

Lecture 1 - How does a single-celled fertilized egg develop into a complex adult? Early embryology, Germ Layers, Embryonic Induction and Cell Differentiation

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Recommended reading: Langman's Medical Embryology, 13th Ed., by T. W. Sadler, 2012 (Lippincott Williams & Wilkins). Previous editions are fine as well. This short book will be useful throughout your medical studies. We will cover Chapters 3 (p. 37-41), Chapters 4, 5 and 6. It would be very helpful if you could leaf lightly through the book before this lecture as there is a new vocabulary to be learned. If not, at least read through these outlines as there are many key words to be learned. If that is still too much, you might prepare by watching these three animations:

<https://www.youtube.com/watch?v=3AOoikTEfeo>

<https://www.youtube.com/watch?v=IGLexQR9xGs>

<https://www.youtube.com/watch?v=yXUv4MPuNTA>

All we want you to remember are the figures shown in the PowerPoint presentation of these first two lectures. Most are from Langman's. The Embryology lab is designed to drive home the outline of Medical Embryology presented here. We will start at the beginning – fertilization – and take you through a general tour of human anatomical development.

Lecture objectives

- To examine how a multitude of differentiated cell types arise from a single egg cell. Will provide a general overview of how the human body is formed.
- To understand how the germ layers – **ectoderm**, **mesoderm** and **endoderm** – are formed and how the main organs are laid out in this body plan. You only need to understand the figures provided.
- To examine how embryonic stem cells form tissues in the embryo.
- To analyze how embryonic development is coordinated with maternal physiology.
- To examine the pathologies that can arise from malfunction at each of these steps of human development through clinical correlations.

Key words:

- **Ectoderm:** external germ layer that gives rise to skin and its derivatives (hair, nails, mammary gland), the central nervous system (CNS), and the neural crest (melanocytes, sympathetic ganglia, adrenal medulla, sensory ganglia of cranial and spinal nerves, pharyngeal arches, and bones of the cranium).
- **Mesoderm:** middle germ layer that gives rise to notochord, muscles, skeleton,

- dermis, kidneys, connective tissue and blood.
- **Endoderm:** internal germ layer that gives rise to the epithelium of the gastrointestinal tract and its derivatives (thyroid, trachea, bronchi, lungs, liver, pancreas).
- **Gastrulation:** coordinated movements of cells in the embryo (**morphogenetic movements**) that generate the three germ layers.
- **Stem cells:** cells that can self-renew in the proper conditions (e.g., when a correct “**niche**” of surrounding cells is provided) and can give rise to differentiated cells via asymmetric divisions.
- **Epithelial-Mesenchymal Transition (EMT).** In **epithelia**, cells are tightly attached to each other by cell-cell junctions (such as **adherens junctions** mediated by cadherin cell adhesion proteins), forming cell sheets. Epithelial cell sheets provide **barriers** between body **compartments**. In **mesenchyme**, individual cells attach to a network of extracellular matrix proteins (such as collagens and fibronectin) and are able to **migrate individually**. At certain points in development, epithelial cells can disperse to become mesenchyme by downregulation of cell adhesion proteins called cadherins (e.g., formation of mesoderm and neural crest) and vice-versa (e.g., formation of mesothelial (serous) membranes such as peritoneum). Epithelial-Mesenchymal Transition (EMT) plays a key role in metastases.

Overview:

Cell differentiation

- The vertebrate body results from coordinated cell movements of cell layers. By the end of gastrulation an embryo with stereotypical antero-posterior (A-P) and dorso-ventral (D-V) polarity is formed (movie and one slide).
- The **differentiated state is stable**. Decisions taken at gastrulation will last a lifetime and determine human anatomy (slide).
- Today is dedicated to a week-by-week overview of human development. The main tissue and organ types are determined during embryogenesis.
- Medical embryology allows you to understand the anatomical basis of the human body and is part of your Medical boards.
- We will examine in some depth the **induction** of cell differentiation by cell-cell signals from **organizing centers** in the embryo.

MEDICAL EMBRYOLOGY

1. THE FIRST WEEK

Fertilization – takes place in the uterine (Fallopian) tubes. **Corona radiata** cells are digested. **Zona pellucida** (transparent zone) is penetrated (Langman's Fig. 3.6).

Cleavage – divisions without intervening cell growth. Langman's Fig. 3.8.

Morula stage – transit through the uterine tube, entry into the uterus at 3 days. Langman's Figs. 3.10, 4.1 and 3.11.

Blastocyst stage and implantation – Fluid accumulates forming the **inner cell mass (ICM, or embryoblast)** and **trophoblast** (slide of 5-day human blastocyst). **In vitro fertilization (IVF)** embryos are implanted into the uterus of women at this stage. The culture methods for embryos were pioneered by Dr. Robert Edwards of Cambridge, England. He received the 2010 Nobel Prize for Medicine for this procedure. Since 1978, four million IVF babies have been born worldwide. Attachment to endometrial epithelium. **Implantation** at the 6th day; the uterine endometrium is invaded by trophoblast, which provides nutrition to the embryo (Langman's Figs. 3.10, 4.1 and 3.11).

- Cells of the inner cell mass of the mouse blastocyst are **pluripotent**. When transplanted into the blastocyst cavity of another embryo, these cells can give rise to all embryonic tissues, including the germ line (slide).
- If cultured in the appropriate conditions, inner cell mass cells can be induced to proliferate without loss of their potency. These are called **embryonic stem (ES)** cell lines. Stem cells are called **pluripotent** because they can give rise to differentiated tissues of ectodermal, mesodermal and endodermal origins.
- The egg cytoplasm has the remarkable ability to reprogram even differentiated cell nuclei to generate an entire embryo. The 1964 **nuclear transplantation** experiments by Sir John Gurdon in the frog *Xenopus* had the important implication that genes are not lost during cell differentiation, just turned off or on during development (slide).
- The 2012 Nobel Prize in Medicine was awarded for **nuclear reprogramming** of adult cells into embryonic stem cells (slide). Sir John Gurdon, who was your teacher's teacher, showed that egg cytoplasm could reprogram differentiated (intestinal epithelial) nuclei giving rise to fertile frogs. Later nuclear transfer

experiments gave rise to cloned mammals from mammalian fibroblasts from the adult. Dr. Shinya Yamanaka showed that introducing four transcription factors is sufficient to generate **induced pluripotent cells (iPS cells)** from skin fibroblasts of mice and humans, as will be explained by Dr. Kathrin Plath.

Coordinating the mother's physiology with embryonic development - at implantation: normal **menstrual cycle**, **Follicle Stimulating Hormone (FSH)**, **Estrogen**, **Luteinizing hormone (LH)**, **Progesterone** production by **Corpus Luteum**, **Human Chorionic Gonadotrophin (HCG)** secreted by the trophoblast maintains the corpus luteum, Corpus Luteum of pregnancy (required for first 3 months of pregnancy). The embryo hijacks, via HCG, the mother's physiology. **Pregnancy tests** detect HCG (Langman's Figs. 3.4, 3.11-13).

Clinical correlation: ectopic pregnancies. Langman's Figs. 4.8, 4.9. Presents as young woman with intense abdominal pains, missed one or two periods, sometimes genital bleeding (and HCG positive).

2. THE SECOND WEEK

The Bilaminar embryonic disc

- The flattened embryonic disc consists of: **epiblast** and **hypoblast**. All cells of the adult body derive from the epiblast epithelium. Formation of the **amniotic cavity** and the **primitive yolk sac**. Langman's Figs. 4.3, 4.4.
- The trophoblast differentiates into **syncytiotrophoblast (multinucleated cells)** and **cytotrophoblast**. The syncytiotrophoblast secretes HCG, maintaining the corpus luteum of pregnancy. Formation of lacunae in the syncytiotrophoblast, connection to maternal capillaries (called sinusoids).
- The second week of development is the **week of twos**: A) the epiblast and hypoblast are formed; B) the trophoblast forms syncytio- and cyto-trophoblast; C) two embryonic cavities, the amnion and the primitive yolk sac, are formed.
- The first menstrual period is missed at the end of 2nd week.

3. THE THIRD WEEK: Gastrulation

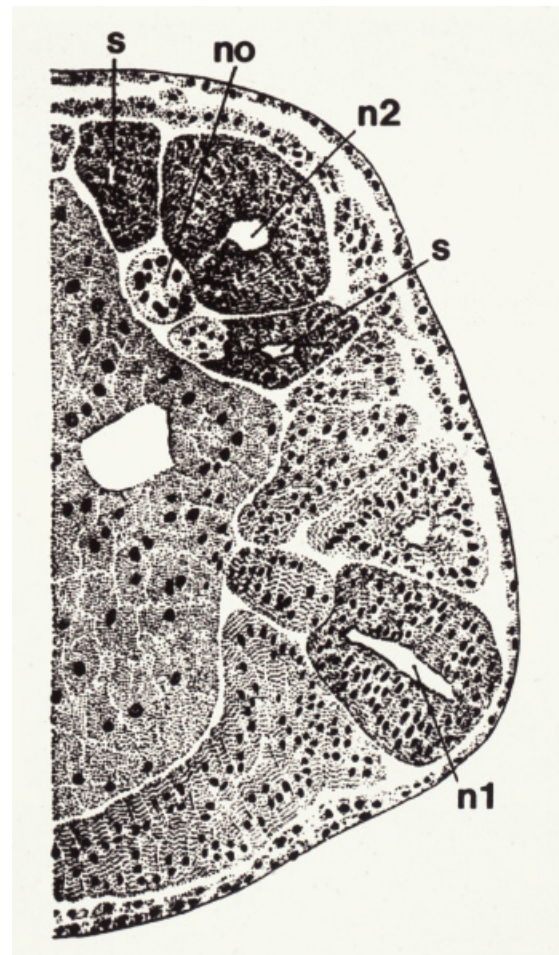
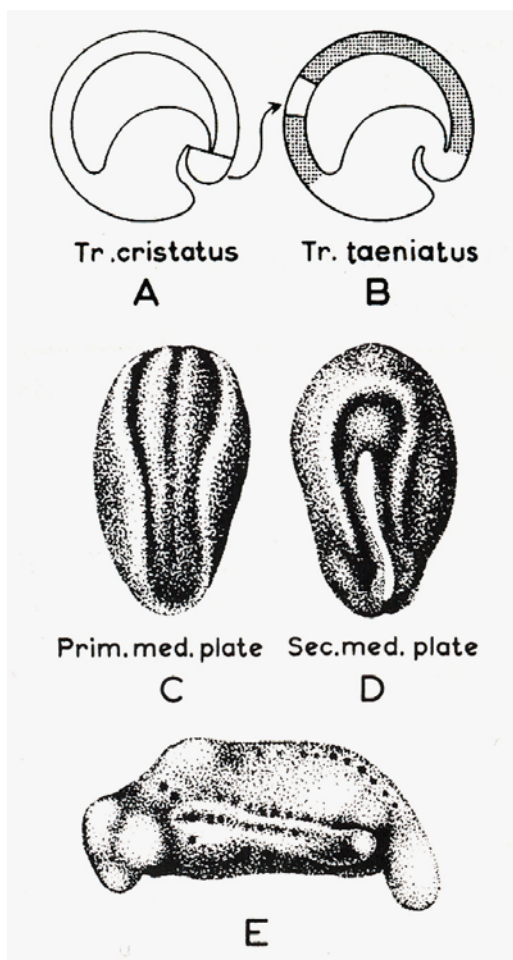
"It is not birth, marriage or death, but gastrulation, which is truly the most important time in your life". Lewis Wolpert, 1986.

Gastrulation results in the formation of the three germ layers: mesoderm,

endoderm and ectoderm. The **morphogenetic movements** of these cell layers shape the body plan and occur only once in your lifetime. Gastrulation is the period when life-long tissue differentiations are chosen. Conjoined or Siamese twins arise during gastrulation (Langman's Fig. 5-5). An "**organizing center**" controls gastrulation. This was discovered in the amphibian embryo in an experiment that showed that cells located in the dorsal blastopore can **induce** changes in the cell differentiation of neighboring cells.

Embryonic Induction:

- Spemann's transplantation experiment (1924) in the amphibian embryo demonstrated that vertebrate development results from a series of cell-cell inductions. A small group of cells (called the organizer center) induces a neural plate and dorsal mesoderm in neighboring cells (slide). Cell differentiation can be triggered by protein factors released by small regions of the embryo. This discovery of **embryonic induction** earned Spemann the 1935 Nobel Prize for Medicine (movie).
- Many other organizing (signaling) centers function at other stages of development (such as the midbrain-hindbrain **isthmus organizer**).
- A demonstration of frog gastrulation and the organizer experiment will be offered in Embryology Labs.



- In humans, the **primitive streak** (the equivalent of the amphibian blastopore) forms in posterior epiblast (Langman's Fig. 6.1). An **epithelial-mesenchymal transition (EMT)** causes delamination from epiblast epithelium. Epiblast cells dissociate from the epithelium and migrate inwards (invaginate) through the primitive streak midline. Cells that migrate internally give rise to the **mesoderm** and definitive **endoderm**. The hypoblast does not contribute to the embryo itself and is replaced by **definitive endoderm** cells from the primitive streak. The epiblast that remains on the outside surface becomes the **ectoderm** (Langman's Fig. 5.2).
- **The primitive node** forms at the anterior of the primitive streak and is a source of **inducing signals** such as **Chordin** (discovered by us) and **Noggin** (discovered by Harland at Berkeley) that induce the **neural plate**. The node is the mammalian equivalent of Spemann's organizing center of other organisms.
- The mesoderm has a stereotypic dorsal-ventral organization, which is generated at the primitive streak (Langman's Fig. 6.8). **Mesoderm organization** is a useful concept that you should grasp (slide), because it explains the architecture of the human body and its organs:
 - 1) **Axial mesoderm** or **notochord** is the dorsal-most mesoderm and is located in the midline.
 - 2) **paraxial mesoderm** (future somite) is formed to the side of the notochord.
 - 3) **intermediate mesoderm** (future kidney and urogenital ducts).
 - 4) **lateral plate mesoderm** (future body wall and gut mesoderm) located in the ventral-most region.
 - 5) **extraembryonic mesoderm**.
- Mesodermal cells invaginate along the primitive streak midline. Anterior primitive streak generates dorsal mesoderm and posterior primitive streak generates ventral mesoderm (I will explain this in the slide provided).
- At later stages the primitive streak becomes progressively shorter until all endoderm and mesoderm has invaginated, and then it disappears. Gastrulation of posterior mesoderm continues into the fourth week, when the primitive streak disappears.

Clinical correlations: Gastrulation defects. Posterior remnants of the primitive streak give rise to **sacroccocygeal teratomas**, the most common tumor in neonates (Langman's Fig. 5.9). **Sirenomelia or caudal dysgenesis:** fusion of lower limbs

due to lack of ventral mesoderm in posterior primitive streak, causing mermaid-like appearance (slide).

Twinning:

- In some cases two gastrula organizing centers are formed, causing the formation of conjoined (Siamese) twins. Siamese twins are always **monozygotic** (identical) as opposed to **dizygotic** (two-egg or fraternal) twins. The incidence of identical twins is 3 in 1000 births, compared to 7 in 1000 births for fraternal twins.
- Identical twins result from separation of early embryonic cells (Langman's Fig. 8.19), for example: A) at the 2-cell stage (resulting in 2 placentas and 2 amniotic cavities), but this is so infrequent that in practice this pattern is not seen in identical twins and is generally considered diagnostic of fraternal twins; B) splitting at the inner cell mass stage before the amnion is formed (one placenta and two amniotic cavities, the most common type of identical twins); C) or separation during gastrulation (one placenta and one amnion). The latter case includes all conjoined twins, which fail to separate completely. By examining the fetal membranes at birth (or by ultrasound), one can deduce the stage of development at which monozygotic twinning occurred.
- Identical twins provide the best illustration of the **self-organizing** nature of vertebrate development. In frogs, if a blastula is cut in half, one can obtain identical twins (slide). There are many checks and balances in cell-cell communication to ensure that a perfectly proportioned baby is formed time after time.

Conclusion: In this bird's eye view of the first three weeks we have learned that:

- 1) The blastocyst contains embryonic stem cells.
- 2) The trophoblast directs the physiology of the mother via HCG.
- 3) The entire adult body derives from epiblast epithelium.
- 4) Organizer centers induce new cell differentiations on neighboring cells, as in Central Nervous System (CNS) induction by the primitive node organizer.
- 5) An invariant body pattern is generated during gastrulation.