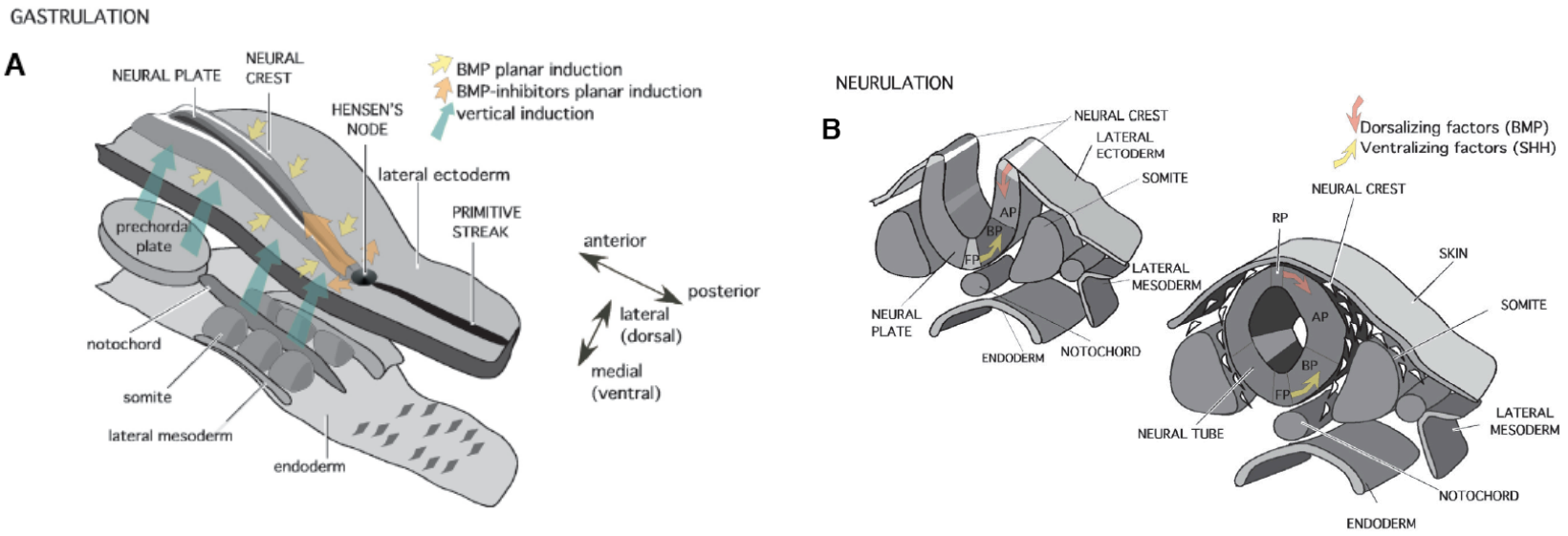
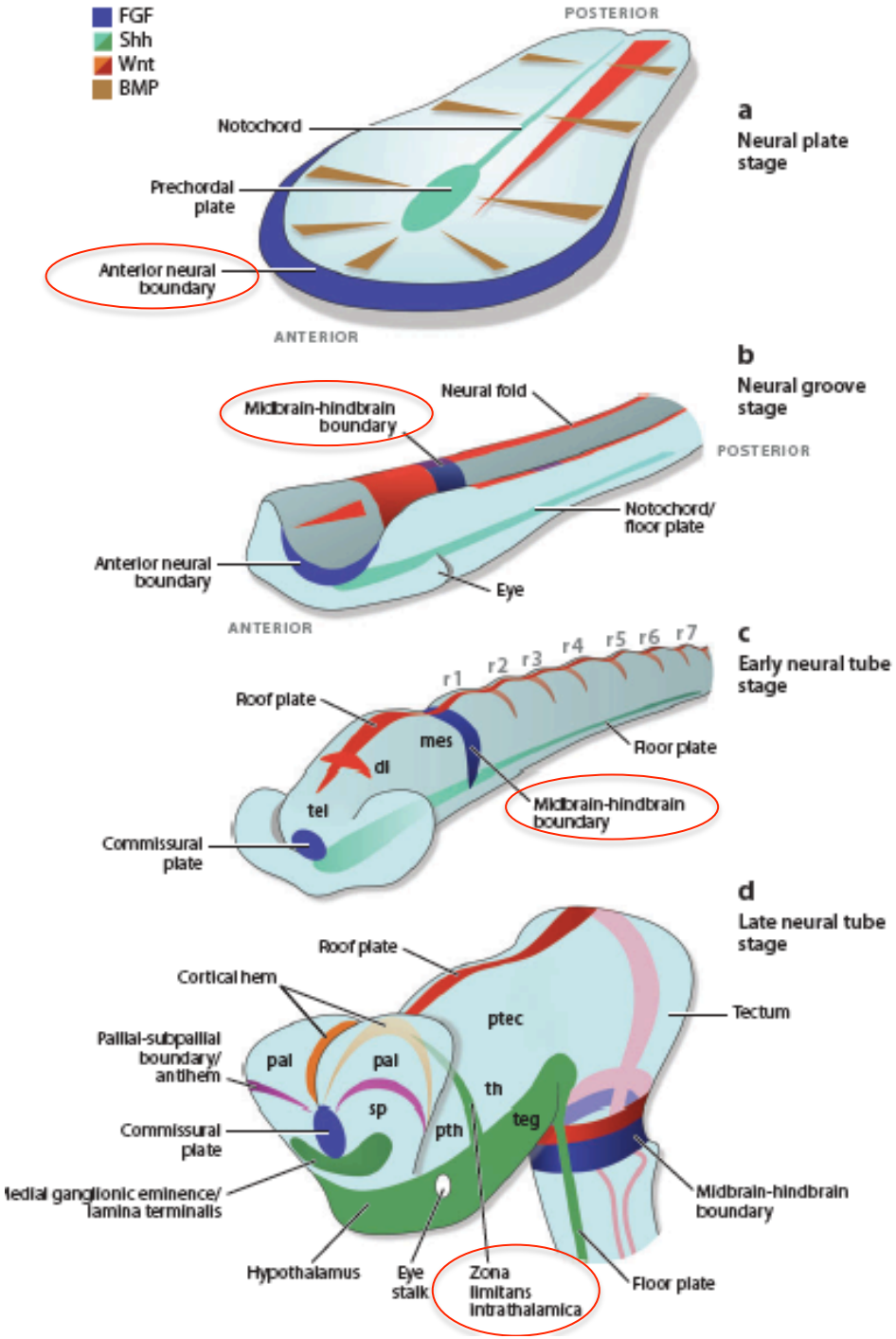
→Inductive factors generate regional specification within the developing brain

✓ regionalization of the CNS occurs in concert with patterning of the entire embryo:
graded signaling of secreted proteins <u>establishes differential expression of transcription Factors (TFs):</u>

1° source of inducing factors: **primay organizer** and its derivatives → crude pattern
2° source of inducing factors: **secondary organizers** within the neuroepithelium→ **refine local neuronal identities**

✓ differential expression of TFs results in the establishment of distinct neural progenitor cells with specific positional identity





ANTERO-POSTERIOR PATTERNING
&
SECONDARY ORGANIZERS

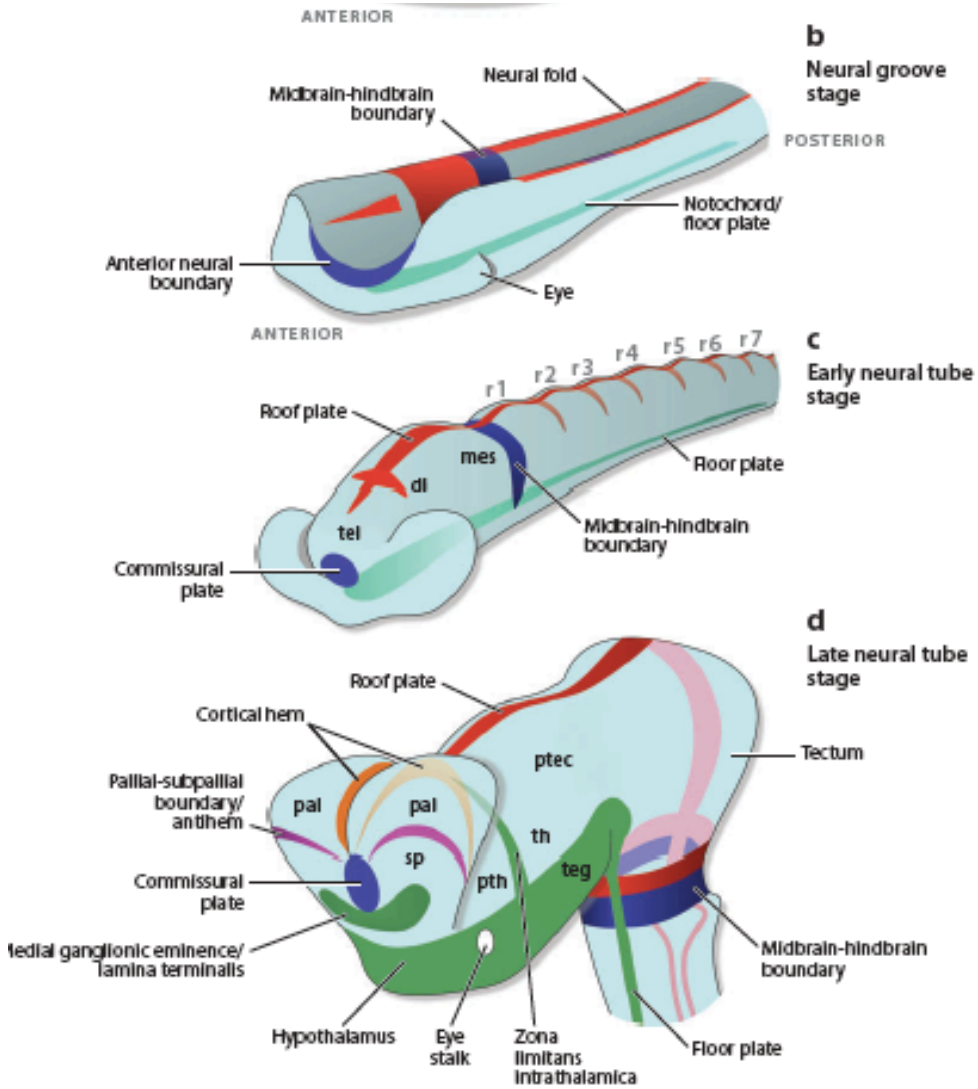
→ ***Organizing centers*** *are established gradually within a given field* ***at the junction between territories*** *that acquire different positional identities*

ANR= The Anterior Neural Boundary/Commissural Plate

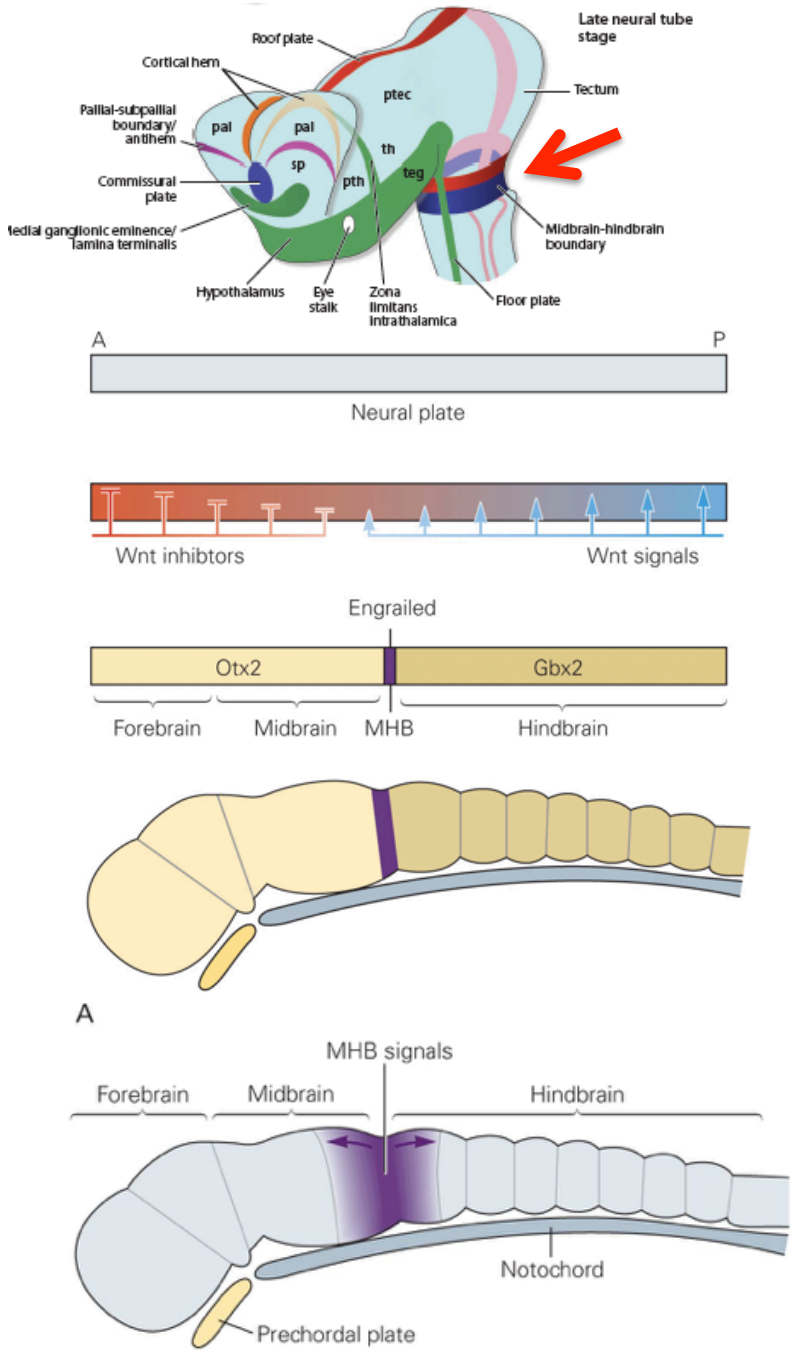
MHB=midbrain-hindbrain boudary

ZLI= Zona limitans intrathalamica

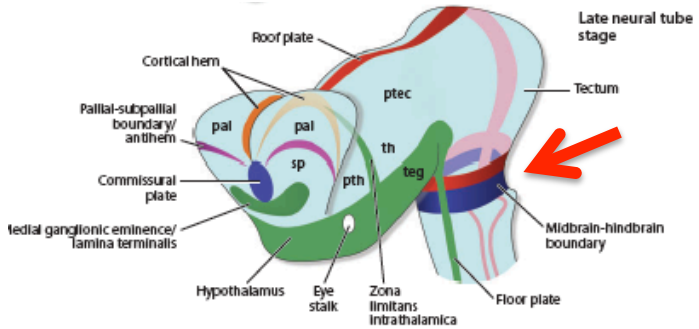
The Midbrain-Hindbrain Boundary (MHB) or Isthmic organizer (IsO)



Development of the **mesencephalon** and **metencephalon** is regulated and coordinated by molecules produced in a **signaling center**, known as **the isthmic organizer MHB or IsO**, which develops at the mes/met boundary, and co-localizes with a morphological constriction of the neural tube called the **isthmic constriction**



- ✓ The MHB/IsO emerges after neural tube closure
- ✓ Wnt signaling establishes the initial A-P polarity of the neural plate
- ✓ The earliest molecular event for the IsO specification is the differential expression in the neural plate of ***Otx2*** in the rostral epithelium and a ***Gbx2*** in the posterior domain
- ✓ The MHB expresses **Engrailed** (**En**)
- ✓ The MHB is source of **secreted signals** that pattern the midbrain and hindrain

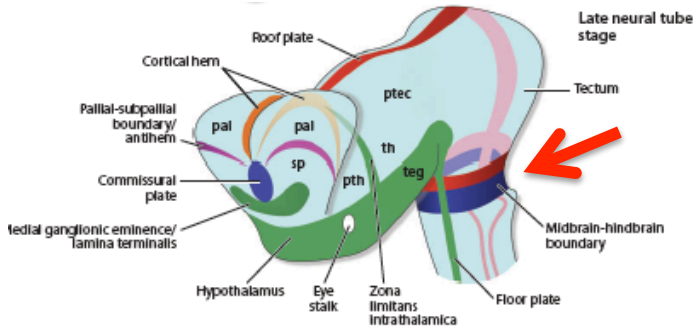


The Midbrain-Hindbrain Boundary (MHB) or Isthmic organizer (IsO)

- IsO activity has been found in **all vertebrate species** that have been studied and it is regarded as a prototypical local organizer of the embryonic brain

- **Functional identification of the IsO:** first identified in the avian embryo

Experimental approaches???



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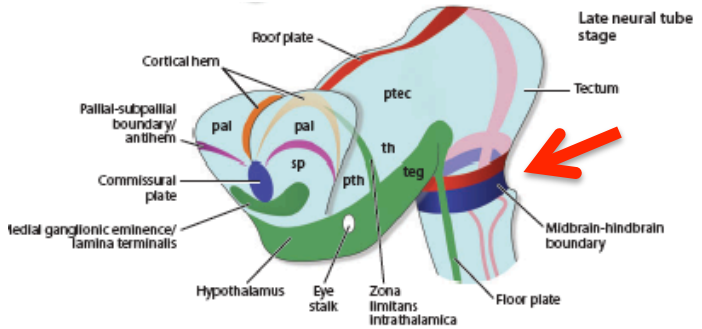
experimental approaches???

<u>Two main approaches</u>

IsO ectopic transplantation

IsO removal

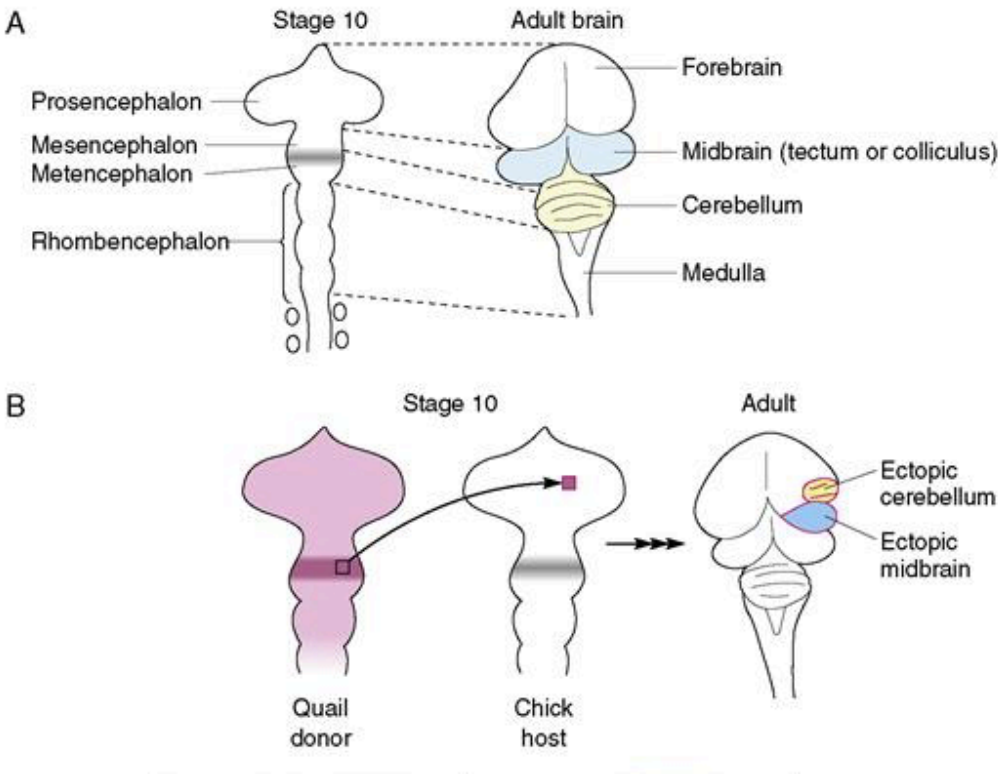


The Midbrain-Hindbrain Boundary (MHB) or Isthmic organizer (IsO)

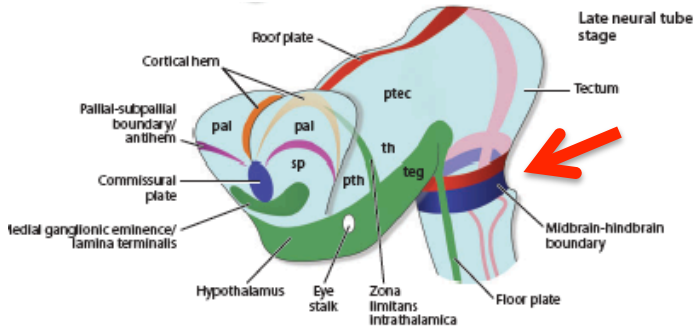
1) **IsO ectopic transplantation**

transplantation of the isthmus to ectopic neural locations → induced a mes-metencephalic fate in the host territories that contacted the graft

(interspecific transplantation paradigm, Developed by Nicole Le Douarin, 1982)



→ *Notably the induction of mes-metencephalic tissue was always* ***polarized*** *(*the caudal side of the induced mesencephalon and the rostral side of the induced metencephalon were in contact with the isthmic graft)



The Midbrain-Hindbrain Boundary (MHB) or Isthmic organizer (IsO)

2) **IsO removal**

complete removal of the isthmus *triggers the* ***loss of the entire mesencephalon*** *and* ***metencephalon***

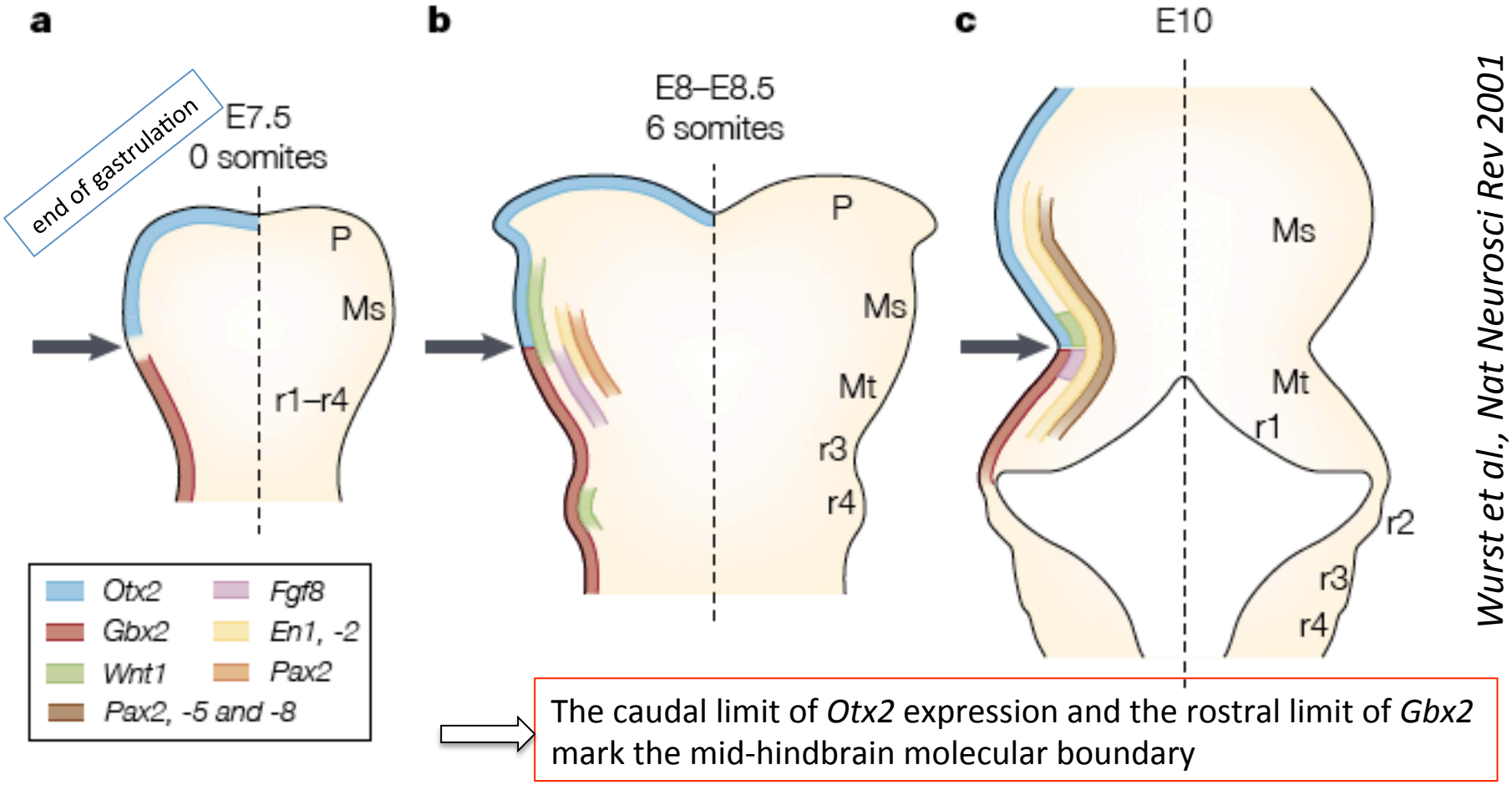
→ the isthmic territory is both **necessary and sufficient** for the development of the mes-metencephalic domain

→ the isthmic territory functions as an **organizing centre**, controlling both the growth and the **ordered rostrocaudal specification of mesencephalic and metencephalic territories**

these results have been extended to mouse and zebrafish embryos, indicating that the mes-metencephalic junction is likely to be crucial for neural tube patterning in all vertebrates

Gene expression at the mid-hindbrain junction (mouse)

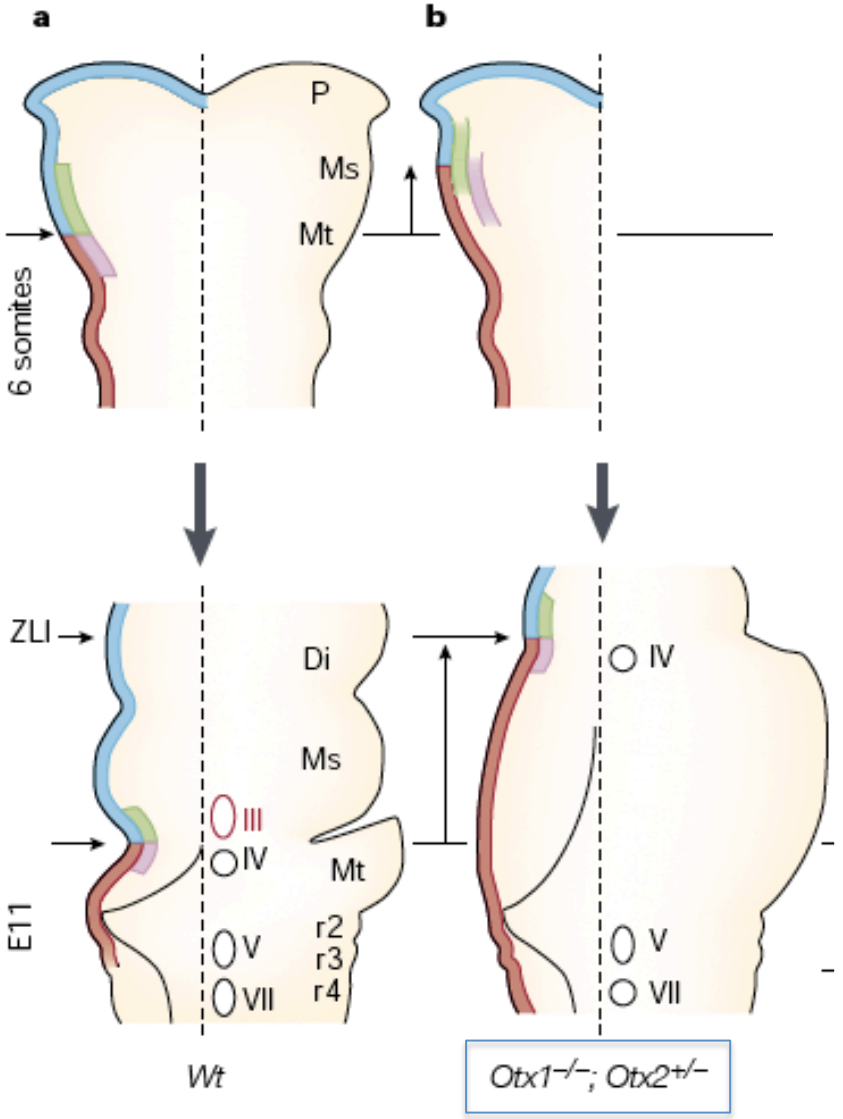
the boundary between the midbrain and hindbrain is roughly positioned during late gastrulation and is **progressively refined** during early somitogenesis



*The spatio-temporal expression profiles of these genes have been **largely conserved throughout evolution** (only subtle differences in the onset of expression).*

The caudal limit of ***Otx2*** expression and the rostral limit of ***Gbx2*** mark the mid-hindbrain molecular boundary

The Midbrain-Hindbrain Boundary (MHB) or Isthmic organizer (IsO)



In Otx2–/+;Otx1–/– or **Otx2–/+;Otx1–/+** mutants:

the IsO is shifted anteriorly below a critical threshold of Otx2 function

Mice with the genotype *Otx2–/+;Otx1–/–* or *Otx2–/+;Otx1–/+* lack a mesencephalon and show an extension of metencephalic tissue, (giant cerebellum)

Otx2 *Wnt1* *Gbx2* *Fgf8* Cranial motor neurons

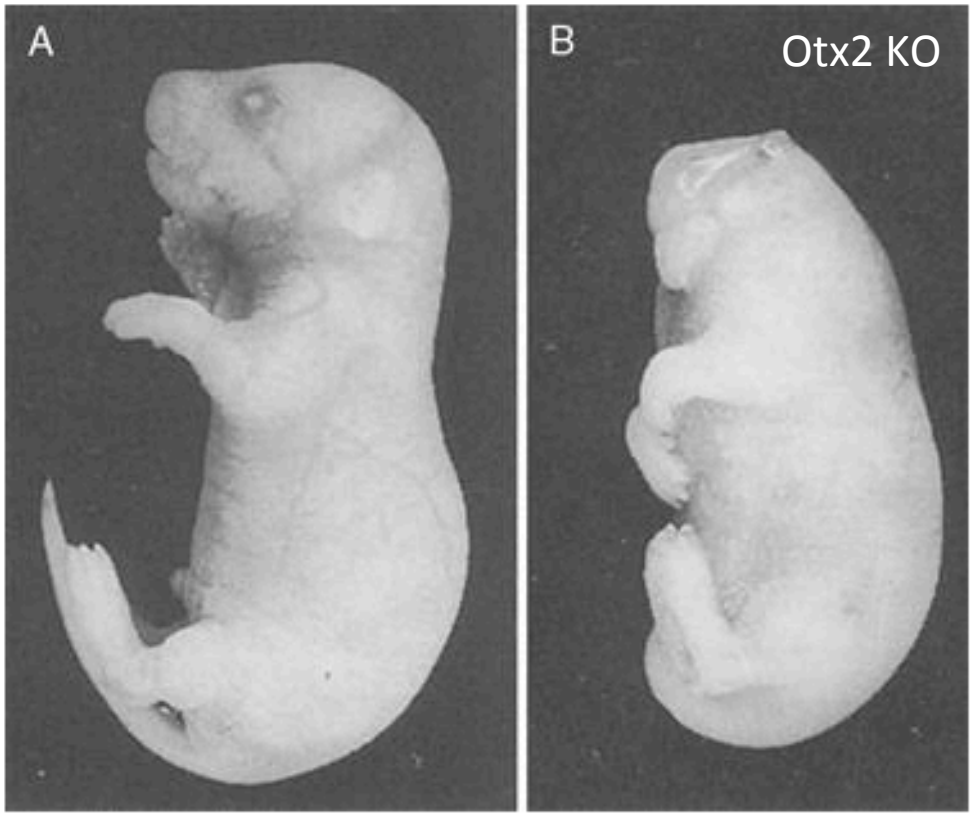
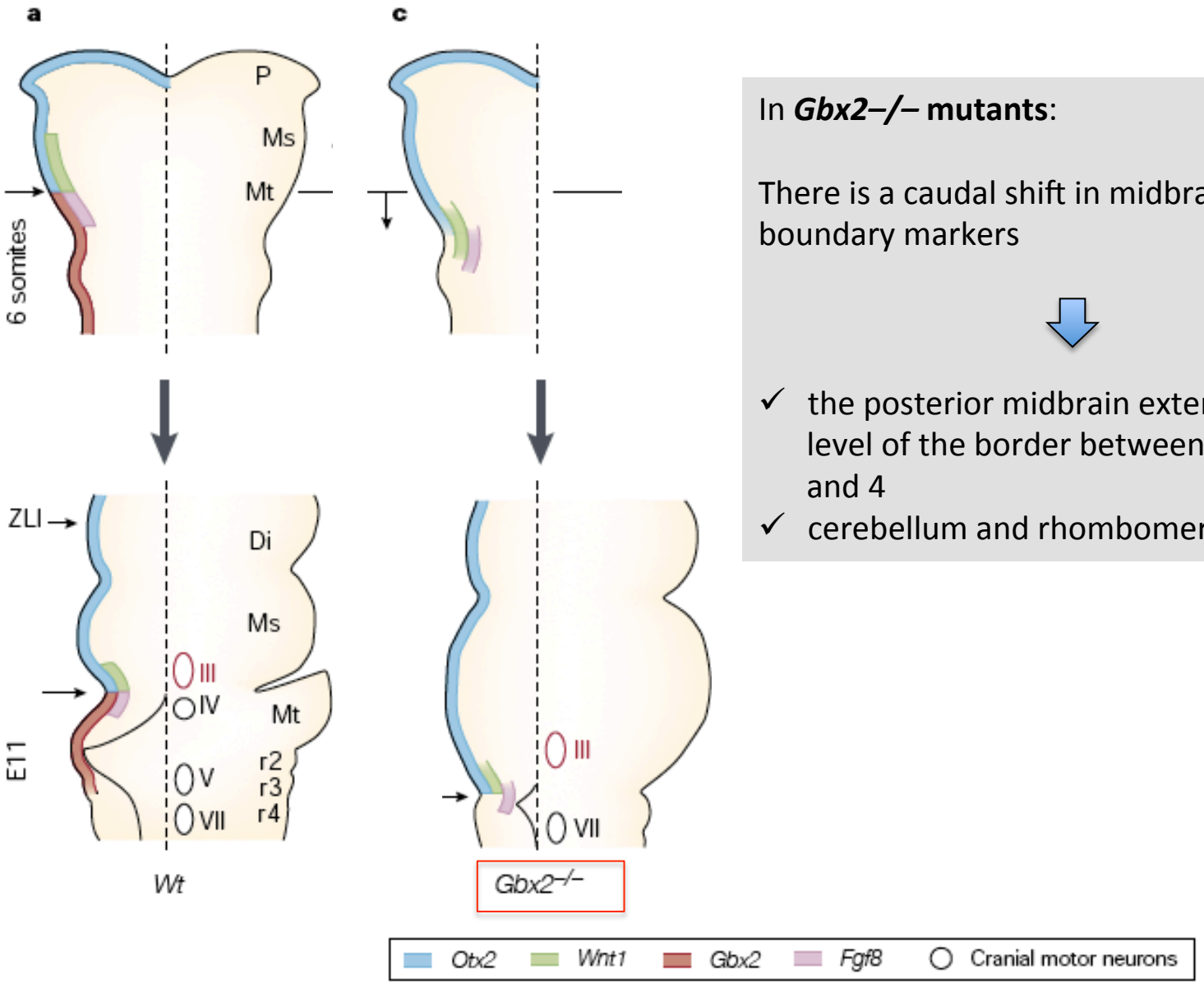


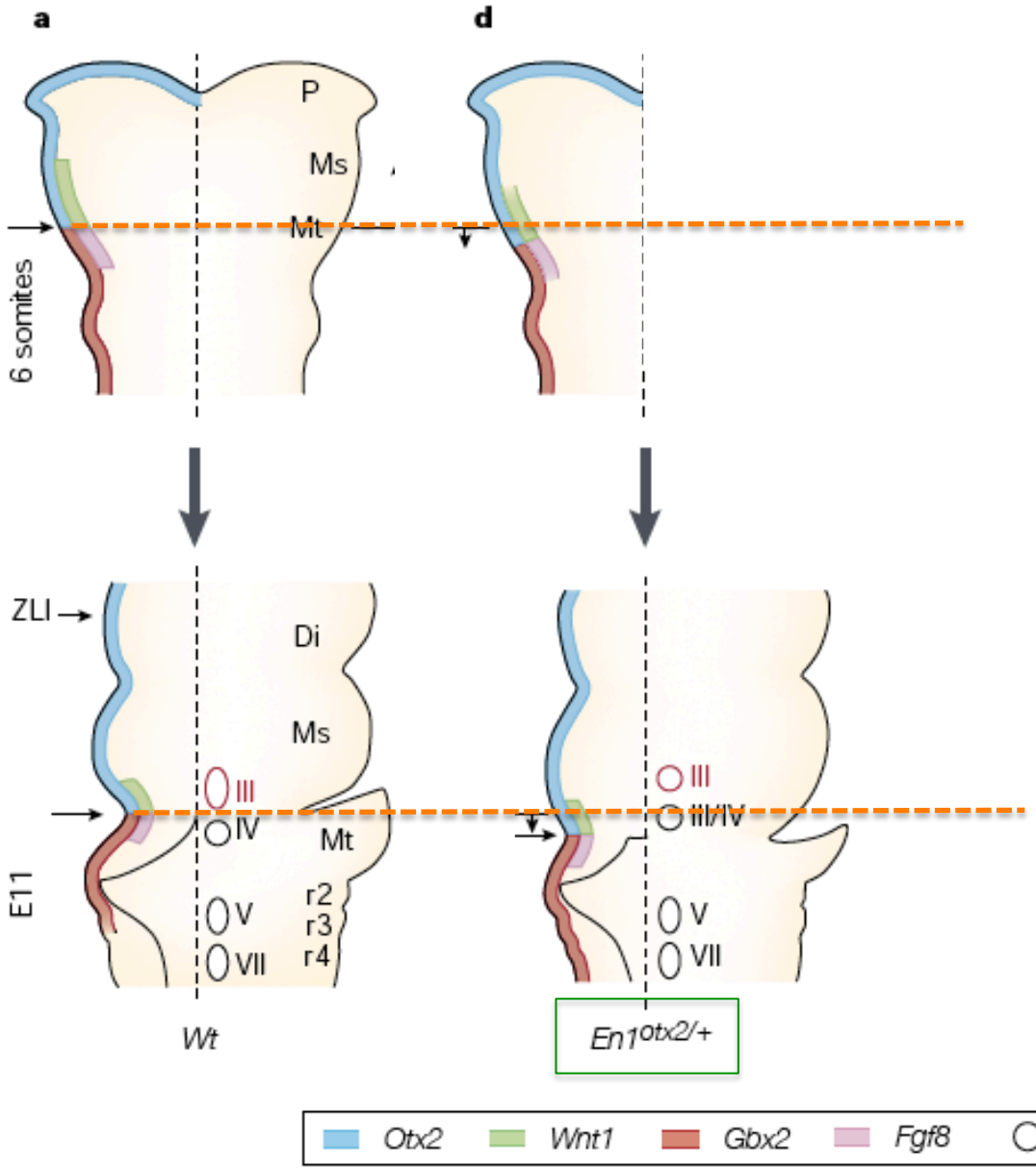
Fig. 2.12 *Otx2* is required for the formation of the mouse head. A dramatic illustration of the importance of the *otx2* gene is the development of the mouse forebrain and rostral head. If the gene is deleted using homologous recombination, embryos without either allele of the gene fail to develop brain regions rostral to rhombomere 3, a condition known as anencephaly. Since many of the bones and muscles of the head are derived from the neural crest, which also fails to form in these animals, the animals lack most of the head in addition to the loss of the brain.From Matsuo et al., 1995



In ***Gbx2–/–* mutants**:

There is a caudal shift in midbrain/hindbrain boundary markers

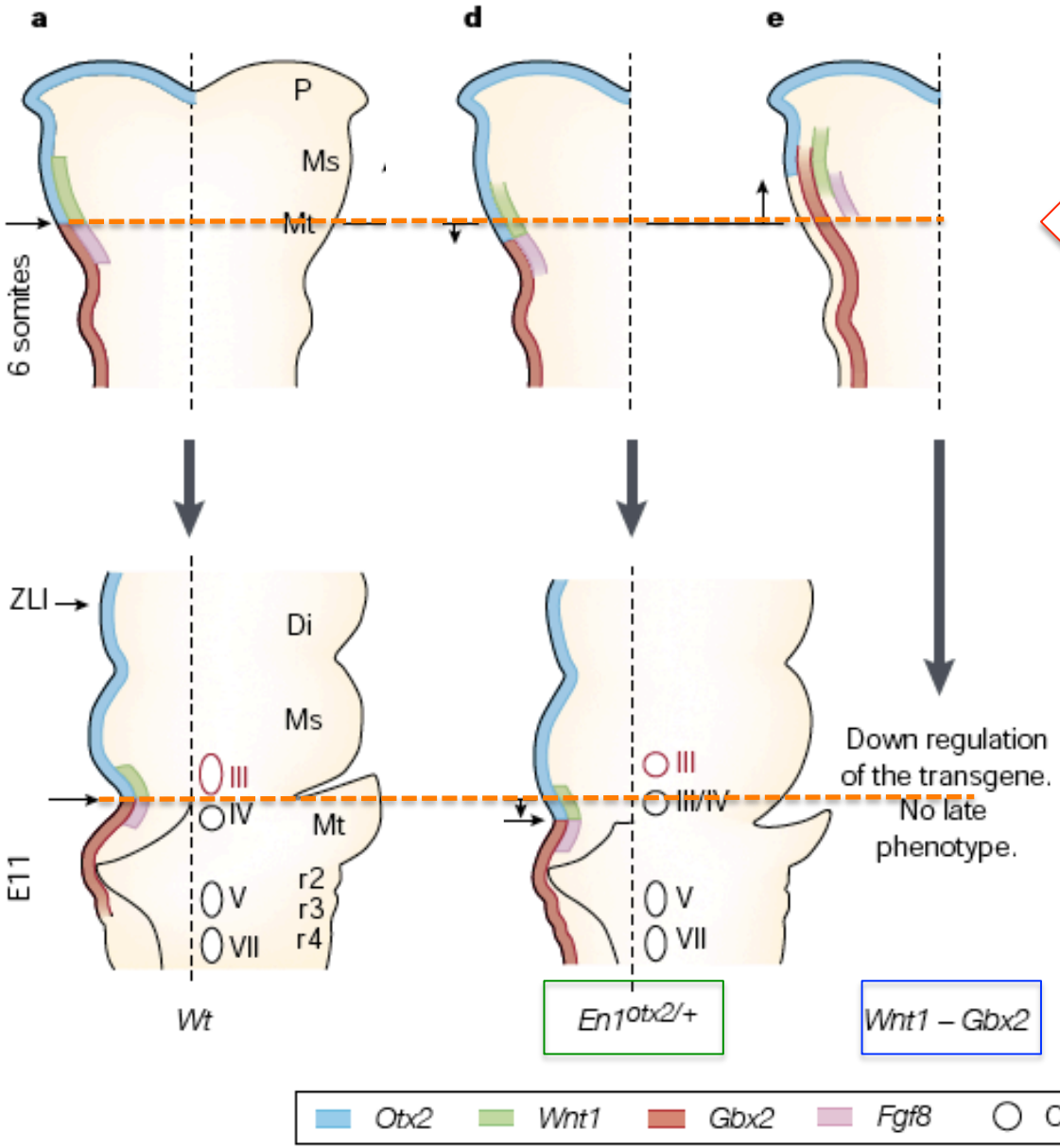
- ✓ the posterior midbrain extends caudally to the level of the border between rhombomeres 3 and 4
- ✓ cerebellum and rhombomeres 1–3 are lost



<u>Otx2 ectopic expression (knock-in):</u>
the caudal limit of *Otx2* expression was shifted caudally into the metencephalon after inserting the *Otx2* gene into the *En1* locus.

- ✓ Induces a redistribution of all IsO markers
- ✓ Can induce mesencephalic fate within the mid-hindbrain territory

→the posterior part of the tectum was enlarged at the expense of the anterior cerebellar vermis.



Gbx2 ectopic expression:

Gbx2 expression was driven rostrally into the midbrain by the *Wnt1* promoter

the position of the IsO appeared transiently shifted into the mesencephalon

The Midbrain-Hindbrain Boundary (MHB) or Isthmic organizer (IsO)

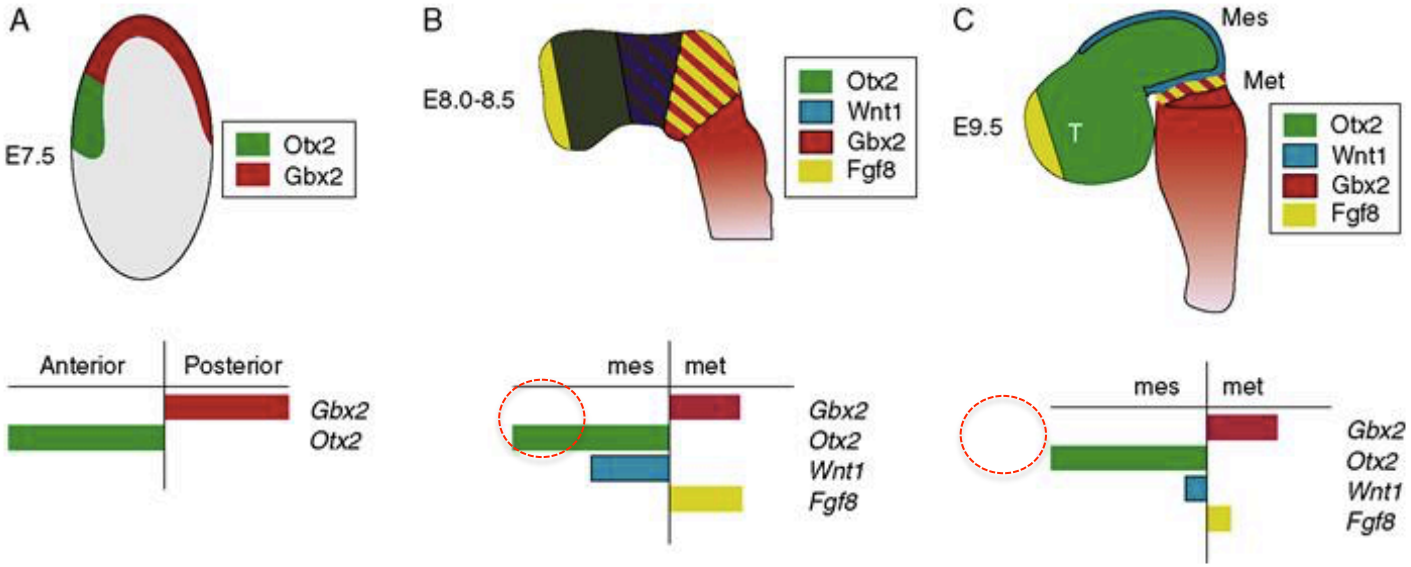
the loss- and gain-of-function experiments converge into a model in which the relative doses of **Otx2** and **Gbx2** proteins control:

- ✓ the **induction and positioning of the IsO,**
- ✓ the **development of mesencephalic** versus **metencephalic fates** in the normal embryo

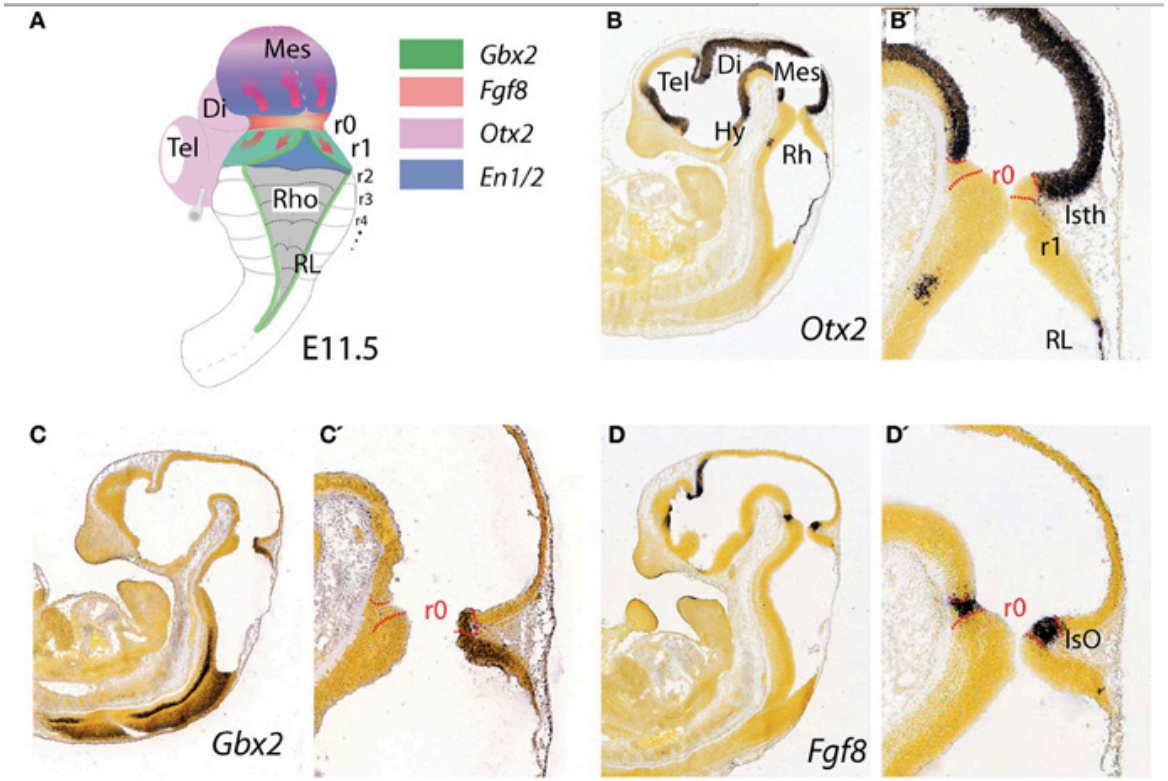
Within the mes/met, Otx2 and Gbx2 act antagonistically and are required for positioning and function of the IsO

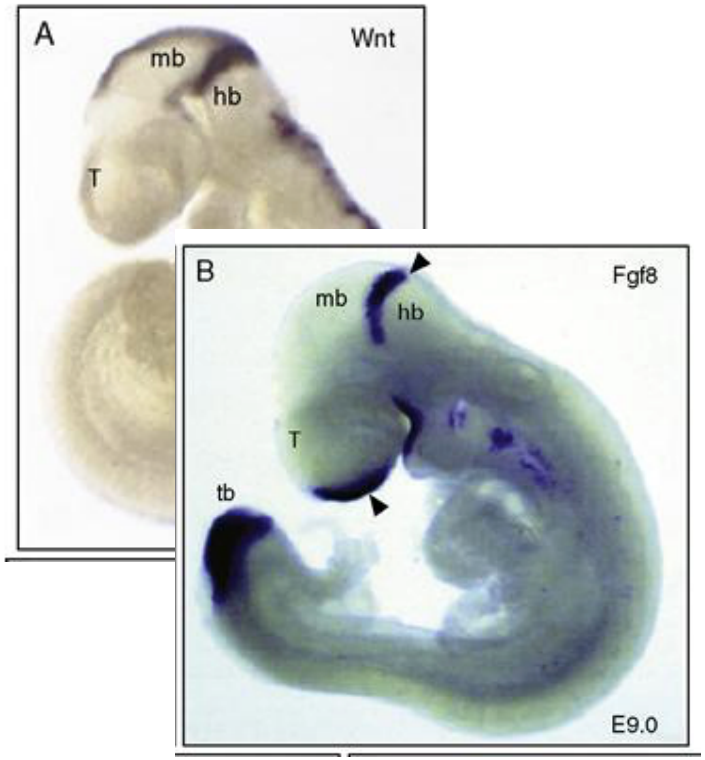
Wnt1 and ***Fgf8*** are expressed in adjacent bands on either side of the IsO

→ candidate **mediators of IsO activity for** <u>induction</u> and <u>maintenance</u> of polarized mesencephalic and metencephalic fates when transplanted ectopically



Molecular characterization of the mid-hindbrain boundary in mice



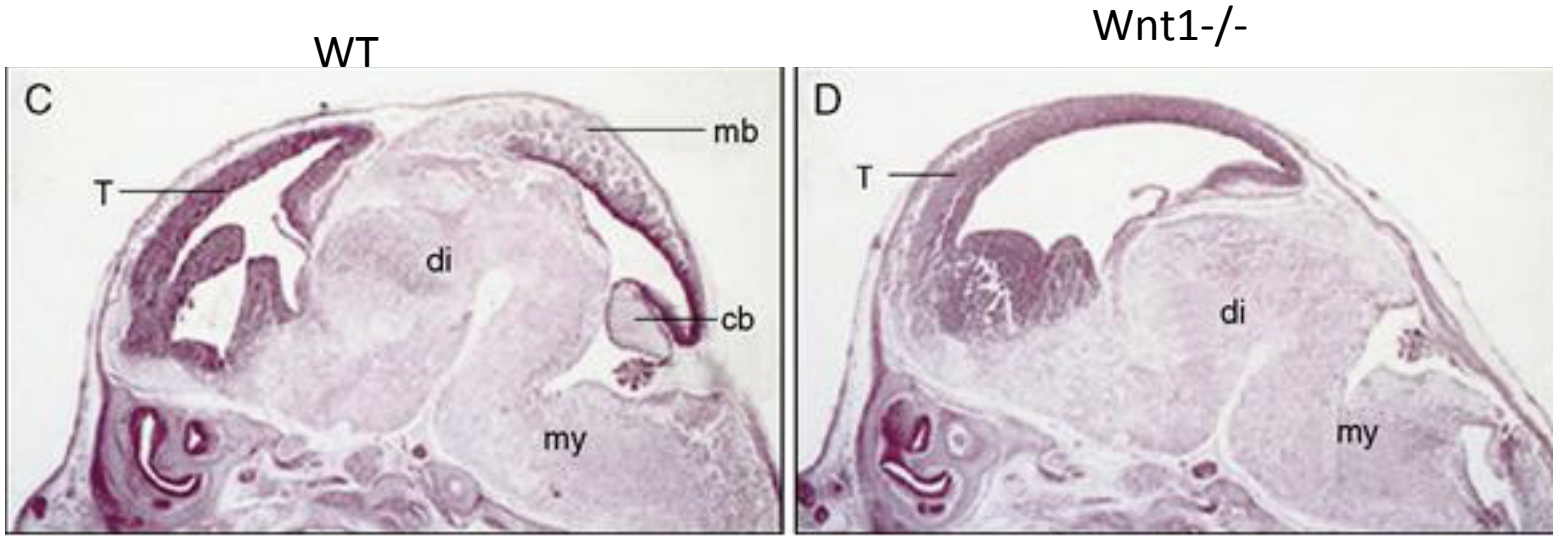


Pictures are taken by the **Allen Brain Atlas**….

Martinez et al., Frontiers in Neuroanatomy, 2013

Wnt1 and Fgf8 are expressed in adjacent bands on either side of the midbrain/hindbrain boundary

Wnt1



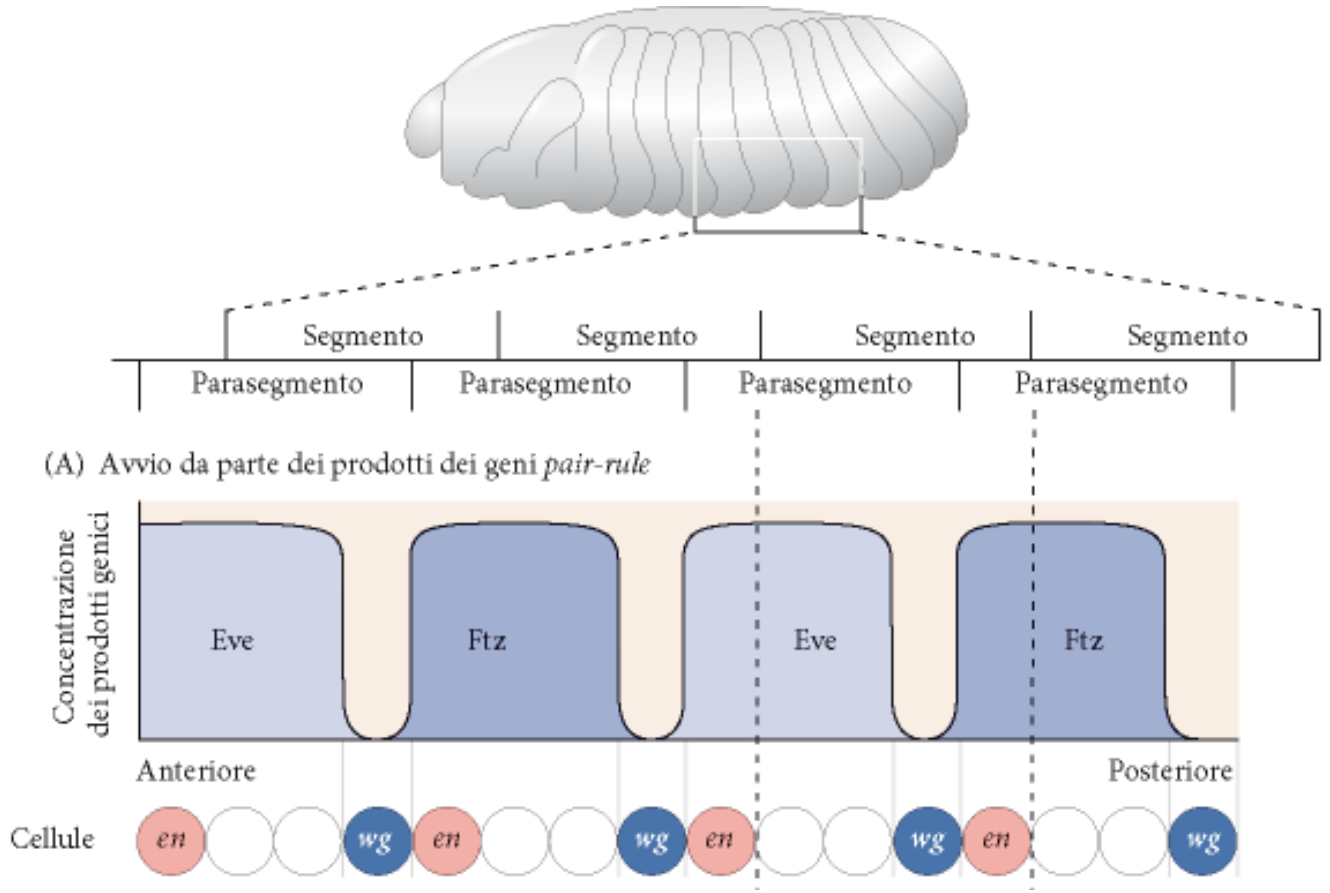
Wnt1-/- mice show:

- Dramatic reduction in midbrain/hindbrain structures
- **Lack of En1** expression in the IsO (En1 KO = phenotype as Wnt1 ko)

→….in GOF experiments, Wnt1 does not mimic organizer activity

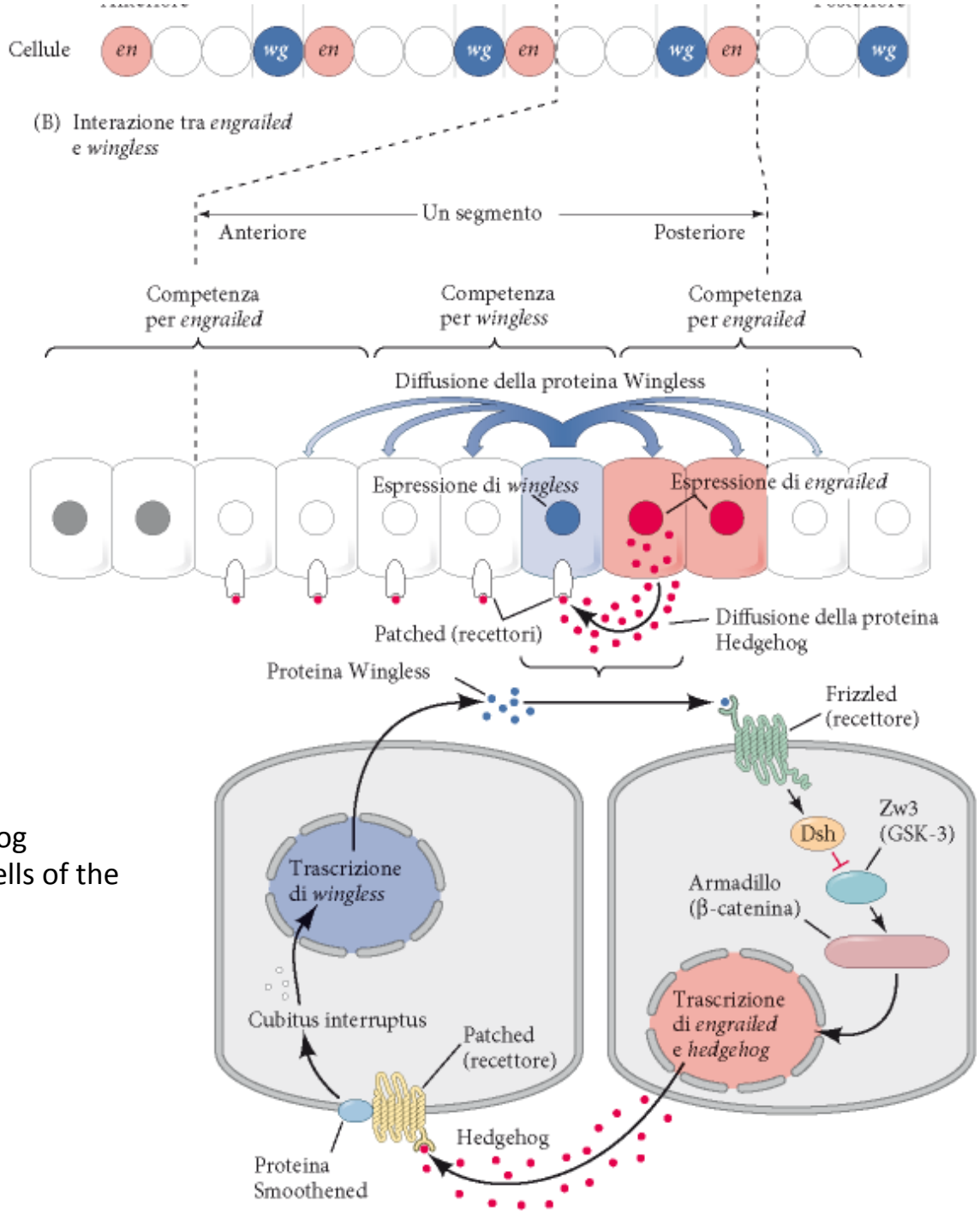
permissive but not instructive….

In Drosophila the homologus of Wnt1 (wg) is necessary for Engrailed expression



Once ***wingless*** and ***engrailed*** expression is established in adjacent cells, this pattern must be maintained to retain the parasegmental periodicity of the body plan established by the pair-rule genes.

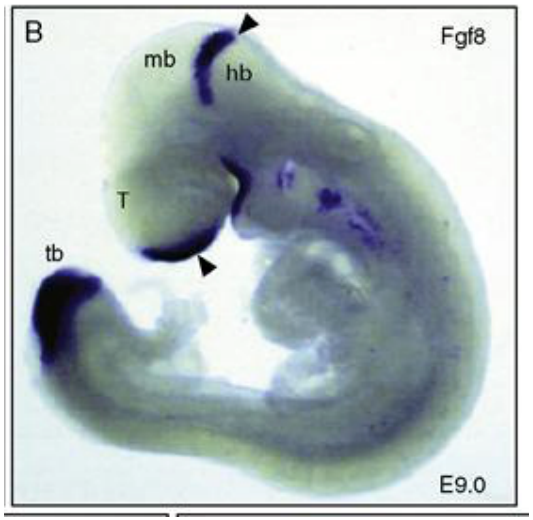
Engrailed- and Wingless-expressing cells interact to maintain the pattern of **en** and **wg** expression



Reciprocal loop

The diffusion of Wingless and Hedgehog provides the gradients by which the cells of the parasegment acquire their identities

FGF8



FGF8 -/- embryos → fail to gastrulate

Fgf8^{neo}/Fgf8^{neo}hypomorphs → survive to birth - *a substantial portion of the midbrain as well as isthmus and cerebellum are deleted – similar to KO for wnt1 and en1*

…but defects can be possibly due to altereation during gastrulation (Meyers et al., NatureGenetics 1998)

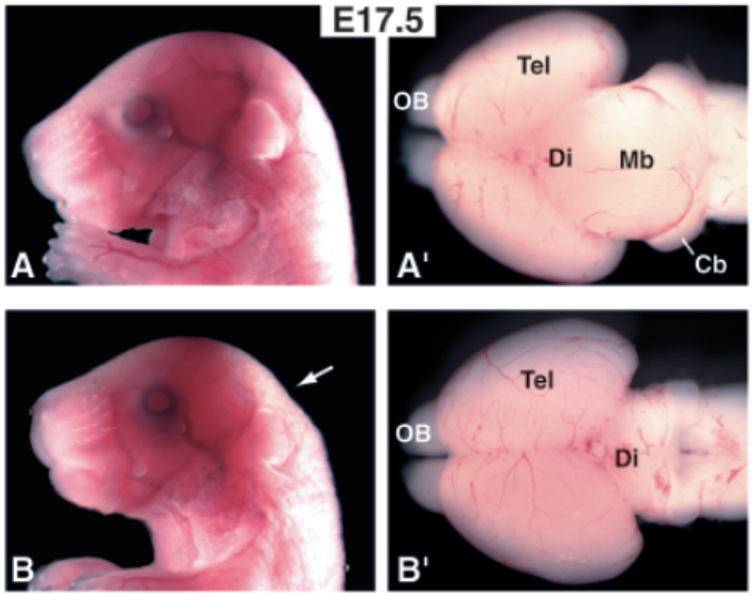
FGF8

Conditional gene inactivation approach

Mouse model to study FGF8 function in IsO

FGF8 MHB KO mutants → conditional KO
En1Cre/+,Fgf8flox
(Chi et al., Development 2003)

En1Cre produces sufficient Cre protein throughout the midbrain/ hindbrain boundary region to eliminate *Fgf8* function by 3 somite stage, when *Fgf8* expression normally commences in a subset of *En1*-expressing cells.

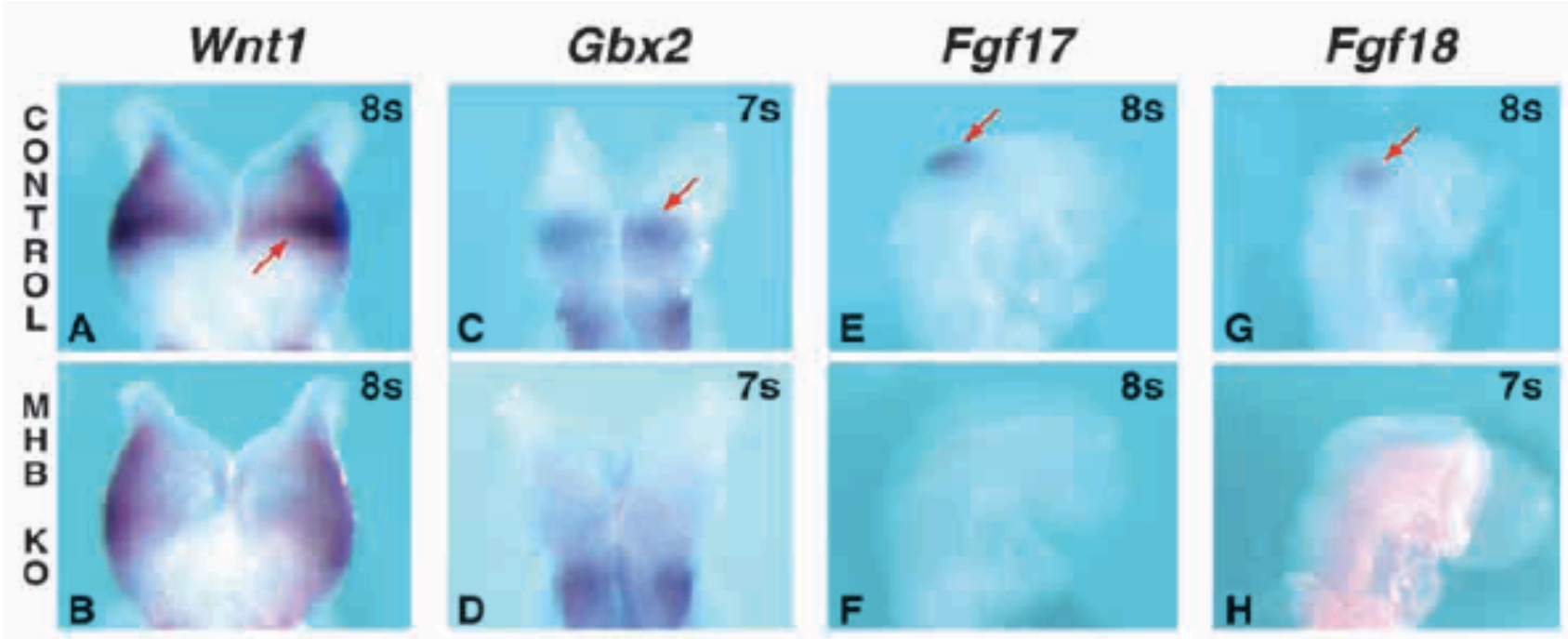


Extensive cell death in the mes/met before E10

***Fgf8* is part of a complex gene regulatory network** *essential for mes/met development*

Analysis of gene expression in *Fgf8* MHB KO mutants

→Four genes are negatively affected by the loss of *Fgf8* function

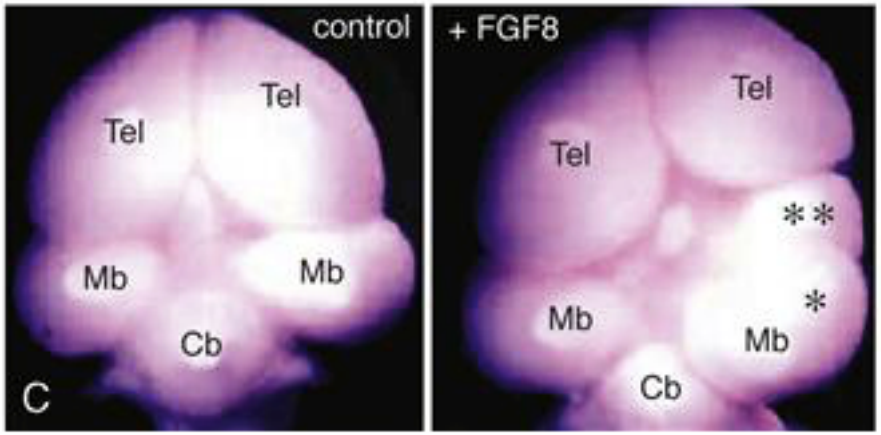


FGF8

FGF8 normally stimulates cell proliferation in the mes/met and maintains gene expression required for IsO activity

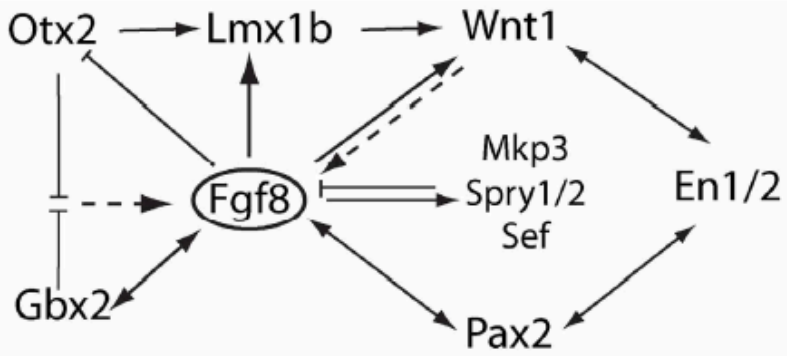
GOF

✓ Beads that are soaked with **Fgf8** and implanted into the diencephalon, mesencephalon or rhombencephalon triggered the <u>ectopic expression of mes-metencephalic markers</u>



FGF8 can mimic the effects of grafts of the mes/met boundary region in the chick, in some cases inducing diencephalic tissue to form complete ectopic midbrains as well as cerebellar tissue (these structures were polarized relative to the position of the beads)

FGF8 → mimics organizer activity

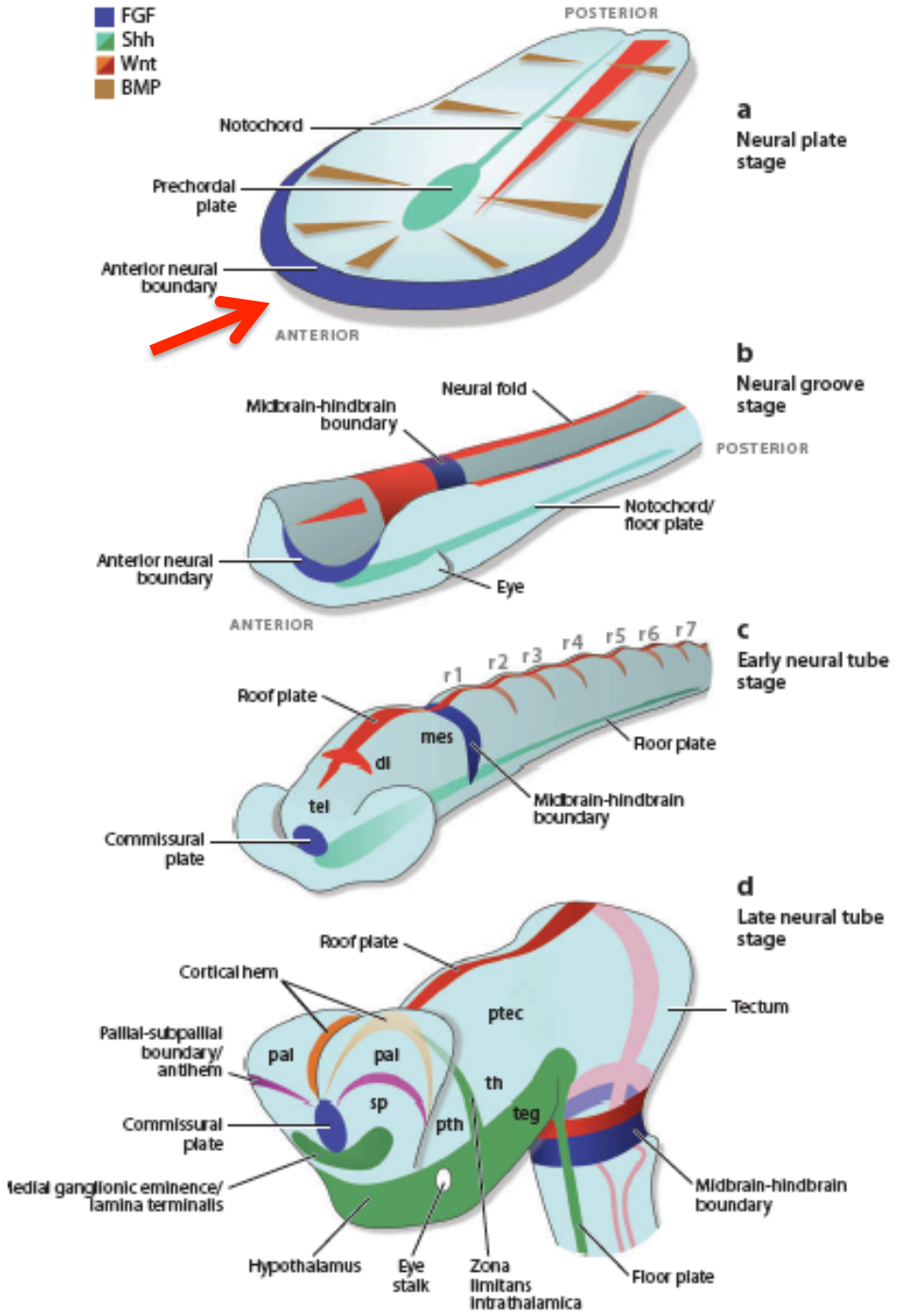


H = homebox
TF= transcription factor
M= morphogen
EP= extracellular protein
E = intracellular enzyme
inh= inhibitor of FGF8 signalling

	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												

FIGURE 4 | The upper scheme represents the functional interaction (induction/inhibition) of genes that, together with *Fgf8,* are involved in the molecular maintenance of isthmic region at E9.5. The table below summarizes the expression intensity and range of genes along the AP axis of the neural tube focusing on the isthmus. The color code depicts their mRNA expression range from the isthmus toward rostral or caudal regions.

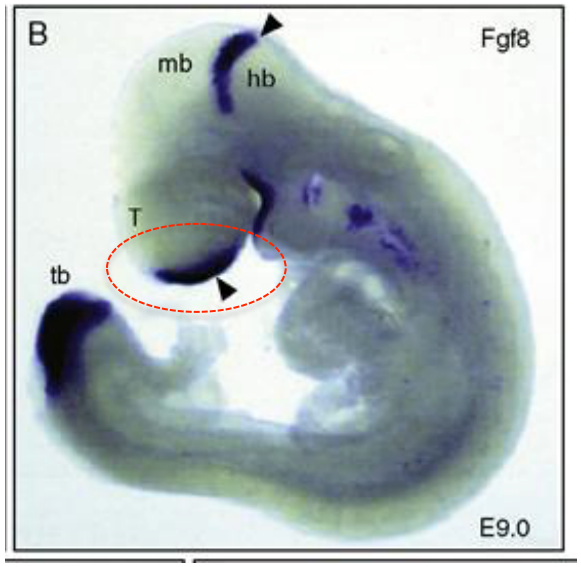
Martinez et al., Frontiers in Neuroanatomy, 2013



ANR= The Anterior Neural Boundary/Commissural Plate

Acts as organizer for the forebrain (neocortical patterning)

FGF8



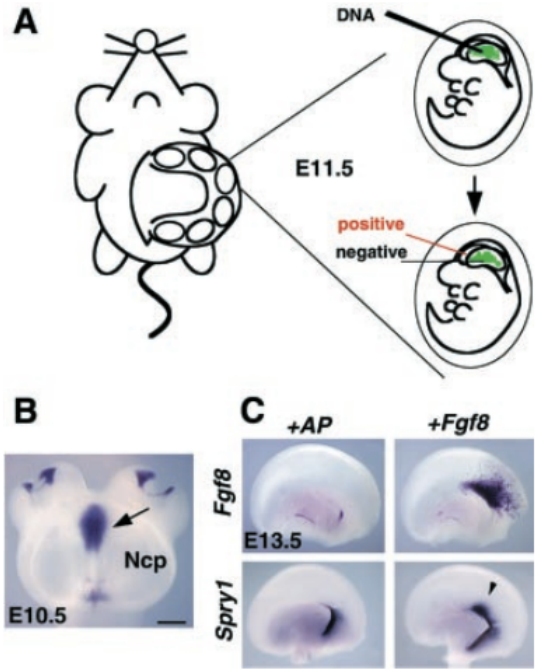
2 NOVEMBER 2001 VOL 294 SCIENCE

Neocortex Patterning by the Secreted Signaling Molecule FGF8

Tomomi Fukuchi-Shimogori and Elizabeth A. Grove*

A classic model proposes that the mammalian neocortex is divided into areas early in neurogenesis, but the molecular mechanisms that generate the area map have been elusive. Here we provide evidence that FGF8 regulates development of the map from a source in the anterior telencephalon. Using electroporation-mediated gene transfer in mouse embryos, we show that augmenting the endogenous anterior FGF8 signal shifts area boundaries posteriorly, reducing the signal shifts them anteriorly, and introducing a posterior source of FGF8 elicits partial area duplications, revealed by ectopic somatosensory barrel fields. These findings support a role for FGF signaling in specifying positional identity in the neocortex.

In utero microelectroporation



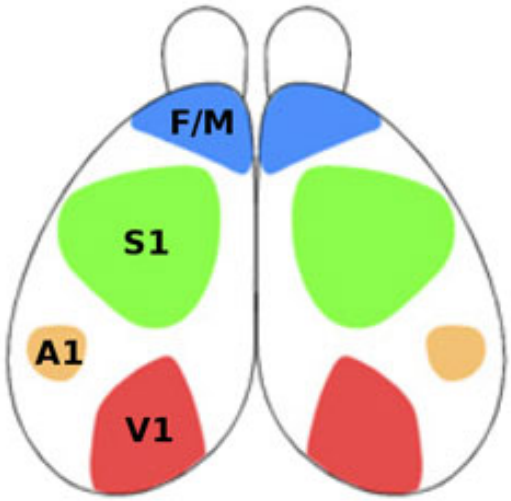
Gene misexpression strategies:

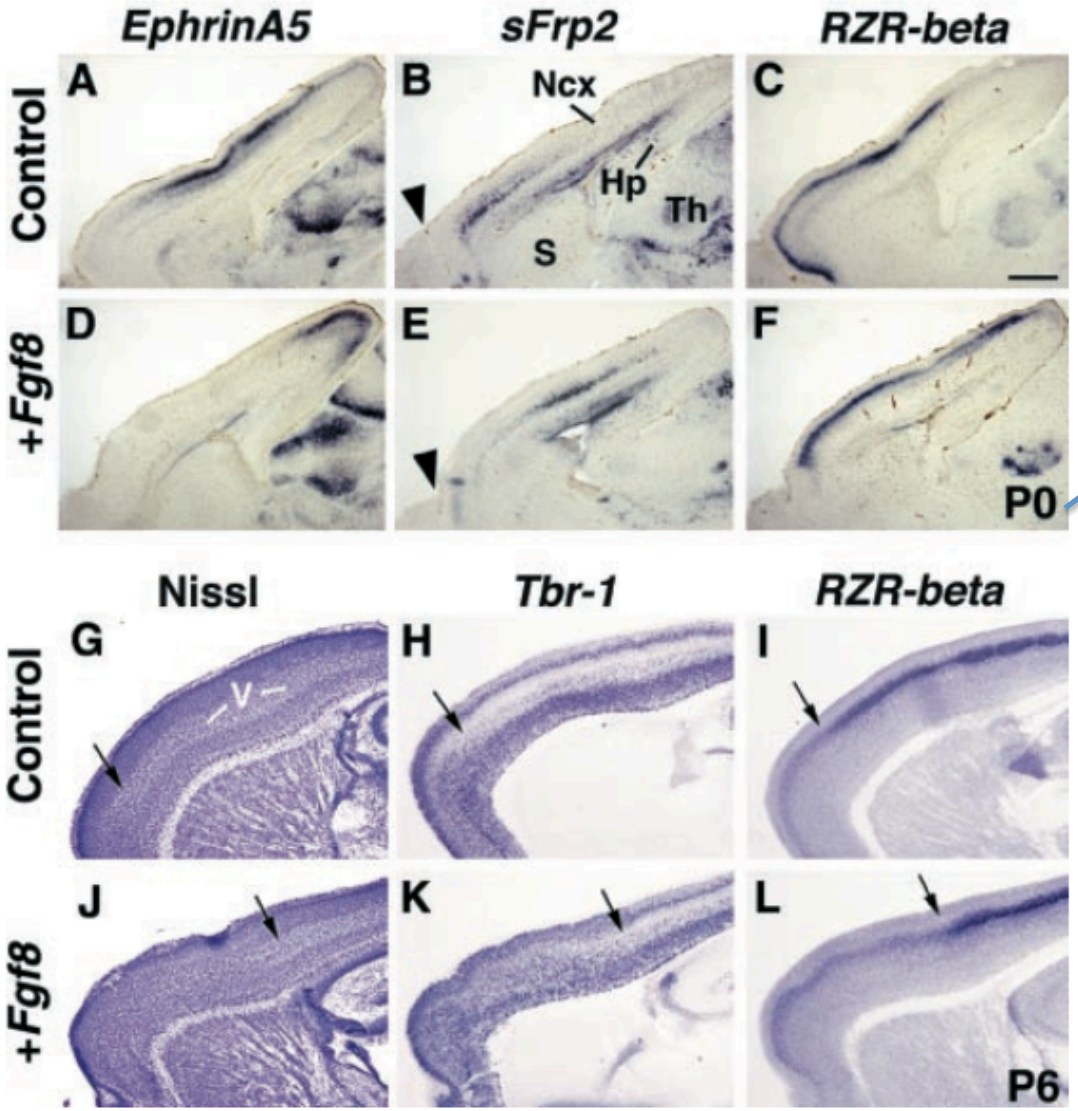
1. augmenting the anterior FGF8 source in the embryonic mouse cerebrum
2. sequestering endogenous FGF8 with a soluble FGF receptor construct
3. introducing a second, posterior source of FGF8

Video in utero electroporation

1. overexpression of FGF8 results in a posterior shift of cortical domains

Embryos were electroporated at embryonic day 11.5 (E11.5)- *early in neocortical neurogenesis, before neocortical area identity is determined* - and analyzed postnatally

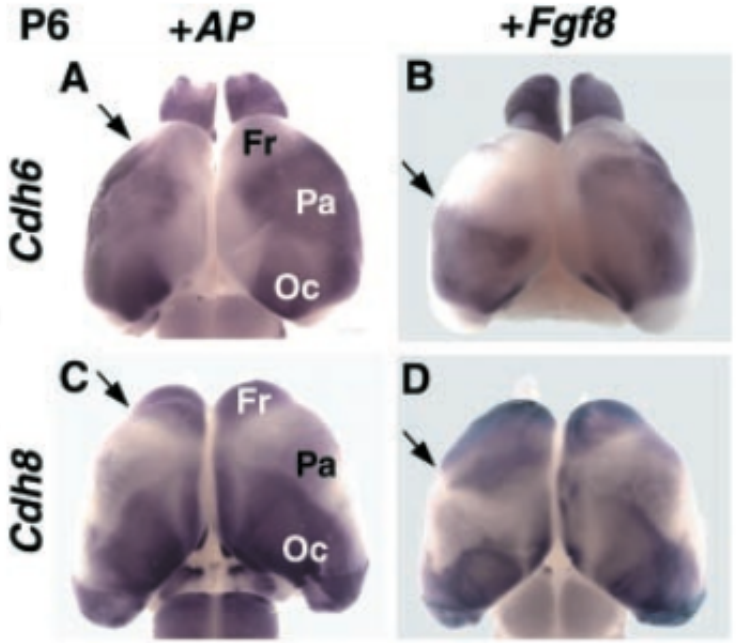




gene expression domains

Cytoarchitectonic boundaries

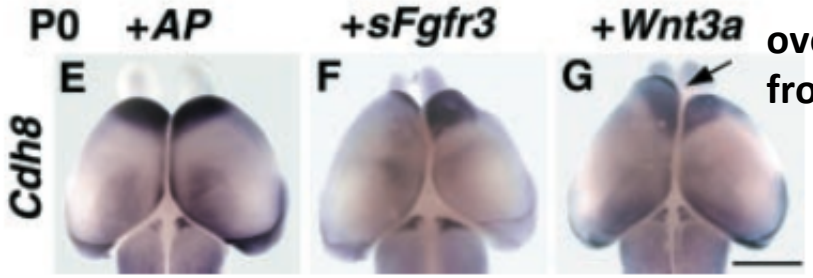
…..and expansion of anterior neocortical domain with a parallel shrinkage of posterior regions



Differential expression

area boundary shifts are not due to a simple growth effect (as in the case of Wnt3a) !

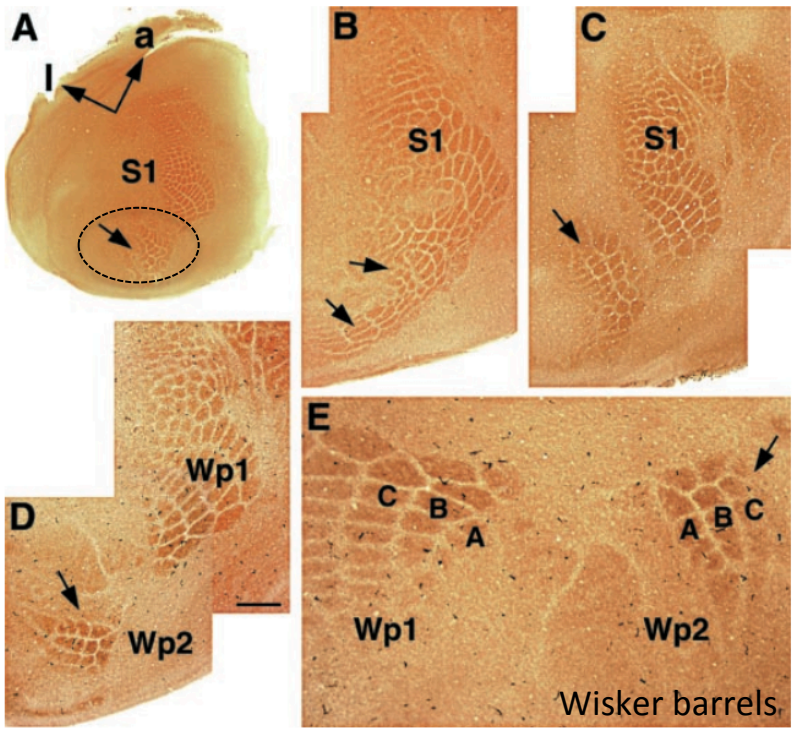
2. Reducing endogenous FGF8 signal shifts cortical area boundaries anteriorly



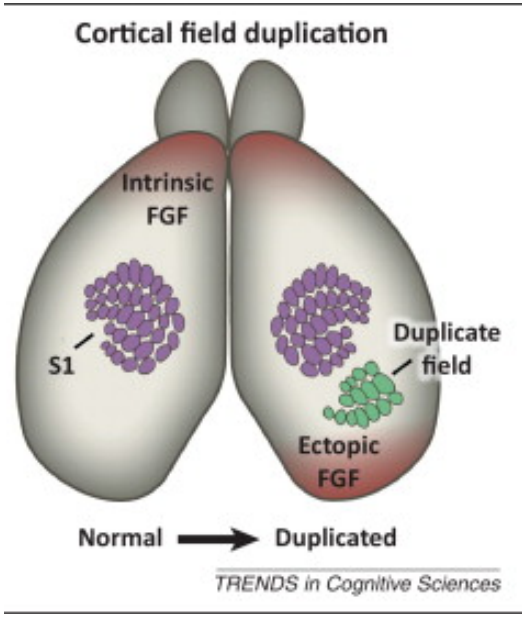
overgrowth of the frontal pole

the left frontal *Cdh8*-high domain is unchanged by *AP* (E), reduced by *sFgfr3* (F),

3. A posterior source of Fgf8 generates ectopic S1 barrels: FGF8 specifies area identity



Fukuchi-Shimogori and Grove Science 2001



FGF8 as a classic diffusible morphogen in neocortex: it *forms a diffusion gradient along the entire anterior to posterior (A/P) axis of the neocortical primordium, and acts directly to impart positional identity, both close to the FGF8 source, and at a distance. (Toyoda et al., Development 2010)*

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Molecular regionalization of the neocortex is disrupted in *Fgf8* hypomorphic mutants

Sonia Garel, Kelly J. Huffman and John L. R. Rubenstein*

Nina Ireland Laboratory of Developmental Neurobiology, Department of Psychiatry, University of California, San Francisco, CA 94143-0984, USA

*Author for correspondence (e-mail: jlrr@cgl.ucsf.edu)

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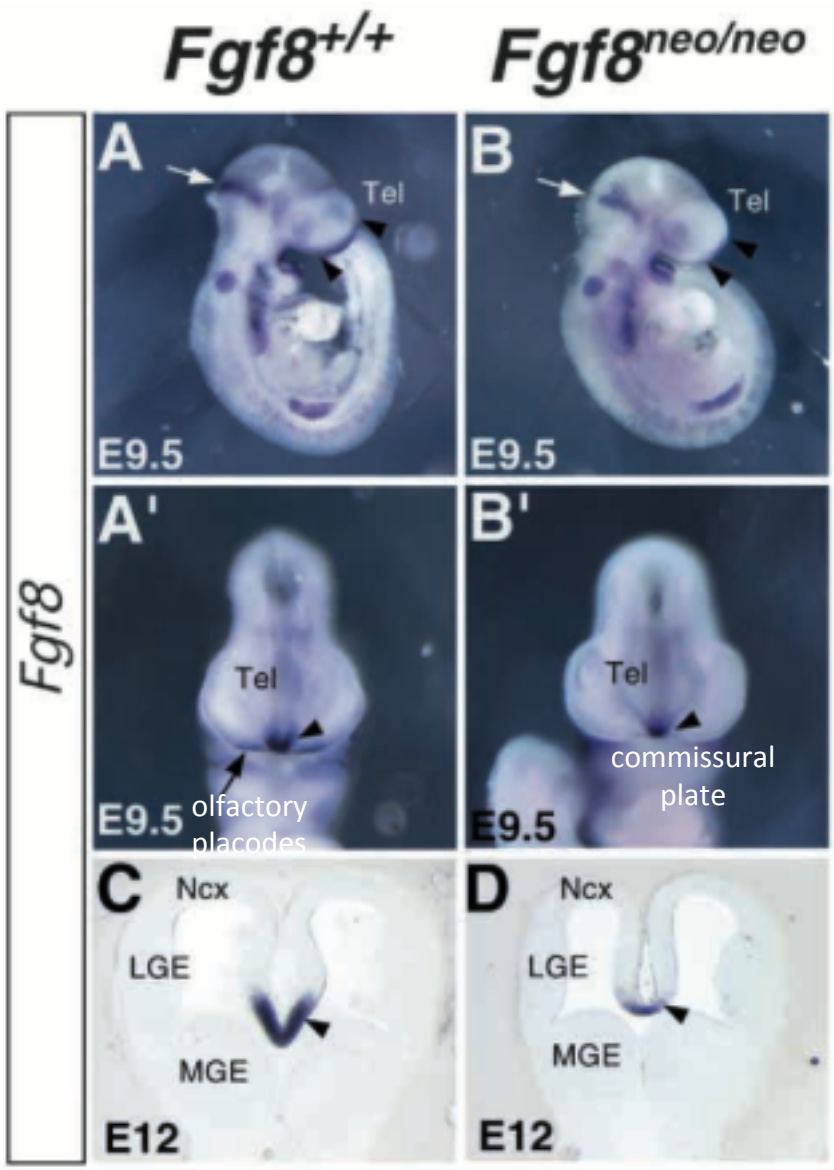
SUMMARY

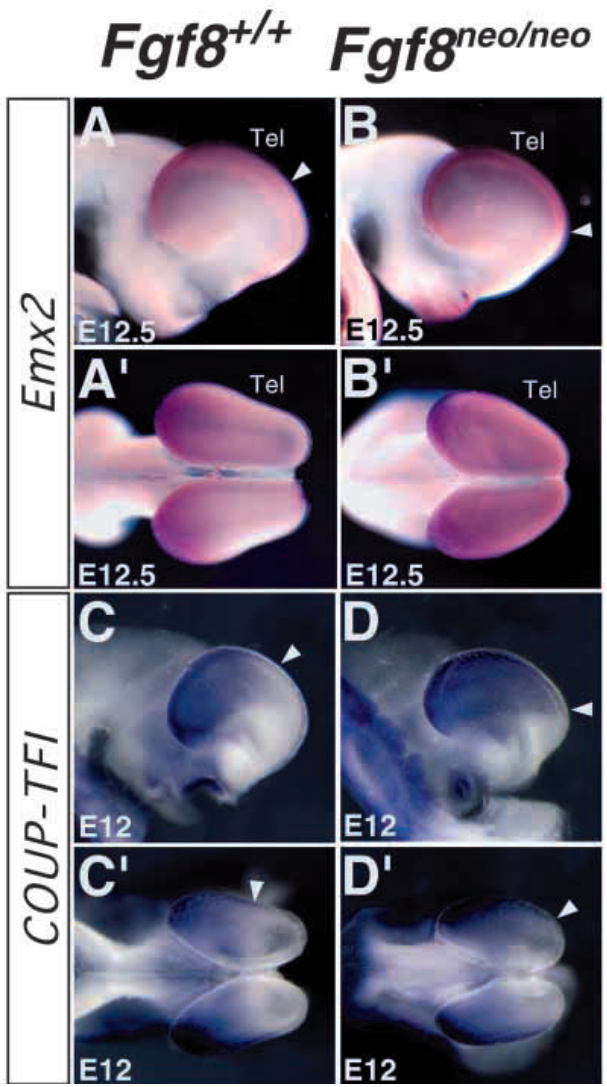
The neocortex is divided into multiple areas with specific architecture, molecular identity and pattern of connectivity with the dorsal thalamus. Gradients of transcription factor expression in the cortical primordium regulate molecular regionalization and potentially the patterning of thalamic projections. We show that reduction of *Fgf8* levels in hypomorphic mouse mutants shifts early gradients of gene expression rostrally, thereby modifying the molecular identity of rostral cortical progenitors. This shift correlates with a reduction in the size of a molecularly defined rostral neocortical domain and a corresponding rostral expansion of more caudal regions. Despite these molecular changes, the topography of projections between the dorsal thalamus and rostral neocortex in mutant neonates appears the same as the topography of wild-type littermates. Overall, our study demonstrates the role of endogenous *Fgf8* in regulating early gradients of transcription factors in cortical progenitor cells and in molecular regionalization of the cortical plate

Key words: *Fgf8*, Neocortex, Regionalization, Topography, Thalamocortical axons

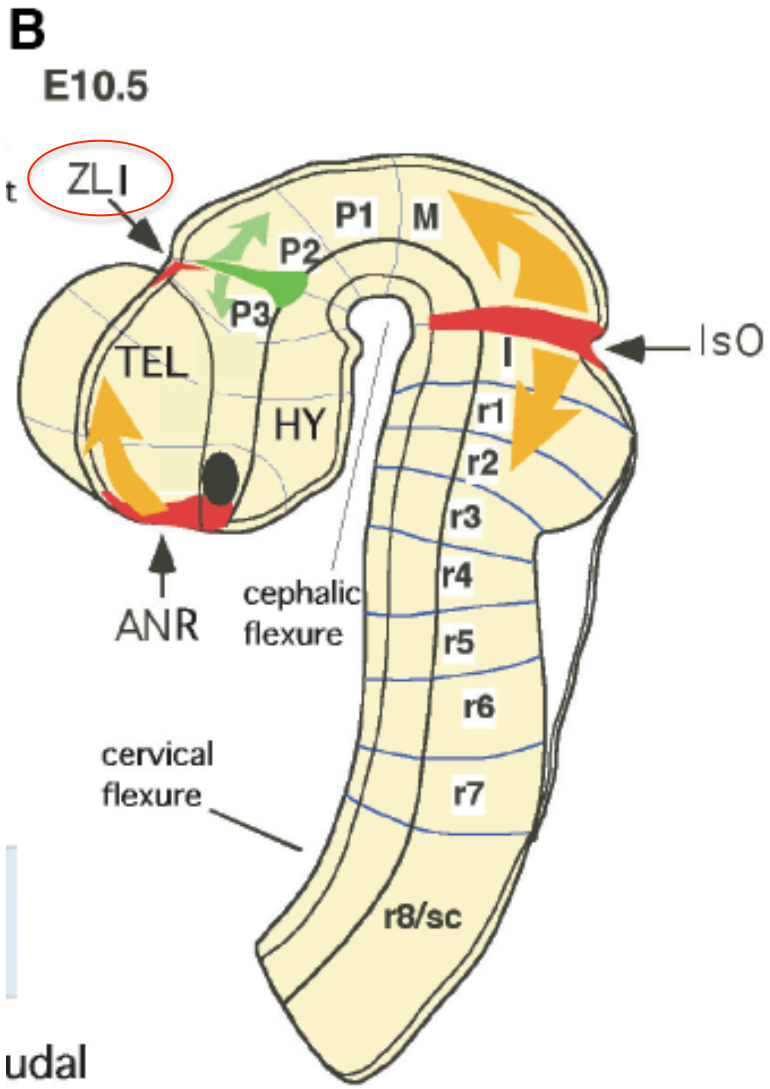
Levels of Fgf8 expression are reduced in Fgf8neo/neo hypomorph embryos



Caudal to rostral gradients of expression are shifted rostrally in the dorsal telencephalon of E12/E13 *Fgf8neo/neo* embryos



ZLI= Zona limitans intrathalamica

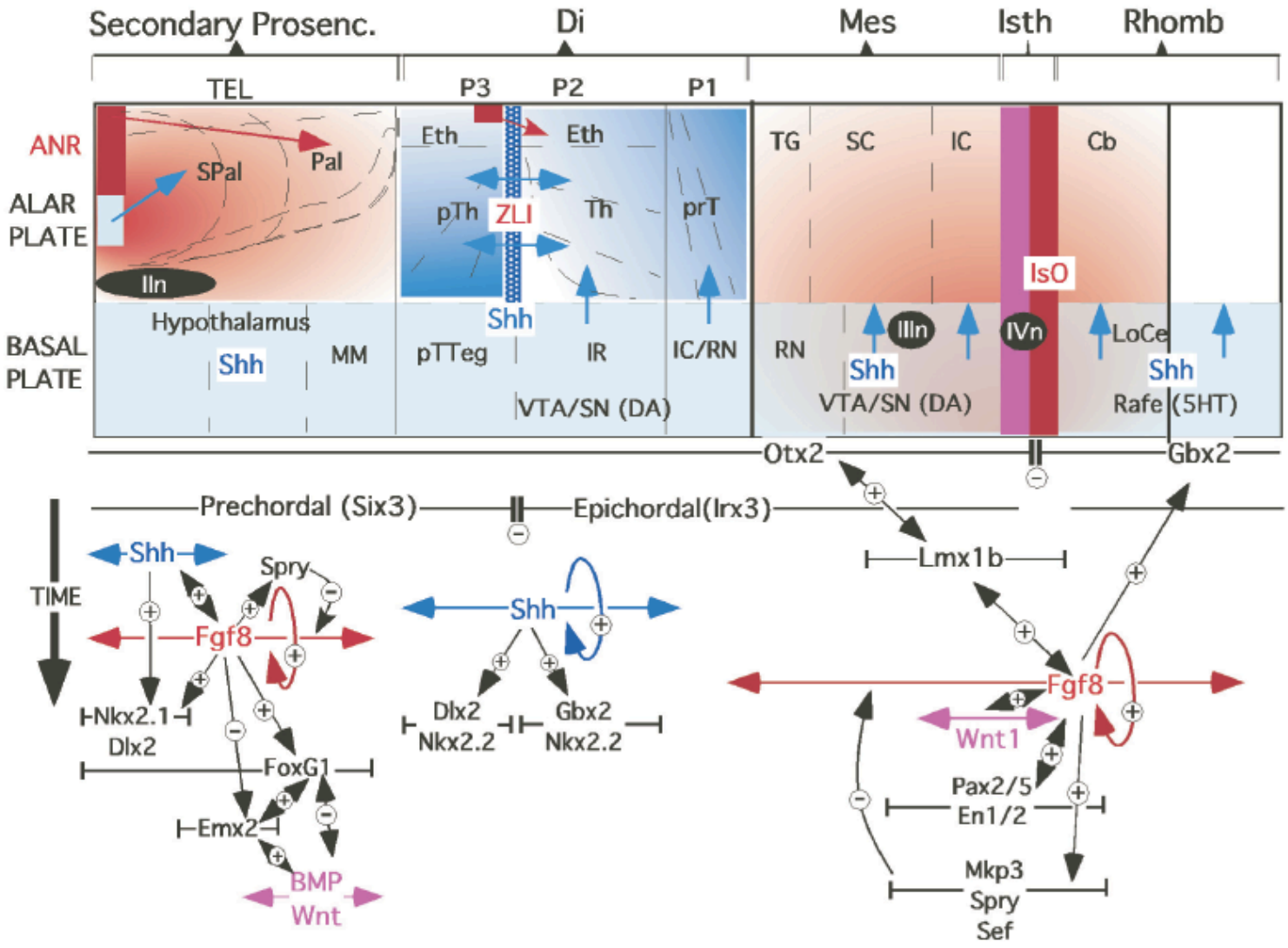


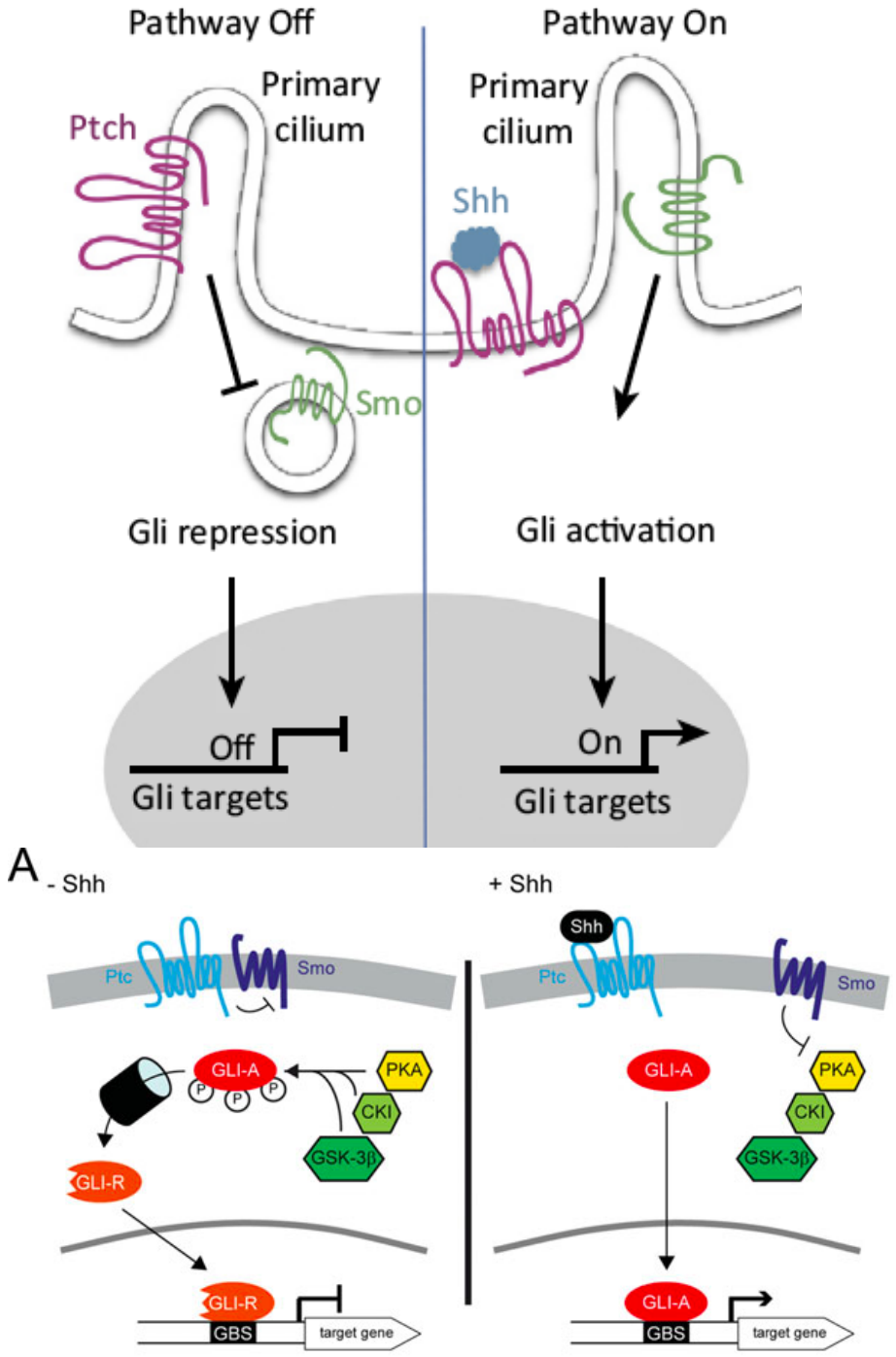
The ZLI is a narrow transverse region between prosomer 2 and 3

ZLI secretes signaling molecules that generate the patterning of the diencephalon

ZLI= Zona limitans intrathalamica

The ZLI is the only structure in the alar plate that expresses signal molecules of the Hedgehog family (Hh)



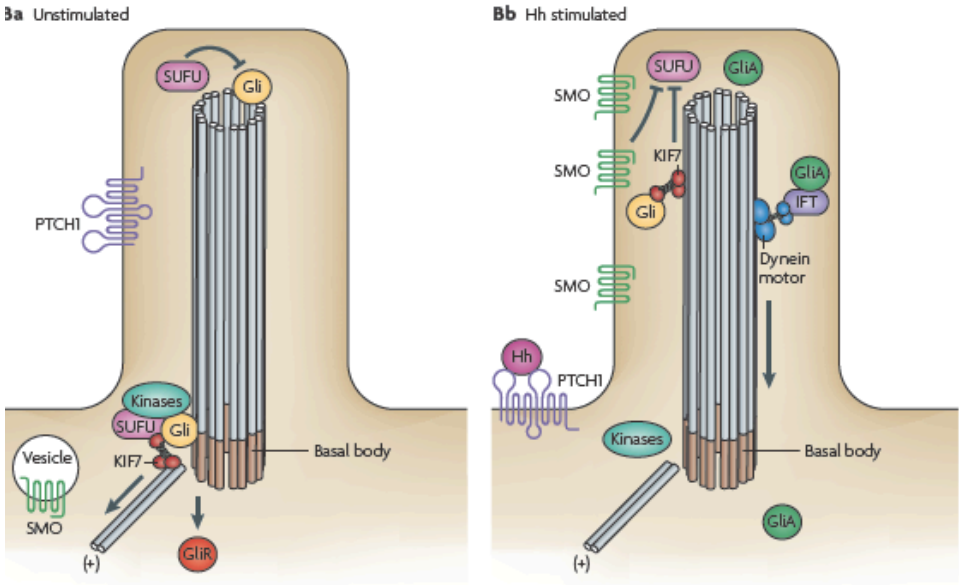


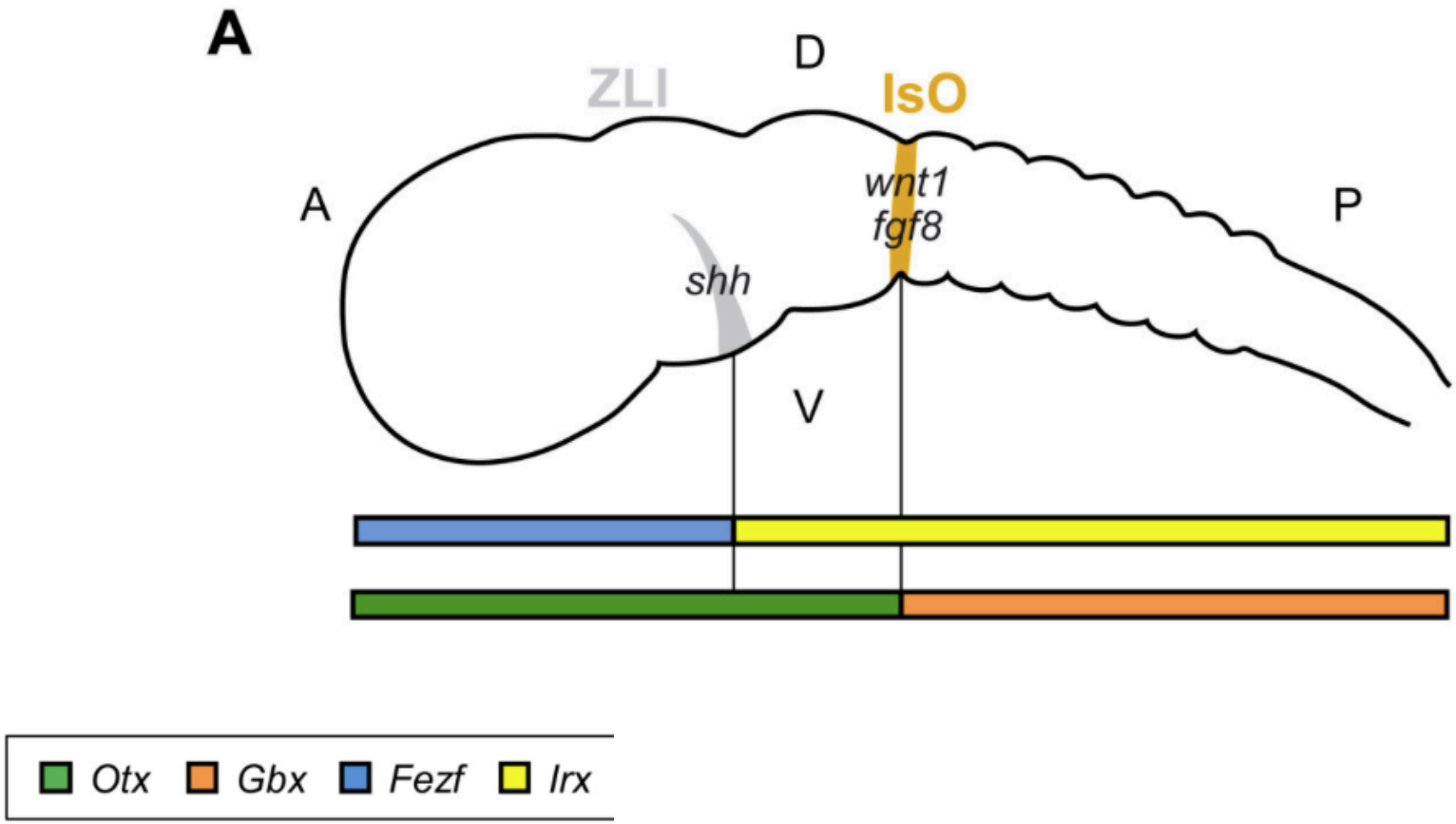
In the absence of Shh:

- the membrane G-protein-coupled receptor (GPCR)-like protein Smoothened (Smo) is tonically inhibited by Patched (Ptch)
- Gli3 is activated
- target gene transcription repressed

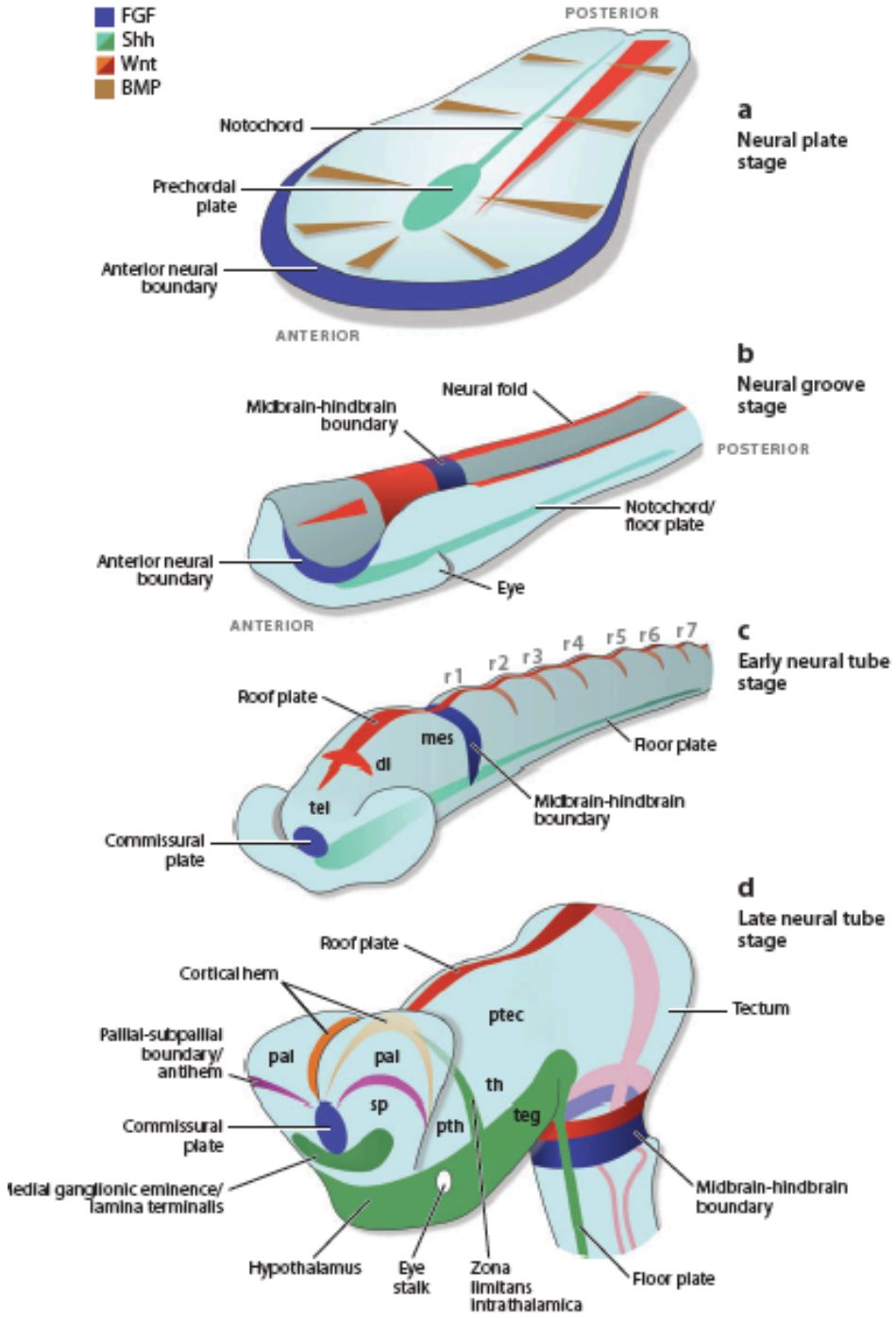
Binding of Shh to Ptch:

- Activation of smo
- Gli1-2 activated
- Target gene transcription activated



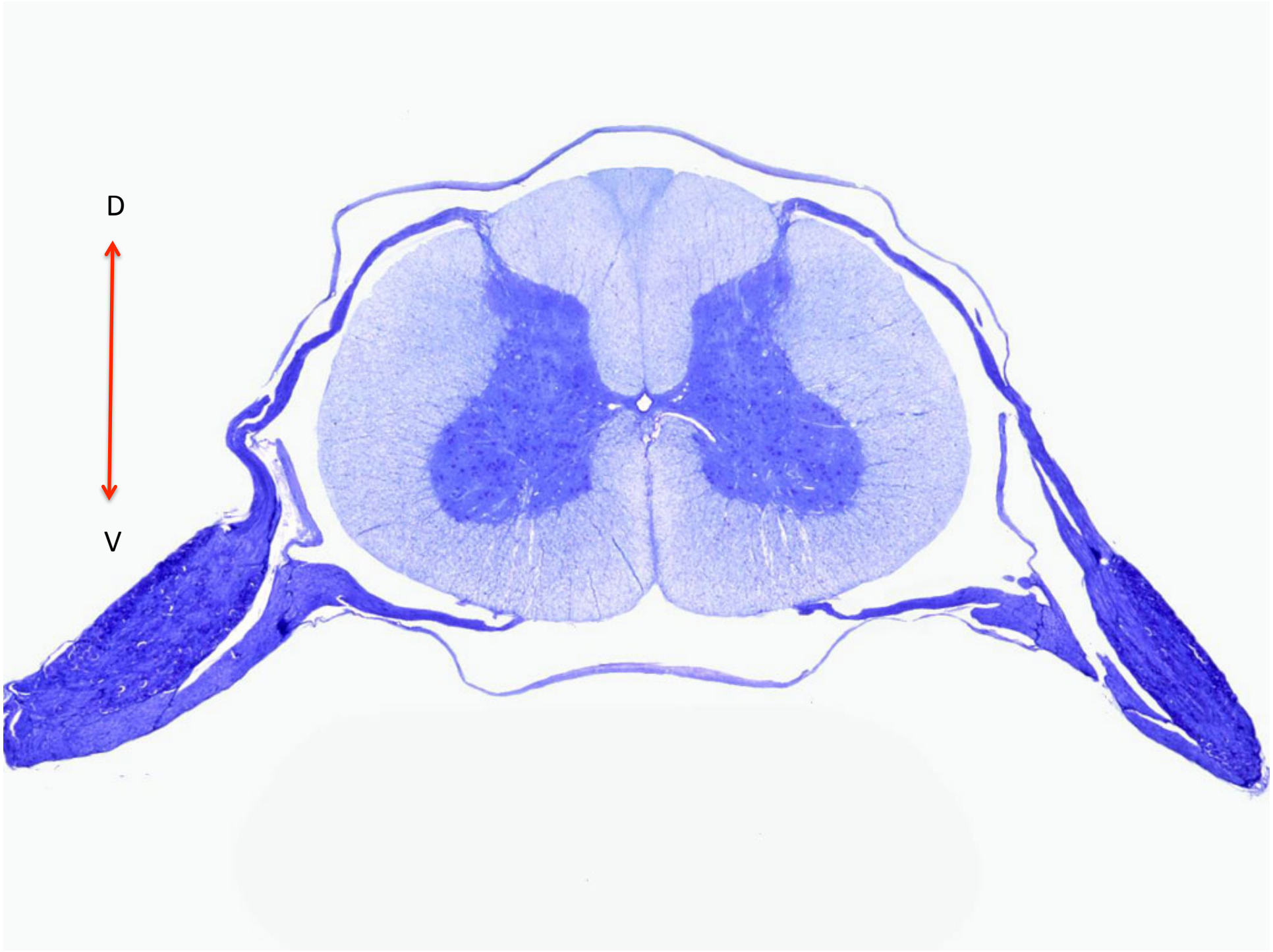


Schematic view of a generalized vertebrate CNS. **(A)** Topological location of the ZLI and IsO organizers and the key genes determining their position in a schematic view of a generalized developing vertebrate central nervous system. The most anterior of the internal brain secondary organizers, the ZLI, develops within the diencephalon, at the interface of the expression of Fezf (blue) and Irx (yellow). The IsO organizer will be located at the midbrain hindbrain boundary (MHB), delimited by the expression of members of the Otx (green) and Gbx (orange) gene families.



- ✓ A limited set of signaling factor families acts in neural patterning

- ✓ Competence of target cells plays an important role in the specificity of neural patterning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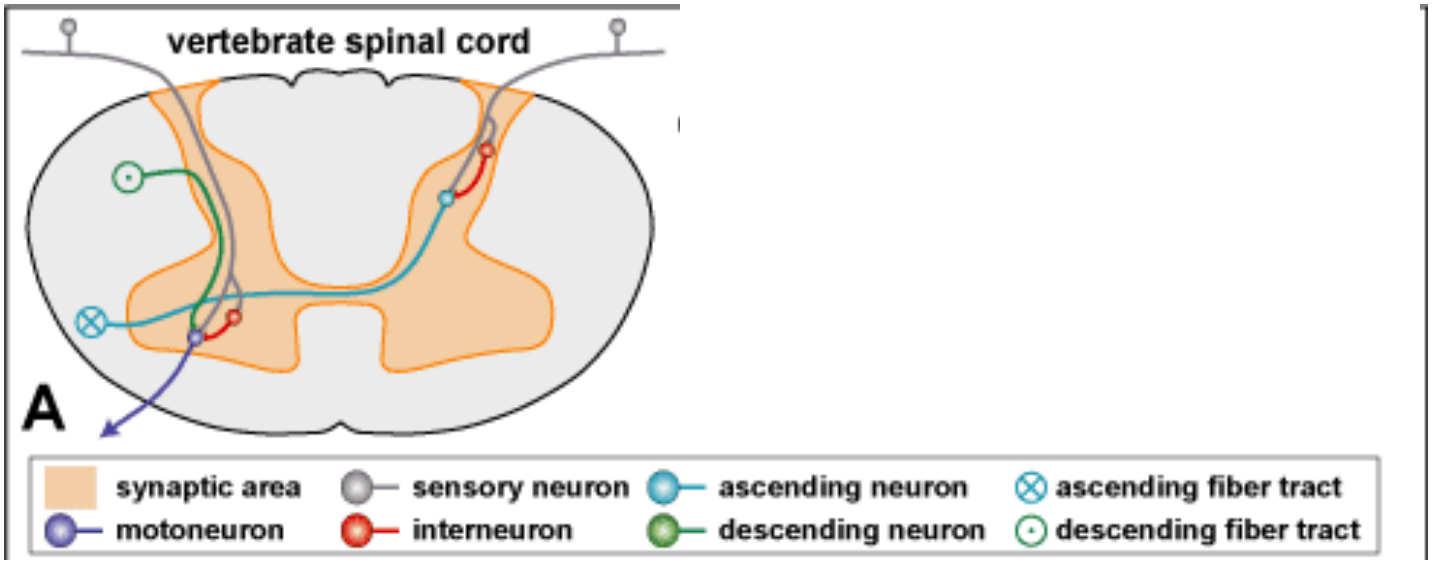
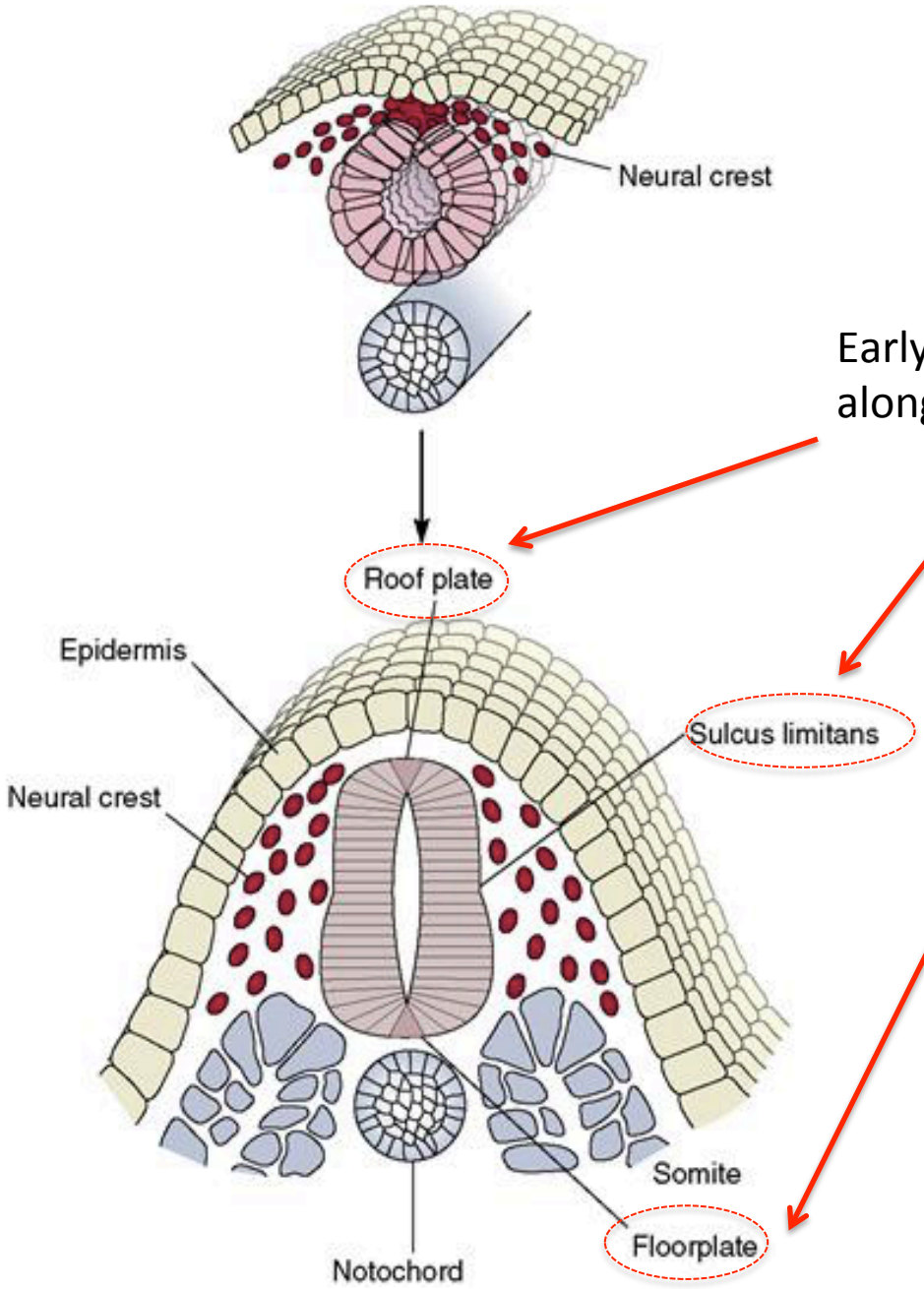


Different neuronal types are located at highly specific position

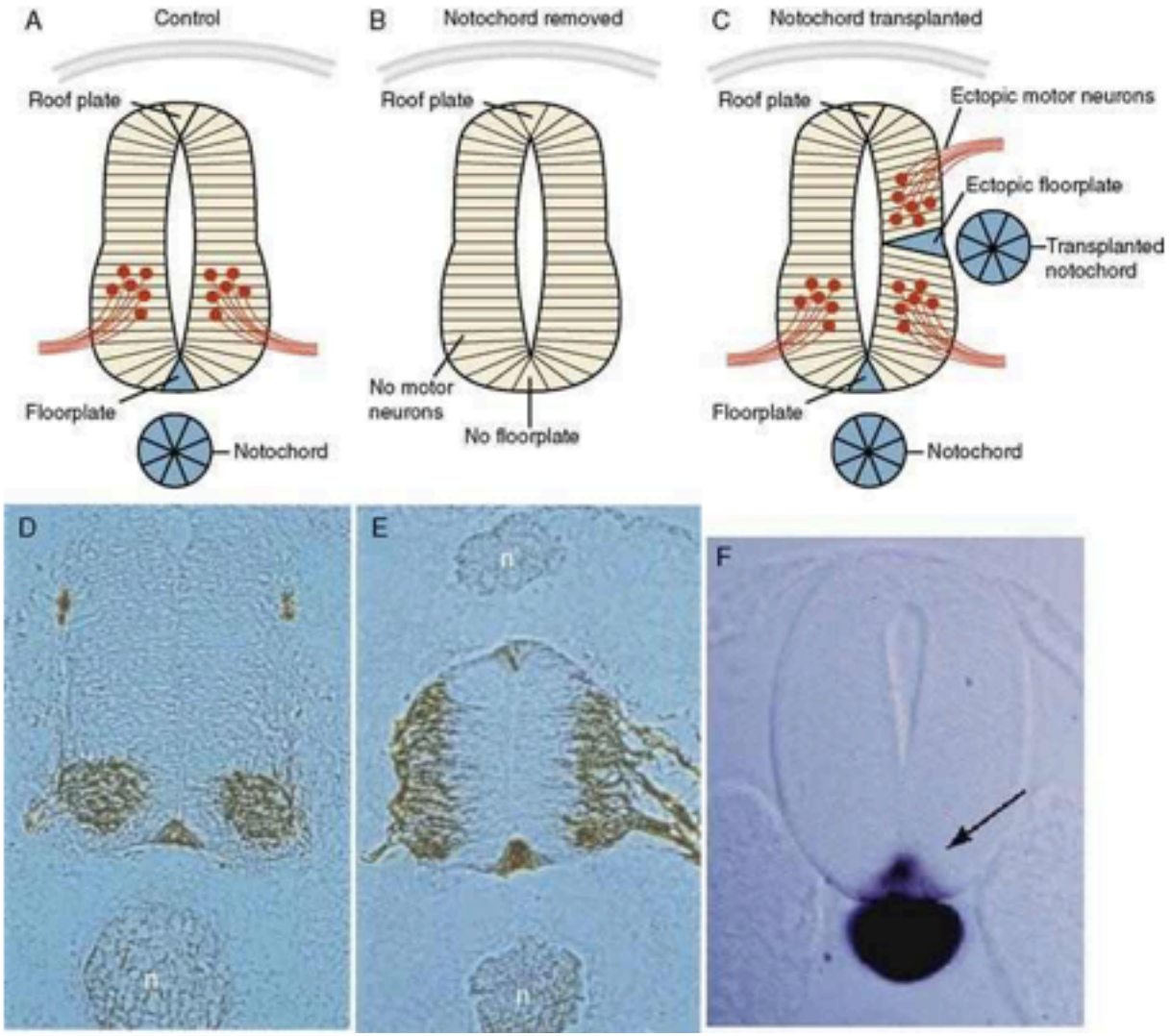
How does dorsal-ventral difference in the spinal cord organization emerge during development?

Dorsal –ventral polarity in the neural tube



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

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



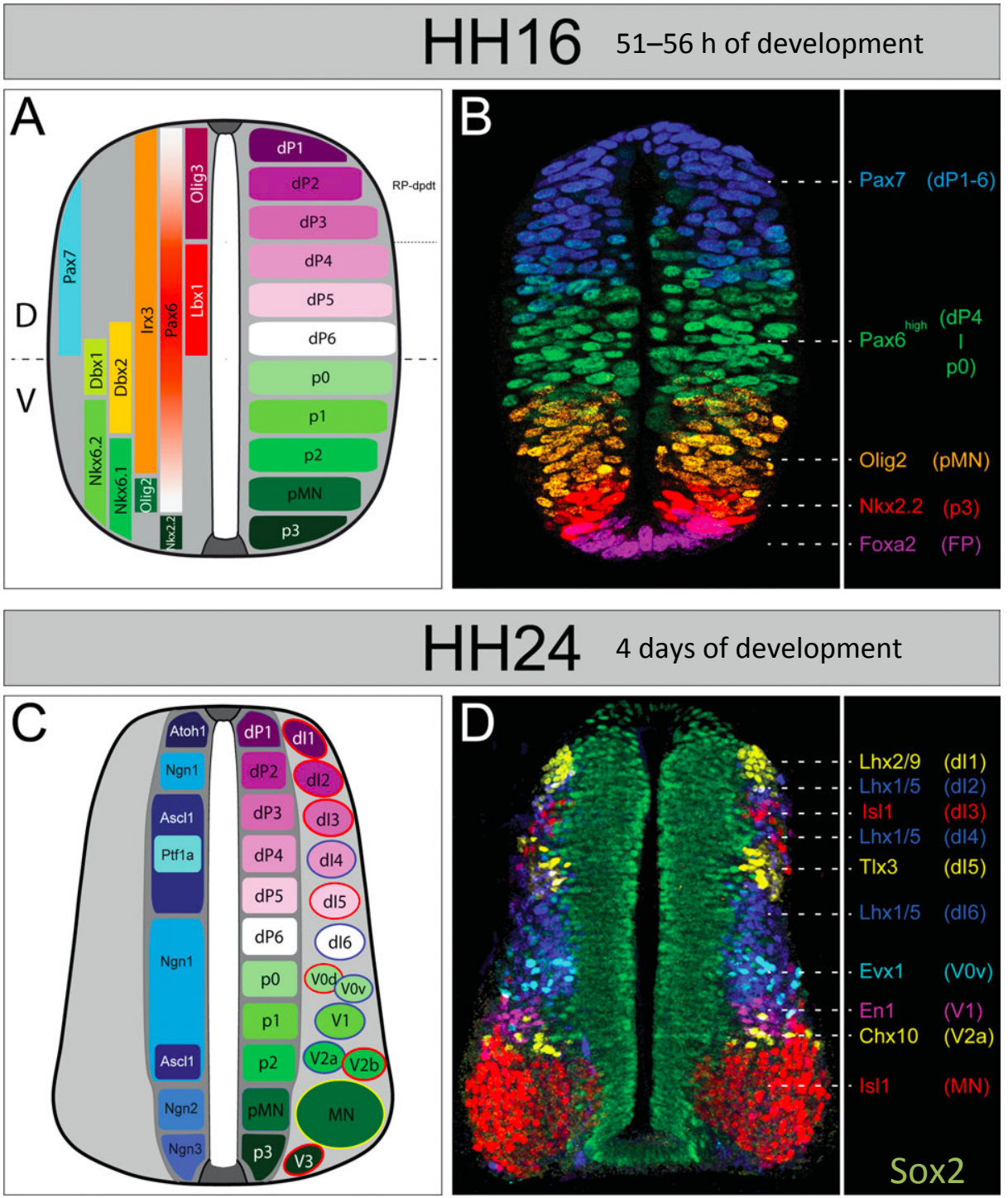
Dorsal–ventral patterning of the vertebrate developing spinal cord

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

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

The pattern of gene expression determine the type of progenitor

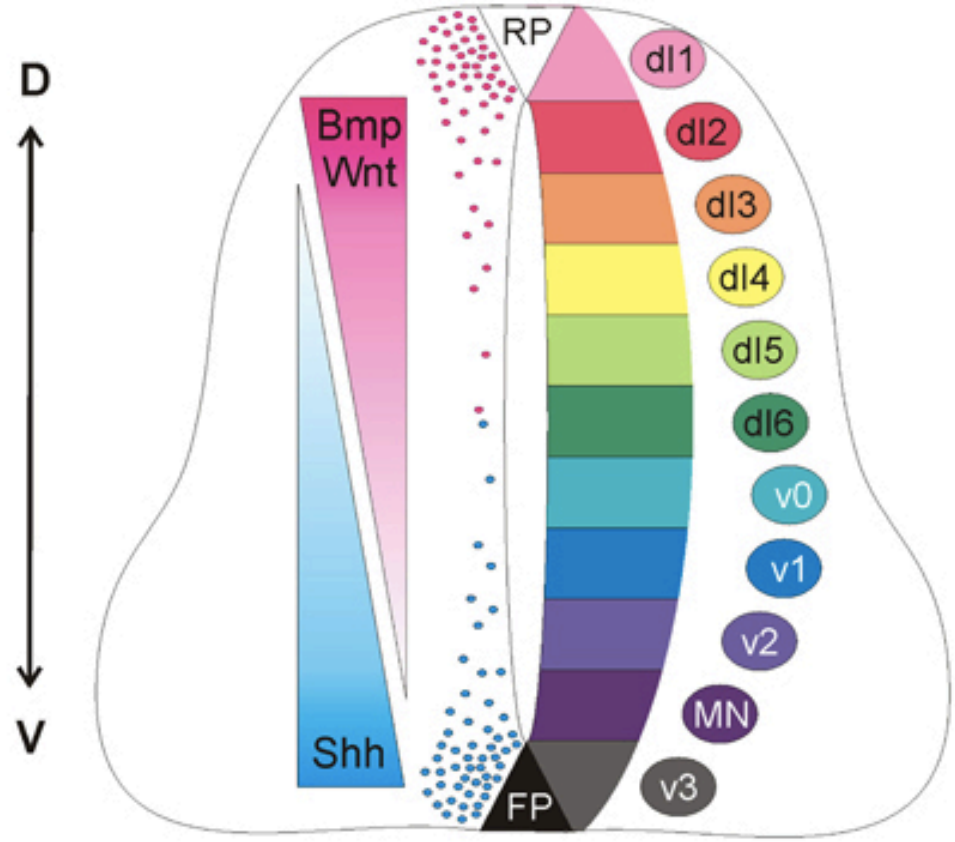
Le Dréau *Dev Neurobiol 2012*



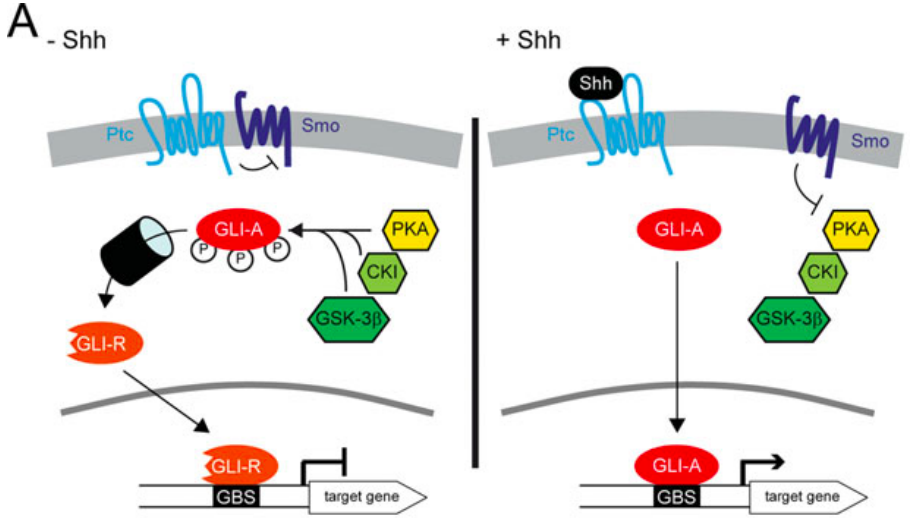
The dorso-ventral patterning

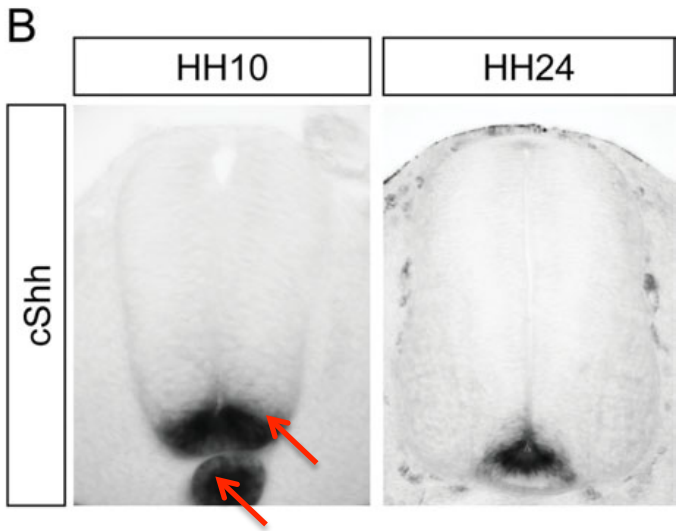
2 opposite signaling centers:

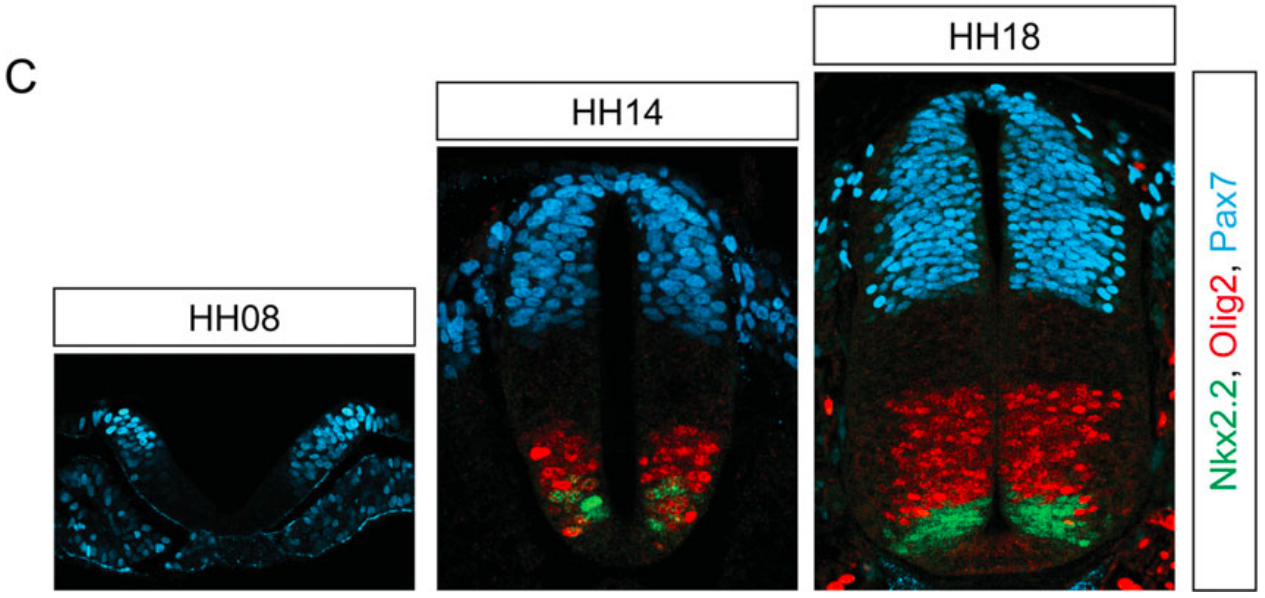
- the roof plate
- the ventral floor plate



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

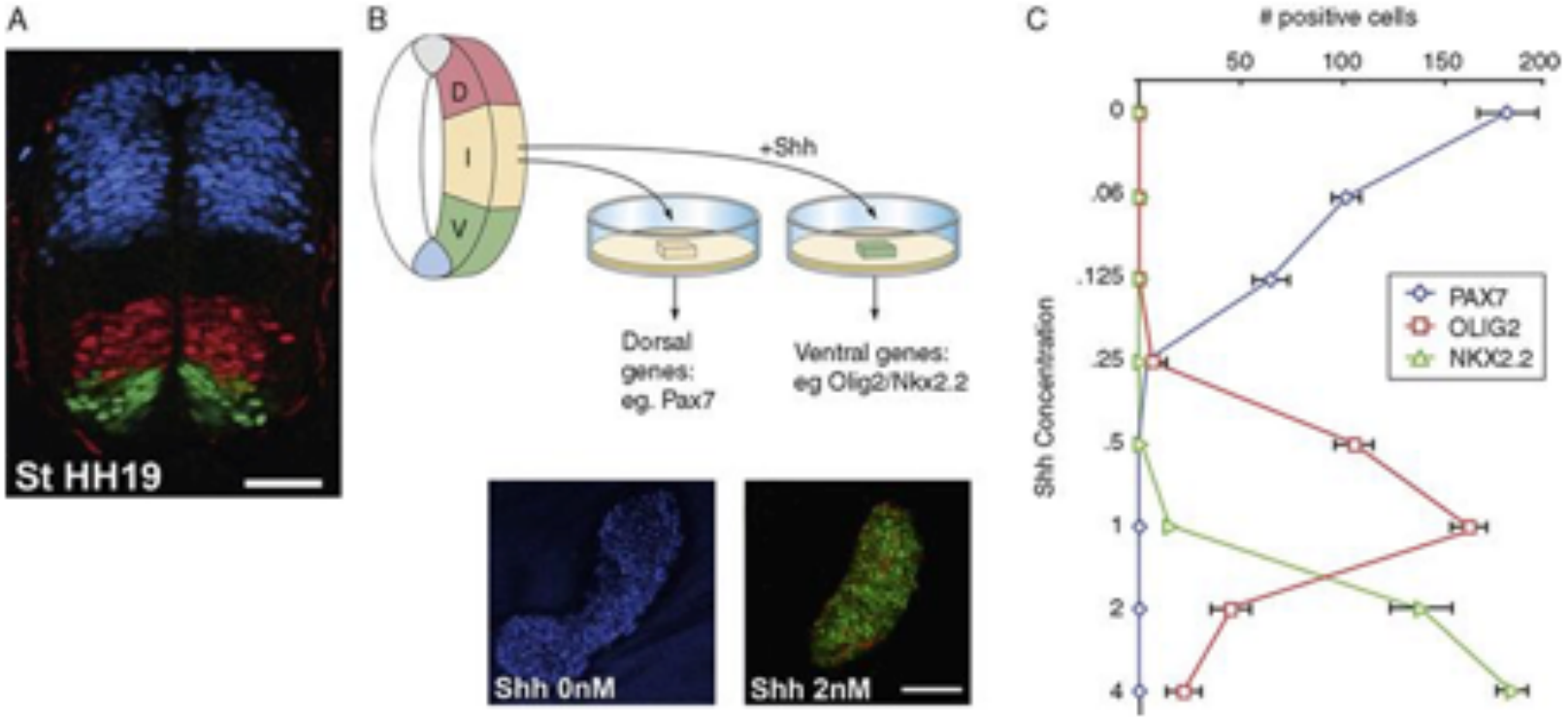






Progressive emergence of ventral neural progenitor domains

In vitro experiments:



Shh is sufficient to ventralize the neural tube during development

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

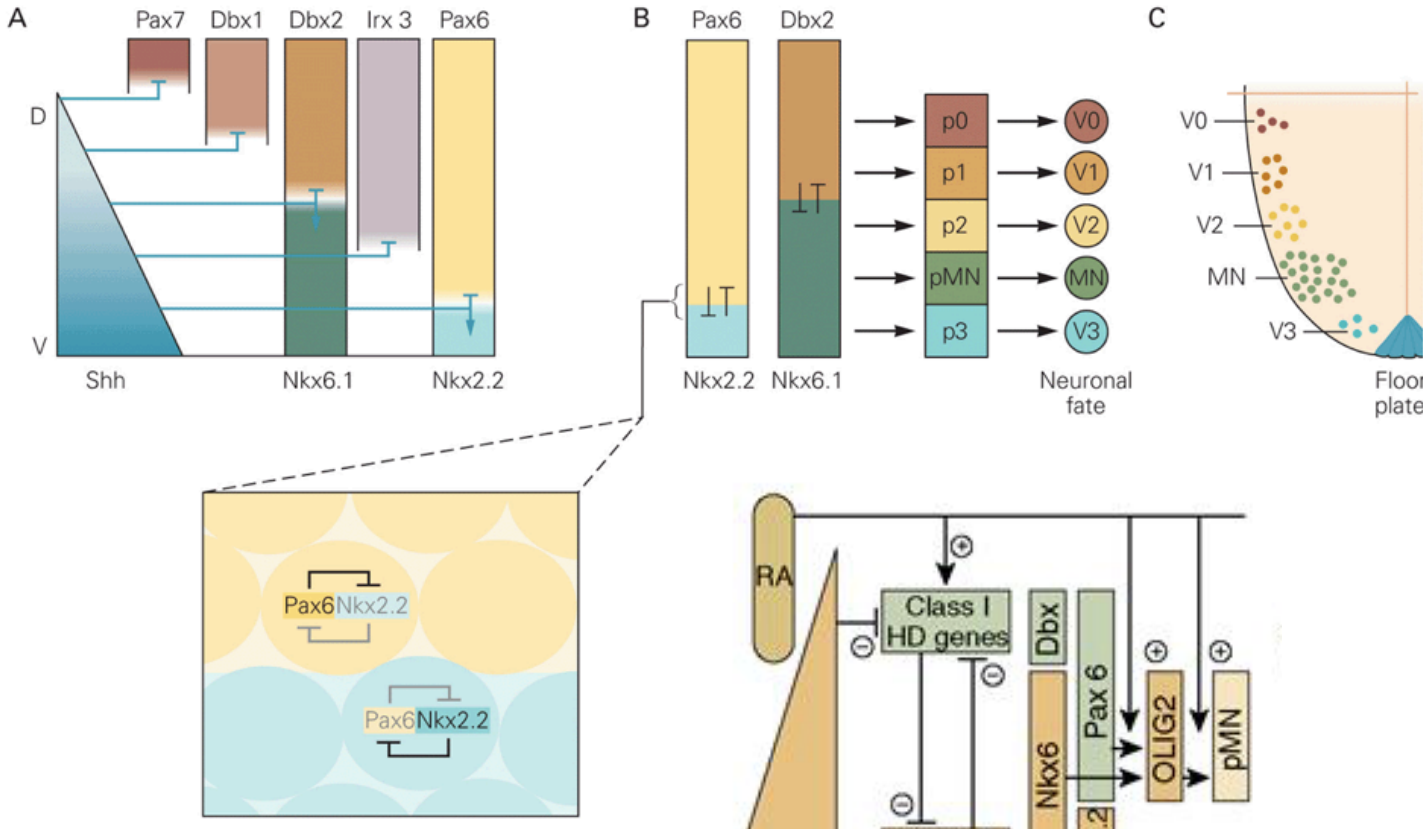


Figure 52-9 A sonic hedgehog signaling gradient controls nei

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

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

Shh is released by Notochord and floor plate

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

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

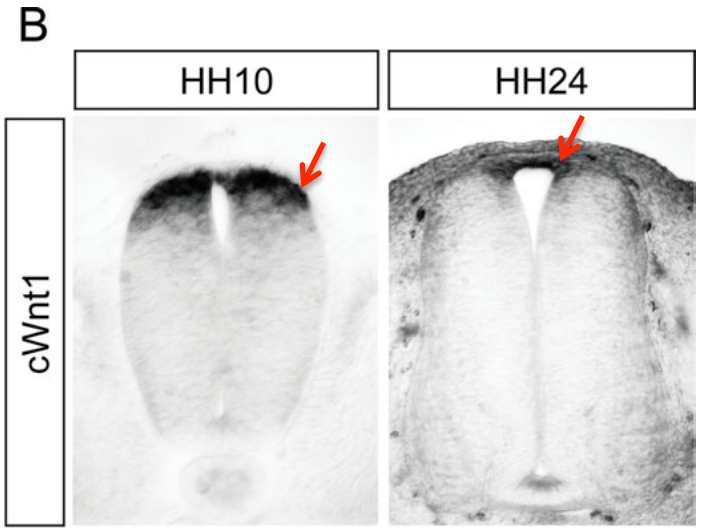
Activation of the Shh pathway is transduced into **regulated levels and duration of Gli activity**

→Increased and sustained concentrations of Shh →Longer maintenances of the Gli intracellular signaling activity→Progenitor cells adopt ventral identities

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

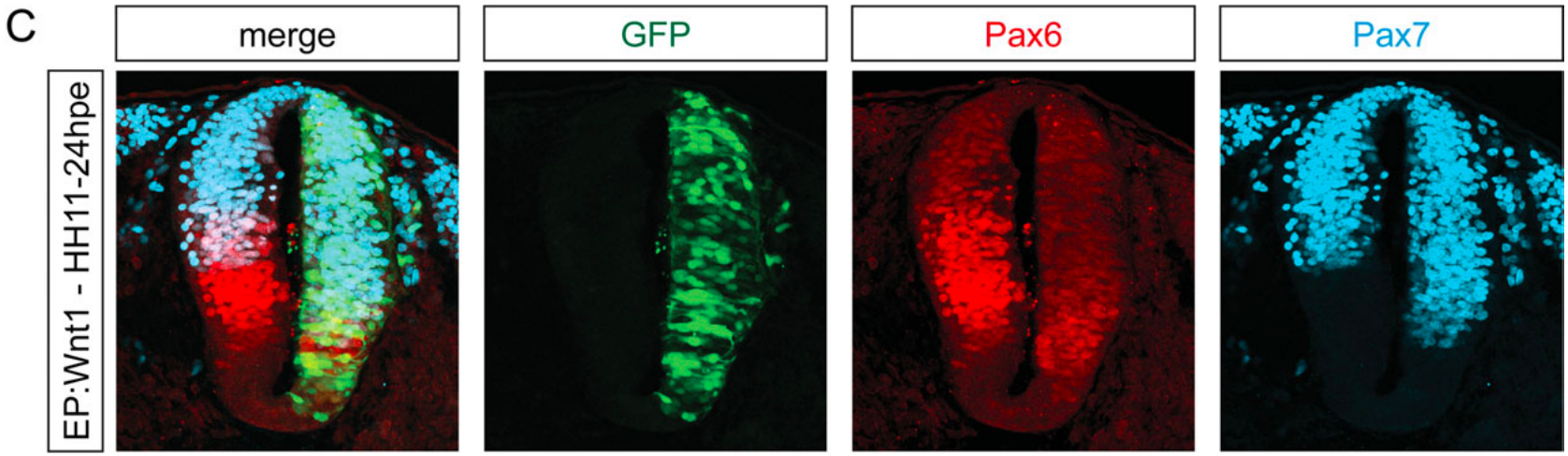
The role of the **Roof plate** in patterning the dorsal neural tube:

I. The effect of Wnt



1. Several members of the Wnt family (including Wnt1 and Wnt3a) are expressed by the RP

2. Activation of Wnt pathway promote dorsal progenitor identities



Experimental approach: In ovo electroporation of a bi-cistronic plasmid encoding Wnt1 and GFP (green) at early neural developmental stages (HH11, 40–45 h of development, 13 somites)

Results:

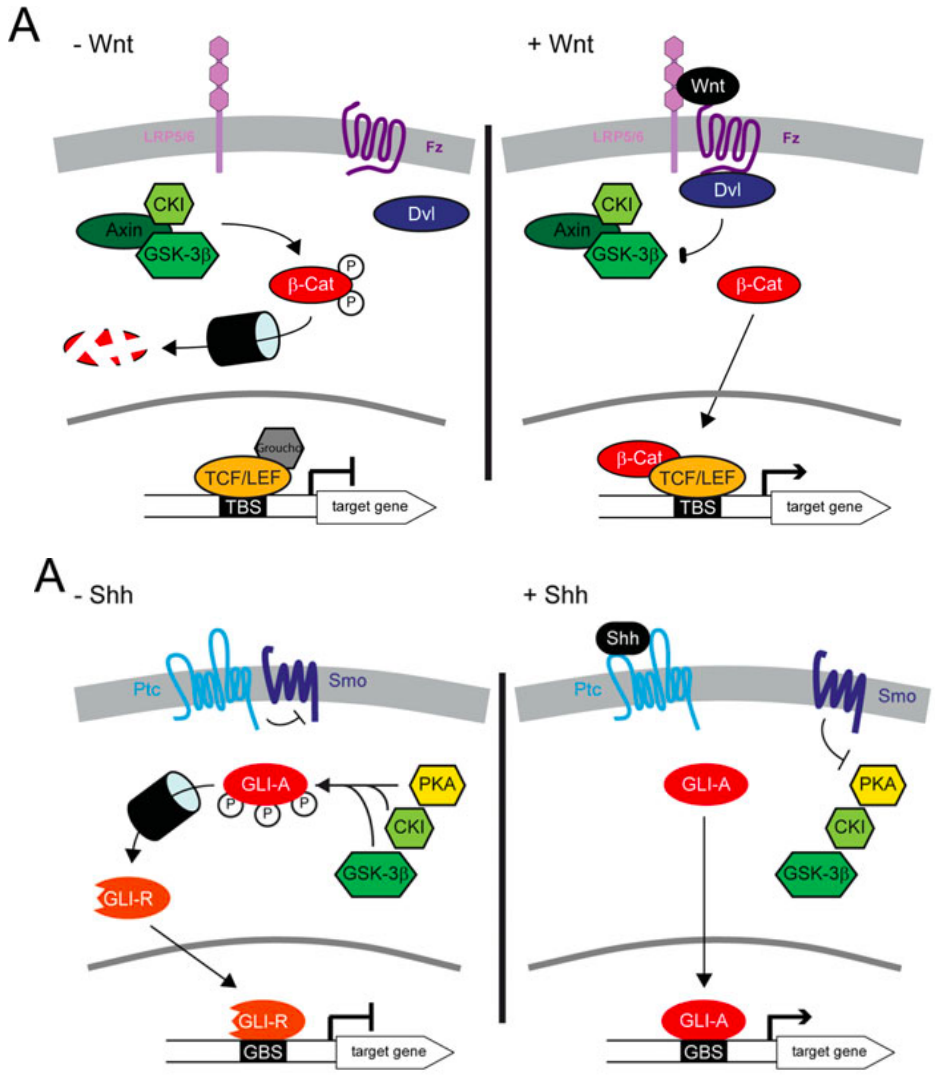
24 h post-electroporation:

- ventral expansion of the expression territory of the dorsal progenitor marker Pax7
- dorsal-to-ventral shift of the expression of the intermediate progenitor marker Pax6

→ overexpression of a stabilized form of b-catenin in the **chick** results in prominent changes in progenitor gene expression along the DV axis - increased generation of dorsal neuronal subtypes with the concomitant loss of ventral motor neurons (Alvarez-Medina et al., 2008)

-->similar data in mouse

Wnt signaling antagonizes Shh activity



Gli proteins are mediators of Hh signaling

Gli3

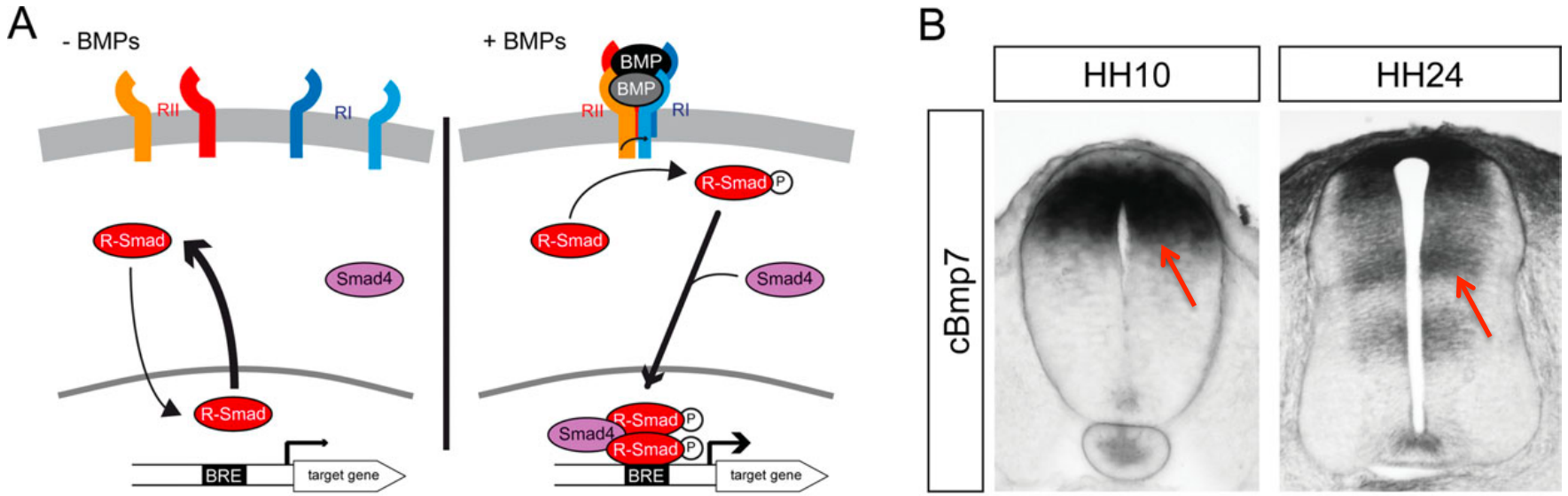
- is expressed in the dorsal spinal cord
- **is a direct target of Wnt/beta catenin**

Truncated Gli3 protein represents the main repressor of Shh signaling

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

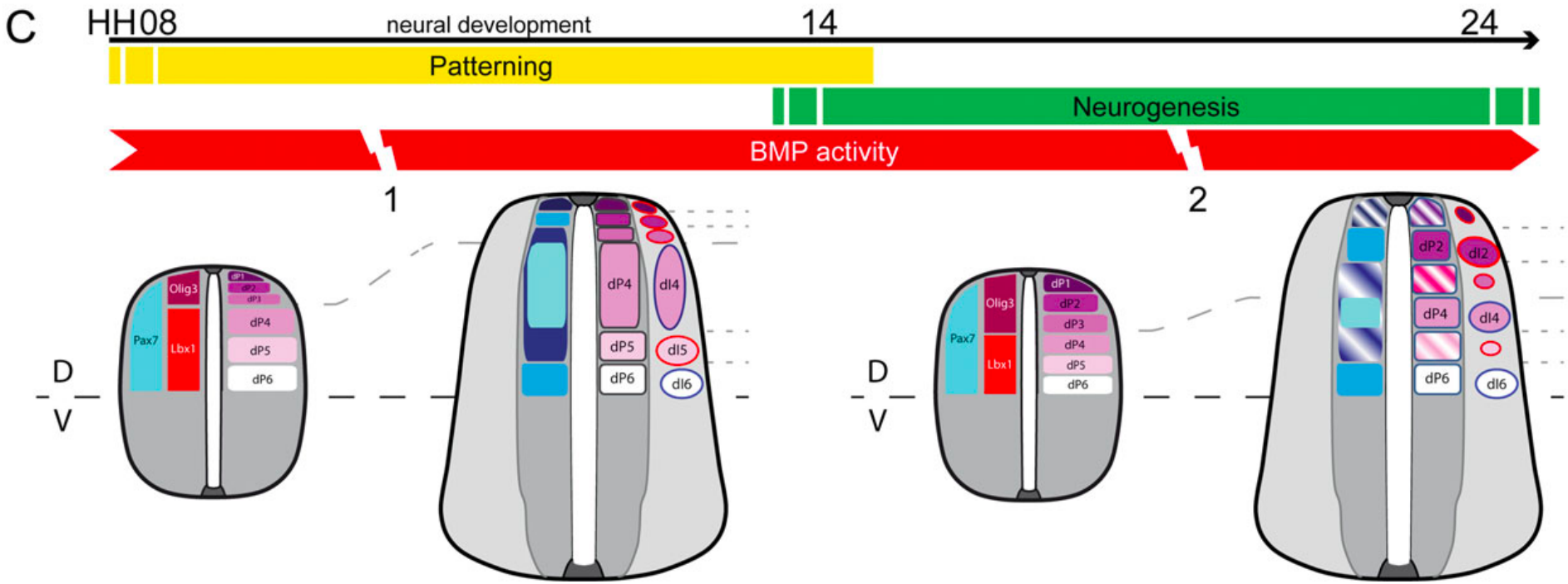
The role of the **Roof plate** in patterning the dorsal neural tube:

II. The effect of BMP



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

After neural tube closure, several BMPs (BMP4, BMP5, and BMP7) are expressed in the RP.



a loss or a reduction in BMP signaling result in:

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

A reduction in the BMP activity occurring later during development:

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

Different BMPs (e.g. BMP4 and BMP7) exert different roles in patterning & neurogenesis…

? Different expression pattern or different intracellular transduction?

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

Take home message :

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

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

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

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

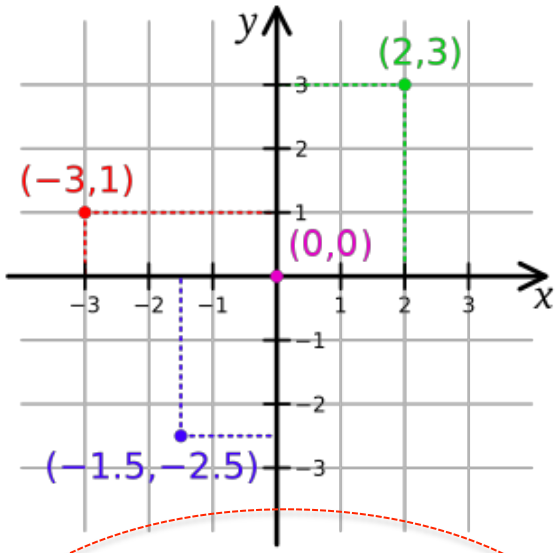
Regionalization → generates diversity in cell types

Precursor cells become regionalized => their potential is based on their **spatial position**

The acquisition of specific neural cell fate depends on the initial spatial coordinates of a precursor cell within the neural plate

The exposure to specific local environmental signals progressively restrict its developmental potential

Environmental signals direct cell fate by activating or repressing the expression of TFs which in turn control the genetic network necessary for differentiation of each neural cell type.



- ✓ Proliferation
- ✓ Migration
- ✓ Specification
- ✓ Differentiation

Regionalization → generates diversity in cell types

Precursor cells become regionalized => their potential is based on their **spatial position**

Development progress with **time**

→ **Positional identity = spatio-temporal identity**