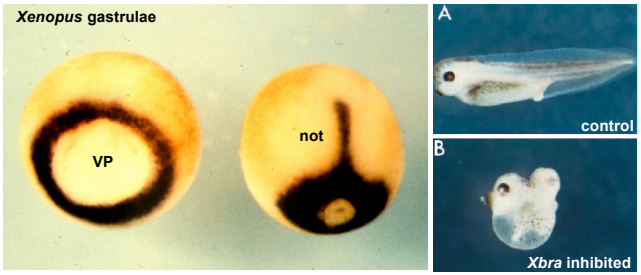
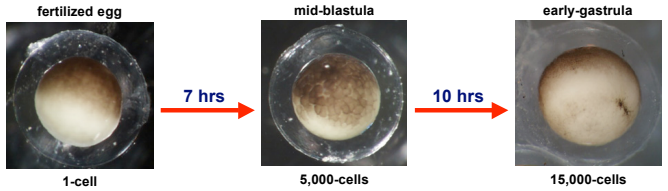
# Maternal Control of Germ-Layer Formation in *Xenopus*




*Xenopus brachyury* (*Xbra*) is expressed in the posterior mesoderm and notochord of gastrulae. The development of these tissues is severely disrupted when *Xbra* is inhibited.

Dr Leslie Dale (B2010) Lecture 1

# The zygotic genome is activated at the mid-blastula transition



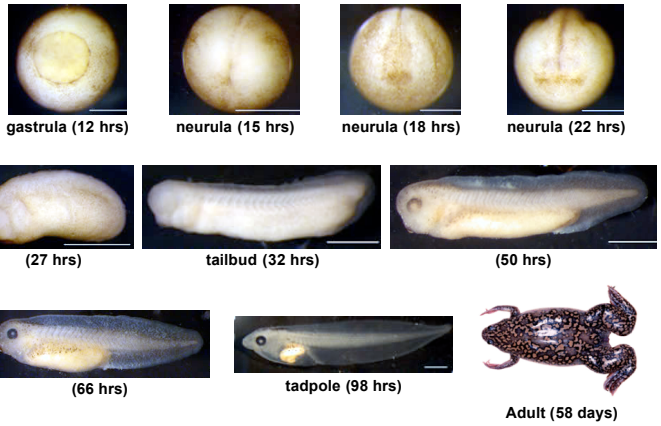

rapid, synchronous, cell divisions
G1 and G2 absent
zygotic genome silent

slow, asynchronous, cell divisions
G1 and G2 present
zygotic genome active

dependent on maternal mRNA

dependent on zygotic mRNA

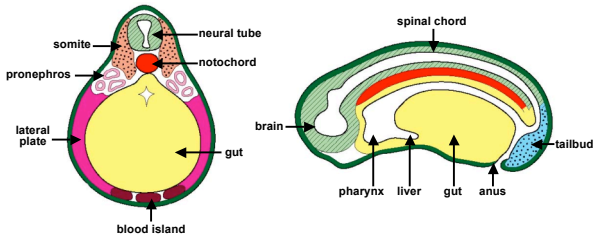
# *Xenopus* development




times at 21°C

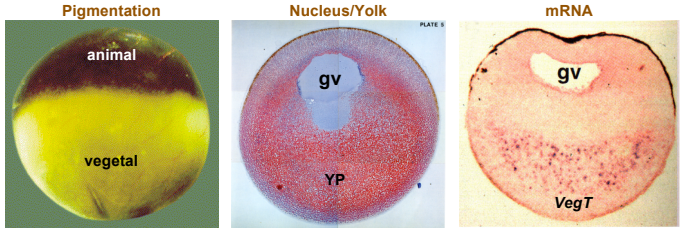
# The amphibian body plan

green = ectoderm
red = mesoderm
yellow = endoderm



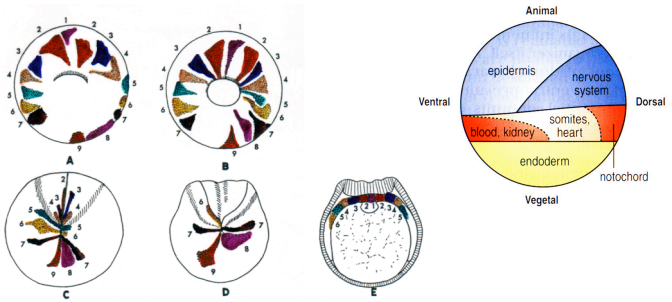

*Slack, Essential Developmental Biology*

## Animal-vegetal polarity in *Xenopus* oocytes



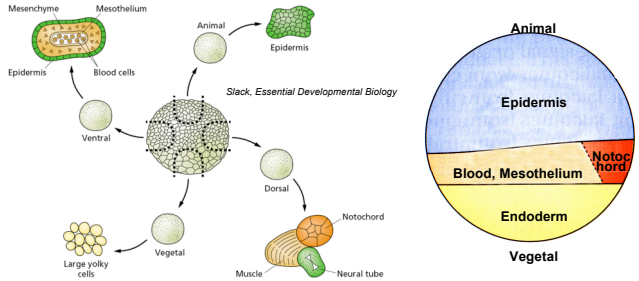

Animal-vegetal polarity is established during oogenesis. The animal hemisphere is darkly pigmented and contains the germinal vesicle (gv), the female haploid nucleus. The vegetal hemisphere is lightly pigmented and contains the largest yolk platelets (YP). A small number of maternal mRNAs are localized to the vegetal hemisphere during oogenesis, including *Vg1*, *VegT* and *Wnt11.* The egg is radially symmetric around this axis.

## Fate mapping amphibian embryos




Fate maps tell us what cells will become at a later stage of development and are an important tool for embryologists. The fate map above was generated by laballing small populations of cells at the early gastrula stage and looking where they were located after the completion of neurulation. It is important to note that the fate map does not tell you that cells are already committed to forming these tissues.

## Specification map of early gastrula

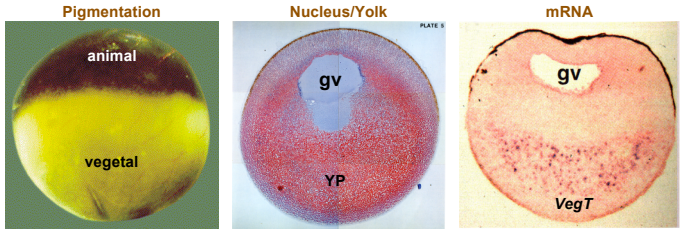






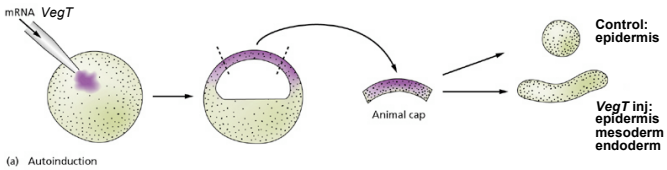
*Xenopus* embryos are packed full of yolk, which provides all their nutritional needs until the tadpole can feed for itself. This allows us to isolate fragments of the embryo and culture them in neutral media that do not affect their development. In this way we can find out how cells are specified at a particular stage of development. Above (right) is a specification map that applies to both blastulae and early gastrulae. Note how the future nervous system forms epidermis in this assay, demonstrating that it is not yet specified.

## Animal-vegetal polarity in *Xenopus* oocytes



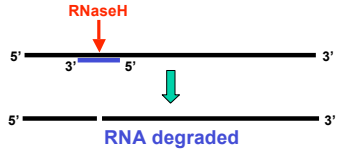

Animal-vegetal polarity is established during oogenesis. The animal hemisphere is darkly pigmented and contains the germinal vesicle (gv), the female haploid nucleus. The vegetal hemisphere is lightly pigmented and contains the largest yolk platelets (YP). **A small number of maternal mRNAs are localized to the vegetal hemisphere during oogenesis, including *Vg1*, *VegT* and *Wnt11.*** The egg is radially symmetric around this axis.

## VegT is Sufficient for Endoderm and Mesoderm Formation

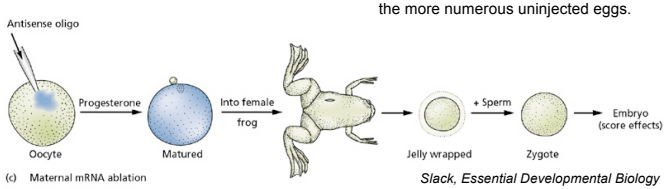



*Xenopus* eggs were injected in the animal hemisphere with *VegT* mRNA and animal caps isolated from blastulae. Control caps only form epidermis, while *VegT* injected caps also form endoderm and mesoderm. This demonstrates that VegT is sufficient for the formation of both of these germ layers.

## Antisense oligonucleotides deplete maternal mRNA




Oligonucleotides (about 20-25 deoxy nucleotides in length) can be injected into *Xenopus* oocytes where they hybridize to the target mRNA. Hybridized mRNA is then degraded by the enzyme RNase H. Injected oocytes, marked with a vital dye, are transferred into a surrogate female, who lays them with a jelly coat that is required for fertilization. The dye allows injected eggs to be detected from amongst the more numerous uninjected eggs.




*Slack, Essential Developmental Biology*

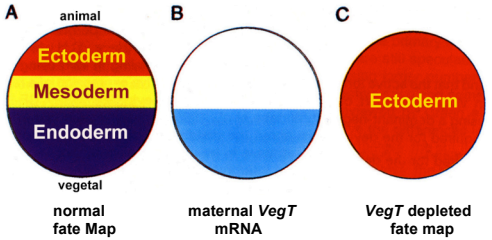
## Depletion of maternal *VegT* disrupts normal development

Injection of low (L) concentrations of oligonucleotide generates embryos with no endoderm but nearly normal mesoderm.

Injection of high (H) concentrations of oligonucleotide generates embryos with no endoderm and practically no mesoderm.

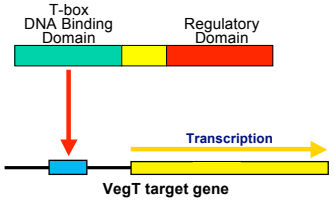

*Zhang et al., Cell 94: 515-524 (1998)*
*Kofron et al., Development 126: 5759-5770 (1999)*
*Xanthos et al., Development 128: 167-180 (2001)*

## Depletion of maternal *VegT* disrupts mesoderm and endoderm formation




*VegT* mRNA is localized to the prospective endoderm, yet both endoderm and mesoderm are disrupted in *VegT* depleted embryos!

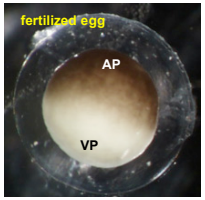
## VegT is a T-box transcription factor



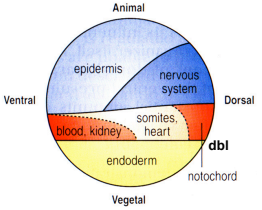

T-box genes are a large family of transcriptional regulators unified by a conserved DNA binding domain. Named after the murine *T-gene* (now known as *brachyury*), which was first identified as a mutation with a short, blunted tail. They also contain a C-terminal regulatory domain required for the formation of active transcriptional complexes

**Target genes for VegT, expressed in vegetal hemisphere of blastulae:**
**Transcription Factors: *bix1*, *bix2*, *bix3*, *bix4*, *mix1*, *mix2*, *sox17***
**Signalling Molecules: *xnr1*, *xnr2*, *xnr4*, *xnr5*, *xnr6*, *derrière***

## Polarity in *Xenopus* embryos

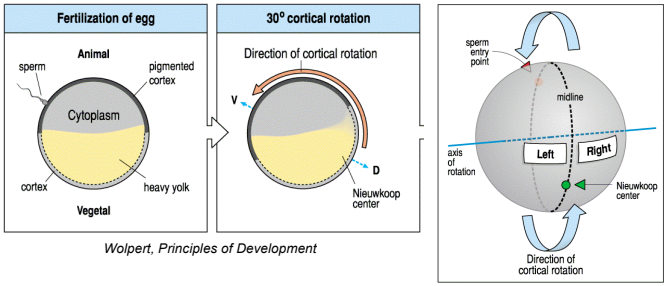







The newly laid egg has clearly animal-vegetal polarity (AP-VP), which was established during oogenesis. However, it is radially symmetric around this axis. Ten hours after fertilization a darkly pigmented arc appears in the future dorsal quadrant of the vegetal hemisphere, the dorsal blastopore lip (dbl). The dorsal mesoderm (notochord) and ectoderm (nervous system) form above this lip while ventral tissues form on the opposite side. **How did this dorsal-ventral axis form?**

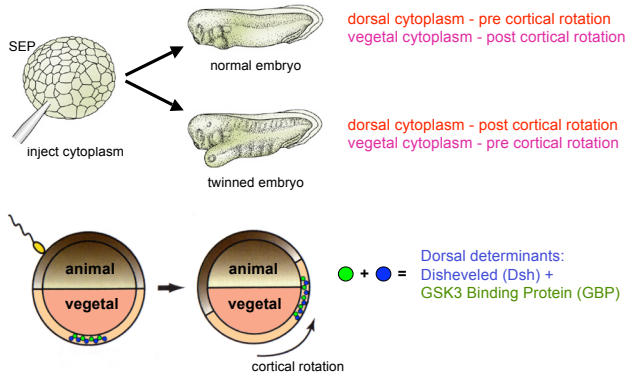
## Cortical rotation breaks radial symmetry



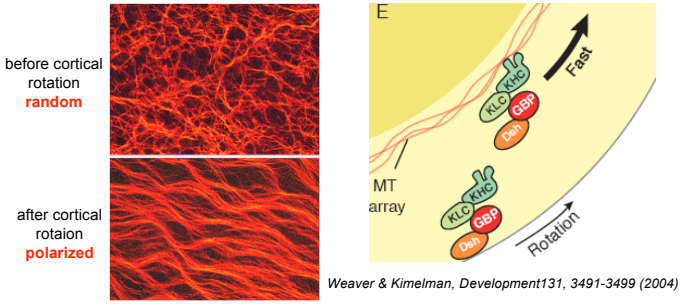

*Wolpert, Principles of Development*

The meridian of maximal rotation passes through animal pole and SEP. It defines the plane of first cleavage, the dorsal-ventral axis and the left-right axis

## Dorsal determinants move from the vegetal pole towards the dorsal marginal zone



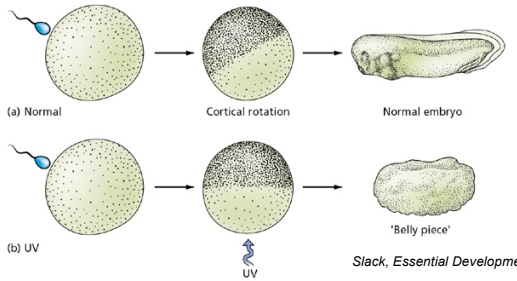
## Cortical rotation and microtubules (MT)




*Weaver & Kimelman, Development131, 3491-3499 (2004)*

MT form in "shear zone" between cortex and deep cytoplasm in the vegetal hemisphere, with the sperm centrosome at the minus-end organizing centre. Dsh & GBP couple to the kinesin light chain (KLC) and move along the MT, from minus to plus end, using the kinesin heavy chain (KHC) motor. Movement (60-90º) is greater than the angle of rotation (30º). MT depolymerizes towards end of first cell cycle releasing Dsh & GBP.

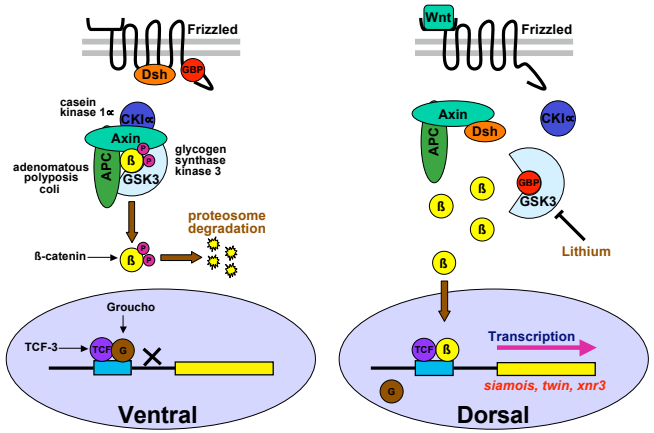
## UV-irradiating the vegetal pole disrupts MT and ventralizes *Xenopus* embryos



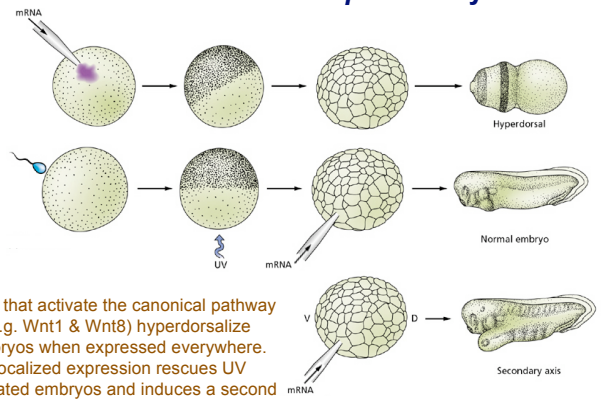

*Slack, Essential Developmental Biology*

UV-light blocks MT formation, during cortical rotation, when vegetal pole is Irradiated 25-30 mins after fertilization, causing loss of dorsal tissues. Embryos form an epidermal bag containing excess blood and mesothelium, and undifferentiated endoderm

## Dsh and GBP are components of the canonical Wnt signalling pathway



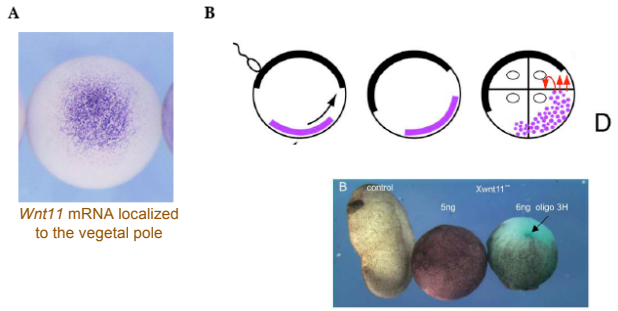

## Wnt dorsalizes *Xenopus* embryos




Wnts that activate the canonical pathway (e.g. Wnt1 & Wnt8) hyperdorsalize embryos when expressed everywhere. Localized expression rescues UV irradiated embryos and induces a second dorsal axis, if expressed ventrally. Wnts mimick the dorsal determinant.

*Slack, Essential Developmental Biology*

## *wnt11* mRNA is enriched on the dorsal side of the embryo following cortical rotation and is required for dorsal development

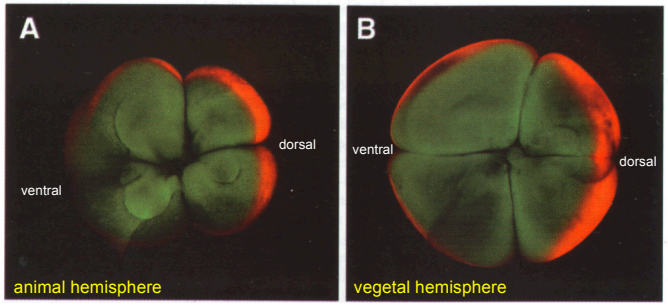



*Wnt11* mRNA localized to the vegetal pole

Depletion of maternal *wnt11* mRNA ventralizes *Xenopus* embryos

*Tao et al. Cell 120: 857-871 (2005)*

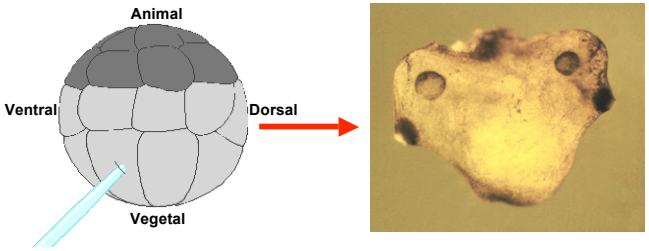
## ß-catenin is enriched on the dorsal side of the 8-cell *Xenopus* embryo




**Larabell et al., J. Cell Biol. 136: 1123-1136 (1997)**

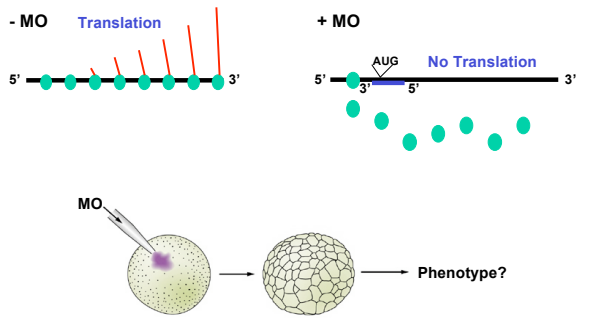
Confocal microscope images of ß-catenin protein (red signal) in 8-cell Xenopus embryo, as detected by specific antibodies. ß-catenin is enriched in the dorsal quadrant and subsequently enters dorsal nuclei of both hemispheres

## Ventral injection of *ß-catenin* mRNA induces a second dorsal axis

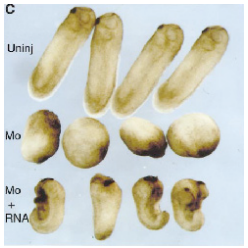



Inject a single ventral-vegetal blastomere, at the 32-cell stage, with *ß-catenin* mRNA. Embryo subsequently forms a second dorsal axis (conjoined twins) with a fully formed head. Ventral-posterior structures (tail & blood) are greatly reduced. ß-catenin has respecified ventral cells as dorsal.

## Antisense morpholino-oligonucleotides (MO) block translation of mRNAs



## ß-catenin depleted embryos lack a dorsal axis




Normal tadpole

Ventralized - no dorsal mesoderm

Injection of *ß-catenin* mRNA - dorsal mesoderm restored

Embryos depleted of maternal *ß-catenin* mRNA, by injection of specific antisense oligonucleotides, are ventralized, lacking all dorsal structures. They are identical to UV-irradiated embryos. Dorsal structures can be rescued by injecting *ß-catenin* mRNA at early cleavage stages.

*Heasman et al., Dev Biol 222: 124-134 (2000)*

## Conclusions

1. **Maternal transcription factors establish both the animal-vegetal and dorsal-ventral axes**
2. **VegT is localized to the vegetal hemisphere and is required for the development of both the endoderm (autonomous) and mesoderm (non-autonomous).**
3. **ß-catenin is enriched on the dorsal side of the embryo, following cortical rotation, and is required for the development of dorsal tissues**
4. **Wnt11 is localized to the dorsal side of the embryo, following cortical rotation, and is responsible for stabilizing ß-catenin in this region.**