Cleavage and Gastrulation in Echinoderms and Amphibians

Cleavage is a series of mitotic cell divisions in which the large cytoplasmic volume of the fertilized egg is reduced in a step-like manner into smaller, nucleated cells. The process of nuclear division is called karyokinesis and the process of cytoplasmic division is called cytokinesis. Karyokinesis and cytokinesis are usually, but not always, tightly coupled. Cleavage-stage cells are called blastomeres.

Eggs of different species differ in the amount and position of yolk they contain. This affects the pattern of cleavage divisions in the embryo. Features of the cleavage pattern that can be readily observed are (1) the patterns of symmetry and placement of cleavage furrows that separate blastomeres, and (2) the degree of completeness of cytokinesis. Complete cytokinesis is called holoblastic cleavage; incomplete cytokinesis is called meroblastic cleavage. During cleavage, the embryo does not grow, nor do cells change positions with their neighbors.

After cleavage is completed, embryos undergo the process of gastrulation in which growth or increase in body size begins. Gastrulation is accomplished by cell movements which provide new opportunities for cell-to-cell interactions and serve to rearrange the positions of cells. This process produces the three germ layers (ectoderm, mesoderm and endoderm), and the axes of the embryo become fixed.

You are going to examine cleavage and the beginning of gastrulation in two holoblastically cleaving species, the starfish and the frog, *Rana pipiens*.

Cleavage and Gastrulation in Echinoderms (Starfish)

As echinoderms, the starfish and the sea urchin have many characteristics in common. One of these is embryogenesis, or the manner in which embryos form and assume their typical anatomy.

Examine the two prepared **Starfish embryology** slides in your collection. One has a collection of whole embryos (W.M. = whole mount); the other contains sectioned embryos. Following the description of the developmental sequence below, find several examples of each stage. Compare your embryos to those pictured in the atlas. Since the embryos are mixed together on the whole mount slide, it would help if you drew embryos at the different stages described below.

Cleavage stages:

1. Cleavages of echinoderm embryos are complete (holoblastic). The first few cleavages are equal, producing blastomeres of similar size. Can you find embryos at the 2, 4, and 8 cell stages? Are the cells the same size?
2. By the 16-cell stage, the blastomeres are of three different sizes: middle-sized mesomeres (forming the animal pole), the large macromeres (forming in the middle); and the small micromeres (forming the cells at the base of the vegetal pole). Can you find embryos with these variations in cell size?
3. The solid ball of cells begins to hollow out at the center, forming a fluid-filled cavity called the blastula cavity or blastocoel. This hollow ball of cells becomes some 1000-2000 cells strong while the overall size of the embryo remains virtually the same.
    - Directly compare embryos at the early stages of cleavage with one at this late stage to see if the overall size remains constant. If your microscope has an eyepiece reticle (measuring bar), use it to quantify the size of the two stages.
    - Compare the diameter of a single blastomere in the one - four-cell embryo (using your eyepiece reticle,, if present) and compare it with the diameter of cells at the final stages of cleavag.
4. After “hatching” from its fertilization membrane, the blastula settles on its vegetal- pole side which flattens into a vegetal plate. Your slides should contain some embryos that were fixed onto the slide “sideways” so this flattened vegetal plate is visible.

Gastrulation stages: see what you can observe; the details will be covered in the next class:

1. The first indication you will see of gastrulation is the "indentation" of some cells into the interior cavity. This happens at the vegetal plate. The first cells that enter the cavity are the micromere descendants. They may not be distinct. They form part of the middle germ layer (the mesoderm).
2. The cells that you WILL see clearly moving into the interior do so as a sheet of cells. These are the macromere descendants. They invaginate as a sheet of cells, forming a deep, narrow pocket, the archenteron (or primitive gut tube). This layer will become the inner or endoderm germ layer. As the archenteron grows into the blastocoel, cells on its leading end (secondary mesenchyme) send out extensions (filopodia) that contact the far animal pole wall and establish connections with it. This contact breaks down to create the second opening of the gut, or the mouth. (Why does this make echinoderms deuterostomes?) What does this make the first opening formed by the original archenteron ingrowth?
3. At the end of gastrulation, the embryo consists of an outer cell layer of cells formed largely from the original animal pole mesomere cells (the ectoderm germ layer), which grows a band or tuft of cilia for locomotion that may be visible, an inner cell layer (the archenteron) formed by the invaginated vegetal plate cells, and a middle layer (mesoderm) in between the other two.

Larval stage: The starfish embryo develops into a larva called a bipinnaria. The corresponding larve in the sea urchin is shaped differently and is called a pluteus.

Cleavage and Gastrulation in Amphibians (Frog *Rana pipiens*)

[Adapted from "Structure and Development of the Vertebrates" by F. Moog and M. Krukowski.]

I. Frog early cleavage, late cleavage and blastula slides

The mature frog's egg is large - one to two millimeters in diameter. Its relatively large size is due to the considerable amount of yolk it contains. In this and many other species, two-thirds of the egg is generally covered by pigment, yellow to black in color, which centers around the animal pole. The other one-third, centered around the vegetal pole, appears cream-colored. Surrounding the egg is a noncellular and nonliving vitelline membrane and a thick jelly coat. Most of the jelly coat has been removed during histological processing. The yolk, which occurs in the form of massive platelets, is concentrated in the vegetal half of the egg. The weight of the yolk platelets causes the egg to float with the vegetal pole downward. You can see these egg structures in your microscope slides labeled **Frog early cleavage** and **Frog late cleavage.** Each contains just a few sections through these stages.

Early Cleavage in *rana pipiens*

Examination of these slides will prepare you for observing the development of living salamander embryos, which are scheduled to arrive for next week's lab. Your early cleavage stage slide does not include a two-cell egg, but the other early cleavages should give you some insight into the first cleavage.

Within an hour after fertilization the pigment shifts upward on one side, leaving a cleared grey crescent extending about halfway around the egg at its "equator" (midway between animal and vegetal poles). Some of the early cleavage slides may have traces of the grey crescent. Does yours? Later the grey crescent disappears, but the local differentiation which it represents persists: gastrulation usually begins at the midpoint of the grey crescent area, and this point establishes th future dorsoventral axis, and the bilateral symmetry of the embryo,.

The first cleavage begins two hours or more after fertilization, depending on temperature and species. The two cells resulting from the division are each called blastomeres. The blastomeres may be outlined with dark pigment which is carried into the egg by the advancing cleavage furrow. The first cleavage furrow starts to form at the animal pole, and runs towards the vegetal pols. It cuts rapidly through the highly cytoplasmic animal half of the egg but pushes laboriously through the vegetal half, indicating that the mass of inert yolk offers great resistance to the advancing partition.

The second cleavage, which is also vertical and begins at the animal pole, runs at right angles to the first,. It begins before the first cleavage plane has reached the vegetal pole within one hour of the first at laboratory temperatures. The pigmented outlines of blastomeres will show clearly that the cleavage furrows do not penetrate the vegetal hemisphere within the egg and that the furrows are rather superficial. (Even though cytokinesis is temporarily incomplete in the vegetal hemisphere, the amphibian cleavage is considered holoblastic and not meroblastic because cytokinesis is completed within several rounds of cell division. Fully separate blastomeres at early cleavage stages are found only in the animal hemisphere.)

The third cleavage is horizontal and cuts above the equator, so that the upper quartet of cells is smaller than the lower. The fourth cleavage is again vertical, the fifth horizontal. Later cleavages are more irregular.

Make a drawing of the sections showing the early cleavage blastomeres. Label the animal and vegetal poles of the embryo and draw the relative sizes of the blastomeres.

Later cleavage stages in *rana pipiens*

These sectioned embryos may be from 8- to 32-cell stages and the sections will be in various planes. It is possible to determine the plane of each section by means of the pigment distribution. Obviously no single section can include all of the cells, but it will be possible to estimate the approximate stage by comparing the cell sizes with the previous one. Note that the better formed blastomeres are always in the animal hemisphere, but that cleavage furrows now appear in the vegetal hemisphere. What are the differences in size between animal pole and vegetal pole cells at this stage?

During the first cleavage divisions the egg remains fairly solid, but in later cleavage a cavity or blastocoel appears as the small cells of the animal region push out to the surface; this shift probably has its basis in a need for more oxygen on the part of the active cells within the mass. The blastocoel remains slightly above the center of the egg into gastrulation. The yolk-laden cells of the vegetal half are too sluggish to remove themselves from the center of the egg, so that the blastocoel of the amphibian is limited to the animal half of the egg.

Make a drawing of your slide showing later cleavage stages. How far does the pigment penetrate into the interior in each?

Blastula stage slide: the end of cleavage: only one embryo section is included on this slide

As the cleavage stage ends, the blastula cavity becomes as large as it will be, and the embryo as a whole is called a blastula stage embryo. The egg does not enlarge at all during cleavage, so that the effect of the process is to reduce the single giant cell to a multitude of tiny cells. Note how small the cells are compared to those in the previous slides. Compare cells at this stage with early cleavage stages and note the dramatic differences in nuclear to cytoplasmic ratio. As the cells repeatedly divide, the amount of nuclear material increases while the cytoplasm in each cell decreases. Nevertheless, cells are of different sizes at animal and vegetal poles. What are the size differences at the end of cleavage?
Make a drawing of your slide showing the relative location and size of the blastocoel in the interior.

II. **Gastrulation:** you are only previewing the major events of gastrulation at this time
The two slides in your collection illustrating amphibian gastrulation are (in sequence) **Frog crescent blastopore** and **Frog yolk plug**. These slides only contain a single section (or two in some cases) on each slide.

Gastrulation patterns start from a very different configuration of cells at the end of cleavage in the amphibian than in the echinoderm. This is generally applicable to distinctions between most types of organisms. All gastrulation involves major movements of cells over the surface and into the interior of the embryo. In amphibians, at the end of cleavage the embryo is a multilayered ball of cells with an asymmetrically placed blastocoel. The small animal pole cells migrate over the surface to cover the entire embryo by the end of gastrulation. These surface cells become the ectoderm as in echinoderms. The invaginating cells become an archenteron tube as in echinoderms.

Frog crescent blastopore slide. The onset of gastrulation is marked by the appearance of a small dark line at one point just a little below the pigmented area; this line is at the center of the former grey crescent. This line is the beginning of the dorsal lip of the blastopore. Identify the dorsal lip. Where does it form in relation to the animal pole and vegetal pole? Its formation is due to the fact that some of the surface cells (containing pigment) have begun to push their way to the interior of the blastula at this point. The inpushing – or invagination--spreads to each side of the original blastopore opening, giving rise to a lip that progresses from a crescent shape to a horseshoe-shape, and finally becomes circular. The pigmented surface cells also expand downward over the surface toward the vegetal pole. They will cover the embryo and form the ectoderm germ layer.

Can you find the blastocoel? Do you see cells that have invaginated from the surface? These are future mesoderm cells. As more cells begin to invaginate, they form a new cavity called the archenteron as in echinoderms. It will become the gut tube lining and will grow until it contacts the other side of the embryo to form a flow-through tube. Draw this section to assist you in identifying the above structures.

Now examine your **frog yolk plug slide** and try to visualize the cell movements that have taken place since the embryo was at the crescent blastopore stage. As the invagination proceeds, the blastopore grows steadily smaller, until only a "plug" of yolk remains exposed. At last the yolk plug itself is drawn inside, and the blastopore is reduced to a slit. Do you see the yolk plug protruding slightly from the blastoporal lips? Can you see the yolk platelets in these cells? The large cavity above the large yolky cells is the archenteron, and these yolky cells will form the future endoderm of the embryo. Distinguish between the remains of the original blastocoel and the archenteron. The blastocoel will soon become completely obliterated. Note that external layer of cells, the presumptive ectoderm, is composed of a thinner layer of cells than in the previous section.

The end result of gastrulation is that the major parts of the future animal have been blocked out and brought into positions in which they can begin to interact to form organ systems. The three germ layers provide the basic material out of which the body organs are built. At a late gastrula stage, the fate of the germ layers has become fixed and is no longer alterable. Thus the effect of gastrulation is not only to place the larger embryonic components in their proper position, but also to fix their fates.