2. FROM FERTILIZATION TO THE THREE LAYERED EMBRYO

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Recommended reading:

William J. Larsen "Human Embryology" 3rd ed., pages 18-20, 29-33, 37-43, 53-61, 65-67 (clinical applications), 67-76

Learning objectives:

The student should be able to:

1. Discuss the anatomical location within the mother's reproductive tract where fertilization and the initial divisons of the fertilized egg occur.
2. Discuss the significance of compaction, the segregation of the blastomeres and formation of the blastocyst.
3. Distinguish between the descendents of the trophoblasts and the inner cell mass.
4. Formulate the concepts of potency and differentiation.
5. Describe the role played by hypoblast and the primitive node (Hensen's node in chicks, dorsal lip of the blastopore in amphibia) in producing signals that establish the axes of the embryo.
6. Describe the establishment of three germ layers with particular attention to the cellular movements through the primitive streak and primitive node.
7. Describe how the concepts of induction and competence are applied to the differentiating tissues of the embryo.

Summary:

This lecture begins with the events that occur immediately following fertilization within the oviduct and the reestablishment of the diploid state. Fertilization takes place in a section of the oviduct (FALLOPIAN TUBE) called the ampulla. It takes the embryo 5 days to reach the lumen of the uterus. During its journey, the zygote undergoes mitotic divisions called CLEAVAGE DIVISIONS; these occur without growth of daughter cells. These cells are now called BLASTOMERES. At the 8 cell stage the embryo undergoes COMPACTION, leaving only a portion of the blastomeres facing the external environment. This creates two cellular lineages: the TROPHOBLASTS which form a portion of the placenta; and the INNER CELL MASS

which forms the embryo proper and the extraembryonic membranes. These latter include the AMNIOTIC MEMBRANE of the amniotic cavity and the extraembryonic mesoderm [EEM] (see Lecture 3). EEM contributes to the placenta (the later will be covered elsewhere).

Once in the uterus, the embryo and the uterine lining recognize each other biochemically, permiting ATTACHMENT of the embryo followed by a carefully controlled IMPLANTATION. These are subjects of a subsequent lecture. At implantation the inner cell mass reorganizes into a two layered embryo; the epithelial EPIBLAST, which will form the embryo and amniotic membrane and the HYPOBLAST, which has an important role in the orientation of the embryonic axes. In the second half of the lecture, we will focus on the events taking place in the epiblast and on its interaction with the hypoblast.

As implantation proceeds, the cellular movements, termed GASTRULATION, establish the three primary germ layers. Gastrulation occurs between days 14 and 19 post-conception. It is a series of rapid, complicated, but coordinated movements of cells from the surface epiblast of the bilaminar embryo into the interior. Because of the complexity of this process, many embryos do not gastrulate correctly. It is estimated that improper gastrulation occurs in one-third of all human embryos. When this happens, a miscarriage may take place, even before the woman realizes that she is pregnant.

Gastrulation movements form the three germ layers: ectoderm, mesoderm and endoderm. While these cellular movements occur, signals originating from different sources will result in establishment of the axes of the embryo.

Glossary:

Blastomeres: cells produced by cleavage divisions of the zygote.
Blastocyst: Formed from the blastomeres. Has a central fluid filled cavity (blastocoel) and is divided into outer trophoblasts and an inner cell mass.
Chordamesoderm: axial (midline) mesoderm which gives rise to the notochord.
Cleavage divisions: Non-synchronous mitotic divisions following fertilization. No growth between cell division cycles. Resulting in cells (blastomeres) of approximately equal size.
Committed: the time point when a cell's fate to a particular lineage is fixed. This does not imply final phenotypic differentiation.
Competence: the ability to respond to an inductive signal. Once a competent cell responds to an inductive signal, it becomes specified.
Epiblast: The inner cell mass forms a two layered embryo. The epiblast is the top layer (facing the placenta) and forms the embryo proper and the amniotic membrane.
Extra-embryonic mesoderm: Tissue derived from the epiblast the contributes to the fetal compartment of the placenta but not the embryo.
Fallopian tube: oviduct of the human, site of fertilization and initial cleavage divisions.
Germ layers: ectoderm, mesoderm and endoderm (see summary).
Hypoblast: Bottom layer (facing the blastocoel) of the 2 layered embryo. Plays a role in establishing polarity but does not contribute cells to the embryo. Also called anterior visceral endoderm (AVE).
Inner cell mass: will give rise to the epiblast and hypoblast. Also called embryoblast in the text.
Induction: the change in a cell or tissue's fate due to a signal from another tissue or cell.

Notochord: midline (axial) mesoderm.
Prechordal plate: a portion of axial mesoderm just cranial to the notochord, will give rise to mesoderm of the head and is also an important signaling center.
Primitive node: most anterior (cranial) aspect of the primitive streak with a role in inducing structures of the trunk.
Primitive streak: site of cell movements from epiblast to form other germ layers.
Trophoblasts: derived from the outer cells of the blastocyst, forms the embryonic/fetal component of the placenta.
Zygote: fertilized egg.

Lecture Notes:

Fertilization and Cleavage

The female reproductive tract is discontinuous (Figure 2-1). The ovary is not connected to the oviduct. The ovulated ovum must be caught by the fingers (fimbria) of the oviduct and then enter the infundibulum (funnel). Fertilization will take place in the distal 1/3 of the next compartment (ampulla) where the zygote will remain for approximately 72 hours. This halt may prevent further interaction with the reservoir of sperm still in the cervical region of the uterus and is regulated by alterations in the patency (opening) of the oviduct. The length of the transit time is important to ensure synchrony between the developmental stage of the embryo and the uterine lining (endometrium). It takes the endometrium (lining of the uterus) several days to be made ready to receive the embryo. Knowledge about the timing requirements for synchronization of the embryo and mother are very important for the success of *in vitro* fertilization.

Cleavage divisions: During transit the embryo remains within its zona pellucida (see Lecture 1) and the corona radiata (several layers of ovarian cells that surrounded the oocyte during its development) until after its entery into the uterine cavity. The traveling embryo goes through several mitotic divisions called CLEAVAGE DIVISIONS (Fig. 2-1). Compared to other classes of vertebrates, cleavage divisions in mammals are very slow with ~1 per day for the first 3-4 days. These divisions increase the number of cells (blastomeres) in the embryo, without any increase in the overall size of the embryo. Cleavage in mammals is asynchronous so there need not be an even-number of cells in the embryo. The timing and positional relationships are important variables in determining developmental destinies. The zygotic genome is turned on in humans between the 4 to 8 cell stage and the maternal message is degraded.

Compaction: Commitment to 2 Cell Lineages

At the 8 cell stage, following the third cleavage, the embryo is transformed from a loosely organized ball of cells into a compact closely adherent cluster (Fig. 2-2). This process is called COMPACTION. Compaction is an extremely important event because at this time (and we shall develop the theme more fully) the **fates** of the cells begin to diverge radically from each other.

Developmental biologists have specific definitions for such terms as fate, potency, determination and differentiation:

fate: normal developmental pathway of unperturbed cell or cell group.

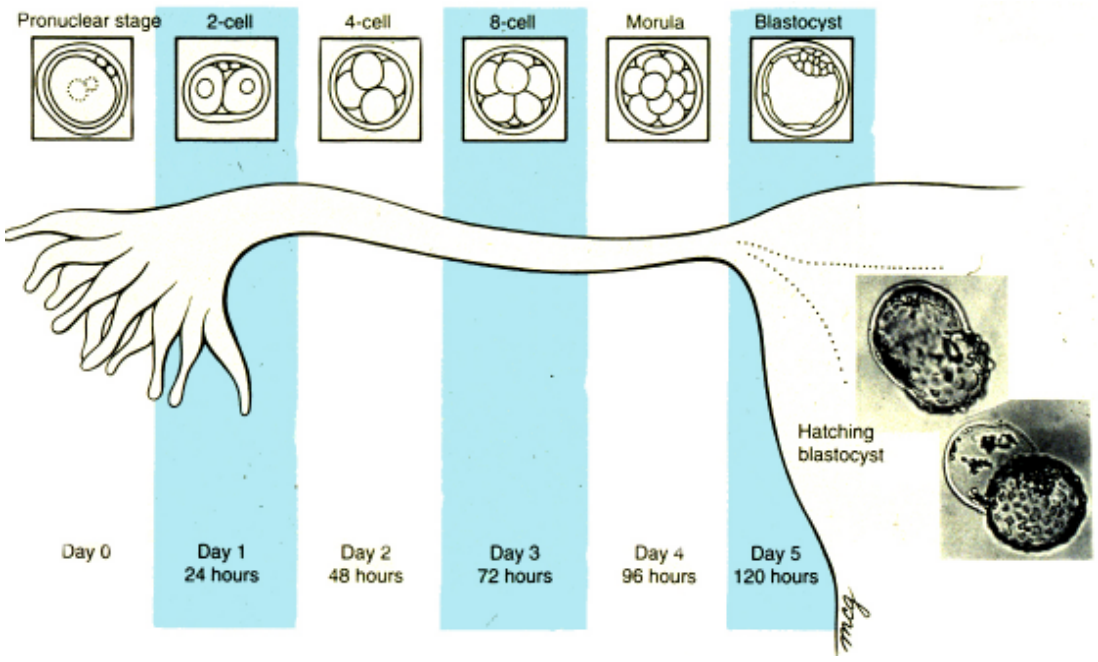

Fig. 2-1. Cleavage and transport down the oviduct. Fertilization occurs in the ampulla of th oviduct. During the first five days, the zygote undergoes cleavage as it travels down the oviduct and enters the uterus. On day 5, the blastocyst hatches from the zona pellucida and is then able to implant in the uterine endometrium.

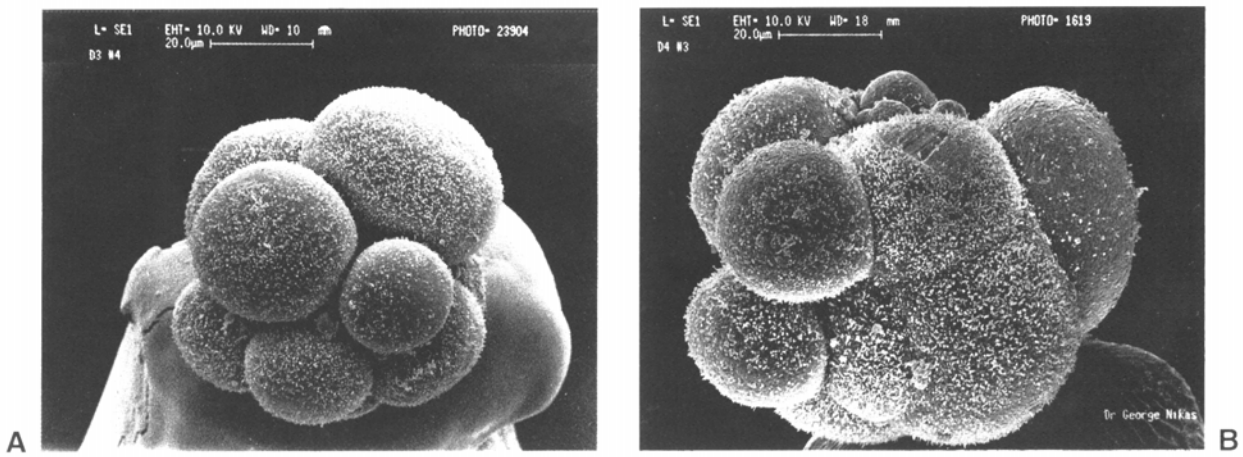

Fig. 2-2. Compaction. **(A)** Scanning electron micrograph of 10-cell human embryo before compaction. Note intercellular clefts. **(B)** Scanning electron micrograph of 10-cell human embryo during process of compaction. Note the absence of intercellular clefts between some of the blastomeres. The zona pellucida was mechanically removed from both embryos.

potency: description of the range of cell types that can arise from an individual cell.

totipotent: describes a single cell which is capable of making the whole embryo - a stem cell. During cleavage divisions there is a loss of potency with time. Stem cells retain high levels of potency. For example, the pluripotential stem cell in the bone marrow gives rise to all of the different kinds of blood cells.

commitment: Cells and tissues of the embryo receive inducing (decision making) signals that guide their fate. A cell or tissue is said to be **competent** if it can respond to such a signal. A cell or tissue is subsequently **committed** to a developmental fate even though no overt morphological change has occurred.

differentiation: overt morphological change that accompanies or follows commitment.

final differentiation: the last step in the development of a cell, resulting in a unipotential cell that will follow the same fate for the rest of its life.

Before compaction the inner faces of the blastomeres contact other blastomeres; the outer faces are exposed to the oviduct. Cells are therefore equally polarized vis-a-vis their environment and are essentially identical and replaceable. For genotyping of embryos for *in vitro* fertilization, single cells can be removed prior to compaction and PCR technology can be used to determine if genetic anomalies exist. The remaining cells will compensate for what is removed.

After the event of compaction cells are divided into an **inner** and **outer** set which have different fates. The inner cells have surfaces that touch only other blastomeres and outer cells have one of their surfaces touching the outer world. The process of compaction is mediated, in part, by the expression of E-cadherin, a Ca^{++} dependent cell adhesion molecule. Treatment of embryos with antibodies to E-cadherin will prevent compaction or, if it has already taken place, will cause decompaction.

Between the 8 – 16 cell stage, deposition of extracellular matrix (ECM) occurs. ECM doesn't just act as glue between cells but can bind growth factors and hence mediate signaling (see below).

Blastocyst Formation: Differentiation of 2 Cell Lineages

By the 16-32 cell stage, the embryo is called a **morula** (Latin, mulberry). The outer cells develop tight junctions which are fluid impermant. The outer cells begin to secrete fluid (using the energy of a Na+-K+ ATPase) which accumulates, forming the **blastocoele** or **blastocyst cavity**. The embryo is now called a **blastocyst** (mature by day 5).

By the 64 cell stage (3.5 days), the embryonic cells have differentiated (i.e., undergone overt morphological changes) into **TROPHECTODERM (TE)** and **INNER CELL MASS (ICM)**. The ICM consists of ~15 cells and will go on to develop into the embryo, yolk sac, amnion and contribute to a portion of the placenta. TE consists of ~45 cells and is derived from the outer cells of the blastocyst. It is divided into a polar cap above the ICM and a mural set/ embryonic pole population (Fig 2.3). The cells of the polar cap will remain diploid (**cytotrophoblasts**); those at the embryonic pole, following contact with the uterus form a multinucleate syncytium (**syncytiotrophoblasts**). (Fig. 2.4). The process of implantation and the roles of these two groups of cells will be discussed in a subsequent lecture.

At day 5, the blastocyst is still within the zona pellucida. Hatching (day 5) (Figure 2-1) is the release of the blastocyst from zona pellucida and subsequent increase in adhesivity that allows implantation (see subsequent lecture).

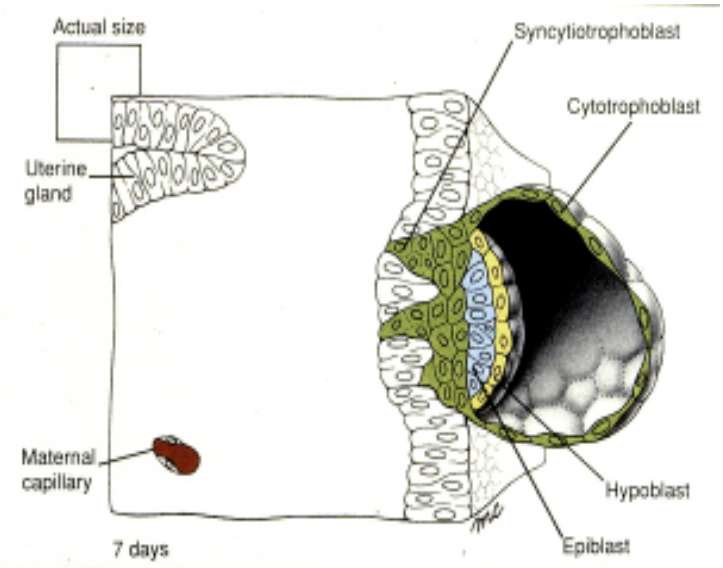

Fig. 2-3. At 7 days, the newly hatched blastocyst contacts the uterine endometrium and begins to implant. The trophoblasts at the embryonic pole of the blastocyst proliferates to form the invasive syncytiotrophoblast, which insinuates itself among the cells of the endometrium and begins to draw the blastocyst into the uterine wall. The germ disc is bilaminar, consisting of hypoblast and epiblast layers.

Formation of Bilaminar Embryo

Less is known about the divergence of cell types within the ICM in the final 24 hours prior to implantation. At this time there is a reorganization of ICM to produce a **bilaminar** embryo (Fig. 2-3). The ICM cells facing away from the blastocoel become the **columnar epiblast;** the layer facing blastocoel, becomes the **cuboidal hypoblast** (in modern developmental biology literature it is also called **visceral or primary endoderm)**. Exactly how the hypoblast forms in humans is not certain. Although not contributing directly to the embryo, it has important roles in establishing embryonic polarity. The distinction between these two derivatives of the ICM may arise from their original positiion within the blastocyst.

The amniotic cavity appears on day 8 as fluid collects between epiblastic cells facing the trophoblasts (Fig. 2-4, 2-5). The cells delaminate and differentiate into the amniotic epithelium (called amnioblasts in Larsen). This will eventually form the **amniotic membrane** and the **amniotic cavity** will surround the entire embryo/fetus (see Lecture 3).

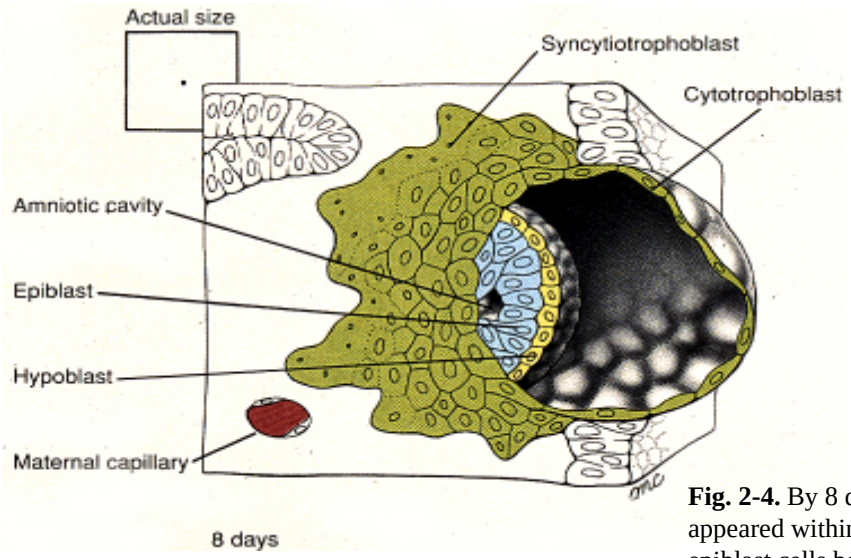

Fig. 2-4. By 8 days, the amniotic cavity has appeared within the epiblast, and some epiblast cells begin to differentiate into the amnioblasts that will form the amniotic membrane. Implantation continues, and the growing syncytiotrophoblast expands to cover more of the blastocyst.

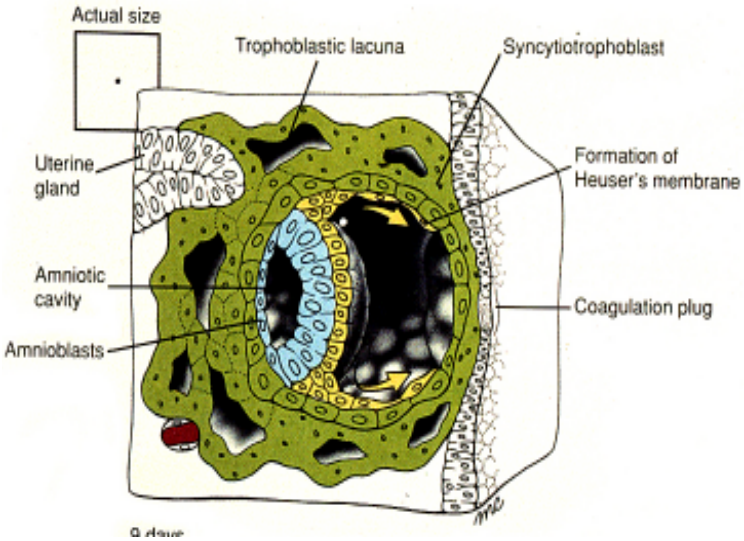

Fig. 2-5. By 9 days, the embryo is completely implanted in the uterine endometrium. The amniotic cavity is expanding, and the hypoblast has begun to proliferate and migrate out over the cytotrophoblast to form Heuser's membrane. Trophoblastic lacunae appear in the syncytiotrophoblast, which now completely surround the embryo. The point of implantation is marked by a transient coagulation plug in the endometrial surface.

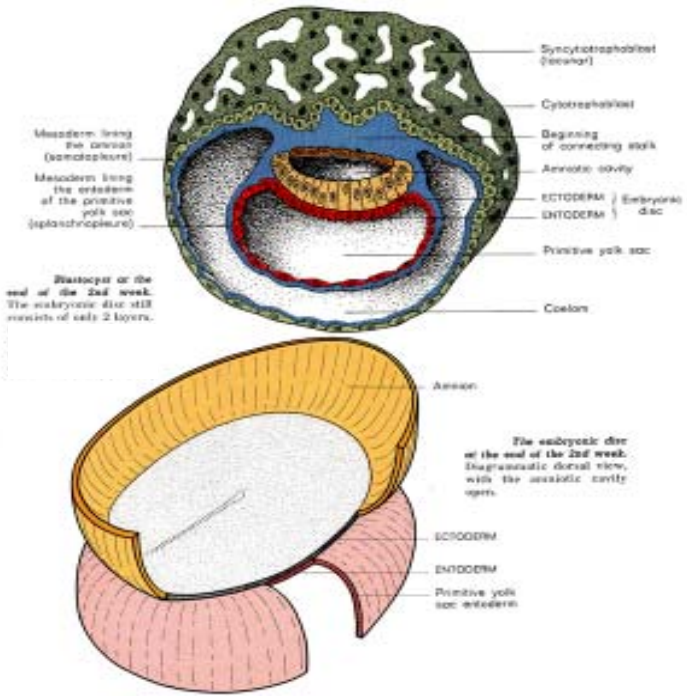

Fig. 2-6. View of the dorsal surface of the bilaminar germ disc through the sectioned amnion and yolk sac. The inset at the upper left shows the relation of the embryo to the wall of the chorionic cavity. The primitive streak, now one day old, occupies 50 percent of the length of the germ disc. The buccopharyngeal and cloacal membranes are present.

Formation of the Primitive Streak

Gastrulation begins on day 14. The first sign of gastrulation is illustrated in Figure 2-6. Cells move from the lateral aspect of the epiblast toward the midline, where they accumulate to form bilateral ridges with an indentation in the center (the primitive groove)(Fig. 2-7). This is the entry site into the space below the epiblast (the primitive pit). These structures constitute the primitive streak; the site of its formation marks the **posterior** pole of the embryo. Shortly after the formation of the streak, at what will be the cranial end of the animal, a special accumulation of cells is evident. This structure is the primitive node or just “node”(Henson’s node in avian species although Hensen named it in rabbit embyros!). Gastrulation is the movement of primitive streak cells over the ridges into the primitive grove and continued migration from the site of entry (Fig. 2-7; also see Fig. 2-9). These migratory cells form the primitive streak/node, endoderm and mesoderm. Those that remain behind are the ectoderm. The primitive streak defines the longitudinal axis and the primitive node defines the cranial end of the embryo. At this point in developmental time the embryo appears bilaterally symmetrical.

Growth of the Primitive Streak

The primitive streak initially elongates cranially. By day 18 the primitive streak begins a "retreat"(due to the more rapid growth of anterior structures) but cells continue to go through the node and streak. As the streak **elongates**, those cells entering the **node** will give rise to endoderm, the prechordal plate (head mesoderm) and chordamesoderm (the axial mesoderm = notochord). As the streak retreats, the **node** will continue to lay down the more caudal aspect of the notochord and cells passing through the **streak** will give rise to the remaining mesoderm of the body.

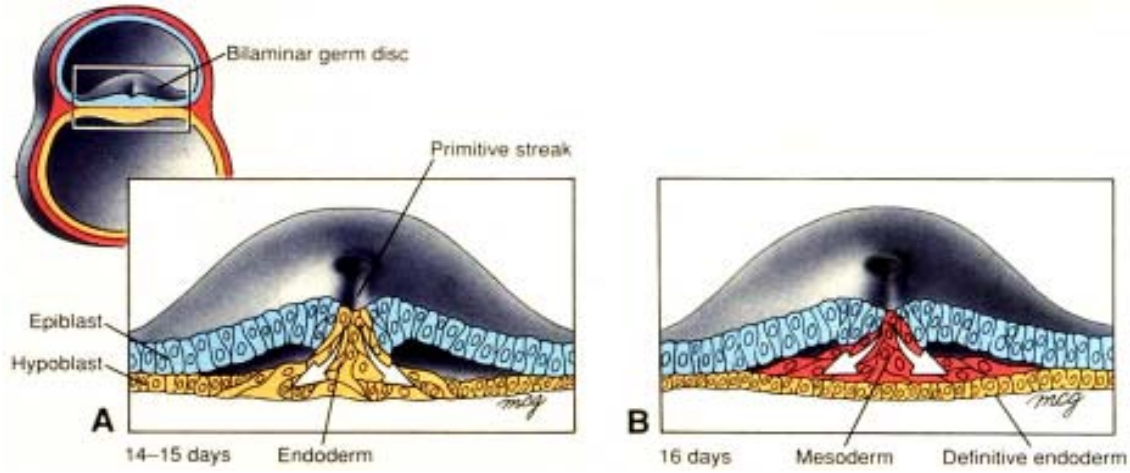

Fig. 2-7. Germ discs sectioned through the region of the primitive streak, showing gastrulation. **(A)** On days 14 and 15, the ingressing epiblast cells replace the hypoblast to form the definitive endoderm. **(B)** The epiblast that on day 16 ingresses and migrates between the endoderm and epiblast layers to form the intraembryonic mesoderm.

Two other structures to note in Figure 2-9 are the buccopharyngeal membrane, the future mouth, and the cloacal membrane, the future urinary and anal openings. At these two regions, no mesoderm is inserted between the overlying ectoderm and the underlying endoderm (at this early stage of embryogenesis).

Gastrulation begins with very few cells (~600) in the epiblast and this population expands enormously as gastrulation proceeds. Cell cycle times average 6hrs although some cells are cycling as fast as every 2hrs. The morphogenetic "movements" described below may reflect in part an increased population size. It is now well established that new cells enter the primitive streak to take up their migratory life.

Requirements for the Migration of Cells

Cells of the epiblast form an epithelium with junctional complexes and expression of adhesion molecules, particularly E-cadherin. Both junctions and adhesion proteins hold the cells together. There are also integrins (see SBPM/D) which mediate the interaction with the extracellular matrix. To migrate, the cells undergo de-epithelialization with the break-up of junctional

complexes and the down-regulation of expression of cell-cell adhesion molecules, particularly E-cadherin (or change in the type of adhesion molecule expressed as some are less adhesive and more "slippery"). There are also changes in the integrins expressed so that cells can interact with the different extracellular matrix molecules. The migrating cells produce the matrix molecules particularly hyaluronic acid, which has a large water shell. This gives individual cells the space to migrate. This is called an **epithelial to mesenchymal** transition. (Mesenchyme is a word for embryonic loose connective tissue, see SBPM/D.)

The Anterior visceral endoderm (AVE, Hypoblast): Role in primitive streak formation

Since the 1930's, it has been widely accepted that the directionality of the primitive streak is largely guided by the underlying hypoblast. Rotation **prior** to formation of the primitive streak results in the re-orientation of the primitive streak axis to follow that of the hypoblast. Rotation of the hypoblast by 90° at the **initiation** of primitive streak formation also results in the reorientation of the streak. Taken together these data suggest that the hypoblast provides **positional information** to the epiblast (by an unknown molecular mechanism).

If the hypoblast is destroyed (in particular, the more posterior aspect), the result is a disorganized primitive streak. If the destruction is extensive, the primitive streak may not form/re-form. These experiments suggested that the hypoblast **might** be necessary not only for the directionality of the primitive streak but also to induce (see discussion on induction below) the uncommitted (naive) epiblast cells to become the primitive streak cells (those that will migrate).

To Be Motile, or Not To Be Motile...

To test the hypothesis stated above the following experiments were performed.

(1) Label a sub-population of epiblast cells with an antibody-gold complex. The complex is **internalized** at 37°C.

(2) **The outcome**: all of the cells that migrate to the interior of the embryo contain gold particles. Those cells that are left behind never contain gold particles.

(3) **Can the cells the "stay-behind cells" be induced to migrate?** Label embryos at 4°C (no internalization). Warm up to 37°C and treat with complement (**protease** which lyses cells that have antibodies bound to their surface).

(4) **Outcome**: the migratory cells are killed. None of the lost cells are replaced; there is no primitive streak. **The two lineages had already been set aside.**

(5) **Interpretation**: Either the hypoblast cannot send a signal to the non-lysed epiblastic cells or the remaining cells can no longer interpret the signals (non-competent).

The molecular signals and signaling pathways that regulate morphogenetic movements are only now becoming understood.

Fate Maps

Construction of a fate map (what cells will become if left in their normal places) of the epiblast cells has been carried out by injecting the cells with vital dyes and tracing their descendants **(Fig. 2-8)**. The major principal established by these experiments is that the location of cells on the epiblast sheet can **predict** what they become. A fate map of the primitive streak shows that the time of migration and place along the streak from which cells "take off" are critical in the future determination of that cell's and its descendants phenotype.

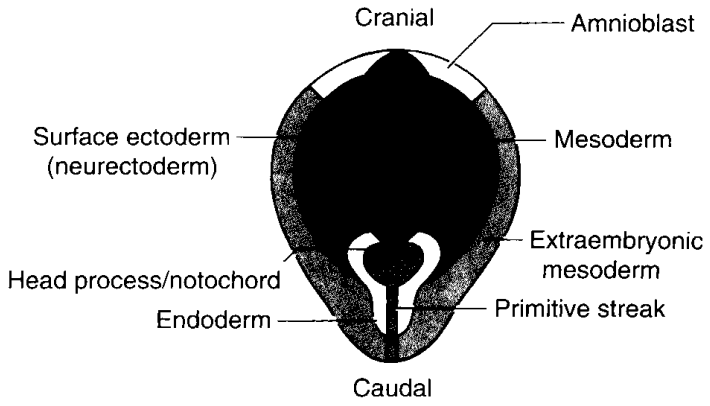

Fig. 2-8. Fate map of the epiblast of a mouse/embryo, showing the zones of epiblast that ingress through the primitive streak and form the major structures of the trilaminar germ disc. This map was deduced on the basis of cell lineage studies, in which epiblast cells were labeled with vital dyes.

Gastrulation movements: Outer cells that pass through the **node** displace the hypoblast and become the **(definitive) endoderm of the future foregut (Fig. 2-9).** The node also contains **progenitors** that form the prechordal plate and axial mesoderm = the notochord. The latter cells migrate cranially along the midline and stop at the buccopharyngeal membrane.

Cells entering the **primitive streak** immediately caudal to the node become the paraxial mesoderm and will form the somites (axial skeleton and all striated muscles) (see Lecture 5). Others from the same "district" migrate laterally and anteriorly, around the buccopharyngeal membrane. These will form the heart (arrow #2 in Fig 2.9).The cells along the more caudal aspect of the primitive streak migrate laterally to form intermediate and lateral mesoderm (see Lecture 5).

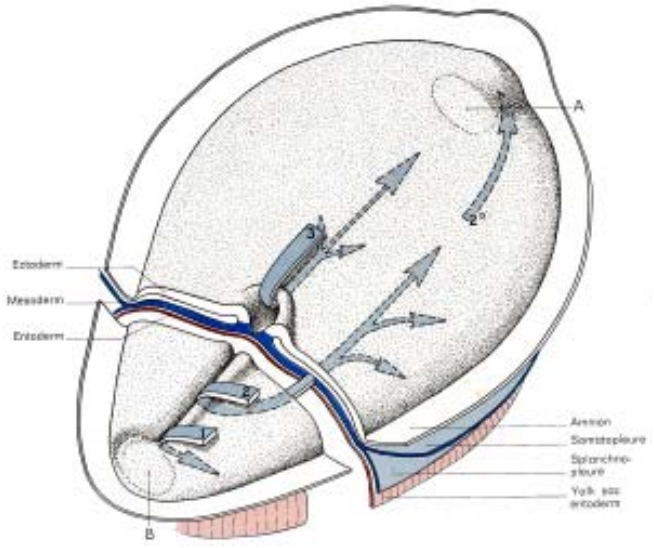

Fig. 2-9
General view of cell migration at the time of gastrulation. The arrows show the direction of ectodermal cell movements:

1: origin of mesoderm of caudal end
2: origin of lateral mesoderm
2a: part of the lateral mesoderm reaches the cephalic end
3: origin of notochordal substance

Letters **A** and **B** indicate two regions where mesoderm is not interposed between ectoderm and entoderm: these are the future pharyngeal (**A**) and cloacal (**B**) membranes.

It is important to understand the temporal changes occurring in the primitive streak. For example, the time period during which the primitive node contains endoderm precursors is very brief (a few hours in the chick embryo). Similarly, it "runs out" of heart progenitors. On the other hand, precursors for the paraxial mesoderm and lateral plate mesoderm persist for the life of the streak.

Extraembryonic Mesoderm

The origin of the extraembryonic mesoderm is still controversial. Prior to primitive streak formation it is thought to come from proliferating epiblast. During gastrulation it is thought that cells that enter the most caudal end of the primitive streak develop into the extraembryonic mesoderm.

Induction

During induction a cell, or set of cells, emits a signal which alters the fate and differentiation pathway of the cells that receive the signal. Induction implies both the **signal** from the inducer and the **competence** on the part of the receiver to respond to the signal. The nature of a signal from a particular cell group can vary over time, and the competence of the responding cells can be altered or lost. In some instances the signaling molecule is "the instruction", while at other times the absence or blockade of a signaling molecule is "the instruction". You will see this in Lecture 4 where receipt of a signal(s) induces the ectoderm to become epidermis while blockade of that signal allows the ectoderm to follow its "default pathway" and become neuronal.

There are two general mechanisms for induction (not mutually exclusive):

1) The signal is a **secreted molecule** (or combination of molecules) for which the responding cell has a receptor. The signal is called a **morphogen** and in many instances a morphogen gradient is established. Based on the morphogen concentration, the responding cells will have different developmental fates. Thus a single signal secreted by a tissue can induce cells to follow different fates depending on their distance from the tissue.

2) Appositional induction requires cell-to-cell contact between the inducing cells and the receiving cells.

Axial Patterning

In experiments on amphibia, Spemann first delineated the concept of induction in 1918. Using an amphibian model he showed that ectodermal cells fated to become epidermis could take on a new fate (become neuronal) if they are transplanted early in gastrulation to the appropriate site. They had the competence to respond to "neuralizing" signals. If however the same experiment is performed but at a later stage of gastrulation, the transplanted cells are no longer competent to become "neural"; they are already committed to become "epidermal".

Further studies on induction were conducted in the 1920's by Spemann and Mangold (Spemann later received the Nobel Prize for this work). They studied the role of the dorsal lip of the blastopore (DLB) (the amphibian homologue of the **node**) in axis formation.

They transplanted a donor DLB (newt species A) onto an ectopic site on a host embryo (newt species B) of the same developmental age (beginning of gastrulation). The embryo with two blastopores developed into a chimeric newt with two <u>complete</u> body axes including two heads/brains (Fig 2-10). On the side with the donor DLB, the tissues forming the additional body

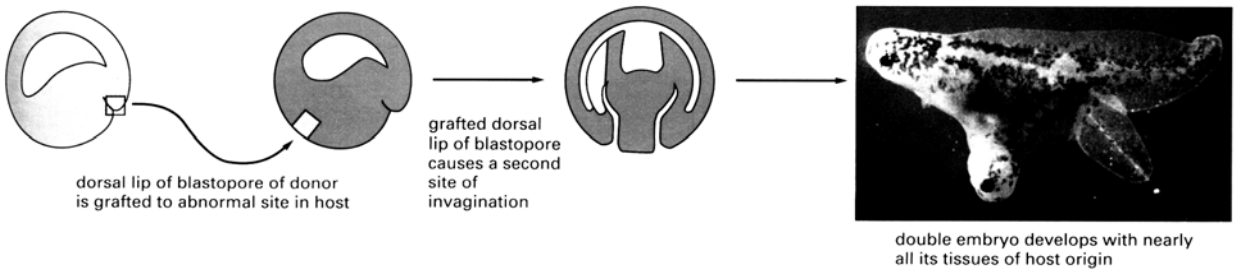

Fig. 2-10. The role of the Organizer. Diagram of an experiment showing that the dorsal lip of the blastopore (Spemann's Organizer) initiates and controls the movements of gastrulation and thereby, if transplanted, organizes the formation of a second set of body structures. The photograph shows a two-headed, two-tailed axolotl tadpole resulting from such an operation; the results are similar for *Xenopus*.

axis were **derived from the host**! Hence the transplanted DLB could change the fate of host cells and the host cells were competent to respond. (Note: The dorsal lip is also known as "Spemann's Organizer.")

Inductive capacity can be altered over time. If the donor DLB is derived from an older (mid-gastrulation) embryo, the 2nd body axis is incomplete (only caudal/tail regions will be respecified). Hence in the amphibian the DLB emits (at least) two different sets of signals in a time dependent fashion - first an anterior signal resulting in head formation (including anterior brain structures) and caudalizing signal resulting in hindbrain and trunk structures.

Similar experiments have now been repeated in mouse. Rosa Beddington and her colleagues in England carried out very elegant work and transplanted the primitive node to ectopic locations during gastrulation. The 2nd node was a true organizer in that it **could induce a second axis** but **only posterior** structures.

Anterior structures. Additional mouse experiments showed that transplantation of the node, anterior epiblast and anterior visceral endoderm (AVE, hypoblast) were all required for induction of anterior structures. Even more recent studies suggest that the AVE is prepatterned before primitive streak formation (for a review of the molecular mechanisms that may be involved in this process [see Lu et al., 2001 Current Opinion in Genes and Development 11:384-392].

Some caution has to be exercised in accepting this mechanism as being true for all mammals as the spatial arranagements for implantation and gastrulation are very different in mice compared to primates. We gastrulate more like an avian embryo.

Dorsal-Ventral Axis

The notochord is, in mammals, the equivalent of dorsal mesoderm in other vertebrates and the lateral plate mesoderm to ventral mesoderm (you will see this in Lecture 3). Hence the primitive streak also marks the dorso-ventral axis. The future ventral side of the embryo is defined in part by the position of the hypoblast in contact with the blastocyst fluid and the future

dorsal side by the ICM (Inner Cell Mass) in contact with the trophoblasts.

Right-left axis

During early organogenesis, the laterality of the body is revealed by the looping of the heart (see Lectures 6 and 7) and rotation of the body axis as well as the asymmetric expression of genes on the left side of the embryo. Without the node, expression of specific "left" genes and distribution of organs (e.g., looping of the heart, *are* randomized (as it is in **situs inversus**).

In mammals (the mouse!) the initial establishment of handedness depends on the formation of motile cilia in cells at the node. The cilia beat counter-clockwise and cause the flow of fluid in the yolk sac to move from right to left. A mutation in the dynein motor of these cilia results in randomization of organ placement (e.g., heart on wrong side) (gene is called *situs inversus viscerum, iv)* versus the normal condition (*situs solitus*).

The secretion of FGF8 from the primitive node and streak and the leftward movement of the ciliary beat restrict the expression of the gene *Nodal*, a secreted signaling molecule, to the left side of the lateral plate mesoderm (Nodal is expressed asymmetrically in all vertebrate classes). After induction of the neural plate (see Lecture 4), *Nodal* and *Lefty -2* continue to be constrained (by the secretion of FGF8) to the left quardrant of the lateral plate mesoderm. *Lefty-1* is similarly constrained to ventral left side of the neural tube. The downstream mechanisms leading to sidedness in humans is still an active area of investigation. Errors in right-left patterning occurs

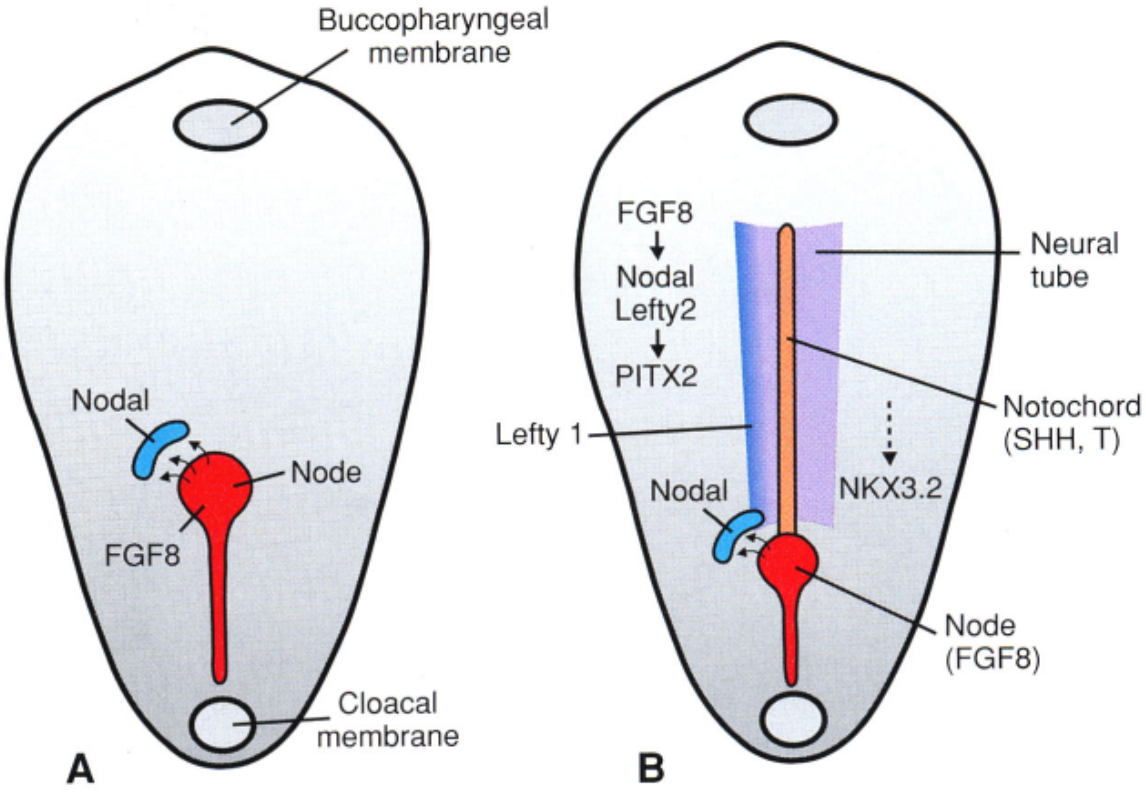

Fig. 2-11

in ~ 1 in 10,000 human births. (See Supp et al, 1998 Cell and Developmental Biology 9:77-87 if interested in more information on genetics of human handedness mutations and clinical outcomes.)

Overview of the Embryo at the End of Gastrulation

There are now three layers:

1) Ectoderm: Its midline portion will become the nervous system (including the retina) and the placodes and their neural and non-neural derivatives (see Lecture 21). The rest of it will become the epidermis.

2) Mesoderm: which is subdivided into four zones: the midline notochord, paraxial somites, the intermediate mesoderm, and the body wall/lateral plate mesoderm which includes heart.

3) Definitive Endoderm: There is no gut yet. Formation of the gut occurs by the folding of the lateral plate mesoderm (see Lectures 3 and 18).

4) The embryonic tissue is still in contact with extra-embryonic tissue. In the next lecture, we will fold the trilaminar disc, thereby creating the body cavities and reducing contact to the connecting stalk = the future umbilical cord.