Early Development in Selected Invertebrates

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"THE GENERAL POLARITY, SYMMETRY, AND PATTERN of the embryo are egg characters which were determined before fertilization," wrote Edwin Grant Conklin in 1920. Indeed, as we will see, the egg cytoplasm plays a major role in determining patterns of cleavage, gastrulation, and cell specification. It does so by interacting with the nuclear genome established at fertilization.

Fertilization gave the organism a new genome and rearranged its cytoplasm. Now the zygote begins the production of a multicellular organism. During *cleavage*, rapid cell divisions divide the cytoplasm of the fertilized egg into numerous cells. These cells undergo dramatic displacements during *gastrulation*, a process whereby they move to different parts of the embryo and acquire new neighbors. During cleavage and gastrulation, the major axes of the embryo are determined and the embryonic cells begin to acquire their respective fates.

While cleavage always precedes gastrulation, axis formation in some species can begin as early as oocyte formation. It can be completed during cleavage (as in *Drosophila*) or extend all the way through gastrulation (as in *Xenopus*). Three body axes must be specified: the anterior-posterior (head-tail) axis; the dorsal-ventral (back-belly) axis; and the left-right axis. Different species specify these axes at different times, using different mechanisms. This chapter will look at the different ways these universal processes take place in some invertebrate embryos.

EARLY DEVELOPMENTAL PROCESSES: AN OVERVIEW

Cleavage

Once fertilization is complete, the development of a multicellular organism proceeds by a process called **cleavage**, a series of mitotic divisions whereby the enormous volume of egg cytoplasm is divided into numerous smaller, nucleated cells. These cleavage-stage cells are called blastomeres. In most species (mammals being the chief exception), both the initial rate of cell division and the placement of the blastomeres with respect to one another are under the control of the proteins and mRNAs stored in the oocyte. Only later do the rates of cell division and the placement of cells come under the control of the newly formed genome.

During the initial phase of development, when cleavage rhythms are controlled by maternal factors, the cytoplasmic volume does not increase. Rather, the zygote cytoplasm is divided into increasingly smaller cells. First the zygote

Hence, studying the period of cleavage, we approach the source whence emerge the progressively branched streams of differentiation that end finally in almost quiet pools, the individual cells of the complex adult organism.

E. E. JUST (1939)

It is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life.

LEWIS WOLPERT (1986)
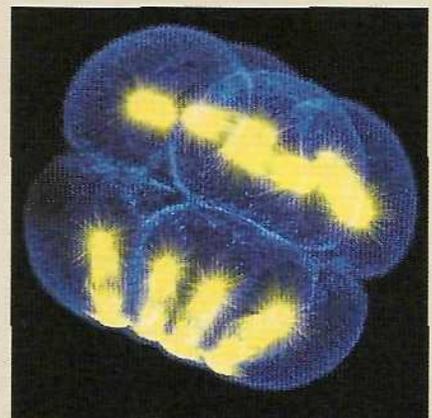
is divided in half, then quarters, then eighths, and so forth. Cleavage occurs very rapidly in most invertebrates, probably as an adaptation to generate a large number of cells quickly and to restore the somatic ratio of nuclear volume to cytoplasmic volume. The embryo often accomplishes this by abolishing the gap periods of the cell cycle (the G_1 and G_2 phases), when growth can occur. A frog egg, for example, can divide into 37,000 cells in just 43 hours. Mitosis in cleavage-stage *Drosophila* embryos occurs every 10 minutes for over 2 hours, and some 50,000 cells form in just 12 hours.

From fertilization to cleavage

As we saw in Chapter 4, fertilization activates protein synthesis, DNA synthesis, and the cell cycle. One of the most important events in this transition from fertilization to cleavage is the activation of **mitosis-promoting factor**, or **MPF**. MPF was first discovered as the major factor responsible for the resumption of meiotic cell divisions in the ovulated frog egg. It continues to play a role after fertilization, regulating the cell cycle of early blastomeres.

Blastomeres generally progress through a biphasic cell cycle consisting of just two steps: M (mitosis) and S (DNA synthesis) (**Figure 5.1**). The MPF activity of early blastomeres is highest during M and undetectable during S. The shift between the M and S phases in blastomeres is driven solely by the gain and loss of MPF activity. When MPF is microinjected into these cells, they enter M. Their nuclear envelope breaks down and their chromatin condenses into chromosomes. After an hour, MPF is degraded and the chromosomes return to S phase (Gerhart et al. 1984; Newport and Kirschner 1984).

What causes this cyclical activity of MPF? Mitosis-promoting factor consists of two subunits. The larger subunit, **cyclin B**, displays the cyclical behavior that is key to mitotic regulation, accumulating during S and being degraded after the cells have reached M (Evans et al. 1983; Swenson et al. 1986). Cyclin B is often encoded by mRNAs stored in the oocyte cytoplasm, and if the translation of this message is specifically inhibited, the cell will not enter mitosis (Minshull et al. 1989).
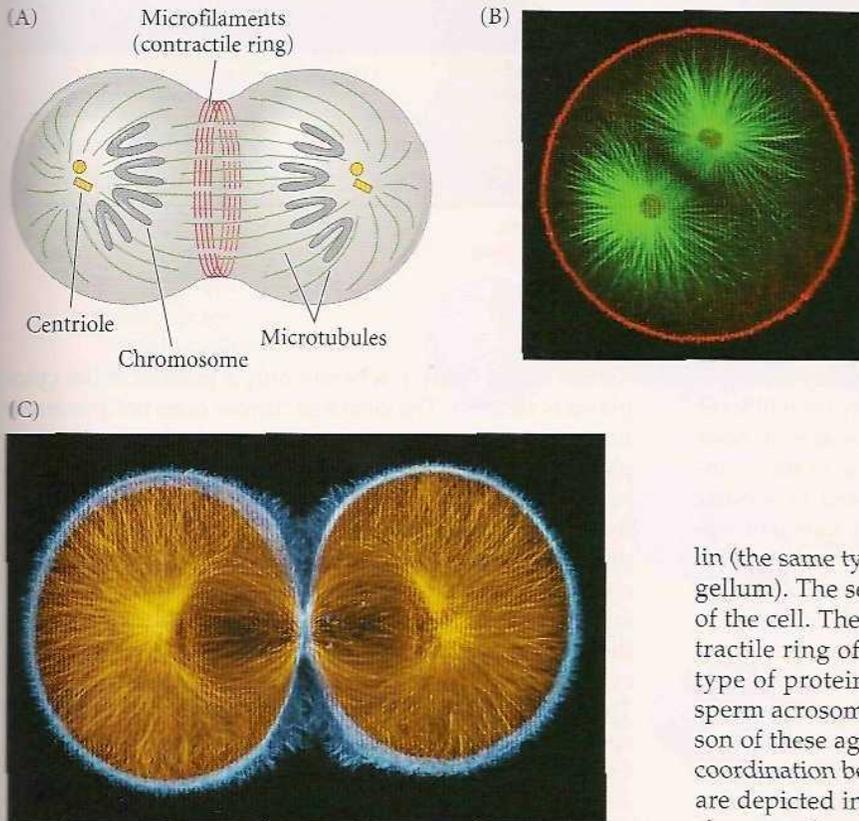
Cyclin B regulates the small subunit of MPF, the **cyclin-dependent kinase**. This kinase activates mitosis by phosphorylating several target proteins, including histones, the nuclear envelope lamin proteins, and the regulatory subunit of cytoplasmic myosin. It is the actions of this small kinase subunit that bring about chromatin condensation, nuclear envelope depolymerization, and the organization of the mitotic spindle. Without the cyclin B subunit, however, the cyclin-dependent kinase subunit of MPF will not function.

The presence of cyclin B is controlled by several proteins that ensure its periodic synthesis and degradation. In most species studied, the regulators of cyclin B (and thus of MPF) are stored in the egg cytoplasm. Therefore, the cell cycle remains independent of the nuclear genome for a number of cell divisions. These early divisions tend to be rapid and synchronous. However, as the cytoplasmic components are used up, the nucleus begins to synthesize them. In several
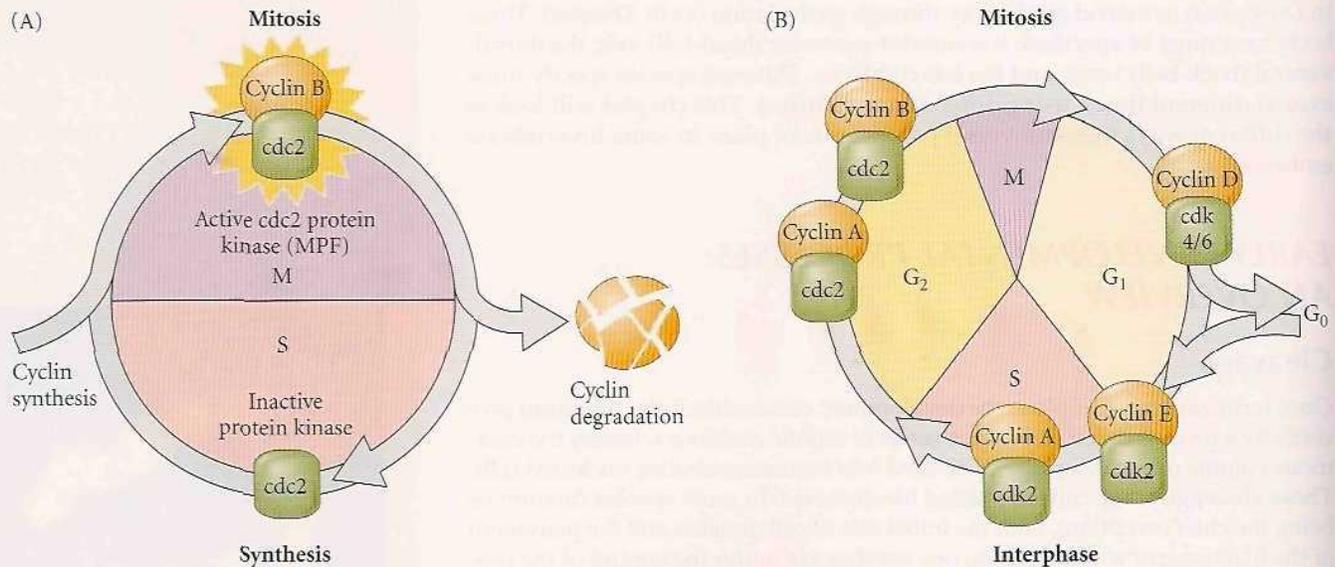
FIGURE 5.1 Cell cycles of somatic cells and early blastomeres. (A) The biphasic cell cycle of early amphibian blastomeres has only two states, S and M. Cyclin B synthesis allows progression to M (mitosis), while degradation of cyclin B allows cells to pass into S (synthesis) phase. (B) The complete cell cycle of a typical somatic cell. Mitosis (M) is followed by an interphase stage. Interphase is subdivided into G_1, S (synthesis), and G_2 phases. Cells that are differentiating are usually taken "out" of the cell cycle and are in an extended G_1 phase called G_0. The cyclins responsible for the progression through the cell cycle and their respective kinases are shown at their point of cell cycle regulation. (B after Nigg 1995.)

(A)

Microfilaments
(contractile ring)

Centriole

Chromosome

Microtubules

(B)

(C)

species, the embryo now enters a **mid-blastula transition**, in which several new properties are added to the biphasic cell divisions of the embryo. First, the "gap" stages (G_1 and G_2) are added to the cell cycle (see Figure 5.1B). *Xenopus* embryos add G_1 and G_2 phases to the cell cycle shortly after the twelfth cleavage. *Drosophila* adds G_2 during cycle 14 and G_1 during cycle 17 (Newport and Kirschner 1982; Edgar et al. 1986). Second, the synchronicity of cell division is lost, because different cells synthesize different regulators of MPF. After numerous synchronous rounds of mitosis, the cells begin to "go their own way." Third, new mRNAs are transcribed. Many of these messages encode proteins that will become necessary for gastrulation. In several species, if transcription is blocked cell division will still occur at normal rates and times, but the embryo will not be able to initiate gastrulation. Many of these new messenger RNAs are also used for cell specification. As we will see in sea urchin embryos, the new mRNA expression patterns of the mid-blastula transition map out territories where specific types of cells will later differentiate.

The cytoskeletal mechanisms of mitosis

Cleavage is the result of two coordinated processes. The first of these is **karyokinesis**, the mitotic division of the cell's nucleus. The mechanical agent of karyokinesis is the mitotic spindle, with its microtubules composed of tubu-

lin (the same type of protein that makes up the sperm flagellum). The second process is **cytokinesis**: the division of the cell. The mechanical agent of cytokinesis is a contractile ring of microfilaments made of actin (the same type of protein that extends the egg microvilli and the sperm acrosomal process). Table 5.1 presents a comparison of these agents of cell division. The relationship and coordination between these two systems during cleavage are depicted in Figure 5.2A, in which a sea urchin egg is shown undergoing first cleavage. The mitotic spindle and contractile ring are perpendicular to each other, and the spindle is internal to the contractile ring. The contractile ring creates a cleavage furrow, which eventually bisects the plane of mitosis, thereby creating two genetically equivalent blastomeres.

The actin microfilaments are found in the cortex (outer cytoplasm) of the egg rather than in the central cytoplasm. Under the electron microscope, the ring of microfilaments can be seen forming a distinct cortical band 0.1 μm wide (Figure 5.2B,C). This contractile ring exists only during cleavage and extends 8–10 μm into the center of the egg. It is responsible for exerting the force that splits the zygote into blastomeres; if the ring is disrupted, cytokinesis stops. Schroeder (1973) likened the contractile ring to an "intracellular purse-string," tightening about the egg as cleavage continues. This tightening of the microfilamentous ring creates the **cleavage furrow**. Microtubules are also seen near the cleavage furrow (in addition to their role in creating the mitotic spindle), since they are needed to bring membrane material to the site of membrane addition (Danilchik et al. 1998).

Although karyokinesis and cytokinesis are usually coordinated, they are sometimes modified by natural or experimental conditions. The placement of the centrioles is critical in orienting the mitotic spindle, and thus the division plane of the blastomeres. Depending on the placement of the centrioles, the blastomeres can separate either into dorsal and ventral daughter cells, anterior and posterior daughter cells, or left and right daughter cells. The spin-

TABLE 5.1 Karyokinesis and cytokinesis

Process	Mechanical agent	Major protein composition	Location	Major disruptive drug
Karyokinesis	Mitotic spindle	Tubulin microtubules	Central cytoplasm	Colchicine, nocodazole[a]
Cytokinesis	Contractile ring	Actin microfilaments	Cortical cytoplasm	Cytochalasin B

[a]Because colchicine has been found to independently inhibit several membrane functions, including osmoregulation and the transport of ions and nucleosides, nocodazole has become the major drug used to inhibit microtubule-mediated processes (see Hardin 1987).

dle can even be at an angle such that one daughter cell is clockwise or counterclockwise to the other. As we will learn Chapter 6, cleavage in insect eggs consists of karyokinesis several times before cytokinesis takes place. In this manner, numerous nuclei exist within the same cell. (The outer membrane of that one big cell will eventually indent to separate the nuclei and form individual cells.)

Patterns of embryonic cleavage

In 1923, embryologist E. B. Wilson reflected on how little we knew about cleavage: "To our limited intelligence, it would seem a simple task to divide a nucleus into equal parts. The cell, manifestly, entertains a very different opinion." Indeed, different organisms undergo cleavage in distinctly different ways. The pattern of embryonic cleavage peculiar to a species is determined by two major parameters: (1) the amount and distribution of yolk protein within the cytoplasm, and (2) factors in the egg cytoplasm that influence the angle of the mitotic spindle and the timing of its formation.

The amount and distribution of yolk determine where cleavage can occur and the relative size of the blastomeres. In general, yolk inhibits cleavage. When one pole of the egg is relatively yolk-free, cellular divisions occur there at a faster rate than at the opposite pole. The yolk-rich pole is referred to as the **vegetal pole**; the yolk concentration in the **animal pole** is relatively low. The zygote nucleus is frequently displaced toward the animal pole. Figure 5.3 provides a classification of cleavage types and shows the influence of yolk on cleavage symmetry and pattern.

At one extreme are the eggs of sea urchins, mammals, and snails. These eggs have sparse, equally distributed yolk and are thus **isolecithal** (Greek, "equal yolk"). In these species, cleavage is **holoblastic** (Greek *holos*, "complete"), meaning that the cleavage furrow extends through the entire egg. With little yolk, these embryos must have some other way of obtaining food. Most will generate a voracious larval form, while mammals will obtain their nutrition from the maternal placenta.

At the other extreme are the eggs of insects, fish, reptiles, and birds. Most of their cell volumes are made up of yolk. The yolk must be sufficient to nourish these animals *throughout embryonic development*. Zygotes containing large accumulations of yolk undergo **meroblastic** cleavage

(Greek *meros*, "part"), wherein only a portion of the cytoplasm is cleaved. The cleavage furrow does not penetrate the yolky portion of the cytoplasm because the yolk platelets impede membrane formation there. Insect eggs have yolk in the center (i.e., they are **centrolecithal**), and the divisions of the cytoplasm occur only in the rim of cytoplasm, around the periphery of the cell (i.e., **superficial cleavage**). The eggs of birds and fish have only one small area of the egg that is free of yolk (**telolecithal** eggs), and therefore the cell divisions occur only in this small disc of cytoplasm, giving rise to the **discoidal** pattern of cleavage. These are general rules, however, and even closely related species have evolved different patterns of cleavage in different environments.

Yolk is just one factor influencing a species' pattern of cleavage. There are also, as Conklin had intuited, inherited patterns of cell division superimposed on the constraints of the yolk. The importance of this inheritance can readily be seen in isolecithal eggs. In the absence of a large concentration of yolk, holoblastic cleavage takes place, and four major patterns of this cleavage type can be observed: *radial*, *spiral*, *bilateral*, and *rotational* holoblastic cleavage. We will see examples of all of these cleavage patterns as this chapter takes a more detailed look at four different invertebrate groups.

Gastrulation

The blastula consists of numerous cells, the positions of which were established during cleavage. During **gastrulation**, these cells are given new positions and new neighbors, and the multilayered body plan of the organism is established. The cells that will form the endodermal and mesodermal organs are brought to the inside of the embryo, while the cells that will form the skin and nervous system are spread over its outside surface. Thus, the three germ layers—outer ectoderm, inner endoderm, and interstitial mesoderm—are first produced during gastrulation. In addition, the stage is set for the interactions of these newly positioned tissues.

Gastrulation usually involves some combination of several types of movements (Figure 5.4). These movements involve the entire embryo, and cell migrations in one part of the gastrulating embryo must be intimately coordinated with other movements that are taking place simultane-

I. HOLOBLASTIC (COMPLETE) CLEAVAGE

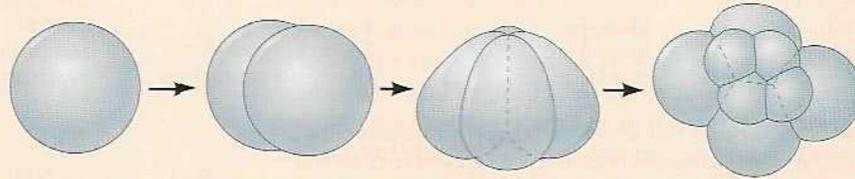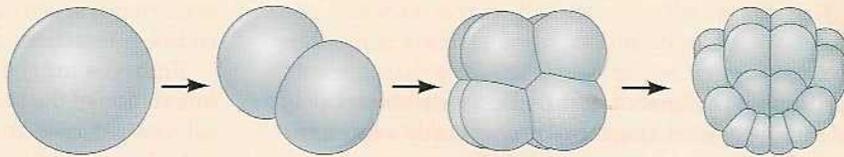### A. Isolecithal
(Sparce, evenly distributed yolk)

1. Radial cleavage
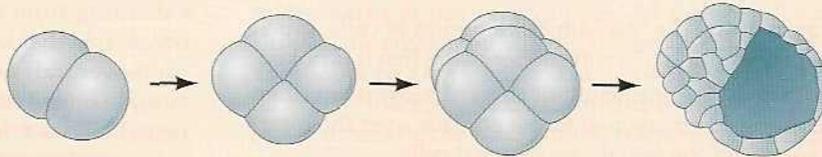Echinoderms, amphioxus
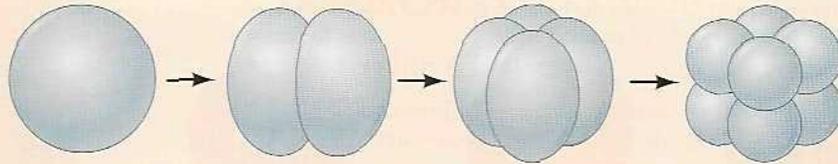
2. Spiral cleavage
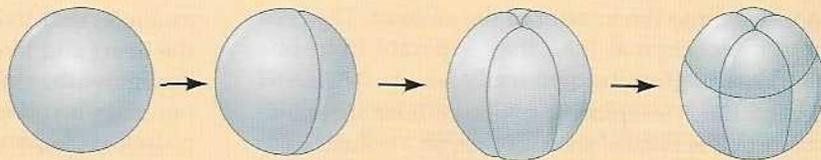Annelids, molluscs,
flatworms

3. Bilateral cleavage
Tunicates

4. Rotational cleavage
Mammals, nematodes

B. Mesolecithal
(Moderate vegetal yolk disposition)
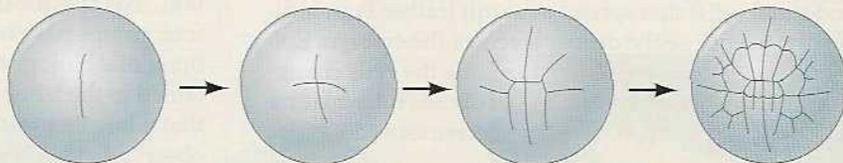
Displaced radial cleavage
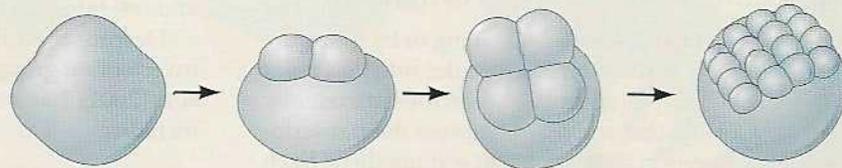Amphibians

II. MEROBLASTIC (INCOMPLETE) CLEAVAGE

A. Telolecithal
(Dense yolk throughout most of cell)

1. Bilateral cleavage
Cephalopod molluscs

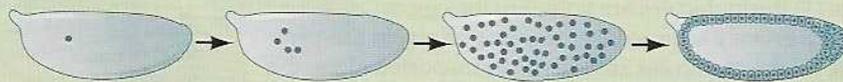2. Discoidal cleavage
Fish, reptiles, birds

B. Centrolecithal
(Yolk in center of egg)

Superficial cleavage
Most insects

FIGURE 5.3   Summary of the main patterns of cleavage.