BMP inhibition initiates neural induction via FGF signaling and Zic genes

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Zic (vertebrate homologues of odd paired genes in drosophila) – zinc –finger proteins control the initial phase during which ectoderm differentiates into neuroectoderm

1. What were the premises to carry out this work???

2 models to explain neural induction emerged by studies on Xenopus and Chicken
1) default model (BMP inhibition)
2) FGF signaling **instructive functions** + BMP inhibition

→Neural fate requires BMP-inhibition – that is clear…**but is BMP inhibition sufficient for neural induction??**

Previous experiments:

Establishment of a paradigm to define validity of default model in frog:
micro-injection of BMP inhibitors in ventral ectoderm (16-32 cell embryos) that normally give rise only to epidermal cells

-these cells are competent for neuralization BUT injection with **SMAD6** or a dominant negative BMP receptor do not neuralize (is inhibition really efficient in these models?)

However…

-low FGF4 + BMP inhibitors → neural induction → these results support a combinatorial model

2. What are the specific objectives and the experimental strategies of the current work ?

1. To clarify the **role of FGF** in neural induction

2. To verify if **BMP inhibition** is not only **necessary** but also **sufficient** to induce neural induction

3. To identify **immediate early targets** for neural induction

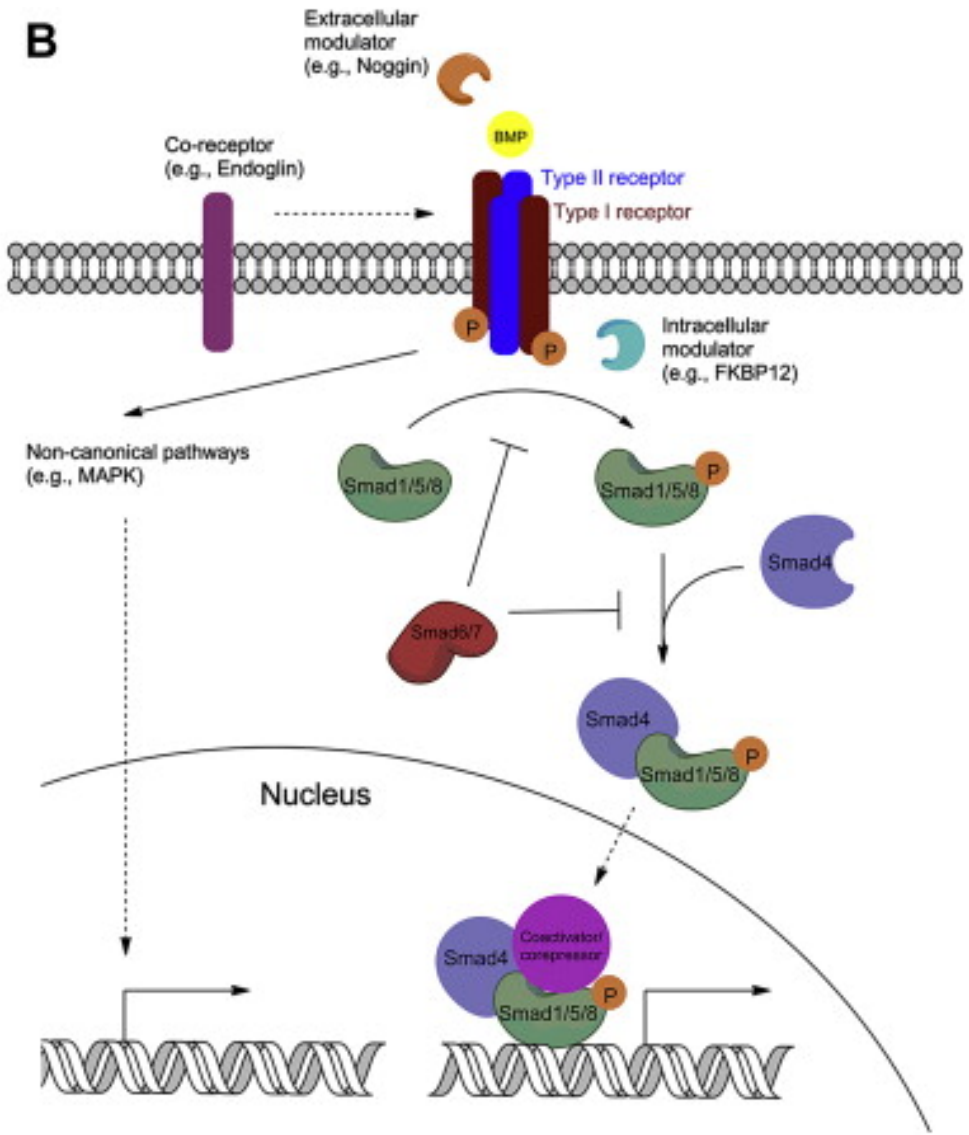
Experimental stategies

- *smad5-somitabun* mRNA injected in the ventralmost animal blastomeres of 16-cell embryos

- Use of cycloheximide (translation inhibitor) in whole embryos to test direct effects on gene transcription

- Loss of function analyses to test the role of Zic1 and Zic3 in neural induction (morpholinos)

Anti-morphic form of SMAD5→ mutated murine Smad5 (similar to zebrafish somitabun mutation: prevent binding to co-Smad 4)



prevent binding of Smad5 to the co-Smad Smad4, but not to Smad5 itself

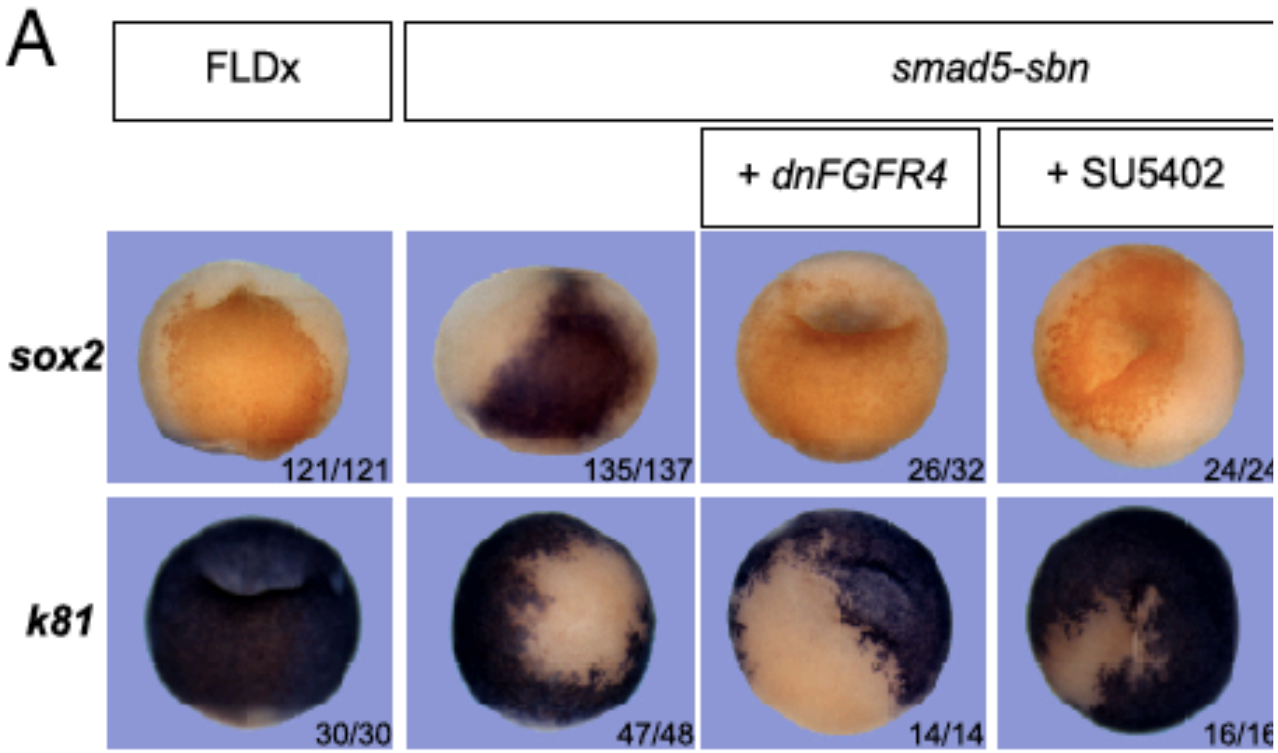
Smad5-sbn could form inactive heteromeric complexes with Smad5, Smad1, and perhaps also Smad8, thus efficiently shutting down BMP signaling at the lowest integration point in the pathway.

1 - In Vivo Neural Induction by BMP Inhibition

→ectopic neural induction

***Experimental approach**: smad5-sbn* mRNA was injected in the ventralmost animal blastomeres of 16-cell embryos
Analysis performed at late gastrula stage

Notes:
1) tracing of cells by fluorescent dextran labelling → only injected domain becomes neuralized= cell-autonomous effect

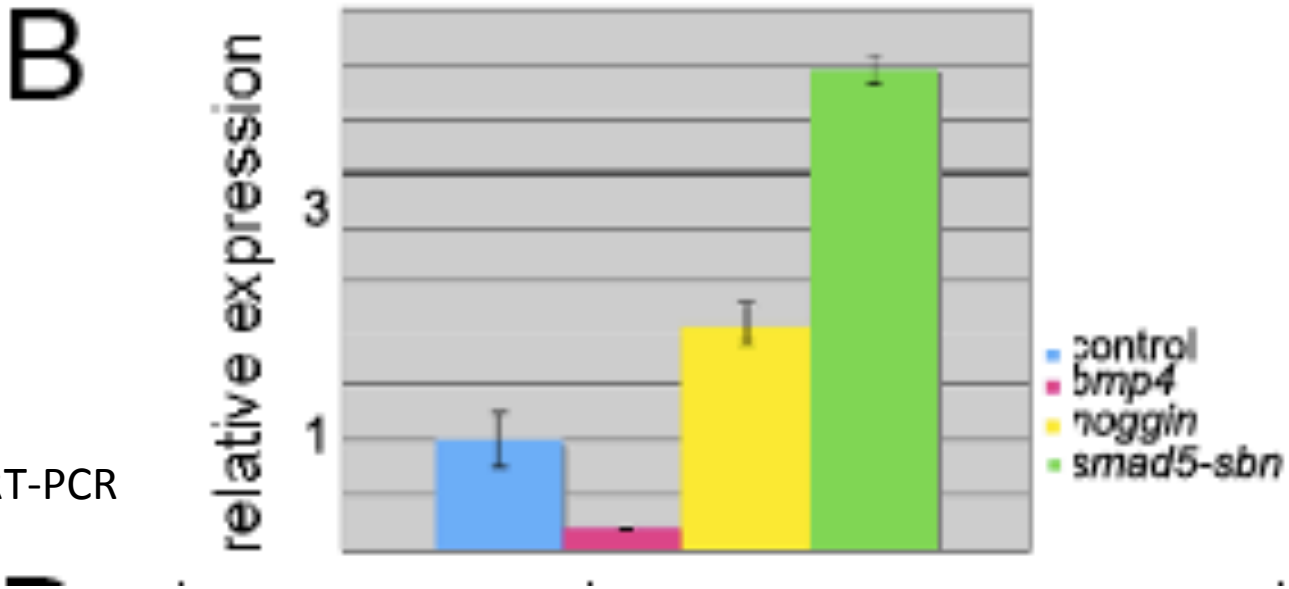


SU5402 is a potent multi-targeted receptor tyrosine kinase inhibitor with **IC50** of 20 nM, 30 nM, and 510 nM for VEGFR2, FGFR1, and PDGF-Rβ, respectively.

→ Neural induction by BMP inhibition depends on the presence of FGF activity.

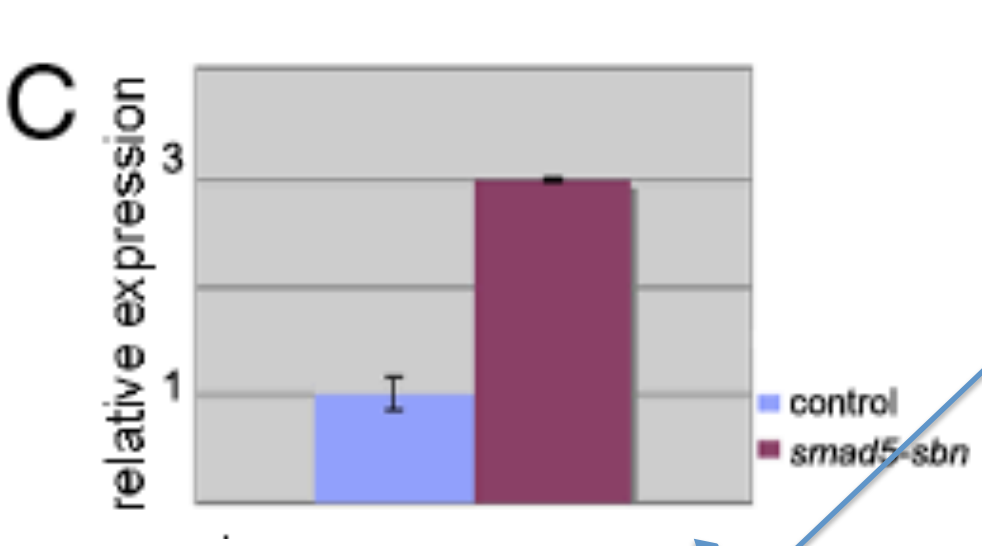
FGF activity could be resident in the ventral ectoderm or **induced by BMP inhibition**

FGF4 expression at stage 10.5
whole embryos

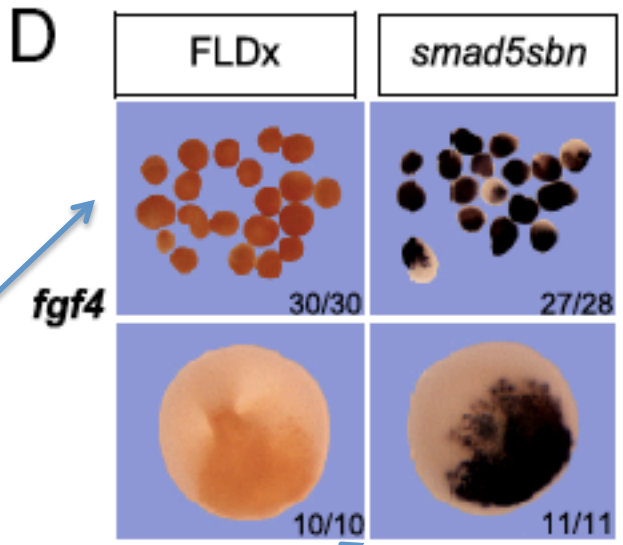


Quantitative RT-PCR

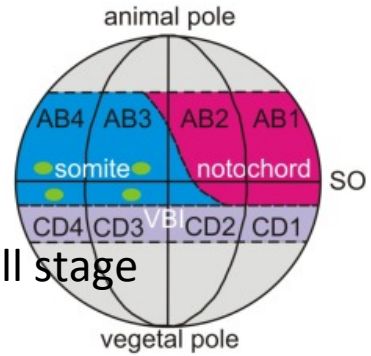
Smad5-sbn activated *FGF4* expression in AB4 descendants and in animal caps



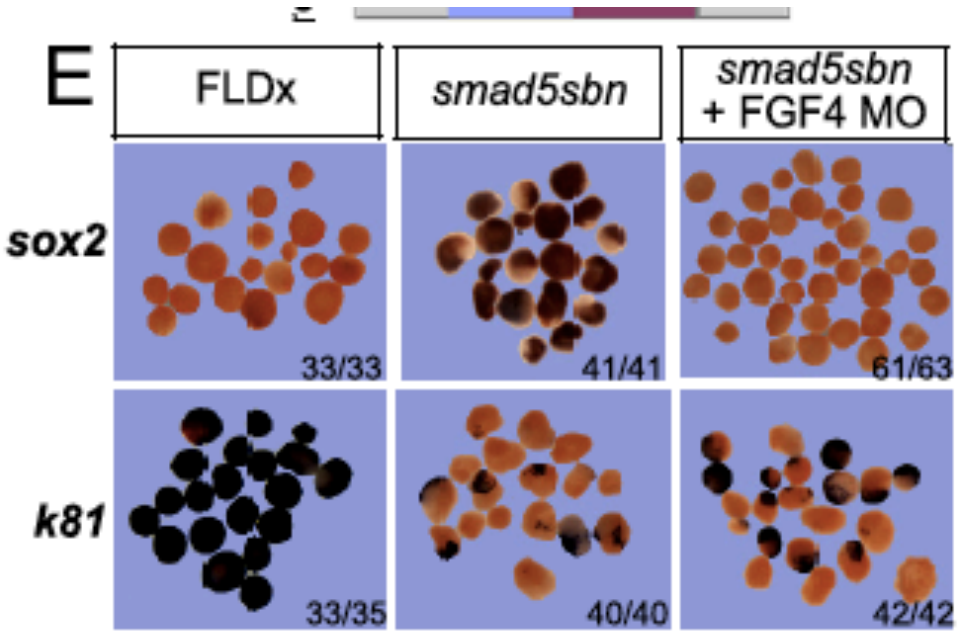
FGF4 expression at stage 10 of **animal caps** taken at late blastula stage from embryos injected in all cells at 4-cell stage



Ventral view
Stage 10 embryos
Injected in AB4 at 16-cell stage

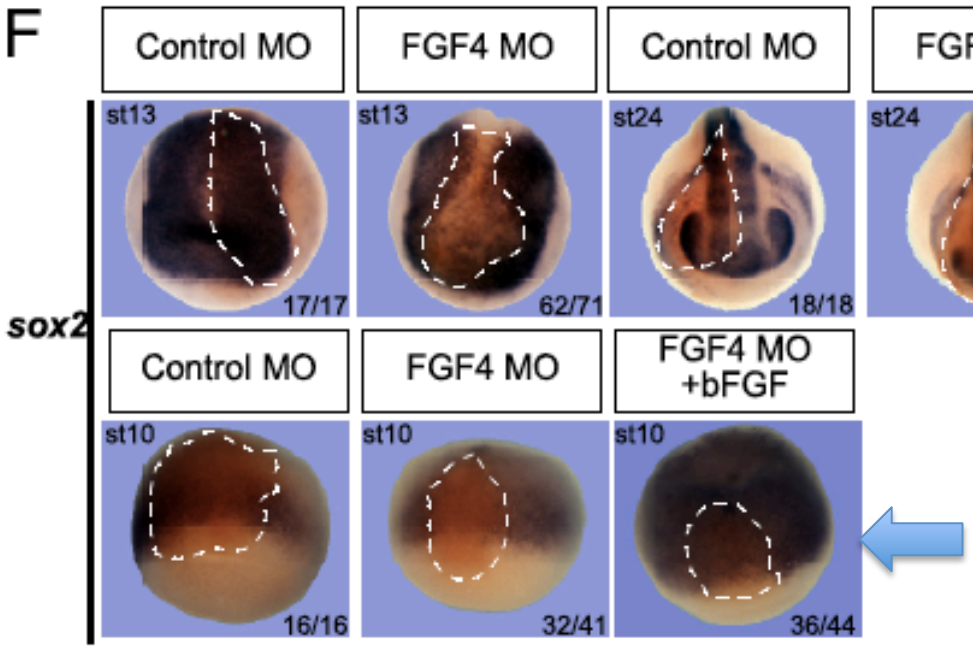


Neural induction by BMP inhibition might depend on induced *FGF4* activity



knocking down FGF4 with a translation-blocking morpholino-modified antisense oligonucleotide

FGF4 knockdown
Suppresses *sox2* activation by Smad5-sbn without restoring *k81* expression



Endogenous *sox2* expression in the developing neural plate was down-regulated upon *FGF4* MO injection in the marginal zone, the normal site of expression of *FGF4* from the early gastrula to tailbud Stage

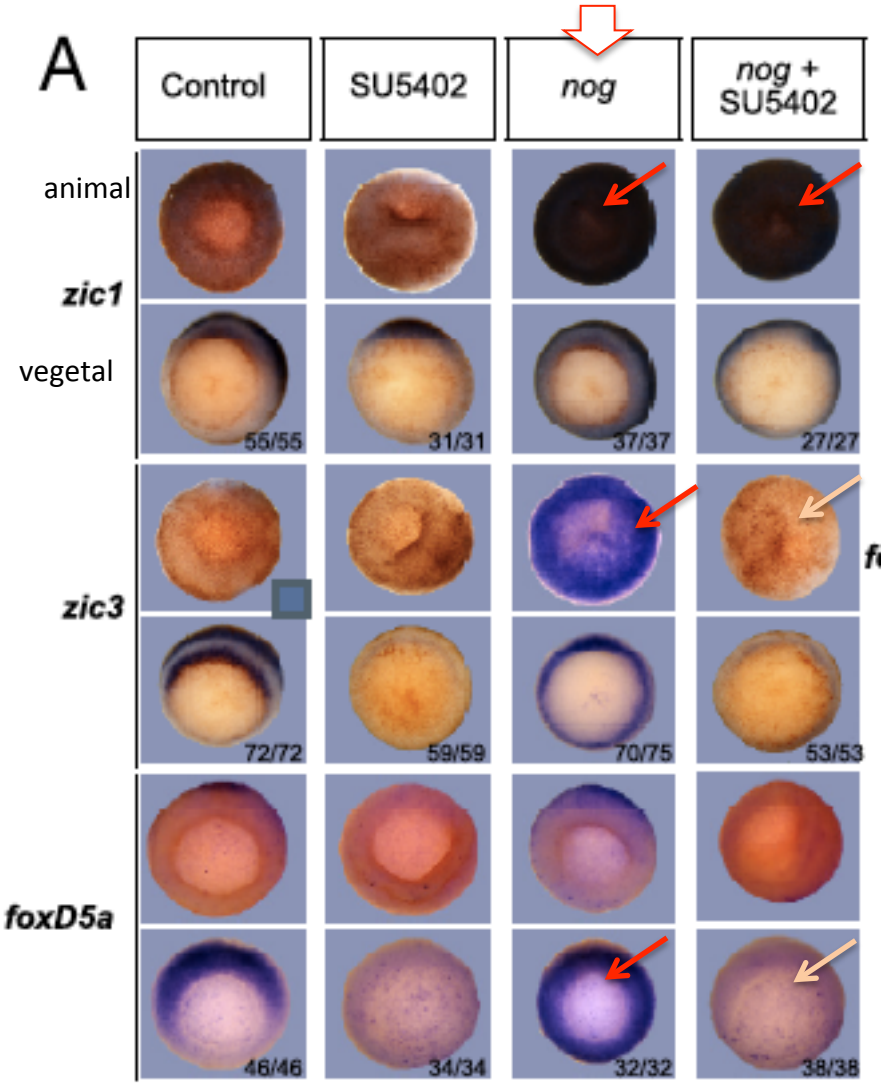
rescue

Recovered Sox2 expression in FGF4 morphant embryos injected at blastula stage with recombinant bFGF protein in the blastocele

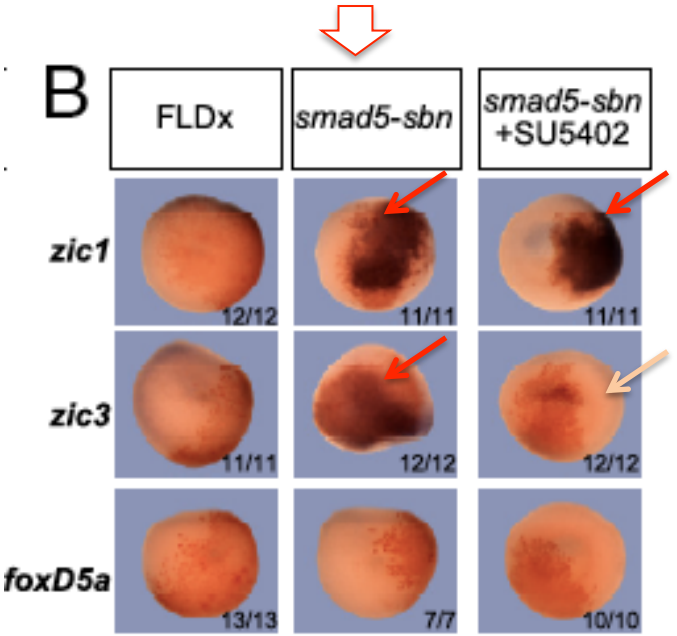
confirming that the lack of neural induction can be due to decreased FGF activity

(no change in BMP inhibitors expression in FGF4 morphant = here the role of FGF in neural induction is independent of BMP inhibition)

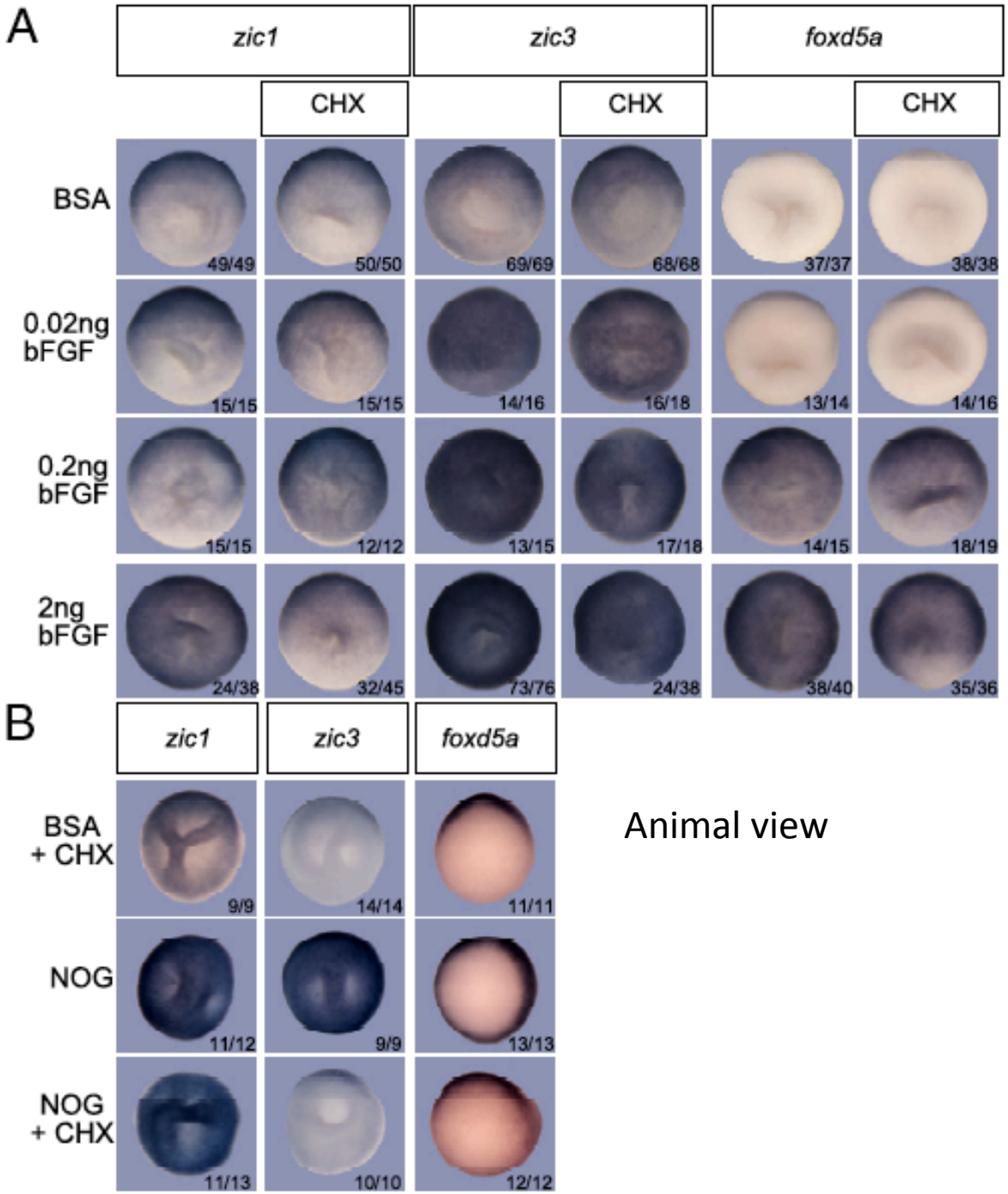
BMP inhibition and FGF signaling control distinct effector genes required for deployment of the neural program



nog mRNA radial injection

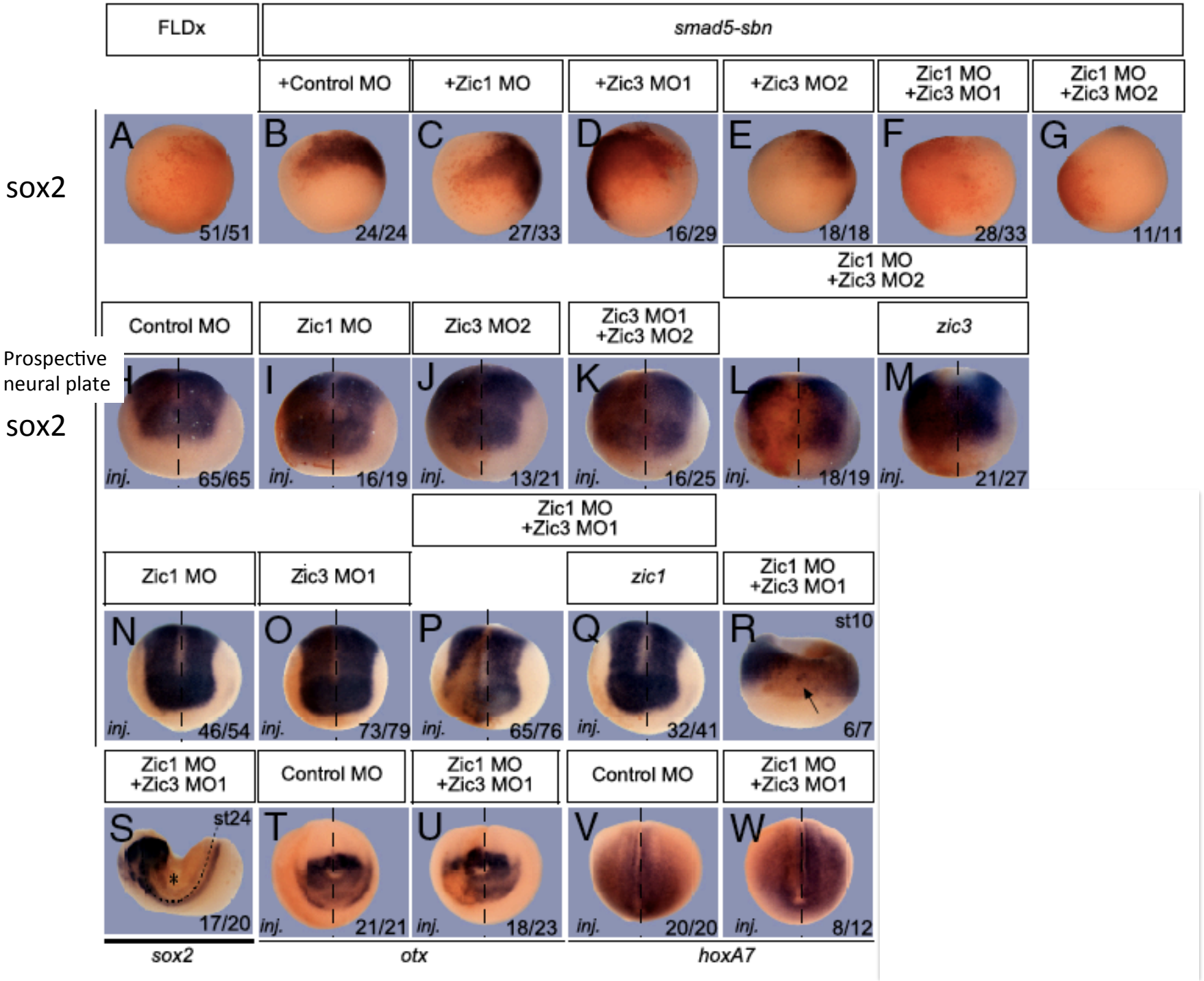


AB4 injection

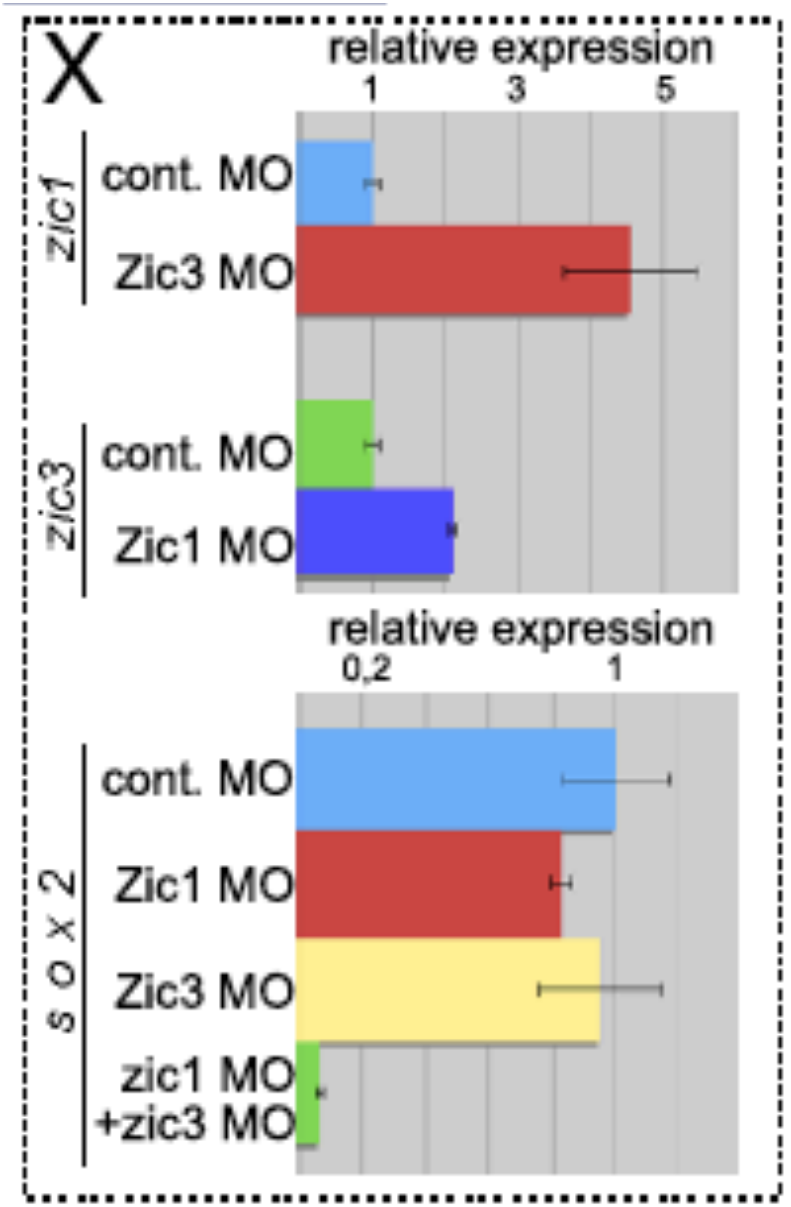


- Gastrulating embryos
- Recombinant bFGF injected in the blastocele
- Translation inhibitor: Cycloheximide (CHX)

Animal view


FLDx
smad5-sbn
+Control MO
+Zic1 MO
+Zic3 MO1
+Zic3 MO2
Zic1 MO +Zic3 MO1
Zic1 MO +Zic3 MO2
sox2
A
51/51
B
24/24
C
27/33
D
16/29
E
18/18
F
28/33
G
11/11
Zic1 MO +Zic3 MO2
Control MO
Zic1 MO
Zic3 MO2
Zic3 MO1 +Zic3 MO2
zic3
Prospective neural plate
sox2
H
inj.
65/65
I
inj.
16/19
J
inj.
13/21
K
inj.
16/25
L
inj.
18/19
M
inj.
21/27
Zic1 MO +Zic3 MO1
Zic1 MO
Zic3 MO1
zic1
Zic1 MO +Zic3 MO1
N
inj.
46/54
O
inj.
73/79
P
inj.
65/76
Q
inj.
32/41
R
st10
6/7
Zic1 MO +Zic3 MO1
Control MO
Zic1 MO +Zic3 MO1
Control MO
Zic1 MO +Zic3 MO1
S
st24
17/20
T
inj.
21/21
U
inj.
18/23
V
inj.
20/20
W
inj.
8/12
sox2
otx
hoxA7

Compensatory mechanisms
between Zic1 and Zic3



4. What are the main findings and general conclusions ?

General conclusions:

- Validation of the default model of neural induction
- Confirmation of **FGF signaling instructive role** in neural induction
- FGF is downstream of BMP inhibition to initiate the neural program= BMP inhibition and FGF signaling are both important and act sequentially
- Both Zic1 and Zic3 are required to initiate the neural program (they act redundantly)