

Fertilization and early embryonic cleavage

Reading (9th ed): Chp 4: 121-127; Chp 5: 159-162; Chp 7: 241-244

Reading (8th ed): Chp 7: 175-180, 205-206; Chp 8: 211-215, 243-246; Chp 10:291-295.

Learning goals: Be able to:

Describe, in a general way, the process of fertilization and the different features of sperm and oocyte.

Explain how the orientation of cleavage planes is determined.

Explain the consequences of cleavage planes on establishment of cell fate.

Describe the onset and consequences of the "mid-blastula transition", and compare its importance in different organisms.

Fertilization

Fertilization involves some spectacular cell biology, but in terms of development it simply provides the stimulus to start the process. It serves three major developmental functions: bringing in the paternal genome, bringing in a new centrosome, and re-starting the cell cycle in the arrested oocyte. Contact of sperm with egg initiates a sequence of pre-programmed responses of the two gametes to each other. These result in: invasion of the egg by the sperm, transmembrane signals that block further sperm entry to prevent polysomy and activate the egg's metabolism, triggering of lipid and macromolecular synthesis in preparation for cleavage, re-initiation of the cell cycle, and finally fusion of sperm and egg nuclei to produce a diploid zygote with a combination of maternal and paternal genetic information.

Interestingly, in almost all animals, the process of oocyte meiosis is not complete when the sperm enters the egg (7.5). In amphibians, fish, and most mammals, the oocyte is arrested in metaphase of meiosis II. Accordingly, the oocyte nucleus still has two copies of each chromosome when the sperm nucleus penetrates the egg! The oocyte has to complete meiosis, shunting off those chromosomes into the last polar body (7.32) before fusing with the sperm nucleus. The fertilized egg then goes immediately into mitosis--the chromosomal material from the egg and sperm replicate separately, and then all the chromosomes line up along the metaphase plate, and go on to complete the first mitotic division of the embryo. Finally, at the now 2-cell stage, each cell has an integrated complete set of information from each parent, and development can proceed.

Cleavage

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;
- 3) form a hollow ball (or disc) of cells called the blastula, and
- 4) shift control of development from maternally derived mRNA's and proteins to embryonically encoded gene products. Also during cleavage, the axes of the embryo (A/P, D/V, L/R) become established; we will discuss this process in more detail later. Embryos of different species differ in the relative timing of these events as well as in their patterns of cleavage, but the developmental functions of cleavage are essentially the same in all embryos.

Mechanics of cleavage

The embryonic cell cycle and its initiation

Since no growth of blastomeres is required between cell divisions, early cleavage can employ a specialized form of the cell cycle, consisting only of S and M phases, with no G1 and G2.

Cytokinesis

Division of the cytoplasmic and membrane components of the cell is accomplished in most animal cells by a contractile ring of actin filaments and bipolar myosin filaments. The position of the contractile ring is always at the intersection of the cortex and a plane through the metaphase plate of the mitotic spindle. Therefore, moving the spindle toward one pole of the cell or the other can cause an unequal cleavage, which we will see examples of below. The position of the cleavage furrow is probably determined by aster microtubules emanating from the centrosomes.

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. Choice of these orientations determines the form of the early embryo. Control of this process must result from control of spindle orientation, since cleavage plane orientation is dictated by the spindle as described above. Spindle orientation is determined by positioning of the centrosomes, which is not well understood. In the normal centrosomal replication cycle, the centriole inherited by each daughter cell after mitosis replicates, and each of the two daughter centrioles migrates 90° away from its sister to opposite points on the nuclear envelope, where they nucleate the spindle poles for the next division. Therefore, the spindle orientation at each cleavage should be orthogonal (perpendicular) to the orientation of the preceding cleavage.

However, this is not always true. In *C. elegans* embryos, spindle orientations can be changed by tethering to a site on the cell cortex, which appears to pull the spindle poles around by their aster microtubules, rotating the spindle in a specific manner and thereby altering the plane of the next cleavage. This rotation has consequences for the patterning of the embryo, because it determines which cells will be adjacent to each other.

Partitioning the contents of the zygote

Non-uniformity of egg cytoplasm gives the embryo a polarity

The cytoplasm of many eggs contains yolk, which will serve as a source of nutrients (and other important molecules) during subsequent cleavage. Many eggs are non-uniform, that is, the cytoplasmic components including yolk have been organized into a specific pattern during oogenesis. These eggs (e.g. amphibia) have an established polarity prior to fertilization, and the animal pole (less yolk, future ectodermal cells) and vegetal pole (more yolk, future endodermal cells) may be visibly distinguishable. In many eggs, additional cytoplasmic reorganization occurs just after fertilization. In mammalian eggs there appears to be little cytoplasmic non-uniformity, and patterning is initiated at a later stage by other mechanisms we will discuss later. In other words, unlike in most organisms, all early cells of the mammalian embryo are equivalent, up until about the 16-cell stage.

Patterns of cleavage

A common cleavage pattern (seen in sea urchins and amphibia) is that the first two synchronous cleavages are meridional, i.e. pass through the animal and vegetal poles, preserving the egg polarity. Third cleavage planes are equatorial, but may be unequal (spindles must be repositioned).

Yolk distribution and extent of cleavages

Whether cleavages go entirely through the embryo (holoblastic) or only partially (meroblastic) depends partly on the amount and distribution of yolk in the egg. Eggs differ markedly in this respect (for more detail, see Gilbert).

Establishing different cell identities

Why cleavage patterns matter: determinants in the cytoplasm of fertilized egg

As cleavage progresses there is little relative movement of cells. This means that non-uniformities in the cytoplasm of the fertilized egg are preserved and stabilized. Because organization of the egg cytoplasm occurs during oogenesis or just after fertilization, cleavage can be responsible for separating these maternally supplied components to specific parts of the embryo. Differential partitioning of localized factors can give blastomeres different developmental potentials. Early embryologists obtained evidence for these factors by culturing individual cells or parts of embryos and observing how they developed, in isolation or in combination. Some cells exhibit autonomous behavior: they maintain their normal fate in isolation. Other cells require the presence of neighboring cells to display their normal fates: these cells show non-autonomous behavior dependent on intercellular signaling, often called induction in embryos.

Thus cleavage segregates cell-autonomous determinants of cell fate, as well as abilities to send and respond to inductive signals.

Establishing cell polarities

C. elegans

The anterior-posterior polarity of the 1-cell embryo depends on a set of cortical components called the PAR proteins; when they are mutant, partitioning of cytoplasmic components is defective. Homologs of these proteins turn out to be important in all animals later in development when cells become polarized, for example in epithelia.

Amphibian cleavage

The first two cleavages in *Xenopus* embryos are meridional, giving a radially symmetric 4-cell stage. The third cleavage is equatorial, but unequal, giving rise to quartets of large cells on the vegetal side and smaller cells on the animal side (animal cap cells). As in *C. elegans*, unequal cleavage is accomplished by moving the spindles of the 4-cell stage toward the animal pole, so that the subsequent cleavage furrows are displaced upward from the equator.

Chick cleavage forms a discoblastula on the surface of the yolk

In the chicken egg, the huge amount of yolk restricts cleavage, such that the cleavage furrows extend only to the surface of the yolk (11.12). Therefore, cleavage gives rise to a flat disk of cells with a cavity between it and the yolk. As dramatically different as this is from other organisms, it has surprisingly few consequences on development.

Mammalian cleavage is different

In mammals, there is apparently little or no pre patterning of the cytoplasm. There is little if any yolk (nutrients are supplied by the placenta), and early cleavages are equal. Compaction, occurring at the 8-cell stage in the mouse, forms a tight ball of cells that is unique to mammals. Patterning of the embryo, with commitments to different cell and tissue fates, begins after the 16-cell stage, and surprisingly (given its complexity), must depend entirely on cell signaling rather than a combination of determinants and signaling, as is true for most organisms.

Common features of cleavage

All these mechanisms of cleavage result in

- 1) partitioning of the egg cytoplasm into many individual cells, and
- 2) establishment of different cell identities, i.e. commitments to different fates. All embryos use similar mechanisms for cell determination, but different embryos use partitioning of cytoplasmic

determinants and cell signaling to different extents, so that commitments occur at different times during the cleavage process.

3) In all embryos, the end product of the cleavage process is a hollow ball (or disc) of cells called the blastula or blastocyst (or discoblastula for fish and birds). The space inside (blastocoel cavity) is created during cleavage. Cells of the blastula 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. In mammals, the tightly packed ball of cells resulting from compaction is called the morula.

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

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 (sometimes called zygotic transcription – a misnomer that has unfortunately gained general use). Thus, most of those early developmental events are controlled by proteins synthesized from transcripts that were actually maternally contributed, not embryonically transcribed. The time at which the embryonic genome takes over control of embryogenesis and initiates extensive transcription varies from species to species. This takeover occurs suddenly in *Xenopus* after the 12th cleavage, at what is called the midblastula transition (MBT). 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). At a particular reproducible point, the ratio of cytoplasm to DNA triggers the transition. Another marked change occurs at the MBT: the rapid cell cycle lengthens (acquiring G1 and G2 phases) and becomes non-uniform. The result is that if cleavage was initially synchronous, it now becomes asynchronous, with region-specific differences in duration of the cell-cycle. A third change is that cells become motile and soon begin the movements of gastrulation. In other embryos (e.g. worms), these three changes all occur but not so suddenly and simultaneously, so that an MBT is less evident. In mammalian embryos, where there appears to be little if any maternally contributed mRNA, embryonic transcription has already begun at the two-cell stage, cleavage begins asynchronously, and some other combination of factors must determine when cells become motile.

Review Questions

- 1) What three major developmental functions does the fertilization process provide to embryos?
- 2) What major developmental functions does cleavage accomplish for the embryo?
- 3) Explain the relation between amount and distribution of yolk and the cleavage patterns seen in various embryos.
- 4) When an embryonic blastomere cleaves (or any other cell divides), what determines where the cleavage furrow forms and the resulting relative sizes of the two daughter cells?
- 5) How are the orientations of cleavages usually determined (i.e. anterior-posterior, left-right, etc.)?
- 6) If a cell has just divided with an A-P orientation, what will be the default orientations of the cleavages of its two daughter cells?
- 7) At what point does embryonic bulk transcription turn on? What else happens at this point?
- 8) How is chick cleavage different from amphibian cleavage and human cleavage?