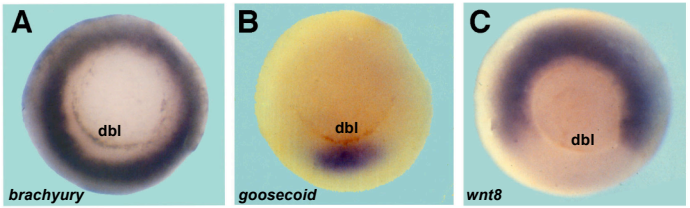
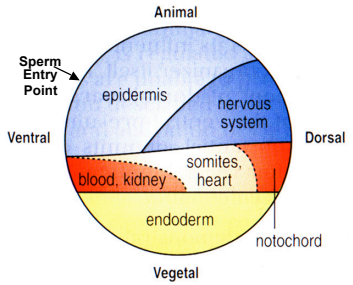
# Mesoderm Formation




Dr L Dale (B2010) Lecture 2

# Fate map of early gastrula




Animal hemisphere forms **ectoderm** (lacks VegT)

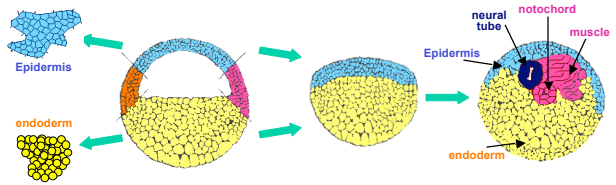
Vegetal hemisphere forms **endoderm** (requires VegT)

Marginal zone forms **mesoderm** (requires VegT in vegetal pole)

How do we explain this non-autonomous requirement for VegT in mesoderm development?

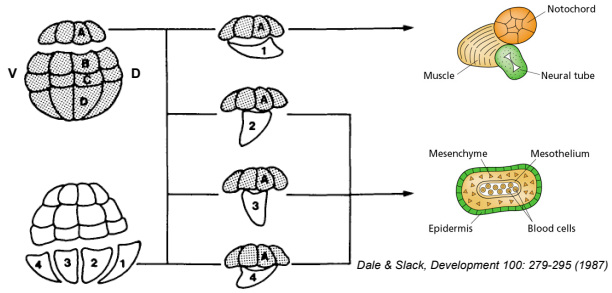
*Wolpert, Principles of Development*

# Mesoderm induction by the vegetal hemisphere




Pieter Nieuwkoop (1969), working with axolotl embryos, grafted blastula stage animal and vegetal poles together and found that the animal cap formed mesoderm. He showed that mesoderm was not formed if gastrula stage fragments were used and suggested that the mesoderm was induced by the vegetal hemisphere during blastula stages. These experiment were repeated on *Xenopus* embryos with identical results. It was subsequently shown that direct cell contact was not required, suggesting that a secreted signalling molecule is responsible.

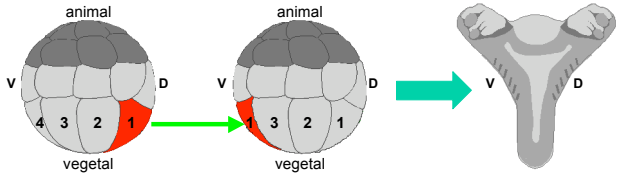
# Only two types of mesoderm are induced




*Dale & Slack, Development 100: 279-295 (1987)*

The animal (A) tier (8 blastomeres) was isolated at the 32-cell stage and recombined with a single blastomere from the vegetal (D) tier. Only the dorsal most blastomere (D1), induced a notochord (and large amounts of muscle) while all remaining blastomeres induced blood, mesenchyme and mesothelium (and in some cases small amounts of muscle). **Hence the D1 blastomere and its descendents have special inductive properties.**

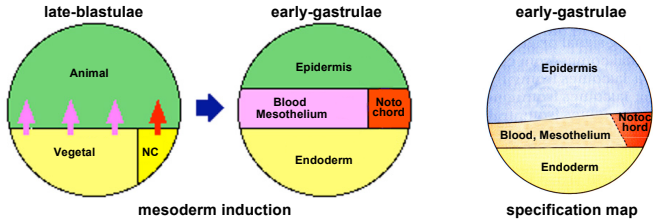
## The D1 blastomere can induce a second dorsal axis




*Gimlich & Gerhart, Dev Biol 104: 117-130 (1984)*

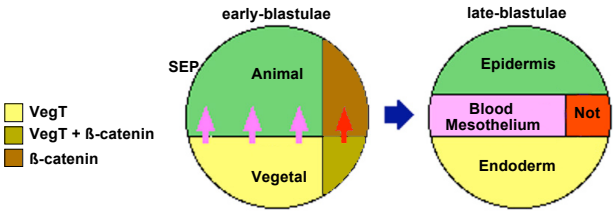
Grafting a single D1 blastomere into the ventral side of the 32-cell stage (replacing blastomere D4) induces a second dorsal axis, forming conjoined twins. The grafted blastomere only forms endoderm, all remaining tissues of the second dorsal axis are formed by the host and have therefore been induced by D1. No other vegetal blastomere can do this. Blastomere C1, directly above D1 will also induce a second dorsal axis when it replaces C4 (on the ventral side), but it forms the second notochord (see lecture 3 for explanation). Because of the special inductive properties of blastomere D1, it was named the "**Nieuwkoop Centre**" in honour of Pieter Nieuwkoop.

## Two mesoderm inducing signals?



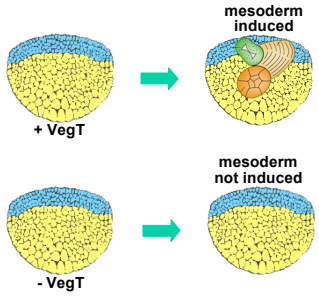

A signal from most of vegetal hemisphere induces ventral-type mesoderm in marginal zone, while a signal from the Nieuwkoop centre induce dorsal-type mesoderm. This simple model explains the specification map of early gastrulae, indicating that it is the result of mesoderm induction during blastula stages. The original model envisaged two independent signals but it was also recognized that different concentrations of a single signal could explain the results.

## Are mesoderm-inducing signals regulated by VegT and ß-catenin?




The ventral signal originates from vegetal cells that express VegT while the dorsal signal originates from the Nieuwkoop centre, which expresses both VegT and ß-catenin. Can this explain the different mesoderm inducing activities of these regions? ß-catenin is also expressed in the dorsal-animal hemisphere and may affect the competence of these cells to respond to mesoderm inducing signals.

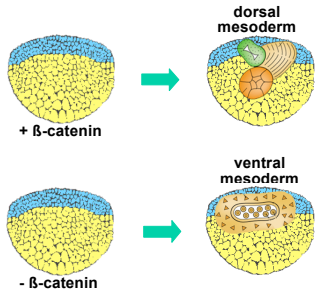
## VegT depleted vegetal poles do not induce mesoderm




Deplete maternal *VegT* mRNA using antisense oligonucleotides (see lecture 1), then remove vegetal pole (VP) and recombine with normal animal cap. Control VP induces mesoderm while *VegT* depleted VP does not. Thus, VegT is necessary for both dorsal and ventral mesoderm inducing activity of VP. This explains the lack of mesoderm in *VegT* depleted embryos (see lecture 1).

*Zhang et al., Cell 94: 515-524 (1998)*

## ß-catenin depleted vegetal poles do not induce dorsal mesoderm




Deplete maternal *ß-catenin* mRNA using antisense oligonucleotides (see lecture 1), then remove vegetal pole (VP) and recombine with normal animal cap. Control VP induces dorsal mesoderm while *ß-catenin* depleted VP induces ventral mesoderm. Thus, ß-catenin is necessary for dorsal, but not ventral, mesoderm inducing activity of VP. This explains the lack of dorsal mesoderm in *ß-catenin* depleted embryos (see lecture 1).

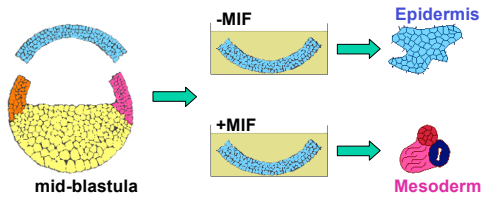
*Heasman et al., Cell 79: 791-803 (1994)*

## VegT and ß-catenin are required for mesoderm induction

**They are not secreted, so cannot be inducing mesoderm directly**

**They are transcription factors, so may activate expression of the inducing factor(s)**

## Animal cap assay for mesoderm-inducing factors




Isolate animal caps from mid-blastulae and incubate in buffered salt solution, adding candidate mesoderm inducing factors (MIF). Alternatively, animal caps can be isolated from embryos injected with mRNA encoding a putative MIF. The cap differentiates as epidermis if the factor has no activity and mesoderm if it does. This assay was first used by Smith (1987) to identify Activin, and Slack et al. (1987) to identify FGF2, as mesoderm inducing factors.

## Mesoderm Inducing Factors

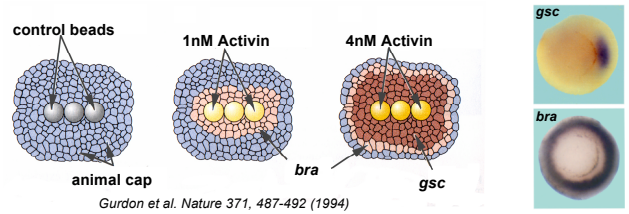
| MIF | Mesoderm Induced |
|---|---|
| Activin | Dorsal (high), Ventral (low) |
| BMPs | Ventral |
| Derrière | Dorsal (high), Ventral (low) |
| XNRs | Dorsal (high), Ventral (low) |
| Vg1 | Dorsal (high), Ventral (low) |

**All of the above are members of the transforming growth factor ß (TGFß) family of extracellular signalling molecules.**

| | |
|---|---|
| FGFs | Muscle (high), Ventral (low) |

**BMP = Bone Morphogenetic Protein, XNR = *Xenopus* Nodal-Related, FGF = Fibroblast Growth Factor, high = high concentration, low = low concentration**

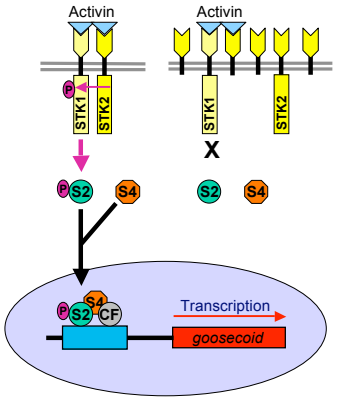
## Concentration-dependent induction of *brachyury* and *goosecoid* by Activin




*Gurdon et al. Nature 371, 487-492 (1994)*

Agarose beads were soaked in solutions of Activin and then sandwiched between two animal caps isolated from mid-blastulae. After a few hours Activin has diffused away from the beads creating a concentration gradient, with high concentrations close to the beads and low concentrations further away. 4nM Activin induces *goosecoid* (*gsc*) expression in cells adjacent to the beads and *brachyury* (*bra*) in cells further away. 1nM Activin is only sufficient to induce *brachyury* in cells adjacent to the bead. Thus cells respond to different concentrations of Activin by activating expression of different sets of genes, a dorsal (*goosecoid*) set at high concentrations and a ventral set (*brachyury*) at low concentrations.

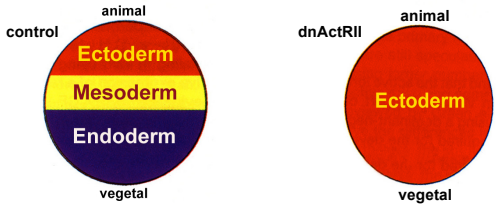
## dnActRII blocks TGF-ß signalling




**Activin** is a homodimer that binds to extra-cellular domain of both a **type I and a type II serine/threonine kinase receptor** (**STK**). This allows STK2 to **phosphorylate**, and activate, STK1. Active STK1 phosphorylates **Smad2** (**S2**), which then forms a complex with **Smad4** (**S4**) and moves into the nucleus. This complex recruits **cofactors** (**CF**) that allow transcription of target genes (e.g. ***gsc*** and ***bra***). A **dominant-negative type II Activin receptor** (**dnActRII**) was created by deleting the kinase domain. dnActRII can still bind Activin and if present at sufficiently high concentrations can out compete normal ActRII. These conditions can be easily achieved when mRNA for dnActRII is injected into *Xenopus* embryos. However, dnActRII is not specific and inhibits signalling by all members of the TGFß family

*Hemmati-Brivanlou & Melton, Nature 359: 609-614 (1992)*

## Dominant-negative ActRII "ectodermalises" *Xenopus* embryos




*Xenopus* embryos injected with dnActRII fail to gastrulate and analysis using molecular probes shows that only ectoderm has formed, both epidermis and neural tissue. Mesoderm and endoderm do not form, a phenotype similar to that of VegT depleted embryos (see lecture 1). Animal caps isolated from dnActRII expressing embryos do not form mesoderm in response to Activin (indeed any TGFß family member) but will form mesoderm in response to FGFs.

## The endogenous mesoderm inducing factor(s) must be localized to the vegetal pole of blastulae and activated by VegT and/or ß-catenin

# Mesoderm Inducing Factors

| MIF | Blastula Expression | |
|---|---|---|
| | Maternal | Zygotic |
| Activin | AP + VP | AP + VP |
| BMPs | AP + VP | AP + VP |
| Derrière | - | VP |
| XNRs | - | VP |
| Vg1 | VP | - |
| FGFs | AP | MZ |

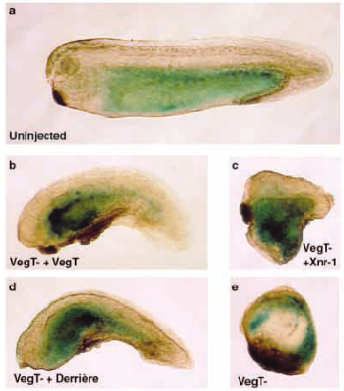
**AP = animal pole, VP = vegetal pole, MZ = marginal zone**

# VegT and ß-catenin activates transcription of *Xnr1*


*Hyde & Old, Development 127: 1221-1229 (2000)*

The promoter of the *Xenopus nodal-related 1* (*Xnr1*) gene contains DNA sequences bound by VegT and ß-catenin, which form transcriptional complexes that activate transcription. VegT alone only promotes low level transcription while VegT + ß-catenin promotes high level transcription. VegT has also been shown to bind to the promoters of *Xnr5* and *derriere*, activating transcription. These genes are not expressed in the absence of VegT.

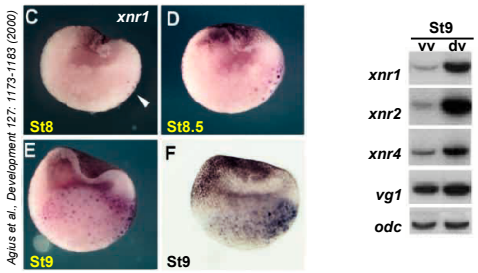
# *VegT* depleted embryos are rescued by injection of *Xnr-1* and *Derrière*




As described in lecture 1, depletion of maternal *VegT* mRNA produces embryos with no endoderm of mesoderm (fig e), a phenotype that can be rescued by injecting embryos *VegT* mRNA (fig b). The phenotype can also be partially rescued by injecting embryos with mRNA for either *Xnr1* (fig c) or *Derrière* (fig d). This suggests that these VegT targets are key the function of VegT during early development. Note that Xnr1 rescues head development while Derrière rescues abdomen and tail development. The experiment whereby both mRNAs were injected into the same *VegT*- embryo was either not done or not reported. Perhaps it would they would give more complete rescue than either mRNA alone!

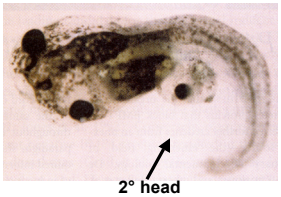
*Kofron et al., Development 126: 5759-5770 (1999)*

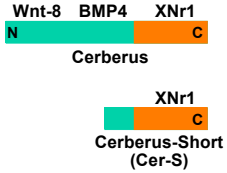
# *Nodal-related* genes are activated in the vegetal half of late-blastulae




*Xenopus nodal-related 1* (*xnr1*) is first detected, using *in situ* hybridization, in the Nieuwkoop centre of mid-blastulae. The signal strengthens during the next few hours and spreads throughout the vegetal hemisphere, but is always more intense in the Nieuwkoop centre. Using PCR we can see that *xnr2* and *xnr4* are also enriched in dorsal-vegetal (dv) blastomeres relative the ventral-vegetal (vv) blastomeres. Transcripts for *vg1* and *ornithine decarboxylase* (*odc*) are uniformly distributed in the vegetal hemisphere

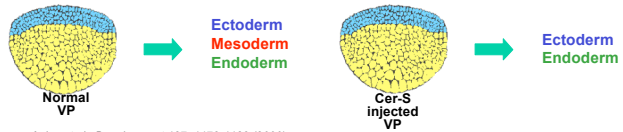
## Cerberus is a secreted inhibitory binding protein for Wnt-8, BMP4, & XNr1




Cerberus (named after the three-headed dog of Greek mythology) is a secreted protein that has the remarkable ability to bind members of three families of secreted signalling molecules; the Wnt, BMP and Nodal families. When *Cerberus* mRNA is injected into ventral blastomeres a fully formed second head is formed (see lecture 3). A C-terminal fragment (Cer-S) was generated that was found to specifically inhibit members of the Nodal family.

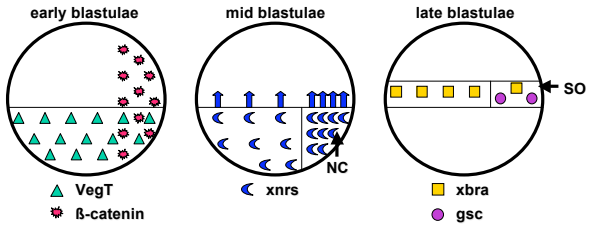
## Mesoderm-inducing signals are blocked by Cer-S




*Agius et al., Development 127: 1173-1183 (2000)*

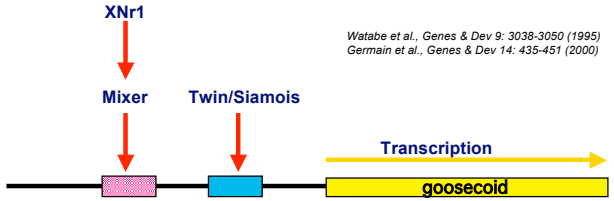
Cerberus (Cer-S) was injected into *Xenopus* embryos and vegetal poles (VP) isolated and grafted onto animal caps from normal embryos. Whereas control VPs induced mesoderm those from Cer-S injected embryos did not. This suggests that a Nodal-related signal, bound by Cer-S, is necessary for mesoderm induction by the vegetal pole. **Nodal-related signals are therefore both necessary and sufficient for mesoderm induction.**

## Model for mesoderm-induction




*VegT* is localized to the vegetal hemisphere during oogenesis and ß-catenin is enriched on the future dorsal side of the embryo as a result of cortical rotation during the first cell cycle. Low level transcription of *XNrs* is activated in the vegetal hemisphere by VegT and high level transcription is activated in the Nieuwkoop centre (NC) by the combined activities of VegT and ß-catenin. Low levels of XNrs induce *brachyury* (*xbra*) expression throughout the marginal zone, while high levels of XNrs induce *goosecoid* (*gsc*) expression is the dorsal marginal zone. This is also known as the Spemann Organizer (SO) - see lecture 3

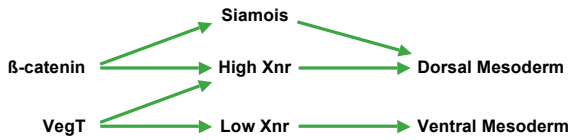
## Both TGF-ß signals and ß-catenin targets are required for *Xgsc* expression




*Watabe et al., Genes & Dev 9: 3038-3050 (1995)*
*Germain et al., Genes & Dev 14: 435-451 (2000)*

Studies on the *goosecoid* promoter have shown that the transcription factor Mixer is responsible for activating goosecoid transcription in response to high levels of TGFß signals. Efficient transcription of *goosecoid* also requires the transcription factors Twin and/or Siamois (two highly homologous proteins), which bind to DNA sequences in the *goosecoid* promoter. Transcription of *twin* and *siamois* is activated directly by ß-catenin (they do not require VegT) and transcripts are localized to the dorsal marginal zone of late blastulae and early gastrulae. **This demonstrates that a combination of ß-catenin and high XNr signalling is required for the formation of the Spemann Organizer.**

## Model for mesoderm-induction in *Xenopus* blastulae




Summary of the role of that maternal transcription factors VegT and ß-catenin in mesoderm formation in amphibian blastulae.

# THE END