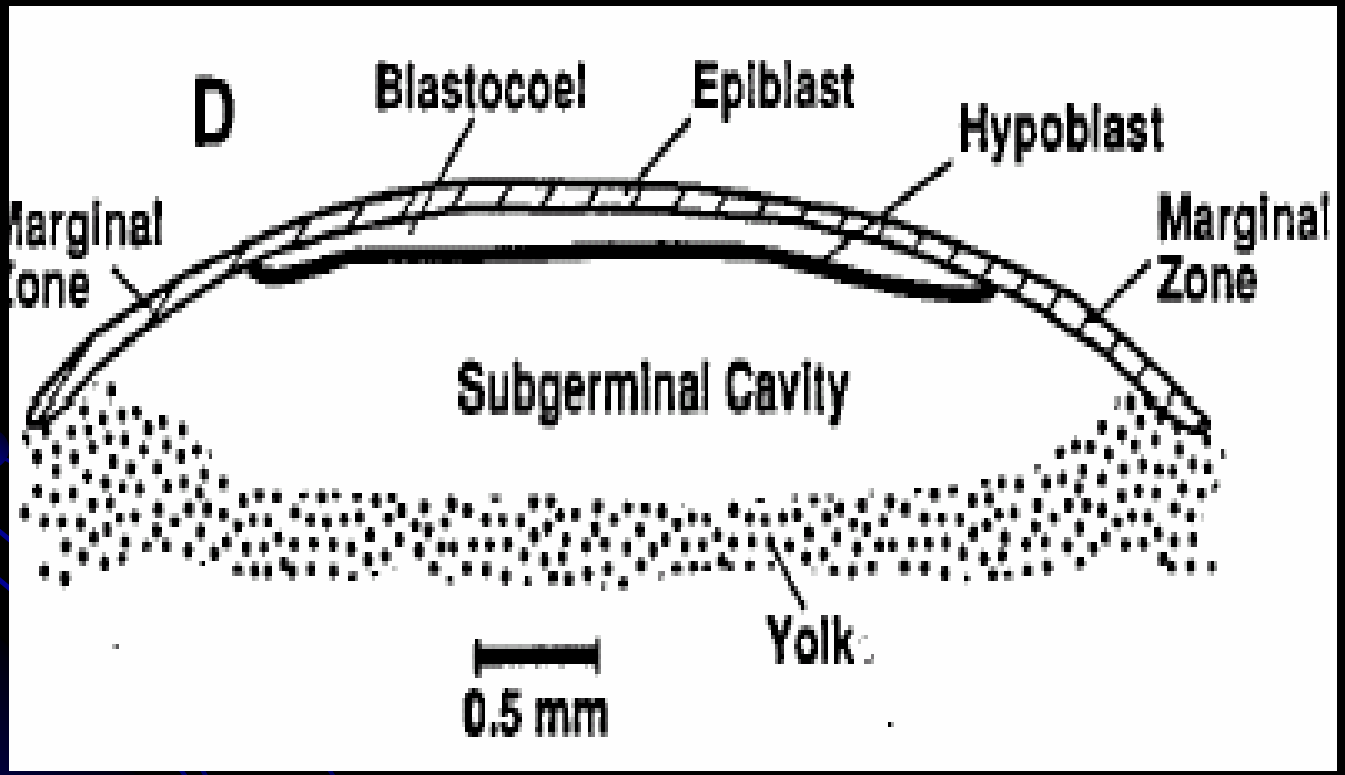
Churchill, a Zinc Finger Transcriptional Activator, Regulates the Transition between Gastrulation and Neurulation

Sheng G, dos Reis M, and Stern C. 2003.

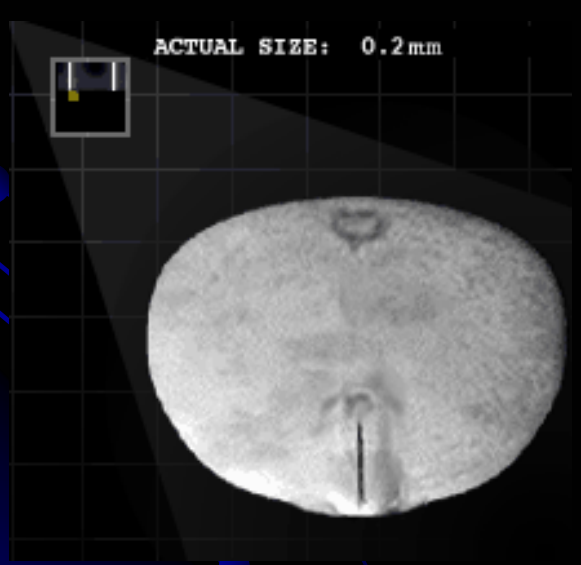
Presented by: Rahmat Muhammad

Early embryogenesis: epiblast formation



The primitive streak

- In mammals, birds and reptiles, the onset of gastrulation is marked by the formation of the **primitive streak** .

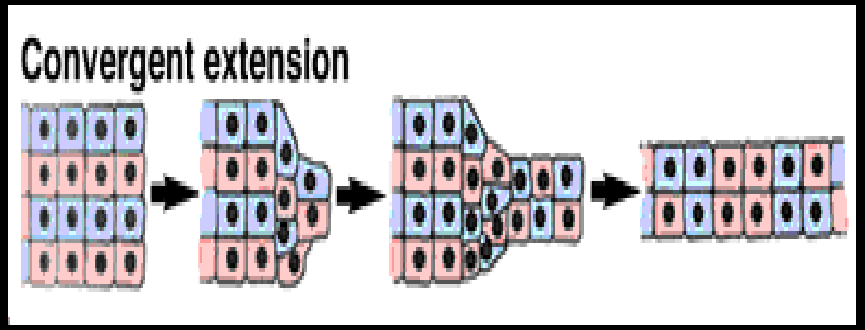


Gastrulation: overview

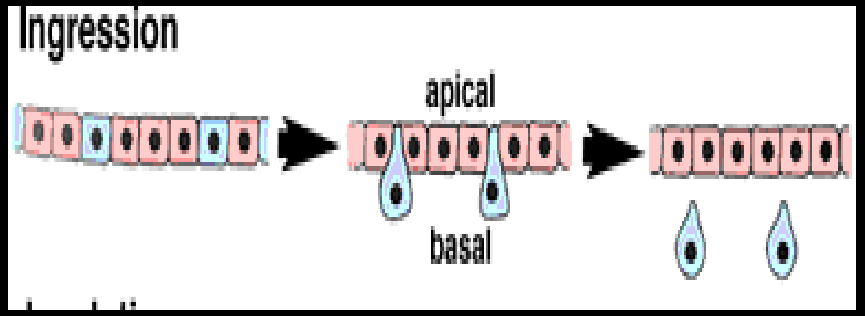
- **Gastrulation** is the process by which the early embryo is transformed into a body consisting of the primary germ layers.

Gastrulation: cell movements

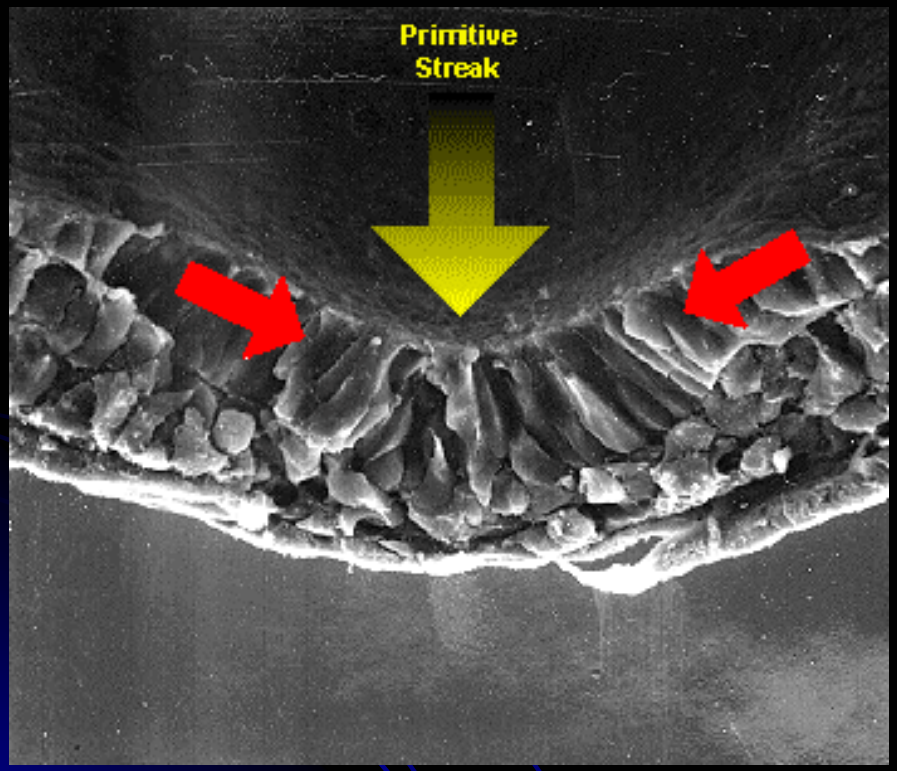
- **Convergent Extension**: rows of cells intercalate, but the intercalation is highly directional.

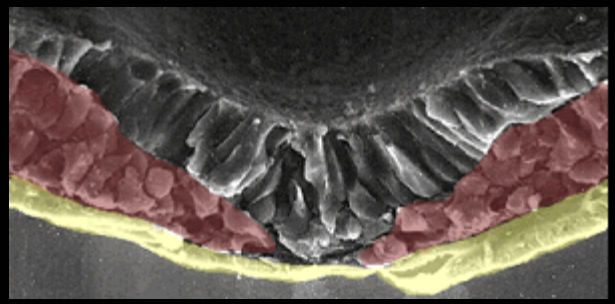


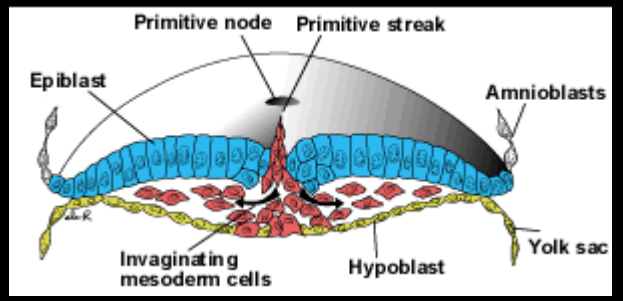
- **Ingression:** individual cells leave an epithelial sheet and become freely migrating mesenchyme cells



Gastrulation: overview

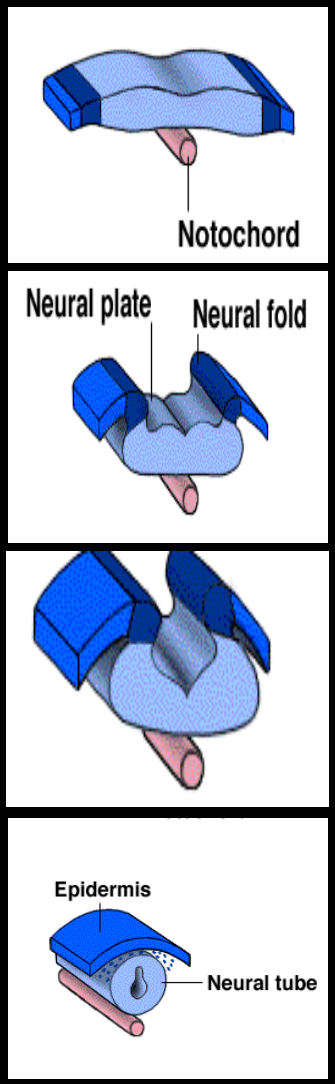






Neurulation: brief overview

- **Neurulation** in vertebrates results in the formation of the **neural tube**, which gives rise to both the spinal cord and the brain.



The many roles of FGFs

- Mesoderm formation and migration
  - *Brachyury* and *Tbx6L*
- Neural induction
  - *Sox3* and *ERNI*
- Caudalization of the neural plate

The hypothesis:

- multiple signaling factors (Streit et al., 2000)
  - FGF is not sufficient for neural induction
  - 5 hour exposure to unknown signals in Hensen's node or FGF8 required to stabilize preneural genes
- FGF alone → epidermal fate
- FGF+ unknown signal → neural fate

Churchill was found in a Differential screen

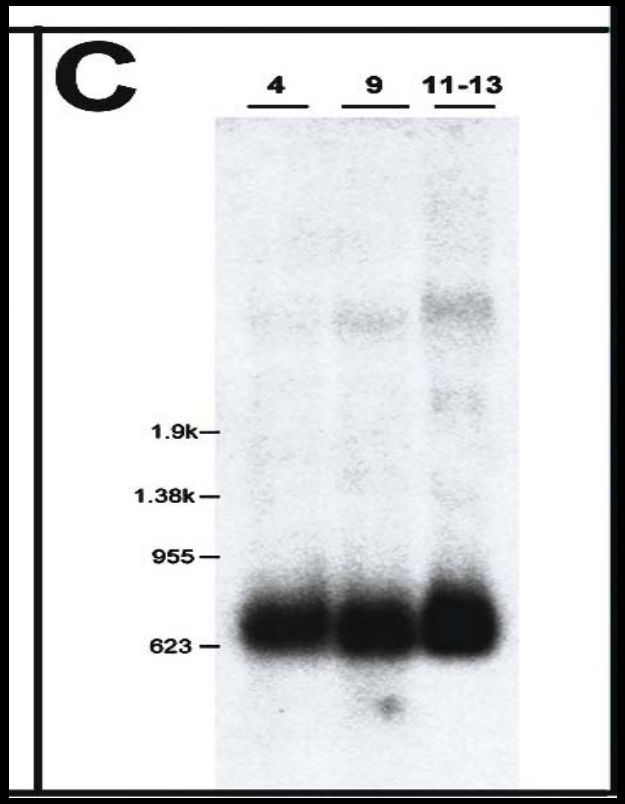
- 112 aa protein containing two putative C4-type zinc fingers

-cxxc----------cxxc----------cxxc----------cxxc--------

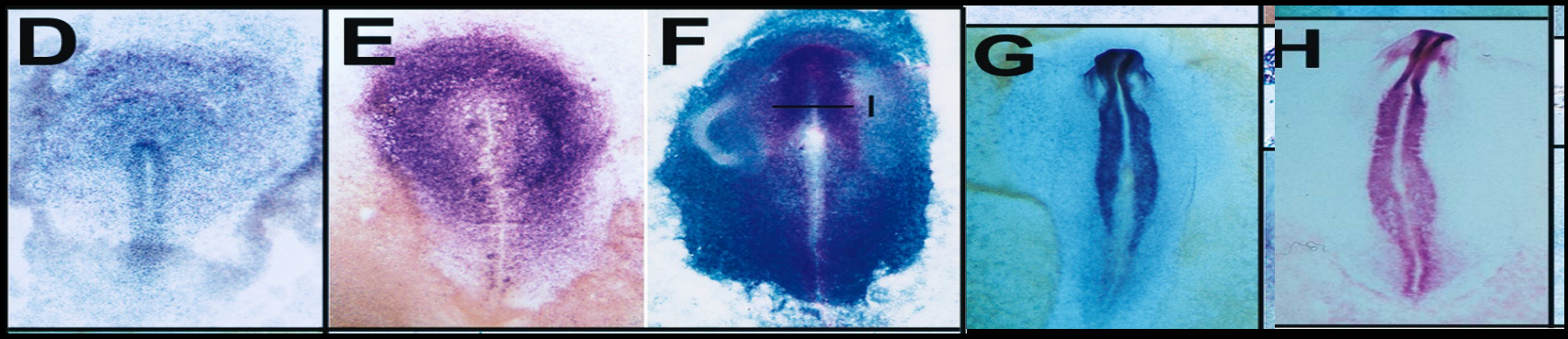
- Homologs in human, rat, *xenopus* etc.
  - Chick ChCh is 75% homologous to *Xenopus* ChCh

Churchill expressed at the right time

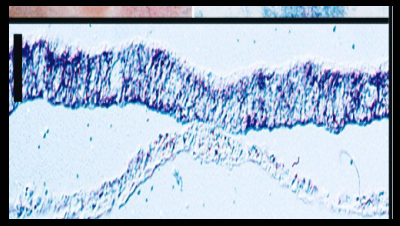
Northerrn blot analysis of chick embryos from stage 4 to stage 13



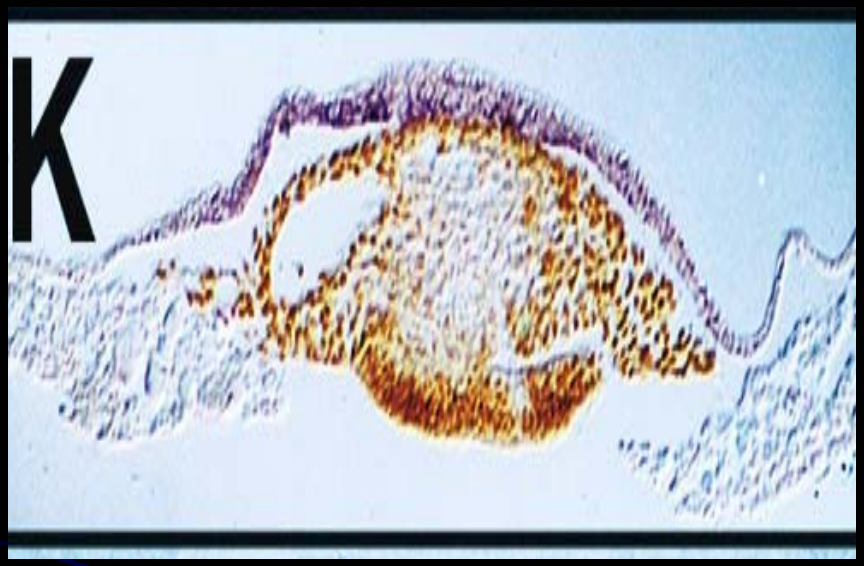
Churchill expressed in the right place

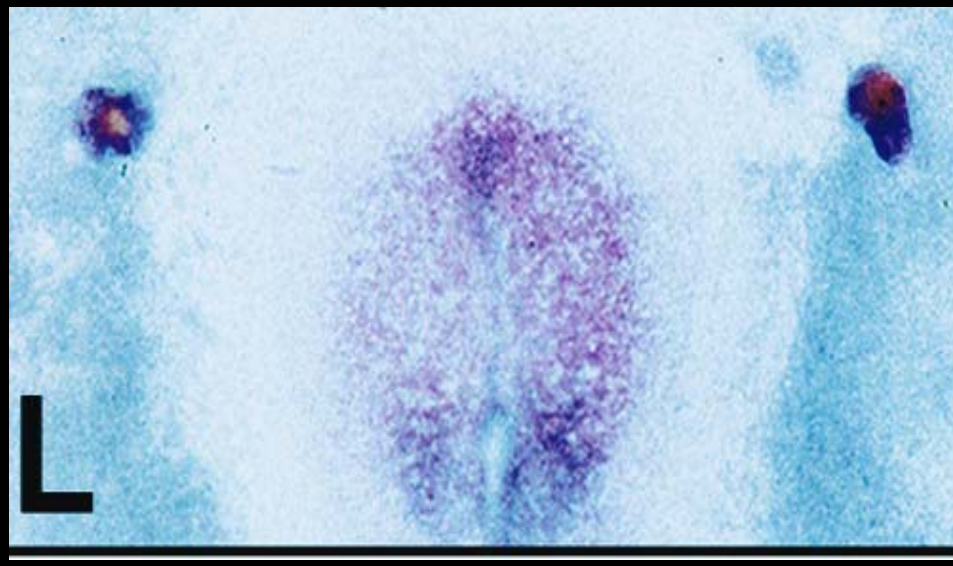


Stage: **4** **4+** **6** **7** **8**



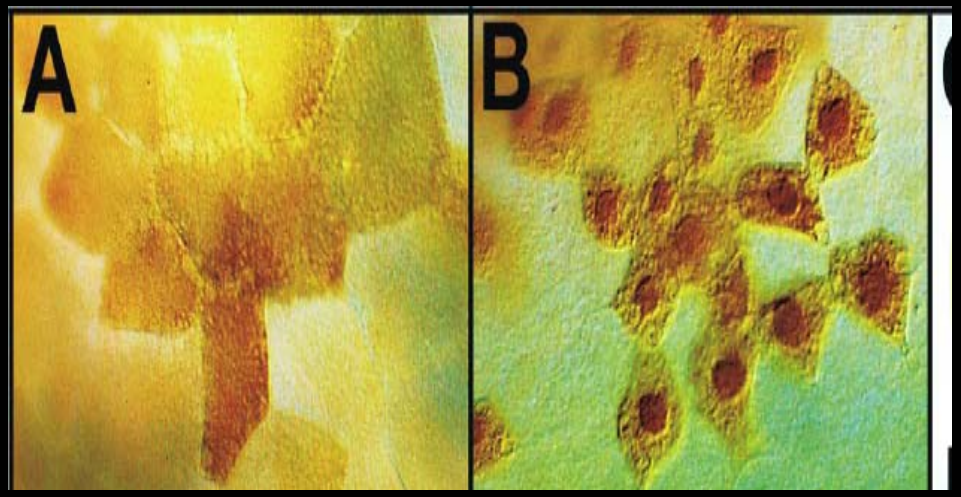
Churchill is regulated by FGF



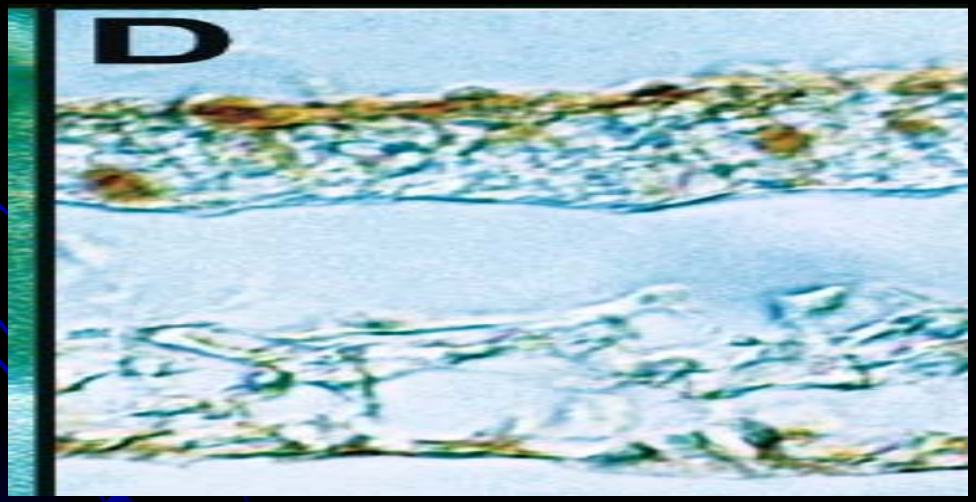


- Graft of quail Hensen's node and heparin beads soaked in FGF4 or FGF8b induced *ChCh* RNA in stage 3+ chick embryos within 4-5 hours of exposure.
- Control beads, chordin, noggin, HGF, Cereberus do not induce *ChCh*

Churchill is localized to the nucleus



Myc-tagged Churchill into *Xenopus*



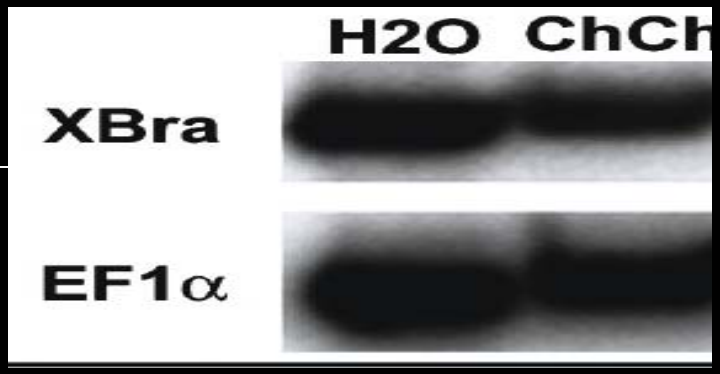
Myc-tagged Churchill into chick embryos

Churchill inhibits FGF signaling

- Misexpression of *ChCh* into 2-4 cell stage Xenopus
  - Phenotype similar to embryos with dominant negative FGF receptor
  - Therefore, ChCh may inhibit FGF signaling

Churchill represses mesoderm marker *Bra*

FGF → *Bra* →



Bra → Phenotype X

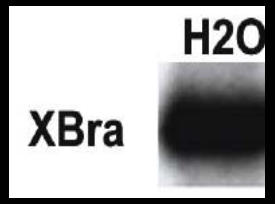
Churchill → Phenotype X

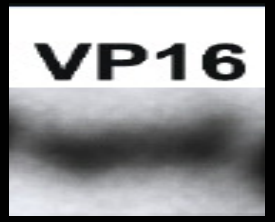
Is Churchill a repressor or activator?

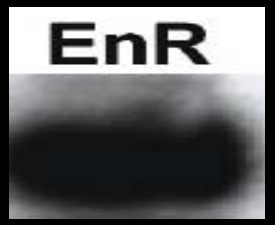
- VP16 is an unusually potent transcriptional activator
- EnR is a potent transcriptional repressor

Normal XBra

Hypothesis	domain	*Xbra* levels
activator	VP16	
activator	EnR	
repressor	VP16	
repressor	EnR	

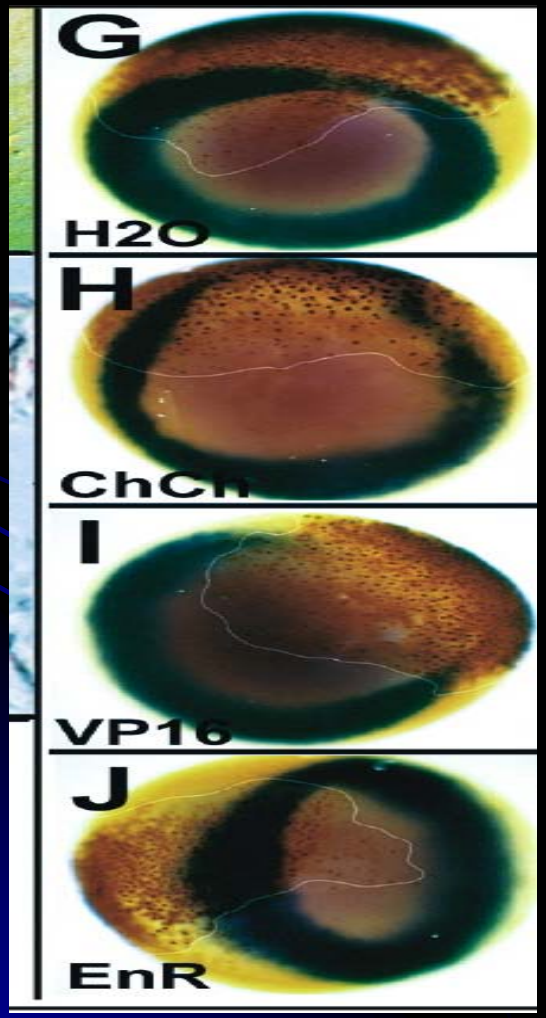




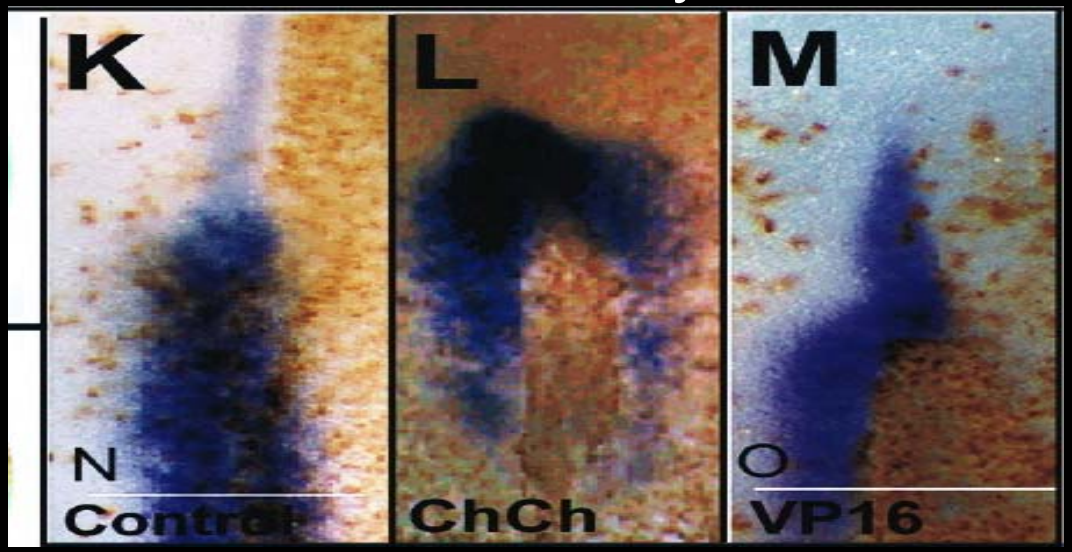


Churchill causes the repression of *Bra* in *Xenopus* and Chick embryos

Xenopus embryos

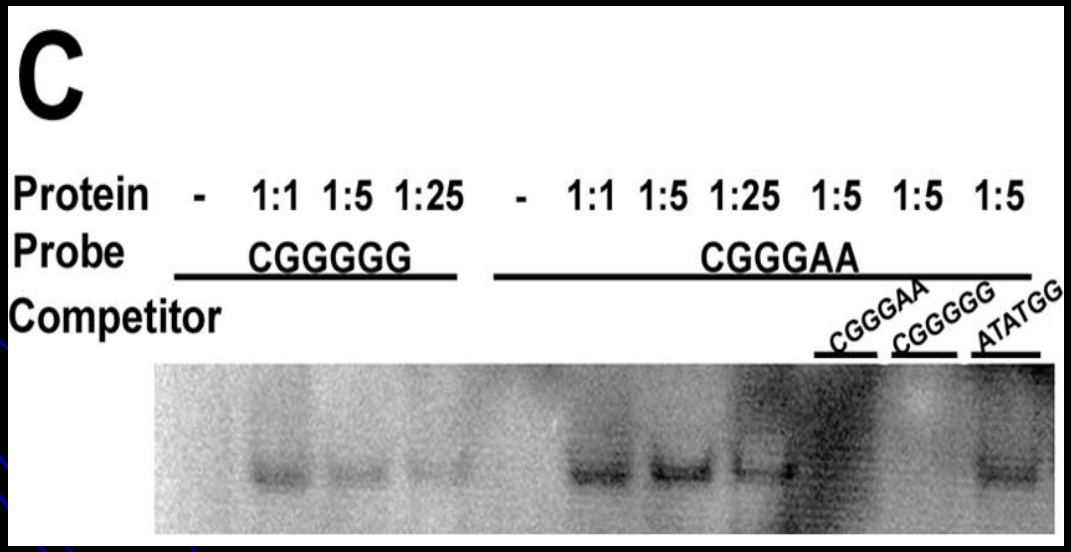


Chick embryos



Churchill binds to specific sequences

- Optimal binding sequences for Churchill protein: CGG(GAT(CAC)
- Gel mobility shift and competition assays



Thus,

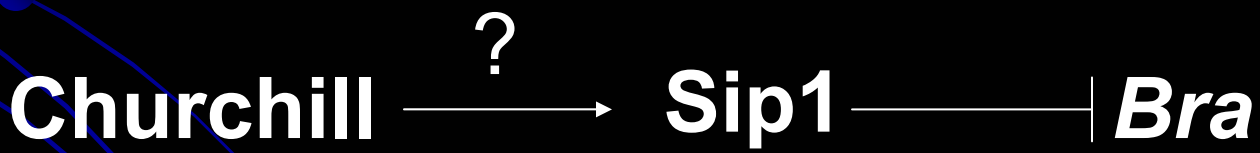
- "…although ChCh can repress the mesoderm markers *Bra* and *Tbx6L*, the above results suggest that it may function as a transcriptional activator…"

So far…

- Churchill inhibits FGF signaling by causing the repression of FGF effectors such as Bra and Tbx6L
- This repression appears to be indirect

Smad-Interacting Protein (Sip1)

- Consequences of ChCh misexpression are very similar to the phenotype of *Xenopus* embryos injected with Sip1
- Sip1 is a direct transcriptional repressor of *XBra*



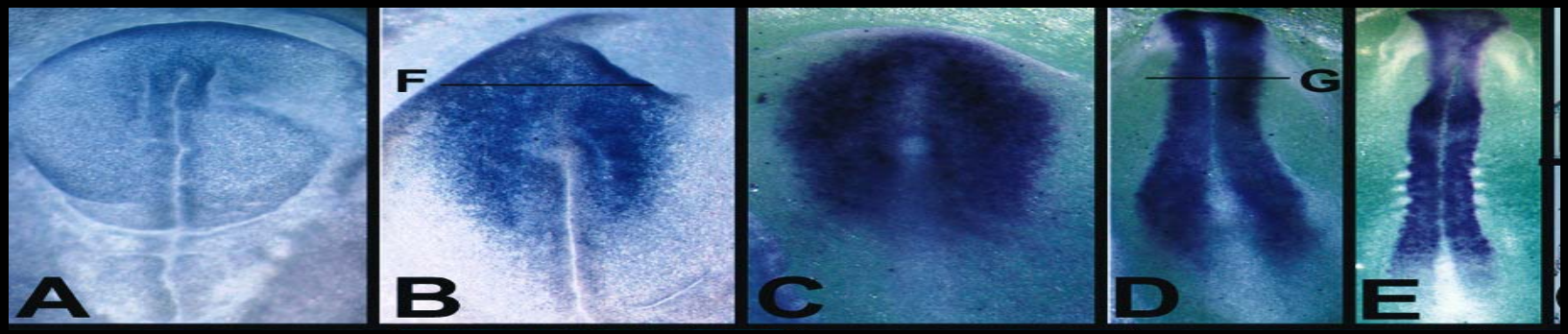
If ChCh activates Sip1:

- Sip1 should contain consensus sequences for ChCh binding
- ChCh and Sip1 should be in the same place at the same time.
- In the absence of ChCh, Sip1 should not be expressed

ChCh and *Sip1* are coexpressed

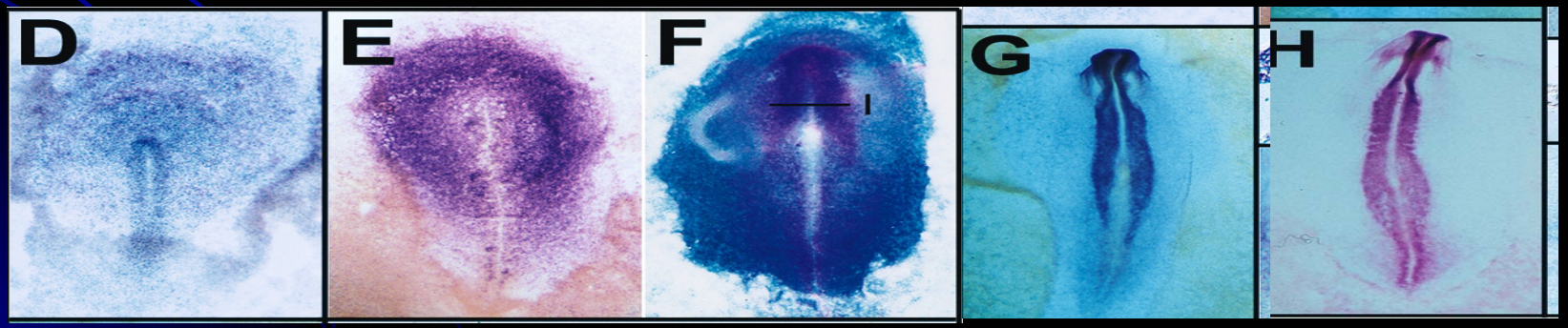
- In situ hybridization with *Sip1* mRNA shows its expression pattern is indistinguishable from that of *ChCh*

Sip1



Stage: 4 4+ 5 7 8

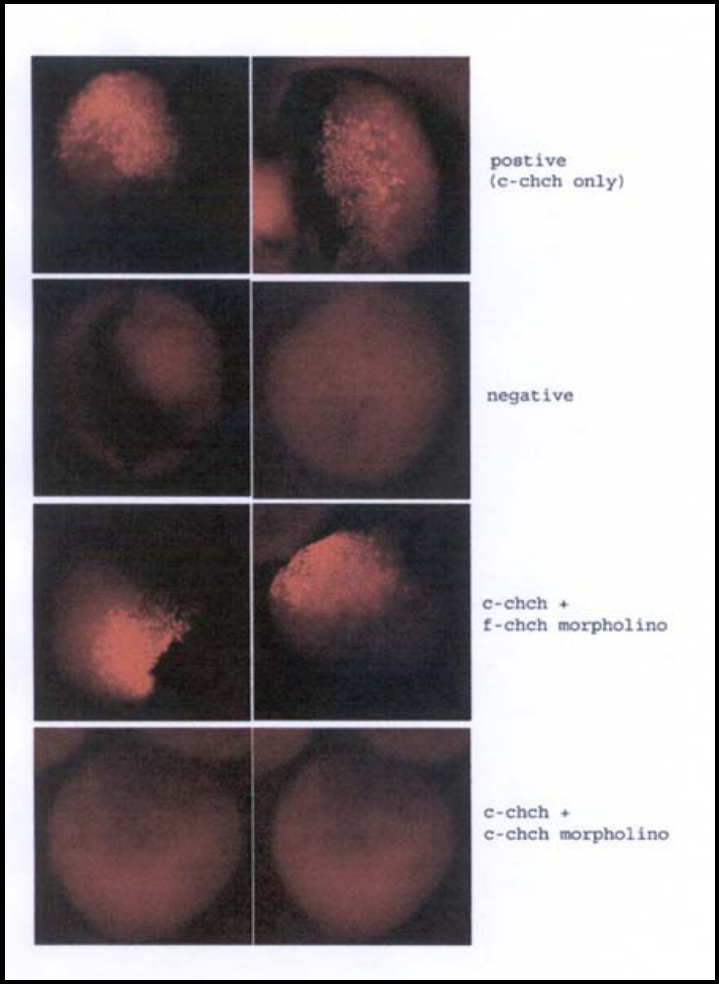
ChCh



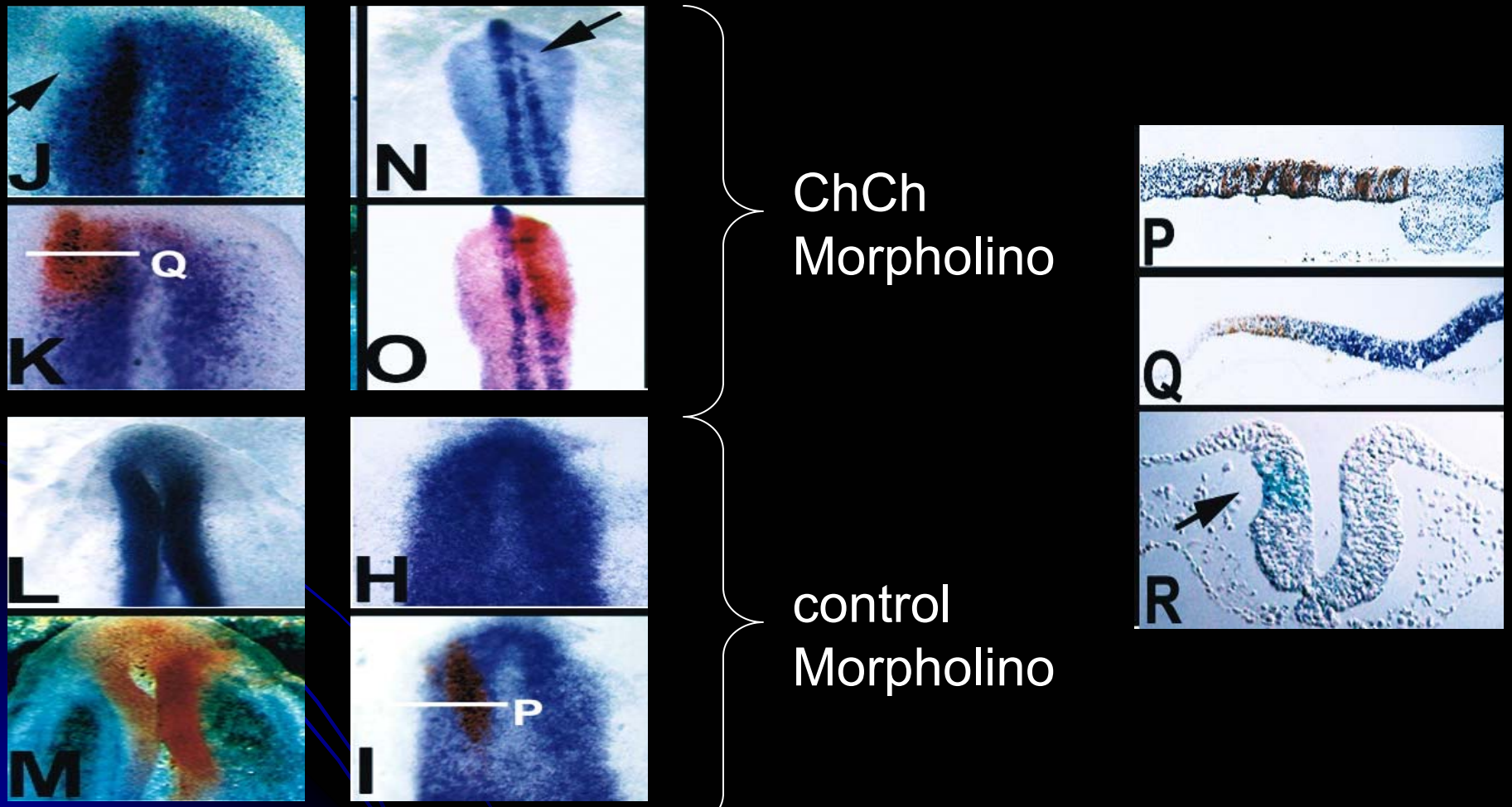
Stage: 4 4+ 6 7 8

Morpholinos against *ChCh*

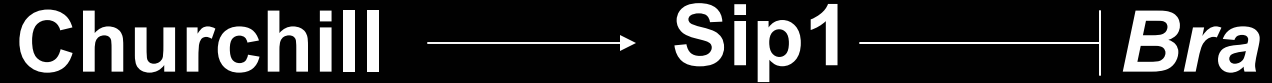
- Fluorescein-labeled morpholino oligonucleotide (MO) against chick *ChCh*
  - Morpholinos block the translation initiation complex or block the nuclear splicing machinery



ChCh required for *Sip1* expression



So far…

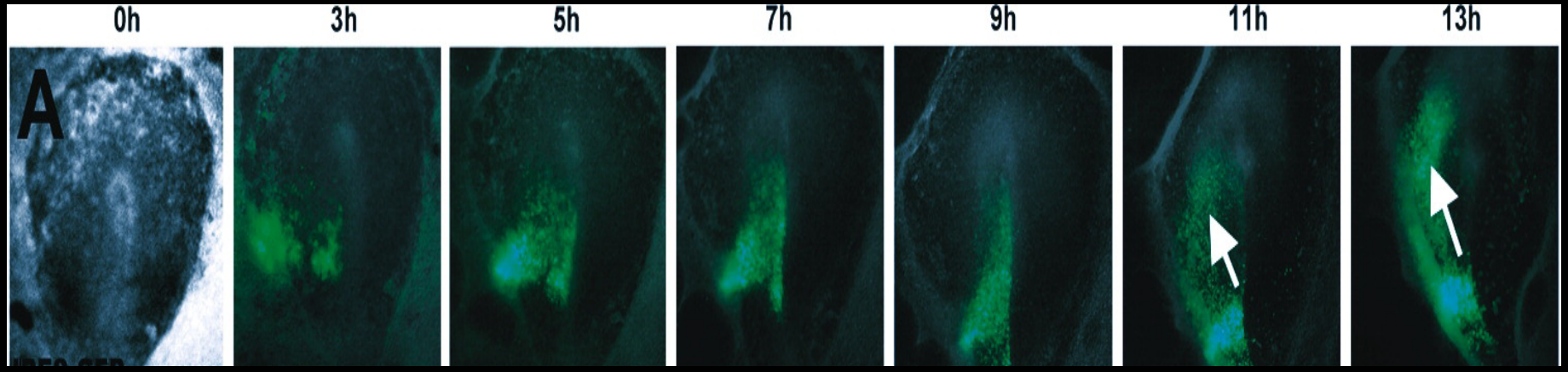


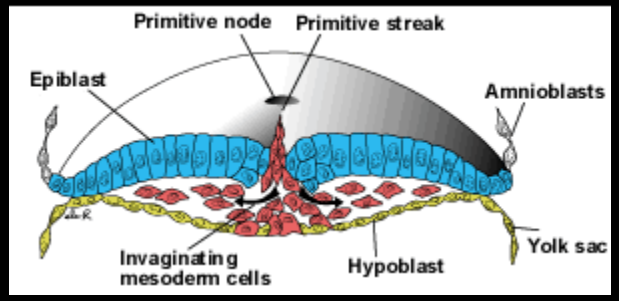
- Bra is an important regulation of mesoderm formation during gastrulation
- What happens when ChCh is misexpressed during gastrulation?

Churchill regulates cell ingression through the primitive streak

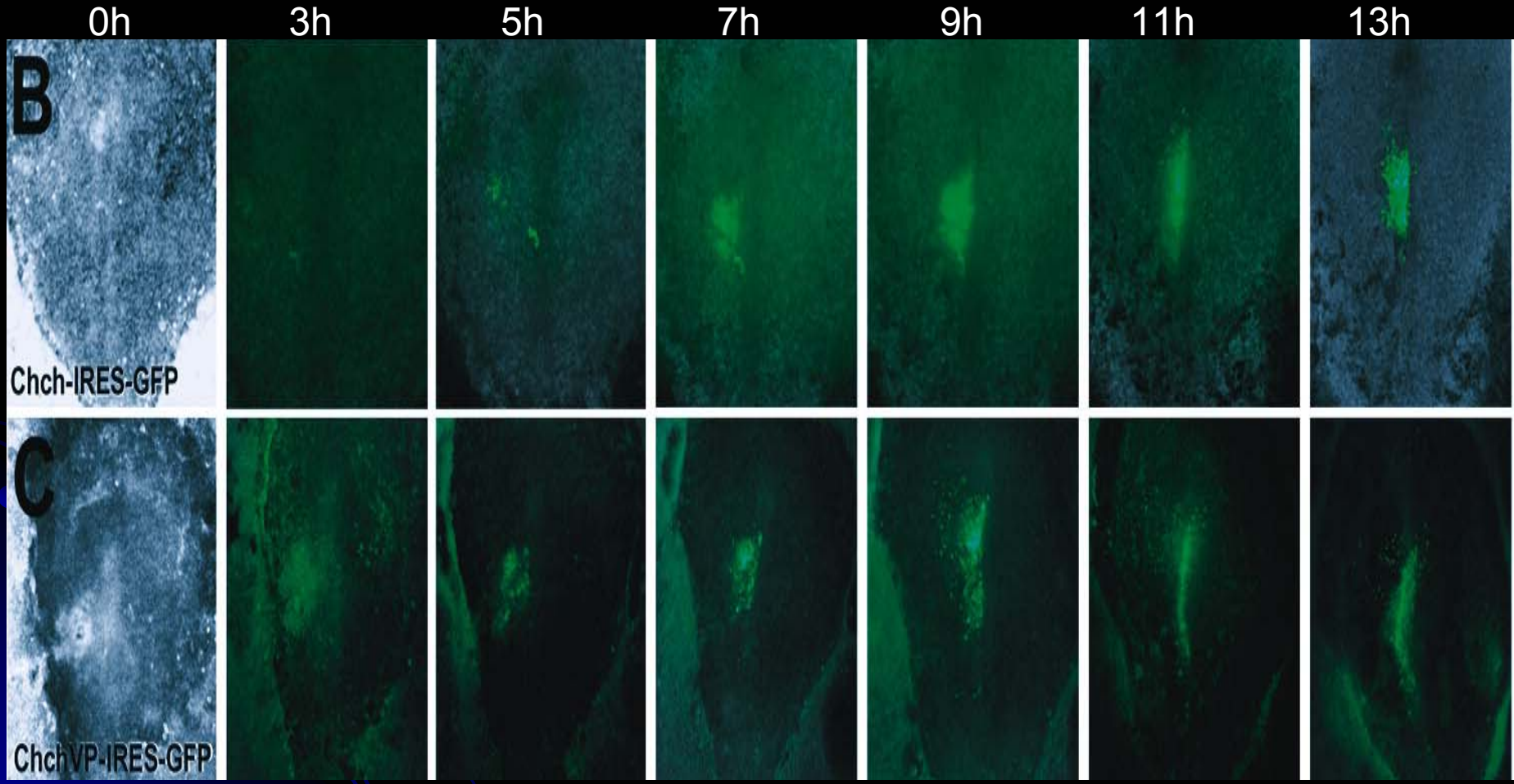
- ChCh-IRES-GFP
- ChChVP16-IRES-GFP
- IRES-GFP
- ChChMutVP-IRES-GFP

IRES-GFP control





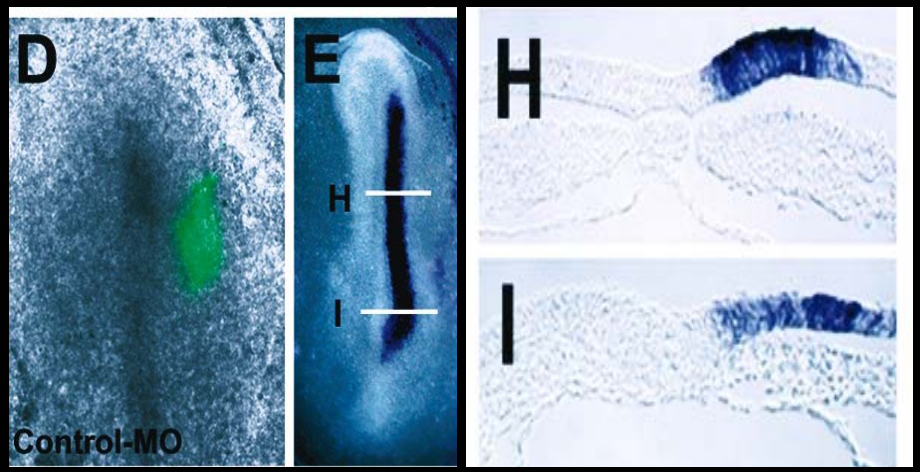
ChCh-IRES-GFP and ChChVP16-IRES-GFP

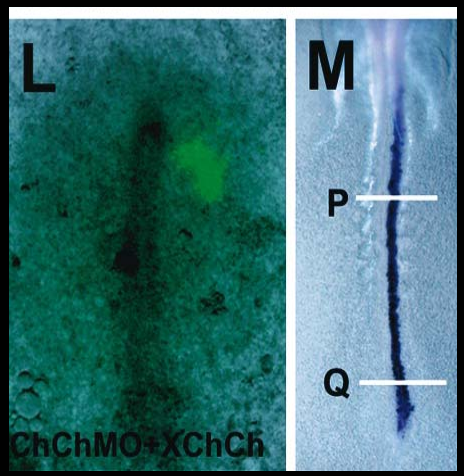


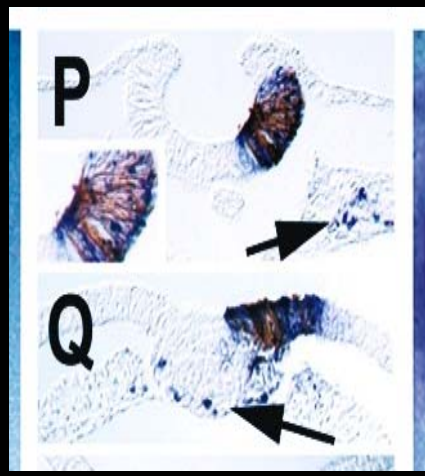
ChCh regulates ingression

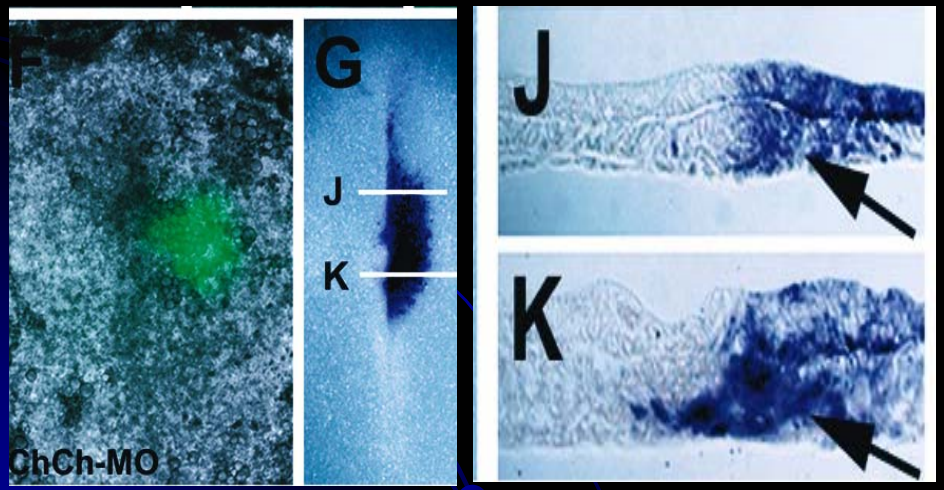
***ChCh* knockdown**

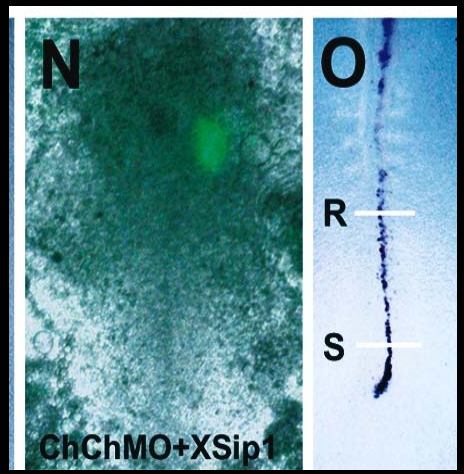
***ChCh* rescue**

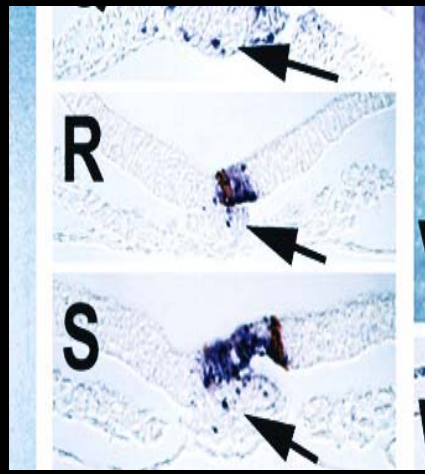






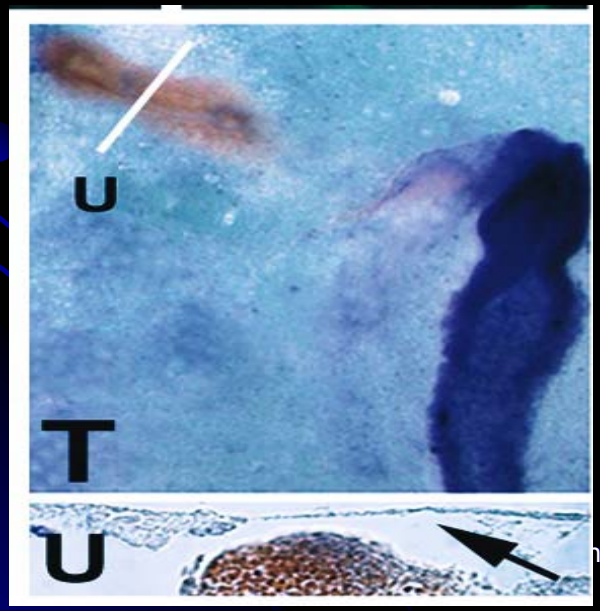


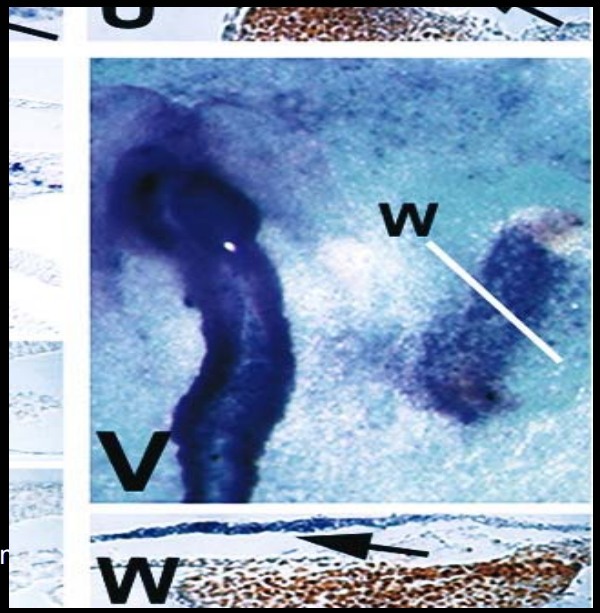




Churchill regulates competence to neural-inducing signals

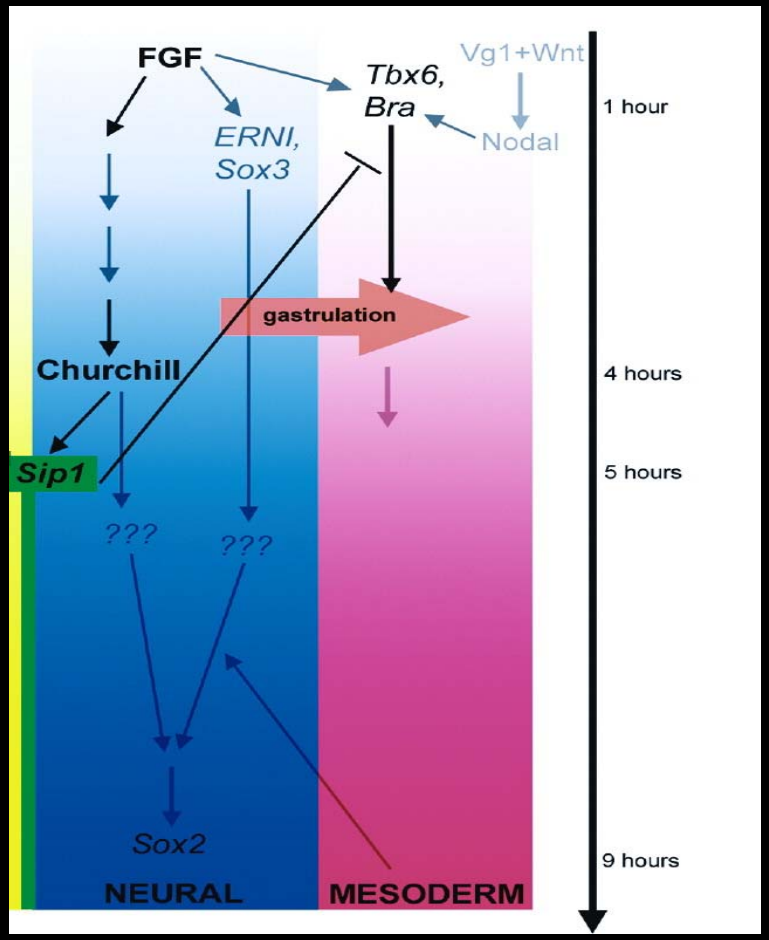
- Does ChCh play a role in sensitizing neural epiblast to neural inducing signals?
- Electroporate ChCh-IRES-GFP to area opaca at stage 4, incubate to stage 5, and graft a quail node





Churchill separates different functions of FGF signaling

- FGF turns on *Bra* and *Tbx6L*
- Churchill induced 4 hours later. Induces Sip1
- Sip1 blocks *Bra* and *Tbx6L*
- Node signals stabilize ‘preneural’ state



Role of Churchill in mesoderm formation

- ChCh activates Sip1
- Sip1 binds to the activated forms of Smad1/5 and Smad2/3
  - Block mesoderm induction/signaling through BMP
- Sip1 inhibits *Bra*
- ChCh ends ingression through the primitive streak

Role of Churchill in neural induction

- Early neural markers expressed 1-2 hr of exposure to node graft
- But cells are not sensitive to neural signals such as chordin until after 5 hr
- The node or FGF sensitizes cells to BMP antagonists by upregulating ChCh
- ChCh upregulates Sip1 which can act as a sensor for BMP signaling
- Early neural markers are then stabilized

Churchill may act with a cofactor

- *ChCh* misexpression in area opaca does not induce Sip1
- Ectopic expression of *ChCh* does not induce or repress any of the makers analyzed
- In *Xenopus*, ChCh localizes to the nucleus only before gastrulation
- Fusion of ChCh with the VP16 domain activator enhances the effects of wild-type ChCh

Upstream of Churchill

- 4 hr lag for induction
- Regulatory region of *ChCh* contains putative binding sites for 12 transcription factors
- Convergence of several pathways?