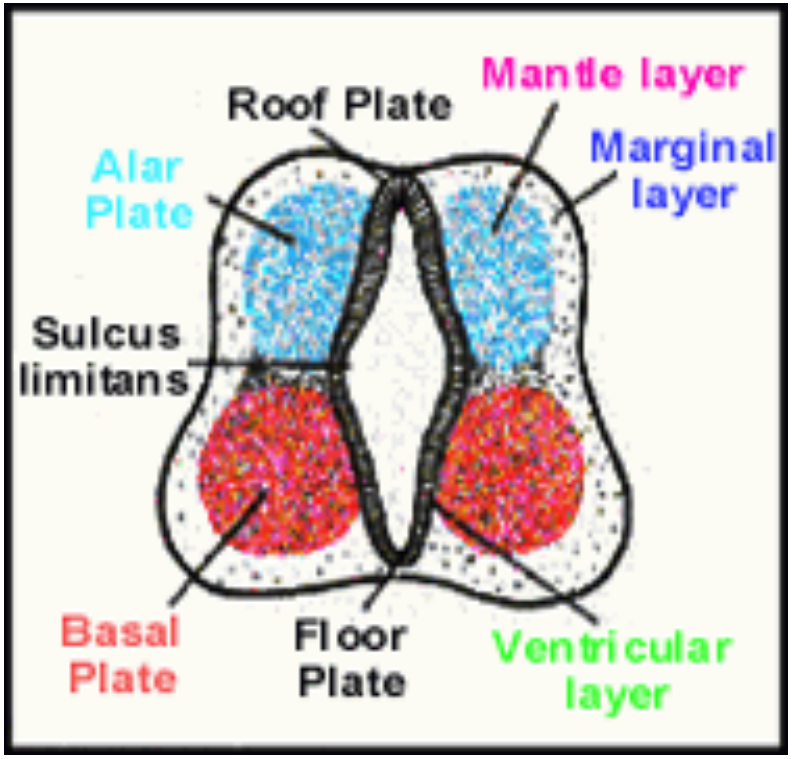
What's in a spinal cord??

1. Neurons (~10%)- interneurons, motoneurons

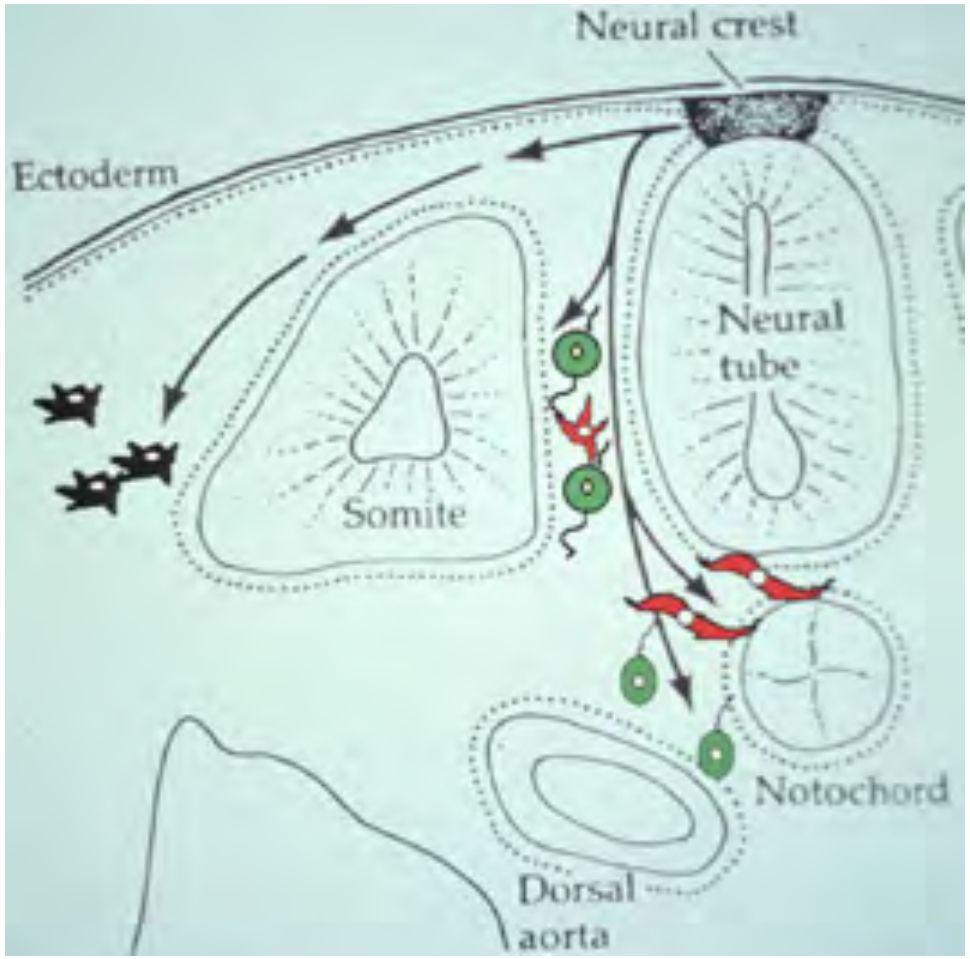
2. Non-neuronal cells (Glial cells) - support cells that are critical for function of the neurons
   a. Astrocytes-make factors that maintain health of neurons (growth factors) and take up or degrade released neurotransmitters.
   b. Oligodendrocytes-myelinate central axons
   c. Macrophages and microglia- immune cells that clean up cellular debris

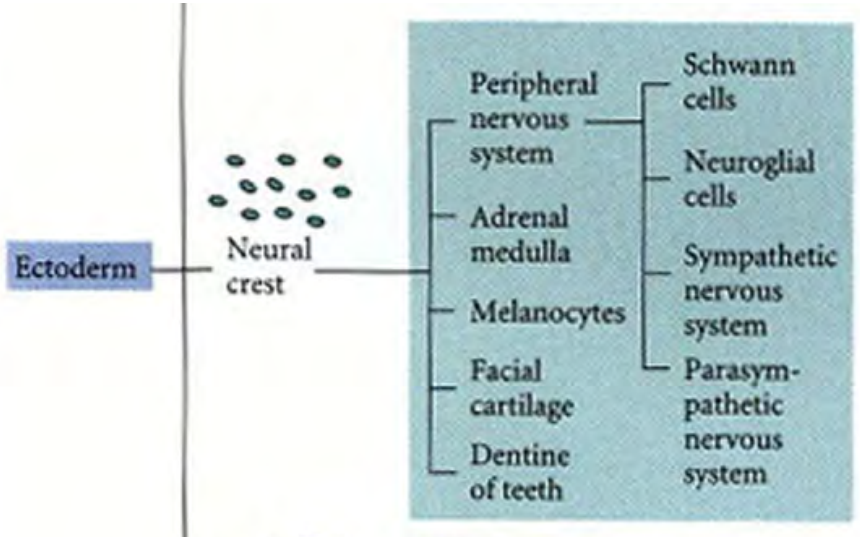
3. Fiber tracts- ascending and descending axon tracts

The cells of the neural tube form 3 layers:
Ventricular layer = undifferentiated, dividing cells
Mantle layer = differentiating neurons (gray matter)
Marginal layer = contains nerve fibers (white matter)



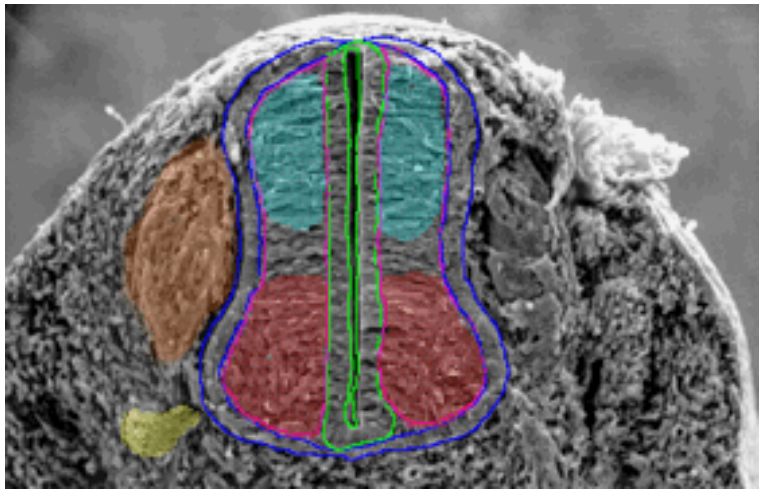
Sensory neurons develop from the neural crest and reside outside of the CNS





Neural crest cells start in the neural tube but then migrate and give rise to numerous cell types including neurons of the peripheral nervous system and melanocytes

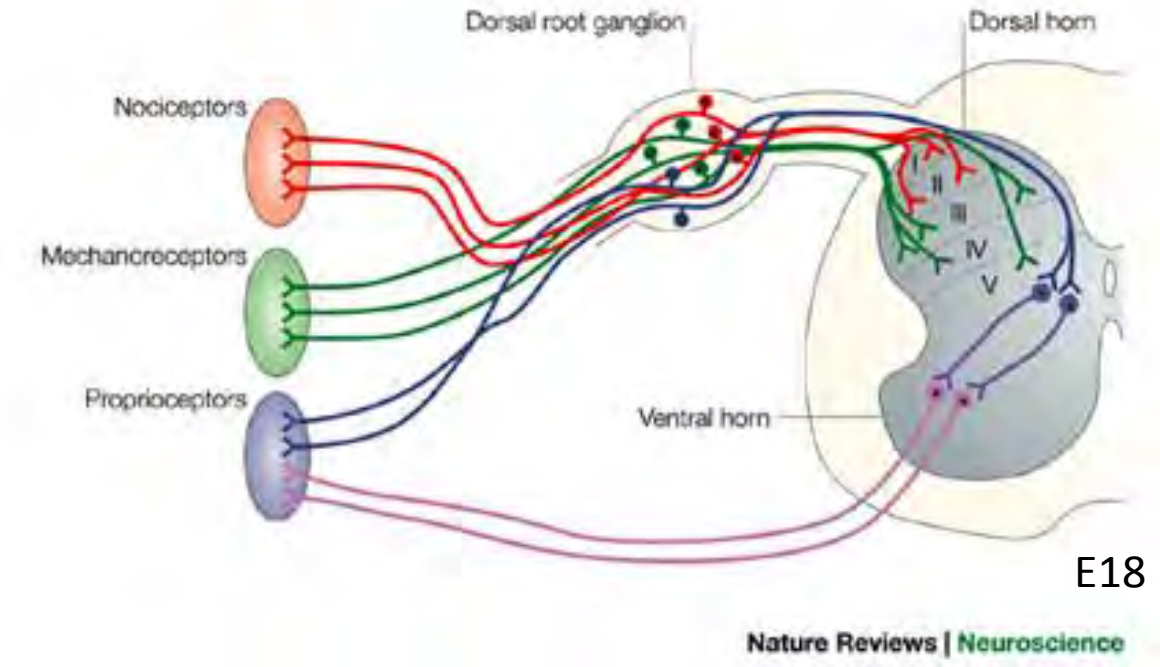
Alar plate (dorsal) = occupied by interneurons (receive sensory input)
Basal plate (ventral) = occupied by motor neurons and interneurons



The dorsal root ganglion (orange) sits outside of the spinal cord and projects sensory axons into the dorsal spinal cord.

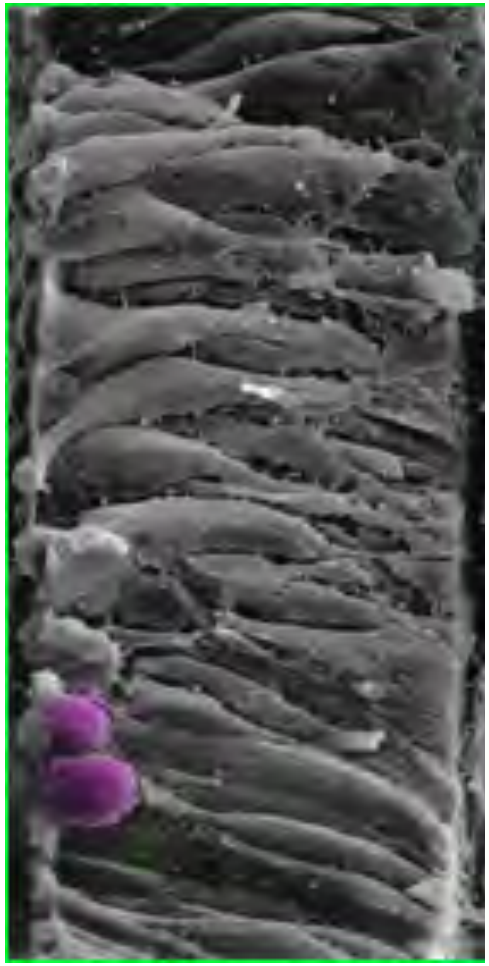
Motor axons exit from the ventral spinal cord (yellow).

Sensory neuron cell bodies reside here

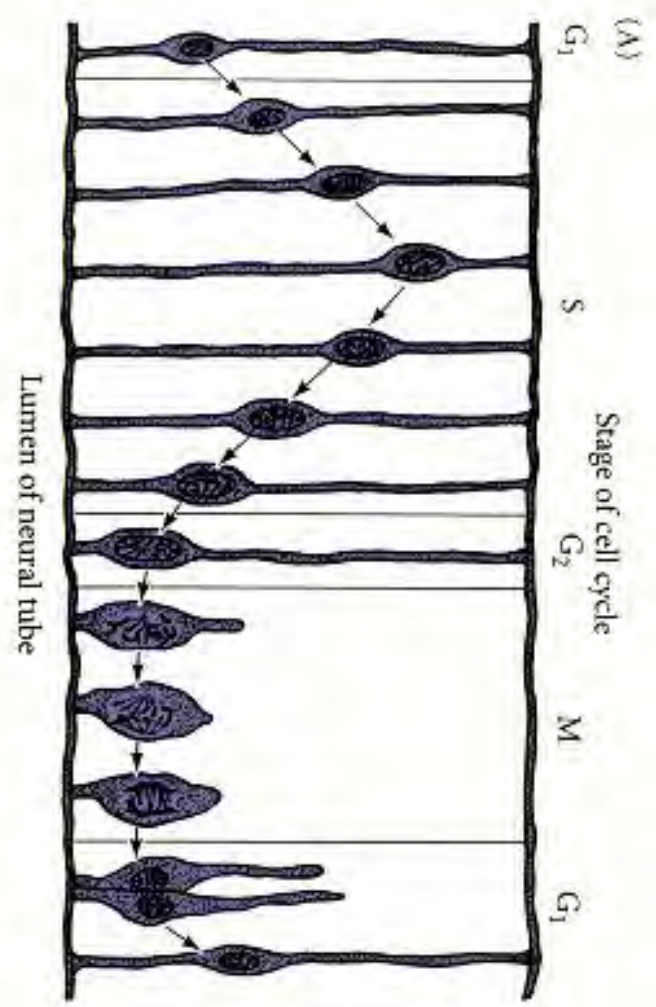


E18

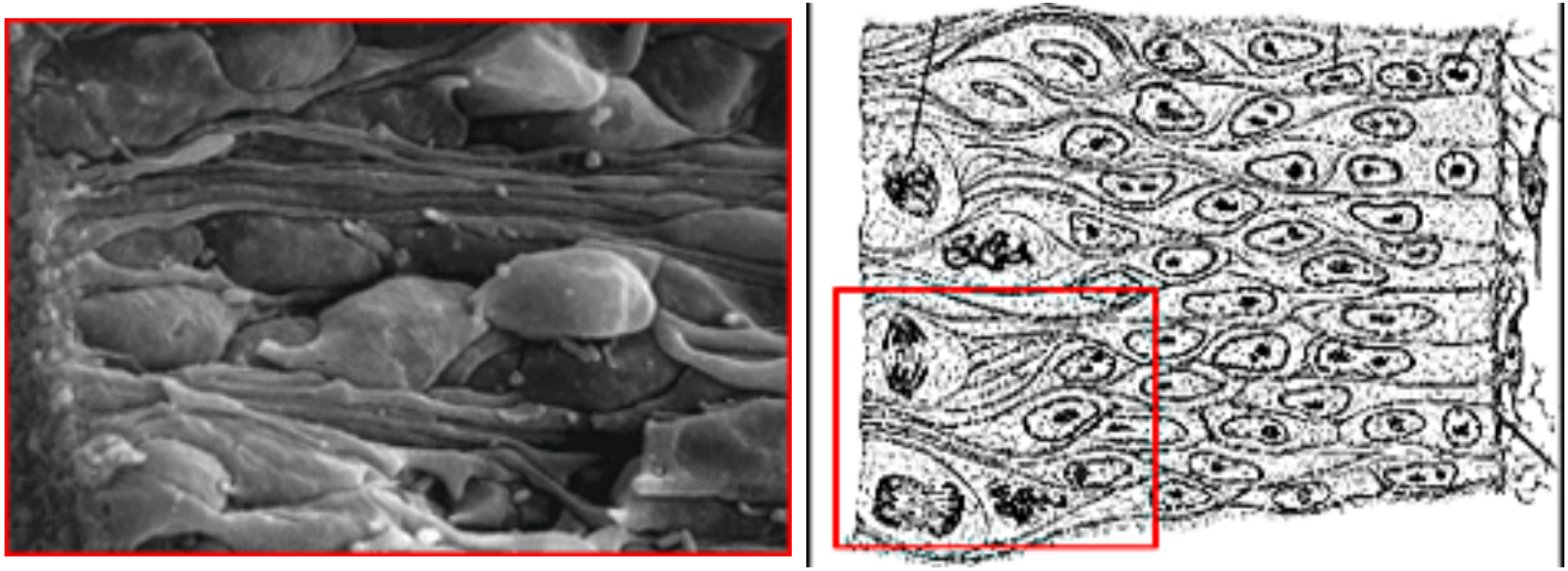
Cells in the spinal cord are pseudostratified epithelial cells.
The nuclei of dividing cells are located at the ventricular surface



lumen

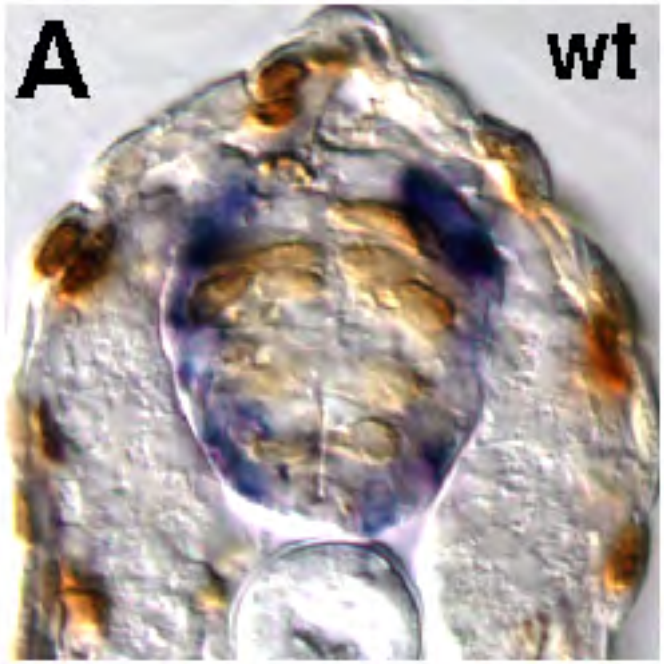


As development proceeds, cells opposite the lumenal border (ventricular zone) begin to differentiate and migrate laterally.



lumen

Example of a section through a zebrafish spinal cord showing both dividing and differentiated cells



Brown = marker for dividing cells

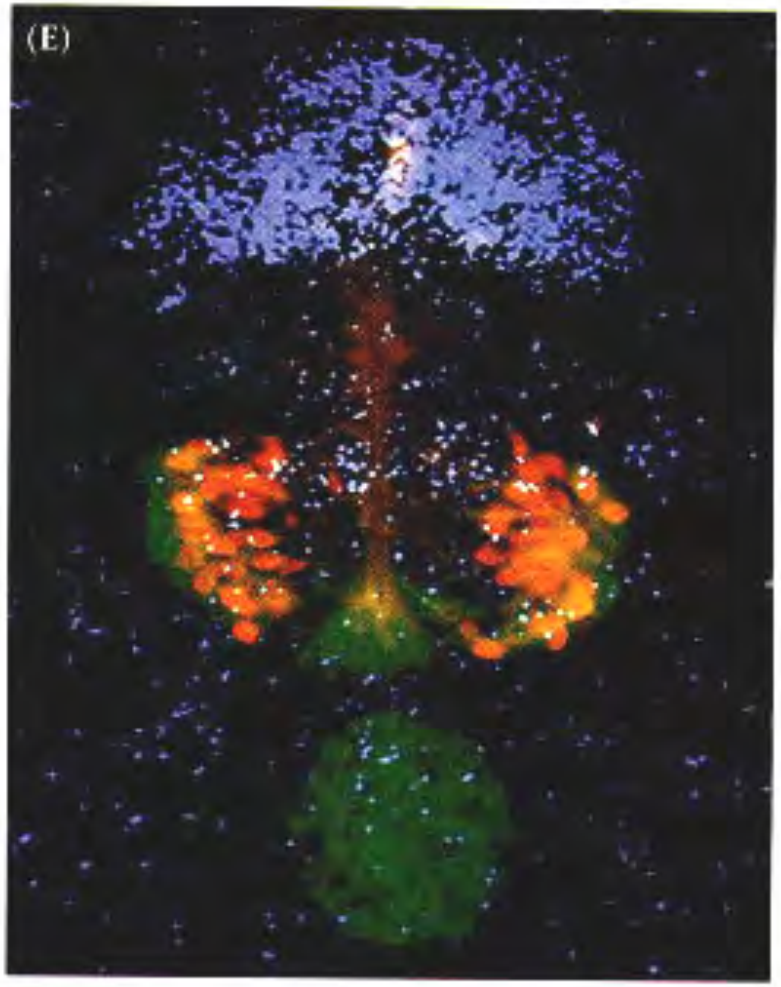
Blue= marker for differentiated neurons

Appel et al. (2001) BMC Developmental Biology 1: 13

How are different cell types determined in the spinal cord?
-Signaling from neighboring tissues

spinal cord
(chick)

notochord



Dorsalin=blue

MN=yellow

Shh =green

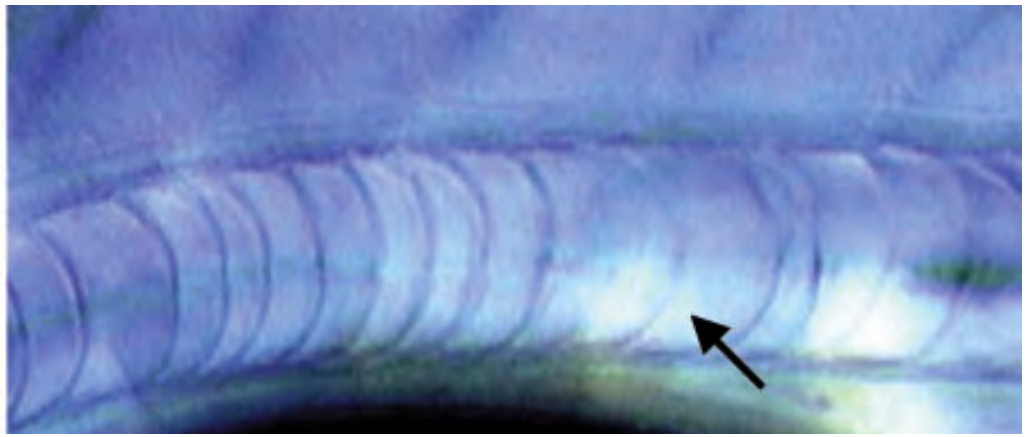
Gilbert

Notochord
(first described by von Baer ~1830s)

Mesodermally derived tissue that gives the embryo rigidity until the vertebral column forms and serves as an important signaling center during development.

After it role in signaling has occurred and once the vertebral column forms, the notochord degenerates.
An exception is in between vertebrae where the notochord cells form the tissue of the intervertebral discs.

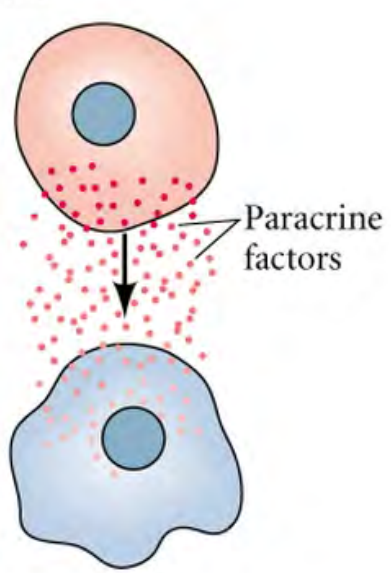
The notochord starts at the midbrain and runs along the length of all chordate animals.



Lateral view of a zebrafish notochord

notochord is an important signaling center during development

Paracrine signaling:



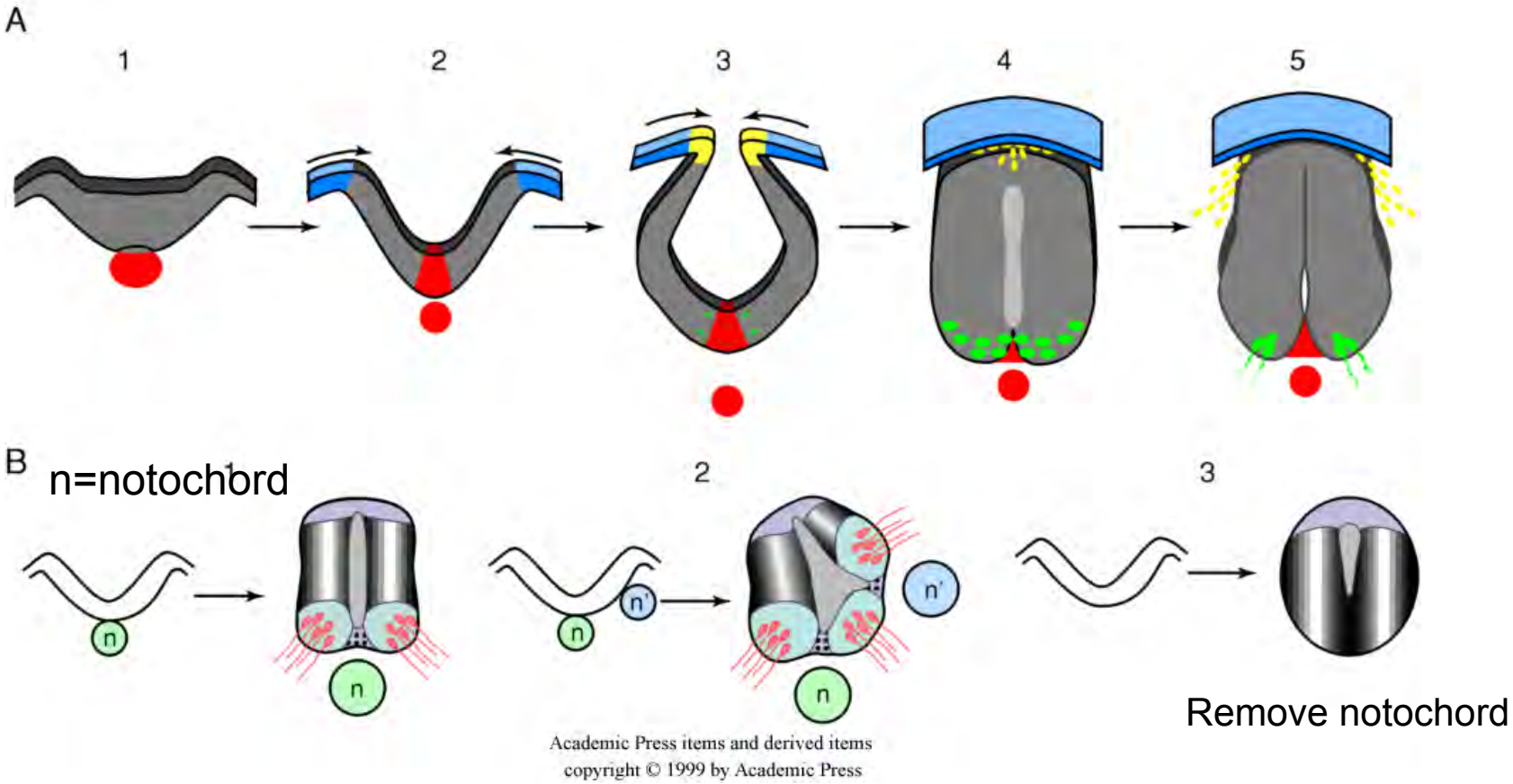
Diffusion of inducers from one cell to another

Induction of numerous organs/tissues is affected by a small set of paracrine factors that are highly conserved throughout the animal kingdom

- **Hedgehog family (Hh)**
- Wingless family (Wnts)
- Transforming growth factor beta family (Tgfb)
- Fibroblast growth factor family (Fgf)

There is considerable debate on how far paracrine factors can operate. Some only act on neighboring cells, but others can diffuse over many cell diameters.

The notochord is essential for ventral spinal cord patterning



Fundamentals of Neuroscience.First edition. Chapter 15

Yamada et al (1991) Cell 64:635-47.

Sonic Hedgehog

Etiology: The first hedgehog gene was identified in the Drosophila segmentation screens that led to the Nobel Prize for Eric Wieschaus, Christiane Nusslein-Volhard and Edward Lewis (1978). In loss of function mutants, denticles (hair-like projections of epidermal origin) are disorganized and reminded the scientists of a hedgehog.

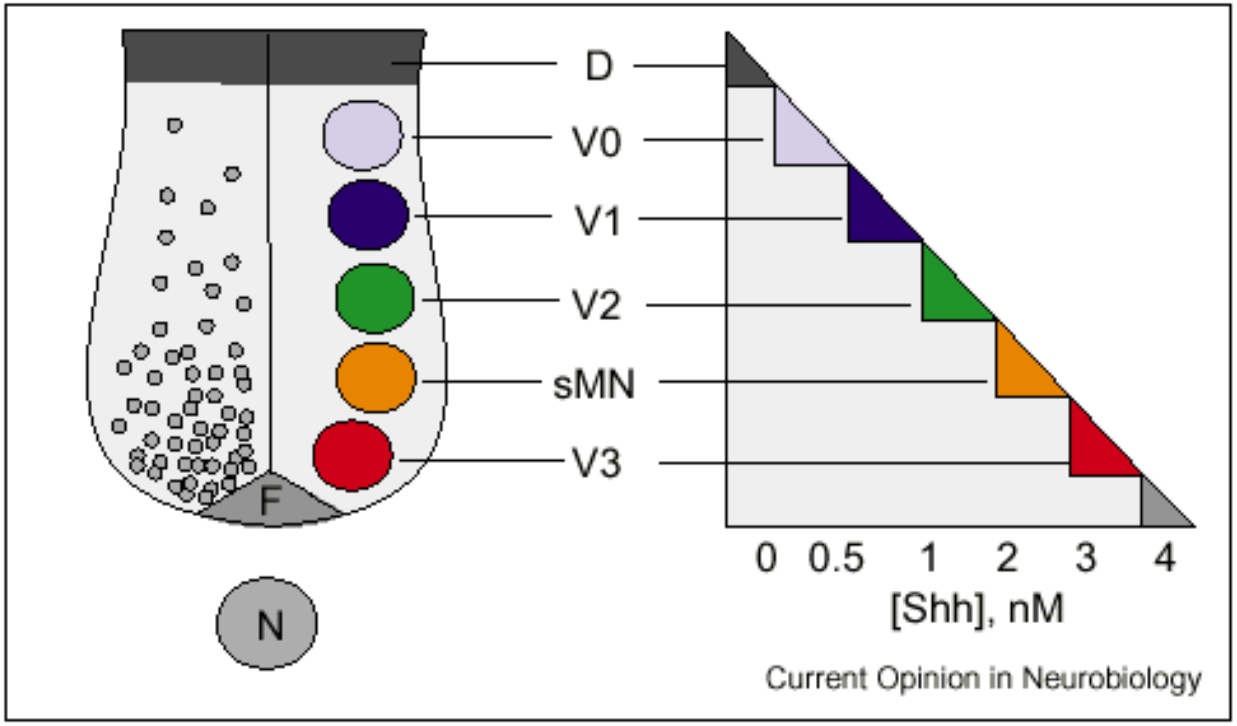
1. Appearance of distinct cell types at defined positions in the ventral neural tube is dependent on inductive signals that derive from the notochord (and subsequently the spinal cord floor plate)

2. These activities appear to be mediated by the secreted protein Sonic Hedgehog (Shh). Shh is both necessary and sufficient in vivo and in vitro to induce the differentiation of most ventral cell types.

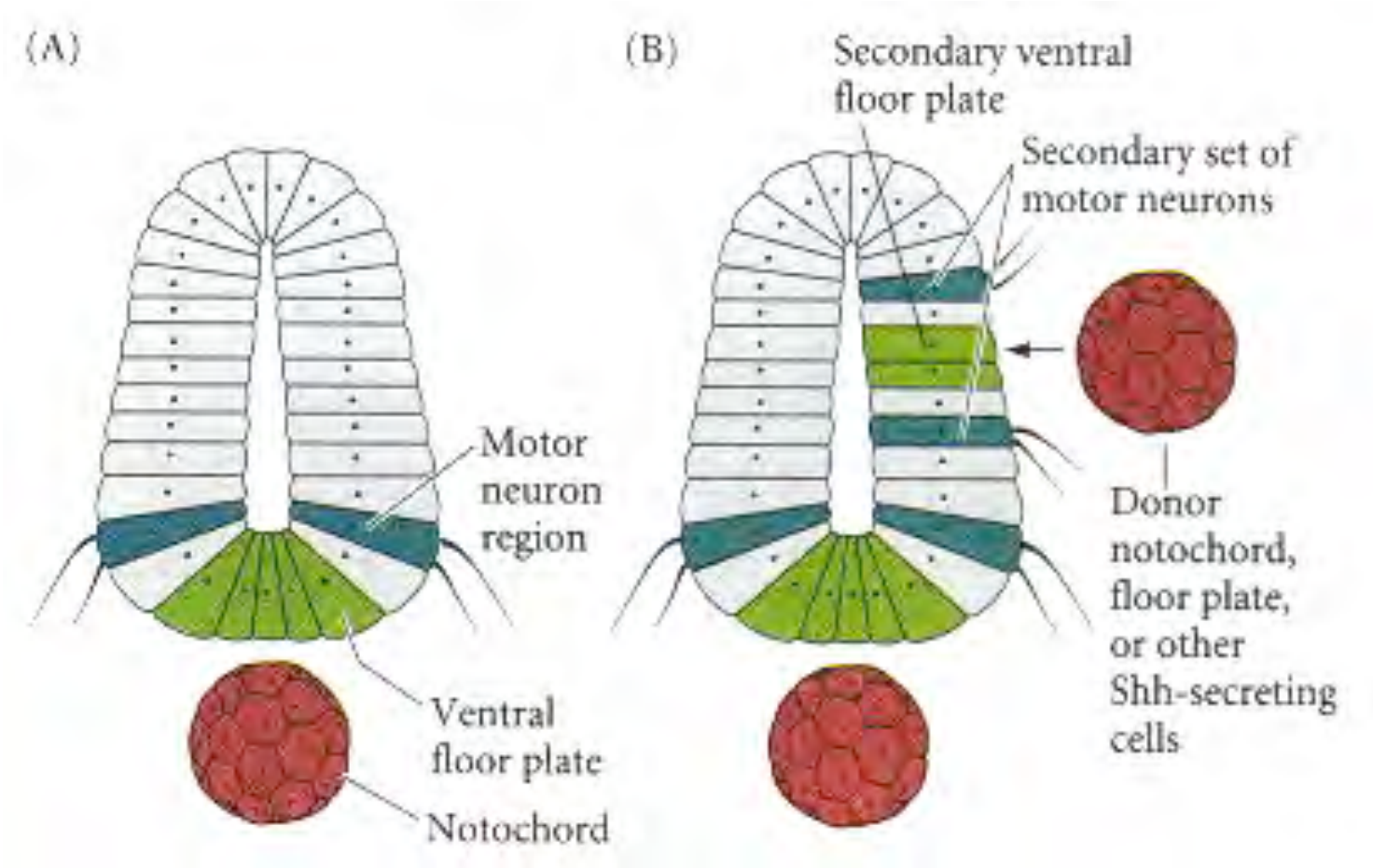
3. Shh is produced by the notochord and floor plate at times when these two cell types exhibit their inductive capabilities

Generation of neuronal diversity in response to graded Shh signaling

Hedgehog genes code for secreted proteins that bind to target receptors and elicit concentration dependent responses.

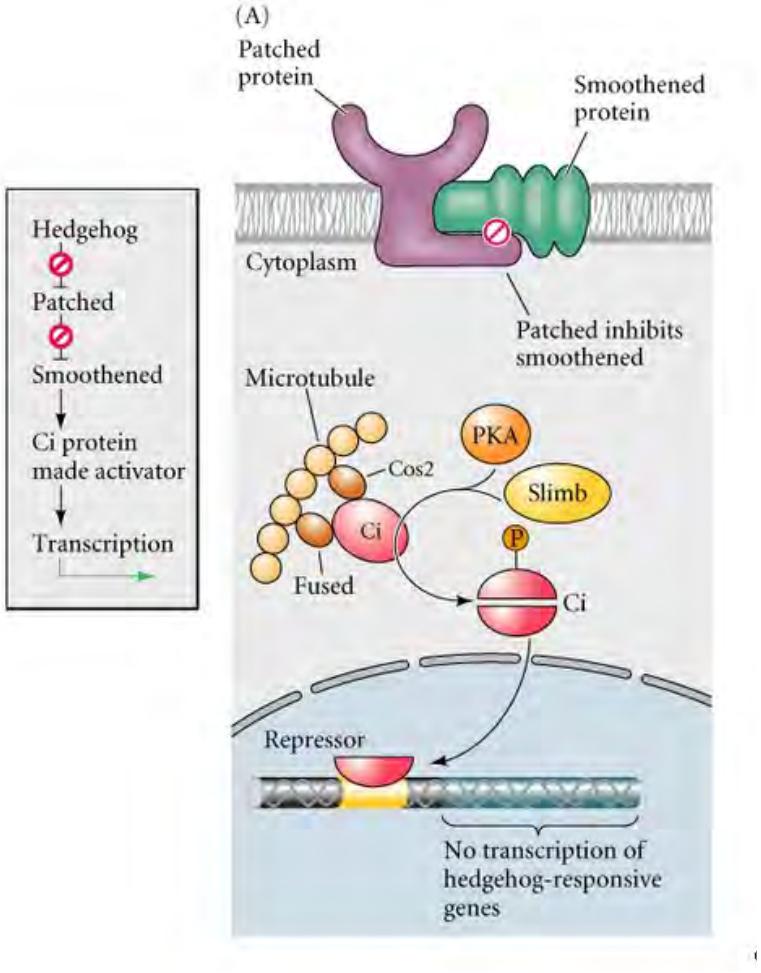


Briscoe, J. and Ericson, J. (2001). Specification of neuronal fates In the ventral neural tube. Current Opinion in Neurobiology 11: 43-49


(A)
Motor neuron region
Ventral floor plate
Notochord
(B)
Secondary ventral floor plate
Secondary set of motor neurons
Donor notochord, floor plate, or other Shh-secreting cells

Gilbert

The Shh signaling pathway



Shh is a diffusible ligand that is cleaved and modified

Shh relieves patched (Ptc) inhibition of the smoothened signaling pathway

PKA and Slimb cleave Ci.

In the presence of Hh, Ci is not cleaved and acts as a transciptional activator.

In vertebrates the Ci proteins are called Gli (1, 2, 3)

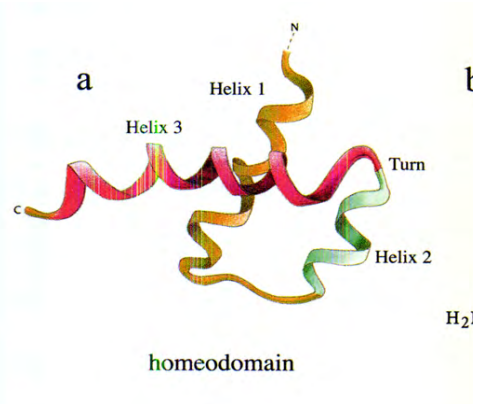
How do neural progenitor cells interpret and respond to small changes in the Shh activity gradient??

1. Data suggests that homeodomain transcription factors are involved

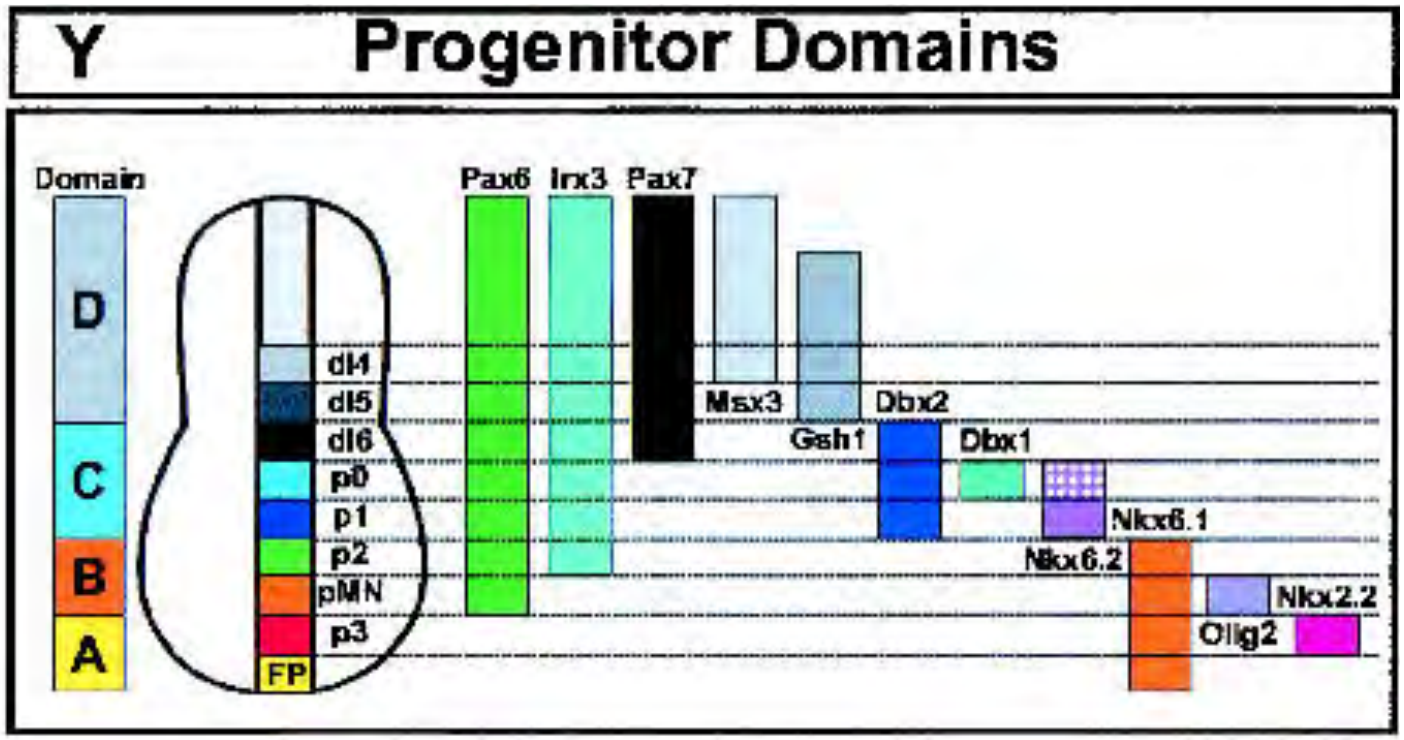
2. Graded Shh signaling sets up 5 domains of progenitor cells by Controlling the expression of a group of homeo-domain proteins

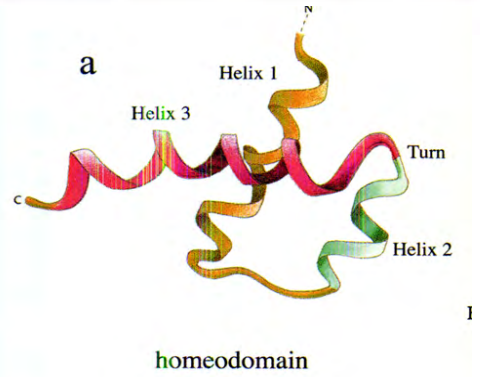
- **class I: repressed by Shh (example Pax 6)**
- **class II: activated by Shh (example Nkx2.2)**

3. Domains are refined and maintained by cross inhibition between homeodomain proteins



Homeobox proteins are expressed in spinal cord domains





dorsal

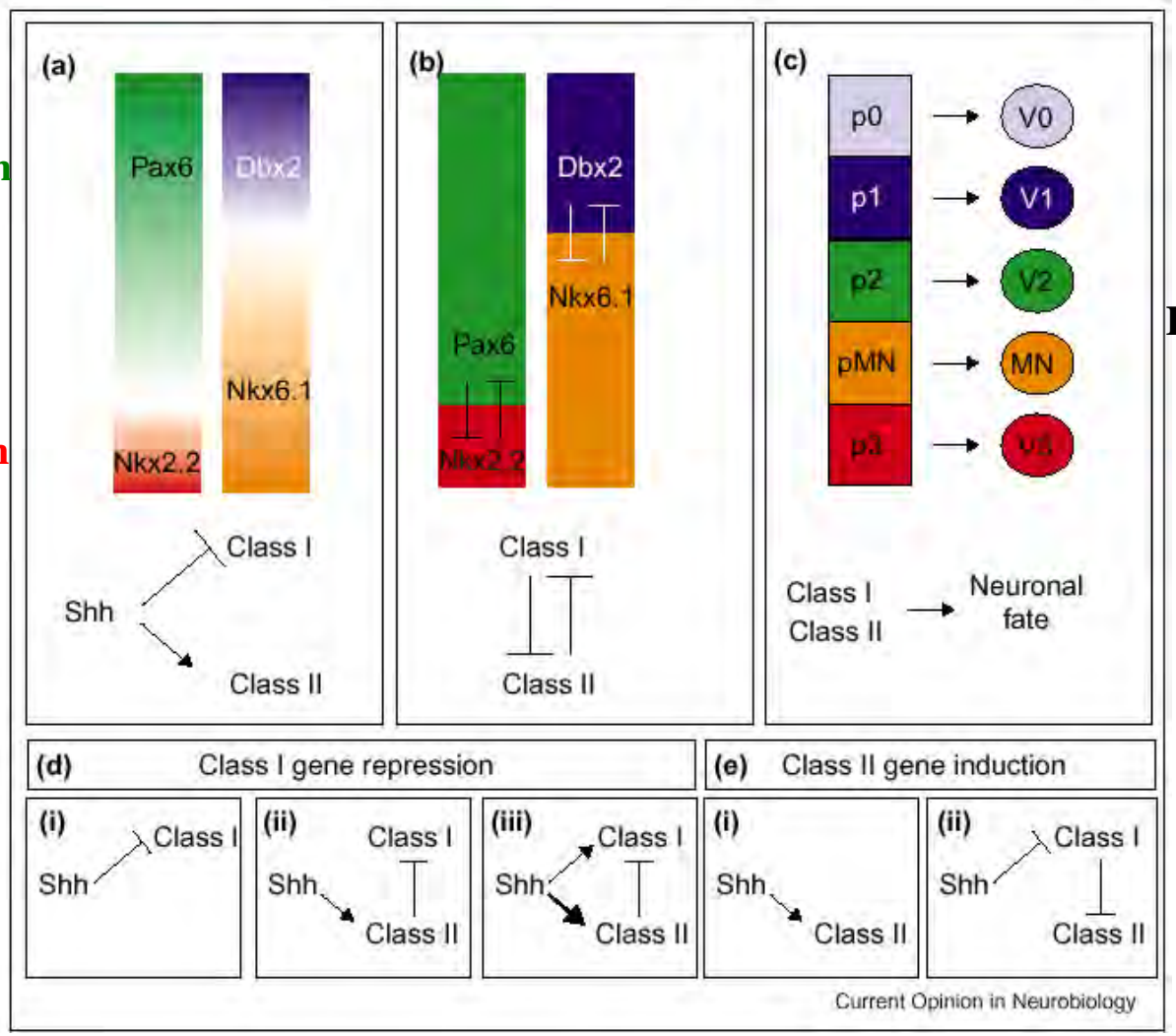
ventral

Shh gradient

Step 1 Step 2 Step 3

Class I Homeodomain proteins

Class II Homeodomain proteins

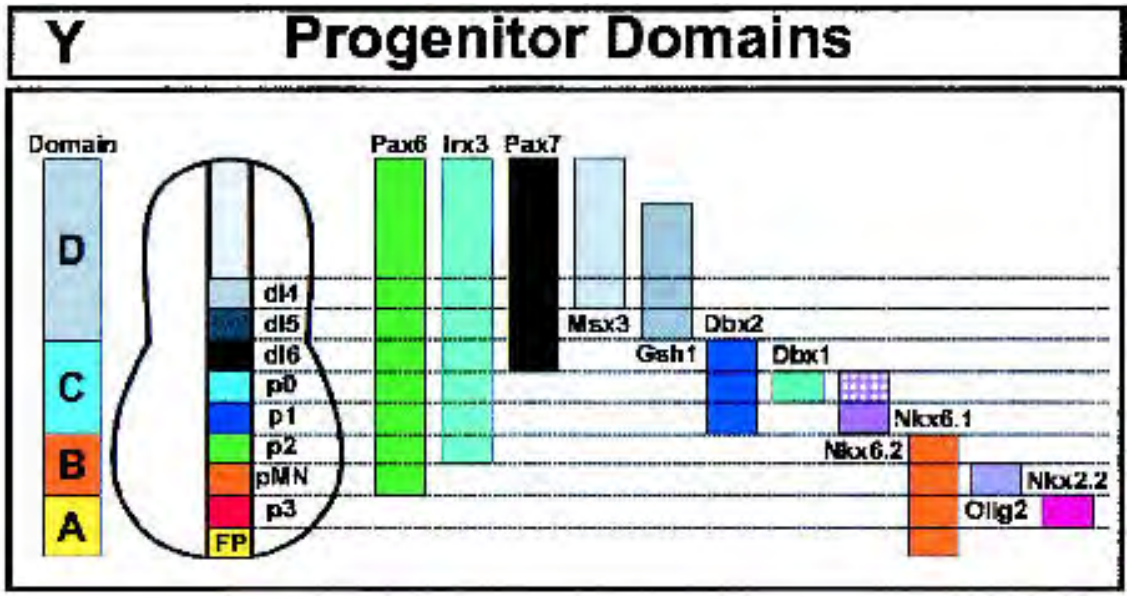


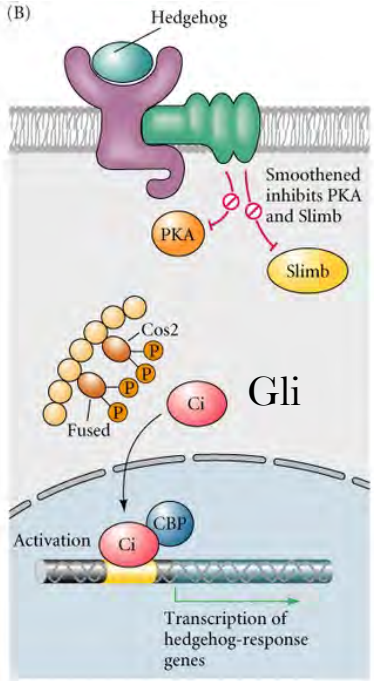
Progenitor domains

possible regulation schemes

Briscoe, J. and Ericson, J. (2001) Specification of neuronal fates in the ventral neural tube. Current Opinion in Neurobiology 11: 43-49

Homeobox proteins are expressed in spinal cord domains

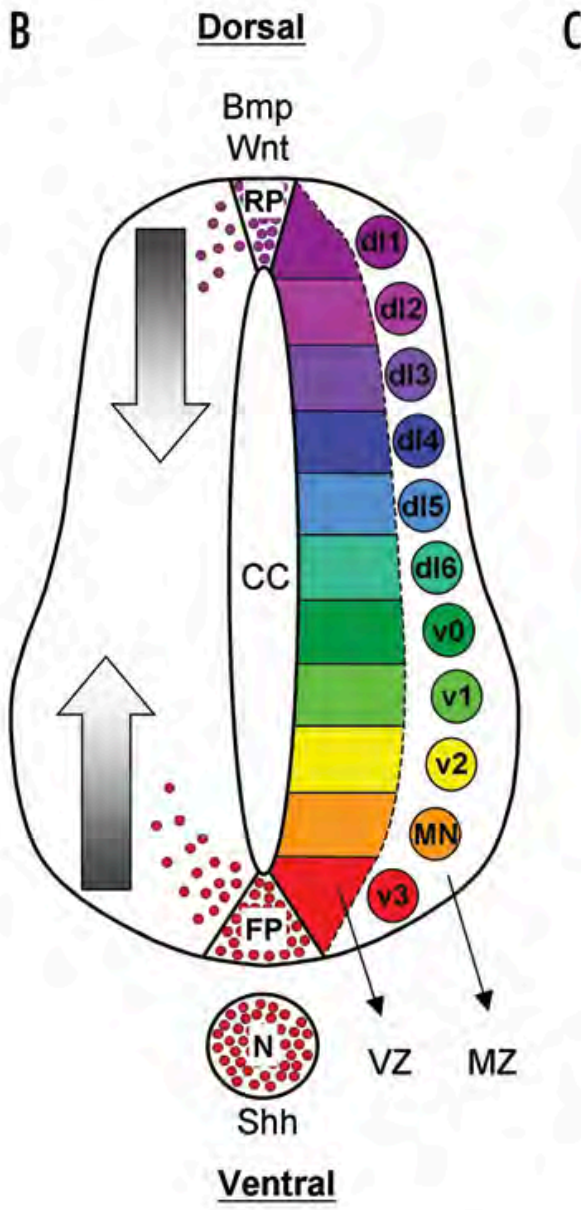




MN domain: Pax6, Nkx6.1, Olig2

Graded Shh translates into graded Gli1/2 activity (transcriptional activators) resulting in homeodomain proteins being expressed (eg Pax6, Nkx6.1, Olig2 in the MN domain). These in turn differentially regulate transcription of other downstream homeodomain genes such as MNR2 and HB9 for motoneurons. These in turn will bind to regulatory regions and regulate genes needed for terminal differentiation.

There are also signals that come from the dorsal spinal cord that contributes to this patterning



Stem Cells

Developmentally relevant signaling factors can induce mouse ES cells to differentiate into spinal motoneurons.

Steps in motoneuron differentiation:

1. Neural ectoderm acquire an anterior fate through regulation of BMPs, FGFs, and Wnt signals

2. Neural ectoderm is posteriorized by signals including retinoic acid

4. In response to ventralizing action of Shh, spinal progenitors acquire a motoneuron fate.

Wichterle et al., (2002) Cell 110: 385

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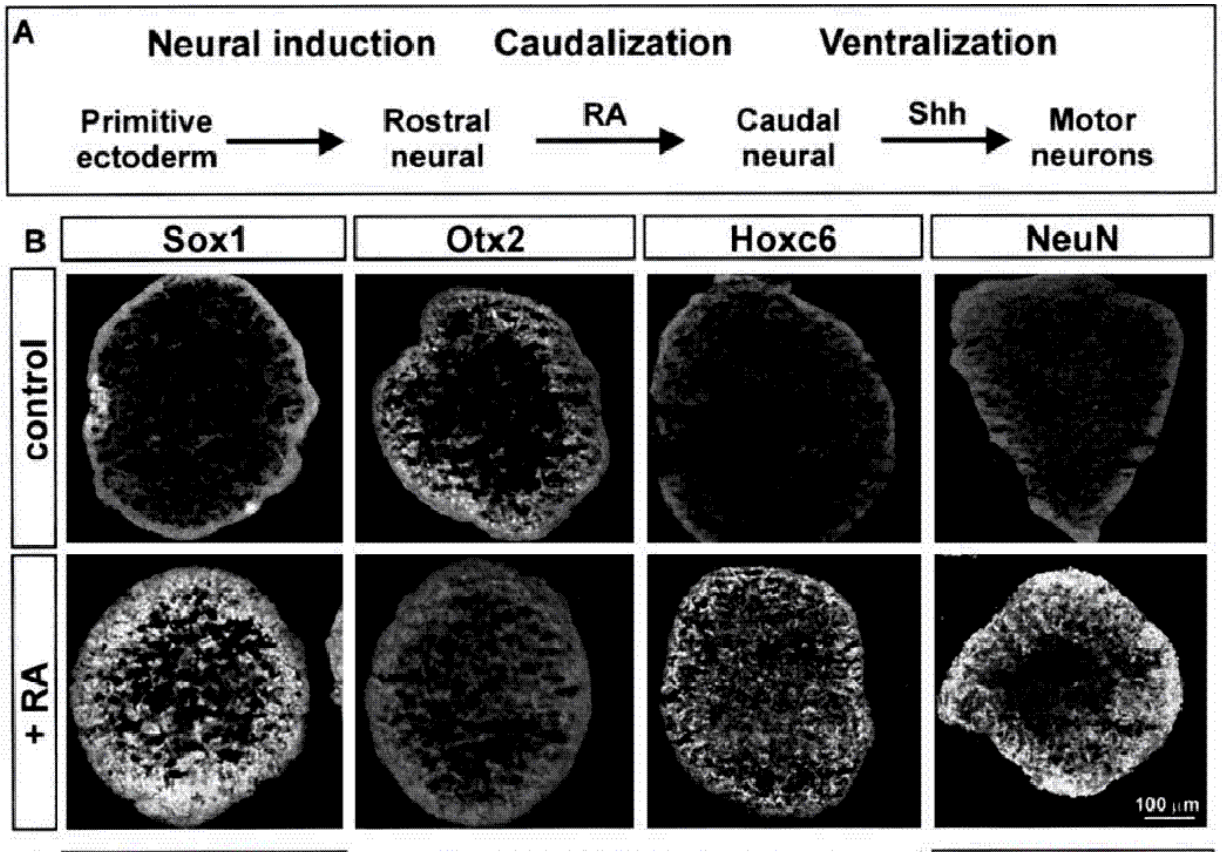
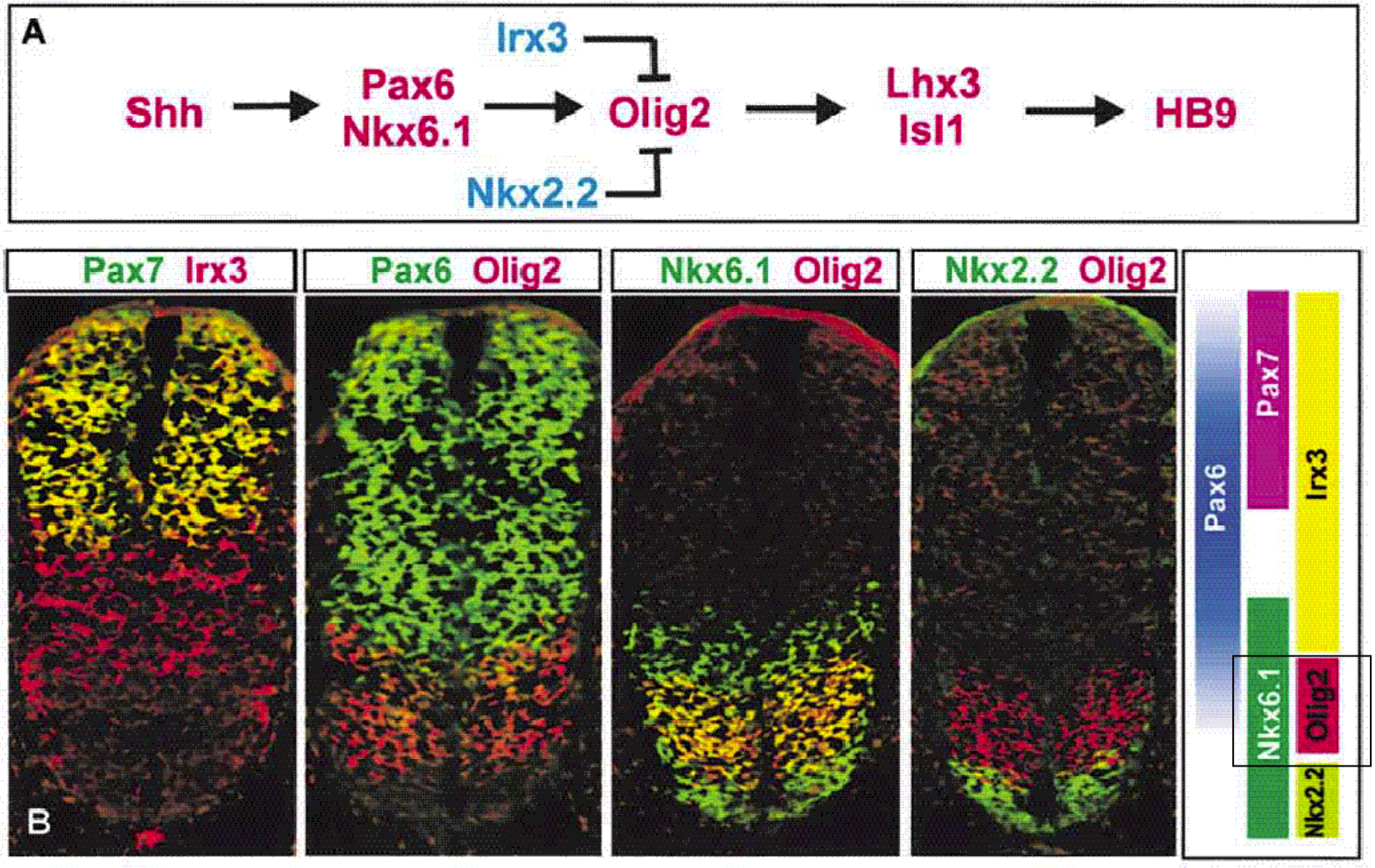


mouse
ES cells grown in aggregate culture form embryoid bodies. Contain about 1000 cells

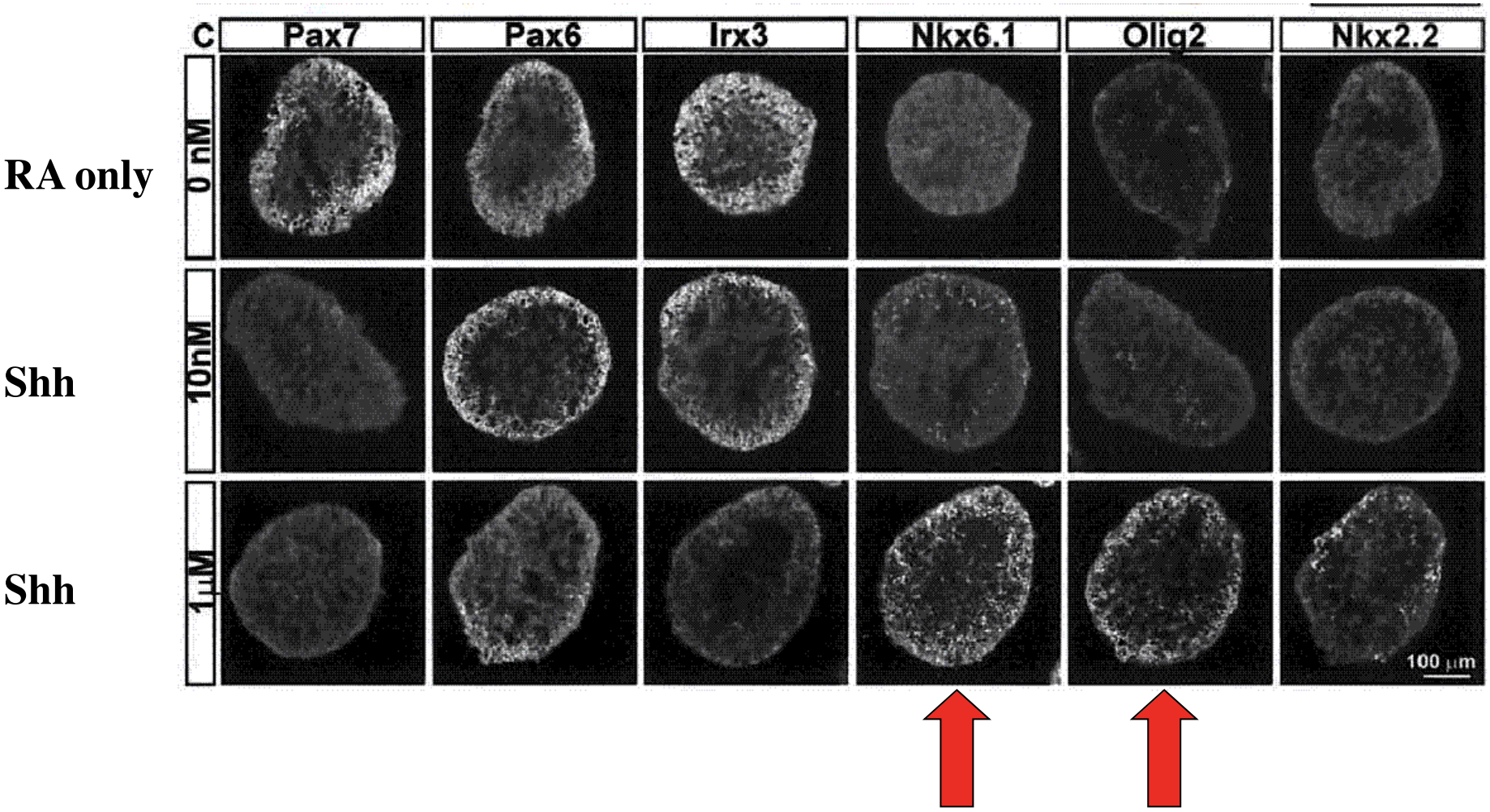
Sox1=panneural marker
Otx2 = anterior marker
Hox6 = posterior marker
NeuN = differentiated neurons

Shh-activated transcription pathway of spinal MN generation

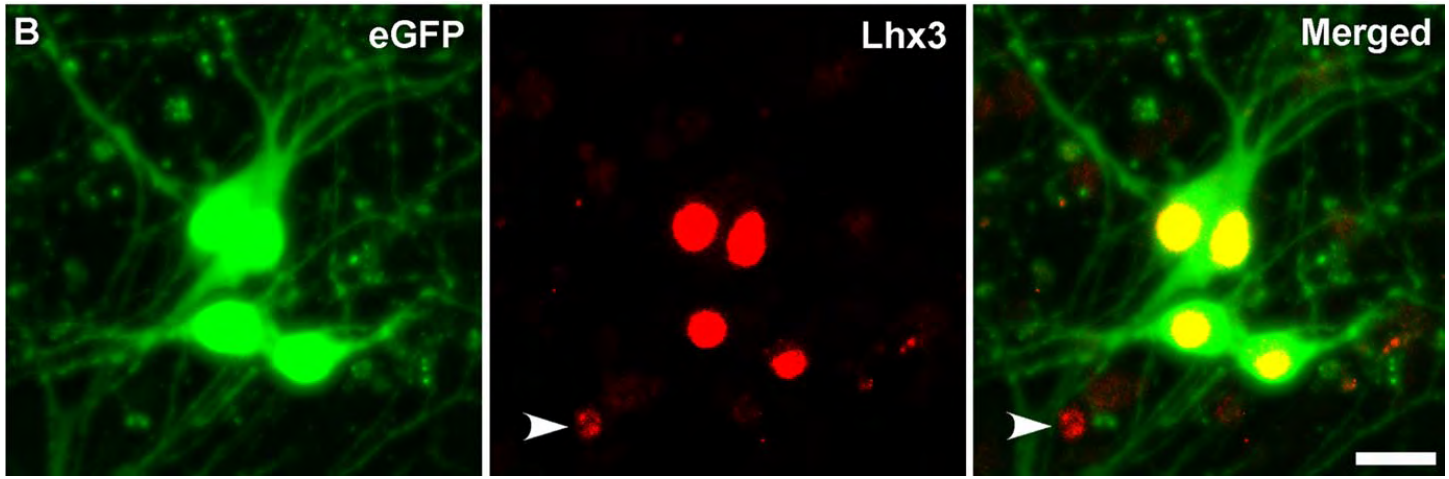


In A: **Red = promotes motoneurons**
Blue = inhibits motoneurons

Transcription factor expression in the presence of Shh

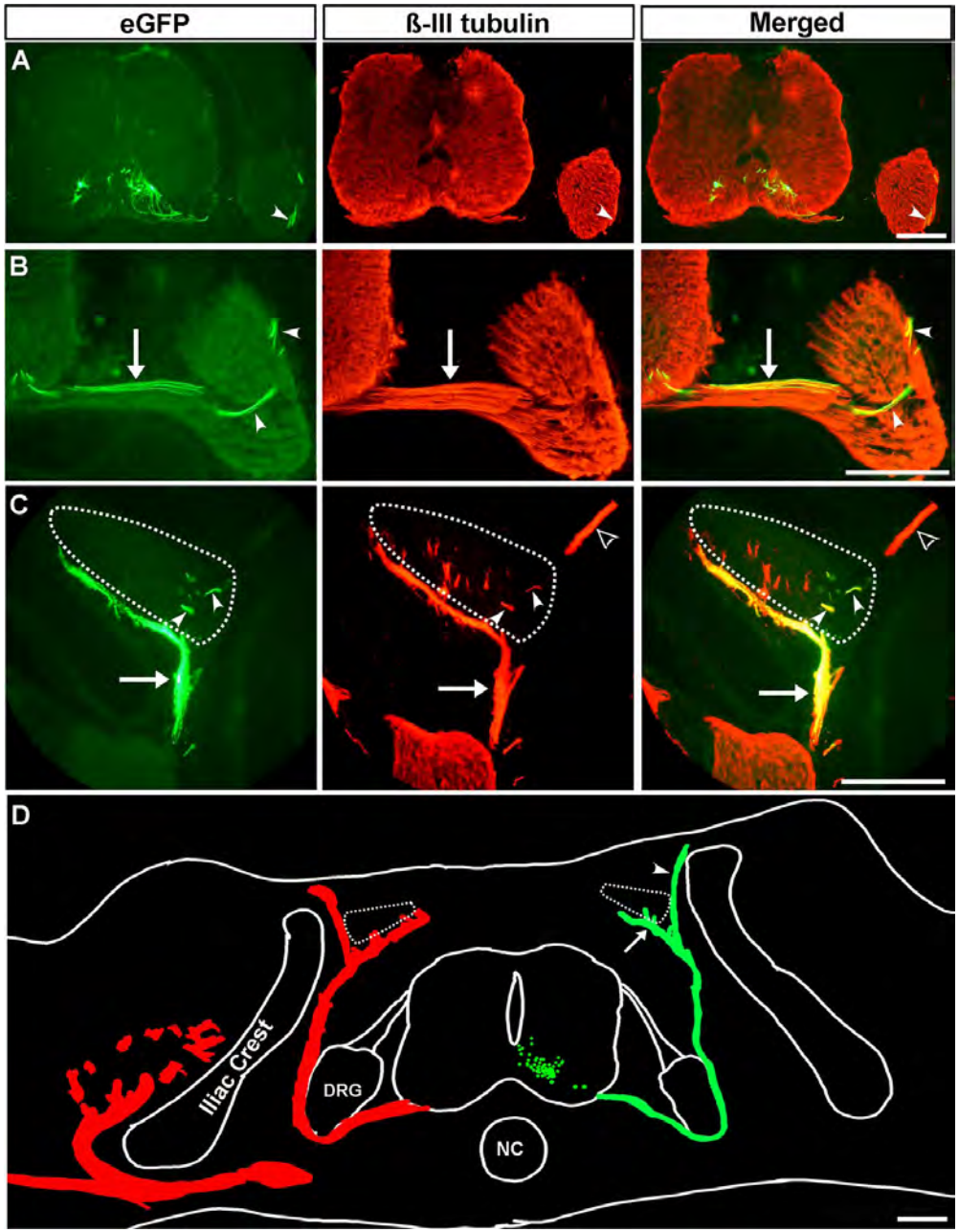


Culture these cells for 5 days see Lhx3 expressing cells are motoneurons (ie express GFP)



Green = HB9::GFP

Soundararajan et al. (2006) JNS 26: 3256

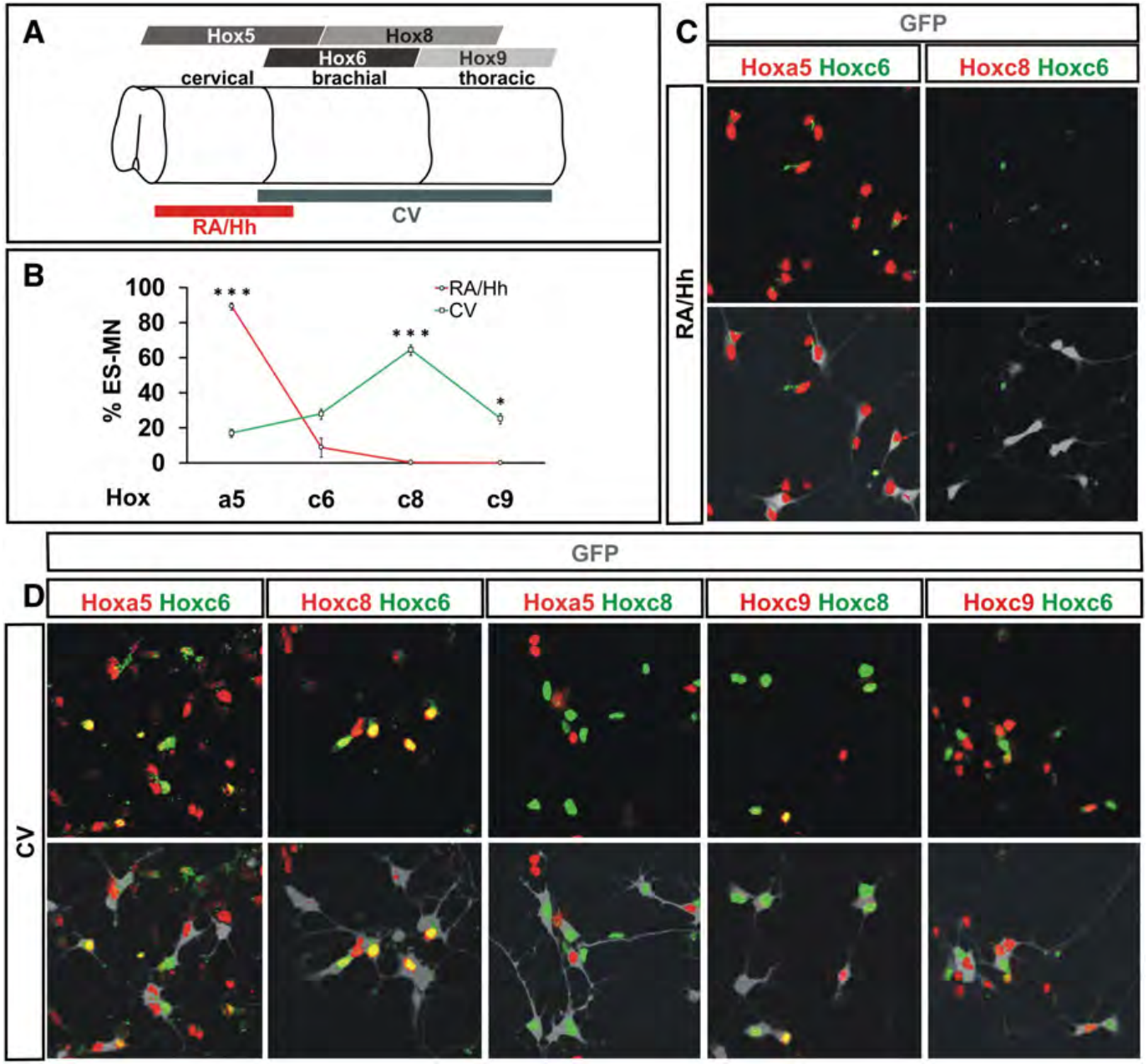


Transplanted ES cell-derived
motoneurons project to
axial muscle

Gene expression, axon projection,
and electrophysiology
all point to these cells
becoming Medial
Motor column neurons

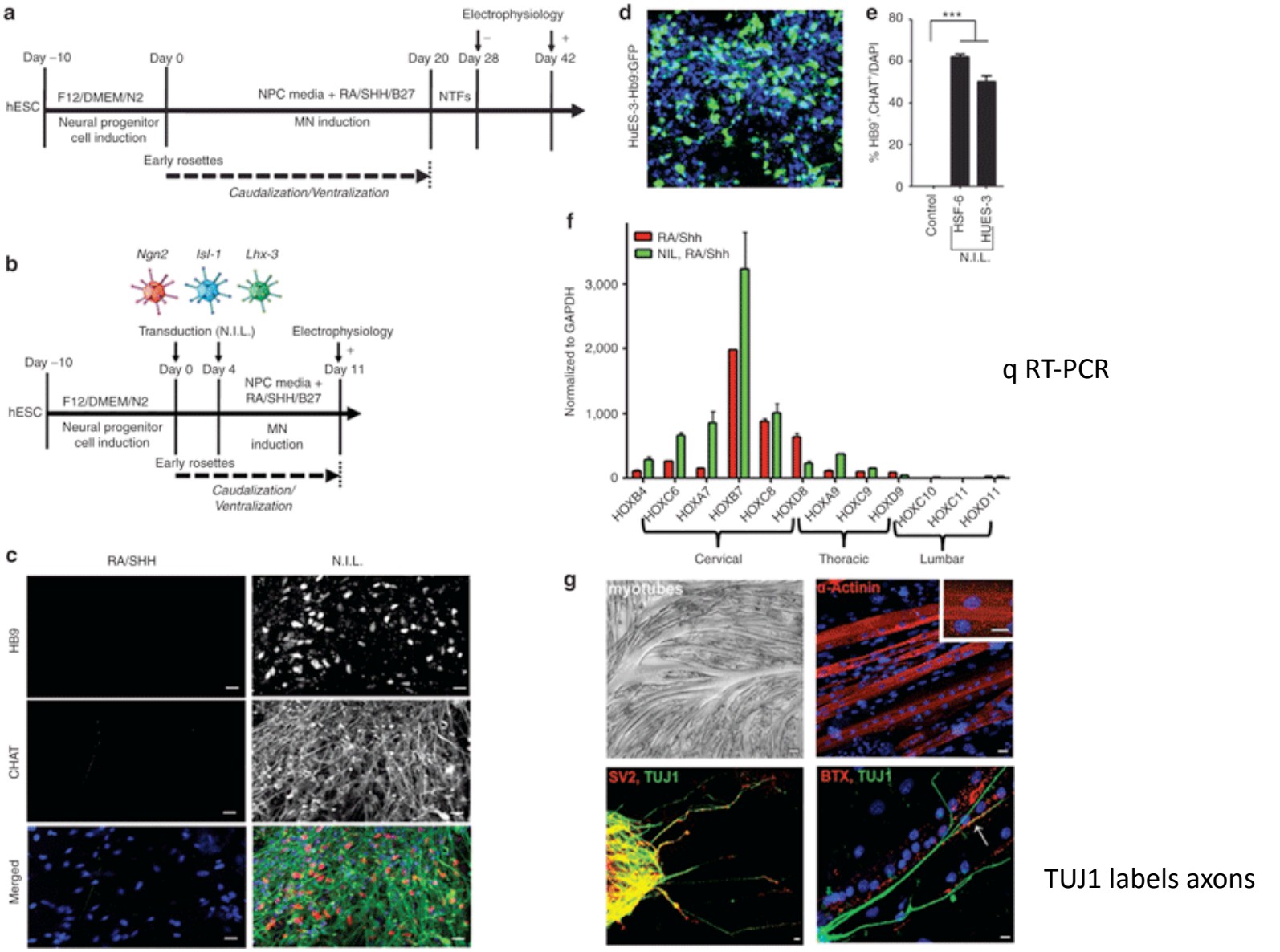
Soundararajan et al. (2006) JNS 26: 3256

Using media without RA allows generation of LMC motoneurons



Peljto et al (2010) Cell Stem Cell: 7

Using virus is a more efficient way to make MNs from ES cells



q RT-PCR

TUJ1 labels axons

Hester et al (2011) Molecular Therapy 19

A motor unit= a MN and all of the fibers it innervates

These fibers are of the same fiber type (1, 2a, 2b) and usually scattered throughout the muscle

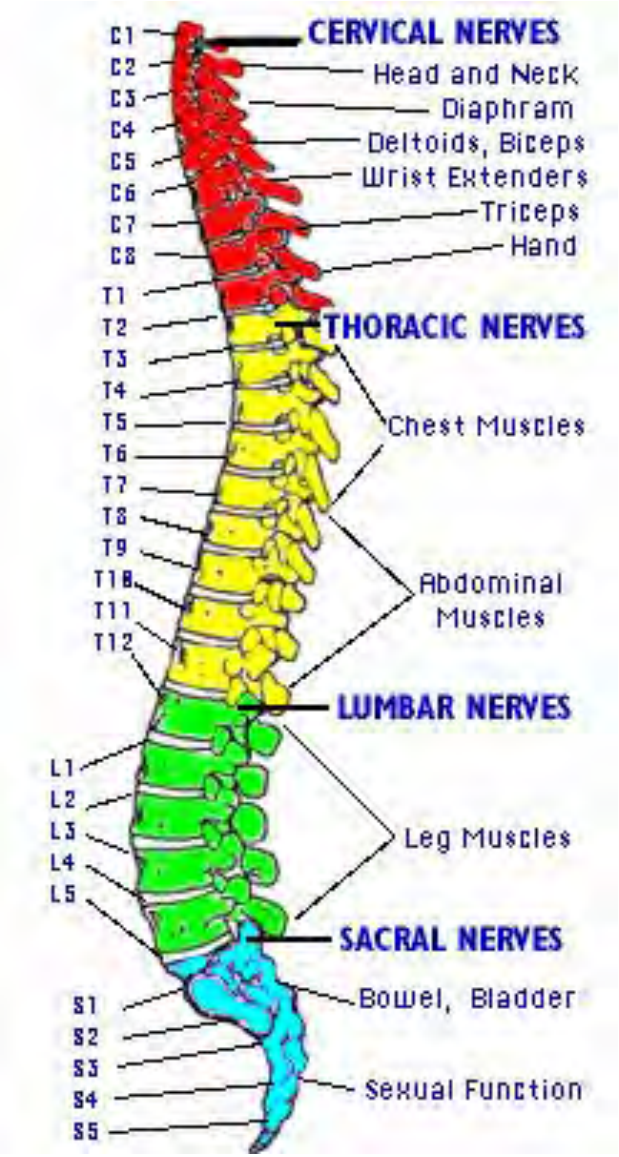
1. MN that innervate the same muscle are grouped in clusters know as pools that occupy stereotyped locations in the spinal cord

2. Motor pools that innervate muscles with related/synergistic functions (say within a limb) are grouped within minicolumns referred to as columels.

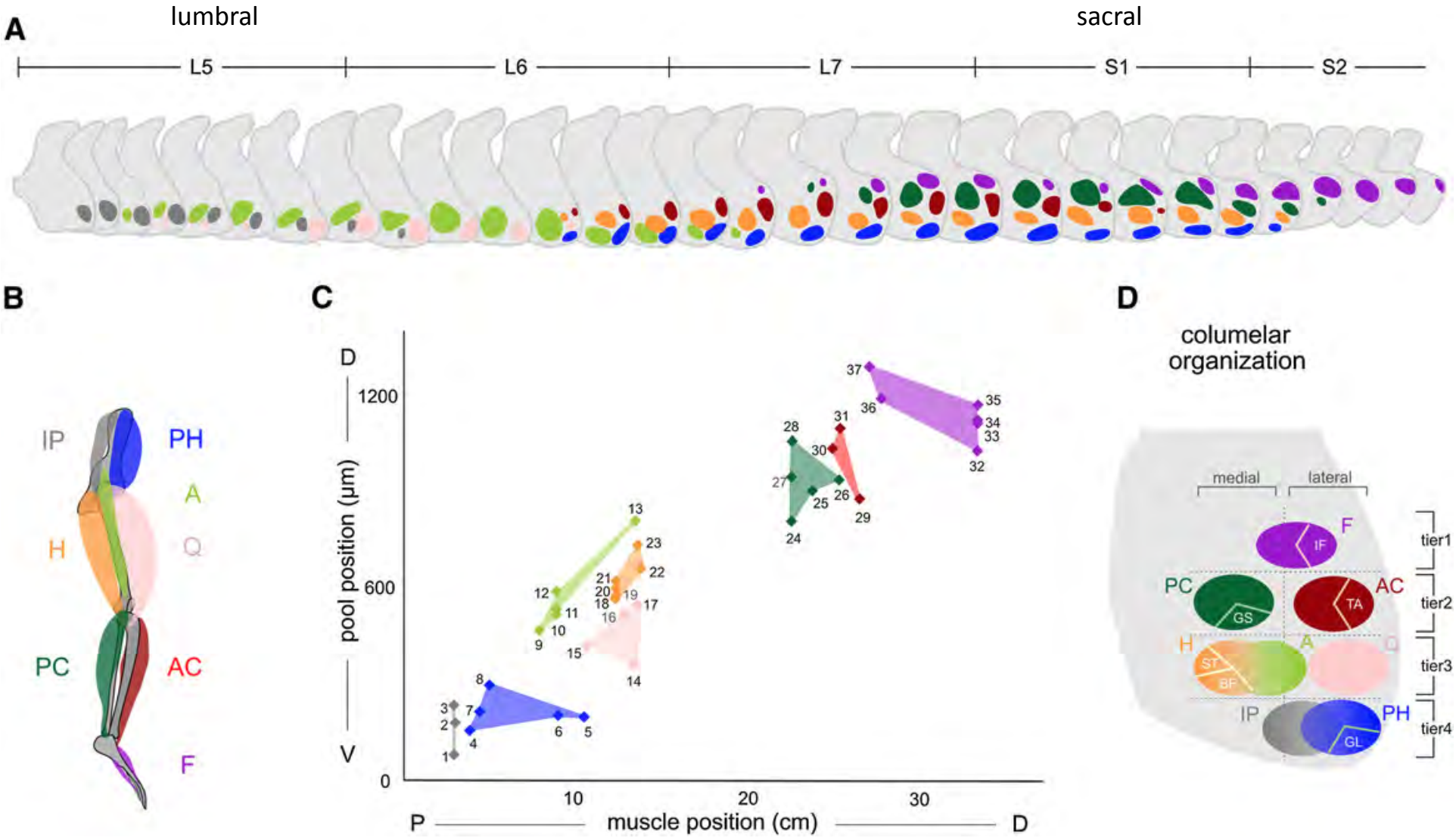
3. The 3 dimensional organization of motor columels reflects the positions of the muscle targets along the DV/ML/AP axis.

4. The clustering of motor neurons into pools also facilitates the formation of gap junction channels between neurons with a common muscle target, thereby enhancing the coherence of motor neuron firing that is thought to stabilize neuromuscular connections

Human spinal cord divisions



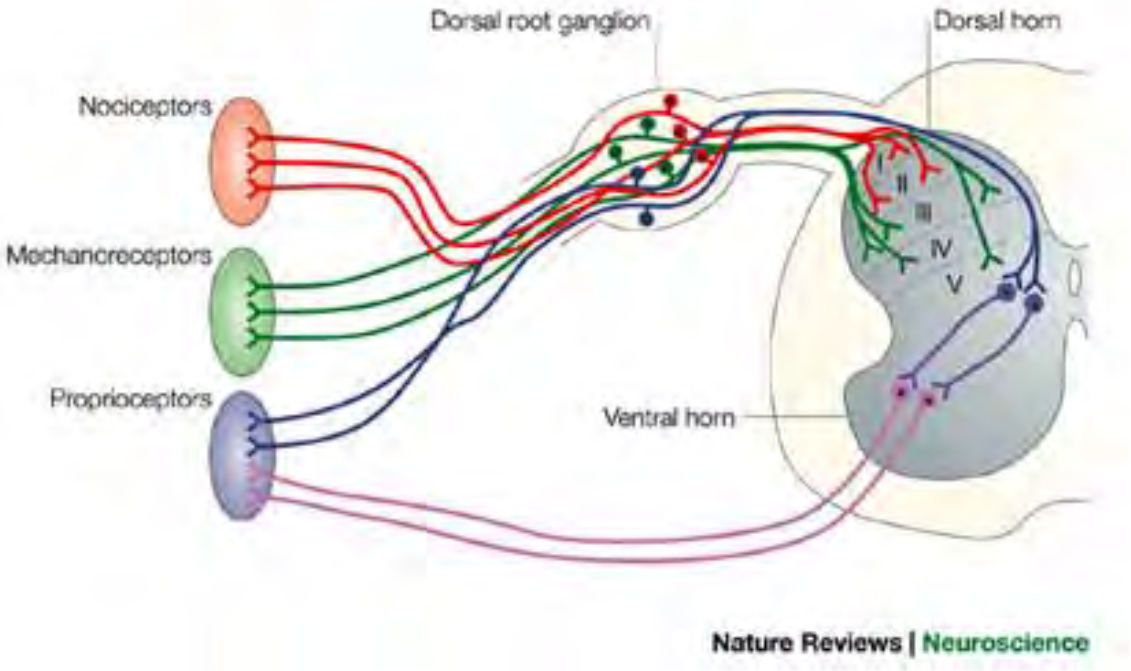
Motoneurons are grouped into pools and columns

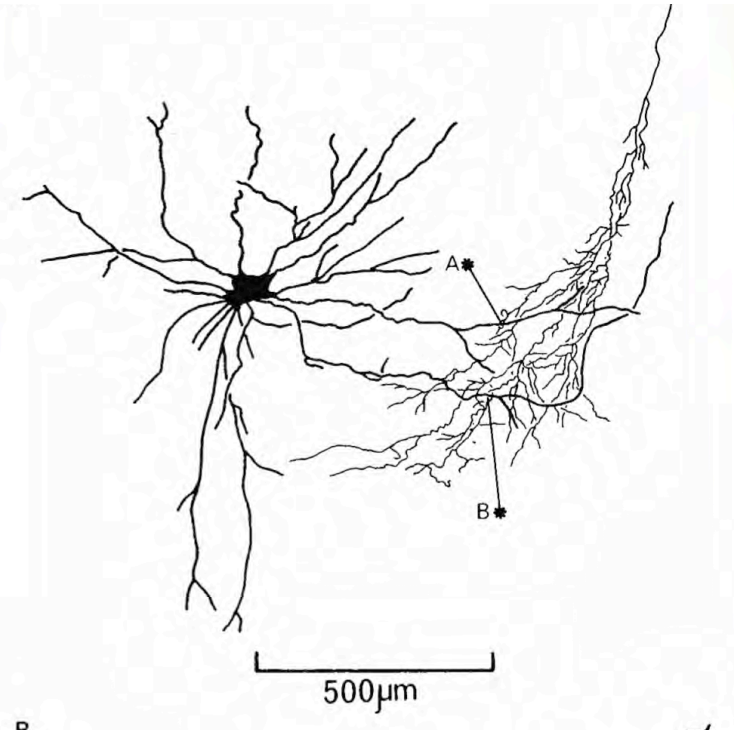


cat

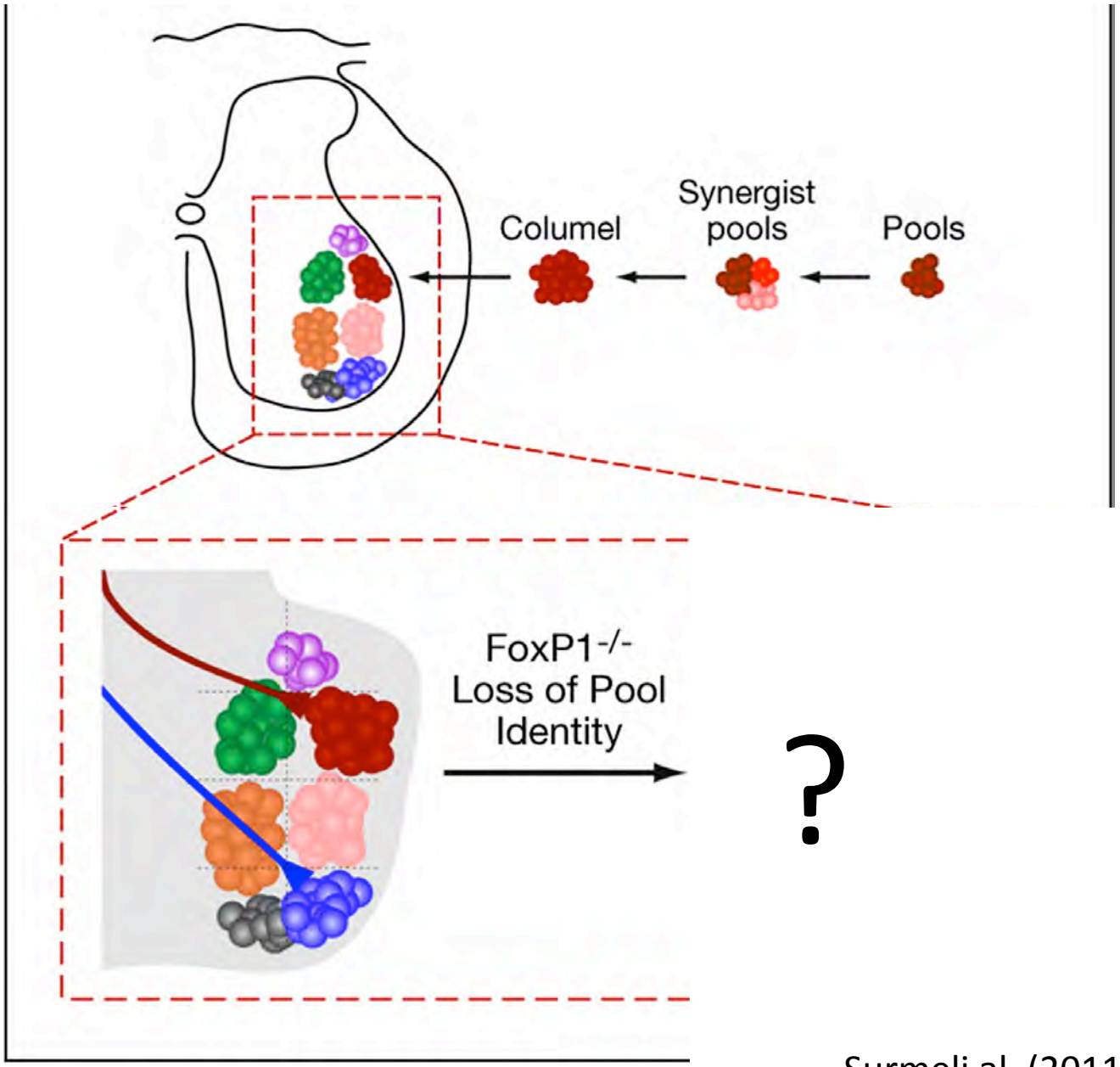
Surmeli al. (2011) Cell 147: 653-665

Axons of proprioceptive sensory neurons connect with MNs late in embryogenesis. Neither MN activity or MN death determine the specificity of these connections.



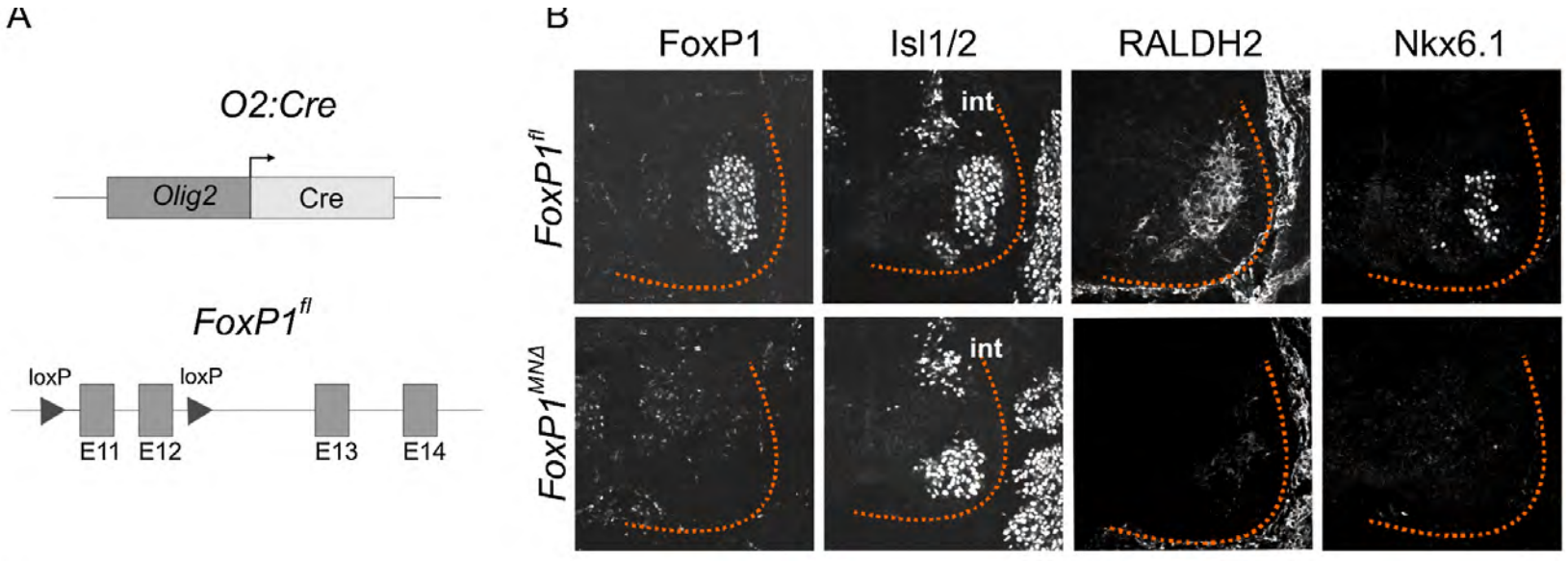


Do motor-sensory connections depend on motor pool organization?



Surmeli al. (2011) Cell 147: 653-665

Inactivation of motor neuron FoxP1 results in a loss of motor pool differentiation

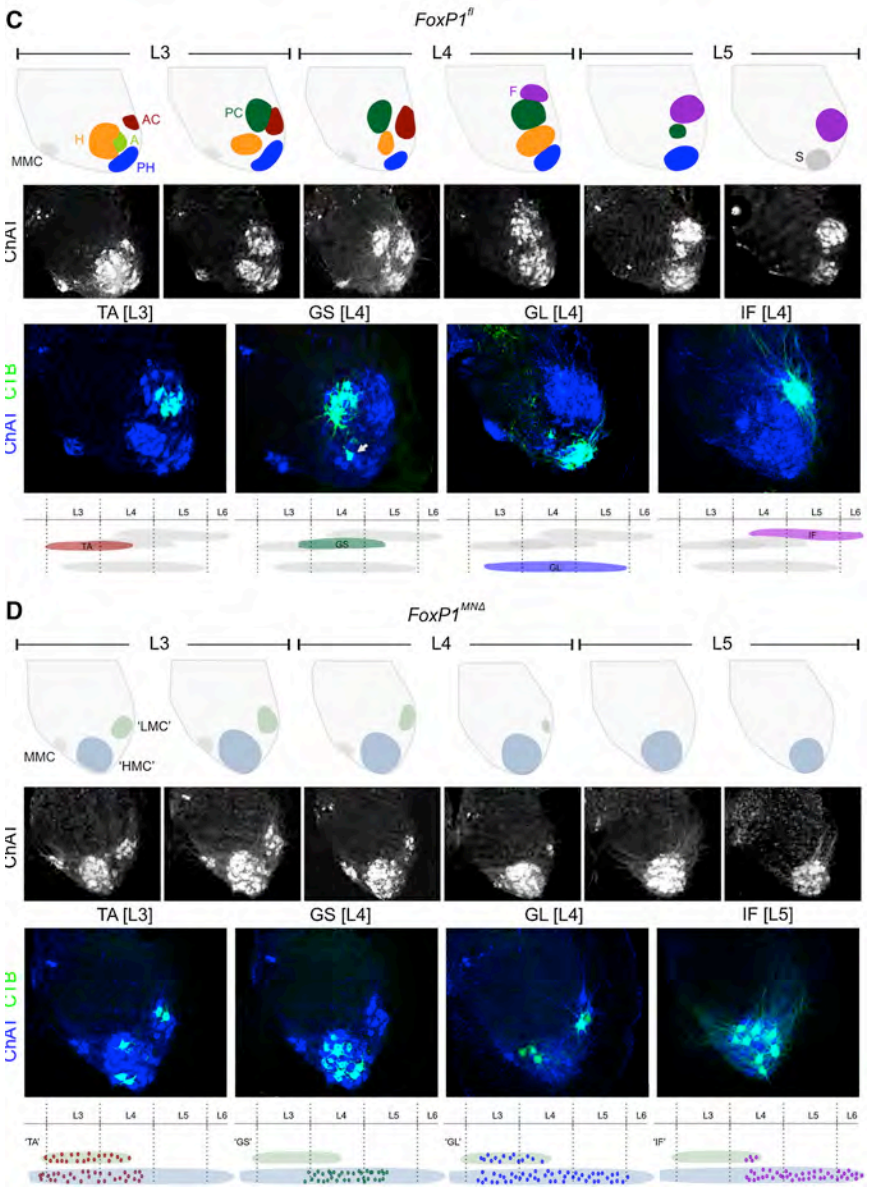


Generic MN character retained but LMC columnar and pool charactor lost

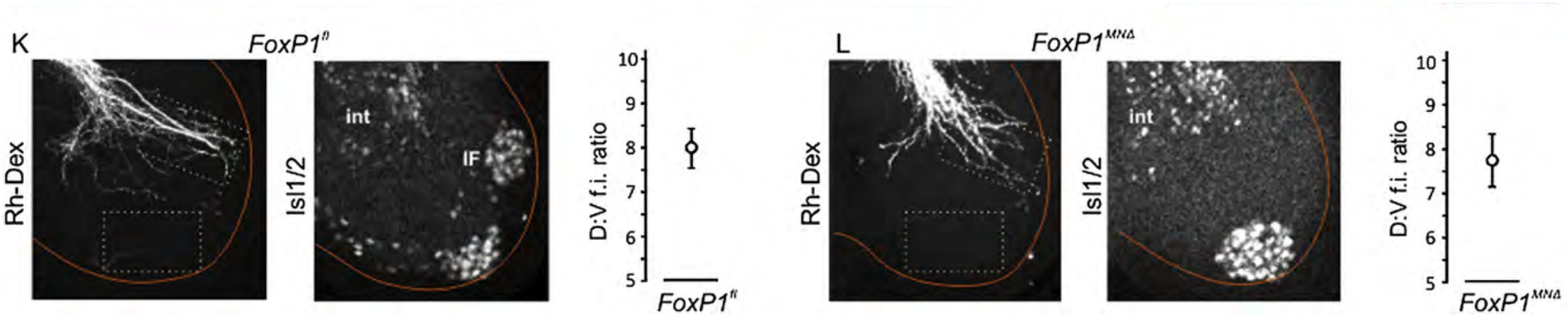
Motor pool disruption in FoxP1 MN mutants

Mice match what seen in cats

Columelar goupings no longer evident. Ventral shift in position of ChAT MNS

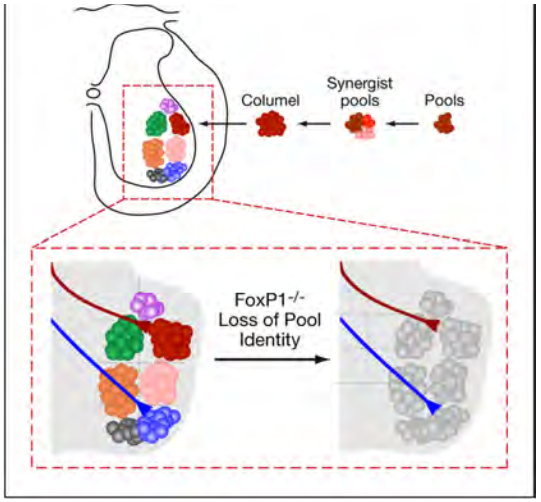


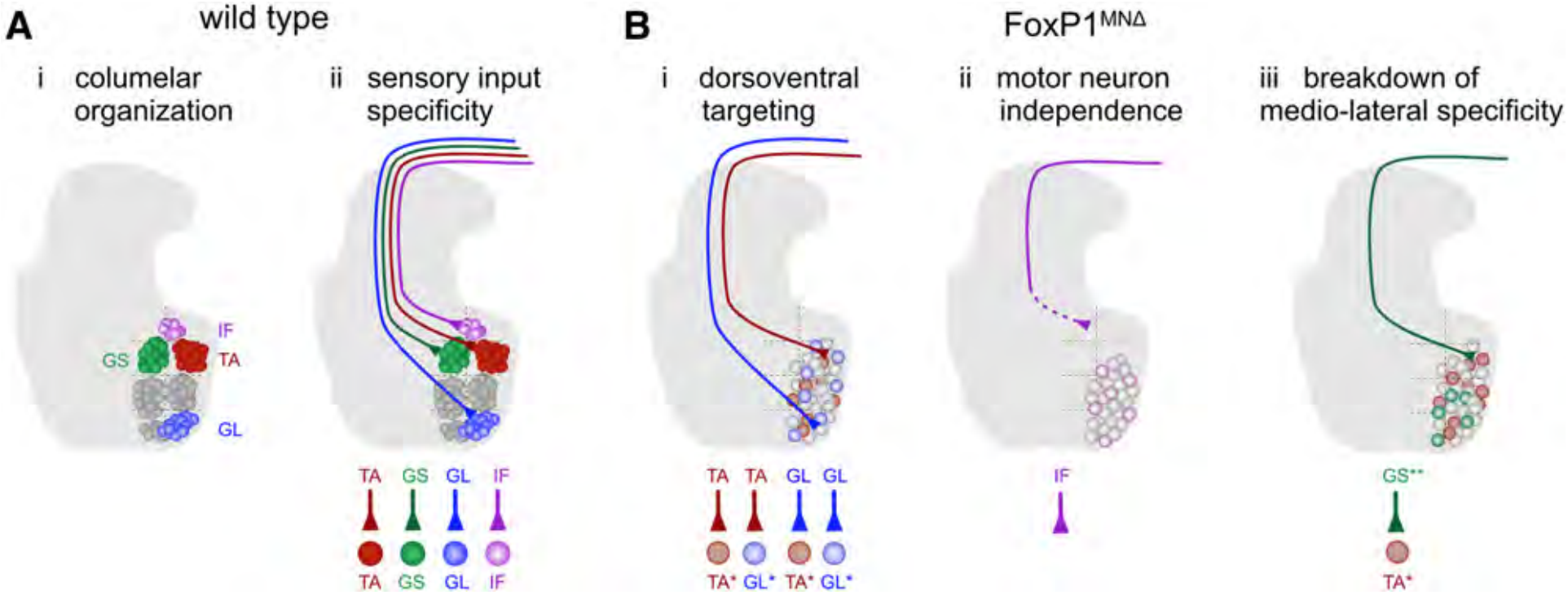
Sensory afferents supplying individual limb muscles target discrete DV tiers without reliance on recognition of MN subtype

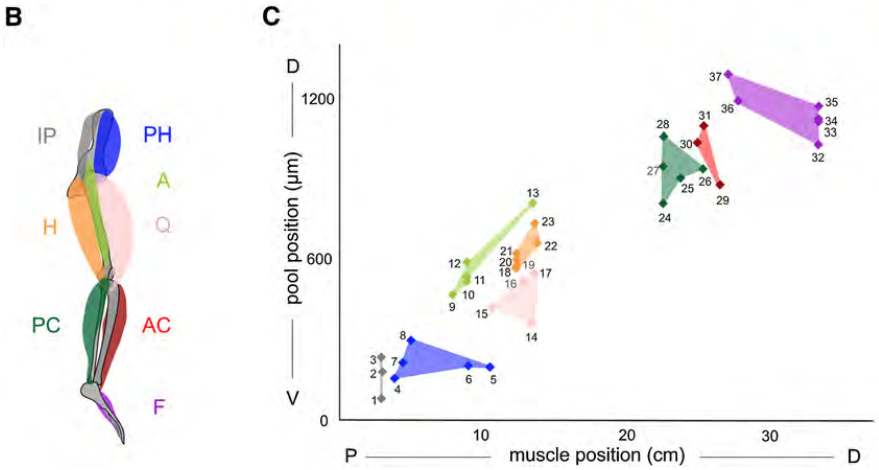


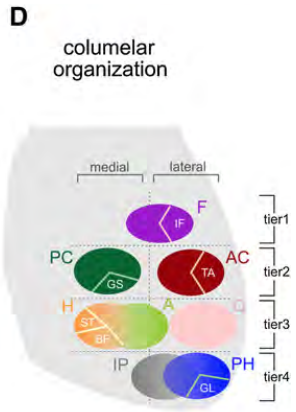
Rh-Dex = rhodamine dextran back label from E18 L5 DRG

Did have some problems with medio-lateral specificity. For example, sensory neurons in the KO mice innervated MNs from antagonistic muscle pools which they normally don't do




A
wild type
i columelar organization
ii sensory input specificity
IF
GS
TA
GL
TA GS GL IF
TA GS GL IF
B
FoxP1MNΔ
i dorsoventral targeting
ii motor neuron independence
iii breakdown of medio-lateral specificity
TA TA GL GL
TA* GL* TA* GL*
IF
GS**
TA*


B
IP
PH
A
H
Q
PC
AC
F
C
D
pool position (µm)
1200
600
0
V
10
20
30
P
muscle position (cm)
D


D
columelar organization
medial
lateral
F
IF
PC
GS
AC
TA
H
A
Q
ST
BF
IP
PH
GL
tier1
tier2
tier3
tier4

Conclusions:

1. Sensory afferents supplying individual limb muscles target DV specific DV tiers irrespective of MN cell type.

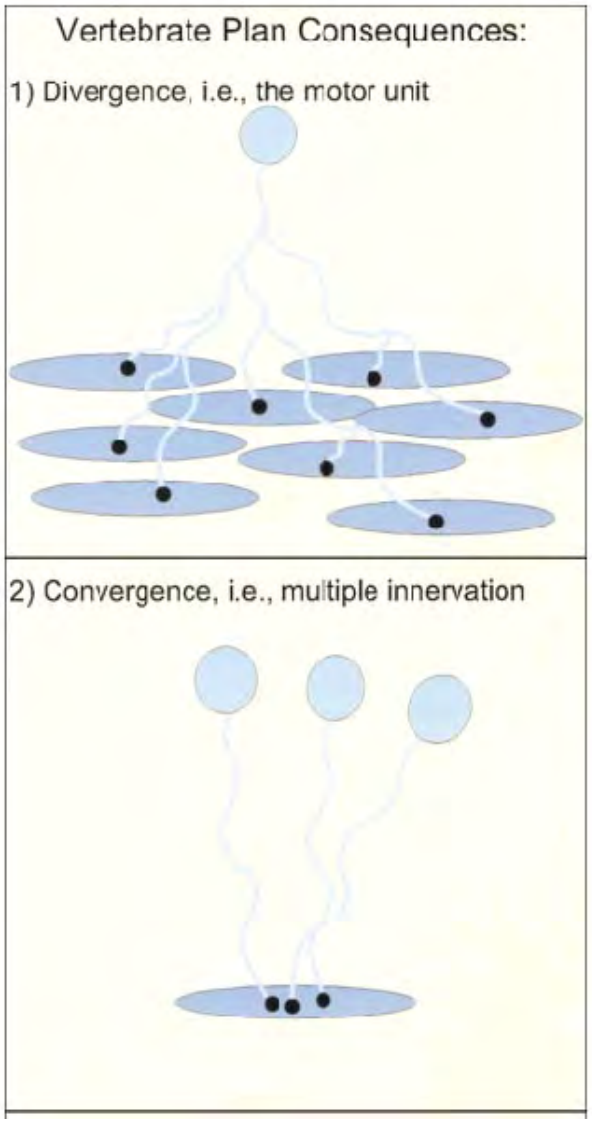
2. This helps explain the precise positioning of MN columels. Argues that this precise position ensures functionality.

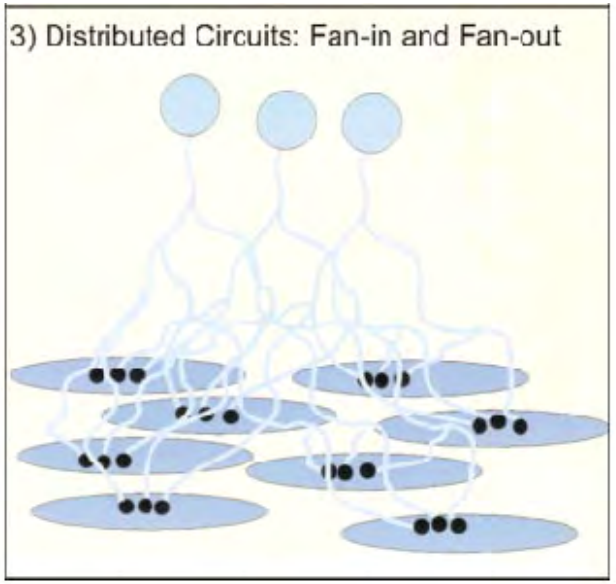
3. What about dendrites that may expand into other tiers and DV locations? Note that initial contacts are on the cell body then they redistribute to dendrites as MN matures.

During development…

Motoneurons innervate more than one muscle fiber

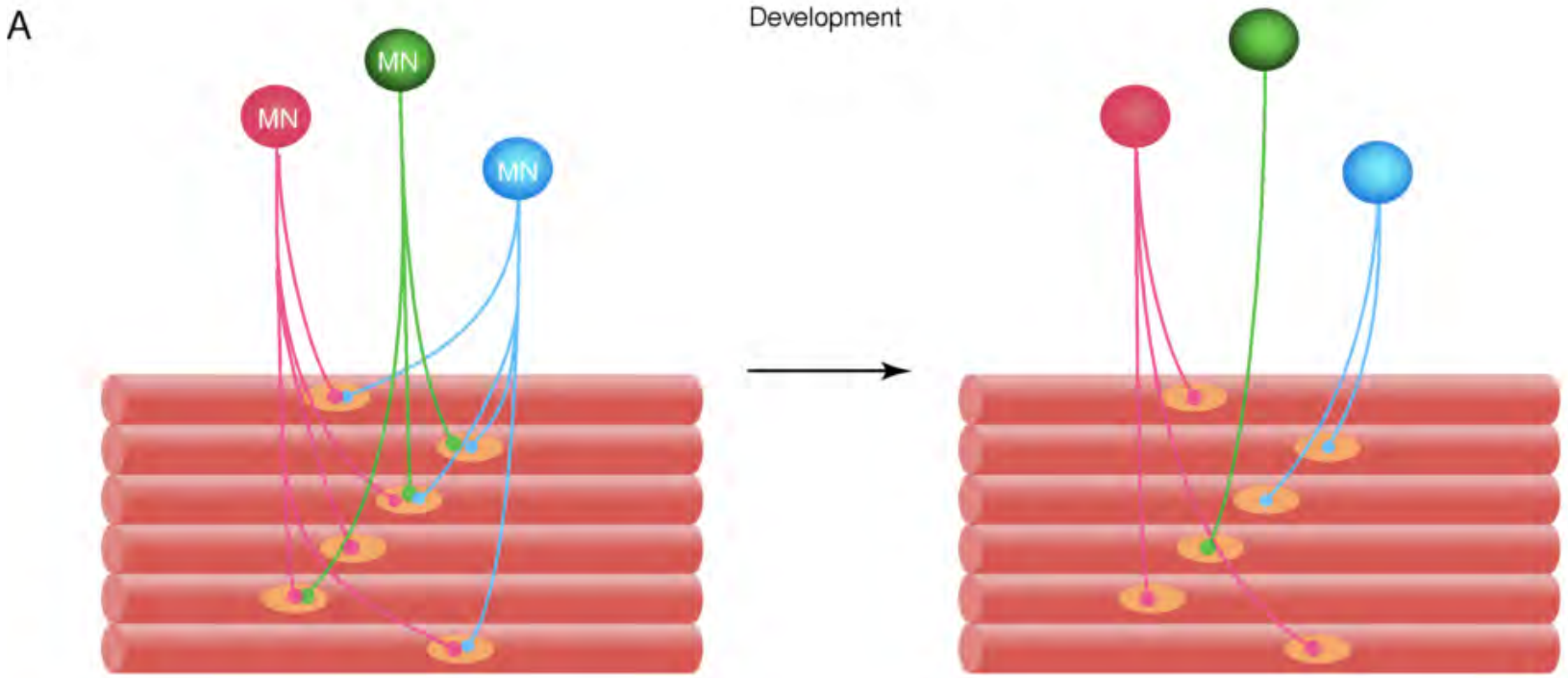
Each muscle fiber is innervated by more than one motoneuron





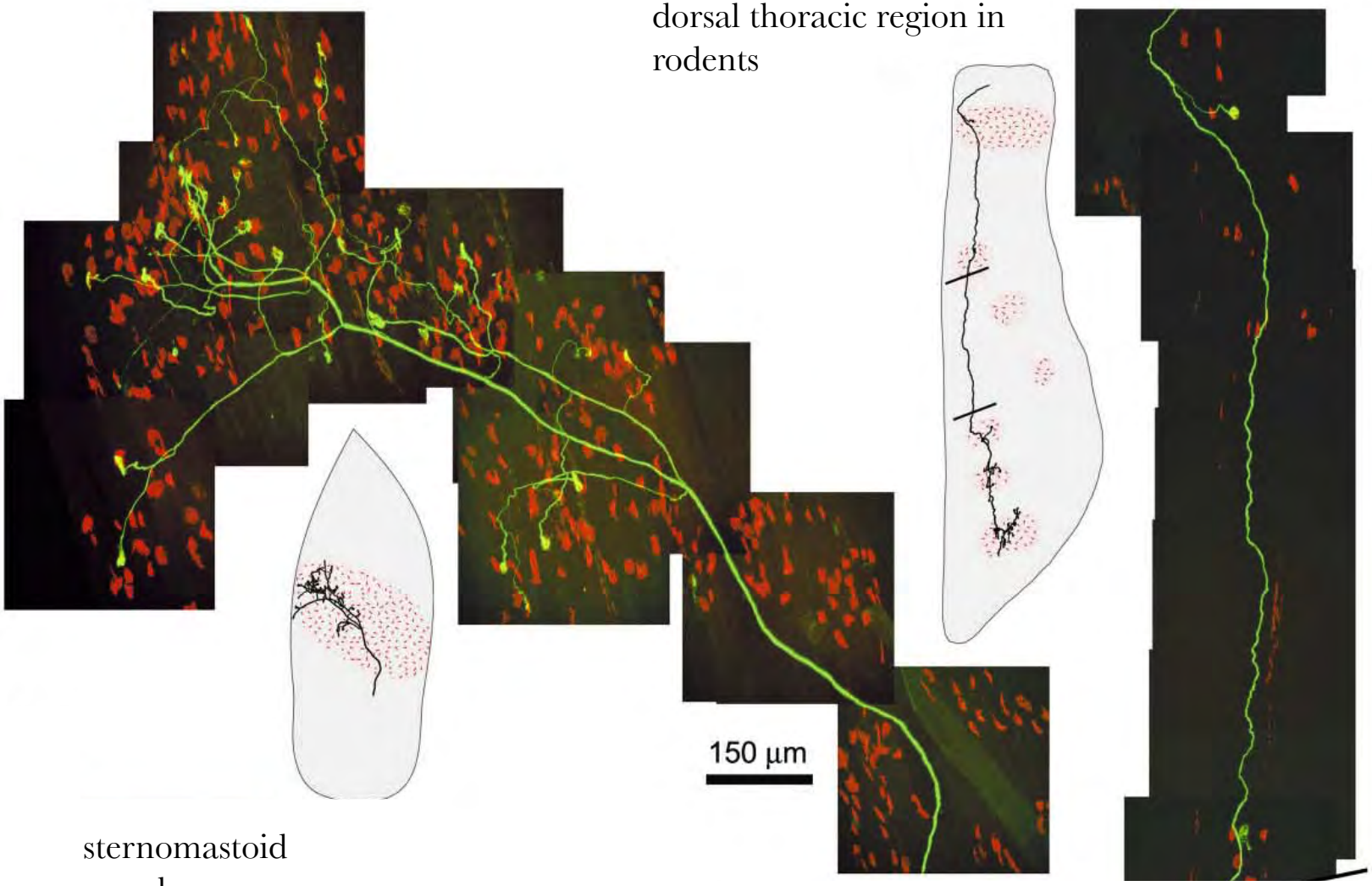
This situation is transient due to synapse elimination and the establishment of unique circuits.

At the vertebrate NMJ, synapse elimination refines connections between populations of pre- and post-synaptic partners



Use transgenic mice to analyze single axons during the process of synapse elimination.

Spinotrapezius muscle
dorsal thoracic region in rodents



sternomastoid
muscle
large muscle on either side of the neck