Chapter 4
Cleavage

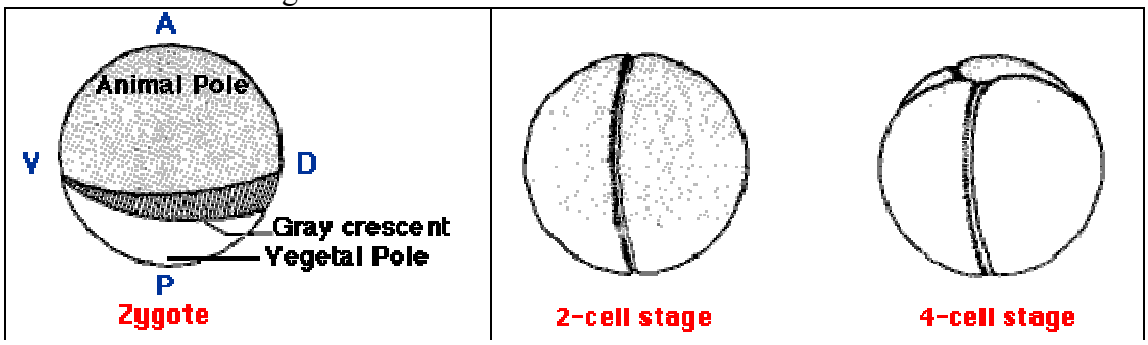
4.1. Basic Concepts

Cleavage is a rapid succession of cell divisions in which the unicellular **zygote** is transformed into the multicellular **blastula** which has a central cavity called the **blastocoele.** There are two consequences to cleavage. Cleavage reduces the volume of the **blastomeres** so they approach the size of typical somatic cells. Secondly, during cleavage, the blastomeres acquire differences that induce them to develop into different cell types. During this time, the embryo does not change its shape, except for acquiring the **blastocoele.** The constituents of the cytoplasm are not displaced during cleavage and qualitative chemical changes are limited. The first cleavage of the zygote results in two daughter cells called **blastomeres**. Both of these blastomeres then divide giving a total of 4 blastomeres which divide to produce 8, then 16, 32, *etc*. In the first few cleavages the blastomeres divide simultaneously, but eventually the synchrony is lost.

There is **no** growth in between cell division. The overall rate of cell division is most rapid during cleavage and slows at the end of cleavage phase. Early in cleavage there are only the **S** and **M** phases, with no G_1 or G_2 phases. This results in no RNA synthesis. Later there is a short G_2 and later a longer G_2 phase, as RNA synthesis resumes.

Cell division consists of division of the nucleus (**karyokinesis**) and division of the cytoplasm (**cytokinesis**). Karyokinesis is controlled by the mitotic apparatus which includes the spindle fibers and the asters, which are all made of microtubules. The nuclear envelope manufactured at each cell division is produced by nuclear envelope precursors that the egg stockpiles in the cytoplasm.

Cytokinesis begins after the chromosomes begin migrating to the cell poles. The cleavage furrow is formed by a mechanism similar to muscle contraction. Large amounts of new plasma membrane are needed during cleavage. In sea urchins, the excess membrane from the exploded cortical granules is used. The location of the mitotic apparatus determines the site of the cleavage furrow.



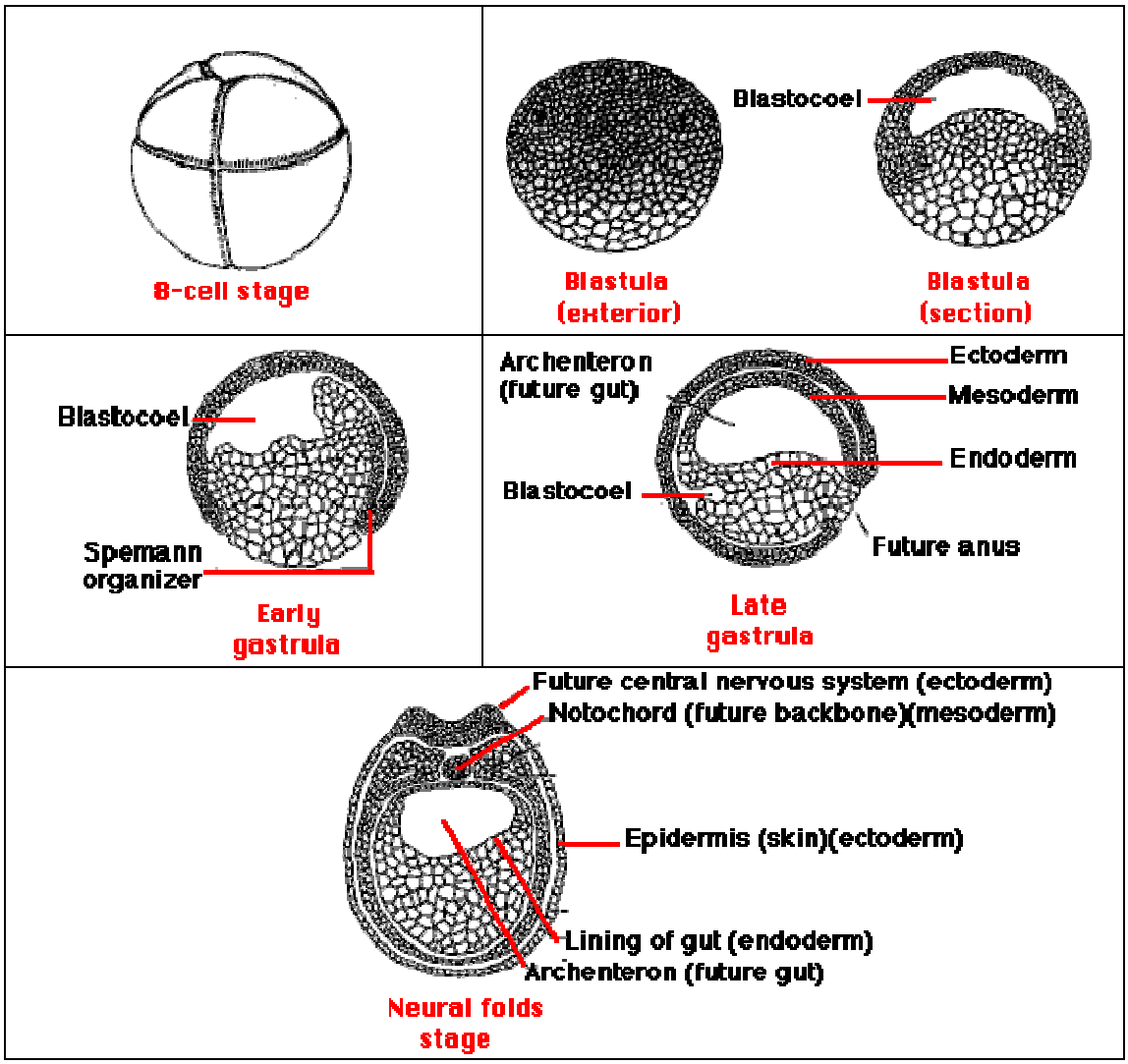


Fig.4.1.Cleavage

4.2. Molecular Aspects

The ratio of nucleus to cytoplasm increases during cleavage. Mitosis increases the number of nuclei and therefore the amount of DNA. Mainly this DNA is synthesized from low molecular weight precursors. Cytoplasmic RNA conversion to DNA accounts for some of the DNA. Some DNA is stored in the cytoplasm (in mitochondria and yolk platelets). Some of this cytoplasmic DNA is used in making nuclear DNA. Synthesis of **ribosomal RNA** is lacking during **frog** cleavage. The nucleoli (sites of rRNA synthesis) are absent during the cleavage stage. Synthesis of rRNA increases dramatically at the start of gastrulation. Synthesis of mRNA **does** occur during **frog** cleavage. However, embryos treated with **Actinomycin D** (which inhibits RNA synthesis) continue cleavage normally. Therefore, it appears that this mRNA produced during cleavage is masked and is not used until later on in development. If cleavage stage **frog** embryos are treated with **puromycin** (which inhibits protein synthesis) development abruptly ceases. Therefore, while the cleavage embryo does not need new mRNA, the existing mRNA must be translated to produce new protein, or development will stop.

4.3. Important Proteins Needed During Cleavage

Nuclear histones are proteins which combine with DNA to make chromatin (and chromosomes). With cell division occurring rapidly there is a demand for histones. In mid cleavage sea urchin embryos, as much as 50% of the newly synthesized protein is located in the nucleus. The mRNA for histones, synthesized during cleavage, is not masked and is translated into protein, as is the case for most other mRNA synthesized during cleavage. Some mRNA for nuclear histones is present in the unfertilized egg. **Tubulin** is a protein used to make microtubules (used as spindle fibers) which appear during mitosis. This mRNA is present in the egg and is unmasked during cleavage.

The enzyme, **ribonucleotide reductase**, converts RNA into DNA. Much of the RNA present in the cytoplasm is converted to DNA and used in making new chromosomes during cleavage. The mRNA which codes for ribonucleotide reductase is present in the unfertilized egg and becomes unmasked at fertilization. **DNA polymerase** is a major enzyme involved in DNA synthesis; it is present in sufficient quantities in the egg and does not increase in quantity during cleavage.

4.4. Patterns of Cleavage

Patterns of cleavage are influenced most by the amount of yolk. **Isolecithal (Oligolecithal)** eggs have limited amounts of yolk. The first cleavage furrow is vertical (meridional) in most animals and the second is at right angles to the 1st and also is vertical. The third cleavage furrow is at right angles to both the 1st and 2nd, and is horizontal.

If the top four blastomeres are directly over the lower four blastomeres then the cleavage is of the **radial** type. *i.e.* Sea cucumbers. As the blastomeres continue to divide they form a solid ball called the **morula**. After further cell division the blastomeres form a hollow **blastula**, the center of which is the **blastocoele**. The cell layer surrounding this cavity are called the **blastoderm**.

In some animals (nemerteans, mollusks, annelids and some planarians) the four top blastomeres are rotated either clockwise or counterclockwise relative to the bottom four blastomeres. This is **spiral cleavage**. In these animals, the mitotic apparatus is oriented obliquely instead of parallel to the long axis of the blastomere. If the spiraling is clockwise as seen from above, it is **dextral**. If the spiraling is counterclockwise, it is **sinistral**. In snails, the direction of cleavage is controlled by a single gene (Dextral is dominant), which lends itself to genetic studies.

Bilateral cleavage is similar to radial cleavage. In radial cleavage all the vertical planes (animal-vegetal axis) divide the embryo into equal parts. However, in bilateral cleavage only the mid-sagittal vertical plane bisects the embryo equally. Mammals and nematodes show **Rotational Cleavage**, in which the first vertical cleavage is followed by another vertical cleavage in one daughter cell and a horizontal cleavage in the other daughter cell.

Moderately & highly telolecithal eggs have a sufficient amount of yolk to provide resistance to cleavage. In Frogs, the 1st cleavage furrow does not appear all the way around the zygote at once, but starts at the animal pole and moves toward the vegetal pole. While the 1st cleavage furrow is forming, the 2nd forms in same manner at right angles to the first and in the vertical plane. The 3rd cleavage furrow appears around the embryo above equator, because the yolk displaces the mitotic apparatus in each blastomere into the animal pole. Blastomeres in vegetal pole divide more slowly than

those in the animal pole, because they contain more yolk. Therefore, the blastomeres in the vegetal pole are larger and called **macromeres** and in the animal pole they are the smaller **micromeres**. The eggs in frogs become completely divided into blastomeres. These are **holoblastic** eggs.

In **Meroblastic eggs** the cleavage furrows do not reach the vegetal pole; this results in a number of blastomeres plus an undivided residue of cytoplasm with some scattered nuclei. The mitotic apparatus is restricted to a small yolk-free zone at the animal pole. This occurs in **discoidal** and **superficial** cleavage. In **discoidal cleavage** there is a disc of function cytoplasm atop the yolk at the animal pole. This is found in elasmobranchs, most bony fish, birds & reptiles. The cleavage is **superficial** in centrolecithal eggs, where the functional cytoplasm forms an outer layer around the yolk. This is seen in insects *etc.*

The **Avian egg** has a small cytoplasmic disk (**blastodisk**) at the animal pole sitting on top of the yolk. Only the cytoplasm and not the yolk will be divided. This is **discoidal** cleavage *(which is a type of meroblastic cleavage)*.

The blastodisk has a central portion, the **blastoderm** (which will contribute to the embryo proper) and a marginal area, the **periblast**, which blends with the underlying yolk. The first two cleavage furrows are at right angles & vertical, but incomplete. The 3rd & 4th are also vertical and incomplete, as well as asynchronous. At about the 32-cell stage horizontal cleavage furrows appear. This results in central blastomeres on the surface which are separate. The marginal blastomeres and those on the bottom are incompletely separated. The central cells increase in numbers by mitosis within the central cells and by cells cut off from the inner ends of the marginal cells. The marginal cells increase by new radial furrows.

As early as the 8 cell stage, some of the yolk material beneath the central part of the blastodisk breaks down, forming the **subgerminal cavity**. The subgerminal cavity does not extend below the periblast. Eventually the nuclei of the marginal cells invade the periblast and it too, undergoes mitosis and forms the **germ wall**. As seen from the top, the periblast is known as the **area opaca** and the central **blastoderm**, which overlies the subgerminal cavity, is the **area pellucida** (*L. transparent*), because the area opaca is more opaque and the area pellucida is more transparent. At about the 256-cell stage the cells at the lower part of the blastoderm split off from the overlying cells, producing a cavity, the **blastocoele**. The upper layer of blastoderm cells is the **epiblast** and the lower layer is the **hypoblast**.

Centrolecithal eggs have a superficial layer of cytoplasm (**periplasm**) and a central island of cytoplasm (**energid**) which contains the nucleus. The nucleus undergoes mitosis, repeatedly and they migrate to the periplasm. In the periplasm the nuclei direct cell division, forming a superficial layer of cells around the central yolk. This is called **superficial cleavage**.

4.5. Determination of Cleavage

Determination is the narrowing of **prospective potencies**. Cell determination can be controlled by intrinsic factors (ooplasmic determinants) or extrinsic factors, like environmental stimuli or cell-cell interaction. The latter is known as **induction**.

Cleavage in embryos is categorized as **determinative** and **indeterminate**. In **Determinative** cleavage, sometimes known as mosaic cleavage, the blastomeres become restricted during first few cleavages. Thus, an embryo will lack structures derived from blastomeres that are removed or destroyed. Determination in these animals (*e.g.*tunicates)

may rely mainly on ooplasmic determinants. In **Indeterminate** cleavage, sometimes known as regulative cleavage, restriction begins later. These embryos can compensate for blastomeres that are removed or destroyed. They rely mostly on cell interactions. Examples include sea urchins and amphibian.

4.6. Nuclear and Cytoplasmic Control During Cleavage Stage

Weisman (1904) put forth the **Germ Plasm Theory**, which stated that every part of the body has a representative particle, a determinant, in the sex cells. During the process of cleavage and development, the determinants end up in the appropriate area of the embryo where they cause the correct part to develop in the correct area. This theory is not actually valid, but there are **ooplasmic determinants**, that do influence the development of embryonic structures.

Horstadius (1936) demonstrated that any one of the first 8 sea urchin blastomeres is capable of forming a complete embryo. More conclusively, Spemann (1928) pulled a loop around the egg of a newt, *Triturus*, confining the nucleus to one half. The two halves remained connected by a cytoplasmic bridge. The half with the nucleus underwent mitosis & cell division while the other half did not. At the 16 or 32 cell stage, a nucleus from the dividing half was now small enough to squeeze across the bridge into the cytoplasmic side. The side with the nucleus from the 4th or 5th generation blastomere was capable of developing into a complete newt, as was the side which had the original nucleus. This demonstrated that the hereditary material of the nucleus is not divided up with each cell division, but that the nucleus of each of the 32 blastomeres has a full compliment of hereditary material.

Seidel (1932) corroborated Spemann's findings by destroying one of the first two nuclei of the centrolecithal Dragonfly eggs with UV radiation and observing that a complete organism still develops.

Briggs & King (1950's) transplanted nuclei (from cells at various stages of development) into activated enucleated frog eggs. They found that nuclei from embryos of all stages and numerous tissues were capable of allowing the enucleated egg to develop into a larva. Nuclei from a carcinoma even worked. The younger the cells from which the nuclei were taken the more success was seen. Nuclei from adult tissues did not cause development if transplanted directly into enucleated frog eggs. But if adult cells are cultured *in vitro* for a time they tend to **de-differentiate** and start mitosis. If these nuclei are used, then better success is achieved, but still, the embryos are abnormal in part. If nuclei from the normal parts of these abnormal embryos are used for transplantation into enucleated eggs the embryos of this 2nd generation did better. Some developed into swimming larvae and a few metamorphosed into **froglets**.

Apparently, the nuclei of cells of even differentiated tissue still have complete genetic ability. Many times cloned animals have deficiencies, even in comparative successes like Dolly the sheep. Nuclei from advanced embryos do change when transplanted into activated eggs. Their volume increases 30 fold, the nucleolus disappears and rRNA synthesis stops. Therefore, the cytoplasm must have some effect on the nucleus and upon controlling the development of the embryo.

Spemann (1938) drew a loop around newt eggs. If the loop divides the uncleaved zygote so that each half gets some of the cytoplasm in the area of the gray crescent, then 2

normal embryos develop. If the loop separates the cytoplasm so that all the gray crescent is in one half and none in the other, then the half with the gray crescent will develop into a normal embryo and the other half will develop into a half embryo with ventral structures only. Therefore, the cytoplasm exerts some control in newt differentiation.
Centrifugation of most animal eggs at moderate speeds causes the egg to become stratified into 3 layers.

1. **Centri*p*etal pole** (pole nearest axis of rotation) contains lipids.
2. **Hyaline layer** contains the nucleus, centrioles and the matrix of the cytoplasm.
3. **Centri*f*ugal** pole is the most dense and contains yolk.

In the urochordate, *Cynthia*, 4 areas in the egg cytoplasm can be distinguished on the basis of the distribution of pigment and yolk in the cytoplasm. These four areas are determined (their developmental fates have been programmed so they can only develop into specific structures). Centrifugation of *Cynthia* eggs redistributes the substances in the cytoplasm. As the embryo develops, structures form in a chaotic way. They are recognizable as specific organ rudiments, but they are out of place.
Again, this demonstrates that the substances in the cytoplasm play an important role in differentiation.
However, the pattern of cleavage may be independent of the cytoplasmic substances inside the egg. For example: If the eggs of the sea urchin *Arbacia* are centrifuged, the red pigment granules accumulate at the centrifugal end of the egg. Depending upon how the egg is oriented during centrifugation the red pigment granules will be at the animal pole, vegetal pole or on one side of the egg. Regardless of where the red pigment granules are, the blastopore always forms in the same place, at the vegetal pole. *(The Blastopore is where involution occurs during gastrulation)*.
Another example is the snail, *Ilyanassa*. Their eggs form a polar lobe, which extends from the egg at the vegetal pole. Therefore, the polar lobe is usually filled with yolk. If eggs are centrifuged upside down the animal pole contains the yolk granules and the vegetal pole contains the hyaline cytoplasm and lipids. The polar lobe still forms at the vegetal pole, even if it is filled with hyaline cytoplasm.
Therefore, something in the egg is not disrupted by centrifugation and it determines where on the egg the polar lobe of *Ilyanassa* and the blastopore in *Arbacia* will form. This "something" is the cortex. Centrifugation does not displace the cortex because of its viscosity. It appears the polarity of the egg is determined by the cortex. If eggs are centrifuged and then left to stand, the cytoplasmic substances will return to their specified areas if there is enough time before cleavage begins.

4.7. Ooplasmic Gradients

Most animal eggs contain gradients of components along their **animal-vegetal** axis and many contain gradients along their **dorsoventral** axis, also. The **sea urchin** egg has a marginal band of pigment granules. If the ooplasm is divided **vertically**, then two small, **complete** larvae develop. If the ooplasm is divided **horizontally**, then two **incomplete** larvae develop. As a result of **sea urchin** cleavage **three sizes of cells** form:

1. **Mesomeres** are medium sized blastomeres at the animal pole. These will produce ectodermal structures (i.e. tuft of cilia).
2. **Macromeres** are large blastomeres, containing red pigment granules. These will develop into endodermal structures (gut) and some ectodermal structures (ciliary

band).

3. **Micromeres** are small blastomeres at the vegetal pole, which develop into mesenchymal structures (skeleton of calcareous spicules.)

If the blastomeres from a 2- or 4-cell stage are isolated from each other they will develop into a complete larva. The first two cleavages are vertical and the resulting blastomeres receive some of each of the cytoplasmic regions from one pole to the other. If whole eggs are exposed to Lithium salts the embryo develops just as if it were only the vegetal half. If whole eggs are exposed to NaSCN (sodium thiocyanate) the embryo develops as though it were only the animal half. These results can be explained by assuming that there are two mutually antagonistic gradients present in the embryo. The interaction between them results in the formation of a normal embryo. These two gradients have their greatest activity in the two poles and their activities diminish as they move away from that pole.Therefore the animal gradient has its center of activity in the animal pole and its weakest activity in the vegetal pole. The vegetal gradient has its center of activity in the vegetal pole and its weakest activity in the animal pole. This is known as the **double gradient model** and was first proposed by **J. Runnstrom**.

If the blastomeres of the two poles are physically separated from one another or if one of the gradients is chemically destroyed, then the embryo does not develop normally. If the animal gradient is weakened or removed the embryo is vegetalized (developing only parts pertaining to the vegetal gradient, *i.e.* gut). If the vegetal gradient is weakened or removed the embryo is animalized (developing only parts pertaining to the animal gradient, i.e. cilia tuft). The existence of these gradients can be further demonstrated by separating the three groups of blastomeres from one another and then recombining them:

1. Isolated mesomeres develop into ectodermal structures
2. Isolated micromeres don't develop
3. Isolated mesomeres plus micromeres form a complete larva
4. Isolated macromeres sometimes develop into a complete larva.
5. Isolated macromeres plus micromeres produce a large gut and a small ectodermal vesicle.

Instead of being inside the ectoderm, the gut is outside the ectoderm. This is known as **exogastrulation** and results from mesenchyme cells migrating to the inside. In this abnormal environment the mesenchymal cells do not produce a skeleton, as is normally the case. Ooplasm gradients can be visualized by observing the reduction of vital dyes in anaerobic conditions. For instance, when the dye, **Janus green**, is reduced under certain conditions it changes from green to red. This color change can be observed and starts in the vegetal pole, spreads to the equator and stops; then it starts in the animal pole and spreads to the equator. If the egg is animalized or vegetalized (chemically or by isolation of blastomeres) then there appears only one center of reduction of Janus green. Or if supernumerary micromeres are implanted on the side of an embryo there appears a center of activity from them as well as from both poles. Keep in mind that it is the genes of the maternal set of chromosomes which determine the peculiarities of the egg during cleavage. The embryonic genes are not expressed until after the cleavage stage.