Lectures 4 and 5

The first steps to forming a new organism
Descriptive embryology 2

Cleavage, Gastrulation, Neurulation and Organogenesis

Early animal development can be divided into the following key events

Fertilisation – cleavage – gastrulation – neurulation – organogenesis

Lecture 3              Lecture 4 and Lecture 5

Cleavage-

Very rapid cell division of the fertilised egg

Features:
Cell cycle involves S phase (DNA synthesis) and M phase (mitosis) but virtually skips G phases (so no growth). This also leads to faster cell division
Embryo does not enlarge- cell division in the absence of overall growth
Cytoplasm is divided up between emerging blastomere cells.

First 5-7 divisions yield a loose grouping of cells called a morula

Fluid filled cavity forms- blastocoel- to give rise to a hollow ball of cells- the blastula

How animals develop is used as part of their classification- it can tell us something about their evolution. For example the following is a classification based on cleavage pattern.

Radial cleavage:
As seen in vertebrates and echinoderms- also called deuterostomes.
Cleavages are equal and symmetrical. This explains why early development in the sea urchin (an echinoderm) is similar to vertebrates

Spiral cleavage: As seen in e.g, mollusca, annelids- also called protostomes. The spindle is at an oblique angle within the cell so cleavage is spiral. Successive cleavage planes are different. Producing a spiral pattern.
A variety of egg designs underlies differences in early development of animals

Does the egg contain yolk proteins for embryo nourishment?

  e.g.  amphibians ✓  birds ✓  mammals X

How much yolk?
e.g. most plentiful in birds, reptiles, many fishes and insects. less in sea urchins

How is the yolk distributed? Basis of differences between frog and chick.

**Cleavage in the frog**

The egg has polarity
Upper pigmented side- animal pole; lower yolky side- vegetal pole.

The movement of molecules in the egg -triggered by fertilisation
→ appearance of “gray crescent”

First two cleavages are vertical

Cleavage 3 is horizontal
Yolk content displaces plane of division toward animal pole.
⇒ More cells and blastocoel in animal half

This stage is the blastula

Cleavage pattern is similar in all animals classified as deuterostomes-
But in eggs with less yolk e.g. sea urchin, the blastomeres are of more even size and the blastocoel is more centrally located

**Cleavage in the chick**

• The entire “yolk” of the chicken egg is the egg cell- packed full of yolk proteins.
• The “white” - a protein rich solution bathing the cell- also used for nutrition
• All the cytoplasm is contained in a tiny disk at the animal pole of the yolk.

Cleavage planes cannot penetrate the dense yolk so only the animal pole cytoplasm is cleaved. This incomplete cleavage is know as meroblastic cleavage (complete cleavage is called holoblastic)

Cleavage produces a cap of cells sitting on top of yolk- called blastoderm

So the blastoderm in the chick is the equivalent of the blastula in the frog- the shape is related to egg architecture (yolk content and distribu
The cavity that forms (blastocoel) divides two layers of cells: the hypoblast and the epiblast. The embryo will emerge entirely from the epiblast.
Therefore the embryo will emerge from a simple flat layer of cells
Cleavage in different species can produce a ball or disk of cells. How does that turn into an organised embryo?

The next step is gastrulation - Literally means “making a gut”

**Gastrulation**
Marked by extensive cell movement where the cells of the blastula become organised into three layers. This 3 layered embryo has a primitive gut.

The details of the movements are different in different organisms (again related to the architecture of the egg) but the outcome is the same- a 3 layered embryo
The 3-layered embryo is called a gastrula

<table>
<thead>
<tr>
<th>Morula</th>
<th>blastula</th>
<th>gastrula</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ball of cells</td>
<td>hollow ball</td>
<td>3-layered hollow ball</td>
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</tbody>
</table>

Gastrulation best studied in the frog.
How could the early embryologists figure out the details of cell movement in a tiny embryo?
1. Frog embryo is not so tiny
2. The obviously visible poles aid visualization of movements
3. They used methods to mark cells and follow their movements and their fate- cell lineage marking / cell fate mapping

**Cell lineage marking / Cell fate mapping**
Mark a cell early in development and observe where it and its descendent cells end up.

This is how cell fate maps are made and how we can follow the movements and fates of all the cells

Gastrulation occurs in all eumetazoa (animals with an organised body plan)
Animals that do not undergo gastrulation are the most “simple” animals with no organs e.g. jelly fish.
“triploblastic” animals – gastrulation produces 3 germ layers.
Some animals are diploblastic (e.g. hydra- ;there are only two layers)

**The 3 layers formed are called the embryonic germ layers**

- **Ectoderm** “ecto”: outer
- **Mesoderm** “meso”: middle
- **Endoderm** “endo”: inside  (Note: the colour scheme used here is the classical scheme for mapping ectoderm (blue), mesoderm (red) and endoderm (yellow))

Eventually these 3 germ layers develop into all the tissues and organs of the adult animal
### The sea urchin- gastrulation on a simple scale

The blastula is a single layered hollow ball

1. Gastrulation begins at the vegetal pole- individual cells detach and enter the blastocoel- first mesenchyme cells (top arrow)

2. The vegetal plate destined to form endoderm- flattens and buckles inward- invagination (arrow)

3. Invagination deepens to form a tube called the Archenteron- this is the primitive gut (arrow).

4. Extensions from the mesenchyme cells called filopodia- drag the archenteron toward the blastocoel wall (arrow)

5. Archenteron fuses with blastocoel wall - forms digestive tube with mouth and anus- 3 layers - mesenchyme forms future skeleton

### Frog gastrulation

Gastrulation is much more complex in the frog because there are more cells and there are large yolk laden cells in the vegetal pole, but the process also produces a 3-layered gastrula with an archenteron as a primitive gut.

In Fig 47.10 panel 1 you see the fate map of where the future ectoderm, mesoderm and endoderm are located at the start of gastrulation. Gastrulation begins when a line of cells begin to change shape and invaginate. This occurs at the earlier site of the gray crescent and is called the dorsal lip of the blastopore.

Panel 2: Future mesoderm cells and endoderm cells at the site of the dorsal lip then begin to roll over the lip and involute into the interior of the embryo.
Inside, these cells move away from the blastopore and become organised into layers of endoderm and mesoderm.

Panel 3: The original cavity - the blastocoel - is collapsed by the movement and a new cavity is created - this is the archenteron and will form the future gut. The yolk rich cells are internalised in the region of the future gut. Meanwhile the remaining cells on the surface that will form the ectoderm (blue) move down around the surface to completely cover the embryo. As in the sea urchin the blastopore is the future site of the anus and the mouth will form at the opposite side.

As a result of all the cell movement the embryo changes shape - elongates as the cells meet and converge inside the embryo

Convergent extension

-like lanes of traffic meeting and converging - leading to elongation (extension)

The important features of gastrulation:

Elaborate cell movement.
Differentiation of cells into 3 broad types: ectodermal, mesodermal and endodermal, placed in the right position with respect to each other.
Allows communication between neighbouring cells that are now set apart as different to each other.

Mechanisms involved in bringing about gastrulation - what is needed?
• changes in cell motility
• changes in cell adhesion (therefore molecules on the outside of the cells).

Gastrulation may vary in detail from organism to organism but the result is similar:

the production of a three layered gastrula with organised groups of cells that interact and co-operate to form the embryo.

The positioning of the cell layers in the gastrula allows cells to interact in new ways.

Gastrulation brought all the cells into the correct position within the embryo to facilitate local development of the organs- organogenesis.
While gastrulation involves massive cell movements, organogenesis involves more local interactions between cells and *morphogenetic changes in tissue and cell shape- and in cell arrangement.*

Note that cell differentiation is happening continuously throughout these processes and cells are getting closer to their final characteristics and functions.

Many of the changes in organogenesis are brought about by Induction:- the influence of one group of cells on another changing the way in which the responding cells develop (more in lecture 8).

The process of Neurulation is a very special type of organogenesis
- It sets aside the cells for and forms the rudiments of the entire nervous system.
- It is the first event in organogenesis.

The first event in neurulation starts as gastrulation is still underway and the future mesoderm cells are moving inside the embryo. A particular group of these migrating mesoderm cells assemble into a rod-like structure along the dorsal midline of the future embryo.
These special mesoderm cells form the notochord and have very special characteristics-they have very potent ability to induce neighbouring cells.

Signals sent from the notochord to the ectoderm lying above induce the ectoderm to thicken and form the neural plate (green on the figure below) the rudiment of the nervous system
See figure 47.12 and images below- note the notochord and the neural plate

The outer edges of the neural plate then begin to fold upward- forming the neural folds. Eventually the two sides of the neural folds fuse to enclose the neural tube (see figure below).
As the neural tube fuses the adjacent surface ectoderm (blue) also fuses to enclose the neural tube, which forms the rudiment of the brain and the spinal chord. The neural tube runs the length of the embryo from anterior where it forms the vesicles of the brain.
By the end of gastrulation and neurulation, the embryo has elongated with a head end and a tail end and has an internalised neural tube running along the dorsal midline. In the head region this will form the brain chambers and elsewhere the spinal chord.

As neurulation proceeds, other organs and structures are also starting to form.

A special set of cells form at the dorsal edge of the neural tube- **the neural crest cells** (see below).
These migrate away from the neural tube to many destinations and form peripheral nerves, teeth, skull bones and other cell types.

Note the coelom in the top figure – this is a fluid-filled cavity which forms by splitting the mesoderm- it is by definition surrounded by mesoderm on all sides. Many of the organs as they form will push into this space - although contained within the peritoneum.

The somites are another set of structures forming at this time.

The somites are mesoderm cells that form into blocks on either side of the neural tube (see figure below). These are transitory structures that later dissociate to form different cell types.

1. The muscle blocks of the body and muscles attached to the skeleton
2. The vertebrae of the back-bone. The cells from within the somite that will form the vertebrae (blue on lower diagram) leave the somite and migrate around the notochord and neural tube. The notochord later degenerates; the only remnants forming the “disks” between the vertebrae.
The somites show that vertebrates are partially segmented.

In organogenesis cells of one germ layer often induce neighbouring cells of another germ layer to change character and begin to form a tissue or part of an organ - this is precisely why bringing the germ layers into the right position during gastrulation is so vital for development of the embryo.

- More about induction and its molecular basis in lecture 8

Gastrulation in the chick

The process looks very different because the early embryo is a flat disk of cells with masses of yolk underneath. The entire embryo will form from one cell layer - the epiblast. Gastrulation involves cells from the epiblast moving underneath through a line at the midline of the disk called the primitive streak. Some of these involuting cells form endoderm and some form mesoderm. The primitive streak marks the future anterior-posterior axis of the embryo. The primitive streak in the chick is equivalent to the blastopore in the frog (site through which the cells involute).

In the chick, organogenesis proceeds much as it does in the frog but appears different because the chick embryo is flat and the frog round.

Organogenesis in the chick: To achieve the same “tube within a tube” (gut within the body wall) structure of other embryos, the edges of the blastodisk (flat chick embryo) fold ventrally to enclose the gut - but the gut is still joined in the middle of the body to the yolk in the yolk sac. See Figure 47.13(a) reproduced below. Note the neural tube, notochord, somites and endoderm of the future gut.
By 2-3 days of development the rudiments of all the major organs is seen in the chick embryo. See Figure 47.13(b) below. A movie showing the beating heat of the chick embryo at this stage was viewed.

A movie of organogenesis in zebrafish was shown.

The figure on the right shows illustrations of embryos during each of the developmental processes that we have discussed.

Note it is a continuous series of processes. A movie of the continuous processes listed above in zebrafish was viewed again.

The story so far has led us from quite different egg designs to very similar embryos among vertebrates— as shown in the figure below for frog (Xenopus), chick, mouse and zebrafish.
This was first noted by Karl Ernst von Baer in the early 19th century— he called the stage when all vertebrate embryos look alike (2nd row above) the **phylotypic stage**— typical of the phylum. At this stage the general characteristics of the phylum are established— notochord, neural tube, somites and the very beginnings of the limbs. Von Baer devised some laws to express this observation: he stated in these laws that general characteristics of a group appear first while those that distinguish a species appear later.

**But why is this? He couldn’t really explain it – there is something he didn’t know but we know today….**

**….organisms share similar developmental mechanisms.**

The mechanisms driving the changes you have just seen will be explored in future lectures

- Lecture 7: cell differentiation
- Lecture 8: cell communication
- Lectures 9-10: developmental genetics

**Key concepts in lectures 4 and 5**

1. Cleavage refers to the very rapid cell division of the fertilised egg. It is marked by little or no protein synthesis and no growth of the embryo.

2. The first divisions form a loose group of cells called a morula.

3. After further divisions a cavity forms (blastocoel) – the embryo is then called a blastula.

4. Cleavage is holoblastic if it divides the entire egg into blastomeres (cells) but meroblastic if only the cytoplasm is divided and the yolk proteins are excluded (e.g. chick).

5. The appearance of cleavage and the resulting embryo depends on the amount and distribution of yolk. Cleavage produces a ball or disk of cells. Gastrulation then turns that ball or disk into an organised embryo.

6. Gastrulation is a fundamental event in all complex animals (eumetazoa) with organs. It leads to the formation of a 3 germ layered embryo (for triploblastic animals)- the three germ layers will give rise to all the future organs and structures.

7. The three germ layers, the ectoderm, mesoderm and endoderm contain 3 broadly different cell types.
8. Gastrulation is characterised by elaborate cell movement so that the three germ layers end up in the correct position with respect to each other.

9. This sets groups of cells apart as different to each other and allows communication between neighbouring cells that will be so important in future events- organogenesis.

10. Changes in cell motility and changes in cell adhesion (therefore molecules on the outside of the cells) are important in bringing about the cell movements of gastrulation.

11. Gastrulation brought all the cells into the correct position within the embryo to facilitate local development of the organs- organogenesis.

12. While gastrulation involved massive cell movements, organogenesis involves more local interactions between cells and *morphogenetic changes in tissue and cell shape, and in cell arrangement.*

13. Many of the changes in organogenesis are brought about by **Induction**- cells of one germ layer often induce neighbouring cells of another germ layer to change character and begin to form a tissue or part of an organ- underlines the importance of bringing the germ layers into the right position during gastrulation.

14. The process of **Neurulation**- a special event in organogenesis that sets aside the cells for and forms the rudiments of the entire nervous system.
   (Make sure you can describe the events involved in neurulation)

15. By the end of gastrulation and neurulation, the embryo has elongated with a head end and a tail end and has an internalised neural tube running along the dorsal midline. In the head region this will form the brain chambers and elsewhere the spinal chord.

16. **The somites** are mesoderm cells that form into blocks on either side of the neural tube- transitory structures that later dissociate to form different cell types including the muscle blocks and the vertebrae.

17. In the chick, organogenesis proceeds much as it does in the frog but appears different because the chick embryo is flat and the frog round.

18. The processes that form the embryo (cleavage, gastrulation and organogenesis) occur as a continuous series of interlinked events- they are not discrete.

19. The early observation by von Baer that all vertebrate embryos look similar at the end of gastrulation and early organogenesis reflects the fact that molecular and cellular events that underlie early development are very similar across all animals.

**Lecture 4 and Lecture 5: Learning outcomes:** you should be able to….
A) State what cleavage is and, using appropriate terminology, describe cleavage in the frog and differences in the chick.
B) State what gastrulation is and describe its fundamental importance in all complex animals. Describe gastrulation in frog but be able to discuss the differences between frog and chick and how the differences in detail are related to structure of the egg- but that the outcome is the same.

C) Distil the important features of gastrulation from the descriptions and relate this to its important outcomes.

D) State what organogenesis is and contrast it with the earlier events of gastrulation.

E) Describe neurulation as a very early event in organogenesis and the importance of this early event as part of establishing the body plan.

**Key terms to be familiar with:** Cleavage, blastomere cells, morula, blastocoeel, blastula, gray crescent, holoblastic cleavage, meroblastic cleavage, blastoderm, epiblast, hypoblast, gastrulation, gastrula, fate map, germ layers, ectoderm, mesoderm, endoderm, invagination, archenteron, blastopore, involution, convergent extension, primitive streak, Henson’s node,