XIII. THE CELL DIVISION

XIII.1. THE MITOSIS

We will discuss about the mechanical events of the **M phase** (or cell division phase) of the cell cycle. This phase, which includes the various stages of nuclear division **(mitosis)** and cytoplasmatic division **(cytokinesis)**, is the culmination of the cell cycle. In a comparatively brief period, the contents of the parental cell, which were doubled by the biosynthetic activities of the preceding interphase, are segregated into two daughter cells.

At the molecular level M phase is initiated by a cascade of protein phosphorylations triggered by the activation of the mitosis-inducing protein kinase MPF, and it is terminated by the dephosphorylations that follow the inactivation of MPF through proteolysis of its cyclin subunits. The protein phosphorylations that occur during M phase are responsible for the many morphological changes that accompany mitosis:

-the chromosomes condense

-the nuclear envelope breaks down

-the endoplasmic reticulum and Golgi apparatus fragment

-the cell loosens its adhesions to other cells and the extracellular matrix

-the cytoskeleton is transformed to bring about the highly organized movements that will segregate the chromosomes and partition the cell.

Because M phase involves a complete reorganization of the cell interior, the number of the proteins that become phosphorylated is thought to be large, and essentially every part of the cell is affected in some way.

XIII.1.1. Mitosis Cells That Are Indentical With Parental Cell

Mitosis traditionally is divided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase.

During **prophase,** the chromatin, which was duplicated during S phase, slowly condenses into chromosomes. The number of chromosomes varies from species, but in humans the diploid chromosomes number is 46. The mitotic chromosome consists of two chromatids that are connected at a region termed the *centromere.* On the surface of the centromere are two *kinetochores* - one associated with each chromatid. The kinetochore is the region of the chromosome to which the forces that result in chromosomal movements appear to act. Prophase also is the period when the cytoplasmic microtubules are broken down as the cell prepares for the reorganization of the cellular microtubules into the mitotic apparatus.

Prometaphase begins at the instant the nuclear envelope disassembles. During prometaphasse, some of the microtubules of the forming spindle apparatus make contact with and become attached to the kinetochores of the condensed chromosomes. Early in prometaphase, the chromosomes are haphazardly scattered around the mitotic spindle. However, the chromosomes begin to undergo a series of movements that result in the alignment of all of the chromosomes at the equator of the spindle.

The arrangement of chromosomes at the midzone of the spindle is sometimes termed the ***metaphase plate configuration***. Under the microscope, **methaphase** aqppears as a period of inactivity or rest as the cell prepares for anaphase.

Anaphase is the period of mitosis when the sister chromatids separate. The onset of anaphase is the time at which the centromere splits in two. At that moment the chromatids begin their migration, or segregation, toward opposite poles of the spindle. The force-genereting mechanism that results in anaphase chromosomal segregation has not been identified, although there are several possible explanations for this motile event. Possibly, one or more of the proteins that compose the kinetochore contain the ATPase activity that allows the movement of chromosomes along the spindle microtubules. Alternatively, force-generating molecules may be associated with the spindle microtubules. Although the details concerning anaphase chromosomal migration are unknown, what is clear is that the microtubules that extend from the spindle pole to the kinetochore must disassemble for anaphase chromosomal movement to occur. If cells are treated with antitumor drugs that inhibit the breakdown of spindle microtubules, anaphase chromosomal segregation is blocked.

The final stage of mitosis is telophase. **Telophase** is identified as the period when the daughter chromatids have completed their segregation to the opposite spindle poles. Other events that occur during telophase are the reformation of a nuclear envelope around each set of chromosomes, the decondensation of chromosomes into chromatin, the dissolution of the mitotic apparatus, and cytokinesis.

Cytokinesis is accomplished by a contractile ring of actin microfilaments that encircles the cortex of the dividing cell in an area of the cell surface that overlies the area where metaphase plate chromosomes were organized. The end result of mitosis is that two progeny cells are produced that are identical in genetic composition with the original parental cell.

XIII.1.2. The Mitotic Apparatus and Contractile Ring are M-Phase Specific Cytoskeletal Structures

The cellular machine that is responsible for directing the events of chromosomal segregation is the mitotic spindle apparatus. Mitosis occurs with such unerring fidelity because the genes that are contained within the chromosomal arms must be divided evenly. However, the genes themselves contribute little to the process of mitosis. Instead, the active roles in mitosis are played by the spindle microtubules, the centrosomes, and the kinetochore region of the chromosomes. The chromosomal arms, which contain the genetic material, are thought to be passive participants in the mitotic event.

The centrosome is responsible for nucleating microtubule growth in most human cells. The centrosome is composed of a centriole pair and a surrounding cloud of amorphous substance called the pericentriolar material. Experimental analysis has demonstrated that the centrosomes microtubule nucleating capacity is contained within the pericentriolar cloud and not the centriole cylinders.

As the cell proceeds through interphase, the centrosome is duplicated and, at the onset of mitosis, the daughter centrosomes migrate to opposite sides of the nucleus where they will serve as the mitotic spindle poles. Along with this migration, the interphase array of cytoplasmic microtubule complex is then replaced by the mitotic aparatus.

The mitotic apparatus is a complex array of microtubules, membranous vesicles, chromosomes, and other cellular proteins. As in interphase, all of the spindle microtubules appear to originate in the pericentriolar material. Three classes of microtubules, all of similar biologic composition, can be identified in the mitotic apparatus. The first class (1), **the astral microtubules**, are arranged in a starlike fashion around each spindle pole. The microtubules are thought to be important in orienting the contractile ring. The second type (2) is the **kinetochore**

microtubule that extends from the centrosome to the kinetochore and is important for directing the chromosomal migrations that occur during mitosis. The final class (3) of spindle microtubule extends from the centrosome past the metaphase plate region, and these microtubule then overlap with microtubules that extend from the opposite spindle pole. These **polar microtubules** are thought to be involved in pushing the two spindle poles apart late in anaphase so that the contractile ring can split the cell in two.

One of the key events in spindle morphogenesis is the attachment of the chromosomes to the spindle microtubules. This occurs only at the kinetochore regions of the mitotic chromosomes, with the chromosomal arms playing no active role in mitosis. This has been demonstrated in cells in which the kinetochores have been experimentally detached from the mitotic chromosomes. In these cells, the detached kinetochores are able to attach to the spindle microtubules and to undergo the entire repertoire of mitotic chromosomal movements. The chromosomal arms in these experimentally treated cells do not associate with the spindle and are displaced to the cell periphery. Therefore, the proteins of the kinetochore region are responsible for the attachment of chromosomes to spindle microtubules as well as for directing the chromosomal migrations that occur during prometaphase and anaphase.

The other major cytoskeletal structure that plays an active role in cell division is the contractile ring. The contractile ring is composed of a belt of actin microfilaments to the plasma membrane. During cytokinesis, the belt of actin microfilaments constricts, which results in the cell being cleaved in half. Molecular biological studies have demonstrated that cytokinesis is dependent on the activity of myosin, suggesting that the events of cytokinesis may occur by a mechanism that is similar to smooth-muscle contraction. Following mitosis, both the contractile ring and mitotic apparatus are diassembled and replaced by the interphase configurations of microtubules and microfilaments.

XIII.2. MEIOSIS

The realization that germ cells are haploid, and must therefore be produced by a special type of cell division, came from an observation that was also among the first to suggest that chromosomes carry genetic information. In 1883 it was discovered that, whereas the fertilized egg of a particular worm contains four chromosomes, the nucleus of the egg and that of the sperm each contain four chromosomes. The chromosome theory of heredity therefore explained the longstanding paradox that maternal and paternal contributions to the character of the progeny seem often to be equal, despite the enormous difference in size between the egg and sperm.

The finding also implied that germ cells must be formed by a special kind of nuclear division in which the chromosome complement is precisely halved. This type of division is called **meiosis,** from the Greek, meaning diminution.

XIII.2.1. MEIOSIS INVOLVES TWO NUCLEAR DIVISIONS RATHER THAN ONE

Meiosis thus consists of two cell divisions following a single phase of DNA replication, so that four haploid cells are produced from each cell that enters meiosis.

Meiotic Chromosome Pairing Culminates in the Formation of the Synaptonemal Complex

Elaborate morphological changes occur in the chromosomes as they pair (*synapse*) and then begin to unpair (*dysinapse*) during the first meiotic prophase. This prophase is traditionally divided into five sequential stages - *leptotene, zygotene, pachytene, diplotene* and *diakinesis* - defined by these morphological changes. The most striking event is the initiation of intimate chromosome synapsis at **zygotene**, when a complex structure called by *synaptonemal complex* begins to develop between the two sets of sister chromatids in each bivalent. **Pachytene** is said to begin as soon as synapsis is complete, and it generally persists for days, until desynapsis begins the **diplotene** stage, in which the chiasmata are first seen.

Genetic recombination requires a close apposition between the recombining chromosomes. The synaptonemal complex, which forms just before pachytene and dissolves just afterward, keeps the homologous chromosomes in a bivalent together and closely aligned, and it has been suggested that it may play a part in the recombination process. It consists of a long ladderlike protein core, on opposite sides of which the two homologues are aligned to form a long linear chromosome pair. The sister chromatids in each homologue are kept tightly packed together, and their DNA extends from the same side of the protein ladder in a series of loops.

It is not known how homologous chromosomes become aligned. It is unlikely that continuous connections all along the interacting chromosomes are involved, since the chromatin of the homologue is well separated from the chromatin of its partner in the synaptonemal complex. It has been proposed that the initial interaction between homologues chromosomes is mediated by complementary DNA base-pair interaction at discrete sites along the chromosomes. This recognition may occur at zygotene or even earlier, when the chromosomes are not very condensed; following chromosome condensation, the formation of the synaptonemal complex would then pack the remaining portions of the chromosomes together.

Recombination Nodules Are Thought to Mediate Chromatid Exchanges

Although the synaptonemal complex may provide the structural framework for recombination events, it probably is not the engine that brings them about. The active recombination process is thought to be mediated instead by **recombination nodules,** which are very large protein-containing assemblies with a diameter of about 90 nm. Recombination nodules sit at intervals on the synaptonemal complex, placed like basketballs on a ladder between the two homologues chromosomes. They are thought to mark the site of a large multienzyme “recombination machine”, which brings local regions of DNA on the maternal and paternal chromatids together across the 100-nm-wide synaptonemal complex.

The evidence that the recombination nodules serve this function is indirect:

(1) The total number of nodules is about equal to the total number of chiasmata seen later in prophase.

(2) The nodules are distributed along the synaptonemal complex in the same way that crossover events are distributed. Like the crossover events themselves, for example, the nodules are absent from those regions of the synaptonemal complex that hold heterochromatin together. Moreover, both genetic and cytological measurements indicate that the occurrence of one crossover event prevents a second crossover event occurring at any nearby chromosomal site.

Chiasmata Play an Important Part in Chromosome Segregation in Meiosis

In addition to resorting genes, chromosomal crossing-over is crucial in most organisms for correct segregation of the two homologues to separate daughter nuclei. This is because the

chiasma created by each crossover event plays a role analogous to that of the centromere in an ordinary mitotic division, holding the maternal and paternal homologues together on the spindle until anaphase I. In mutant organisms that have a reduced frequency of meiotic chromosomes crossing-over, some of the chromosome pairs lack chiasmata. These pairs failed to segregate normally, and a high proportion of the resulting gametes contain too many or too few chromosomes-an example of nondisjunction.

There are at least two major differences in the way chromosomes separate a meiotic division I and in normal mitosis. (1) During normal mitosis (and meiotic division II, which resembles a normal mitosis) the sister chromatids are held together only at the centromere; the kinetochores (protein complexes associated with the centromeres) on each sister chromatid have attached kinetochore fibers pointing in opposite directions, so that the chromatids are drawn into different daughter cells at anaphase. At methaphase I of meiosis, by contrast, the kinetochores on both sister chromatids appear to have fused so that their attached kinetochore fibers all point in the same direction and the arms of the sister chromatids are closely apposed; moreover, the homologous maternal and paternal chromosomes are held together at the chiasmata. (2) During normal mitosis (and meiotic division II) the movement of chromatids to the poles is triggered by a mechanism that detaches the two sister kinetochores from each other (thus beginning anaphase), allowing the sister chromatids to segregate into different daughter cells. In anaphase I of meiosis, however, movement to the poles is initiated by the disruption of the poorly understood forces keeping the arms of sister chromatids together and by the simulataneous dissolution of the chiasmata linking the homologous maternal and paternal chromosomes; consequently, the sister chromatids remain paired, but the maternal and paternal homologues segregate into different daughter cells.

Pairing of the Sex Chromosomes Ensures That They Also Segregate

We have explained how homologous chromosomes pair during meiotic division I so that they segregate accurately between the daughter cells. But what about the **sex chromosomes**, which in male mammals are not homologous? Females have two X chromosomes, which pair and segregate like other homologues. But males have one X and one Y chromosome, which must pair during the first metaphase of meiosis if the sperm are to contain either one Y or one X chromosome and not both or neither. The necessary pairing is made possible by a small region of homology between the X and Y at one end of these chromosomes. In this region the two chromosomes pair and cross over during the first meiotic prophase. The chiasma corresponding to this small amount of genetic recombination is sufficient to keep the X and Y chromosomes paired on the spindle so that only two types of sperm are normally produced: sperm containing one Y chromosome, which will give rise to male embryos, and sperm containing one X chromosome, which will give rise to female embryos.

XIII.2.2. MEIOTIC DIVISION II RESEMBLES A NORMAL MITOSIS

After the long prophase I (which can occupy 90% or more of meiosis) has ended, two successive cell divisions, without an intervening period of DNA synthesis, bring meiosis to an end. The entire first meiotic cell cycle, which ends with an initial meiotic cell division, is called *meiotic division I,* and it is far more complex and requires much more time than the second meiotic cell cycle, called *meiotic division II.* Even the preparatory DNA replication during the first cell cycle tends to the take much longer than a normal S phase, and cells can then spend

days, months, or even years in the first meiotic prophase, depending on the species and the gamete being formed. Although it is traditionally called prophase, this prolonged phase of meiotic division I resembles the G_2 phase of an ordinary cell division in that the nuclear envelope remains intact and disappears only when the spindle fibers begin to form as prophase I gives way to metaphase I.

After the end of meiotic division I, nuclear membranes re-form around the two daughter nuclei and a brief interphase begins. During this period the chromosomes may decondense somewhat, but usually they soon recondense and prophase II begins. As there is no DNA synthesis during this interval, in some organisms the chromosomes seem to pass almost directly from one division phase into the next. In all organisms prophase II is brief: the nuclear envelope breaks down as the new spindle forms, after which metaphase II, anaphase II, and telophase II usuallly follow in quick succesion. As in an ordinary mitosis, a separate set of kinetochore fibers forms on each sister chromatid, and these two sets of fibers extend in opposite directions. Moreover, the two sister chromatids are kept together on the metaphase plate until they are released by the sudden separation of their kinetochores at anaphase. Thus division II, unlike division I, closely resembles a normal mitosis. The difference is that one copy of each chromosome is present instead of two homologues. After nuclear envelopes have formed around the four haploid nuclei produced at telophase II, meiosis is complete. The principles of meiosis are the same in plants and animals; and in males and females. But the production of gametes involves more than just meiosis, and the other processes required vary widely among organisms and are very different for eggs and sperm. We shall focus our discussion of gametogenesis mainly on vertebrates.

XIII.3. SPERMATOGENESIS

Sperm are produced continuously in many mammals, and the process is called **spermatogenesis**. In human males, spermatogenesis occurs in the seminiferous tubules of the testes and continues from the onset of puberty until death.

So, immature germ cells, called **spermatogonia,** are located around the outer edge of these tubules next to the basal lamina, where they proliferate continuously by ordinary cell division cycles. Some of the daughter cells stop proliferating and differentiate into **primary spermatocytes.** These cells enter the first meiotic prophase, in which their paired homologous chromosomes participate in crossing-over, and then proceed with division I of meiosis to produce two **secondary spermatocytes,** each containing 22 duplicated autosomal chromosomes and either a duplicated X or duplicated Y chromosome. The two secondary spermatocytes proceed through meiotic division II to produce four **spermatids,** each with a haploid number of single chromosomes. These haploid spermatids then undergo morphological differentiation into sperm, which escape into lumen of the seminiferous tubule. The sperm subsequently pass into the epididymis, a coiled tube overlying the testis, where they are stored and undergo further maturation.

XIII.4. OOGENESIS

Ovum production, or **oogenesis**, begins before birth, accelerate at puberty, and ends at menopause.

Oogenesis varies in different species, but the general stages are similar. Primordial germ cells migrate to the forming gonad to become **oogonia**, which proliferate by ordinary cell

division cycles for a period before differentiating into **primary oocytes**. At this stage the first meiotic division begins: the DNA replicates so that each chromosome consists of two chromatids, the homologous chromosomes pair along their long axes, and crossing-over occurs between the chromatids of these paired chromosomes. After these events the cell remains arrested in prophase of division I of meiosis for a period lasting from a few days to many years, depending on the species. During this long period, the primary oocytes synthesize a coat and cortical granules and, in the case of large nonmammalian oocytes, they accumulate ribosomes, yolk, glycogen, lipid, and the mRNA that will later direct the synthesis of proteins required for early embryonic growth and the unfolding of the developmental program.

The next phase of oocyte development is called **oocyte maturation** and usually does not occur until sexual maturity, when it is stimulated by hormones. Under these hormonal influences the cell resumes its progress through division I of meiosis: the chromosomes recondense, the nuclear envelope breaks down (this is generally taken to mark the beginning of maturation), and the replicated homologous chromosomes segregate at anphase I into two daughter nuclei, each containing half the original number of chromosomes. To end division I, the cytoplasm divides asymmetrically to produce two cells that differ greatly in size: one is a small **polar body**, and the other is a large **secondary oocyte**, the precursor of the egg. At this stage each of the chromosomes is still composed of two sister chromatids. These chromatids do not separate until division II of meiosis, when they are partitioned into separate cells by a process that is identical to a normal mitosis. After this final chromosome separation at anaphase II, the cytoplasm of the large secondary oocyte again divides asymmetrically to produce the mature **egg** (or **ovum**) and a second small polar body each with haploid number of single chromosomes. Because of these two asymmetrical divisions of their cytoplasm, oocytes maintain their large size despite undergoing the two meiotic divisions. Both of the polar bodies are small, and they eventually degenerate.

As we describe previous, the secondary oocyte continues the meiosis II, but is arrested at metaphase. Experimental evidence suggests that the meiotic arrest at metaphase II is due to the activity of the protein product of the c-mos protooncogene. The mechanism of action of the c-mos protein is thought to be through the stabilization of cyclin. If cyclin is not degraded, then the activity of MPF kinase cannot be reversed; as a result, the cell is blocked in metaphase. At fertilization, the c-mos protooncogene product, sometimes called cytostatic factor (CSF), is inactivated and cyclin is degraded. Meiosis is completed, and a second asymmetric division occurs, generating a mature fertilized egg and second polar body.

Not all of the primary oocytes at birth survive until puberty. There are roughly 2 million in the ovaries at birth; by the time of puberty, about 400,000 remain. The rest of the primary oocytes degenerate, a process called atresia. And from 400,000 primary oocytes, just 400-500 will finish the first meiosis and form the secondary oocyte.