Emx2 patterns the neocortex by regulating FGF positional signaling

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Presented by Sally Kwok

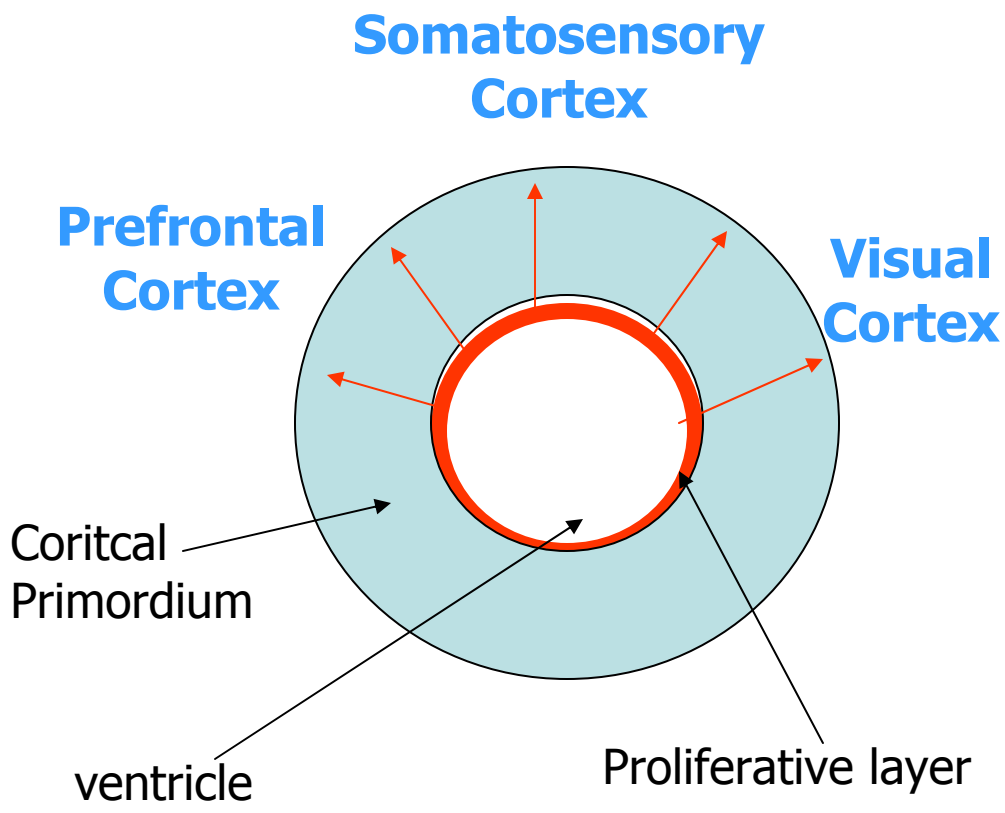
Background

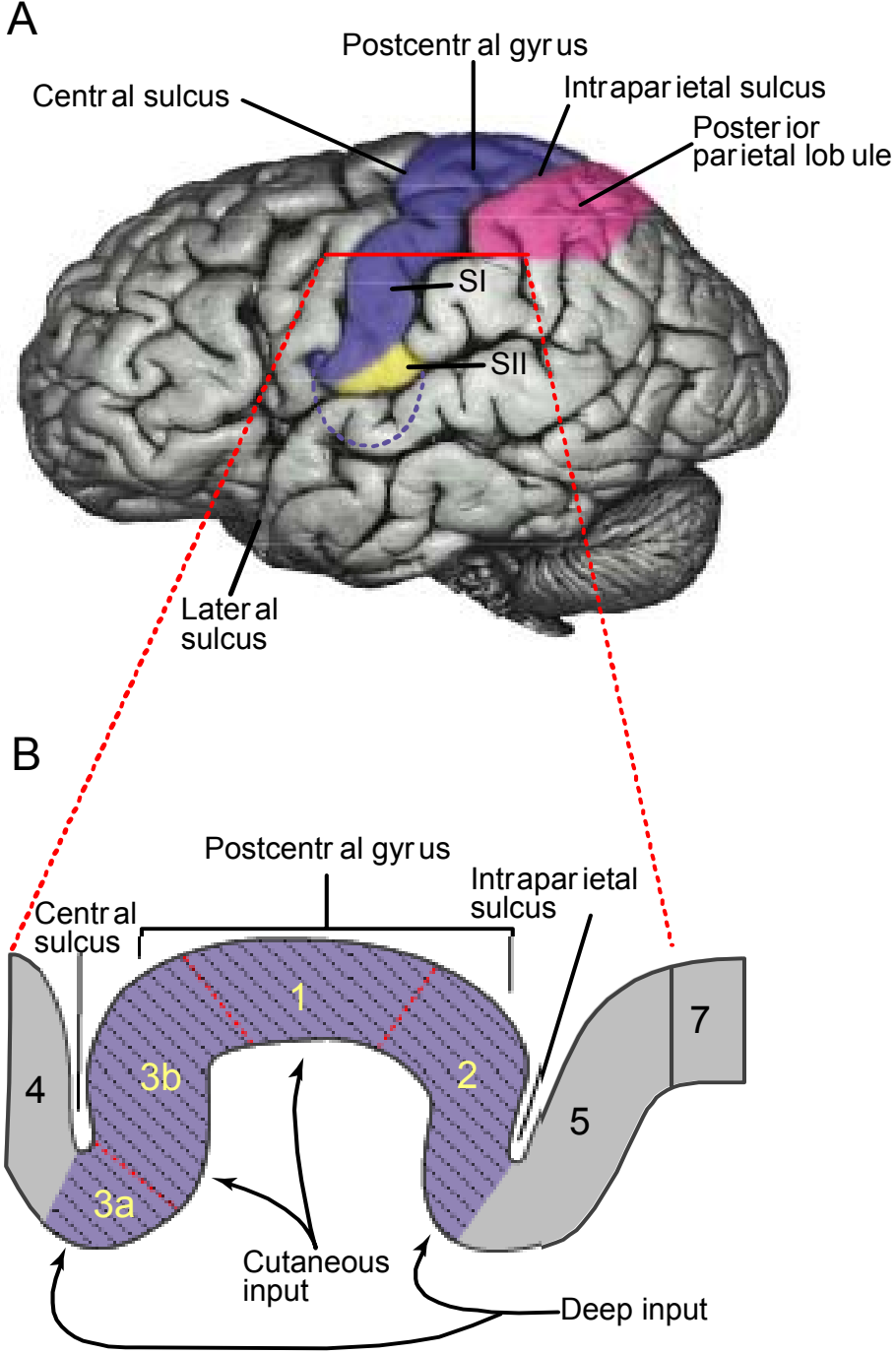
- Cerebral cortex has anatomically and functionally distinct areas.
- The areas create a map, similar across the species.
- Question: How is the immense population of neurons that constitute the cerebral neocortex distributed to appropriate layers of distinctive cytoarchitectonic areas?

Model:

- Rakic, P. in Yale proposed radial unit hypothesis. According to this hypothesis, the lining of the embryonic cerebral ventricle consists of proliferative units that provide a proto-map of prospective cytoarchitectonic areas. The output of the proliferative units is translated via glial guides to the expanding cortex …. final number for each area can be modified through interaction with afferent input (thalamic inputs).

So.....

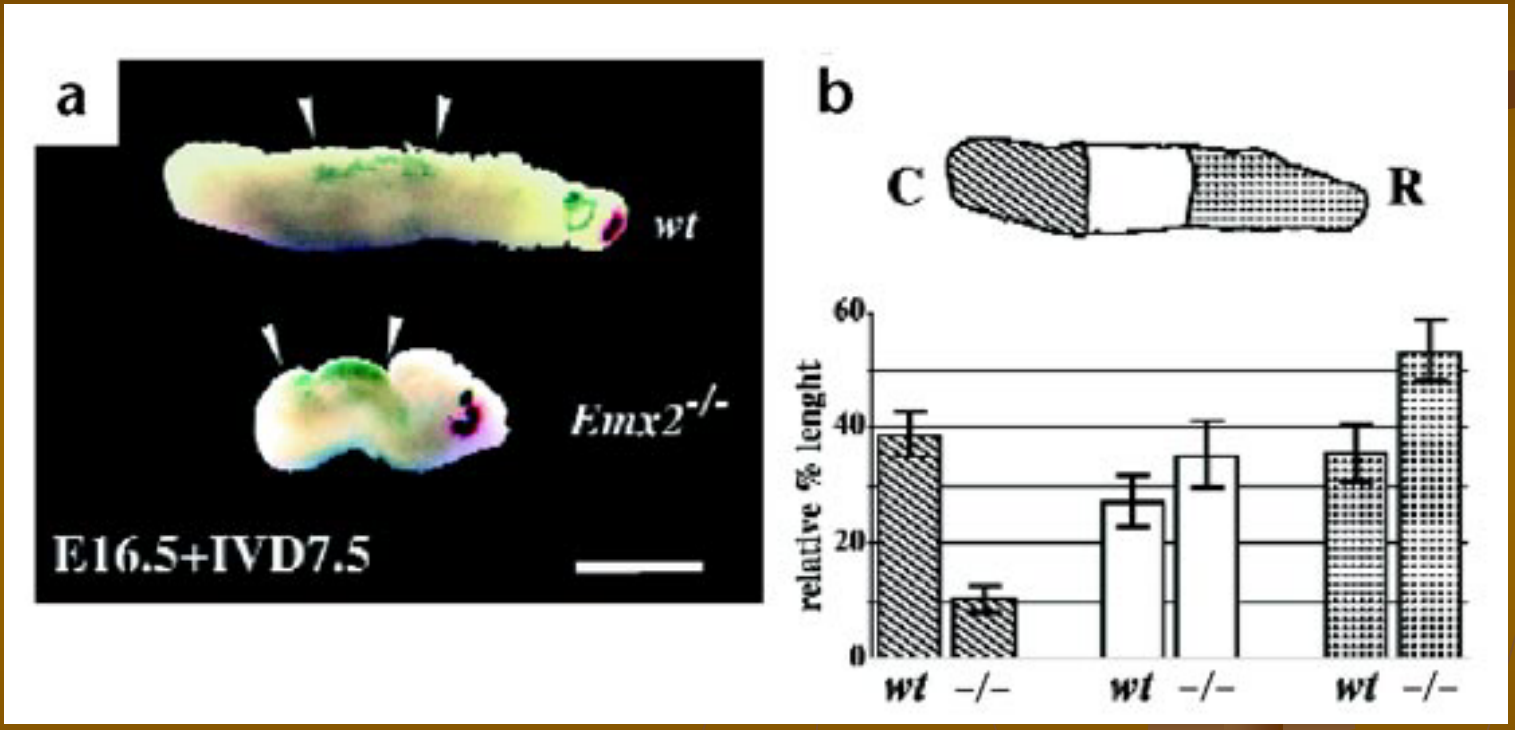

Somatosensory Cortex
Prefrontal Cortex
Visual Cortex
Coritcal Primordium
ventricle
Proliferative layer


A
Postcentral gyrus
Central sulcus
Intraparietal sulcus
Posterior parietal lobule
SI
SII
Lateral sulcus
B
Postcentral gyrus
Central sulcus
Intraparietal sulcus
1
2
3b
3a
4
5
7
Cutaneous input
Deep input

Recent studies

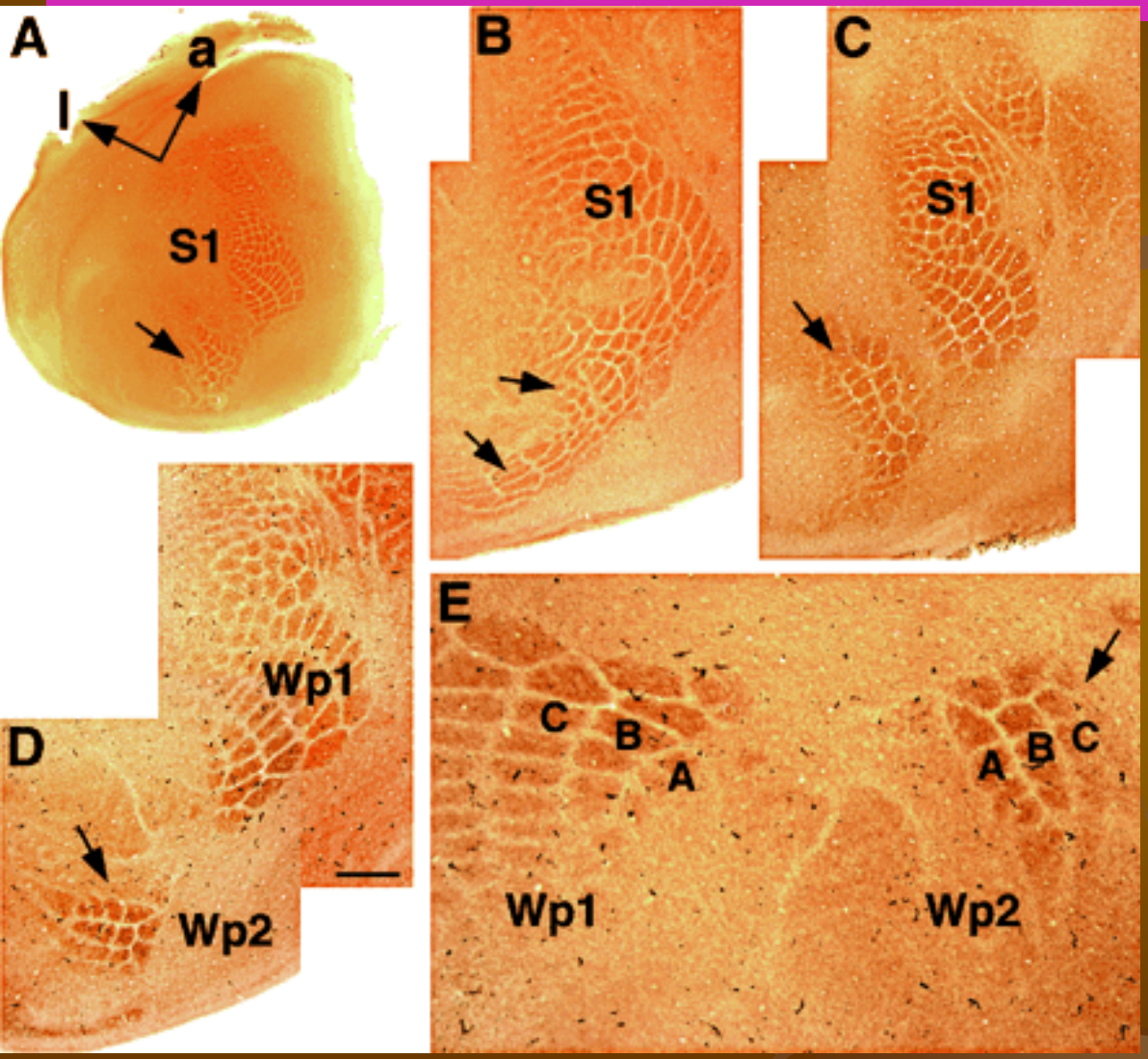
- Area map is patterned similar to those used elsewhere is the embryo: i.e. signaling molecules secreted from signaling centers establish positional info
- Expression of transcription factors (TF) leads to the patterned tissues differentiation.
- Reported the TF that are critical to cotical area patterning-→ consistent with protomap

Study by Mallamaci et al….



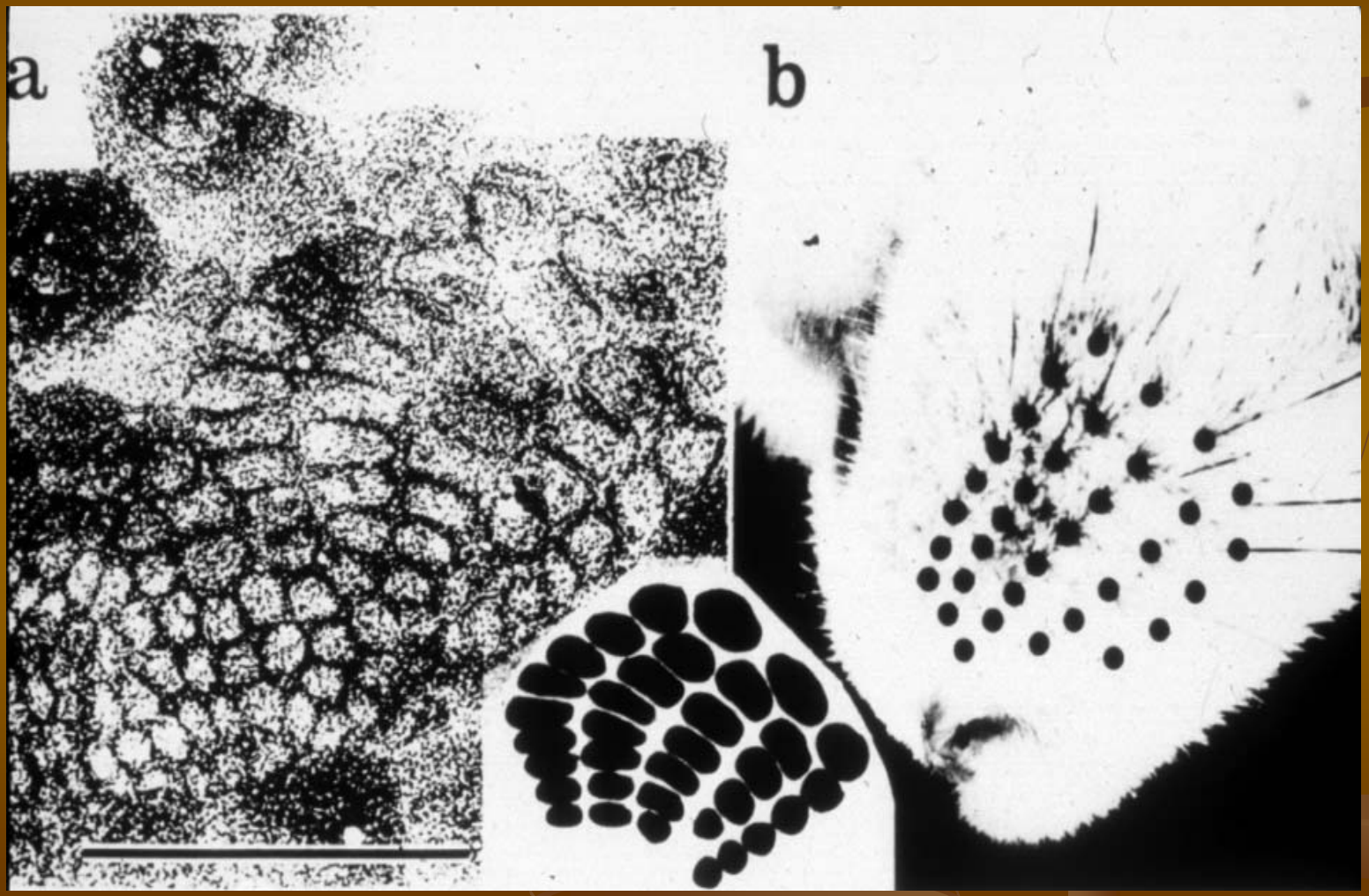
In *Emx2* -/- mice, found that the normal spectrum of cortical areal identities was encoded in these mutants, but areas with caudal–medial identities were reduced and those with anterior–lateral identities were relatively expanded in the cortex.

Previous Study by the same group…



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.

Barrel Field



Key finding

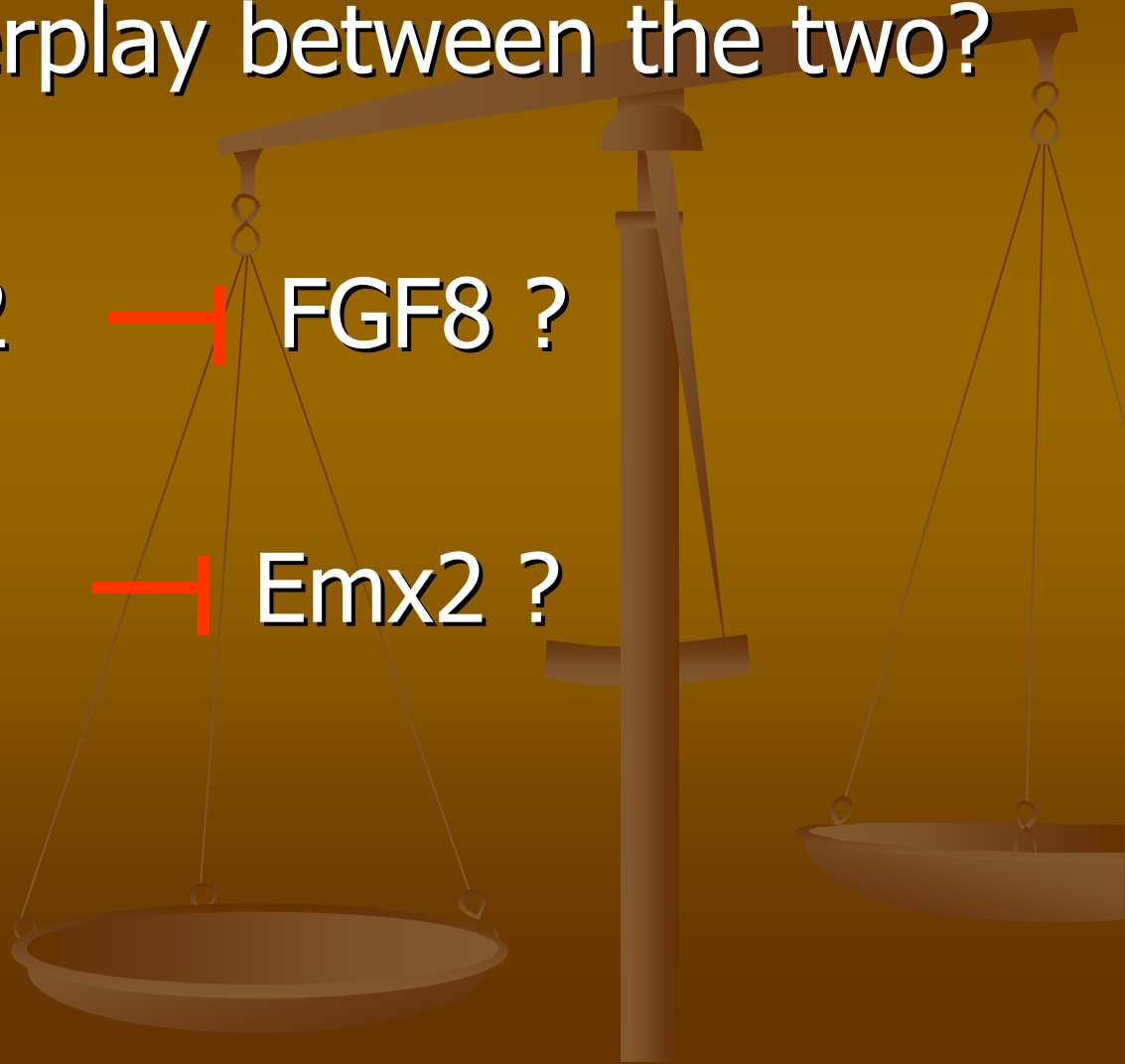
- FGF8 (growth factor) and Emx2 (TF) are closely related in patterning function along the anterior/posterior (A/P) axis.
- Increase in FGF8 or depleting Emx2 ---→ strikingly similar area map shift!
- Anterior domains enlarged and posterior domains shifted back and shrunken.
- The two show complementary expression pattern.

Question:

What is the interplay between the two?

Emx2 ⊣ FGF8 ?

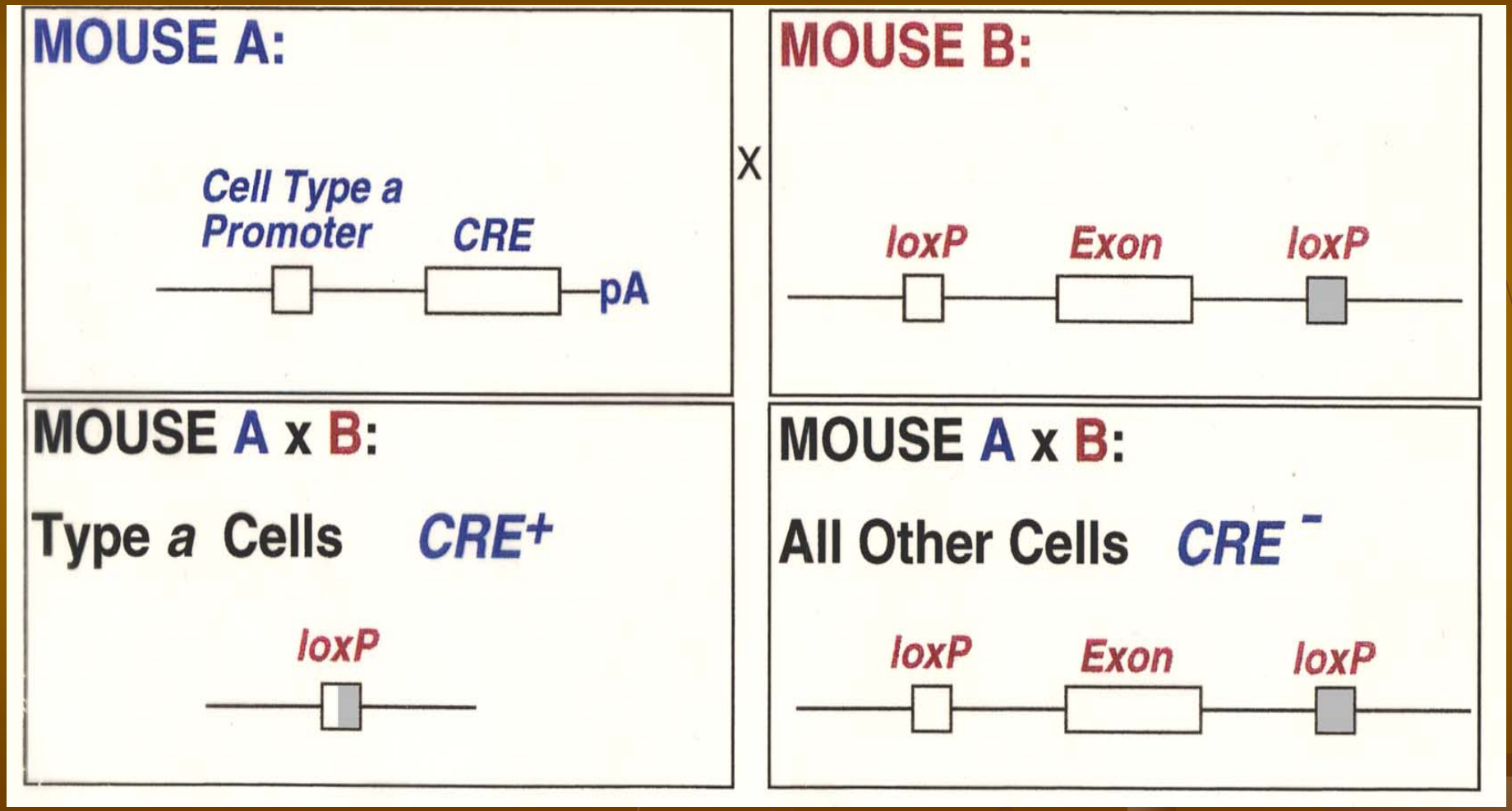
FGF8 ⊣ Emx2 ?

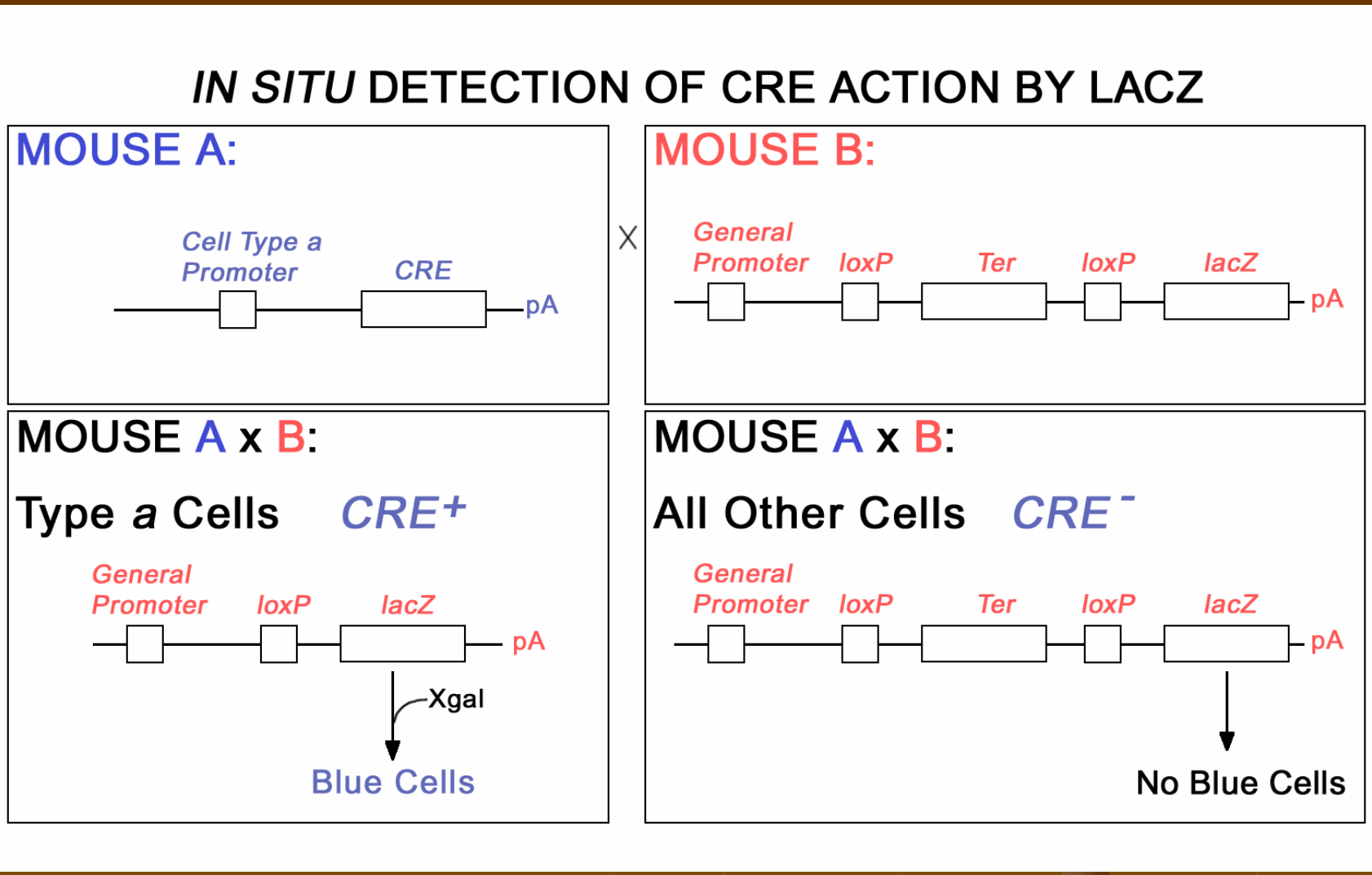


Methods:

- Used D1 mice for their increased survival of electroporation.
- Cre/LoxP reporter system of R26R mouse line.
- In-situ hybridization
- In-utero and ex-utero electroporation
- Counterstaining: staining used in conjunction with another reagent with greater specificity to provide more contrast and structure of the tissue.
- Histochemistry: detection of chemical composition of tissues by means of specific staining reactions (eg cytochrome oxidase).

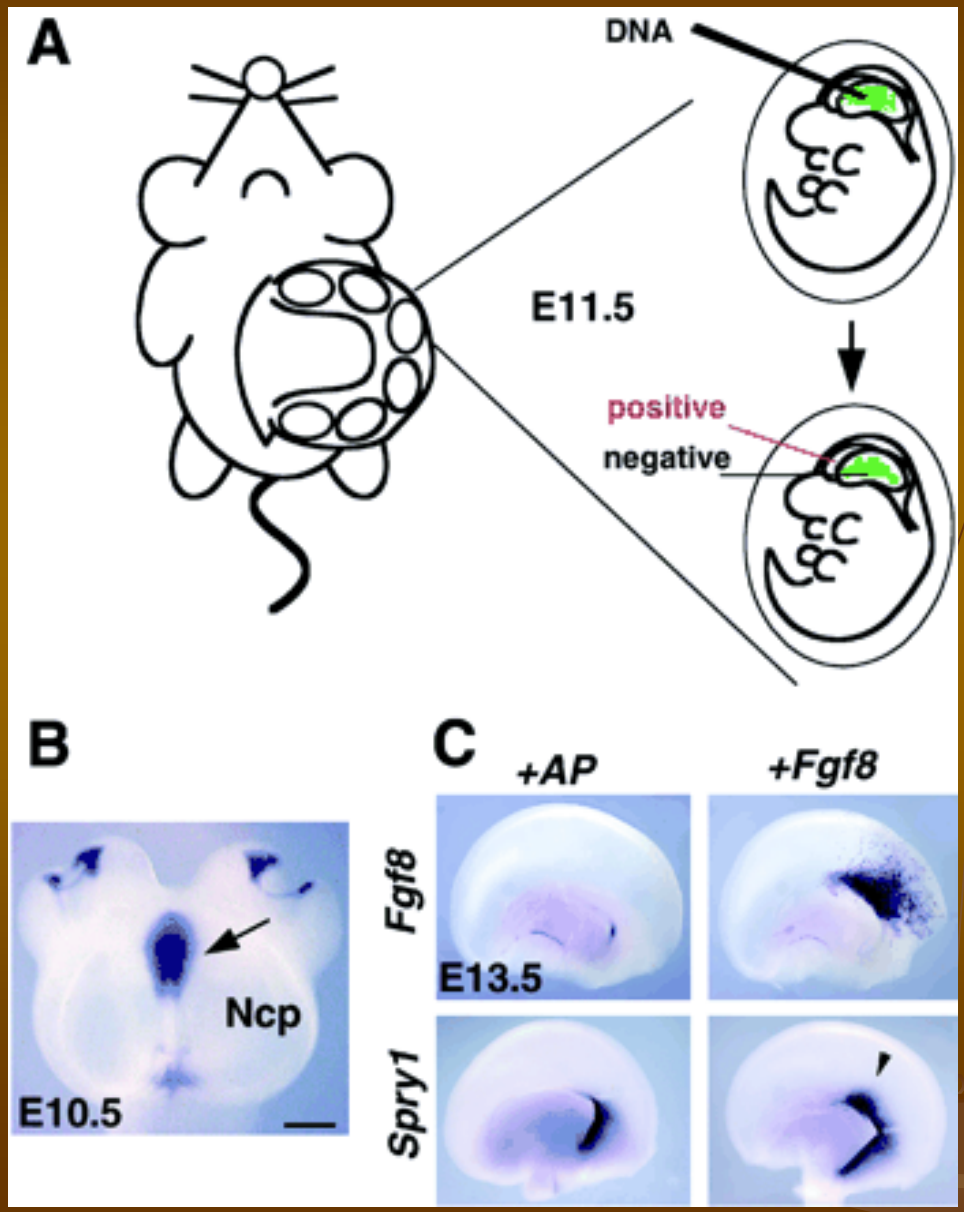
Cre/LoxP reporter system





In our case, the Cre protein is introduced later, not in the genome.

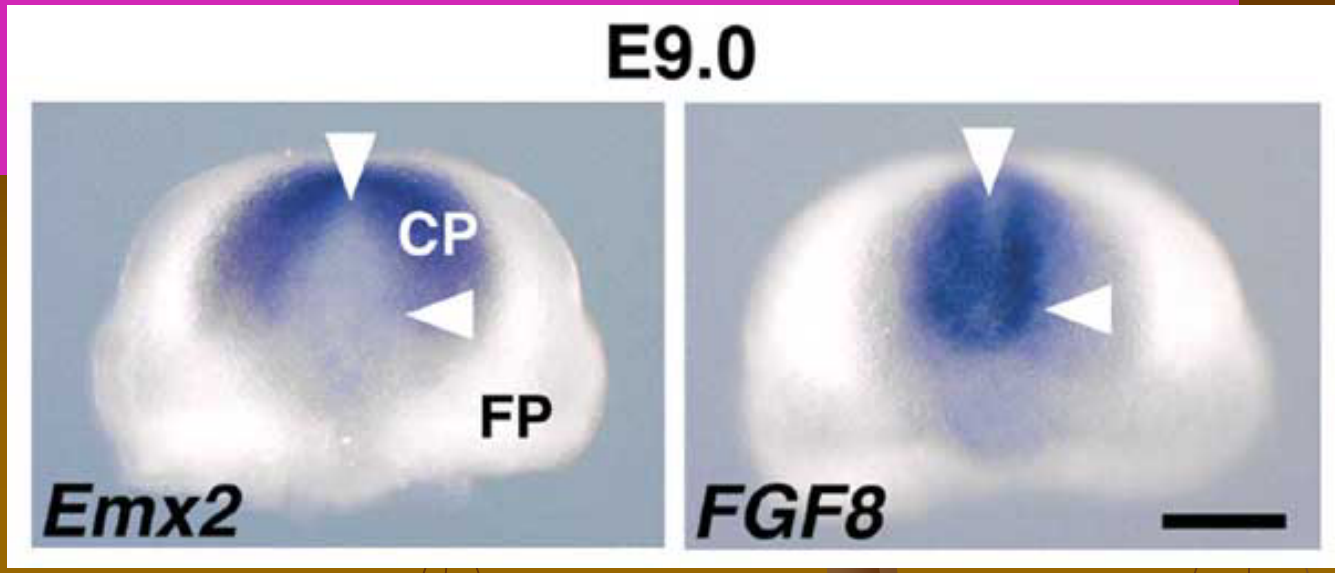
In-utero and ex-utero electroporation

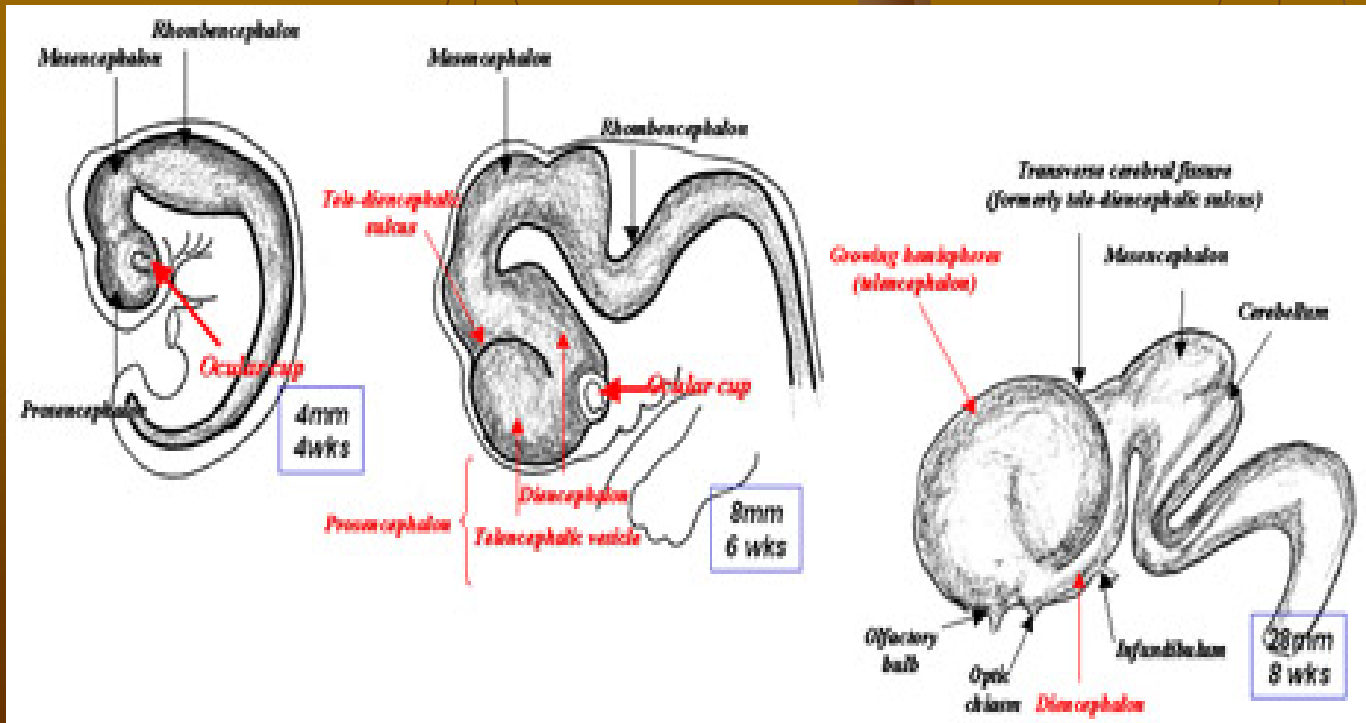


- IUEP is only 50% accurate and thus, post hoc confirmation is needed. EUEP is ~100% accurate because no uterine wall obscures the embryo. Explants were maintained in vitro.

Results:

FGF8 and Emx2 are expressed in complementary pattern at E9. Emx2 is surrounding the anterior domain in which FGF8 is expressed.



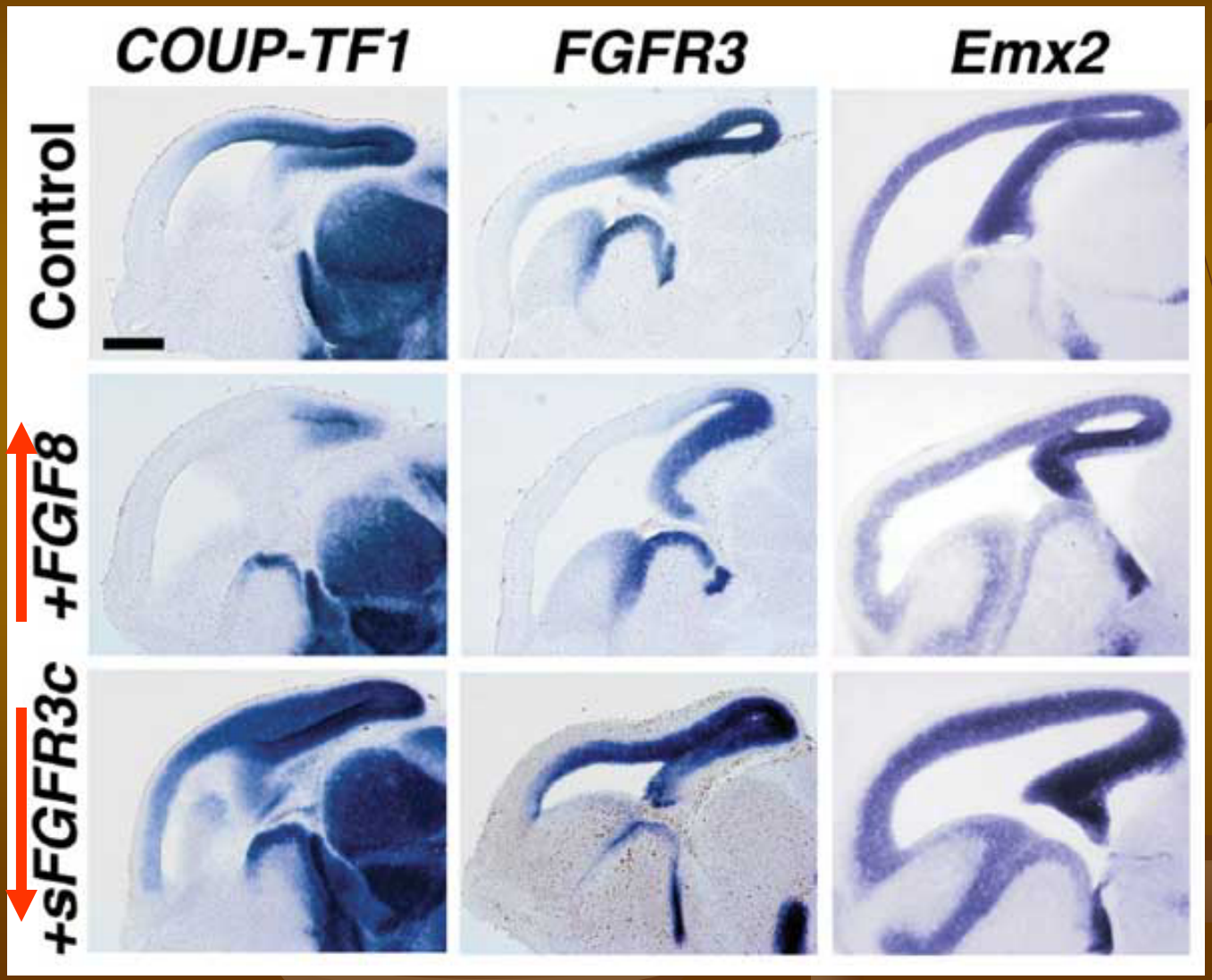


FGF8 regulates Emx2 gradient?

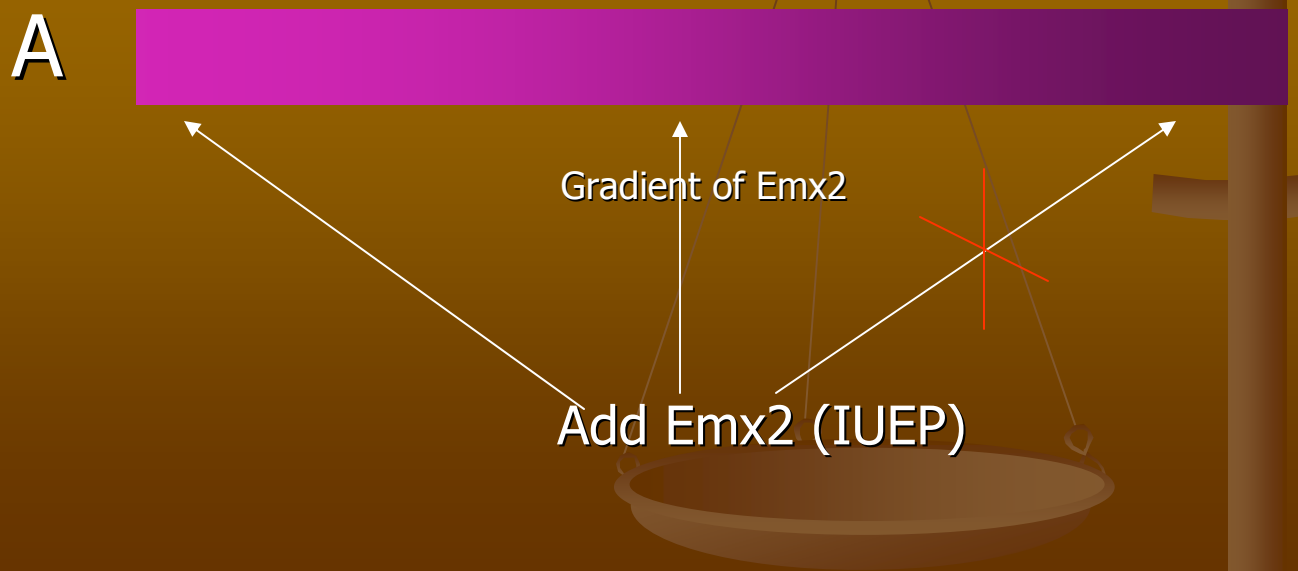
COUP-TF1: TF important in area patterning; FGFR3 WT receptor.

IUEP FGF8 into Ant. Cortical primordium → Posterior shift of Emx2, COUP-TF1, and FGFR3

IUEP truncated FGF8 R into Ant. Cortical primordium → anterior shift of Emx2, COUP-TF1, and FGFR3



- If Emx2 gradient indeed promotes different area fates along A/P axis, creating ecpotic Emx2 peaks in the gradient should alter the area map.
- But the effect of Emx2 is maxed out at the posterior cortex. So, focus on anterior and central, but not the posterior regions.



First, confirm that IUEP can be regionally targeted by electroporating AP at E10.5 (latest to affect patterning) and analyze 1d after.

EP cells are densely packed soon after IUEP.

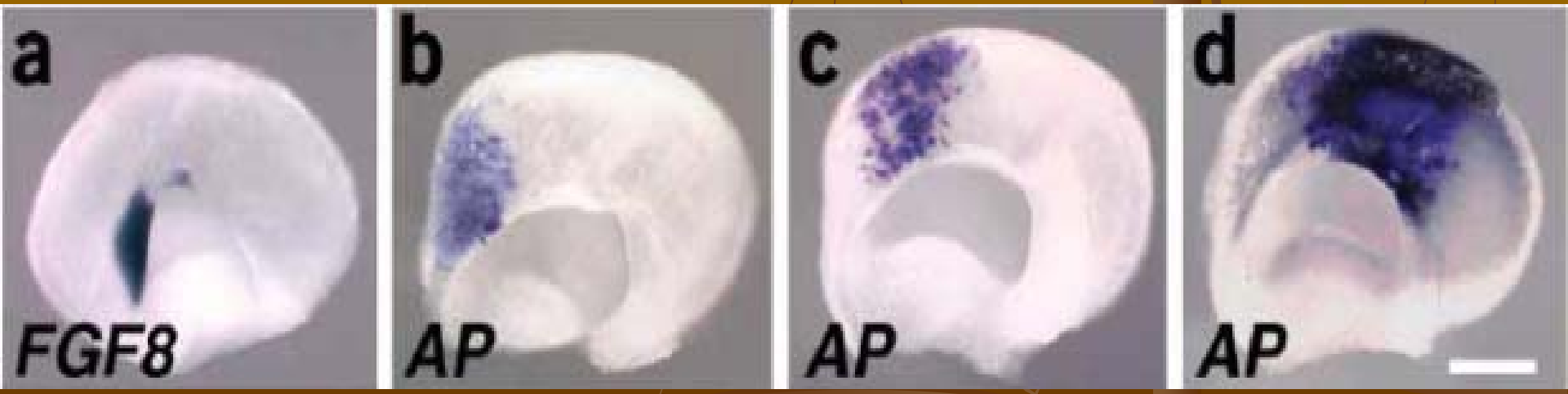
A

Completely overlapping

Partially overlapping

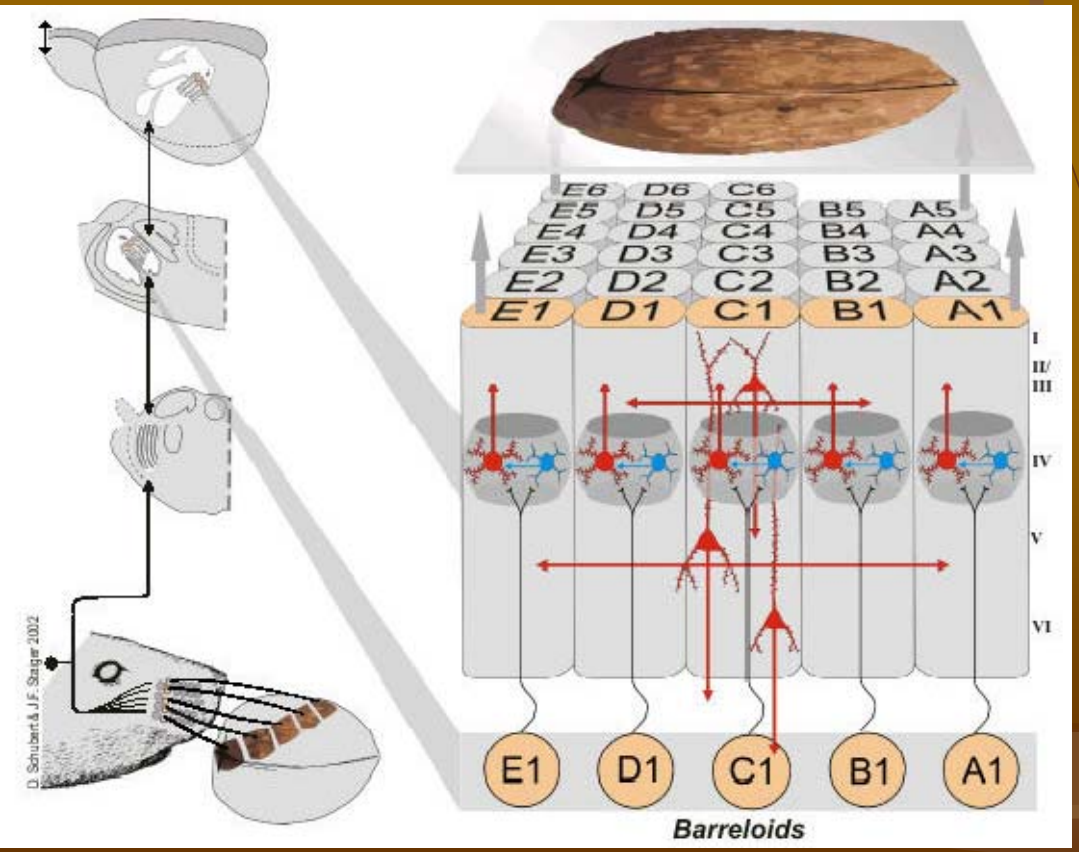
Not overlapping

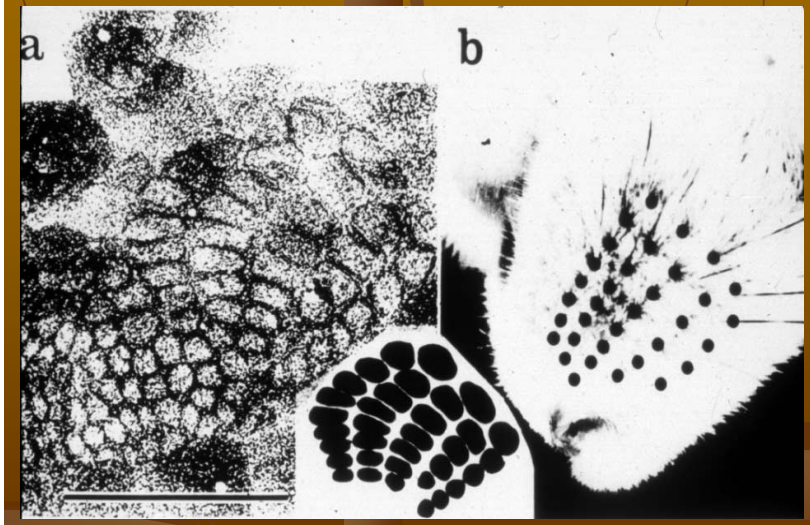
P



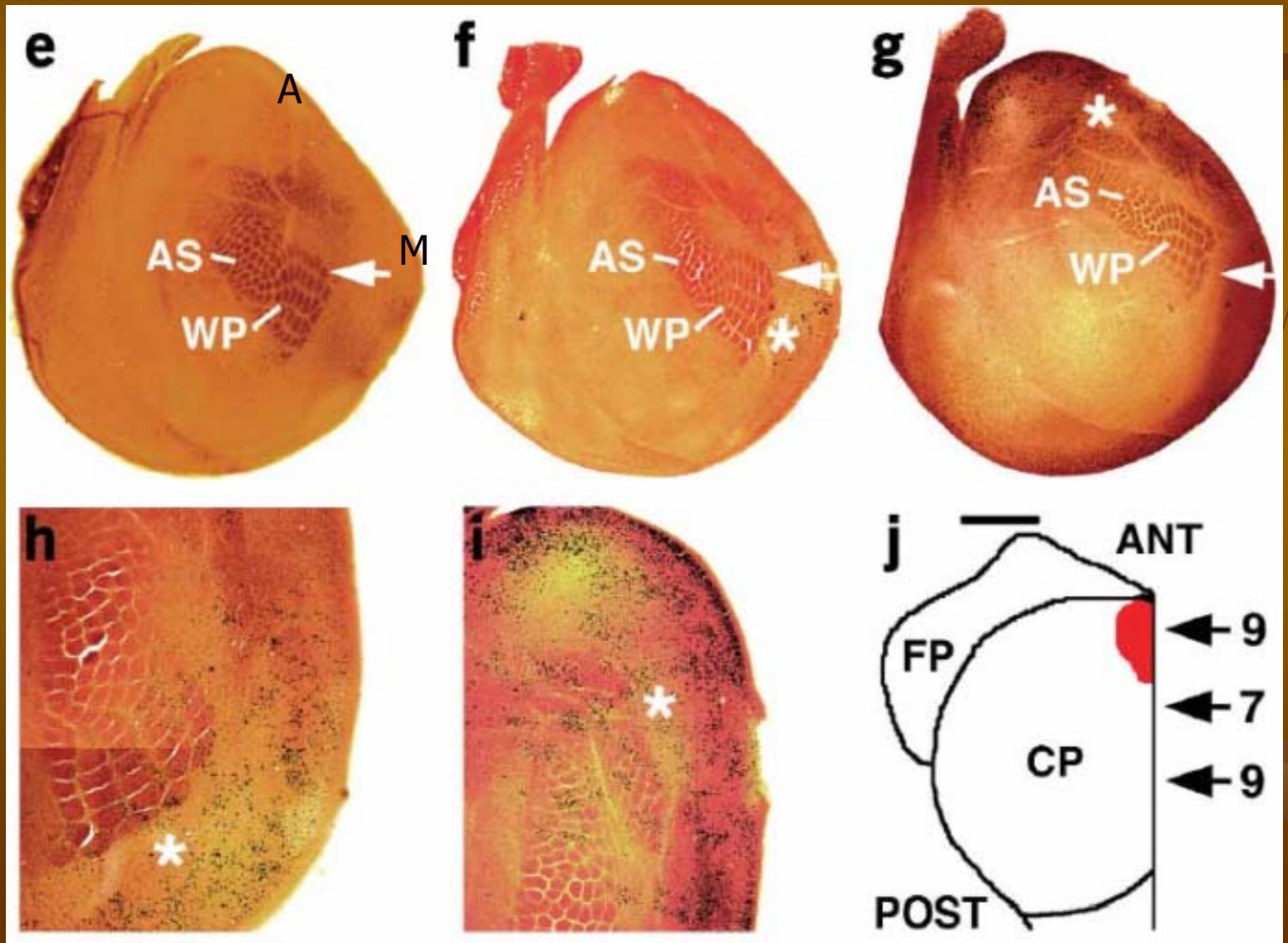
-- To check the EP site post hoc at P6, co-EP Emx2 with Cre at E10.5 of R26R mouse and do X-gal histochemistry.

-- CO stain, counterstained with X-gal to show region of EP.





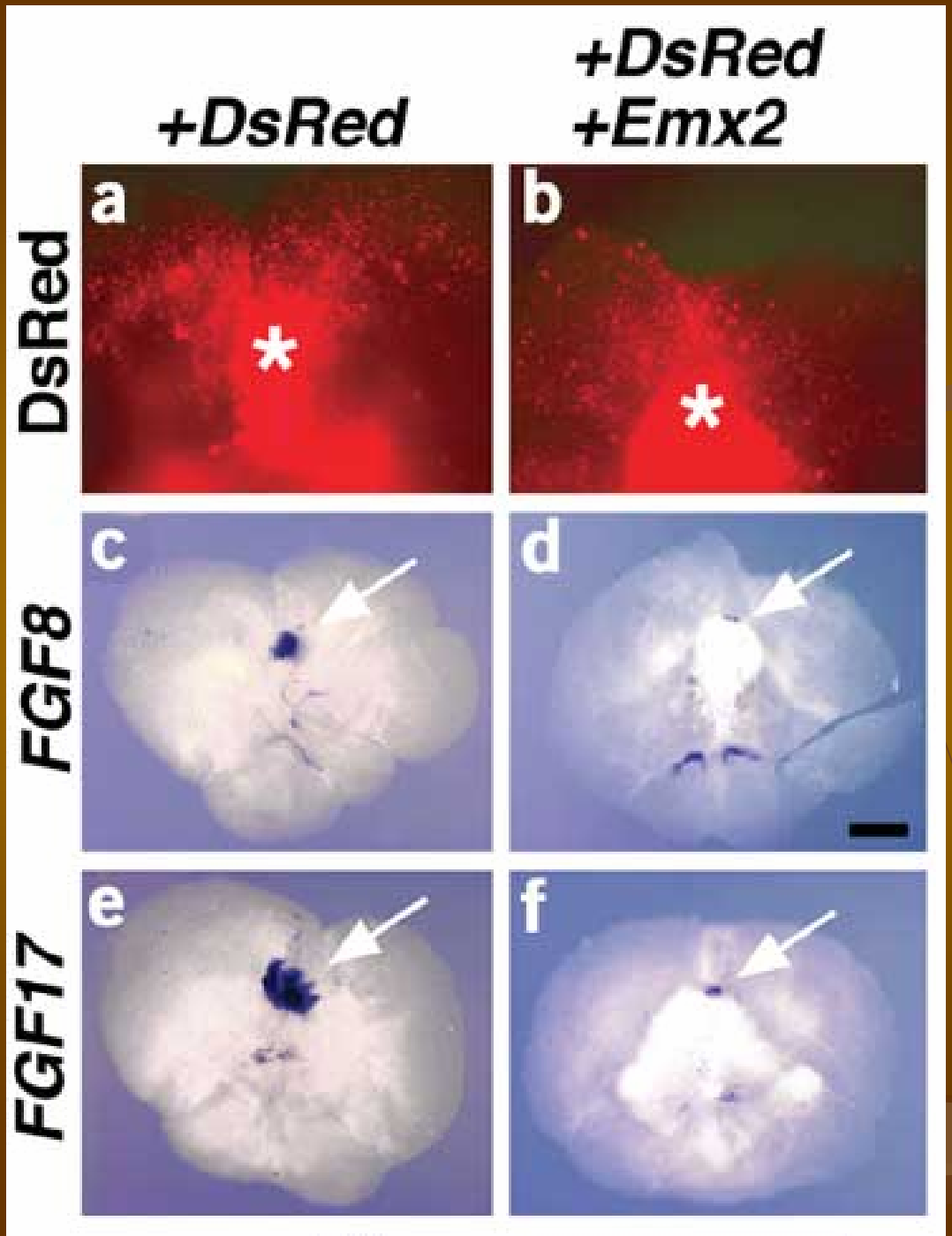
-- P6, cells in layer IV are not as dense, diluted in cortical growth.
-- e: control, h,I = larged f,g AS: anterior snout, WP: whisker pad, #: target replication, red: source of FGF8; (f similar site as d), g (b+c): ant., near mid-pt
Emx2 overexpression shifted the barrel field into anterior half of neocortex!!!!



What do the results mean?

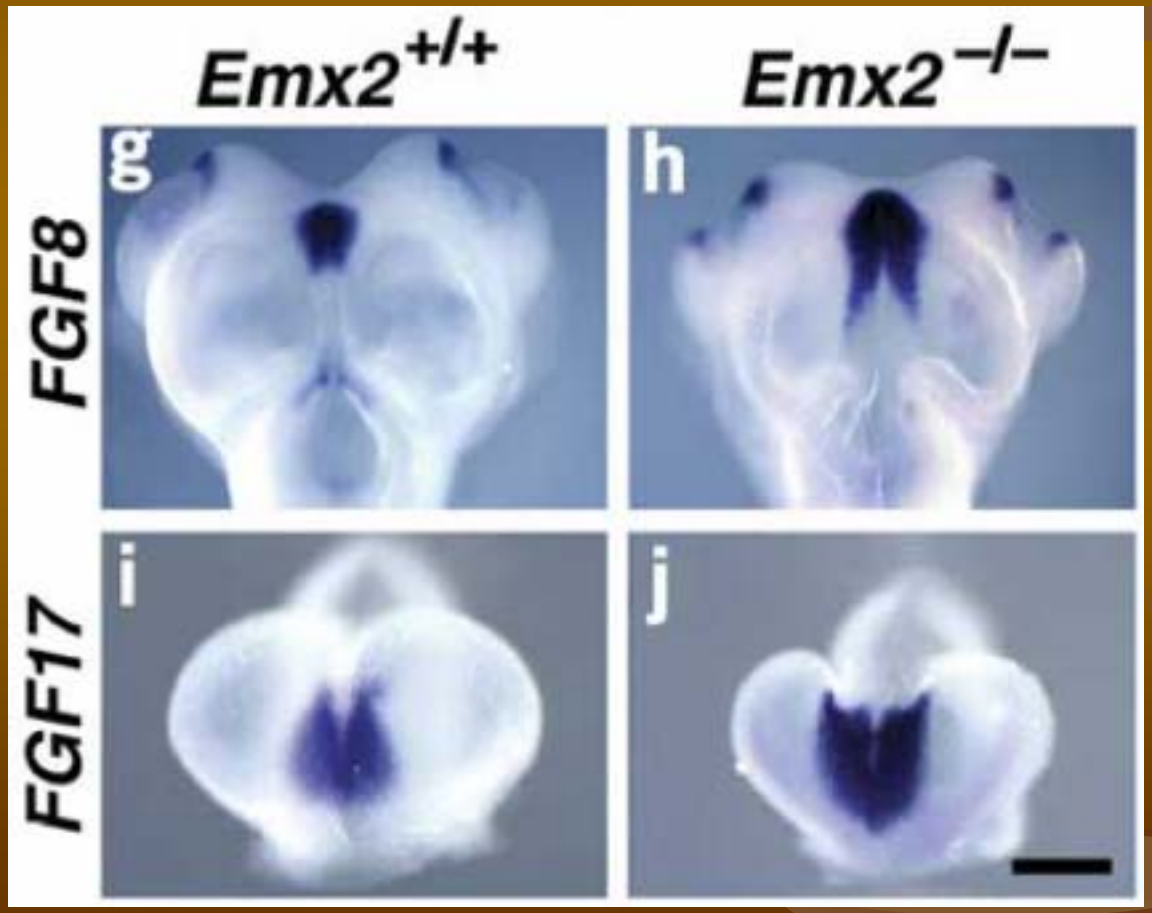
- Excess anterior Emx2 transforms frontal cortex into the normally more central barrel fields → increase posterior identity.
- Emx2 can alter area map only if it overlaps FGF8.
- S1 lies about in the middle of the Emx2 gradient, excess Emx2 at these central sites should promote a more posterior fate, or force S1 to shift or disappear IF Emx2 gradient specifies area fate. But S1 did not change, → Emx2 gradient does not specify area fate.
- Previous studies showed that similar S1 shift was observed when $[FGF8]_{\downarrow}$ (sFGFR3c), hypothesis:

  Emx2 regulates size and signaling capacity of FGF8 source in anterior cortical primordium.



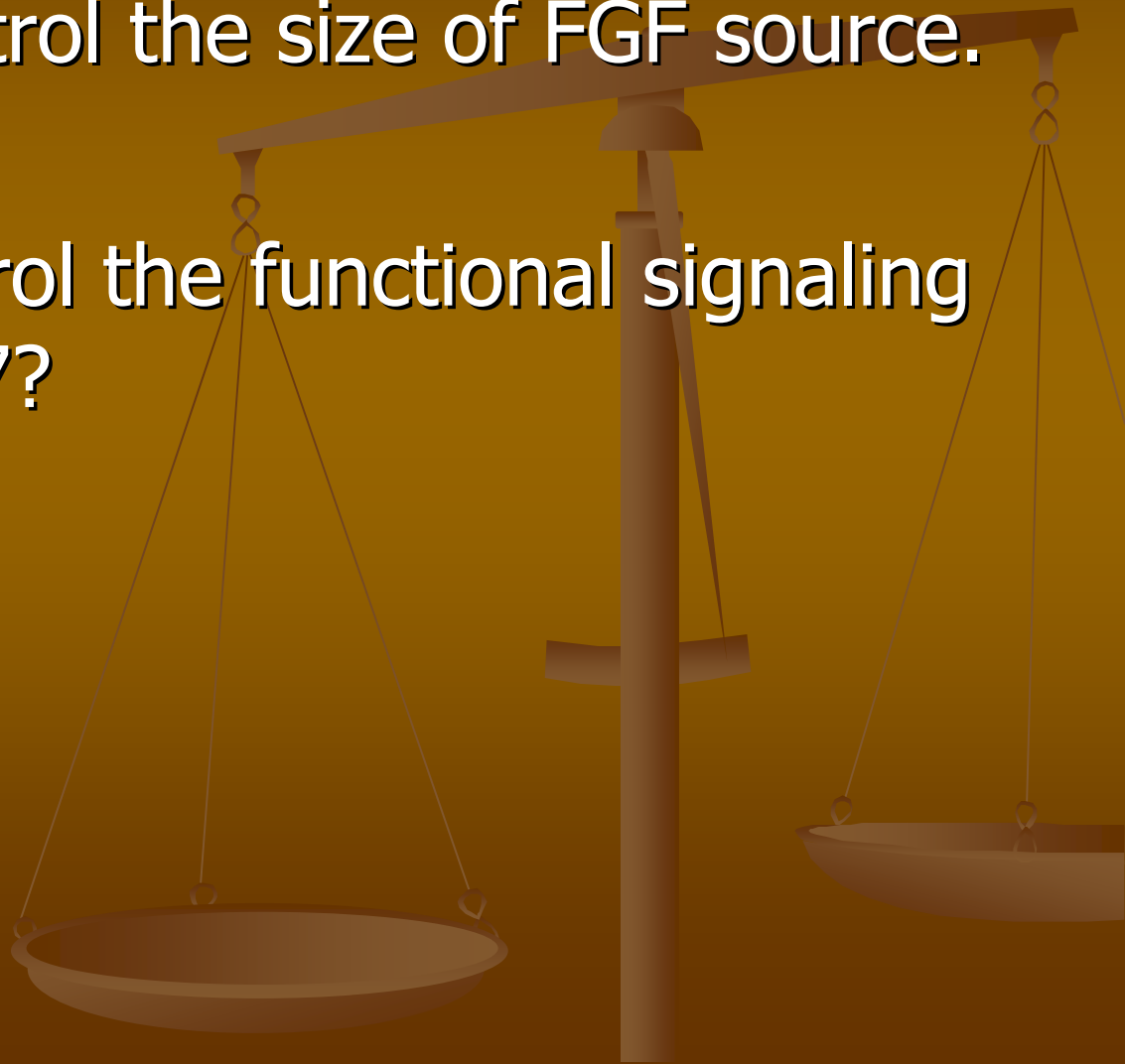
- FGF8 subfamily has more protein, but these 2 have similar effect on neocortex. So, sFGFR3c will sequester both.
- Red: EUEP site
- DsRed alone allows high expression of FGF8/17, as in vivo.
- Emx2 down regulates FGF8/17 expression in the ant. C.P.

An opposite experiment was carried out to determine whether loss of Emx2 increases FGF8/17.

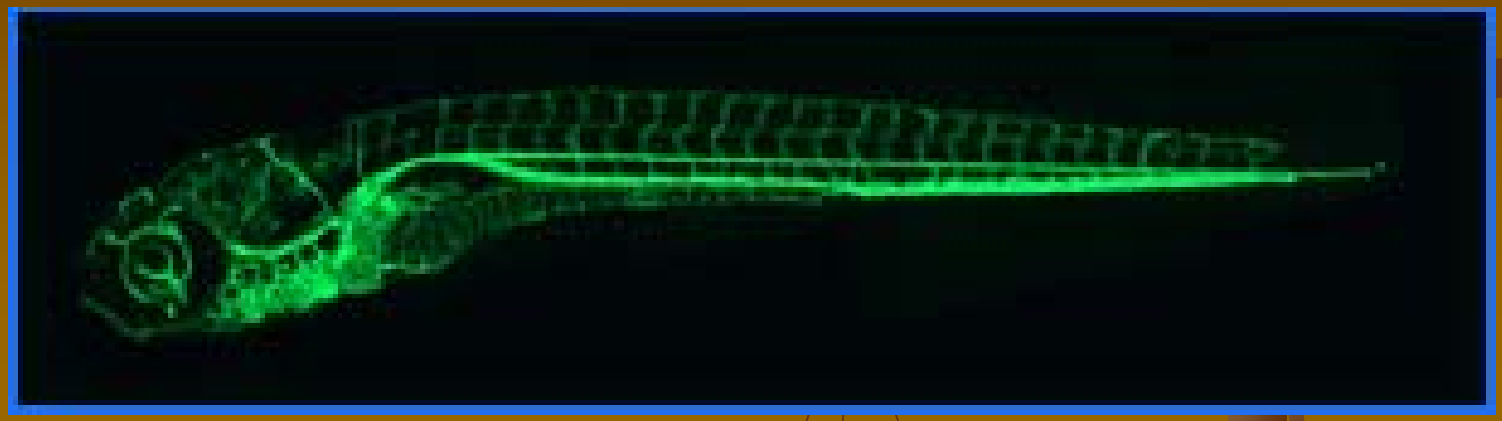


- Homozygous mutants used
- FGF8/17 domains are larger in abs size and proportionally.

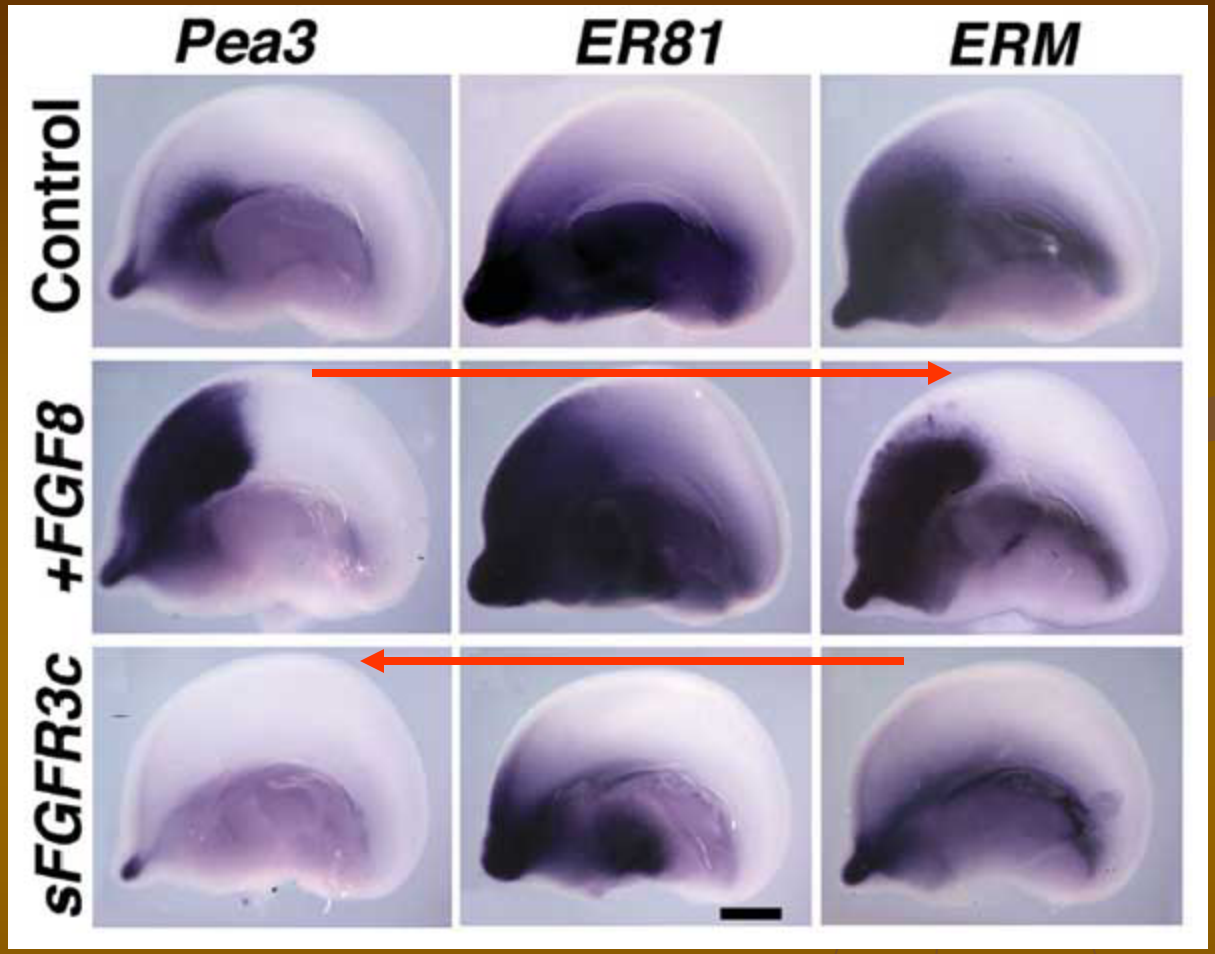
- Emx2 DOES control the size of FGF source.
- Does Emx2 control the functional signaling byb FGF8 and 17?



To identify the read-out of FGF8/17 protein activity in mouse CP.



- Known: zebrafish, Pea3 subfamily of ETS TF is tightly controlled by FGF8.
- How about the mouse homolog of Pea3, ERM and ER81?



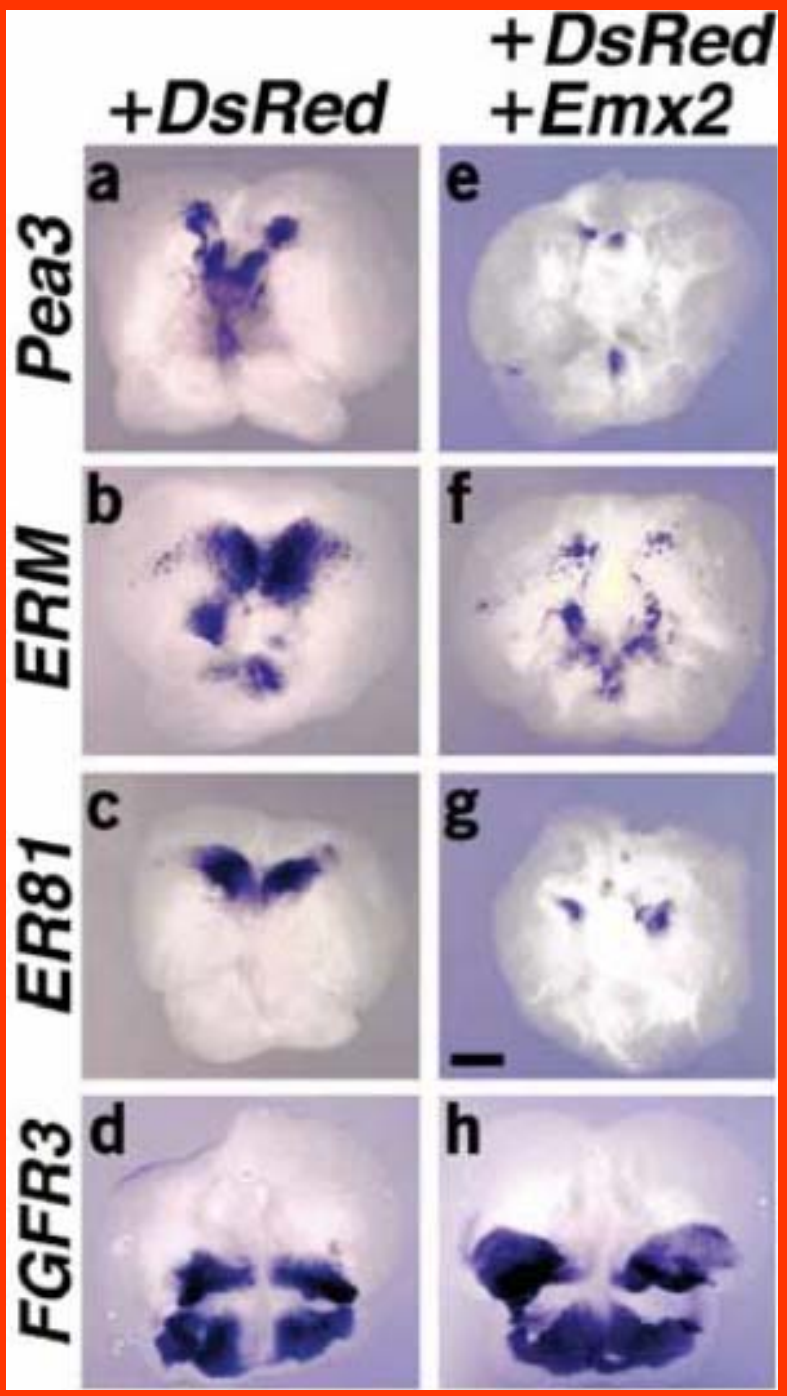
Cortical Hemispheres viewed from the medial face. A: left

In-situ hybridization 2 d after IUEP

All 3 found in the anterior half of the CP

3 domains have diff posterior boundaries

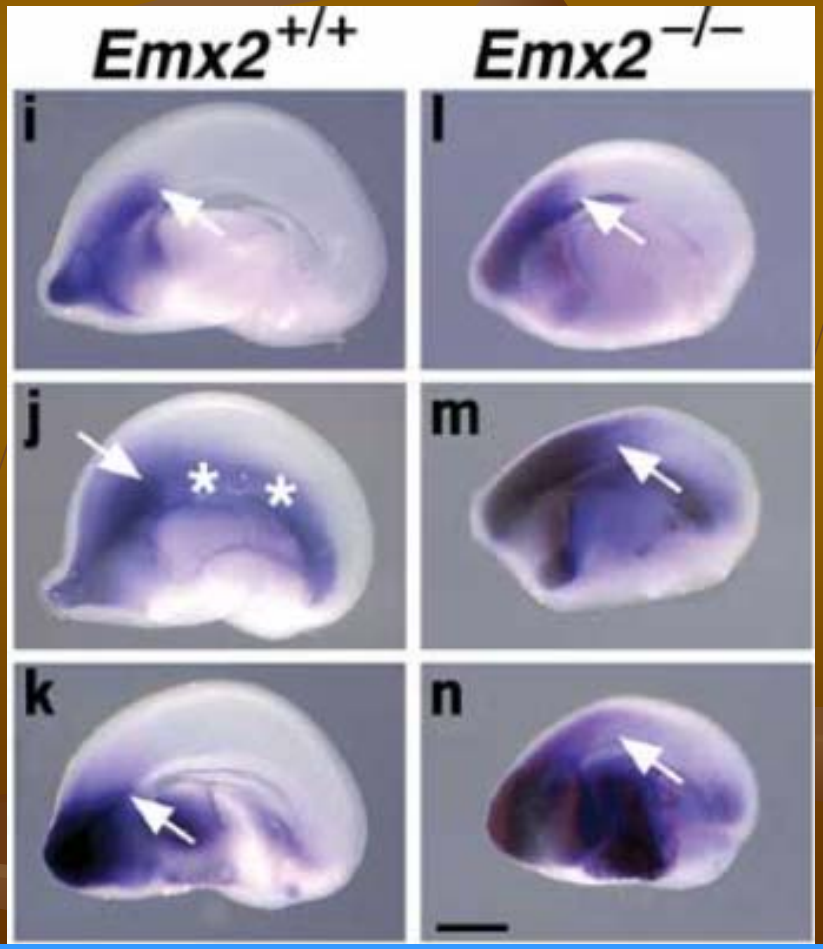
In mouse, Pea3 subfamily is regulated by FGF signaling.



But, overexpression of Emx3 in explants decreased the expression of the three

Also, it shifted FGFR3 expression toward anterior limit

All Emx2 mutants showed expansion of expression of the three



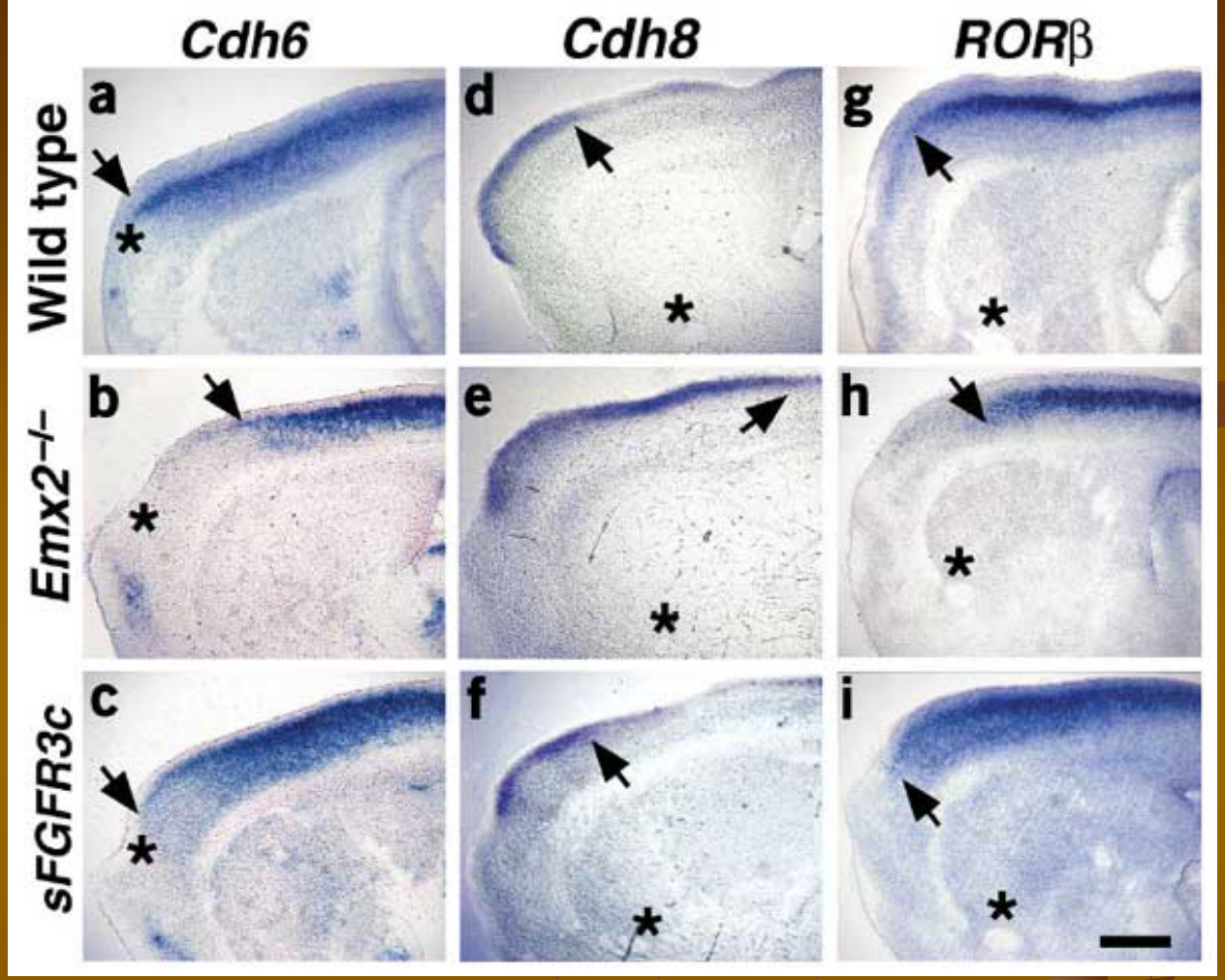
Which means…..

- FGF8 signaling is lower than in control explant at the presence of Emx2
- From FGFR3 result, Emx2 did not show a cell-autonomous effect in promoting a posterior identity as it did not upregulate gene expression of FGFR3.
- Emx2 seems to act globally to shift expression boundaries (like a decrease in FGF8/17)

This leads to a hypothesis that domain shift in neocortex of Emx2 mutant mice (not viable beyond birth) could be caused by excess FGF8/17.

No Emx2→excess FGF8/17 →domain shift

If ↓FGF8/17 → rescue domain shift in $Emx^{-/-}$?



- Look at E18.5 mutant, and regional expression pattern of RORβ, Cadherins 6 & 8.
- Mutant: the boundaries of Cdh6 and RORβ shifted posteriorly with Cdh8's exp expanded posteriorly.
- ↓ FGF8/17 in mutant by introducing sFGFR3c, boundaries go back to WT.

- Rescue was more complete in lateral than medial CP → truncated receptor did not sequester all excess FGF ligand.
- FGF8/17 can regulate the position of neocortex gene expression boudaries without Emx2.
- Emx1 can't substitute Emx2's cortical patterning. Thus, its expression in mutant does not partially rescue area pattern.

Conclusion

- Excess Emx2 decreases FGF8 level and vice versa.
- FGF8/17 manipulations can shift area pattern in the absence of Emx2.
- Emx2 is crucially involved in neocortical area patterning by controlling the extent of a primary signaling source (gives rise to FGF8/17).

Discussion

- Since the FGF8/17 expression does not spread throughout the cortex in Emx2 null mutant, indicating that Emx2 interacts with other factors to control the source.
- Other studies showed that Gli3, a zinc finger TF is part of this regulatory network.
- GOF study showed that Emx2 can't transform area fate. Its gradient is neither nece nor suff to regulate patterning directly.
- Emx2 regulates FGF8/17 and FGF8 regulates Emx2 expression also → feedback mechanism