Early events of neural development

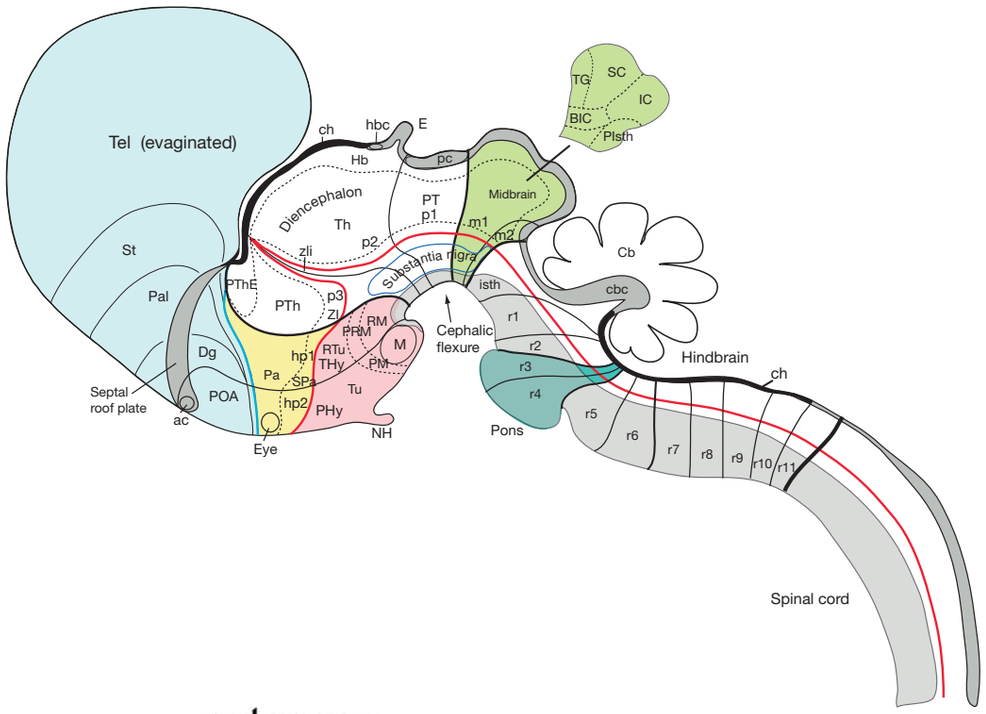
Goals:
1) to discuss the origins of cells in the nervous system
2) to discuss how neural stem cells generate diverse cell types in the nervous system

The next four lectures will cover:
Induction (Jan 22)...emergence of the nervous system
Regionalization (Jan 24)...acquisition of positional information of neural cells
Discussion of a journal article (Jan 26)

Neuronal fate specification (Jan 29)
Cell division and cell lineage (Jan 31)
Discussion of a journal article (Feb 2)

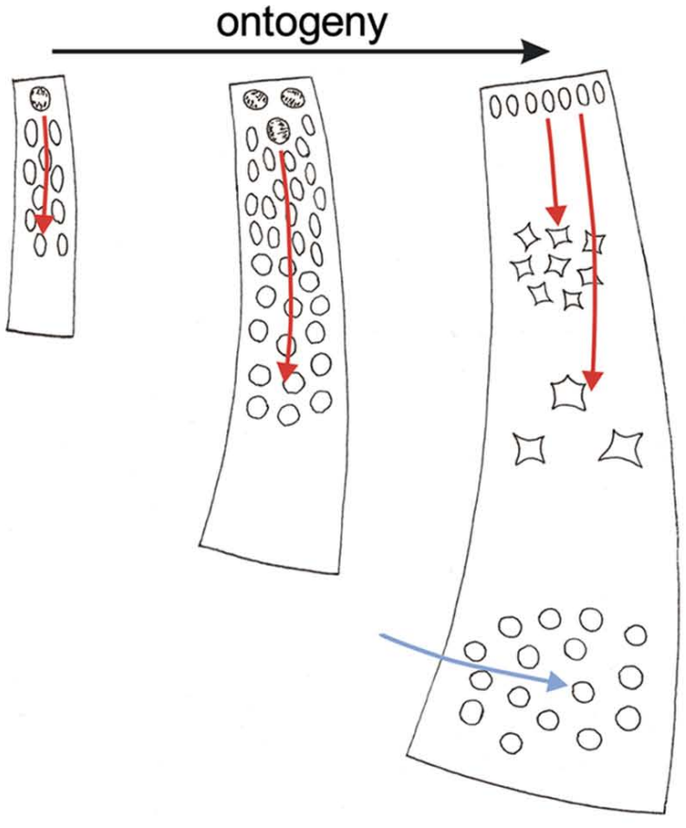
We will deal with glia later in the course!

How does the neural tissue acquire more precise positional identity?



Puelles (2013)

Specialized “patterning centers” are formed **within** the neural tissue and secrete signaling molecules that further separate the brain into distinct regions or segments.



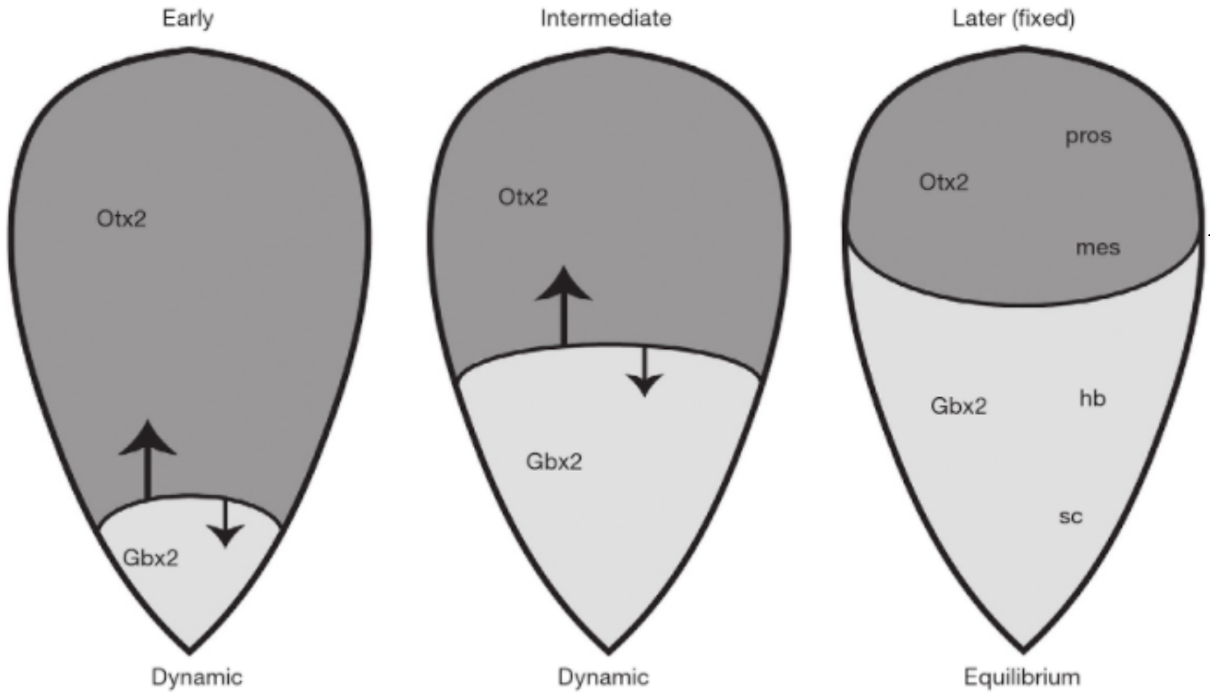
Nieuwenhuys (2011)

Neurons generally migrate radially (perpendicular to the surface of the ventricle), thus preserving the positional relationship between stem/progenitor cells and postmitotic neurons.

As a result, regional/segmental organization of early nervous system dictates the regional/segmental organization of the adult nervous system.

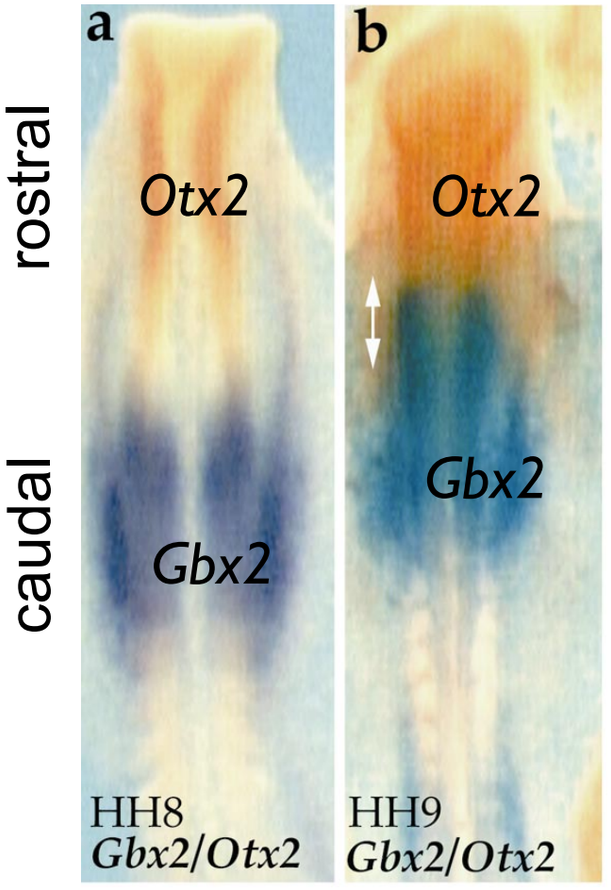
At the cellular level, positional information of individual neural stem/progenitor cells controls the types of neurons and glia they produce.

Activation-transformation results in coarse rotro-caudal patterning of the brain



midbrain-hindbrain border

Puelles 2015



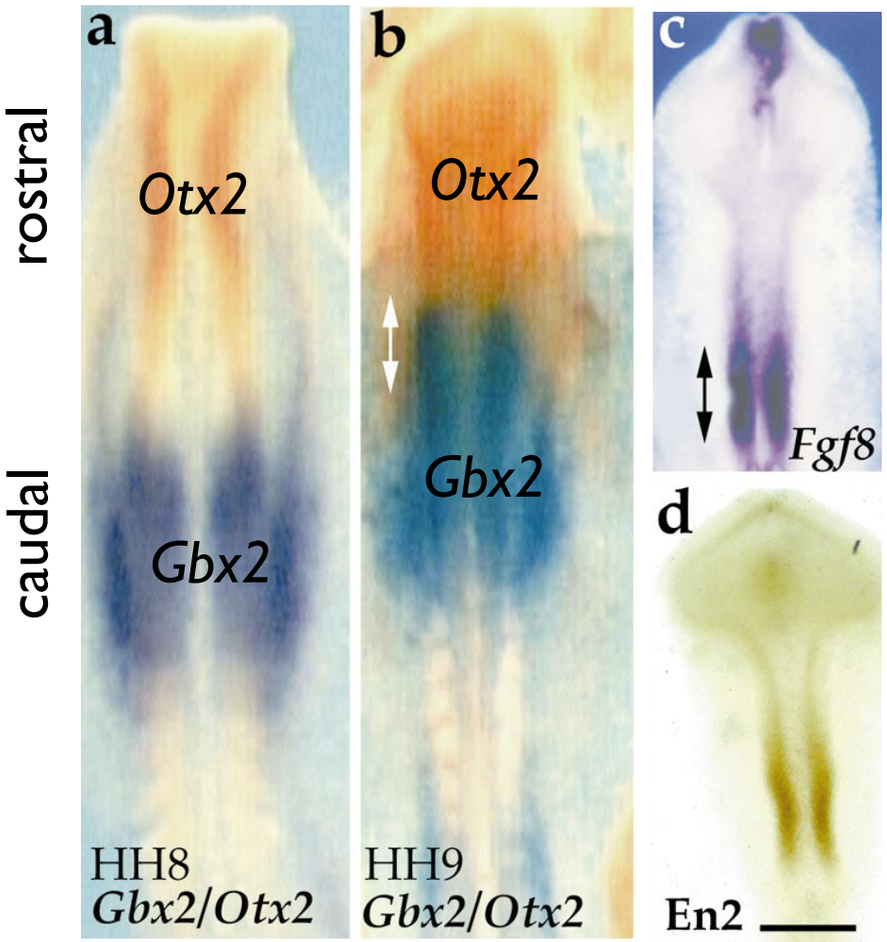
Garda et al., 2001

-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.

Interface between the Otx2- and Gbx2-expression domains becomes the rostral end of the hindbrain

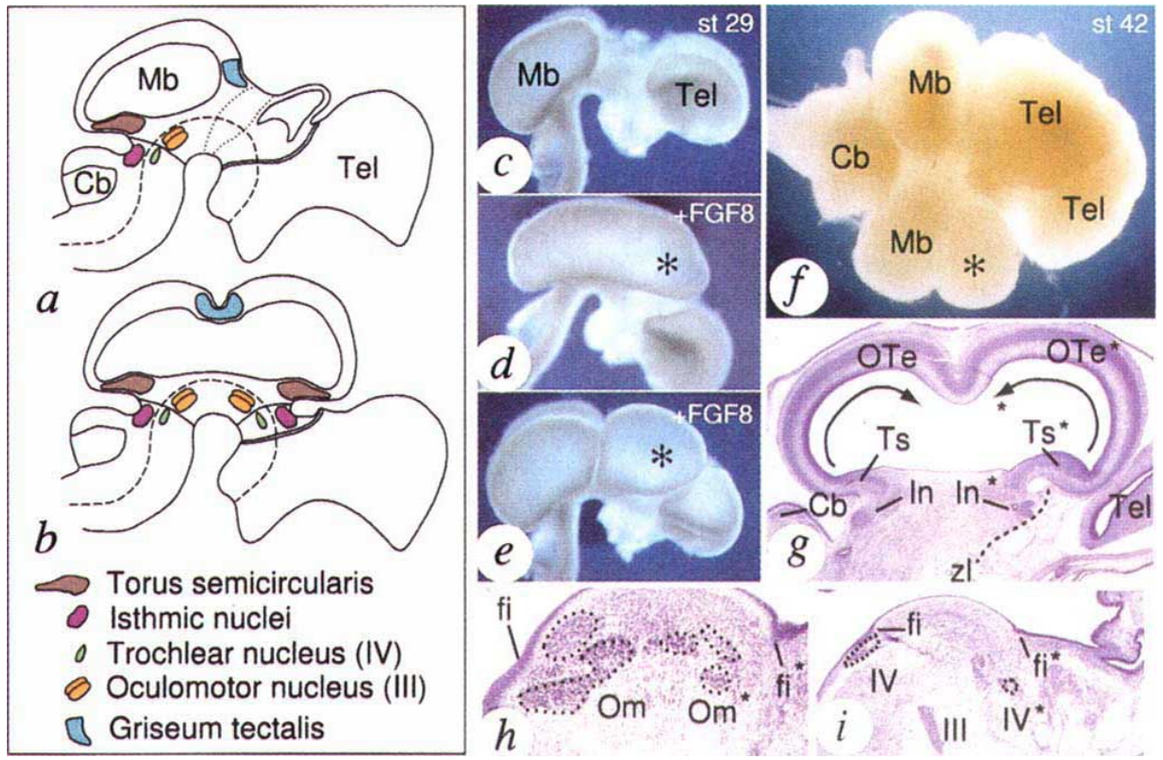


Garda et al., 2001

Co-expression of *Otx2* and *Gbx2* induced a new domain that expresses Fibroblast growth factor 8 (*Fgf8*) and the transcription factor Engrailed 2 (En2).

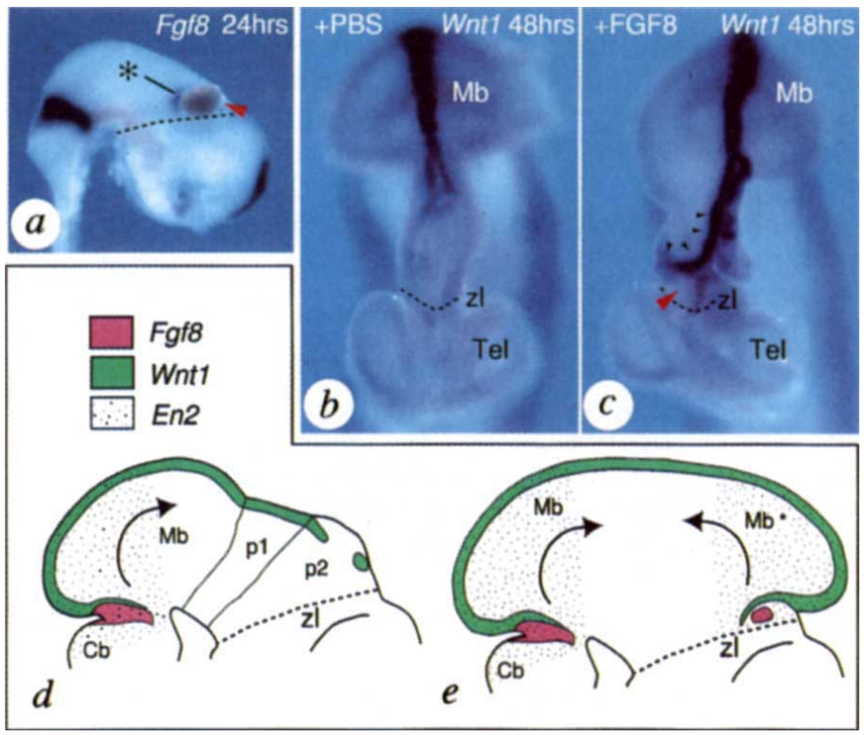
The *Fgf8*-expressing domain is called the isthmus (isthmic organizer), which forms the rostral end of the hindbrain and later contributes the cerebellum.

FGF8 induces an ectopic midbrain when expressed in the caudal diencephalon



-Implanting an FGF8-soaked bead or transplanting an isthmic tissue into the caudal diencephalon induced an ectopic midbrain in the host tissue.

-The ectopic midbrain was present in a mirror image orientation relative to the normal midbrain.



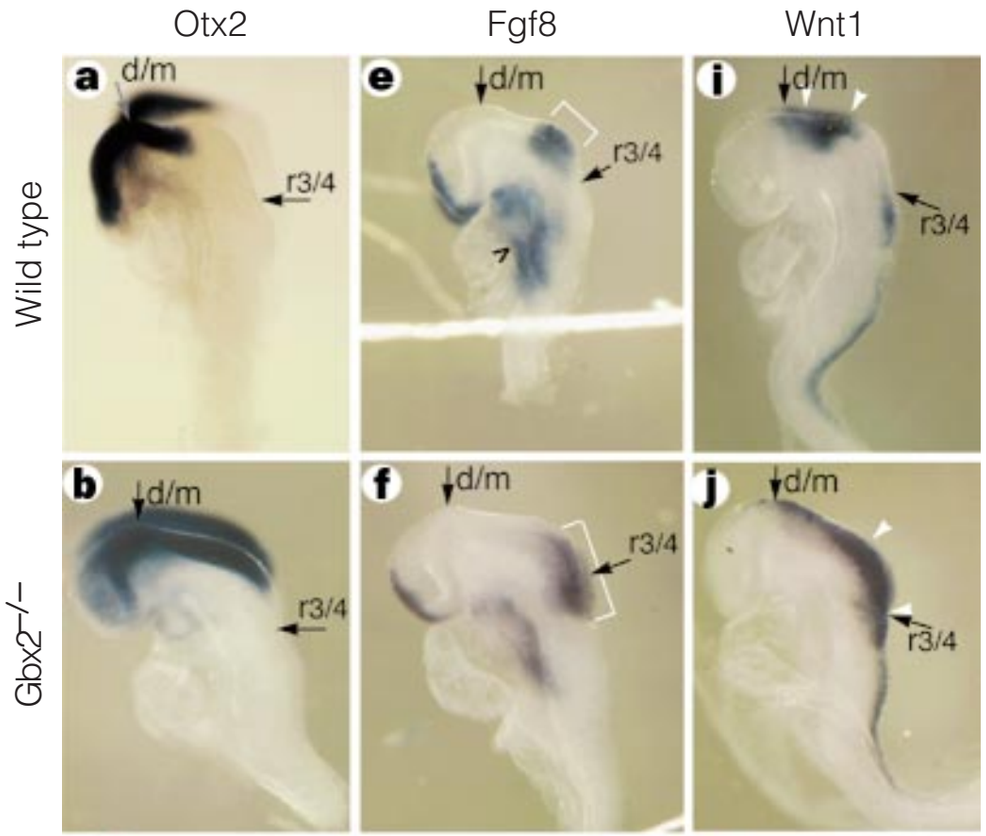
Crosby et al., 1996

Primary vs secondary organizers

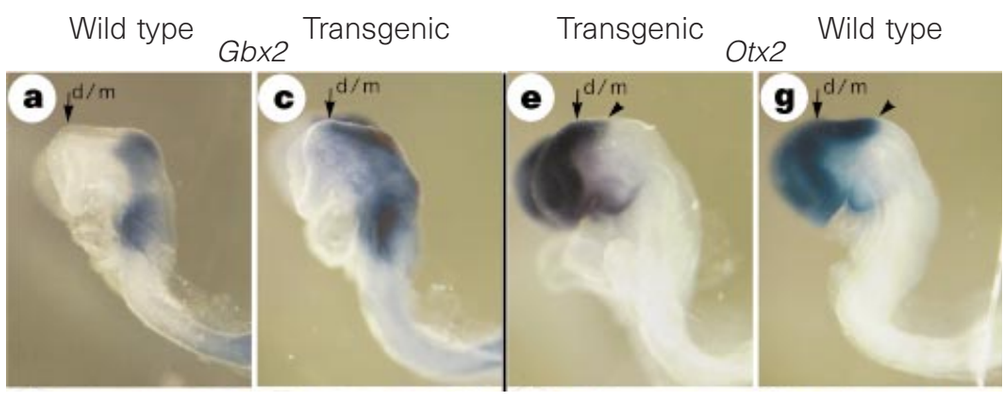
primary organizer:
The Spemann organizer (e.g. dorsal lip in Xenopus, (Hansen's) node in chick and mouse) induces a neural tissue in the ectoderm that would otherwise form the epidermis. BMP inhibitors are the responsible molecules for this role.

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

GBX2 and OTX2 suppress each other's expression



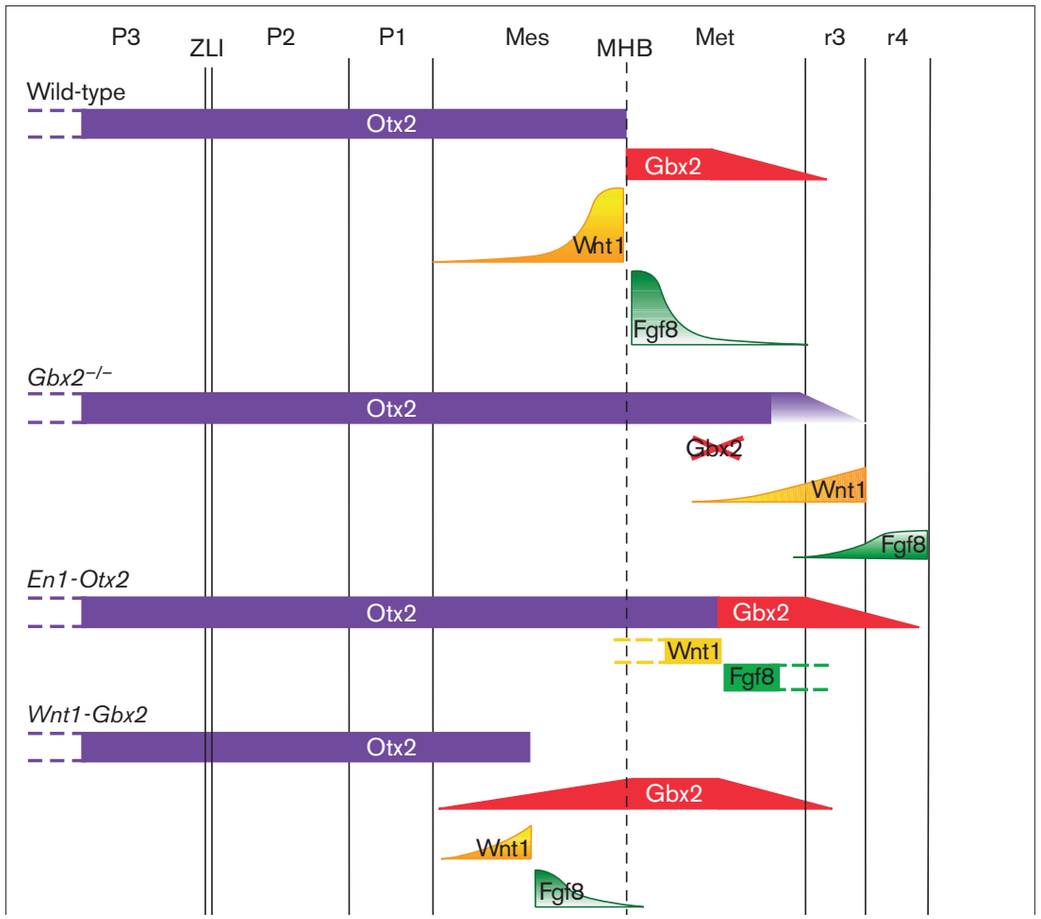
-In *Gbx2* knockout mice, expression of *Otx2* expands caudally (b).
-Fgf8 expression shifts caudally so that its rostral border of matches the caudal border of *Otx2* expression (f).
-*Wnt1* is normally expressed in the caudal midbrain (i). Its expression expands caudally similar to *Otx2* (j).



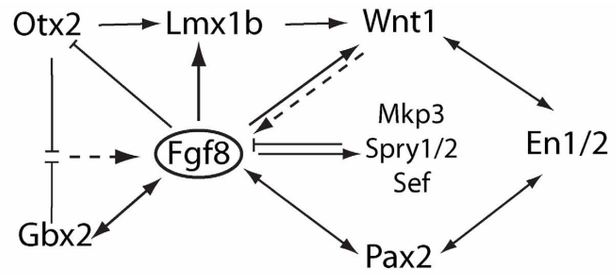
-When *Gbx2* is over-expressed, caudal border of *Otx2* expression is shifted rostrally (g).

Millet et al., 1999

Gene interactions in the isthmus



Joyner et al., 2000



H = homebox
TF= transcription factor
M= morphogen
EP= extracellular protein
E = intracellular enzyme
inh= inhibitor of FGF8 signalling

	Otx2	Gbx2	Wnt1	Lmx1b	En1	En2	Pax2	Fgf8	Mkp3	Spry1	Spry2	Sef
	H/TF	H/TF	M/EP	H/TF	H/TF	H/TF	H/TF	M/EP	E/Inh	E/Inh	E/Inh	E/Inh
Di	+++											
	+++											
Mes	+++					+					+	
	+++		+++	+++	++	++			+	++	++	++
Isth		+++		+	+++	+++	+++	+++	+++	+++	+++	+++
R_1		+++			++	++	++		++	++	++	++
R_2												
R_3												

R: rhombomere (discussed later)

Martinez et al., 2013

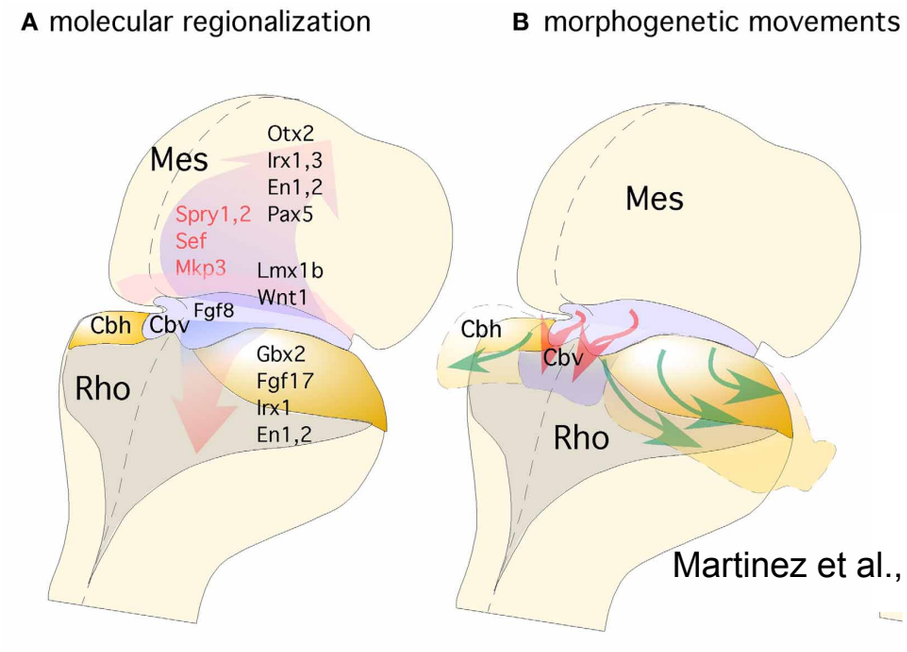
-Mutual suppression between two transcription factors results in the formation of a secondary organizer that produces a signaling molecule.

-Signaling molecules produced by a secondary organizer regulates regional identity of the brain by controlling the differential expression of various transcription factors.

-Both FGF8 and Gbx2 are important for repressing the expression of Otx2.

-Each of these transcription factors determines the gene expression “landscape” (=identity) of neural progenitor cells.

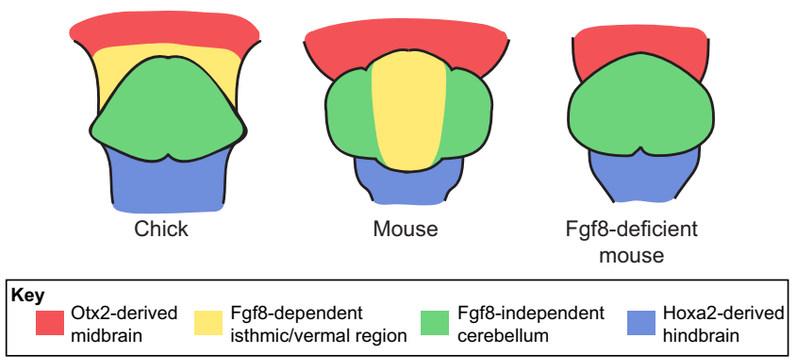
Embryonic origins of the cerebellum



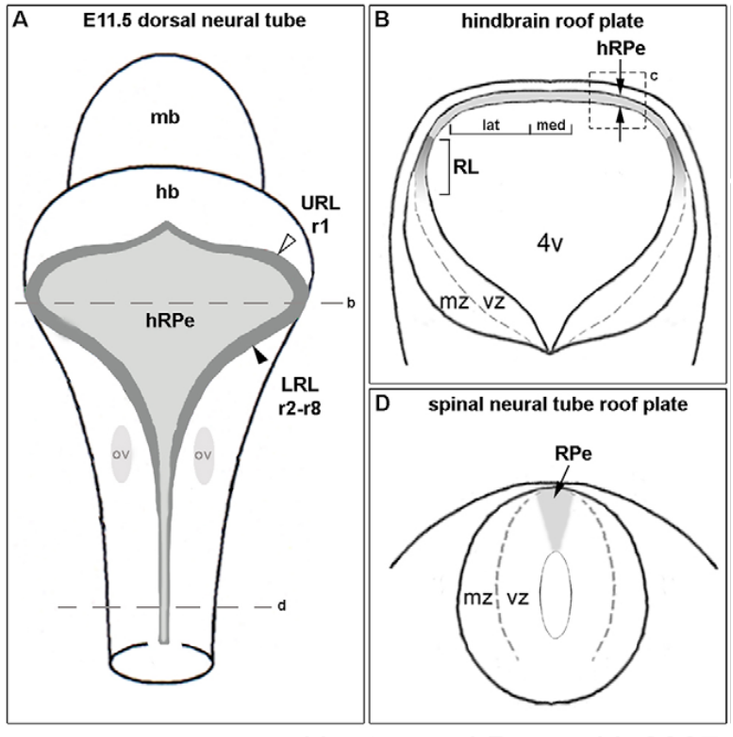
Martinez et al., 2013

vermis (in mice): isthmus and "r1"
hemisphere: "r1"

Formation of the cerebellum depends on *Fgf8* and *Gbx2*.



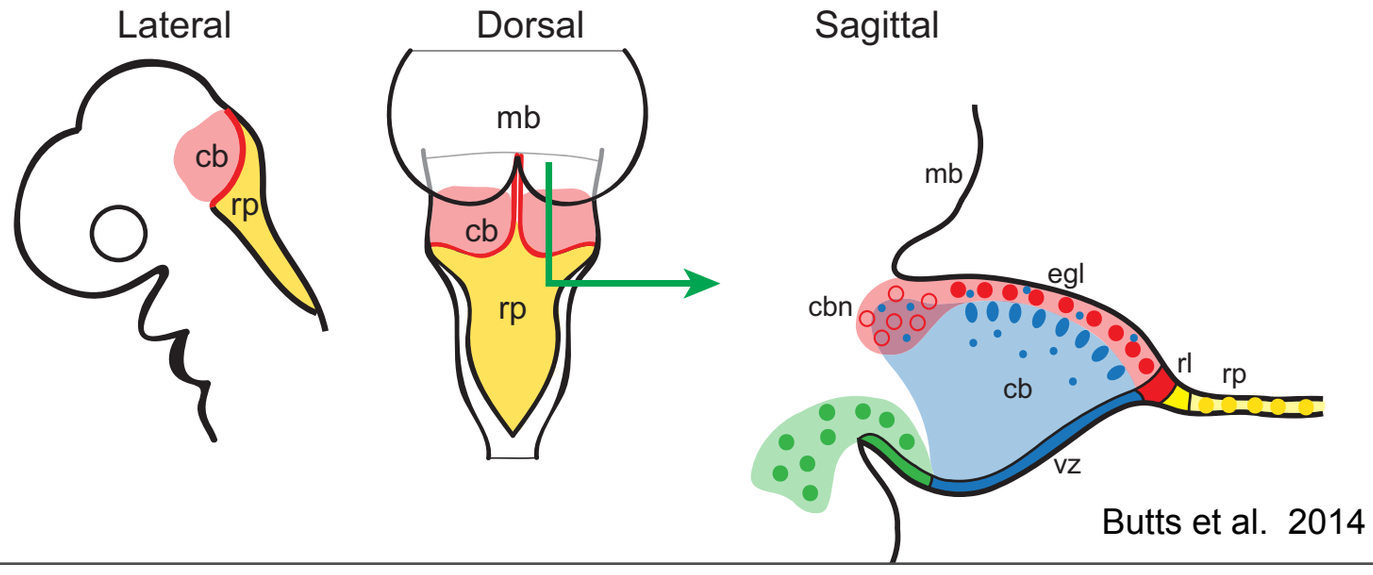
Butts et al. 2014



Hunter and Dymecki, 2007

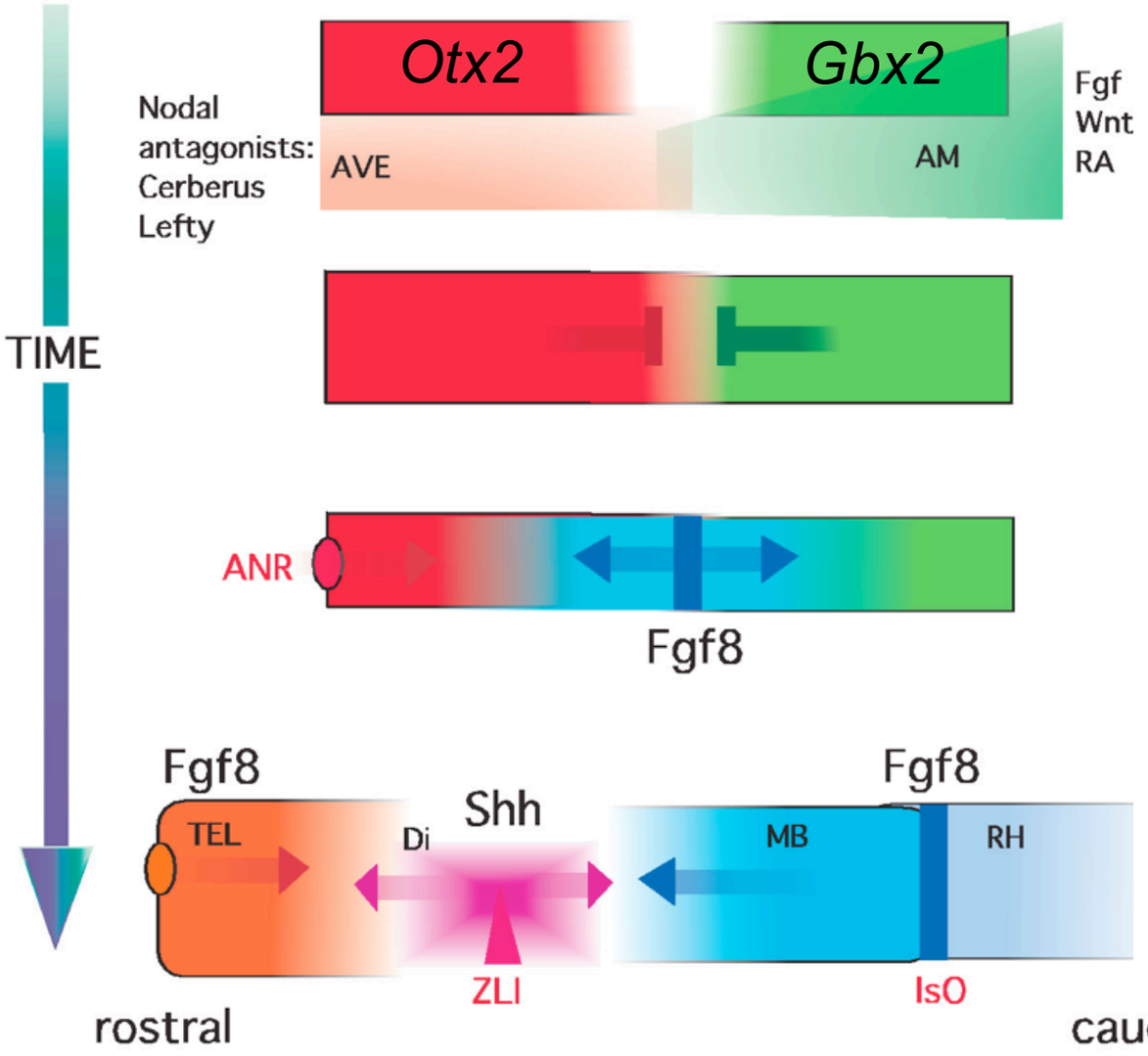
Granule cells are produced in a special progenitor zone, the external granular layer (EGL). EGL is derived from the upper rhombic lip, originated from r1. EGL progenitor cells undergo many rounds of symmetric divisions and produce granule neurons (source of medulloblastoma).

GABA neurons (including Purkinje cells) are derived from the ventricular zone (blue below).



Butts et al. 2014

Multiple secondary organizers contribute to anterior-posterior patterning of the brain



Vieira et al., 2010

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

Anterior neural ridge (ANR)

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

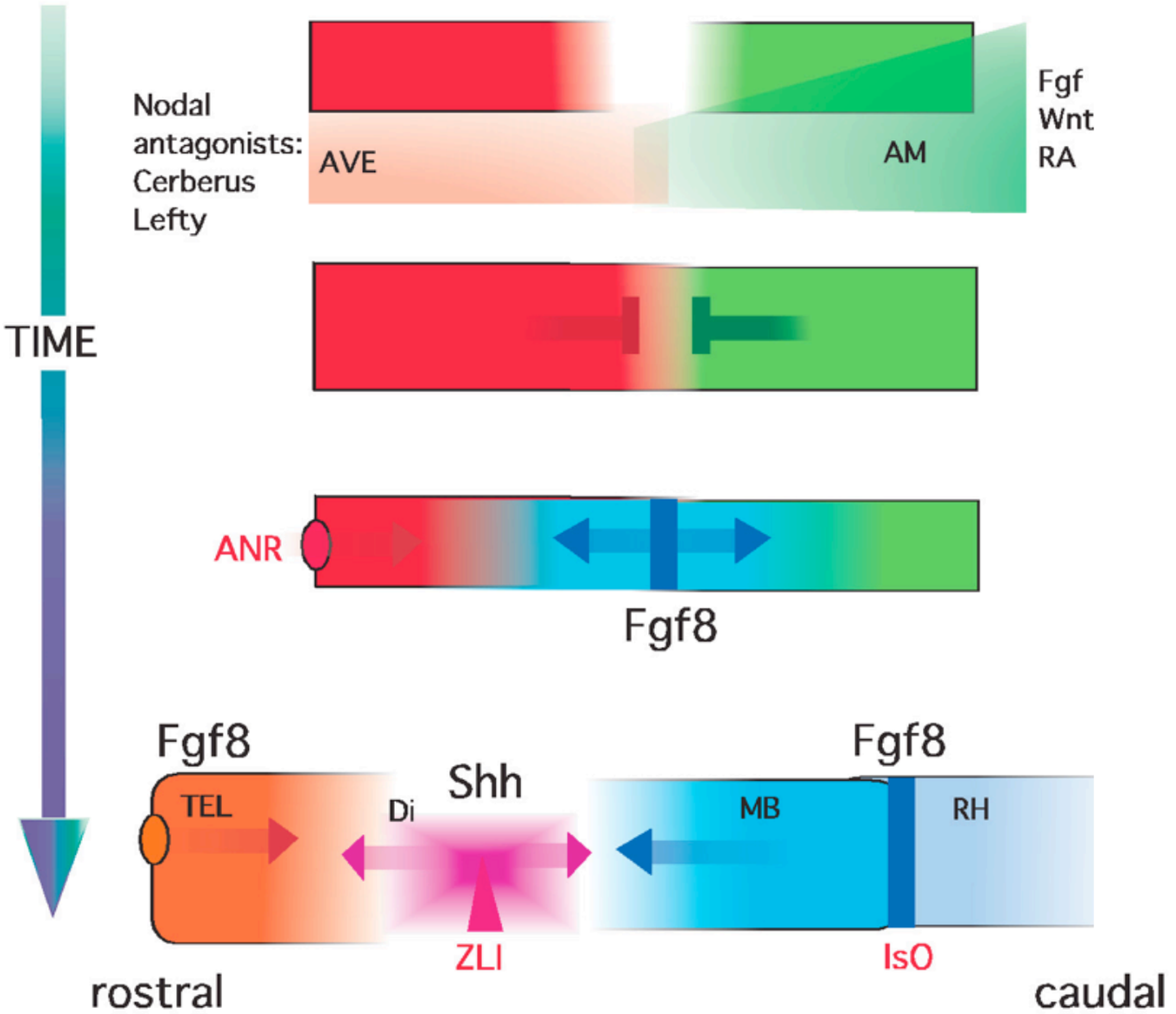
-ANR requires the underlying anterior visceral endoderm (AVE) for its formation

-ANR is a source of FGF8.

-Ectopic FGF8 in more caudal tissue induces rostral forebrain phenotypes.

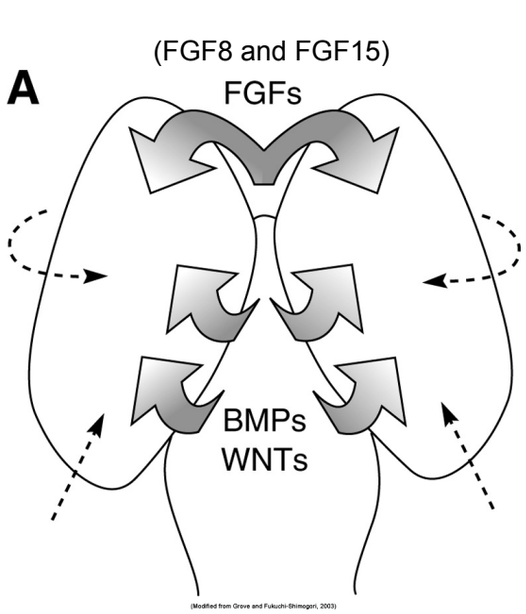
-ANR and FGF8 are required for the telencephalic identity

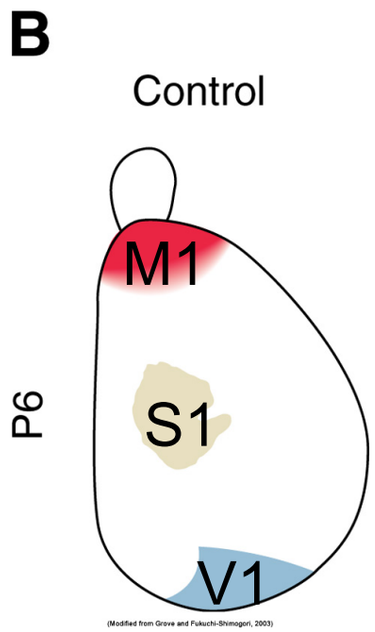
-FGF8 also regulate the AP polarity of the cerebral cortex at later stages (discussed later).

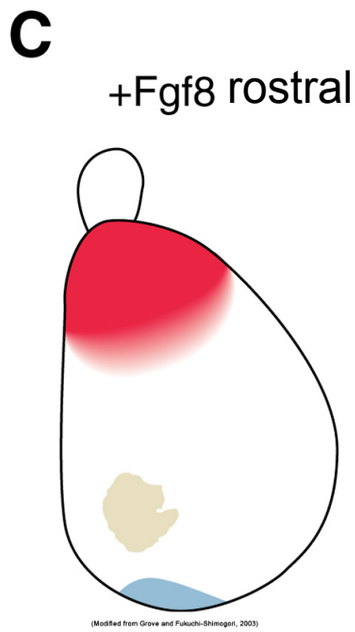


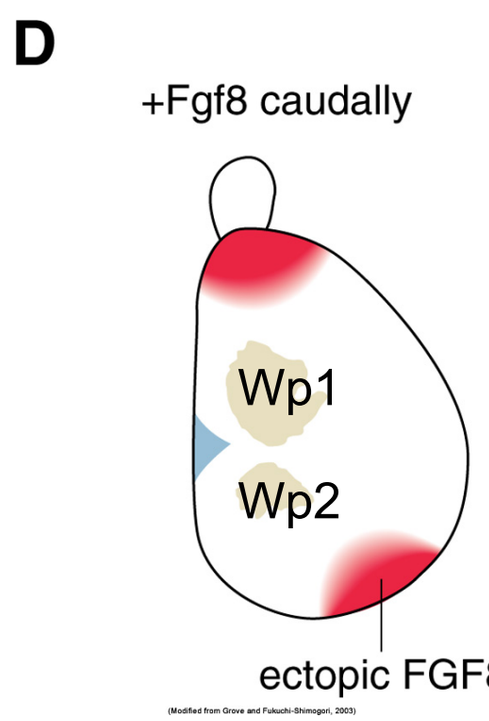
Vieira et al., 2010

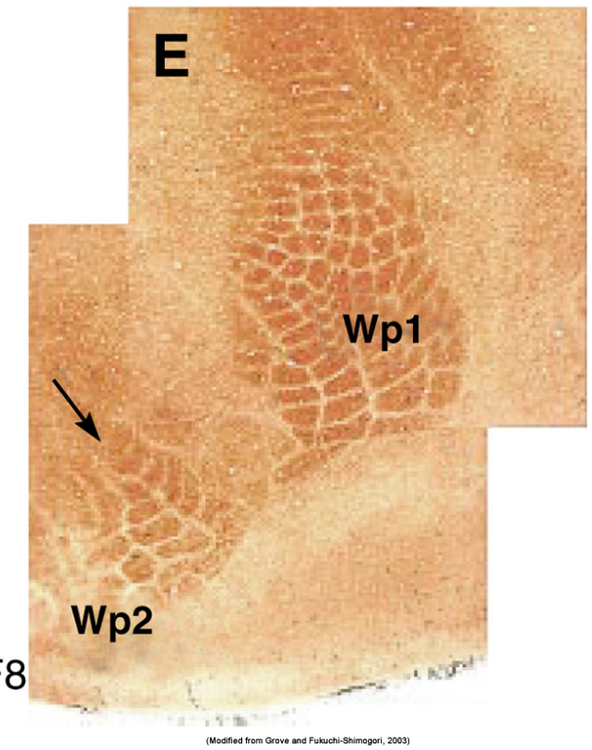
FGF8 patterns the cerebral cortex

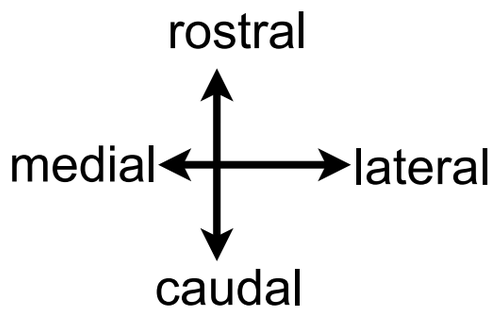










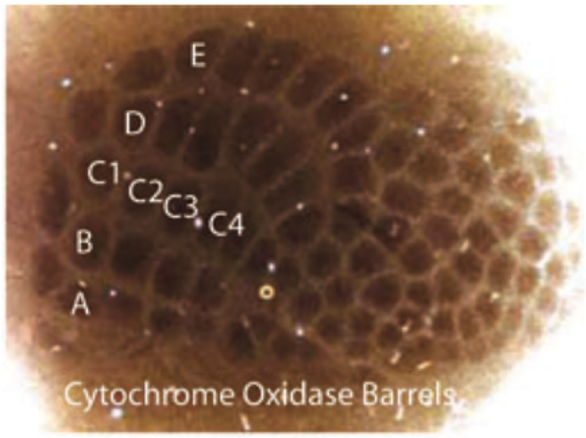


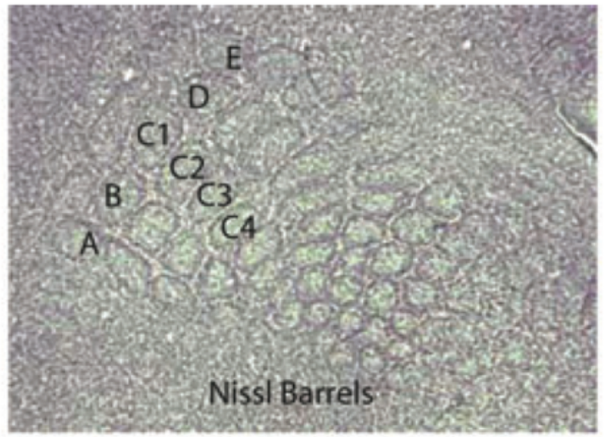
Wp1: original somatosensory map
Wp2: new somatosensory map

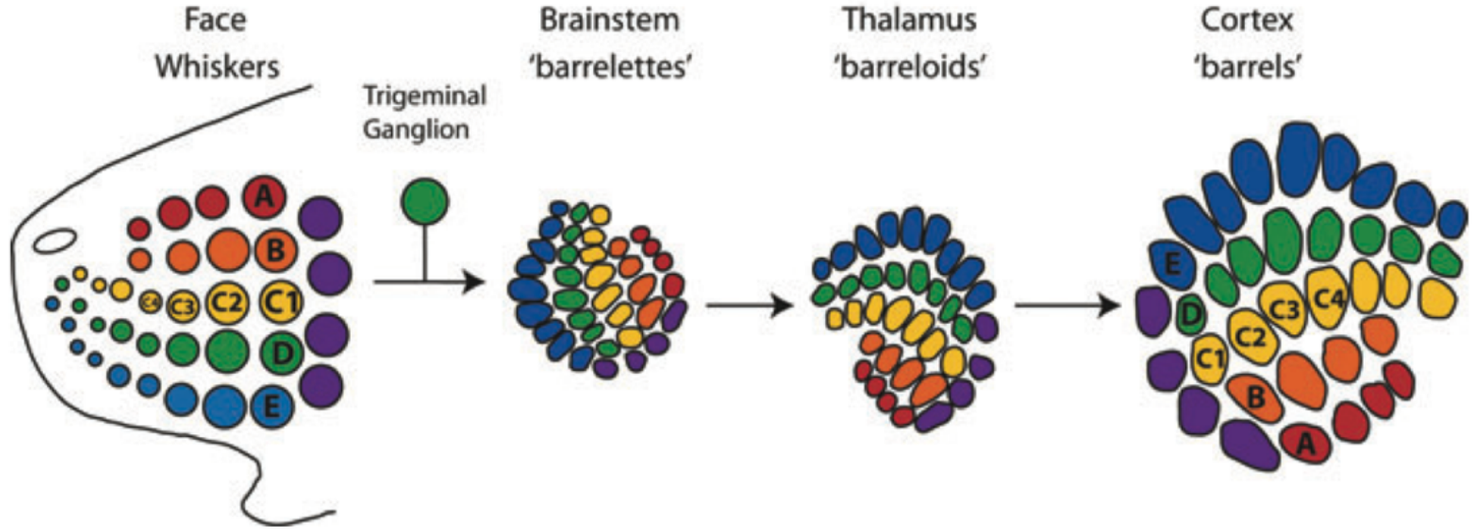
-Ectopic FGF8 expression in the rostral cortex by in utero electroporation results in the expansion of rostral cortical areas, such as the motor area (red)

-Ectopic FGF8 expression in the caudal cortex causes the formation of duplicated, mirror image of the somatosensory map.

Primary somatosensory area of rodents can be identified histologically





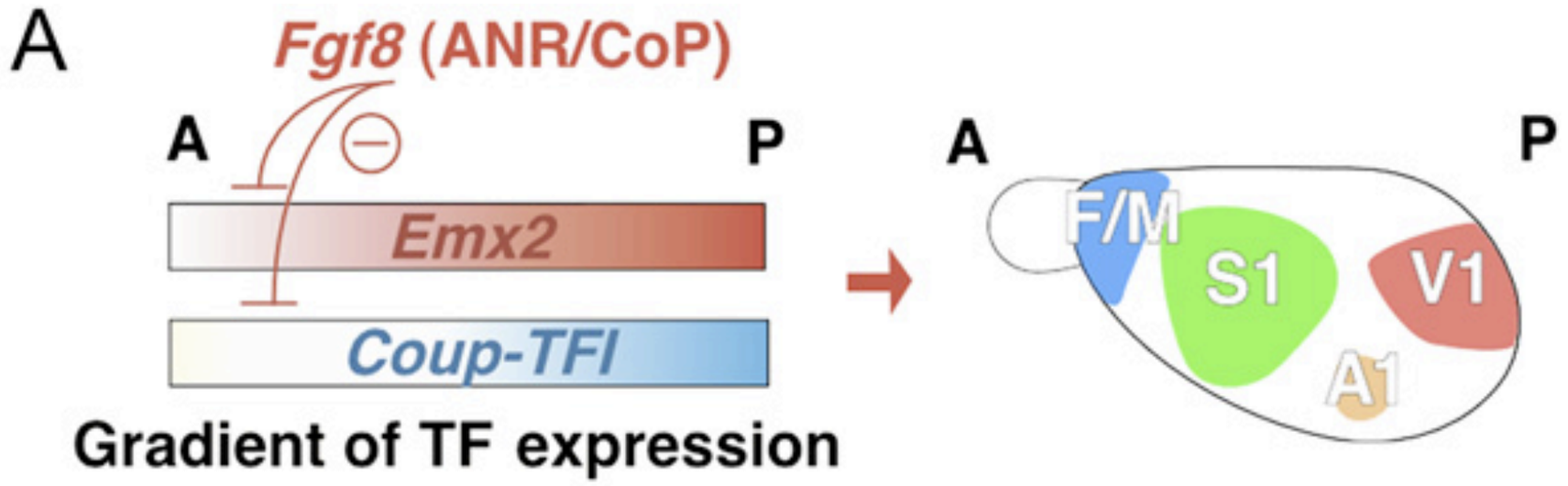


Li and Crair (2011)

Neurons conveying somatosensory information from each whisker are clustered in the brainstem (“barrelettes”) and the thalamus (barreloids).

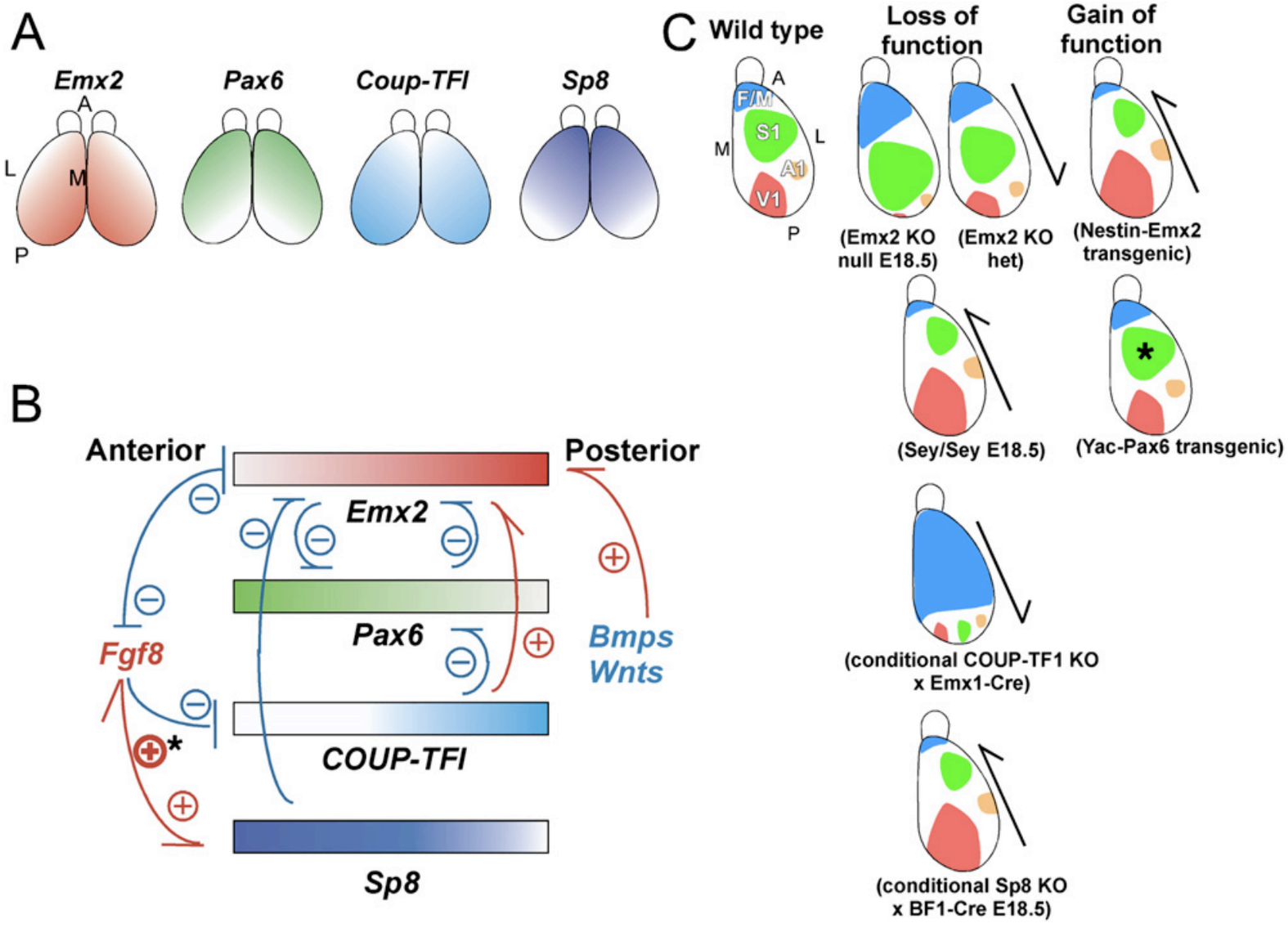
Terminals of thalamocortical axons originated from each barreloid are clustered in primary somatosensory area, which is required for aggregation of layer 4 neurons. This results in the formation of “barrels”, which are histologically distinct.

FGF8 suppresses caudally-expressed transcription factors, Emx2 and Coup-TF1



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

FGF8 regulates cortical patterning by controlling gene expression network



Analysis of knockout and over-expression mice show that *Emx2* and *Coup-TF1* impart the caudal cortical area identity.

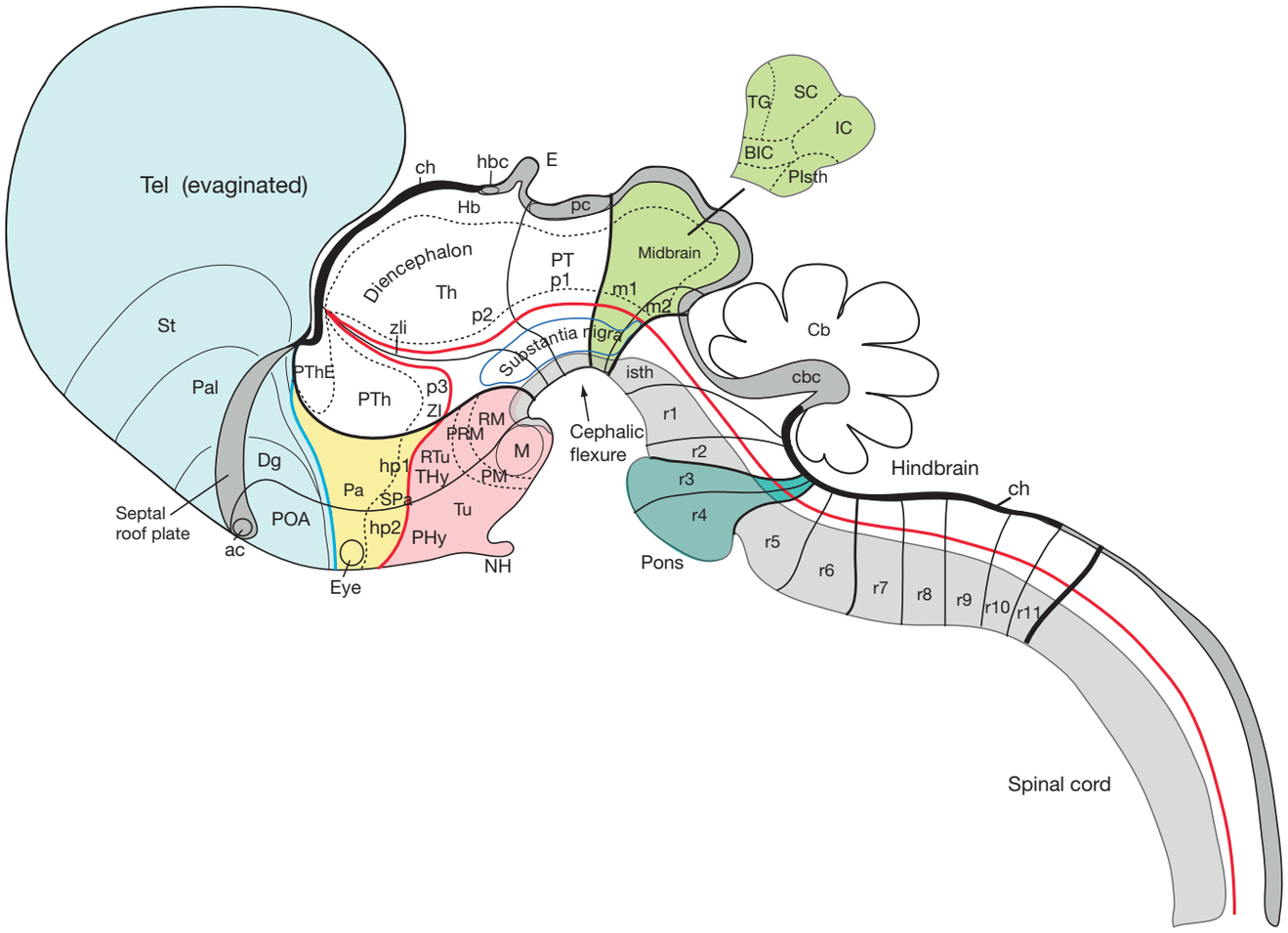
FGF8 suppresses the caudal cortical area identity by suppressing the expression of *Emx2* and *Coup-TF1* in anterior cortical progenitor cells.

Other transcription factors such as *Pax6* and *Sp8* are also involved in this gene regulation network.

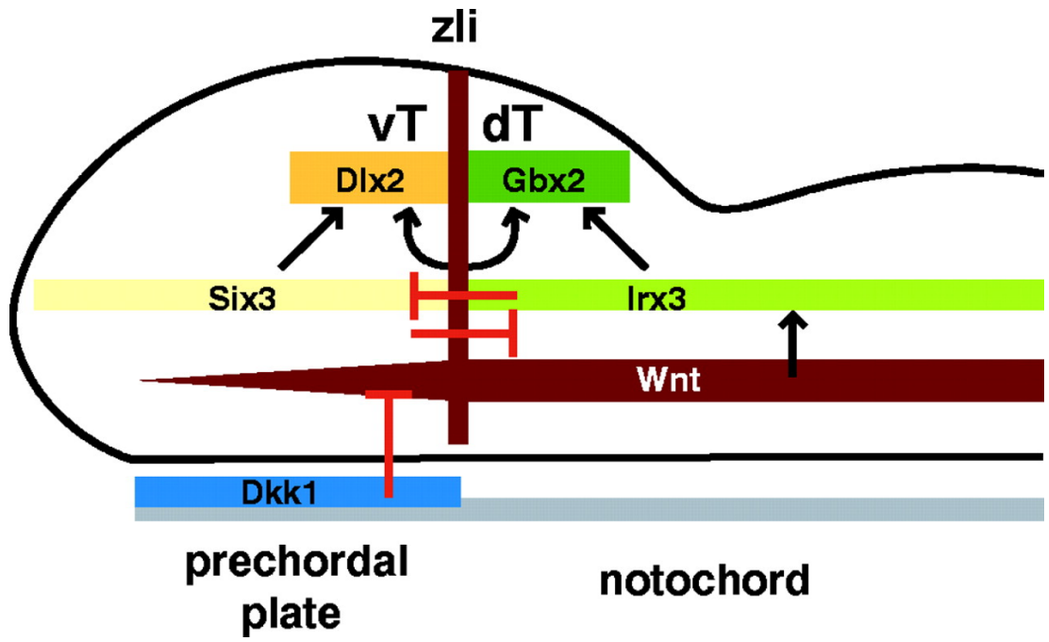
O'Leary et al. (2007) Neuron 56: 252-269

The zona limitans intrathalamica (ZLI)

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



ZLI is formed at the interface of two domains (*Six3* vs *Irx3*) that are established by differential Wnt signaling coming from the paraxial mesoderm.



ZLI produces Sonic Hedgehog (SHH). In addition to conferring ventral identity to neural progenitor cells (discussed later), SHH from the ZLI is critical for rostro-caudal patterning of the thalamus.

Summary 1 (secondary organizers and rostro-caudal identity of the neural tissue)

As the neural identity is established in early embryos, differential Wnt signaling originating from paraxial mesoderm, establish gross rostro-caudal identity of neural tissue by controlling expression of several transcription factors (e.g. *Otx2* and *Gbx2, Six3* and *Irx3*).

Mutual repression between these transcription factors produces an expression interface, which becomes a secondary organizer.

Secondary organizers secrete molecules (e.g., FGF8, SHH) that further refine the grossly patterned nervous system into smaller domains.

e.g. FGF8 from the isthmus....midbrain
FGF8 from the ANR...cerebral cortex
SHH from the ZLI...thalamus

Each of the established structures possesses proper rostro-caudal polarity.

How does the same molecule (FGF8) induces different brain regions (midbrain and cortex)?

Through regionalization, neural progenitor cells establish positional identity (=gene expression network appropriate for their location). Together with their temporal identity, many different types of neurons are generated at the right time and place.

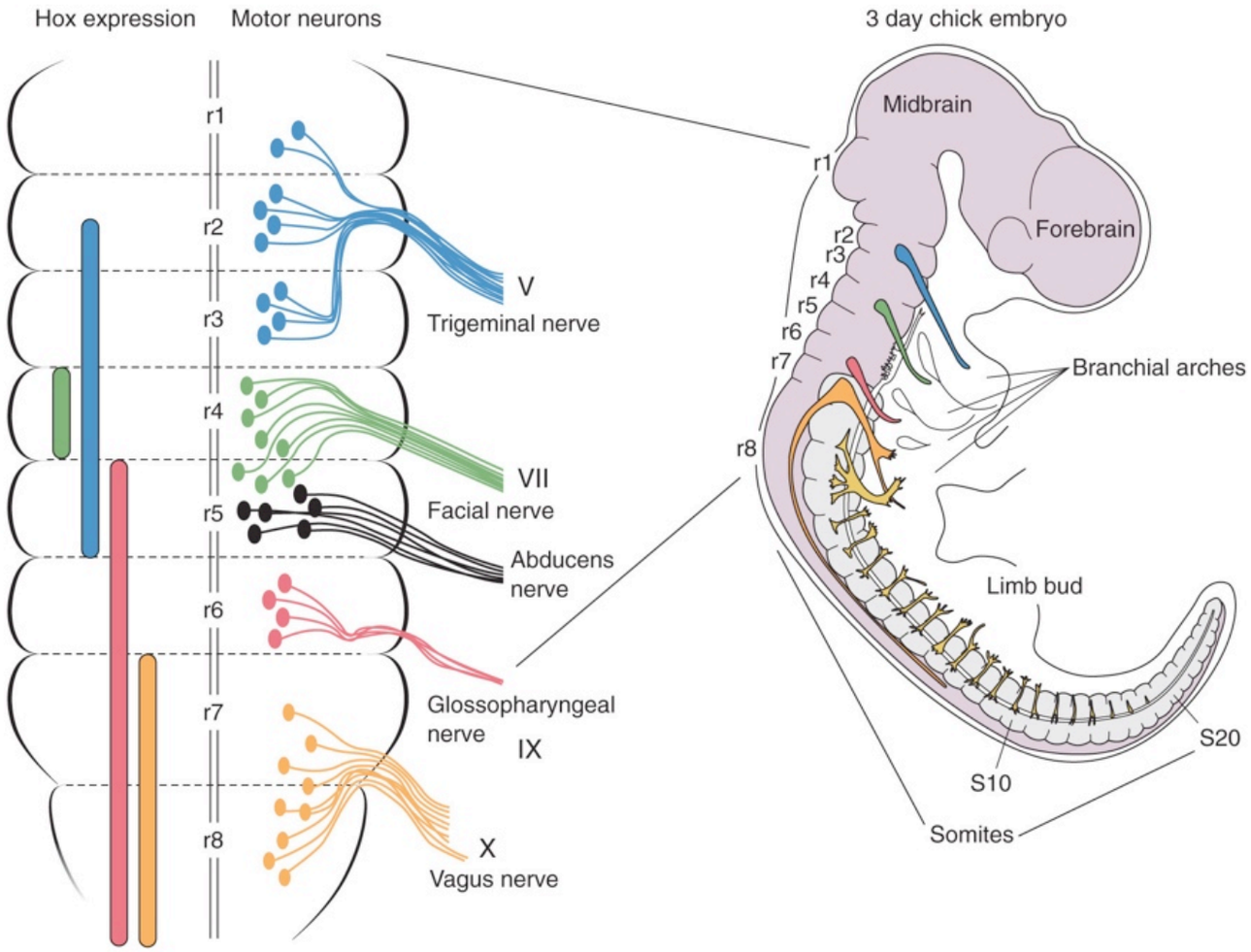
Two more stories

1. Hndbrain patterning and segmentation
   -roles of Hox genes
   -roles of retinoic acid, another mesoderm-derived signaling molecule
   -origin of concepts and key genes in Drosophila genetics

2. Dorso-ventral patterning of the brain and spinal cord
   -involvement of two more secondary organizers, floor plate and roof plate
   -opposing roles of SHH and BMP in dorso-ventral “segmentation” of the spinal cord

Segmental organization of the hindbrain

-Early embryonic hindbrain is divided into morphological units called rhombomeres.
-Progenitor cells in each rhombomere produce specific classes of neurons and branchial neural crest cells
-A special class of transcription factors called *Hox* genes regulate the segmental identity of rhombomeres. They show overlapping and nested patterns of expression, which is regulated by RA, FGF and Wnts).



(just FYI) Origins of various hindbrain structures from rhombomers

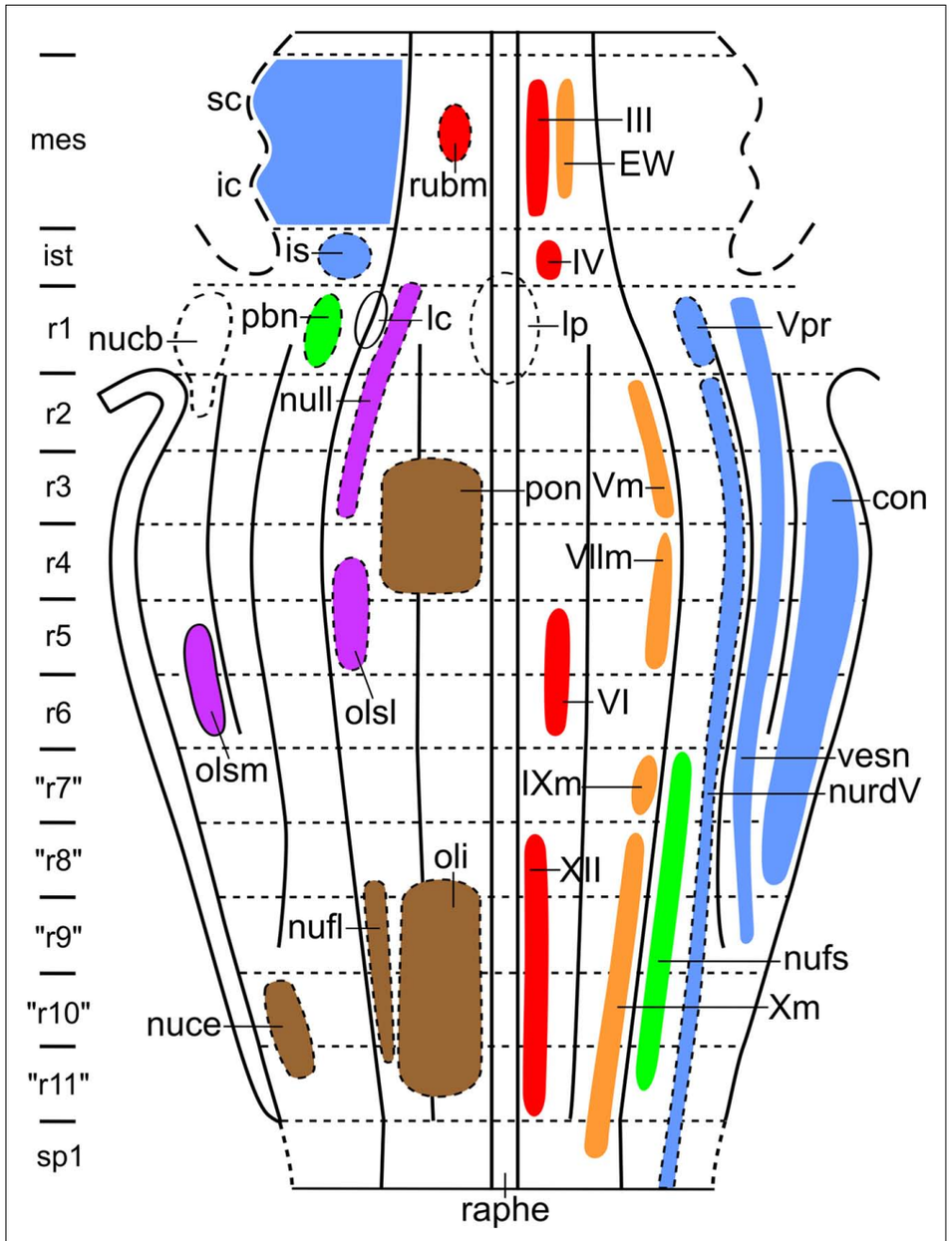


FIGURE 10 | Provisional topological chart of the brainstem of amniotes, showing the zonal and segmental allocation of cell masses, as determined by Puelles and collaborators. In order to avoid crowding, primary sensory and primary motor nuclei are shown to the right, whereas centers of higher order are shown to the left.

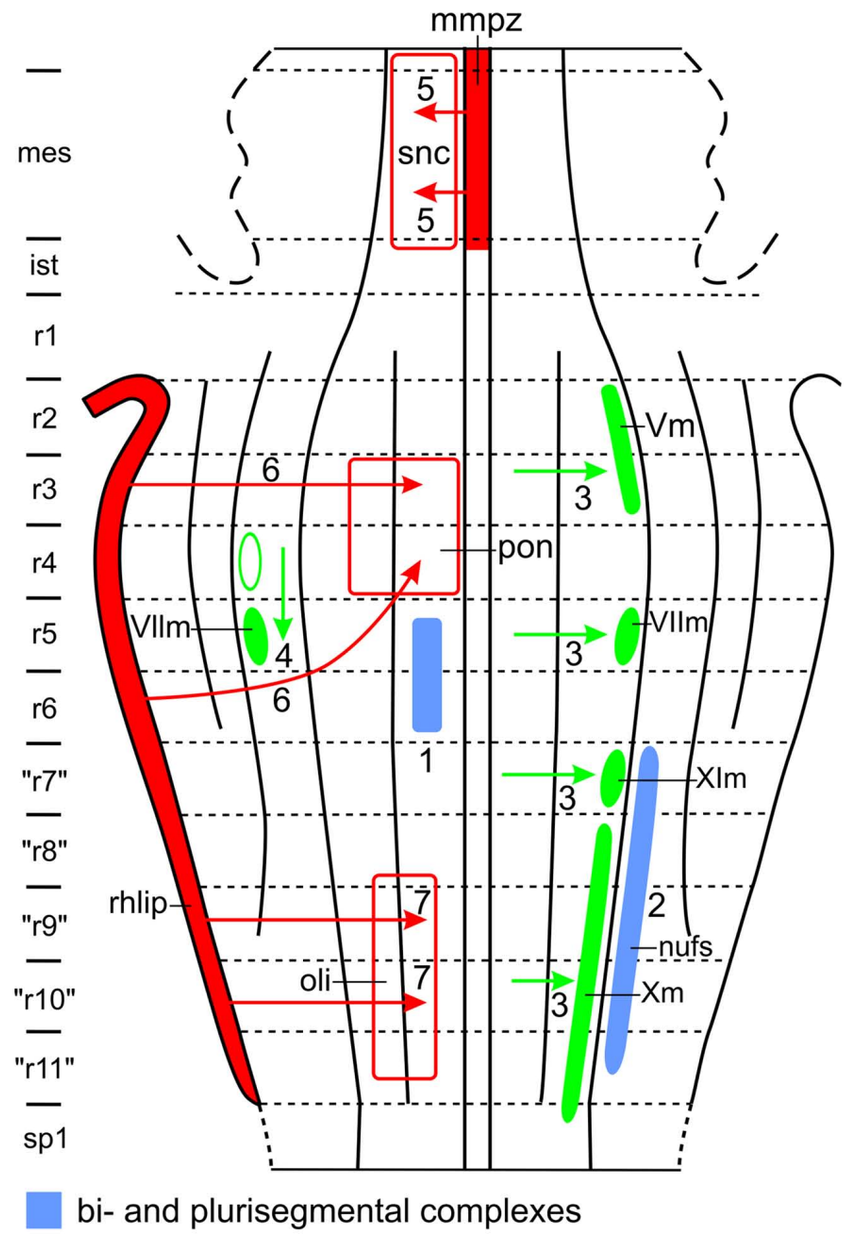


FIGURE 11 | Features which may complicate the morphological interpretation of topological charts. For explanation, see text.

Dopaminergic neurons of the pars compacta of the substantia nigra are generated in the floor plate of the midbrain.

Neurons of pontine nuclei and inferior olive migrate from different levels of the lower rhombic lip.

Nieuwenhuys (2011)

How were Hox genes discovered?

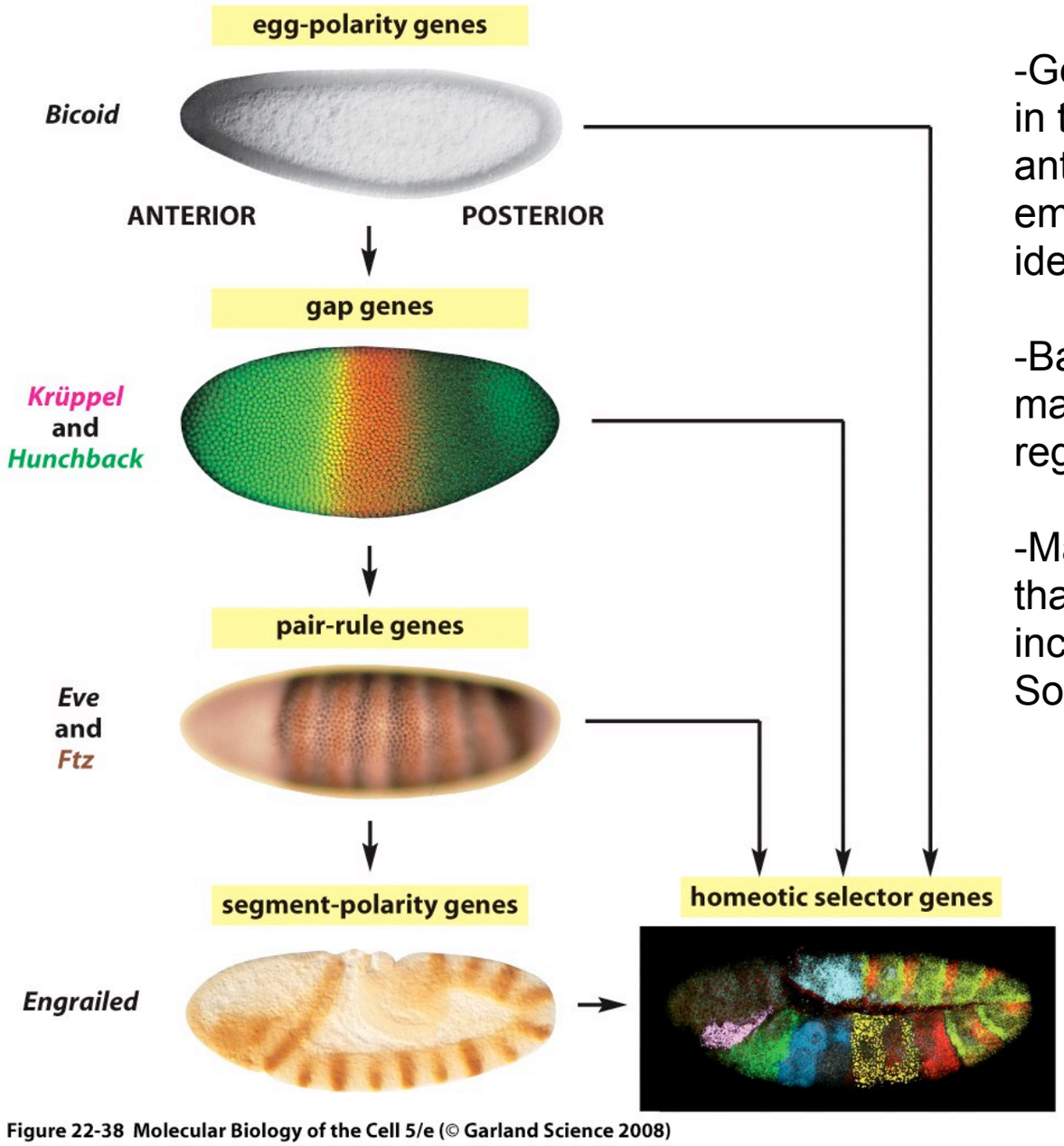


Figure 22-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

-Genetic screens pioneered by Nüsslein-Volhard and Wiechaus in the 1980s identified a hierarchy of genes that establish anterior-posterior polarity of Drosophila embryos and divide the embryo into a specific number of segments with different identities.

-Basic ideas of the identified gene regulatory cascade apply to many other aspects of animal development, including the regionalization of the vertebrate nervous system.

-Many genes identified in this screen have vertebrate homologs that are important in the patterning of the neural tissue. These include Hox and other homeobox genes, Wnt genes as well as Sonic hedgehog.

A-P patterning of Drosophila embryos

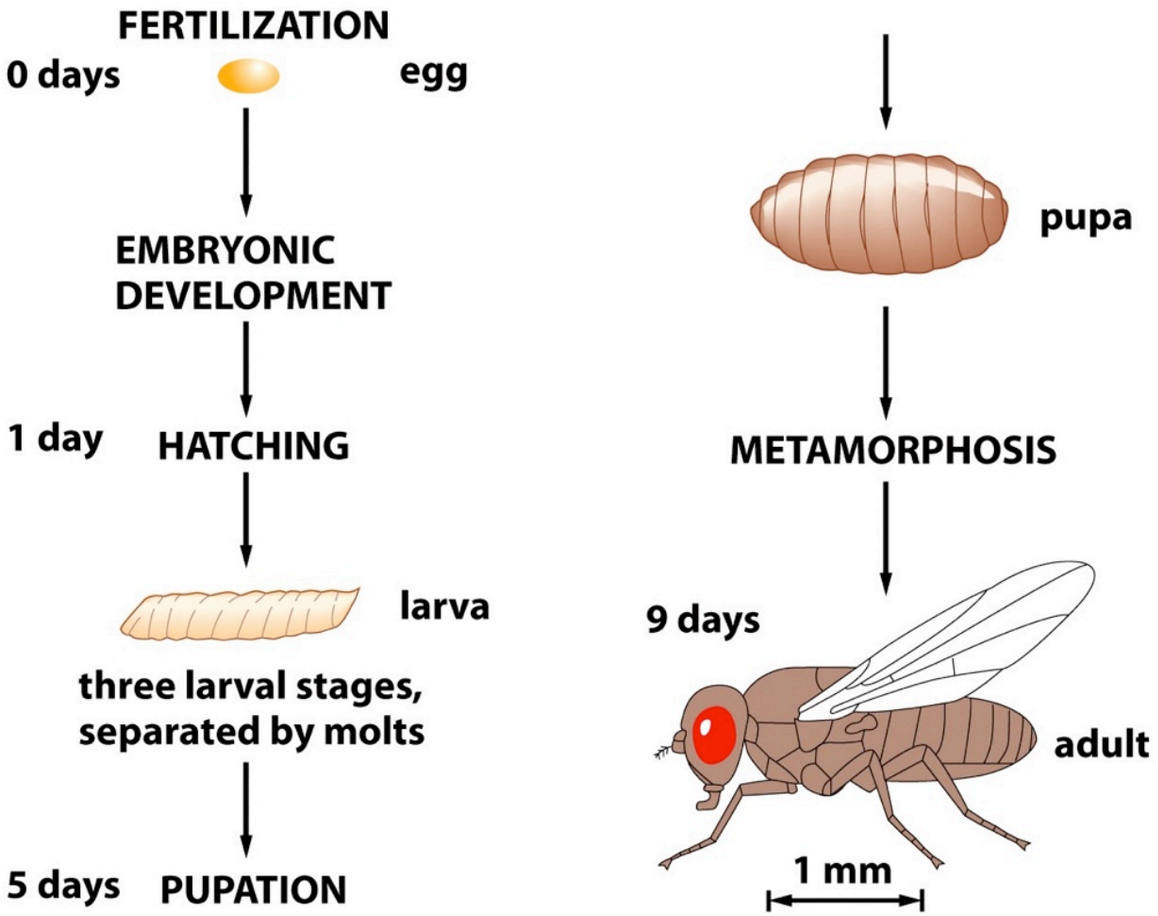


Figure 22-25 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The fly consists of a head (with mouth, eyes, antennae), three thoracic segments (T1-3) and 8-9 abdominal segments (A1-9)

The segmentation starts to develop in early embryos

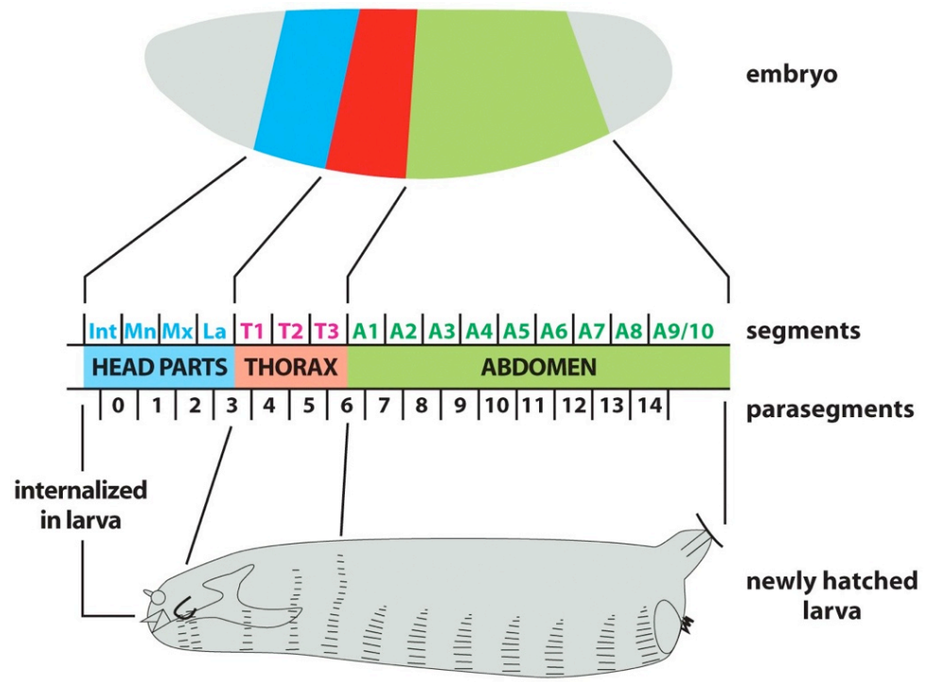
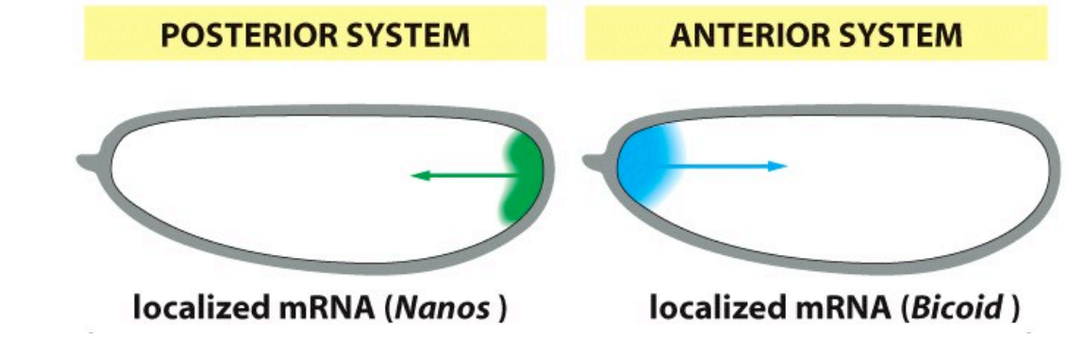


Figure 22-27 Molecular Biology of the Cell 5/e (© Garland Science 2008)

A-P polarization starts in unfertilized oocytes



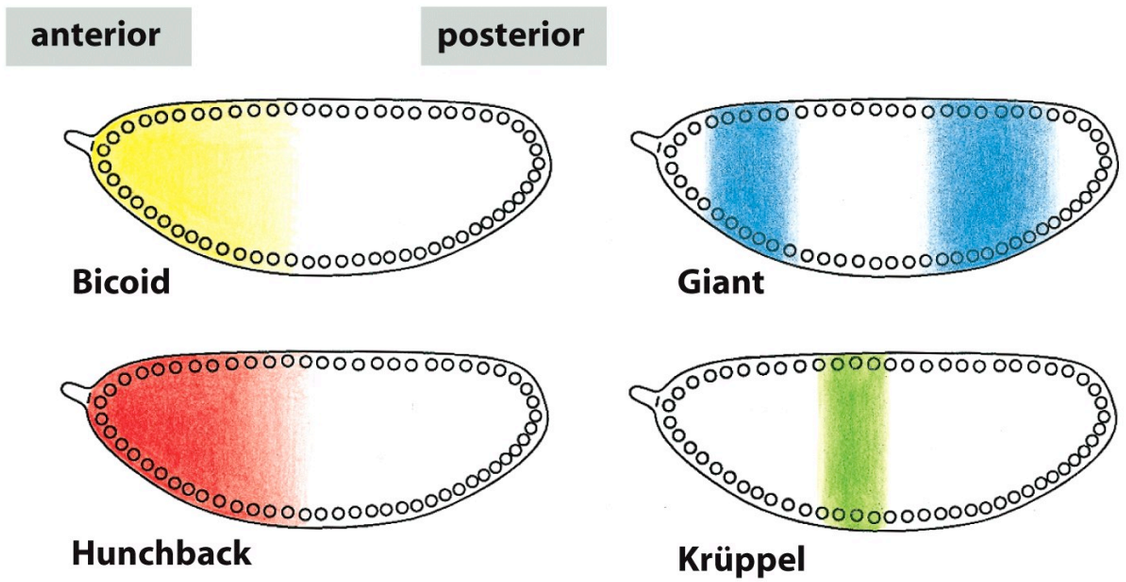


Figure 7-53 Molecular Biology of the Cell 5/e (© Garland Science 2008)

bicoid and *nanos* mRNAs are near the anterior and posterior end of the oocyte, respectively (**egg-polarity genes**)

Bicoid protein diffuses and forms a concentration gradient, regulating the graded expression of Hunchback

Hunchback, Krüppel and Giant are products of the **gap genes**, which mark out coarse subdivisions of the embryo

Pair-rule genes are required for alternative body segments

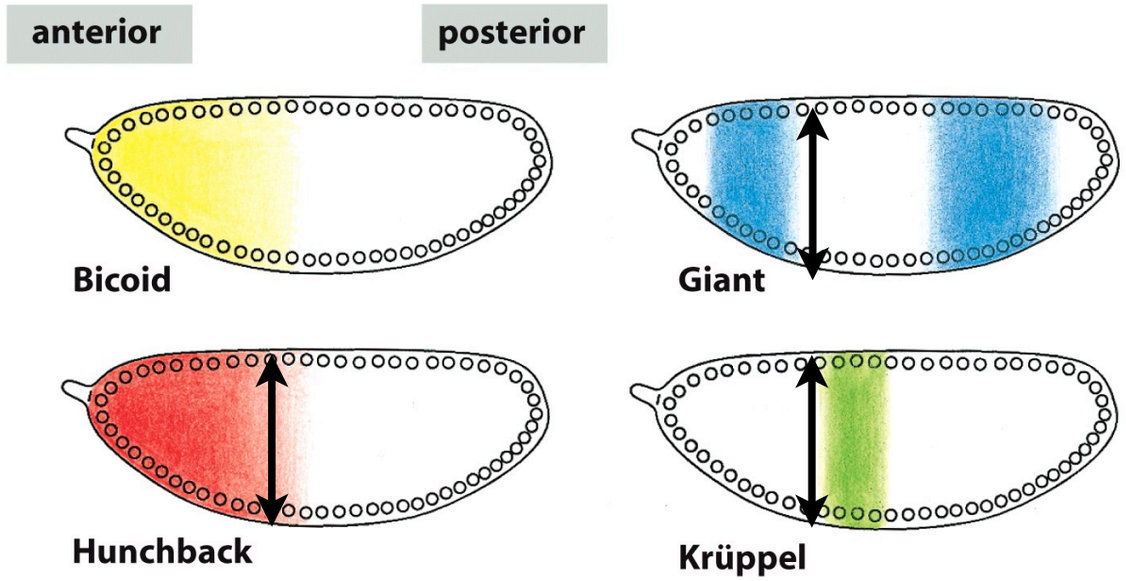


Figure 7-53 Molecular Biology of the Cell 5/e (© Garland Science 2008)

eve expression

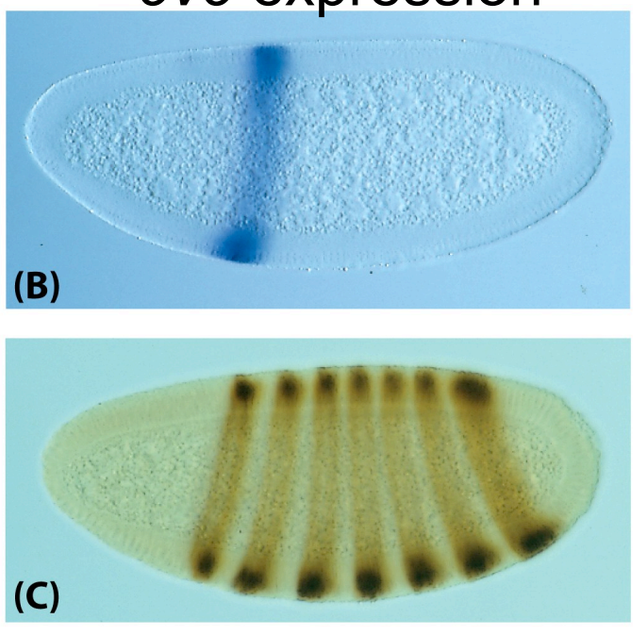


Figure 7-55bc Molecular Biology of the Cell 5/e (© Garland Science 2008)

Expression of *even-skipped (eve)* and *fushi tarazu (ftz)* are under the combinatorial regulation of gap genes

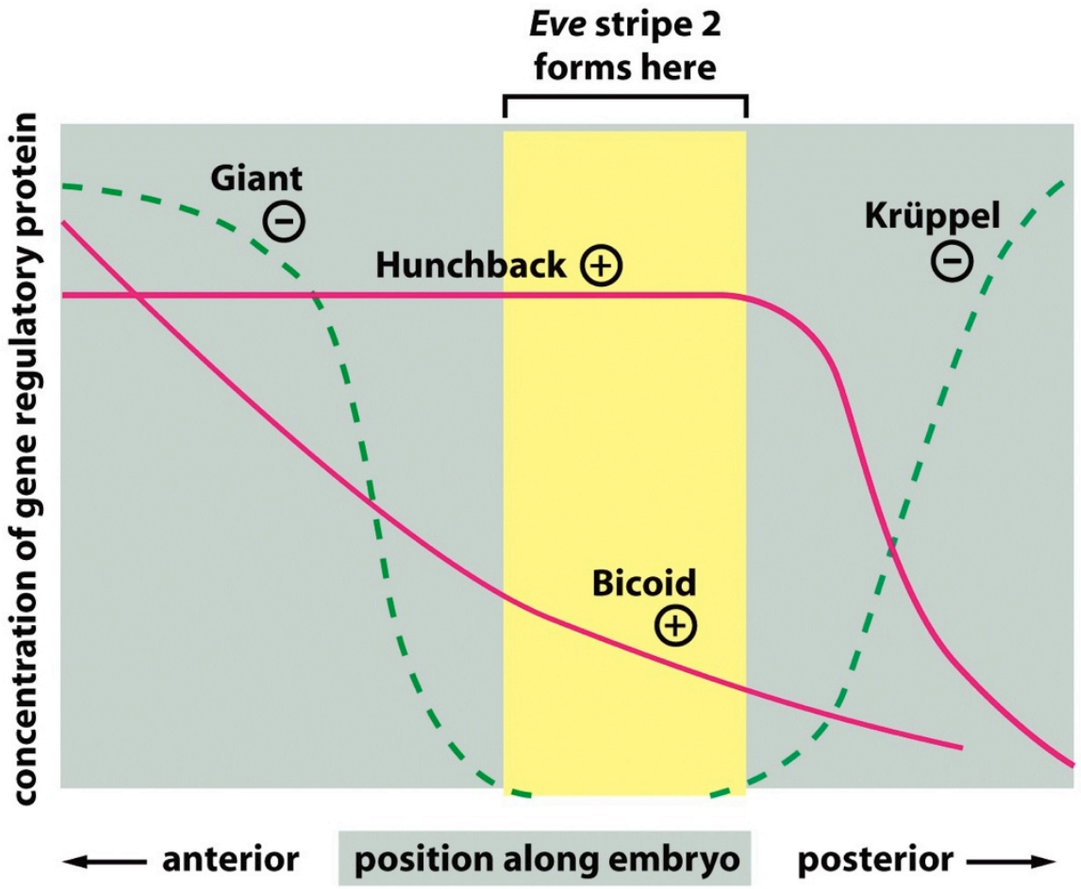


Figure 7-57 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Interactions of transcription factors with graded expression can generate a sharp border.

Segment polarity genes organize the A-P pattern of individual segment

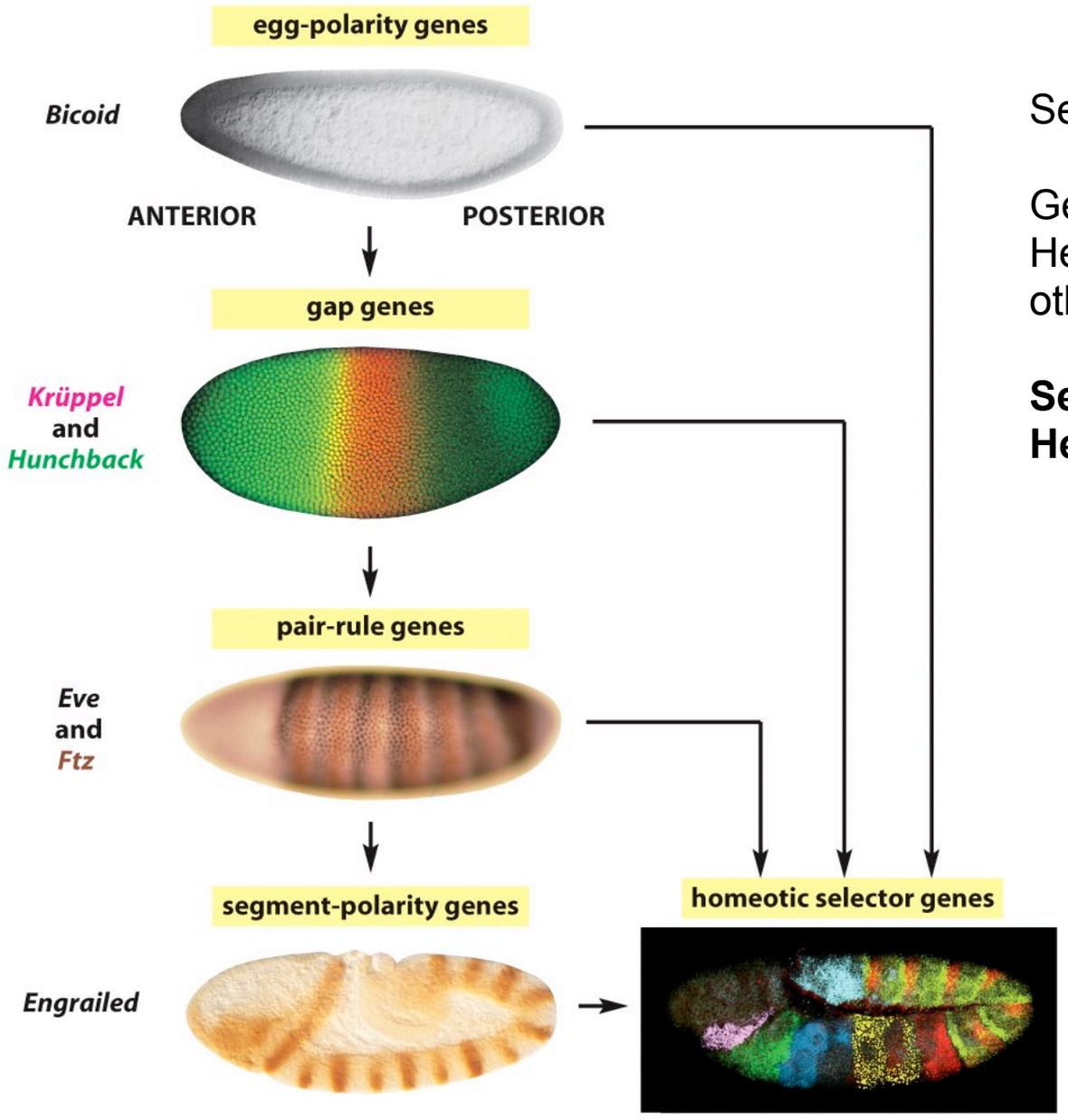
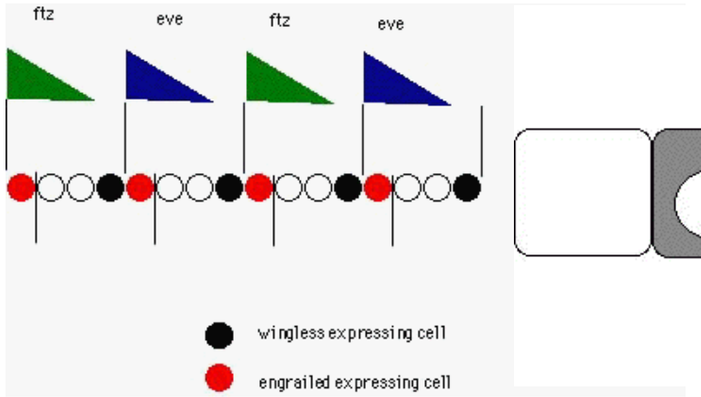


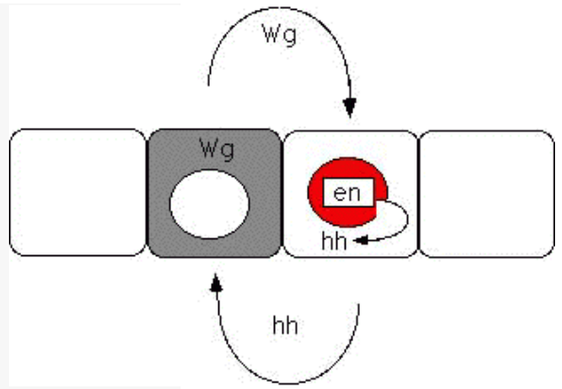
Figure 22-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Segment polarity genes stabilize boundary between segments.

Genes encoding two secreted proteins, Wingless and Hedgehog, are segment polarity genes. They promote each other's expression as well as a transcription factor Engrailed.

Search for vertebrate homologs for Wingless and Hedgehog identified Wnts and Shh.





Homeotic mutations

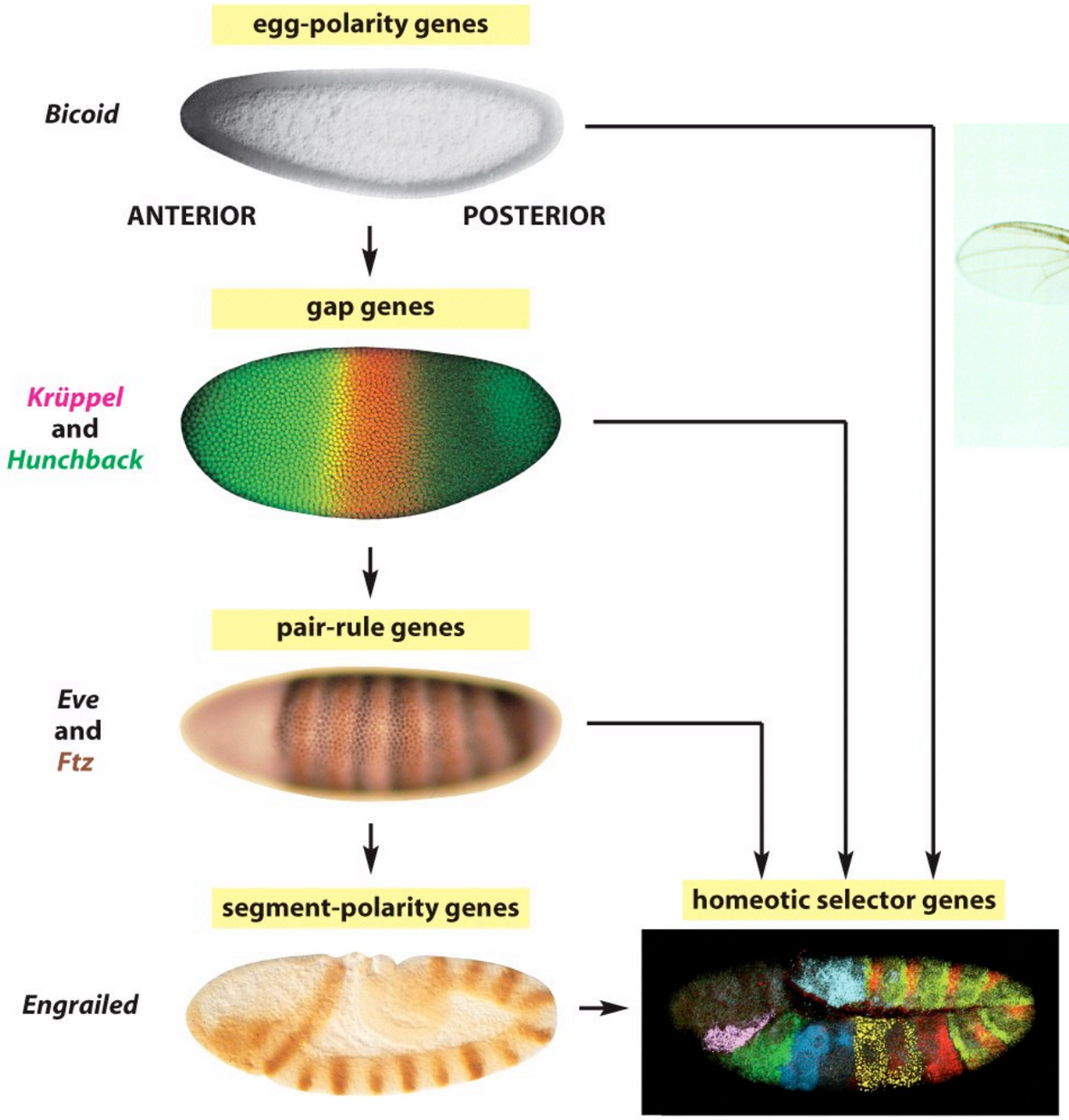
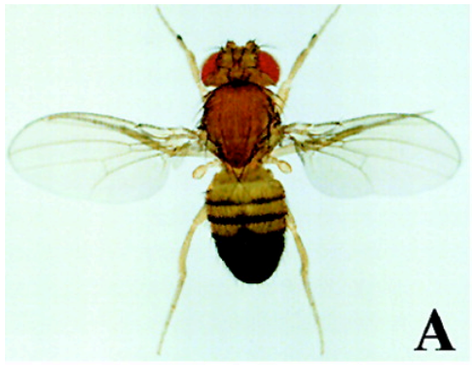


Figure 22-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)



extra pair of wings

legs sprout from the head instead of antennae

Antennapedia

Mutations that transform parts of the body into structures appropriate for other positions are called **homeotic mutations.**

Homeotic selector genes code for DNA-binding proteins

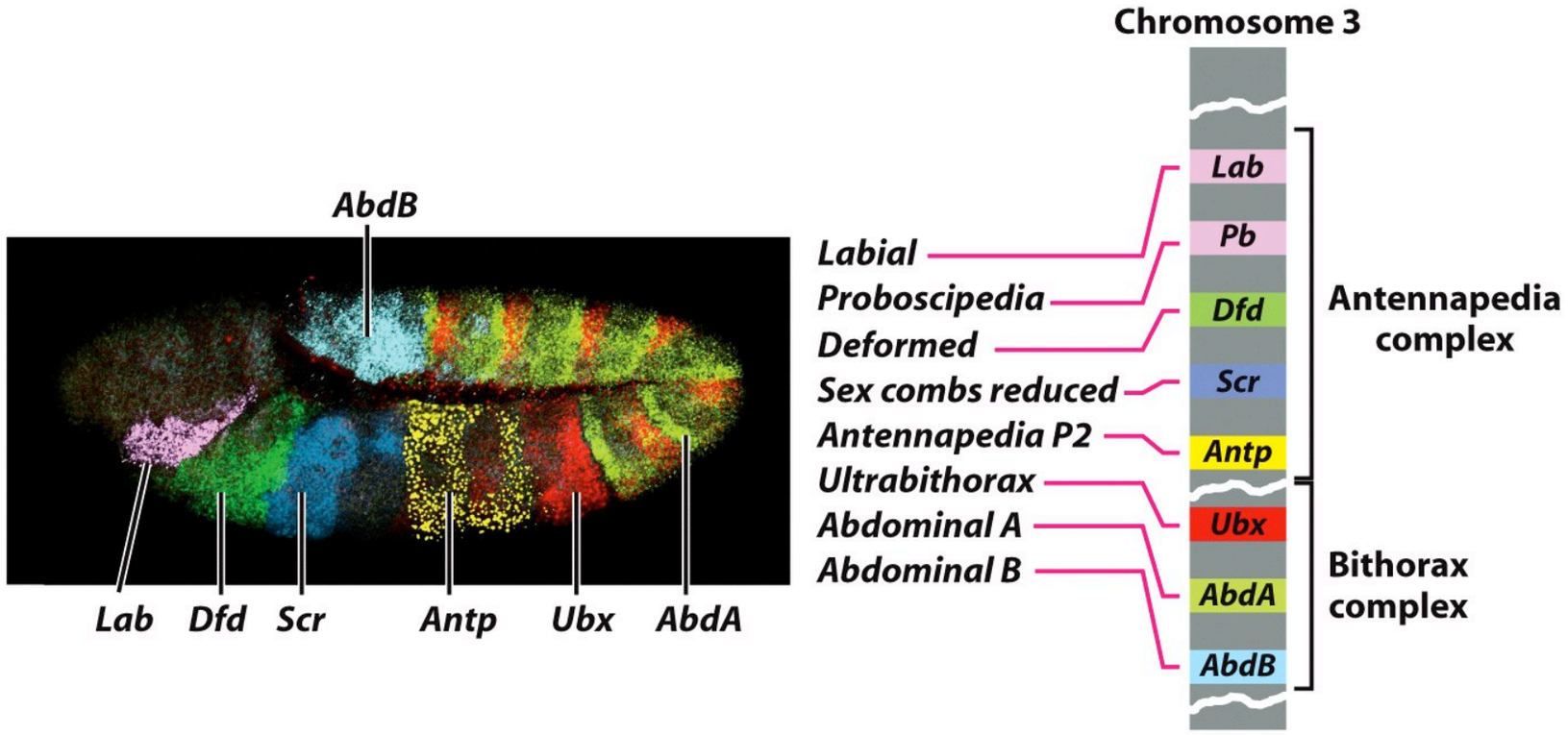


Figure 22-44 Molecular Biology of the Cell 5/e (© Garland Science 2008)

These proteins contain 60 amino acids of a conserved DNA-binding domain called the homeodomain.

These genes are located in two clusters (*Antennapedia* complex and *Bithorax* complex) on chromosome 3.

The order of genes on the chromosome corresponds almost entirely to the order in which they are expressed along the AP axis of the body (**co-lineality**).

Hox gene complexes in mice and flies

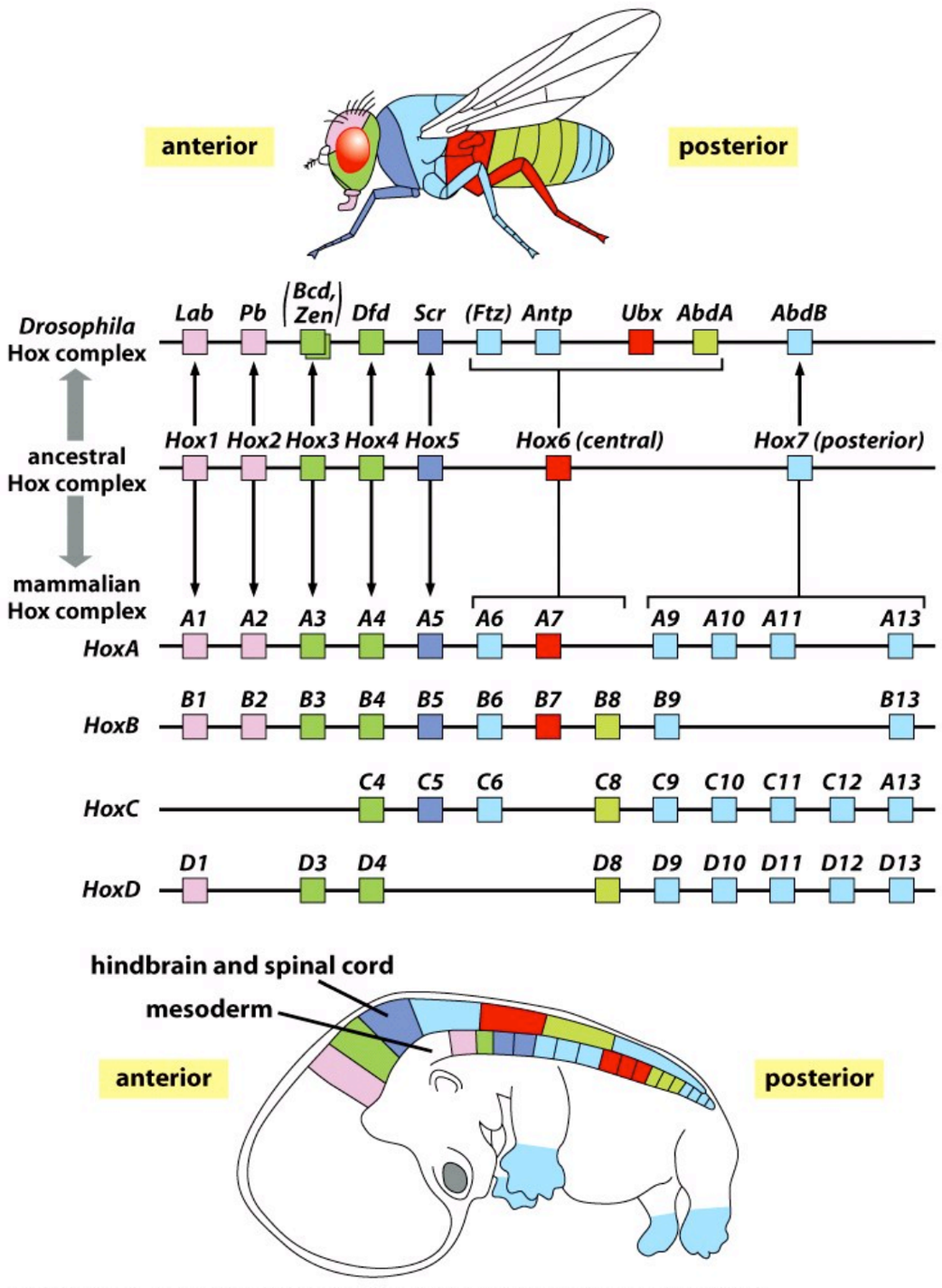


Figure 22-46 Molecular Biology of the Cell 5/e (© Garland Science 2008)

After the discovery of *Hox* genes in the fly, mammalian homologs were identified.

In the mouse, there are four complexes, *HoxA*, *HoxB*, *HoxC* and *HoxD* complexes, each on different chromosomes.

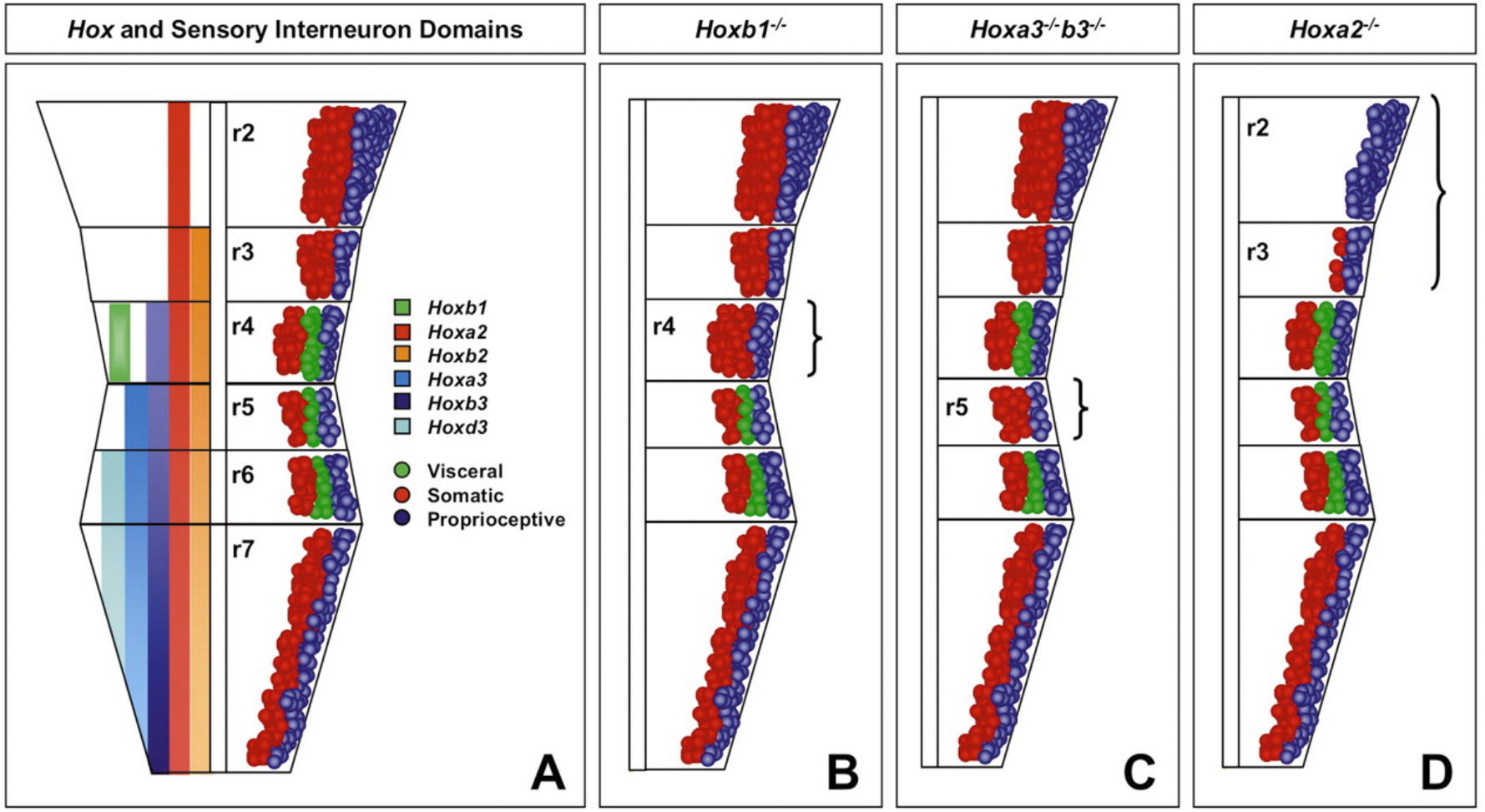
Each of the four complexes is the equivalent of the Drosophila set.

Members of each complex are expressed in a head-to-tail series along the AP axis, just as in Drosophila.

In neural cells of the CNS, *Hox* genes are expressed only in the hindbrain and spinal cord.

There are many transcription factors that have a homeodomain (**homeobox genes**). Only a subset of these are encoded by *Hox* genes. Non-*Hox* homeobox genes are differentially expressed in the forebrain and midbrain (*Otx2*, *Emx2*, *Gbx2* are all homeobox genes).

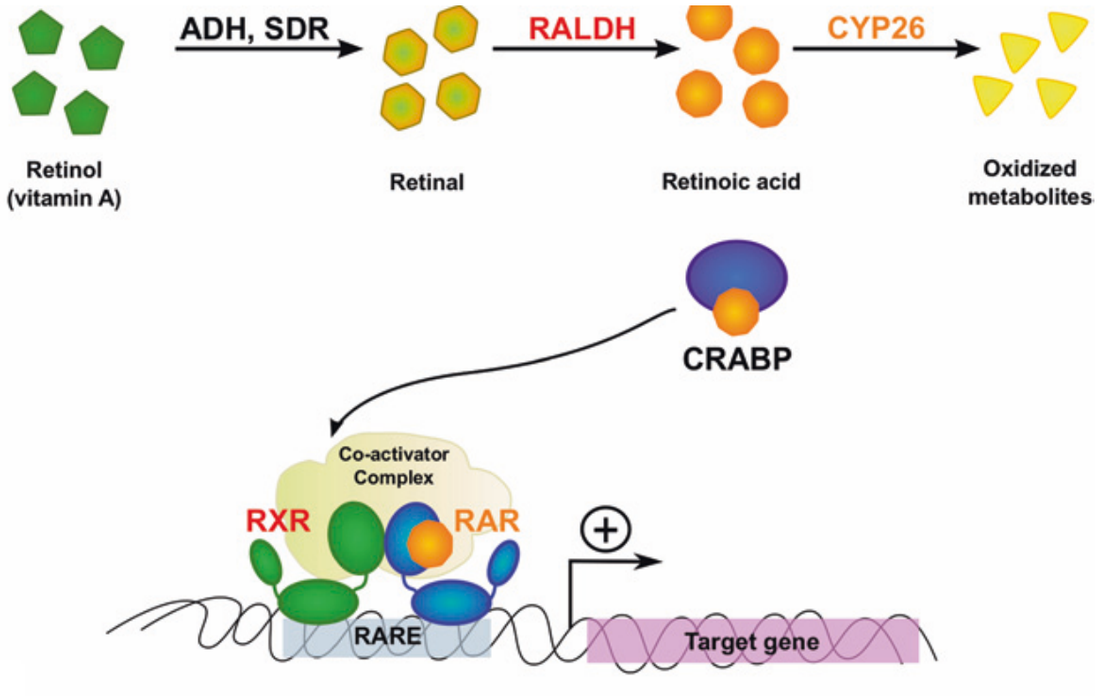
Hox genes are required for segment-specific differentiation program in the hindbrain



Gaufo, Wu, Capecchi (2004)

Mutation of a *Hox* genes or a combination of “paralogous” *Hox* genes (e.g., a3+b3) causes a loss of specific types of neurons in a specific rhombomere.

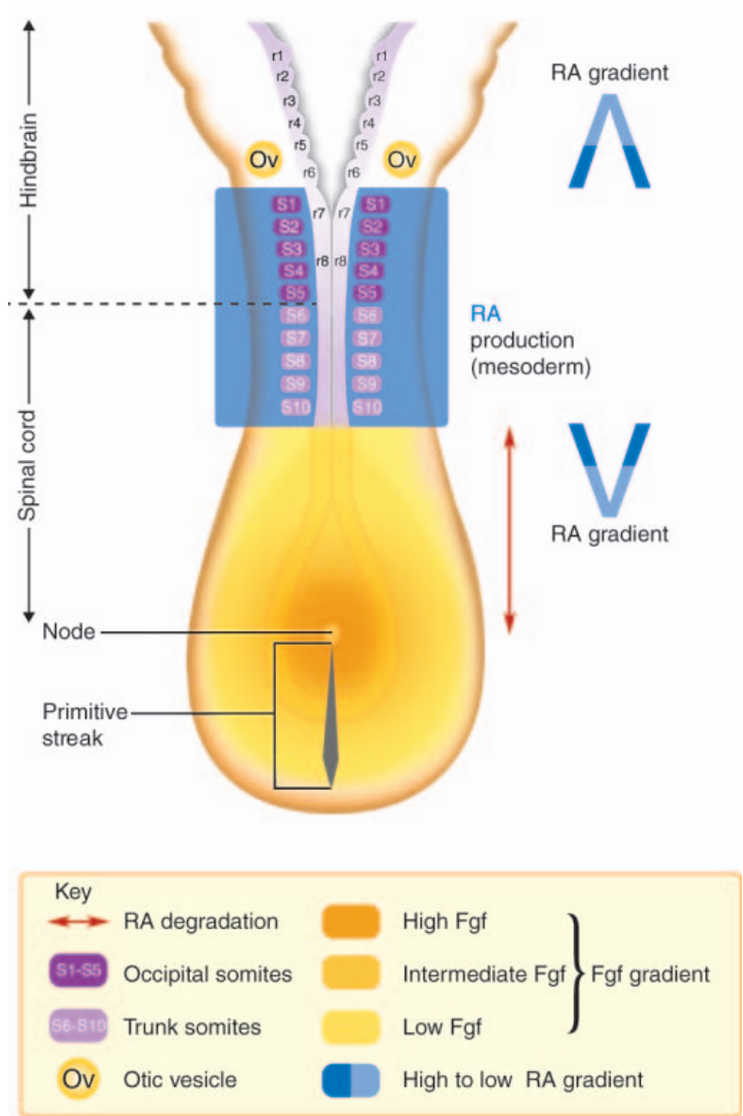
Retinoic acid (RA) regulates Hox gene expression



-RA is synthesized from vitamin A by two sets of enzymes.

-RA binds to a receptor RAR, which heterodimerizes with RXR.

-The RA-RAR-RXR complex binds to a specific target sequence (RARE) and activates transcription.

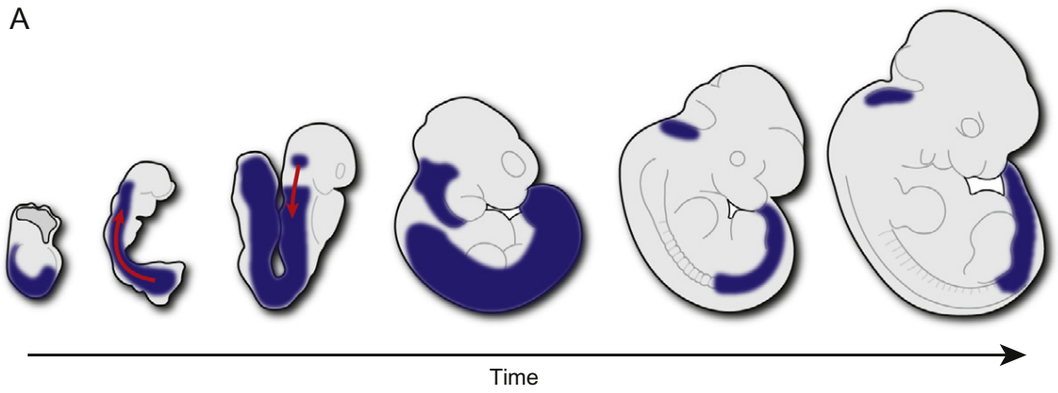


Deschamps and van Nes (2005)

-RA is produced in paraxial mesoderm and has the highest level in the caudal hindbrain.

Segmentally restricted Hox gene expression is controlled by temporally dynamic gene regulation

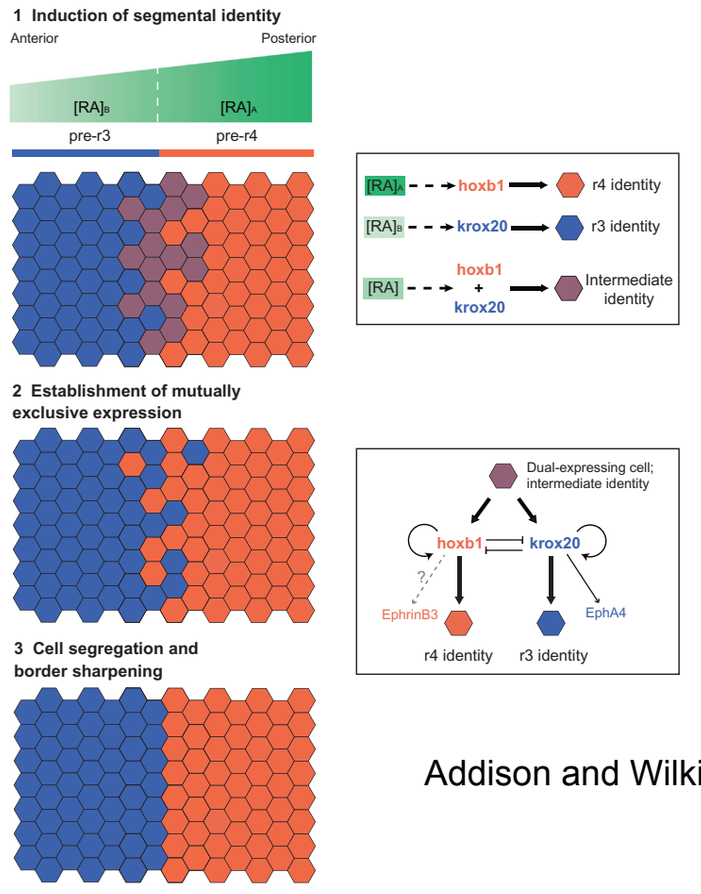
Hoxb1 regulation



Krumlauf (2016)

-Initial expression is established by the caudal-high RA signaling. FGF8 from the isthmus and the RA-degrading enzyme Cyp26a1 suppress *Hoxb1* in r1 and r2.

Expression is suppressed in r3 and r5 by a zinc-finger ranscription factor Krox20.

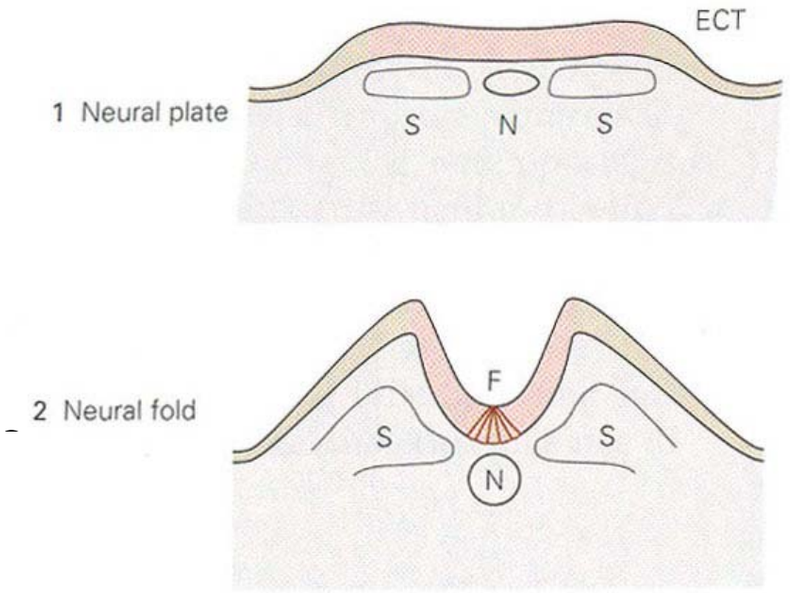


Addison and Wilkinson (2016)

Expression is enhanced by other *Hox* genes like *Hoxa1* and *Hoxb1*.

-In the end, expression becomes specific to r4.

Dorsoventral (DV) patterning



Similar to early AP patterning, early DV patterning is imposed by signals that come from outside of the nervous system.

medial (future ventral): notochord

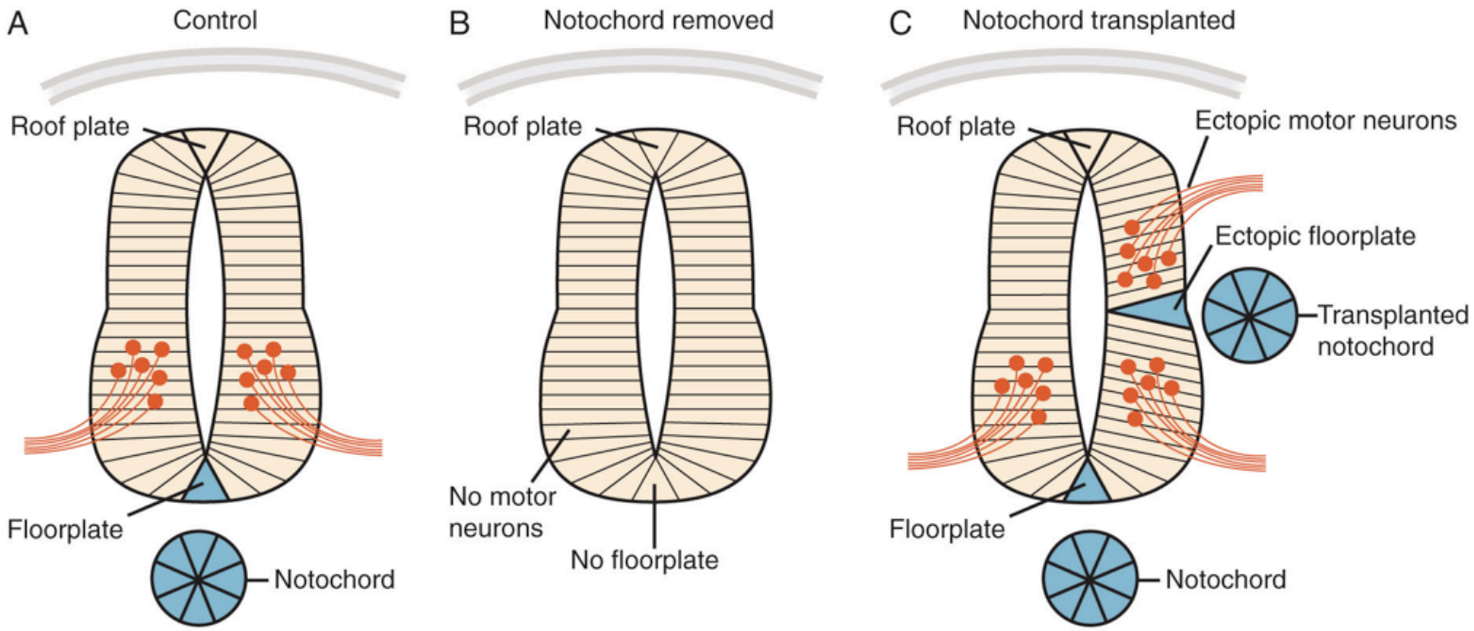
lateral (future dorsal): epidermis

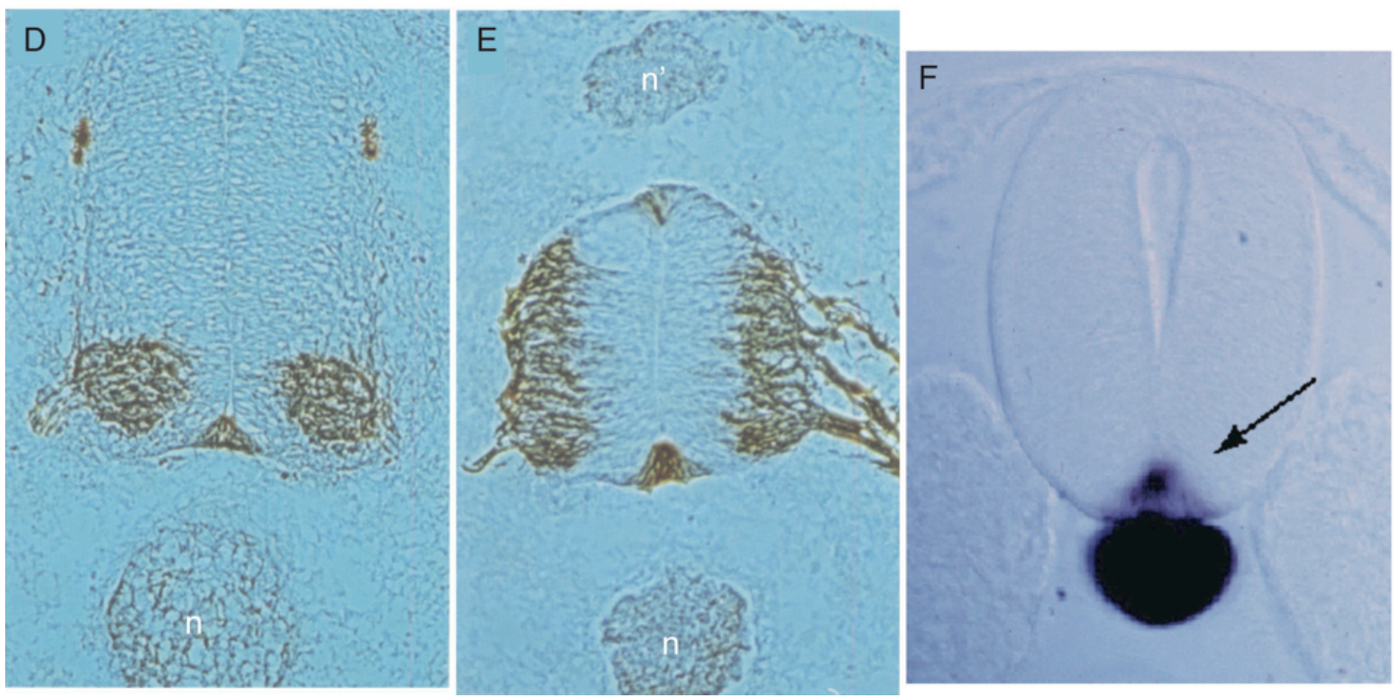
Notochord induces the floor plate at the ventral end of the neural tube.

Epidermis (surface ectoderm) induces roof plate at the dorsal end of the neural tube.

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

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



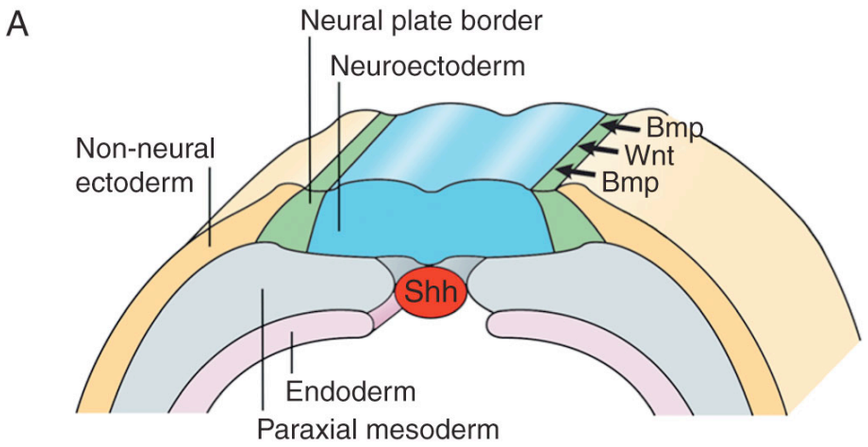


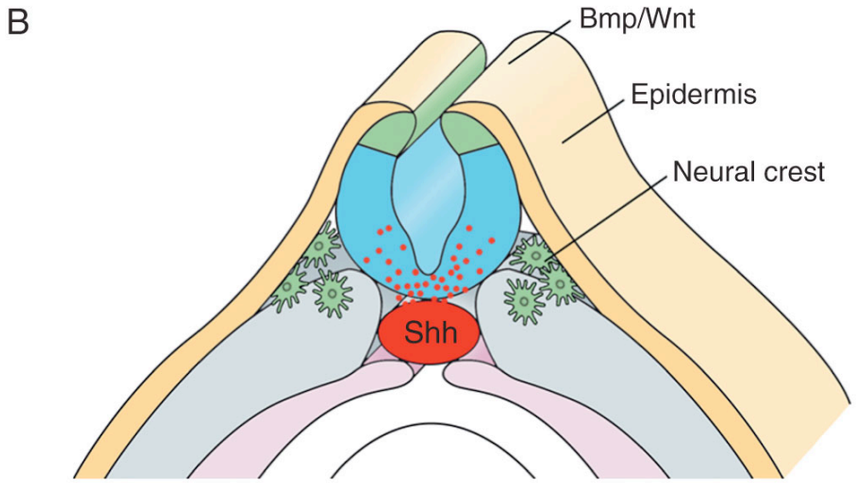
B. Removal of the notochord results in the loss of the floor plate and motor neurons.

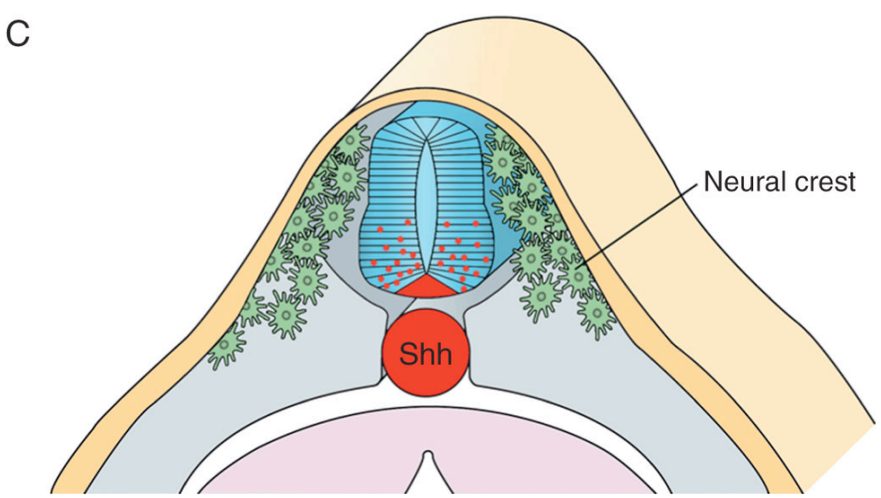
C. Ectopic transplantation of the notochord near the dorsal neural tube induces a second floor plate and ectopic motor neurons.

The floor plate can be considered as another secondary organizer.

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





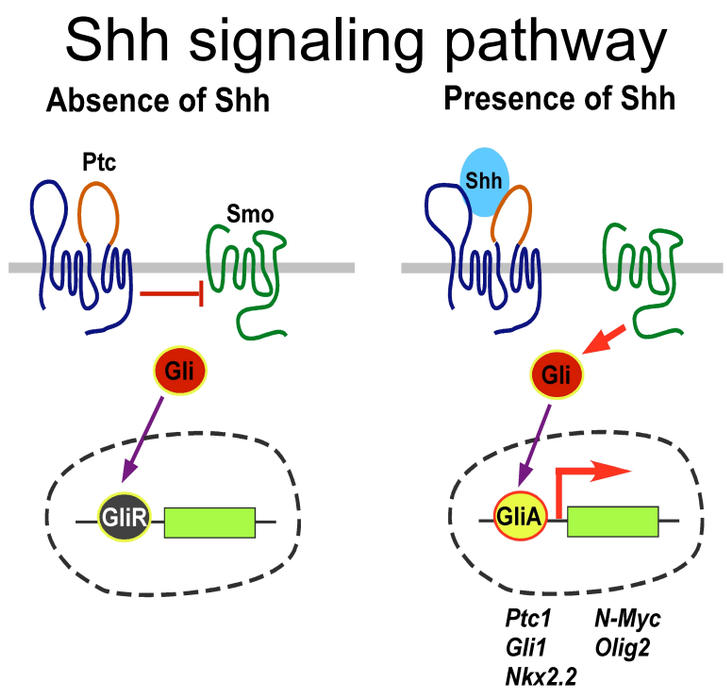


Shh is a homologue of the Drosophila segment polarity gene, *Hedgehog*.

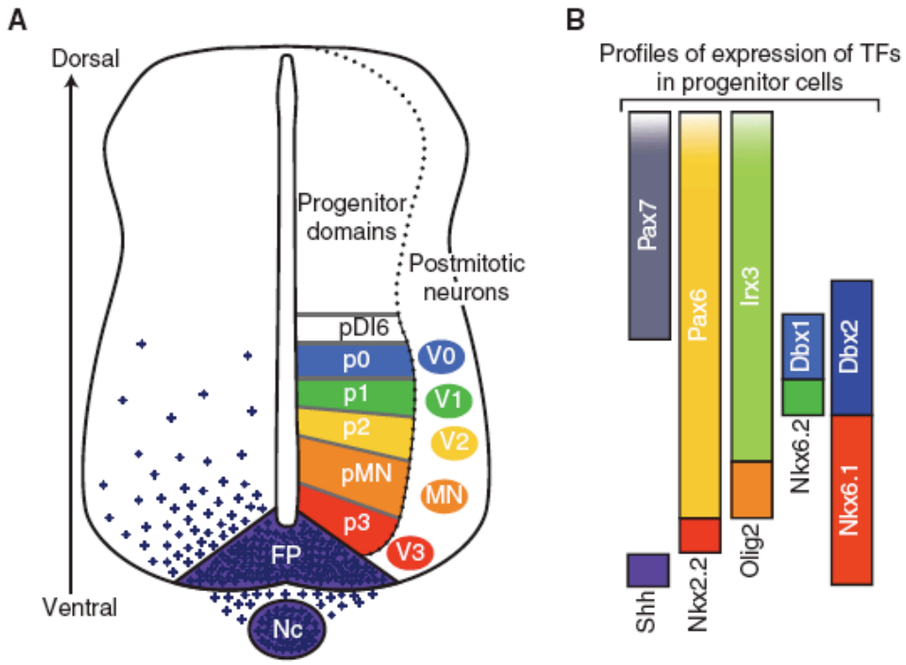
Notochord-derived SHH induces the expression of *Shh* in the floor plate.

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

Roof-plate derived BMP antagonizes SHH signaling.



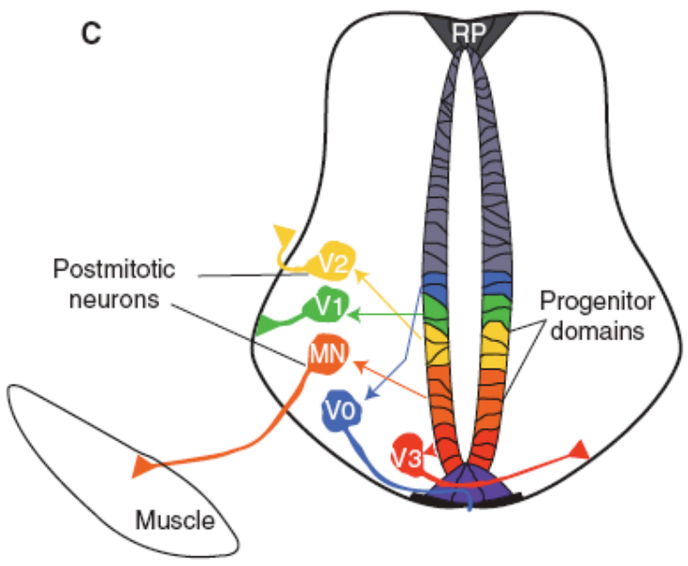
Graded SHH signaling controls differential gene expression in ventral spinal cord



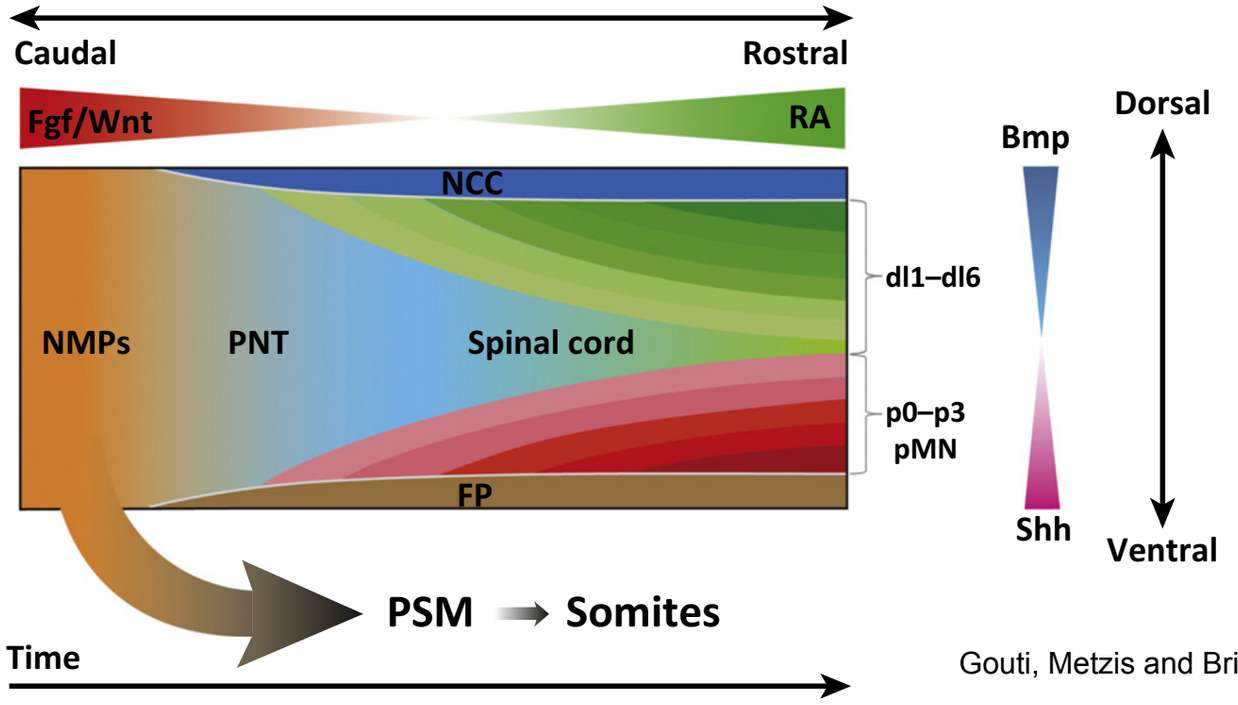
Initially, expression of various transcription factors in progenitor cells is graded.

By the time neurogenesis starts, mutual suppression between these transcription factors results in the formation of boundaries of progenitor cell domains.

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



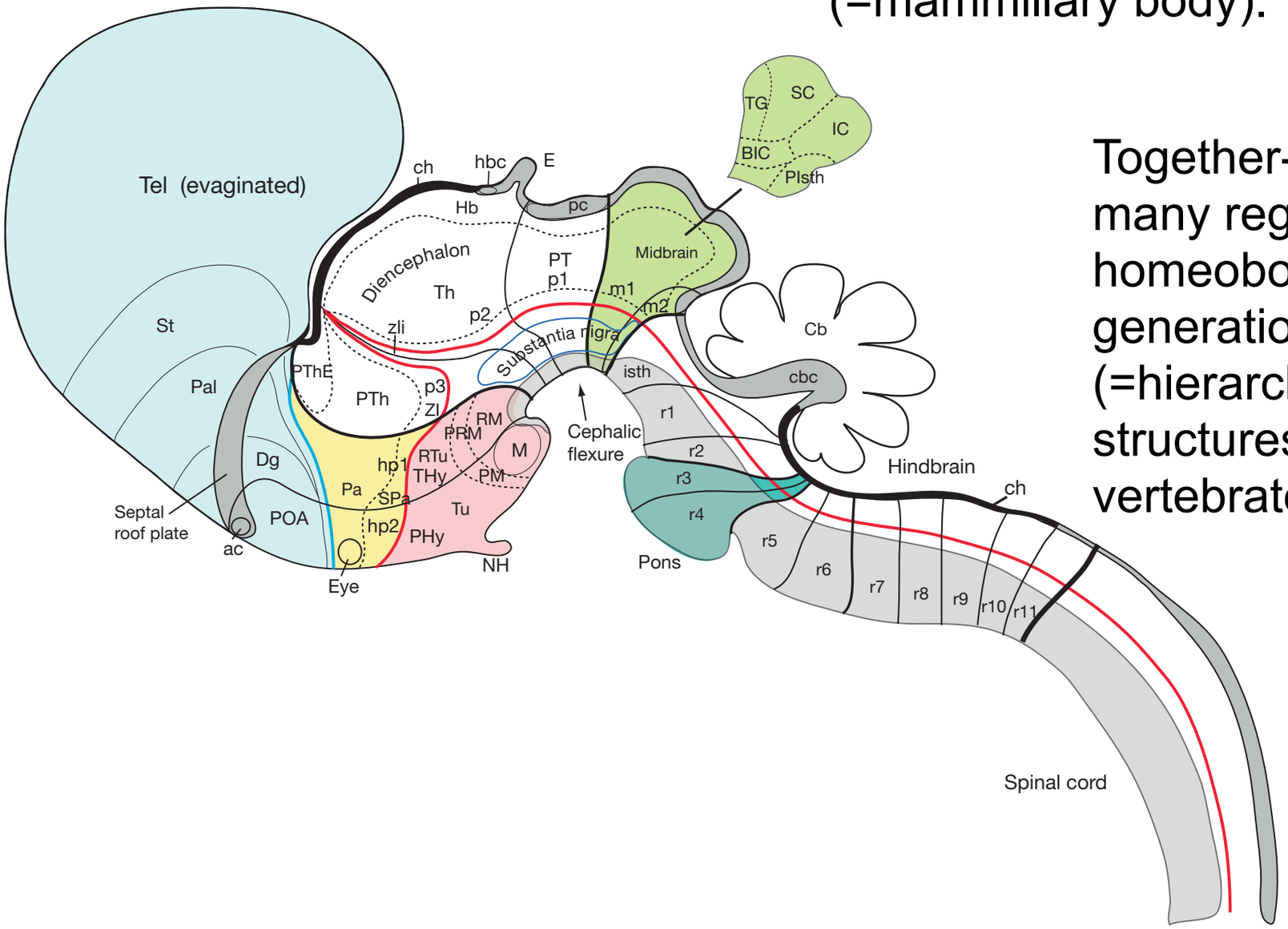
Ribes and Briscoe (2009)



Gouti, Metzis and Briscoe (2015)

SHH ventralizes the brain, too

Location of SHH expression and expression of SHH-target genes allowed defining the border between alar and basal plate (red line, “sulcus limitans”) as well as the rostral end of the ventral-most part of the brain (=mammillary body).



Together-with the expression patterns of many regulatory genes, most notably homeobox genes, may allow the generation of logical ontology (=hierarchical classification) of forebrain structures that can be applied to all vertebrate species.

Puelles (2013)

Summary 2

1. Hndbrain patterning and segmentation

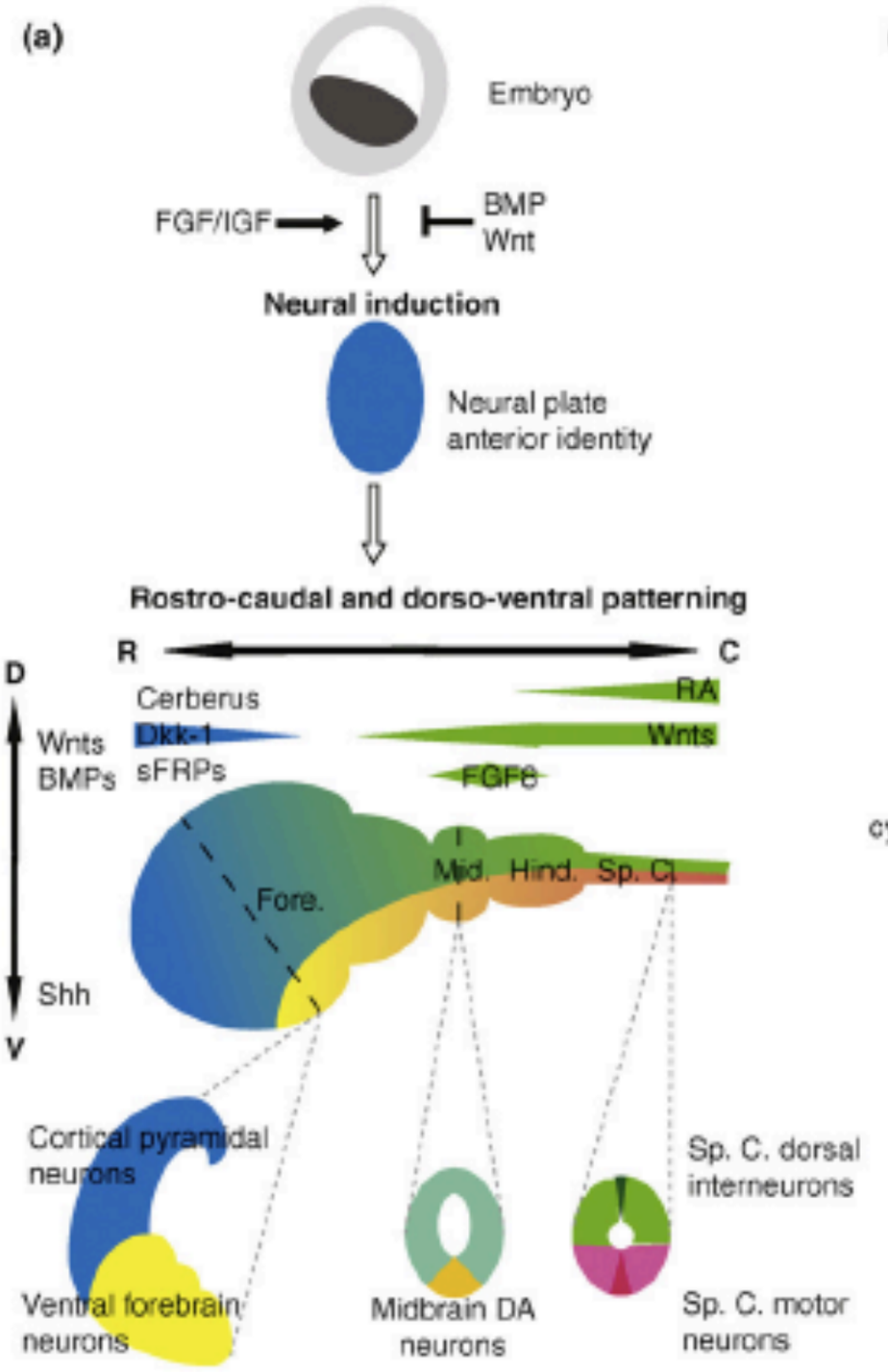
-Hindbrain is segmentally organized into rhombomeres.
-*Hox* genes are expressed in the hindbrain and spinal cord in nested patterns.
-*Hox* genes are required for segmental identity of rhombomeres.
-Segmental expression of *Hox* genes is controlled by a combination of extrinsic signals such as retinoic acid and intrinsic gene regulatory network.
-Genetic studies of anterior-posterior patterning of Drosophila embryos found basic concepts of gene regulation as well as the actual genes that are conserved across species. These genes include *Hox* and other homeobox genes, *Wnt*s and *Shh*.

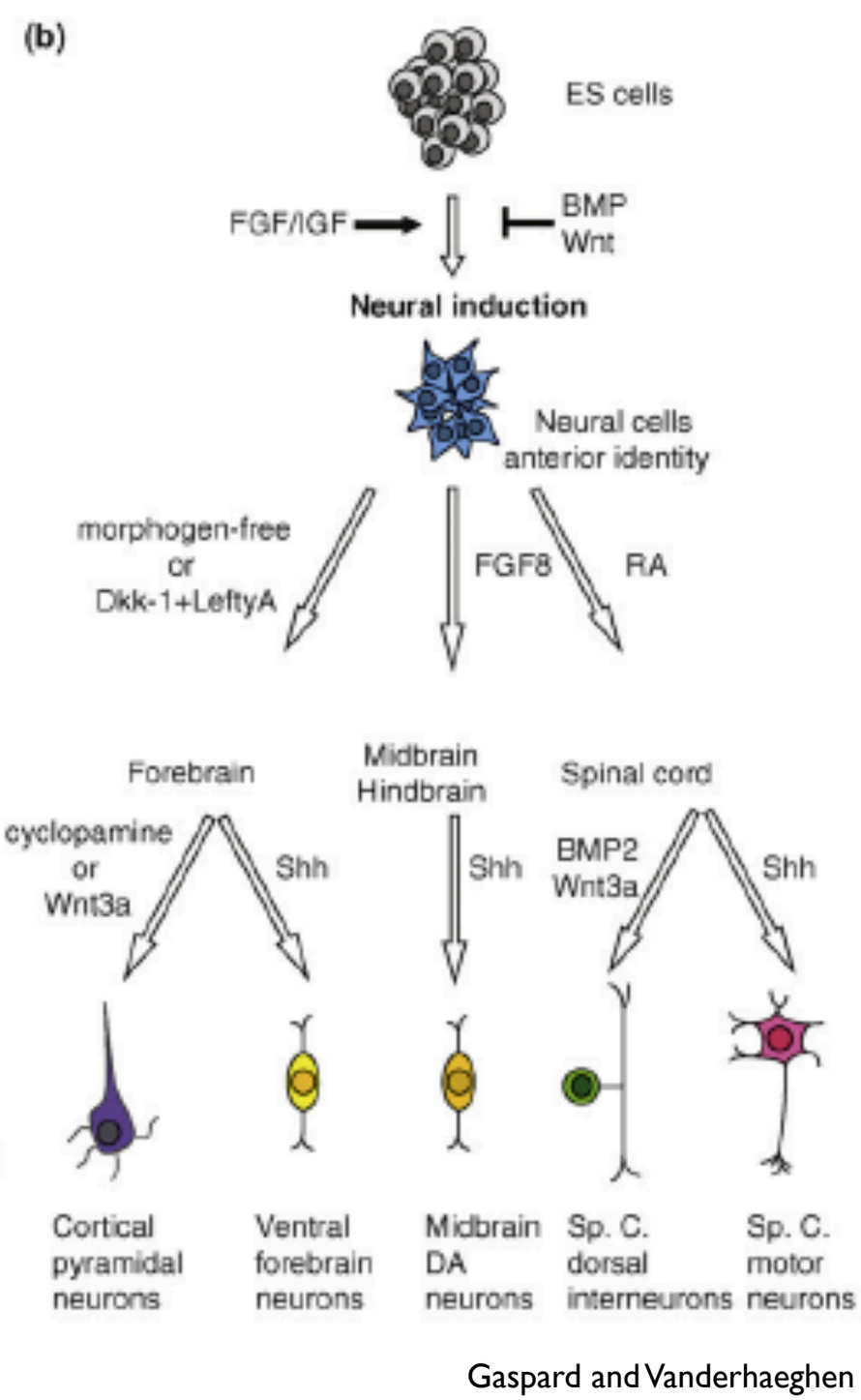
2. Dorso-ventral patterning of the brain and spinal cord

-Two secondary organizers, floor plate and roof plate, impart dorso-ventral patterning of the neural tube, in which opposing gradients of SHH and BMP signaling generates dorso-ventral “segmentation” of the spinal cord.

Findings in *in vivo* neural patterning have been applied to the generation of specific types of neurons from pluripotent stem cells.

In vitro generation of specific types of neurons from ES cells





Gaspard and Vanderhaeghen (2010)