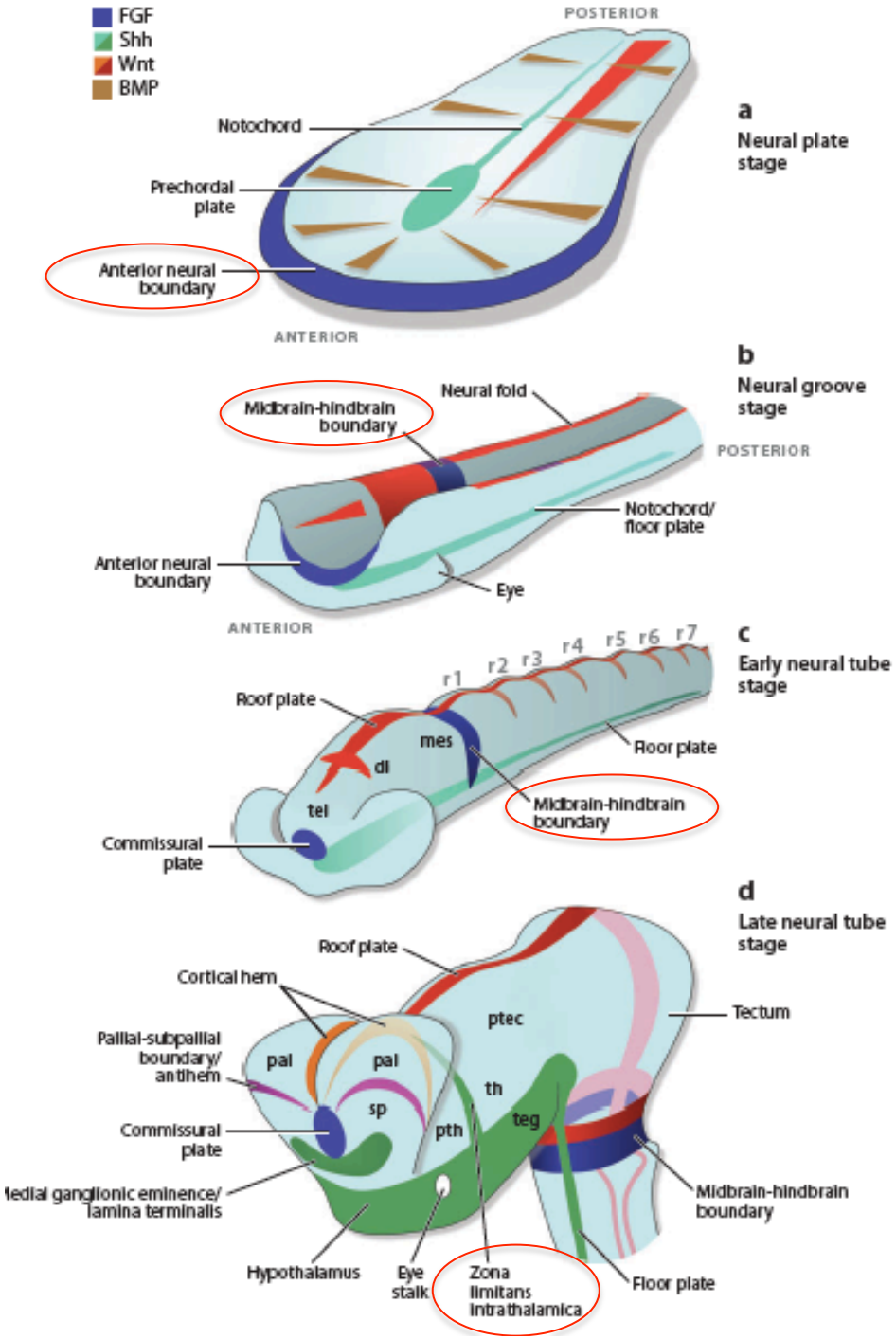
# ANTERO-POSTERIOR PATTERNING
# &
# SECONDARY ORGANIZERS

# SECONDARY ORGANIZERS

- Are secondary to those that operate throughout the embryo during gastrulation
- Develop within the neuroepithelium at given genetic boundaries
- Source of inducing factors that control differential expression of TFs resulting in the establishment of distinct neural progenitor cells with specific positional identity.
- Regulate the identity and regional polarity of neighboring neuroepithelial regions




ANTERO-POSTERIOR PATTERNING
&
SECONDARY ORGANIZERS

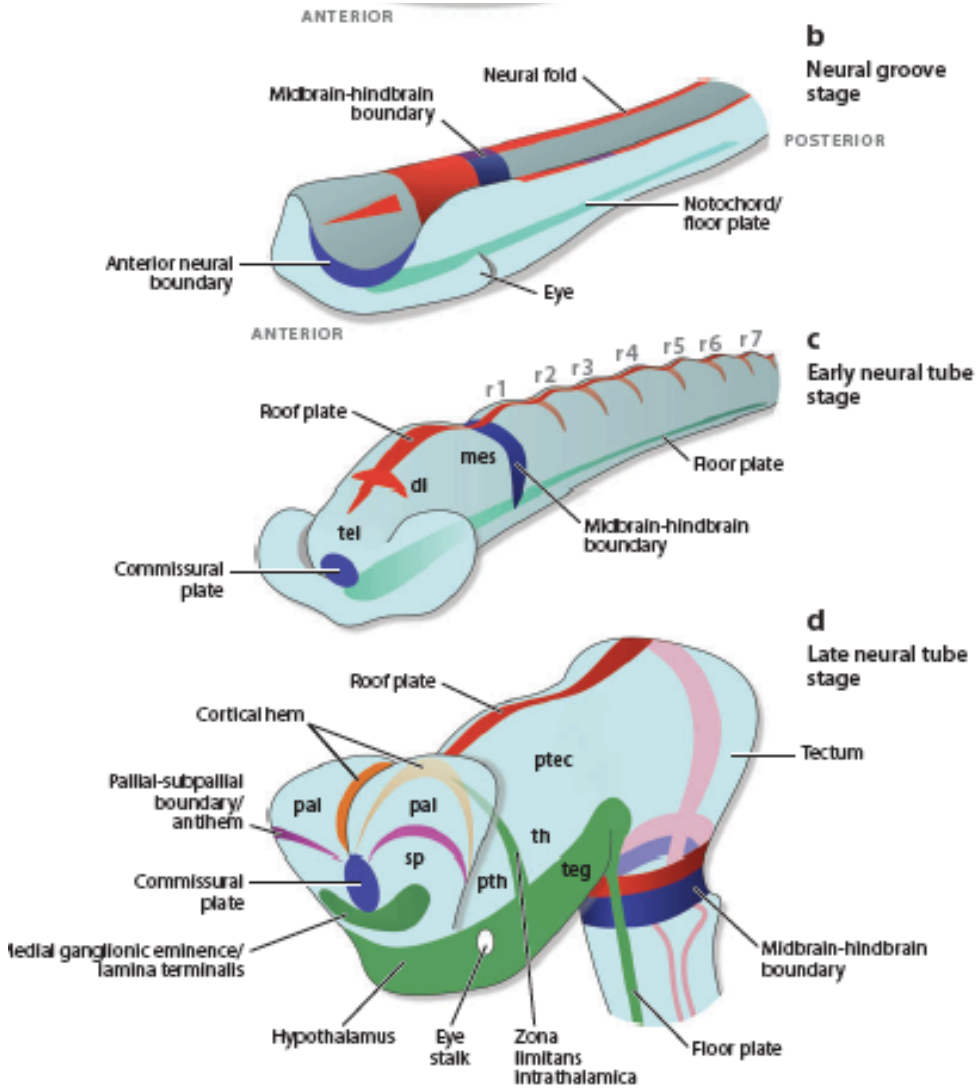
→ ***Organizing centers*** *are established gradually within a given field* ***at the junction between territories*** *that acquire different positional identities*

**ANR= The Anterior Neural Boundary/Commissural Plate**

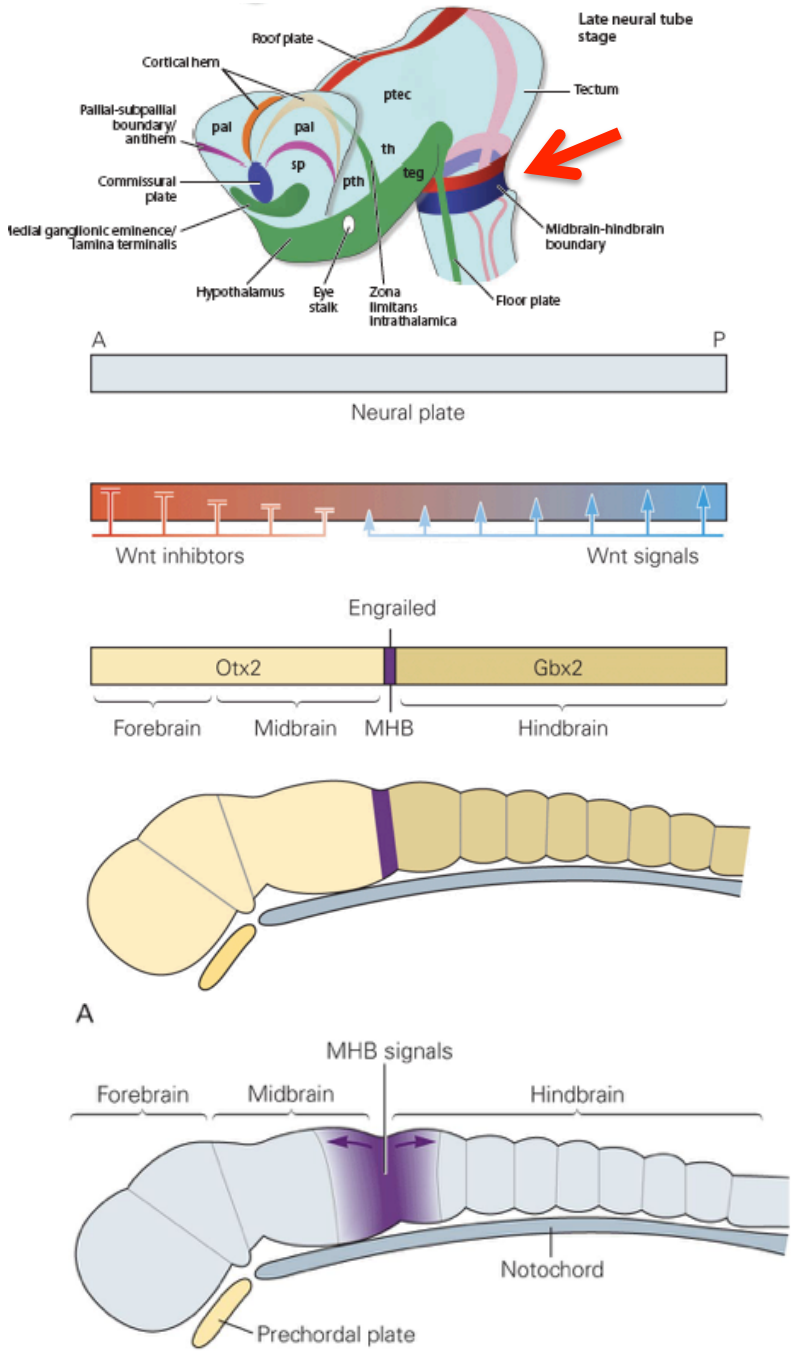
**MHB=midbrain-hindbrain boudary**

**ZLI= Zona limitans intrathalamica**

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

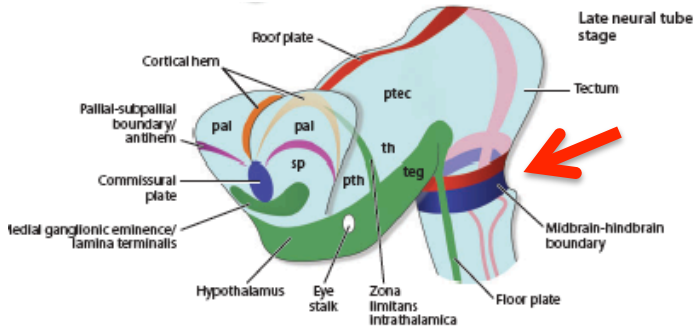



Development of the **mesencephalon** and **metencephalon** is regulated and coordinated by molecules produced in a **signaling center**, known as **the isthmic organizer MHB or IsO**, which develops at the mes/met boundary, and co-localizes with a morphological constriction of the neural tube called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




## What determines the A-P position of MHB formation?

- ✓ 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

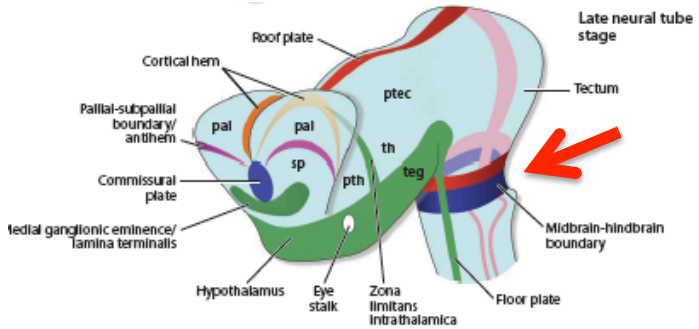



**The Midbrain-Hindbrain Boundary (MHB) or Isthmic organizer (IsO)**

- 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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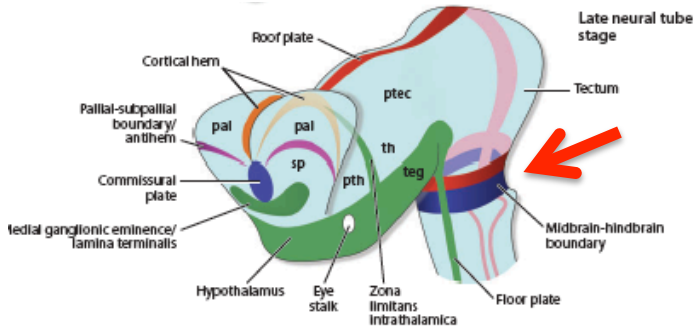
experimental approaches???

<u>Two main approaches</u>

**IsO ectopic transplantation**

**IsO removal**

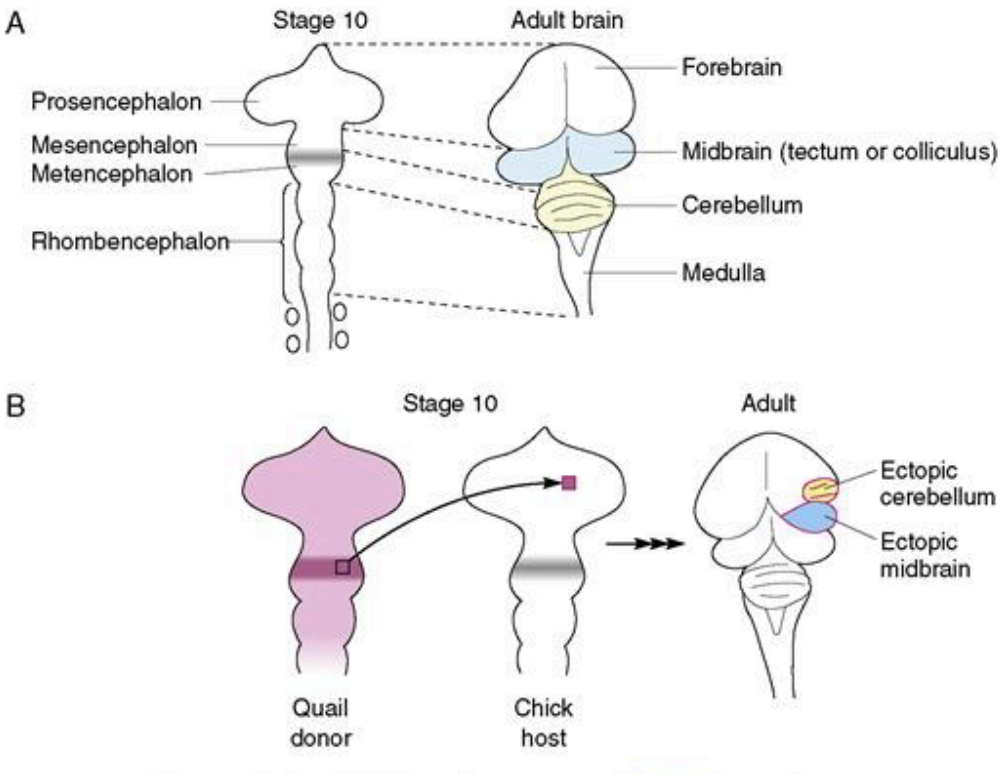



**The Midbrain-Hindbrain Boundary (MHB) or Isthmic organizer (IsO)**

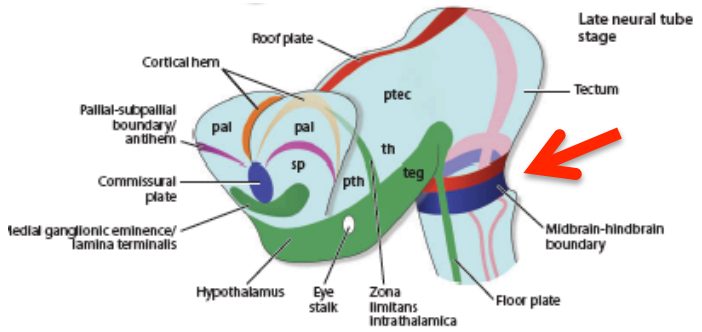
## 1) **IsO ectopic transplantation**

**Transplantation of the isthmus to ectopic neural locations →**
*induction of 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*

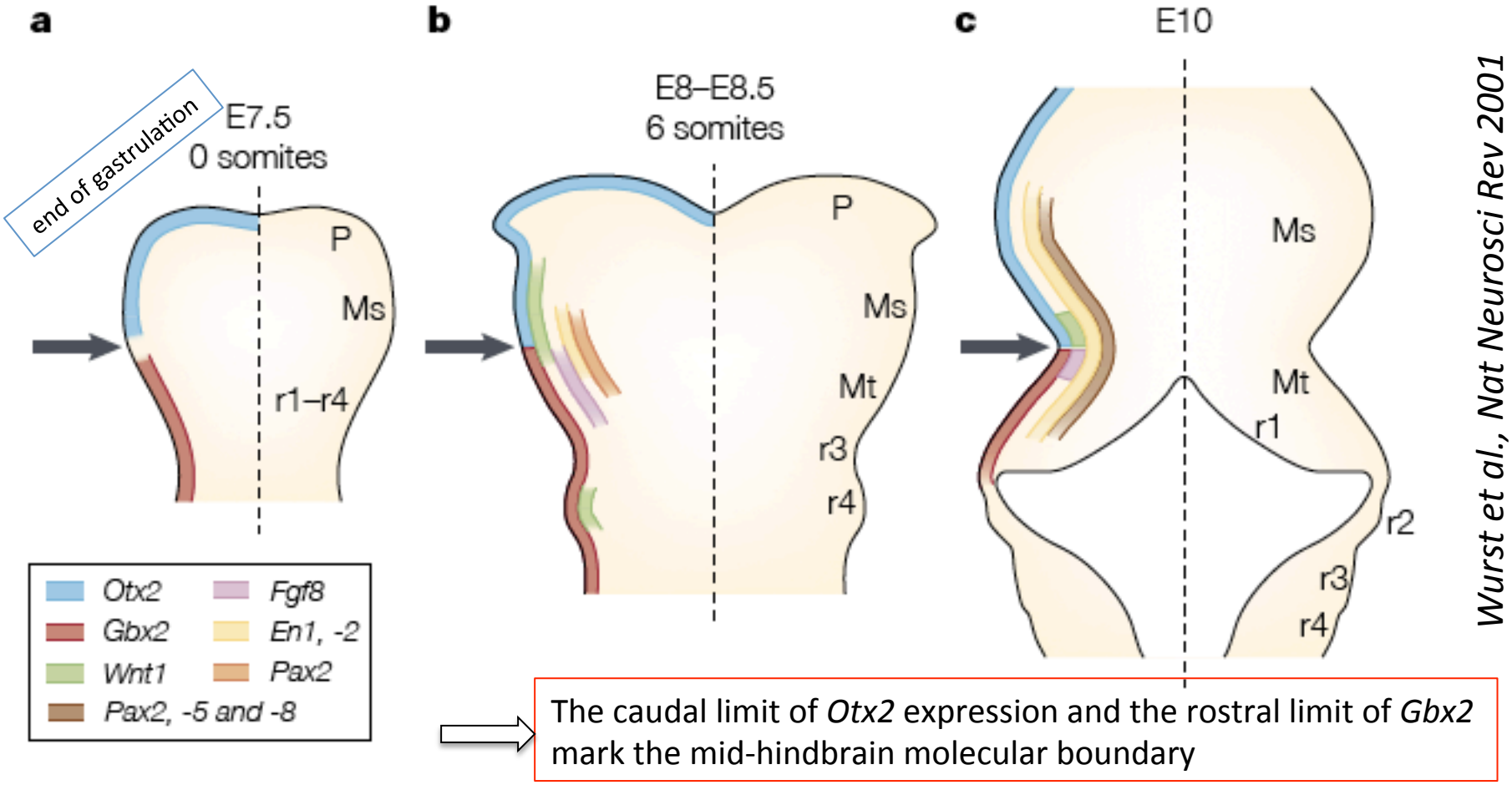
→ the isthmic territory is both **necessary and sufficient** for the development of the mes-metencephalic domain

→ 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)

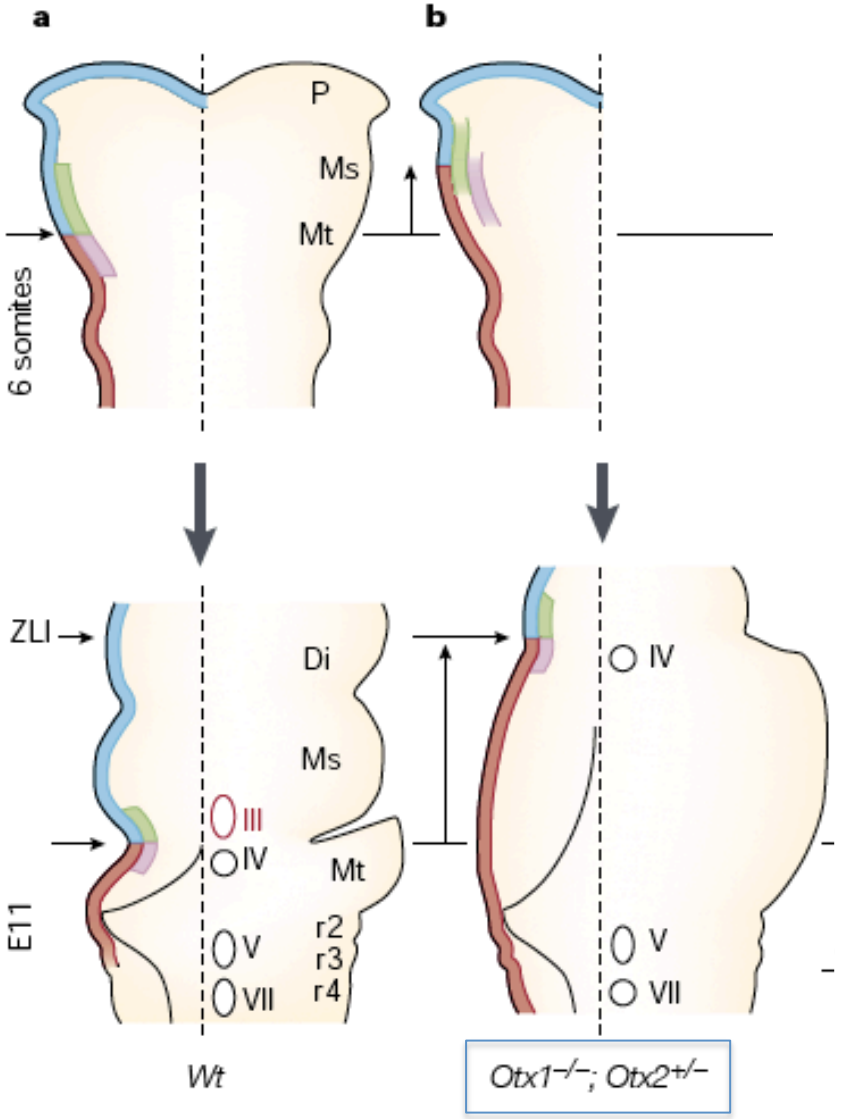
the boundary between the midbrain and hindbrain is roughly positioned during late gastrulation and is **progressively refined** during early somitogenesis




*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

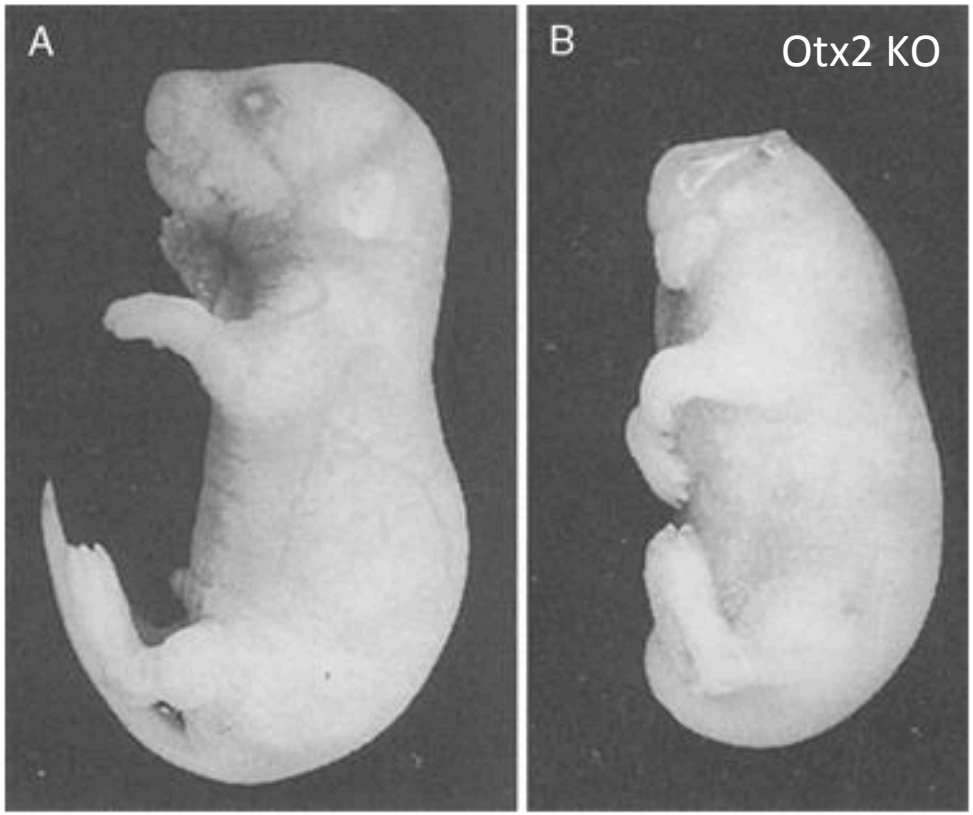
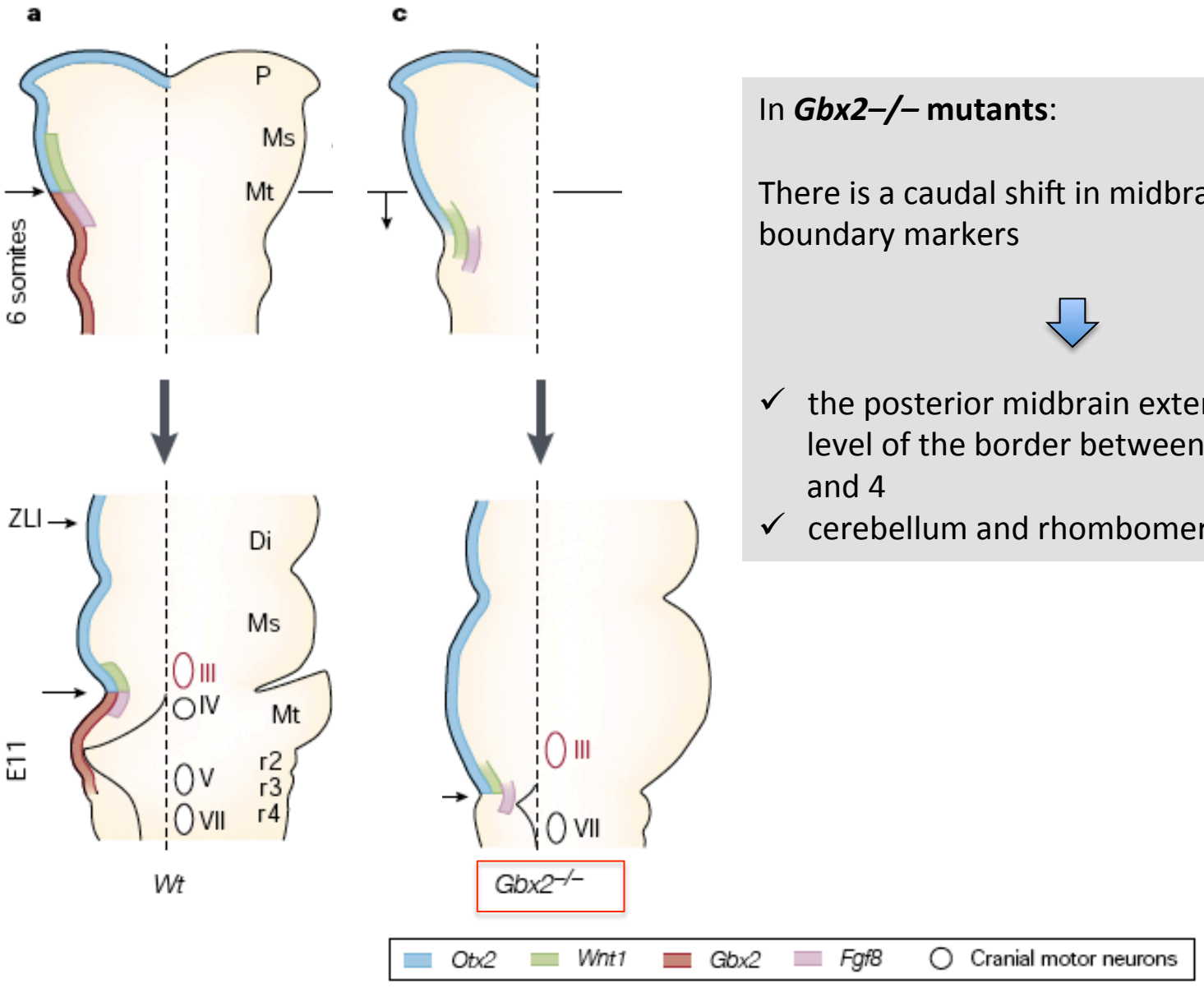


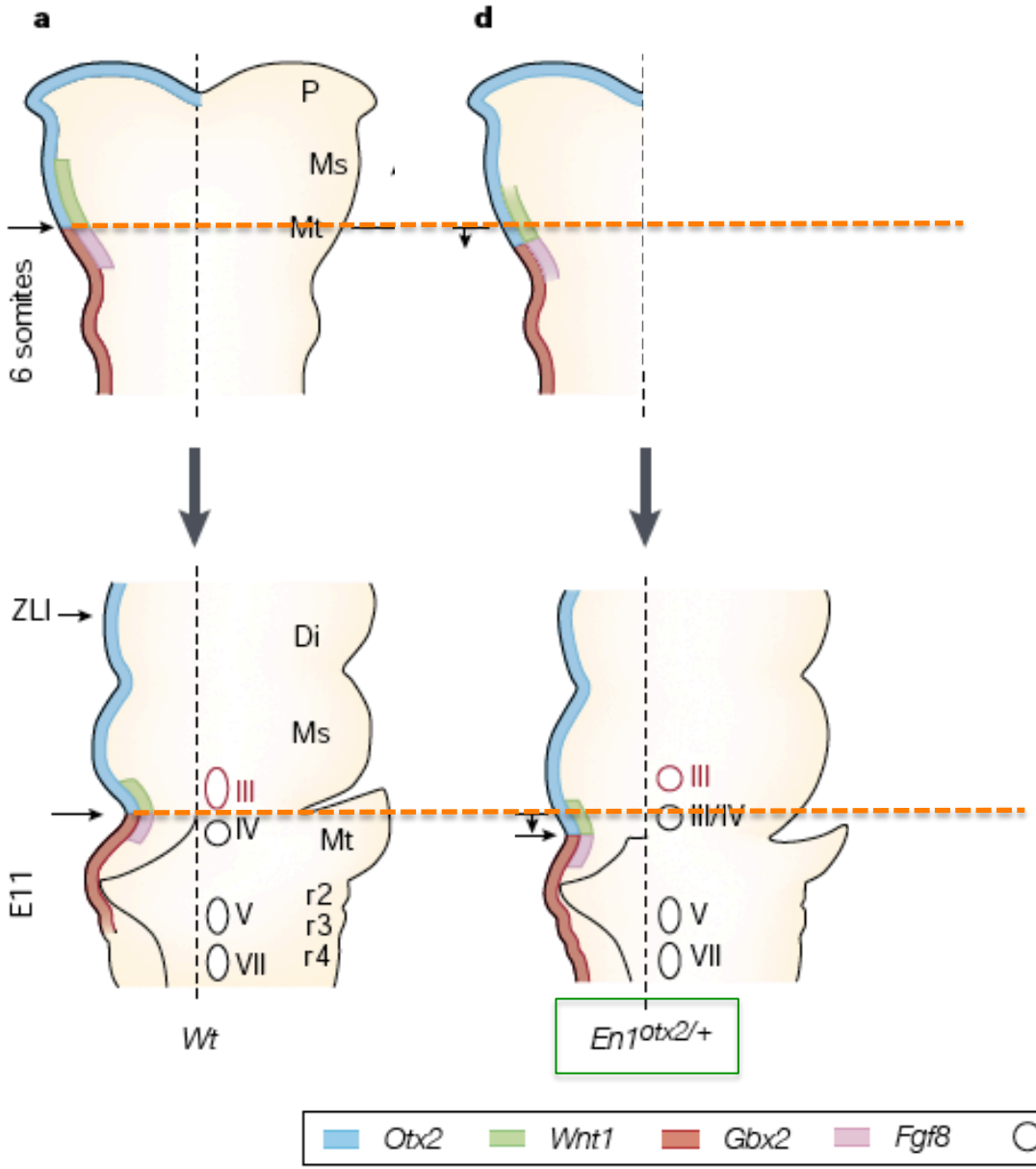

**Fig. 2.12** *Otx2* is required for the formation of the mouse head. A dramatic illustration of the importance of the *otx2* gene is the development of the mouse forebrain and rostral head. If the gene is deleted using homologous recombination, embryos without either allele of the gene fail to develop 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

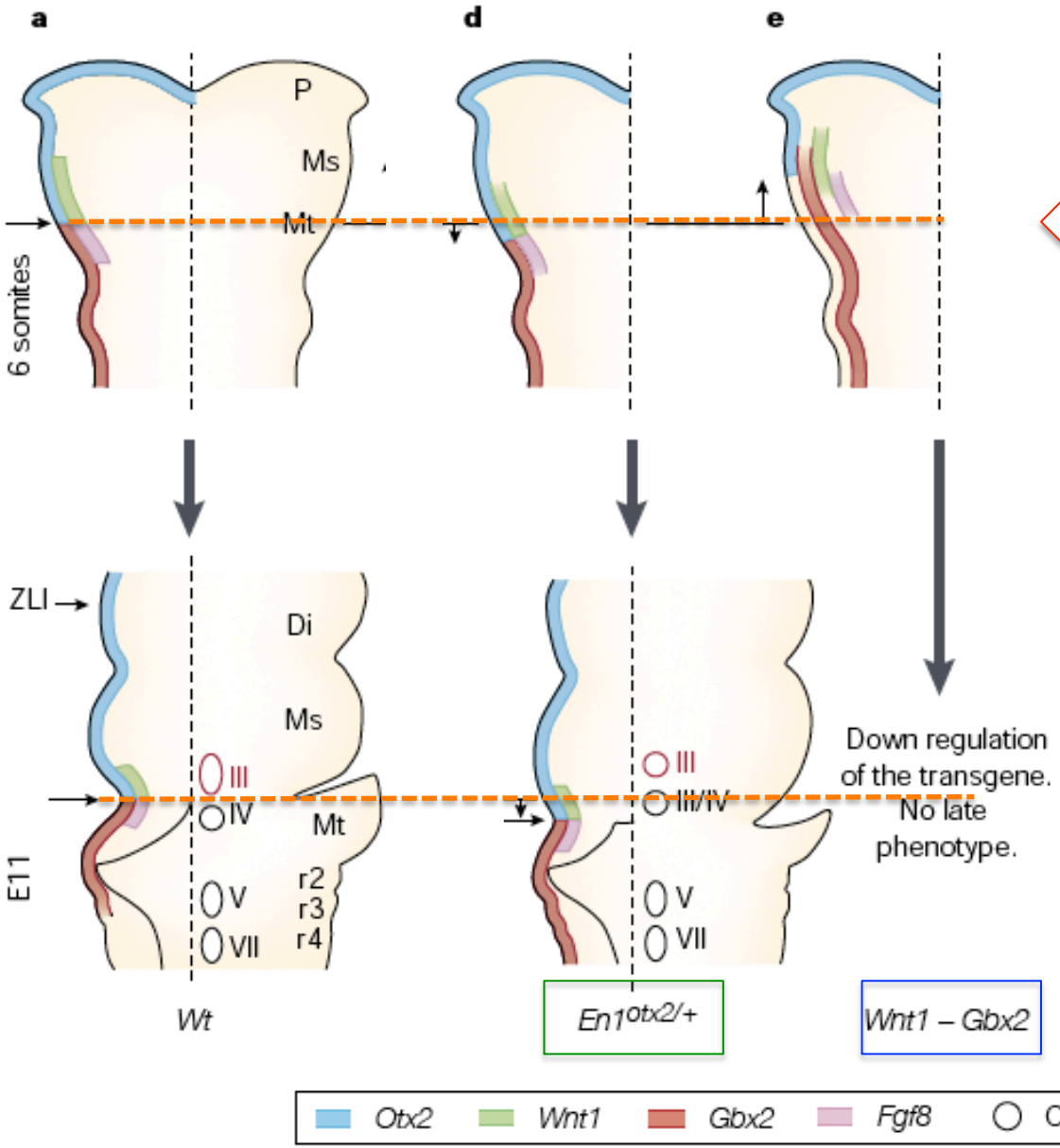
- ✓ the posterior midbrain extends caudally to the level of the border between rhombomeres 3 and 4
- ✓ cerebellum and rhombomeres 1–3 are lost




**Otx2 ectopic expression (knock-in):** 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

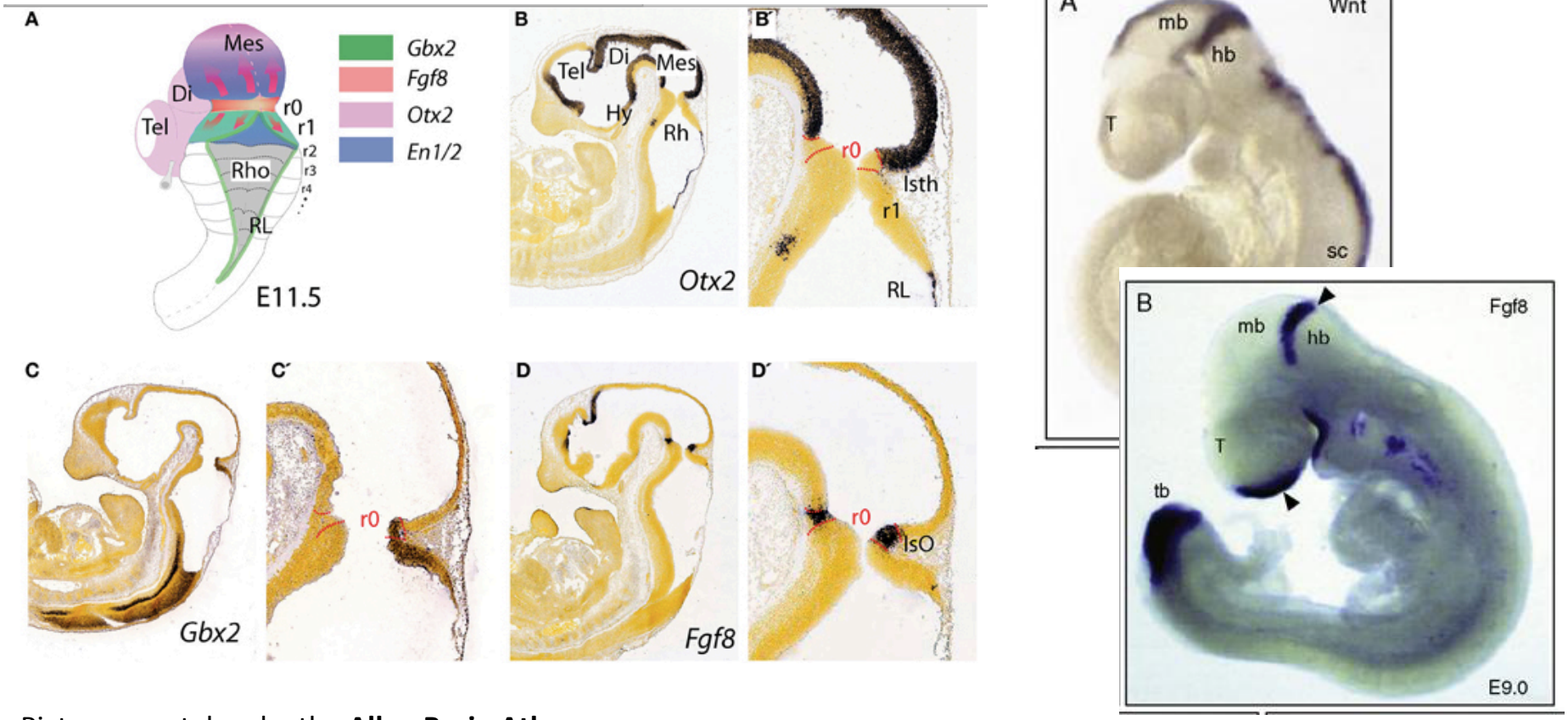
**The Midbrain-Hindbrain Boundary (MHB) or Isthmic organizer (IsO)**

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

# Molecular characterization of the mid-hindbrain boundary in mice



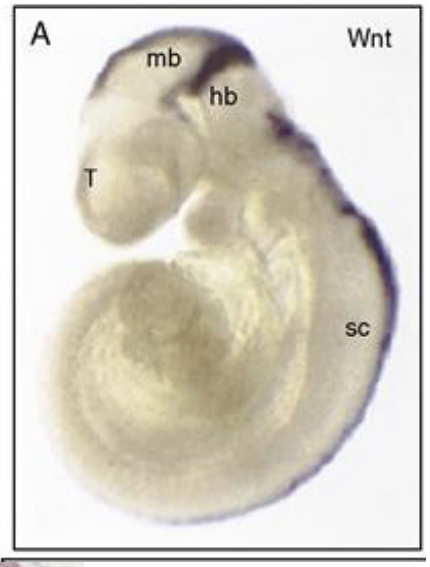

Pictures are taken by the **Allen Brain Atlas**….

*Martinez et al., Frontiers in Neuroanatomy, 2013*

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

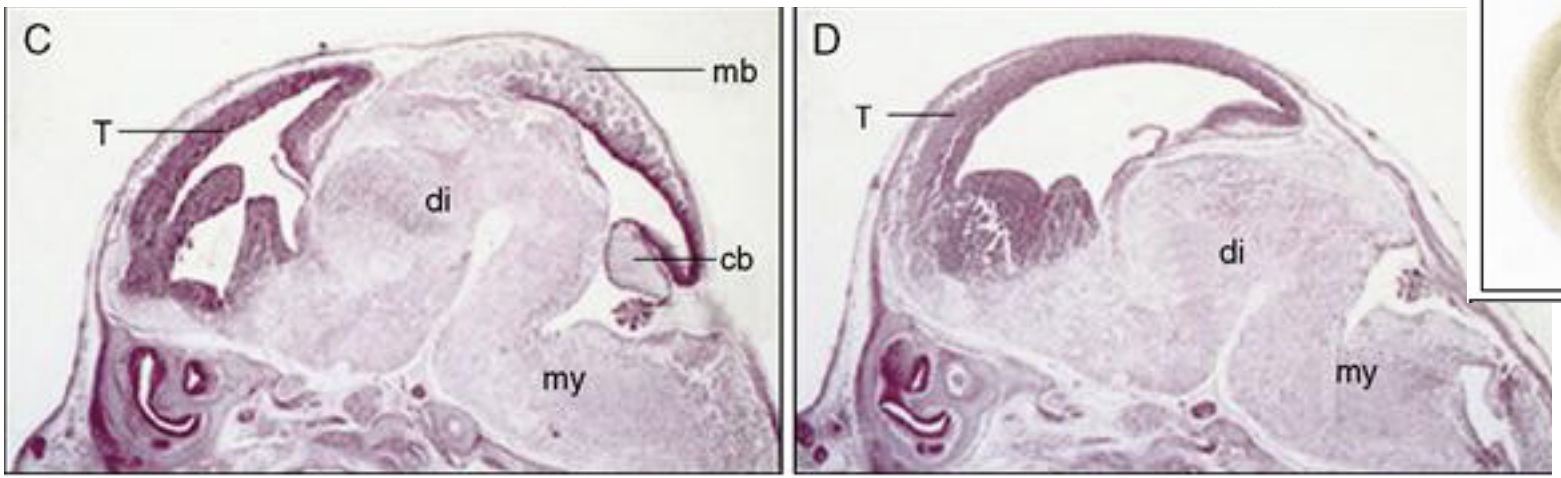
→ candidate **mediators of IsO activity for** induction and maintenance of polarized mesencephalic and metencephalic fates when transplanted ectopically

**Wnt1**




WT

Wnt1-/-



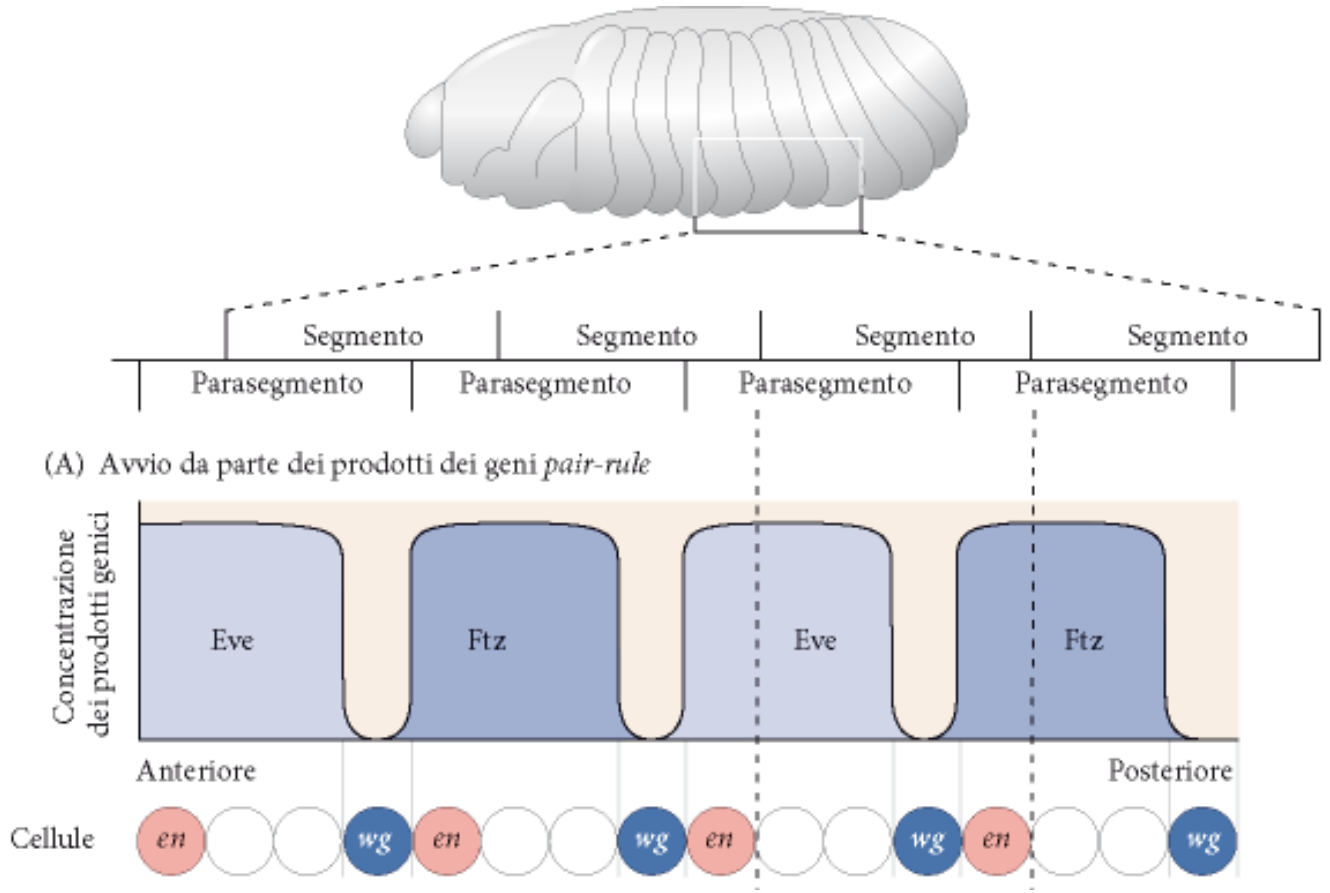

Wnt1-/- mice show:

- Dramatic reduction in midbrain/hindbrain structures

- **Lack of En1** expression in the IsO (En1 KO = phenotype as Wnt1 ko)

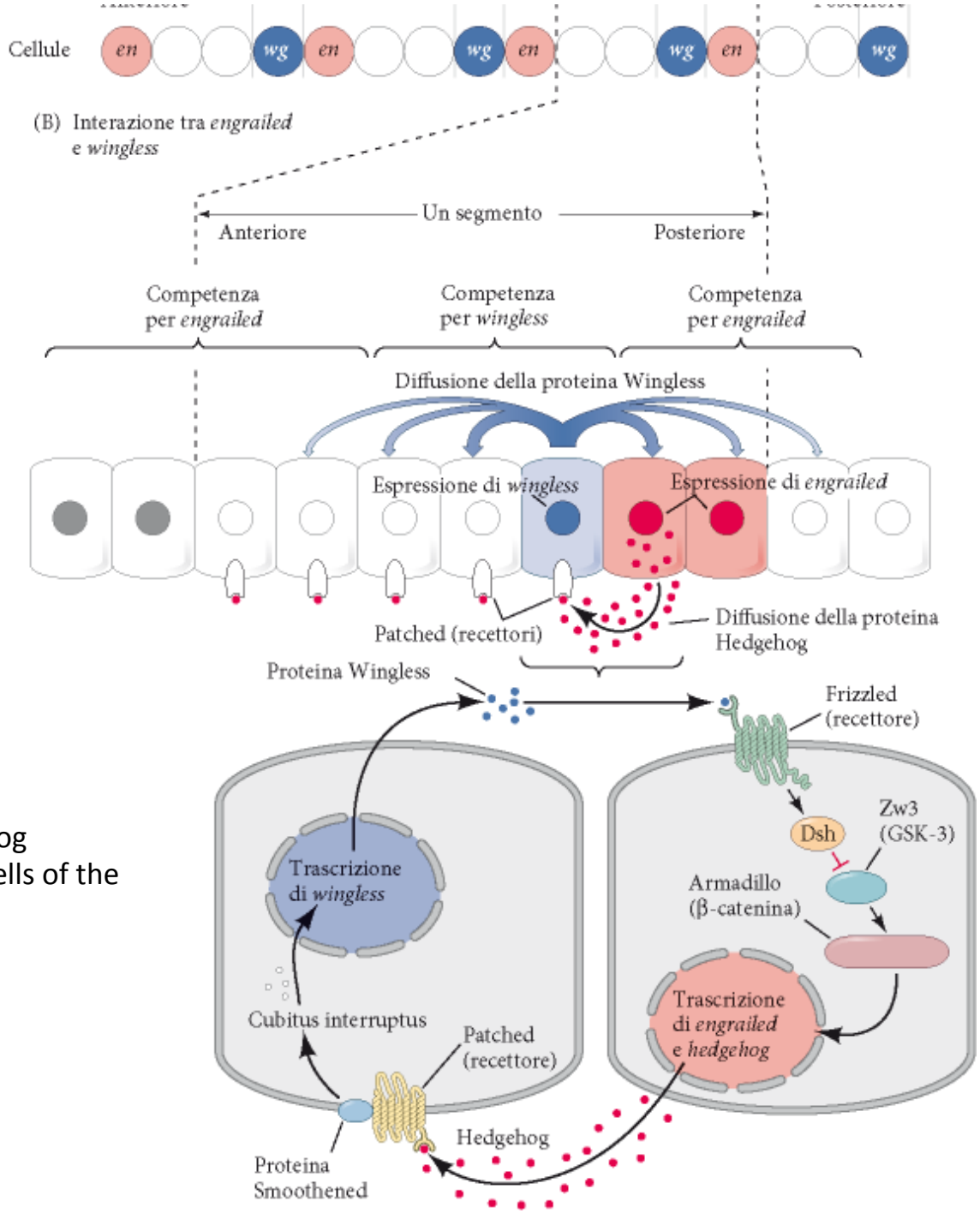
→….in GOF experiments, Wnt1 does not mimic organizer activity (**permissive but not instructive…. )**

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.

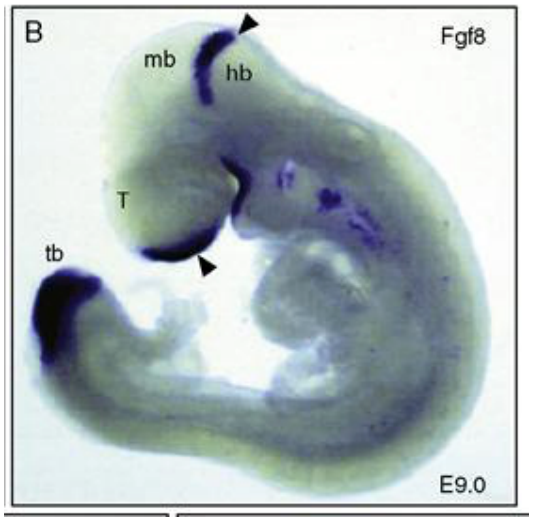
Engrailed- and Wingless-expressing cells interact to maintain the pattern of **en** and **wg** expression




## 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 due to altereation during gastrulation (Meyers et al., NatureGenetics 1998)

how can be addressed this concern ???

**FGF8**

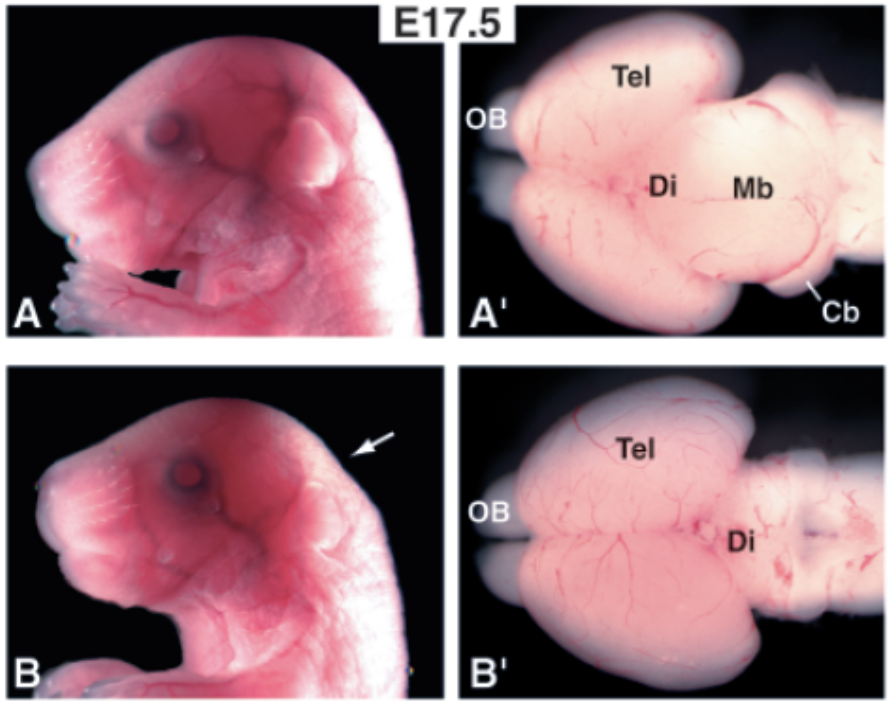
## Conditional gene inactivation approach

Mouse model to study FGF8 function in IsO

→ FGF8 MHB KO mutants → conditional KO *En1Cre/+,Fgf8flox*

*(Chi et al., Development 2003)*

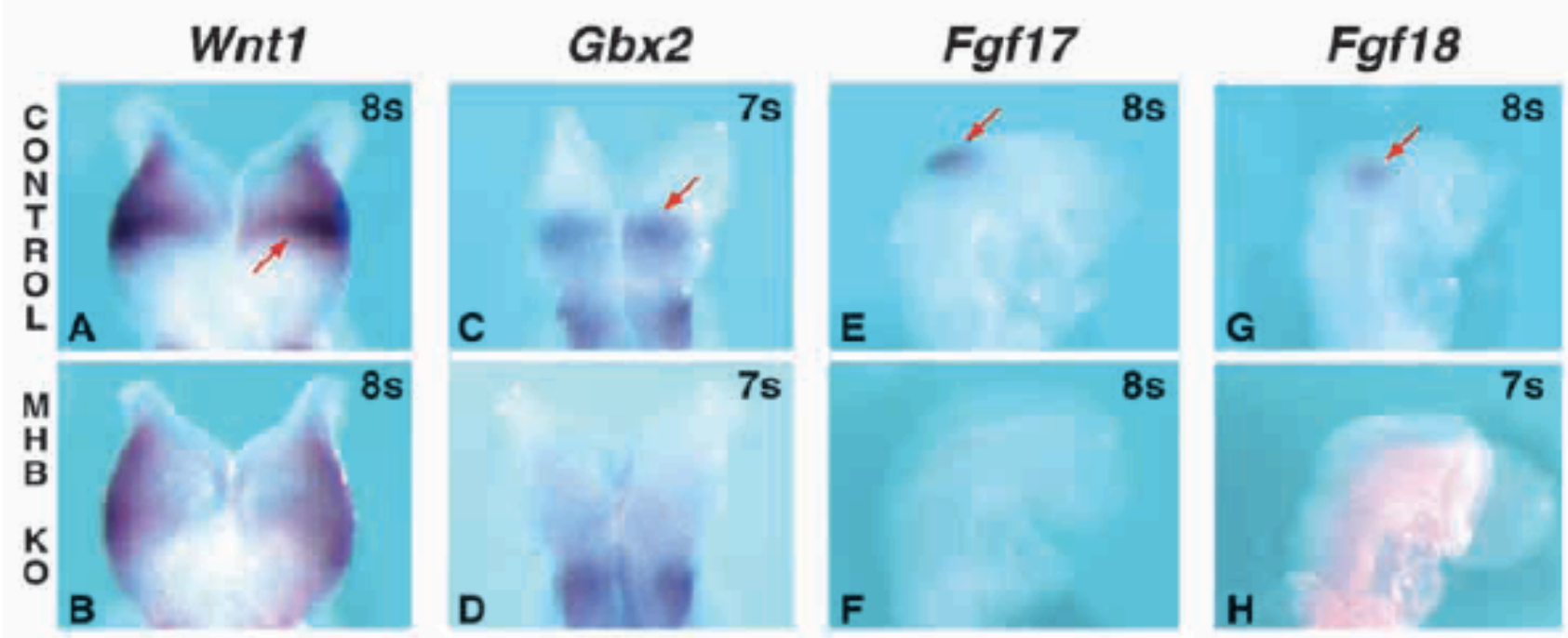
*En1Cre* produces sufficient Cre protein throughout the midbrain/ hindbrain boundary region to eliminate *Fgf8* function by 3 somite stage, when *Fgf8* expression normally commences in a subset of *En1*-expressing cells.




*Extensive cell death in the mes/met before E10*

**Analysis of gene expression in *Fgf8* MHB KO mutants**

→ Ectopic cell death
→Genes are negatively affected by the loss of *Fgf8* function



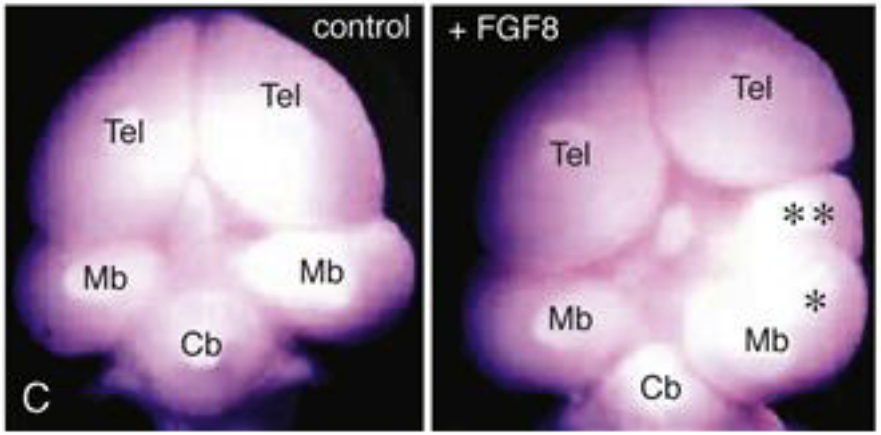

***Fgf8* is part of a complex gene regulatory network** *essential for mes/met development*

**FGF8**

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

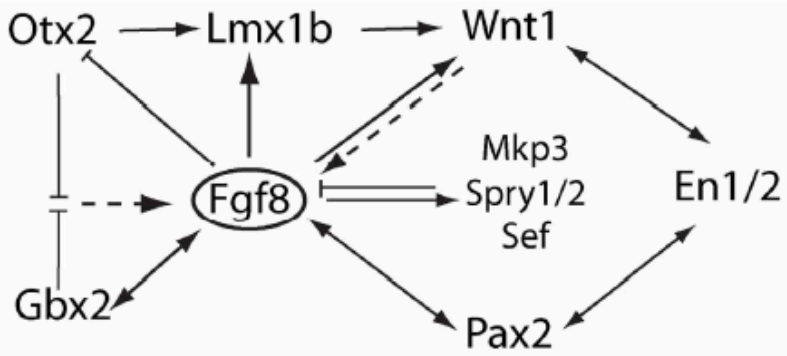
**GOF**

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




**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 | +++ | | | | | | | | | | | |
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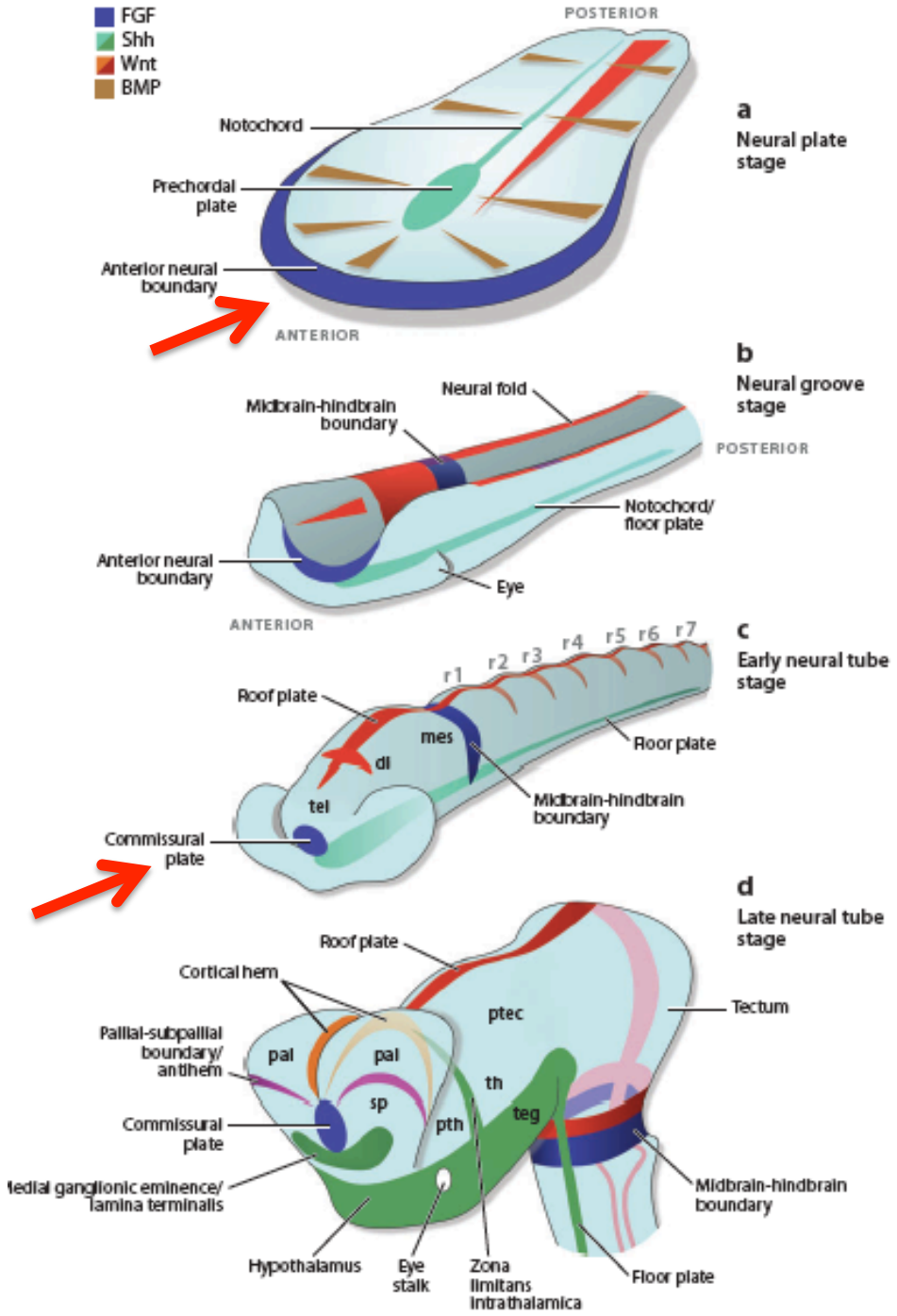
| | +++ | | +++ | +++ | ++ | ++ | | | + | ++ | ++ | ++ |
| Isth | | +++ | | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| $R_1$ | | +++ | | | ++ | ++ | ++ | | ++ | ++ | ++ | ++ |
| $R_2$ | | | | | | | | | | | | |
| $R_3$ | | | | | | | | | | | | |

**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.

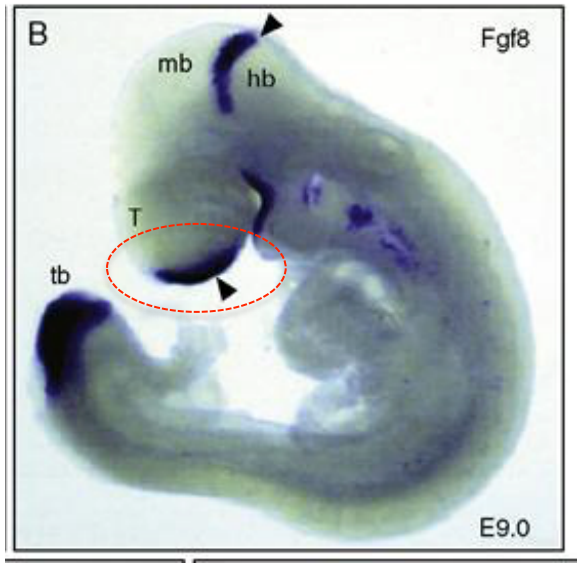
*Martinez et al., Frontiers in Neuroanatomy, 2013*




**ANR= The Anterior Neural Boundary/Commissural Plate**

Acts as organizer for the forebrain (neocortical patterning)

FGF8



2 NOVEMBER 2001 VOL 294 SCIENCE

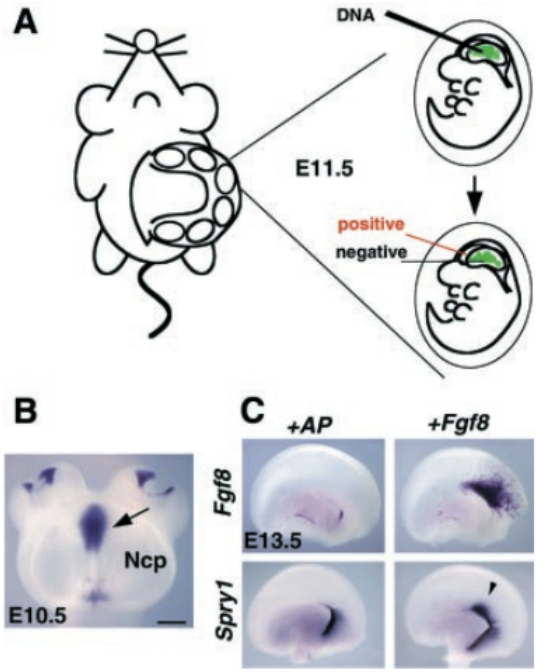


# Neocortex Patterning by the Secreted Signaling Molecule FGF8

**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



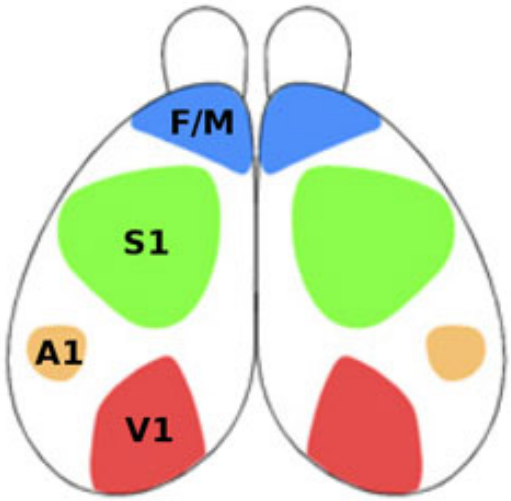

Gene misexpression strategies:

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

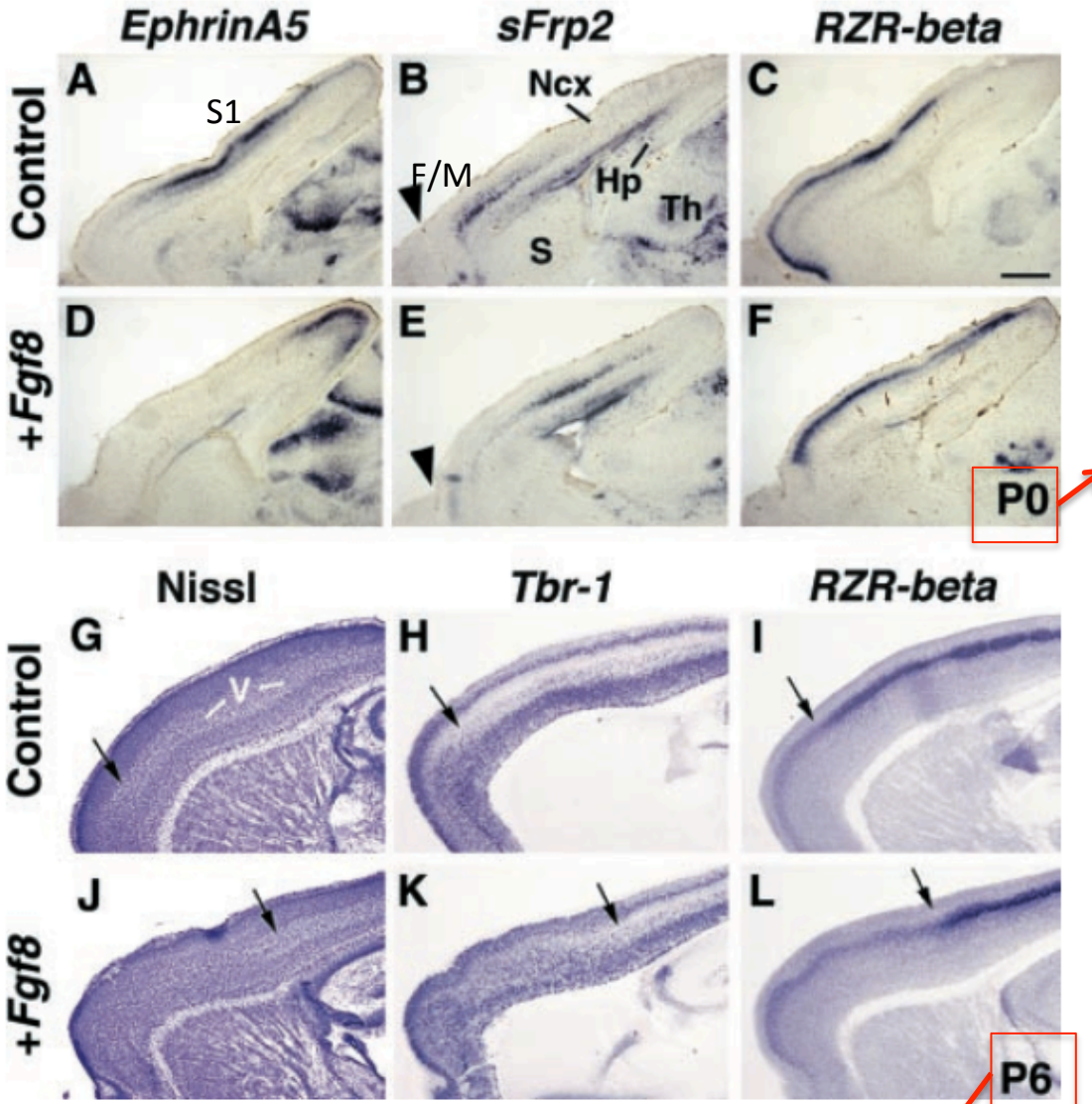
***Video in utero electroporation***

# 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




boundary between somatosensory and motor cortex

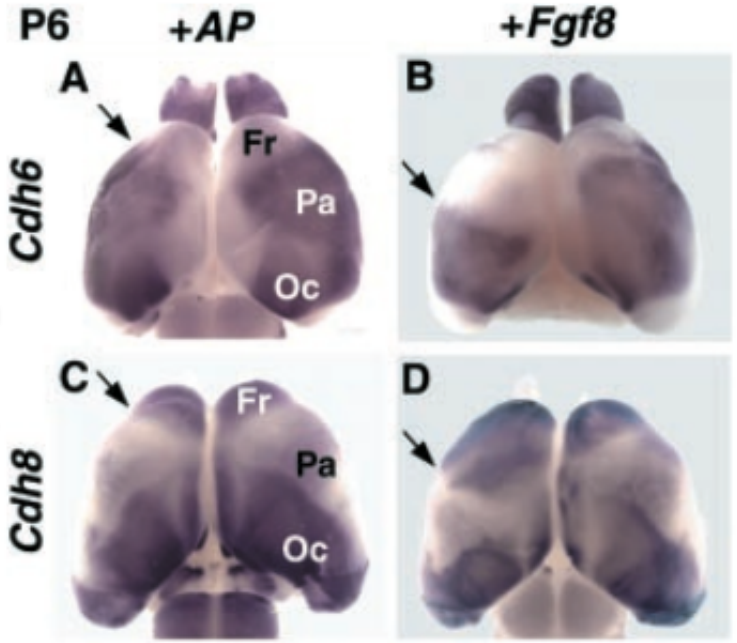



Cytoarchitectonic boundaries

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

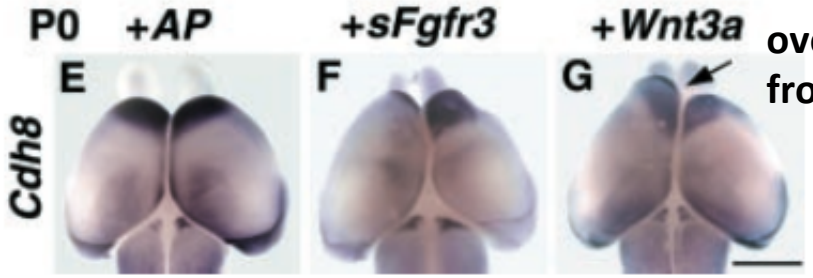
Fr=frontal
Pa=parietal
Oc=occipital

Differential expression




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

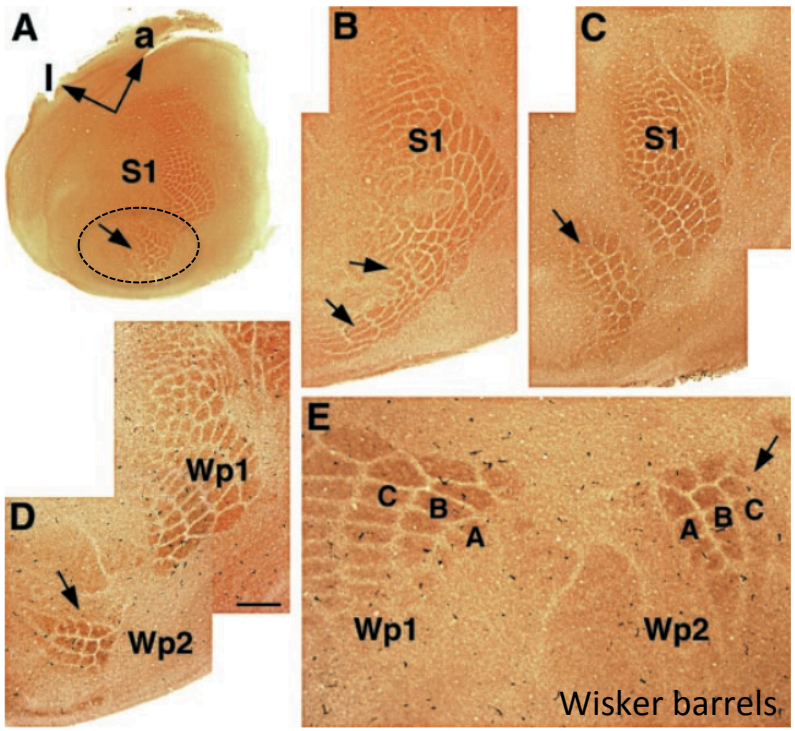
**2. Reducing endogenous FGF8 signal shifts cortical area boundaries anteriorly**




**overgrowth of the frontal pole**

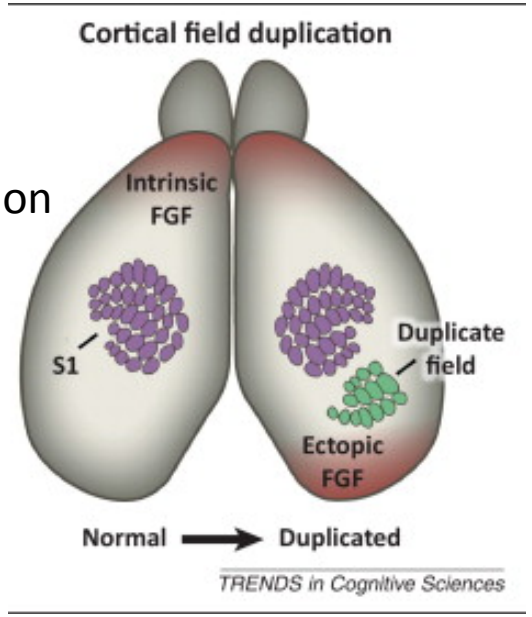
the left frontal *Cdh8*-high domain is unchanged by *AP* (E), reduced by *sFgfr3* (F),

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




Fukuchi-Shimogori and Grove Science 2001

Mirror-image duplication




*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)*

Development 130, 1903-1914

doi:10.1242/dev.00416



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

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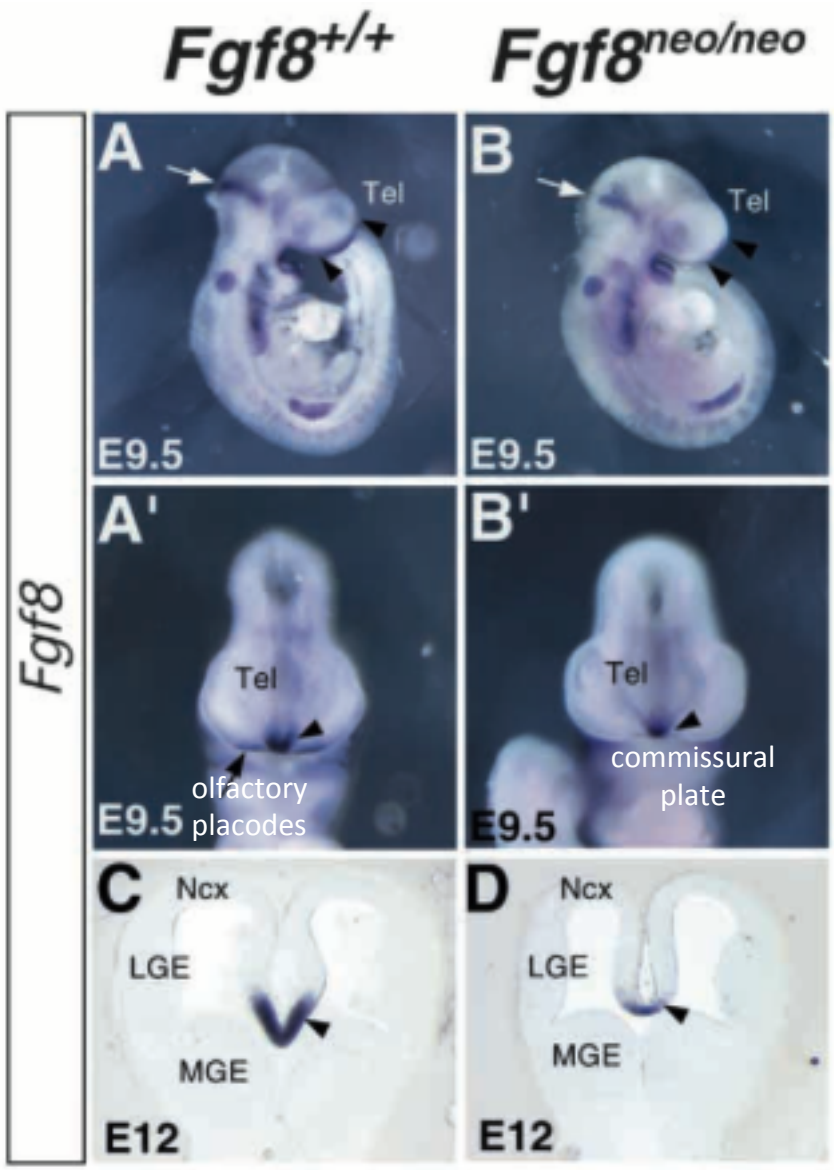
*Accepted 31 January 2003*

## SUMMARY

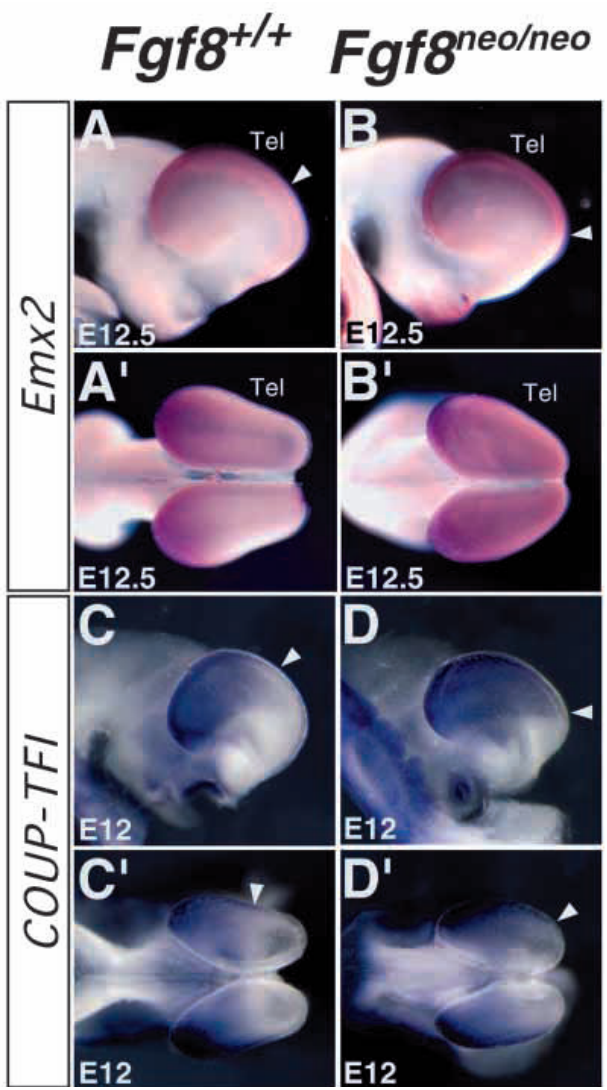
**The neocortex is divided into multiple areas with specific architecture, molecular identity and pattern of connectivity with the dorsal thalamus. Gradients of transcription factor expression in the cortical primordium regulate molecular regionalization and potentially the patterning of thalamic projections. 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

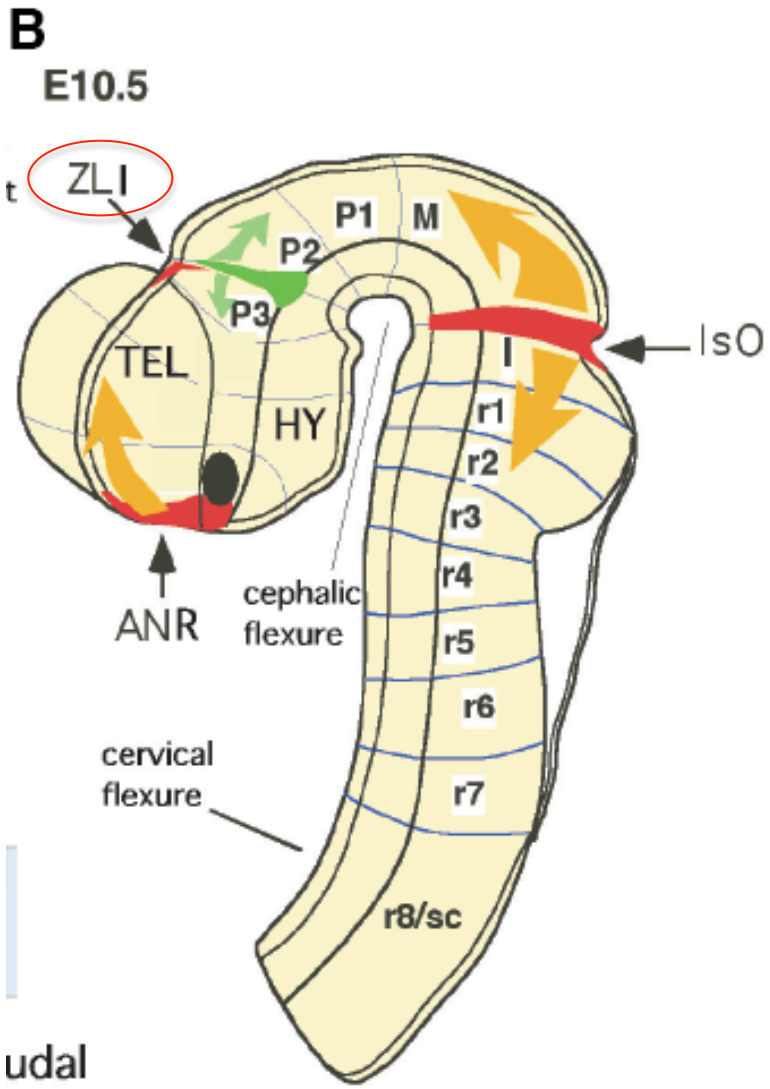
Levels of Fgf8 expression are reduced in Fgf8neo/neo hypomorph embryos




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



**ZLI= Zona limitans intrathalamica**

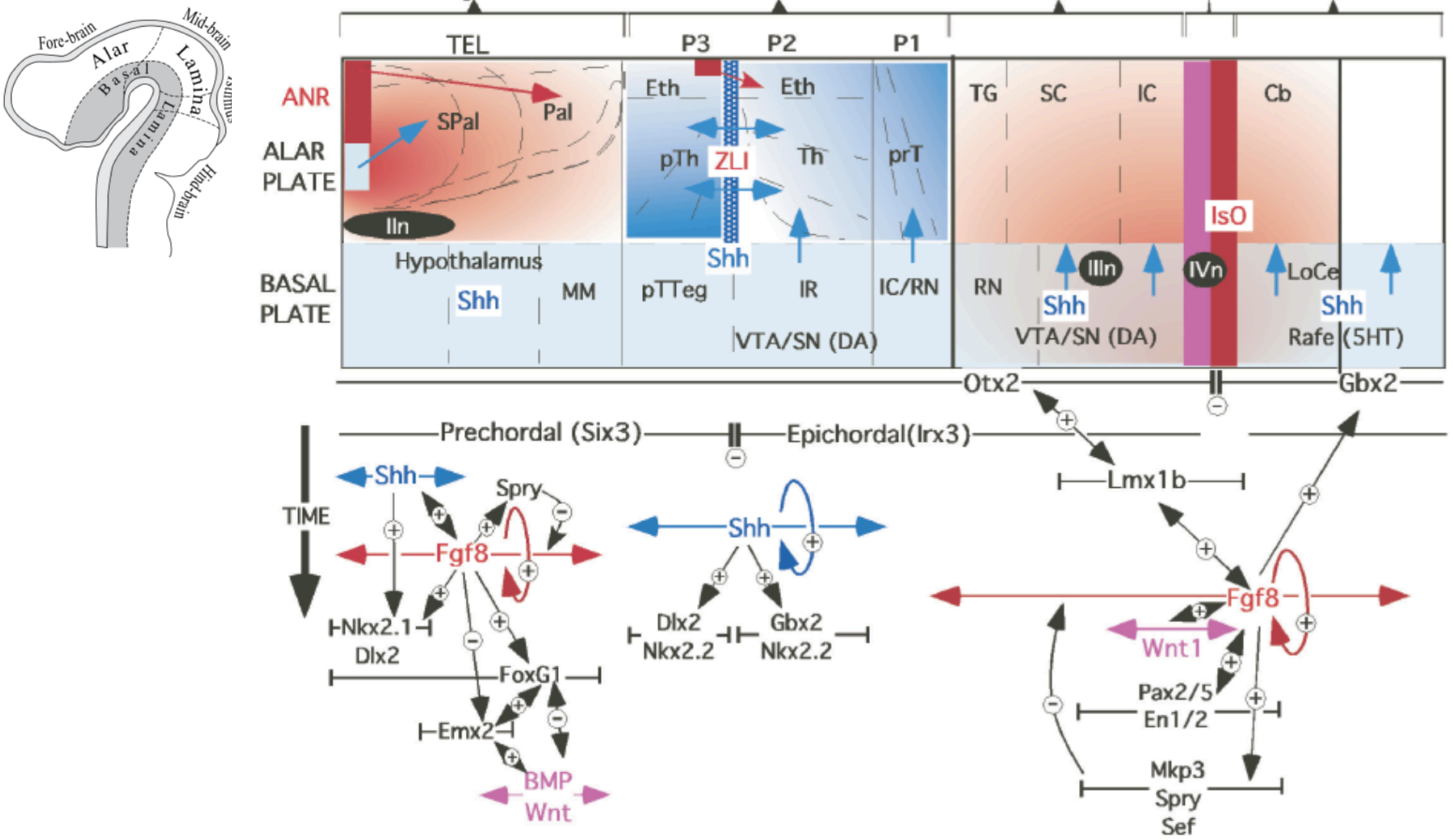


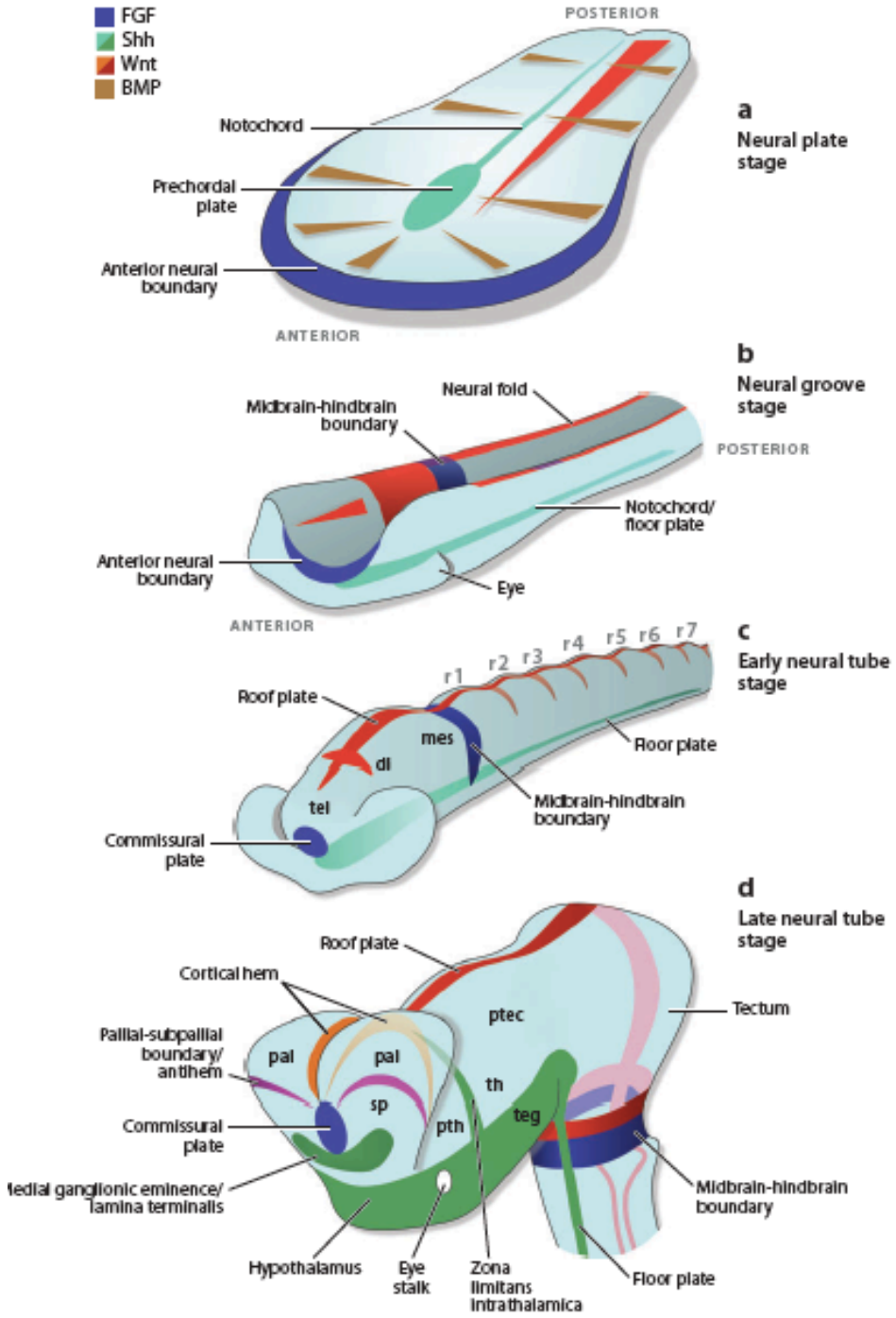

The ZLI is a narrow transverse region between prosomer 2 and 3

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)






- ✓ 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