Regionalization of the nervous system 2

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Key concepts for part 1-secondary organizers

-Early AP patterning of vertebrate neural tissue is closely linked to neural induction

-There are four major steps in regionalization of the brain:

1. **Ectodermal cells acquire neural identity (neural induction)**
   BMP inhibition results in the formation of anterior neural tissue.

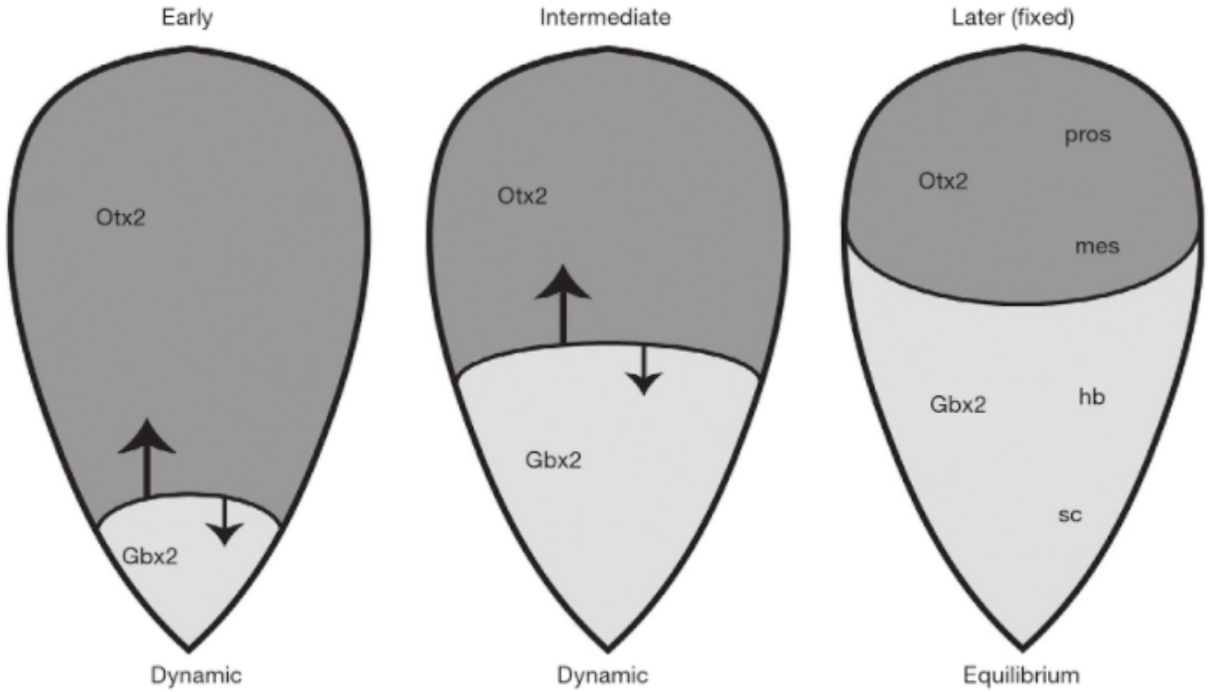
2. **Adoption of <u>crude</u> positional character (anterior vs posterior)**
   Opposition between caudalizing factors and their inhibitors (especially Wnts and Wnt inhibitors) establish crude AP patterning.

3. Formation of cell populations ("secondary organizers") <u>within</u> the neural tissue that secretes signaling molecules (morphogens)

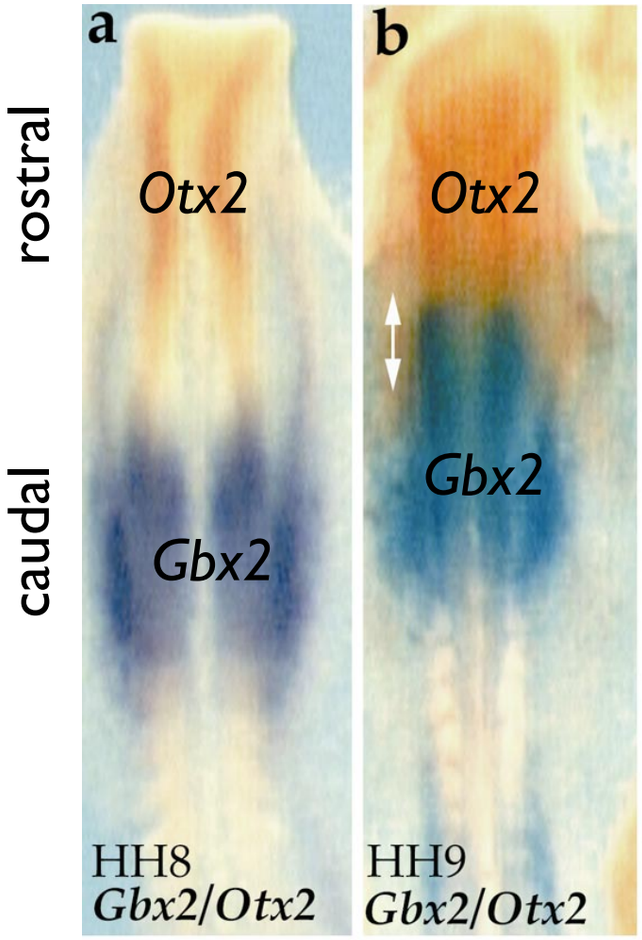
4. These secondary organizers modulate and refine initial regional patterning such that the differential gene expression subdivides the neural plate into discrete territories that prefigure the various structures of the mature CNS.

-Same molecular pathways (Wnt, BMP, FGF, RA, Shh, etc.) play a role in more than one steps at different times and places.

Activation-transformation results in coarse AP patterning



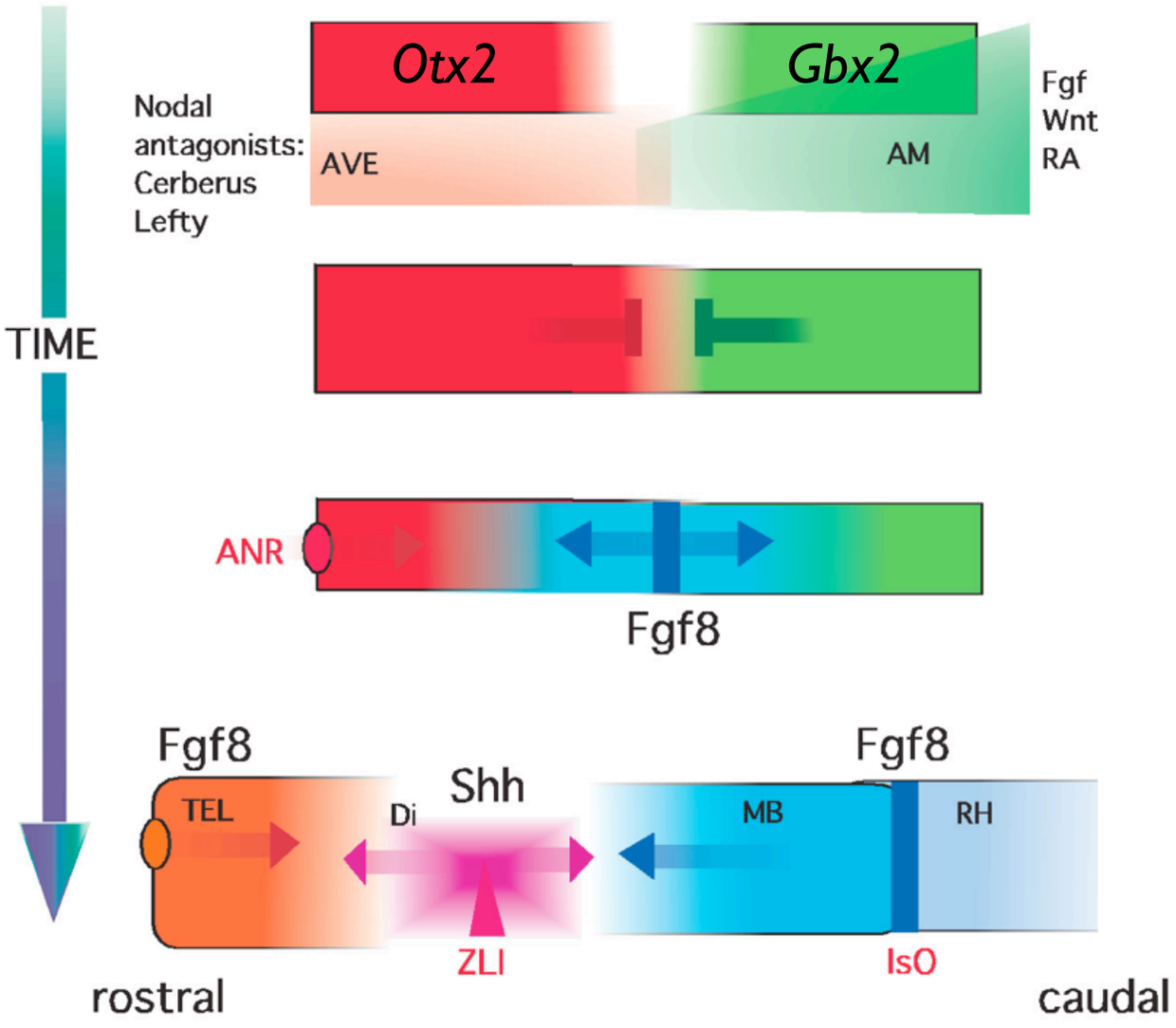
Puelles 2015



Garda et al., 2001

-Initially, almost the entire brain is dominated by expression of the transcription factor *Otx2*.
-"Transformation" results in expansion of the expression domain of *Gbx2*, a transcription factor expressed in the caudal brain.
-Expression of *Otx2* and *Gbx2* overlaps, which results in the formation of the border between the midbrain and the hindbrain.

Secondary organizers are formed within the neural tissue



Vieira et al., 2010

ANR: anterior neural ridge
ZLI: zona limitans intrathalamica
IsO: isthmic organizer

-At the border between *Otx2* and *Gbx2* domains, a new domain forms that expresses a secreted protein FGF8.

-This domain is called the **isthmic organizer (IsO)** and later becomes the cerebellum.

-At the front end of the brain, another domain forms that also expresses FGF8.

-This domain is called the **anterior neural ridge (ANR)**.

-Within the diencephalon, a third domain is formed that expresses Sonic hedgehog (Shh).

-This domain is called the **zona limitans intrathalamica (ZLI)**.

Anterior neural ridge (ANR)

-formed at the junction between most anterior neural tissue (anterior commissure later forms here) and non-neural ectoderm

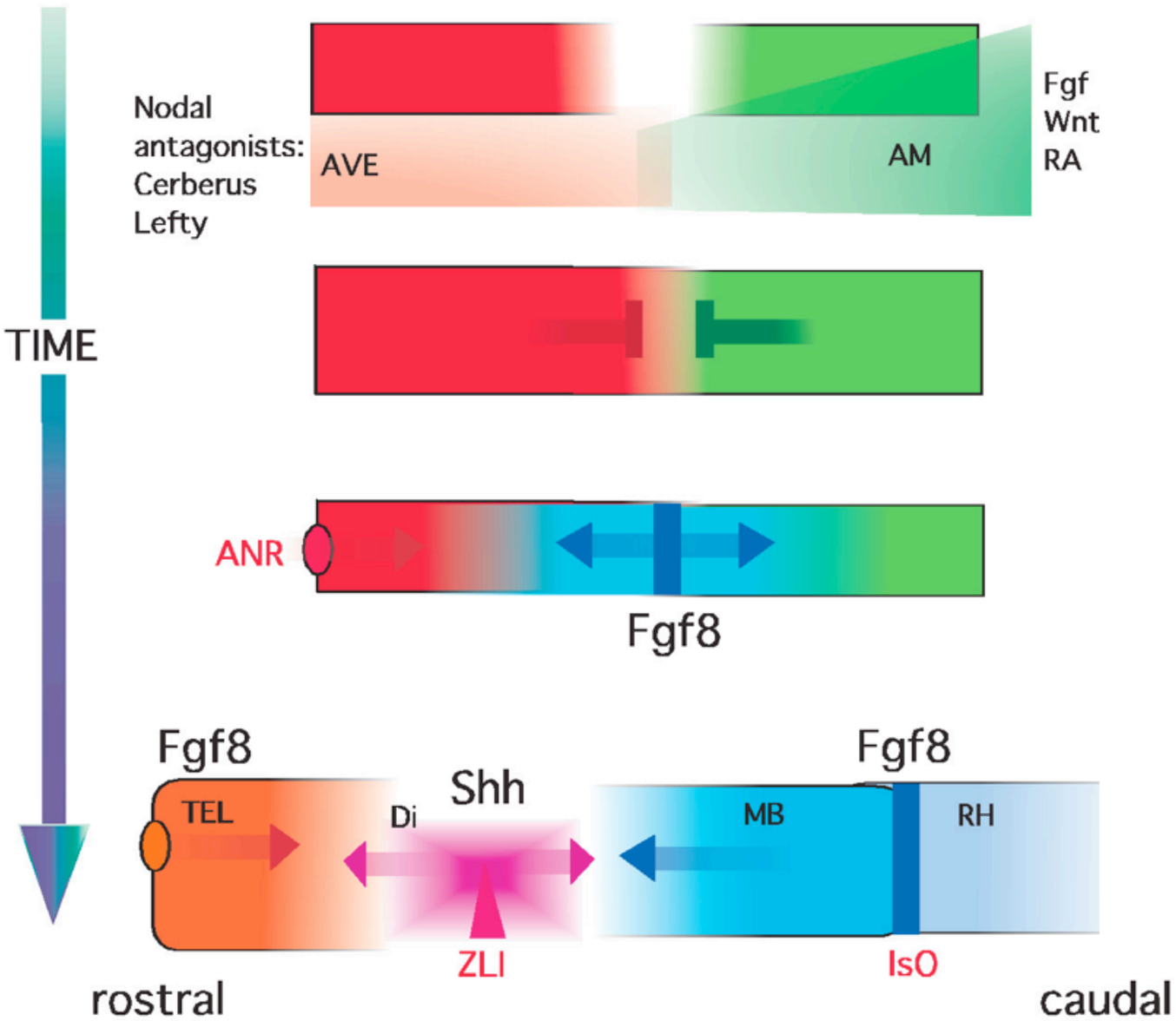
-requires anterior visceral endoderm (AVE) for its formation

-expresses FGF8

-ectopic FGF8 induces rostral forebrain phenotypes in more caudal tissue

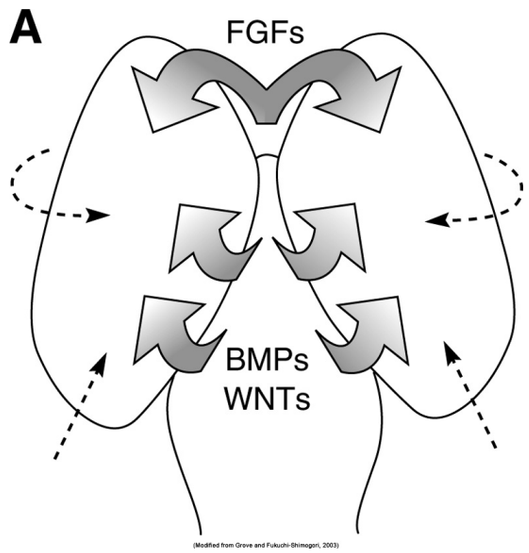
-FGF8 is required for the telencephalic identity

-FGF8 also regulate the AP polarity of the cerebral cortex.



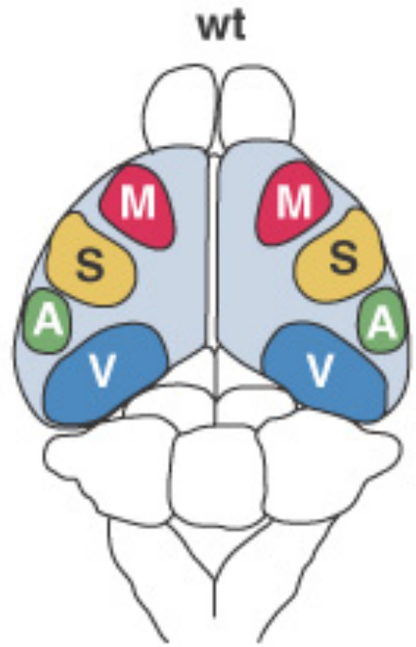
Vieira et al., 2010

ANR polarizes the cerebral cortex later in development



Sanes Fig.2.29

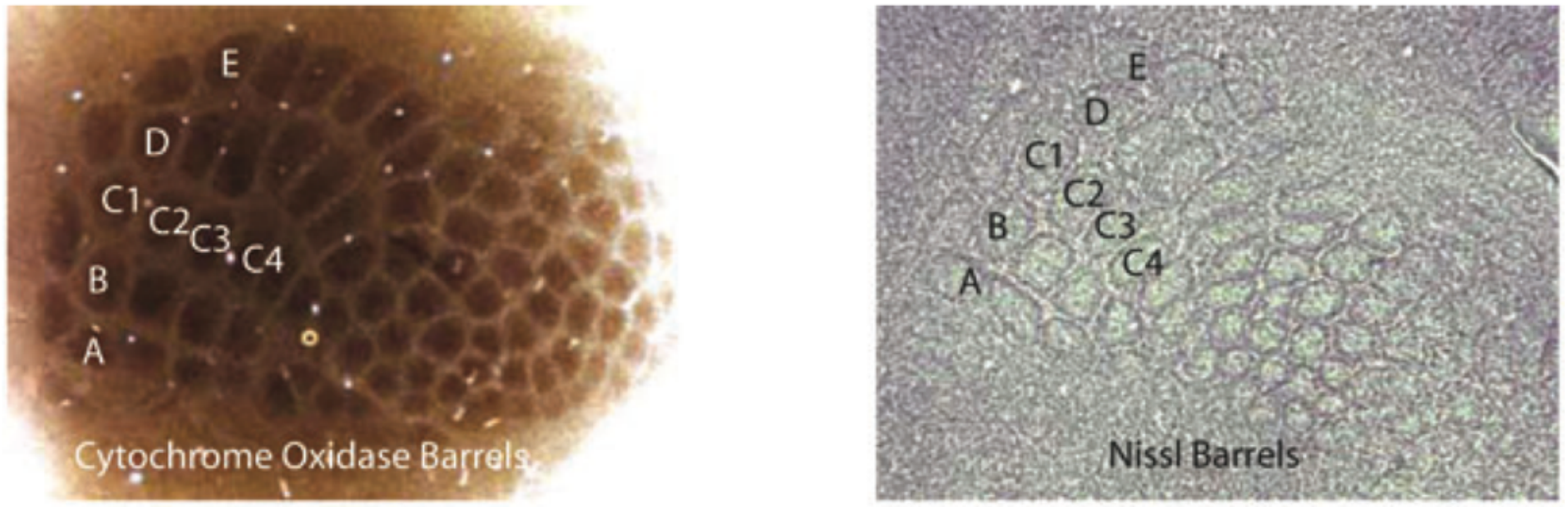
FGF8 is expressed in the ANR, which is located at the anterior pole of the future cerebral cortex in mammals.

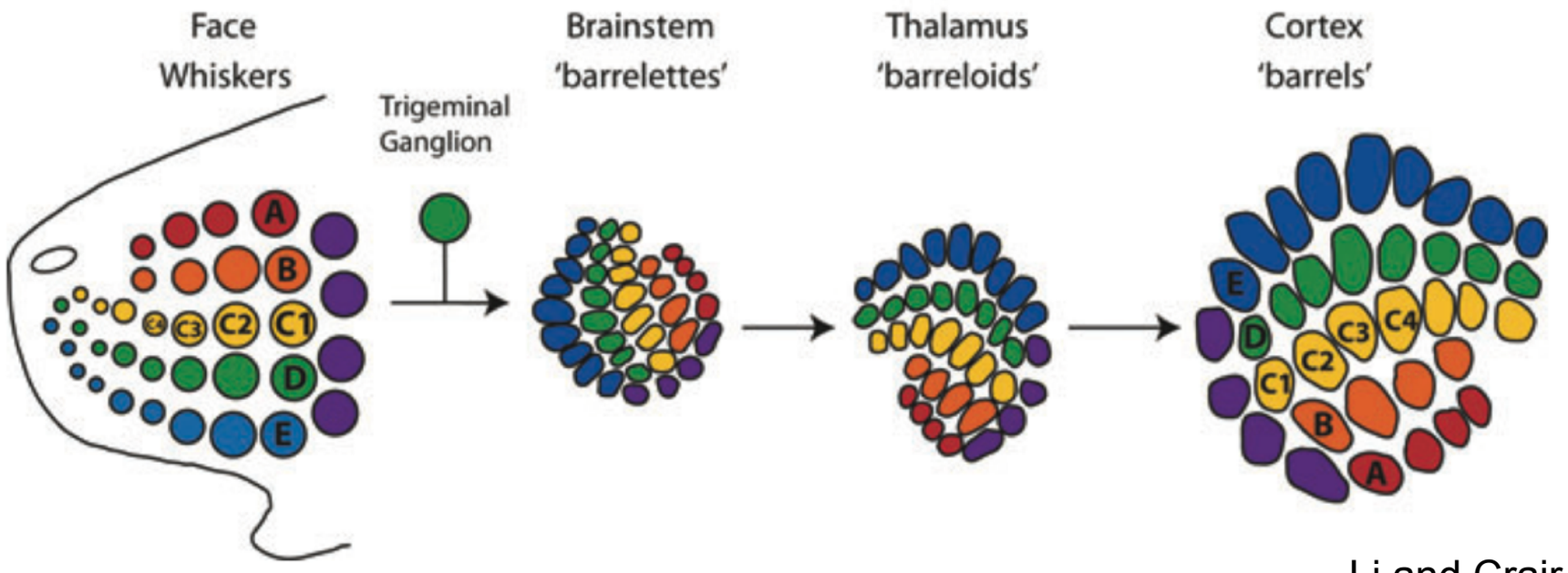


Sanes Fig.2.28

Areas in the mature cortex
M: motor area
S: somatosensory area
A: auditory area
V: visual area

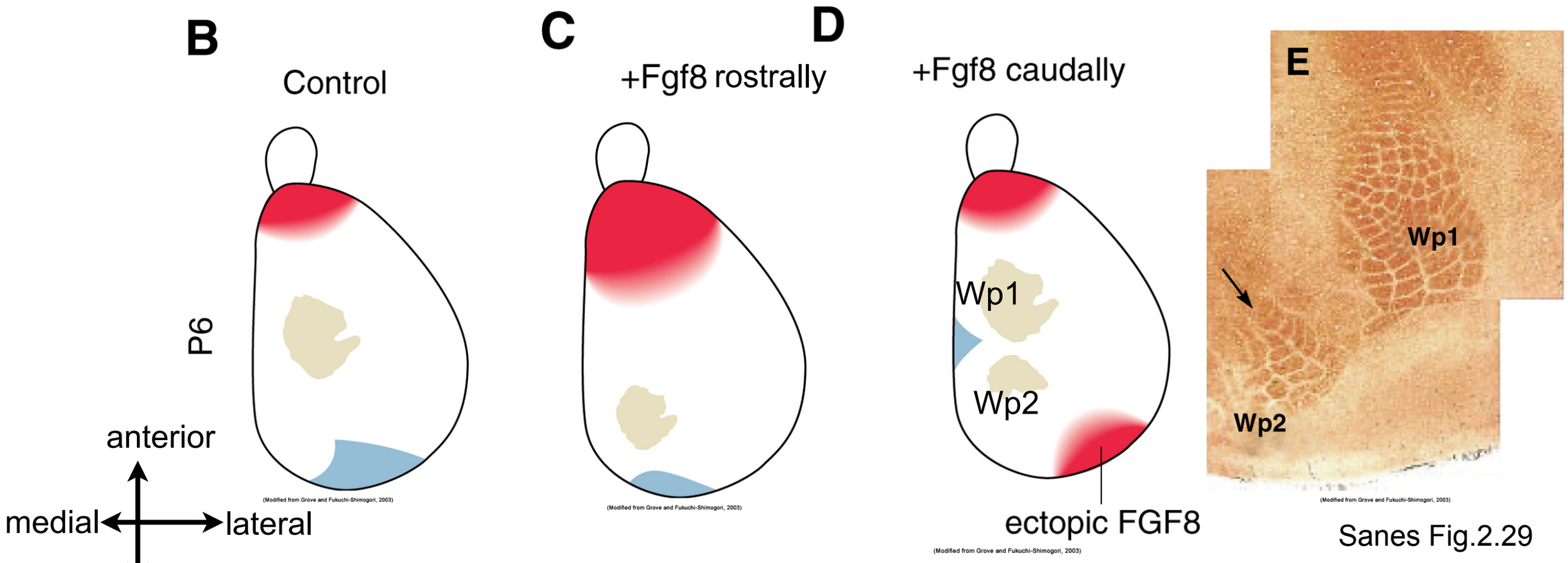
Primary somatosensory area of rodents can be identified histologically





Li and Crair (2011)

Ectopic expression of FGF8 changes areal organization of the cerebral cortex

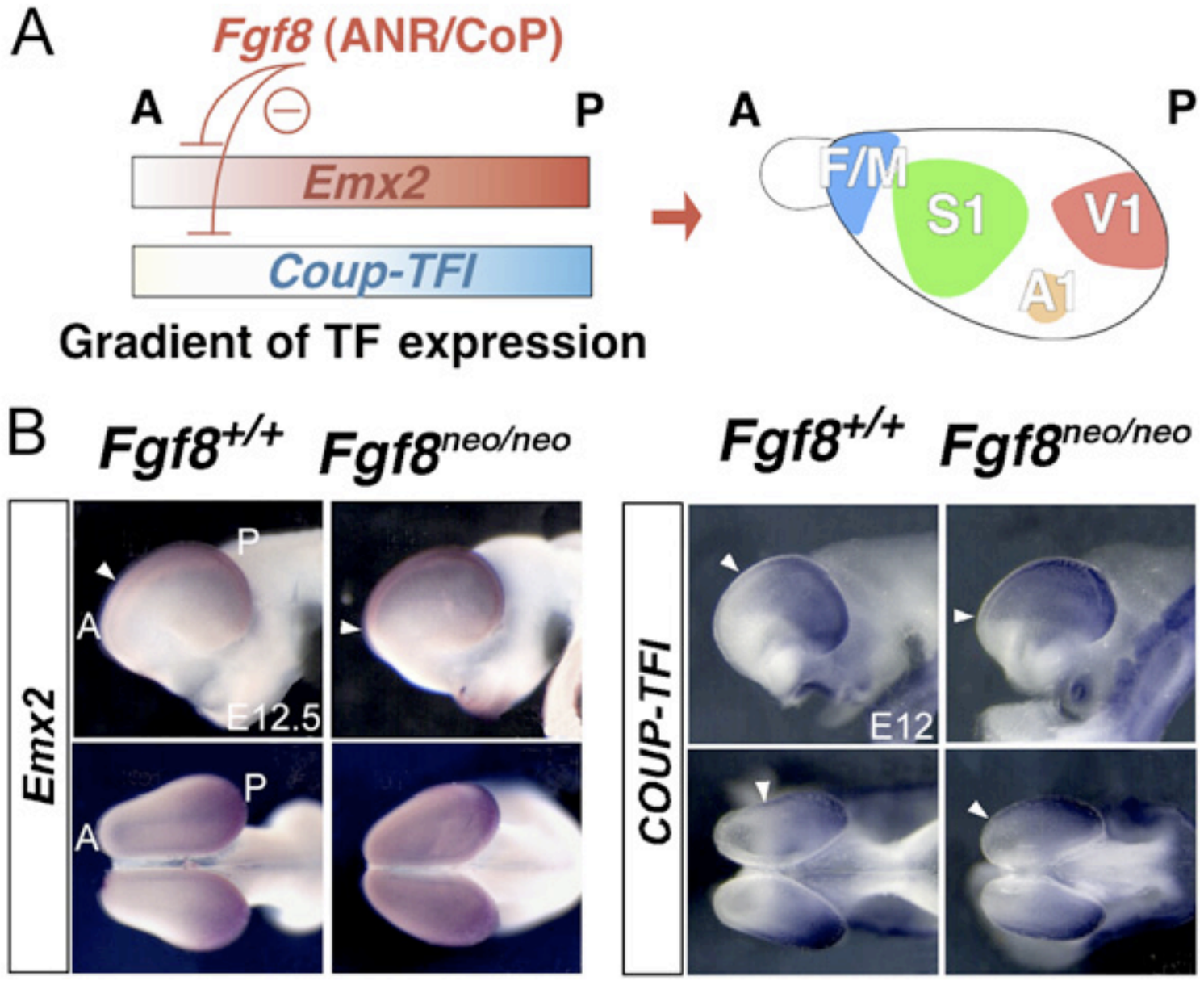


Sanes Fig.2.29

Wp1: original somatosensory map
Wp2: new somatosensory map

C: Increasing FGF8 expression in the anterior cortex by in vivo electroporation results in the expansion of anterior cortical areas, such as the motor area (red)
D: Ectopic FGF8 expression by in vivo electroporation in the caudal cortex causes the formation of duplicated, mirror image of the somatosensory map.

FGF8 controls differential gene expression in cortical progenitor cells



FGF8 suppresses the expression of *Emx2* and *Coup-TF1*, two transcription factors expressed in anterior-low, posterior-high gradients in the immature cortex in mice.

Decreasing the expression of FGF8 results in increased expression of *Emx2* and *Coup-TF1*

O'Leary et al. (2007) Neuron 56: 252-269

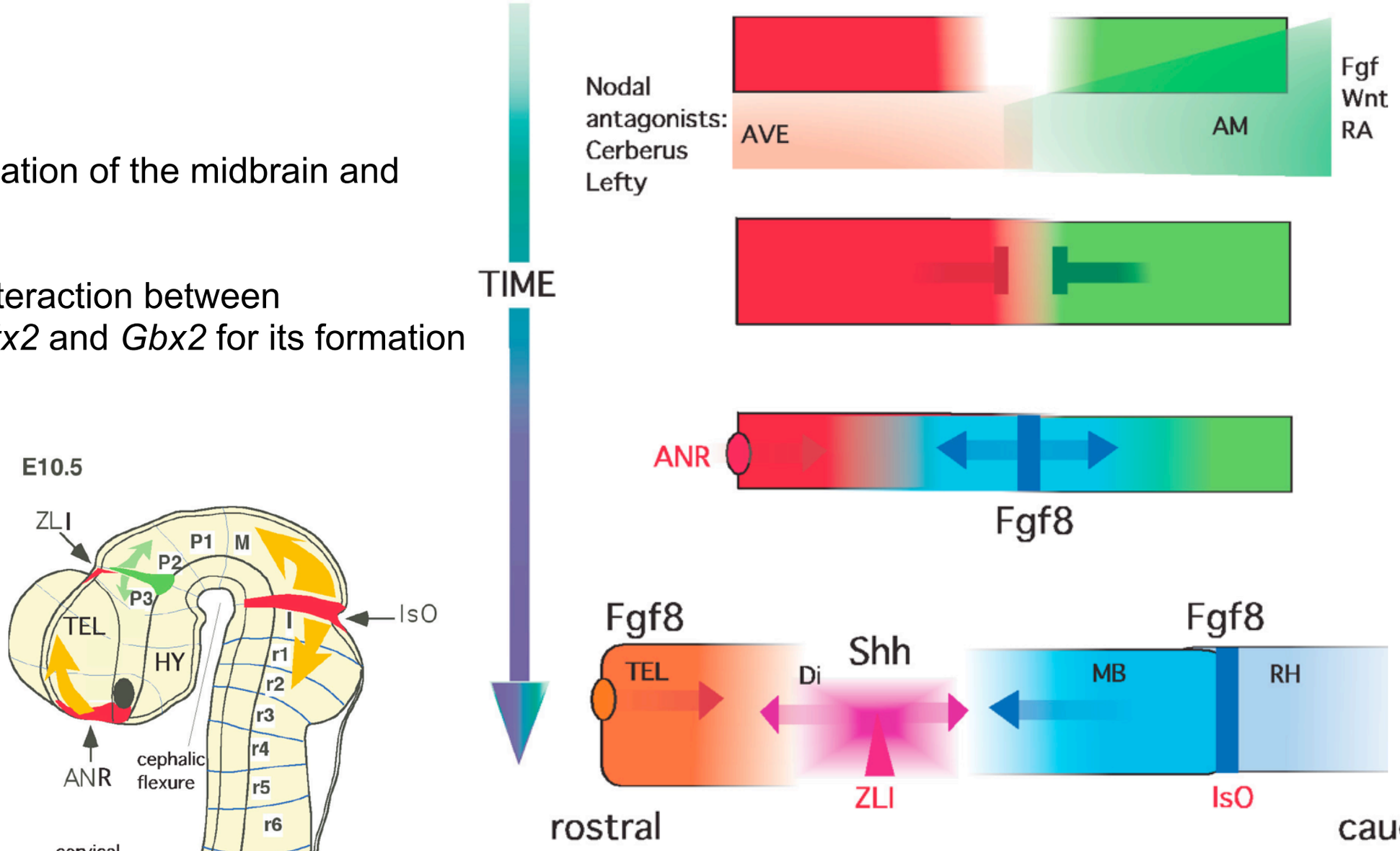
Isthmic organizer (IsO)

-formed at the junction between midbrain and hindbrain

-expresses FGF8

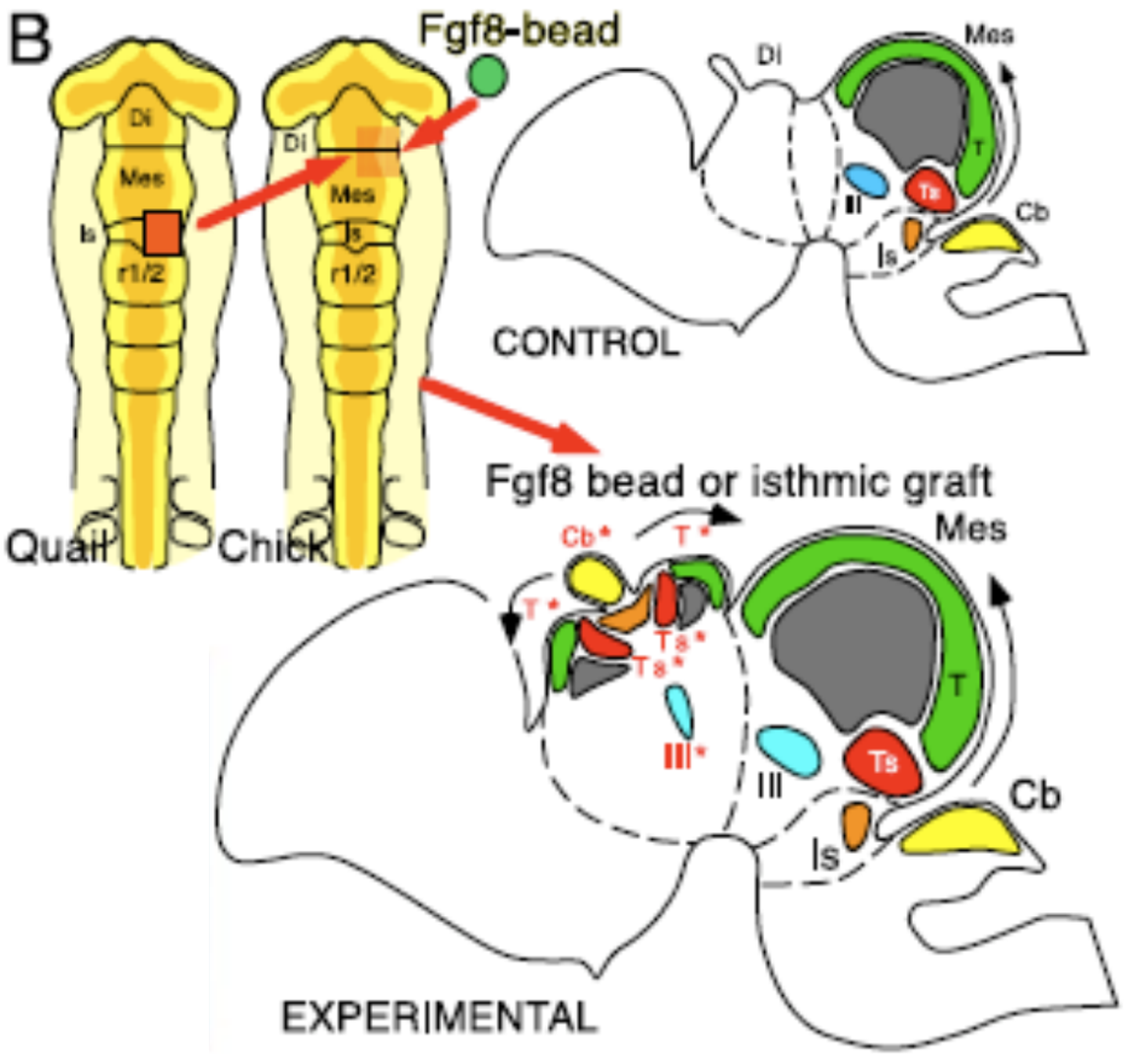
-controls the regionalization of the midbrain and anterior hindbrain

-requires repressive interaction between transcription factors *Otx2* and *Gbx2* for its formation



Vieira et al., 2010

Grafted IsO or FGF8-soaked beads induced midbrain and cerebellum in the host forebrain



-reminiscent of Spemann's organizer transplant experiment

(re-patterning of the surrounding neuroepithelium upon transplantation)

-FGF8-soaked beads mimic the effects of IsO transplantation.

-The induced ectopic midbrain is correctly polarized, suggesting that FGF8 has a role in patterning the midbrain, as it has a patterning role in the neocortex

Eschevaria et al. 2003

Primary vs secondary organizers

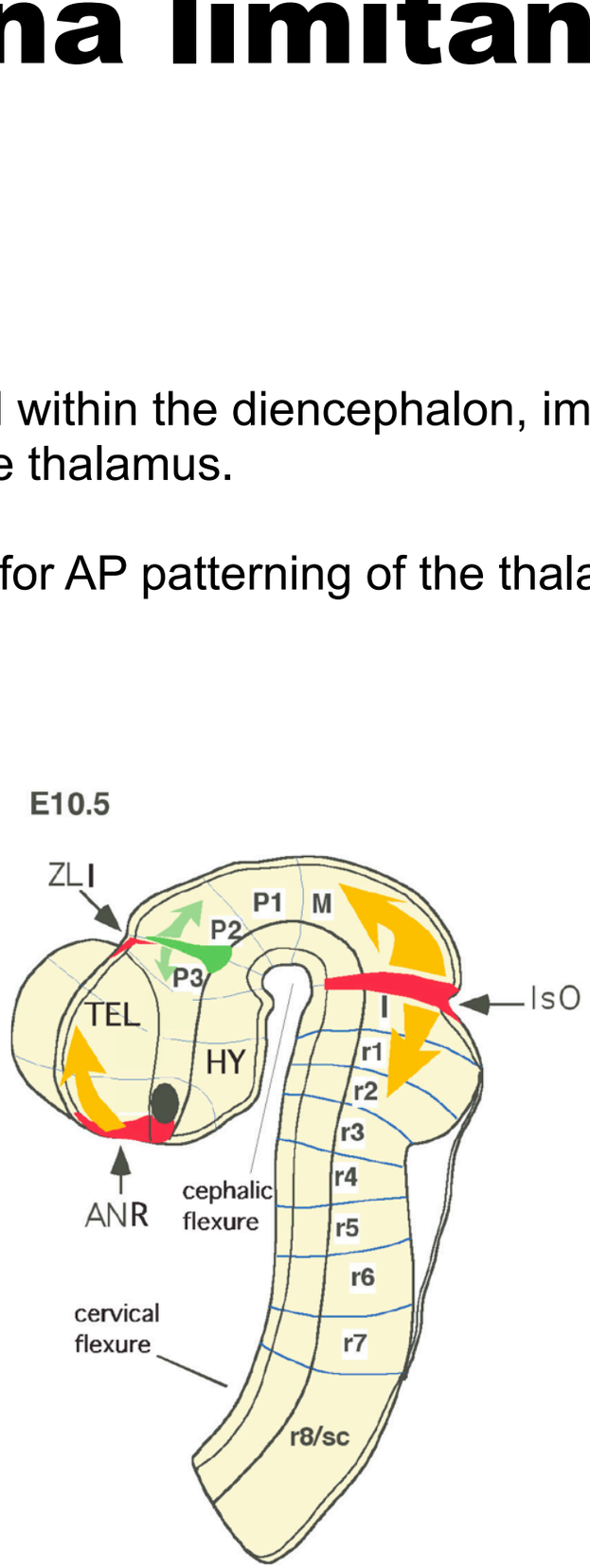
primary organizer:
The Spemann organizer (e.g. dorsal lip in Xenopus, node in mice) induces a neural tissue in the ectoderm that would otherwise form the epidermis. BMP inhibitors are the responsible molecules for this role.

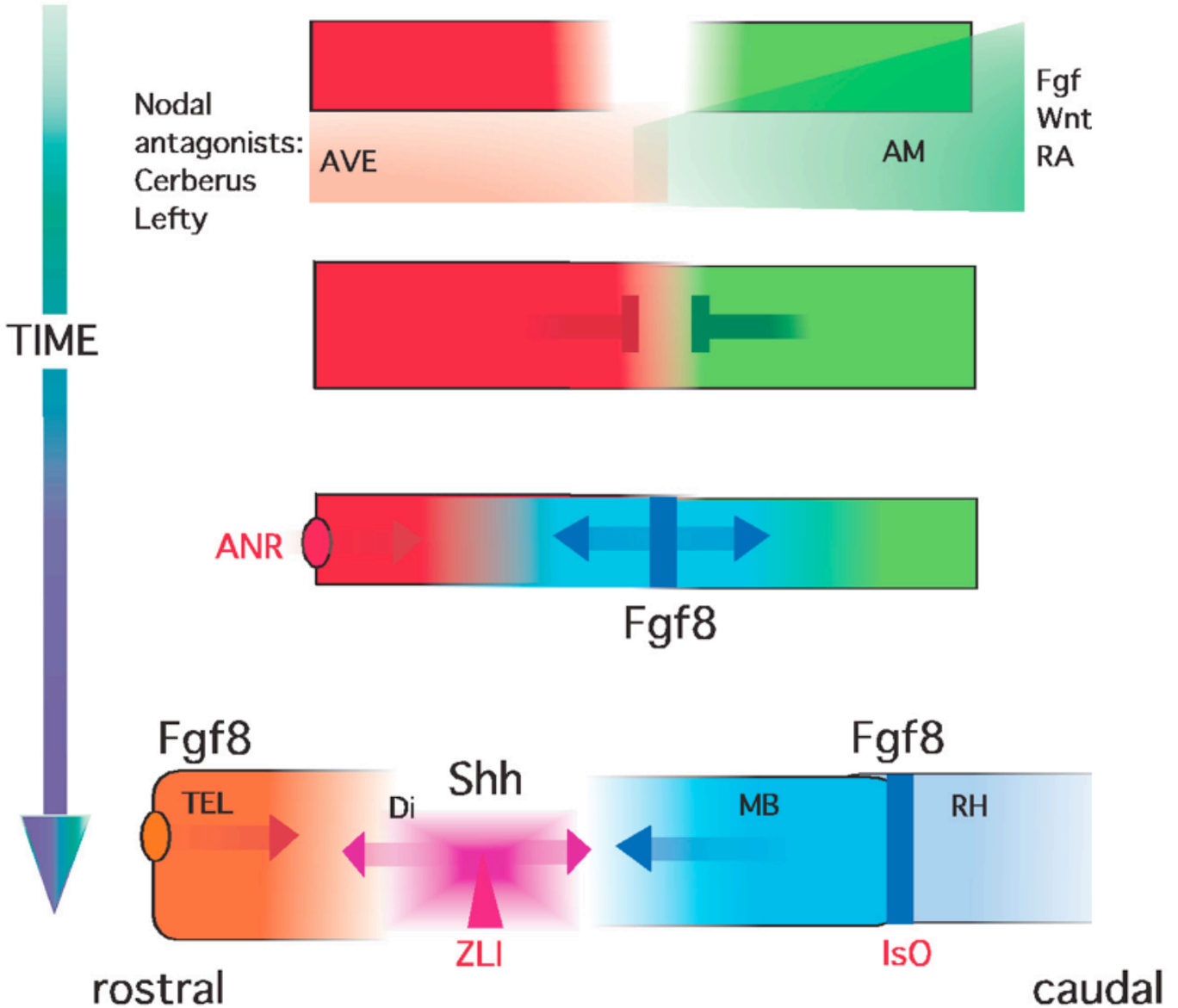
secondary organizer:
The isthmic organizer induces midbrain in the neural tissue that would otherwise form the caudal diencephalon. FGF8 is the responsible molecule for this role.

Zona limitans intrathalamica (ZLI)

ZLI is located within the diencephalon, immediately anterior to the thalamus.

ZLI is critical for AP patterning of the thalamus.





Vieira et al., 2010

Summary of part 1

Secondary organizers that control anterior-posterior patterning:

-are formed within the neural tissue that secretes signaling molecules (morphogens)

-examples: anterior neural ridge (ANR), isthmic organizer (IsO), zona limitans intrathalamica (ZLI)

-secrete signaling molecules like FGF8 and Shh

-modulate and refine initial regional patterning such that the differential gene expression subdivides the neural plate into discrete territories that prefigure the various structures of the mature CNS.

ANR (FGF8): cerebral cortex
IsO (FGF8): midbrain, cerebellum
ZLI (Shh): thalamus

Dorsal-ventral patterning is also controlled by secondary organizers.
-floor plate (ventral-most)
-roof plate (dorsal-most)
-Formation of both requires signals from surrounding, non-neural tissues

Key concepts for part 2- segmental organization of the hindbrain

Hindbrain is divided into anterior-posterior segments called rhombomeres.

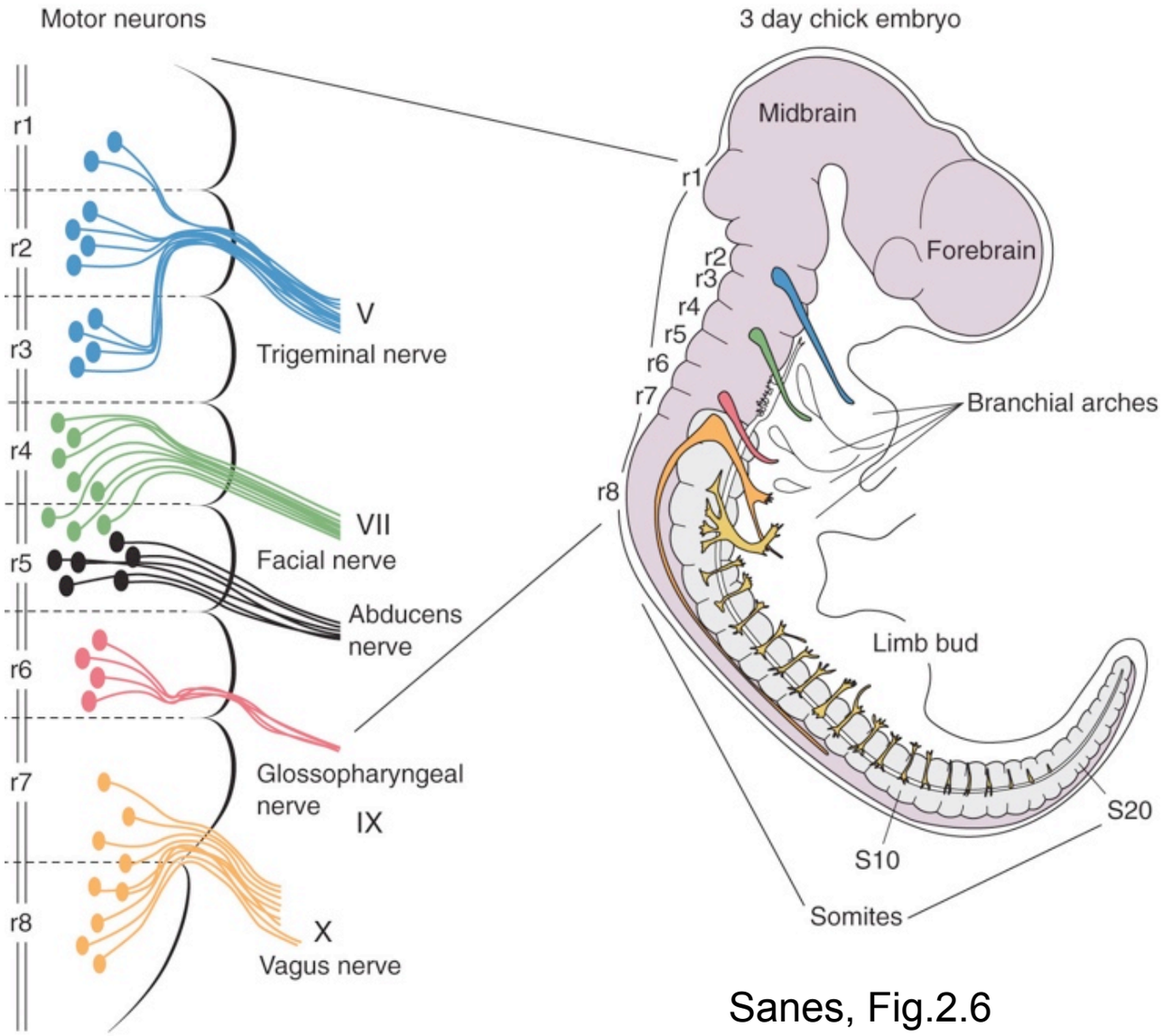
Specific gene regulation and cell segregation allow each rhombomere to have its own cellular identity.

Hox genes are expressed in segmental patterns that match rhombomere borders.

Expression of *Hox* genes are regulated by signals from the somite, including retinoic acid (RA) and FGF.

Hox genes regulate the segmental identity of the hindbrain.

Hindbrain is divided into rhombomeres

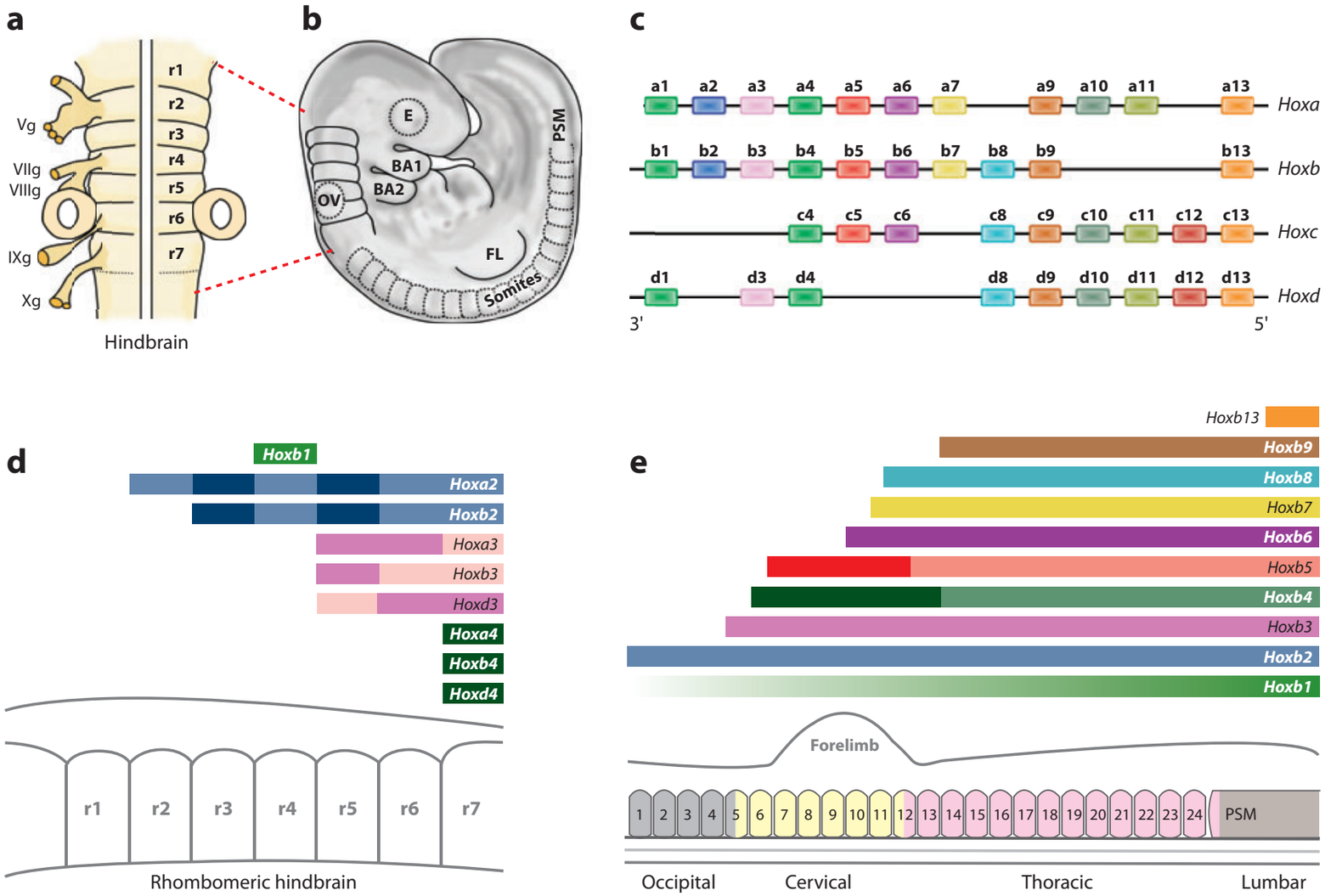


Sanes, Fig.2.6

Hindbrain is segmented in a progressive process and is divided into discrete units called **rhombomeres.**

Cell sorting mechanisms create segregated groups of cells that adopt distinct characteristics (e.g., cranial nerve nuclei)

Segmental expression patterns of mammalian Hox genes in the hindbrain and spinal cord

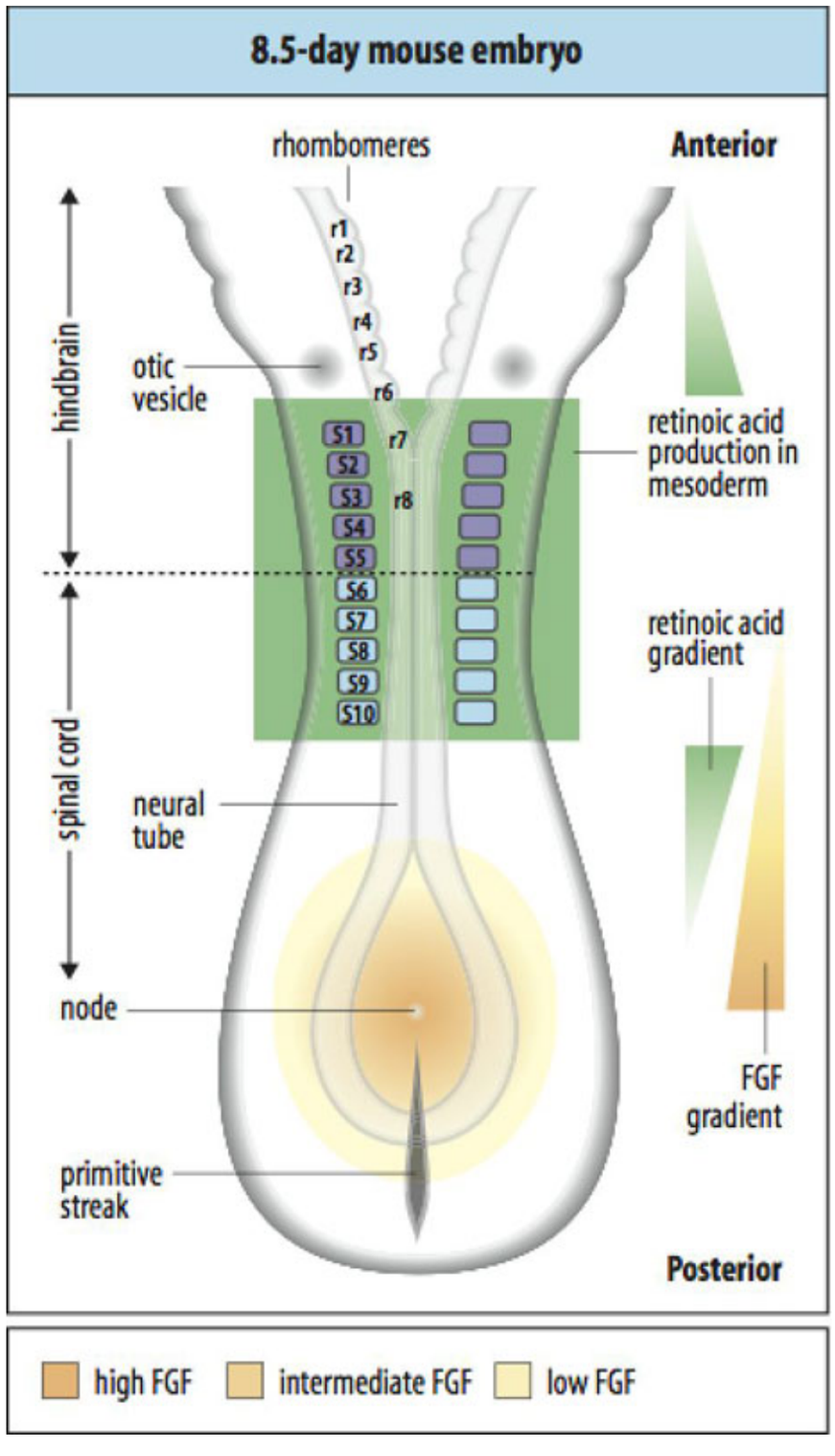


Alexander et al. (2009)

<u>Colineality:</u> Anterior expression borders of *Hox* genes are correlated with the positions of their locations on the chromosome (the more 3' the gene is, the more anterior the border of expression is).

The most anterior border of *Hox* gene expression is between r1 and r2.

How is the expression of Hox gene regulated?



Signals from the isthmus prevents *Hox* gene expression in the most anterior rhombomere.

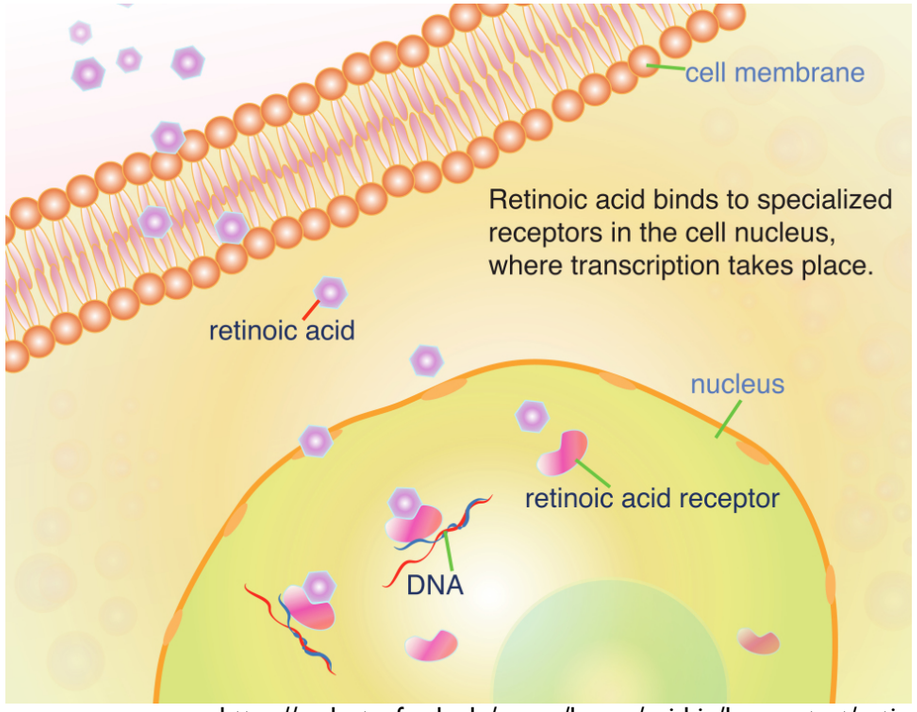
Initial activation of *Hox* genes in the hindbrain and spinal cord is mediated by morphogens acting in a graded manner along the AP axis.

Both retinoic acid (RA) and FGFs are expressed in paraxial mesoderm (later forming the somite).

Exposure of hindbrain progenitors to elevated RA in chick leads to an expansion of caudal rhombomeres at the expense of rostral, while inhibition of RA expands rostral and depletes caudal rhombomeres.

Regulation of *Hox* gene expression by RA and FGF can be considered as part of the “transformation” mechanisms during regionalization.

How does RA regulates Hox gene expression and hindbrain segmentation?

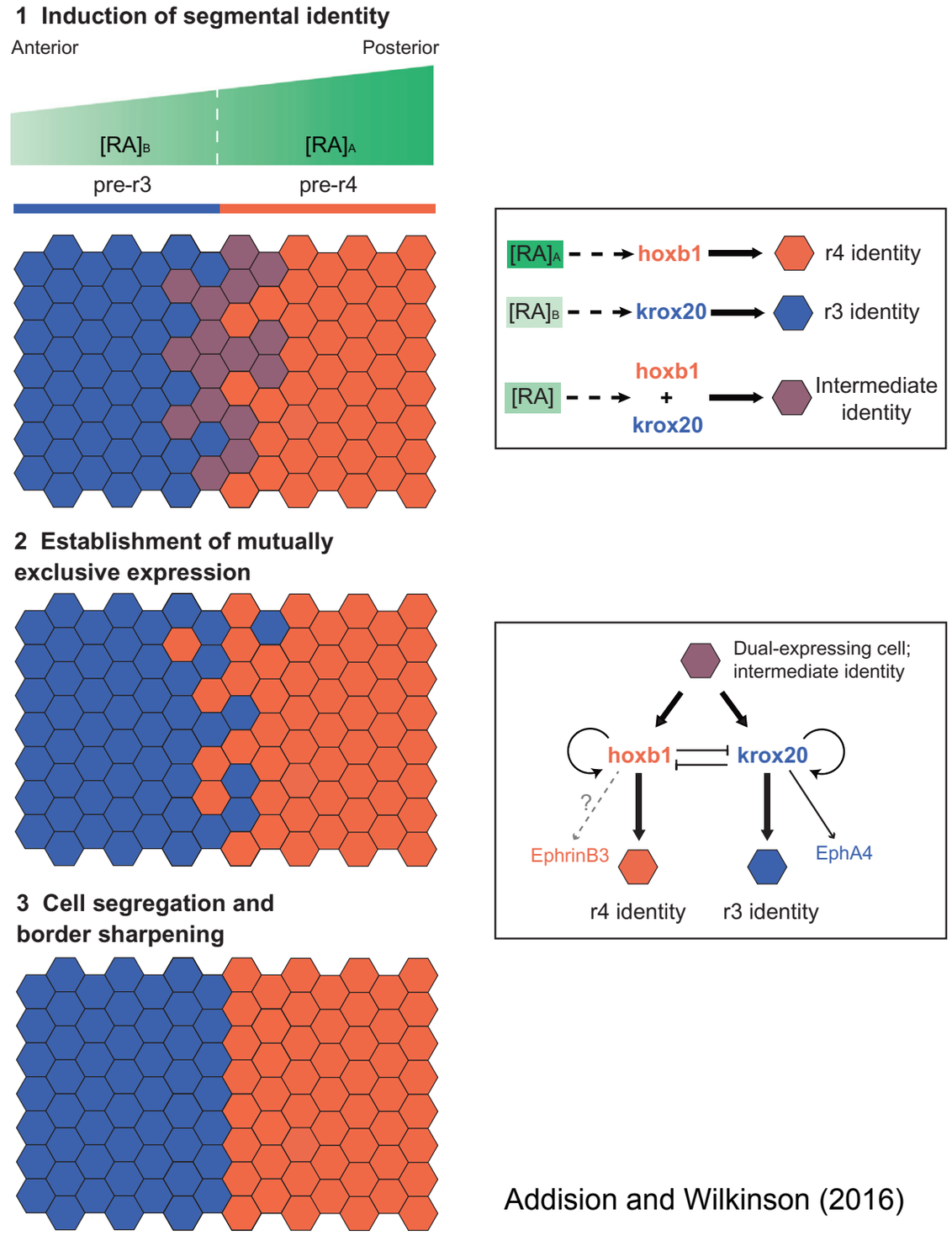


https://web.stanford.edu/group/hopes/cgi-bin/hopes_test/retinoic-acid-ra/

RA binds to specific “nuclear receptors”, which bind DNA and activate transcription of various other transcription factors including many *Hox* genes (e.g., *Hoxb1*).

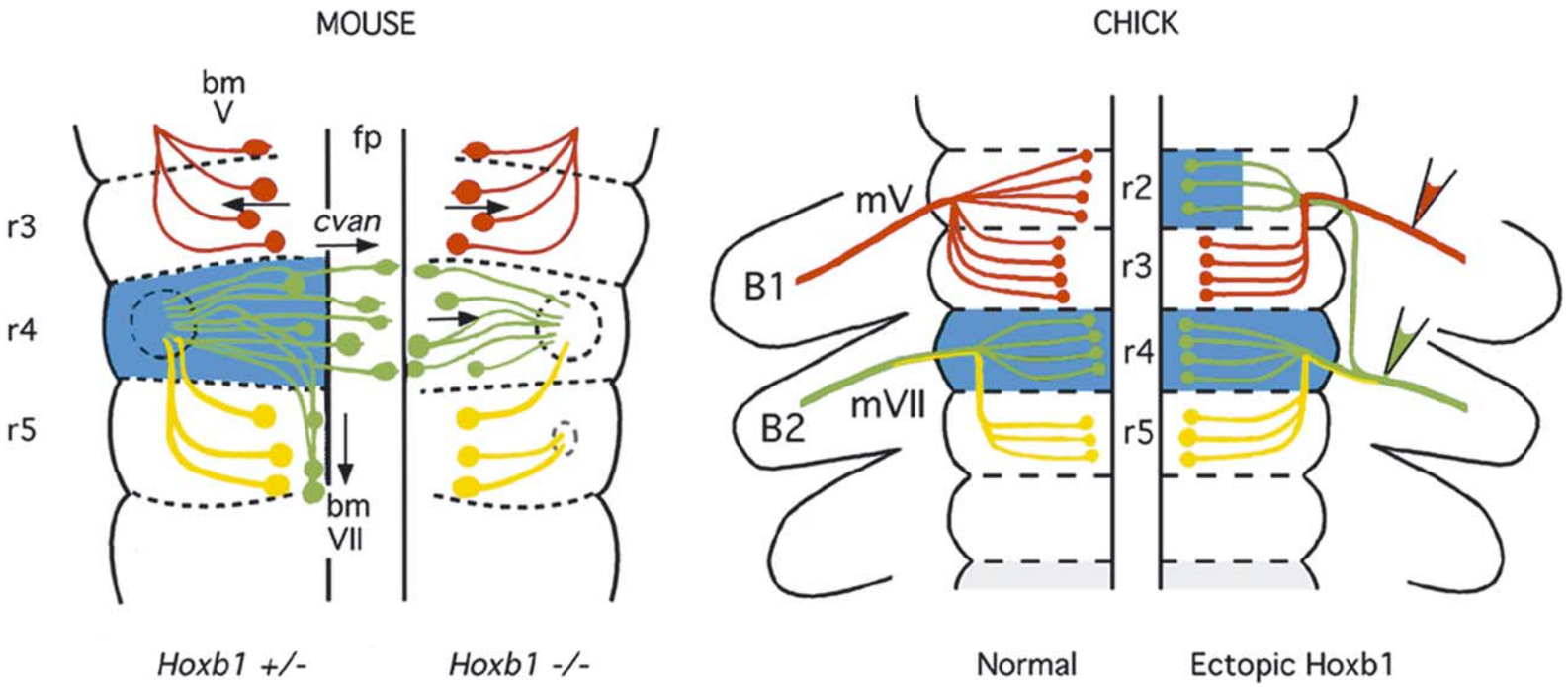
Mutual repression between two transcription factors sharpens boundaries of gene expression.

Transcription factors also regulates genes (e.g. Ephs and Ephrins) that contribute to segregation of cells between gene expression boundaries.



Addision and Wilkinson (2016)

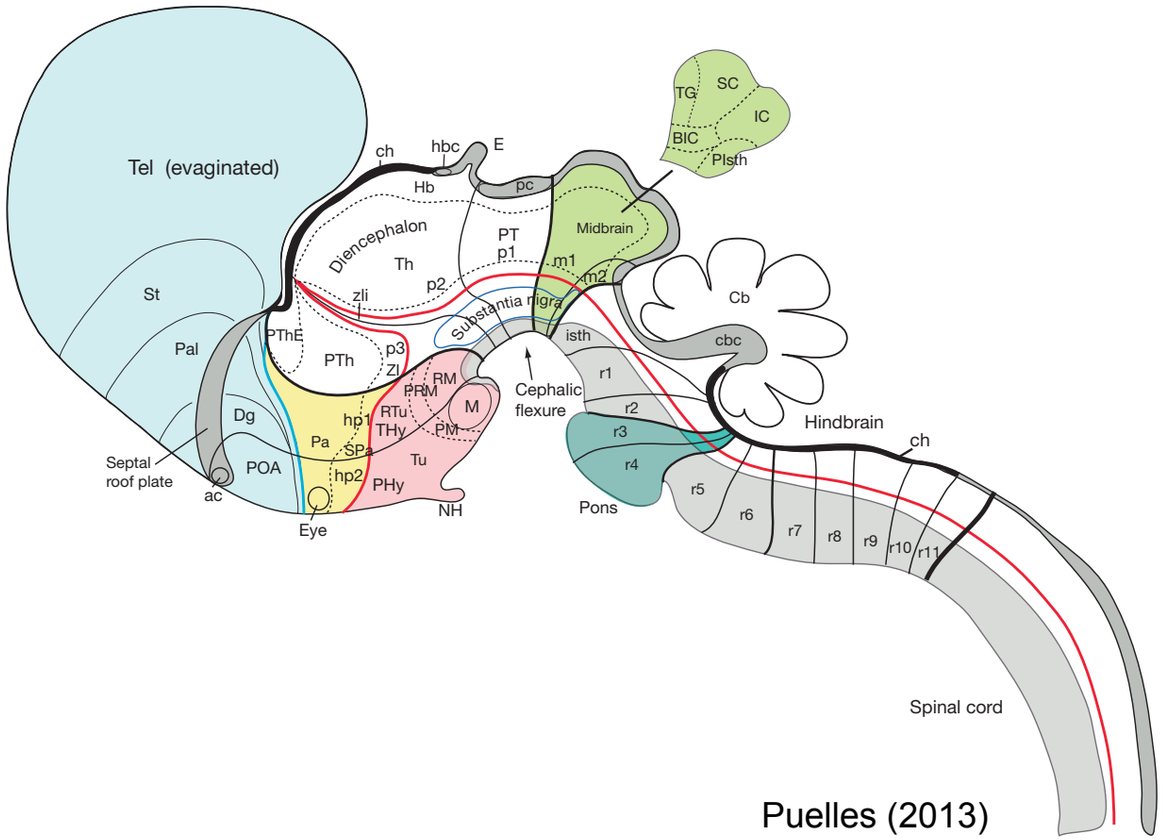
Hox genes regulate the segmental identity of the hindbrain



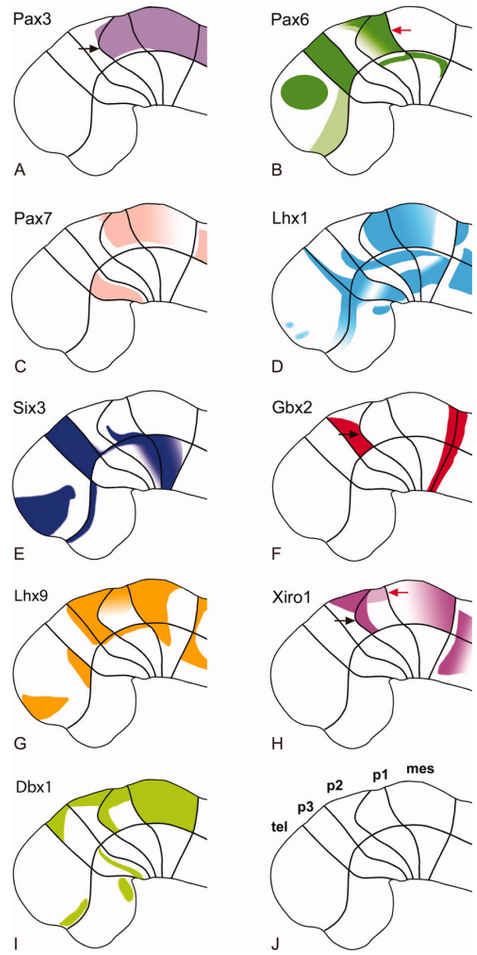
In *Hoxb1* knockout mice, motor neurons generated in r4 (innervating facial muscles) take an abnormal migration pattern that resemble those generated in r2.

In chick where *Hoxb1* mis-expression in r2, motor neurons in r2 grow axons to in the target of motor neurons from r4.

Is the rostral part of the brain segmented like the hindbrain?



Puelles (2013)



Moroa et al. (2016)

Based on morphology and gene expression pattern, forebrain and midbrain can be further subdivided into “prosomeres” (Puelles and Rubenstein, 1993, 2003).

Many homeobox genes that do not belong to the *Hox* gene family are differentially expressed in the forebrain and midbrain across species.

Summary of part 2- segmental organization of the hindbrain

Hindbrain is divided into anterior-posterior segments called rhombomeres.

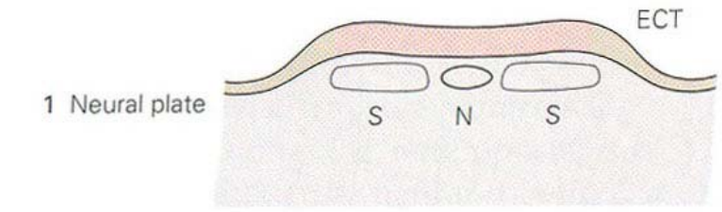
Specific gene regulation and cell segregation allow each rhombomere to have its own cellular identity.
-e.g., motor neurons that form specific cranial nerves (trigeminal, facial, etc.)

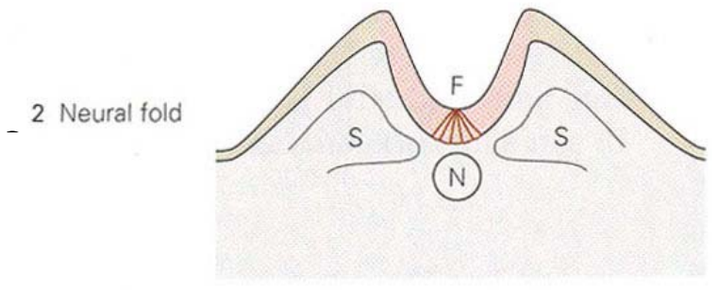
Hox genes are expressed in segmental patterns that match rhombomere borders.
-colineality with regard to anterior expression borders

Expression of *Hox* genes are regulated by signals from the somite, including retinoic acid (RA) and FGF.
-"transformation signal"
-RA binds to receptors, which then bind to DNA to activate transcription of downstream genes.
-Mutual interactions between transcription factors sharpens borders of gene expression

Hox genes regulate the segmental identity of the hindbrain.
-Deletion or mis-expression of *Hox* genes in vertebrate hindbrain causes phenotypes similar to homeotic mutations in flies.

Key concepts for part 3- dorsoventral patterning of the neural tissue



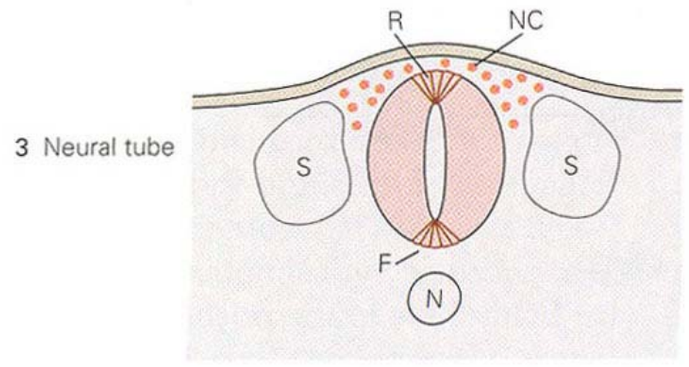


Dorsoventral (DV) patterning starts at the neural plate stage.

Similar to early AP patterning, early DV patterning is imposed by signals that come from outside:

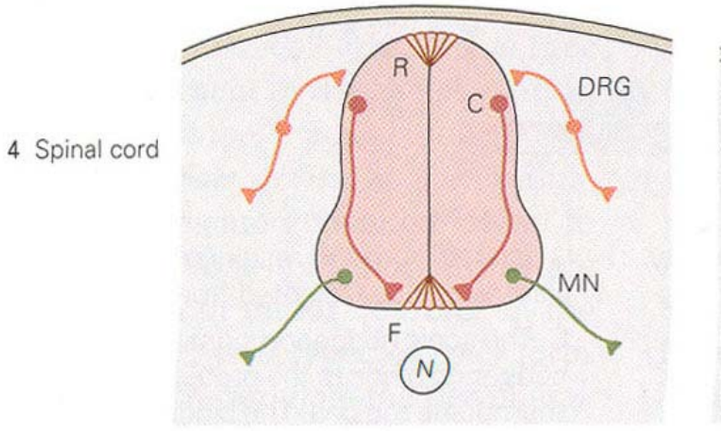
medial (future ventral): notochord

lateral (future dorsal): ectoderm



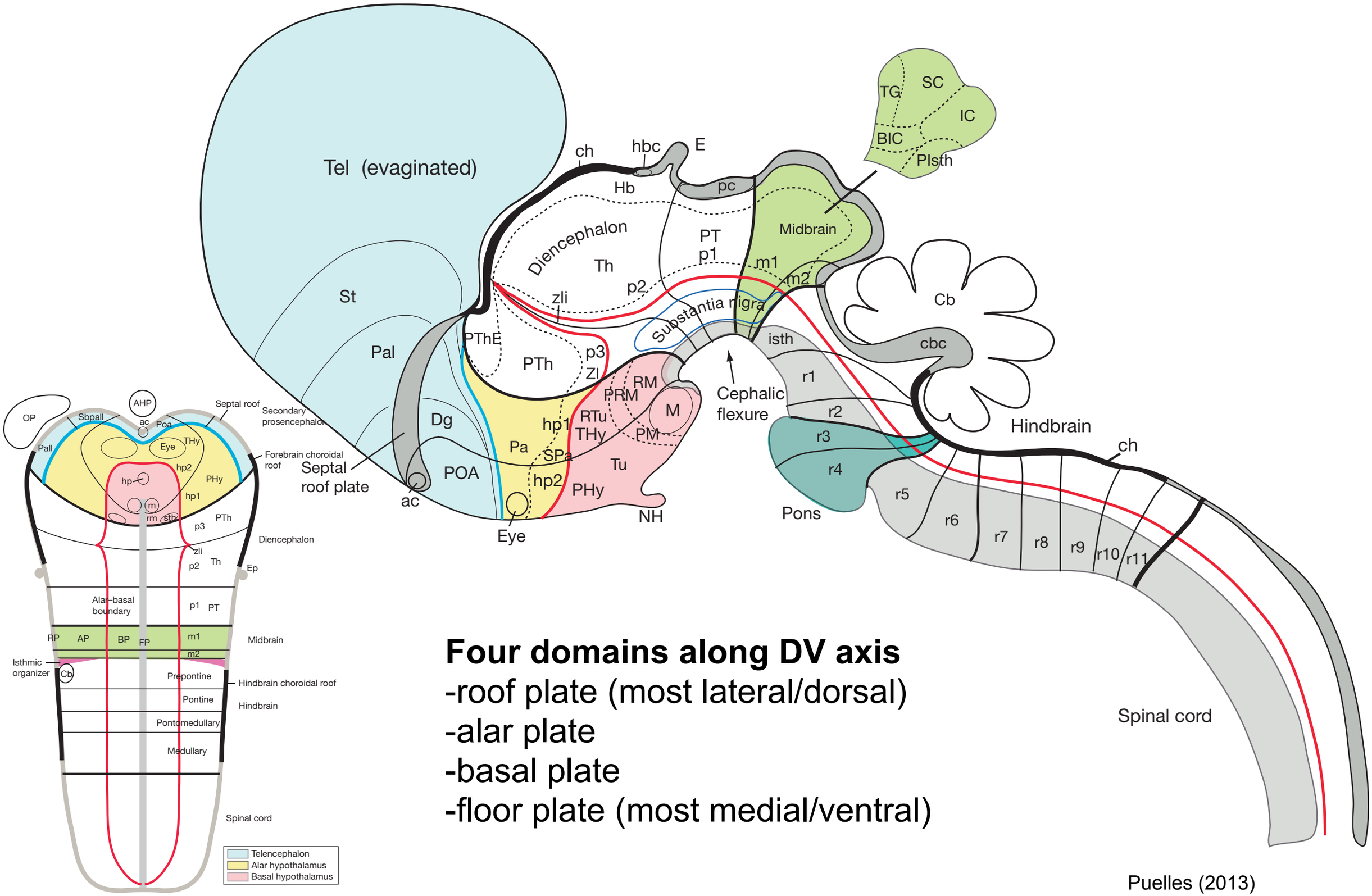
Notochord induces the floor plate at the midline (future ventral end.

Epidermis (surface ectoderm) induces roof plate at the lateral edge (future dorsal end.



Floor plate and roof plate secrete signaling molecules that antagonize with each other and further pattern the neural tube into domains along DV axis.

DV patterning



Four domains along DV axis

-roof plate (most lateral/dorsal)

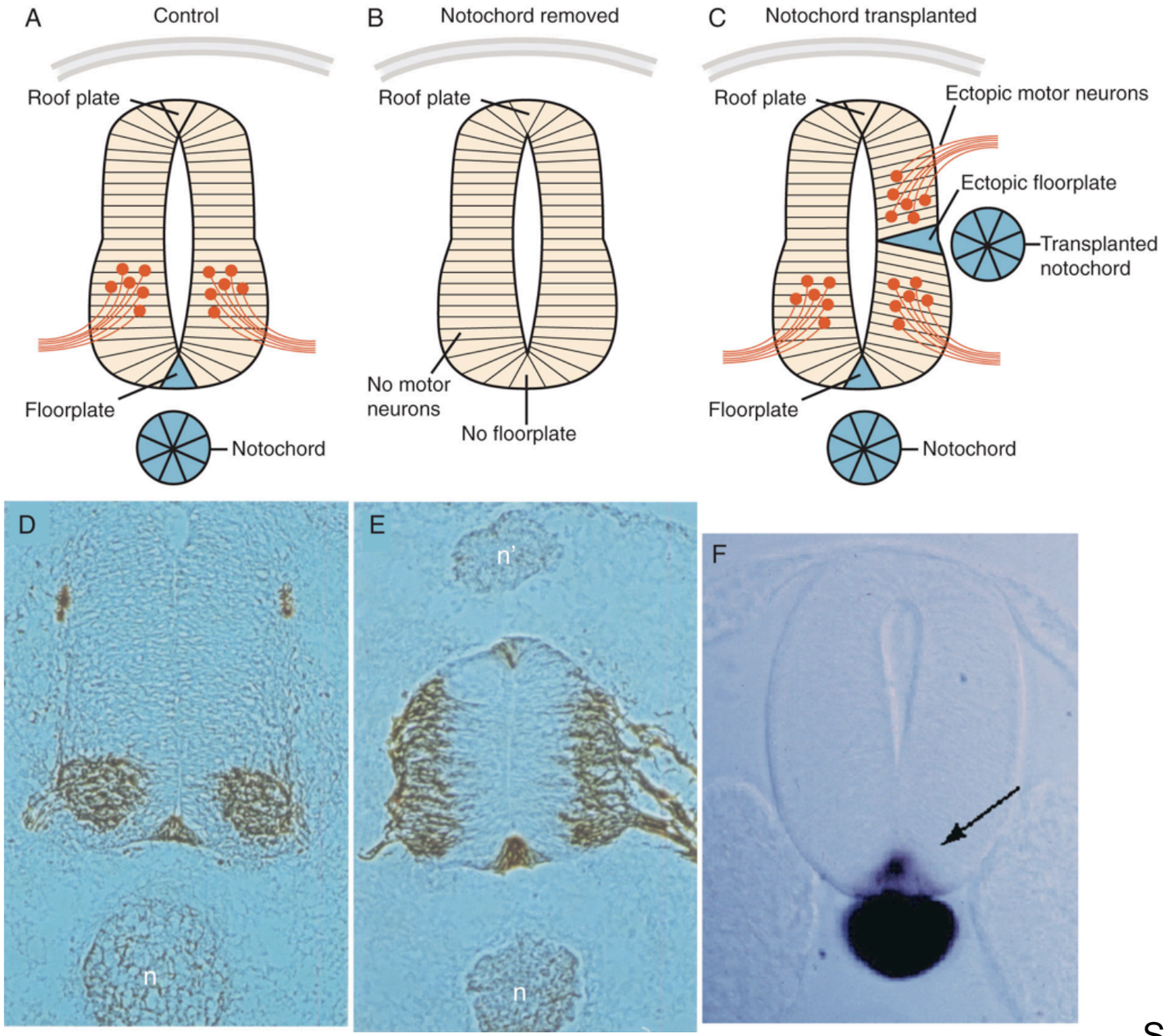
-alar plate

-basal plate

-floor plate (most medial/ventral)

Puelles (2013)

The notochord controls the dorsal-ventral polarity of the neural tube

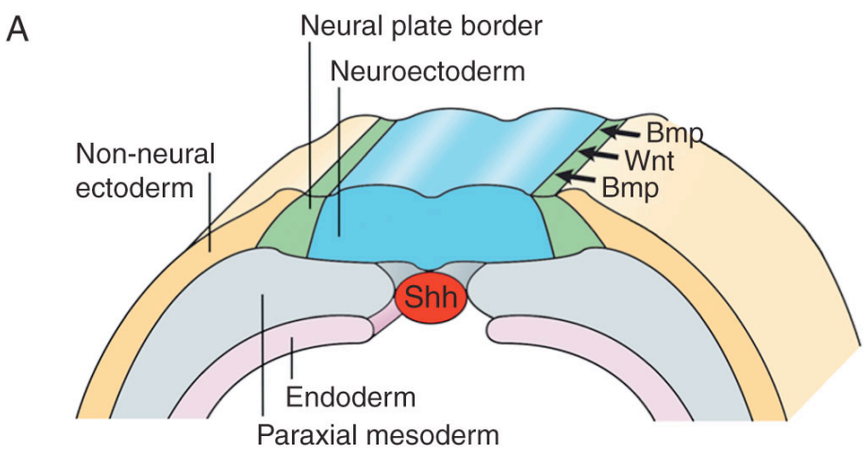


B. Removal of the notochord results in the loss of the floor plate and motor neurons.

C. Ectopic transplantation of the notochord near the dorsal neural tube induces a second floor plate and ectopic motor neurons.

Sanes, Fig.2.22

Sonic hedgehog (Shh) controls the ventral identity of the neural tube



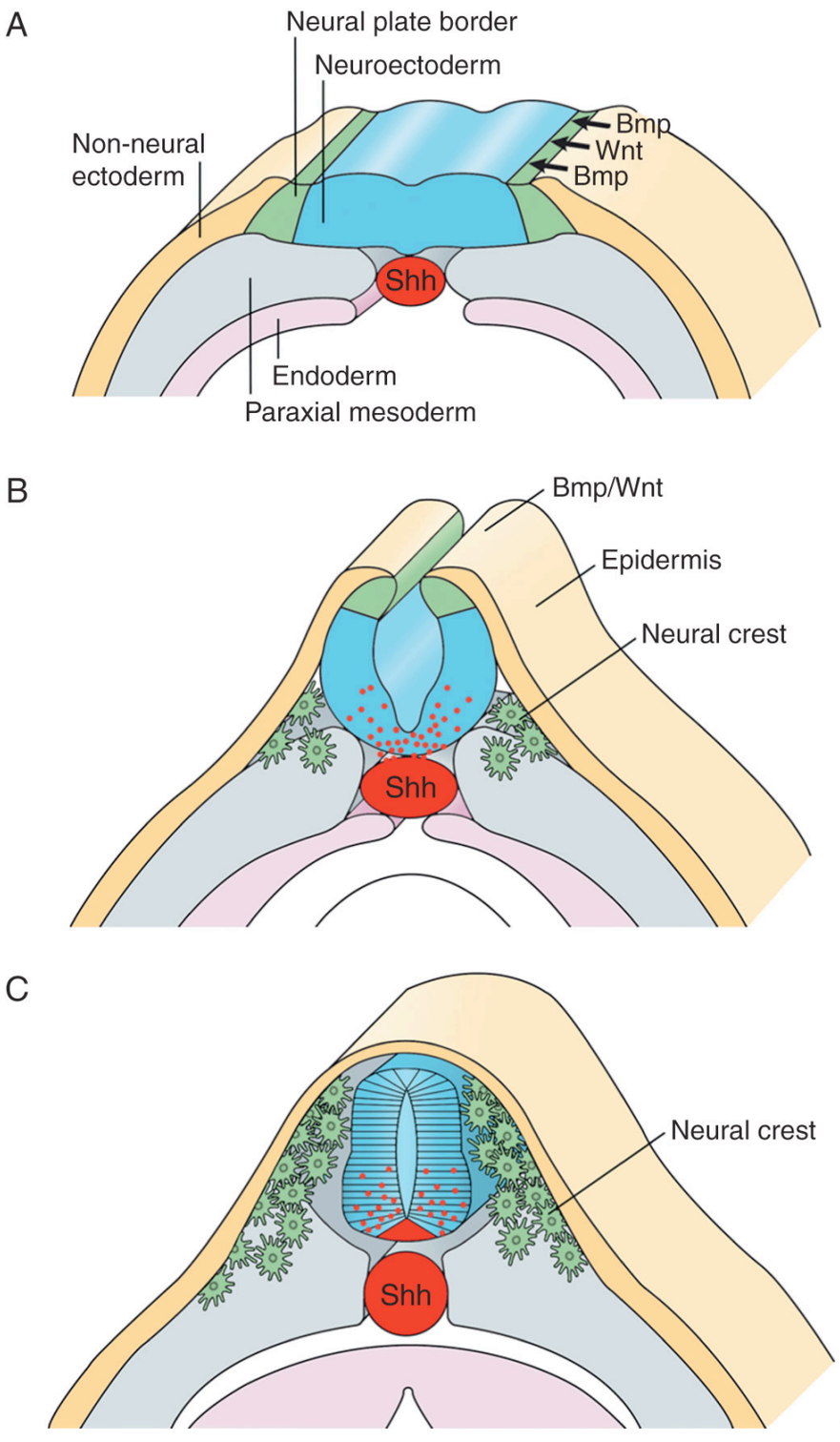
Shh is a homologue of the Drosophila segment polarity gene, *Hedgehog*.

Notochord-derived Shh induces the expression of Shh in the floor plate.

Shh is secreted and form a concentration gradient along the DV axis.

Sanes, Fig.2.26

Shh and BMP signals antagonize each other in DV patterning



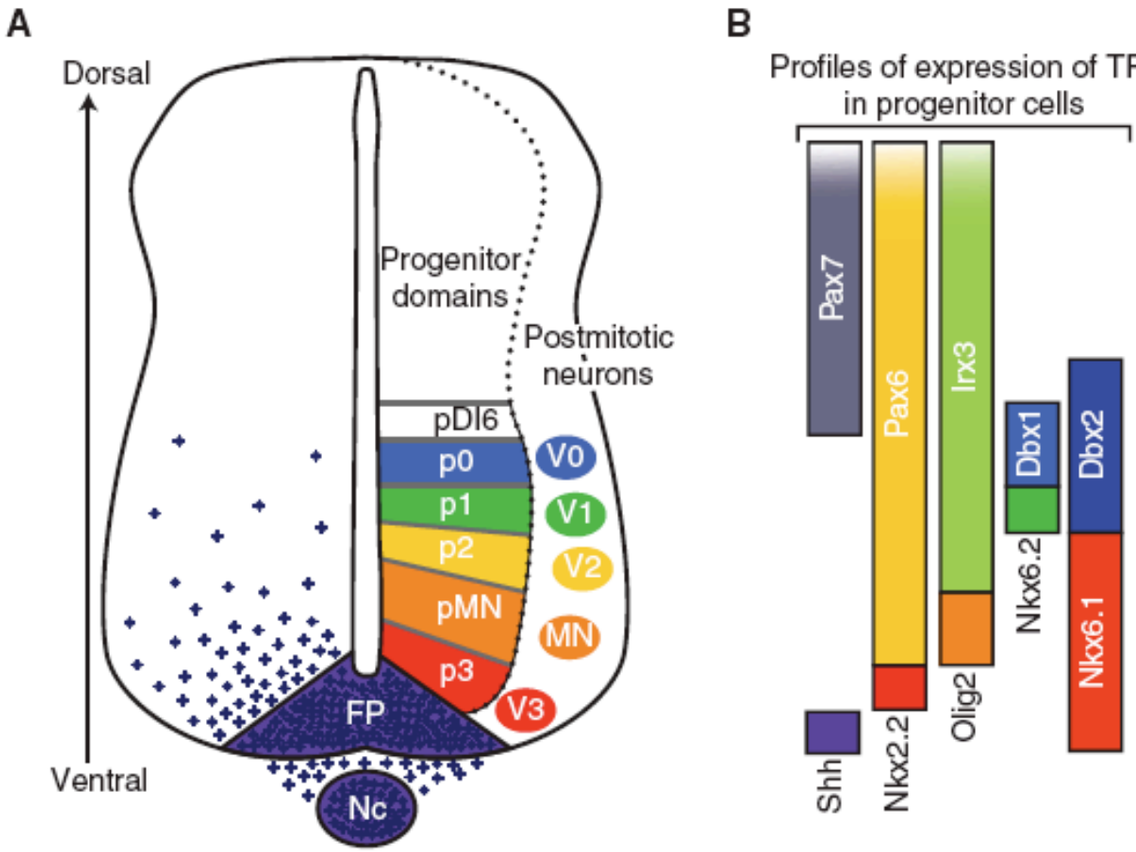
Non-neural ectoderm produces BMPs, which induces the expression of BMPs in the roof plate.

Shh and BMP signals antagonize each other to define the identity of neural progenitor cells along the dorsal-ventral axis

Neural crest is derived from the lateral border of the neural plate (green), which contribute to many cell types including neurons of the **peripheral nervous system** (e.g., the sensory neurons and autonomic neurons, Schwann cells).

Sanes, Fig.2.26

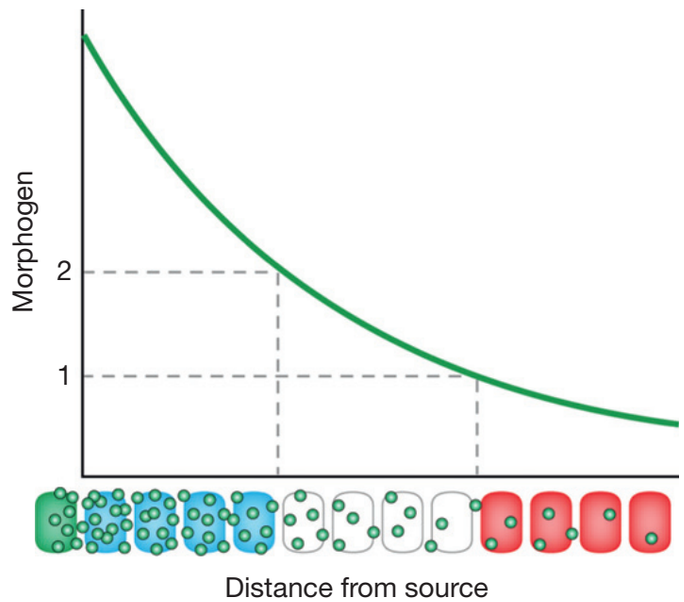
Sonic hedgehog (Shh) controls the ventral identity of the neural tube



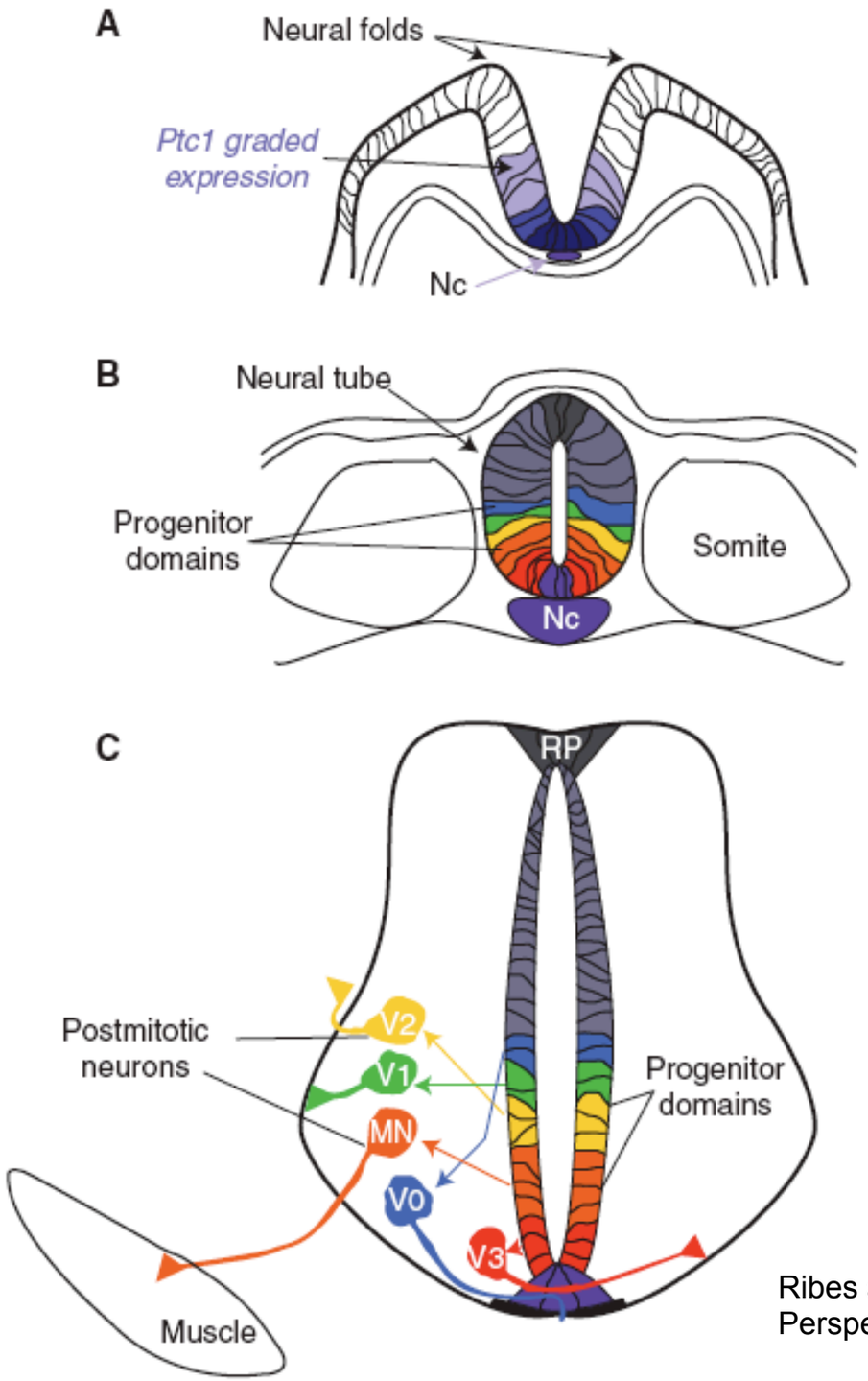
Ribes and Briscoe, (2009) Cold Spring Harbor Perspectives in Biology

Different dorsal-ventral domains of neural progenitor cells are defined by differential expression of transcription factors.

The differential expression patterns of transcription factors are established by graded Shh signaling and subsequent of sharpening of the expression borders through mutual repression of the expression of transcription factors.



Sonic hedgehog (Shh) controls the ventral identity of the neural tube



Establishment of spatial organization of neurons from ventral progenitor cells in the spinal cord is regulated by Shh signaling.

Distinct subtypes of interneurons (V0-V3) and motor neurons (MN) are generated from each of the six progenitor domains.

Ribes and Briscoe, (2009) Cold Spring Harbor Perspectives in Biology

Regionalization -summary

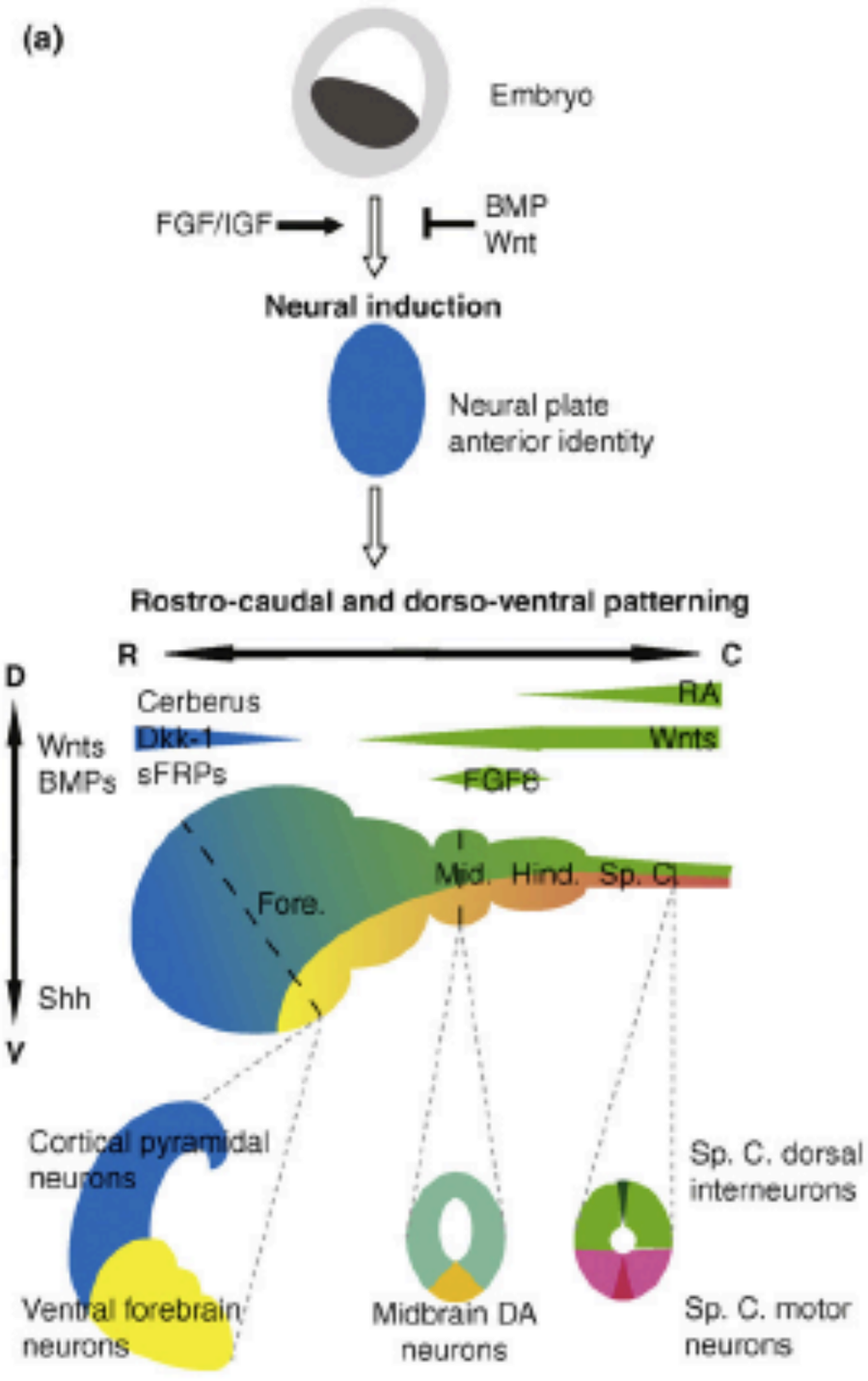
Anterior-posterior difference in the future nervous system is formed early in development, and the early mechanism is linked tightly to neural induction.

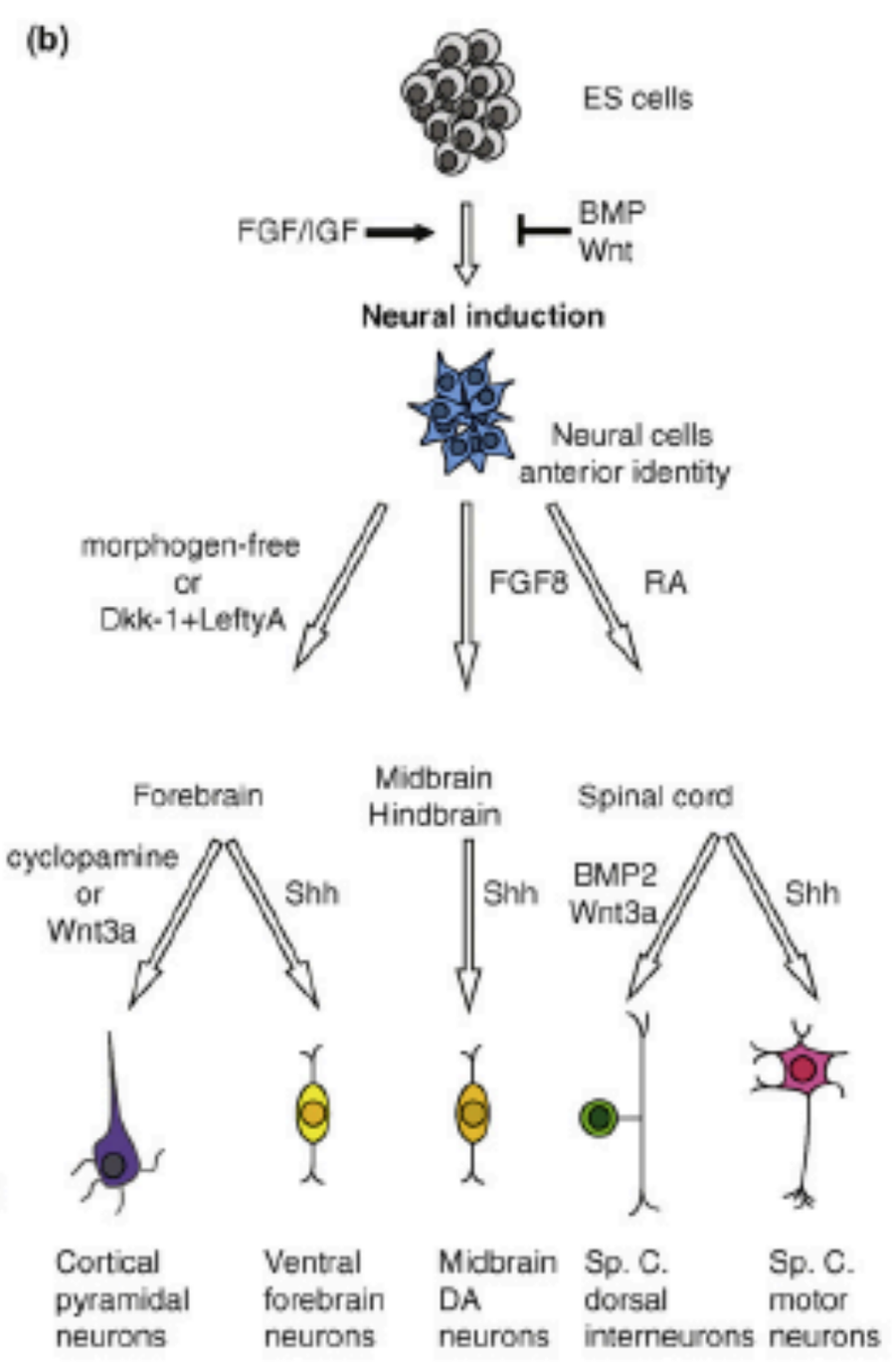
During and after gastrulation, **organizer region** and its mesodermal derivatives (notochord, prechordal plate) send signals for neural induction (e.g., BMP inhibition) and the anterior fate (e.g., Wnt inhibition). Anterior mesoendodermal tissue (e.g., AVE) also antagonizes caudalizing factors (Fgf, RA, Wnt, etc.). This initial mechanism roughly divides the nervous system into anterior and posterior halves.

”**Secondary organizers**” are formed in the neural tissue and produce signaling molecules. These signals, together with mutual suppression of transcription factor expression, further refine the grossly patterned nervous system into smaller domains of neural progenitor cells (both along AP and DV axes). Establishment of these distinct progenitor pools is crucial for the generation of different types of neurons.

AP patterning of the Drosophila embryos is a prototype for the research on vertebrate nervous system, and has provided a number of important genes whose vertebrate homologues play crucial roles.

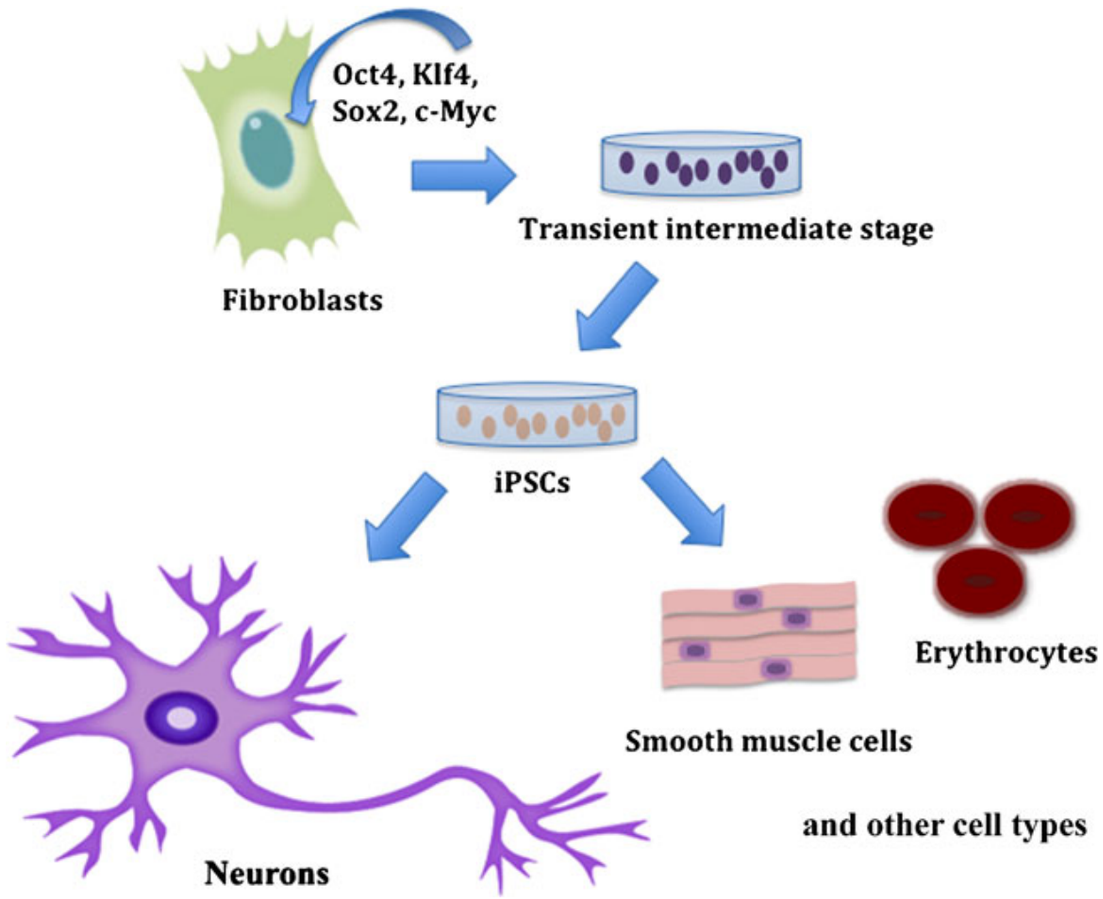
How can we apply the knowledge to ES cell-mediated neurogenesis?





Gaspard and Vanderhaeghen (2010)

From fibroblasts to neurons
-via iPS cells
(induced pluripotent stem cells)



-Similar strategies are used to generate specific types of neurons from ES cells and iPS cells.

-There are methods that bypass the formation of iPS cells, either directly generating neural stem cells or even neurons.

Use of iPSCs in translational neuroscience

Therapeutic use: transplantation of iPSCs-derived neurons or glial cells into damaged brain

replacement

chaperone effects

Modeling of human diseases

generate iPSCs from patients with various neurological or psychiatric disorders

differentiate iPSCs in vitro into neurons or glia and identify defects of patient-derived cells

screen for drugs that ameliorate the defects

3D culture system has allowed the formation of “organoids” that mimic various parts of the actual brain.