The Eukaryotic Cell Cycle

The cell cycle is the series of events that take place in a cell that cause it to divide into two daughter cells. The division cycle of most cells consists of four coordinated processes: cell growth, DNA replication, distribution of the duplicated chromosomes to daughter cells, and cell division. In bacteria, cell growth and DNA replication take place throughout most of the cell cycle, and duplicated chromosomes are distributed to daughter cells in association with the plasma membrane.

In eukaryotes, however, the cell cycle is more complex and consists of four discrete phases. Although cell growth is usually a continuous process, DNA is synthesized during only one phase of the cell cycle, and the replicated chromosomes are then distributed to daughter nuclei by a complex series of events preceding cell division. Progression between these stages of the cell cycle is controlled by a conserved regulatory apparatus, which not only coordinates the different events of the cell cycle but also links the cell cycle with extracellular signals that control cell proliferation.

Phases of the Cell Cycle

The increased size and more complex organization of eukaryotic genomes over those of bacteria required radical changes in the process by which the two replicas of the genome are partitioned into the daughter cells during cell division. This division process is diagrammed as a **cell cycle,** consisting of five phases.

The Five Phases

G1 is the primary growth phase of the cell. For many organisms, this encompasses the major portion of the cell's life span. During G1 the cell is metabolically active and continuously grows but does not replicate its DNA.

G1 is followed by S phase (synthesis) during which DNA replication takes place. **S** is the phase in which the cell synthesizes a replica of the genome.

G2 is the second growth phase, in which preparations are made for genomic separation. During this phase, mitochondria and other organelles replicate, chromosomes condense, and microtubules begin to assemble at a spindle. G1, S, and G2 together constitute **interphase,** the portion of the cell cycle between cell divisions.

M is the phase of the cell cycle in which the microtubular apparatus assembles, binds to the chromosomes, and moves the sister chromatids apart. Called **mitosis,** this process is the essential step in the separation of the two daughter genomes. Although mitosis is a continuous process, it is traditionally subdivided into four stages: prophase, metaphase, anaphase, and telophase.

C is the phase of the cell cycle when the cytoplasm divides, creating two daughter cells. This phase is called **cytokinesis.** In animal cells, the microtubule spindle helps position a contracting ring of actin that constricts like a drawstring to pinch the cell in two. In cells with a cell wall, such as plant cells, a plate forms between the dividing cells.

Duration of the Cell Cycle

The time it takes to complete a cell cycle varies greatly among organisms. Cells in growing embryos can complete their cell cycle in under 20 minutes; the shortest known animal nuclear division cycles occur in fruit fly embryos (8 minutes). Cells such as these simply divide their nuclei as quickly as they can replicate their DNA, without cell growth. Half of the cycle is taken up by S, half by M, and essentially none by G1 or G2. Because mature cells require time to grow, most of their cycles are much longer than those of embryonic tissue. Typically, a dividing mammalian cell completes its cell cycle in about 24 hours, but some cells, like certain cells in the human liver, have cell cycles lasting more than a year. During the cycle, growth occurs throughout the G1 and G2 phases (referred to as "gap" phases, as they separate S from M), as well as during the S phase. The M phase takes only about an hour, a small fraction of the entire cycle. Most of the variation in the length of the cell cycle from one organism or tissue to the next occurs in the G1 phase. Cells often pause in G1 before DNA replication and enter a resting state called **G0 phase;** they may remain in this phase for days to years before resuming cell division. At any given time, most of the cells in an animal's body are in G0 phase. Some, such as muscle and nerve cells, remain there permanently; others, such as liver cells, can resume G1 phase in response to factors released during injury.

For a typical rapidly proliferating human cell with a total cycle time of 24 hours, the G1 phase might last about 11 hours, S phase about 8 hours, G2 about 4 hours, and M about 1 hour. Other types of cells, however, can divide much more rapidly. Budding yeasts, for example, can progress through all four stages of the cell cycle in only about 90 minutes. Even shorter cell cycles (30 minutes or less) occur in early embryo cells shortly after fertilization of the egg. In this case, however, cell growth does not take place. Instead, these early embryonic cell cycles rapidly divide the egg cytoplasm into smaller cells. There is no G1 or G2 phase, and DNA replication occurs very rapidly in these early embryonic cell cycles, which therefore consist of very shorts phases alternating with M phases.

Cells at different stages of the cell cycle can also be distinguished by their DNA content. For example, animal cells in G1 are diploid (containing two copies of each chromosome), so their DNA content is referred to as *2n (n* designates the haploid DNA content of the genome). During S phase, replication increases the DNA content of the cell from *2n* to *4n,* so cells inS have DNA contents ranging from *2n* to *4n.* DNA content then remains at 4n for cells in G2 and M, decreasing to *2n* after cytokinesis. Experimentally, cellular DNA content can be determined by incubation of cells with a fluorescent dye that binds to DNA, followed by analysis of the fluorescence intensity of individual cells in a flow cytometer.

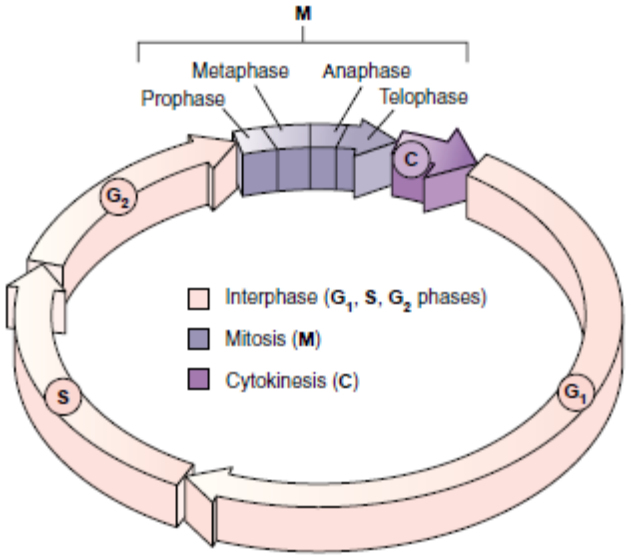


Fig: phases of cell cycle

Regulation of the Cell Cycle by Cell Growth and Extracellular Signals

The progression of cells through the division cycle is regulated by extracellular signals from the environment, as well as by internal signals that monitor and coordinate the various processes that take place during different cell cycle phases. An example of cell cycle regulation by extracellular signals is provided by the effect of growth factors on animal cell proliferation. In addition, different cellular processes, such as cell growth, DNA replication, and mitosis, all must be coordinated during cell cycle progression. This is accomplished by a series of control points that regulate progression through various phases of the cell cycle.

A major cell cycle regulatory point in many types of cells occurs late in G1 and controls progression from G1 to S. This regulatory point was first defined by studies of budding yeast *(Saccharomyces cerevisiae),* where it is known as START. Once cells have passed START, they are committed to entering S phase and undergoing one cell division cycle. However, passage through START is a highly regulated event in the yeast cell cycle where it is controlled by external signals, such as the availability of nutrients, as well as by cell size. For example, if yeasts are faced with a shortage of nutrients, they arrest their cell cycle at START and enter a resting state rather than proceeding to S phase. Thus START represents a decision point at which the cell determines whether sufficient nutrients are available to support progression through the rest of the division cycle. Polypeptide factors that signal yeast mating also arrest the cell cycle at START, allowing haploid yeast cells to fuse with one another instead of progressing to S phase. In addition to serving as a decision point for monitoring extracellular signals, START is the point at which cell growth is coordinated with DNA replication and cell division. The importance of this regulation is particularly evident in budding yeasts in which cell division produces progeny cells of very different sizes: a large mother cell and a small daughter cell. In order for yeast cells to maintain a constant size, the small daughter cell must grow more than the large mother cell does before they divide again. Thus cell size must be monitored

in order to coordinate cell growth with other cell cycle events. This regulation is accomplished by a control mechanism that requires each cell to reach a minimum size before it can pass START. Consequently, the small daughter cell spends a longer time in G1 and grows more than the mother cell.

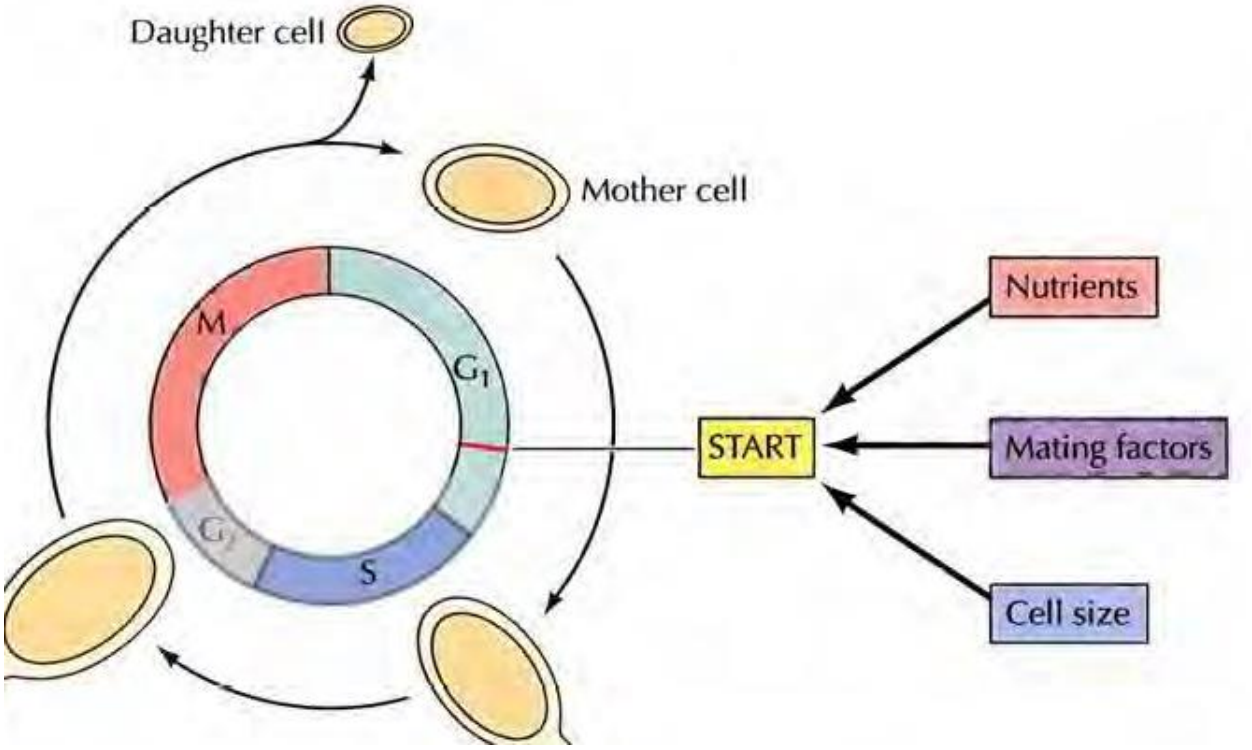


FIGURE. Regulation of the cell cycle of budding yeast: The cell cycle of *Saccharomyces cerevisiae* is regulated primarily at a point in late G1 called START. Passage through START is controlled by the availability of nutrients, mating factors, and cell size. Note that these yeasts divide by budding. Buds form just after START and continue growing until they separate from the mother cell after mitosis. The daughter cell formed from the bud is smaller than the mother cell and therefore requires more time to grow during the G1 phase of the next cell cycle. Although G1 and S phases occur normally, the mitotic spindle begins to form during S phase, so the cell cycle of budding yeast lacks a distinct G2 phase.

Mitosis or M phase

The mitosis (Gr., mitos=thread) occurs in the somatic cells and is meant for multiplication of cell number during embryogenesis and blastogenesis of plants and animals. Fundamentally it remains related with the growth of an individual from zygote to adult stage. M phase is the most dramatic period of the cell cycle, involving a major reorganization of virtually all cell components. During mitosis (nuclear division), the chromosomes condense, the nuclear envelope of most cells breaks down, the cytoskeleton reorganizes to form the mitotic spindle, and the chromosomes move to

opposite poles. Chromosome segregation is then usually followed by cell division (cytokinesis).

Stages of Mitosis

Although many of the details of mitosis vary among different organisms, the fundamental processes that ensure the faithful segregation of sister chromatids are conserved in all eukaryotes. These basic events of mitosis include chromosome condensation, formation of the mitotic spindle, and attachment of chromosomes to the spindle microtubules. Sister chromatids then separate from each other and move to opposite poles of the spindle, followed by the formation of daughter nuclei. Mitosis is conventionally divided into four stages-prophase, metaphase, anaphase, and telophase.

The beginning of prophase is marked by the appearance of condensed chromosomes, each of which consists of two sister chromatids (the daughter DNA molecules produced in S phase). These newly replicated DNA molecules remain intertwined throughout S and G2, becoming untangled during the process of chromatin condensation. The condensed sister chromatids are then held together at the centromere, which is a DNA sequence to which proteins bind to form the kinetochore-the site of eventual attachment of the spindle microtubules. In addition to chromosome condensation, cytoplasmic changes leading to the development of the mitotic spindle initiate during prophase. The centrosomes (which had duplicated during interphase) separate and move to opposite sides of the nucleus. There they serve as the two poles of the mitotic spindle, which begins to form during late prophase. In higher eukaryotes the end of prophase corresponds to the breakdown of the nuclear envelope. However, this disassembly of the nucleus is not a universal feature of mitosis and does not occur in all cells. Some unicellular eukaryotes (e.g., yeasts) undergo so-called closed mitosis in which the nuclear envelope remains intact. In closed mitosis the daughter chromosomes migrate to opposite poles of the nucleus, which then divides in two. In these cells the spindle pole bodies are embedded within the nuclear envelope, and the nucleus divides in two following migration of daughter chromosomes to opposite poles of the spindle.

Following completion of prophase, the cell enters prometaphase- a transition period between prophase and metaphase. During prometaphase the microtubules of the mitotic spindle attach to the kinetochores of condensed chromosomes. The kinetochores of sister chromatids are oriented on opposite sides of the chromosome, so they attach to microtubules emanating from opposite poles of the spindle. The chromosomes shuffle back and forth until they eventually align on the metaphase plate in the center of the spindle. At this stage, the cell has reached metaphase.

Most cells remain only briefly at metaphase before proceeding to anaphase. The transition from metaphase to anaphase is triggered by breakage of the link between sister chromatids, which then separate and move to opposite poles of the spindle. Mitosis ends with telophase, during which nuclei reform and the chromosomes decondense. Cytokinesis usually begins during late anaphase and is almost complete by the end of telophase, resulting in the formation of two interphase daughter cells.

Prophase: Formation of the Mitotic Apparatus

When the chromosome condensation initiated in G2 phase reaches the point at which individual condensed chromosomes first become visible with the light microscope, the first stage of mitosis, **prophase,** has begun. The appearance of thin thread like chromosomes marks the first phase of mitosis. The condensation process continues throughout prophase; consequently, some chromosomes that start prophase as minute threads appear quite bulky before its conclusion. Ribosomal RNA synthesis ceases when the portion of the chromosome bearing the rRNA genes is condensed. During prophase the cell becomes spheroid, more retractile and viscous.

Assembling the Spindle Apparatus. The assembly of the microtubular apparatus that will later separate the sister chromatids also continues during prophase. In animal cells, the two centriole pairs formed during G2 phase begin to move apart early in prophase, forming between them an axis of microtubules referred to as spindle fibers. By the time the centrioles reach the opposite poles of the cell, they have established a bridge of microtubules called the spindle apparatus between them. In plant cells, a similar bridge of microtubular fibers forms between opposite poles of the cell, although centrioles are absent in plant cells.

During the formation of the spindle apparatus, the nuclear envelope breaks down and the endoplasmic reticulum reabsorbs its components. At this point, then, the microtubular spindle fibers extend completely across the cell, from one pole to the other. Their orientation determines the plane in which the cell will subsequently divide, through the center of the cell at right angles to the spindle apparatus. In animal cell mitosis, the centrioles extend a radial array of microtubules toward the plasma membrane when they reach the poles of the cell. This arrangement of microtubules is called an **aster.** Although the aster's function is not fully understood, it probably braces the centrioles against the membrane and stiffens the point of microtubular attachment during the retraction of the spindle. Plant cells, which have rigid cell walls, do not form asters.

Linking Sister Chromatids to Opposite Poles. Each chromosome possesses two kinetochores, one attached to the centromere region of each sister chromatid. As prophase continues, a second group of microtubules appears to grow from the poles of the cell toward the centromeres. These microtubules connect the kinetochores on each pair of sister chromatids to the two poles of the spindle. Because microtubules extending from the two poles attach to opposite sides of the centromere, they attach one sister chromatid to one pole and the other sister chromatid to the other pole. This arrangement is absolutely critical to the process of mitosis; any mistakes in microtubule positioning can be disastrous. The attachment of the two sides of a centromere to the same pole, for example, leads to a failure of the sister chromatids to separate, so that they end up in the same daughter cell.

Metaphase: Alignment of the Centromeres

The second stage of mitosis, **metaphase,** is the phase where the chromosomes align in the center of the cell. When viewed with a light microscope, the chromosomes appear to array themselves in a circle along the inner circumference of the cell, as the equator girdles the earth. An imaginary plane perpendicular to the axis of the spindle that passes through this circle is called the *metaphase plate.* The metaphase plate is not an actual

structure, but rather an indication of the future axis of cell division. Positioned by the microtubules attached to the kinetochores of their centromeres, all of the chromosomes line up on the metaphase plate. At this point, which marks the end of metaphase, their centromeres are neatly arrayed in a circle, equidistant from the two poles of the cell, with microtubules extending back towards the opposite poles of the cell in an arrangement called a spindle because of its shape.

Anaphase: Chromatid segregation

Of all the stages of mitosis, **anaphase** is the shortest and the most beautiful to watch. It starts when the centromeres divide. Each centromere splits in two, freeing the two sister chromatids from each other. The centromeres of all the chromosomes separate simultaneously, but the mechanism that achieves this synchrony is not known. Freed from each other, the sister chromatids are pulle rapidly toward the poles to which their kinetochores are attached. In the process, two forms of movement take place simultaneously, each driven by microtubules.

First, *the poles move apart* as microtubular spindle fibers physically anchored to opposite poles slide past each other, away from the center of the cell. Because another group of microtubules attach the chromosomes to the poles, the chromosomes move apart, too. If a flexible membrane surrounds the cell, it becomes visibly elongated. Second, *the centromeres move toward the poles* as the microtubules that connect them to the poles shorten. This shortening process is not a contraction; the microtubules do not get any thicker. Instead, tubulin subunits are removed from the kinetochore ends of the microtubules by the organizing center. As more subunits are removed, the chromatid-bearing microtubules are progressively disassembled, and the chromatids are pulled ever closer to the poles of the cell. When the sister chromatids separate in anaphase, the accurate partitioning of the replicated genome—the essential element of mitosis—is complete.

Telophase: Reformation of the Nuclei

The end of polar migration of the daughter chromosomes marks the beginning of telophase; which in turn is terminated by the reorganization of two nuclei and their entry into the G1 phase of interphase. In general terms the events of prophase occur in reverse sequence during this phase. In **telophase,** the spindle apparatus disassembles, as the microtubules are broken down into tubulin monomers that can be used to construct the cytoskeletons of the daughter cells. A nuclear envelope forms around each set of sister chromatids, which can now be called chromosomes because each has its own centromere. The chromosomes soon begin to uncoil into the more extended form that permits gene expression. One of the early group of genes expressed are the rRNA genes, resulting in the reappearance of the nucleolus.

During prophase, microtubules attach the centromeres joining pairs of sister chromatids to opposite poles of the spindle apparatus. During metaphase, each chromosome is drawn to a ring along the inner circumference of the cell by the microtubules extending from the centromere to the two poles of the spindle apparatus. During anaphase, the poles of the cell are pushed apart by microtubular sliding, and the sister chromatids are drawn to opposite

poles by the shortening of the microtubules attached to them. During telophase, the spindle is disassembled, nuclear envelopes are reestablished, and the normal expression of genes present in the chromosomes is reinitiated.

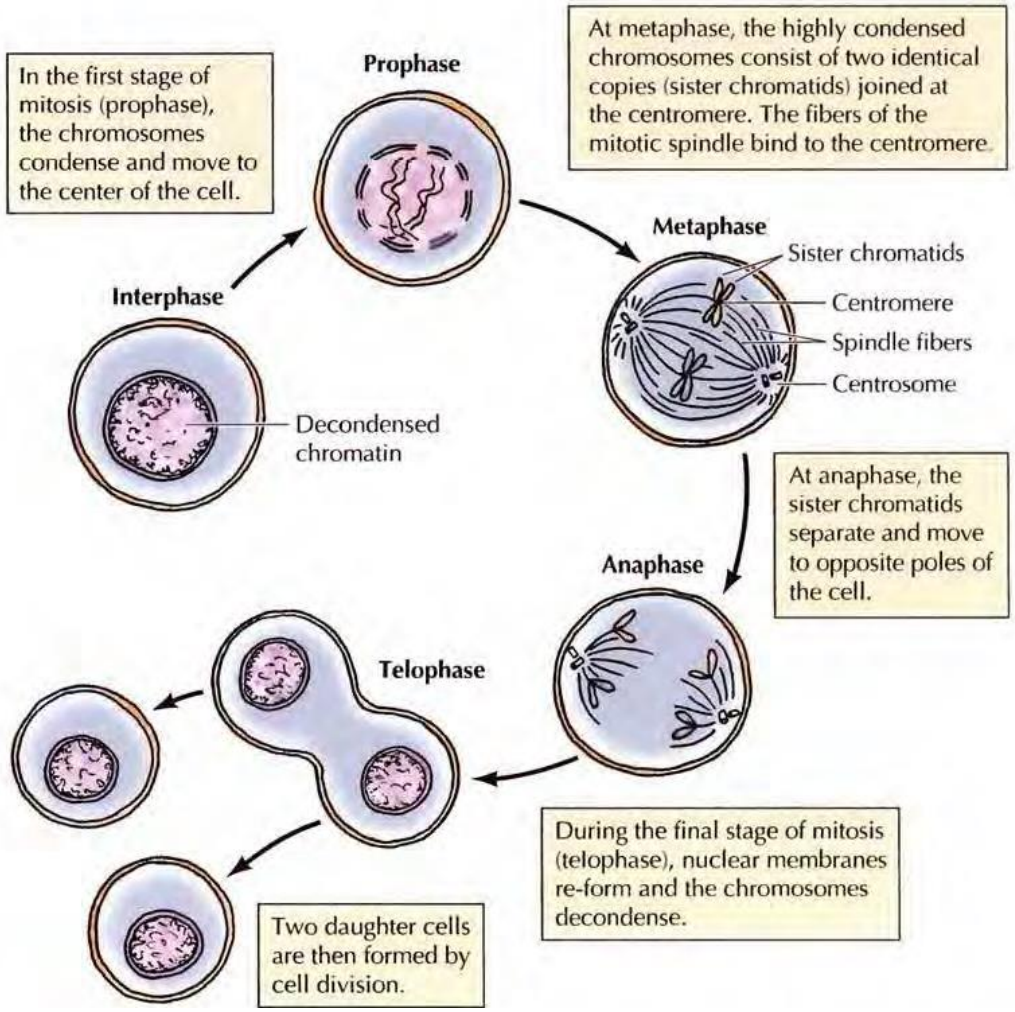


Fig. chromosomes during mitosis

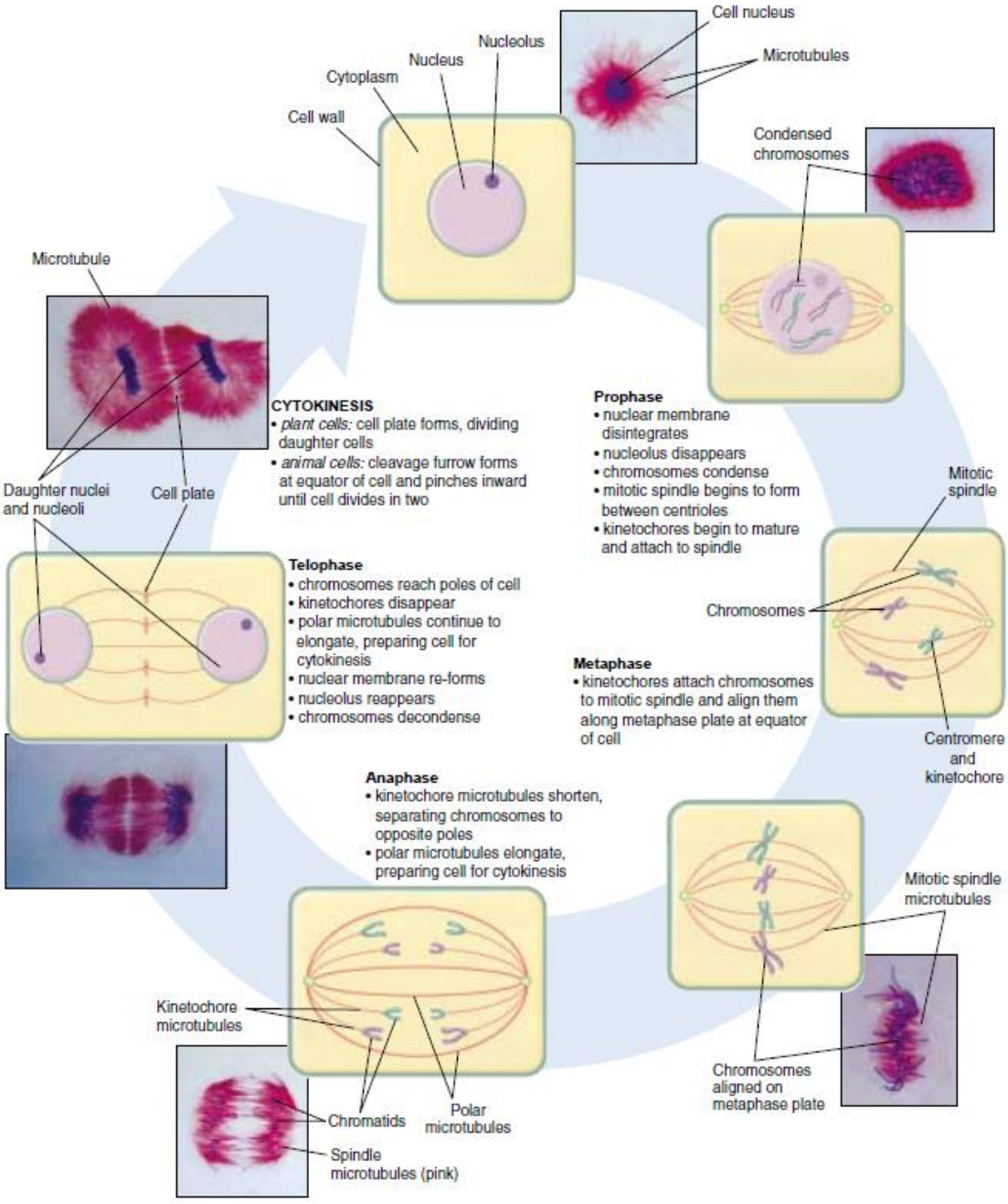


Fig. stages of mitosis

Meiosis

Meiosis is a specialized kind of cell cycle that reduces the chromosome number by half, resulting in the production of haploid daughter cells. Unicellular eukaryotes, such as yeasts, can undergo meiosis as well as reproducing by mitosis. Diploid *Saccharomyces cerevisiae*, for example, undergo meiosis and produce spores when faced with

unfavorable environmental conditions. In multicellular plants and animals, however, meiosis is restricted to the germ cells, where it is key to sexual reproduction. Whereas somatic cells undergo mitosis to proliferate, the germ cells undergo meiosis to produce haploid gametes (the sperm and the egg).

The Process of Meiosis

In contrast to mitosis, meiosis results in the division of a diploid parental cell into haploid progeny, each containing only one member of the pair of homologous chromosomes that were present in the diploid parental cell. This reduction in chromosome number is accomplished by two sequential rounds of nuclear and cell division (called meiosis I and meiosis II), which follow a single round of DNA replication. Like mitosis, meiosis I initiates after S phase has been completed and the parental chromosomes have replicated to produce identical sister chromatids. The pattern of chromosome segregation in meiosis I, however, is dramatically different from that of mitosis. During meiosis I, homologous chromosomes first pair with one another and then segregate to different daughter cells. Sister chromatids remain together, so completion of meiosis I results in the formation of daughter cells containing a single member of each chromosome pair (consisting of two sister chromatids). Meiosis I is followed by meiosis II, which resembles mitosis in that the sister chromatids separate and segregate to different daughter cells. Completion of meiosis II thus results in the production of four haploid daughter cells, each of which contains only one copy of each chromosome.

The First Meiotic Division

Meiosis-I: Divided into four stages-prophase-I, metaphase-I, anaphase-I, and telophase-I.

Prophase I

In prophase I of meiosis, the DNA coils tighter, and individual chromosomes first become visible under the light microscope as a matrix of fine threads. Because the DNA has already replicated before the onset of meiosis, each of these threads actually consists of two sister chromatids joined at their centromeres. In prophase I, homologous chromosomes become closely associated in synapsis, exchange segments by crossing over, and then separate.

An Overview

Prophase I is traditionally divided into five sequential stages: leptotene, zygotene, pachytene, diplotene, and diakinesis.

Leptotene. Chromosomes condense tightly.

Zygotene. A lattice of protein is laid down between the homologous chromosomes in the process of synapsis, forming a structure called a *synaptonemal complex*.

Pachytene. Pachytene begins when synapsis is complete (just after the synaptonemal complex forms), and lasts for days. This complex, about 100 nm across, holds the two replicated chromosomes in precise register, keeping each gene directly across from its partner on the homologous chromosome, like the teeth of a zipper. Within the synaptonemal complex, the DNA duplexes unwind at certain sites, and single strands of DNA form base-pairs with complementary strands *on the other homologue.* The synaptonemal complex thus provides the structural framework that enables crossing over

between the homologous chromosomes. As you will see, this has a key impact on how the homologues separate later in meiosis.

Diplotene. At the beginning of diplotene, the protein lattice of the synaptonemal complex disassembles. Diplotene is a period of intense cell growth. During this period the chromosomes decondense and become very active in transcription.

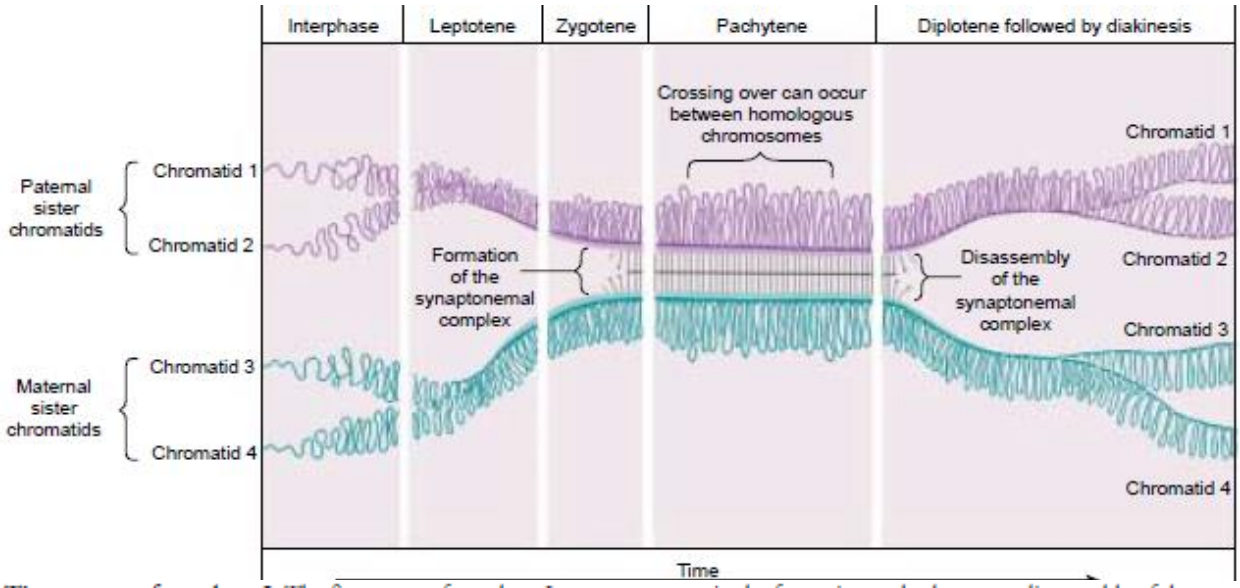
Diakinesis. At the beginning of diakinesis, the transition into metaphase, transcription ceases and the chromosomes recondense.

Synapsis During prophase, the ends of the chromatids attach to the nuclear envelope at specific sites. The sites the homologues attach to are adjacent, so that the members of each homologous pair of chromosomes are brought close together. They then line up side by side, apparently guided by heterochromatin sequences, in the process called synapsis.

Crossing Over Within the synaptonemal complex, recombination is thought to be carried out during pachytene by very large protein assemblies called **recombination nodules.** A nodule's diameter is about 90 nm, spanning the central element of the synaptonemal complex. Spaced along the synaptonemal complex, these recombination nodules act as large multienzyme "recombination machines," each nodule bringing about a recombination event. The details of the crossing over process are not well understood, but involve a complex series of events in which DNA segments are exchanged between nonsister or sister chromatids. In humans, an average of two or three such crossover events occur per chromosome pair. When crossing over is complete, the synaptonemal complex breaks down, and the homologous chromosomes are released from the nuclear envelope and begin to move away from each other. At this point, there are four chromatids for each type of chromosome (two homologous chromosomes, each of which consists of two sister chromatids). The four chromatids do not separate completely, however, because they are held together in two ways: (1) the two sister chromatids of each homologue, recently created by DNA replication, are held near by their common centromeres; and (2) the paired homologues are held together at the points where crossing over occurred within the synaptonemal complex.

Chiasma Formation Evidence of crossing over can often be seen under the light microscope as an X-shaped structure known as a **chiasma** (Greek, "cross"; plural, **chiasmata**). The presence of a chiasma indicates that two chromatids (one from each homologue) have exchanged parts. Like small rings moving down two strands of rope, the chiasmata move to the end of the chromosome arm as the homologous chromosomes separate.

- **Synapsis is the close pairing of homologous chromosomes that takes place early in prophase I of meiosis. Crossing over occurs between the paired DNA strands, creating the chromosomal configurations known as chiasmata. The two homologues are locked together by these exchanges and they do not disengage readily.**



Time course of prophase I. The five stages of prophase I represent stages in the formation and subsequent disassembly of the synaptonemal complex, the protein lattice that holds homologous chromosomes together during synapsis.

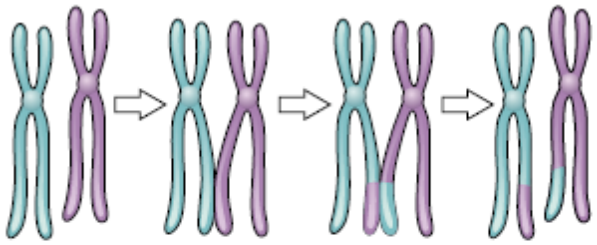


Fig. Result of crossing over

Metaphase I

By metaphase I, the second stage of meiosis I, the nuclear envelope has dispersed and the microtubules form a spindle, just as in mitosis. During diakinesis of prophase I, the chiasmata move down the paired chromosomes from their original points of crossing over, eventually reaching the ends of the chromosomes. At this point, they are called terminal chiasmata. Terminal chiasmata hold the homologous chromosomes together in metaphase I, so that only one side of each centromere faces outward from the complex; the other side is turned inward toward the other homologue. Consequently, spindle microtubules are able to attach to kinetochore proteins only on the outside of each centromere, and the centromeres of the two homologues attach to microtubules originating from opposite poles. This one-sided attachment is in marked contrast to the attachment in mitosis, when kinetochores on *both* sides of a centromere bind to microtubules. Each joined pair of homologues then lines up on the metaphase plate. The orientation of each pair on the spindle axis is random: either the maternal or the paternal homologue may orient toward a given pole.

- **Chiasmata play an important role in aligning the chromosomes on the metaphase plate. Completing Meiosis**

After the long duration of prophase and metaphase, which together make up 90% or more of the time meiosis I takes, meiosis I rapidly concludes. Anaphase I and telophase I

proceed quickly, followed—without an intervening period of DNA synthesis—by the second meiotic division.

Anaphase I

In anaphase I, the microtubules of the spindle fibers begin to shorten. As they shorten, they break the chiasmata and pull the centromeres toward the poles, dragging the chromosomes along with them. Because the microtubules are attached to kinetochores on only one side of each centromere, the individual centromeres are not pulled apart to form two daughter centromeres, as they are in mitosis. Instead, the entire centromere moves to one pole, taking both sister chromatids with it. When the spindle fibers have fully contracted, each pole has a complete haploid set of chromosomes consisting of one member of each homologous pair. Because of the random orientation of homologous chromosomes on the metaphase plate, a pole may receive either the maternal or the paternal homologue from each chromosome pair. As a result, the genes on different chromosomes assort independently; that is, meiosis I results in the **independent assortment** of maternal and paternal chromosomes into the gametes.

Telophase I

By the beginning of telophase I, the chromosomes have segregated into two clusters, one at each pole of the cell. Now the nuclear membrane re-forms around each daughter nucleus. Because each chromosome within a daughter nucleus replicated before meiosis I began, each now contains two sister chromatids attached by a common centromere. Importantly, *the sister chromatids are no longer identical,* because of the crossing over that occurred in prophase I. Cytokinesis may or may not occur after telophase I. The second meiotic division, meiosis II, occurs after an interval of variable length.

The Second Meiotic Division

After a typically brief interphase, in which no DNA synthesis occurs, the second meiotic division begins. Meiosis II resembles a normal mitotic division. Prophase II, metaphase II, anaphase II, and telophase II follow in quick succession.

Prophase II. At the two poles of the cell the clusters of chromosomes enter a brief prophase II, each nuclear envelope breaking down as a new spindle forms.

Metaphase II. In metaphase II, spindle fibers bind to both sides of the centromeres.

Anaphase II. The spindle fibers contract, splitting the centromeres and moving the sister chromatids to opposite poles.

Telophase II. Finally, the nuclear envelope re-forms around the four sets of daughter chromosomes.

The final result of this division is four cells containing haploid sets of chromosomes. No two are alike, because of the crossing over in prophase I. Nuclear somes. The cells that contain these haploid nuclei may develop directly into gametes, as they do in animals. Alternatively, they may themselves divide mitotically, as they do in plants, fungi, and many protists, eventually producing greater numbers of gametes or, as in the case of some plants and insects, adult individuals of varying ploidy.

- **During meiosis I, homologous chromosomes move toward opposite poles in anaphase I, and individual chromosomes cluster at the two poles in telophase I. At the end of meiosis II, each of the four haploid cells contains one copy of every**

chromosome in the set, rather than two. Because of crossing over, no two cells are the same. These haploid cells may develop directly into gametes, as in animals, or they may divide by mitosis, as in plants, fungi, and many protists.

Unique Features of Meiosis

Meiosis has three unique features

The mechanism of cell division varies in important details in different organisms. This is particularly true of chromosomal separation mechanisms, which differ substantially in protists and fungi from the process in plants and animals that we will describe here. Meiosis in a diploid organism consists of two rounds of division, mitosis of one. Although meiosis and mitosis have much in common, meiosis has three unique features: synapsis, homologous recombination, and reduction division.

Synapsis

The first unique feature of meiosis happens early during the first nuclear division. Following chromosome replication, *homologous chromosomes,* or *homologues*, *pair all along their length.* The process of forming these complexes of homologous chromosomes is called **synapsis**

Homologous Recombination

The second unique feature of meiosis is that *genetic exchange occurs between the homologous chromosomes* while they are thus physically joined. The exchange process that occurs between paired chromosomes is called **crossing over.** Chromosomes are then drawn together along the equatorial plane of the dividing cell; subsequently, homologues are pulled by microtubules toward opposite poles of the cell. When this process is complete, the cluster of chromosomes at each pole contains one of the two homologues of each chromosome. Each pole is haploid, containing half the number of chromosomes present in the original diploid cell. Sister chromatids do not separate from each other in the first nuclear division, so each homologue is still composed of two chromatids.

Reduction Division

The third unique feature of meiosis is that *the chromosomes do not replicate between the two nuclear divisions*, so that at the end of meiosis, each cell contains only half the original complement of chromosomes. In most respects, the second meiotic division is identical to a normal mitotic division. However, because of the crossing over that occurred during the first division, the sister chromatids in meiosis II are not identical to each other. Meiosis is a continuous process, but it is most easily studied when we divide it into arbitrary stages. The stages of meiosis are traditionally called meiosis I and meiosis II. Like mitosis, each stage is subdivided further into prophase, metaphase, anaphase, and telophase. In meiosis, however, prophase I is more complex than in mitosis.

In meiosis, homologous chromosomes become intimately associated and do not replicate between the two nuclear divisions.

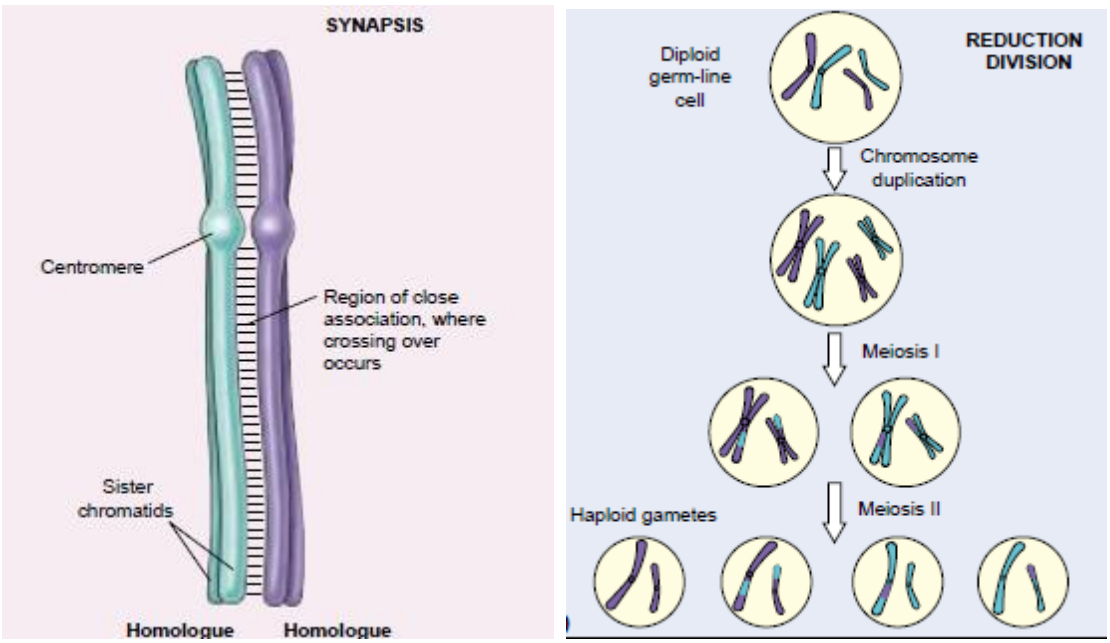


Fig.Unique features of meiosis (*a*) Synapsis draws homologous chromosomes together, creating a situation where the two chromosomes can physically exchange parts, a process called crossing over. (*b*) Reduction division, by omitting a chromosome duplication before meiosis II, produces haploid gametes, thus ensuring that chromosome number remains stable during the reproduction cycle.

The Structure of DNA

DNA Is a Double Helix That Stores Genetic Information

DNA was first isolated and characterized by Friedrich Miescher in 1868. He called the phosphorus-containing substance "nuclein." Not until the 1940s, with the work of Oswald T. Avery, Colin MacLeod, and Maclyn McCarty, was there any compelling evidence that DNA was the genetic material. Experiments conducted by Oswald T. Avery, Colin MacLeod, and Maclyn McCarty on *Streptococcus pneumoniae* revealed that DNA from the virulent strain carried the genetic information for virulence. Then in 1952, experiments by Alfred D. Hershey and Martha Chase, in which they studied the infection of bacterial cells by a virus (bacteriophage) with radioactively labeled DNA or protein, removed any remaining doubt that DNA, not protein, carried the genetic information.

Another important clue to the structure of DNA came from the work of Erwin Chargaff and his colleagues in the late 1940s. They found that the four nucleotide bases of DNA occur in different ratios in the DNAs of different organisms and that the amounts of certain bases are closely related. These data, collected from DNAs of a great many different species, led Chargaff to the following conclusions:

1. The base composition of DNA generally varies from one species to another.
2. DNA specimens isolated from different tissues of the same species have the same base composition.

3. The base composition of DNA in a given species does not change with an organism's age, nutritional state, or changing environment.
4. In all cellular DNAs, regardless of the species, the number of adenosine residues is equal to the number of thymidine residues (that is, A = T), and the number of guanosine residues is equal to the number of cytidine residues (G = C). From these relationships it follows that the sum of the purine residues equals the sum of the pyrimidine residues; that is, A +G =T + C.

These quantitative relationships, sometimes called "Chargaff's rules," were confirmed by many subsequent researchers. They were a key to establishing the three dimensional structure of DNA and yielded clues to how genetic information is encoded in DNA and passed from one generation to the next.

To shed more light on the structure of DNA, Rosalind Franklin and Maurice Wilkins used the powerful method of x-ray diffraction to analyze DNA fibers. They showed in the early 1950s that DNA produces a characteristic x-ray diffraction pattern. From this pattern it was deduced that DNA molecules are helical with two periodicities along their long axis, a primary one of 3.4 A^o and a secondary one of 34 A^o. The problem then was to formulate a three-dimensional model of the DNA molecule that could account not only for the x-ray diffraction data but also for the specific A = T and G = C base equivalences discovered by Chargaff and for the other chemical properties of DNA.

James Watson and Francis Crick relied on this accumulated information about DNA to set about deducing its structure. In 1953 they postulated a three-dimensional model of DNA structure that accounted for all the available data. It consists of two helical DNA chains wound around the same axis to form a right-handed double helix. The hydrophilic backbones of alternating deoxyribose and phosphate groups are on the outside of the double helix, facing the surrounding water. The furanose ring of each deoxyribose is in the C-$2^/$ endo conformation. The purine and pyrimidine bases of both strands are stacked inside the double helix, with their hydrophobic and nearly planar ring structures very close together and perpendicular to the long axis. The offset pairing of the two strands creates a **major groove** and **minor groove** on the surface of the duplex. Each nucleotide base of one strand is paired in the same plane with a base of the other strand. Watson and Crick found that the hydrogen bonded base pairs G with C and A with T, are those that fit best within thē structure, providing a rationale for Chargaff's rule that in any DNA, G = C and A = T. It is important to note that three hydrogen bonds can form between G and C, symbolized G = C but only two can form between A and T, symbolized A=T.

When Watson and Crick constructed their model, they had to decide at the outset whether the strands of DNA should be **parallel** or **antiparallel**—whether their $3^/$,$5^/$-phosphodiester bonds should run in the same or opposite directions. An antiparallel orientation produced the most convincing model, and later work with DNA polymerases provided experimental evidence that the strands are indeed antiparallel, a finding ultimately confirmed by x-ray analysis. To account for the periodicities observed in the x-ray diffraction patterns of DNA fibers, Watson and Crick manipulated molecular models to arrive at a structure in which the vertically stacked bases inside the double helix would be 3.4 A apart; the secondary repeat distance of about 34 A was accounted for by the

presence of 10 base pairs in each complete turn of the double helix. The two antiparallel polynucleotide chains of double-helical DNA are not identical in either base sequence or composition. Instead they are **complementary** to each other. Wherever adenine occurs in one chain, thymine is found in the other; similarly, wherever guanine occurs in one chain, cytosine is found in the other. The DNA double helix, or duplex, is held together by two forces: hydrogen bonding between complementary base pairs and base-stacking interactions. The complementarity between the DNA strands is attributable to the hydrogen bonding between base pairs. The base-stacking interactions, which are largely nonspecific with respect to the identity of the stacked bases, make the major contribution to the stability of the double helix. Moreover, the model suggested a mechanism for the transmission of genetic information.

- The structure of DNA described by Watson and Crick was a remarkable achievement, but not perfect In their initial paper, they assumed that both A-T and G-C were paired by two hydrogen bonds, missing the third hydrogen bond in G-C base pairs.

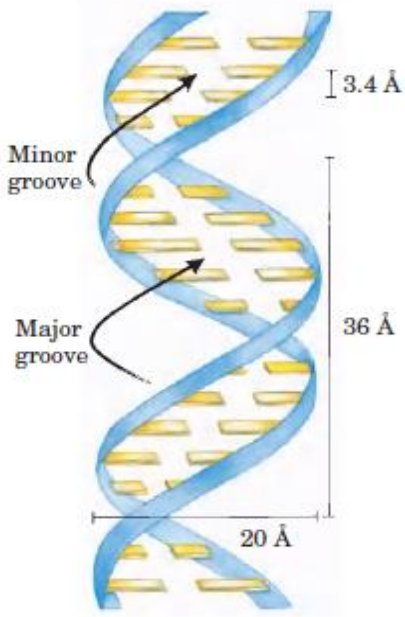


Fig. Watson and Crick model for the structure of DNA

Identification of DNA as the Genetic Material

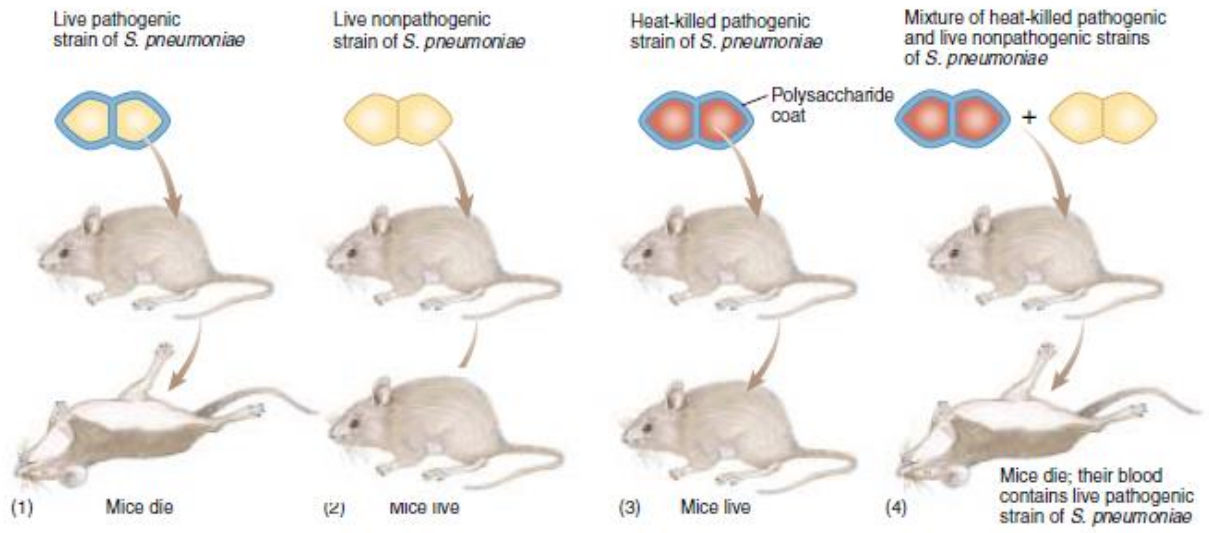
Understanding the chromosomal basis of heredity and the relationship between genes and enzymes did not in itself provide a molecular explanation of the gene. Chromosomes contain proteins as well as DNA, and it was initially thought that genes were proteins. The first evidence leading to the identification of DNA as the genetic material came from studies in bacteria.

The Griffith Experiment: Hereditary Information Can Pass between Organisms

The identification of the nucleus as the repository of hereditary information focused attention on the chromosomes, which were already suspected to be the vehicles of Mendelian inheritance. Specifically, biologists wondered how the **genes,** the units of hereditary information studied by Mendel, were actually arranged in the chromosomes. They knew that chromosomes contained both protein and deoxyribonucleic acid (DNA). Which of these held the genes? Starting in the late 1920s and continuing for about 30 years, a series of investigations addressed this question. In 1928, British microbiologist Frederick Griffith made a series of unexpected observations while experimenting with pathogenic (disease-causing) bacteria. When he infected mice with a virulent strain of *Streptococcus pneumonia* bacteria (then known as *Pneumococcus*), the mice died of blood poisoning. However, when he infected similar mice with a mutant strain of *S. pneumoniae* that lacked the virulent strain's polysaccharide coat, the mice showed no ill effects. The coat was apparently necessary for virulence. The normal pathogenic form of this bacterium is referred to as the S form because it forms smooth colonies on a culture dish. The mutant form, which lacks an enzyme needed to manufacture the polysaccharide capsule, is called the R form because it forms rough colonies. To determine whether the polysaccharide coat itself had a toxic effect, Griffith injected dead bacteria of the virulent S strain into mice; the mice remained perfectly healthy. As a control, he injected mice with a mixture containing dead S bacteria of the virulent strain and live coatless R bacteria, each of which by itself did not harm the mice. Unexpectedly, the mice developed disease symptoms and many of them died. The blood of the dead mice was found to contain high levels of live, virulent *Streptococcus* type S bacteria, which had surface proteins characteristic of the live (previously R) strain. Somehow, the information specifying the polysaccharide coat had passed from the dead, virulent S bacteria to the live, coatless R bacteria in the mixture, permanently transforming the coatless R bacteria into the virulent S variety.

Transformation is the transfer of genetic material from one cell to another and can alter the genetic makeup of the recipient cell.

Hereditary information can pass from dead cells to living ones, transforming them.



Griffith's discovery of transformation. (*1*) The pathogenic of the bacterium *Streptococcus pneumoniae* kills many of the mice it is injected into. The bacterial cells are covered with a polysaccharide coat, which the bacteria themselves synthesize. (*2*) Interestingly, an injection of live, coatless bacteria produced no ill effects. However, the coat itself is not the agent of disease. (*3*) When Griffith injected mice with dead bacteria that possessed polysaccharide coats, the mice were unharmed. (*4*) But when Griffith injected a mixture of dead bacteria with polysaccharide coats and live bacteria without such coats, many of the mice died, and virulent bacteria with coats were recovered. Griffith concluded that the live cells had been "transformed" by the dead ones; that is, genetic information specifying the polysaccharide coat had passed from the dead cells to the living ones.

The Avery and Hershey-Chase Experiments: The Active Principle Is DNA

The Avery Experiments

The agent responsible for transforming *Streptococcus* went undiscovered until 1944. In a classic series of experiments, Oswald Avery and his coworkers Colin MacLeod and Maclyn McCarty characterized what they referred to as the "transforming principle." They first prepared the mixture of dead S *Streptococcus* and live R *Streptococcus* that Griffith had used. Then Avery and his colleagues removed as much of the protein as they could from their preparation, eventually achieving 99.98% purity. Despite the removal of nearly all protein, the transforming activity was not reduced. Moreover, the properties of the transforming principle resembled those of DNA in several ways:

1. When the purified principle was analyzed chemically, the array of elements agreed closely with DNA.
2. When spun at high speeds in an ultracentrifuge, the transforming principle migrated to the same level (density) as DNA.
3. Extracting the lipid and protein from the purified transforming principle did not reduce its activity.
4. Protein-digesting enzymes did not affect the principle's activity; nor did RNA-digesting enzymes.
5. The DNA-digesting enzyme DNase destroyed all transforming activity. The evidence was overwhelming. They concluded that "a nucleic acid of the deoxyribose type is the fundamental unit of the transforming principle of *Pneumococcus* Type III"—in essence, that DNA is the hereditary material.

The Hershey–Chase Experiment

Avery's results were not widely accepted at first, as many biologists preferred to believe that proteins were the repository of hereditary information. Additional evidence supporting Avery's conclusion was provided in 1952 by Alfred Hershey and Martha Chase, who experimented with **bacteriophages,** viruses that attack bacteria. Viruses consist of either DNA or RNA (ribonucleic acid) surrounded by a protein coat. When a *lytic* (potentially cell-rupturing) bacteriophage infects a bacterial cell, it first binds to the cell's outer surface and then injects its hereditary information into the cell. There, the hereditary information directs the production of thousands of new viruses within the bacterium. The bacterial cell eventually ruptures, or lyses, releasing the newly made viruses.
To identify the hereditary material injected into bacterial cells at the start of an infection, Hershey and Chase used the bacteriophage T2, which contains DNA rather than RNA. They labeled the two parts of the viruses, the DNA and the protein coat, with different radioactive isotopes that would serve as tracers. In some experiments, the viruses were grown on a medium containing an isotope of phosphorus, ^{32}P, and the isotope was incorporated into the phosphate groups of newly synthesized DNA molecules. In other experiments, the viruses were grown on a medium containing ^{35}S, an isotope of sulfur, which is incorporated into the amino acids of newly synthesized protein coats. The ^{32}P and ^{35}S isotopes are easily distinguished from each other because they emit particles with different energies when they decay. After the labeled viruses were permitted to infect bacteria, the bacterial cells were agitated violently to remove the protein coats of the infecting viruses from the surfaces of the bacteria. This procedure removed nearly all of the ^{35}S label (and thus nearly all of the viral protein) from the bacteria. However, the ^{32}P label (and thus the viral DNA) had transferred to the interior of the bacteria and was found in viruses subsequently released from the infected bacteria. Hence, the hereditary information injected into the bacteria that specified the new generation of viruses was DNA and not protein.

Avery-MacLeod-McCarty experiment showed that DNA isolated from one bacterial strain can enter and transform the cells of another strain, endowing it with some of the inheritable characteristics of the donor. Avery's experiments demonstrate conclusively that DNA is Griffith's transforming material. The Hershey-Chase experiment showed that the DNA of a bacterial virus, but not its protein coat, carries the genetic message for replication of the virus in a host cell. The hereditary material of bacteriophages is DNA and not protein.

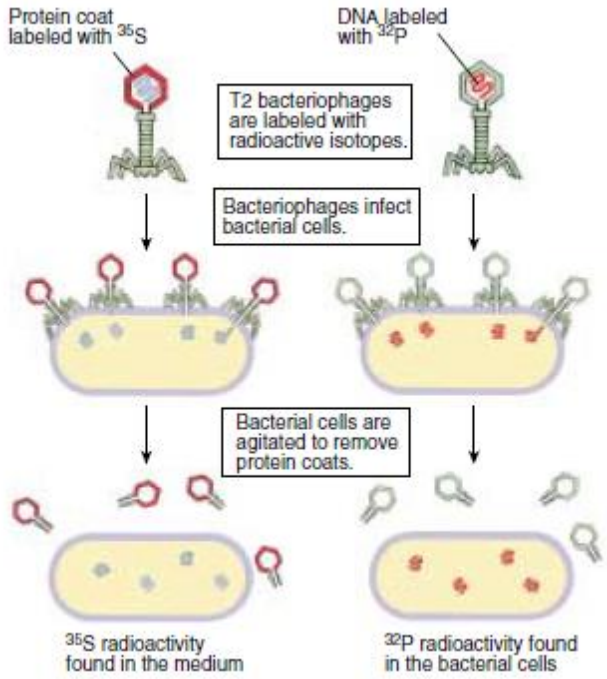


Fig. The Hershey and Chase experiment. Hershey and Chase found that ^{35}S radioactivity did not enter infected bacterial cells and ^{32}P radioactivity did. They concluded that viral DNA, not protein, was responsible for directing the production of new viruses.

DNA Can Occur in Different Three-Dimensional Forms

DNA is a remarkably flexible molecule. Considerable rotation is possible around several types of bonds in the sugar–phosphate (phosphodeoxyribose) backbone, and thermal fluctuation can produce bending, stretching, and unpairing (melting) of the strands. Many significant deviations from the Watson-Crick DNA structure are found in cellular DNA, some or all of which may be important in DNA metabolism. These structural variations generally do not affect the key properties of DNA defined by Watson and Crick: Strand complementarity, antiparallel strands, and the requirement for A=T and G=C base pairs.
Structural variation in DNA reflects three things: the different possible conformations of the deoxyribose, rotation about the contiguous bonds that make up the phosphodeoxyribose backbone, and free rotation about the C-1′–*N*-glycosyl bond.

Because of steric constraints, purines in purine nucleotides are restricted to two stable conformations with respect to deoxyribose, called syn and anti. Pyrimidines are generally restricted to the anti conformation because of steric interference between the sugar and the carbonyl oxygen at C-2 of the pyrimidine.

The Watson-Crick structure is also referred to as **B-form DNA** or B-DNA. The B form is the most stable structure for a random-sequence DNA molecule under physiological conditions and is therefore the standard point of reference in any study of the properties of DNA. Two structural variants that have been well characterized in crystal structures are the **A** and **Z forms**. The A form is favored in many solutions that are relatively devoid of water. The DNA is still arranged in a right-handed double helix, but the helix is wider and the number of base pairs per helical turn is 11, rather than 10.5 as in B-DNA. The plane of the base pairs in A-DNA is tilted about 20^0 with respect to the helix axis. These structural changes deepen the major groove while making the minor groove shallower. The reagents used to promote crystallization of DNA tend to dehydrate it, and thus most short DNA molecules tend to crystallize in the A form.

Z-form DNA is a more radical departure from the B structure; the most obvious distinction is the left handed helical rotation. There are 12 base pairs per helical turn, and the structure appears more slender and elongated. The DNA backbone takes on a zigzag appearance. Certain nucleotide sequences fold into left-handed Z helices much more readily than others. Prominent examples are sequences in which pyrimidines alternate with purines, especially alternating C and G or 5-methyl-C and G residues. To form the left handed helix in Z-DNA, the purine residues flip to the syn conformation, alternating with pyrimidines in the anti conformation. The major groove is barely apparent in Z-DNA, and the minor groove is narrow and deep.

Whether A-DNA occurs in cells is uncertain, but there is evidence for some short stretches (tracts) of Z-DNA in both bacteria and eukaryotes. These ZDNA tracts may play a role (as yet undefined) in regulating the expression of some genes or in genetic recombination.

Comparison of A, B and Z form of DNA

	A form	**B form**	**Z form**
Helical sense	Right handed	Right handed	Left handed
Diameter	26 A^0	20 A^0	18 A^0
Base pairs per helical turn	11	10.5	12
Helical rise per base pair	2.6 A^0	3.4 A^0	3.7 A^0
Base tilt normal to the helical axis	20^0	6^0	7^0
Glycosyl bond conformation	Anti	Anti	Anti for C & T syn for A & G

DNA Replication

DNA replication is a semi-conservative process in which each parental strand serves as a template for the synthesis of a new complementary daughter strand. The central enzyme invoJved is DNA polymerase, which catalyzes the joining of deoxyribonucleoside 5'-triphosphates (dNTPs) to form the growing DNA chain. However, DNA replication is

much more complex than a single enzymatic reaction. Other proteins are involved, and proofreading mechanisms are required to ensure that the accuracy of replication is compatible with the low frequency of errors.

DNA Polymerases

DNA polymerase was first identified in lysates of *E. coli* by Arthur Kornberg in 1956. The ability of this enzyme to accurately copy a DNA template provided a biochemical basis for the mode of DNA replication that was initially proposed by Watson and Crick, so its isolation represented a landmark discovery in molecular biology. Ironically, this first DNA polymerase to be identified (now called DNA polymerase I) is not the major enzyme responsible for *E. coli* DNA replication. Instead, polymerase I is principally involved in repair of damaged DNA, and it is now clear that both prokaryotic and eukaryotic cells contain multiple different DNA polymerases that play distinct roles in DNA replication and repair. In prokaryotic cells, DNA polymerase Ill is the major polymerase responsible for DNA replication.

Eukaryotic cells contain 3 DNA polymerases (ex., o, and e) that function in replication of nuclear DNA. A distinct DNA polymerase (y) is localized to mitochondria and is responsible for replication of mitochondrial DNA. All known DNA polymerases share two fundamental properties that have critical implications for DNA replication (Figure 6.1). First, all polymerases synthesize DNA only in the 5' to 3' direction, adding a dNTP to the 3' hydroxyl group of a growing chain. Second, DNA polymerases can add a new deoxyribonucleotide only to a preformed primer strand that is hydrogen bonded to the template; they are not able to initiate DNA synthesis *de novo* by catalyzing the polymerization of free dNTPs. In this respect, DNA polymerases differ from RNA polymerases, which can initiate the synthesis of a new strand of RNA in the absence of a primer.

The Need for a Primer

One of the features of DNA polymerase III is that it can add nucleotides only to a chain of nucleotides that is already paired with the parent strands. Hence, DNA polymerase cannot link the first nucleotides in a newly synthesized strand. Instead, another enzyme, an RNA polymerase called **primase,** constructs an **RNA primer,** a sequence of about 10 RNA nucleotides complementary to the parent DNA template. DNA polymerase III recognizes the primer and adds DNA nucleotides to it to construct the new DNA strands. The RNA nucleotides in the primers are then replaced by DNA nucleotides.

The Two Strands of DNA Are Assembled in Different Ways

Another feature of DNA polymerase III is that it can add nucleotides only to the 3′ end of a DNA strand (the end with an —OH group attached to a 3′ carbon atom). This means that replication always proceeds in the 5′ → 3′ direction on a growing DNA strand. Because the two parent strands of a DNA molecule are anti-parallel, *the new strands are oriented in opposite directions* along the parent templates at each replication fork. Therefore, the new strands must be elongated by different mechanisms! The **leading strand,** which elongates *toward* the replication fork, is built up simply by adding nucleotides continuously to its growing 3′ end. In contrast, the **lagging strand,** which elongates *away from* the replication fork, is synthesized discontinuously as a series of

short segments that are later connected. These segments, called **Okazaki fragments,** are about 100 to 200 nucleotides long in eukaryotes and 1000 to 2000 nucleotides long in prokaryotes. Each Okazaki fragment is synthesized by DNA polymerase III in the 5′ → 3′ direction, beginning at the replication fork and moving away from it. When the polymerase reaches the 5′ end of the lagging strand, another enzyme, **DNA ligase,** attaches the fragment to the lagging strand. The DNA is further unwound, new RNA primers are constructed, and DNA polymerase III then jumps ahead 1000 to 2000 nucleotides (toward the replication fork) to begin constructing another Okazaki fragment. If one looks carefully at electron micrographs showing DNA replication in progress, one can sometimes see that one of the parent strands near the replication fork appears single-stranded over a distance of about 1000 nucleotides. Because the synthesis of the leading strand is continuous, while that of the lagging strand is discontinuous, the overall replication of DNA is said to be **semi-discontinuous.**

The Replication Process

The replication of the DNA double helix is a complex process that has taken decades of research to understand. It takes place in five interlocking steps:

1. Opening up the DNA double helix. The very stable DNA double helix must be opened up and its strands separated from each other for semi-conservative replication to occur.

Stage one: Initiating replication. The binding of **initiator proteins** to the replication origin starts an intricate series of interactions that opens the helix.

Stage two: Unwinding the duplex. After initiation, "unwinding" enzymes called **helicases** bind to and move along one strand, shouldering aside the other strand as they go.

Stage three: Stabilizing the single strands. The unwound portion of the DNA double helix is stabilized by **single-strand binding protein,** which binds to the exposed single strands, protecting them from cleavage and preventing them from rewinding.

Stage four: Relieving the torque generated by unwinding. For replication to proceed at 1000 nucleotides per second, the parental helix ahead of the replication fork must rotate 100 revolutions per second! To relieve the resulting twisting, called torque, enzymes known as topisomerases—or, more informally, **gyrases**— cleave a strand of the helix, allow it to swivel around the intact strand, and then reseal the broken strand.

2. Building a primer. New DNA cannot be synthesized on the exposed templates until a primer is constructed, as DNA polymerases require 3′ primers to initiate replication. The necessary primer is a short stretch of RNA, added by a specialized RNA polymerase called *primase* in a multi-subunit complex informally called a *primosome.* Why an RNA primer, rather than DNA? Starting chains on exposed templates introduces many errors; RNA marks this initial stretch as "temporary," making this error-prone stretch easy to excise later.

3. Assembling complementary strands. Next, the dimeric DNA polymerase III then binds to the replication fork. While the leading strand complexes with one half of the polymerase dimer, the lagging strand is thought to loop around and complex with the other half of the polymerase dimer. Moving in concert down the parental double helix,

DNA polymerase III catalyzes the formation of complementary sequences on each of the two single strands at the same time.

4. Removing the primer. The enzyme DNA polymerase-I now removes the RNA primer and fills in the gap, as well as any gaps between Okazaki fragments.

5. Joining the Okazaki fragments. After any gaps between Okazaki fragments are filled in, the enzyme DNA ligase joins the fragments to the lagging strand.

DNA replication involves many different proteins that open and unwind the DNA double helix, stabilize the single strands, synthesize RNA primers, assemble new complementary strands on each exposed parental strand—one of them discontinuously—remove the RNA primer, and join new discontinuous segments on the lagging strand.

Eukaryotic DNA Replication

In eukaryotic cells, the DNA is packaged in nucleosomes within chromosomes (figure 14.18). Each individual zone of a chromosome replicates as a discrete section called a **replication unit,** or **replicon,** which can vary in length from 10,000 to 1 million base-pairs; most are about 100,000 base-pairs long. Each replication unit has its own origin of replication, and multiple units may be undergoing replication at any given time, as can be seen in electron micrographs of replicating chromosomes. Each unit replicates in a way fundamentally similar to prokaryotic DNA replication, using similar enzymes. The advantage of having multiple origins of replication in eukaryotes is speed: replication takes approximately eight hours in humans cells, but if there were only one origin, it would take 100 times longer.

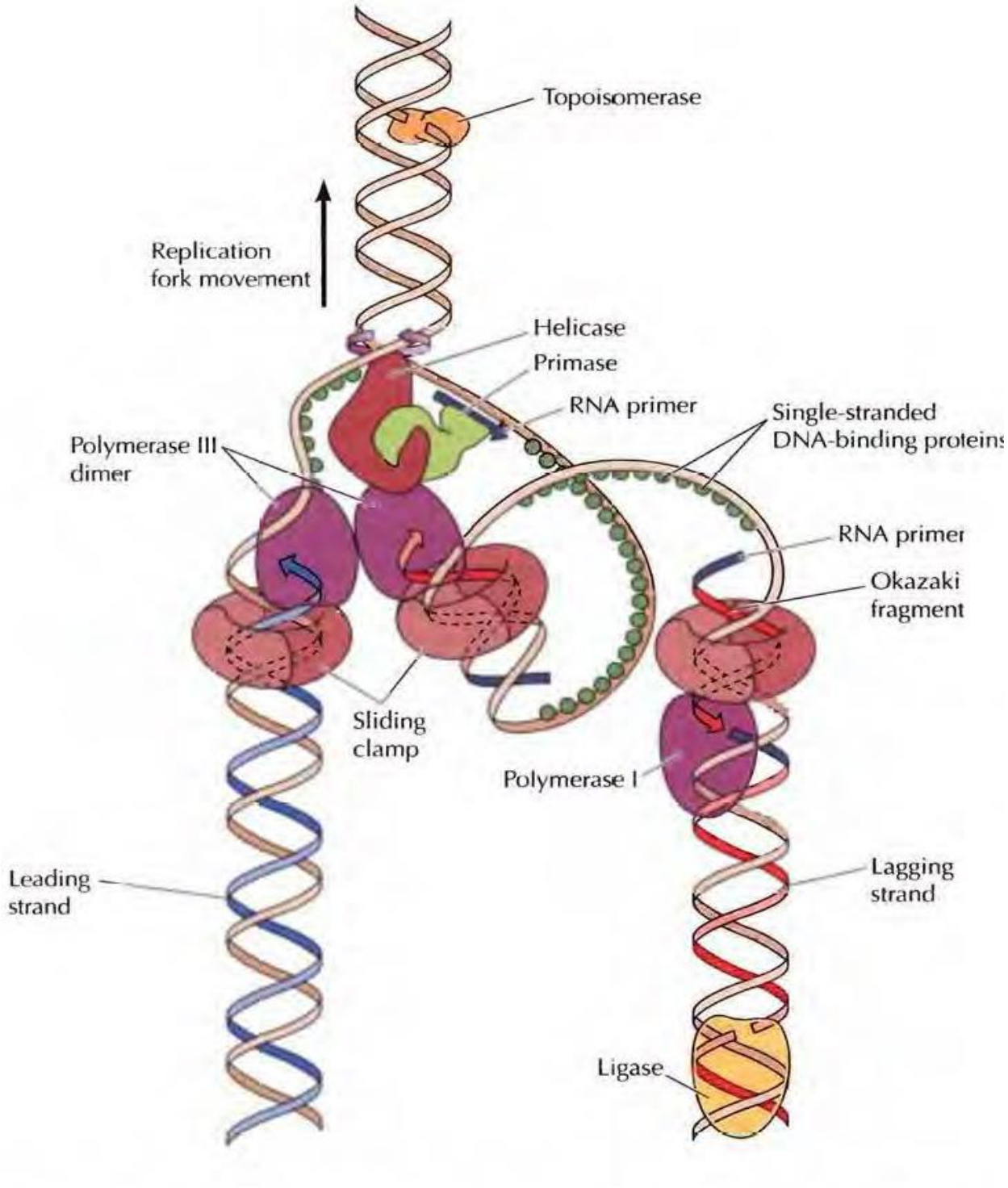


FIGURE: Model of the *E. coli* replication fork Helicase, primase, and two molecules of DNA polymerases Ill carry out coordinated synthesis of both the leading and lagging strands of DNA. The lagging strand template is folded so that the polymerase responsible for lagging strand synthesis moves in the same direction as overall movement of the fork. Topoisomerase acts as a swivel ahead of the fork. Behind the fork, RNA primers are removed by ON A polymerase I and Okazaki fragments are joined by DNA ligase.

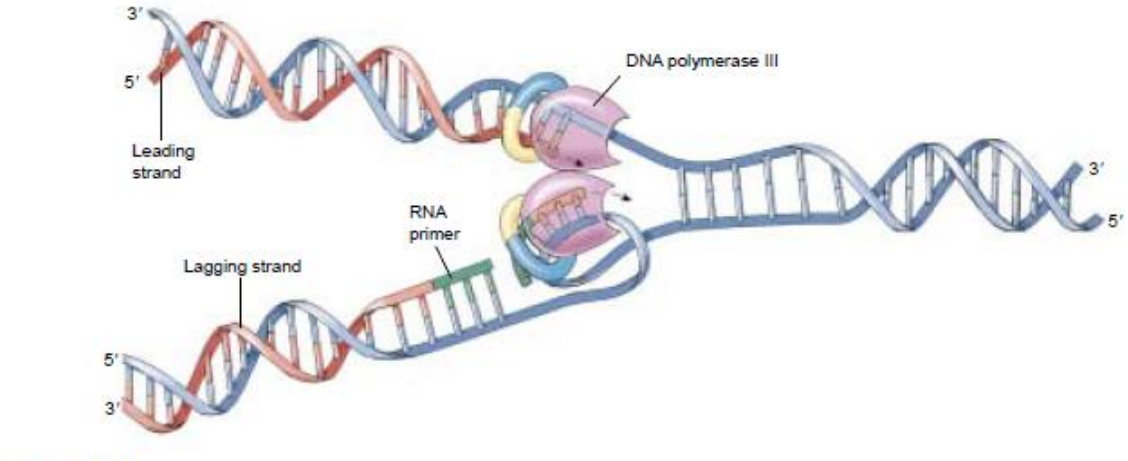


Fig. How DNA polymerase III works