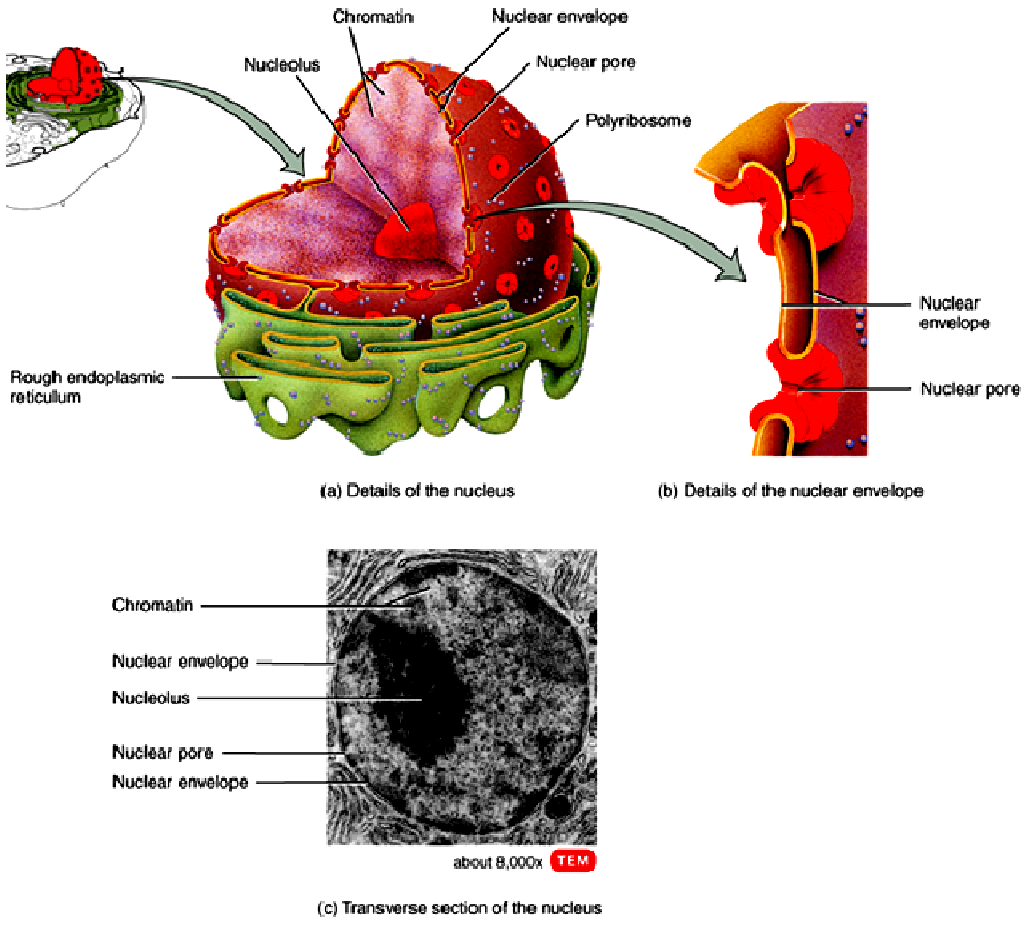
Nuclear Physiology

The Nucleus

The nucleus is the hallmark of eukaryotic cells; the very term eukaryotic means having a "true nucleus".



The Nuclear apparatus

The Nuclear Envelope

The nucleus is enveloped by a pair of membranes enclosing a lumen that is continuous with that of the **endoplasmic reticulum**. The inner membrane is stabilized by a meshwork of intermediate filament proteins called **lamins**. The nuclear envelope is perforated by thousands of **nuclear pore complexes** (**NPC**s) that control the passage of molecules in and out of the nucleus.

Chromatin

The nucleus contains the **chromosomes** of the cell. Each chromosome consists of a single molecule of DNA complex with an equal mass of proteins. Collectively, the DNA of the nucleus with its associated proteins is called **chromatin**. Most of the protein consists of multiple copies of 5 kinds of **histones**. These are basic proteins, bristling with positively charged **arginine** and

lysine residues. (Both Arg and Lys have a free amino group on their **R group**, which attracts protons (H^+) giving them a positive charge.) Just the choice of amino acids you would make to bind tightly to the negatively-charged **phosphate groups** of DNA. Chromatin also contains small amounts of a wide variety of **nonhistone proteins**. Most of these are **transcription factors** (e.g., the **steroid receptors**) and their association with the DNA is more transient.

Nucleosomes

Two copies of each of four kinds of histones, H2A, H2B, H3 and H4 form a core of protein, the **nucleosome** core. Around this is wrapped about 147 base pairs of DNA. From 20–60 bp of DNA link one nucleosome to the next. Each **linker** region is occupied by a single molecule of **histone 1** (**H1**). The binding of histones to DNA does not depend on particular nucleotide sequences in the DNA but does depend critically on the amino acid sequence of the histone. Histones are some of the most conserved molecules during the course of evolution. Histone H4 in the calf differs from H4 in the pea plant at only 2 amino acids residues in the chain of 102.

The formation of nucleosomes helps somewhat, but not nearly enough, to make the DNA sufficiently compact to fit in the nucleus. In order to fit 46 DNA molecules (in humans), totalling over 2 meters in length, into a nucleus that may be only 10 μm across requires more extensive folding and compaction. Interactions between the exposed "tails" of the core histones causes nucleosomes to associate into a compact fibre 30 nm in diameter. These fibres are then folded into more complex structures whose precise configuration is uncertain and which probably changes with the level of activity of the genes in the region.

Histone Modifications

Although their amino acid sequence (primary structure) is unvarying, individual histone molecules do vary in structure as a result of chemical modifications that occur later to individual amino acids. These include adding **acetyl** groups (CH_3CO-) to lysines, phosphate groups to serines and threonines and methyl groups to lysines and arginines Although 75–80% of the histone molecule is incorporated in the core, the remainder at the **N-terminal** dangles out from the core as a "tail". The chemical modifications occur on these tails, especially of H3 and H4. Most of theses changes are reversible. For example, acetyl groups are **added** by enzymes called **histone acetyltransferases** (**HAT**s) and **removed** by **histone deacetylases** (**HDACs**). More often than not, acetylation of histone tails occurs in regions of chromatin that become active in gene **transcription**. This makes a kind of intuitive sense as adding acetyl groups neutralizes the positive charges on Lys thus reducing the strength of the association between the highly-negative DNA and the highly-positive histones.

Methylation, which also neutralizes the charge on lysines (and arginines), can either stimulate or **inhibit** gene transcription in that region. Methylation of lysine-4 in H3 is associated with **active** genes while methylation of lysine-9 in H3 is associated with **inactive** genes. (These include those **imprinted genes** that have been **permanently inactivated** in somatic cells). and adding phosphates causes the chromosomes to become more but not less compact as they get ready for mitosis and meiosis. In any case, it is now clear that histones are a dynamic component of chromatin and not simply inert DNA-packing material.

Histone Variants

We have genes for 8 different varieties of histone 1 (H1). Which variety is found at a particular linker depends on such factors as the type of cell, where it is in the **cell cycle,** and its stage of differentiation. In some cases, at least, a particular variant of H1 associates with certain transcription factors to bind to the **enhancer** of specific genes turning off expression of those genes. Some other examples of histone variants include H3 is replaced by **CENP-A** ("centromere protein A") at the nucleosomes near centromeres. Failure to substitute CENP-A for H3 in this regions blocks centromere structure and function. H2A may be replaced by the variant H2A.Z at the boundaries between euchromatin and heterochromatin. All the "standard" histones are replaced by variants as **sperm** develop. In general, the "standard" histones are incorporated into the nucleosomes as new DNA is synthesized during **S phase** of the cell cycle. Later, some are replaced by variant histones as conditions in the cell dictate.

Euchromatin versus Heterochromatin

During **interphase**, little can be seen of chromatin structure (except for special cases like the **polytene chromosomes** of *Drosophila* and some other flies). However, the density of the chromatin (that is, how tightly it is packed) varies throughout the nucleus: Dense regions are called heterochromatin while less dense regions are called euchromatin.

Heterochromatin is found in parts of the chromosome where there are few or no genes, such as **centromeres** and **telomeres**. It is is densely-packed and greatly enriched with **transposons** and other "junk" DNA. Heterochromatin is replicated late in **S phase of the cell cycle** has reduced crossing over in **meiosis**. Those genes present in heterochromatin are generally inactive; that is, not **transcribed** and show **increased methylation** of the cytosines in CpG islands of the DNA, **decreased acetylation** of histones and **increased methylation of lysine-9 in histone H3**, which now provides a binding site for **heterochromatin protein 1** (HP1), which blocks access by the **transcription factors** needed for gene transcription.

Euchromatin is found in parts of the chromosome that contain many genes. It is loosely packed in loops of **30-nm fibers**. These are separated from adjacent heterochromatin by **insulators**. The loops are often found near the **nuclear pore complexes** because these are gene transcripts destined for the cytosol). The genes in euchromatin are active and thus show **decreased methylation** of the cytosines in CpG islands of the DNA, **increased acetylation** of histones and **decreased methylation** of lysine-9 in histone H3.

Nucleosomes and Transcription

Transcription factors cannot bind to their **promoter** if the promoter is blocked by a nucleosome. For at least some genes, one of the first functions of the assembling **transcription factors** is to slide the nucleosome father along the DNA molecule exposing the gene's promoter so that the transcription factors can then bind to it. The actual transcription of protein-coding genes is done by **RNA polymerase** II (RNAP II). In order for it to travel along the DNA to be transcribed, a complex of proteins removes the nucleosomes in front of it and then replaces them after RNAP II has transcribed that portion of DNA and moved on.

The Nucleolus

During the period between cell divisions, when the chromosomes are in their extended state, one or more of them (10 in human cells) have loops extending into a spherical mass called the nucleolus. Here are synthesized three (of the four) kinds of **RNA molecules** (28S, 18S, 5.8S) used in the assembly of the large and small subunits of **ribosomes**. The 28S, 18S, and 5.8S ribosomal RNA is transcribed (by **RNA polymerase I**) from hundreds to thousands of tandemly-arranged **rDNA genes** distributed (in humans) on 10 different chromosomes. The rDNA-containing regions of these 10 chromosomes cluster together in the nucleolus. (In **yeast**, the 5S rRNA molecules as well as transfer RNA molecules are also synthesized (by **RNA polymerase III**) in the nucleolus). Once formed, rRNA molecules associate with the dozens of different ribosomal **proteins** used in the assembly of the large and small subunits of the ribosome. But all proteins are synthesized in the cytosol and all the ribosomes are needed in the cytosol to do their work so there must be a mechanism for the transport of these large structures in and out of the nucleus. This is one of the functions of the nuclear pore complexes.

Nuclear Pore Complexes (NPCs)

The nuclear envelope is perforated with thousands of pores. Each is constructed from a number (30 in yeast; probably around 50 in vertebrates) different proteins called **nucleoporins**. The entire assembly forms an aqueous channel connecting the cytosol with the interior of the nucleus ("nucleoplasm"). When materials are to be transported through the pore, it opens up to form a channel some 25 nm wide, large enough to get such large assemblies as ribosomal subunits through. Transport through the nuclear pore complexes is **active**; i.e., it requires energy, many different carrier molecules each specialized to transport a particular cargo and docking molecules in the NPC.

All proteins are synthesized in the cytosol and those needed by the nucleus must be imported into it through the NPCs. Probably each of these proteins has a characteristic sequence of amino acids called a **nuclear localization sequence** (NLS) that targets it for entry. They include all the **histones** needed to make the nucleosomes, all the **ribosomal proteins** needed for the assembly of ribosomes, all the **transcription factors** (e.g., the steroid receptors) needed to turn genes on (and off) and all the **splicing factors** needed to process pre-mRNA into mature mRNA molecules; i.e., to cut out **intron** regions and splice the **exon** regions.

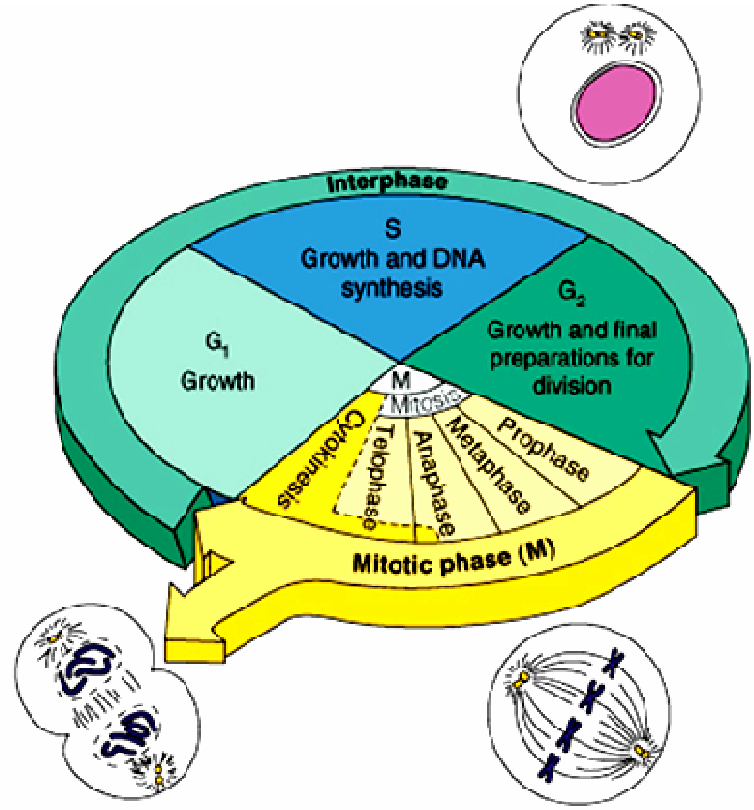
Molecules and macromolecular assemblies exported from the nucleus include the **ribosomal subunits** containing both rRNA and proteins **messenger RNA** (mRNA) molecules (accompanied by proteins) **transfer RNA** (tRNA) molecules (also accompanied by proteins) and **transcription factors** that are returned to the cytosol to await reuse. Both the RNA and protein molecules contain a characteristic **nuclear export sequence** (NES) needed to ensure their association with the right carrier molecules to take them out to the cytosol.

Nucleoplasm

The term "nucleoplasm" is still used to describe the contents of the nucleus. However, the term disguises the structural complexity and order that seems to exist within the nucleus. For example, there is evidence that **DNA replication** and **transcription** occur at discrete sites within the nucleus.

The Cell Cycle

Despite differences between prokaryotes and eukaryotes, there are several common features in their cell division processes. Replication of the DNA must occur. Segregation of the "original" and its "replica" follow. Cytokinesis ends the cell division process. Whether the cell was eukaryotic or prokaryotic, these basic events must occur. Cytokinesis is the process where one cell splits off from its sister cell. It usually occurs after cell division. The **Cell Cycle** is the sequence of growth, DNA replication, growth and cell division that all cells go through. Beginning after cytokinesis, the daughter cells are quite small and low on ATP. They acquire ATP and increase in size during the **G_1 phase** of Interphase. Most cells are observed in **Interphase**, the longest part of the cell cycle. After acquiring sufficient size and ATP, the cells then undergo **DNA Synthesis** (**replication** of the original DNA molecules, making identical copies, one "new molecule" eventually destined for each new cell) which occurs during the **S phase.** Since the formation of new DNA is an energy draining process, the cell undergoes a second growth and energy acquisition stage, the **G_2 phase**. The energy acquired during G_2 is used in cell division.



The cell cycle

Regulation of the cell cycle is accomplished in several ways. Some cells divide rapidly (beans, for example take 19 hours for the complete cycle; red blood cells must divide at a rate of 2.5 million per second). Others, such as nerve cells, lose their capability to divide once they reach maturity. Some cells, such as liver cells, retain but do not normally utilize their capacity for division. Liver cells will divide if part of the liver is removed. The division continues until the liver reaches its former size. Cancer cells are those which undergo a series of rapid divisions such that the daughter cells divide before they have

reached "functional maturity". Environmental factors such as changes in temperature and pH, and declining nutrient levels lead to declining cell division rates. When cells stop dividing, they stop usually at a point late in the G_1 phase, the R point (for restriction).

Control of the Cell Cycle

The passage of a cell through the cell cycle is controlled by proteins in the cytoplasm. Among the main players in animal cells are **Cyclins**, a **G_1 cyclin** (cyclin D), **S-phase cyclins** (cyclins E and A) and **mitotic cyclins** (cyclins B and A). Their levels in the cell rise and fall with the stages of the cell cycle. **Cyclin-dependent kinases (Cdks)**, a **G_1 Cdk** (Cdk4), an **S-phase Cdk** ((Cdk2) and an **M-phase Cdk** (Cdk1). Their levels in the cell remain fairly stable, but each must bind the appropriate cyclin (whose levels fluctuate) in order to be activated. They add phosphate groups to a variety of protein substrates that control processes in the cell cycle. The **anaphase-promoting complex (APC)**. (The APC is also called the **cyclosome**, and the complex is often designated as the **APC/C**.) The APC/C triggers the events leading to destruction of the **cohesins** thus allowing the sister chromatids to separate thus degrading the mitotic cyclin B.

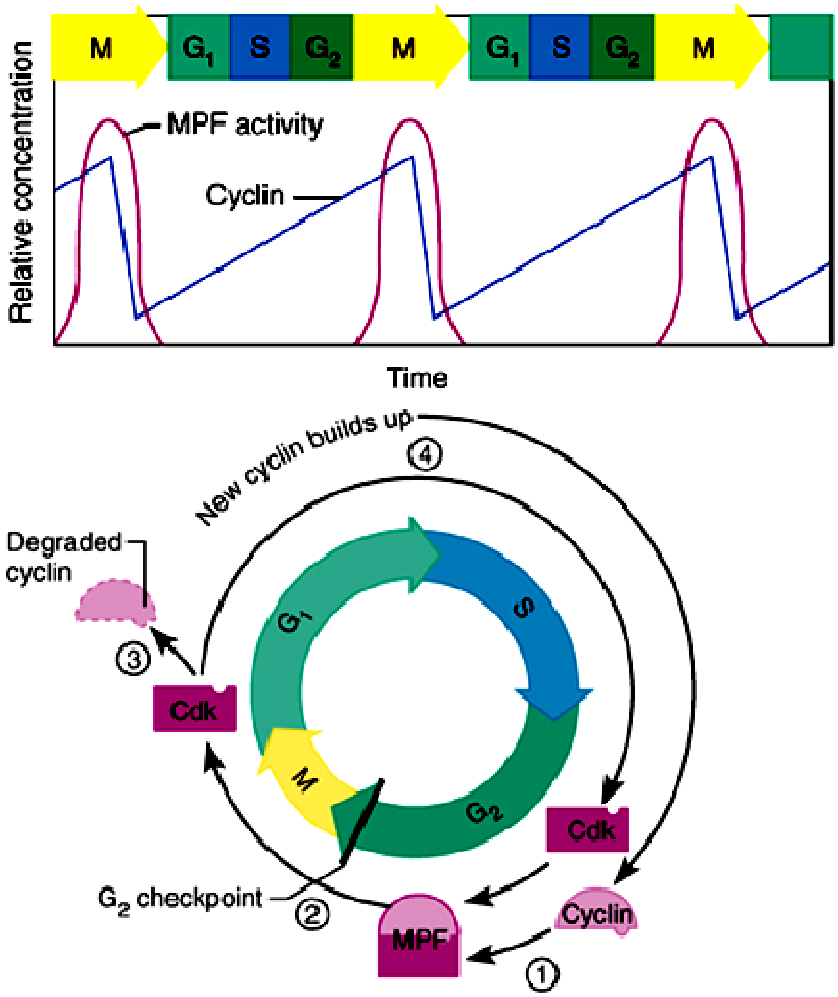


Fig.8.2b: Variations of the levels of cyclins and MPF activities during cell cycle.

A rising level of **G_1-cyclins** bind to their Cdks and signal the cell to prepare the chromosomes for replication. A rising level of **S-phase promoting factor** (**SPF**) which includes cyclin A bound to Cdk2 enters the nucleus and prepares the cell to duplicate its DNA (and its centrosomes). As DNA replication continues, cyclin E is destroyed, and the level of mitotic cyclins begins to rise (in G_2). **M-phase promoting factor** (the complex of mitotic cyclins with the M-phase Cdk) initiates the assembly of the mitotic spindle and the breakdown of the nuclear envelope as well as condensation of the chromosomes. These events take the cell to **metaphase** of mitosis. At this point, the M-phase promoting factor (**MPF**)activates the **anaphase-promoting complex** (**APC/C**) which allows the sister chromatids at the metaphase plate to separate and move to the poles (anaphase), completing mitosis; This destroys **cyclin B**. It does this by attaching it to the protein **ubiquitin** which targets it for destruction by **proteasomes** and turns on synthesis of G_1 cyclin for the next turn of the cycle. This degrades **geminin**, a protein that has kept the freshly-synthesized DNA in S phase from being re-replicated before mitosis. This is only one mechanism by which the cell ensures that every portion of its genome is copied once and only once during S phase. Some cells deliberately cut the cell cycle short allowing repeated S phases without completing mitosis and/or cytokinesis. This is called **endoreplication.** The special behaviour of the chromosomes in **meiosis I** requires some special controls. Nonetheless, passage through the cell cycle in meiosis I (as well as meiosis II, which is essentially a mitotic division) uses many of the same players, e.g., **MPF** and **APC**. (In fact, MPF is also called **m**aturation-**p**romoting **f**actor for its role in meiosis I and II of developing oocytes.

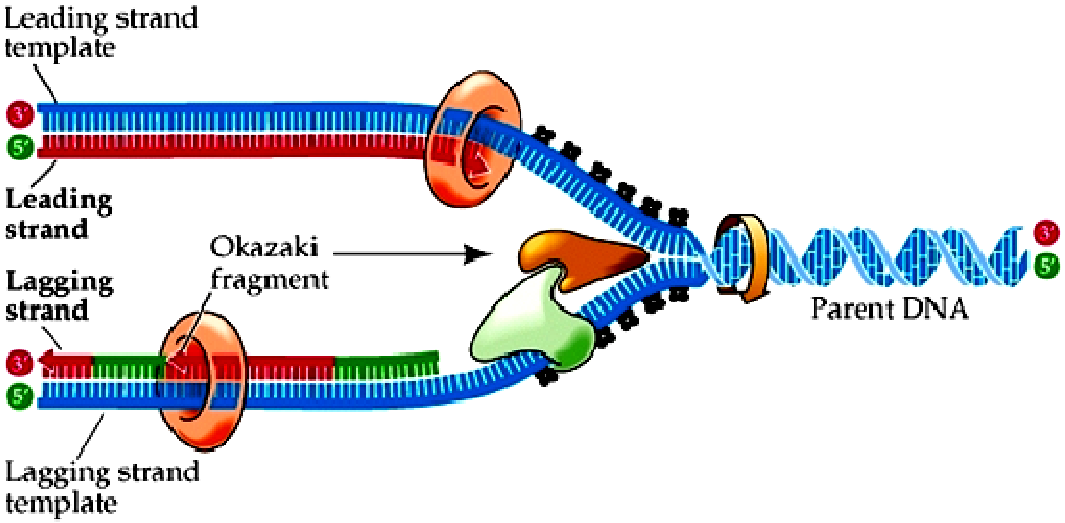
Checkpoints: Quality Control of the Cell Cycle

The cell has several systems for interrupting the cell cycle if something goes wrong. A check on completion of S phase. The cell seems to monitor the presence of the **Okazaki fragments** on the lagging strand during DNA replication. The cell is not permitted to proceed in the cell cycle until these have disappeared. **DNA damage** checkpoints sense DNA damage before the cell enters S phase (a G_1 checkpoint), during S phase, and after DNA replication (a G_2 checkpoint). The **spindle checkpoints** detect any failure of spindle fibres to attach to **kinetochores** and arrest the cell in **metaphase** (M checkpoint), detect improper alignment of the spindle itself and block **cytokinesis** and trigger **apoptosis** if the damage is irreparable. All the checkpoints examined require the services of a complex of proteins. Mutations in the genes encoding some of these have been associated with cancer; that is, they are **oncogenes**. This should not be surprising since checkpoint failures allow the cell to continue dividing despite damage to its integrity. Many times a cell will leave the cell cycle, temporarily or permanently. It exits the cycle at G_1 and enters a stage designated G_0 (G zero). A G_0 cell is often called "quiescent", but that is probably more a reflection of the interests of the scientists studying the cell cycle than the cell itself. Many G_0 cells are anything but quiescent. They are busy carrying out their functions in the organism. e.g., secretion, attacking pathogens. Often G_0 cells are terminally differentiated: they will never re-enter the cell cycle but instead will carry out their function in the organism until they die. For other cells, G_0 can be followed by re-entry into the cell cycle. Most of the lymphocytes in human blood are in G_0. However, with proper stimulation, such as encountering the appropriate antigen, they can be stimulated to re-enter the cell cycle (at G_1) and proceed on

to new rounds of alternating **S phases** and **mitosis**. G_0 represents not simply the absence of signals for mitosis but an active repression of the genes needed for mitosis. Cancer cells cannot enter G_0 and are destined to repeat the cell cycle indefinitely.

DNA Replication

Before a cell can divide, it must duplicate all its DNA. In eukaryotes, this occurs during S phase of the **cell cycle**. The Steps are as follows: A portion of the double helix is unwound by a **helicase** and a molecule of a **DNA polymerase** binds to one strand of the DNA and begins moving along it in the 3' to 5' direction, using it as a template for assembling a **leading strand** of nucleotides and reforming a double helix. In eukaryotes, this molecule is called DNA polymerase delta (δ). Because DNA synthesis can only occur 5' to 3', a molecule of a second type of DNA polymerase (epsilon, ε, in eukaryotes) binds to the other template strand as the double helix opens. This molecule must synthesize discontinuous segments of polynucleotides (called Okazaki fragments). Another enzyme, **DNA ligase I** then stitches these together into the **lagging strand**.



Replication Fork

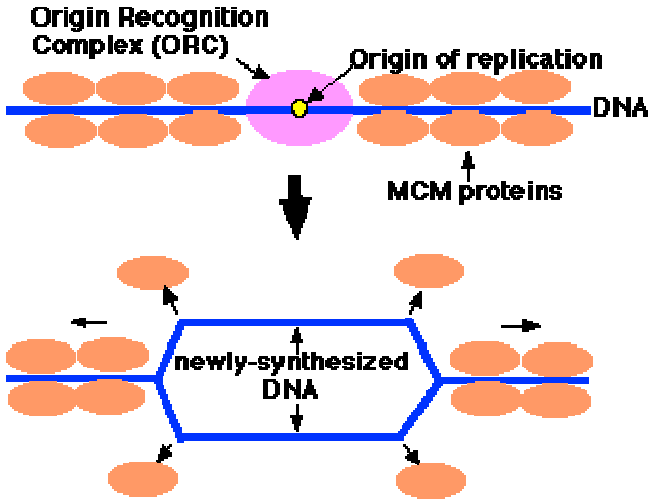
When the replication process is complete, two DNA molecules identical to each other and identical to the original have been produced. Each strand of the original molecule has remained intact as it served as the template for the synthesis of a complementary strand.

This mode of replication is described as **semi-conservative**: one-half of each new molecule of DNA is old; one-half new. **Watson** and **Crick** had suggested that this was the way the DNA would turn out to be replicated. Proof of the model came from the experiments of **Meselson** and **Stahl**.

The average human chromosome contains 150 x 10^6 nucleotide pairs which are copied at about 50 base pairs per second. The process would take a month (rather than the hour it actually does) but for the fact that there are many places on the eukaryotic chromosome where replication can begin. Replication begins at some replication origins earlier in S phase than at others, but the process is completed for all by the end of S phase. As replication nears completion, "bubbles" of newly replicated DNA meet and fuse, finally forming two new molecules. When a cell in G_2 of the **cell cycle** is fused with a cell in S phase, the DNA of the G_2 nucleus does not begin replicating again even though

replication is proceeding normally in the S-phase nucleus. Not until mitosis is completed, can freshly-synthesized DNA be replicated again. Two control mechanisms have been identified, one **positive** and one **negative**. This redundancy probably reflects the crucial importance of precise replication to the integrity of the genome.

In order to be replicated, each origin of replication must be bound by an **O**rigin **R**ecognition **C**omplex of proteins (**ORC**). These remain on the DNA throughout the process. Accessory proteins called **licensing factors**. These accumulate in the nucleus during G_1 of the cell cycle. They include CDC-6 and CDT-1, which bind to the **ORC** and are essential for coating the DNA with **MCM proteins**. Only DNA coated with MCM proteins (there are 6 of them) can be replicated. Once replication begins in S phase, CDC-6 and CDT-1 leave the ORCs (the latter by **ubiquination** and destruction in **proteasomes**). The MCM proteins leave in front of the advancing replication fork.



Regulation of translation

G_2 nuclei also contain at least one protein called **geminin** that prevents assembly of MCM proteins on freshly-synthesized DNA (probably by sequestering Cdt1). As the cell **completes mitosis**, geminin is degraded so the DNA of the two daughter cells will be able to respond to licensing factors and be able to replicate their DNA at the next S phase. Some cells deliberately cut the cell cycle short allowing repeated S phases without completing mitosis and/or cytokinesis. This is called **endoreplication**. How these cells regulate the factors that normally prevent DNA replication if mitosis has not occurred is still being studied

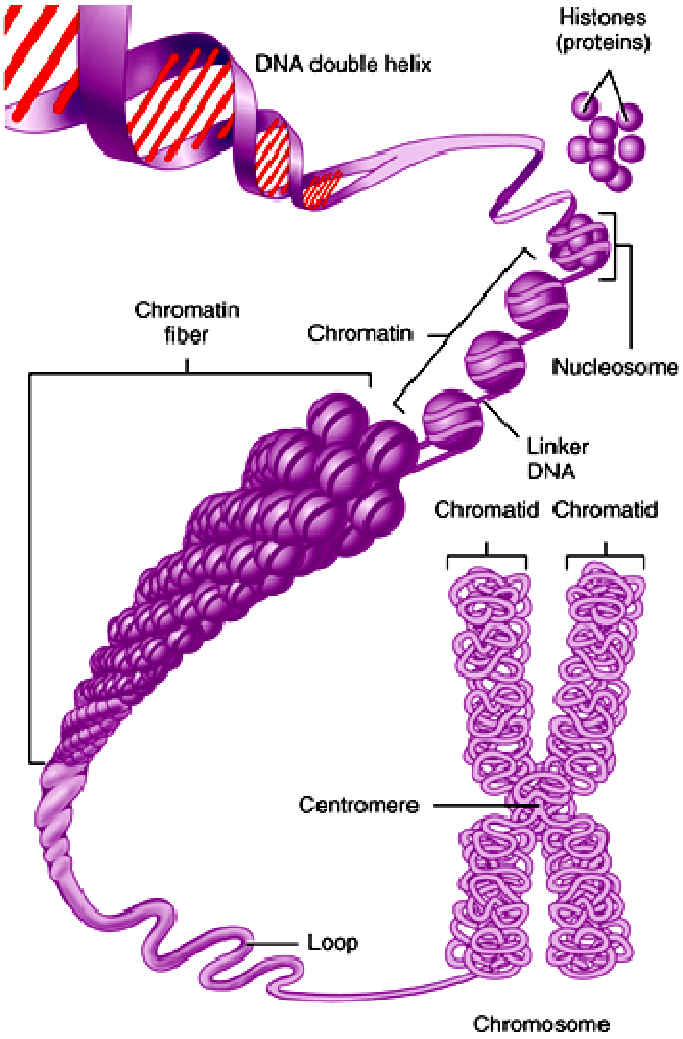
Cell Division

Due to their increased numbers of chromosomes, organelles and complexity, eukaryote cell division is more complicated, although the same processes of replication, segregation, and cytokinesis still occur.

Mitosis

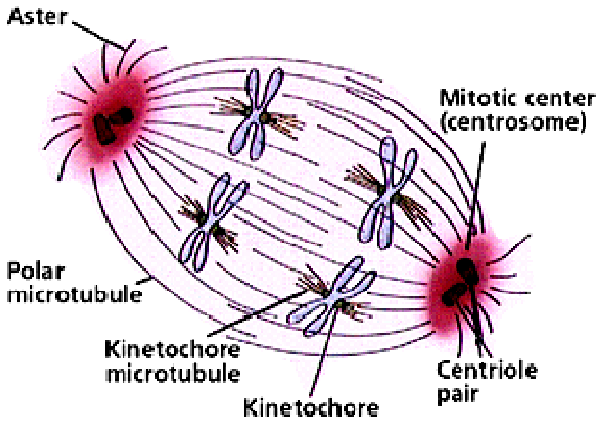
Mitosis is the process of forming (generally) identical daughter cells by replicating and dividing the original chromosomes, in effect making a cellular xerox. Commonly the two processes of cell division are confused. Mitosis deals only with the segregation of the chromosomes and organelles into daughter cells.

Eukaryotic chromosomes occur in the cell in greater numbers than prokaryotic chromosomes. The condensed replicated chromosomes have several points of interest. The **kinetochore** is the point where microtubules of the spindle apparatus attach. Replicated chromosomes consist of two molecules of DNA (along with their associated **histone proteins**) known as **chromatids**. The area where both chromatids are in contact with each other is known as the **centromere** the **kinetochore**s are on the outer sides of the centromere. Remember that chromosomes are condensed **chromatin** (DNA plus histone proteins).



Structure of a eukaryotic chromosome

During mitosis replicated chromosomes are positioned near the middle of the cytoplasm and then segregated so that each daughter cell receives a copy of the original DNA (if you start with 46 in the parent cell, you should end up with 46 chromosomes in each daughter cell). To do this cells utilize microtubules (referred to as the **spindle apparatus**) to "pull" chromosomes into each "cell". The microtubules have the 9+2 arrangement discussed earlier. Animal cells (except for a group of worms known as nematodes) have a **centriole**. Plants and most other eukaryotic organisms lack centrioles. Prokaryotes, of course, lack spindles and centrioles; the cell membrane assumes this function when it pulls the by-then replicated chromosomes apart during binary fission. Cells that contain centrioles also have a series of smaller microtubules, the **aster**, that extend from the centrioles to the cell membrane. The aster is thought to serve as a brace for the functioning of the spindle fibres.



Structure and main features of a spindle apparatus

The phases of mitosis are sometimes difficult to separate. Remember that the process is a dynamic one, not the static process displayed of necessity in a textbook.

Prophase

The two **centrosomes** of the cell, each with its pair of centrioles, move to opposite "poles" of the cell. The **mitotic spindle** forms. This is an array of spindle fibres, each containing about 20 **microtubules**. Microtubules are synthesized from tubulin monomers in the cytoplasm and grow out from each centrosome. The chromosomes become shorter and more compact.

Prometaphase

The **nuclear envelope** disintegrates because of the dissolution of the **lamins** that stabilize its inner membrane. A protein structure, the **kinetochore**, appears at the **centromere** of each chromatid. With the breakdown of the nuclear envelope, spindle fibres attach to the kinetochores as well as to the arms of the chromosomes. For each **dyad**, one of the kinetochores is attached to one pole, the second (or sister) chromatid to the opposite pole. Failure of a kinetochore to become attached to a spindle fibre interrupts the process.

Metaphase

At metaphase all the **dyads** have reached an equilibrium position midway between the poles called the **metaphase plate**. The chromosomes are at their most compact at this time.

Anaphase

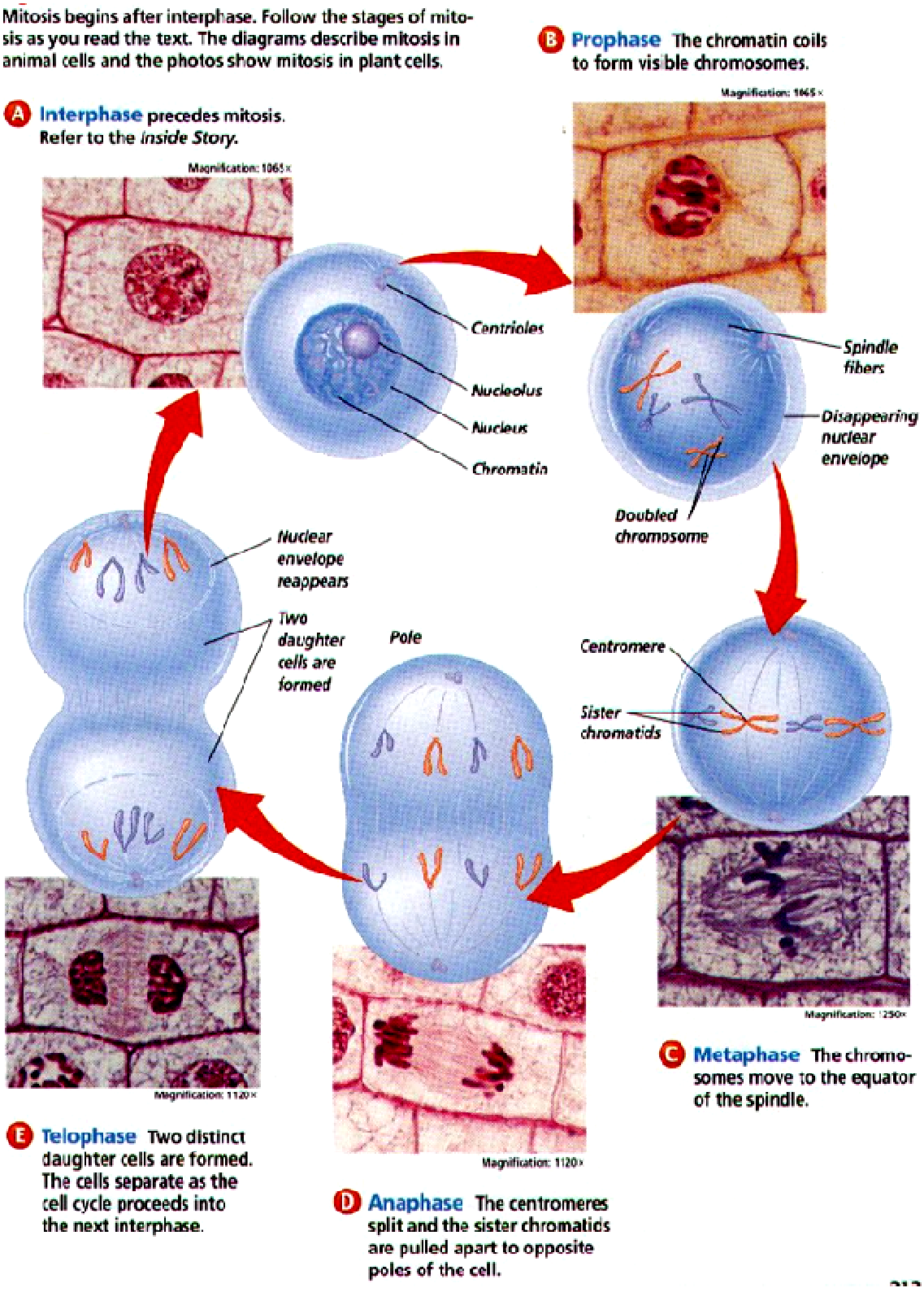
The sister **kinetochores** suddenly separate and each moves to its respective pole dragging its attached chromatid (chromosome) behind it. Separation of the sister chromatids depends on the breakdown of the **cohesins** that have been holding them together. It works like this. **Cohesin** breakdown is caused by a protease called **separase** (also known as **separin**). Separase is kept **inactive** until late metaphase by an inhibitory **chaperone** called **securin**. Anaphase begins when the **anaphase promoting complex** (APC) destroys securin (by tagging it for deposit in a **proteasome**) thus ending its inhibition of separase and allowing separas to break down the cohesins.

Telophase

A nuclear envelope reforms around each cluster of chromosomes and these return to their more extended form.

Cytokinesis

Mitosis is the process of separating the duplicates of each of the cell's chromosomes. It is usually followed by division of the cell. However, there are cases (**cleavage** in the insect embryo is an example) where the chromosomes undergo the mitotic process without division of the cell. Thus a special term, **cytokinesis,** for the separation of a cell into two daughter cells. In **animal cells**, a belt of **actin filaments** forms around the perimeter of the cell, midway between the poles. The interaction of actin and a **myosin** (not the one found in **skeletal muscle**) tightens the belt, and the cell is pinched into two daughter cells. In **plant cells**, a membrane-bounded **cell plate** forms where the metaphase plate had been. The cell plate, which is synthesized by the Golgi apparatus, supplies the plasma membrane that will separate the two daughter cells. Synthesis of a new **cell wall** between the daughter cells also occurs at the cell plate.



The Mitotic Cycle

Meiosis

Sexual reproduction occurs only in **eukaryotes**. During the formation of **gametes**, the number of **chromosomes** is reduced by half, and returned to the full amount when the two **gametes fuse** during **fertilization**.

Ploidy

Haploid and diploid are terms referring to the number of sets of chromosomes in a cell. **Gregor Mendel** determined his peas had two sets of alleles, one from each parent. **Diploid organisms** are those with two (di) sets. Human beings (except for their gametes), most animals and many plants are diploid. We abbreviate diploid as 2n. Ploidy is a term referring to the number of sets of chromosomes. **Haploid organisms/cells** have only one set of chromosomes, abbreviated as n. Organisms with more than two sets of chromosomes are termed polyploid. Chromosomes that carry the same genes are termed **homologous chromosomes**. The **alleles** on homologous chromosomes may differ, as in the case of **heterozygous** individuals. Organisms (normally) receive one set of homologous chromosomes from each parent.

Meiosis is a special type of nuclear division which segregates one copy of each homologous chromosome into each new "gamete". Mitosis maintains the cell's original ploidy level (for example, one diploid 2n cell producing two diploid 2n cells; one haploid n cell producing two haploid n cells; etc.). Meiosis, on the other hand, reduces the number of sets of chromosomes by half, so that when gametic recombination (**fertilization**) occurs the ploidy of the parents will be reestablished.

Most cells in the human body are produced by mitosis. These are the **somatic** (or vegetative) line cells. Cells that become gametes are referred to as **germ line cells.** The vast majority of cell divisions in the human body are mitotic, with meiosis being restricted to the **gonads.**

Phases of Meiosis

Two successive nuclear divisions occur, **Meiosis I** (Reduction) and **Meiosis II** (Division). Meiosis produces 4 haploid cells. Mitosis produces 2 diploid cells. The old name for meiosis was reduction/ division. Meiosis I reduces the ploidy level from 2n to n (reduction) while Meiosis II divides the remaining set of chromosomes in a mitosis-like process (division). Most of the differences between the processes occur during Meiosis I.

Prophase I

The lengthy and complex events of **prophase I** can be broken down into 5 stages.

Leptotene: All the chromosomes condense, pairing. homologous dyads (pairs of sister chromatids) find each other and align themselves from end to end with the aid of an axial element (that contain **cohesins**). How the non-sisters recognize their shared regions of DNA homology is uncertain. **Double-stranded breaks** (DSBs) often occur in the DNA of the chromatids, and these may be necessary for the homologues to find each other.

Zygotene: The **synaptonemal complex** begins to form. DNA strands of non-sister chromatids begin the process of recombination. How they are able to do so across the synaptonemal complex, which is over 100 nm thick, is unknown.

Pachytene: Synapsis is now complete. **Recombination nodules** appear (at least in some organisms, including humans). They are named for the idea that they represent points where DNA recombination is occurring. There must be at least one for each **bivalent** if meiosis is to succeed. There are often more, each one presumably representing the point

of a crossover. They contain enzymes known to be needed for DNA recombination and repair. The steps in recombining DNA continue to the end of pachytene.
Diplotene: DNA recombination is complete. The **synaptonemal complex** begins to break down. The chromatids begin to pull apart revealing **chiasmata**. At first the chiasmata are located at the sites of the recombination nodules, but later they migrate towards the ends of the chromatids.
Diakinesis: In some organisms, the chromosomes **decondense** and begin to be transcribed for a time. This is followed by the chromosomes **recondensing** in preparation for **metaphase I.** In creatures where this does not occur, the chromosomes condense further in preparation for metaphase I.

Metaphase I

Metaphase I is when tetrads line-up along the equator of the spindle. Spindle fibres attach to the centromere region of each homologous chromosome pair. Other metaphase events as in mitosis.

Anaphase I

Anaphase I is when the tetrads separate, and are drawn to opposite poles by the spindle fibres. The centromeres in Anaphase I remain intact.

Telophase I

Telophase I is similar to Telophase of mitosis, except that only one set of (replicated) chromosomes is in each "cell". Depending on species, new nuclear envelopes may or may not form. Some animal cells may have division of the centrioles during this phase.

Prophase II

During Prophase II, nuclear envelopes (if they formed during Telophase I) dissolve, and spindle fibres reform. All else is as in Prophase of mitosis. Indeed Meiosis II is very similar to mitosis.

Metaphase II

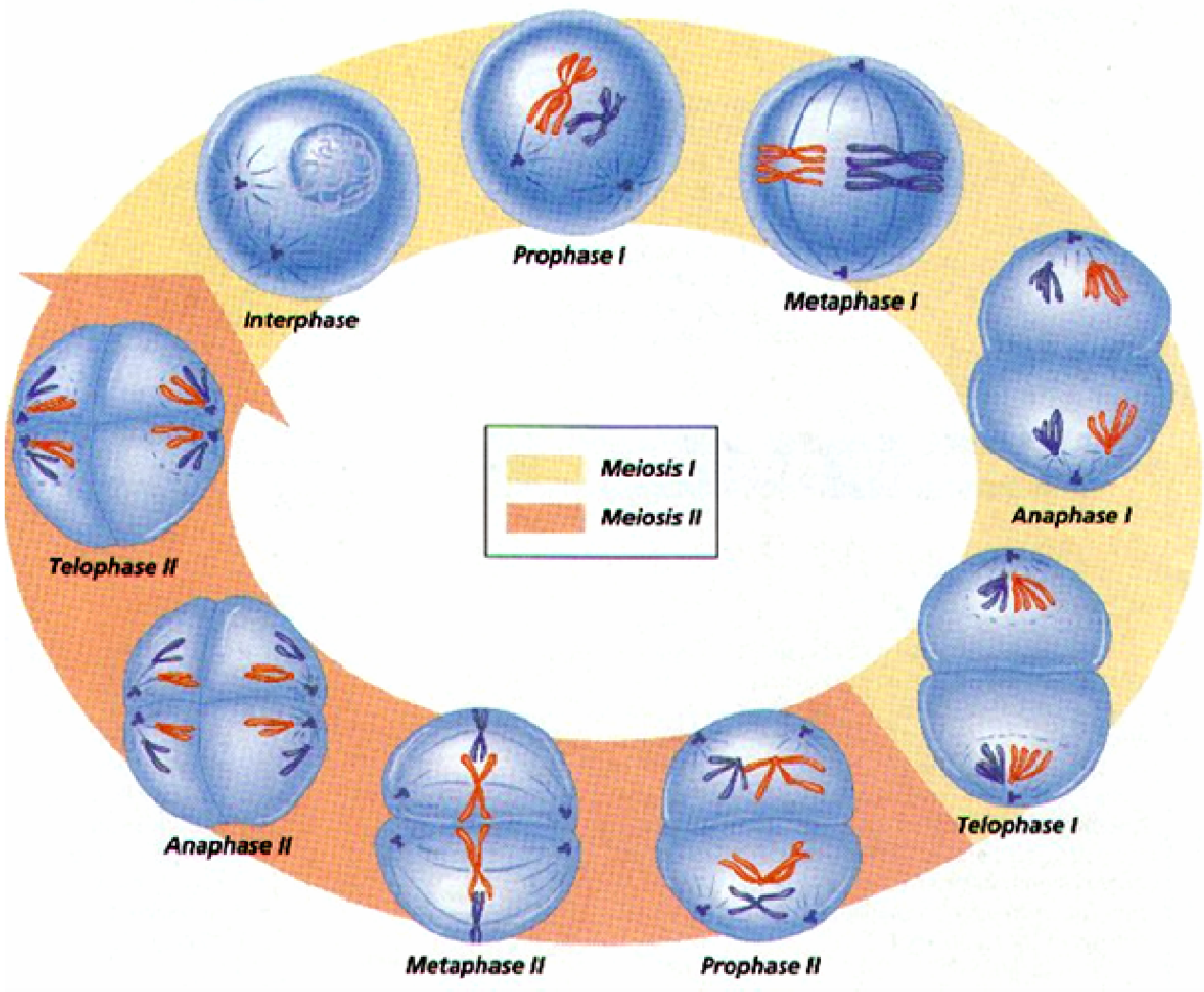
Metaphase II is similar to mitosis, with spindles moving chromosomes into equatorial area and attaching to the opposite sides of the centromeres in the kinetochore region.

Anaphase II

During Anaphase II, the centromeres split and the former chromatids (now chromosomes) are segregated into opposite sides of the cell.

Telophase II

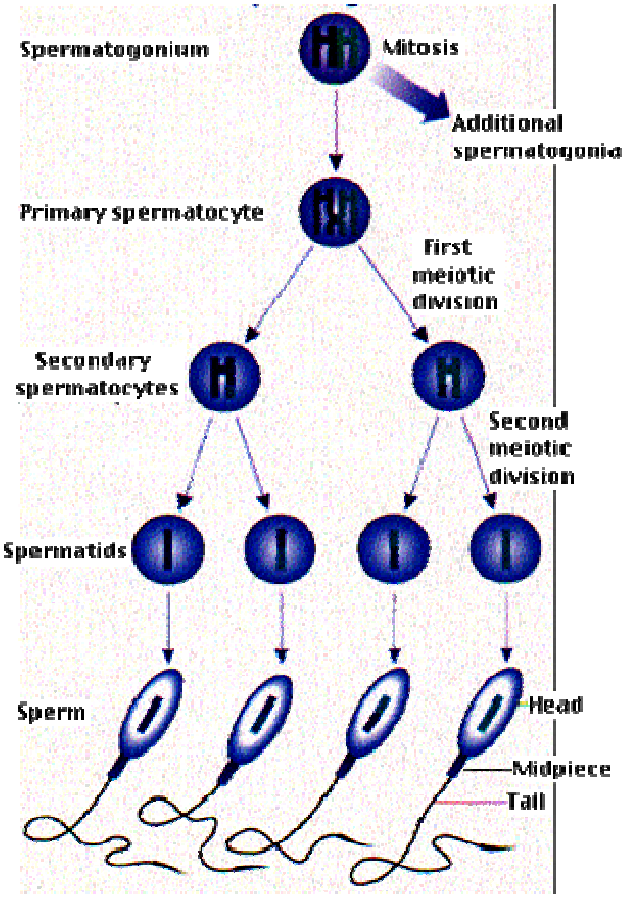
Telophase II is identical to Telophase of mitosis. Cytokinesis separates the cells.



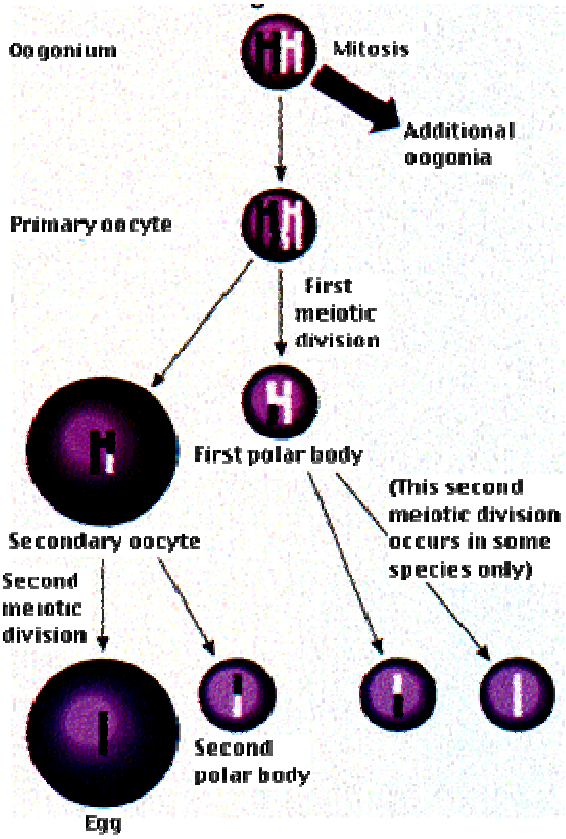
The Meiotic Cell Cycle

Gametogenesis

Gametogenesis is the process of **forming gametes** (by definition haploid, n) from diploid cells of the germ line. **Spermatogenesis** is the process of forming **sperm cells** by meiosis (in animals, by mitosis in plants) in specialized organs known as **gonads** (in males these are termed **testes**). After division the cells undergo differentiation to become sperm cells. **Oogenesis** is the process of forming an **ovum** (egg) by meiosis (in animals, by mitosis in the gametophyte in plants) in specialized gonads known as **ovaries**. Whereas in spermatogenesis all 4 meiotic products develop into gametes, oogenesis places most of the cytoplasm into the large egg. The other cells, the polar bodies, do not develop. This all the cytoplasm and organelles go into the egg. Human males produce 200,000,000 sperm per day, while the female produces one egg (usually) each **menstrual cycle**.



Spermatogenesis



Oogenesis

Gametogenesis

Spermatogenesis

The walls of the seminiferous tubules consist of **diploid** spermatogonia, **stem cells** that are the precursors of sperm. **Spermatogonia** divide by **mitosis** to produce more spermatogonia or differentiate into **spermatocytes**. Meiosis of each spermatocyte produces 4 haploid **spermatids.** This process takes over three weeks to complete. Then the spermatids differentiate into **sperm,** losing most of their cytoplasm in the process. For simplicity, the figure shows the behaviour of just a single pair of homologous chromosomes with a single crossover. With 22 pairs of **autosomes** and an average of two crossovers between each pair, the variety of gene combinations in sperm is very great.

Oogenesis

Egg formation takes place in the **ovaries**. In contrast to males, the initial steps in egg production occur prior to birth. By the time the foetus is 25 weeks old, all the **oogonia** that she will ever possess maybe have been formed by **mitosis**. By the time she is born, thousands of these diploid cells have developed into **primary oocytes**, begun the first steps of the first meiotic division (meiosis I) and then stopped. No further development occurs until years later when the girl becomes **sexually mature**. Then the **oocytes** recommence their development, usually one at a time and once a month. The **primary oocyte** grows much larger and completes the meiosis I, forming a large **secondary oocyte** and a small **polar body** that receives little more than one set of chromosomes. Which chromosomes end up in the egg and which in the polar body is entirely a matter of

chance. In humans (and most vertebrates), the first polar body does not go on to meiosis II, but the secondary oocyte does proceed as far as **metaphase** of meiosis II and then stops. Only if **fertilization** occurs will meiosis II ever be completed. Entry of the sperm restarts the cell cycle breaking down **MPF** (M-phase promoting factor) and turning on the **anaphase promoting complex** (APC). Completion of meiosis II converts the **secondary oocyte** into a fertilized **egg** or **zygote** (and also a second polar body). As in the diagram for spermatogenesis, the behaviour of the chromosomes is greatly simplified. These events take place within a **follicle**, a fluid-filled envelope of cells surrounding the developing egg. The ripening follicle also serves as an **endocrine gland**. Its cells make a mixture of **steroid hormones** collectively known as **oestrogen**. Oestrogen is responsible for the development of the secondary sexual characteristics of a mature woman, e.g., a broadening of the pelvis, development of the breasts, growth of hair around the genitals and in the armpits and the development of **adipose tissue** leading to the more rounded body contours of adult women. Oestrogen continues to be secreted throughout the reproductive years of women During this period, it plays an essential role in the monthly **menstrual cycle**. In March 2004, a group of researchers reported (Johnson, J., *et al*., **Nature**, 11 March 2004) compelling evidence that in mice, at least, oocytes continue to be produced throughout life (from a small population of germ line stem cells).