Genetic information, stored in the chromosomes and transmitted to daughter cells through DNA replication, is expressed through transcription to RNA and, in the case of messenger RNA (mRNA), subsequent translation into proteins. The pathway of protein synthesis is called translation because the "language" of the nucleotide sequence on the mRNA is translated into the "language" of an amino acid sequence. The process of translation requires a genetic code, through which the information contained in the nucleic acid sequence is expressed to produce a specific sequence of amino acids.

THE GENETIC CODE

The correspondence between the sequence of bases in a codon and the amino acid residue it specifies is known as the **genetic code**. Its near universality among all forms of life is compelling evidence that life on Earth arose from a common ancestor. The genetic code is a dictionary that identifies the correspondence between a sequence of nucleotide bases and a sequence of amino acids. Each individual "word" in the code is composed of three nucleotide bases. These genetic words are called codons. There are four possible bases (U, C, A, and G) that can occupy each of the three positions in a codon, and hence there are 4^3-64 possible codons. Of these codons, 61 specify amino acids (of which there are only 20) and the remaining three, UAA, UAG, and UGA, are **Stop codons** that instruct the ribosome to cease polypeptide synthesis and release the resulting transcript.

A. Codons

Codons are presented in the mRNA language of adenine (A), guanine (G), cytosine (C), and uracil (U). Their nucleotide sequences are always written from the 5'-end to the 3'-end. The four nucleotide bases are used to produce the three-base codons. There are, therefore, 64 different combinations of bases, taken three at a time (a triplet code).

1. How to translate a codon: This table (or "dictionary") can be used to translate any codon and, thus, to determine which amino acids are coded for by an mRNA sequence. For example, the codon 5'-AUG-3' codes for methionine. [Note: AUG is the initiation (start) codon for translation.] Sixty-one of the 64 codons code for the 20 common amino acids.

2. Termination ("stop" or "nonsense") codons: Three of the codons, UAG, UGA, and UAA, do not code for amino acids, but rather are termination codons. When one of these codons appears in an mRNA sequence, synthesis of the polypeptide coded for by that mRNA stops.

Characteristics of the genetic code

Usage of the genetic code is remarkably consistent throughout all living organisms. It is assumed that once the standard genetic code evolved in primitive organisms, any mutation that altered its meaning would have caused the alteration of most, if not all, protein sequences, resulting in lethality. Characteristics of the genetic code include the following:

1. Specificity: The genetic code is specific (unambiguous) that is, a particular codon always codes for the same amino acid.

2. Universality: The genetic code is virtually universal, that is, its specificity has been conserved from very early stages of evolution, with only slight differences in the manner in which the code is translated. (An exception occurs in mitochondria)

3. Degeneracy: The genetic code is degenerate (sometimes called redundant). Although each codon corresponds to a single amino acid, a given amino acid may have more than one triplet coding for it. For example, arginine is specified by six different codons. Only Met and Trp have just one coding triplet.

4. Non-overlapping and comma less: The genetic code is non-overlapping and comma less, that is, the code is read from a fixed starting point as a continuous sequence of bases, taken three at a time. For example, AGCUGGAUACAU is read as AGC/UGG/AUA/CAU without any "punctuation" between the codons.

Consequences of altering the nucleotide sequence:

Changing a single nucleotide base on the mRNA chain (a "point mutation") can lead to any one of three results.

1. Silent mutation: The codon containing the changed base may code for the same amino acid. For example, if the serine codon UCA is given a different third base—U—to become UCU, it still codes for serine. This is termed a "silent" mutation.

2. Missense mutation: The codon containing the changed base may code for a different amino acid. For example, if the serine codon UCA is given a different first base—C—to become CCA, it will code for a different amino acid, in this case, proline. The substitution of an incorrect amino acid is called a "missense" mutation.

3. Nonsense mutation: The codon containing the changed base may become a termination codon. For example, if the serine codon UCA is given a different second base—A—to become UAA, the new codon causes termination of translation at that point, and the production of a shortened (truncated) protein. The creation of a termination codon at an inappropriate place is called a "nonsense" mutation.

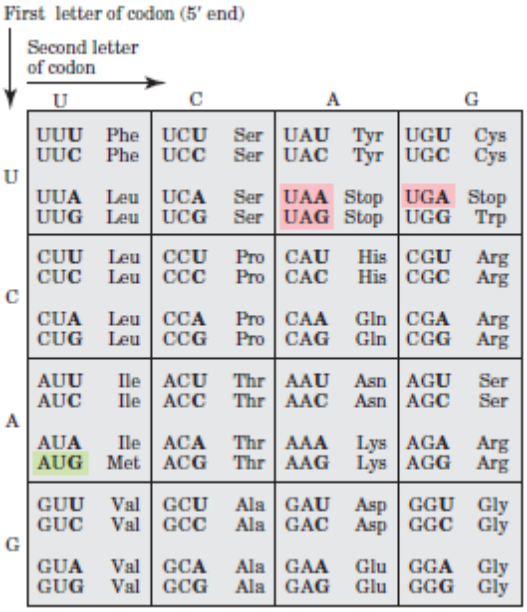
4. Other mutations: These can alter the amount or structure of the protein produced by translation.

a. Trinucleotide repeat expansion: Occasionally, a sequence of three bases that is repeated in tandem will become amplified in number, so that too many copies of the triplet occur. If this happens within the coding region of a gene, the protein will contain many extra copies of one amino acid. For example, amplification of the CAG codon leads to the insertion of many extra glutamine residues in the huntingtin protein, causing the neurodegenerative disorder.

b. Splice site mutations: Mutations at splice sites can alter the way in which introns are removed from pre mRNA molecules, producing aberrant proteins.

c. Frame-shift mutations: If one or two nucleotides are either deleted from or added to the coding region of a message sequence, a frame-shift mutation occurs and the reading frame is altered. This can result in a product with a radically different amino acid sequence, or a truncated product due to the creation of a termination codon. If three nucletides are added, a new amino acid is added to the peptide or, if three nucleotides are deleted, an amino acid is lost. Loss of three nucleotides maintains the reading frame, but can result in serious pathology. For example, cystic fibrosis (CF), a hereditary disease that primarily affects the pulmonary and digestive systems, is most commonly

caused by deletion of three nucleotides from the coding region of a gene, resulting in the loss of phenylalanine.



Amino acid	Number of codons	Amino acid	Number o codons
Met	1	Tyr	2
Trp	1	Ile	3
Asn	2	Ala	4
Asp	2	Gly	4
Cys	2	Pro	4
Gln	2	Thr	4
Glu	2	Val	4
His	2	Arg	6
Lys	2	Leu	6
Phe	2	Ser	6

Fig. Dictionary of amino acid code words in mRNA Table: degeneracy of genetic code

Wobble hypothesis

Examination of codon-anticodon pairings led Crick to conclude that the third base of most codons pairs rather loosely with the corresponding base of its anticodon; to use his picturesque word, the third base of such codons (and the first base of their corresponding anticodons) "wobbles." Crick proposed a set of four relationships called the **wobble hypothesis**:

1. The first two bases of an mRNA codon always form strong Watson-Crick base pairs with the corresponding bases of the tRNA anticodon and confer most of the coding specificity.
2. The first base of the anticodon (reading in the $5^{-} \rightarrow 3^{-}$ direction; this pairs with the third base of the codon) determines the number of codons recognized by the tRNA. When the first base of the anticodon is C or A, base pairing is specific and only one codon is recognized by that tRNA. When the first base is U or G, binding is less specific and two different codons may be read. When inosine (I) is the first (wobble) nucleotide of an anticodon, three different codons can be recognized—the maximum number for any tRNA.
3. When an amino acid is specified by several different codons, the codons that differ in either of the first two bases require different tRNAs.
4. A minimum of 32 tRNAs are required to translate all 61 codons (31 to encode the amino acids and 1 for initiation).

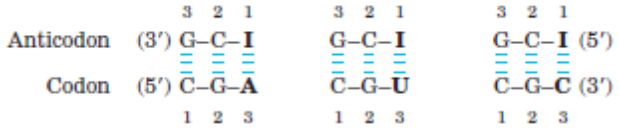


Fig. pairing relationship of codon anticodon

Wobble Allows Some tRNAs to Recognize More than One Codon

When several different codons specify one amino acid, the difference between them usually lies at the third base position (at the 3^{-} end). For example, alanine is coded by the triplets GCU, GCC, GCA, and GCG. The codons for most amino acids can be symbolized by $XY^{A}{}_{G}$ or $XY^{U}{}_{C}$. The first two letters of each codon are the primary determinants of specificity, a feature that has some interesting consequences. Transfer RNAs base-pair with mRNA codons at a three-base sequence on the tRNA called the **anticodon**. The first base of the codon in mRNA (read in the $5^{-} \rightarrow 3^{-}$ direction) pairs with the third base of the anticodon. If the anticodon triplet of a tRNA recognized only one codon triplet through Watson-Crick base pairing at all three positions, cells would have a different tRNA for each amino acid codon. This is not the case, however, because the anticodons in some tRNAs include the nucleotide inosinate (designated I), which contains the uncommon base hypoxanthine. Inosinate can form hydrogen bonds with three different nucleotides (U, C, and A, although these pairings are much weaker than the hydrogen bonds of Watson-Crick base pairs (G=C and A=U). In yeast, one $tRNA^{Arg}$ has the anticodon (5^{-}) ICG, which recognizes three arginine codons: (5^{-})CGA, (5^{-})CGU, and (5^{-})CGC. The first two bases are identical (CG) and form strong Watson-Crick base pairs with the corresponding bases of the anticodon, but the third base (A, U, or C) forms rather weak hydrogen bonds with the I residue at the first position of the anticodon.

The Genetic Code

■ The particular amino acid sequence of a protein is constructed through the translation of information encoded in mRNA. This process is carried out by ribosomes.

■ Amino acids are specified by mRNA codons consisting of nucleotide triplets. Translation requires adaptor molecules, the tRNAs, that recognize codons and insert amino acids into their appropriate sequential positions in the polypeptide.
■ The base sequences of the codons were deduced from experiments using synthetic mRNAs of known composition and sequence.
■ The codon AUG signals initiation of translation. The triplets UAA, UAG, and UGA are signals for termination.
■ The genetic code is degenerate: it has multiple codons for almost every amino acid.
■ The standard genetic code is universal in all species, with some minor deviations in mitochondria and a few single-celled organisms.
■ The third position in each codon is much less specific than the first and second and is said to wobble.
■ Translational frameshifting and RNA editing affect how the genetic code is read during translation.

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.

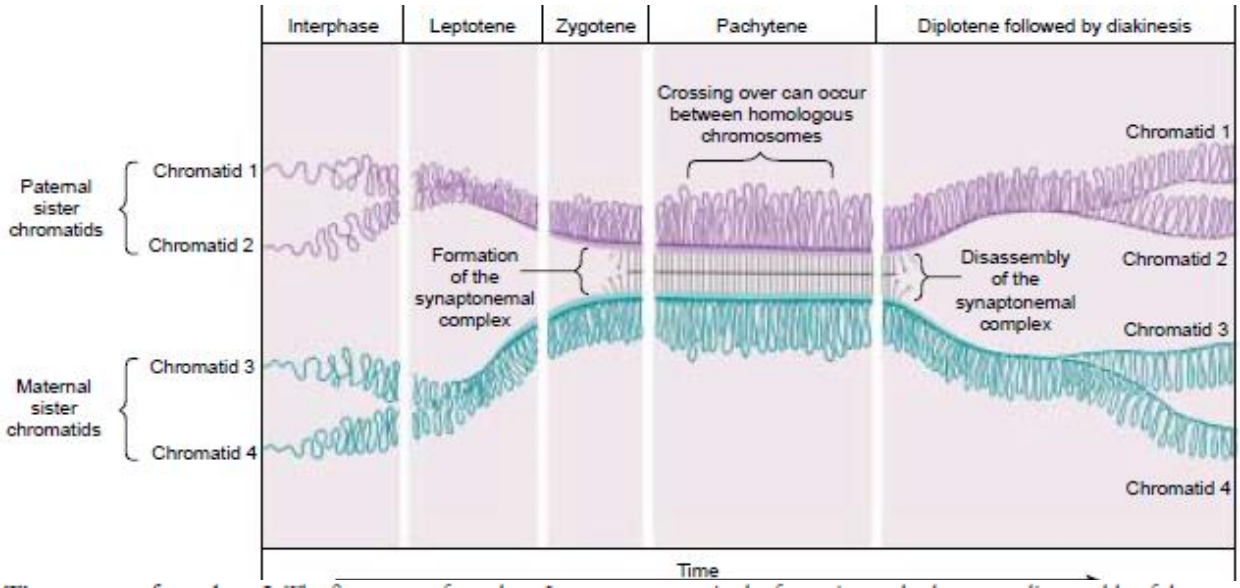
Diakinesis. At the beginning of diakinesis, the transition into metaphase, transcription ceases and the chromosomes recondense.

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.

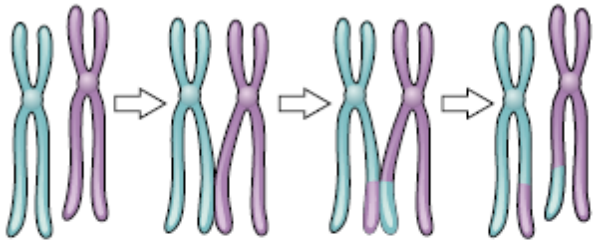


Fig. Result of crossing over

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.**

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.

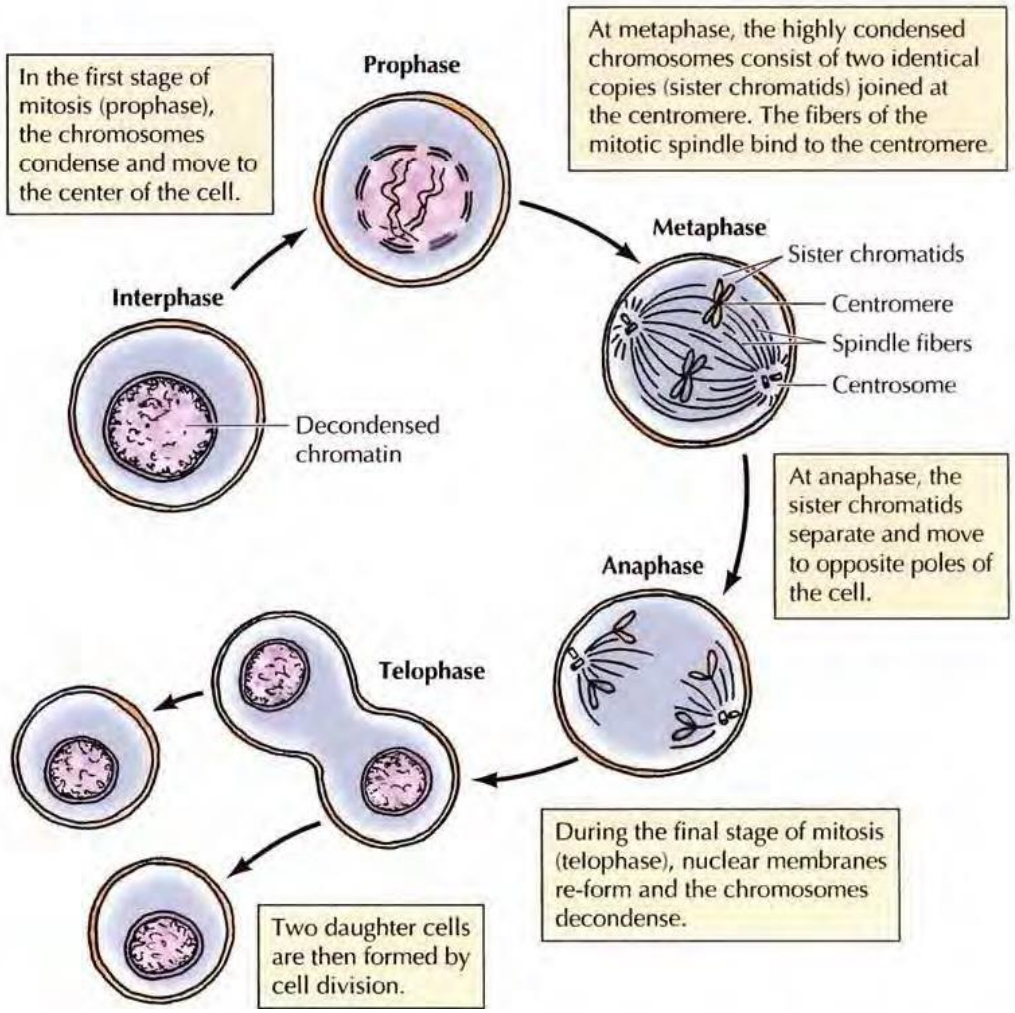


Fig. chromosomes during mitosis

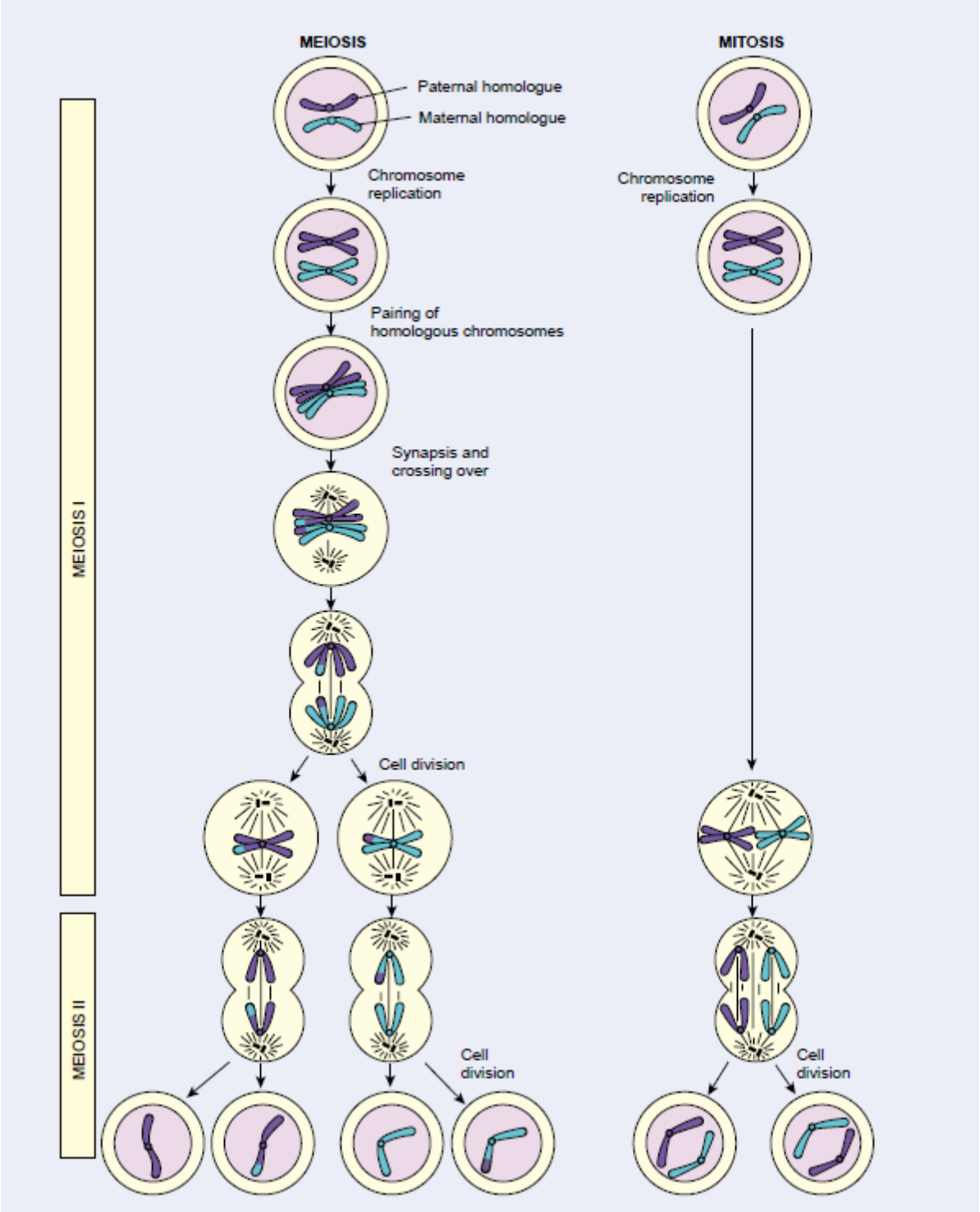


Fig. A comparison of meiosis and mitosis. Meiosis involves two nuclear divisions with no DNA replication between them. It thus produces four daughter cells, each with half the original number of chromosomes. Crossing over occurs in prophase I of meiosis. Mitosis involves a single nuclear division after DNA replication. It thus produces two daughter cells, each containing the original number of chromosomes.

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.

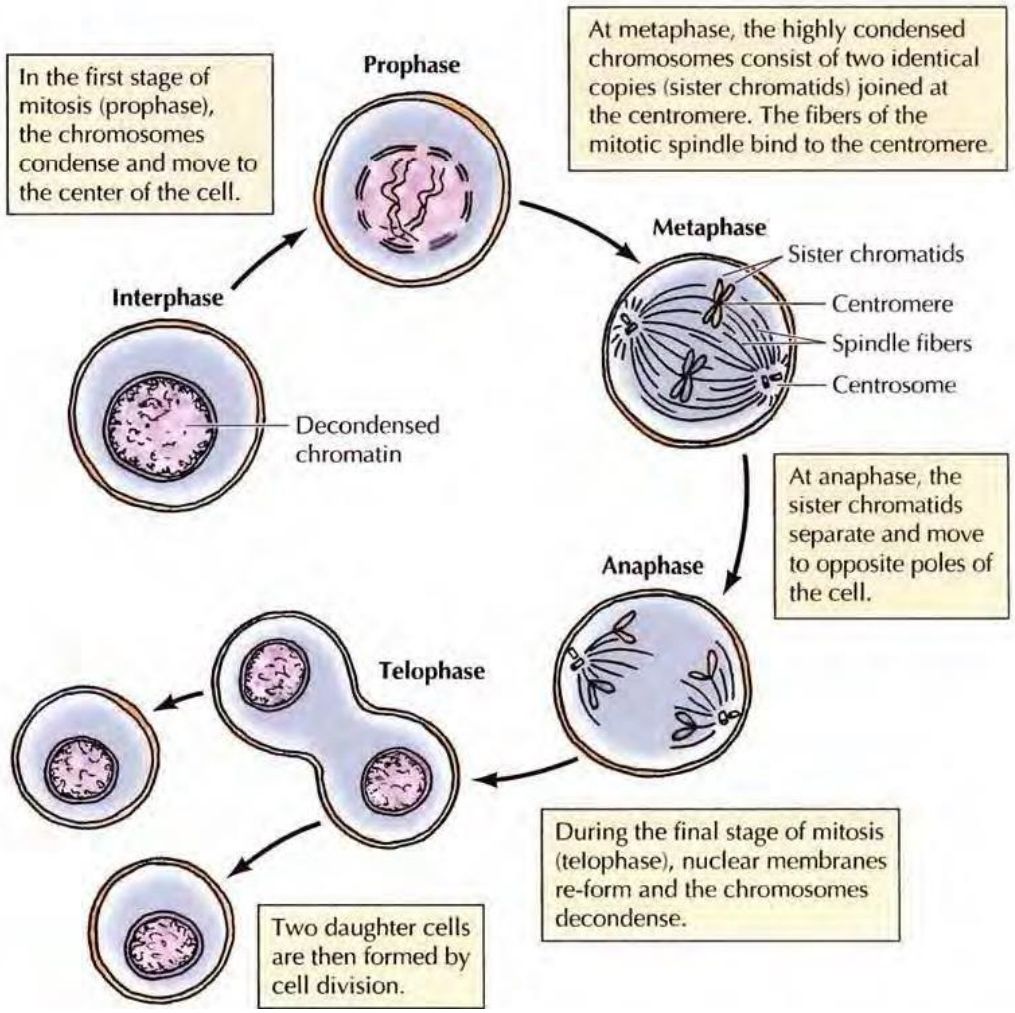


Fig. chromosomes during mitosis

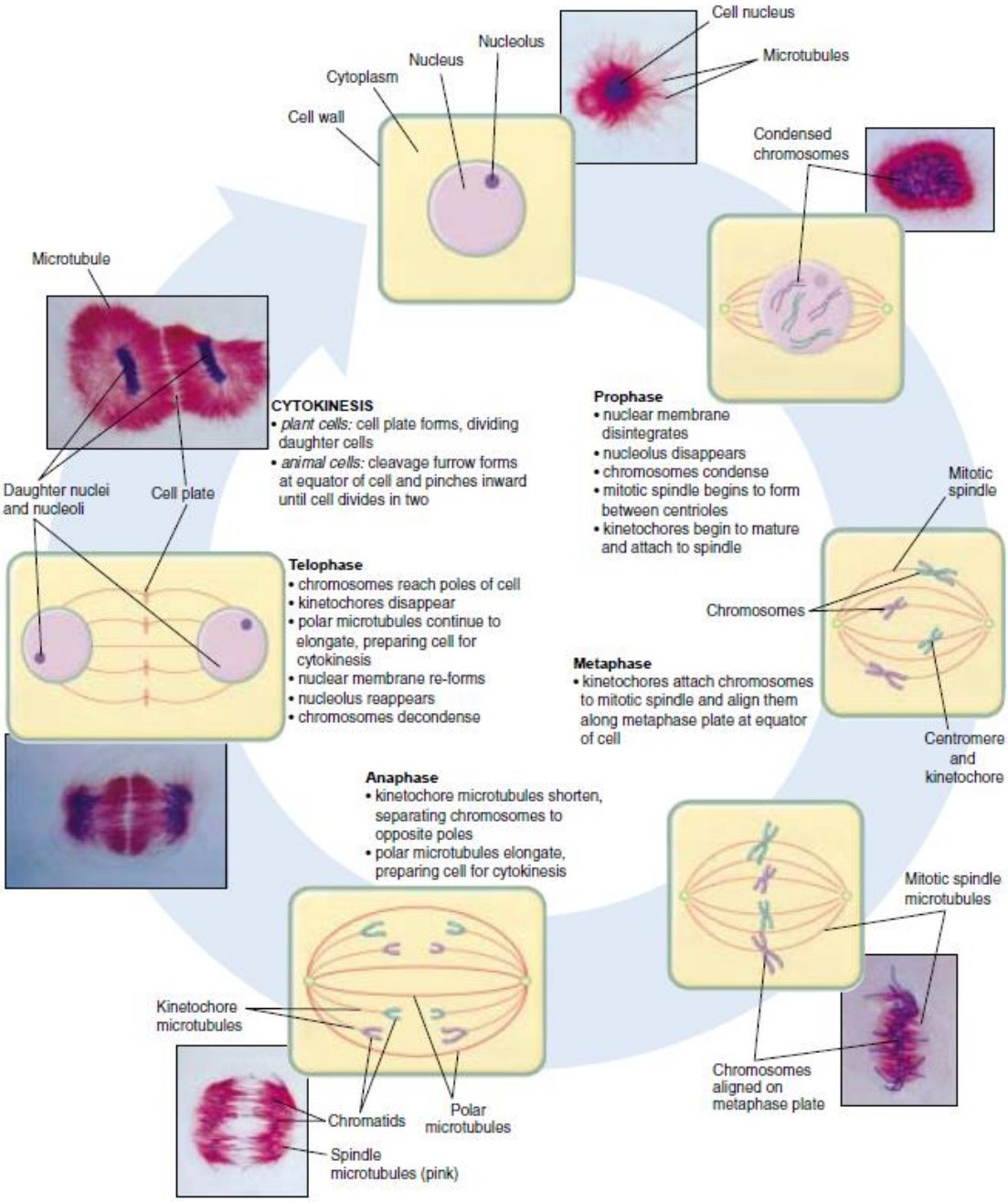


Fig. stages of mitosis

The structure of tRNA In 1965, after a 7-year effort, Robert Holley reported the first known base sequence of a biologically significant nucleic acid, that of yeast **alanine tRNA(tRNAAla**.
Almost all known tRNAs, as Holley first recognized, may be schematically arranged in the so-called cloverleaf secondary structure. Comparisons of tRNAs from various species have revealed many common structural features:
1. Each is a single chain containing between *73 and 93 ribonucleotides*.
2. They contain *many unusual bases,* typically between 7 and 15 per molecule. Some of these bases are methylated or dimethylated derivatives of A, U, C, and G formed by enzymatic modification of a precursor tRNA. Some methylations prevent the formation of certain base pairs, thereby rendering such bases accessible for interactions with other bases. In addition, methylation imparts a hydrophobic character to some regions of tRNAs, which may be important for their interaction with synthetases and ribosomal proteins.
3. The molecule is L-shaped.
4. About half the nucleotides in tRNAs are base-paired to form double helices. The four helical regions are arranged to form two apparently continuous segments of double helix.
5. The 5^- end of a tRNA is phosphorylated. The 5^- terminal residue is usually pG.
6. An activated amino acid is attached to a hydroxyl group of the adenosine residue in the amino acid-attachment site, located at the end of the 3^- CCA component of the acceptor stem. This single-stranded region can change conformation in the course of amino acid activation and protein synthesis.
7. The anticodon loop, which is present in a loop near the center of the sequence, is at the other end of the L, making accessible the three bases that make up the anticodon.

Transfer RNAs Have Characteristic Structural Features
Transfer RNAs are relatively small and consist of a single strand of RNA folded into a precise three-dimensional structure. The tRNAs in bacteria and in the cytosol of eukaryotes have between 73 and 93 nucleotide residues, corresponding to molecular weights of 24,000 to 31,000. Mitochondria and chloroplasts contain distinctive, somewhat smaller tRNAs. Cells have at least one kind of tRNA for each amino acid; at least 32 tRNAs are required to recognize all the amino acid codons (some recognize more than one codon), but some cells use more than 32. Yeast alanine tRNA ($tRNA^{Ala}$), the first nucleic acid to be completely sequenced, contains 76 nucleotide residues, 10 of which have modified bases. Comparisons of tRNAs from various species have revealed many common structural features. Eight or more of the nucleotide residues have modified bases and sugars, many of which are methylated derivatives of the principal bases. Most tRNAs have a guanylate (pG) residue at the 5^- end, and all have the trinucleotide sequence CCA(3^-) at the 3^- end. When drawn in two dimensions, the hydrogenbonding pattern of all tRNAs forms a cloverleaf structure with four arms; the longer tRNAs have a short fifth arm, or extra arm. In three dimensions, a tRNA has the form of a twisted L. Two of the arms of a tRNA are critical for its adaptor function. The **amino acid arm** can carry a specific amino acid esterified by its carboxyl group to the 2^-- or 3^- hydroxyl group of the A residue at the 3^- end of the tRNA. The **anticodon arm** contains the anticodon. The other major arms are the **D arm**, which contains the unusual nucleotide dihydrouridine (D), and the **TψC arm**, which contains ribothymidine (T), not usually present in RNAs, and pseudouridine, which has an unusual carbon–carbon bond between the base and ribose. The D and TψC arms contribute important interactions for the overall folding of tRNA molecules, and the TψC arm interacts with the large subunit rRNA.

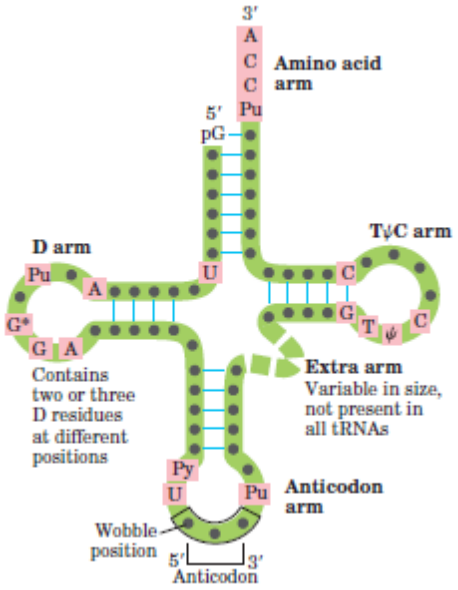


FIGURE General cloverleaf secondary structure of tRNAs. Transfer RNAs vary in length from 73 to 93 nucleotides. Extra nucleotides occur in the extra arm or in the D arm. At the end of the anticodon arm is the anticodon loop, which always contains seven unpaired nucleotides. The D arm contains two or three D (5,6-dihydrouridine) residues, depending on the tRNA. In some tRNAs, the D arm has only three hydrogen-bonded base pairs.

v