Neural Induction

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University of Minnesota

Free ice cream!

Neuroscience Ice Cream Social
Thursday, Sept 20
3:00pm
NHH atrium

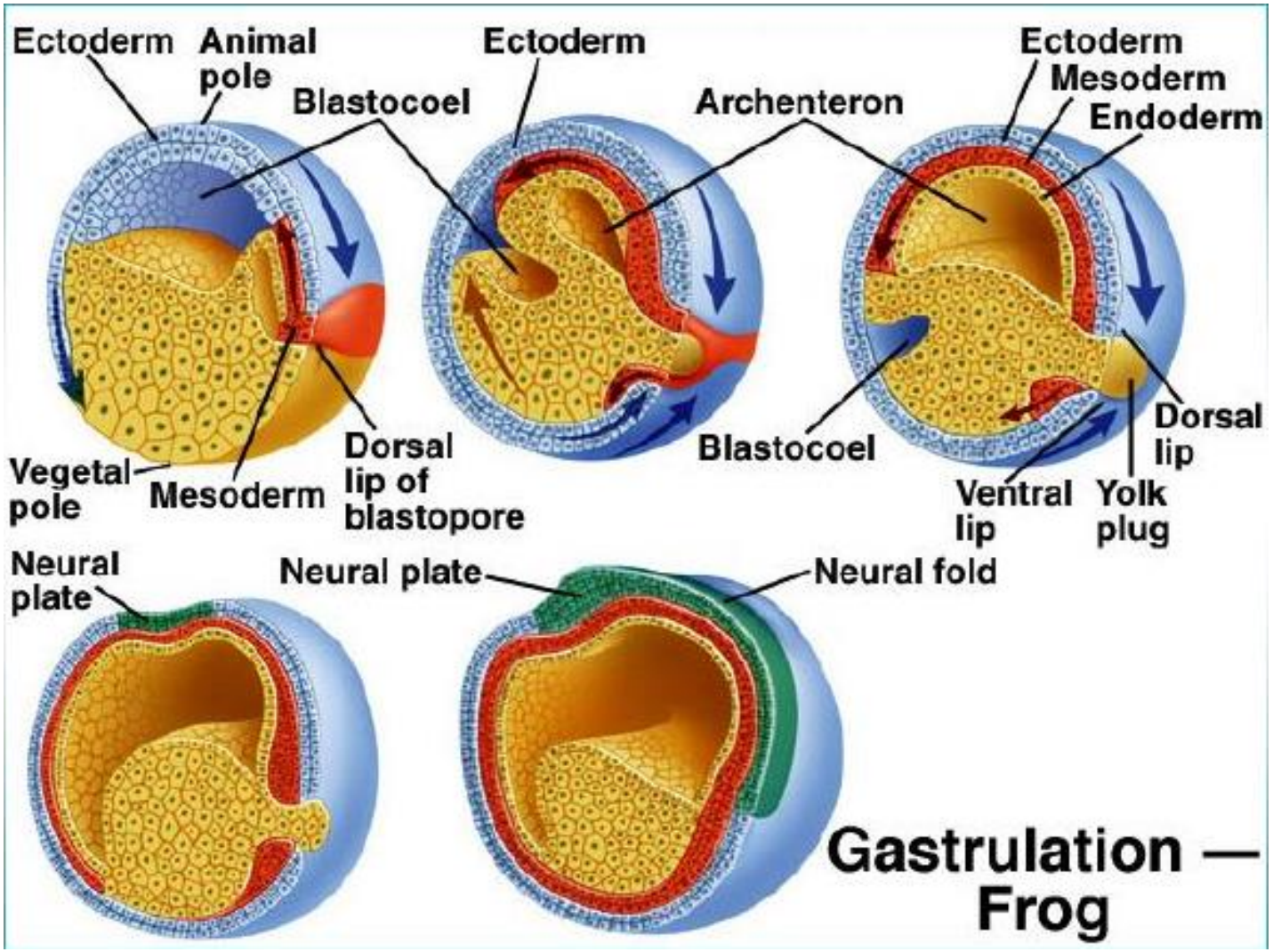
Ice cream served by the neuro faculty!

Gastrulation looks different in different vertebrate species.

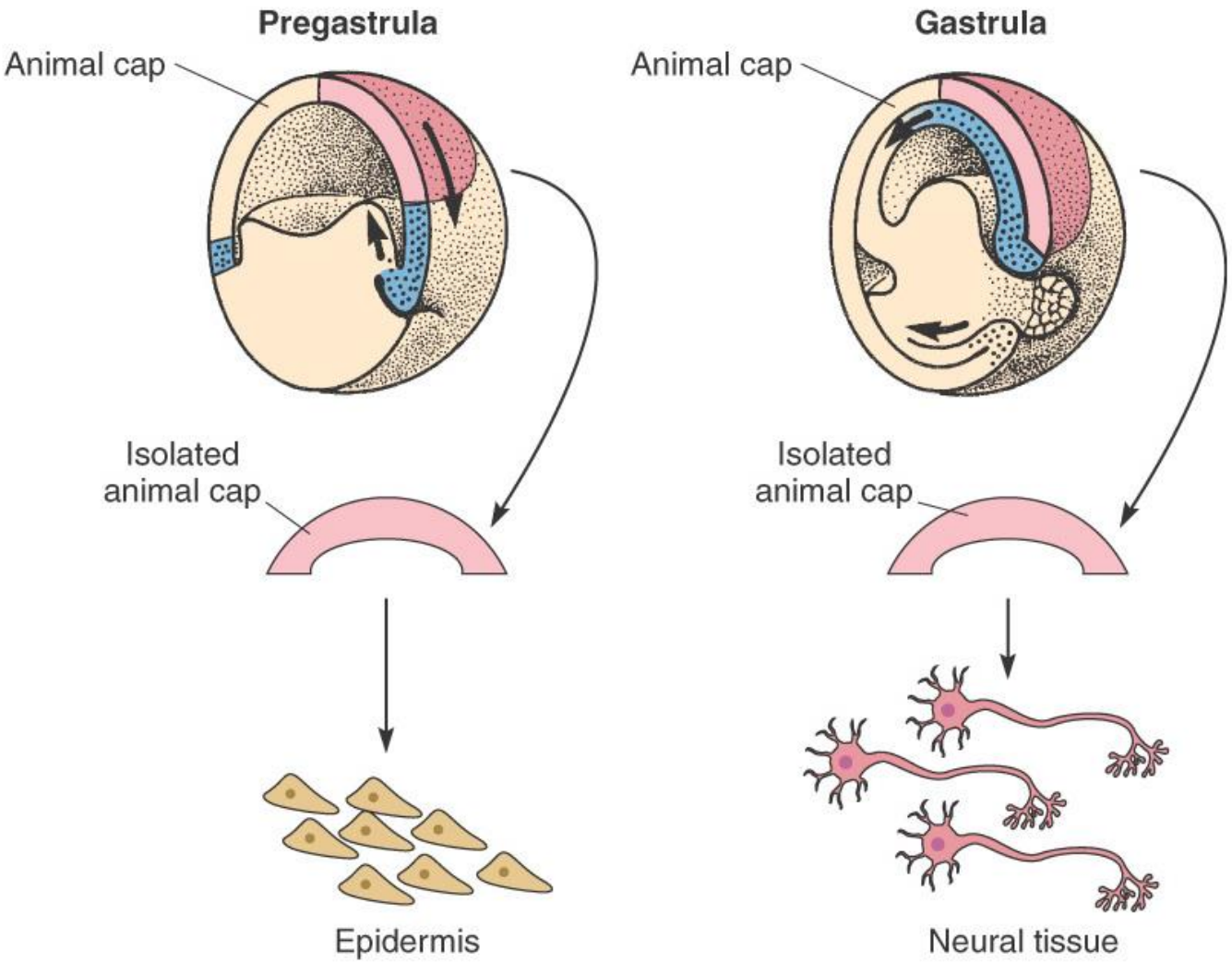
Ingression of cells from the epiblast…

- in reptiles, birds and mammals is through the primitive streak.
- in fish is around the embryonic shield.
- in amphibians is through the blastopore.

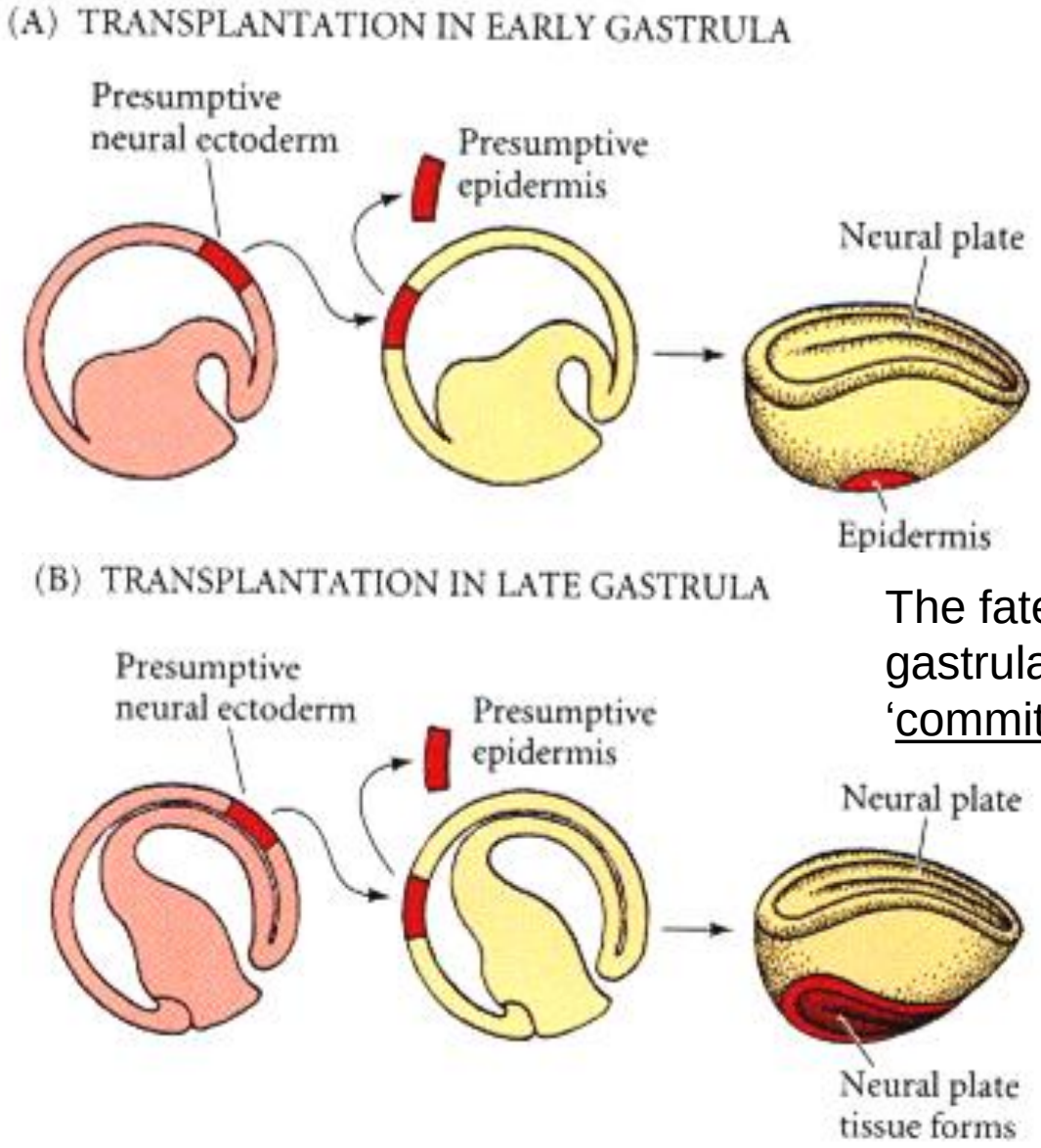
Much of the research on neural induction has been done on amphibian embryos.



Ectoderm changes following gastrulation.



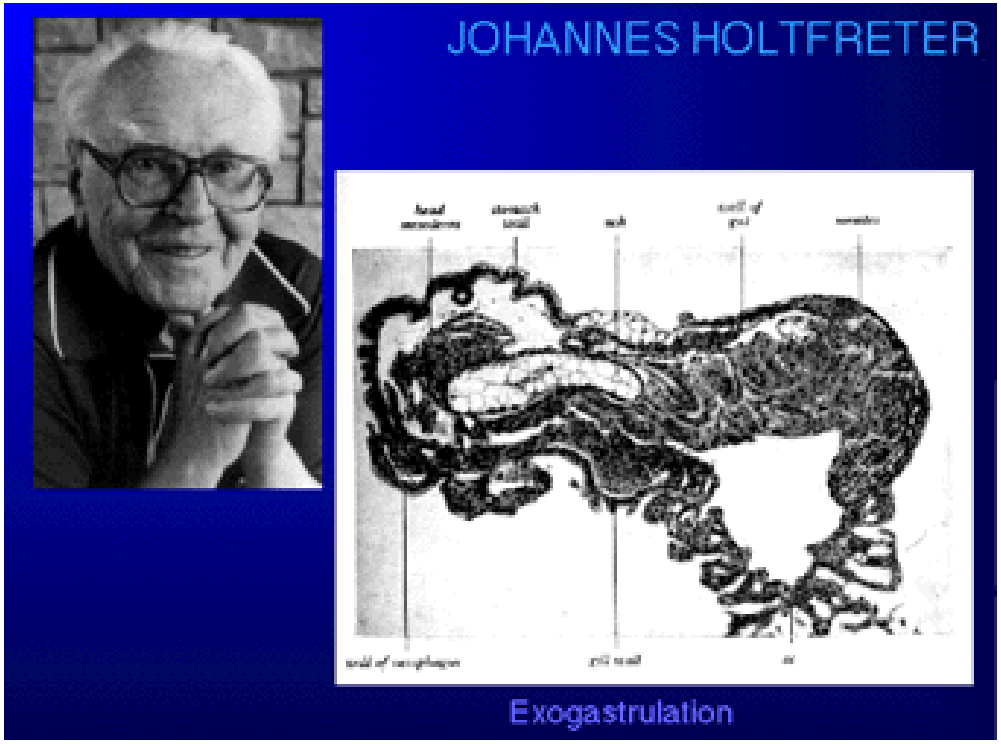
The change in ectoderm is inherent in the cells and is remembered even with a change in environment .



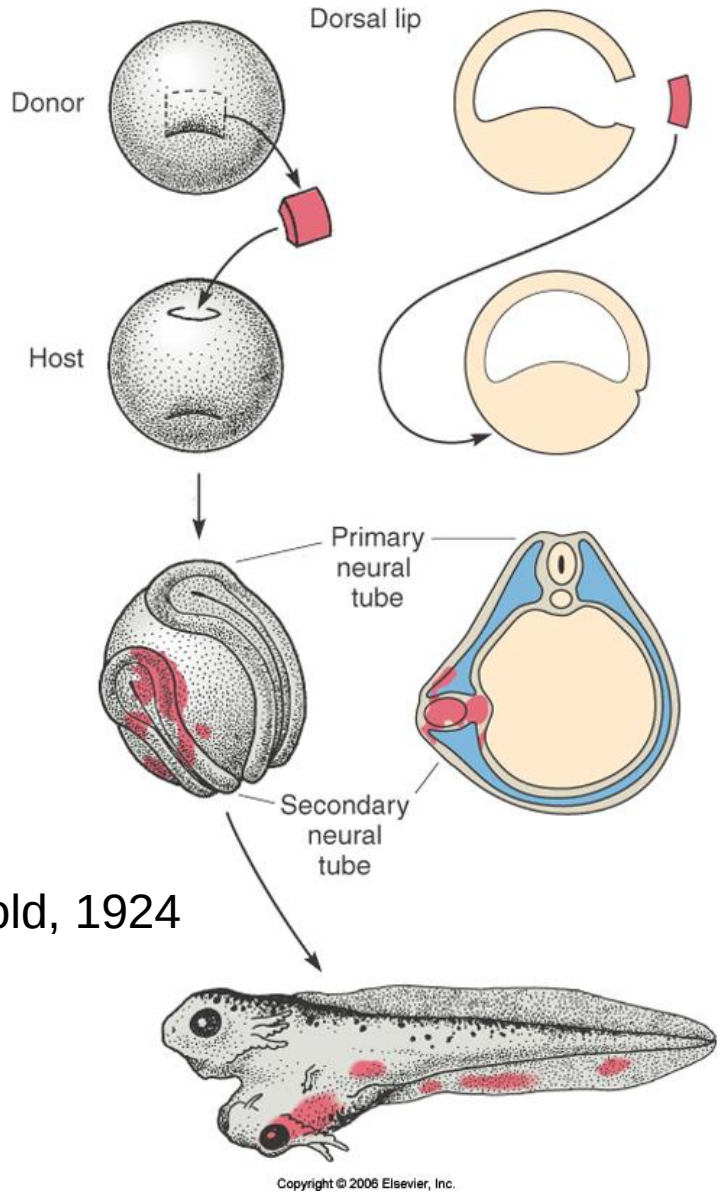
The fate of this tissue after gastrulation is said to be 'committed' or 'determined'.

Interaction of mesoderm with ectoderm is required for nervous system induction.

Holtfreter (1933) showed that frog embryos placed in a high salt solution will exogastrulate (mesoderm evaginates instead of invaginates). The nervous system failed to develop in these embryos.



The organizer induces nervous system.



Spemann and Mangold, 1924

The organizer induces nervous system.

Host cells changed their fate in response to the transplanted cells.

Spemann and Mangold called the dorsal lip of the blastopore the organizer and the effect induction.

The organizer from frog functions in chick and the top of the primitive streak (Hensen's node) from chick functions in frog.

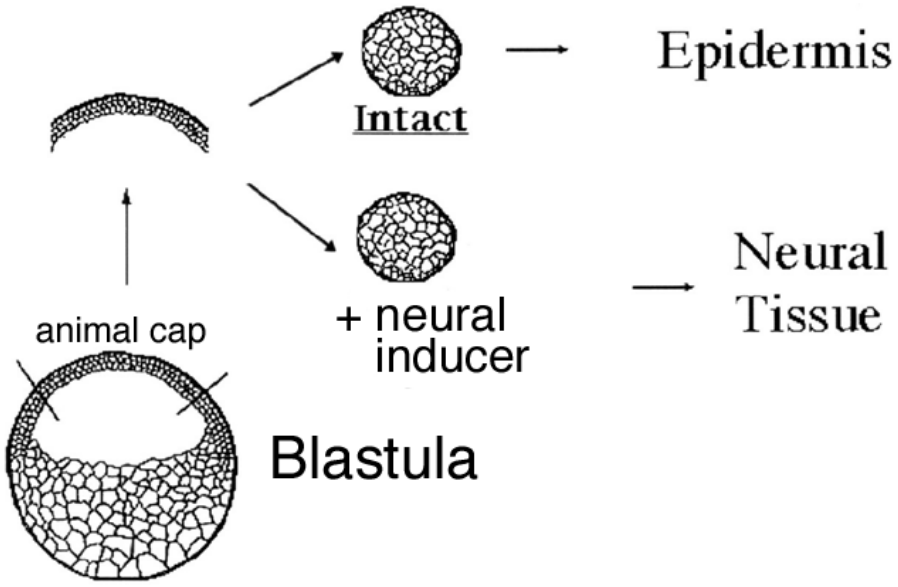
Mangold did the key experiments as a student in Spemann's lab.

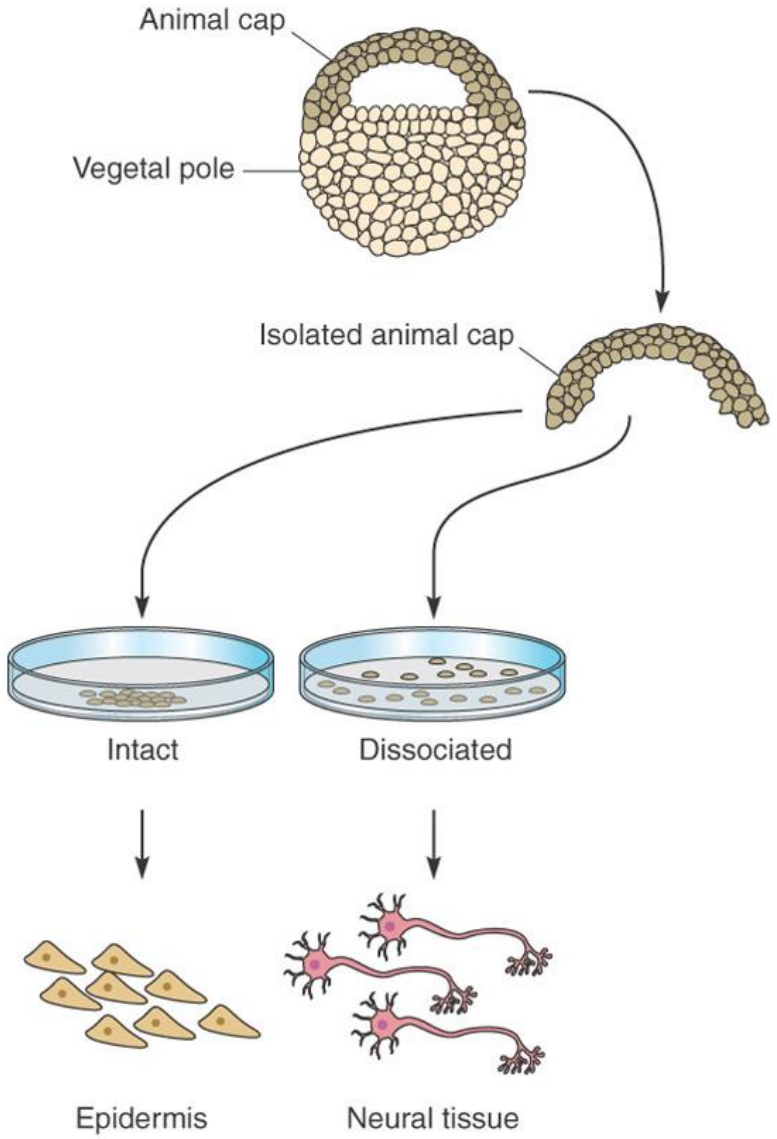
Mangold was killed in an accident, and Spemann was awarded the Nobel Prize in 1935.

A 50 yr search for ‘the’ inducing agent failed…

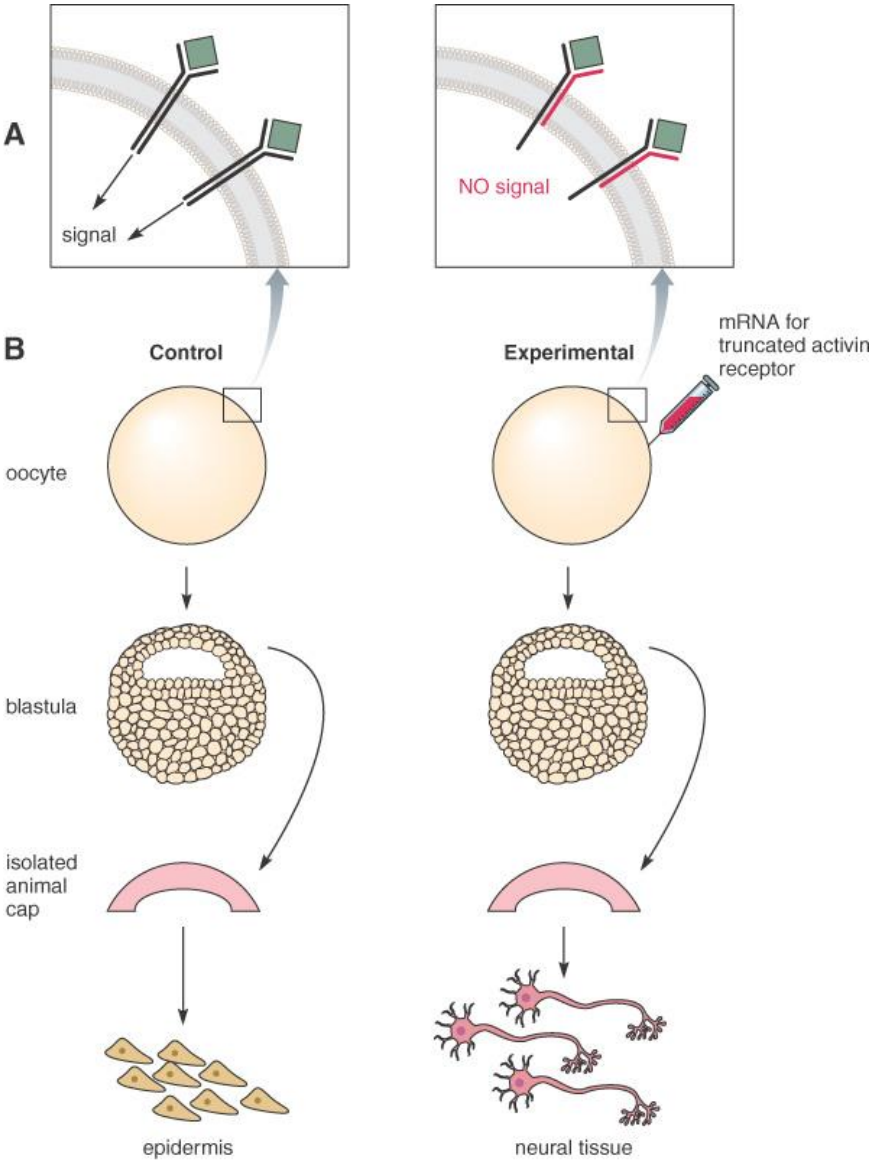
- Found that the inducing agent is present if the organizer cells are dead.

- Found that the inducing agent survived freezing, boiling and acid treatment, but was destroyed by heating to 150°.

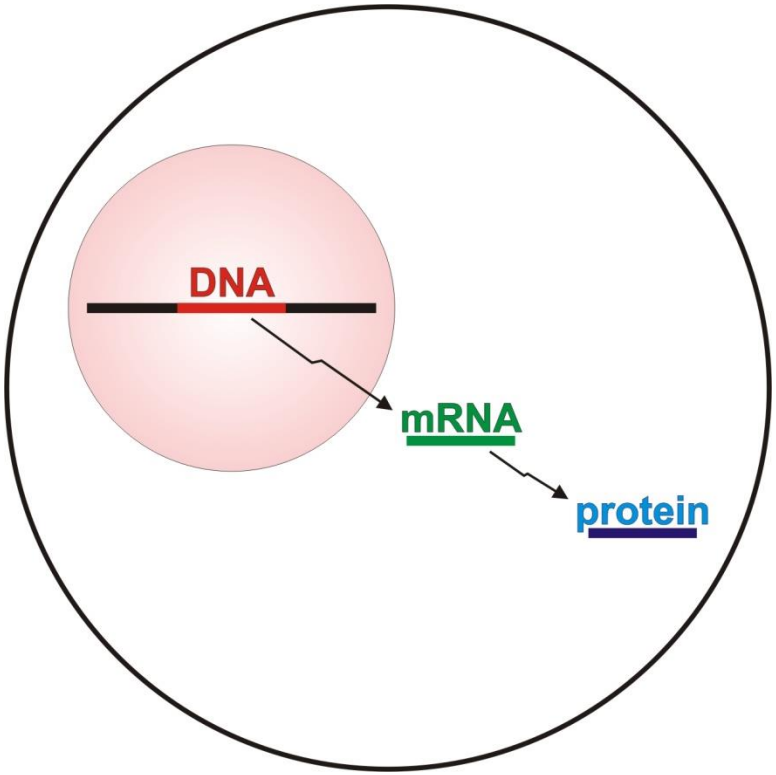



Animal cap
Vegetal pole
Isolated animal cap
Intact
Dissociated
Epidermis
Neural tissue

Misexpression of a dominant negative TGF-β receptor in early gastrula ectoderm neuralized the transfected cells.

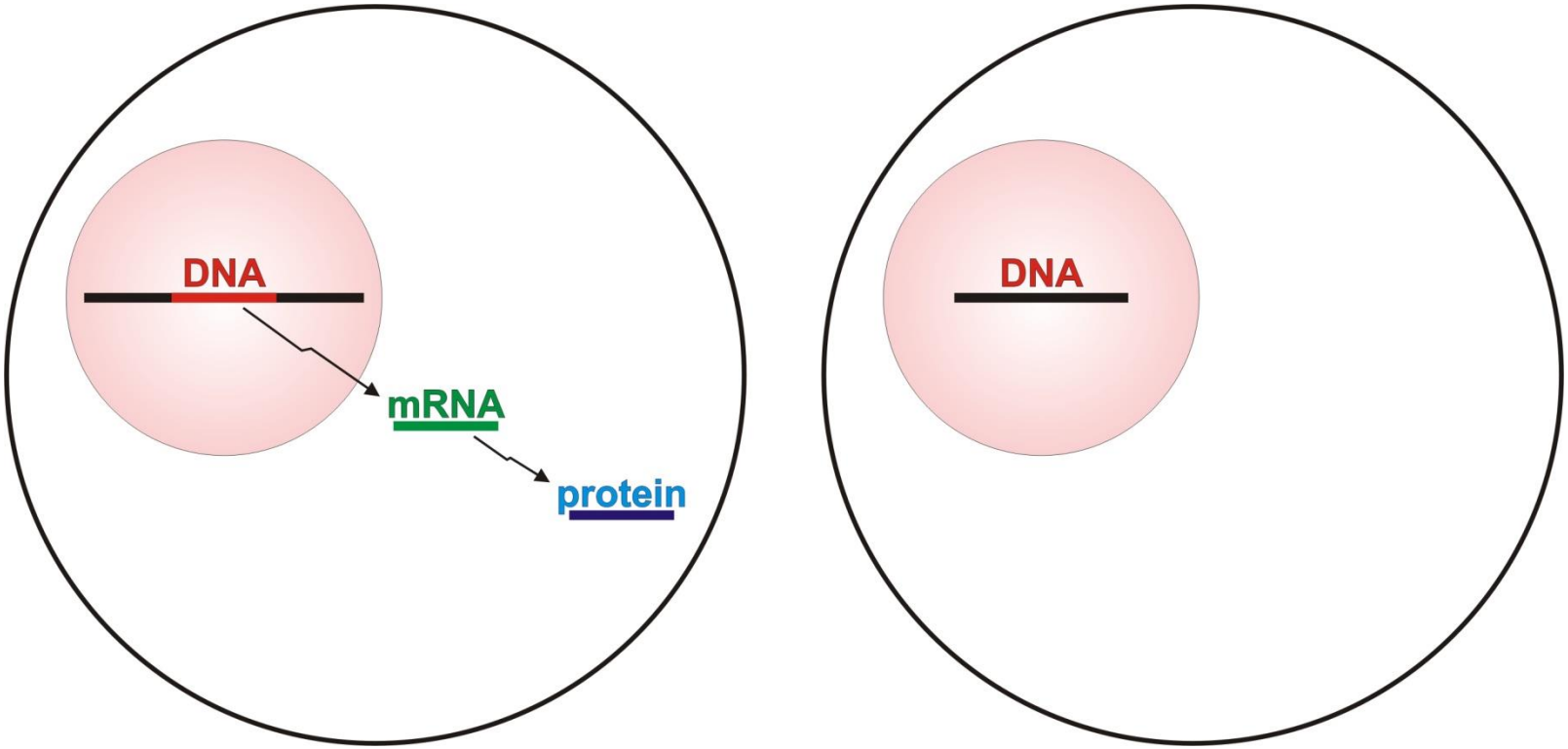


How do we block protein function?



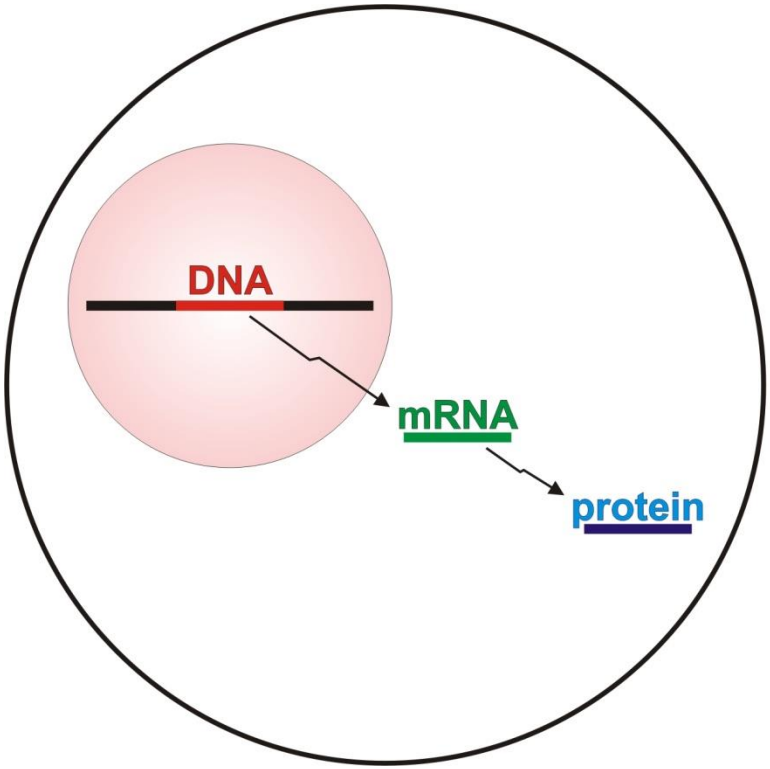
How do we block protein function?

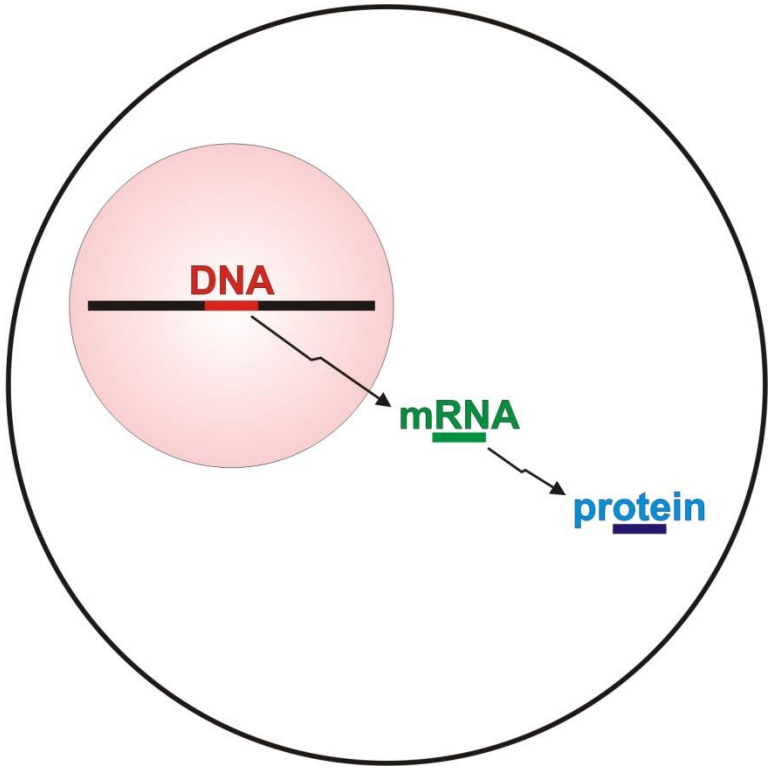
- Eliminate the gene that encodes for the protein (e.g. transgenic knockout)



How do we block protein function?

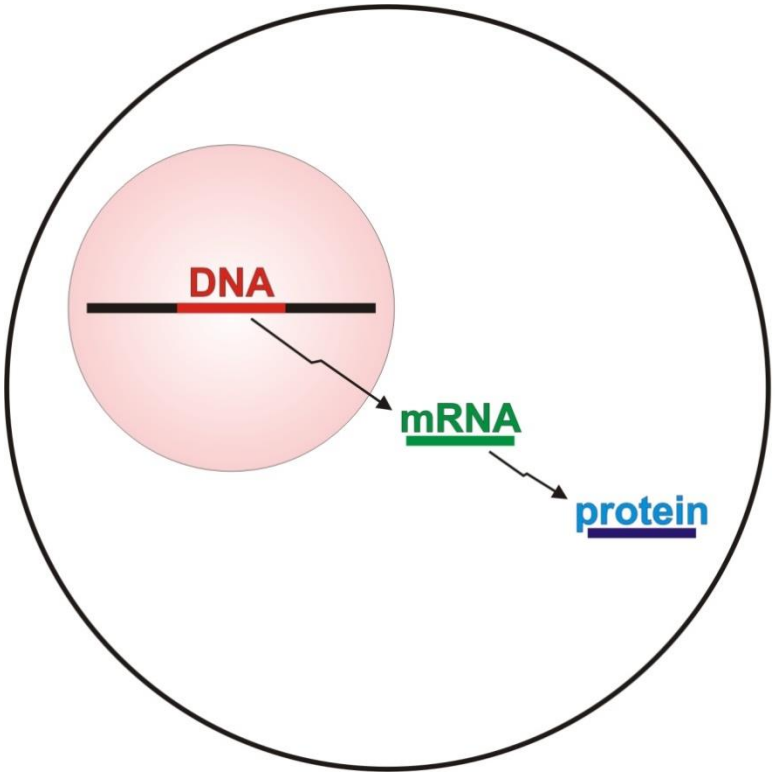
- Induce a mutation in the gene that results in a non functional protein

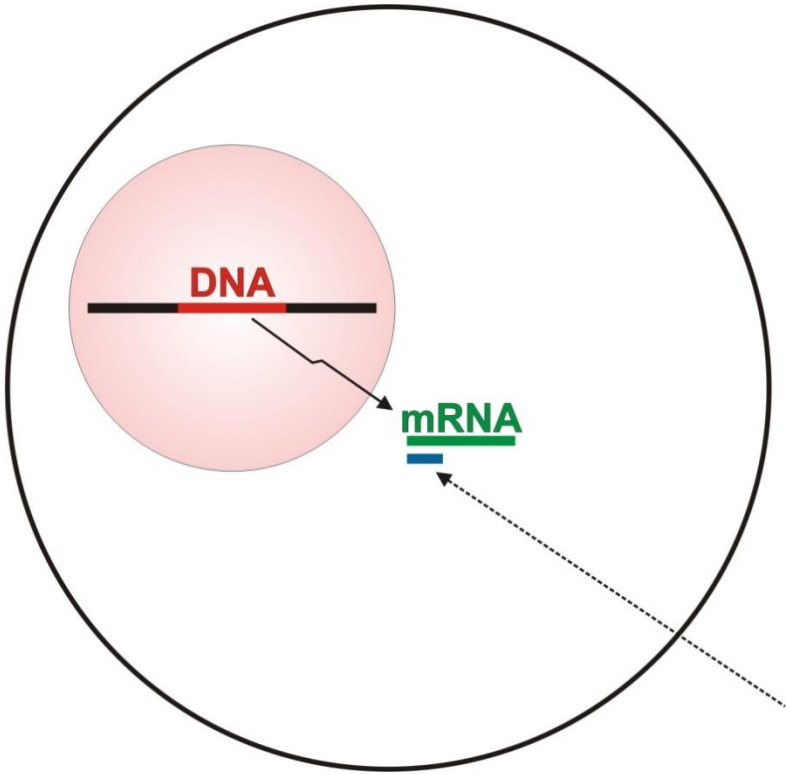




How do we block protein function?

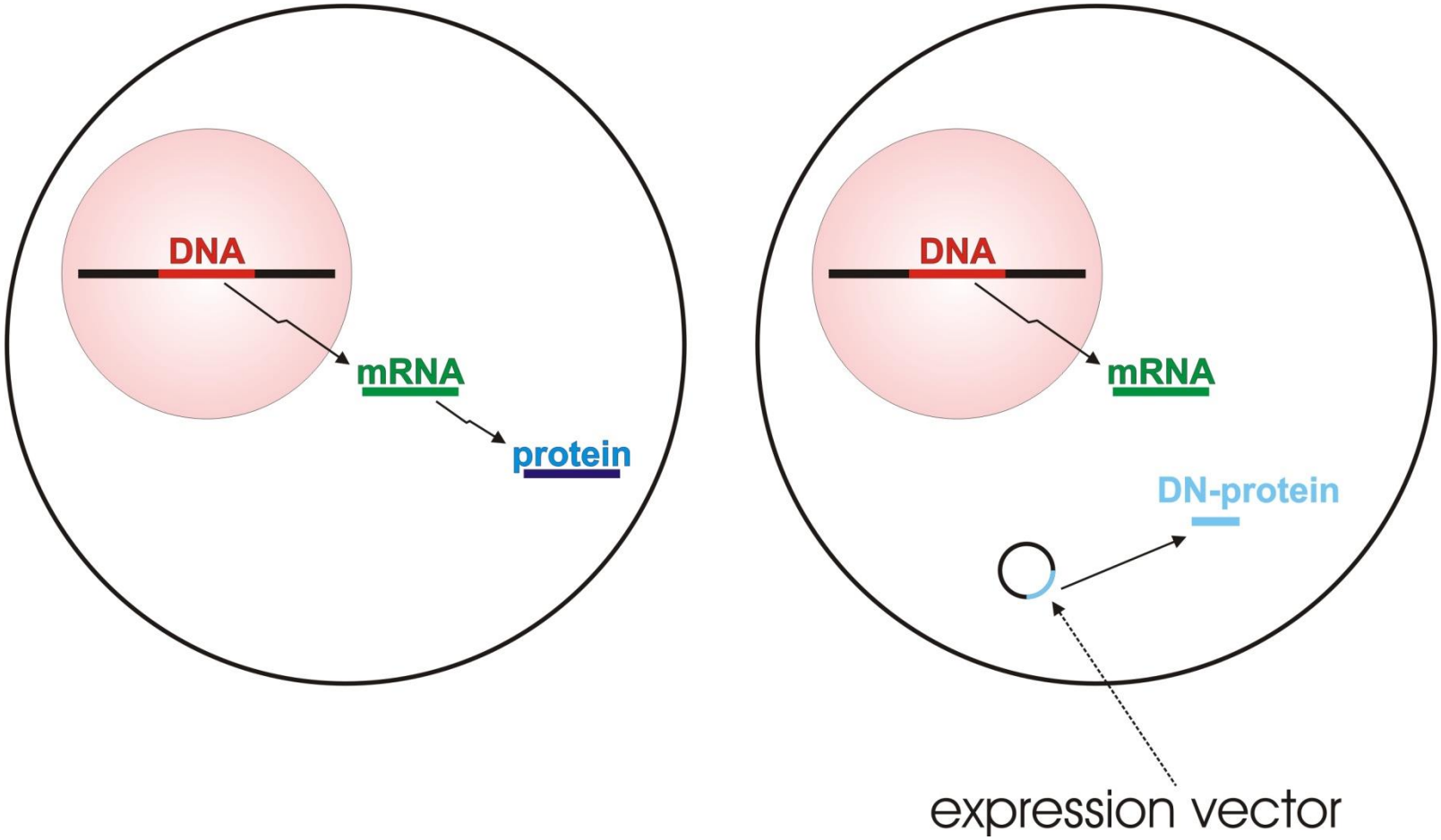
- Block translation (e.g. antisense via an oligonucleotide, morpholino or siRNA)





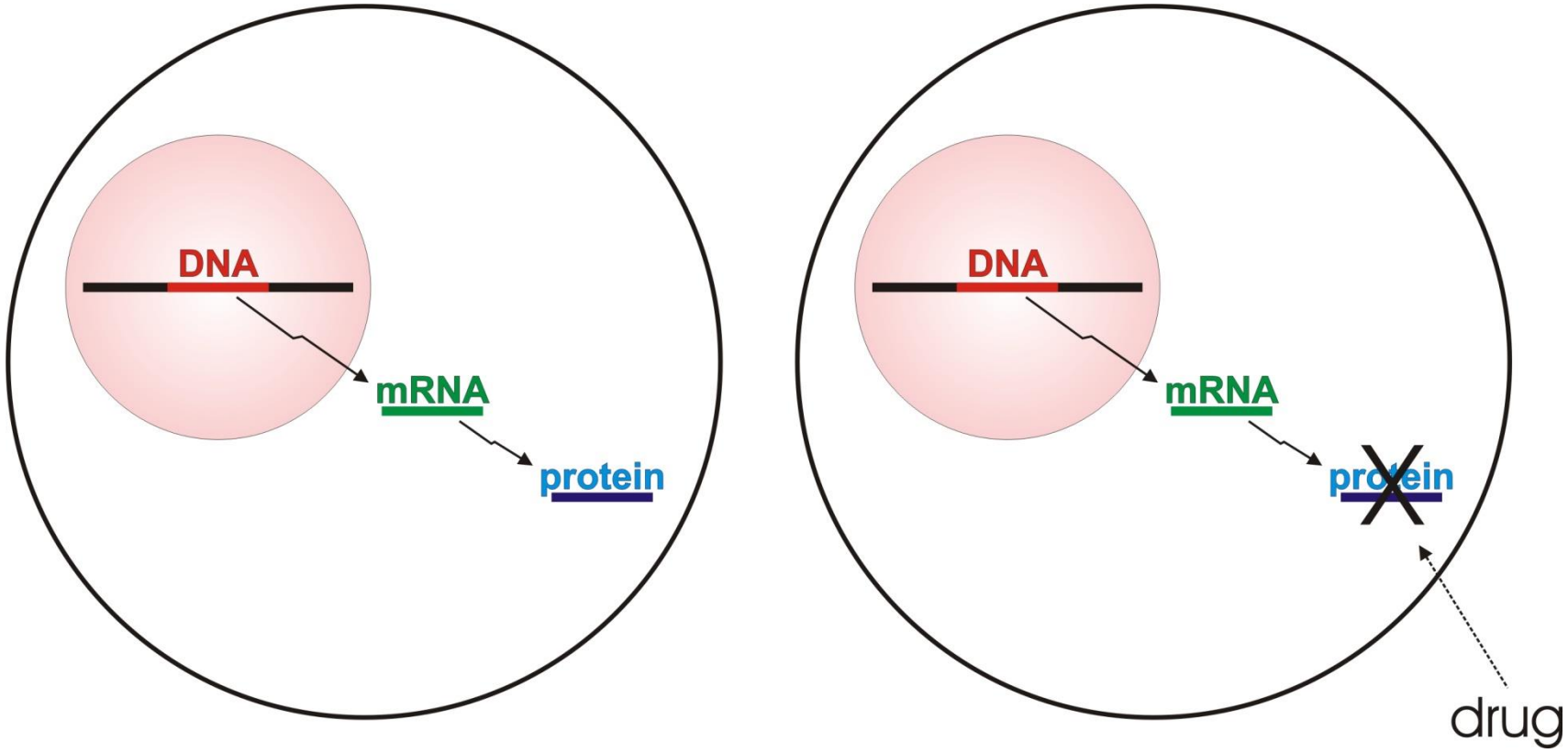
How do we block protein function?

- Out compete the native protein by expressing a ‘dominant negative’ protein

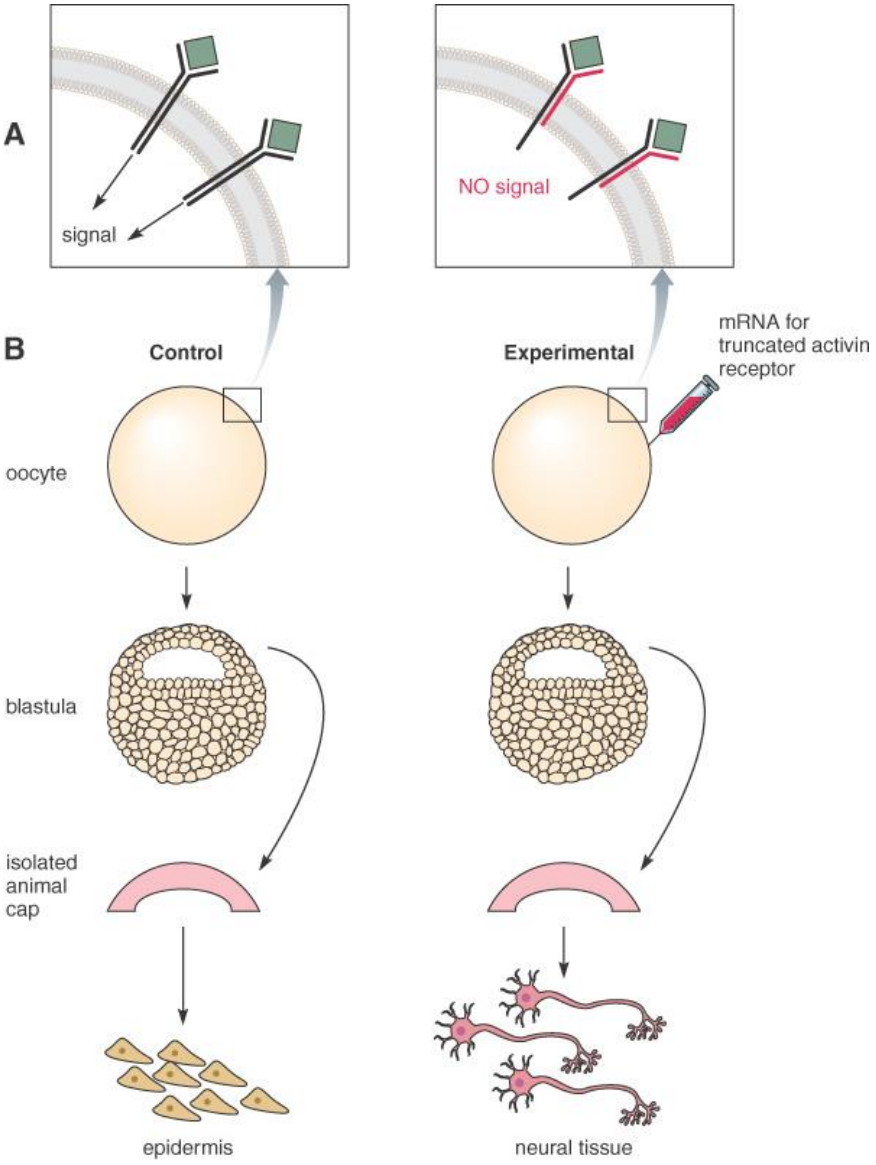


How do we block protein function?

-Directly interfere with the function of a protein
(e.g. drug, antibody or function blocking peptide)

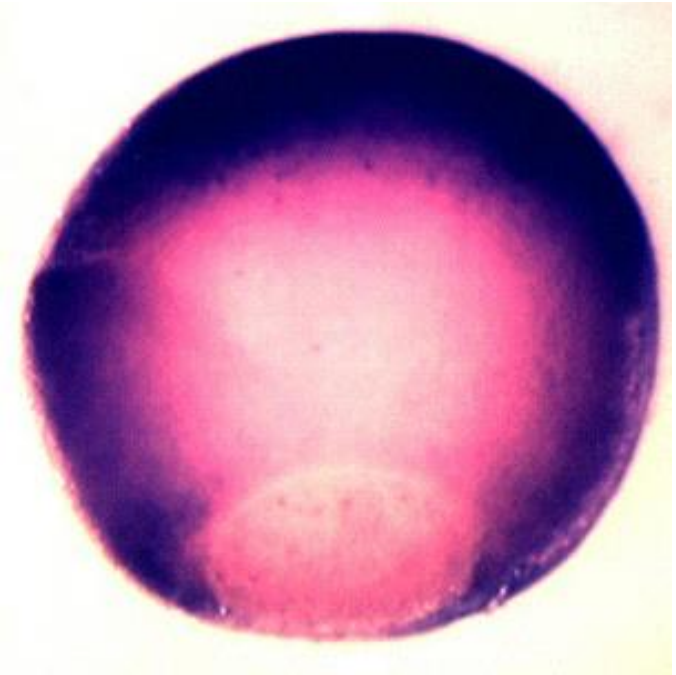


Misexpression of a dominant negative TGF-β receptor in early gastrula ectoderm neuralized the transfected cells.

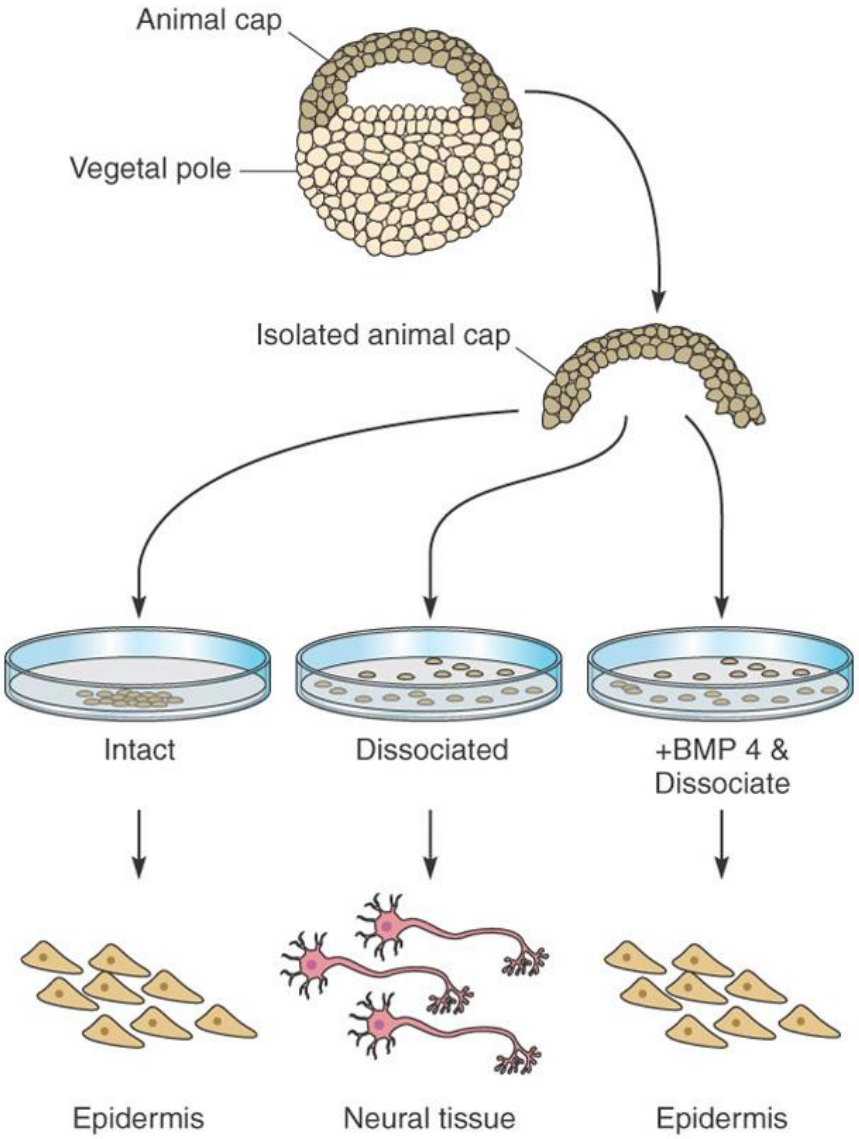


Bone morphogenetic protein 4 (BMP4), a TGFβ-family member, is expressed by all cells of the ectoderm in the blastula and early gastrula stage embryo.

Expression of BMP4 is lost by cells induced to be nervous system.



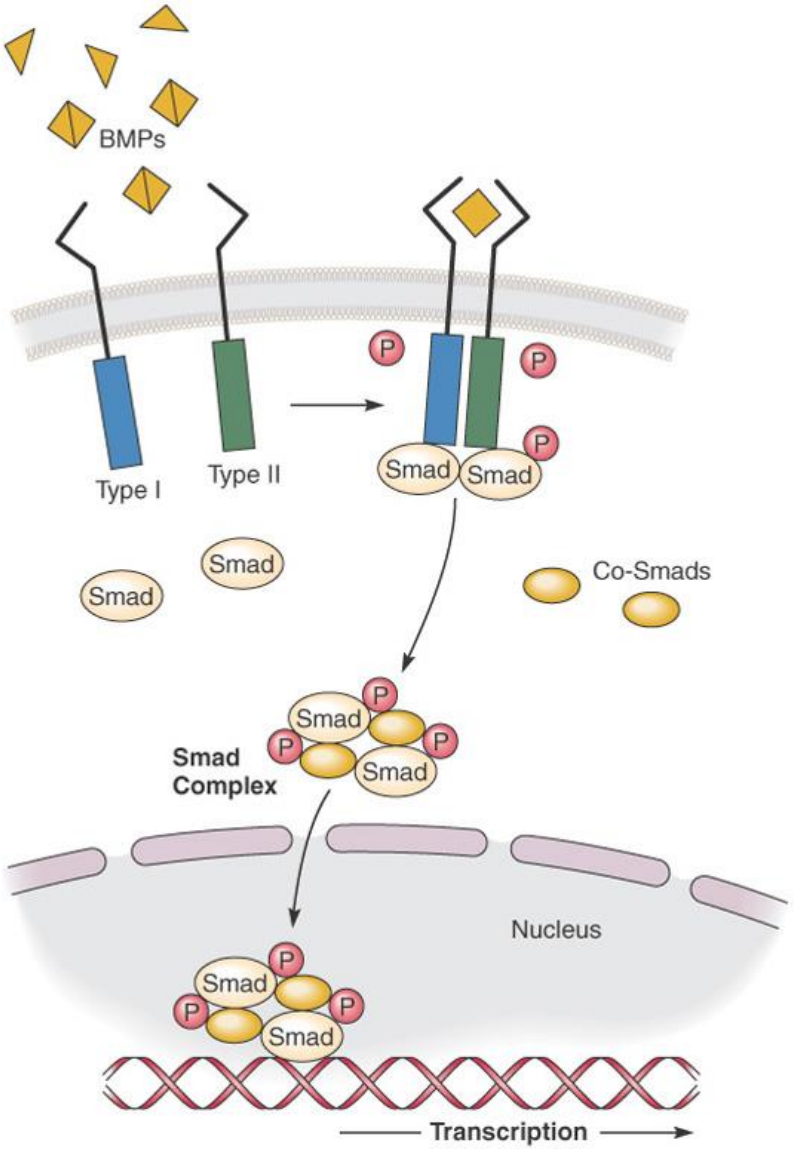
BMP4 blocks neuralization and promotes an epidermal fate .



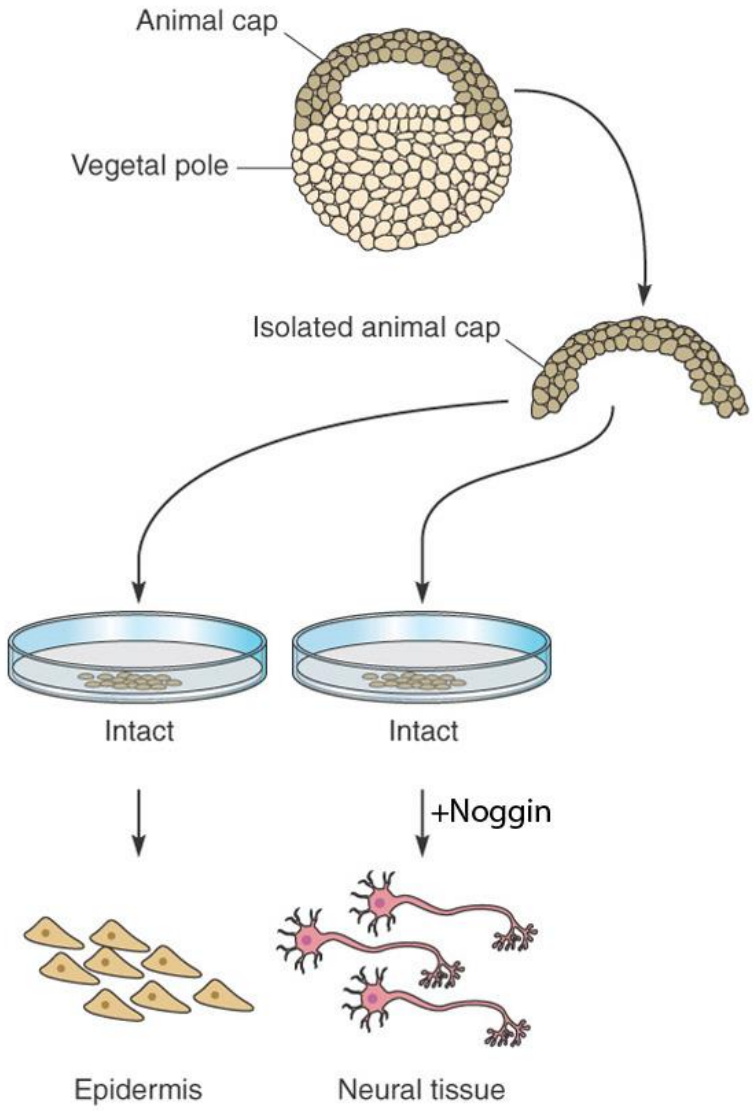
BMP4 blocks neuralization and promotes an epidermal fate .

- Treatment of blastula stage embryos with antisense BMP4 neuralized ectoderm.
- Neuralization failed to occur in dissociated animal cap cells with misexpressed constitutively active Smad1 or Smad5.

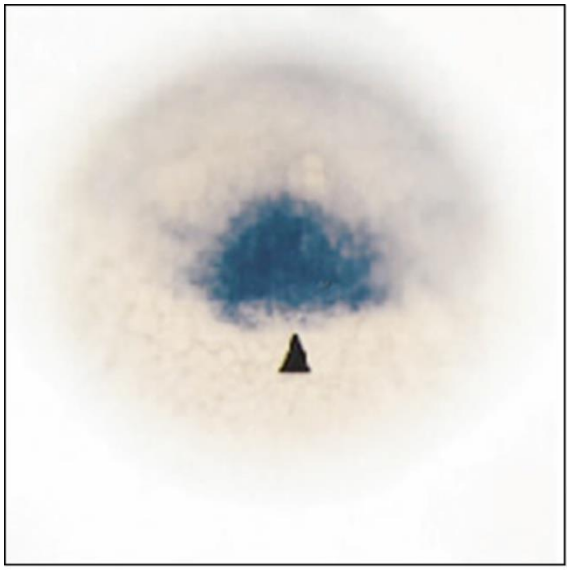
BMP Signaling



Three proteins were independently identified, Noggin, Chordin & Follistatin that could neuralize animal cap cells.

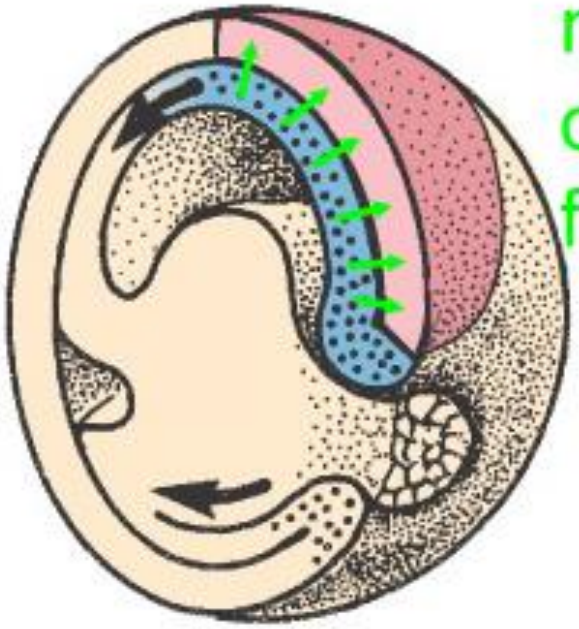


Noggin, Chordin & Follistatin are secreted proteins expressed by the organizer and later by notochord.

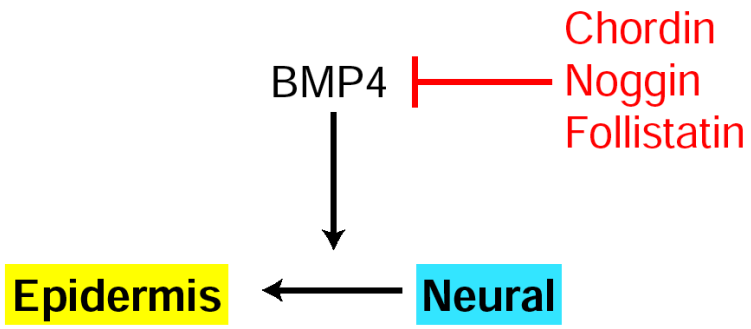


Chordin expression by the organizer

Noggin, Chordin & Follistatin bind BMP4 and inhibit its signaling .



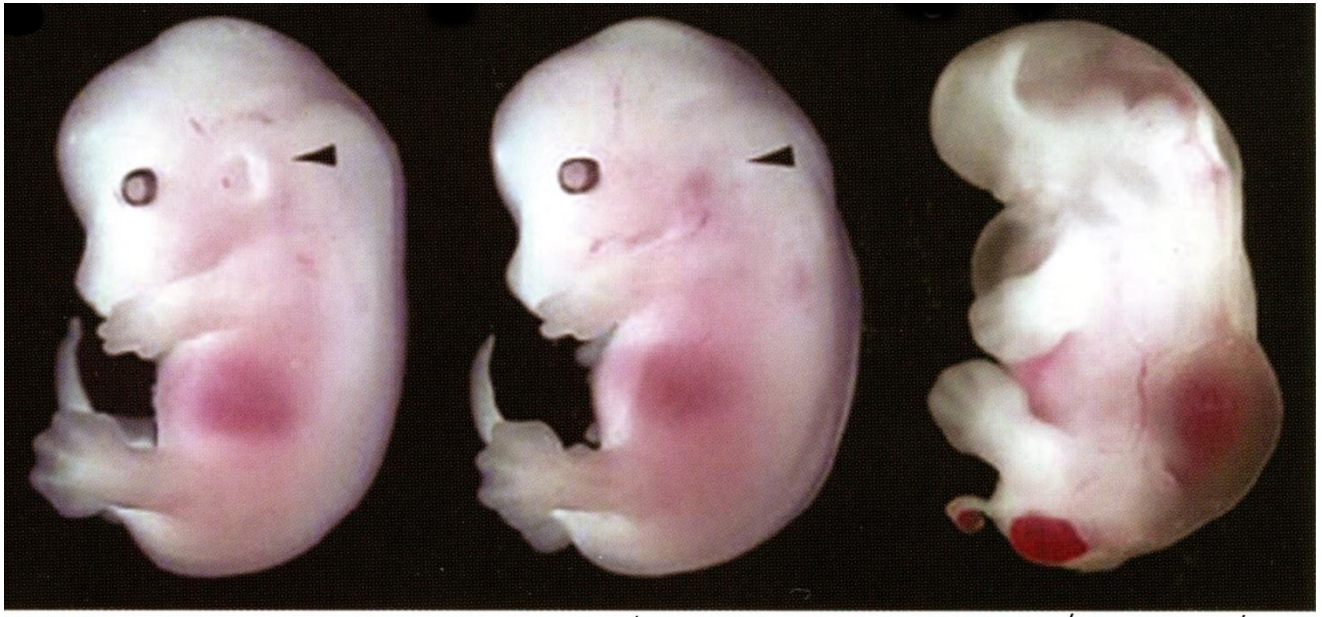
noggin
chordin
follistatin



Knockout of the *Noggin*, *Chordin* or *Follistatin* Genes

- A single knockout of the *noggin*, *chordin* or *follistatin* gene in mice had minimal effect on nervous system development.
- A double knockout of *noggin* and *chordin* resulted in very little nervous system developing…

  however, some did develop… maybe due to follistatin.

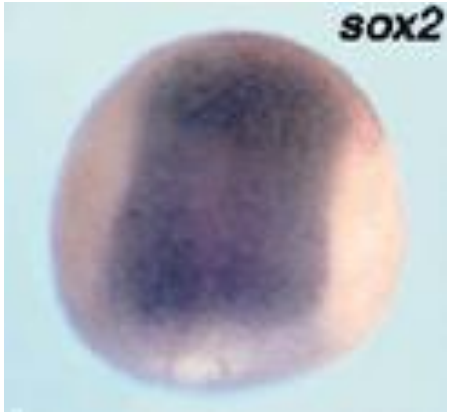


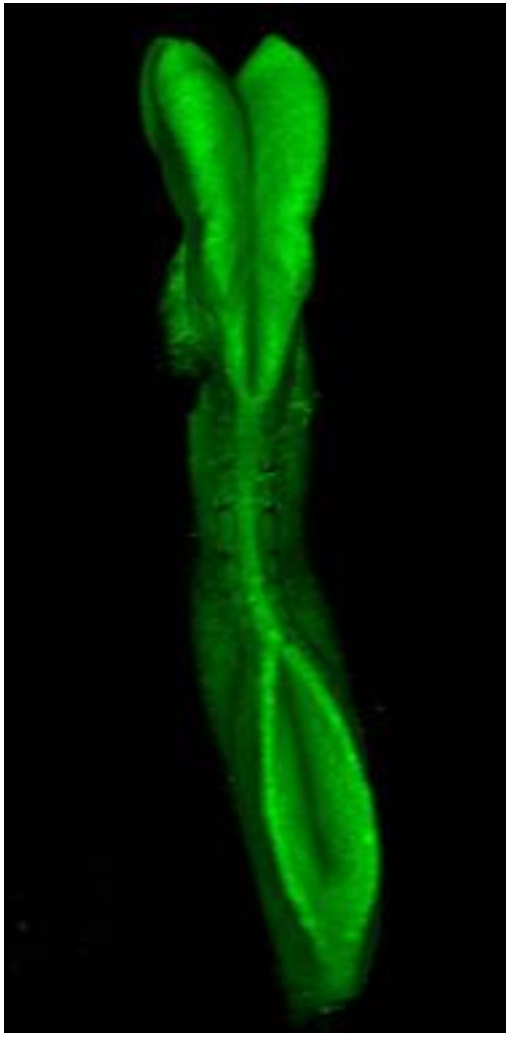
wildtype noggin$^{-/-}$ noggin$^{-/-}$ chordin$^{-/-}$

FGF Signaling and Neural Induction

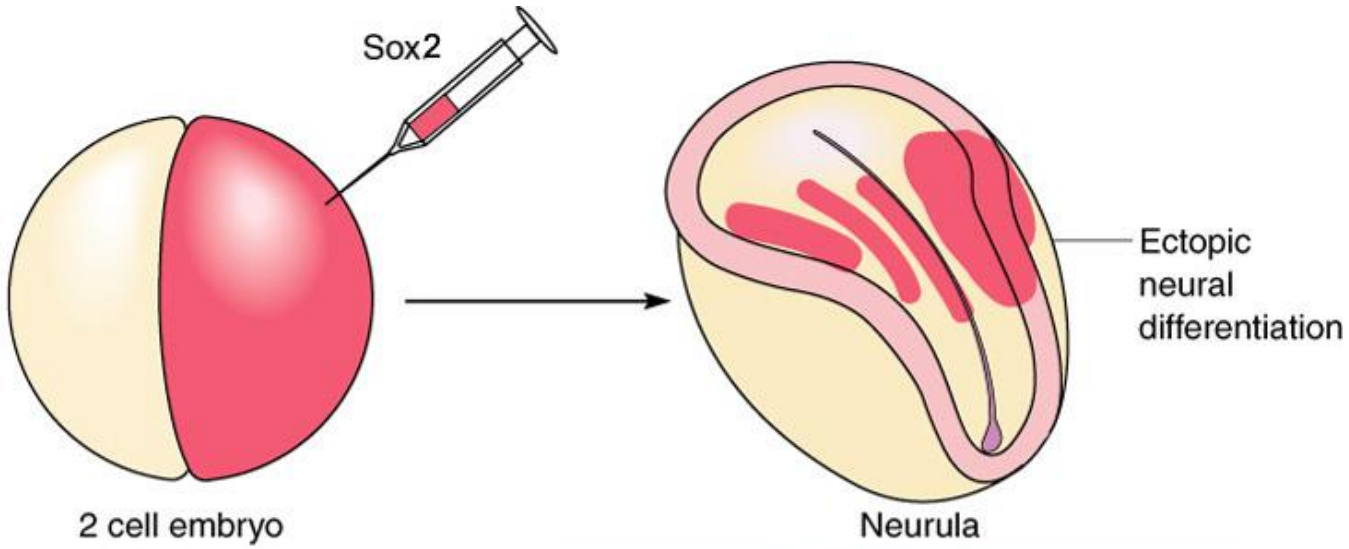
- Fibroblast growth factor (FGF) is expressed by the organizer.
- Noggin, chordin & follistatin can neuralize ectoderm only if FGF signaling is active. Blocking FGF expression or misexpression of a DN-FGF receptor in animal caps made them unresponsive to the three factors.
- FGF signaling can induce Sox2 expression.

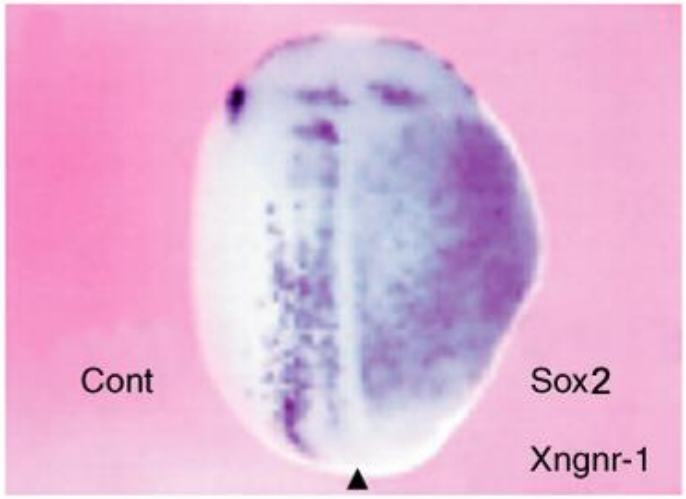
Expression of the transcription factor Sox2 defines neuralized cells .





Misexpression of Sox2 neuralized ectoderm .

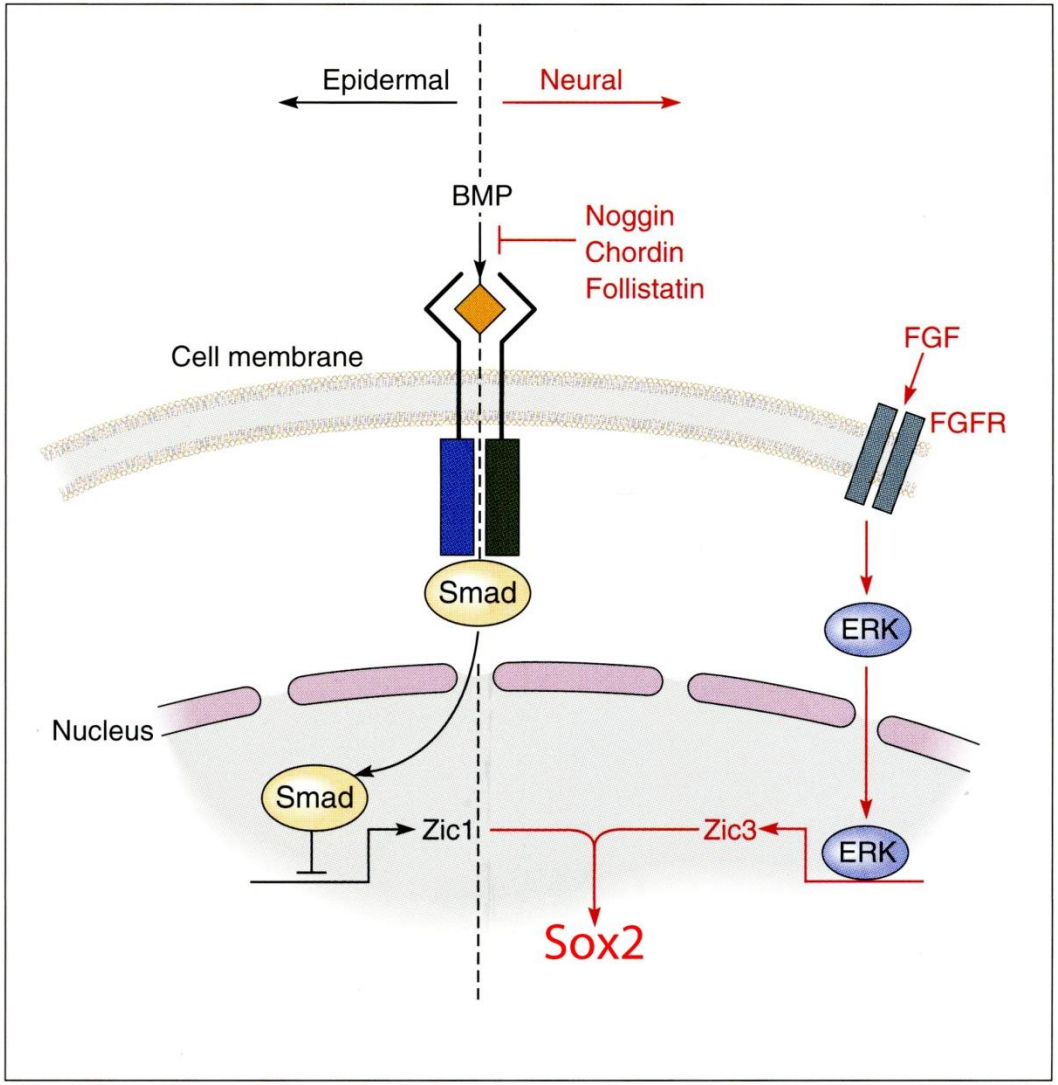




BMP4 signaling represses Sox2 expression.

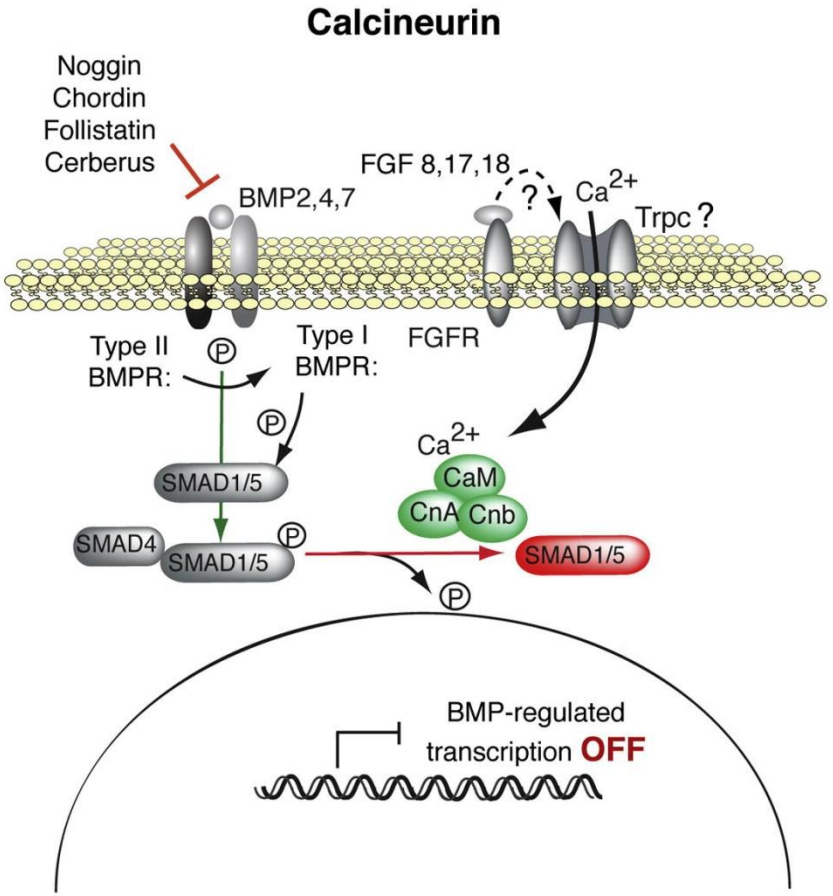
BMP4 —| Sox2

The Working Model



FGF Signaling also activates calcineurin, which regulates neural induction.

- FGF opens Ca^{++} channels; Ca^{++} activates calcineurin; active calcineurin directly dephosphorylates BMP-activated Smads.



Cho A, et al., 2014, Neuron 82:109

The neural tube develops differently along it's length.

- Mesoderm from different rostrocaudal positions has different inductive powers. More rostral mesoderm induces more rostral nervous system structures (e.g. brain); more caudal mesoderm induces more caudal nervous system structures (e.g. spinal cord).