Lecture 3

Early Cleavage Patterns

Cleavage and Gastrulation are two critical stages of embryogenesis. ie ~18% of fertilized embryos fail to complete cleavage and become successfully implanted.

1. After fertilization, early development in most animals starts with a series of rapid cell divisions called CLEAVAGE that serves several purposes:
   a. rapidly increases cell number, dividing the cytoplasm of the egg into smaller and smaller cells called blastomeres
      i. Length of "cell cycle" is regulated in most cell types.
      ii. In the early embryo it's much shorter- many divisions very rapidly, ie. in *Drosophila* cleavage-stage embryos, mitosis every 10 minutes for >2 hours
      iii. How so fast? No growth phases (just mitosis and DNA synthesis) & relies on stored material- no transcription. So faster, and no volume increases
      iv. Transition from fertilization to cleavage caused by activation of MPF (mitosis-promoting factor). Active MPF dependent on presence of cyclin B, which accumulates during S and is then degraded after cells reach M phase.
      v. Cyclin regulators stored in egg cytoplasm, so cell cycle doesn't require zygotic transcription for numerous cell divisions. We'll talk about this more when we discuss the Mid-blastula transition.
   b. distributes egg contents, often asymmetrically, to the cells

2. What controls the process of cleavage?
   c. The cleavage plane forms perpendicular to the mitotic spindles (microtubules made of tubulin).
   d. The contractile ring (microfilaments made of actin) that forms creates a cleavage furrow.
   e. Placement of the mitotic spindle is regulated by attachment sites that form on the inside of the cell membrane. The composition of the sites is unknown, but in some animals is controlled by cytoplasmic factors made by mom. This is an example of a MATERNAL EFFECT, in which the phenotype of the EMBRYOS is controlled by the genotype of the MOM.

3. Different organisms have different patterns of cleavage.
   a. the amount and location of yolk in the egg
   b. factors in the egg cytoplasm influence the angle of the mitotic spindle and the timing of its formation.
      1. Placement of mitotic spindles determines cleavage planes:
         • MERIDIONAL cuts through embryo poles like a geographic meridian
         • EQUATORIAL cuts through the area of greatest diameter
      2. timing of mitotic spindle formation
         • cleavages can be SYNCHRONOUS, occurring at the same time. ie. sea urchin
         • or ASYNCHRONOUS, occurring at different times. ie. mammals
      3. symmetric (equal) vs. asymmetric (unequal) cell divisions:
         • 4 cells in animal tier divide to form 8 mesomeres of same size
• 4 cells in vegetal tier divide to form 4 macromeres and 4 micromeres

4. **Mammalian cleavage is unique in several ways:**
   a. **First,** initial cell divisions in mammalian are slow (12-24 hours) compared to embryos that develop outside the mother (ie. flies 10 minutes), presumably because mammalian embryos are protected.
   b. **Second,** mammals have a **ROTATIONAL** cleavage pattern. For radial cleavage (ie. sea urchins), the first two cleavages are **MERIDIONAL** (cleavage plane goes through the embryo poles). In mammals, the first cleavage is **MERIDIONAL,** but during the second cleavage, one blastomere divides meridionally and the other blastomere divides equatorially.
   c. **Third,** Mammalian cleavage is **asynchronous** from an early timepoint. In other words, mammalian blastomeres do not all divide at the same time. So, embryos frequently contain **odd numbers of cells,** ie. 3 or 6 blastomeres, instead of exponential 2-to 4-to 8-cell stages.
   d. **Fourth,** unlike most other animals, **zygotic transcription occurs during early cleavage,** and produces proteins necessary for subsequent cleavages to occur. In mice, this switch from maternal to zygotic control occurs at the 2-cell stage!
   e. **Fifth,** at the 8 cell stage the mammalian embryo undergoes **COMPACTION.** The outer cells form **tight junctions** with each other, while the inner cells form **gap junctions,** enabling small molecules and ions to pass between them. **Compaction requires the cell adhesion protein E-cadherin.**
   f. The 16 cell stage embryo is called the **morula,** and it has a small group of internal cells surrounded by a larger group of outer cells. This environment of outer vs. inner cells sets the stage for differentiation in development.
   g. Most of the descendents of the outer cells form the **TROPHOBLAST (trophectoderm),** while the inner cells become the **INNER CELL MASS.** These cells will give rise to all parts of the embryo proper and some extraembryonic structures, while the trophoblast cells will participate in placenta formation and implantation. **This distinction between trophoblast and inner cell mass cell fate is the first differentiation event in mammalian development.**
   h. The **BLASTOCYST** forms at 32-64 cells by the outer (trophoblast) cells secreting fluid into the morula to form a cavity called the **BLASTOCOELO.**
   i. **Hatching from the zona pellucida** is prevented until the blastocyst reaches the uterus. Premature hatching/implantation in the oviduct known as ectopic or **tubal pregnancy** can cause a life-threatening hemorrhage.

5. **After fixed number of divisions, Cleavage ends in a controlled manner called the MID-BLASTULA TRANSITION (MBT)**
   a. you can imagine that if it ended haphazardly there would be huge differences in cell numbers among embryos of the same species.
   b. **Cell divisions=9-10 (sea urchin), 12 (frog), 10 (fish), flies (13)**
   c. it seems to be controlled by the **nuclear/cytoplasmic ratio,** which is almost 0 in a fertilized egg with tons of cytoplasm, but approaches 1 in cells after many nuclei are made without extra cytoplasm.
   d. during the MBT the cell cycle lengthens and often G1 is used for the first time.
   e. the **embryo starts to synthesize its own RNA** (often maternal RNA is actively destroyed at this time). Remember this is a difference between mammals and other animals
   f. the **cells become motile** (prelude to gastrulation).
Gastrulation

1. **GASTRULATION** is a complex series of cell movements that:
   a. rearranges cells, giving them new neighbors. These rearrangements put cells in a new environment, with the potential to receive new signals.
   b. results in the formation of the 3 GERM LAYERS (do not confuse with germline) that will form most of the subsequent embryo: ECTODERM, ENDODERM and MESODERM.

2. While all animals gastrulate, gastrulation can seem very different in different organisms (mostly due to geometric differences due to yolk content and distribution), but like cleavage the similarities in terms of mechanism and outcome suggest that the same controls operate across species.

3. Changes in the shape or adhesive properties of single cells in concert can rearrange the entire embryo.
   a. Cell shape changes, mediated by cytoskeleton lead to indentation of a flat sheet of cells, which can then lead to invagination of a tube or ball of cells.
      • Contraction of the adhesion belt
   b. Other changes in cellular shape drive elongation or shortening of a flat sheet of cells-
      Can provide motive force for complex rearrangements.
   c. Cells can alter their surface proteins & thus alter relative stickiness to different groups of neighbors or to ECM secreted by those neighbors. Changes in adhesion lead to cell migrations etc.

4. Changes in cell shape or adhesiveness occur in specific regions of the body. i.e. individual cells have individual identities or fates very early

5. Just as with cleavage, if we understand the possible ways cells can move and rearrange, we can mix and match to get gastrulation in different organisms.
   a. Individual cells move by:
      i. MIGRATION - movement of individual cells over other cells or matrix
      ii. INGRESSION - movement of individual cells or small groups from an epithelium into a cavity
   b. Groups of cells move by:
      i. INVAGINATION - local inward buckling of an epithelium
      ii. INVOLUTION - inward movement of a cell layer around a point or edge
      iii. EPIBOLY - spread of an outside cell layer to envelop a yolk mass or deeper layer
      iv. DELAMINATION - splitting 1 cell sheet into 2 or more parallel sheets.
      v. CONVERGENT EXTENSION - elongation of a cell layer in one dimension with shortening in another

6. Gastrulation in the sea urchin embryo, a "simple" example.
   a. We start with a single-cell thick hollow ball of ~1000 cells, surrounding the central blastocoel cavity.
   b. First actors- primary mesenchyme cells (descendents of micromeres).
      i. Lose adhesion to immediate neighbors and the hyaline layer & gain affinity for the basal lamina and extracellular matrix on the inside of the sphere, which leads to detachment from the epithelium at vegetal pole & crawling along inside of blastocoel like amoebae.
      ii. Migrate along the extracellular matrix using filopodia to detect chemical cues.
iii. Home in on special places in the interior, where they apparently have a higher affinity for the surface.

iv. These cells go on to fuse and form the spicules made of calcium carbonate that serve as the larval skeleton (this is a mesoderm cell fate).

c. Apical constriction and changes in the extracellular matrix create a **dome-shaped invagination** at the vegetal pole.
   i. Invagination = archenteron (primitive gut), opening = blastopore
   ii. Cells change shape (ends facing the blastocoel enlarge while apical end contract) driving a dome-shaped invagination.
   iii. The extracellular matrix changes: secretion of sulfated proteoglycans (CSPG’s) into inner lamina of hyaline layer critical, sulfated proteoglycans absorb water and cause swelling, which in turn causes inward buckling.

d. Convergent extension **extends dome (archenteron) into long tube** = primitive gut.

e. **Secondary mesenchyme cells** at the leading edge reach out with filopodia, apparently looking for place where they’re programmed to adhere, and draw the gut tube to this position.
   • These secondary mesenchyme cells go on to form muscles. (mesoderm)

f. End of the tube fuses with the surface epithelium to form the MOUTH. The original site of invagination forms the ANUS. The tube that makes up the gut = endoderm.

g. Entire gastrulation process takes as little as 9 hours in this type of sea urchin!

2. Frog gastrulation: Similar to the sea urchin, but more complex.
   a. Frog eggs differ from sea urchin in many ways. Most obvious at vegetal pole. The blastula is not a hollow ball of cells, but is a solid hemisphere of yolky cells.
   b. Like sea urchin, frog blastula have a blastocoel. **Functions of the blastocoel**.
   c. One way to understand gastrulation is to look at a “fate map” that shows what the progeny of cells present NOW will become LATER. In frogs this shows that all outside cells of the blastula will form ectoderm or endoderm, while the mesoderm will form from inside cells. Again, compare this situation to a fate map of the sea urchin blastula, where all these three tissue types are generated from outside cells.
   d. **Gastrulation starts by invagination of marginal endoderm cells to form the BLASTOPORE LIP**. This occurs 180° opposite from the point of sperm entry, near the equator of the embryo. The invaginating cells are called BOTTLE CELLS and have a distinctive shape. Just as we saw with the dome-shaped invagination of the archenteron during sea urchin gastrulation, apical constriction drives invagination of the blastopore here. The cavity that forms here is also the ARCHENTERON.
   e. **Next, the MARGINAL ZONE CELLS (cells at the junction between the animal and vegetal hemispheres) begin involution at 2 levels:**
   i. the outside cells involute to form the roof of the archenteron, which will form the endoderm
   ii. the deep or inside cells involute to form mesodermal derivatives. This movement is dependent on fibronectin in the extracellular matrix, which is secreted by the ectoderm of the blastocoel roof shortly before gastrulation. This was determined by an experiment that involved injecting a synthetic fibronectin peptide competitor into the blastocoel. The mesodermal precursor cells bind the synthetic fibronectin competitor so can’t recognize the normal fibronectin-lined traffic route along the blastocoel roof. The archenteron fails to form and these mesodermal precursors remain at the surface. [We’ll talk about this experiment Thurs when we finish the steps of frog gastrulation]