EMBRYOLOGY, DIFFERENTIATION, MORPHOGENESIS AND GROWTH

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Contents

Summary

Embryology deals with the study of embryogenesis, which includes the developmental processes between fertilization and birth or hatching, and can be divided into three distinct stages, cleavage, gastrulation and organogenesis. During cleavage, the single-celled zygote undergoes rapid biphasic mitotic cell divisions to generate thousands of almost identical blastomeres in a relative short period of time. Then during gastrulation extensive cellular movements and rearrangements lead to the formation of the germ layers, *i.e.*, the outer ectoderm, the inner endoderm and the mesoderm sandwiched between them. During organogenesis, differential gene expression-mediated cell differentiation generates hundreds of distinct cell types, and signal transduction pathways-mediated induction and cell adhesion molecule-mediated cell adhesion as well as the extracellular matrix (ECM) act to form tissues from cells, construct organs from tissues, and assemble organ systems from organs. Growth, especially increase in size, rarely occurs during cleavage and gastrulation. Subsequent organogenesis and some adult organs undergo several ways of growth including cellular multiplication, increase in cellular size, and increase in amount of the ECM materials such as bone and shell. As a normal component of development, apoptosis or programmed cell death occurs to remove unneeded cells and thus is essential for the formation of particular structures during embryogenesis.

1. Introduction – Life is a Cycle

Life begins with fertilization, the process of the fusion of the male and female gametes, which includes the eventual fusion of the pronuclei. For most of animals including sea urchins, frogs, fishes and humankind, the entry of the sperm into the egg triggers and activates egg metabolism and development. For some insects such as the fruit fly, the egg starts its metabolism and development even before the sperm fertilizes the egg. The developing organism between fertilization and birth or hatching is called an embryo. The developmental process of an embryo is called embryogenesis, which can be divided into several stages. The first embryonic stage immediately following fertilization is called cleavage, which is characterized by rapid mitotic division of the fertilized egg, also called the zygote, and generation of thousands of smaller cells, also called blastomeres. A sphere or ball of blastomeres is called blastula by the end of the cleavage. The next embryonic stage is gastrulation that involves extensive movements and rearrangements of embryonic cells and produces the three germ layers, *i.e.*, the outer ectoderm, the middle mesoderm and the inner endoderm. The embryonic stage following gastrulation is organogenesis. During organogenesis, the cells from different germ layers start to differentiate and interact, and different types of cells, tissues, organs and organ systems are subsequently formed. Following organogenesis in most of animals, the embryos or fetuses are born or hatched, indicative of the end of embryogenesis. For some metazoans, the young organisms that are just born or hatched are not able to reproduce and must undergo metamorphosis to become sexually mature

adults. Some animals such as frogs and the fruit fly undergo larval stages for feeding or dispersal. The mature adult males and females then produce sperms and eggs through gametogenesis. When the sperm fertilizes the egg, the new zygote is formed again, and the new life also starts again.

Embryology deals with the study of embryogenesis. As organisms also undergo other developmental processes such as larval development and metamorphosis, gametogenesis and adult development, embryology was superseded by developmental biology that deals with all processes of biological development. As part of developmental biology, embryology has a long history that can be traced back to Aristotle who did the very first embryological experiment to examine the embryonic development of chicken over 2, 000 years ago. However, modern developmental biology has a short history of just over 100 years since 1894 when German experimental embryologist Wilhelm Roux and others established the field. In other words, modern developmental biology includes not only the descriptive embryological studies, but also the experimental, genetic and molecular studies of all biological developmental processes. The remaining part of this chapter will first discuss characteristics of early development including cleavage, gastrulation and axis formation; then cellular differentiation and its underlying differential gene expression; morphogenesis and its underlying induction and signal transduction pathways, cell adhesion and cell adhesion molecules, and extracellular matrix (ECM); and finally cellular growth, isometric growth, allometric growth, and apoptosis.

2. Cleavage

2.1. Biphasic Cell Divisions during Cleavage

As soon as fertilization is complete, cleavage takes place, *i.e.*, the zygote undergoes rapid mitotic cell divisions and produces thousands of cells or blastomeres. During cleavage, the ratio of the nucleus and the cytoplasm increases, for the oocyte cytoplasm is divided into smaller and smaller cells, rather than increased in size. The rate of the cell division during cleavage is so remarkable that thousands of blastomeres can be generated in a relative short period of time. This rapid cell division without growth is facilitated by the biphasic cell division cycle that has only M (mitosis) and S (DNA synthesis) phases without growing gap phases (G1 and G2).

MPF (mitosis-promoting factor) plays a critical role during cleavage. Specifically MPF is most active during the M phase but is immeasurable during the S phase. MPF has two subunits: the large subunit is cyclin B whose concentration oscillates, *i.e.*, highest during M and lowest during S; and the small subunit is the cyclin-dependent kinase (Cdk) cdc2 that is regulated by cyclin B. Cdc2 phosphorylates histones, the nuclear lamin proteins and the regulatory subunit of cytoplasmic myosin and in turn triggers mitosis.

2.2. Cleavage Patterns

Two factors are known to determine cleavage patterns that manifest in different organisms: the amount and distribution of the cytoplasmic yolk protein that controls where cleavage occurs as well as the size of blastomeres, and maternal effect factors

that regulate the formation of the mitotic spindle. The yolk-concentrated part of the egg is called the vegetal pole where the cleavage furrow usually cannot reach because the dense yolk proteins inhibits cleavage, whereas the opposite of the vegetal pole is the animal pole where cleavage occurs because it has no or low concentration of yolk. Organisms such as snails, nematodes, sea urchins and mammals possess isolecithal eggs that the whole eggs are divided. This kind of cleavage is called holoblastic. Because there is little yolk in their egg, these organisms have evolved either to have special larval forms for feeding or to have the placenta to obtain food and nutrition directly from their maternal parents. Holoblastic cleavage also occurs in frogs that have mesolecithal eggs with moderate vegetal yolk disposition, where cleavage begins at the animal pole and then extends slowly towards the vegetal pole because the vegetal yolk impedes cleavage. Conversely, fishes, reptiles and birds have telolecithal eggs that only a small part of the egg is cleaved because the majority of the egg cytoplasms are filled with yolk for embryonic development. This kind of cleavage is called meroblastic. Insects such as the fruit fly have centrolecithal eggs (*i.e.*, having yolk in the center of the egg), and undergo superficial cleavage because the dense yolk in the center restricts the cleavage furrow within the periphery of the egg.

For many organisms, the cell division cycle (cleavage rhythm) as well as relative position of blastomeres is initially regulated by maternal effect genes, *i.e.*, the proteins and mRNAs deployed in the egg， while the zygotic genome only starts to function in the middle of cleavage.

2.3. Midblastula Transition (MBT)

The early cleavage cell division is not only rapid but also synchronous for some organisms such as insects, frogs, and fishes. The synchronicity of early cell divisions is possible for these organisms because cyclin B is often stored in their eggs so that their cell division cycles can be independent of their zygotic genomes for a limited time until the cyclin B and other necessary oocyte cytoplasmic components are exhausted. Then, a number of processes characteristic of midblastula transition (MBT) occur: (1). Gap phases G1 and G2 are added to the cell division cycle so that the cell cycle is lengthened. For instance, both G1 and G2 phases are added to the cell cycle shortly after the 12^{th} division in the *Xenopus laevis* embryo. G1 is added to the cell cycle during the 14th division and G2 during the 17^{th} division in the *Drosophila melanogaster* embryo. (2).The synchronicity of the cell divisions is abolished. This is because different cells produce different MPF regulators so that the times for the entry and exit of the M phase are different. (3). The zygotic genome starts to function, *i.e.*, the zygotic genes are being transcribed and translated. Many of these proteins are required for gastrulation. (4). The blastomeres start to move around to acquire new neighboring cells. However, MBT is observed only in some animal embryos. For instance, mammalian embryos do not undergo MBT.

3. Gastrulation, Cellular Movements and Formation of Three Germ Layers

Gastrulation follows cleavage. During gastrulation, extensive cellular movements and rearrangements lead to formation of the three germ layers. The cells fated to become the endoderm and the mesoderm enter the inside of the embryo, whereas the ectodermal

cells cover the outside of the embryo. In general, some combination of several types of cell movements is involved gastrulation.

3.1. Modes of Cell Movements

While different animals display distinct patterns of gastrulation, there are only several modes of embryonic cell movements as follows:

Invagination refers to the infolding of a cell sheet into the embryo. The formation of the blastopore, the first opening of the embryo, is through invagination.

Involution refers to the inturning or inward movement of a cell sheet over the basal surface of an outer cell layer.

Ingression refers to the migration of individual cells from the surface layer into the inside of the embryo. Both involution and ingression is likely involved in the formation of the zebrafish hypoblast.

Delamination refers to the splitting or migration of one cell sheet into two cell sheets. For instance, delamination occurs during formation of the hypoblast in mammals and birds.

Epiboly refers to the expansion of one cell sheet over other cells. Epiboly is involved in the formation of the ectoderm.

Convergent extension refers to the rearrangement of cells mediating dorsal-ward narrowing of the tissue and subsequently their lengthening along the anterior-posterior axis. This contributes to the elongation of the body axis.

3.2. Ectoderm and its Derivatives

The ectoderm, the outer germ layer, gives rise to the epidermis (the surface ectoderm), the central nervous system (the neural tube) and the neural crest cells (Figure 1). The neural crest cells migrate extensively to generate numerous cells/structures as follows: (1). the neurons and glial cells of the sensory, sympathetic and parasympathetic nervous systems; (2). the epinephrine-producing medulla cells of the adrenal gland; (3). the pigment-containing cells of the epidermis; and (4). many of the skeletal and connective tissue components of the head. Accordingly, the neural crest cells have been called “the fourth germ layers” by some developmental biologists. In addition, the ectoderm also gives rise to the placodes, which contribute to the formation of the sensory organs (e.g. lens and inner ears) and cranial nerves.

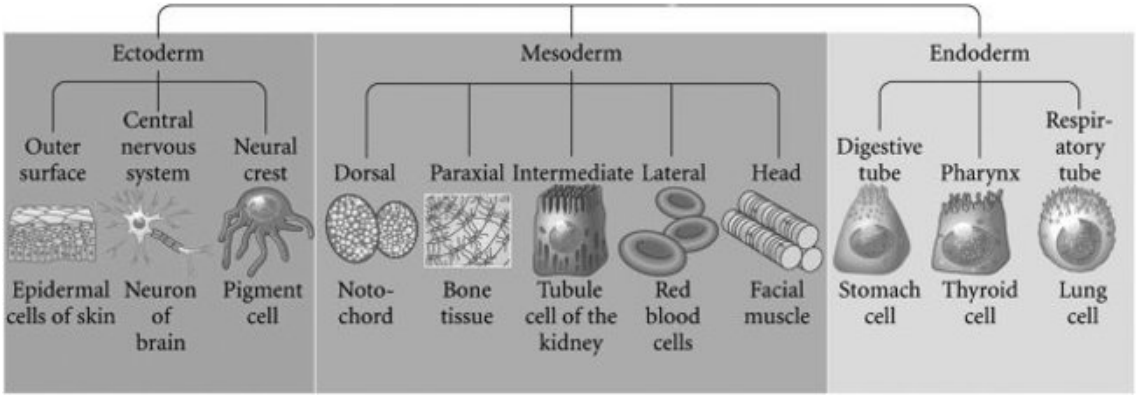

Figure 1. Structures derived from the ectoderm, the mesoderm and the endoderm (Modified from S. F. Gilbert, 2006).

3.3. Mesoderm and its Derivatives

The mesoderm, the middle germ layer, gives rise to all the organs between the ectodermal wall and the endodermal tissues (Figure 1). The vertebrate trunk mesoderm at the neurula can be further divided into four regions. The chordamesoderm, the axial or the central region of the trunk mesoderm, develops into the notochord, the transient rod-like structure that separates the embryos into right and left halves. Recently studies showed that the notochord secrets *Sonic hedgehog* (*shh*) and acts as the primary ventral neural tube patterning center. The two paraxial mesoderms or somitic mesoderms flanking the chordamesoderm develop into somites, blocks of mesodermal cells on either sides of the neural tube. Somites further give rise to many tissues of the back including bone, muscle, cartilage and dermis. The intermediate mesoderm gives rise to the urogenital systems, *i.e.*, the kidneys, the gonads and their associated ducts. Finally, the lateral plate mesoderm, the farthest away from the notochord, develops into the heart, blood vessels and blood cells of the circulating system, the lining of the body cavities, the mesodermal components of the limbs and a series of extraembryonic membranes.

BMP4 (bone morphogenetic protein 4) is known to play a role in patterning the four regions of the mesoderm. BMP4 acts as a morphogen and forms a side-to-center gradient, *i.e.*, the higher concentrations of BMP4 are found in more lateral mesoderm than in more central mesoderm. Different concentrations of BMP4 activate expression of different members of the Forkhead (Fox) transcription factor family. For instance, *foxf1* is expressed in the lateral plate mesoderm, while *foxc1* and *foxc2* are expressed in the paraxial mesoderm; and these *fox* transcription factors in turn activate expression of specific target genes that are responsible for the formation of the lateral plate mesoderm and the paraxial mesoderm, respectively.

3.4. Endoderm and its Derivatives

The endoderm, the inner germ layer, has two major functions (Figure 1). The first function is to induce the formation of some mesodermal organs including the notochord, the heart, the blood vessels and the mesodermal germ layer. The second function of the endoderm is to generate the linings of two tubes of the vertebrate body: the digestive tube that is extending the length of the body and the respiratory tube that is derived from

the digestive tube and eventually develops into two lungs. The digestive tube buds give rise to the liver, gallbladder and pancreas. The pharynx is the region of the digestive tube anterior to the point where the respiratory tube branches off.

It is noteworthy that the derivatives of the three germ layers described above are most for vertebrate embryos. Invertebrate embryos have different derivatives. For instance, invertebrate embryos do not produce the notochord.

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3.5. Cell Migration

Following gastrulation and neurulation, there are several types of cells that migrate from the sites of their origins to the sites where they differentiate to form different tissues. For instance, the primordial germ cells migrate from the endoderm to the gonads where they develop into sperms or eggs; and the vertebrate blood cell precursors migrate several times and eventually move in the liver and the bone marrow. The most amazing type of the migrating cells are the neural crest cells. The dorsalmost region of the neural tube is the origination site of the neural crest cells, which then take several different migrating paths to form numerous types of cells or tissues as described above. For instance, some trunk neural crest cells migrate ventrally through the anterior of the sclerotome to develop into dorsal root ganglia, sympathetic neurons, adrenal medullar cells and Schwann cells; and other trunk neural crest cells migrate dorsolaterally between the epidermis and the dermamyotome to develop into pigment cells (melanocytes).

4. Axis Formation

The animal body plan is defined by three axes, *i.e.*, the anterior-posterior axis (AP axis), the dorsal-ventral axis (DV) and the left-right axis (LR), which are specified and developed during embryogenesis.

4.1. Anterior-posterior Axis (AP axis)

The AP axis refers to the line extending from the anterior end (for instance, the head) to the posterior end (for instance, the tail). In the fruit fly, two signaling centers, the anterior organizing center and the posterior organizing center, specify its AP axis. The anterior organizing center is located at the anterior end of the embryo. The maternal effect factor, Bicoid protein acts as a morphogen and forms a concentration-based anterior to posterior gradient across the embryo. At the anterior end, Bicoid activates transcription of the anterior gap genes such as *hunchback* and also represses translation of the posterior gap gene *caudal*. The posterior organizing center is located in the posterior end of the embryo. The maternal effect factor, Nanos protein functions as a morphogen and forms a concentration-based posterior to anterior gradient across the embryo. At the posterior end, Nanos acts as a transcription factor to activate the posterior gap gene *caudal* and also as translational repressor to inhibit translation of *hunchback*.

The AP axis formation is rather different in vertebrates. For instance, in zebrafish, the interaction of the FGF, Wnt and retinoic acid (RA) signals specifies the AP axis. FGF

and Wnt signals suppress the expression of the anterior genes such as the head-specifying gene *otx2* and *cyp26* that encodes retinoic acid degradation enzyme, retinoic acid-4-hydroxylase, in the future posterior end and restrict the expression of these anterior genes in the future anterior end. Wnt, FGF and retinoic acid also activate the expression of the posterior genes such as *hoxb1* and *meis3* in the future posterior end. Thus the distinct zones of anterior gene expression and posterior gene expression regulated by FGF, Wnt and retinoic acid signaling underlie the zebrafish AP formation.

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Biographical Sketch

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