The Basic and Clinical Consequences of Genetic Disorders in Humans

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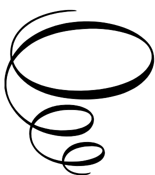
By

Ajit Kumar Saxena

Cambridge Scholars Publishing



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Professor Ajit Kumar Saxena
All India Institute of Medical Sciences

PART I

CHAPTER 1

INTRODUCTION TO GENETICS

In this chapter the author illustrates an understanding of the basic principles of genetics, heredity and Mendel's Law of inheritance. It is a brief and clear text about the first, second, third and fourth Laws of Mendel along with the Law of Segregation and the concept of Lamarck's and Darwin's perceptions

1.1 History of genetics and heredity

Gregor Johann Mendel was born in Czechoslovakia in 1822. He worked for several years as a gardener during his childhood and later on managed to initiate experiments on garden pea plants. He tried to establish first the numerical relationships among traits appearing in the offspring and second, he had the idea that hereditary material is unchanged during segregation of allele, and there is an independent assortment of traits during inheritance to offspring. His experiment was supported by Lamarck's that the plants respective offspring retained the essential traits of the parents and are not influenced by the environment.

Mendel selected *Pisum sativum* because the parental plants are known to possess seven constant and differentiating characteristic features in pea plants that are easily recognized in one of two forms - 1) flower colour–purple or white, 2) position of flower–axial or terminal,) length–long or short, 4) structure of seed–round or wrinkled, 5) appearance of flower colour–yellow or green, 6) pod shape–inflated or constricted, and 7) pod colour–yellow or green. This idea gave birth to heredity based on four different principles of hereditary, recognized as Mendel's Law, in 1900, and he is commonly known as the father of genetics.

1.2 Mendel's Law of Segregation

Mendel's hypothesis consists of four different parts. **The first law** states that with the concept of alleles "alternative forms of genes are responsible for variations in inherited characteristic traits." **The second law** states that for "every character (trait) i.e., height, colour, texture etc. an organism inherits two genes, one from the father and one from the mother suggesting that somatic cells are produced from two gametes; one allele comes from the maternal and one from the paternal side. These alleles may be the same (true-breeding) or different (hybrid) individuals." **The third law** is an extension of the second law that declares that if the two alleles differ from each other then one will be the dominant allele expressed completely in the organism while the other will act as the recessive allele and have no distinguishable effect on the organism. **The fourth law** suggests that two genes for each character segregate during gamete formation. The chromosome number is changed from the diploid number to the haploid number during meiosis. The genes are sorted into separate gametes ensuring variation and the process depends on a "recombination of genes" in a random manner. Data after statistical analysis show even two identical offspring require variations given trillions of years. The most important principle of Mendel's law of independent assortment is where the appearance of one trait act does not affect the development of another, and other scientists simplify the emergence of traits i.e., mixing one trait always resulted in a 3:1 ratio between dominant and recessive phenotypes with two traits showing 9:3:3:1 (ratios). In humans, his experimental model was unsuccessful because of the non-passionate use of the scientific manner of "genetic disorders." Mendel's statistical analysis concluded with four important assumptions on the basis of characteristic features such as colour, shape and texture of the offspring subjected to the simple genetic structure of a pea: 1) inheritance of each trait is determined by "units" or "factors" that are transmitted to the offspring unchanged–later these factors are designated as genes, 2) an individual inherits one such unit from each parent for each feature (trait), 3) a trait or feature may not show up in an individual but can still be passed on to the next generation, and 4) these factors (genes) for each trait segregate themselves during gamete production.

1.3 Darwin's Perception

Mendel's hypothesis of heredity and evolution was unmatched by those of Darwin. He, (Darwin) believed that inheritance is based on acquired characteristics and he presented the famous theory of continuous evolution.

Mendel, in contrast, rejected both the idea of inheritance of acquired characteristics (mutations) as well as the concept of continuous evolution. Although Darwin won the battle in the nineteenth century, no space was left in the next decades for the acceptance of the true scientific laws of heredity discovered by Mendel. Further work in genetics was continued by Darwin's critics, Bateson, Hugo de Vries, Johannsen and Nilsson. Last, Darwin's theory of evolution was accepted by the world regarding variations and inheritance of acquired characteristics

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CHAPTER 2

THE CONCEPTS OF CELL AND TISSUE CULTURE

The concept of in vitro cell and tissue culture development and maintenance technology for living cells has become essential for medical biotechnology. In this chapter, the author includes the principles of primary and secondary cell culture including cell line culture. Details of the procedures and instruments used in cell culture are listed such as the Laminar air flow, CO_2 incubator and phase contrast inverted trinocular microscope with culture flask or vessels. Detailed illustrations for the maintenance of cell cultures with safety requirements during experimentation and cryopreservation techniques are also shown.

2.1. Introduction

In biological sciences cells develop differentiation and proliferation from a small number to a larger amount in an artificial environment by providing a specially prepared nutrient medium under sterile conditions, the term is defined as *culture* and is also called tissue culture. Other synonyms also refer to cell culture, organ culture, explants culture or cell suspensions and the terms are often used interchangeably depending upon laboratory conditions and requirements. Primary cell cultures will have a short life span in culture, whereas continuous cell lines by definition may grow for a longer period of time or if cryopreserved.

2.2 Primary Culture

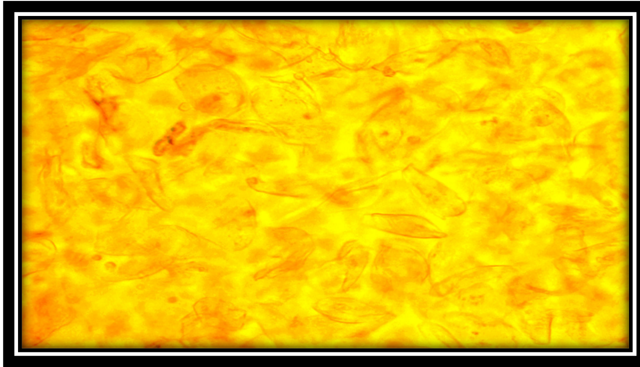
Primary culture includes the maintenance of cell growth, and the differentiation of cells from the parental tissue such as the kidney, liver (hepatocytes) and germ cells after using the mechanical or enzymatic

methods for the dissociation of cells in a sterile culture medium using suitable glass or plastic containers. The primary cell culture is of two types depending upon the kind of cells in the culture: A) anchorage-dependent or adherent cells—the cells for proliferation require substratum for attachment to set on and act as a bed for cell growth, called anchorage-dependent cells. The adherent-dependent cells are usually derived from tissues of organs such as the kidney or liver where they are immobile and embedded in connective tissue. B) Suspension culture or independent cells—this is also known as short-term cell culture in which cells do not require attachment for growth or do not attach to the surface of the culture vessels, that is, anchorage to independent cells. Generally, cell suspension cultures are derived from lymphocytes (blood).

Secondary Cell Cultures: When confluency is reached during primary cultures from the parent culture it is called sub-cultured, also known as secondary culture. Sub-culture (or passage) refers to the transfer of cells from one culture flask to another until used for study or stored in liquid nitrogen after cryopreservation. **Sub-culturing** is defined as the periodical splitting of cells and providing fresh nutrients (media) for the continuous proliferation in new or parent flasks (cell lines), after the confluency of cells removing the growth media and dissociating the adhered cells in a new flask using 0.01% trypsin for the next co-culture, known as secondary cell cultures.

2.3 Cell Line Culture

Cells are allowed to grow continuously depending upon whether they have a limited life span or they are immortal in an in vitro culture system. Based on the life span of cell lines they are categorized into two types: A) finite cell lines—those cell lines which have a limited life span and go through a limited number of cell generations (generally 20–80 population doublings) are known as finite cell lines. These cell lines show the property of contact inhibition, density limitation and anchorage dependence with the growth rate for doubling being approximately 24–96 hours. B) continuous cell lines—these cell lines are transformed cells under laboratory conditions or in vitro culture, tissue-engineered derived cells. Genetically, these cell lines show the characteristic features of ploidy (either aneuploidy or heteroploidy) with a lack of contact inhibition and anchorage dependence. They grow and proliferate in a monolayer with or without suspension and have a faster growth rate (12–24 hours). Monolayer cultures are defined as when the bottom of the culture vessel is covered by a continuous layer of cells, usually,



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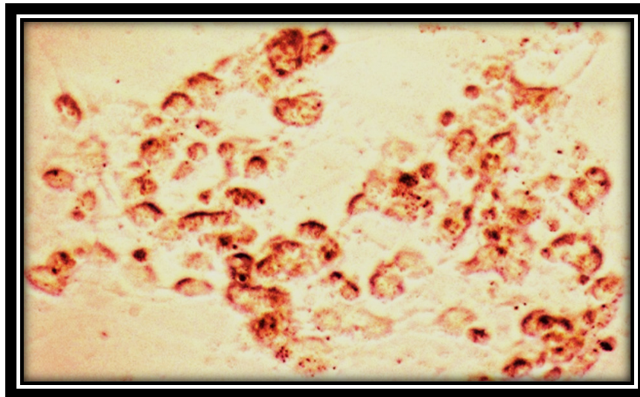

B

Figures 1 A & B. Primary cell culture from amniotic fluid, and (B) MCF-7 cell lines (adenocarcinoma of human breast cancer)

one cell in thickness, and they are referred to as monolayer cultures as shown in the figure below (Figures 1 A & B). The majority of cell lines grow as monolayers and some of the non-adhesive cells, for example, leukaemic or certain cells can be kept mechanically in suspension or propagated in suspension culture. The advantages are as follows: 1) the process of propagation or cell differentiation is faster, 2) the frequent replacement of the medium is not required, 3) suspension cultures have a short lag period, 4) trypsin action is not a prerequisite, 5) homogenous

suspension of cells is attained and 6) the maintenance of suspension cultures is easy but bulk production of cells is difficult to attain.

2.4 Procedures for cell and tissue culture

A. Laminar flow. There are two types of laminar flow hoods, vertical and horizontal. The vertical hood, also known as a biology safety cabinet, is best for working with hazardous organisms since the aerosols that are generated in the hood are filtered out before they are released into the surrounding environment. Horizontal hoods are designed so that the air flows directly at the operator, hence they are not useful for working with hazardous organisms but they are the best protection for cultures. Both types of hoods have a continuous displacement of air that passes through HEPA filters. The hoods are equipped with a short-wave UV light that can be turned on for a few minutes to sterilise the surfaces of the hood and turned off whilst working because it may damage the skin or eyes.

B. CO_2 Incubators. The cells are grown in an atmosphere of 5–10% CO_2 because the medium used is buffered with sodium bicarbonate/carbonic acid and the pH must be strictly maintained. Culture flasks and vials should have loosened caps to allow for sufficient gas exchange. Cells should be left out of the incubator for as little time as possible and the incubator doors should not be opened for very long.

C. Research microscopes. The phase-contrast inverted microscopes and fluorescence microscopes are used for visualizing the growing and differentiating cells. Microscopes should be kept covered to avoid moisture and before using the microscope or whenever an objective is changed, check that the phase rings are aligned.

D. Culture Flasks. Anchorage-dependent cells require a nontoxic, biologically inert and optically transparent surface that will allow cells to attach and allow movement for growth. The most convenient vessels are specially treated polystyrene plastic that is supplied sterile, disposable and available in different sizes (T-25, T-75 and T-150) with screw caps (cm^2 of surface area). Suspension cells are shaken, stirred or grown in vessels identical to those used for anchorage-dependent cells.

2.5 Maintenance of Cell Culture

The maintenance of cell and tissue culture is a difficult task because cells have to be examined daily to observe the morphology, the colour of the

medium and the cell density. A tissue culture logbook should be maintained that is separate from the regular laboratory notebook. The logbook is required to maintain the name of the cell line, the medium components and any alterations to the standard medium, the dates on which the cells were split and a calculation for the doubling time of the confluence of the culture. The growth of the cell should be examined every three days because it completes almost three cell cycles, and the cells undergo a quiescent period (lag phase) depending on the cell types. The cells will then go into exponential growth where they show the highest metabolic activity and enter into the stationary phase i.e., the confluency of the cell population is attained. The harvesting of cells takes place when the cells have reached a maximum density and suppress cell growth. Generally, cells are harvested during a semi-confluent state and are still in the log phase. Usually, the cells are passaged three times a week and suspension cultures are fed by dilution into a fresh medium. Adherent cultures that do not need to be divided can simply be fed by removing the old medium and replacing it with a fresh medium. A mechanical disruptor can be a rubber spatula that may be used to remove the adherent cells quickly because disruptive cells may die (cell death). Other alternative uses of proteolytic enzymes (trypsin, collagenase, or pronase, usually in combination with EDTA), causes cells to detach from the growth surface. This procedure is fast and reliable but can damage the cell surface by digesting the exposed cell membrane proteins. Cells should be examined under a wide field phase-contrast inverted research microscope; then apply centrifuge, remove supernatant and pellet and resuspend into a fresh medium with a cell density of $x10^6$ to maintain cell proliferation. These physiological conditions are required to maintain cultures; temperature (37^0C), pH 7.2–7.5 and osmolality of medium with an associated bicarbonate concentration with CO_2 tension in equilibrium. Sometimes, visible light shows an adverse effect on cell growth due to the production of toxic metabolites in some media, hence cells should be cultured either in the dark or in daylight. Nowadays ready-made media is available on the market like DMEM, MEM, RPMI 1640, and TC199, commonly used for the short-term as well as for primary or cell line cultures having trace elements (iron, selenium), Ions (sodium, potassium, calcium, bicarbonate) with a concentration of thirteen amino acids. The most important component of culture media is a foetal bovine serum in 5–7% as required for growth-promoting activities because it contains hormones and growth factors that promote healthy cell growth. Often antibiotics (0.001ul/ml penicillin) are required to protect the cultures from bacterial contamination during damp conditions. The simple viability of the cells can also be assessed quantitatively using trypan blue, which is excluded from

live cells but accumulates in dead cells using a hemacytometer under a microscope.

2.6 Safety Requirements

The following safety precautions are required while handling pipette aids to prevent ingestion; keep aerosols down; do not smoke, wash hands before leaving the lab, and decontaminate the work surfaces with disinfectant (before and after), autoclave all waste and use the biological safety cabinet whilst working. The HEPA filters of the cabinet should not be overloaded because it protects you from airborne cells and viruses released during experimental activities.

2.7 Cryopreservation of cells

Most mammalian cells can be stored at temperatures below -130°C for many years. The viability of the cells after cryopreservation depends on their ability to cope with the variety of stresses imposed on them during the freezing and thawing procedures. This is a relevant technique used for cell and tissue culture in laboratories. It allows keeping the cells or tissue at a very low temperature (-135°C). Generally, this technique is helpful for medical science to preserve living tissues that need to be surgically implanted or transplanted into a human body, for example, the cornea, heart, sperm and ova (sperm bank). At this low temperature, either to decrease or almost with the total cessation of metabolic processes of the living cells/ tissue you can say that this is the "preservation of life." The basic concept behind cryopreservation is the reduced risk of microbial contamination, genetic drift and morphological changes and cross-contamination with other cell lines.

The basic principle of successful cryopreservation and revival is a slow freeze and quick thaw, and different cell lines should be cooled at a rate of -1°C to -3°C per minute and thawed quickly by incubation in a 37°C water bath for 3–5 minutes. Healthy cultures show the viability of >90% and no signs of microbial contamination. Because these cultures should be in the log phase of growth the required high concentration of serum is >20% (or more). The most common use of cryoprotectant is DMSO (dimethyl sulfoxide) or glycerol to help protect the cells from rupture by the formation of ice crystals. At the rate of freezing in the presence of DMSO (10%), cell lines can be cryopreserved in a suspended state for indefinite periods provided a temperature of less than -135°C is maintained. Such ultra-low

temperatures can only be attained by specialized electric freezers or more usually by immersion in liquid or vapour phase nitrogen.

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CHAPTER 3

FUNDAMENTALS OF GENETIC DISORDERS

Author-discussed clinical aspects of karyotypes and their relevance for diagnosis of genetic disorders. Karyotyping is a simple procedure for the study of chromosomal aberrations requiring a '*bird's eye view*' of an individual's genetic information. The most common type of syndromic and non-syndromic genetic disorders is illustrated in the text with phenotype and genotype (karyotype) presentations such Down's syndrome, Patau syndrome, Edwards' syndrome, Cri Du Chat syndrome, Klinefelter syndrome, Turner syndrome and Hypogonadism

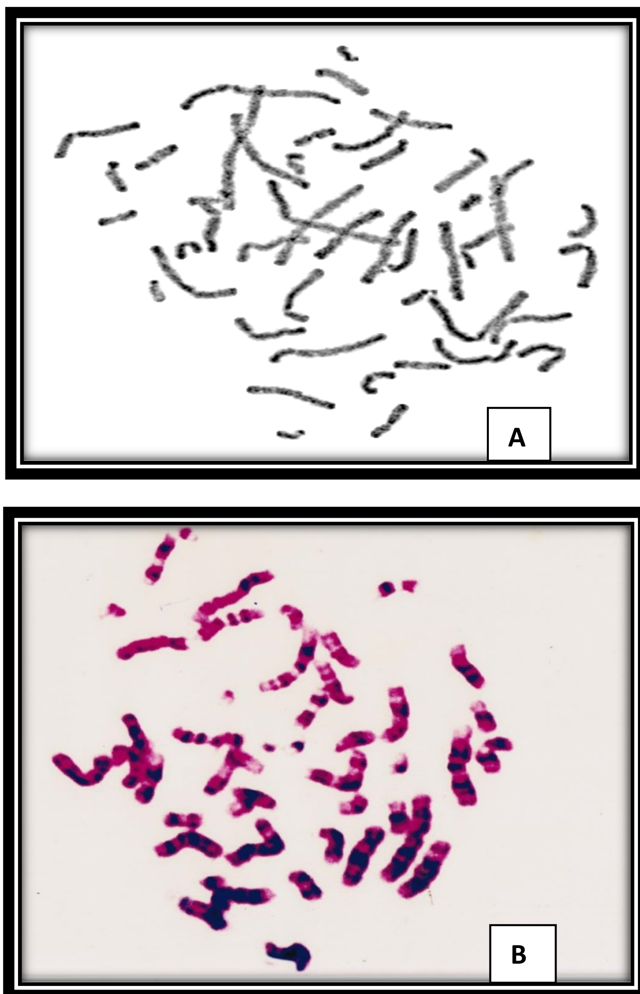
3.1 Introduction

Cytogenetics is a promising branch of cell science that deals with the architecture as well as the functional aspects of chromosome behaviour, in other terms defined as the hereditary unit of all living beings. The individual characteristics of chromosomes are based on routine analysis after using different kinds of banding procedures for the localization of a chromosome or its component. Among all GTG banding, Giemsa-trypsin is more common for the identification of euchromatin and heterochromatin segments of chromosomes designated as bands that appear in the form of dark or light on chromosomes. C-banding (centromeric) and NOR banding is also used for the identification of centromeric and nucleolar organizer regions. During the advancement of technology, molecular cytogenetics becomes relevant for diagnosis using fluorescent in situ hybridization (FISH) probes and comparative genomic hybridization using microarrays as an advanced version of FISH. In whole chromosome paintings using specific DNA, multiple probes were used for the identification of microdeletion in a specific region of the chromosomes. This technique is quite relevant but costly in medical practice for undergraduates as well as postgraduate students learning about genetic disorders.

3.2 Structure of the human chromosome

The word 'chromosome' is derived from Greek *chroma*, colour and *soma*, body i.e., the 'colour body' that appears inside after staining with particular dyes, called **chromosomes.** It is a highly complex and dynamic structure of DNA and protein inside the cells. The human chromosome is tightly coiled by DNA-bound proteins, which serve to package the genes and consists of nucleotide sequences that regulate cellular functions. Morphologically, chromosomes vary in different species. The DNA molecule may be circular or linear and can be composed of 10^3 to 10^8 nucleotides sequences consisting of a long chain. Typically, eukaryotic cells with a nucleus have large, defined chromosomes and prokaryotic cells without a nucleus have small circular chromosomes, although there are many different views by scientists for genomic differentiation. Furthermore, cells may contain more than one type of chromosome, for example, mitochondria in most eukaryotes and chloroplasts in plants have their own small chromosomes. The human chromosome also contains DNA-bound proteins commonly known as chromatin that regulate many functions. The number of chromosomes is fixed, and the identity of the individual organism and the human cell contains 44 autosomes, and the XY sex chromosome in a diploid cell with a nucleus contains about 6×10^9 base pairs of DNA. Chromosomes are the structural and functional unit of cells—divided and replicated in such a fashion to pass genetic material successfully to their daughter cells ensuring that there is no genetic diversity, for the survival of their progeny.

The name chromosome was coined in 1888 by Heinrich von Waldeyer and in the twentieth century, genetics was developed by Grygorii Levitsky, who defined the karyotype for the first time as the phenotypic appearance of the somatic chromosomes. Tjio and Levan (1956) confirmed that 46 chromosomes exist in a normal human cell. The recombination of chromosomes plays a vital role in genetic diversity during disease progression like cancer, congenital malformations and infertility, where it can be observed that complex structural and numerical karyotypic variations are associated with genomic instability leading to the progression of such genetic disorders. Chromosomes may exist as either duplicated or unduplicated forms and contain two copies joined by a centromere. During the duplication of chromosomes at metaphase, they appear as four-arm structures. Healthy living cells show elongated and fragile chromosomes, which can be read or visualized under the wild field phase-contrast advanced microscope. The complete morphological features appear in mitosis during the s-phase of the cell cycle, where it begins duplication and condensation to reduce in size (~ 5 μm) structures. After staining, this can be observed under a light microscope.



Figures-2 A & B. GTG banded Metaphase Chromosomes Stained with Giemsa

These duplicated chromosomes are sister chromatids, called dyads, and are held together throughout the structure, named the centromere, containing 1–10 million base pairs of repetitive short DNA sequences. The centromere is a complex structure consisting of more than a hundred different types of proteins and is responsible for the attachment to the spindle fibres and

separation of the sister chromatids during mitosis, which proceeds then into anaphase. Each chromosome has two arms, the short arm is called the p arm and the longer is the q arm. Figures 2 A & B show the metaphase after staining with the trypsin-Giemsa method and reveal a series of alternating light and dark bands of euchromatin and heterochromatin, respectively called GTG banding, required for gene mapping. Gene mapping is an essential phenomenon for the identification of genes assigned to a specific locus in a chromosome. Each chromosome has been characterised by a specific GTG banding pattern for the identification of gene loci, and high-resolution banding shows 400–2000 bands dependent on the AT or GC-rich content of DNA during cell differentiation.

3.3 Classification of human chromosomes

In eukaryotes, nuclear chromosomes are packed by proteins into a condensed structure called chromatin. This allows the very long DNA molecules to fit into the cell nucleus. The structure of chromosomes and chromatin varies through the cell cycle. Chromosomes are the essential unit for cellular division and must be replicated, divided and passed successfully to their daughter cells so as to ensure genetic diversity and survival of their progeny. Chromosomes may exist as either duplicated or unduplicated chromosomes and are single linear strands, whereas duplicated chromosomes (copied during the synthesis phase) contain two copies joined by a centromere. Compaction of the duplicated chromosomes during mitosis and meiosis results in the classic four-arm structure. Chromosomal recombination plays a vital role in genetic diversity. If these structures are manipulated incorrectly, through processes known as chromosome instability and genetic translocation, the cell may undergo mitotic catastrophe resulting in a failure to survive, or it may aberrantly evade the process of apoptosis or may lead to the progression of cancer. In prokaryotes and viruses, the term genophore is more appropriate when no chromatin is present.

3.4 Relevance of Karyotypes and genetic disorders

The word karyotype originates from Greek and means ‘nucleus.’ In a eukaryotic cell, the number and appearance of chromosomes are constant and is a characteristic feature of a species of an individual organism. Karyotyping is a complex procedure for chromosome analysis that provides a ‘bird’s eye view’ of an individual's genetic information. There is either a missing or extra copy of chromosome material that may be responsible for various problems during embryonic development that fall under the

common category of ‘birth defects’ such as learning difficulties, infertility and recurrent pregnancy loss in humans. In 1882, the chromosome was first observed in salamander and was described by Walther Flemming. The preparation and study of karyotypes is part of the cytogenetic study but the procedure for human karyotypes is very complicated for human cells and requires a high-resolution phase-contrast upright triangular microscope to evaluate the morphological features of an individual chromosome. The basic characteristic feature of the karyotype is based on the common banding (GTG) pattern, arm ratio between the short arm (p) and the long arm (q) of the chromosomes and position of the centromeres (kinetochore). In the early stages of mitosis or the meiosis of cell division, the chromatin strands become more and more condensed, where the transcriptional event stops. This compact form makes the individual chromosomes visible and they form the classic four-arm structure of a pair of sister chromatids attached to each other at the centromere (Figure 3).

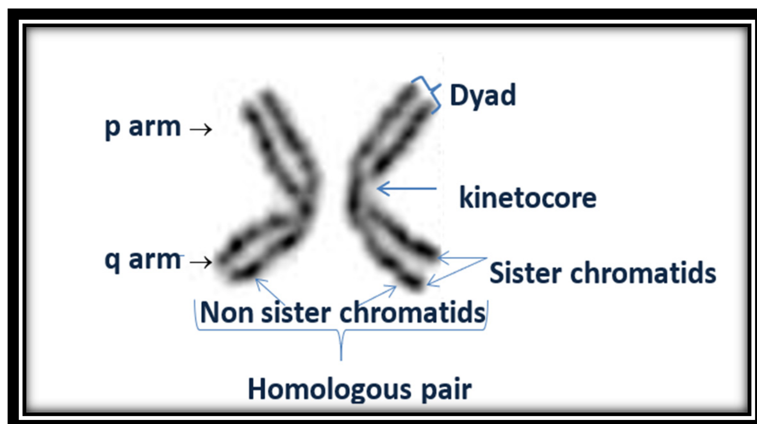


Figure 3. Dyad Structure showing Sister and Non-Sister Chromatids.

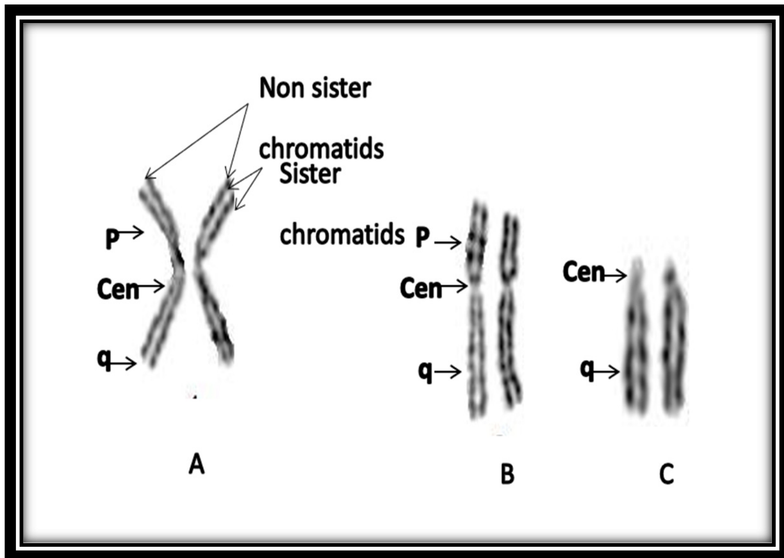
3.5 Types of Chromosomes and Their Classification

The structure of human chromosomes and chromatin varies throughout the cell cycle and form the essential units for cell division and must be replicated. They carry genetic information to their daughter cells so as to ensure the survival of their progeny. In eukaryotes, nuclear chromosomes are packaged by proteins into a condensed structure called chromatin. This allows the very long DNA molecules to fit into the cell nucleus. Chromosomes may exist as either duplicated or unduplicated chromosomes

and are single linear strands, whereas duplicated chromosomes (copied during the synthesis phase) contain two copies joined by a centromere. Structural deformities of the chromosome (recombination) play a significant role in genetic diversity through mutation processes, known as chromosomal instability, and the cell may undergo mitotic catastrophe resulting in cell death, or it may aberrantly evade apoptosis leading to the progression of cancer. In prokaryotes and viruses, the term genophore is more appropriate when no chromatin exists without DNA. However, a group of molecular biologists working on chromosome science use the term chromosome regardless of chromatin content. Prokaryotic DNA is usually arranged like a circle, which is tightly coiled in on itself, sometimes accompanied by one, or more, smaller, circular DNA molecules, called plasmids. These small circular genomes are also found in mitochondria and chloroplasts, reflecting their bacterial origins. The simple genophores are found in viruses and show RNA as genetic material that exists in a short linear fashion with or without structural proteins. The structure of chromatin varies significantly during different stages of the cell division in the cell cycle, according to the requirements of the DNA synthesis. The author illustrates briefly the different forms of chromatin in different stages of cell division, where the chromatin is distinguished either in the form of euchromatin or heterochromatin simply with a GTG banding pattern.

Interphase Chromatin. During the interphase of cell division, two types of chromatin can be distinguished, named euchromatin and heterochromatin. Euchromatin consists of active DNA throughout life and is expressed in the form of protein, while heterochromatin consists of inactive DNA during ontogenetical development, but some scientists believe it is also active in the early life of foetal development, although it is a debatable question. Heterochromatin can be further distinguished into a) constitutive heterochromatin which is expressed once during the embryonic period and is located around the centromere with repetitive conserved DNA sequences, and b) facultative heterochromatin which are rarely expressed and cannot be distinguished at this stage because they appear in the nucleus as a homogeneous tangled mix of DNA and protein. The shorter arms are called p arms and the longer arms are called q arms. This is the only natural context in which individual chromosomes are visible under a microscope. During mitosis, microtubules grow from centrosomes located at opposite ends of the cell and also attach to the centromere at specialized structures called kinetochores, one of which is present on each sister chromatid. A special DNA base sequence in the region of the kinetochores provides, along with conserved proteins, longer-lasting attachment in this region. The microtubules then pull the chromatids apart toward the centrosomes, so that

each daughter cell inherits one set of chromatids. Once the cells have divided the uncoiled chromatids and DNA can again be transcribed in a highly condensed form of chromosomes having giant DNA structures within a nucleus. The centromere is a region of DNA typically found near the middle of a chromosome, where two identical sister chromatids are held together. The centromere is also involved at the metaphase stage, where spindle fibres play an important role in the attachment of sister chromatids. These are connected in a metacentric, submetacentric, acrocentric or telocentric manner as shown in Figures 4 A, B & C.



Figures 4 A, B & C. Types of Human Chromosomes During Cell Division (metaphase), (B) sub-metacentric, and (C) acrocentric chromosome (Cen = Centromere, p = short arm, q= long arm).

Metacentric chromosomes, having two equally long arms, belong to the A group and F group in a karyotype. In some cases, a metacentric chromosome is also formed by balanced translocation due to the fusion of two acrocentric chromosomes to form one metacentric chromosome.

Submetacentric chromosome arms (p and q) are unequal in length and the largest number of chromosomes belong to B, C and E groups while preparing karyotypes.

Acrocentric. The human genome includes ten acrocentric chromosomes—13, 14, and 15, belonging to the D-group and 21 and 22 belong to the F group, respectively. The short arm (p) is very small and difficult to observe and contains genetic material including repetitive DNA sequences which can be visualized using silver staining in the form of nucleolar organizing regions (NORs) which are round. It is apparent that the evolutionary sequence is a reduction of two acrocentric chromosomes in great apes to one metacentric chromosome in humans. NORs have a significant role in cancer patients as well as in children with undescended testis (cryptorchidism case) and could be used as a diagnostic marker as illustrated in Chapter 13.

Telocentric. A **telocentric** chromosome's centromere is located at the terminal end of the chromosome. Telomeres may extend from both ends of the chromosome. For example, the standard house mouse karyotype has only acrocentric chromosomes. It is important to note that human cells do not possess telocentric chromosomes.

Structure of the Centromere. The centromeric DNA is normally in a heterochromatin state, which is essential for the recruitment of the cohesion complex that mediates sister chromatid cohesion after DNA replication as well as coordinating sister chromatid separation during anaphase. In this chromatin, the normal histone H3 protein is replaced by a specific variant, CENPA, in humans. The presence of CENPA is believed to be important for the assembly of the kinetochore on the centromere. CENPC has been shown to localise almost exclusively to these regions of CENPA-associated chromatin fibre. These histone proteins are found to be enriched for H4K20me3 and H3K9me3 which are known heterochromatic modifications. In humans, the primary centromeric repeat unit is called α-satellite (or an alphoid), although a number of other sequence types are found in this region. Point centromeres are smaller and more compact. DNA sequences are both necessary and sufficient to specify centromere identity and function in organisms with point centromeres. In rare cases in humans, neocentromeres can form at new sites on the chromosome. There are currently over 90 known human neocentromeres identified on 20 different chromosomes. The formation of a neocentromere must be coupled with or followed or preceded by the inactivation of the centromere since chromosomes with two functional centromeres (dicentric chromosomes) will result in chromosome breakage during mitosis. In some unusual cases, human neocentromeres have been observed to form spontaneously on fragmented chromosomes. Some of these new positions were originally euchromatic and lacked alpha satellite DNA. Centromere proteins are the autoantigenic target for some anti-nuclear antibodies such as anti-centromere antibodies.

The human karyotype consists of a constant number of 46 chromosomes out of which 44 are autosomes and two are sex chromosomes including X and Y. These 46 chromosomes are divided into seven groups designated as the A group which contains metacentric (1–3), the B group which contains submetacentric (4–5), the C group which contains sub-metacentric 6–12 including X, the D-group which contain acrocentric 13–15, the E group which contains submetacentric 16–18, the F group which contains acrocentric 19–20 and the G-group which contains 21–22 and Y. These sex chromosomes determine one's gender i.e., females have two X chromosomes, while males have one X-chromosome and one Y-chromosome, a representative karyotype of 44, XY (male) and 44, XX (female), (Seabright, 1971), according to the recommendations of the International System for Chromosome Nomenclature (ISCN 2013). Figure 5 shows that the morphological features of each human chromosome are well defined and are documented below -

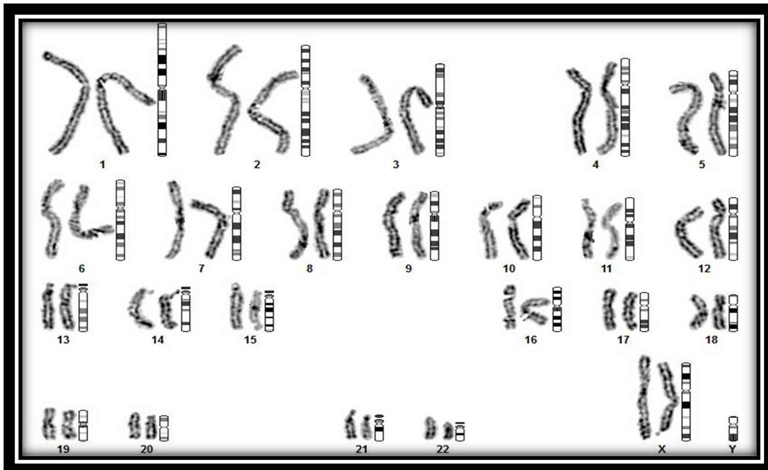


Figure 5. Normal Karyotype with 46, XX (ISCN 2016).

3.6 Chromosome Variation in Humans and other Species

Before a cell gets ready to divide by mitosis, each chromosome is duplicated during the S- phase of the cell cycle. As mitosis begins, the duplicated chromosomes condense into short (~ 5 µm) structures which can be stained and easily observed under the light microscope. These duplicated chromosomes are called dyads. In humans, the centromere contains 1–10