

Morphogenesis: Gastrulation and Neurulation

Reading 9th ed: Chp 7: 244-251; Chp 9: 333-339

Reading 8th ed: Chp 10: 291-302, 337-342; Chp 12: 373-380

Learning goals Be able to:

Define the terms blastula, blastocoel, blastopore, archenteron, gastrula.

Explain the difference between epithelial and mesenchymal cells, and why transitions between the two cell types are a fundamental part of development.

Explain what gastrulation, in general, accomplishes for an embryo.

Predict what happens in the absence of gastrulation (with regard to cell type specification and differentiation).

Compare gastrulation movements in different species and hypothesize why the differences (and similarities) exist.

Compare the movements of gastrulation to the movements (and the purpose of) neurulation.

Gastrulation reorganizes the blastula into a three-layered embryo with a recognizable body plan. In contrast to cleavage, the primary mechanisms of gastrulation involve cell movements; cell divisions occur but are less important to the morphogenetic process. In this class we will discuss aspects of gastrulation in some favorite model organisms; don't worry about details, but rather focus on what gastrulation accomplishes for the embryo. Central to this topic are the characteristics of *epithelia* and *mesenchyme*, and the *epithelial/mesenchymal transition*, which we will encounter examples of throughout the course. After an embryo gastrulates, the next set of morphogenetic movements are called neurulation. This is the process of forming a hollow tube of neural cells, and segregating this tube of cells from the overlying epithelial cells. Obviously, both of these processes are crucial for setting up the normal structure of an embryo, and the two processes also share some features, discussed below.

Epithelia and mesenchyme

There are two basic states of cell organization and behavior termed epithelial and mesenchymal. Both are found during development in all three germ layers.

Many developmental events involve conversion of one state to the other.

Epithelial cells:

Regular in shape, arranged in layer or sheet, often only one cell thick, called an epithelium. Major feature is polarity.

Bounded on one side (basal surface) by a basal lamina, thin mat of ECM secreted by the cells. Further from cells may be a thicker basement membrane.

Opposite side is the apical surface; differs for different epithelia. Often secretory; small protrusions called microvilli increase surface area. Different transport proteins in apical and basal plasma membranes allow transfer of specific molecules across an epithelium.

Three general types of junctions hold epithelial cells together, allowing them to communicate: 1) Tight junctions prevent leakage between cells. 2) Gap junctions allow passage of small molecules (< 1 kD). 3) Other structural junctions include transmembrane linker proteins, e.g. cadherins.

Mesenchymal cells:

Irregular in shape, mobile, non-polar, separated from each other by ECM.

Move by interacting with ECM components.

We will discuss only these two primary types of cell organization, though other intermediate organizations are also found in tissues.

Terms to describe morphogenetic movements of cells (G,8.4):

Epithelia can move as a sheet by invaginating to form a pocket (like making an indentation in a balloon), or involuting (flowing, as a sheet, "around a corner") to form one cell layer inside another.

Epithelia can change shape in several additional ways that are used in the movements of morphogenesis. They can delaminate from a sheet, which means that a whole row of cells releases its connections and forms a new sheet adjacent to the old sheet of cells. Or, individual epithelial cells can release from their junctions to ingress into an adjacent space (when this happens, the epithelial cells become mesenchymal). They can also undergo a process called convergent extension, which allows a flat sheet of cells to form a column of cells by transiently releasing and re-intercalating cells in different positions (radial intercalation). The last movement is called epiboly, which works by intercalating new cells into a preexisting sheet so that it spreads (medio-lateral intercalation: basically the opposite of convergent extension).

Finally, mesenchymal cells can migrate individually through ECM or along a basal lamina.

All these movements depend on adhesion molecules at the cell surface that are connected to the cytoskeleton in the cell interior, such as cadherins and integrins. These connections, and other cytoskeletal rearrangements also account for changes in cell shape, for example, changing a roughly spherical cell into a wedge shaped cell, something that is important during neurulation.

Examples of gastrulation

In learning about gastrulation, keep in mind what's being accomplished for the embryo:

- 1) conversion of a ball of cells into an embryo with three layers of different kinds of cells (endoderm, mesoderm and ectoderm)
- 2) bringing previously separated cell types into contact, allowing new inductive signals that affect later cell determination and differentiation; and
- 3) establishing the rudimentary body plan of the animal: "a tube within a tube," (endoderm in the interior, ectoderm on the surface, and mesoderm in the middle)

Gastrulation in *Xenopus* and chick are similar despite some fundamental differences in the arrangement of the two embryos, so we will use these systems to think about how cells move and change shape during development.

The *Xenopus* blastula is several cells thick, with the blastocoel (fluid filled cavity) in the animal hemisphere separating smaller ectodermal cells from larger (yolky) endodermal cells. Gastrulation begins shortly after the so called mid-blastula transition (MBT).

1) Blastopore forms on future dorsal side of the embryo, in region of the "gray crescent" between animal and vegetal hemispheres just below bottom of the blastocoel. Cells in this region change shape to become bottle cells, causing invagination. (10.7)

2) Subsequent involution over dorsal lip of blastopore forms gut cavity (archenteron), displacing the blastocoel. Bottle cells, at advancing tip of the archenteron, lead the way. But advancement is driven by mobile deep cells of the involuting marginal zone (IMZ), which migrate along the interior surface of the blastocoel and pull more cells from the animal cap inside. (10.10, 10.12)

3) Meanwhile, blastopore region spreads laterally in both directions around to the ventral midline (opposite the original blastopore) until surface cells are involuting from all sides. They surround a yolk plug of endodermal cells, which becomes smaller and eventually closes as the ectodermal layer spreads by convergent extension (epiboly) to enclose the vegetal hemisphere. (10.11)

The embryo now has three layers: ectoderm, which has spread to cover the whole surface, endoderm, which is now all inside, and mesoderm, derived from the marginal zone cells that have moved inside, between ectoderm and endoderm. Epiboly involves cell rearrangements as well as cell division.

By the end of gastrulation, anterior/posterior organization has been laid out in the three cell layers: future head ectoderm and mesoderm on the roof of the archenteron near the former animal pole, future

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spinal cord lying along dorsal side of the archenteron toward original blastopore; future anus where blastopore closed over yolk plug.

Description of this process came from experiments using dyes to fate map the movements of cells. Tissue removal (extirpation) and grafting experiments provided information on where some of the guidance and the forces are coming from, and showed that much of the process is region-autonomous, but molecular controls of these movements are still poorly understood.

Chick:

Blastula formation

The blastula is distinguishable from the cleavage stage embryo in that it has formed two layers, an upper epithelial layer, the epiblast, and a lower layer, the hypoblast, which will give rise to supporting tissues around the embryo, but does not give rise to embryonic tissues. The epiblast will form all three germ layers of the embryo (compare this geometry to the amphibian embryo to see the obvious differences). The blastocoel in chick is the space between epiblast and hypoblast (11.15).

Gastrulation in chick involves the transformation of epithelial cells into mesenchymal cells, as in amphibian gastrulation. Principal events are:

Cells from the entire epiblast converge to the posterior midline as blastodisc lengthens and thickens (convergent extension).

Cells from the posterior marginal zone move anteriorly, forming a ridge called the primitive streak. The streak elongates anteriorly, and terminates in a thickening called Hensen's node (equivalent to the dorsal lip of the blastopore in amphibians). Hensen's node is where cells begin to migrate into the embryo, primarily by ingression.

Gastrulation movements begin at the node, and progress towards the posterior of the embryo. Thus, the chick has a pronounced anterior-posterior gradient of development.

As in frogs, the endoderm cells move in first, then mesoderm. Cells that will form the dorsal mesoderm (notochord, somites, head) migrate in first, followed by cells that will form the intermediate mesoderm of organs (kidney, gonads), and lastly the cells that will form the lateral plate mesoderm (heart, blood). Prospective ectoderm stays on the outside, as in all embryos. (11.16, 11.17)

Comparison to amphibian gastrulation

Node is equivalent to the dorsal lip of the blastopore in frogs, the site where gastrulation begins (and has the "organizer" activity we will discuss in the next lecture).

Cells of the node and dorsal lip become "chordamesoderm"; give rise to notochord. The geometry of the two embryos is quite different, the cells move by slightly different mechanisms, but the end result is the same.

Mammals: this section is for your interest only—you can also see Gilbert, pp. 351-355

Blastula formation

At the 8-cell stage, the embryo compacts into the "morula" stage. At this stage, there are only two inner cells that will give rise to the actual embryo, surrounded by a sphere of outer cells connected by tight junctions, now called the trophoblast, which will give rise to supporting extraembryonic structures (amnion and parts of the placenta).

The blastocoel cavity forms at this stage, and the embryo will derive from only the inner cell mass (derived from the two cells mentioned above).

Human embryos have the same set of extraembryonic membranes as chicks, with similar functions. The most notable in humans is the chorion, since it fuses with the uterine wall to contribute to the formation of the placenta, which connects the embryo with the circulation of the mother to provide nutrients (hence no need for yolk) and remove waste (11.33). The amnion is also important, providing a cushioned environment (the amniotic sac) for the embryo.

Gastrulation

As in chick, primitive streak begins to form in posterior (caudal) half of the germ disc in the epiblast, and extends anteriorly, culminating in the node (same as Hensen's node).

Presumptive endoderm cells ingress through the groove first, pushing aside the hypoblast to form the embryonic endoderm (11.34).

Presumptive mesoderm cells ingress next. As in the chick the earliest mesoderm cells form anterior and dorsal structures, while later mesoderm cells form more posterior structures and also migrate laterally to form lateral plate mesoderm.

Neurulation

Neurulation is the formation of a hollow, dorsal neural tube, which gives rise to both the spinal cord and the brain. Neurulation begins with the formation of a neural plate, a thickening of the dorsal ectodermal region of the embryo, caused when cuboidal epithelial cells become columnar. Then the edges of the plate fold and rise, meeting in the midline to form a tube.

--As a result of the neurulation process, the apical surface of the epithelial cells is inside, facing the lumen.

--Failure of the neural tube to close results in major developmental defects -- anencephaly if the anterior neural tube fails to close (forebrain degenerates) and spina bifida if posterior closure is defective (about 1/500 live births) (12.5).

Mechanical details of neurulation.

The bending and folding of the neural plate is essentially independent of the overlying ectoderm.

--Within the neural plate, the cells become columnar. The midline neural plates cells (median hinge point cells) are responsible for convergent extension that intercalates many layers into fewer layers, creating an elongated, folded neural tube that extends almost the entire length of the embryo.

--Prospective neural plate cells attempt to crawl under the epidermal cells and epidermal cells push toward the midline. These movements are dependent on the block of mesodermal cells that underly the neural tube, the notochord.

--Lateral hinge point cells (at either end of the prospective tube), also help to initiate the bending of the cells into a tube by virtue of changes in cell shape.

In general, the shape changes of cells (e.g. heightening, wedge shape) rely on cytoskeletal changes within the cells: microtubules allow cells to elongate, while the apical constriction required for the folding up of the cells is due to actin.

In addition, cell adhesion molecules help to guide the adhesion of prospective neural cells together: the entire ectoderm initially expresses E-cadherin, but presumptive neural cells stop expressing E-cadherin and begin expressing N-cadherin. Since cadherins bind homotypically, the N-cadherin expressing cells segregate away from the E-cadherin expressing cells, and become the neural tube (12.6). Without the differentially expressed cadherins, the neural tube would not be able to adhere its two sides together, and separate properly from the overlying ectoderm.

Take-home lessons

1) Gastrulation involves movement and reorganization of cells. In contrast to the cleavage stage, cell division is not the major determinative or morphogenetic influence, although it is occurring throughout gastrulation.

2) The gut (inner tube) forms from the archenteron, which displaces the blastocoel. Its formation is the basis for an important evolutionary distinction that divides animals into two groups. In deuterostomes (secondary mouth), including all vertebrates as well as echinoderms (such as sea urchins), the blastopore becomes the

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anus and the mouth forms later. In protostomes (first mouth; all other invertebrates), the blastopore becomes the mouth.

3) The three primary germ layers are formed: endoderm, the inside layer lining the gut, ectoderm, the outside layer that will give rise to the skin and nervous system, and most importantly a new cell layer, the mesoderm, between them, which will form tissues in sea urchin such as the spicules and muscle, and in vertebrates the muscles, blood vessels, and organs such as heart, kidney, and gonads.

4) Although the three germ layers become important later, the major distinction during gastrulation is between the two major states of cell organization: epithelia, which are prevalent in the endodermal and ectodermal layers, and mesenchyme, which is prevalent in the mesoderm.

Because a major outcome of gastrulation is formation of mesoderm from an epithelial blastula, there is a transition of epithelial cells into mesenchymal cells (EMT), as mesodermal precursors leave epithelial sheets and ingress into the blastocoel cavity. We will see other examples of EMT in neurulation and organ development. The cellular and molecular mechanisms of this process are of interest to cancer biologists, because these normal transformations are closely related to the changes that transform epithelial cells to carcinomas. The reverse transformation of mesenchyme to epithelia also occurs, as we shall see later.

5) Neurulation involves some similar movements to gastrulation, derived from changes in cell shape and cell adhesions. Without convergent extension, apical constriction, and sorting of cells dependent on adhesive properties, the neural tube could not form. Remember, all the changes discussed here have to do with rearranging cells in the embryo, but do not actually have a direct effect on the fates of the cells. Cells are fated to become neuronal before neurulation actually happens.

Review Questions

- 1) What are the important distinguishing characteristics of epithelial and mesenchymal cells?
- 2) What are the general roles of cell adhesion molecules in the formation and behavior of epithelia?
- 3) What are some of the major mechanism by which epithelia change shape during gastrulation? What molecules do you suppose are involved in regulating these changes?
- 4) What happens to the blastocoel during gastrulation?
- 5) How does the archenteron form, and what does it become?
- 6) How does the chick primitive streak originate, and what structure forms at its anterior end? What structure is this functionally equivalent to in amphibian embryos? How could you prove they are similar?
- 8) What are the differences between the movements of epiboly and convergent extension in terms of what they accomplish for the embryo?