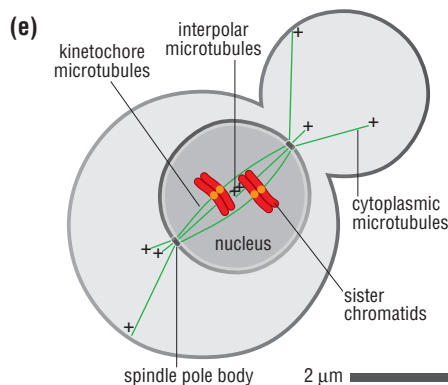
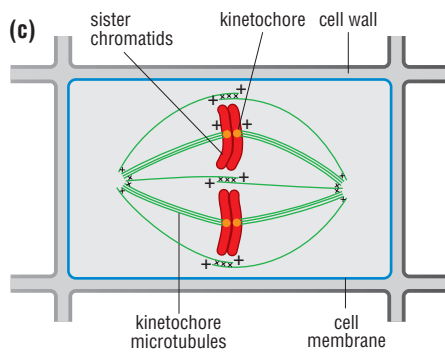
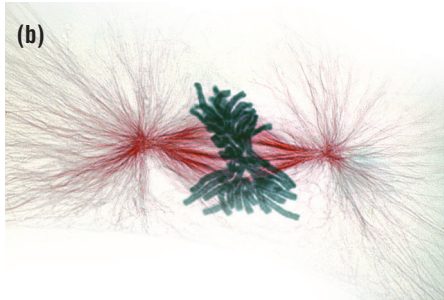
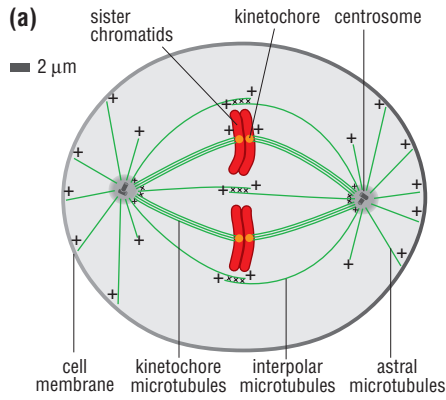


6-0 Overview: The Mitotic Spindle



Chromosome segregation depends on the mitotic spindle

The central function of mitosis is to segregate the two sets of chromosomes that are present in the cell after S phase. Chromosome segregation is carried out by a complex and beautiful machine—the mitotic spindle—that pulls the sister chromatids apart and moves a complete set of chromosomes to each pole of the cell, where they are packaged into daughter nuclei.

The mitotic spindle is based on a bipolar array of *microtubules*, each of which is a polarized protein polymer with one end, the so-called minus end, embedded in a spindle pole and the other end, the plus end, pointing outward from the pole. Plus ends from one pole overlap with plus ends from the other, resulting in an antiparallel array in the spindle midzone (Figure 6-1). Microtubules are highly dynamic polymers that continuously grow and shrink, and in the spindle this behavior is regulated by many different proteins that bind to the sides or ends of microtubules. These include the *motor proteins*, which can travel along microtubules and have important roles in the assembly and stability of the microtubule array and the movement of chromosomes on the spindle.

The sister chromatids are active participants in spindle assembly and function. Each chromatid carries a kinetochore, a multiprotein complex that attaches the chromatid to microtubules connected to a spindle pole (see section 5-0). In addition, proteins in the kinetochore help generate forces that drive chromosome movement. Motors and microtubule-regulatory proteins in the chromatid arms also help govern microtubule growth and spindle assembly.

The mitotic spindle must be bipolar

Although some features of spindle structure vary in different organisms (see Figure 6-1), the underlying logic is always the same. The microtubule array must be bipolar, and the chromatid pairs must be oriented on the spindle such that each sister is connected to an opposite spindle pole. This is known as *bi-orientation*. The bilateral symmetry in spindle structure and chromosome attachment is critical to the ability of the spindle to pull apart chromatid pairs and transport a complete set of chromosomes to each end of the cell. Any defects in spindle bipolarity or chromosome bi-orientation lead to potentially lethal errors in chromosome segregation.

All spindles are bipolar, but the structure of the poles differs in different organisms. In most somatic animal cells, each spindle pole is focused in a large multiprotein organelle called the *centrosome*, which organizes spindle microtubules and also helps position the spindle within the cell (see Figure 6-1a and b). Some cell types, including those of higher plants (see Figure 6-1c and d) and the oocytes of many vertebrates, do not contain centrosomes and depend on the self-organizing properties of microtubules and microtubule-associated proteins to generate the two poles. In budding yeast, the spindle is constructed entirely within the nucleus, which remains intact throughout mitosis, and is organized by protein organelles called *spindle pole bodies* that are embedded in the nuclear envelope (see Figure 6-1e).

Figure 6-1 Anatomy of the mitotic spindle (a) Basic features of the mitotic spindle of somatic animal cells in metaphase. The microtubules (green) have one end (the minus end) embedded in the centrosome and the other (plus end) pointing outward. Numerous motors and other proteins (represented by Xs) cross-link the minus ends of microtubules at the spindle poles and the plus ends of interpoles microtubules in the spindle midzone. Additional motor and other proteins link astral microtubules to the cell cortex, kinetochore microtubules to the kinetochore, and interpoles microtubules to the chromatid arms (not shown). For simplicity, this diagram includes only two sister-chromatid pairs and a fraction of the thousands of microtubules that typically exist in the spindle. (b) Light micrograph of a centrosome-based spindle (stained red) from a salamander. (c) The mitotic spindle in plant cells is similar to that of animal cells except that there are no centrosomes at the spindle poles and no astral microtubules. (d) Light micrograph of an acentrosomal spindle from the African blood lily. (e) In budding yeast, the metaphase spindle is composed of microtubules that emanate from the nuclear face of a pair of spindle pole bodies embedded in the nuclear envelope. For simplicity, only two of the 16 sister-chromatid pairs are shown. The budding yeast spindle is composed of only about 18 intra-nuclear microtubules from each pole: one for each sister-chromatid pair and one or two interpoles microtubules. A small number of astral microtubules radiate out from the spindle poles and attach to the cell cortex to help position the nucleus in the bud neck. Panels (b) and (d) courtesy of Andrew Bajer.

The spindle contains three classes of microtubules. Kinetochore microtubules connect the spindle poles to kinetochores on the sister chromatids; in animal cells multiple kinetochore microtubules bundle together to form *kinetochore fibers*. Interpolar microtubules link the two spindle poles by interdigitating with each other in the midzone of the spindle. Astral microtubules extend from the poles away from the spindle and are typically involved in anchoring and positioning the spindle in the cell. Astral microtubules are generally found only in cells that use centrosomes or spindle pole bodies to form the spindle poles.

Multiple mechanisms drive spindle assembly

The mitotic spindle is assembled in early mitosis in parallel with the changes in chromosome structure that were discussed in Chapter 5. The two key problems in spindle assembly are how to construct a bipolar array of microtubules that surrounds the sister chromatids, and how to attach sister-chromatid pairs to the array with the correct bi-orientation.

In all eukaryotes, construction of a bipolar spindle depends in large part on the ability of the spindle components to self-organize. Motor and other proteins interact with microtubules to organize them into two antiparallel arrays in which the plus ends of each array overlap in the center (Figure 6-2a). The minus ends are cross-linked by other microtubule-associated proteins to form a pair of spindle poles. Spindle self-organization also depends on proteins associated with the sister chromatids, so that the microtubule array is built around them. As the microtubules grow, some plus ends become attached to kinetochores, thus connecting chromatids to the poles. Self-organization is the only mechanism of spindle assembly in cells lacking centrosomes, such as the plant cells shown in Figure 6-1c and d.

In many other cells, including those in humans, spindle microtubules grow out from the centrosomes, which act as prefabricated microtubule-organizing centers (Figure 6-2b). The centrosome is duplicated before mitosis, and upon mitotic entry the two centrosomes move apart to provide the poles of the spindle. As in an acentrosomal spindle, motor and other proteins cross-link the antiparallel microtubule array between the poles and also help focus microtubule minus ends in the centrosomes. Chromatids are attached to the spindle by a process known as search and capture, in which the plus ends of some of the microtubules radiating out from the centrosomes attach to kinetochores. These centrosome-dependent mechanisms are not essential, however, as animal cells can assemble spindles even when their centrosomes have been inactivated.

Errors in sister-chromatid attachment can sometimes occur during spindle assembly. Both sister kinetochores, for example, can become attached to the same spindle pole. How is the correct bi-orientation of sister chromatids achieved? By mechanisms that are discussed later in this chapter, the kinetochore monitors the orientation of microtubule attachment—and corrects any errors that occur.

This chapter provides an overview of the key principles in mitotic spindle assembly, with an emphasis on the centrosome-dependent mechanisms of animal cells, which are the best understood. The spindle machinery of microtubules, their associated regulators, and the centrosome and the kinetochore are introduced in sections 6-1 to 6-5. In the remainder of the chapter we will see how these components interact to assemble a bipolar spindle, how bi-orientation is achieved, and how the motor and other proteins generate forces that align chromatids in the center of the spindle to prepare the cell for anaphase.

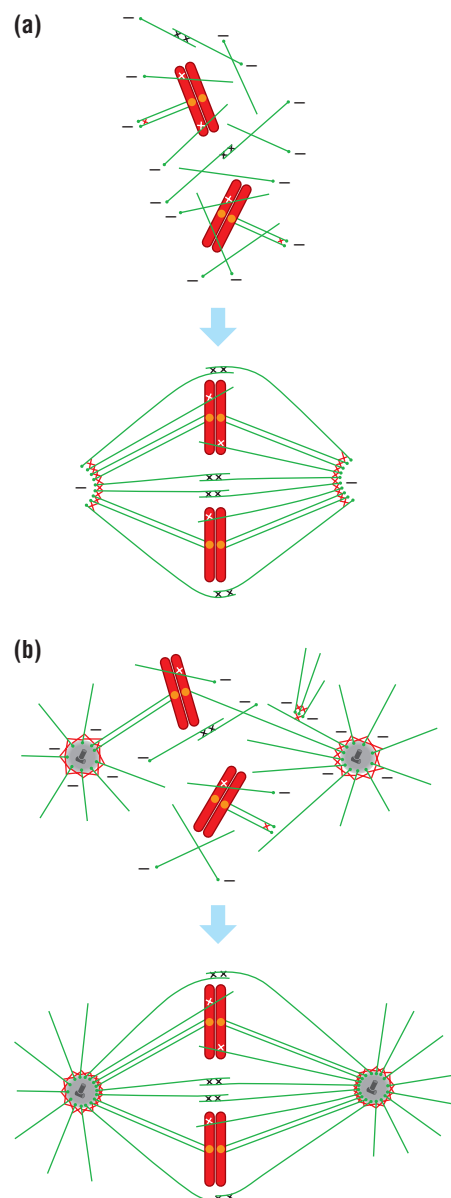


Figure 6-2 General mechanisms of spindle assembly (a) Spindle assembly depends on microtubule self-organization, whereby microtubules form in the vicinity of the sister chromatids and then become organized by motors and other proteins into a bipolar array. This process depends on numerous motor proteins (Xs), including motors that cross-link antiparallel plus ends (black), motors that focus minus ends at the poles (red), and chromatid-associated motors that help orient the array (white). (b) In most animal cells centrosomes facilitate the self-organization process shown in panel (a). Rapidly growing and shrinking microtubules that radiate from the centrosomes search the space between the poles and bind to kinetochores. Many sister-chromatid pairs are initially attached by this search and capture mechanism. Centrosomal microtubules can also capture preformed microtubule bundles and pull them into the poles. Bi-orientation eventually results when a pair of sister kinetochores is connected to both poles.

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