

Lecture 7 (Knight): Cleavage

Reading: pp. 223-228, 229-234, 247-257, 303-304, 345-347, 354-358

Cleavage is the first stage of embryogenesis. The processes that occur during cleavage accomplish several things: 1) partition the contents of the zygote into many cells (called blastomeres), with no increase in total volume; 2) begin to establish different cell identities and separate certain cells from each other; and 3) shift control of development from maternally derived mRNA's and proteins to embryonically encoded gene products. Embryos of different species differ in the relative timing of these events as well as in their patterns of cleavage.

Mechanics of cleavage

The embryonic cell cycle and its initiation.

Since no growth of blastomeres is required between cell divisions, cleavage employs a specialized form of the cell cycle, consisting only of S and M phases, with no G1 and G2. Mitosis-promoting factor (MPF) is one of the key components of the basic cell-cycle oscillator.

- Arrest of oocyte is due to a protein called cytotstatic factor (CSF), which blocks degradation of cyclin,
- After fertilization, the increase in intracellular Ca^{++} causes destruction of cyclin as well as CSF.
- MPF consists of two components, cyclin B and cdc2. The cyclin B component is cyclic, accumulating in S and degrading after M has begun.

Cytokinesis.

- Accomplished by a contractile ring of actin filaments and bipolar myosin filaments (G,8.3).
- Spindle position determines equal or unequal cleavage
- Cleavage furrow is determined by aster microtubules emanating from the centrosomes, but the molecular mechanism is still unclear.
- Cytokinesis cannot happen until mitosis is complete; it is activated when MPF is
- The cell cycle oscillator's affect on the cell cortex and cleavage machinery is independent of the nucleus.

Spindle orientation determines cleavage plane orientation.

- In many embryos, the early cleavages are highly stereotyped, such that cleavage plane orientations in successive divisions occur in the same relationship in all embryos (G,8.5).
- Spindle orientation is determined by positioning of the centrosomes (not understood)
- Because of the way the centrosomes divide, the spindle orientation at each cleavage is usually, though not always, orthogonal to the orientation of the preceding cleavage.
- Screens in *C. elegans* have identified maternal-effect lethal mutations that affect spindle orientations in early cleavages. The mutations defined six genes, which were named the *par* genes, for partitioning-defective.
- The *par* genes also affect partitioning of cytoplasmic components (see below).
- Subsequent cloning and characterization showed that these genes encode "novel" proteins (the PAR proteins; though *Drosophila* and mammalian homologs have now been found)

--These proteins become segregated (localized) to specific regions of the cortex at the one-cell stage and function to further segregate factors in the cytoplasm as well as to control early spindle orientations.

Non-uniformity of egg cytoplasm gives the embryo a polarity.

--Yolk content in the cytoplasm (a source of nutrients) is non-uniform in many eggs.

--Eggs with yolk in one region of the egg have an established polarity prior to fertilization which impacts their development.

--In contrast, in mammalian eggs there appears to be little cytoplasmic non-uniformity, and patterning is initiated at later stages.

Establishment of cell identities is dependent on cytoplasmic determinants and/ or signaling

As cleavage progresses there is little relative movement of cells. Because organization of the egg cytoplasm occurs during oogenesis or just after fertilization, an egg has different factors located throughout its cytoplasm. Early embryologists speculated that these factors could determine the fate of cells. To test this hypothesis, they cultured individual cells or parts of embryos and observed how they developed, in isolation or in combination (cut and paste experiments).

Tunicates (sea squirts).

The cytoplasm in some species of tunicates is "color coded," that is, it is actually possible to see reproducible differences in appearance of the cytoplasm in different parts of the egg.

Cleavage partitions these different components into different blastomeres, always in the same way (G,1.7).

--By ablating (destroying, say with a hot needle) one or another of these cells and observing the tissues that were missing from the resulting embryos, embryologists showed that the fates of the cells that descended from these blastomeres correlated with the types of cytoplasm they contained.

--Furthermore, if cells were separated into pairs with similar cytoplasm at the 8-cell stage and cultured in sea water, each of the pairs developed into a different set of tissues. In other words, they were already partially determined, committed to a certain set of cell fates.

--Embryos that develop in this way were called mosaic embryos, because the early embryo was like a mosaic of differently specified cells.

C. elegans

In *C. elegans*, the divisions are asymmetric from the start. The first cleavage creates two different sized blastomeres with different fates due to the segregation of cytoplasmic components.

--P granules segregate specifically to germ line cells (the P lineage) in early cleavages, as seen by antibody staining (8.43).

--The granules are partitioned by an actin-based process. They contain regulatory proteins and RNAs, which include germ-line determinants.

-- Not all cell fates are determined by segregation; there are many signaling events in the early embryo that are required for correct fate determination.

Sea urchin

--The animal and vegetal halves of the embryo show different and limited developmental potentials after the third cleavage, which separates them. Interestingly, however, recombination of micromeres (small cells at the vegetal pole) with the animal half, allows formation of a recognizable larva. That means the micromeres are inducing formation of other tissues from cells that by themselves are only able to form ectodermal tissues.

Although segregation of cytoplasmic components is more generally important in invertebrate embryos, there are some examples in most vertebrates as well. For example, in the unfertilized *Xenopus* egg, Dsh, a member of the Wnt pathway is localized to the vegetal hemisphere of the embryo (more on this later). In mammals, however, there is apparently little or no prepatterning of the cytoplasm, and patterning of the embryo depends entirely on cell signaling rather than segregated determinants.

Passing control to the embryonic genome: blastulation and the mid-blastula transition

The end product of the cleavage process is a hollow ball (or disc) of cells called the blastula or blastocyst. The space inside (blastocoel cavity) is created during cleavage. The outer cells of the blastula (sometimes called a morula) become tightly coupled by tight junctions so that ions and small molecules cannot leak to the outside. The coupled cells then pump Na⁺ ions and secrete proteins into the interior of the embryo, and water is drawn in by osmosis to form the blastocoel (A, 21-4).

The mature eggs of most non-mammalian species contain large amounts of protein and RNA synthesized maternally during oogenesis. The stored proteins and those derived from translation of maternal RNA's after fertilization are sufficient to support almost all embryonic processes during the early stages of rapid cleavage, and there is relatively little transcription of the embryonic genome.

At some point during the blastula stage, most embryos undergo a sort of mid-blastula transition (MBT). This takeover occurs suddenly in *Xenopus* after the 12th cleavage, and in *Drosophila* after the 14th mitotic cycle. Its timing is somehow determined by the ratio of cytoplasmic volume to cellular DNA (which decreases by an average factor of two at each cleavage).

There are three consequences of the MBT:

- the embryonic genome initiates extensive transcription
- the rapid cell cycle lengthens (acquiring G1 and G2 phases) and becomes non-uniform
- cleavage becomes asynchronous with region-specific differences in duration of the cell-cycle.

Specific examples of cleavage in model systems

Mammals

In mammals, cleavage is rotational, asynchronous, and slow (12 to 24 hours per division) (11.21).

While the egg is dividing and forming the blastocyst, it is moving down the fallopian tube to the uterus, where it will implant (11.20). Interestingly, unlike most other animals, the embryonic genome begins to function as early as the 2-cell stage.

Blastulation

--At the 8 cell stage, the loosely connected blastomeres undergo compaction (11.22). The cells are now tightly packed together, and the outside cells are connected via tight junctions

(preventing leakage of ions and small molecules).

--At the 16-cell stage, there are only two inner cells, all the rest are outer cells, connected by tight junctions. At this point, the embryo is called a morula.

--At about the 30 cell stage, the blastocoele cavity forms and the embryo is organized into the inner cell mass, which will form the embryo and some extraembryonic tissues, and the trophoblast, the outer ring of tightly joined epithelial cells, which will form the chorion (embryonic part of the placenta).

--The inner cell mass then reorganizes into two layers, the epiblast (embryonic tissues only) and hypoblast (extraembryonic).

Chick

--Cleavage is meroblastic (incomplete--the yolk does not cleave, Fig 11.8E) and discoidal (the cleaving cells lie on top of the yolk in the form of a flat, circular disk)

--cleavage divisions are synchronous for the first four only, then become asynchronous

--as the cells divide and become several layers thick, they secrete fluid towards the yolk, creating the subgerminal cavity that now separates the future embryo from the yolk.

--After several cleavages, the group of cells sitting atop the yolk is called the blastoderm. These cells are linked by tight junctions, characteristic of epithelial cells.

-- Next, the embryo moves into the blastula stage: it has formed an upper layer, the epiblast, and a lower layer, the hypoblast.

Amphibia

--Amphibian eggs are meroblastic like chick, but the whole embryo does divide. Because of the yolk concentrated in the vegetal hemisphere, the cell divisions proceed more rapidly in the animal hemisphere (10.1)

--This leads to the production of large vegetal macromeres and small animal micromeres

-- A blastocoele cavity becomes apparent at about the 32-cell stage, although it actually begins to form earlier.