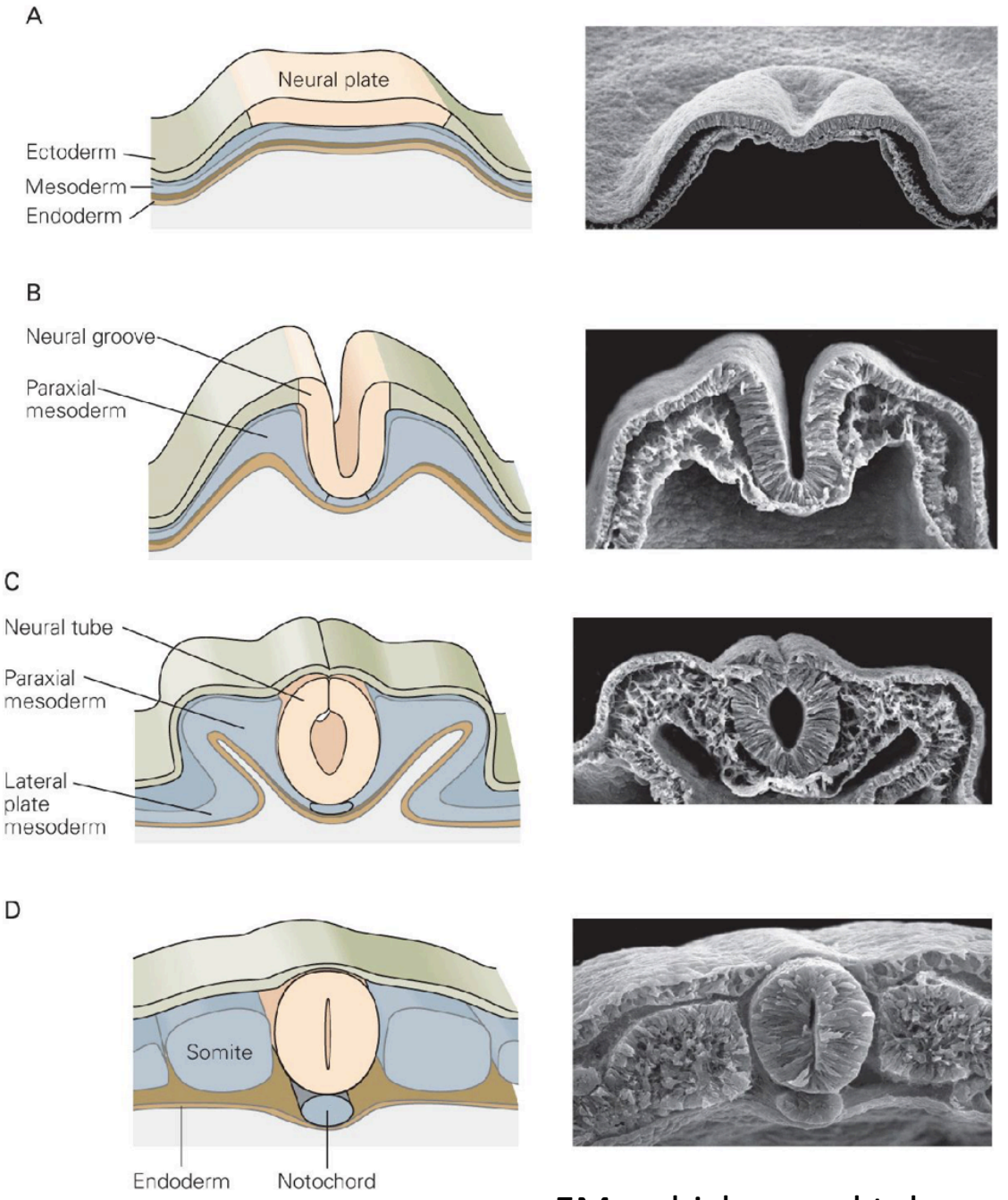
Neural induction and early patterning in vertebrates

Vertebrates

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

The **neural crest cells** originate at the dorsalmost region of the neural tube →PNS

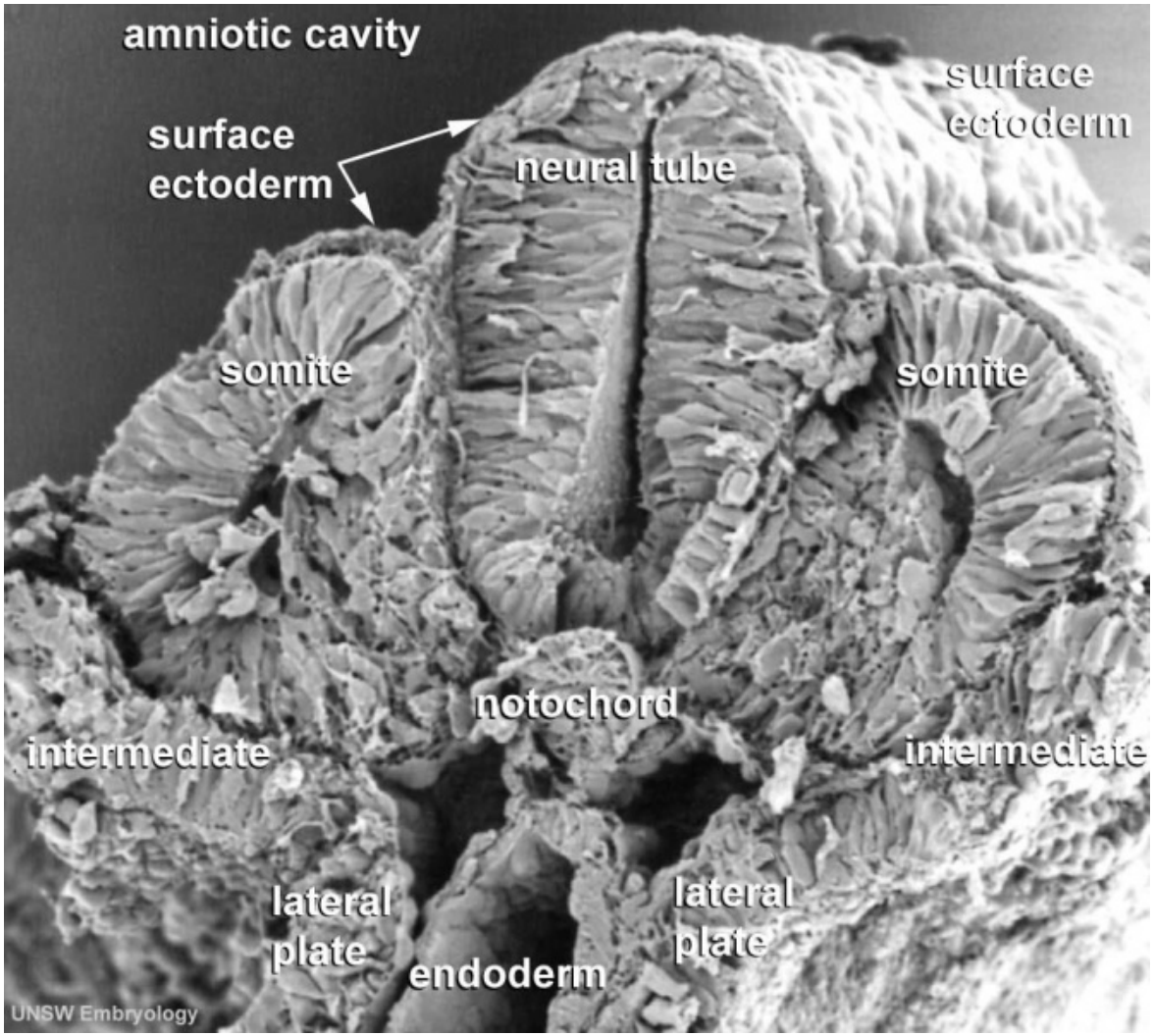
Ectodermal cells at the most anterior edge of the neural–epidermal boundary give rise to **placodes** that will form sensory organs as well as some **cranial sensory ganglia**



EM – chick neural tube

Week 4 Carnagie stage 11

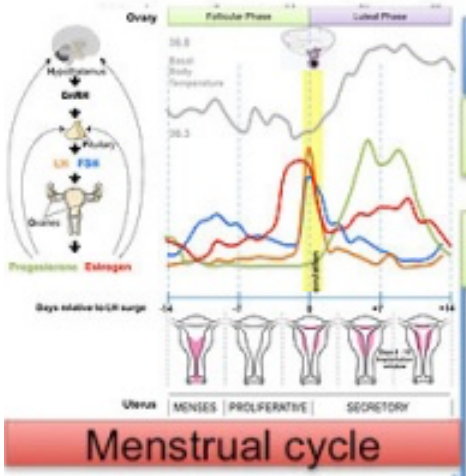
Human



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

Human Development Timeline



Menstrual cycle

Human Pregnancy - 9 months

First Trimester | Second Trimester | Third Trimester

Embryonic | Fetal

Embryonic Period (stages 1 to 23) | Fetal

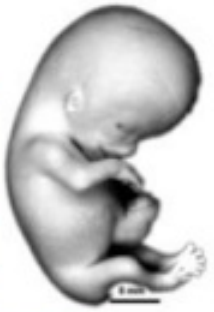
Last Menstrual Period

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

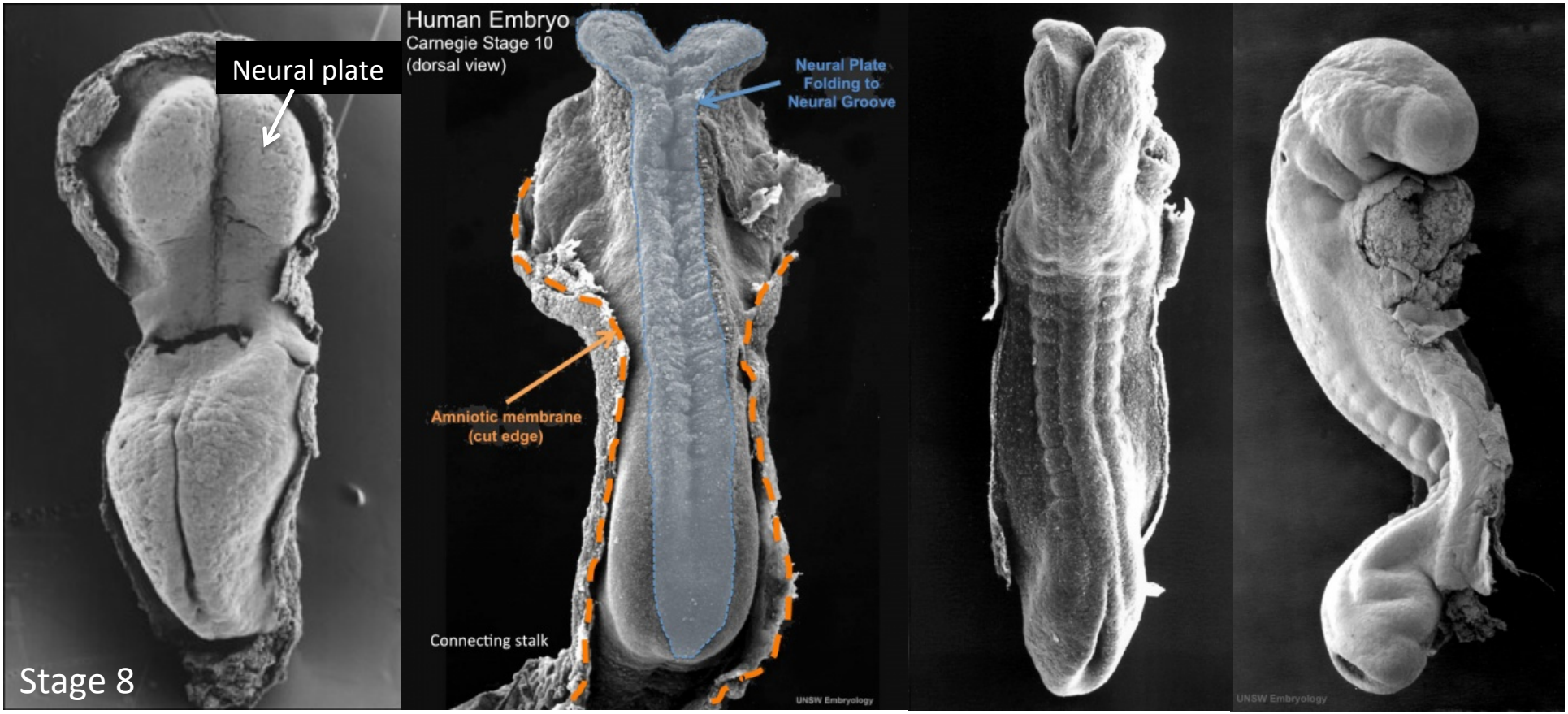
zygote
morula
blastocyst
bilaminar
trilaminar

Events

implantation
blastocyst hatching
gastrulation
folding
somitogenesis
cardiogenesis
neurogenesis
placodes

Human

Week 3 Week 4



Establishment of the neuroectoderm in vertebrates

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

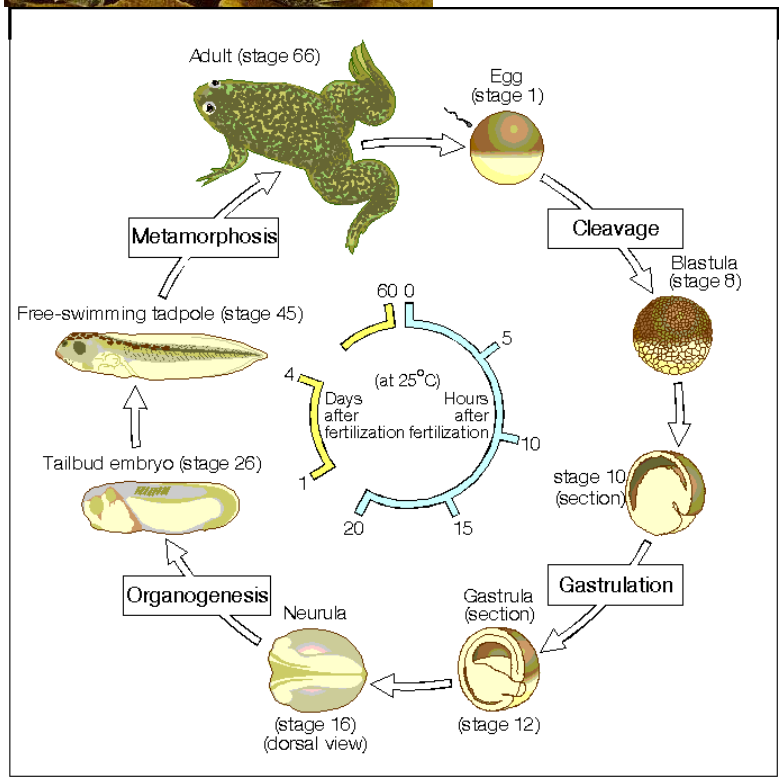
Neural induction in vertebrates

The development of the nervous system is triggered by signals from a powerful **'organizing' region** of the early embryo during gastrulation.

Neural induction was originally discovered and given conceptual definition by experimental embryologists working with **amphibian embryos.**

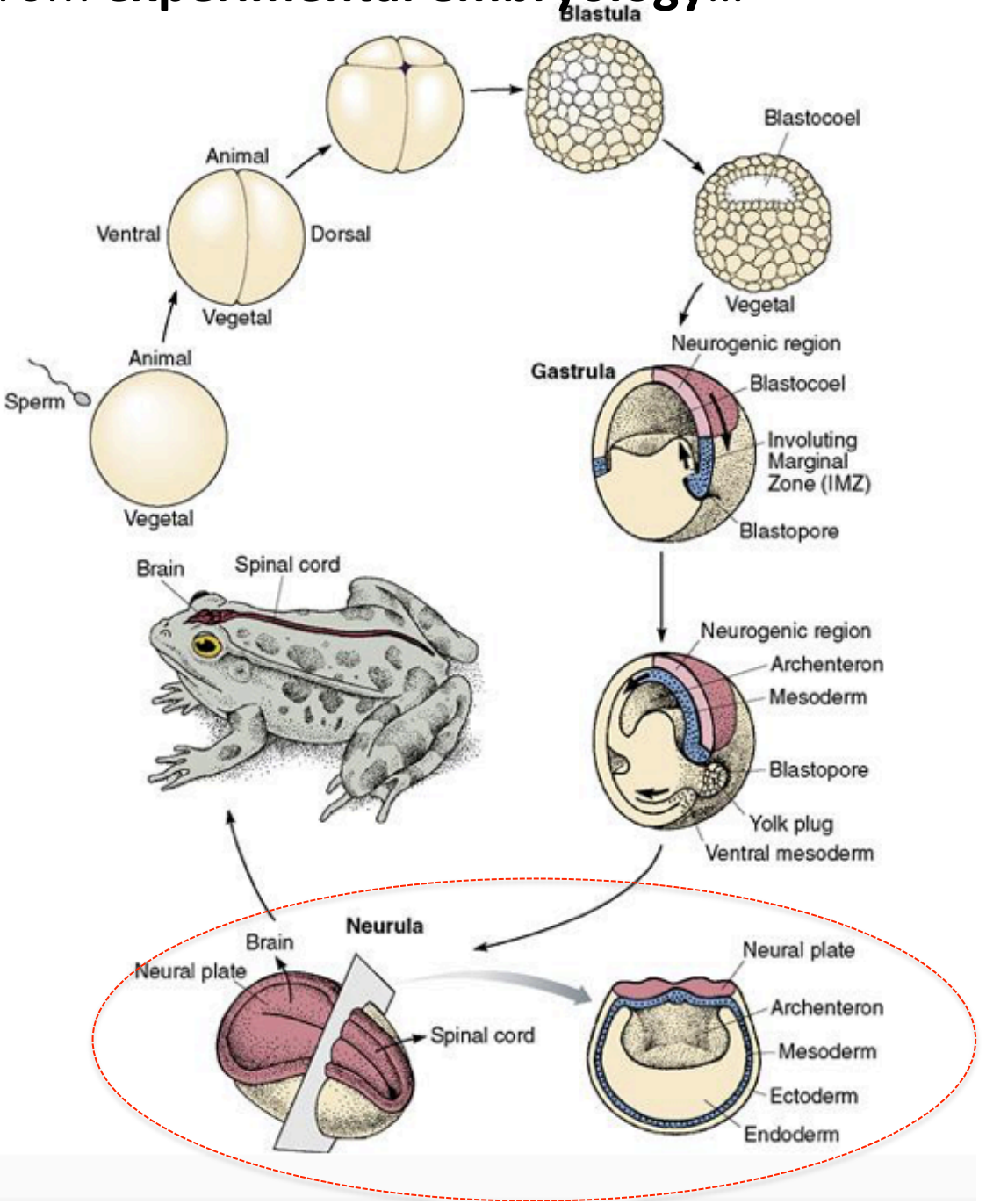
Many events involved in neural induction are characteristic of **all vertebrates** although the timing and geometry vary across phylogeny

Amphibia and some lessons from **experimental embryology**…



Life cycle

VIDEO neurulation



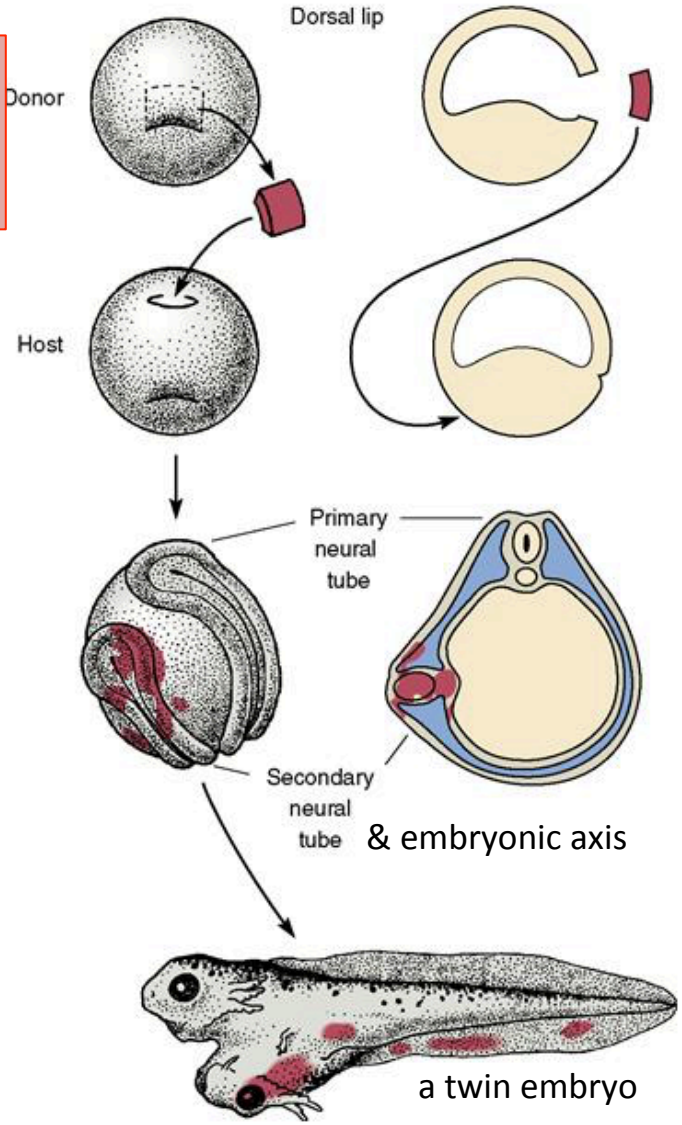
1. The Spemann & Mangold Experiment (1924)

1935 – Nobel Prize to Hans Spemann

VIDEO

newt embryos
at the gastrulation stage

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

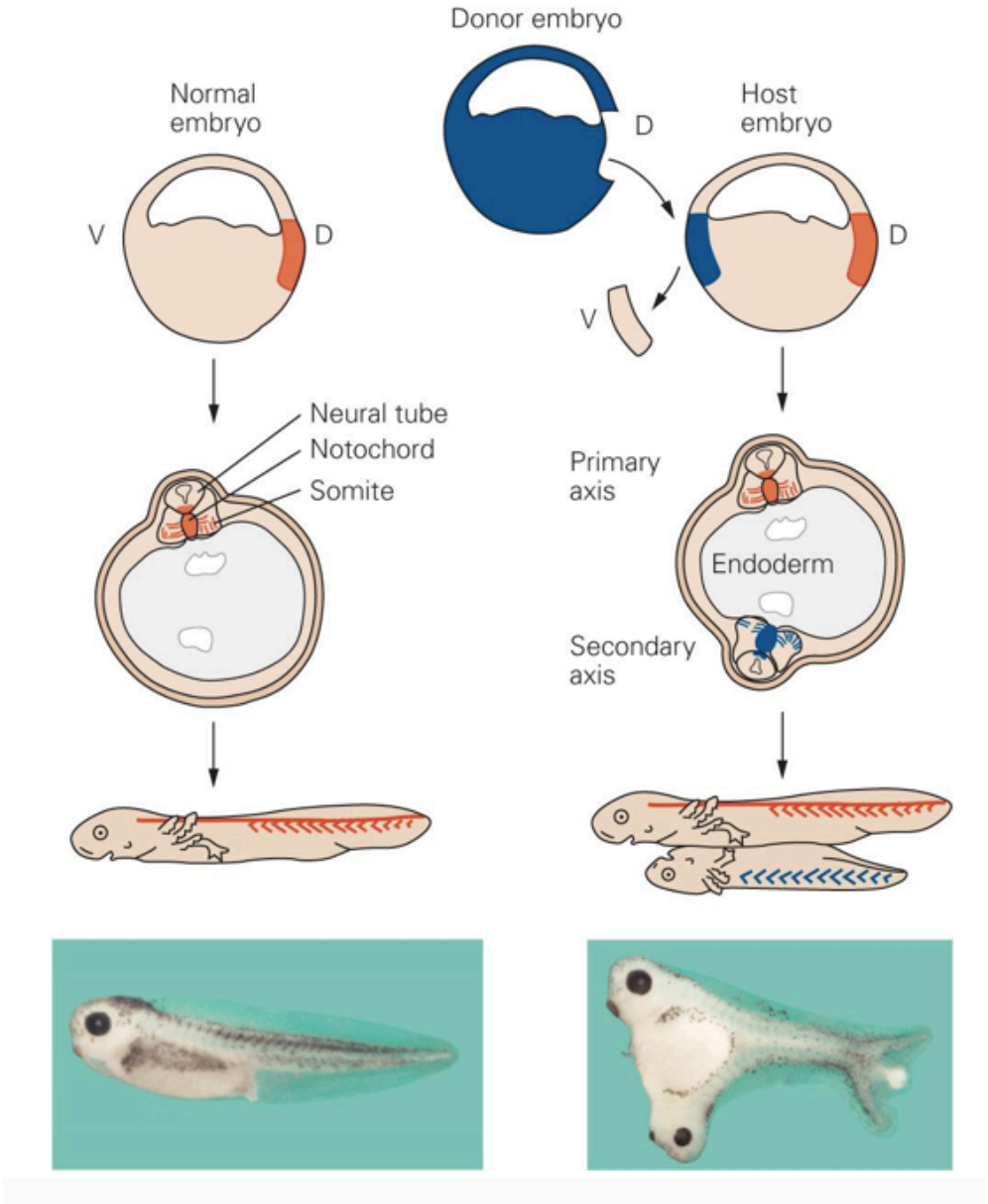


Embryonic Induction

→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

Transplanted organizer cells:

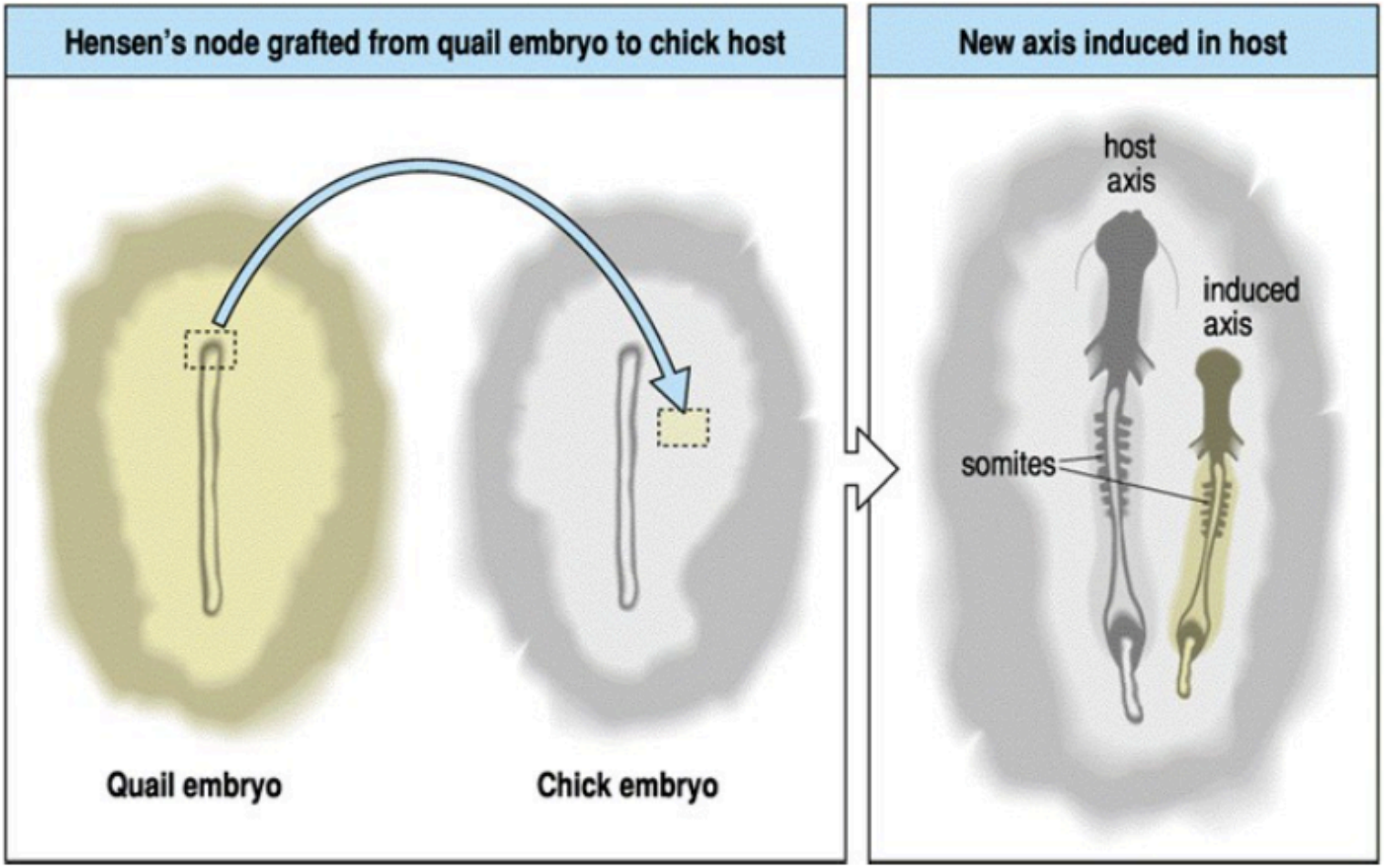
- follow their own developmental program (midline mesoderm tissue: notochord and somites)

- 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

Evolutionary conservation of the organizer:

Transplantation experiments were reproduced in fish – chick and mouse embryos…



2. Development of the Animal Cap Explants and Assays

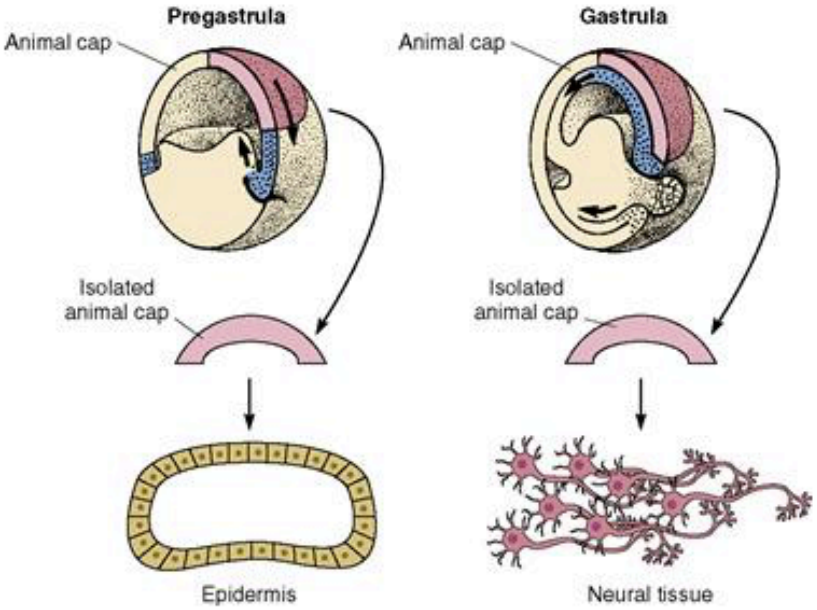


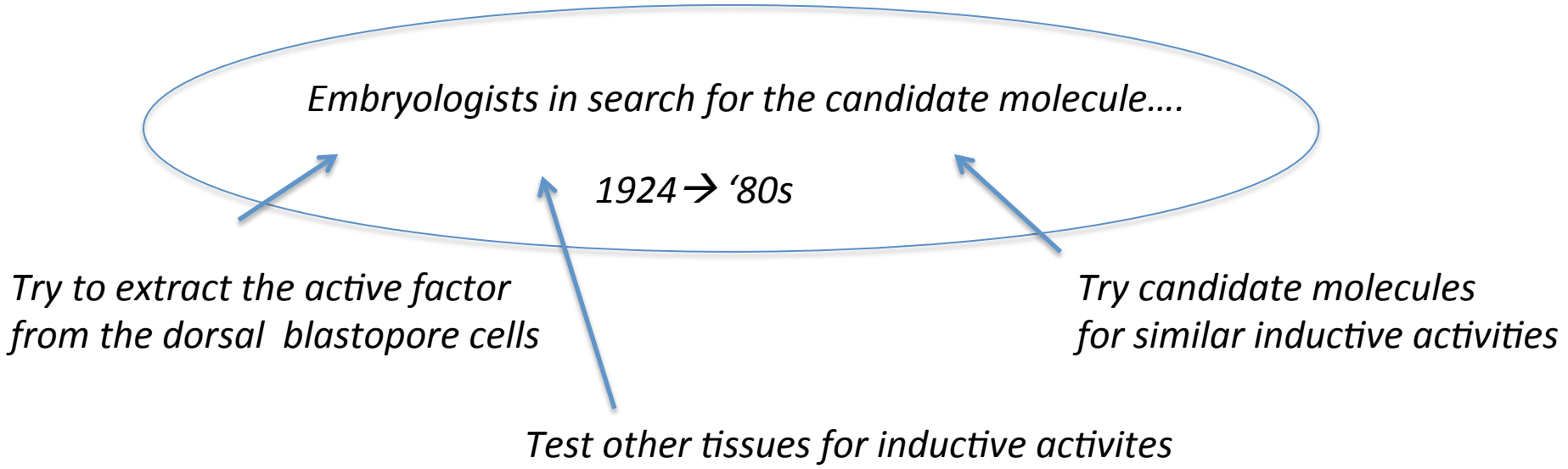
Fig. 1.11 Isolation of fragments of embryos at different stages of development demonstrates when tissue becomes committed to the neural lineage. If the animal cap is isolated from the rest of the embryo (left), the cells develop as epidermis, or skin. If the same region of the embryo is isolated a few 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 development (Amphibian embryos)

Results: cell types differentiate depending on the stage and co-culture tissue

1° Hypothesis: the ectoderm forms epidermis as a default state → the organizer and resulting notochord, through secreted soluble molecules, **instruct neural-plate differentiation** in the overlying ectoderm.

What is the molecular nature of the **neural inducer?**



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

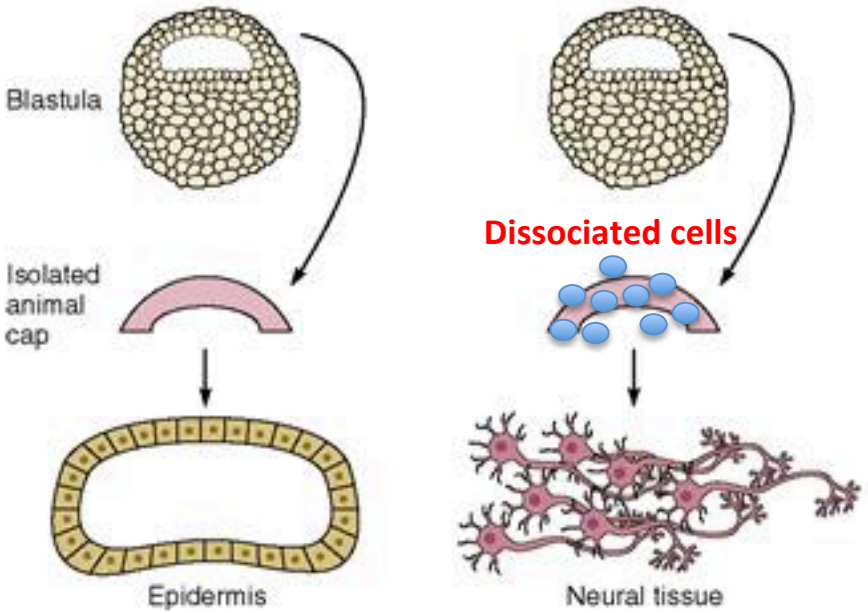
Neural differentiation of Xenopus laevis ectoderm takes place after disaggregation and delayed reaggregation without inducer.

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.



?

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

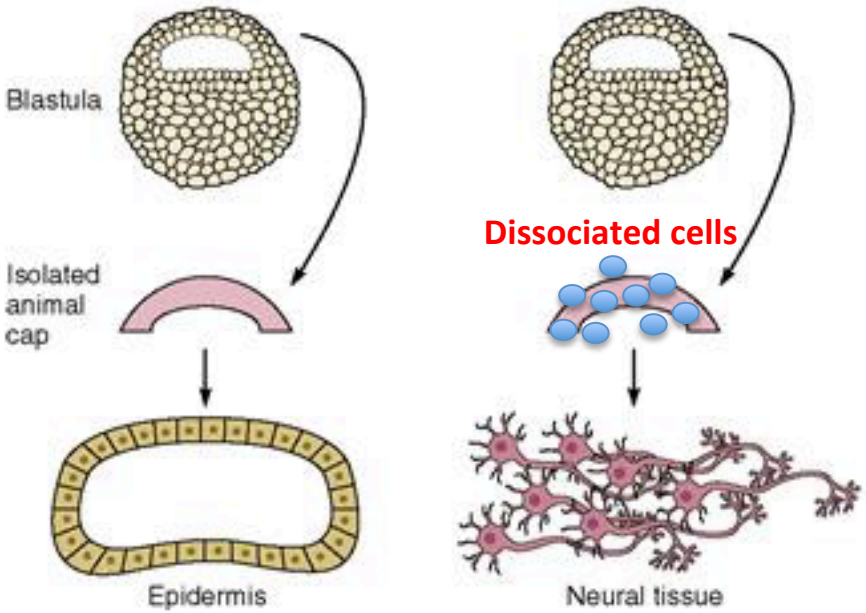
Neural differentiation of Xenopus laevis ectoderm takes place after disaggregation and delayed reaggregation without inducer.

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.



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

1° Hypothesis: : the ectoderm forms epidermis as a default state → 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 consists of an **antagonist**, 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 cells

The inducer is a de-repressor of neural fate

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

What experimental results demonstrate that a particular signal induces a response ?

LOCATION
(be at the right place at the right time)

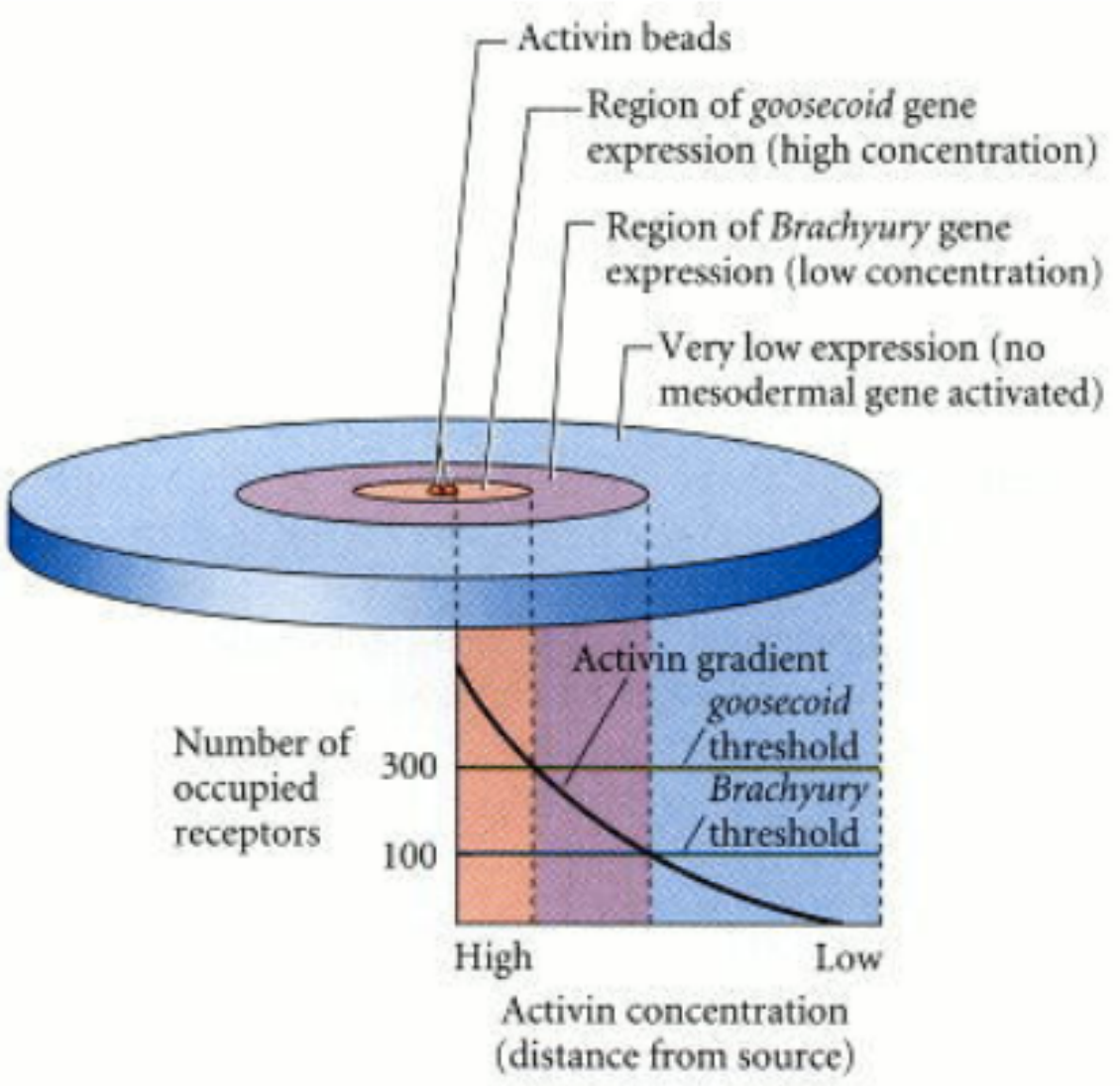
NECESSITY
(when signal is blocked – no response)

SUFFICIENCY
(the signal is able induce the response
peraphs ectopically)

Interpretation of Activin gradient by *Xenopus* animal cap cells

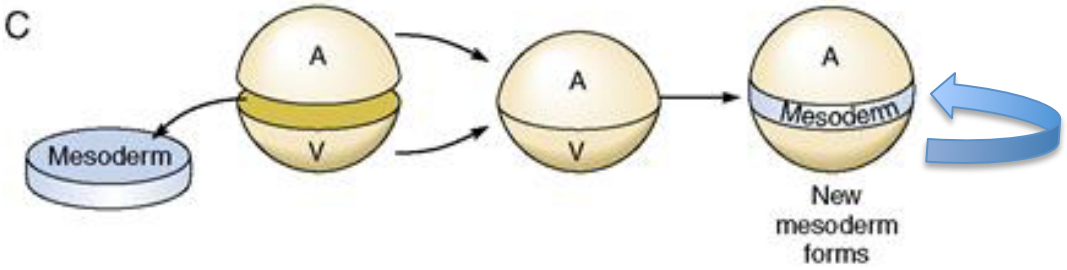
high concentrations of Activin activate the ***goosecoid*** gene (→dorsal mesoderm= Notochord, muscle..) …some neural tissue…

lower concentrations of activin activate the ***brachyury*** **gene** (→ventromedial mesoderm=blood, connective tissue)

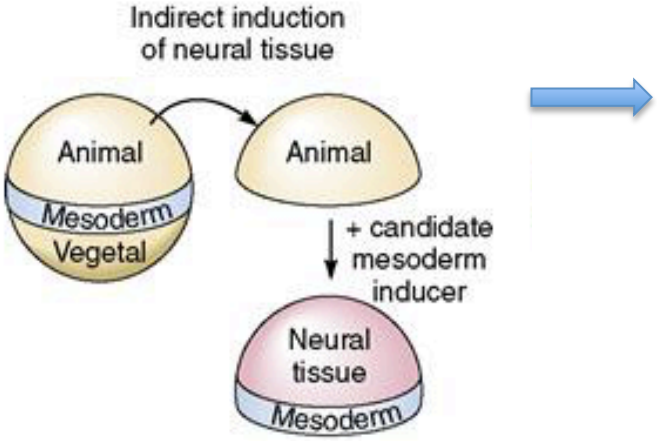


Candidate n°1: Activin

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**

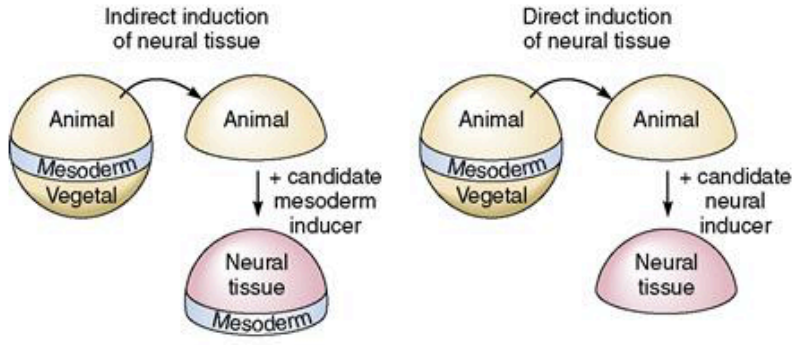


Fig. 1.14 Indirect neural induction versus direct neural induction. The organizer transplant experiments show that the involuting mesoderm has the capacity to induce neural tissue in the cells of the animal cap ectoderm. When assaying for the factor released from mesoderm that is responsible for this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

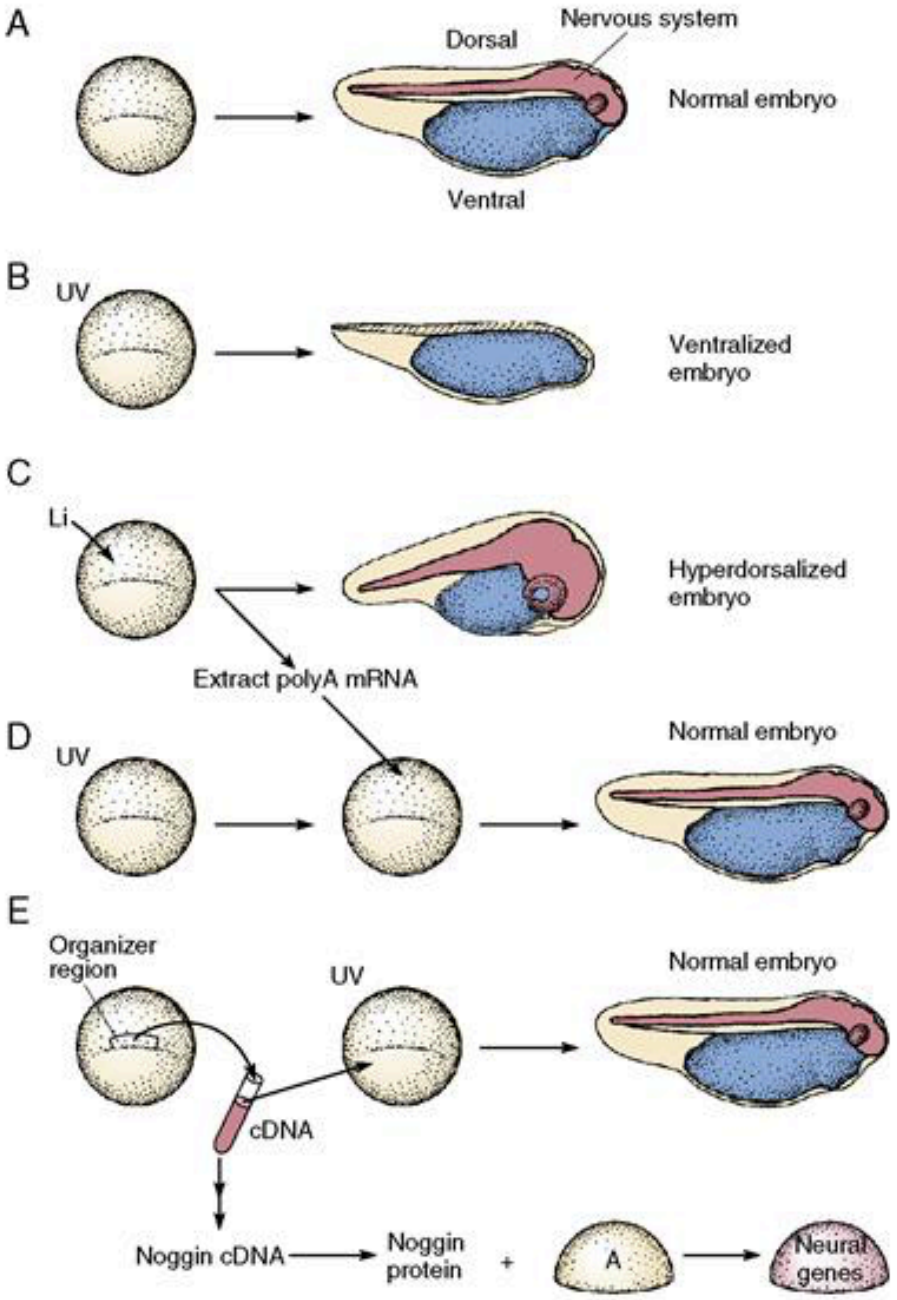
RESEARCH ARTICLE

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:



Screening cDNA libraries for their neural-inducing activity

Noggin = the first *bonafide* endogenous direct neural inducer

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

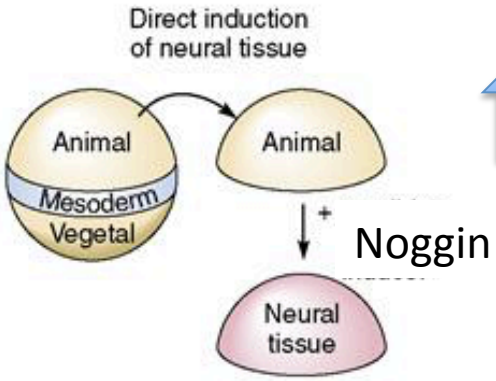
noggin expression begins at the **late blastula stage** in the **prospective dorsal mesoderm** and continues in the gastrula stage **organizer**.

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**

How to prove the direct neural induction by noggin ???

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

- analysis of specific markers (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.

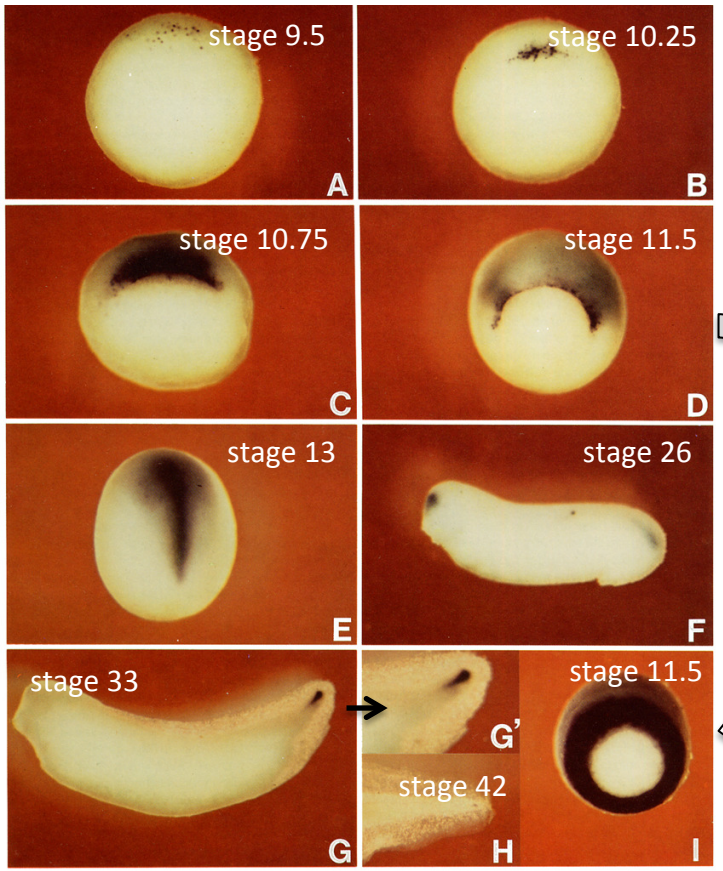
Sasai Y[1], Lu B, Steinbeisser H, Geissert D, Gont LK, De Robertis EM.

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.

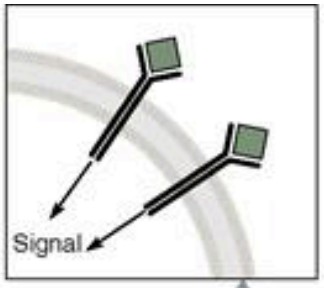
Hemmati-Brivanlou A[1], Melton DA.

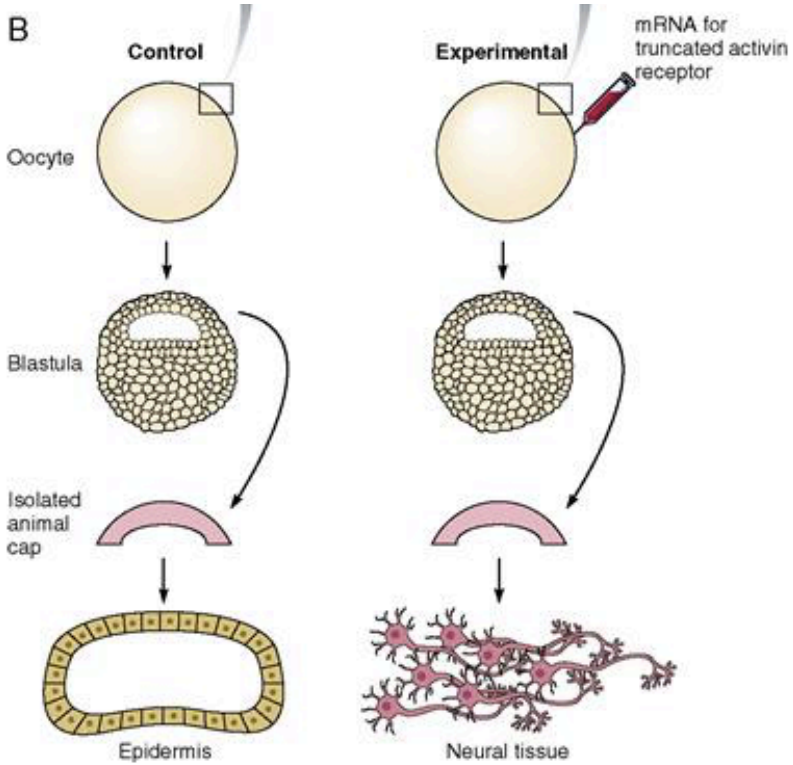
⊕ Author information

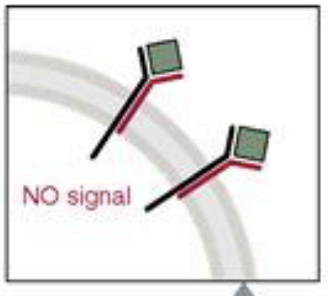
Abstract

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

*broad acting

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]

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.

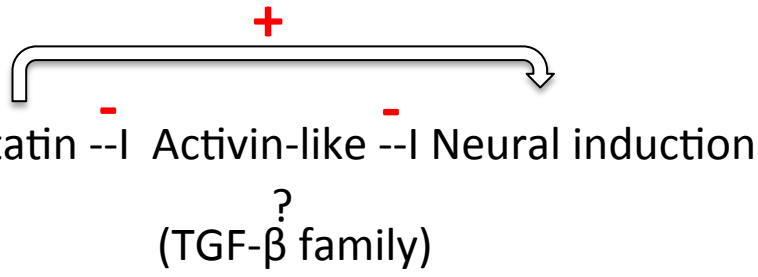
⊕ Author information

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

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.

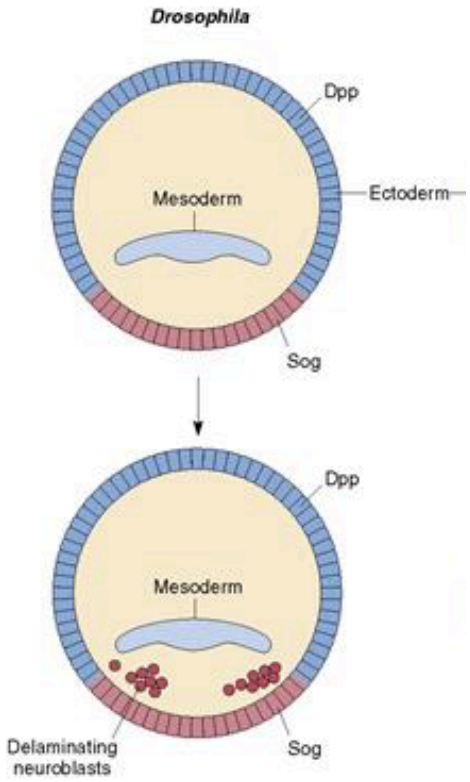
A conserved system for dorsal-ventral patterning in insects and vertebrates involving sog and chordin.

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**

decapentaplegic = homologus of **BMP-4**
(member of the TGF-β family)

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.

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

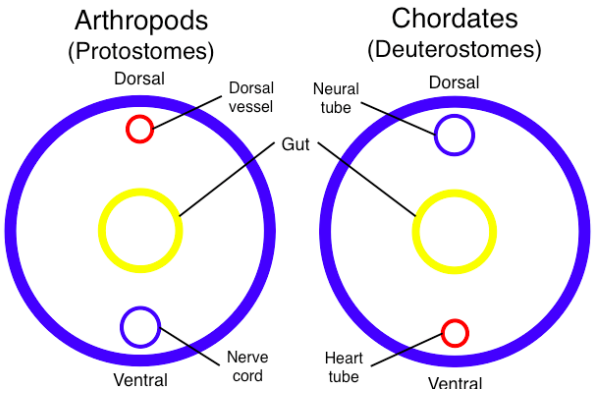
Erratum in

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.

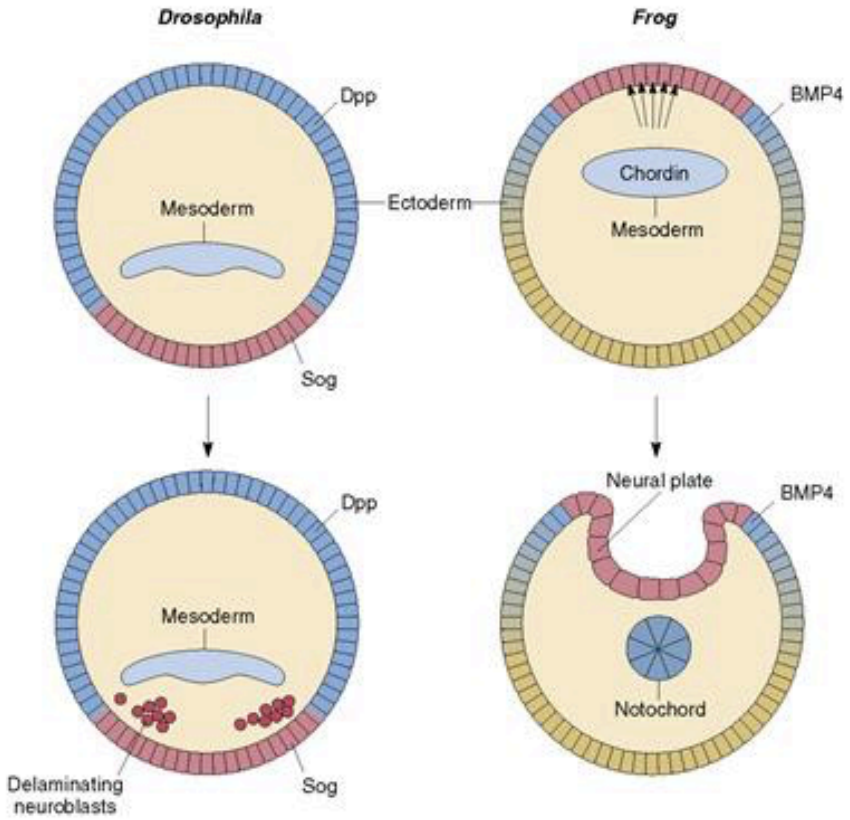
Evolutionary conservation of molecular circuitry underlying neural induction



The embryonic axis is flipped in Arthropods compared to Chordates:
the epidermis forms in the dorsal regions, whereas the neural tissue arises from a ventral position.

Dpp=decapentaplegic

Sog=short gastrulation

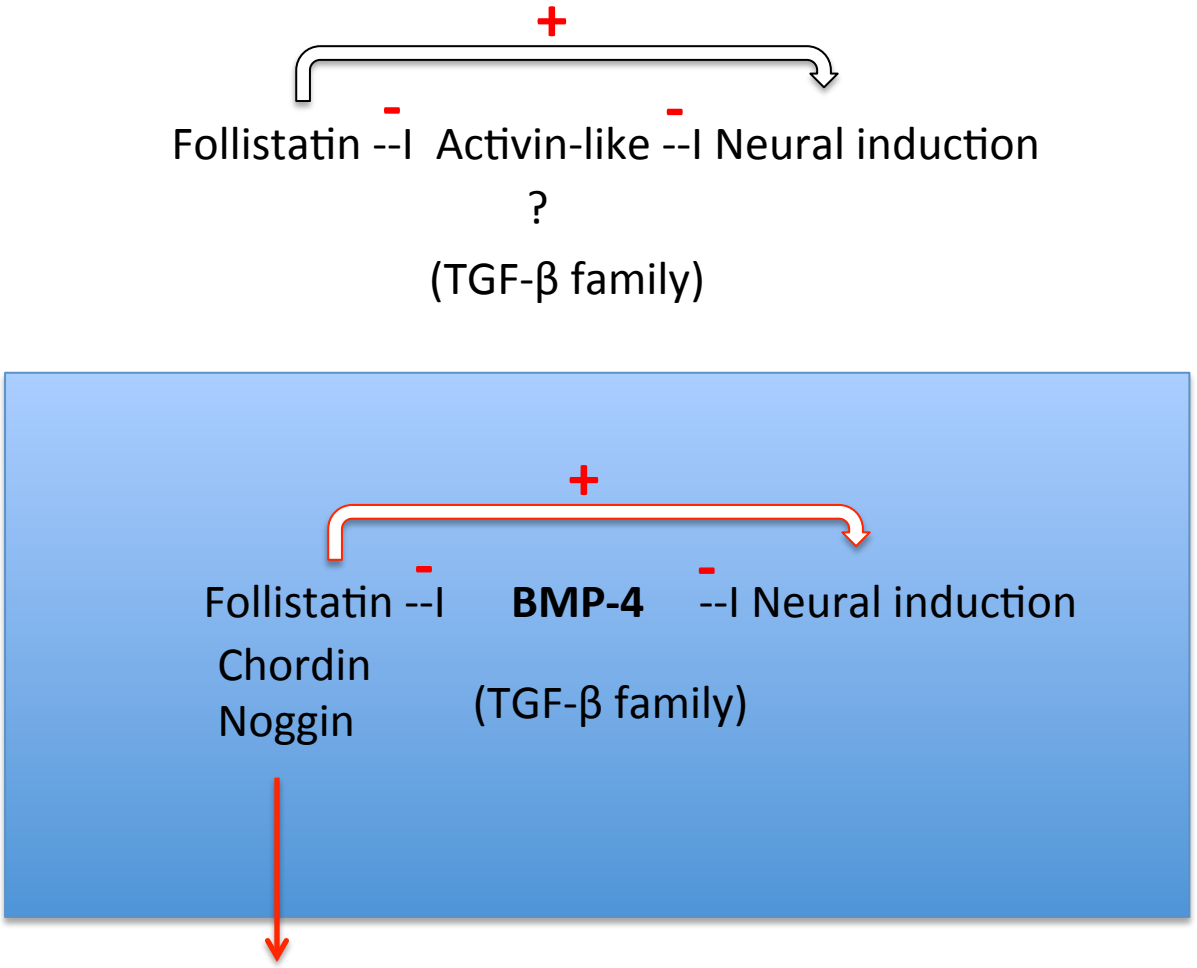


BMP4=bone morphogenetic protein 4

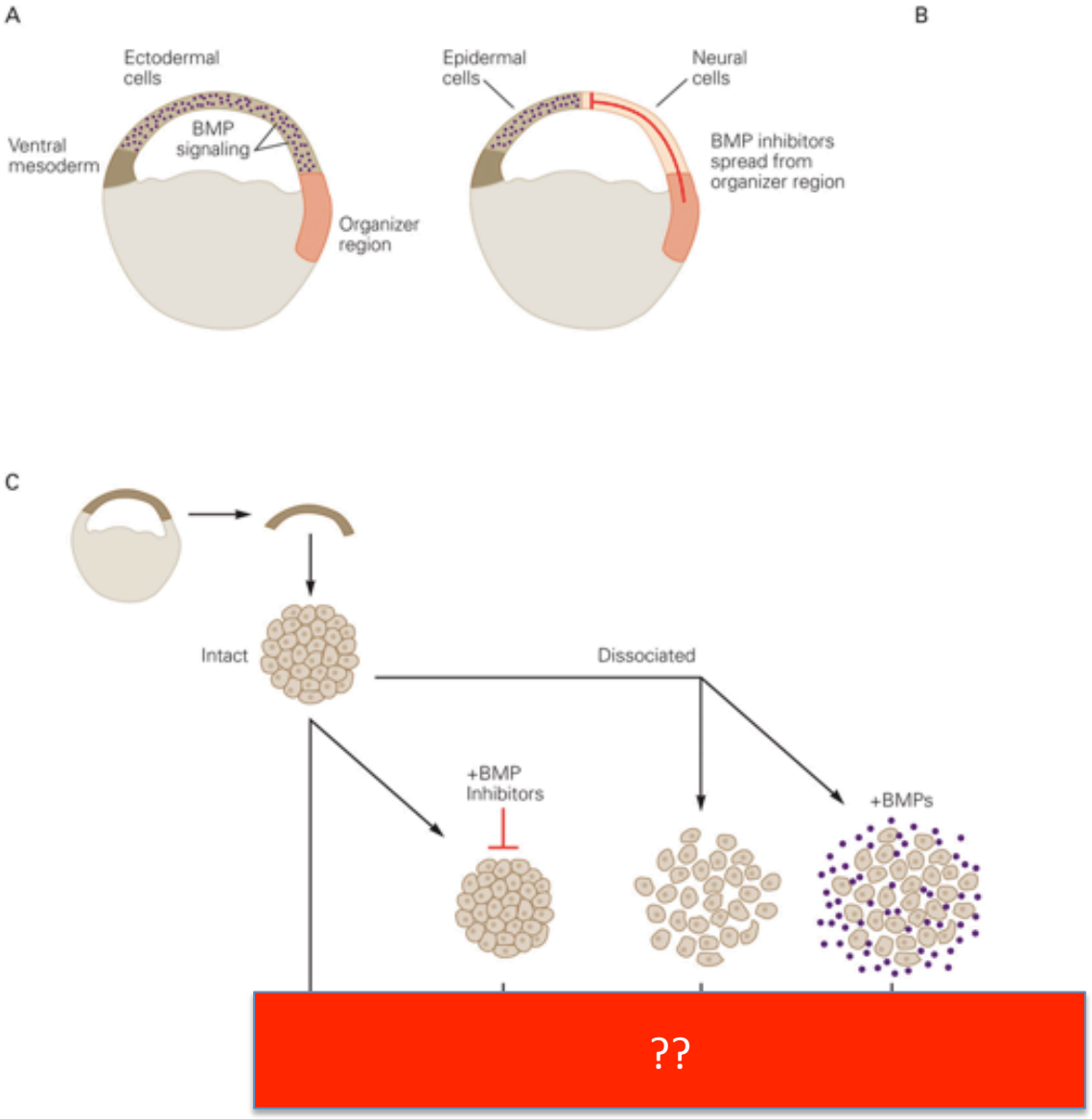
Chordin

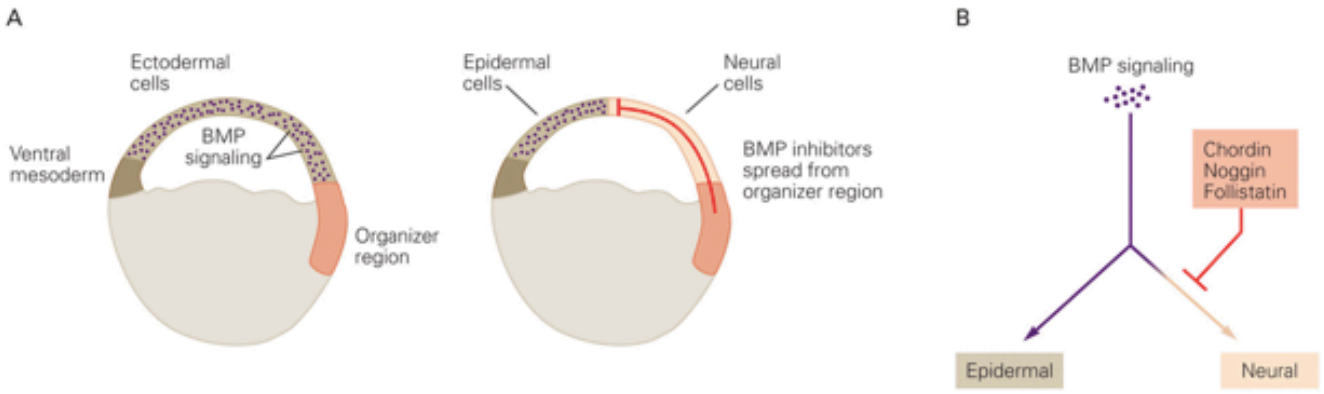
in drosophila

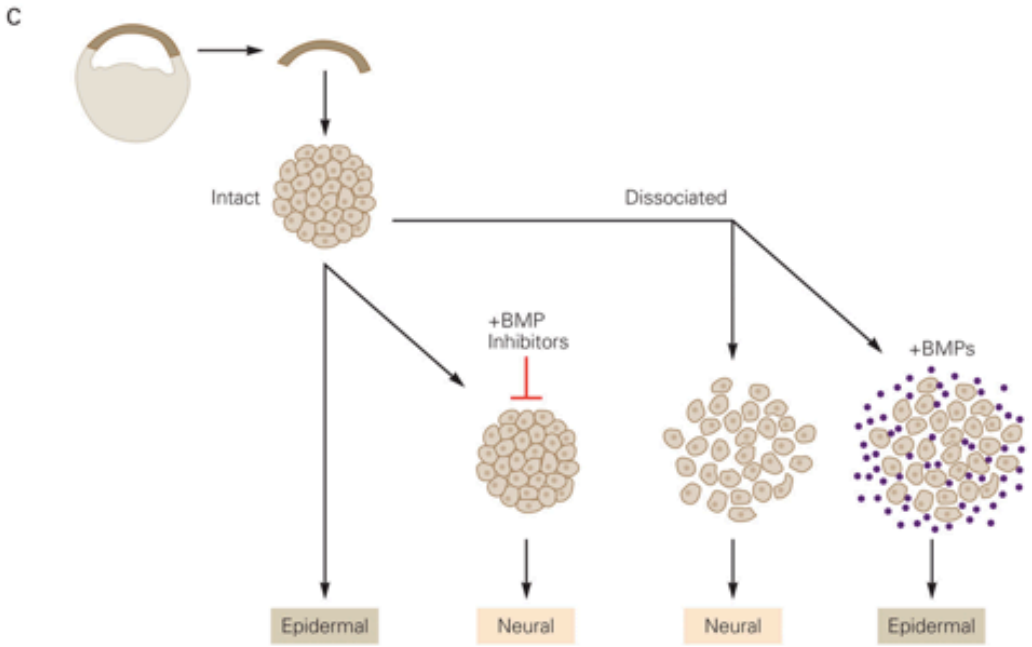
in the frog



All potent extracellular inhibitors of TGFbeta family signaling
→ Bind with high affinity to the ligands and prevent them from activating their cognate receptors


A
Ectodermal cells
Ventral mesoderm
BMP signaling
Organizer region
B
Epidermal cells
Neural cells
BMP inhibitors spread from organizer region
C
Intact
Dissociated
+BMP Inhibitors
+BMPs
??


A
Ectodermal cells
Ventral mesoderm
BMP signaling
Organizer region
Epidermal cells
Neural cells
BMP inhibitors spread from organizer region
B
BMP signaling
Chordin
Noggin
Follistatin
Epidermal
Neural


C
Intact
Dissociated
+BMP Inhibitors
+BMPs
Epidermal
Neural
Neural
Epidermal

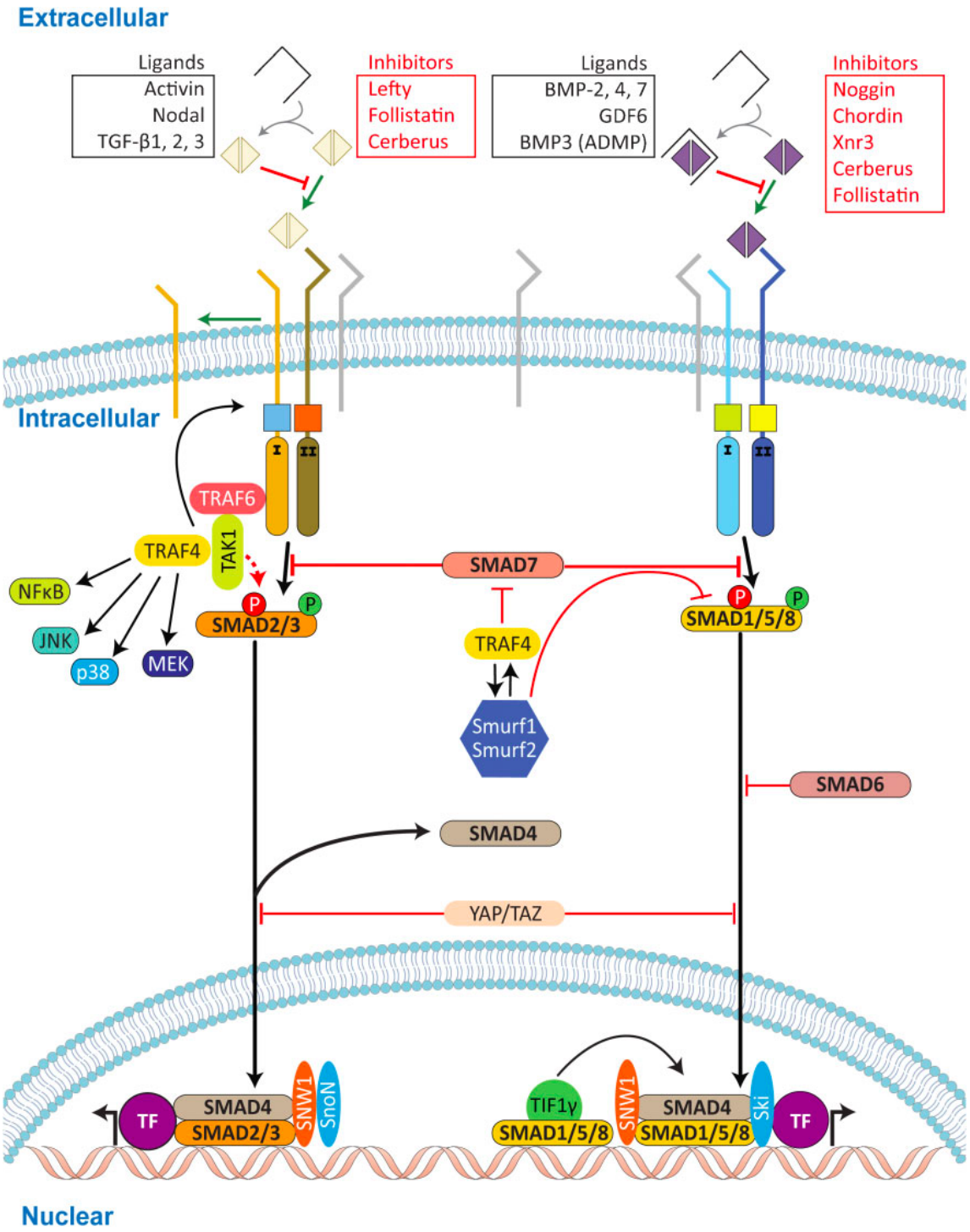
BMP4,2,7

Animal cap cells pass through 2 competence phases sequentially:

1) mid and late blastula: activin/nodal signaling → mesoderm derivatives

2) gastrula -early neurula: BMP signaling→epidermis

Neural fate occurs when animal cap cells avoid both activin and BMP signals



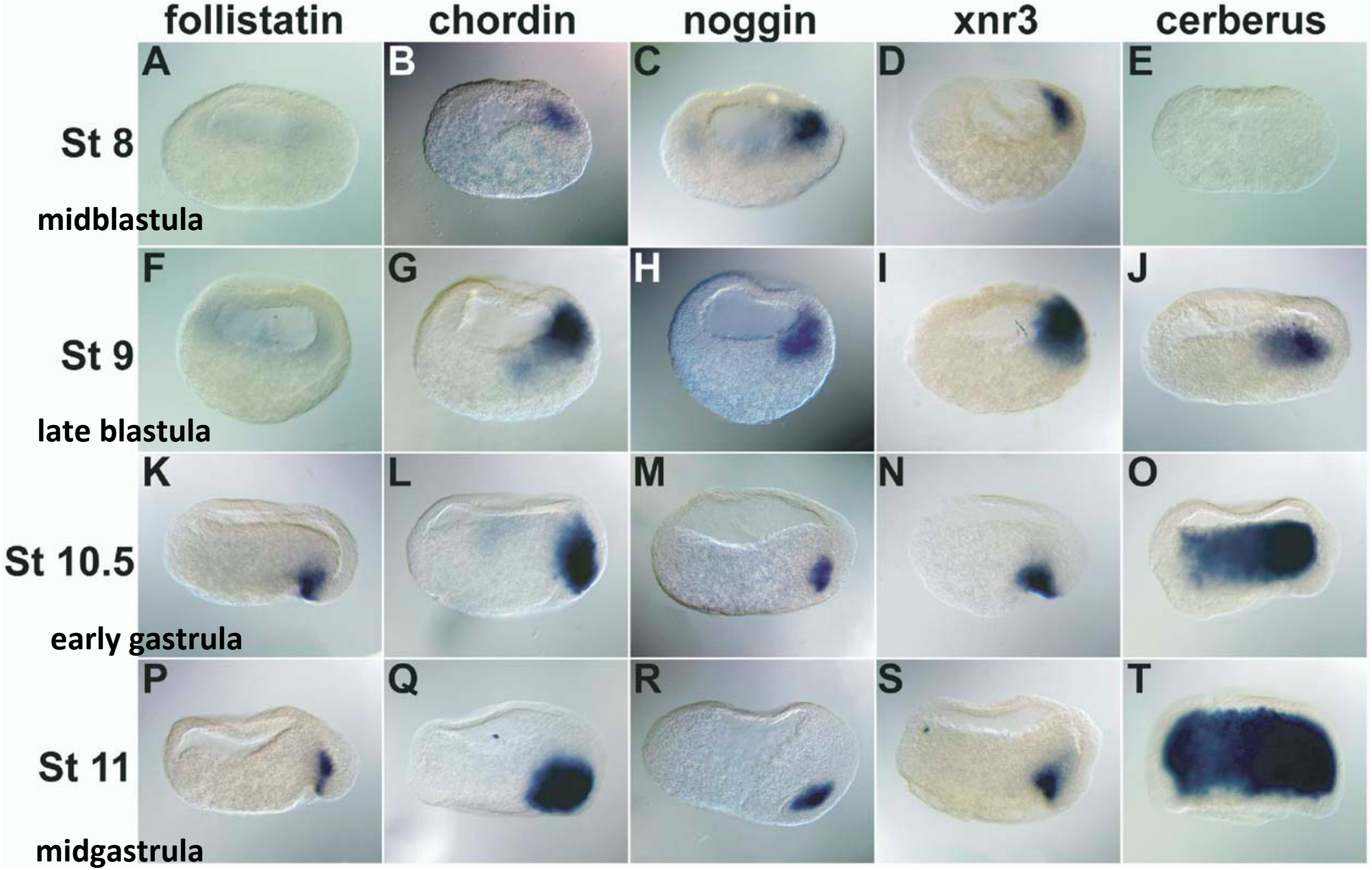
The transforming growth factor-*β* (TGF*β*) pathway: 2 main branches

co-inhibition of both SMAD2/3 and SMAD 1/5/8 branches induces neural fate more potently than each alone…similarly to dominant negative activin receptor

Inhibithors downstream of receptor activator:

- Phosphorylation of linker region of R-Smad via MAPK, GSK3*β*, and CDKs.
- Smad6 and Smad7
- YAP/TAZ complex regulates Smad nuclear translocation

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

TABLE 1 | Secreted Inhibitors of the BMP Pathway

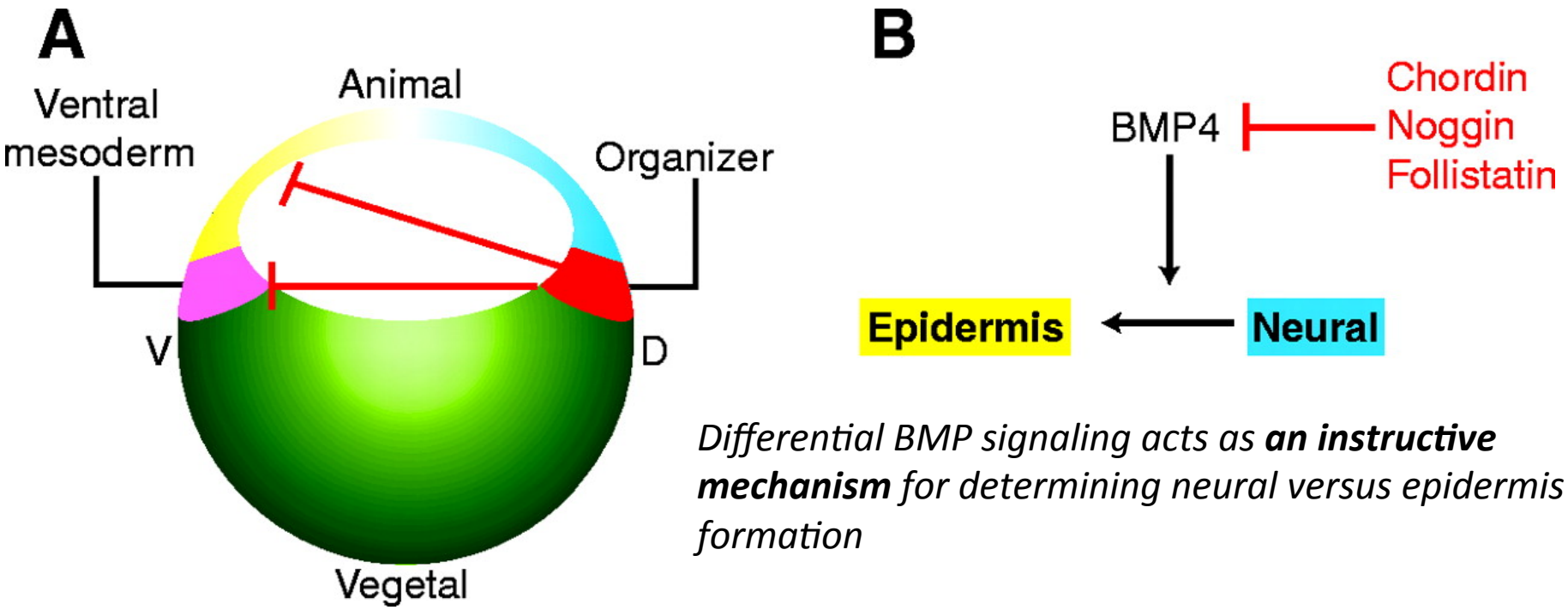
Gene	Inhibits	Species	Gastrula Expression[1]	Features-Comments	References
Chordin	BMP-2,4,7	Mouse *Xenopus* Zebrafish Chicken	Node (m) Organizer (x,z) Node and rostral mesendoderm (c)		26 30 28 31
CHL/chordin-like	BMP-4,5,6	Mouse	No	3 CR domains	32
Noggin	BMP-2,4,7 GDF-5	Mouse *Xenopus* Zebrafish	Node (m) Organizer (x,z) Axial mesendoderm (c)	3 noggin-like genes found in Zebrafish	25 33 34 35 36 37
Follistatin	BMP-2,4,7,11 GDF-8,11 Activin	Mouse *Xenopus* Chick	Node (m) Organizer (x) Node, mesendoderm, caudal neural plate (c)		27 38 39 40 37
FSRP proteins: FLRG, Flik	BMP-2,6,7 Activin	Mouse Chicken	FLRG: e7.0 by Northern (m) Flik-1: node (c)	Follistatin related	41 42 43 44 45
Cerberus	BMP-4 xNr-1,2 Wnt-8	*Xenopus* Mouse (Cer1) Chicken	Anterior endoderm (x) Anterior visceral endoderm (m) Hypoblast, Ant. Endoderm, Prechordal plate (c)		46 47 48 49 50

TABLE 1 | Secreted Inhibitors of the BMP Pathway

Gene	Inhibits	Species	Gastrula Expression[1]	Features-Comments	References
Coco	BMP-4 Activin xNr-1 Wnt-8	*Xenopus*	Gradient from animal to vegetal Strongest expression in ectoderm	Cerberus/dan related	51
Dan	BMP-2,4,7 GDF-5,6,7	Mouse *Xenopus*	No No		52 50 53 54
Caronte	BMP-4,7	Chicken	Mesoderm flanking the node		55 56
Lefty1 Lefty2	Nodal	Mouse Chicken	Notochord/midline (Lefty1; m,c) Mesoderm (Lefty2; m,c)		57,58
Dante	ND	Mouse	Node	No full-length cDNA reported	53
PRDC	ND	Mouse	ND	Cerberus/Dan-like	59
Drm/Gremlin	BMP-2,4	Mouse *Xenopus*	No No		50 60 53
Neuralin-1	BMP-4,5 TGF-β1,2	Mouse	Emerging neural plate	3 CR domains	61 32
CTGF	BMP-4 TGF-β1	*Xenopus*	Weak expression	1 CR domain	62
Kielin	ND	*Xenopus*	Axial mesoderm	27 CR domains	63

.....and more ...

The `default model' in Xenopus



*Differential BMP signaling acts as **an instructive mechanism** for determining neural versus epidermis formation*

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**:

SUFFICIENCY

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

LOCATION

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**

NECESSITY

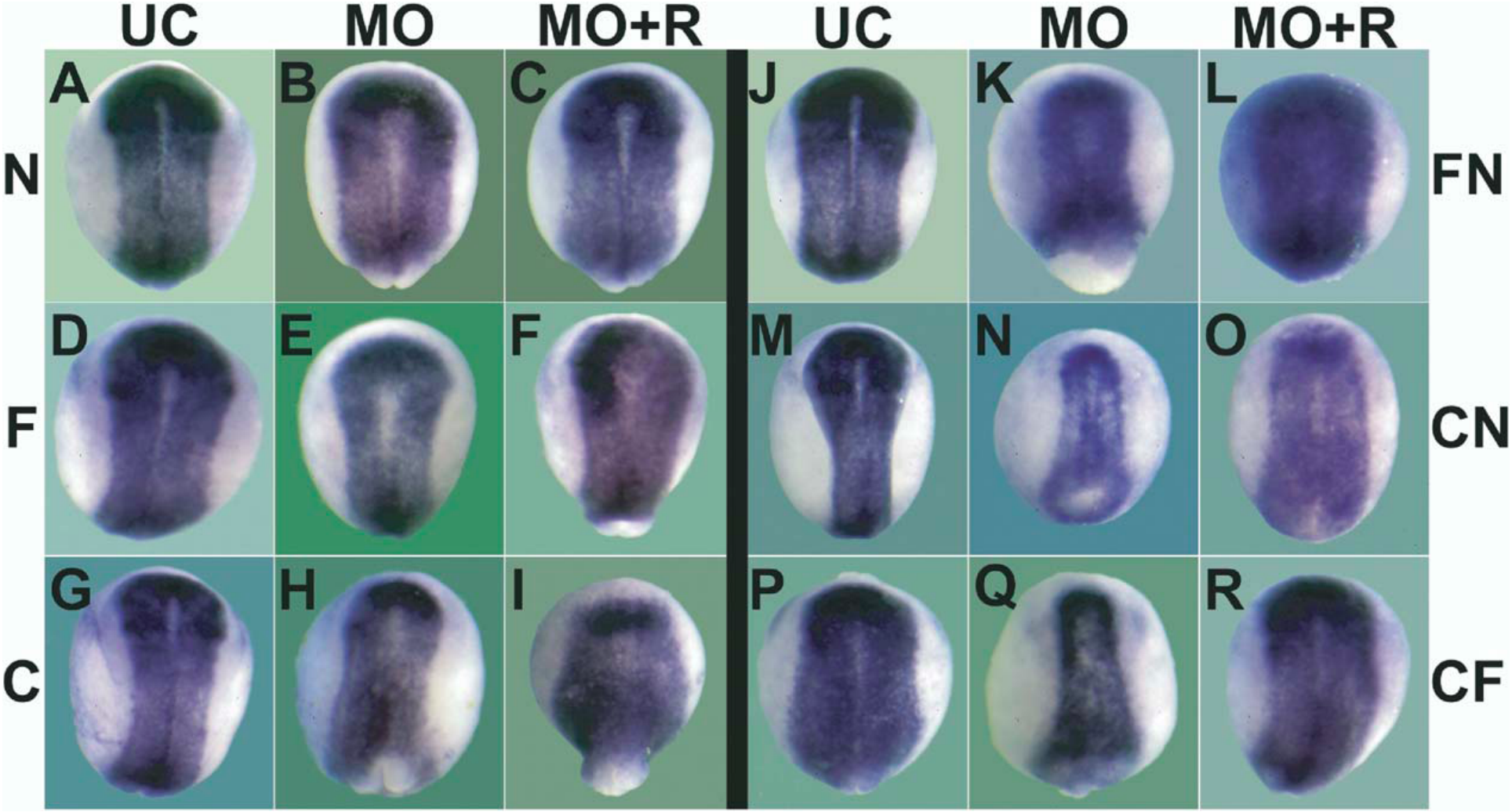
Loss of function assay

Morpholino based knock down
approaches to inhibit translation of mRNA

Xenopus tropicalis

Loss of function assay with Morpholinos

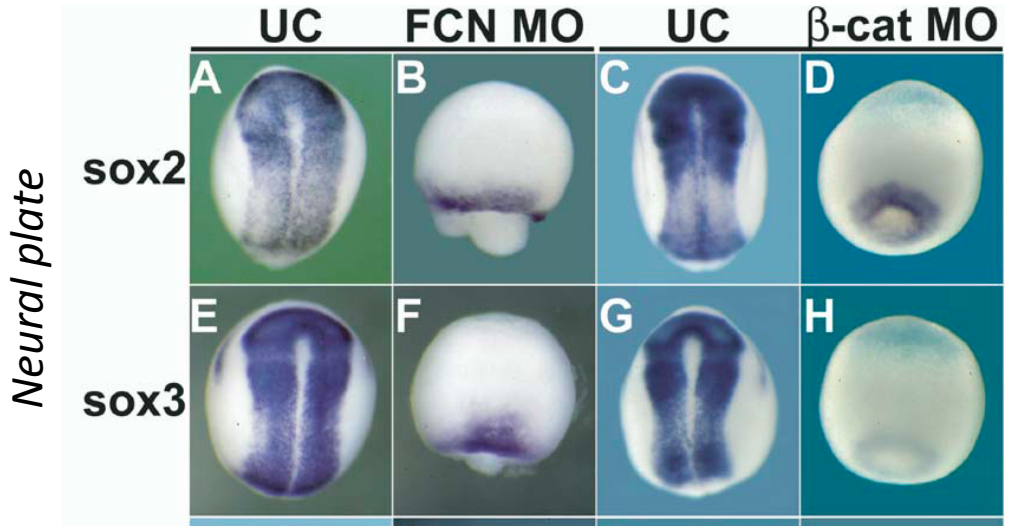
Sox2 expression in neurula (st 14–15) embryo



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

Molecular redundancy of neural induction



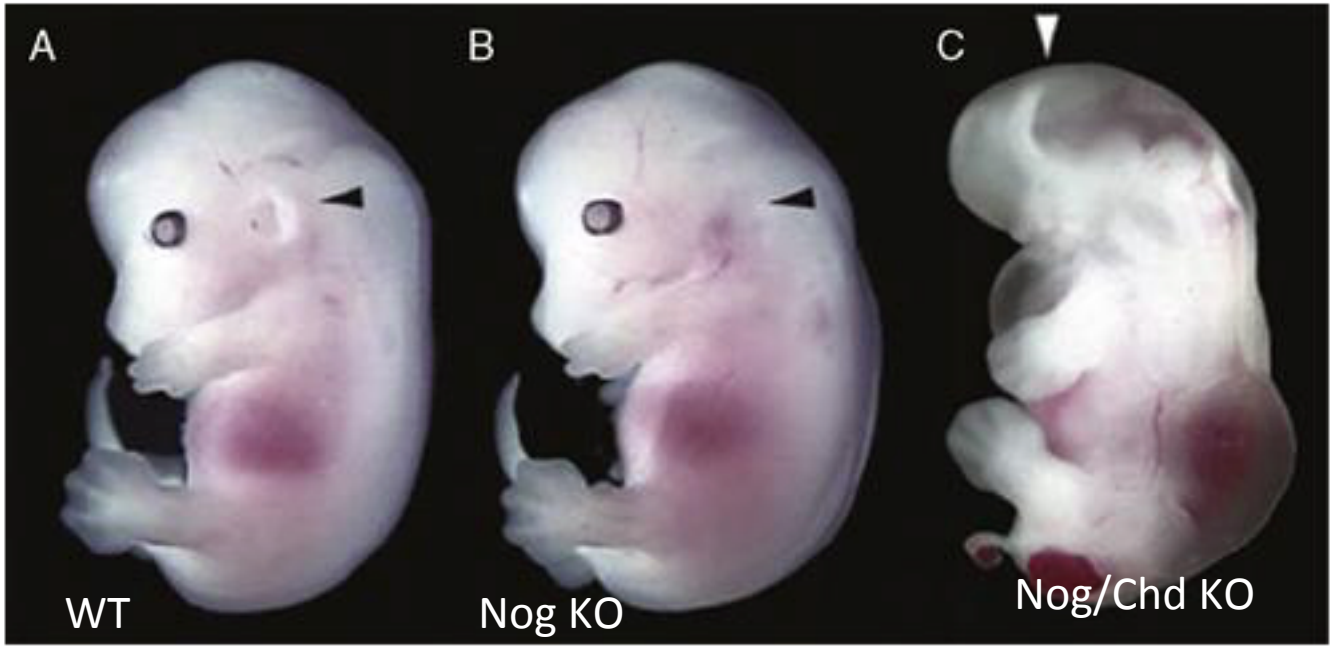
Deletion of 3 BMP antagonists from Spemann's organizer
leads to a chatastrophic loss of dorsal structure:
lack a morphological neural plate at the neural stage
and dorsal mesoderm structures

→ Conclusive in vivo evidence that BMP inhibitors
are essential for neural induction

Beta-catenin MO – prevent formation of the Speman organizer

Khokha et al., Developmental Cell 2005

Molecular redundancy in neural induction in mice



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

Bachiller et al., 2000

Beyond the default model:

- Is BMPs inhibition sufficient for neural induction?
- The default model may be too simplistic to describe neural induction
- Other factors involved?

FGF

blocking BMP signalling cell autonomously by electroporating SMAD6 is not sufficient to induce Sox3 expression in competent chick epiblasts.

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

Data from fish – frog – chick… indicate FGF signaling restricts BMP gene expression and is required for expression of BMP inhibitors

Is there an instructive role for FGF signaling in neural induction?

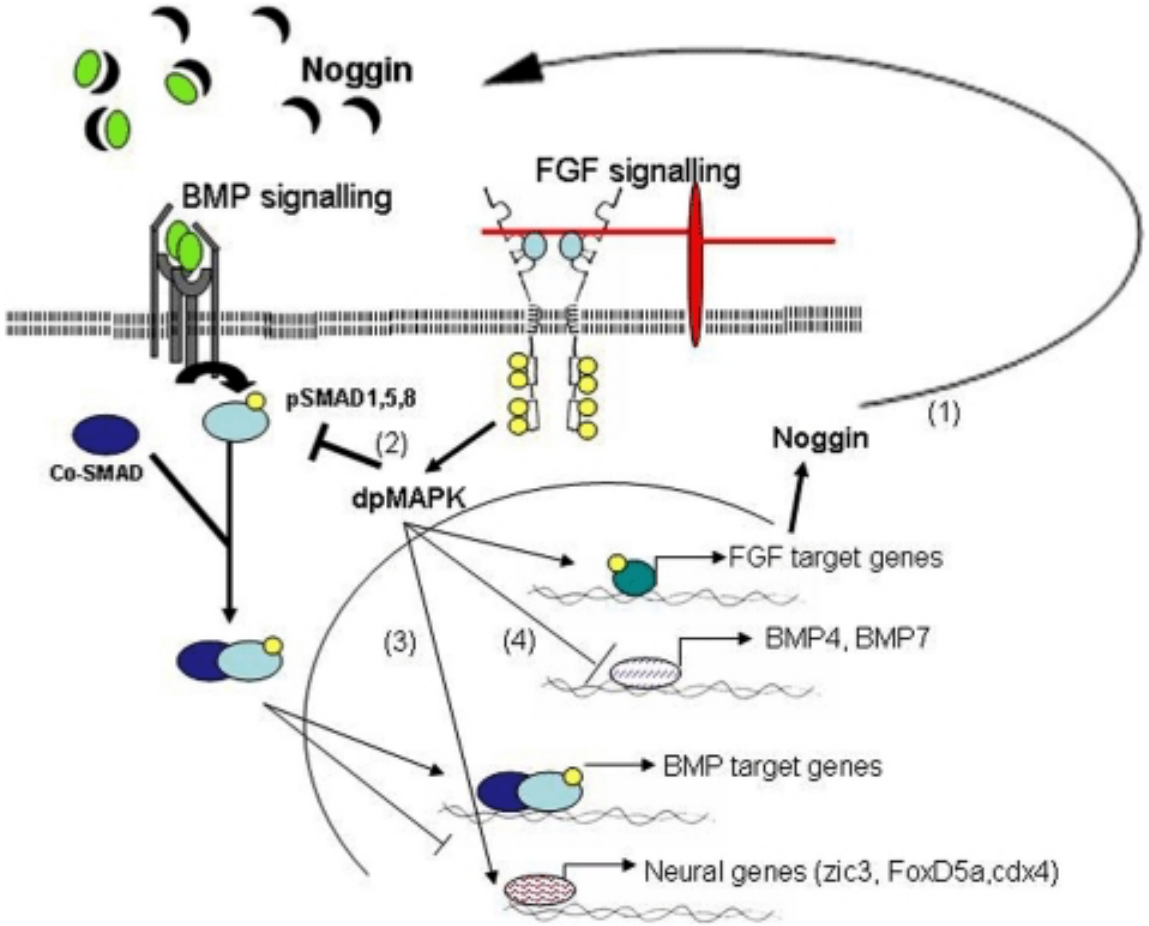
How can FGF impact on neural induction?

(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) FGF signalling can directly activate the transcription of a set of neural genes

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



FGF signaling in vertebrate development
Pownall and Isaacs - 2010

Experiments in frogs suggest the existence of a possible combinatorial mode:

micro-injection of cell-autonomously acting BMP inhibitors in ventral ectodermal cells (Smad6 or a dominant-negative BMP Receptor) of the 16- or 32-cell embryo
→ normal development – the cells do not become neural

the epidermal-to-neural switch occurs only when **a low amount of FGF4 is combined with those BMP inhibitors.**

Efficiency of BMP inhibition?

it could be that these reagents are just less efficient at inhibiting BMP signalling

Use of an effective inhibitor→ **Smad5-somitabun**= a dominant negative form of SMAD5, based on the zebrafish mutant *somitabun,* that forms nonfunctional multimeric complexes with SMAD1, 5, and 8 to shut down the BMP signalling pathway

Overexpressing this mutant SMAD5 in ventral epidermis was capable of inducing neural tissue cell autonomously in early neurula frog embryos.

NAS

BMP inhibition initiates neural induction via FGF signaling and Zic genes

Leslie Marchal, Guillaume Luxardi, Virginie Thomé, and Laurent Kodjabachian[1]

Institut de Biologie du Développement de Marseille Luminy, UMR 6216, CNRS-Université de la Méditerranée, 13288 Marseille Cedex 09, France

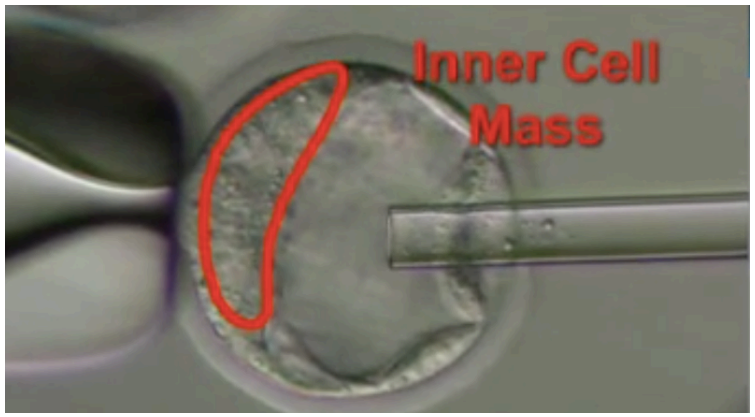
Edited by Igor B. Dawid, National Institute of Child Health and Human Development, Bethesda, MD, and approved August 19, 2009
(received for review June 11, 2009)

Article discussion….

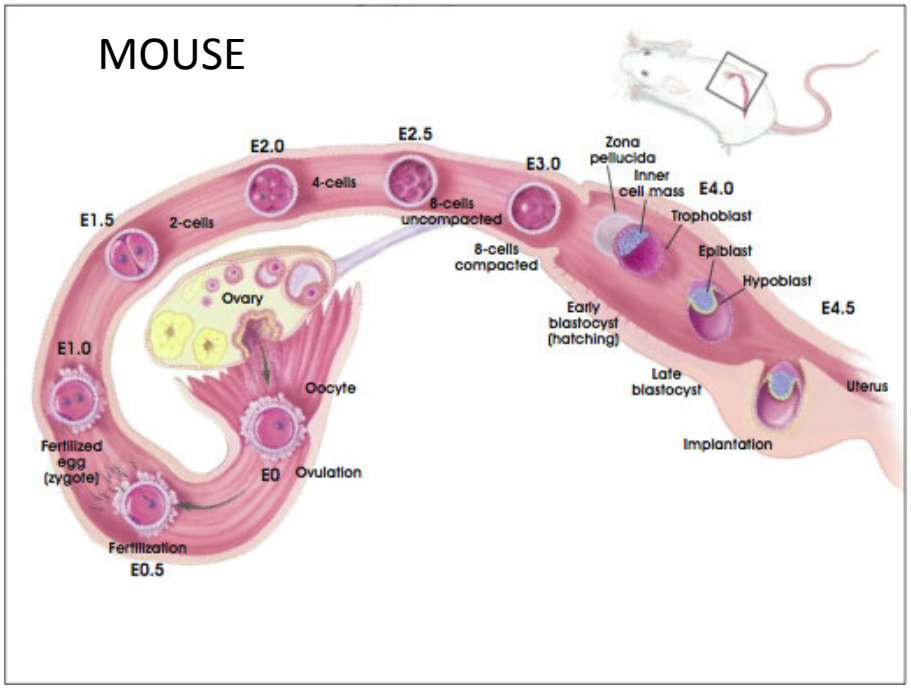
Neural induction in mammalian ESCs

ESCs=embryonic stem cells
1981 Gail Martin
Martin Evans and Matthew Kaufman

Mouse ESCs derived from the inner cell mass of the blastocyst of preimplantation embryos



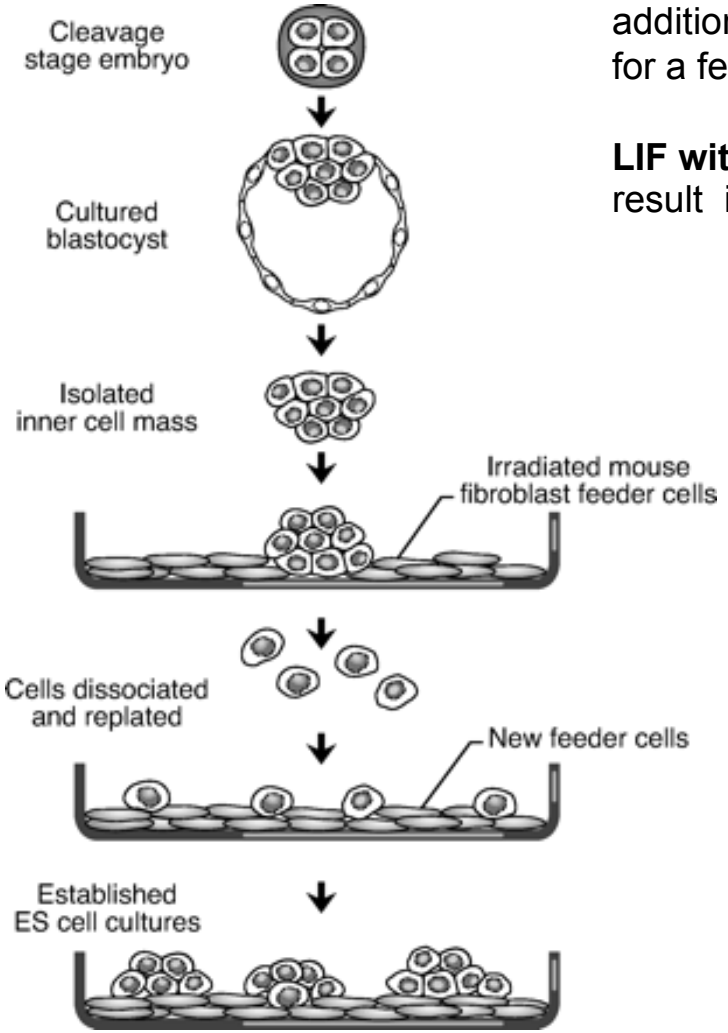
Self-renewal
Pluripotency
Express embryonic TFs
(i.e. Oct-4; Sox2; Nanog)



ESCs: an in vitro platform
to test hypoteses and investigate mechanisms controlling embryonic fate determination

Watch the video on moodle

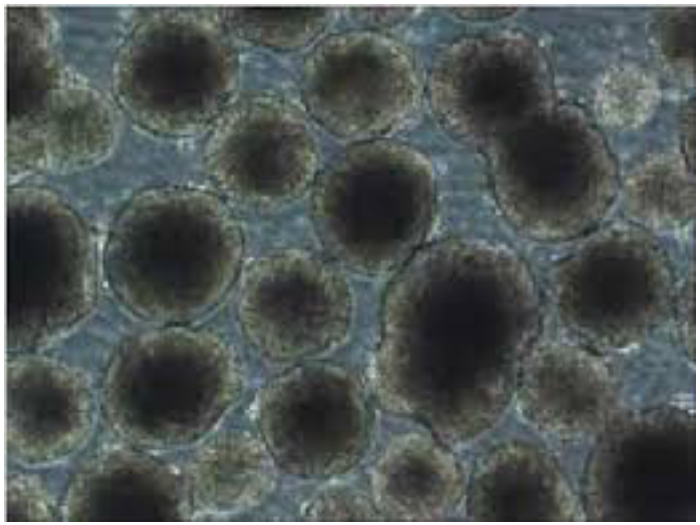
Mouse ES cell culture



Feeder layer was crucial for **isolation** of ES from blastocyst

addition of **leukemia inhibitory factor (LIF**) replaces the need for a feeder (LIF is required for maintenance in **undifferentiated** state)

LIF withdrawal and growth of ES cells in **suspension** result in **embryoid bodies** and differentiation



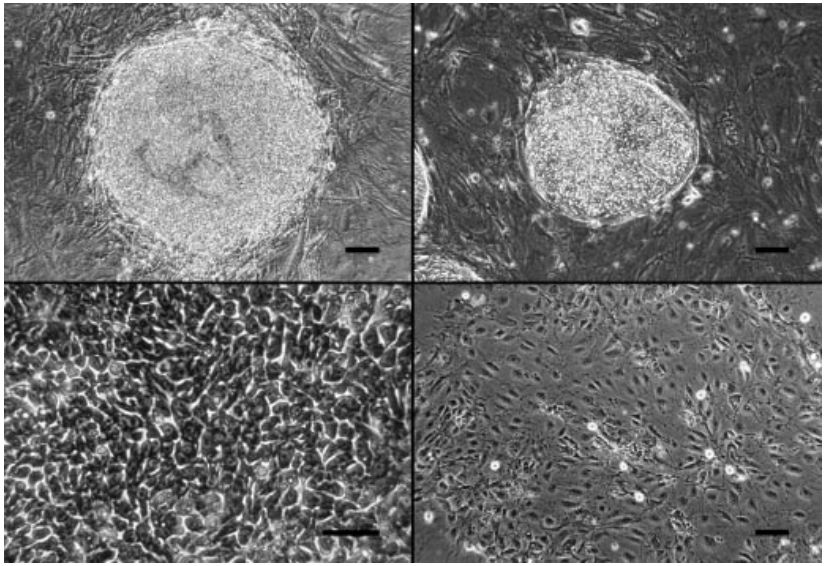
Science-1998

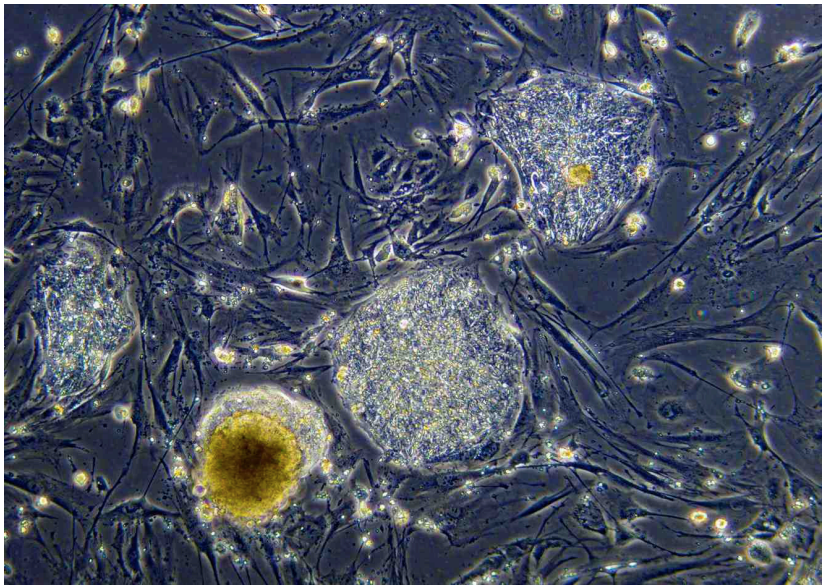
REPORTS

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

Human blastocyst-derived, pluripotent cell lines are described that have normal karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize other early lineages. After undifferentiated proliferation in vitro for 4 to 5 months, these cells still maintained the developmental potential to form trophoblast and derivatives of all three embryonic germ layers, including gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm). These cell lines should be useful in human developmental biology, drug discovery, and transplantation medicine.





Legal problems
Politics
Moral
Founding

Important to know!

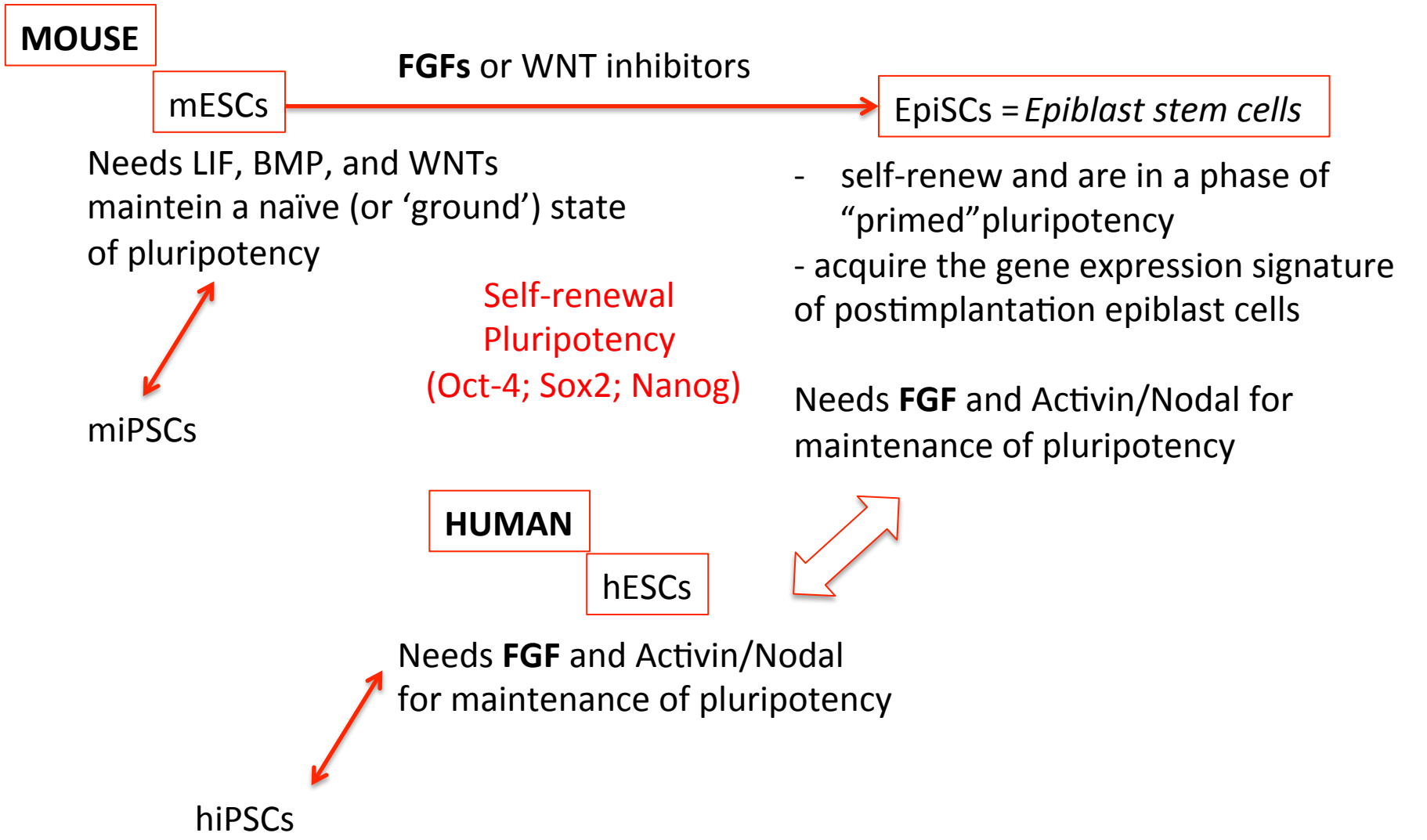
Human ES cells do not come from aborted fetuses

ES cells derive from leftover in vitro fertilization (IVF) embryos
(6days after fertilization – contain about 50 cells – no tissue differentiation)

One blastocyst may produce a number of cell lines that can be kept in culture for years.

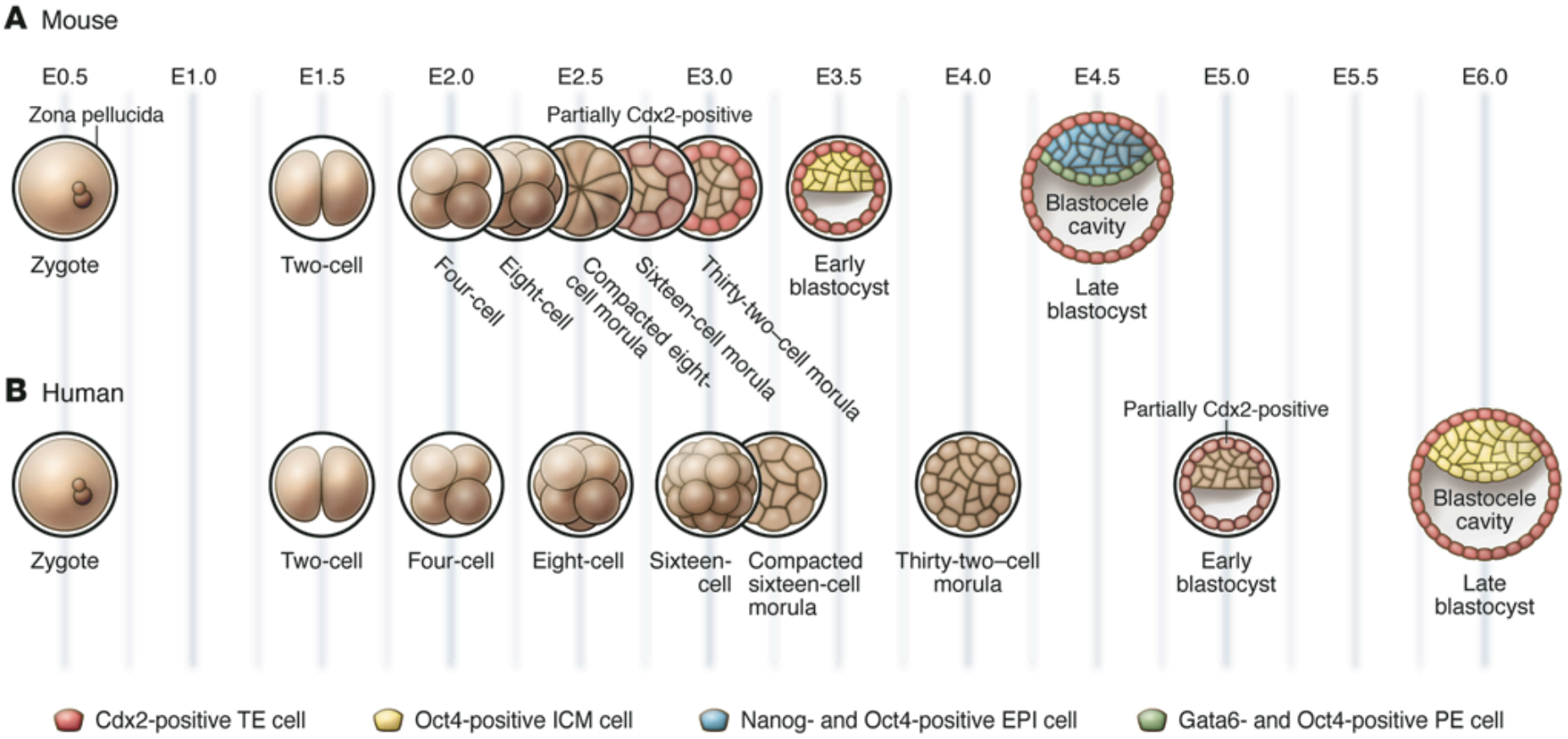
Cells adapted to proliferate in tissue culture represent only a proxy for the in vivo situation (properties of cells in the embryo)

Both mouse and human ESCs are derived from the inner cell mass of preimplantation blastocysts … are mESC and hESCs the same?



mESCs represent an early stage of development compared to hESCs

Stages of mouse and human preimplantation development



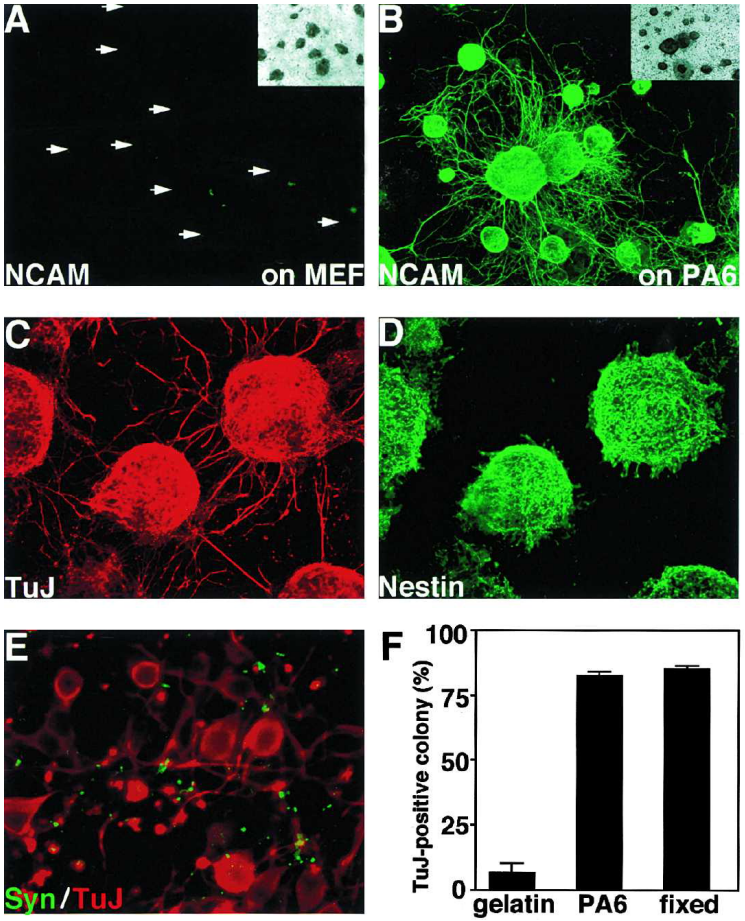
EPI= epiblast
TE=trophoectoderm
PE=primitive endoderm

3 blastocyst lineages

Neural Induction in Mouse ESCs

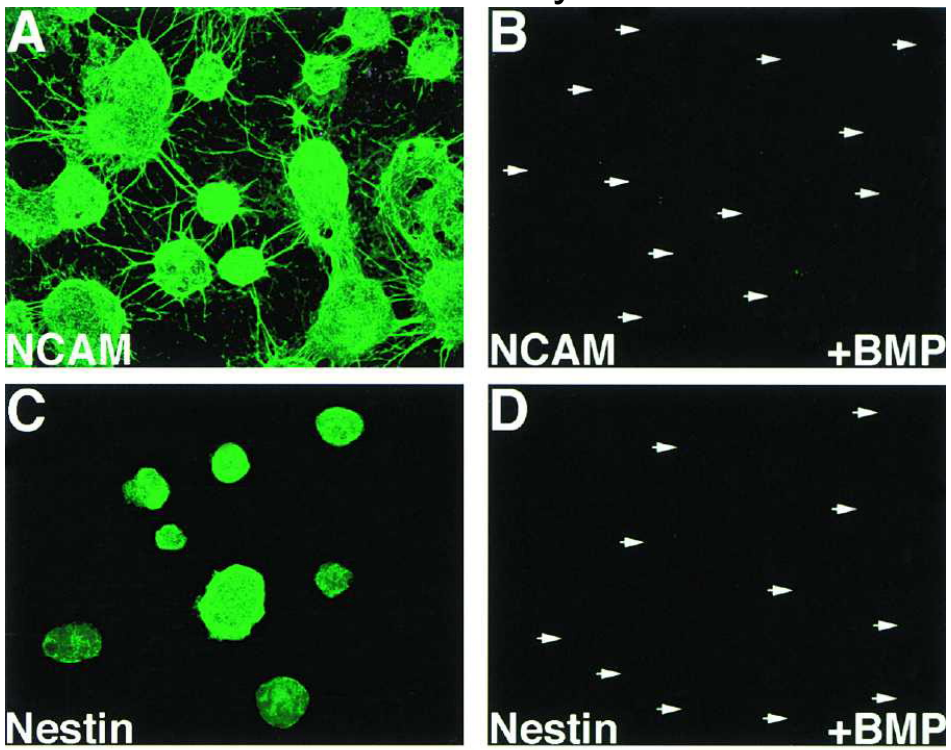
Does the default model of neural induction work also in mammals?

- ESCs + Stromal cell lines possessing SDIA (stromal cell–derived inducing activity)



Kawasaki et al., Neuron 2000

BMP4 Negatively Regulates Neural Induction by SDIA



PA6 cells for 8 days

Neural Induction in Mouse ESCs

-Chemically defined serum-free, feeder layer-free, low-density culture conditions are sufficient for neural differentiation of ES cells.

ARTICLE

Direct Neural Fate Specification from Embryonic Stem Cells

A Primitive Mammalian Neural Stem Cell Stage Acquired through a Default Mechanism

Vincent Tropepe[‖], Seiji Hitoshi, Christian Sirard[#], Tak W Mak, Janet Rossant, Derek van der Kooy

[‖] Present address: Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142.

[#] Present address: Brain Tumor Research Center, Montreal Neurological Institute, Montreal, Quebec H3A 2B4, Canada.

Abstract

Little is known about how neural stem cells are formed initially during development. We investigated whether a default mechanism of neural specification could regulate acquisition of neural stem cell identity directly from embryonic stem (ES) cells. ES cells cultured in defined, low-density conditions readily acquire a neural identity. We characterize a novel primitive neural stem cell as a component of neural lineage specification that is negatively regulated by TGFβ-related signaling. Primitive neural stem cells have distinct growth factor requirements, express neural precursor markers, generate neurons and glia in vitro, and have neural and non-neural lineage potential in vivo. These results are consistent with a default mechanism for neural fate specification and support a model whereby definitive neural stem cell formation is preceded by a primitive neural stem cell stage during neural lineage commitment.

→ conversion into nestin expressing neural precursors (enhanced by inhibition of BMP signaling with Noggin or Cerberus and Smad4 KO ESCs)

Neuron 2001

Neural Induction in Mouse ESCs and the Role of FGF Signaling

A role for FGF in neural induction on mESCs ?

FGF signaling manipulations on ESCs suggest a role for FGF…

BUT:
-mESCs require FGF signaling to progress to a primed state of pluripotency before they acquire the competence for neural induction

- FGF signaling has been shown to inhibit rather than promote neural induction in EpiSCs

- Inhibition of the TGF*β*/BMP signaling promotes neural commitment from EpiSCs

--→ Default model works in mouse ESCs!

FGF signaling conceivably regulates the competence of mESCs for germ layer differentiation, rather than neural induction per se

Neural Induction in Human ESCs/IPSCs and the Role of FGF Signaling

hESCs do not survive as single cells

Embryoid bodies in absence of exogenous factors differentiate into neural tissue (→Default pathway)

FGF?

- small-molecule inhibitors of FGF signaling reduced the number of cells expressing PAX6 (but FGF inhibitors were not added in the initial 4 days of differentiation)

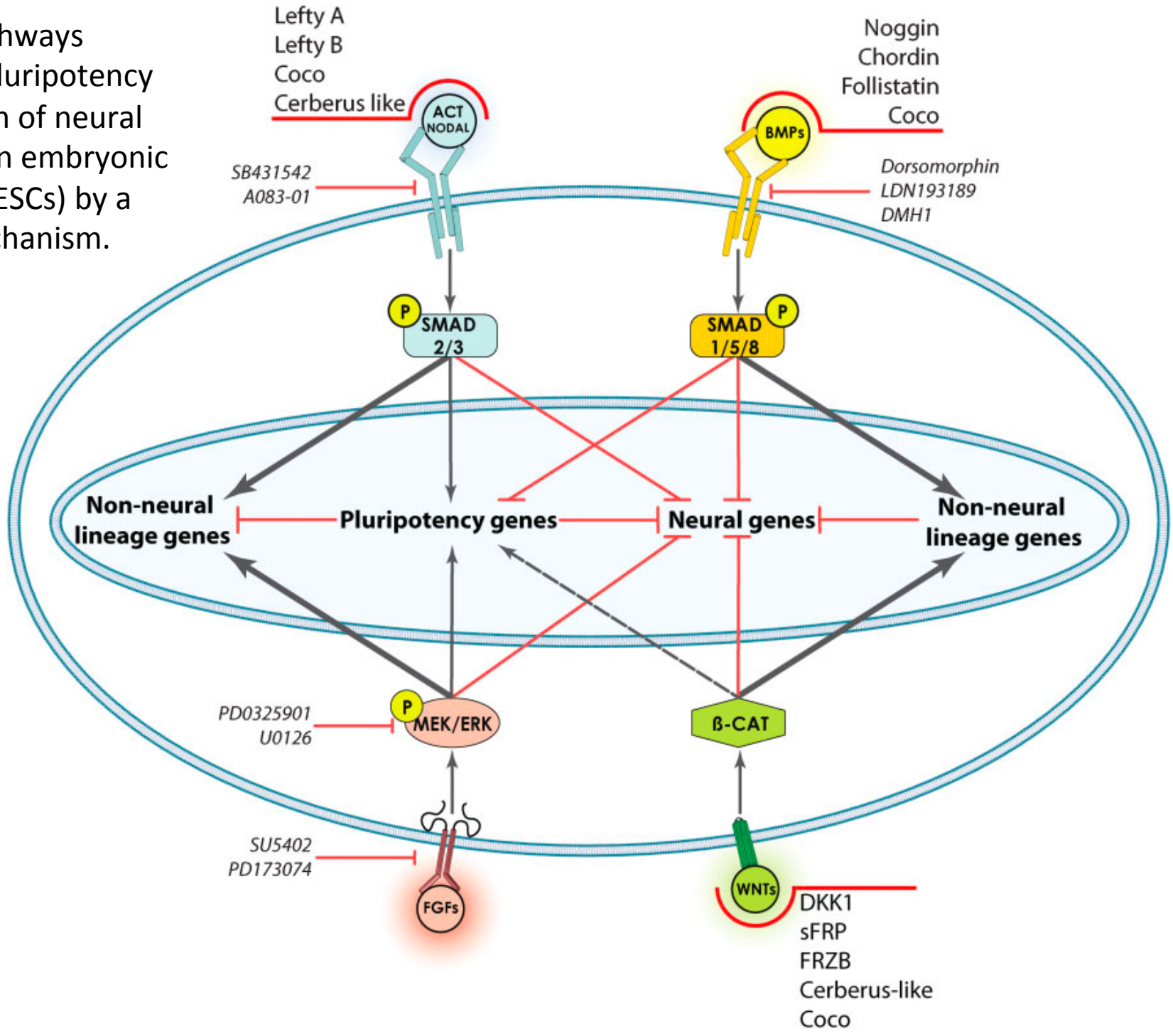
-FGF increases the size of neural colonies without changing the efficiency of neural induction

-neuralized hESCs displayed low levels of BMP-SMAD1/5/8 signaling, presumably because of the high-level expression of several soluble BMP antagonists

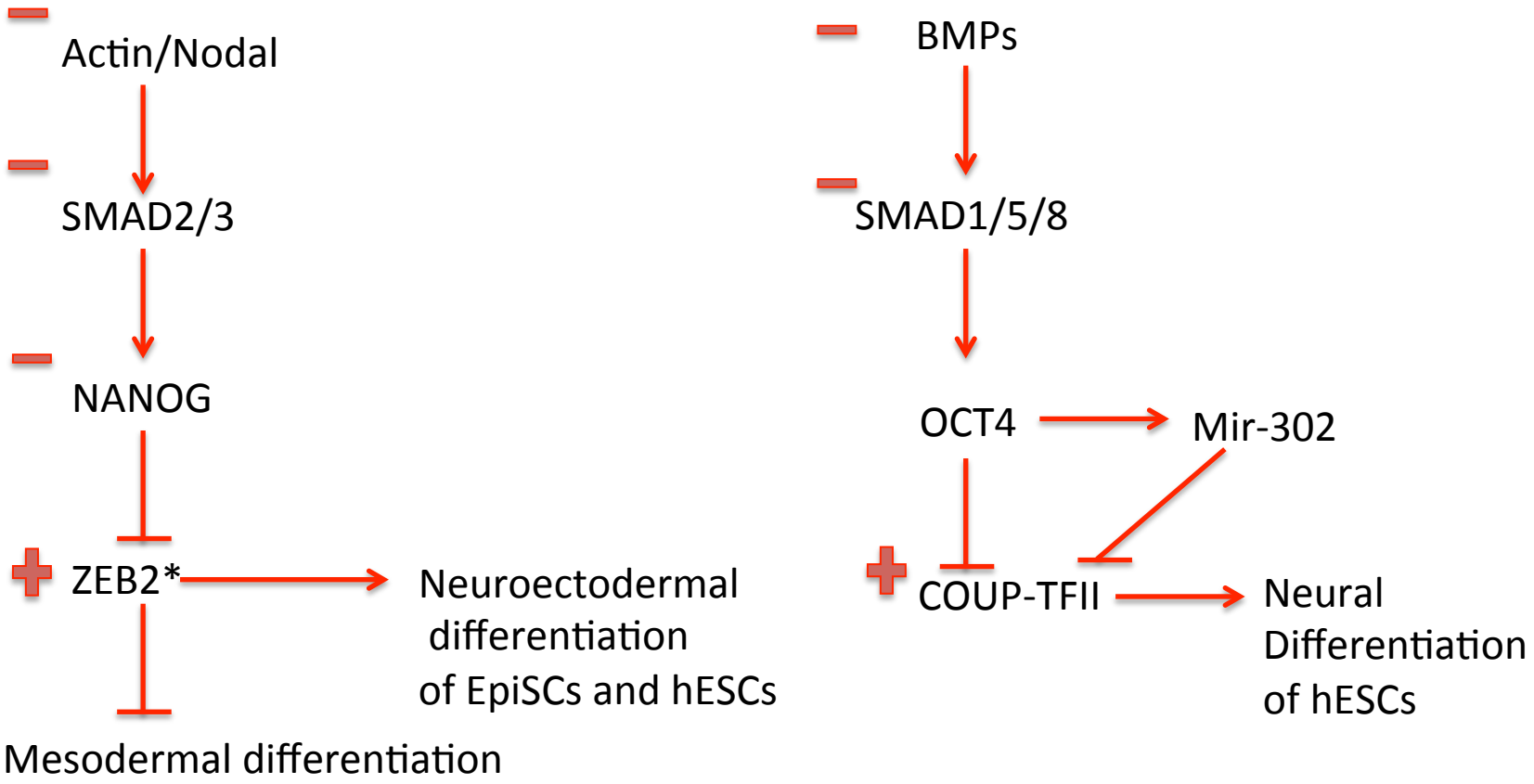
FGF → survival and/or proliferative role in the early neuroepithelium

In the absence of exogenous morphogens, hESC colonies take on a neural fate of anterior character in line with the default model → no specfic need for FGF for neuralization

Signaling pathways involved in pluripotency and induction of neural fate in human embryonic stem cells (hESCs) by a ‘default’ mechanism.



Downstream Mechanisms of Default Neural Induction in Mouse EpiSCs and Human ESCs



*SMAD-binding protein