

Mitosis, Meiosis and the Cell Cycle

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Objectives

By the end of the session the student should be able to:

- ☞ Define the meaning of chromosomes
- ☞ State how a karyotype is constructed.
- ☞ Distinguish between diploidy, heteroploidy and polyploidy
- ☞ Name the phases of the cell cycle
- ☞ Give examples of cells that normally have high, and others that have low, mitotic rates
- ☞ List the main factors that control the cell cycle
- ☞ Give examples of drugs acting at different phases of the cell cycle
- ☞ Name the important events and functional significance of meiosis

Practical Skills Objectives

- ☞ Classify chromosomes in a karyotype according to size and centromere position.
- ☞ Identify metacentric, submetacentric and acrocentric chromosomes

Clinical applications

Cancer

- a disorder in the control of the cell cycle
- cytostatic drugs for the treatment of cancers

Recommended Reading

- ☞ Chapter 17: The cell cycle: DNA, Replication, Mitosis and Cancer
- ☞ Chapter 18: Sexual reproduction, Meiosis and Genetic Recombination

Mitosis and Meiosis

- **Mitosis** is the process of cell division in which the daughter cells receive **identical copies** of DNA, which are also identical to that of the mother cell.
- **Meiosis** is the process of cell division that results in the formation of cells containing **half the amount** of DNA contained in the parent cell, and having **different copies** of DNA from one another.
- The cytoplasm and organelles are usually shared approximately equally between the daughter cells.

Chromosomes

- Before cell division, whether in mitosis or meiosis, the DNA replicates itself. Each chromatin strand is also replicated. During cell division, the chromatin strands become coiled (condensed) to form chromosomes.
- Each chromosome is, therefore, a duplicate structure, consisting of two **chromatids** joined by a **centromere**.
- Chromosomes are visible as discrete structures only during cell division
- During mitosis, each daughter cell receives one chromatid of each chromosome.

In this electron micrograph of a whole chromosome, Note that:

- The chromosome consists of two **chromatids**
- The chromatids are joined at the **centromere**
- The chromatids are composed of chromatin strands, similar to those in nuclei, but packed in a regular fashion.

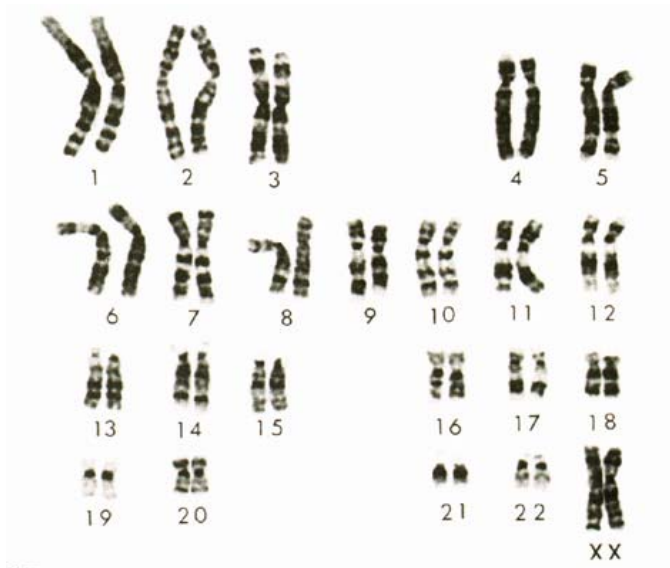


Chromosome 1

Do not confuse the two chromatids that constitute a chromosome with **homologous pairs** of chromosomes. The figure illustrates chromosome pair number 1.

The genes contained on the chromosomes are also paired.

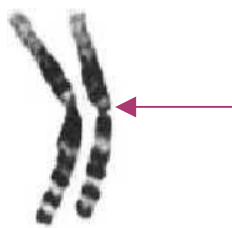
Chromosomes are arranged in a karyotype for the purpose of analysis.



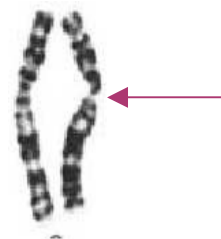
Classification of the chromosomes is based on their size, centromere position and banding patterns that are specific for each chromosome. There are 23 pairs of chromosomes. One pair is the sex chromosomes, in this case XX. The other 22 pairs are termed autosomes.

Chromosomes are classified according to centromere position:

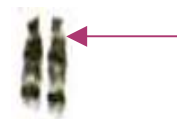
Metacentric



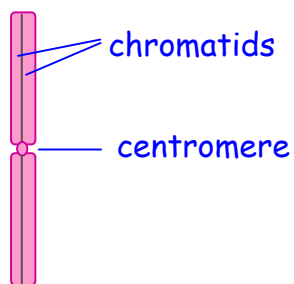
Sub-metacentric



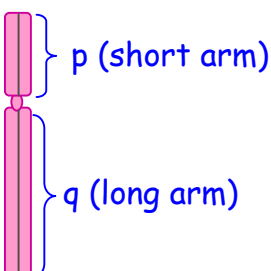
Acrocentric



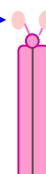
- Note also some other points of nomenclature:



When the chromosomes are elongated, the chromatids are very close to one another and might not appear as separate structures.



- By convention the short arms of chromosomes are designated as "p" and the long arms as "q".
- The short arms of the acrocentric chromosomes are very short and have satellites



The **acrocentric chromosomes** have their centromere very close to one end. Their short arms are very small and have tiny satellites. The acrocentric chromosomes are chromosomes 13, 14, 15, 21 and 22.



The satellites contain the nucleolus **organisers** that form the **nucleolus** in interphase nuclei.

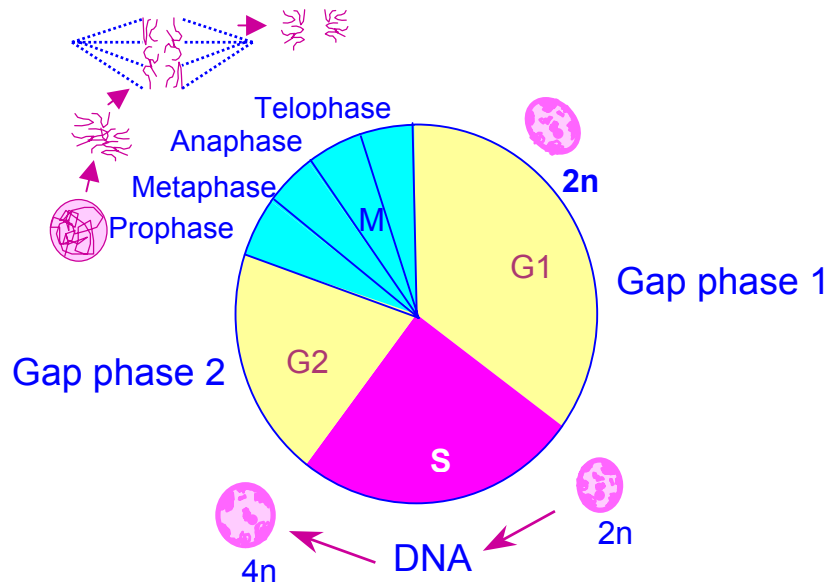
"**High resolution chromosome analysis**" is a special technique in which the chromosomes are long and the banding more detailed to enable more precise identification of small regions of chromosomes.

The Cell Cycle

The cell cycle is the series of events that occur in dividing cells between the completion of one mitotic division and the completion of the next division.

Mitosis occupies only a small proportion of the whole cell cycle.

The time taken to complete a cell cycle is very variable.



The cell cycle begins with the formation of a new cell following mitosis.

- The nucleus of the cell contains $2n$ amount of DNA
- **DNA replication** is a crucial event in the cell cycle.
- Prior to cell division, whether mitosis or meiosis, the DNA replicates itself to form two identical copies.
- This occurs during the **S (synthesis) phase**. By the end of the S phase the cell nucleus contains $4c$ amount of DNA.
- The S phase is preceded and followed by two gap phases, *G1* and *G2* respectively, during which synthesis of the cytoplasm occurs, and the cell performs its own specific functions.

The cell cycle is interrupted by three "checkpoints"

- The *G1* checkpoint, at end of *G1*
 - provides the trigger for DNA synthesis
- The *G2* checkpoint, at end of *G2*
 - Ensures that replication is complete, and
 - Provides trigger to proceed to mitosis
- The "Spindle Assembly" checkpoint at the end of metaphase
 - Ensures spindle formation
 - Provides trigger for attachment of chromatids to the spindle

Factors affecting the cell cycle

1. General metabolic factors

- Temperature, pH, nutrients and metabolites. The effect of these factors is seen in tissue culture. When cells in culture become very numerous, there is reduced nutrient availability and increased amounts of metabolites, and the rate of mitosis is reduced.
- AMP, cyclic GMP and Ca^{2+} vary with the phases of the cell cycle and are important **internal regulatory factors**

2. Intrinsic molecules within the cytoplasm

- These are regulatory molecules produced during specific phases of the cell cycle. They include:
 - ☛ **Cyclins**
 - Proteins that fluctuate in the cell cycle
 - ☛ **Cyclin dependent kinase (Cdk)**
 - Enzymes that interact with cyclins
 - ☛ **Cyclin-Cdk complexes**
 - Trigger the passage through "checkpoints"
 - Are phosphorylated / dephosphorylated
- **G1 cyclin and G1 Cdk** trigger the transition from G1 to S
- **Mitotic cyclin & mitotic Cdk**: These are or mitosis-promoting factors (MPF) that trigger the transition from G2 to M phase

3. Extrinsic factors - interaction with other cells

- ☛ **Growth factors** are necessary for stimulating cell proliferation . Some growth factors act mainly, but not exclusively, on certain cells as indicated by their names.

e.g. Platelet derived growth factor (PDGF)
 Epidermal growth factor (EGF)
 Fibroblast growth factor (FGF)
 Transforming growth factor (TGF)
 Interleukin-2 (IL-2).

- ☛ **Chalones** are substances produced by some cells that **inhibit** mitosis. When tissues are injured, the fibroblasts proliferate to heal the wound. Normally fibroblasts do not divide because they are inhibited by chalones. Tissue injury stops chalone secretion and stimulates mitosis.

4. Other factors

- **Plasma membrane receptors** - bind to Growth factor
- **Protein kinases** - activate certain substrate proteins e.g. receptor tyrosine kinase
- **G-proteins** - proteins that are activated by binding to GTP e.g. Ras protein
- **Transcription factors** - proteins that activate transcription of specific genes e.g. *c-myc*, *c-jun*, *c-fos*

Oncogenes

- ☞ **Oncogenes** are genes that cause tumours and uncontrolled cell proliferation by interfering with the cell cycle control system.
- ☞ Most oncogenes arise by mutation of normal genes, called **proto-oncogenes** that help to regulate the cell cycle
- ☞ Examples of oncogenes are *src*, *myc*, and *ras* oncogenes.
- ☞ Their counterpart proto-oncogenes are denoted by the prefix *c-* e.g. *c-src*, *c-myc*, *c-ras* proto-oncogenes
- ☞ Oncogenes may arise by mutation of any of the 6 classes of intermediate proteins involved in the cell cycle

Cytostatic Drugs

Drugs that interfere with the cell cycle act are called cytostatic drugs. They may be clinically useful to stop proliferation of cancer or other malignant cells .

Cytostatic drugs act at specific phases of the cell cycle.

Some cytostatic drugs act on the **S phase** and inhibit DNA synthesis

- Methotrexate
- Fluorouracil
- Mercaptopurine

Some cytostatic drugs cause cells to accumulate in **G2**

- Mitomycin C
- Adriamycin
- Cyclophosphamide

Some drugs inhibit the formation of the mitotic spindle

- Colchicine is used in the laboratory to interrupt mitosis in metaphase, but does not act as a mitotic inhibitor when taken orally

In vitro control of the cell cycle

☞ **Mitogens** stimulate some cells to divide by mitosis

Many of these are plant extracts:

- Phytohaemagglutinin
- Pokeweed mitogen
- Concanavilin A

☞ **Colcemid** (a derivative of colchicine) is commonly used to interrupt mitosis, causing the dividing cells to accumulate in metaphase

Synchronisation of cells in culture

Cells in tissue culture enter into mitosis randomly. At any particular point, some cells are in *G*₁, some in *S*, some in *G*₂ and some in mitosis.

Cells in tissue culture may be synchronised so that they all enter mitosis simultaneously. This can be achieved by inhibiting DNA synthesis. Two ways of achieving this are:

☞ **By treatment with methotrexate**

- This drug inhibits DNA synthesis.
- Cells accumulate at the beginning of the *S* phase.

☞ **Thymidine block**

- Thymidine is one of the four nucleotides of which DNA is formed. If excess thymidine is applied to the medium instead of all four nucleotides, DNA synthesis will be interrupted.
- Cells accumulate at the beginning of the *S* phase.

When the cells are washed after about 18 hours and re-suspended in normal culture medium, they complete synthesis and proceed synchronously through *G*₂ and mitosis.

Sites of mitosis in the adult

In the foetus, babies and growing children mitosis occurs in most tissues. In adults, however, most tissues do not proliferate but mitosis occurs regularly at the following sites:

- Red bone marrow - for production of blood cells (erythropoiesis)
- Lymphoid tissue - formation of lymphocytes (lymphopoiesis)
- Testes - for spermatogenesis (production of spermatozoa)
- Epidermis - replacement of superficial skin cells
- Hair follicles - hair growth
- Gastro-intestinal tract - renewal of epithelium

In adults mitosis does not normally occur in:

- Neurons
- Muscle cells

In the adult, many cells are normally quiescent but may be stimulated to undergo mitosis. Such cells are said to be in " G_0 " phase of the cell cycle.

- Fibroblasts in connective tissue are normally quiescent but are stimulated into the cell cycle following tissue injury fibroblasts.
- Hepatocytes (liver cells) normally have a very slow rate of turnover. The liver contains some cells with large tetraploid (4n) nuclei (cells in G_2) and binucleate cells (cells that have not yet undergone cytokinesis).

Stages of Mitosis

1. Prophase

- The chromosomes gradually condense and appear as strands that become thicker and shorter;
- The nuclear envelope breaks up.

2. Metaphase

- The chromosomes are condensed;
- A mitotic spindle is formed of microtubules;
- Microtubules attach to the centromeres on chromosomes and to the centrioles at opposite poles of the cell

3. Anaphase

- The chromatids separate and move to opposite poles

4. Telophase

- The chromatids are at opposite poles of the cells
- The nuclear envelope is formed

5. Cytokinesis

- Division of the cytoplasm mediated by actin filaments

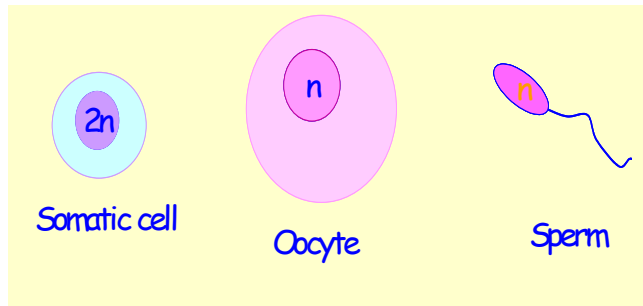
MEIOSIS

Meiosis is the basis of sexual reproduction and involves two important events, which are both directed towards creating genetic diversity.

a. **Reduction division** - the chromosome number is halved from diploid (2n) to haploid (n). Fertilization involves the fusion of male and female gametes, each containing a haploid set of chromosomes, to form a zygote with a diploid set of chromosomes.

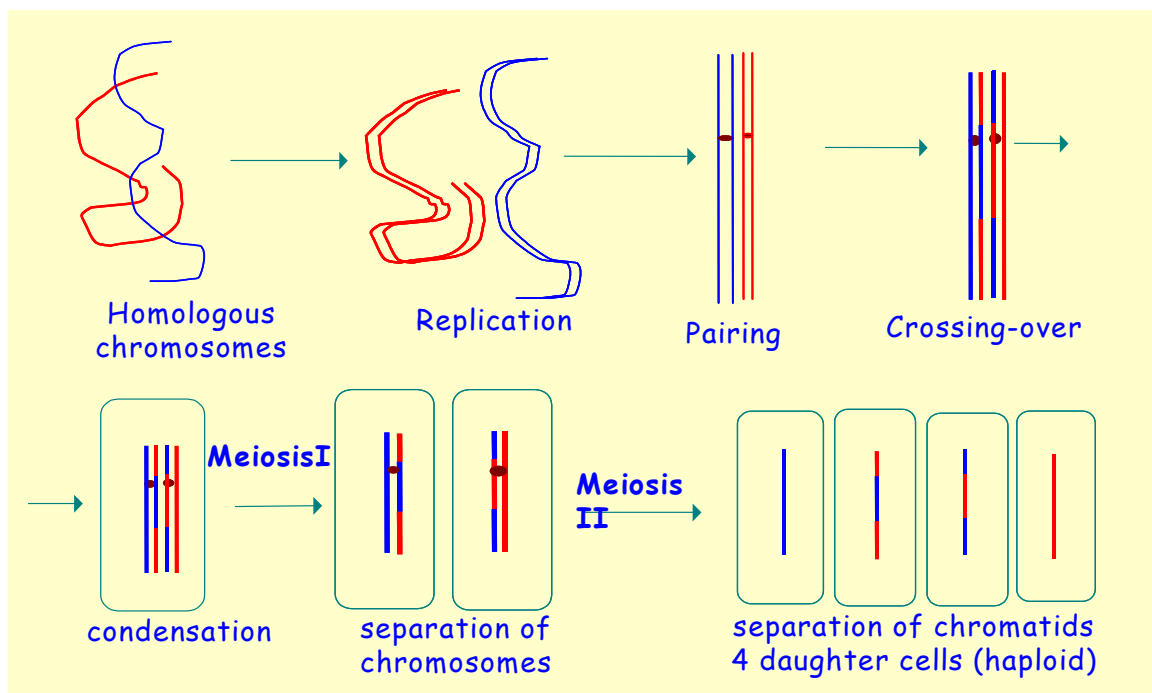
b. **Crossing-over and recombination** - the exchange of chromosome segments between homologous pairs ensures that there is a reshuffling of genes and, therefore, genetic diversity among the offspring. Every

individual has pairs of chromosomes derived from the mother and the father. Genetic recombination ensures that a recombination of maternal and paternal chromosomes and genes are passed on to the offspring.



Meiosis occurs only in the germ cell precursors - cells that form spermatozoa and oocytes (ova). These are the only cells that have a haploid set (n) of chromosomes, while somatic cells are diploid ($2n$).

Meiosis involves two successive divisions, producing four germ cells with different chromosome combinations.



Remember the important features of Meiosis:

- ☞ Occurs in the **germ cells** to form gametes.
- ☞ Reduces the chromosome number to **haploid (n)**
- ☞ Produces "**pairing and crossing-over**" of segments between parental chromosomes
- ☞ Ensures reshuffling of genes and genetic variation
- ☞ Occurs in two successive cell divisions: **Meiosis I and Meiosis II**
- ☞ Is preceded by DNA synthesis and replication (S phase)

Pairing and crossing-over of chromosomes result in genetic recombination. Each chromosome, and, therefore, each germ cell, acquires a different and unique combination of genes. This is an important means of ensuring genetic variation among the offspring.

Pairing and crossing-over (recombination) occur in the prophase of meiosis I, a complex process that also involves chromosome condensation (shortening). It is divided into five stages. Their apparently complex names are in fact Greek descriptions of the appearance of the paired chromosomes:

Leptotene (= thin threads)

Zygotene (= adjoining threads)

Pachytene (= thick threads)

Diplotene (= double threads)

Diakinesis (= cutting across)

Crossing over involves breakage and re-union of the chromatin strands. The sites of crossing-over are termed synaptonemal complexes. As diakinesis occurs and the paired chromosomes separate, the chromosomes remain temporarily attached at sites known as chiasmata, which may represent the original sites of crossing over.

Meiosis I involves metaphase, anaphase and telophase. It results in two daughter cells, each of which receives one of each pair of homologous chromosomes. Each chromosome consists of two chromatids.

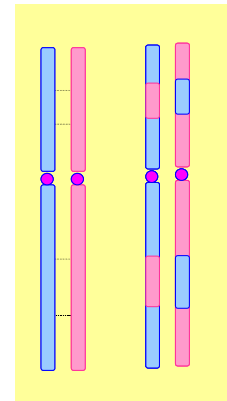
Meiosis II involves the separation of the chromatids.

- It is similar to mitosis with metaphase, anaphase and telophase
- It results in 4 haploid daughter cells.

Clinical Applications

Cancer cells undergo uncontrolled cell proliferation. As such, they are defects of the control of the cell cycle. Oncogenes are mutations in the genes that normally control the cell cycle.

Chemotherapy of cancers is aimed towards interrupting the cell cycle and preventing the cancer cells from proliferating. As a side effect, however, also the normal sites of cell proliferation are affected resulting in hair loss, intestinal disorders, anaemia and infertility.



Chromosome analysis is a routine examination in genetic abnormalities. Chromosome analysis requires a source of dividing cells. This is usually obtained from lymphocytes in the peripheral blood which are stimulated to proliferate using a **mitogen** (e.g. phythaemagglutinin) and accumulation of cells in mitosis using **colchicine**. Cell synchronization is used to increase the number of cells at the beginning of mitosis.

Errors of Meiosis

Chromosome **non-disjunction** is the failure of separation of chromosomes during meiosis I or of chromatids during meiosis II. This defect gives rise to trisomy, in which the embryo has three homologous chromosomes instead of a pair. The commonest trisomies are **trisomy 21** (Down syndrome), **trisomy 18** (Edward syndrome) and **trisomy 13** (Patau syndrome). Trisomies of other chromosomes are rare.

Non-disjunction is a defect of the meiotic spindle, in which microtubules fail to attach to the kinetochore of one of the chromosomes. As a result one cell receives both chromosomes of a pair while the other has a missing chromosome. Germ cells with a missing chromosome may give rise to monosomies but these are very rare.

Many genetic syndromes involving **deletions** (loss) or **duplications** of a chromosome segment, and **translocation** or exchange of chromosome segments between two chromosomes arise during meiosis. All these abnormalities involve chromosome breakage and re-union, events that occur during the prophase of meiosisI.
