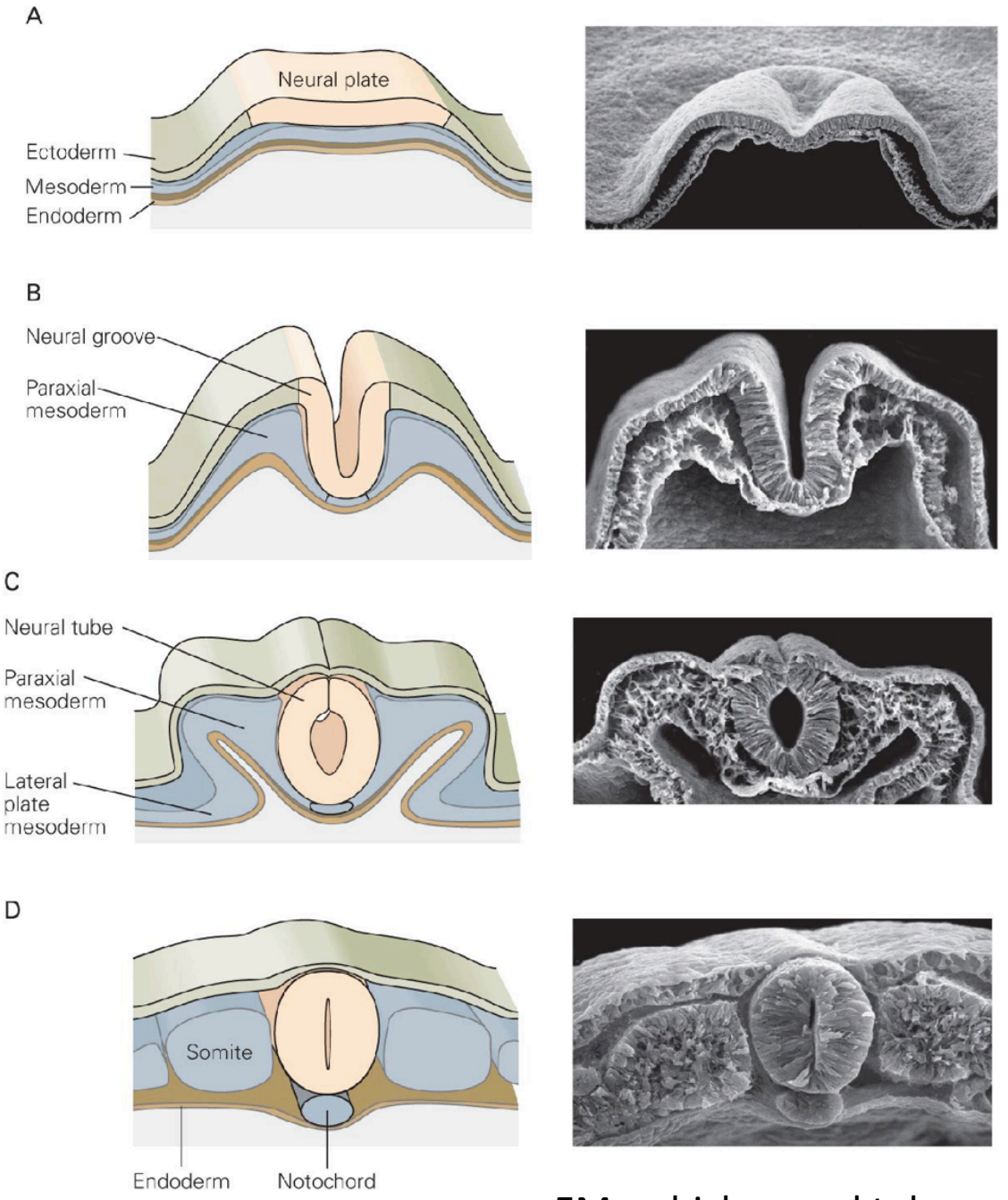
Vertebrates

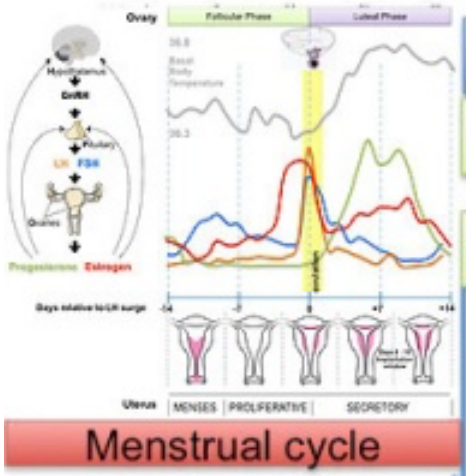
The ectoderm gives rise to the columnar epithelium of the neural plate = the precursor of the CNS and PNS

When and how embryonic tissue becomes **committed** to the neural fate?



EM – chick neural tube

Human Development Timeline



Menstrual cycle

Human Pregnancy - 9 months

First Trimester | Second Trimester | Third Trimester

Embryonic | Fetal

Embryonic Period (stages 1 to 23) | Fetal

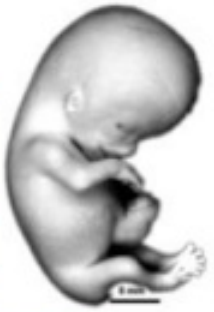
Last Menstrual Period

Fertilization

Positive

Week 1 to 4 | Week 5 to 8

-2 | -1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8



Fertilization

Week 1 | Week 2 | Week 3 | Week 4

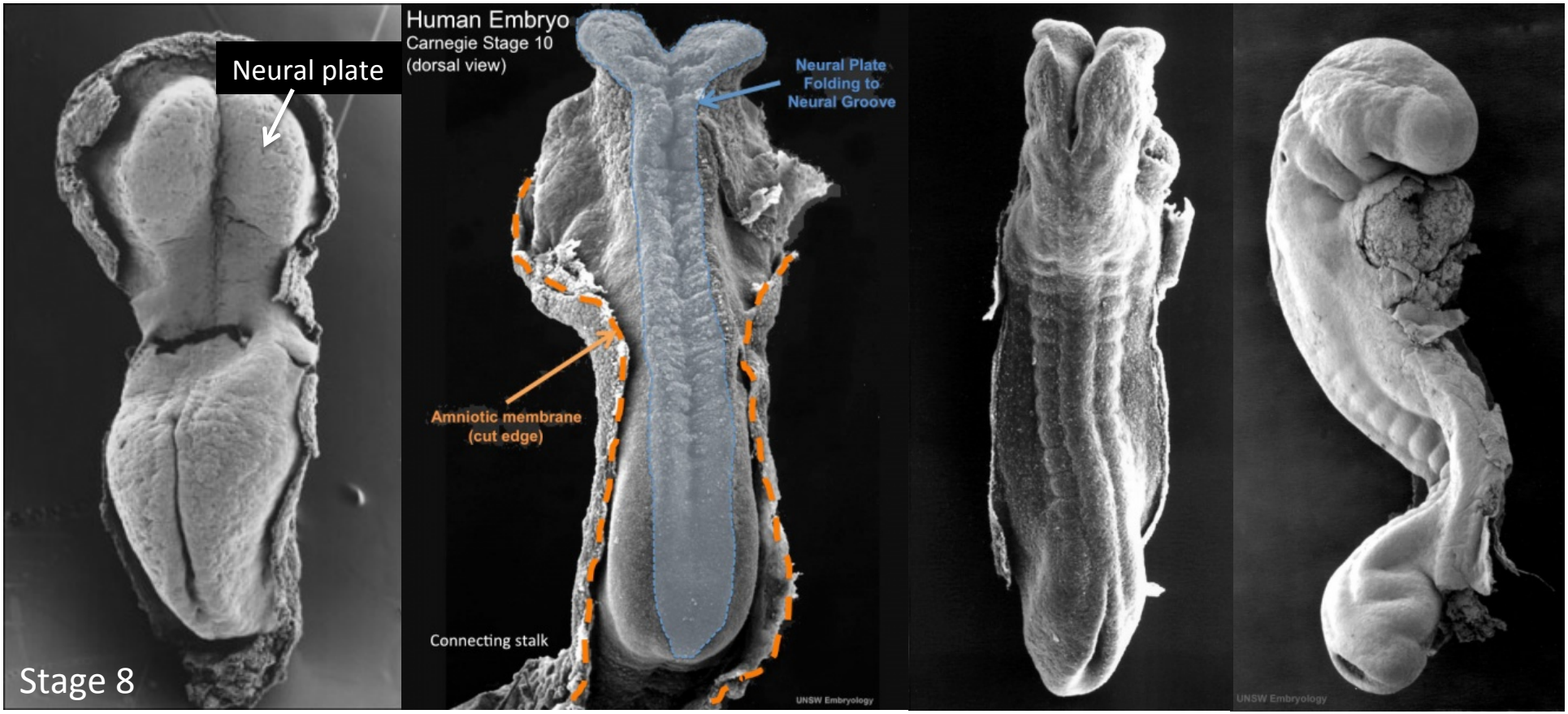
zygote
morula
blastocyst
bilaminar
trilaminar

Events

implantation
blastocyst hatching
gastrulation
folding
somitogenesis
cardiogenesis
neurogenesis
placodes

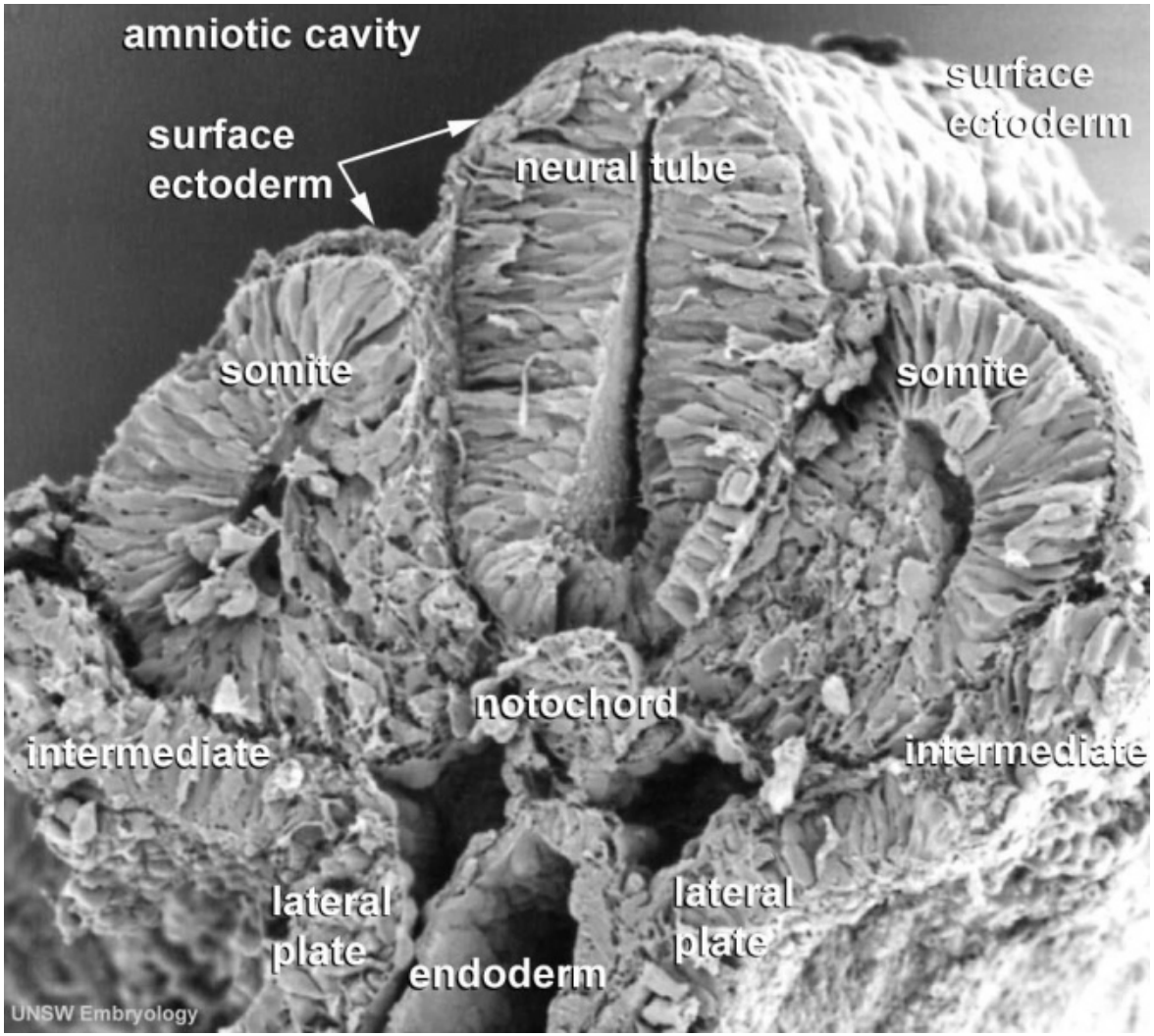
Human

Week 3 Week 4



Week 4 Carnagie stage 11

Human

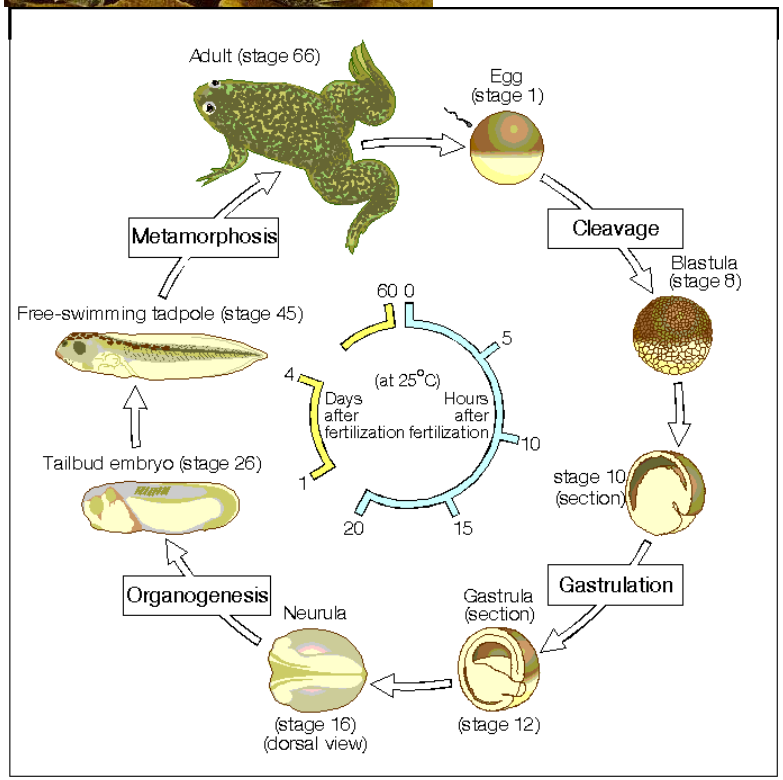


Time line – comparison based on Carnagies stages

Species	Stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Human	Days	1	2-3	4-5	5-6	7-12	13-15	15-17	17-19	20	22	24	28	30	33	36	40	42	44	48	52	54	55	58
Mouse	Days	1	2	3	4	5	6	7.0	8.0	9.0	9.5	E10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16
Rat	Days	1	3.5	4-5	5	6	7.5	8.5	9	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5

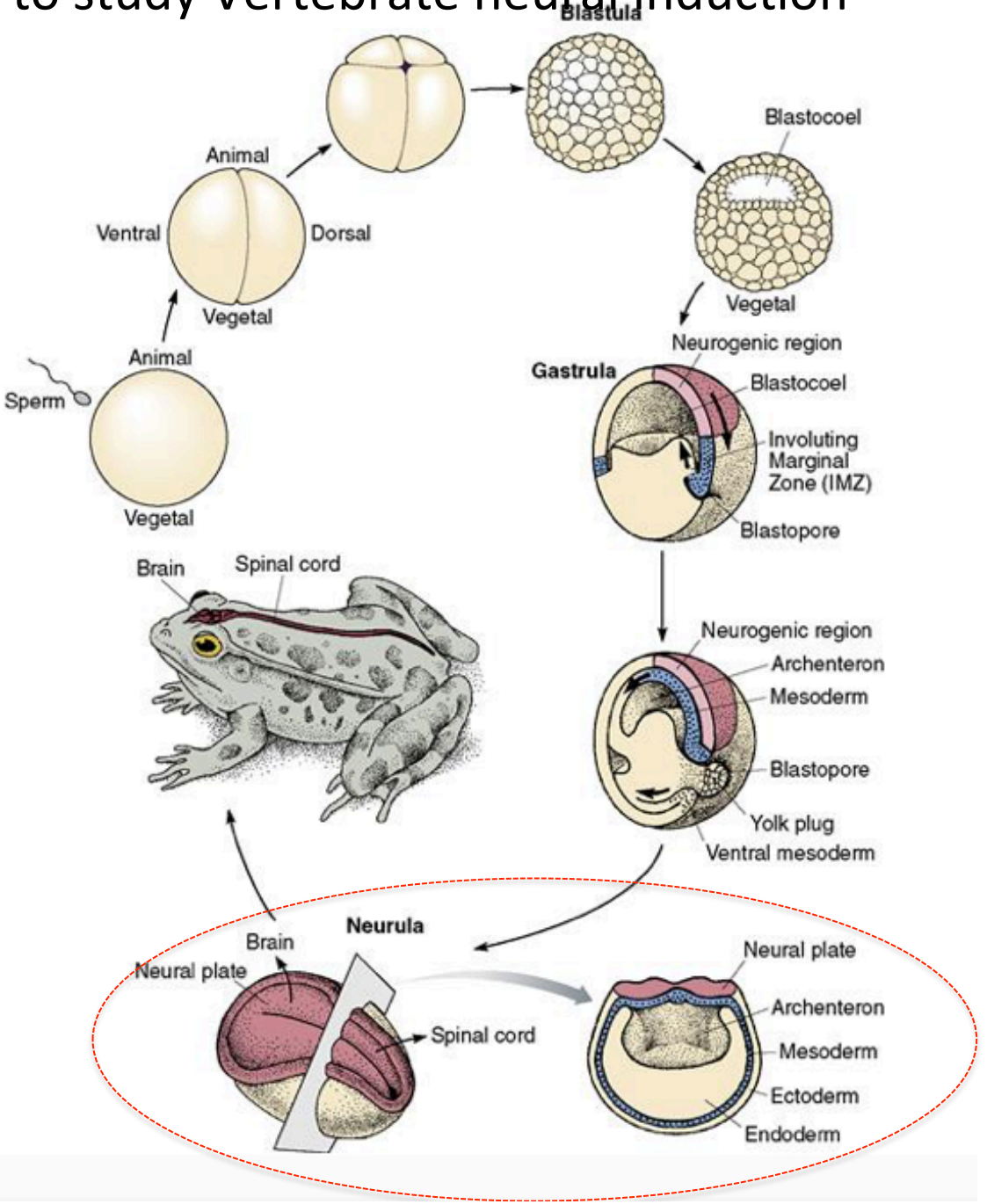
When and **how** embryonic tissue becomes **committed to the neural fate**?

Amphibia as a model system to study Vertebrate neural induction



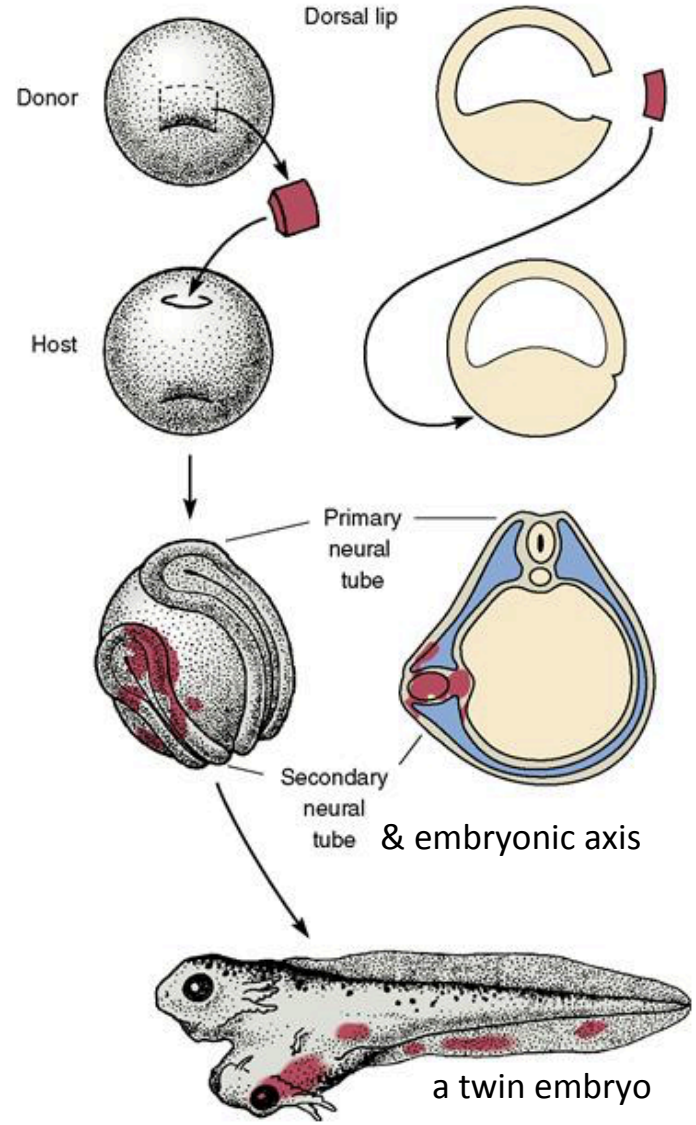
Life cycle

VIDEOS



Spemann & Mangold Experiment (1924)

1935 – Nobel Prize to Hans Spemann



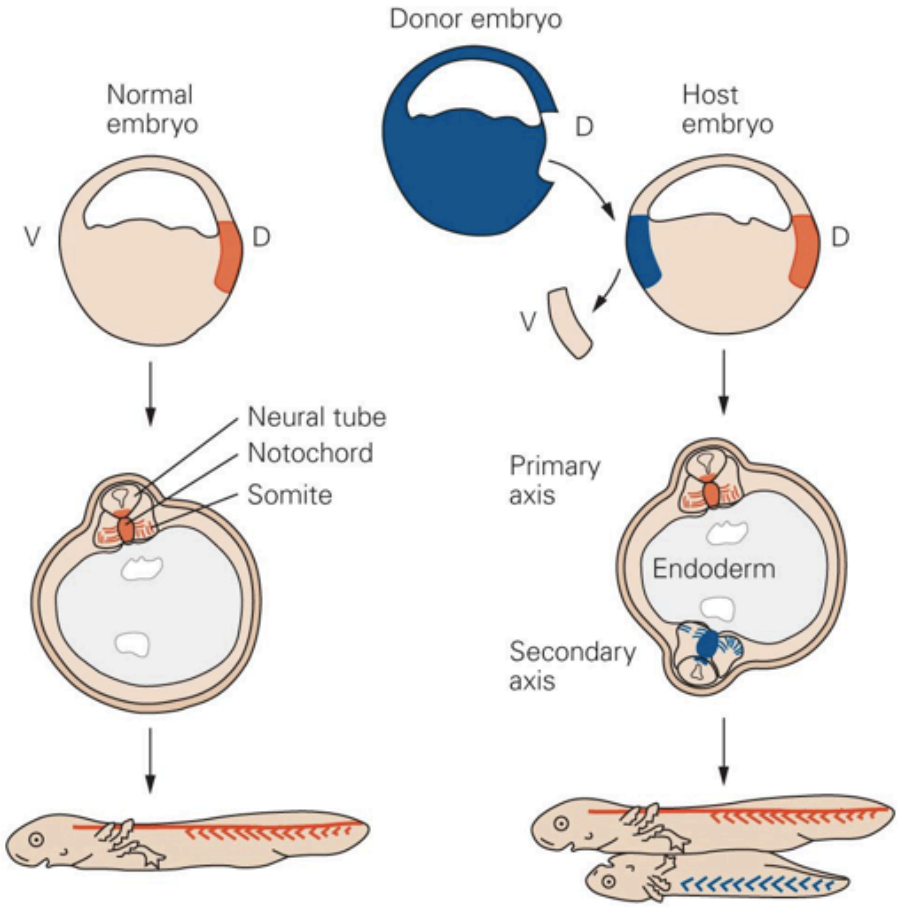
White (Triturus) cristatus and the dark T. taeniatus or T. alpestris

Embryonic Induction

Cell and tissue fate can be determined by signals received from other cells

Fig. 1.12 Spemann and Mangold transplanted the dorsal lip of the blastopore from a pigmented embryo (shown as red) to a nonpigmented host embryo. A second axis, including the neural tube, was induced by the transplanted tissue. The transplanted dorsal blastopore lip cells gave rise to some of the tissue in the secondary axis, but some of the host cells also contributed to the new body axis. They concluded that the dorsal lip cells could "organize" the host cells to form a new body axis, and they named this special region of the embryo the organizer.

X. laevis embryos



E. De Robertis

VIDEOS

Transplanted organizer cells:

- follow their own developmental program (midline mesoderm tissue: notochord and somites)

- induce host cells to change their fate forming a second embryonic axis
  → neuralization
  → dorsalization

cells can adopt their developmental fate according to their position when instructed by other cells

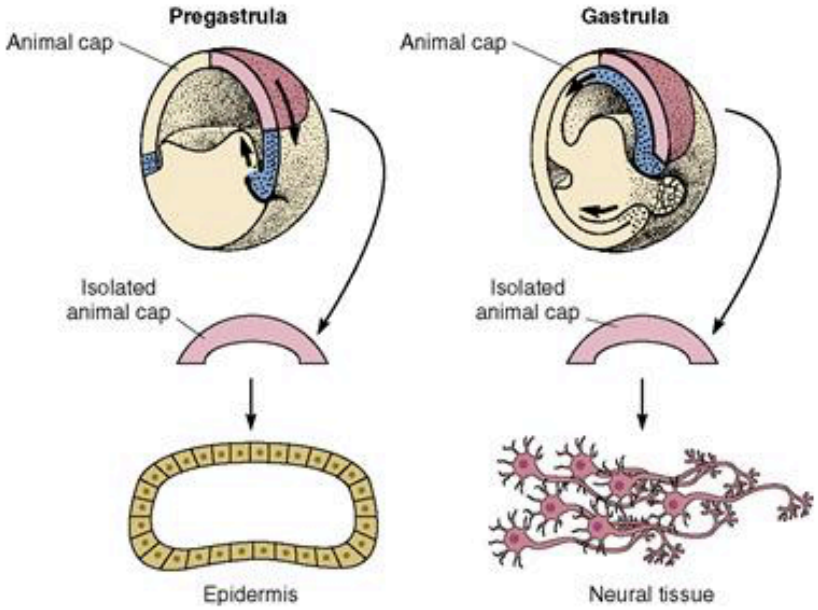


Fig. 1.11 Isolation of fragments of embryos at different stages of development demonstrates when tissue becomes committed to the neural lineage. If the animal cap is isolated from the rest of the embryo (left), the cells develop as epidermis, or skin. If the same region of the embryo is isolated a few hours later, during gastrulation (right), it will develop into neural tissue (shown in the figure as red neurons). Experiments like these led to the idea that the neural lineage arises during gastrulation.

Experimental approach: isolation and culture of tissue fragments at different stages of developmen (Amphibian embryos)

Results: cell types differentiate depending on the stage

When and **how** embryonic tissue
becomes **committed to the neural fate**?

When?

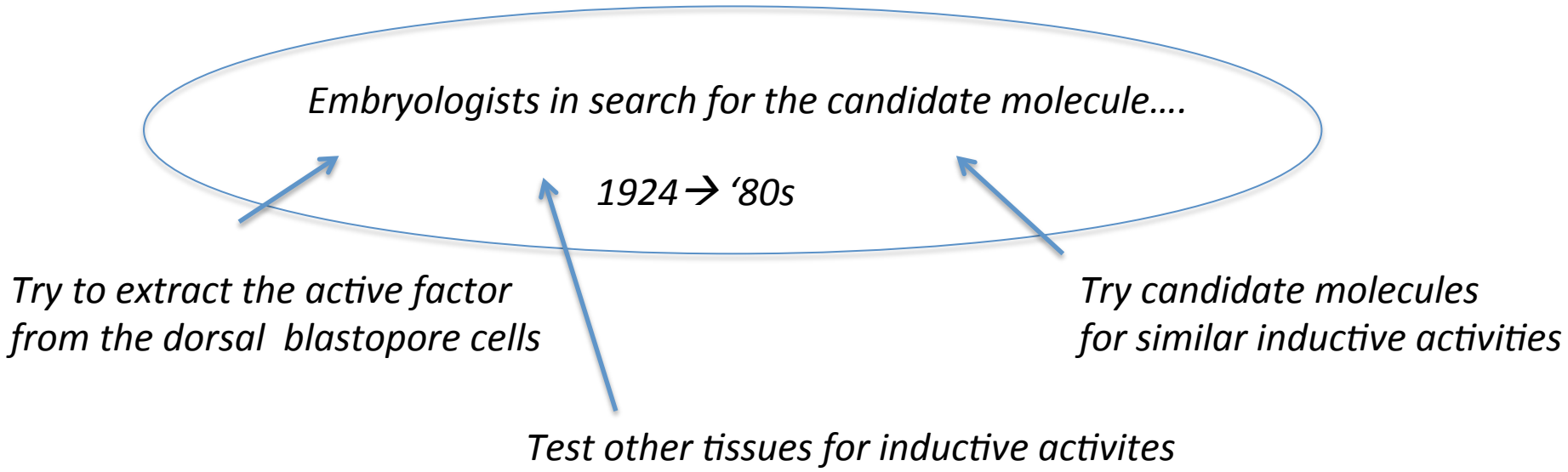
during gastrulation

How?

interaction with neighboring tissues

The molecular nature of the **neural inducer**

1° Hypothesis: the organizer and resulting notochord, through secreted soluble molecules, **instruct neural-plate differentiation** in the overlying ectoderm.



Cell Differ Dev. 1989 Dec;28(3):211-7.

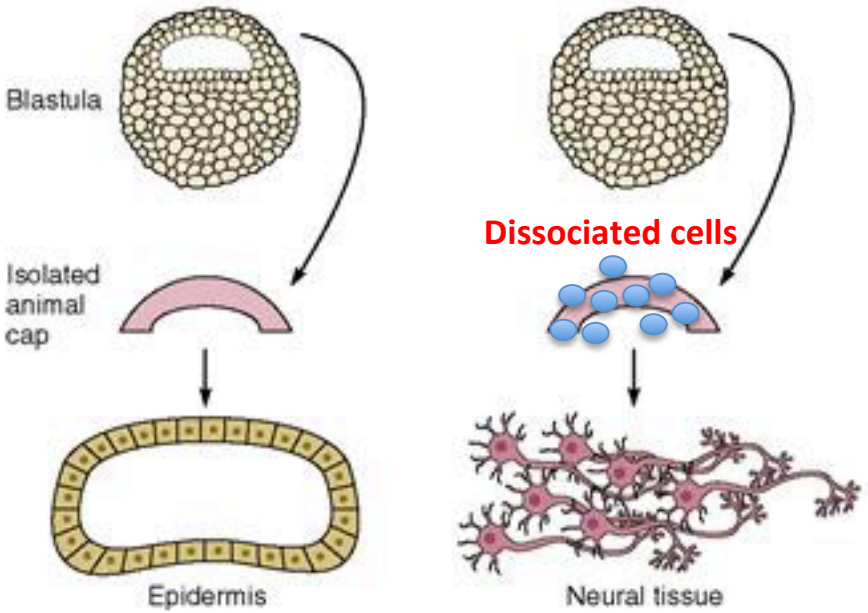
Neural differentiation of Xenopus laevis ectoderm takes place after disaggregation and delayed reaggregation without inducer.

Grunz H[1], Tacke L.

⊕ Author information

Abstract

When Xenopus blastula or early gastrula ectoderm is disaggregated and cells are kept dispersed for up to 5 h prior to reaggregation, the resulting spheres will differentiate into large neural structures. In contrast, dissociated and immediately reaggregated ectoderm will only differentiate into ciliated epidermis (so-called 'atypical epidermis'). Ectoderm treated with mesoderm-inducing XTC-conditioned medium during the period of reaggregation immediately after disaggregation will only form one- or two-cell types (notochord and somites) only. Ectoderm treated with XTC-factor prior to disaggregation will differentiate into a large variety of cell types.



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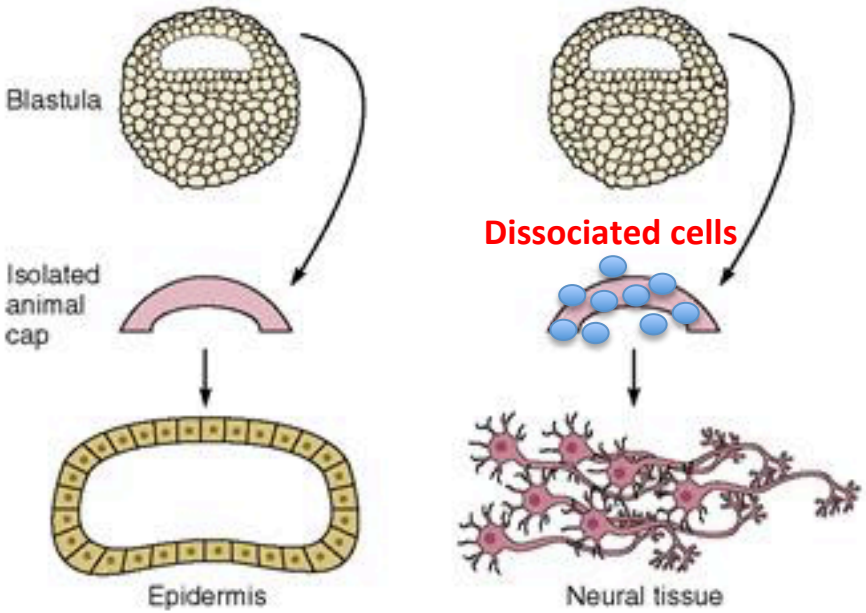
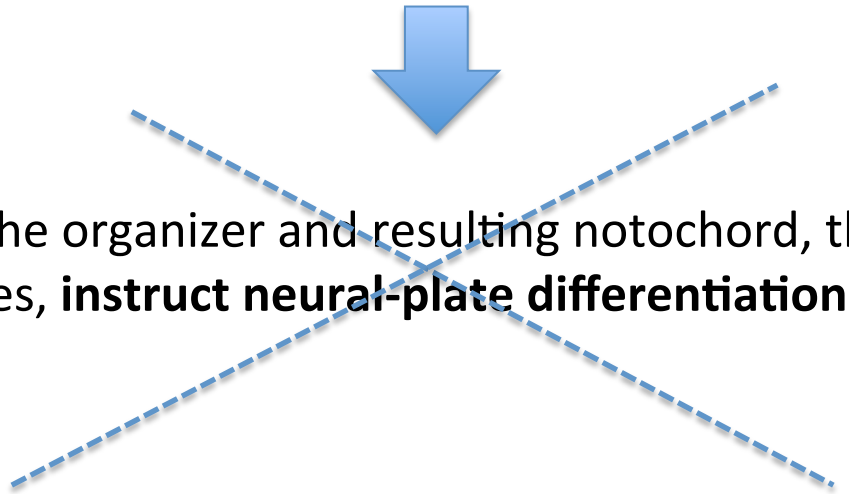


The **absence**, not the presence, of an intercellular signal was necessary for neural differentiation

Neural fate might indeed be the '**default' fate** of ectodermal cells

The molecular nature of the **neural inducer**



1° Hypothesis: the organizer and resulting notochord, through secreted soluble molecules, **instruct neural-plate differentiation** in the overlying ectoderm.

2° Hypothesis: the signal released by the organizer to cause neuralization and dorsalization is **not to a direct inducer**, but instead consists of **antagonists**, which block **inhibitors** that prevent the dorsalization/ neuralization of adjacent tissue.

The default model

The **default fate** of ectodermal cells is **neural differentiation**

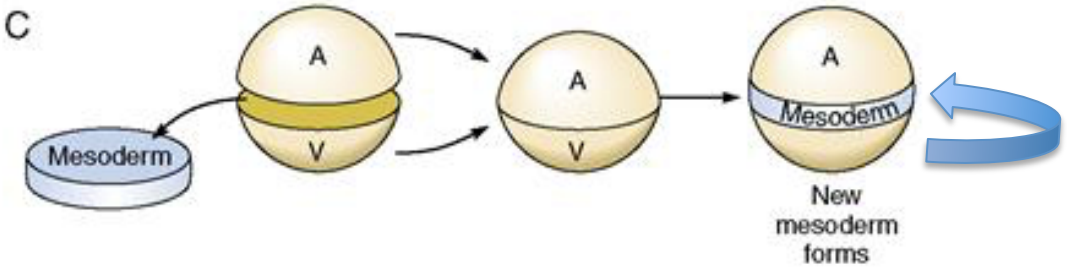
This fate is prevented by signals from neighboring ectodermal cells

The inducer is a de-repressor of neural fate

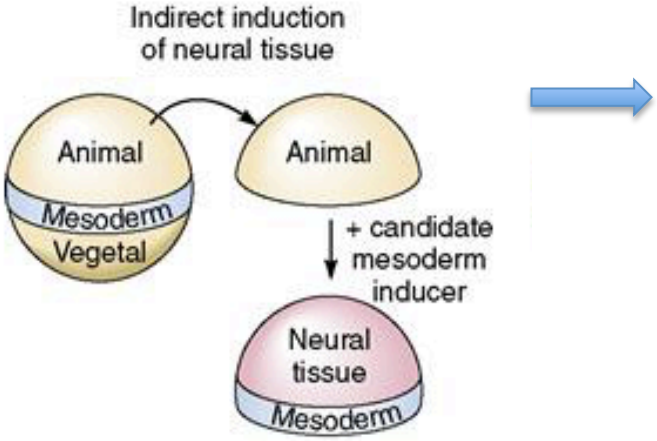
- What ectodermal signals repress neural differentiation?
- What does organizer tissue provide to overcome the effects of the repressor?

Candidate n°1: Activin

In the *Xenopus* blastula, the cells in the middle of the embryo become mesodermal by responding to **activin** (or an activin-like compound) produced in the vegetal hemisphere.



activin



At the blastula stage Activin can promote formation of neural tissue….but this occurs through **an indirect effect** by the dorsal mesoderm (which is induced by activin)

In the gastrula, activin is ineffective at promoting the formation of neural tissue, since the gastrula ectoderm loses competence to form mesoderm in response to activin

…Activin **is not an authentic neural inducer**

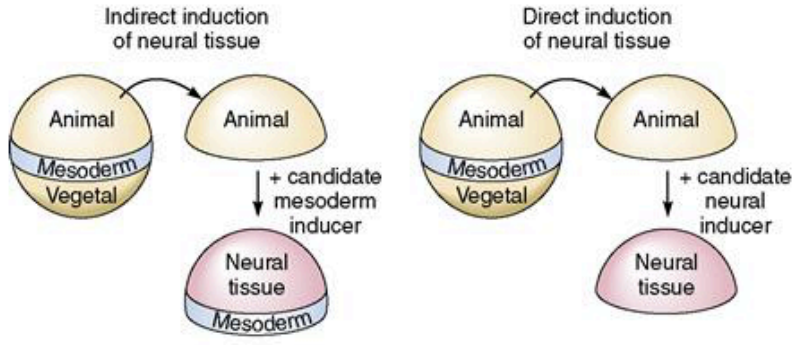


Fig. 1.14 Indirect neural induction versus direct neural induction. The organizer transplant experiments show that the involuting mesoderm has the capacity to induce neural tissue in the cells of the animal cap ectoderm. When assaying for the factor released from mesoderm that is responsible for this activity, it was important to distinguish between the direct and indirect

Main criteria for the activities of an **authentic neural inducer**

1) It should be able to induce neural tissue from animal cap ectoderm in the absence of dorsal mesoderm → **direct induction**

2) competent ectoderm should be responsive to the neural inducer at the gastrula stage (when dorsal mesoderm can still induce neural tissue)

3) It must be present at the right **time** and **place** to account for normal neural development

4) elimination of its activity should block normal neural development

Science 1993

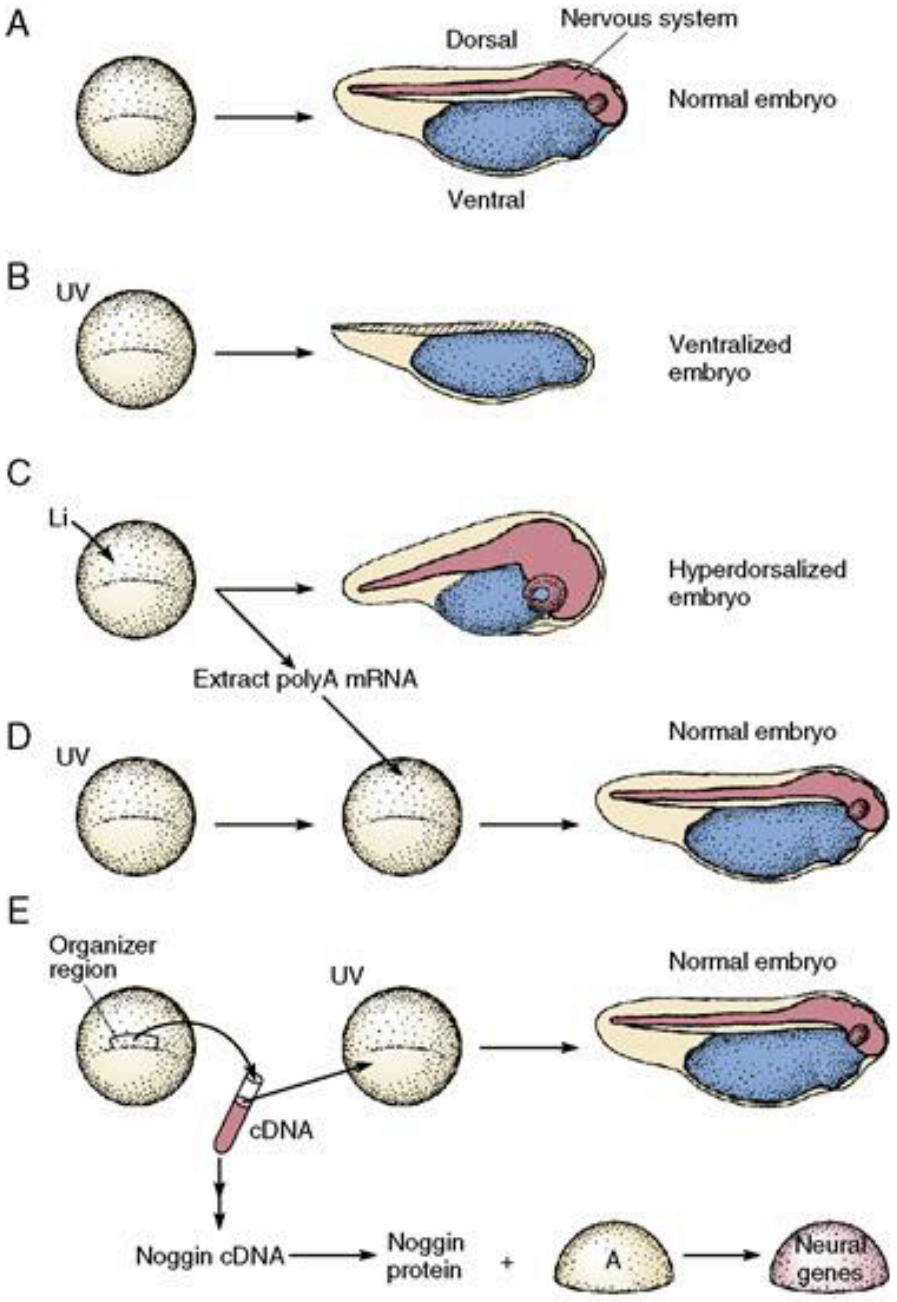
RESEARCH ARTICLE

Neural Induction by the Secreted Polypeptide Noggin

Teresa M. Lamb, Anne K. Knecht, William C. Smith, Scott E. Stachel, Aris N. Economides, Neil Stahl, George D. Yancopolous, Richard M. Harland*

The Spemann organizer induces neural tissue from dorsal ectoderm and dorsalizes lateral and ventral mesoderm in *Xenopus*. The secreted factor noggin, which is expressed in the organizer, can mimic the dorsalizing signal of the organizer. Data are presented showing that noggin directly induces neural tissue, that it induces neural tissue in the absence of dorsal mesoderm, and that it acts at the appropriate stage to be an endogenous neural inducing signal. Noggin induces cement glands and anterior brain markers, but not hindbrain or spinal cord markers. Thus, noggin has the expression pattern and activity expected of an endogenous neural inducer.

Experimental strategy used to isolate noggin:



Noggin

- **Is expressed by the organizer** (at the right **time** and **place** to be a neural inducer):

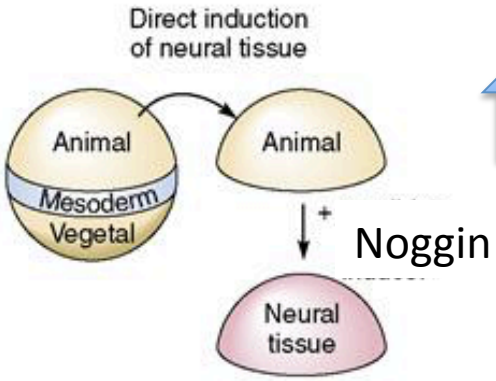
noggin expression begins at the **late blastula stage** in the **prospective dorsal mesoderm** and continues in the gastrula stage **organizer**.

Later, noggin is expressed in the organizer derivatives, the head mesoderm, and **notochord**→ the notochord directly underlies the neural plate and has been shown to be a **potent neural inducer**

Direct neural induction by noggin

1° set of experiments:
medium containing Xenopus noggin → <u>added to **blastula** animal caps</u>

- analysis of specific transcripts (neural and mesoderm)



induction

NCAM
Beta-tubulin (isoform expressed in hindbrain and spinal cord)
XIF3 (neurally expressed intermediate filament gene)

~~Muscle actin~~
~~Goosecoid~~ (early mesoderm marker)
~~Brachyury (Xbra)~~ (early mesoderm marker)

Results→ noggin induces neural tissue (+NCAM, XIF3, Beta-tubulin) in the absence of mesoderm (activin at this stage induces both mesoderm and neural markers)

<u>Similar results were obtained by adding noggin to the gastrula</u>

Cell. 1994 Dec 2;79(5):779-90.

Xenopus chordin: a novel dorsalizing factor activated by organizer-specific homeobox genes.

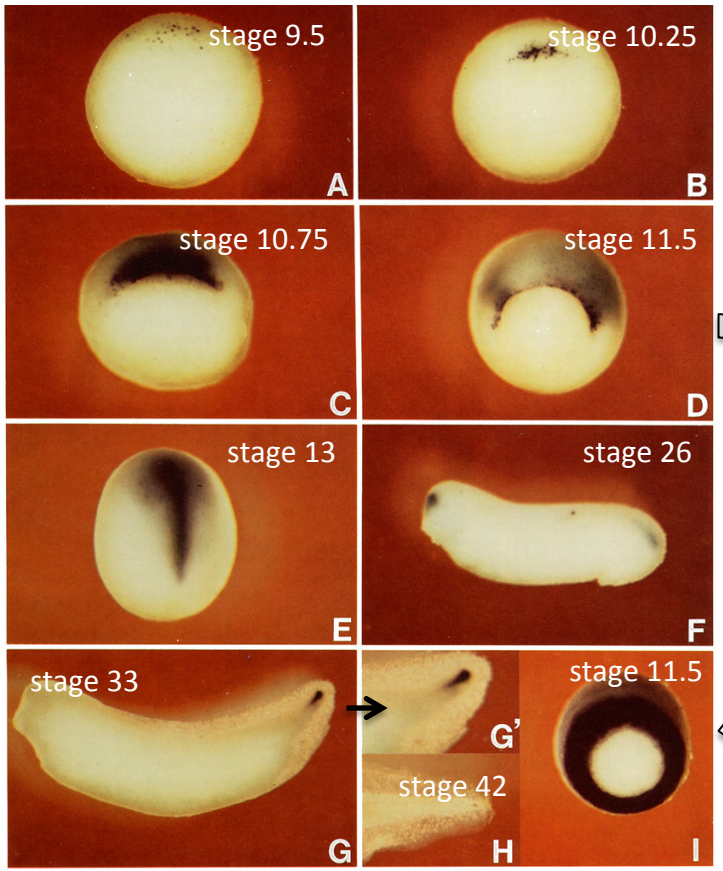
Sasai Y[1], Lu B, Steinbeisser H, Geissert D, Gont LK, De Robertis EM.

Author information

Abstract

A Xenopus gene whose expression can be activated by the organizer-specific homeobox genes goosecoid and Xnot2 was isolated by differential screening. The chordin gene encodes a novel protein of 941 amino acids that has a signal sequence and four Cys-rich domains. The expression of chordin starts in Spemann's organizer subsequent to that of goosecoid, and its induction by activin requires de novo protein synthesis. Microinjection of chordin mRNA induces twinned axes and can completely rescue axial development in ventralized embryos. This molecule is a potent dorsalizing factor that is expressed at the right time and in the right place to regulate cell-cell interactions in the organizing centers of head, trunk, and tail development.

Digoxygenin-labeled antisense *chordin* RNA was hybridized to embryos



Like noggin, chordin is a secreted protein that is expressed by the organizer region during the period when the neural induction occurs

chordin Is Expressed in Regions with Head, Trunk, and Tail Organizer Activity

(A)–(D) and (I) are vegetal views, dorsal side is at the top. (E) is viewed from the dorsal side with anterior at top. (F)–(H) are lateral views.

← LiCl-treated embryo

Cell. 1994 Apr 22;77(2):273-81.

Inhibition of activin receptor signaling promotes neuralization in Xenopus.

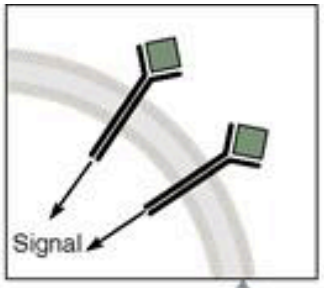
Hemmati-Brivanlou A[1], Melton DA.

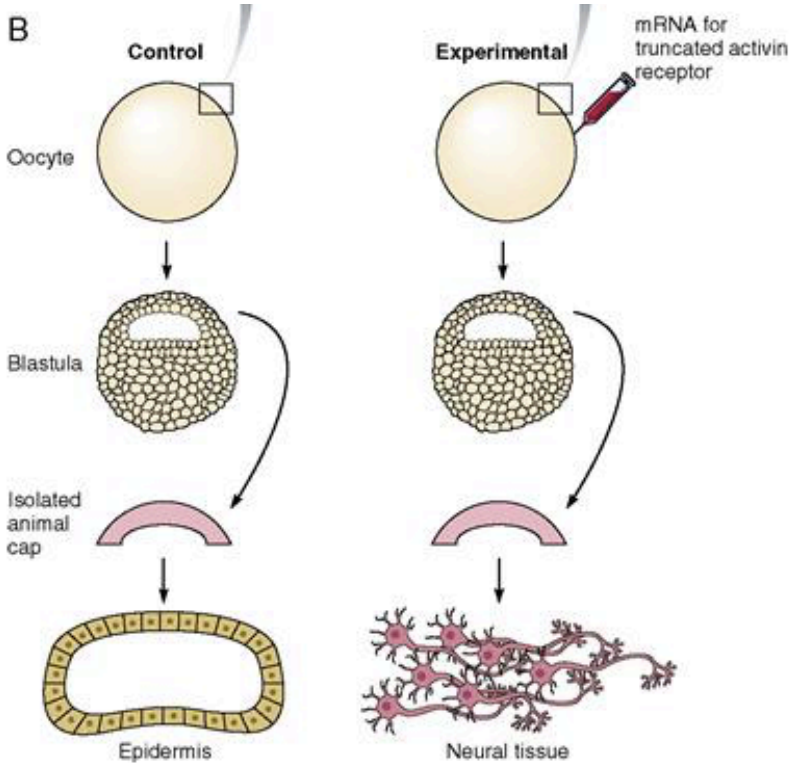
⊕ Author information

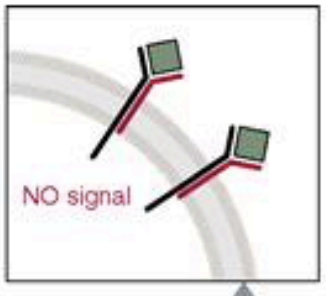
Abstract

Expression of a truncated activin type II receptor, which blocks signaling by activin, neuralizes explants of embryonic cells that would otherwise become epidermal cells. This neuralization is direct and does not require the presence of mesoderm. The induced neural tissue expresses general molecular markers of the central nervous system as well as an array of neural markers along the anteroposterior axis. In the context of the whole embryo, expression of this truncated activin receptor diverts prospective ectoderm and endoderm to a neural fate. We propose that inhibition of the activin type II receptor signaling causes the cells of Xenopus embryos to adopt a neural fate. These results, along with previous experiments performed in Drosophila, suggest that the formation of the nervous system in vertebrates and invertebrates occurs by a common strategy.

PMID: 8168134 [PubMed - indexed for MEDLINE]







Truncated Activin type II receptor (dominant negative) promotes neuralization

↓

Activin-like molecule (TGF-β family) inhibitor of neuralization

Cell. 1994 Apr 22;77(2):273-81.

Inhibition of activin receptor signaling promotes neuralization in Xenopus.

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Follistatin= Key regulator in adult reproductive system → by inhibition of Activin

Cell. 1994 Apr 22;77(2):283-95.

Follistatin, an antagonist of activin, is expressed in the Spemann organizer and displays direct neuralizing activity.

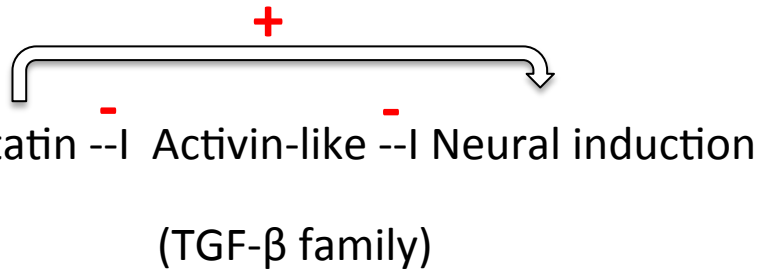
Hemmati-Brivanlou A[1], Kelly OG, Melton DA.

⊕ Author information

Abstract

In the accompanying paper, we show that the expression of a dominant negative activin receptor can convert prospective ectoderm into neural tissue, which suggests that activin is an inhibitor of neuralization. Here we report the isolation and characterization of an activin antagonist, follistatin, that can induce neural tissue directly in vivo. Follistatin RNA is localized in the Spemann organizer and notochord, tissues known to be potent neural inducers. We demonstrate that follistatin RNA and protein are able to block the activity of activin in embryonic explants. Furthermore, we show that follistatin RNA directly neuralizes ectodermal explants in the absence of detectable mesoderm. Thus, follistatin is present at the correct time and location to play a role in neural induction in vivo.

PMID: 8168135 [PubMed - indexed for MEDLINE]



Nature. 1995 Jul 20;376(6537):249-53.

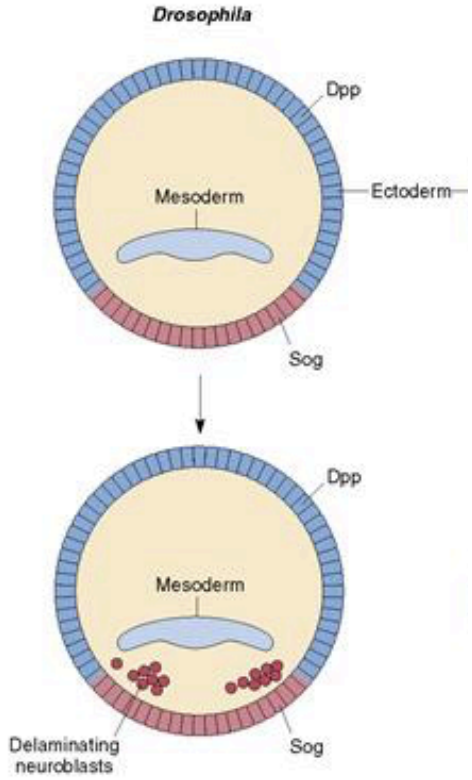
A conserved system for dorsal-ventral patterning in insects and vertebrates involving sog and chordin.

Holley SA[1], Jackson PD, Sasai Y, Lu B, De Robertis EM, Hoffmann FM, Ferguson EL.

Author information

Abstract

Dorsal-ventral patterning within the ectoderm of the Drosophila embryo requires seven zygotic genes, including short gastrulation (sog). Here we demonstrate that sog, which is expressed in the ventrolateral region of the embryo that gives rise to the nerve cord, is functionally homologous to the chordin gene of Xenopus, which is expressed in the dorsal blastopore lip of the embryo and in dorsal mesoderm, in particular the notochord. We show by injections of messenger RNA that both sog and chordin can promote ventral development in Drosophila, and that sog, like chordin, can promote dorsal development in Xenopus. In Drosophila, sog antagonizes the dorsalizing effects of decapentaplegic (dpp), a member of the transforming growth factor-beta family. One of the dpp homologues in vertebrates, bmp-4, is expressed ventrally in Xenopus and promotes ventral development. We show that dpp can promote ventral fates in Xenopus, and that injection of sog mRNA counteracts the ventralizing effects of dpp. These results suggest the molecular conservation of dorsoventral patterning mechanisms during evolution.



Sog

→ functional homologus of **chordin**

→ antagonizes dorsalizing effect of **decapentaplegic**
(a member of the TGF-β family)

↓

homologus of **BMP-4**

Nature. 1995 Jul 20;376(6537):249-53.

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Nature. 1995 Jul 27;376(6538):333-6.

Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in Xenopus.

Sasai Y[1], Lu B, Steinbeisser H, De Robertis EM.

Author information

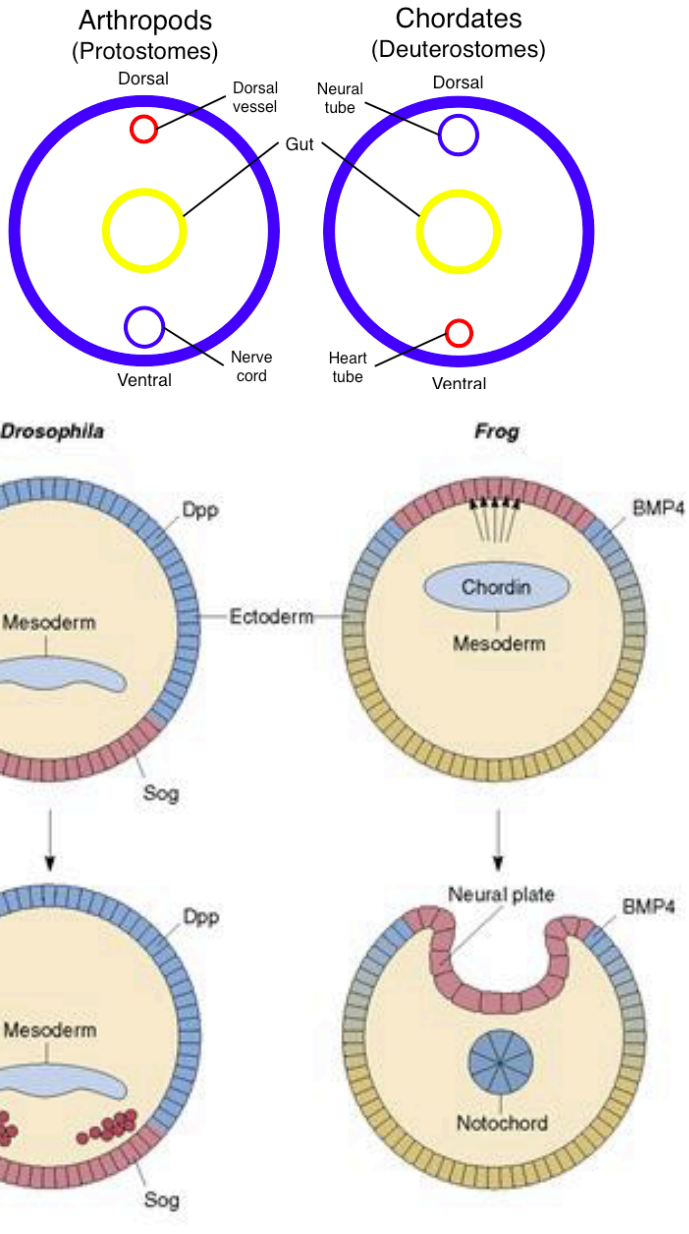
Erratum in

Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in Xenopus. [Nature. 1995]
Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in Xenopus. [Nature. 1995]

Abstract

In Drosophila the amount of neurogenic ectoderm, from which the central nervous system (CNS) derives, is regulated by a dorsal-ventral system of positional information in which two secreted molecules of antagonistic functions, decapentaplegic (dpp) and short-gastrulation (sog), play fundamental roles. The vertebrate homologue of dpp is either bmp-4 or bmp-2 (ref. 5), and the homologue os sog is chd (s-chordin). In Xenopus the CNS is induced by signals emanating from the organizer, and two proteins secreted by the organizer, noggin and follistatin, have been shown to induce neural tissue in animal-cap assays. Here we report that Chd, another organizer-specific secreted factor, has neuralizing activity and that this activity can be antagonized by Bmp-4. Inhibition of the function of the endogenous Bmp-4 present in the animal cap also leads to neural differentiation. We suggest that conserved molecular mechanisms involving chd/sog and bmp-4/dpp gene products pattern the ectoderm in Xenopus and in Drosophila.

Vertebrates and Invertebrates use similar molecules to pattern the dorso-ventral axis



Dpp=decapentaplegic

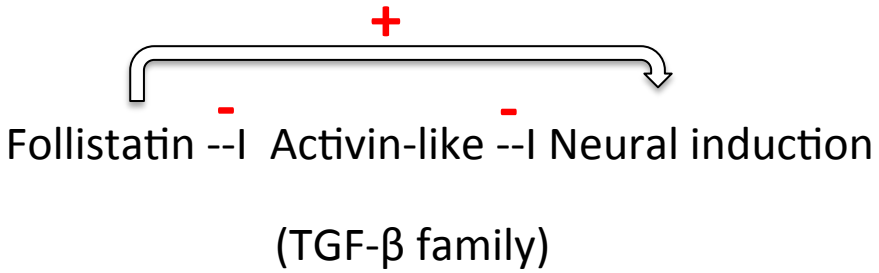
Sog=short gastrulation

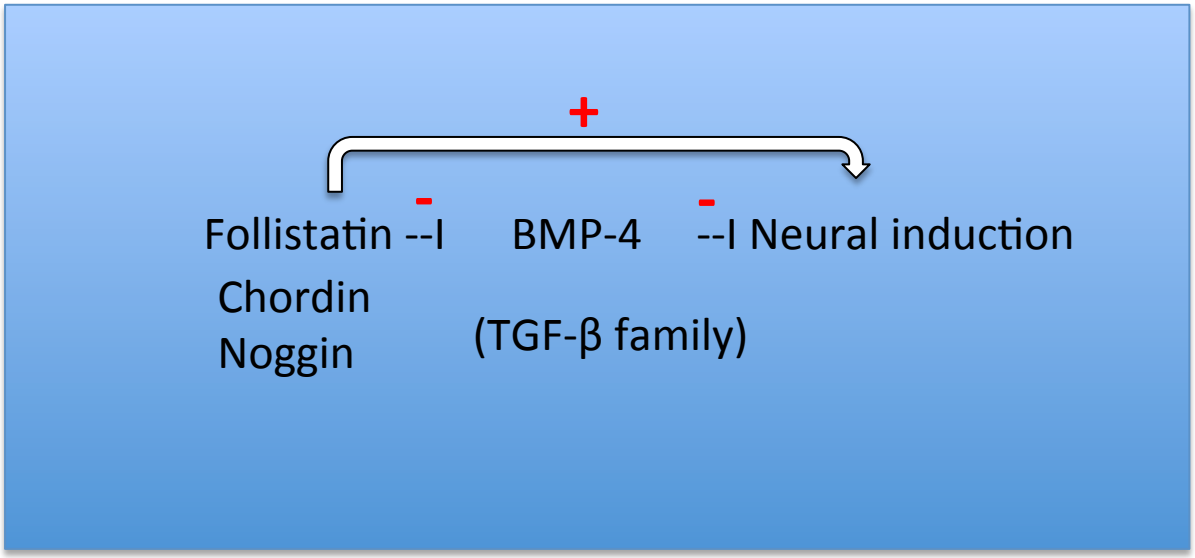
in drosophila

BMP4=bone morphogenetic protein 4

Chordin

in the frog


+
-
-
Follistatin --I
Activin-like --I Neural induction
(TGF-β family)


+
-
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Follistatin --I
BMP-4
--I Neural induction
Chordin
Noggin
(TGF-β family)

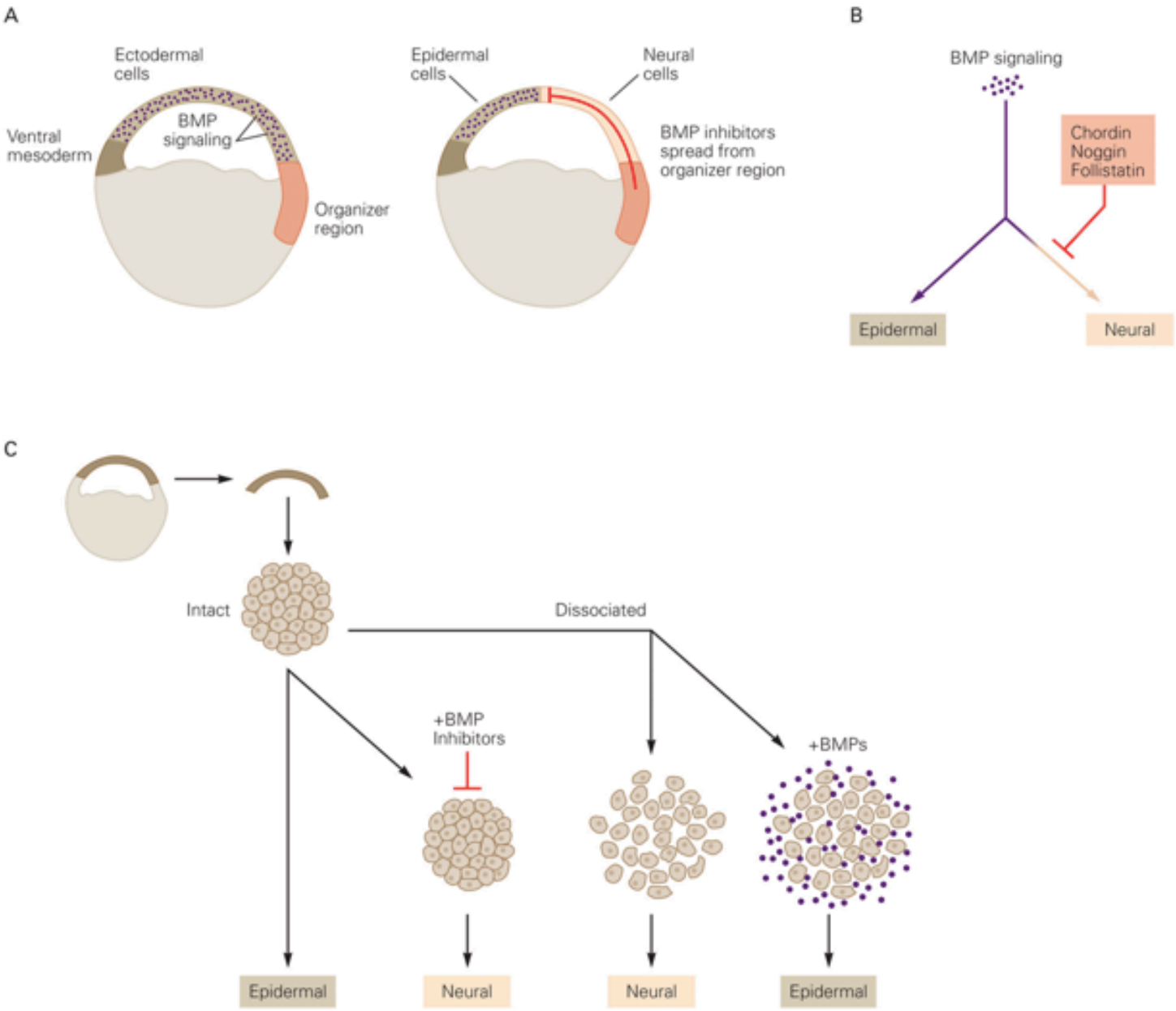
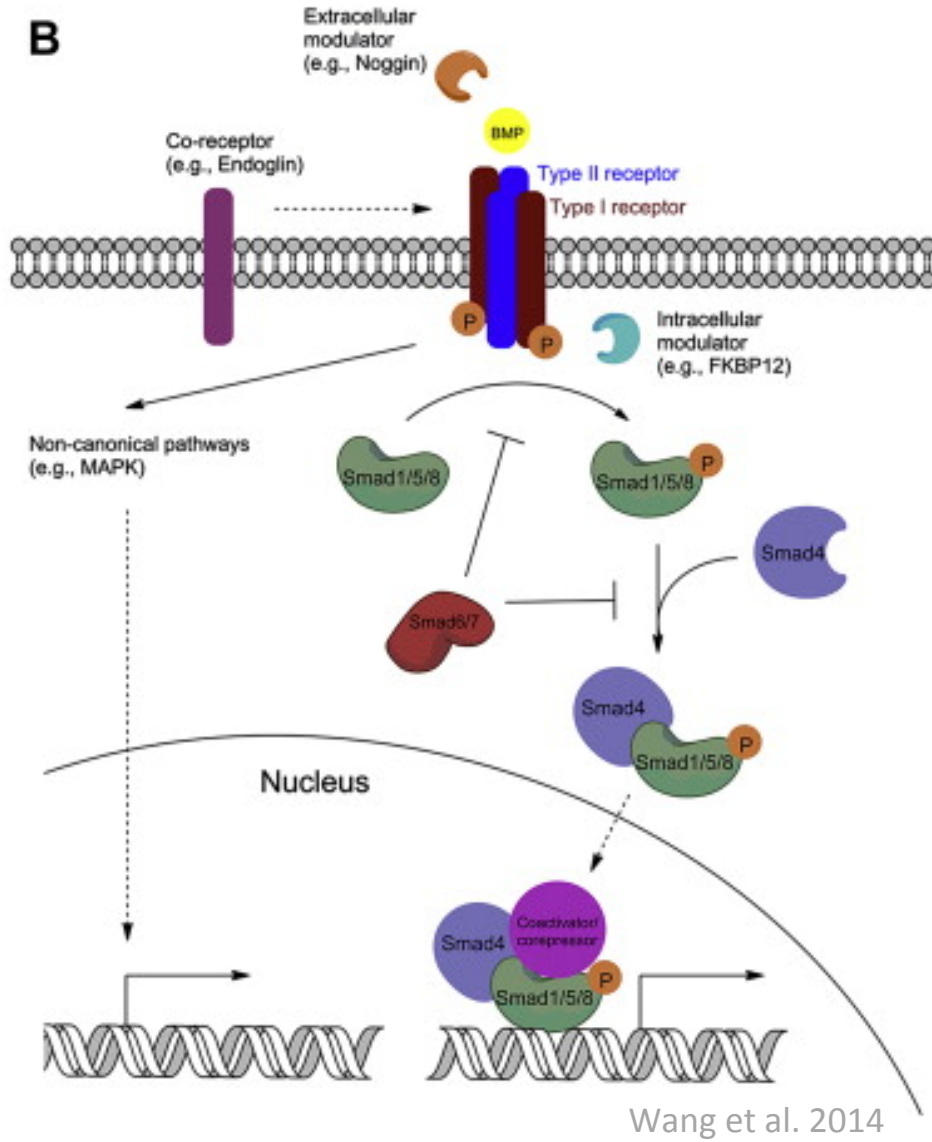


Figure 52-4 Inhibition of bone morphogenetic protein (BMP) signaling initiates neural induction.

BMP signaling pathway



Wang et al. 2014

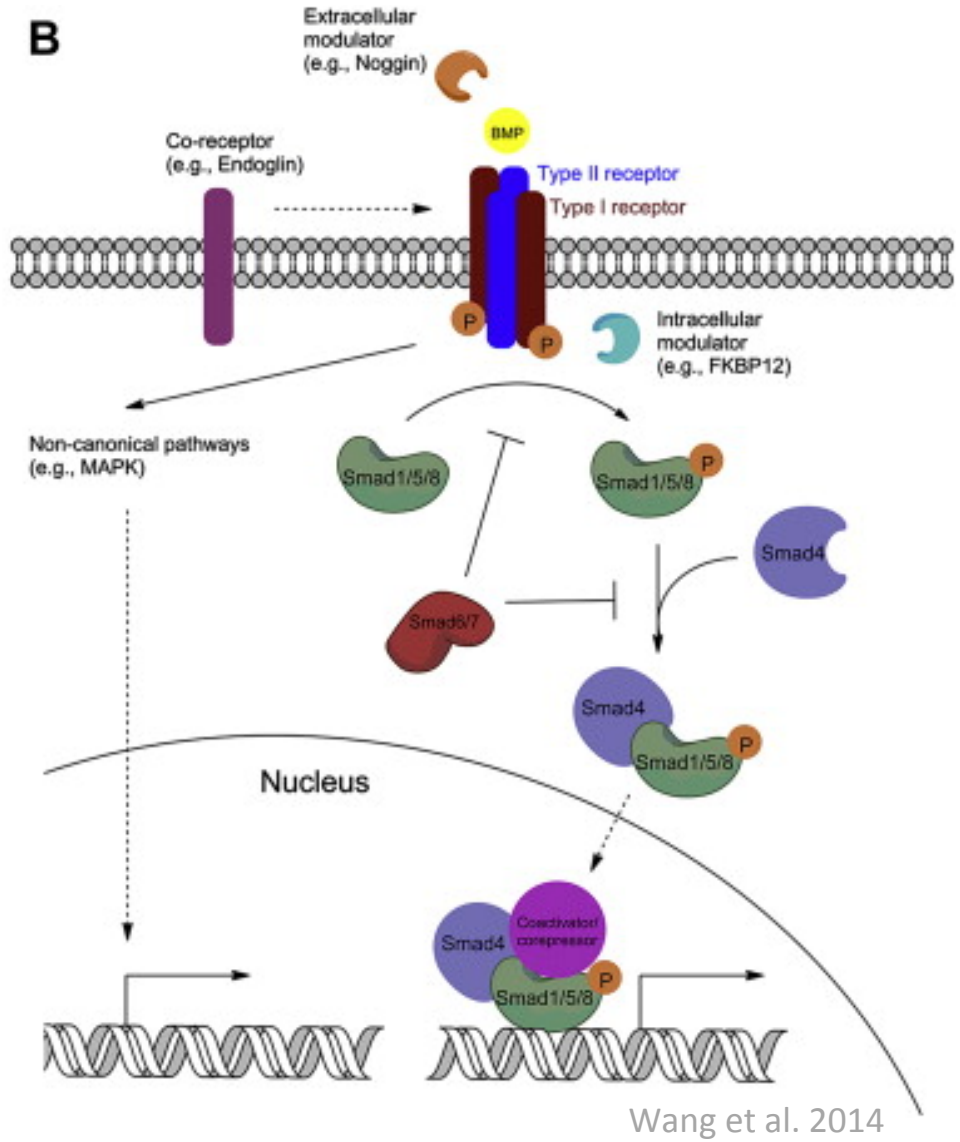
BMP receptors =serine/ threonine receptor kinases

BMPs→ belong to the Transforming Growth Factor-β (TGF-β) superfamily of proteins
(represent the largest sub-group of the TGF-β superfamily → 20 growth factors)

<u>Canonical pathway</u>:

1. BMPs bind to type I or type II receptors → form a heterotetrameric complex
2. The constitutive active type II receptor transphosphorylate type I receptor
3. Type I receptor phosphorilates the R-Smads (1-5-8)
4. Phosphorilated Smad1/5/8 associate with co-Smad4
5. P-Smad1/5/8-Smad4 complex translocates to the nucleus, where it further associates with coactivators or corepressors to regulate gene expression.

BMP signaling pathway



Wang et al. 2014

BMP receptors =serine/ threonine receptor kinases

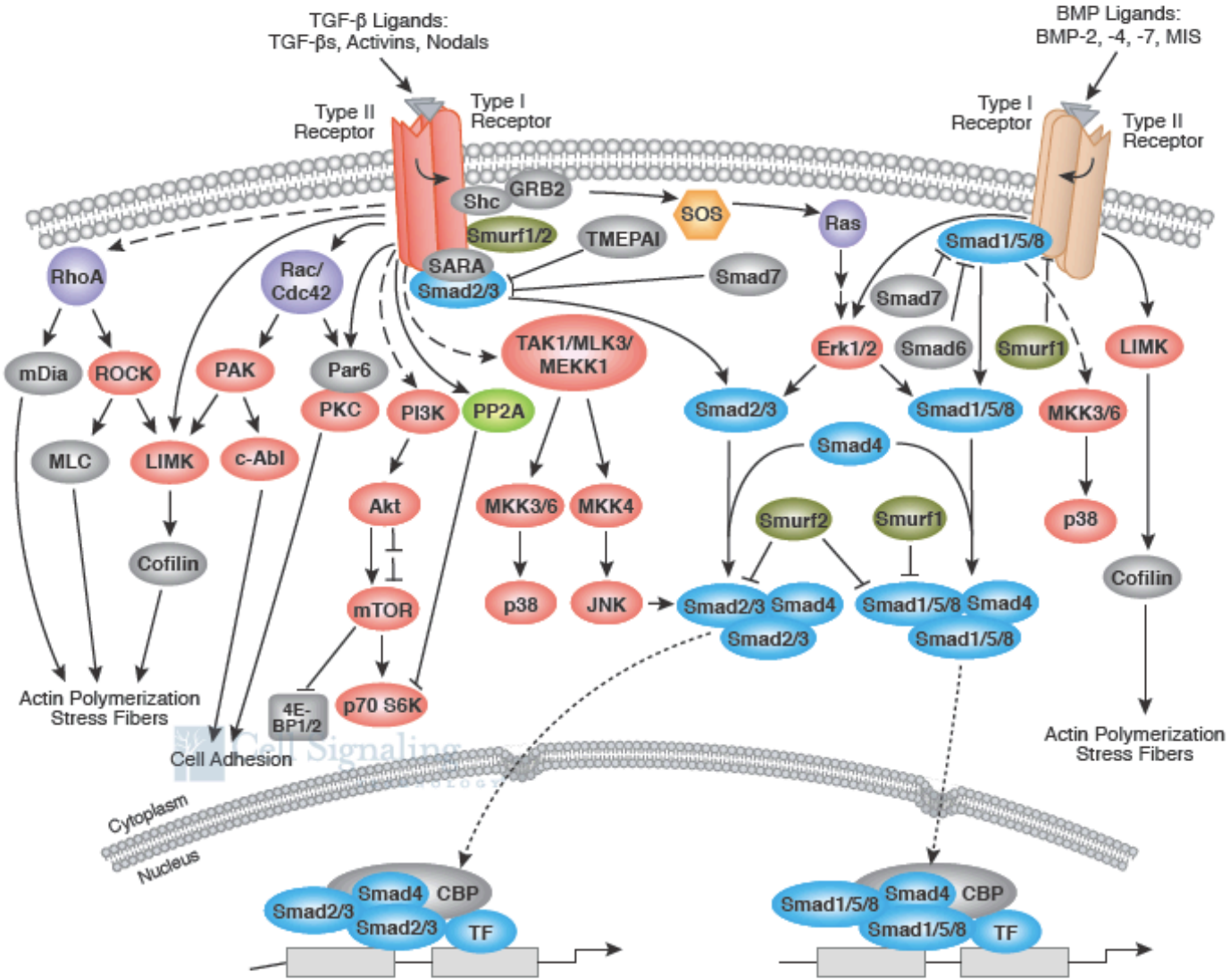
<u>BMP signaling modulation</u>:

1.Extracellularly : e.g., Noggin, Chordin, Follistatin→ antagonists

2. Intracellularly (e.g., FKBP12, microRNAs, phosphatases, and I-Smads6/7)

3. by co-receptors in the plasma membrane (e.g., Endoglin→ vascular growth and disease).

TGF-β Signaling …what we know now



Transcription Factors:

AP-1	homeodomain
bZIP	Sp1
RUNX	nuclear receptors
Fox	IRF-7
bHLH	

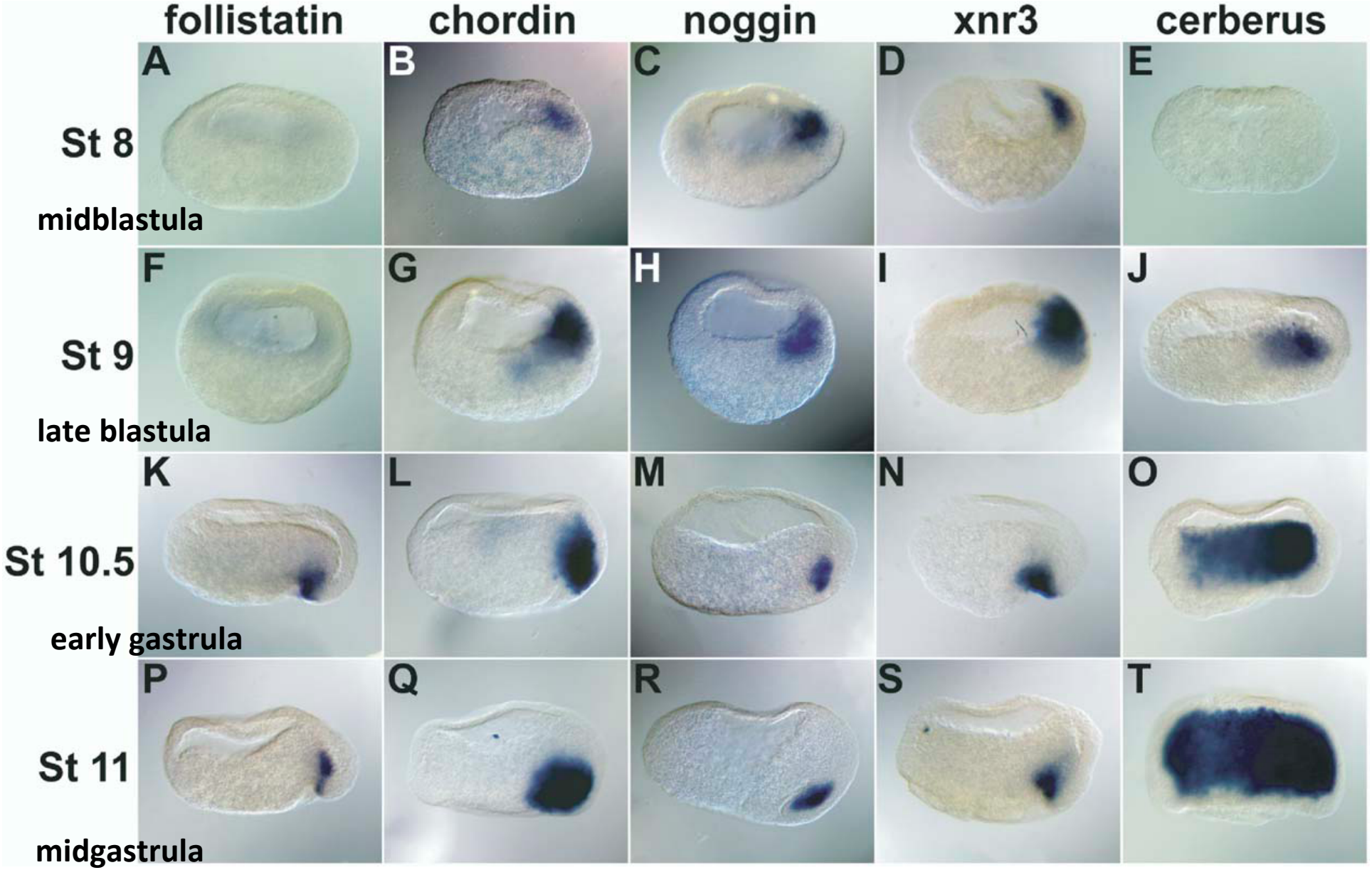
Corepressors:

c-Ski/SnoN
Evi1
TGIF
SIP1
Tob (BMP only)
HDAC4/5

Coactivators:

CBP/p300
SMIF
MSG1
ARC105

BMP Antagonists Expressed in Spemann's Organizer

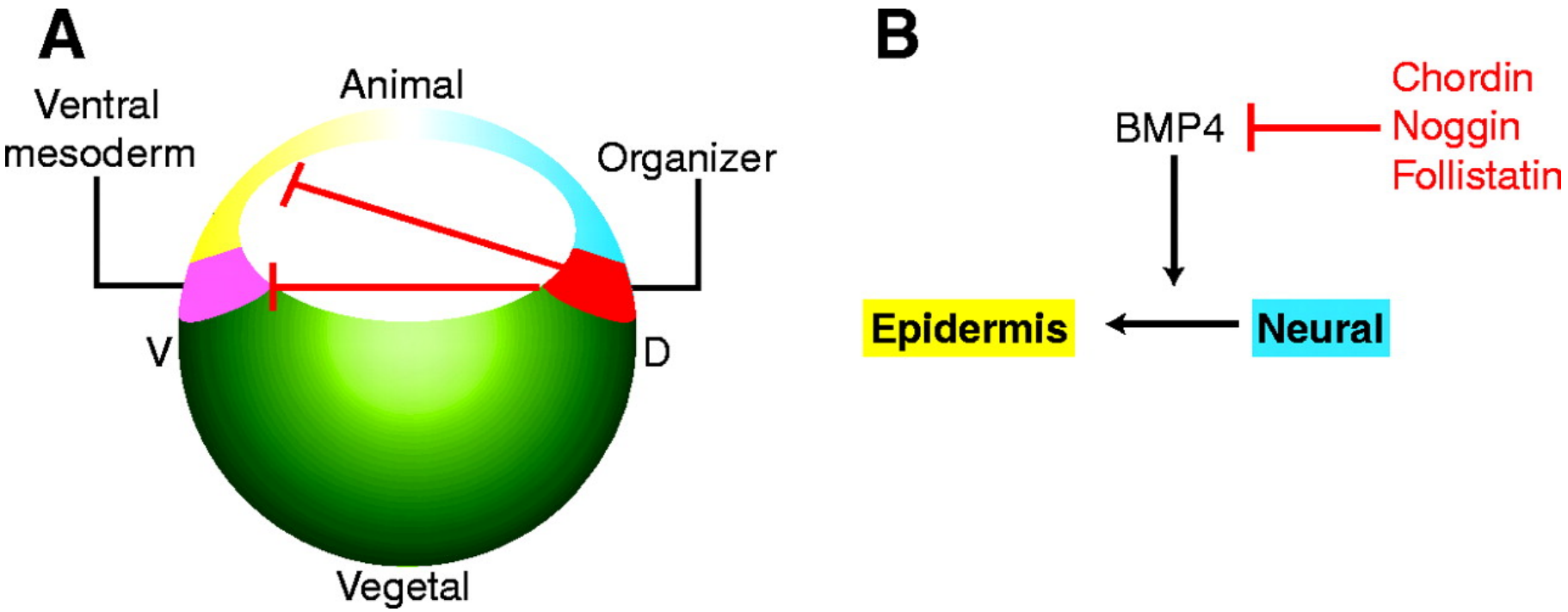


Xenopus tropicalis

Dorsal is to the right with the animal pole toward the top of the figure

Xnr3=Xenopus nodal-related-3

The `default model' in Xenopus



Prospective territories are: organizer in red, ventral mesoderm in pink, neural tissue in blue, epidermis in yellow and yolky endoderm in green.

Claudio D. Stern Development 2005;132:2007-2021

Main criteria for the activities of an **authentic neural inducer**:

1) the molecule should be able to induce neural tissue from animal cap ectoderm in the absence of dorsal mesoderm → **direct induction**

2) competent ectoderm should be responsive to the neural inducer at the gastrula stage, when dorsal mesoderm can still induce neural tissue

3) must be present at the right time and place to account for normal neural development

4) **elimination of its activity should block normal neural development**

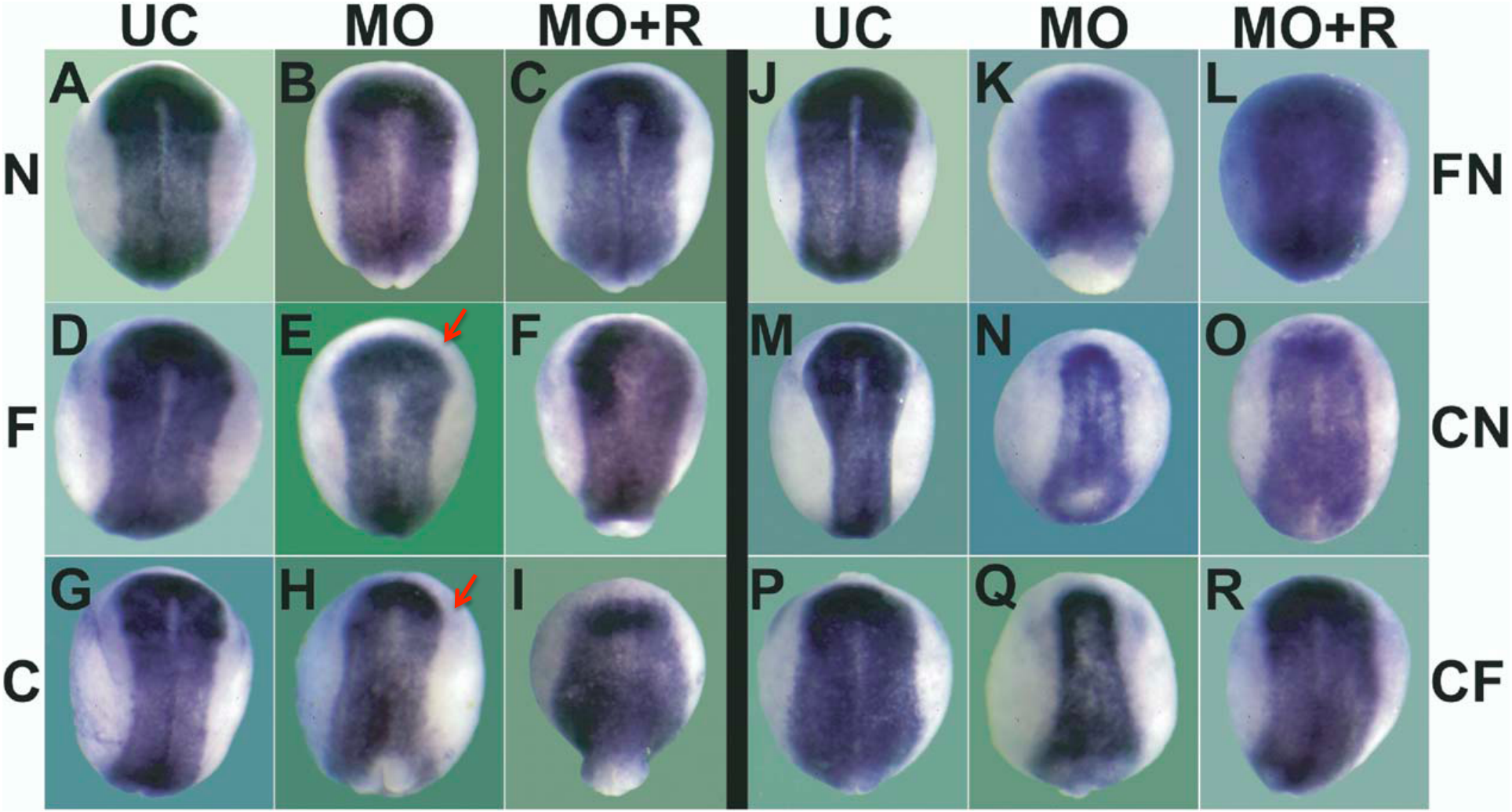
Loss of function assay

Morpholino based knock down
approaches to inhibit translation of mRNA

Xenopus tropicalis

Loss of function assay with Morpholinos

Sox2 expression in neurula (st 14–15) embryo



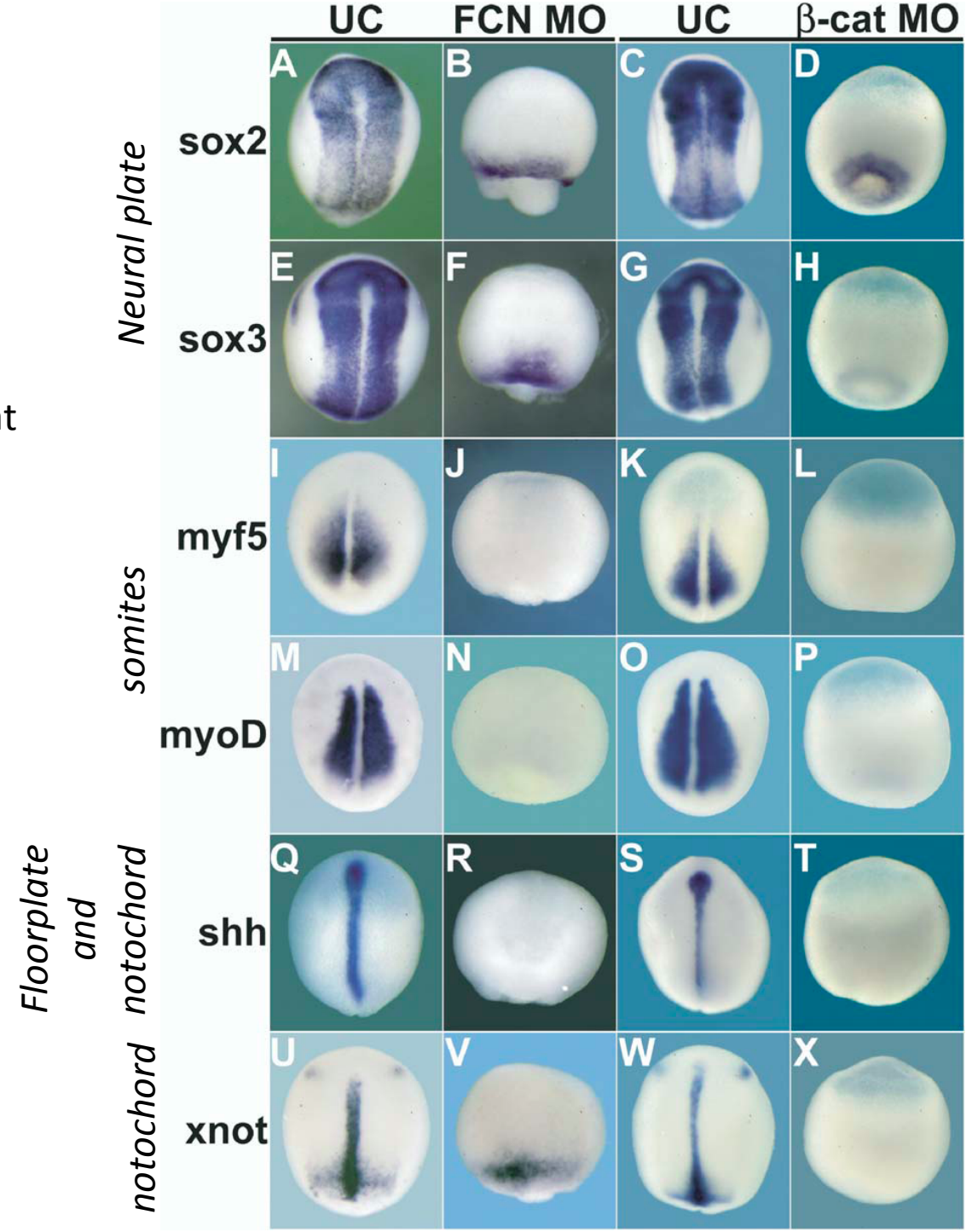
F = follistatin, C = chordin, N =noggin, UC = uninjected sibling control embryos, MO = morphant, and MO + R = morphant rescued with **pufferfish** noggin mRNA.

Khokha et al….R. Harland, Developmental Cell 2005

Deletion of 3 BMP antagonists
from Spemann's organizer leads
to a chatastrophic loss
of dorsal structure

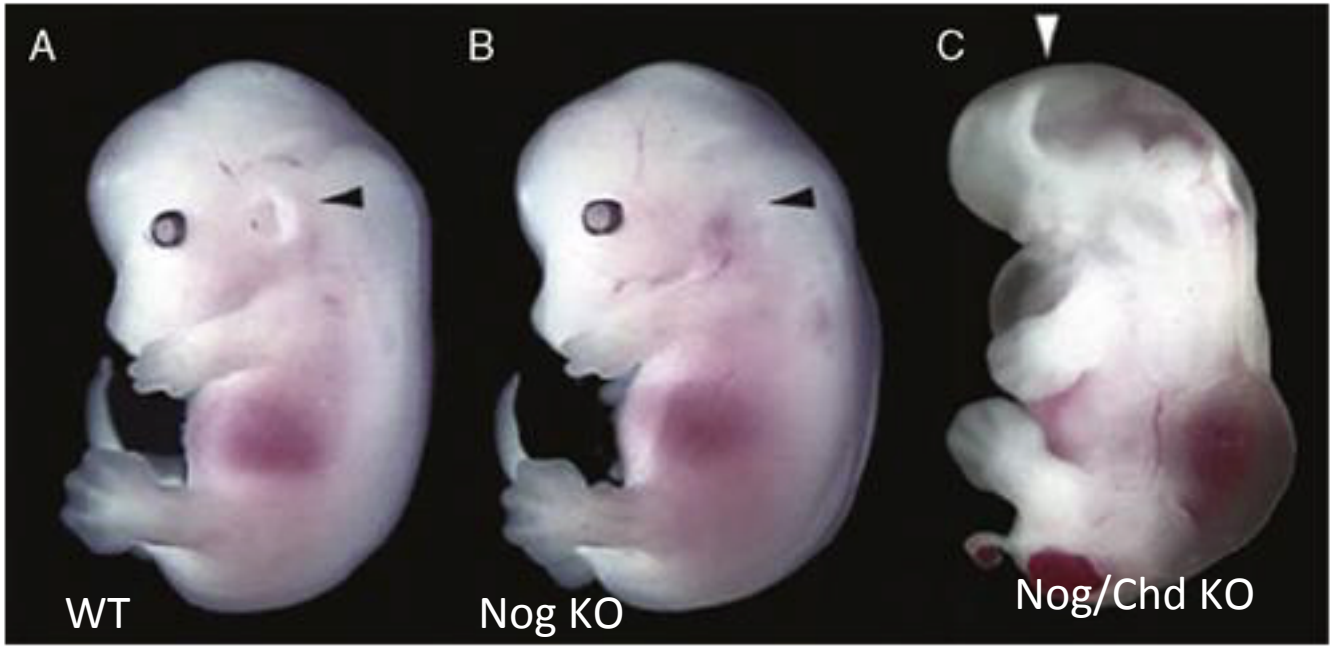
Lack a morphological neural plate at the neural stage and dorsal mesoderm structures

Conclusive in vivo evidence that BMP inhibitors are essential for neural induction
and
dorsal mesoderm, somites and notochord



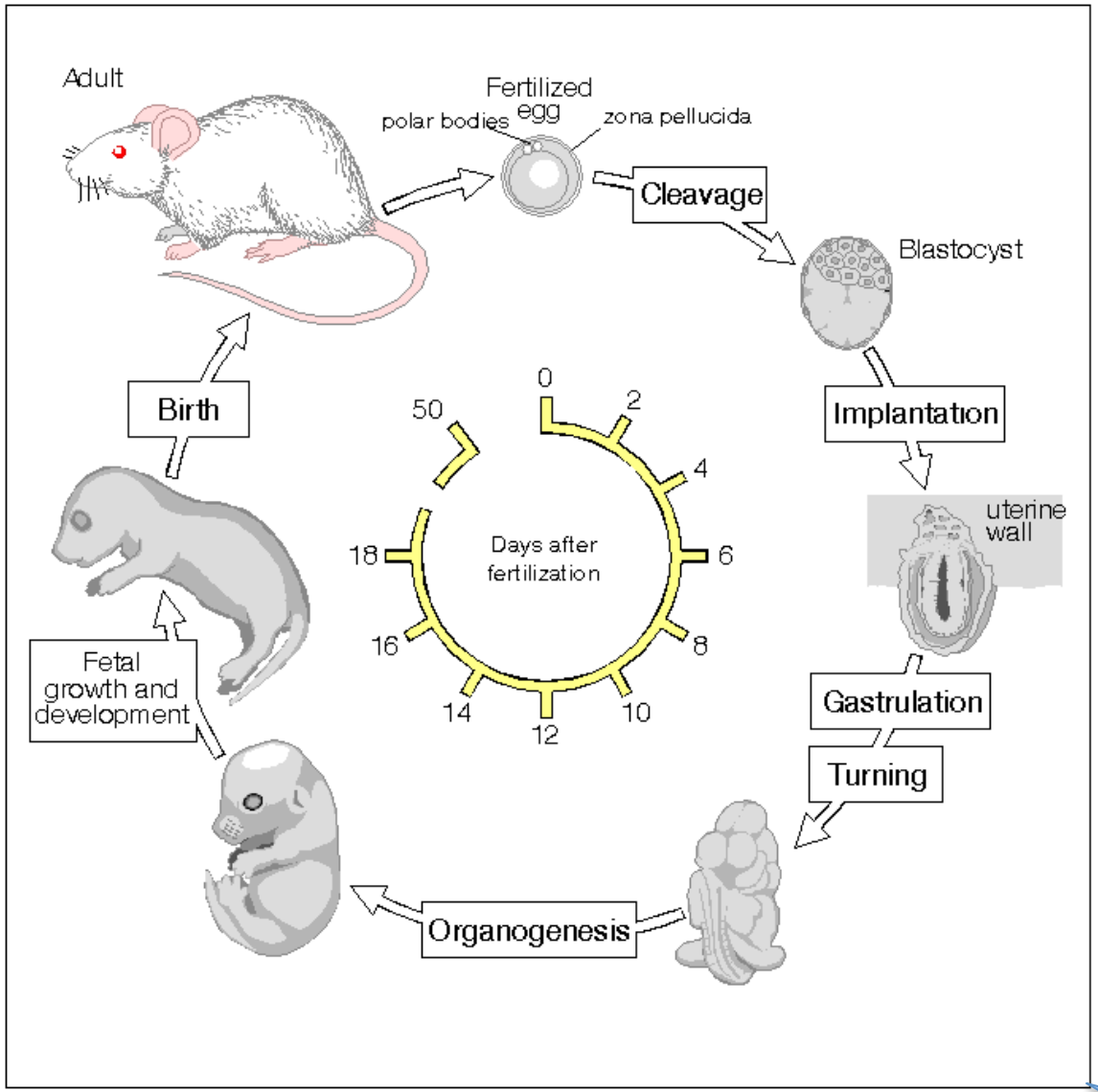
Khokha et al., Developmental Cell 2005

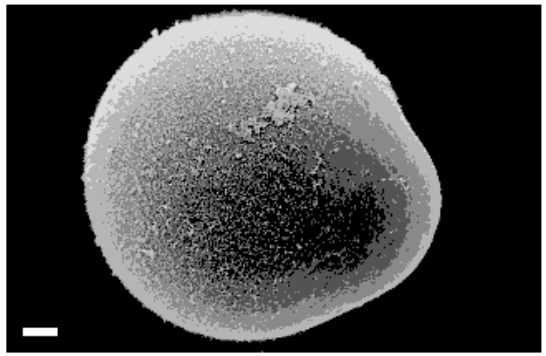
BMP patterning system and genetic redundancy in mice

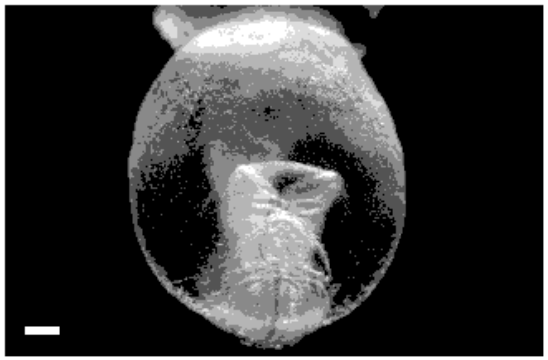


Genetic redundancy→ to be taken into account in programming a LOF approach

Bachiller et al., 2000


Adult
Fertilized egg
polar bodies
zona pellucida
Cleavage
Blastocyst
Implantation
uterine wall
Gastrulation
Turning
Organogenesis
Fetal growth and development
Birth
Days after fertilization
0
2
4
6
8
10
12
14
16
18
50





Watch video

Beyond the default model

- The default model may be too simplistic to describe neural induction: In amniotes, the expression of BMP ligands and antagonists is not entirely consistent with this model…

- Other factors involved:

FGFs:

- *In chicks, blocking FGF signalling can lead to a loss of neural markers like Sox3*

(In the urochordate Ciona intestinalis, FGF is an important neural inducing signal)

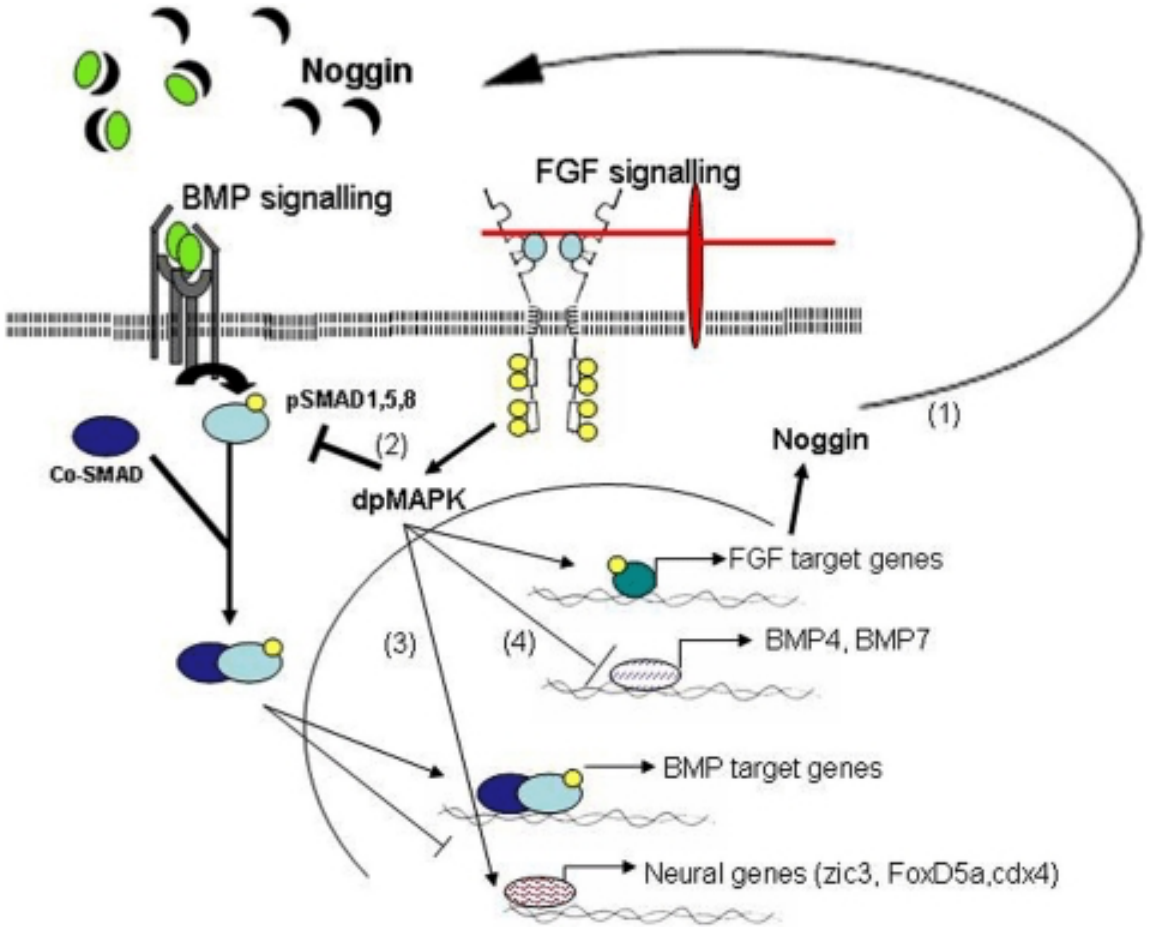
FGF impact on neural induction

(1) FGF signalling is required for the expression of Noggin, which acts outside the cell to bind and inhibit the activity of BMP ligands

(2) FGF signalling results in the phosphorylation of SMAD1, 5, 8 in a central domain, which inhibits its ability to move to the nucleus or activate the transcription of BMP target genes

(3) <u>FGF signalling can directly activate the transcription of a set of neural genes</u>

(4) FGF can inhibit the expression of genes coding for BMP ligands



FGFs: positive effectors of vertebrate neural induction → instructive activity

BMP inhibition initiates neural induction via FGF signaling and Zic genes

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Edited by Igor B. Dawid, National Institute of Child Health and Human Development, Bethesda, MD, and approved August 19, 2009
(received for review June 11, 2009)