Introduction

3. Books
This module is based on the following books:

4. Summary
In this module you will study the process of gastrulation. Gastrulation occurs during early embryonic development in animals. In this process the endoderm and the mesoderm move from the outer surface of the embryo to the inside.
- “Gastrulation is the process of highly coordinated cell and tissue movement, whereby the cells of the blastula are dramatically rearranged” (Gilbert).
- “Gastrulation is the process, whereby endoderm and mesoderm move from the outside to the inside of the embryo, giving rise to internal organs” (Wolpert).

At the end of gastrulation:
- mesoderm and endoderm are located inside the embryo.
- ectoderm is completely covering the outside of the embryo.
- endoderm and mesoderm give rise to internal organs.

5. Aims and objectives
After studying this module you should be able to:
- describe the aims of gastrulation
- describe the 5 different mechanisms of gastrulation
- relate the 5 different mechanisms to examples in the animal kingdom
- understand and recognize the 3-dimensional movements of cells during the gastrulation processes
- describe what tissue and organs are derivatives of the endoderm, mesoderm and ectoderm

6. Formation of mesoderm
Depending on the species, mesoderm is formed either before or during gastrulation.
**Before gastrulation:** For example in the frog (amphibia) the formation of the mesoderm starts around the 64-cell stage. It continues until the onset of gastrulation.
**During gastrulation:** In sea urchins (echinoderms), for example, the formation of mesoderm is part of the gastrulation process.

7. Mechanisms of gastrulation
There are 5 different kinds of gastrulation movements. One or more of these mechanisms can be responsible for gastrulation. This depends on the species.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiboly</td>
<td>Extension of a sheet of cells causing movement of this sheet over presumptive deeper layers</td>
</tr>
<tr>
<td>Invagination</td>
<td>Indentation of a region of cells resulting in the formation of a ‘dimple’</td>
</tr>
</tbody>
</table>
8. **Q1 Mechanisms**  
You are asked to put the names of the gastrulation processes to the figures.

9. **Convergent extension**  
In most species very extensive cell movements take place during gastrulation. Often cells 'crawl' in-between each other (intercalate) and elongate (extend). This process is called convergent extension.  
Two types of convergent extension are known: radial and medio-lateral.  
1) **Radial intercalation**: A double (or multiple) layer of cells intercalate in such a way that a single layer of cells is formed. As a result, the surface of the layer of cells is enlarged. Occurs in for example embolic movements in the ectoderm (e.g. in *Xenopus*, *Zebrafish*).  
2) **Medio-lateral intercalation**: Cells of a single layer intercalate medio-laterally. Due to this type of intercalation, the cells form an elongated structure. Occurs in for example the notochord.

10. **Main menu**

11. **Q2 Gastrulation mechanisms**  
*Last question, next page to the menu, to be answered when you completed the module.*

<table>
<thead>
<tr>
<th>mechanism</th>
<th>Xenopus</th>
<th>Zebrafish</th>
<th>Fruit Fly</th>
<th>Chick</th>
<th>Sea Urchin</th>
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</thead>
<tbody>
<tr>
<td>Ingression</td>
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<tr>
<td>Invagination</td>
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<td>Epiboly</td>
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</table>
Echinoderms

1. Sea Urchin
   Echinoderm: the Sea Urchin

2. Early blastula
   This is an early blastula stage embryo (left) and its schematic drawing (right).
   ![Image of early blastula]

   The embryo is a hollow sphere (blastula) with a large cavity, the blastocoel. The cells surrounding the cavity are connected to each other by tight junctions. These cells form an epithelial layer, called the blastoderm. All cells have cilia on their external surface. Because of ciliary movements the embryo can rotate. The external face of the cells is covered by the hyalin layer. This is a layer of extracellular matrix proteins. At the blastocoelic side, the cells are covered with an extracellular layer. This layer is the basal lamina.

3. Q1 Vegetal plate
   **Sea Urchin: late blastula**
   After the formation of a blastuloel, the cells at the vegetal side elongate and form a ‘thicker’ layer of cells. This layer is called the vegetal plate. 
   *Indicate the vegetal plate in the figure.*

4. Late blastula
   **Sea Urchin: late blastula**
   At the animal pole, some cells developed very long cilia. This group of cilia is called the apical tuft.

5. Fate map
   A fate map illustrates what the different parts of the embryo will form later in (normal) development. This is a fate map of a late blastula stage. The cells that will later form the mesoderm are all derivatives (descendants) of the micromeres. Micromeres are small cells that result from unequal cleavage during early animal development. In Sea Urchin embryos the micromeres are located at the vegetal pole.

6. Onset of gastrulation
   In the sea urchin the gastrulation starts in the vegetal plate in individual cells. Some cells of the vegetal plate loose their affinity for their neighbouring cells and for the hyalin layer. Moreover, their affinity for the basal lamina is increased. These cells change shape and form filopodia.
Finally, these cells leave their epithelial configuration and migrate into the blastocoel. This process is called ingression.

After ingression, the cells that migrated into the blastocoel are called primary mesenchyme cells (PMC). In normal development about 64 PMC are formed. All PMC are derivatives of the micromeres.

7. Microscopic image
In the figures you can see both a schematic drawing of ingression and a microscopic photograph of the ingressed PMC.

8. Informational cues for migration
After ingression, the PMC migrate towards a specific position inside the blastocoel. On the next page is an enlargement of the marked area. You will be asked to name some visible structures.

9. Q3 Filopodia

1 =

2 =

3 =

4 =
10. **Q4 Masking of ECM**
Studies indicate that basal lamina proteins play an important role in defining the direction of migration of the primary mesenchyme cells.
Before the onset of gastrulation the ECM molecules inside the blastocoel are 'masked' with an injection of antibodies against ECM.
*What processes do you expect to be hindered?*

11. **Migration of PMC**
After ingression, the PMC move to two ventro-lateral regions in the blastocoel. Here they form calcium carbonate spicules (spines).
The two clusters remain connected by a small row of PMC, called dorsal and ventral chain respectively. The PMC form filopodia with which they 'probe' the surrounding cells and the extracellular matrix (ECM).

12. **Q2 Naming the structures of PCMs**
Put the names to the four structures in the figure.

1 =

2 =

3 =

4 =

13. **Photomicrograph**
This picture is a photomicrograph of a gastrula stage embryo. In this embryo the PMC are stained (colored) with a PMC-specific antibody. The picture shows the ventrolateral clusters and one of their connecting chains.
14. **Q5 Next processes**
After the ingress of the primary mesenchyme cells, the remaining cells of the vegetal plate start to change in shape. Cells flatten and actin filaments that are located at the apical side of some cells constrict. Because of this, a small indentation (dimple) is formed. **What is the name of this gastrulation mechanism?**

15. **Blastopore**
Invagination marks the onset of the formation of the archenteron (primitive gut). The indentation or opening of the archenteron is called the blastopore. The blastopore will form the anus of the adult sea urchin.

16. **Q6 Animals where blastopore forms anus**
*What are animals with these types of development called?*

Blastopore forms mouth:

Blastopore forms anus:

17. **Formation of the archenteron (1)**
Formation of the blastopore solely results from changes in cell shape due to apical constriction. This is confirmed by computer simulations.

18. **Formation of the archenteron (2)**
The hyalin layer consists of an inner and outer layer. The microvilli of the vegetal plate cells extend through the hyalin layer. Their cytoplasm contains secretory vesicles with the enzyme chondroitin sulfate proteoglycan (CSPG).
19. Formation of the archenteron (3)
At the onset of archenteron formation, the secretory vesicles secrete Chondroitin Sulfate Proteoglycan (CSPG) into the inner lamina of the hyalin layer. The CSPG absorbs water and consequently the inner lamina swells. The outer lamina to which it is attached, does not swell. This causes bending of the hyalin layer and consequently the attached epithelium moves further inward.

20. Elongation of the archenteron
After invagination the archenteron starts to elongate. The endodermal cells lining the archenteron undergo convergent extension by medio-lateral intercalation.

This elongates the archenteron tube.

21. Diameter archenteron
How does the process of convergent extension affect the diameter of the archenteron tube?

22. Final extension of archenteron
The onset of the final extension is characterized by yet another ingress of cells, the secondary mesenchyme cells (SMC). This ingress occurs at the top of the archenteron. The secondary mesenchyme cells remain on top of the archenteron. They form long filopodia with which they 'probe' the basal lamina of the blastocoel.

The filopodia stick to one specific site at the animal side. Specificity for this site is based on differences in affinity to ECM proteins (differential adhesiveness). Once 'stuck' to this site the filopodia contract. This way the top of the archenteron is pulled up.
23. Fate of secondary mesenchyme cells
After the formation of the mouth, the secondary mesenchyme cells disperse into the blastocoel. They form mesodermal organs such as the skeletal rods.

24. Q8 Summary formation archenteron
Formation of the archenteron can be divided into four steps. In chronological order, enter the process belonging to each step.

1)
2)
3)
4)

25. Summary archenteron formation
Formation of the archenteron can be divided into four steps.
1) ingresson
2) invagination
3) convergent extension
4) final extension
As a last step, the mouth is formed by breaking through the ectoderm. This finalizes the formation of the archenteron (digestive system).

26. Gastrulation Sea Urchin
We've discussed the gastrulation of the Sea Urchin. Which two gastrulation mechanisms are important during Sea Urchin development?

1.
2.
Amphibians

1. **African clawed frog**
   Amphibian: African Clawed Frog (*Xenopus laevis*)

2. **Fertilization**
   The spermium enters at the animal half (hemisphere) of the oocyte. The point of entrance is called the 'sperm entry point' (SEP). In this drawing it is located at the backside of the egg. The side where the SEP is located is the future ventral side of the animal. The opposite side is the future dorsal side.

3. **Q1 Dorsal side**
   What is the dorsal side of this oocyte?

4. **First cleavage**
   The first cleavage is through the sperm entry point and the future dorsal side. It divides the embryo in a left and right side.

5. **Gastrulation start**
   Gastrulation starts at the opposite side of the sperm entry point, just below the Spemann Organizer. The Spemann Organizer is a signaling center on the dorsal side of the amphibian embryo. Signals from this center can organize new antero-posterior and dorso-ventral axes.

   Reread Wolpert, pages 110-113 to refresh your memory about:
   - cortical reaction
   - cortical rotation
   Reread Wolpert, pages 135, 167-169, for more information about the Spemann Organizer.

6. **Q2 Gastrulation**
   In *Xenopus* embryos, the presumptive endoderm is located in the vegetal hemisphere. These cells are rich in yolk. Yolk is used as 'food' by the developing embryo.

   Write the labels in the figure.

7. **Gastrulation end**
   During gastrulation, the endoderm and mesoderm become located inside the embryo. After gastrulation, the outside of the embryo is completely covered with ectoderm.
8. **Q3 Invagination**
Gastrulation starts with an invagination. This invagination is located at the dorsal side of the embryo, just vegetal from the marginal region, vegetal from the Spemann organizer. The invagination is driven by bottle-shaped cells inside the embryo.
*What is the name of the ‘dimple’ resulting from invagination?*

9. **Q4 Inward movement**
Invagination is followed by inrolling of cells.
*What is the name of this gastrulation mechanism?*

10. **Onset of gastrulation**
Invagination is followed by inrolling of presumptive endoderm and mesoderm.
The dorsal mesoderm converges and extends by medio-lateral intercalation. Later on, the dorsal mesoderm will form the notochord.

11. **Intercalation**
After gastrulation, the ectodermal cells cover the surface. The enlargement of the outer surface is caused by radial intercalation (1 of the 2 types of convergent extension).
Invagination is followed by inward movement of cells.
*What is the name of this gastrulation mechanism?*

12. **Summary of processes**
Gastrulation starts with an invagination. Invagination is followed by inward movement of cells: involution.
The enlargement of the outer surface is caused by radial intercalation (1 of the 2 types of convergent extension). This is called epiboly.

13. **‘Real’ egg**
The involution that started at the dorsal side (A) extends laterally (B). Finally it starts at the ventral side (C). This way a complete ring of involuting cells is seen (D).
In the last stages some endodermal cells remain visible. These cells are called the yolk plug.

14. **Q6 Endoderm**
Where in the egg (C) is the endoderm situated?

15. **Q7 Ectoderm**
Where in the egg (C) is the ectoderm situated?

16. **Q8 Ectoderm**
What is the dorsal side of this egg (C)?

17. **Gastrulation movie**
This movie shows a ventral view of the *Xenopus* embryo.
18. Q9 Labels
This figure shows a cross section of a gastrula stage *Xenopus* embryo. 
*Write the labels in the figure.*

19. Q10 Order stages
The pictures below show the tissue movements that take place during gastrulation of *Xenopus*. They are, however, not in the correct sequence. 
*Indicate the correct order with numbers 1-6.*

20. Q11 Blastocoel
*In which pictures is the blastocoel visible?*

21. Q12 Archenteron
*In which pictures is the archenteron visible?*

22. Gastrulation movie
This movie shows a ventral view of the *Xenopus* embryo, and gives another look at involution and epiboly.

23. Q13 Labels after gastrulation
The figure below shows a ventral view of the *Xenopus* embryo. 
*Write the correct labels next to the picture.*

24. Q14 Gastrulation processes
We've discussed *Xenopus* gastrulation. 
*Which three gastrulation processes are important in Xenopus?*

1. 

2. 

3. 
Fishes

1. Zebrafish
   Fish: Zebrafish (*Danio rerio*)
   The zebrafish is a small fish that lives at relatively high temperatures of around 25-28 °C.

2. Early development
   ![](image)
   In the figure above you see the early cleavages of the zebrafish embryo. The first cleavages occur every 15 min. These cleavages are confined to the animal half of the embryo. In this way a mound of cells is formed that sit on top of a large yolk cell. Below some of these stages under light microscopy.

3. The 10th cycle
   ![](image)
   Until the 10th cell cycle, the blastomeres remain on top of the yolk cell and divide synchronously. At the 10th cell cycle, the blastoderm can be divided into two layers of cells:
   - the outer enveloping cells and
   - the deep cells.

4. Q1 First cell movement
   After the 10th cell cycle, the division of cells become asynchronous and the cells begin to move. The first cell movement is the movement of cells over the yolk cell.
   *What is the name of this gastrulation mechanism?*
5. **Q2 Second cell movement**
   Once cell movement has reached about 50% of the diameter of the embryo, a thickening occurs at the entire margin of the blastoderm. This thickening is called the germ ring.
   The endodermal and mesodermal cells are derivatives of the deep layer.
   At the germ ring, the presumptive endodermal and mesodermal cells turn inwards.
   *What is the name of this gastrulation mechanism?*

6. **Epiblast and hypoblast**

   Due to involution, the deep cells form two layers of cells:
   - an outer layer called **epiblast**,
   - an involuted layer called **hypoblast**.
   The envelope layer lays on top of the epiblast.
   The epiblast forms the outer epithelium and the neural tissue. The hypoblast forms the chordamesoderm and the adjacent mesodermal structures.

7. **The embryonic shield**
   At the future dorsal side of the embryo a localized thickening is formed: the **embryonic shield**. This shield is functionally equivalent to the dorsal involuting cells of the *Xenopus* embryo (the Spemann Organizer).

8. **Q3 Third cell movement**
   After involution, the hypoblast cells converge anteriorly and dorsally and extend in anterior direction.
   *What is the name of this gastrulation mechanism?*

9. **Q4 Third cell movement**
   After this movement, the hypoblast cells converge anteriorly and dorsally and extend in anterior direction.
   *What is this type of movement known as?*

   Answer: extension by intercalation
10. Three movements
The hypoblast cells that involute at the dorsal side converge anteriorly and dorsally and extend in anterior direction (medio-lateral intercalation).
The hypoblast cells form the chordamesoderm, the primordium of the notochord.

11. Embryonic shield in microscopy
Convergent extension can be shown by visualizing cells that express the gene no tail. No tail is expressed in presumptive notochord cells. In the figures you see no tail-expressing cells in two different stages of gastrulation.

12. From 1 cell to 100% epiboly
This movie starts to show a zebrafish embryo at the 1-cell stage.

13. Q5 Labels
In the figure below, epiboly has reached about 50% of the diameter of the embryo.
What are the names of the specified structures?

1 =

2 =

3 =

14. Q5 Mechanisms
We’ve discussed Zebrafish gastrulation.
Which two gastrulation mechanisms are important during Zebrafish development?

1.

2.
../Insects

1. **Fruit Fly**
   Insect: Fruit Fly (*Drosophila melanogaster*)
   An adult fly laying an egg.

2. **Early development**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 nucleus</td>
<td>1 nucleus</td>
</tr>
<tr>
<td>4 nuclei</td>
<td><em>Drosophila</em> embryos undergo superficial cleavages.</td>
</tr>
<tr>
<td>Many nuclei</td>
<td>This way many nuclei are formed within a single cell.</td>
</tr>
<tr>
<td>256 nuclei</td>
<td>At 256 nuclei, the nuclei migrate to the periphery of the embryo.</td>
</tr>
<tr>
<td>Syncytium</td>
<td>The embryo is now called a syncytial blastoderm.</td>
</tr>
<tr>
<td>1024 nuclei</td>
<td>At 1024 nuclei, the outer membrane of the embryo folds inwards between the nuclei.</td>
</tr>
<tr>
<td>Cellular blastoderm</td>
<td>Eventually, each somatic nucleus is partitioned off into a single cell. Now the cells are arranged into a single-layered jacket around the yolky core of the egg. This is called the cellular blastoderm stage.</td>
</tr>
</tbody>
</table>

3. **Q1 Onset of gastrulation**

At the ventral side, by ingestion an indentation is formed along the anterior-posterior midline. This indentation is called the ventral furrow.

*What is the name of the gastrulation mechanism leading to the formation of the ventral furrow?*
4. **Q2 Onset of gastrulation (2)**
   In the ventral furrow, the presumptive mesodermal cells move inward.
   *What is the name of the gastrulation mechanism responsible for the inward movement of cells in *Drosophila*?*

5. **Visualization of presumptive mesodermal cells**
   This process can be visualized by following *twist* expression. The *twist* gene is expressed in all presumptive mesodermal cells. Within 30 min., all presumptive mesodermal cells have moved inside the embryo.

6. **Lateral view gastrulation**
   The figure shows a lateral view of the *Drosophila* embryo. Gastrulation begins when the presumptive mesoderm invaginates in the ventral region. First the ventral furrow is formed. Then an internalized tube is formed. Cells leave the tube and migrate under the ectoderm. Later they form the mesodermal organs.

7. **Q3 Mechanisms**
   We've discussed *Drosophila* gastrulation. *Which two gastrulation mechanisms are important during *Drosophila* development?*
   1. 
   2. 
Birds

1. Chicken
   Bird: Chicken (*Gallus*)
   Adult rooster.

2. Early cleavages (1)
   The early cleavages in the chick embryo create a blastodisc (= blastoderm) above the enormous yolk cell. As cleavages continue, the central cells of the blastodisc become separated from the yolk cell by the subgerminal cavity.

3. Early cleavages (2)
   The cells above the subgerminal cavity appear clear and therefore this area is called the area pellucida (PELLUCIDUS = transparant). The cells at the margin of the area pellucida appear unclear and are called the area opaque (OPACUS = non-transparant).

4. Q1 Onset of gastrulation
   From the area pellucida individual cells move into the subgerminal cavity. What is the name of this gastrulation mechanism?

5. Primary hypoblast cells
   From the area pellucida individual cells ingress into the subgerminal cavity. They form small islets of cells called the primary hypoblast cells.

6. Secondary hypoblast cells
   At the posterior margin of the blastoderm a group of cells migrates into anterior direction. These are the secondary hypoblast cells.

7. Epiblast
   As the secondary hypoblast cells migrate they incorporate the primary hypoblast cells. The resulting layer is called the secondary hypoblast or endoblast.
   Now the blastoderm consists of two layers:
   - the epiblast, and
   - the secondary hypoblast.
   The primary and secondary hypoblast do not contribute to the embryo proper. It forms part of the external membranes (for example the yolk sac).
8. Early primitive streak
As the secondary hypoblast moves anteriorly, some cells at the posterior side in the epiblast thicken. This thickened area is the (early) primitive streak.

9. Primitive streak (1)
At the place of thickening of the epiblast the following occurs:
- ingression of mesodermal cells from the epiblast into the blastocoel, and
- migration of cells from the lateral regions of the posterior epiblast towards the center.

10. Primitive streak (2)
The thickening narrows and moves anteriorly to form the definitive primitive streak.

11. Hensen’s node (1)
The primitive streak moves anteriorly (progression) until about 60-75% of the length of the area pellucida. It marks the anterior-posterior axis.

12. Hensen’s node (2)
At the anterior end of the primitive streak a regional thickening is seen. This thickening is called Hensen’s node.

13. Hensen’s node (3)
Having reached 60-75% of the length of the area pellucida, the primitive streak regresses toward the posterior end.

14. Q2 Primitive streak
You are asked for the labels in the figure of page 8.
15. **Movement through Hensen’s node (1)**
During the progression of the primitive streak, cells migrate through:
- Hensen’s node, and
- the primitive streak

Cells that move through Hensen’s node migrate anteriorly. They form the anlage of the foregut, head-mesoderm and the notochord.
Cells that move through the primitive streak form the majority of the endoderm and mesoderm.
All cell movements through Hensen’s node and the primitive streak are movements of individual cells, not of a sheet of cells like in *Xenopus*.

16. **Movement through Hensen’s node (2)**
The presumptive endodermal cells move deeper than the presumptive mesodermal cells.
The endodermal cells displace the hypoblast cells to the sides. They form the endoderm and most of the external membranes. The rest of the external membranes is formed by the hypoblast.

17. **Q3 Similarities**
The primitive streak of chick is functionally equivalent to a structure in *Xenopus* and in zebrafish.
In these structures (presumptive) mesodermal cells migrate into the blastocoel.
*Which structure in Xenopus and zebrafish are we referring to?*

*Xenopus:*

*Zebrafish:*

18. **Mechanisms**
We’ve discussed chicken gastrulation.
*Which gastrulation mechanism is important during chick development?*