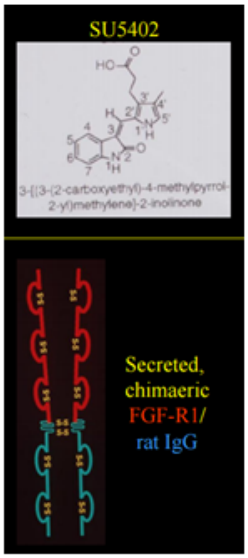
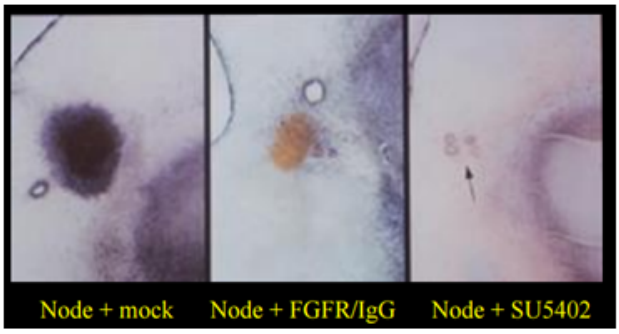


Two ways to block FGF (inside and outside cell):

1. The chemical SU5402 inhibits the FGF receptor from being phosphorylated.
2. Artificial version of FGF receptor made which is secreted. It acts outside the cell to bind and sequester FGF, preventing contact with the cell.



Both methods give the same results...

- Transplant control node and there is a strong ERNI and Sox3.
- Transplant node with chimeric receptor and get no ENRI and sox3 expression (brown stain is the node).
- Transplant node with beads soaked in SU5402 and get no ENRI and sox3 expression.

In absence of FGF signalling - no induced ERNI or sox3. In fact... if you leave in the various nodes for 13-16 hours, without FGF signalling you don't get the sox2 late marker expression neither. FGF is really crucial.

Find that ERNI and sox3 are actually expressed in events before gastrulation. And FGF8 is expressed in the layer underneath in the hypoblast.

Transplant hypoblast from really young embryo and into an older embryo. Get transient expression of several markers after 5 hours. V. early, so neural induction is occurring earlier than was originally though. The markers are lost by 12 hours, suggesting that FGF is crucial for initiation but is not sufficient to induce whole nervous system.

Found a gene (ERNI)... know when it is expressed... know what regulated it... WHAT DOES IT DO?? Series of experiments to try an establish this... (this is really difficult when you don't know anything about the protein).

Preview from Notesale.co.uk
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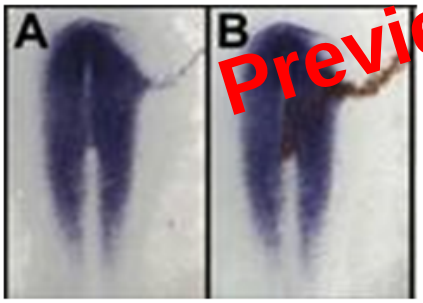
Looking at the structure... is coiled domain potentially used for binding? Does it bind to itself (dimerization)?
Use of report proteins and GFP to test for dimerization

Yes - ERNI does bind to itself via its coiled region.
But we know a protein forms a dimer, and via what part, then it can be used to make a dominant -ve.



Blue = sox2
Brown = electroporation of dominant -ve.

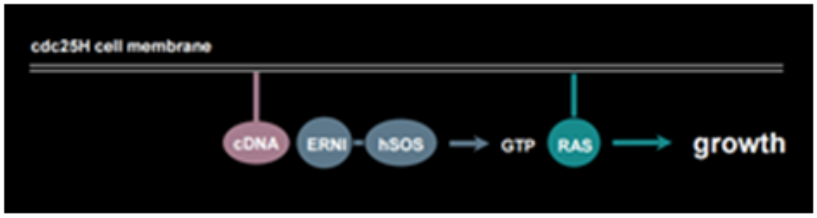
When ERNI is inhibited in the electroporation line, then sox2 gets expressed outside its normal domain of the neural plate.

Take the dimerization domain and remove everything else. This causes that region to bind all the endogenous protein, inhibiting it.

Point mutations are also useful, e.g. mutating the phosphorylation site to prevent phosphorylation.

So... Does ERNI act like an inhibitor of Sox2 expression? Therefore maybe the embryo has an endogenous inhibitor of ERNI in the right plate.

Time to find binding partners!
Cytotrap 2-hybrid system



This system uses a 'bait' - in this case the target protein ERNI.

- Using a library of cDNAs (from early chick np), grow yeast on a stunted growth medium and transfect with one library clone per yeast cell.
- The yeast will only grow if the 'bait' is bound by one of the proteins in the library.
- Bound 'bait' allows GTP activation of RAS allowing the yeast to grow.
- If not binding partners found then the yeast die.

Pick yeast that survive to form yeast colonies that have proteins which interact with the target protein. (have to sort out ones which are false positive, which are the majority).