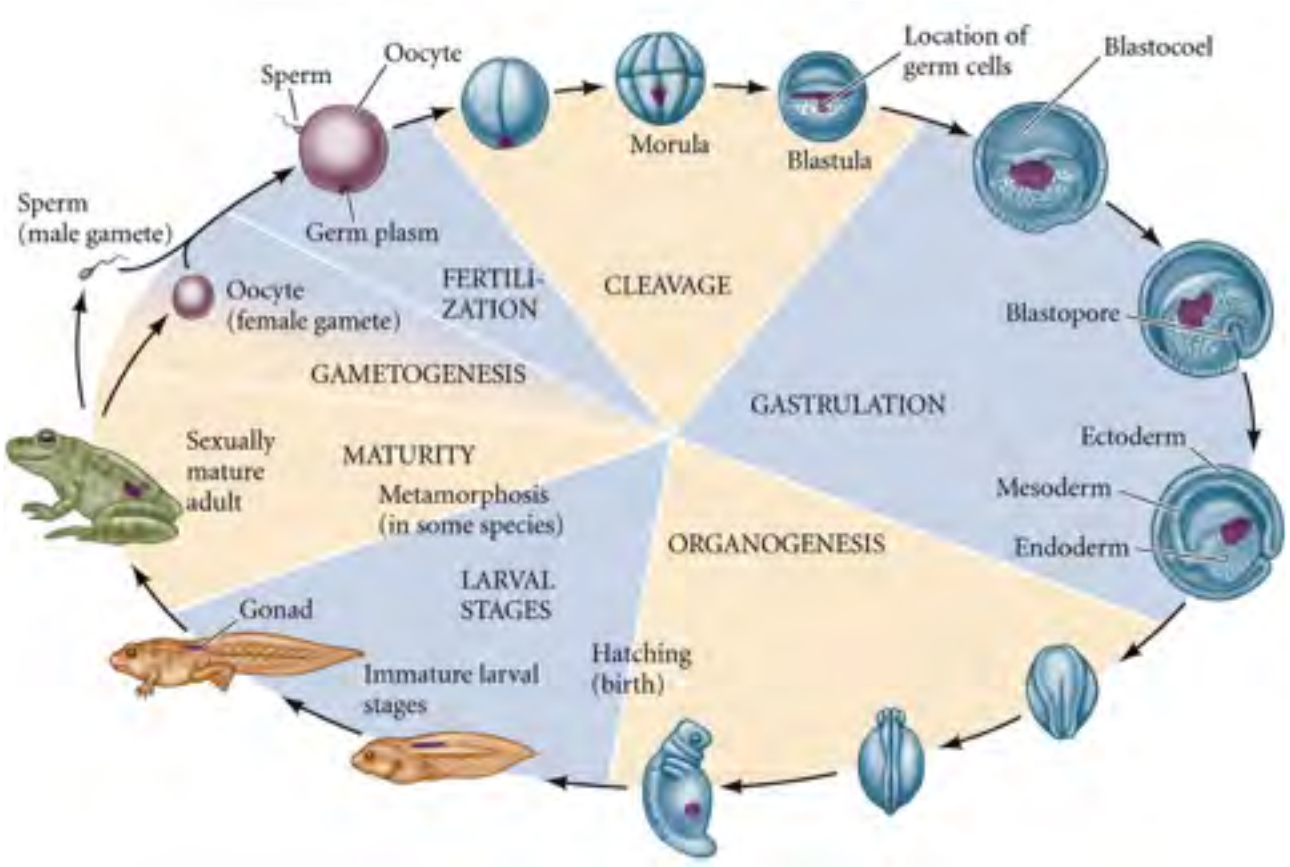
Lecture 2- The frog, *Xenopus laevis*, the African clawed toad



- Fertilisation- 1 cell
- Undergoes cleavage
- Generate a large number of cells
- Forms blastula
- 6 hours after fertilisation-10000 cells
- Rapid cell division
- Cell division stops
- Cells move in respect to each other- gastrulation
- 10 hours
- Move in coordinated fashion to generate embryo
- Organogensis- generate different organs that make tadpole

Blastula- early stage embryo consisting of a sphere of cells surrounding inner fluid filled cavity blastocoel in **frog and fish.**

Blastocyst- **mammalian** blastula. the blastocoel is expanded and the inner cell mass is positioned on one side of the ring of trophoblast cells.

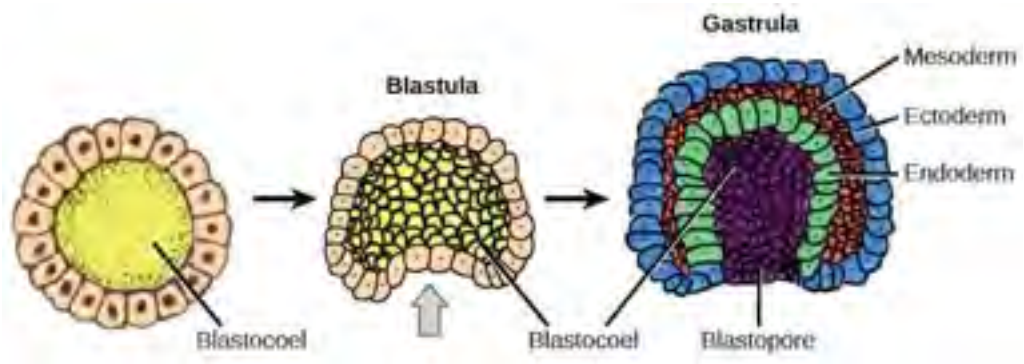
Blastocoel- fluid filled cavity that forms an animal hemisphere of early amphibian and echinoderm embryos, or between the epiblast and hypoblast of avian, reptilian and mammalian blastoderm-stage embryos.

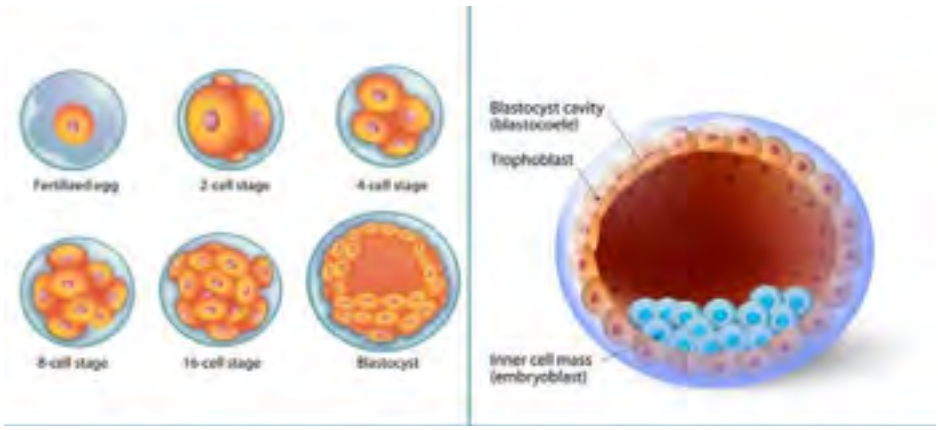
Blastopore- the invagination point where gastrulation begins. In deuterostomes this marks the site of the anus. In protostomes this marks the site of the mouth.

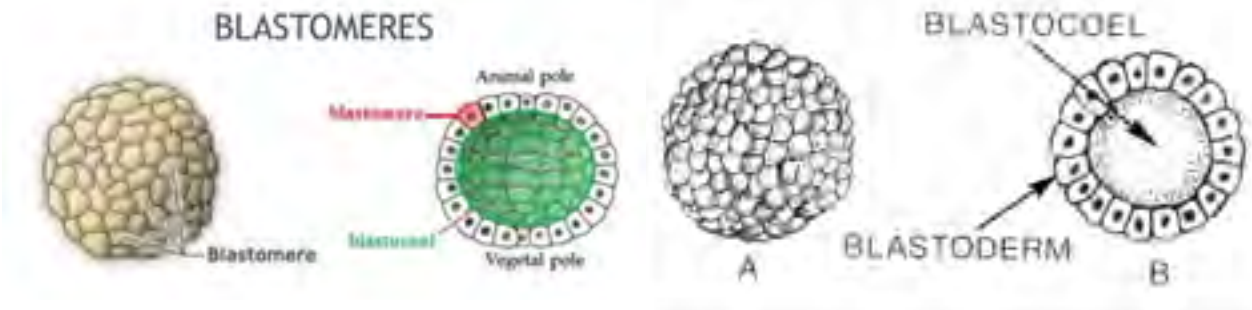
Blastoderm- single layer of embryonic epithelial tissue that makes up the blastula.

Blastodisc - small region at the animal pole of the telolecithal eggs of **fish** and **chicks**, containing the yolk free cytoplasm where cleavage can occur and that gives rise to the embryo. Following cleavage the blastodisc becomes the blastoderm.

Blastomere- a cleavage-stage cell resulting from mitosis.

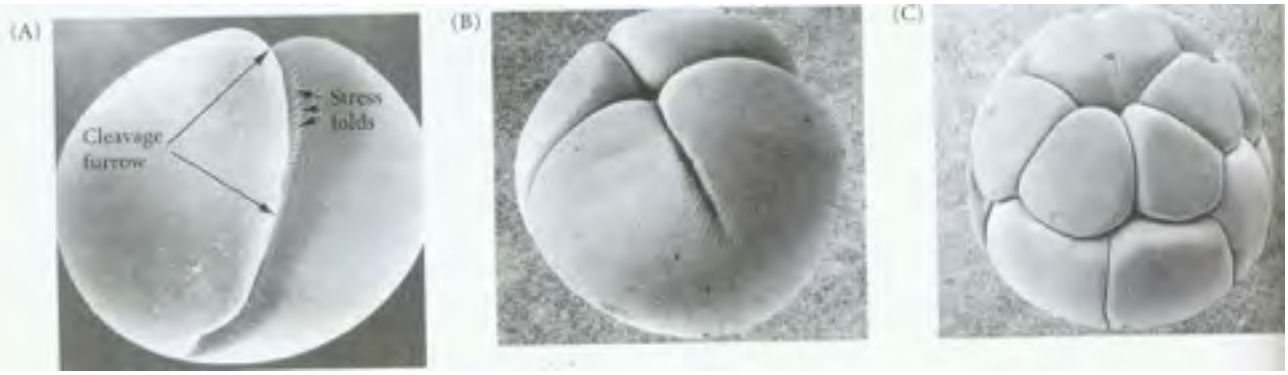




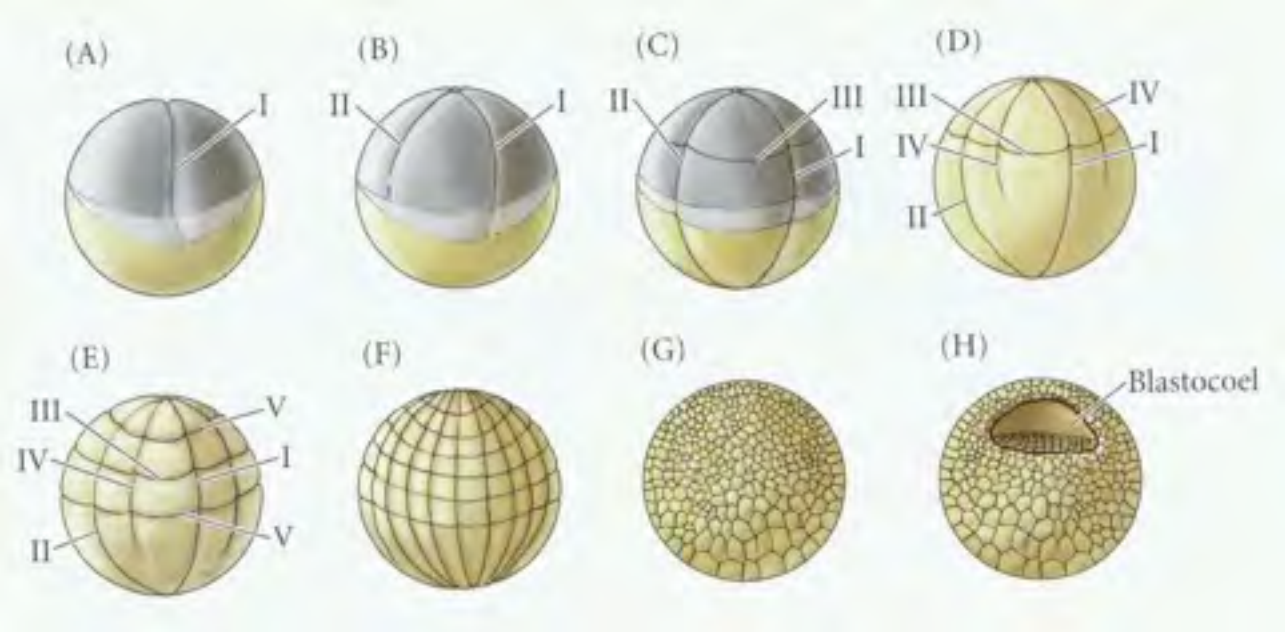


Scanning electron micrographs of early *Xenopus* embryos

Cleavage furrow- generate more cells



Positions of initial cleavage furrows



(A)

- Grey cresent- top of figure- animal pole
- Bottom- vegetal pole- primary access down through egg- animal to vegetal
- Yolk more concentrated at vegetal pole
- Cell division starts from animal pole
- Large cells take a while for cleavage furrow to pass through whole single cell to generate 2 cells
- Starts with animal pole
- Yolk in vegetal pole inhibits cytokinesis - cell division

(B)

- Cell division occurs at right angles to first cell division from animal pole
- 2nd cell division starts before 1st finished
- 3rd cell division takes place- equatorially
- Right angle to first 2 cell divisions
- Divide embryo across
- Displaced towards animal pole due to inhibition by yolk
- Animal pole cells are smaller than vegetal cells
- Subsequent cells divisions right angles to divisions
- Carry on rapidly

(F)

- Blastomeres dividing
- Large number of cells
- Gradient in the size of the cells but divide at same rate
- Smaller- animal pole
- Larger- vegetal pole

(H)

- 128 cell stage- Fluid filled cavity— blastocoel forms
  - Functions: permit cell migration during gastrulation and prevent cells underneath interacting prematurely with cells above it
- All cells are known as blastomeres in blastula
- Gradient of cell sizes
- Cleavage burrows right angles to each other- large cell- large amount DNA replicated
- Cell divisions are 30 mins apart
- Bacteria- smaller cells- smaller genome-20 mins cells division
- Rapid rate of cell division

SOCRATIVE

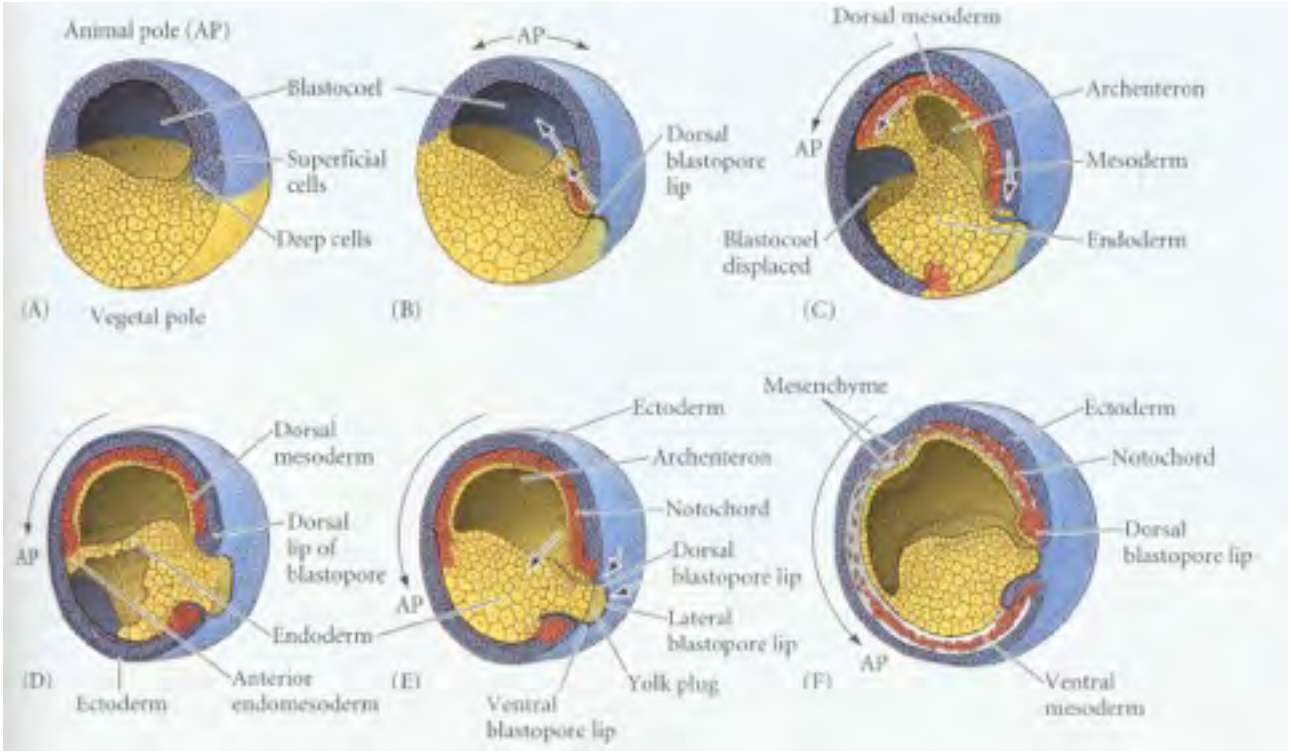
Diameter of zygote- 2mm

Frog is a toad

Diameter of blastula after cleavage - 2mm - no growth from zygote to blastula- single cell that is organised- development not growth

20,000 genes in human genome

Gastrulation



Reorganisation of cells

(A,B)

Early gastrulation

- Blastula animal cells are small than vegetal pole
- Cells fold into interior into the blastocoel
- Move in a coordinated pattern
- Important in development
- Fold in from dorsal surface- back of the animal- opposite point of sperm entry
- Generate dorsal blaspheme lip- cell fold over into interior- this is where organiser is
- Organiser- coordinates process- tells cells to fold and where they will be in embryo and what they will develop into

- Colour code
- Different germ layers
- Blue- ectoderm- outside germ layer
- Yellow- endoderm- inside germ layer- lines the gut
- Red- mesoderm- between ectoderm and endoderm

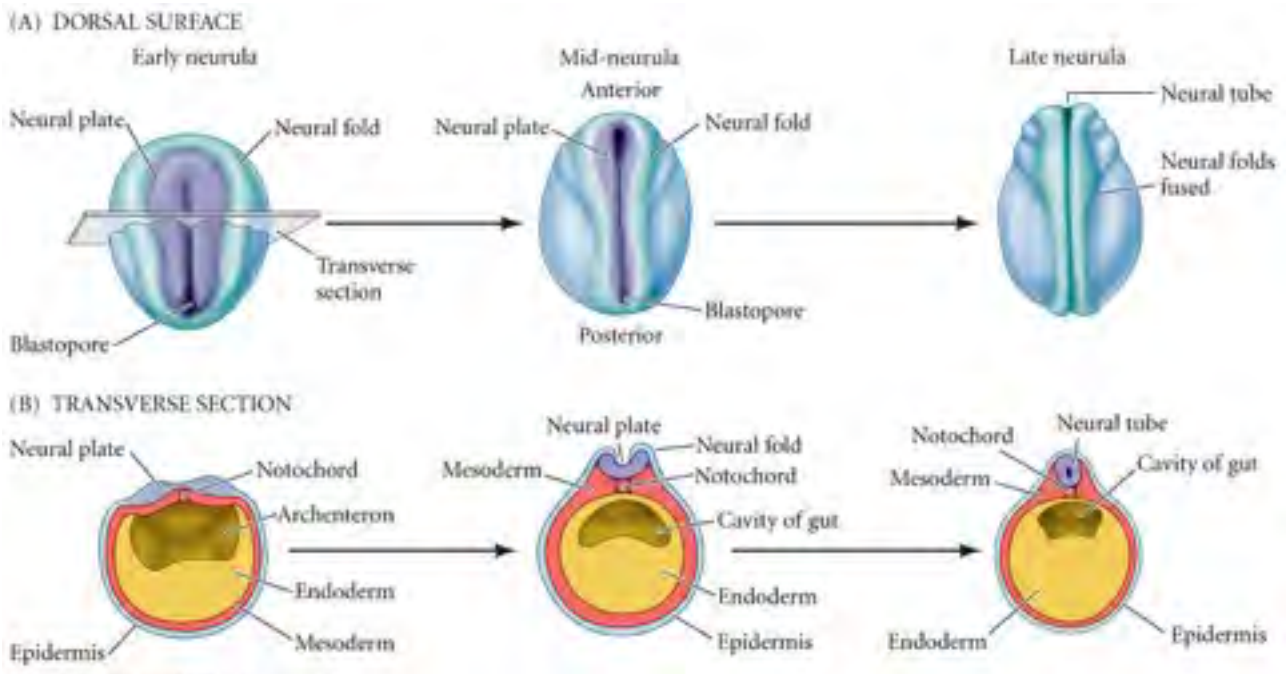
- Animal pole cells- ectoderm
- Vegetal pole cells - endoderm

- Cells fold into interior
- Another cavity forms- archenteron (primitve gut)- form the lumen of the gut
- Blastocoel is obliterated
- Embryo is surrounded by ectoderm, endoderm has been internalised and mesoderm cells are between the 2 germ layers

(F)

- Opening of fluid filled cavity to the outside - anus of animal
- Mouth opposite end of gut
- Form on opposite side
- Endoderm comes into contact with ectoderm
- Single to each other
- This is where the mouth forms
- Mouth forms secondary
- Anus forms first
- Frog and humans are deuterostomes- anus first, mouth second

<u>Neurulation</u>



(A)

- Dorsal surface
- Anterior- top

- Posterior- tail/bottom

- Neurulation forms central nervous system
- Cells on dorsal surface form neural plate
- Neural plate edges thicken and move upwards to form neural folds
- u shaped neural groove appears in centre of the plate
- neural folds migrate towards midline of embryo fusing to form neural tube below ectoderm
- Cells fold into the interior to form central nervous system

(B)
- Cross section
- Ectoderm on outside
- Endoderm on inside
- Mesoderm between
- Circle - rod of mesdormernal tissues- runs under surface of the back of the animal
- Notochord- prove signal to overlying ectoderm telling it to be the nervous system
- Ectoderm thickens and buckles inwards
- folds up and forms the neural tube
- Ectoderm seals over tube

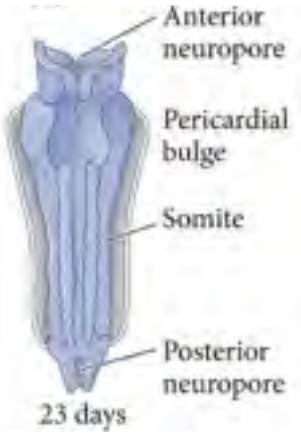
- Notochord provides signal
- Sonic hedgehog signal - telling overlying ectoderm to form nervous system

- Neurala zips up from anterior to posterior
- Ectoderm seals over the top
- There are ends on the tube that aren't sealed
- Difficult to achieve
- Sometimes don't seal over- if they don't- nervous tissue exposed to outside
- Nervous tissue will degenerate

<u>Neuropore closure failure</u>

- Failure to close causes problems
- Posterior neuropore doesn't close over- spina bifida
- Anterior neuropore doesn't close over- anencephaly

Somites- blocks of mesoderm repeating down through vertebrate body giving rise to repeated structures in our body

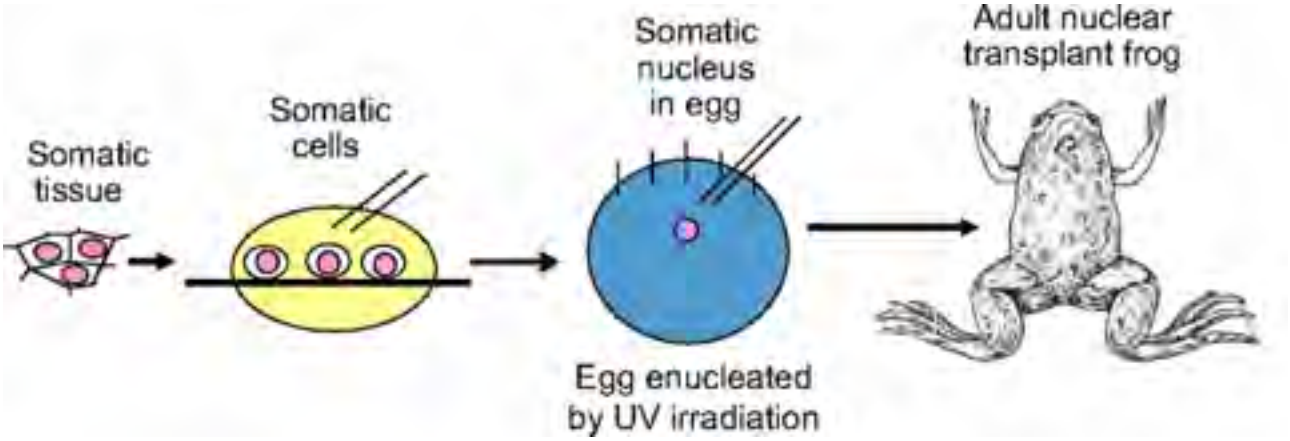


Xenopus experiments

Xenopus laevis cleavage

- A temperature gradient across the embryo results in variation of cell division rates across the embryo.
- Cells divide autonomously (no cell-to-cell coupling.)- at own rate- no communication
- Despite manipulation embryos grow fine - adjust for the consequences and are viable- shows way in which embryo develops- communication and control in developmental process
- Despite disturbances

Vertebrate Cloning



- King in 1952 with blastula stage nuclei in *Rana pipiens*
- Take blastula stage nuclei
- Isolate nuclei from blastula stage cells- blastomeres
- Put them into enucleated oocyte
- Allow to develop

Gurdon 1962

Experiment:

- Destroyed the nuclei of frog (Xenopus laevis) eggs by exposing the eggs to UV light. Then then transplanted the nuclei from cells of frogembryos and tadpoles into the enucleated eggs.

Results:

- When a transplanted nuclei came from an early embryo, whose cells are relatively undifferentiated, most of the recipients eggs developed into tadpoles.
- But when the nuclei came from the fully differentiated intestinal cells of a tadpole, fewer than 2% of the eggs developed into normal tadpoles, and most of the embryos stopped developing at a much earlier stage.

Conclusions:

- Demonstrated the nucleus of fully differentiated cell was capable to correcting the development of cells in organism
- Fully differentiated cells had genetic instructions for development of cells

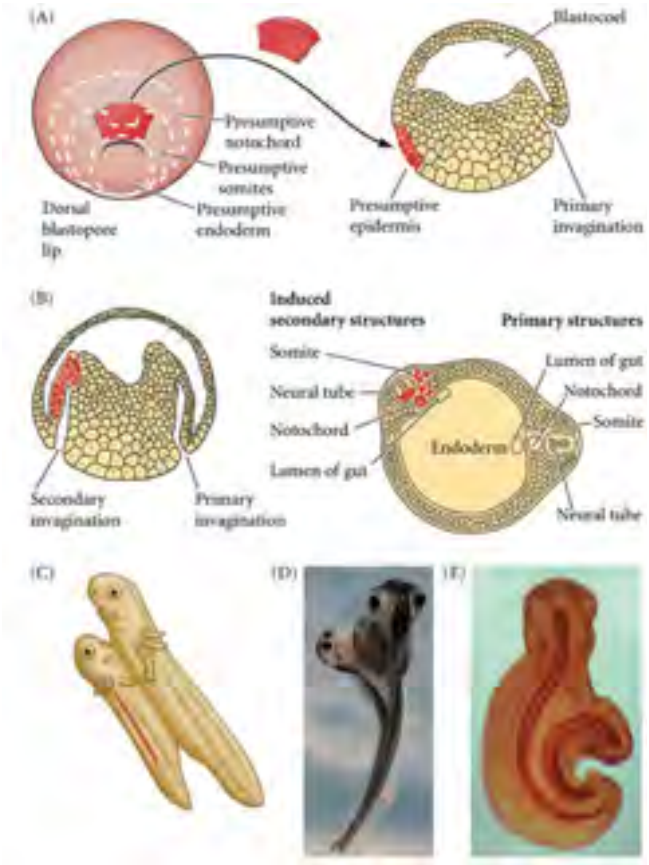
Highly regulative development

Demonstrates organisner

Speemann and Mangold

Original experiment- not on xenophus but in other amphibian

- *Experiment:*
  Transplanted a piece of the dorsal lip from a pigmented newt gastrula to the ventral side of a non-pigmented newt gastrula to investigate the induced ability of the dorsal lip.



Results:

- The recipient embryo formed a second notochord and neural tube in the region of the transplant, and eventually most of a second embryo developed.
- Examination of the interior of the double embryo revealed that the secondary structures were formed partly, but not wholly, from recipient tissue.
- Therefore, two sites of gastrulation and neurulation occurred

Conclusions:

- This forms second site of gastrulation
- Dark red region- organiser
- forms a 2nd archenteron, then 2nd embryonic axis
- both donor and host tissue have neural tube, notochord and somites
- Conjoined tadpoles
- Graft cells (red)- organises cells around it to form 2nd embryo- provide signals for gastrulation- cascade of signalling events
- Similar signals- humans and frogs

Xenopus embryos

Embryos are:

- Large
- Accessible- can follow fertilisation process easily externally
- Robust - can manipulate and they recover and continue developing
- Well-studied- a to of information collected on them to advance further studies

- Xenopus is important for identifying signalling molecules
- Chordin- produced by the organiser to coordinate gastrulation event
- Genetic approach important for animal development
- *Xenopus laevis* is an allotetraploid as a result of an evolutionarily recent hybridisation
- Species farmed as a result of evolutionary reason- fusion event of 2 species coming together to form new species
- Consequence- 2 related species- many genes are present in multiple copies- copies of equivalent genes from 2 species
- Frog- diploid- 2 copies of genes
- for genetic need both copies for phenotype
- If we have extra copy of gene in genomes- to see phenotype - need to activate all 4 copies- difficult in genetic terms
- Organism not good for genetics

Xenopus laevis (left)

Xenopus tropicalis (right)

- Consequence of genetic redundancy- genome of this species was sequenced in 2016
- Other model species genes sequenced earlier- easier for genetics

- Difficult to sequence in this animal due to all the duplication- techniques developed to deal with this

Xenopus (Silurana) tropicalis, a *Xenopus* that is amenable to genetics

- Alternative species - could apply genetic approaches to it
- The only diploid species in the genus
- Smaller adult & generation time of 4-6 months
- Transgenesis procedures developed
- (But CRISPR-Cas9 is a powerful new approach)
- Genome sequence completed 2010