<Further Development 1_2>

# The Cell Biology of Cell Mitosis and Embryonic 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.[i] 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. The zygote is divided first 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 G1 and G2 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 more than 2 hours, and some 50,000 cells form in just 12 hours.

# From fertilization to cleavage

As we will see in Chapter 7, 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 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).

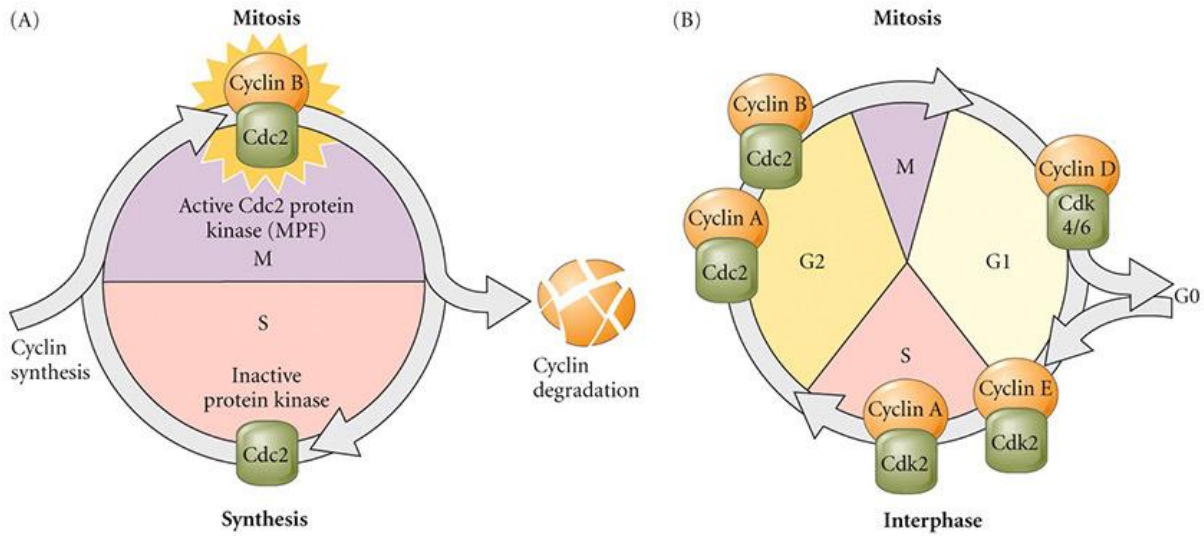


**Figure 1** Cell cycles of somatic cells and early blastomeres. (A) The biphasic cell cycle of early amphibian blastomeres has only two states, S and M. Cyclin B synthesis allows progression to M (mitosis), whereas degradation of cyclin B allows cells to pass into S (synthesis) phase. (B) The complete cell cycle of a typical somatic cell. Mitosis (M) is followed by an interphase stage. Interphase is subdivided into G1, S (synthesis), and G2 phases. Cells that are differentiating are usually taken "out" of the cell cycle and are in an extended G1 phase called G0. 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.)

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** (**CDK**). 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 species, the embryo now enters a **mid-blastula transition** (**MBT**), in which several new properties are added to the biphasic cell divisions of the embryo. First, the "gap" stages (G1 and G2) are added to the cell cycle (see Figure 1B). *Xenopus* embryos add G1 and G2 phases to the cell cycle shortly after the twelfth cleavage. *Drosophila* adds G2 during cycle 14 and G1 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

when we look at sea urchin embryos in Chapter 10, 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 tubulin (the same type of protein that makes up the sperm flagellum). The second process is **cytokinesis**, the division of the cell itself. 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 1** compares these agents of cell division. The relationship and coordination between these two systems during cleavage are depicted in **Figure 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.

**Table 1**

| 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).

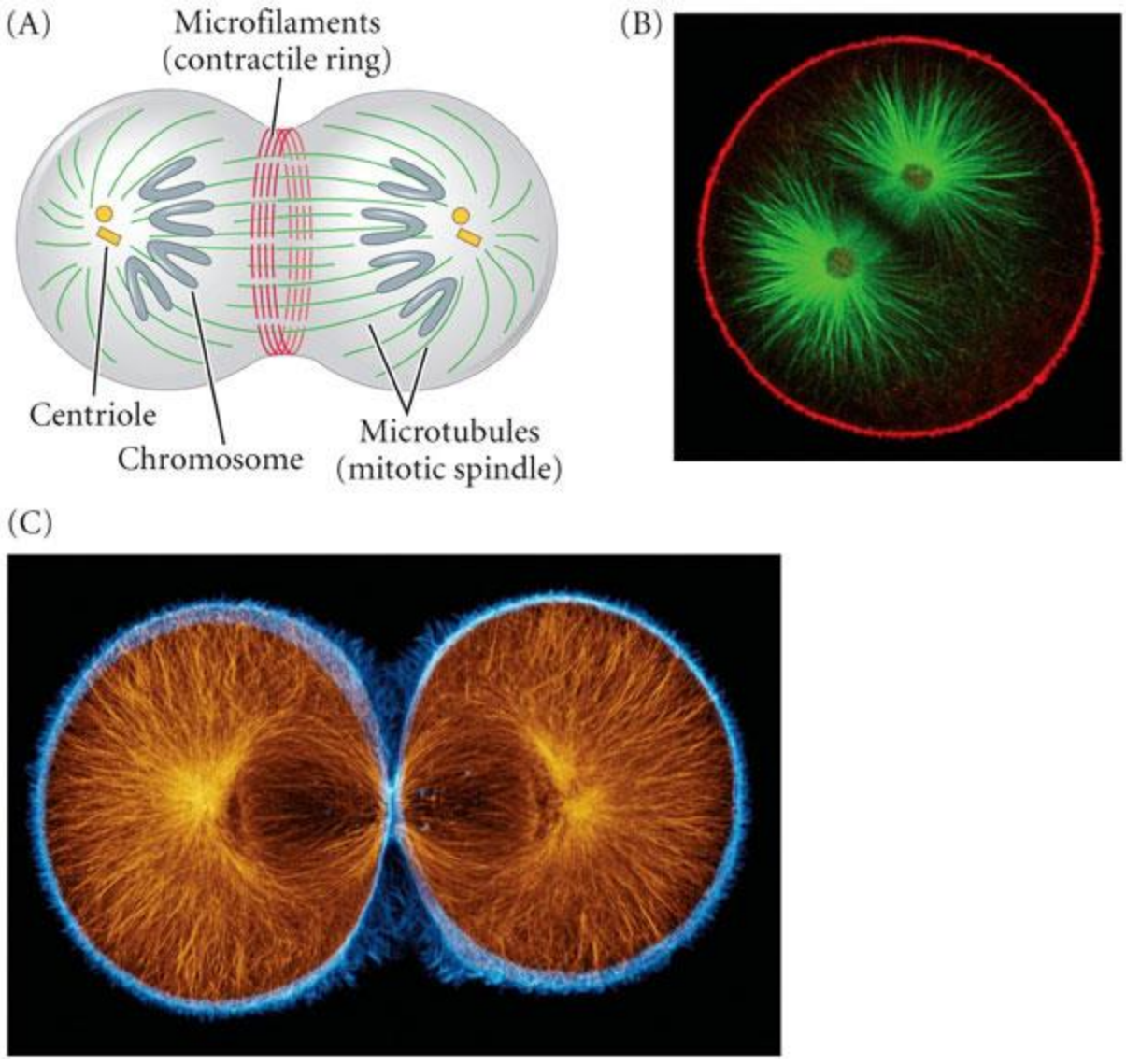


**Figure 2** Roles of microtubules and microfilaments in cell division. (A) Diagram of first-cleavage telophase in a sea urchin egg. The chromosomes are drawn to the centrioles by microtubules while the cytoplasm is being pinched in by the contraction of microfilaments. (B) Confocal fluorescent image of an echinoderm embryo undergoing first cleavage (early anaphase). Microtubules are stained green, actin microfilaments are stained red. (C) Confocal fluorescent image of a sea urchin embryo at the very end of first cleavage. Microtubules are orange; actin proteins (both unpolymerized and in microfilaments) are blue. (B,C courtesy of G. von Dassow and the Center for Cell Dynamics.)

The actin microfilaments are found in the cortex (outer cytoplasm) of the egg rather than in the central cytoplasm. Under the confocal microscope, the ring of microfilaments can be seen forming a distinct cortical band 0.1 μm wide (**Figure 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 spindle can even be at an angle such that one daughter cell is clockwise or counterclockwise to the other. As we will learn Chapter 9, cleavage in insect eggs consists of karyokinesis several times before cytokinesis takes place, so that numerous nuclei exist within the same cell. The outer membrane of that one large cell eventually indents, separating the nuclei and forming individual cells.

# Small GTPases in regulating mitosis

Rho GTPases, small proteins that split GTP into GDP, regulate numerous cellular functions by modulating both the microtubule and actin-myosin components of the cytoskeleton. Rho GTPases generally come in two forms: the active, GTP-bound, state; and the inactive, GDP-bound state. The coordination of microtubule and actin microfilament assembly and disassembly that ensures that each mitotic stage occurs in the correct temporal sequence (and not prematurely) is largely regulated by the specific activation the Rho GTPases, especially RhoA, Rac, and Cdc42. During karyokinesis, these processes include cell cortex stiffening, mitotic spindle formation, and attachment of the spindle microtubules to the kinetochore. During cytokinesis, these GTPases play important roles in establishing the plane of cell division, the assembly and activation of the contractile ring, membrane ingression, and the separation of the two cells. The spatial and temporal activation of RhoA and Cdc42 during cell division is critical for normal mitotic progression and completion.

The functions of the Rho GTPases are sometimes antagonistic and sometimes cooperative. At the beginnings of meiotic prophase, RhoA becomes activated by cyclins, and it is responsible for stiffening the actin cortex of the cell and rounding the cell in preparation for division. During metaphase, cdc42 migrates to the spindle and kinetichores and becomes critical for properly attaching the chromosomes to the tubulin fibers. Moreover, it coordinates the positioning of the spindle fibers and the cortical cytoplasm to orient the direction of cell division. Later, RhoA becomes critical for determining the site of membrane ingression to initiate cytokinesis. It becomes active at those sites that initiate the furrowing of animal cells. It promotes actin and myosin II assembly and functioning through the activation of formins. (This, we will later see, is critical in snail coiling.)

Throughout mitosis, a third Rho GTPase, Rac1, must be inactive. Rac1 generally promotes membrane expansion at the edge of the cell, and this would be deleterious for mitosis. Thus, the coordinated activity of the Rho GTPases is a critical part of normal embryonic cell cleavage.

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[i] A blastomere is a cell derived from cleavage in an early embryo. A blastula is an embryonic stage composed of blastomeres; a mammalian blastula is called a blastocyst. The cavity within the blastula is the blastocoel. (A blastula that lacks a blastocoel is called a stereoblastula.) The invagination where gastrulation begins is the blastopore.