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BASIC ELEMENTS OF CYTOGENETICS

Dr. Bülbin Sunar

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BASIC ELEMENTS OF CYTOGENETICS

MARMARA ÜNİVERSİTESİ
KÜTÜPHANE VE DOKÜMANTASYON
DAİRE BAŞKANLIĞI

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Kütüphane ve Dokümantasyon Daire Başkanlığı
0010456

MARMARA ÜNİVERSİTESİ YAYINEVİ

To the loving memory of my mother
Hikmet Sunar

MARMARA ÜNİVERSİTESİ
KÜT. PÖK. D. BSK.
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10456

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'Science may be learned by rote,
but wisdom not'

—Laurence Sterne

MARMARA ÜNİVERSİTESİ 1883

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FOREWORD

I was very honoured by Dr. Bülbin Sunar's request that I review her manuscript and write a foreword. After carefull reading and reviewing; I am even more pleased to recommend this book to students and interested people in the field of genetics.

The subject of the book "Basic Elements of Cytogenetics" is a very essential part of Medical Biology and Genetics course for the undergraduate medical students.

I am very happy to write this foreword for our one of the young, eager and hard working colleague It has been known for a long time that there is a definite relationship between genetics and various biochemical abnormalities and congenital human diseases.

I think, this book is so well organized and logically arranged that medical students and even the interested geneticists in training will find it usefull.

I am proud that our young asistant Professor, -in the early stage of the development of our medical school- was able to provide us with a brief account of the knowledge and understanding of basic essentials of cytogenetics.

Dr. Ali Ertugrul
Professor of Pediatric Cardiology
Director of Pediatrics Unit at Marmara University Hospital, Chairman of Internal Medicine Division at Faculty of Medicine, Marmara University

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PREFACE

The series, "Medical Biology and Genetics Department Lecture Notes", was launched at the beginning of our first teaching year at Marmara University Faculty of Medicine as a study guide for undergraduate medical students.

This study guide, second of the series, was written to be used in conjunction with the lectures given in the second semester of Medical Biology and Genetics. It contains the basic essentials of cytogenetics with an emphasis on the role of cell division and chromosome studies in biology and medicine. For those interested students, further reading references were added to the end of each chapter.

ISTANBUL
May 1987

Bülbin Sunar

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ACKNOWLEDGEMENTS

I am greatly indebted to my former Dean Professor Ali Ertuğrul for initiating the project and to my past and present colleagues for encouraging me on its realization. I am particularly greatful to my Dean Professor Atıf Aktaş, Professor Kaya Emerk, Professor Yıldızhan Yayla and Associate Professor Türay Yardımcı for their valuable quidance and help.

Finally, I would like to thank the staff of Marmara University, in particular Mehmet Aydın for his careful typing of my illegible manuscript and Javad Amirahmadi for his patience in redrawing the illustrations.

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1

INTRODUCTION

1.1. HISTORICAL DEVELOPMENT OF CYTOGENETICS

In 1665, R. Hooke first identified the cell as the basic structural unit of all animal and plant tissue. Almost two centuries past before the process of cell division was described by R. Virchow, who showed that cells always arise from other cells. It was Flemming, however, who gave the first detailed account of somatic cell division; describing the conversion of the nuclear material, the "chromatin", into longitudinally split threads [the term chromosome(s) was introduced later] and used the world "MITOSIS" to describe the process. The reduction division during gametogenesis was observed by Boveri in 1887 and was later called "MEIOSIS". The rediscovering of Mendel's breeding experiments with peas helped Walter Sutton link chromosomes to Mendelian hereditary. The inescapable significance of the regular transmission of chromosomes and the inheritance of biological characteristics led to the formulation of the chromosome theory of inheritance, the Sutton-Boveri hypothesis (1), and thus the founding of cytogenetics at the beginning of the twentieth century.

Important contributions to support the chromosome theory of inheritance accumulated in subsequent years. McClung and Stevens in 1902 (2) and 1905 (3) respectively, correlated sex determination with sex chromosomes the demostrations of Morgan (1910) and Sturtevant (1913) showed that specific chromosomes carry specific genes (4); and the linear arrangement of genes along the chromosomes (5); all gave meaningful insight into Janssen's (6) interperetion of chiasmata, observed during meiosis as the visual evidence of genetic recombination. In 1931, McClimtock (7) and her colleague showed for the first time microscopic evidence of chromosome breakage and reunion during recombination. Later, she showed that some chromosome segments are "mobile" in the genome of *zea mays*, for which she recieved the Nobel Prize in 1983. Now it is known that Transposons (*) in Eukaryotic cells probably move by using an RNA intermediary.

(*) Transposons: Any discrete and characteristic genetic unit (DNA sequence) that may be transferred from one cell to another and be inserted into any of multiple sites in the recipient cell's plasmid - or chromosomal DNA, with associated rearrangement of the recipient's DNA.

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There had been many attempts to establish the exact number of human chromosomes until 1956 when Tjio and Levan using modern tissue culture techniques showed that the correct number of human chromosomes was 46 (8). First Garrod (9) then Beadle and Tatum in 1941 (10) developed the idea that there is a relationship between genetic defect, biochemical abnormality, and human disease. This was shown to be true in the case of Alkaptonuria which is caused by a rare recessive mutation inherited according to Mendelian rules. Beadle and Tatum's work on *E. coli* led to the important discovery of one gene being responsible for one enzyme. They concluded that each mutant cell that could be restored to its normal growth by the addition of a single compound, carried a single gene defect that impaired the production of an enzyme necessary for a single metabolic step.

Let us explore our increasing knowledge of human genetics and cytogenetics in the following chapters.

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2

THE MECHANISMS OF INHERITANCE

2.1. ASEXUAL REPRODUCTION

Asexual reproduction involves the mitotic division of cells during which the complete adult number of chromosomes is exactly replicated and passed on, so that offspring are genetically identical to the parent. It is more common in plants than in animals. Examples include binary fission and spore formation, budding and vegetative reproduction. In an asexually reproducing line of descendants, or clone, a good combination of genes can arise only if all the genes concerned undergo the necessary changes or mutations in the line of descent.

I- Binary Fission

It is seen in bacteria and many protozoa including *Amoeba*. After the parent cell has grown to a certain optimum size, the nucleus undergoes mitotic division and the cytoplasm undergoes cleavage into roughly equal halves. The stimulus which causes division is not fully understood, but it is thought to involve the nucleus to cytoplasm ratio and the surface area to volume ratio of the parent cell (see the sequence of events in cell division). These rations are important because the nucleus can control only a certain volume of cytoplasm and exchanges taking place over a certain surface area can only supply a certain volume of cytoplasm. When these ratios fall below a certain critical value, and other conditions are suitable, binary fission occurs. These basic principles are also thought to be involved in the control of some cell divisions in multicellular organisms.

In most cases cells grow till they reach a certain size, then they divide and this process continues for each generation. Generaly, in an adult cell there is an optimal equilibrium between nucleus volume and cytoplasmic volume as can be shown;

$$\frac{V_n}{V_c} ; \frac{2V_n}{2V_c} ; \frac{4V_n}{4V_c} \text{ which leads to } NP = \frac{V_n}{V_c - V_n}$$

This relationship is called Nucleoplasmic Index and can be abbreviated as NP. V_n is the nucleus volume and V_c is the cell volume. This equilibrium is valid not only for volume, but it is also applicable for the chemical processes taking place in the cell as well. Therefore, whenever there is an increase in one of the components of the equilibrium, this change of balance appears as an initiator for cell division.

ii- Spore Formation

It is more common in plants than in animals and is characteristic of saprophytic and parasitic microorganisms, especially bacteria and fungi. For parasites and saprophytes the production of vast numbers of easily dispersible spores ensures the successful transference to a new food source.

iii- Budding

Budding occurs in the lower animals such as the *coelenterates*. *Hydra* first forms a bud of cells by mitotic cell division and then a body wall. Next it forms a body cavity continous with that of the parent, tentacles, and eventually a mouth. The point of connection with the parent is sealed and the new individual drops off to live an individual existance.

iv- Vegetative Reproduction

During vegetative reproduction, which occurs only in plants some part of the plant body seperates from the parent plant and then grows into another complete plant. Such reproduction is seen in the fragmentation of filamentous *algae* and in *Angiosperms*.

2.1.1. Cell Cycle: Prokaryotic

The term cell cycle refers to the set of events which occur during the division of a parent cell to form two daughter cells. In prokaryotic cell division the subdivisions of the cycle have not yet been well characterised. In eukaryatic cells, however, there is a well-defined set of cellular events whose order in the cycle is fixed, but whose rate of progression through the cycle may vary.

The division of a prokaryotic cell involves the replication of its single circular chromosome and of any plasmid it may contain and the duplication of the cellular organalles including the plasma membrane and cell wall constituents. The rate at which bacterial division occurs is dependent on the nutrient vlaue of the medium in which the cells are growing. Deprivation of essential nutrients leads to a decrease in the rate of iniation of replication, but not in the rate of replication itself.

The replication frequency is remarkably constant for most prokaryotic cells (about 40 min). Once a cell has committed itself to copying its DNA, the process will continue to completion regardless of any nutritional change imposed during

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that period (20-220 min). In the two examples shown in Fig. 2.1. the distinction is made between a long cycle and a short cycle. A long cycle occurs when the initation frequency is slow. Thus when replication has commenced, no new initiation sites appear until the cell has replicated itself and the division events have occured. The length of time taken for new initation sites to appear is variable and dictates the total cycle time. A short cycle occurs when new initation sites appear even before the current round of replication has finished. Thus when the cell has divided, replication is again already in progress.

The initation of replication is brought about by proteins. If the cells are grown in the absence of a key nutrient such as tryptophan, protein synthesis stops suddenly. Although DNA replication and cell division continue, no new round of replication is initiated until protein synthesis starts again. Thus, it is likely that the initiators are proteins which interact with the DNA and render it in the correct state for binding DNA polymerase.

The division of a cell occurs when the total mass is sufficient for two cells and replication has been completed. The time taken for the division process is relatively constant and is a about 20 min.

2.1.2. Cell Cycle: Eukaryotic

The division of a eukaryotic cell by mitosis is a process in which the replication and equal division of the chromosomes (karyokinesis), accompanied by the division of the cell into two daughter cells (cytokinesis) takes place. It ensures the constancy of genetic information in all the somatic cells of an individual. Mitosis involves a number of phases of the cell cycle, the sum of which may add up to as long as 24 hours for a single cell division.

INTERPHASE refers to that period of the cycle in which the nuclear chromatin is dispersed throughout the nucleus and no chromosomal features are detectable. There are 4 sub-sections of interphase; G_0, G_1, S and G_2 as seen in Fig. 2.1.

PROPHASE succeeds interphase and is that period after G_2 when condensed chromosomes are first detectable.

MITOSIS (M) follows prophase and is the period during which chromosome preparation occurs culminating in the formation of two nuclei and finally two daughter cells. There are 3 distinct sub-periods of mitosis known as metaphase, anaphase and telophase.

THE G_0 PHASE is an interval of variable length which a cell may enter immediately after having undergone division. When in G_0 a cell is said to be quiescent, that is in a non-dividing state. Many tissues in the adult organism are in this arrested stage and undergo no DNA replication and very little, if any, RNA synthesis. The commitment by a cell to enter this state is influenced by such factors as nutrient failure, cell-cell contact inhibition and intracellular negative regulators

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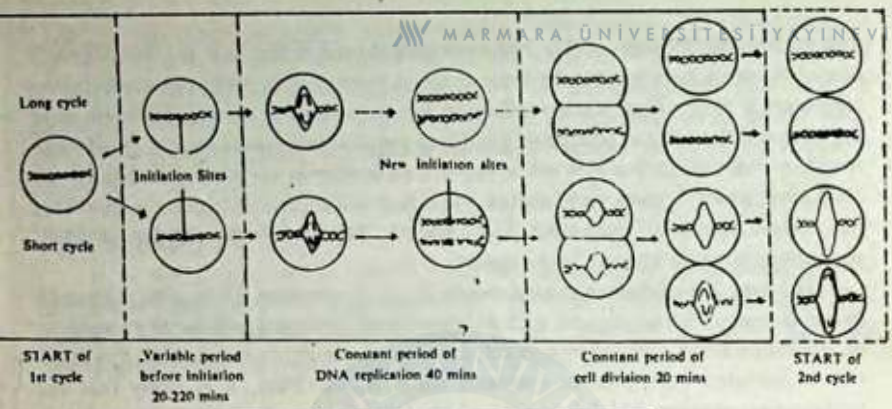

THE PROKARYOTIC CELL CYCLE

↑

CELL DIVISION

↓

THE EUKARYOTIC CELL CYCLE

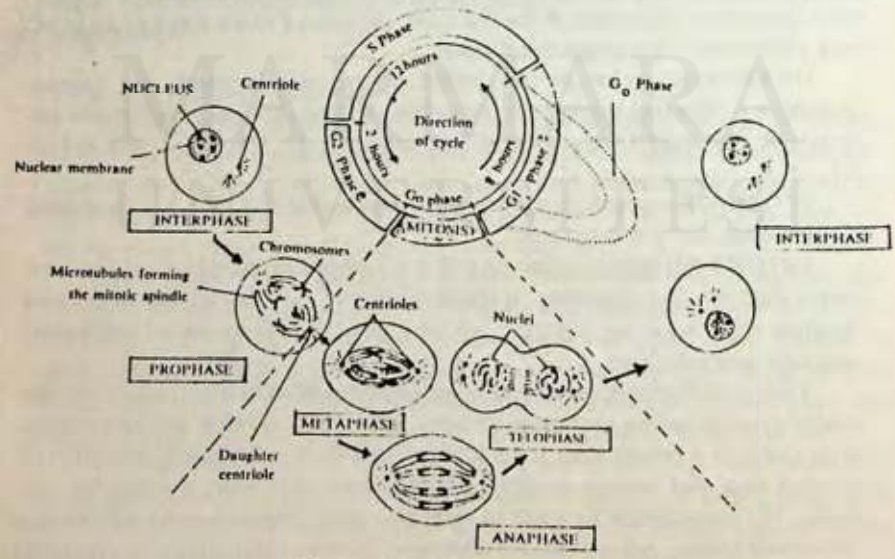

Fig. 2.1. The cell Cycle.

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(e.g. cyclic AMP under certain circumstances). When a cell in G_0 is challenged by certain hormones and other growth factors it may be stimulated to emerge from G_0 into the G_1 phase.

THE G_1 PHASE is the interval during which the cell commits itself to initiate DNA synthesis. It is variable in length (from a few hours to even days or longer) and during this period the cell prepares itself for the succeeding S interval in which the synthesis will occur. It is not known why the length of this state is so variable but when hormones are added to cells in the G_1 period, the length of the interval can often be reduced. This may be explained by a hormone-induced increase in the rate of production of certain key molecules. Frequently the hormone must be present in the cell or at its surface for as much as 8 hours before DNA replication can commence. It is not known why a continued hormone presence is required. When the cell is totally prepared by the G_1 events it enters the S phase.

THE S PHASE is the interval during which the DNA content doubles prior to division into daughter cells. This phase varies slightly from cell to cell and is about 6-8 hours long. DNA replication may be demostrated by measuring the incorporation of tritiated thymidine into DNA during this period. RNA synthesis also occurs. If tritiated-cytidine is added to cells in the S phase, the radioactive RNA appears first in the nucleus and then in the cytoplasm. While RNA synthesis occurs throughout interphase, it is at its highest level during the S phase. The cell may be arrested in the S phase by the addition of inhibitors such as actinomycin D which blocks RNA polymerase activity by intercalating between the DNA strands. Thus the cell cycle cannot continue until DNA replication has been completed. When the synthesis of nucleic acids and the doubling of most other cell constituents has taken place, the cell progresses to G_2.

THE G_2 PHASE is the interval of interphase during which no DNA replication and very little if any RNA synthesis takes place; that is, it is a "gap" period. A cell in G_2 carries twice the chromosomal content of a normal diploid cell. The length of G_2 is about 2-6 hours and is relatively constant from one cell type to another. During G_2 condensation of the chromatin occurs and the nuclear membrane disappears. At the junction of G_2 and M the cell enters the prophase state.

IN PROPHASE the highly condensed chromosomes actually consist of two intertwined but distinct entities called *chromatids*. Each chromatid is a complete copy of double-stranded DNA with its associated proteins. During prophase the centrioles, which have divided to form smaller daughter centrioles, move apart to form opposing poles. The complex bundle of microtubular projections which can now be seen radiating between the centrioles is known as the mitotic spindle. This is formed by microtubules which are organised between the centrioles to form a sort of egg-shaped scaffold. As the cell enters mitosis (M) the chromosomes become associated with this scaffold and the nuclear membrane is no longer visible. The chromosomes are attached to the microtubules at their centromeric regions.

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MITOSIS (M) is characterised by three distinct phases: metaphase, anaphase and telophase.

IN METAPHASE the chromosomes line up along the mitotic spindle to form the metaphase plate. The mechanism of this orienting operation is unknown.

IN ANAPHASE the chromosomes split into their constituent chromatids. One chromatid from each pair moves to one or the other end of the mitotic spindle. The movement of the chromatids, now called daughter chromosomes, is mediated by microtubule contraction but the precise way in which this occurs in unkown. When chromatid seperation is completed the cell enters telophase.

IN TELOPHASE the chromatids de-condense to form disperse chromatin and nuclear membrane forms. The mitotic spindle collapses and the plasma membrane invaginates from either side to produce two daughter cells. Each daughter cell then enters a new cycle at G_0 or G_1 and the process is repeated.

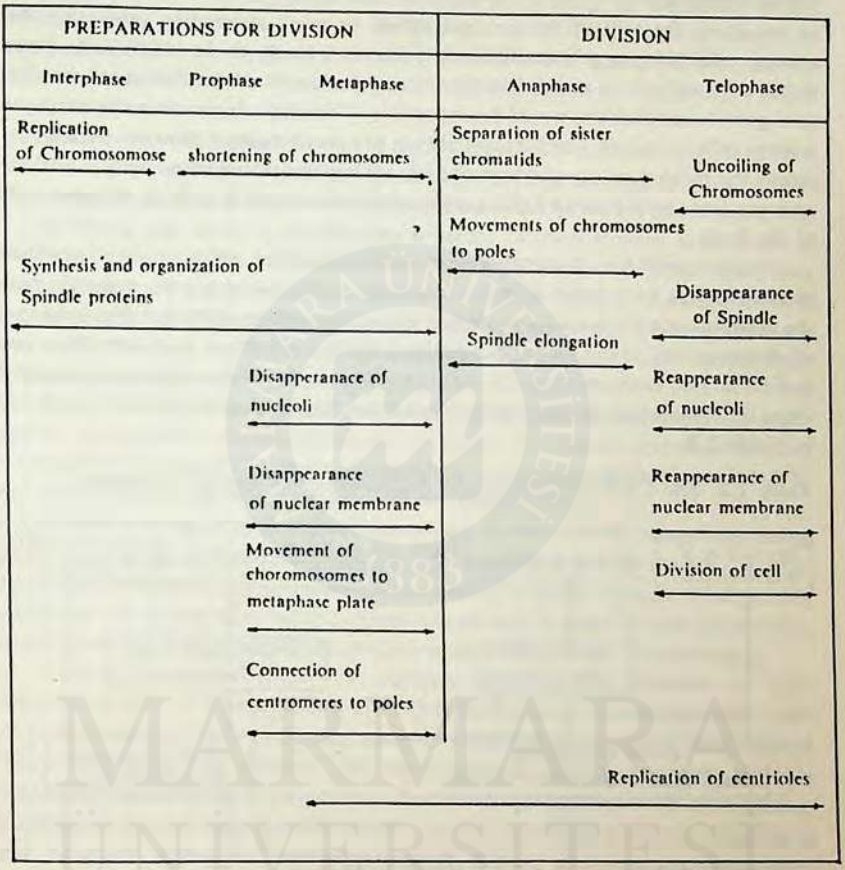
2.1.3 The sequence of events in cell division

Many aspects of cell division are not explained by a simple description of events taking place in cell as it progresses through its cycle from one interphase to another. It must be clear that the cell cycle is not a single process, but rather is a complicated affair in which the nucleus, cytoplasm, and their constituent parts take their turn in playing their roles. Just as a watch runs only when all of its parts function properly, the cell completes its division to form daughter cells only when all of the elements are in a state of readiness at the proper time in the cycle and when preceeding events have prepared the cell for the events to follow. For example, if the spindle is prevented from forming, the chromatids cannot segregate from each other, no anaphase movement occurs, and the cell cannot partition the cytoplasm by cell-plate formation. Table 2.1. summarizes the sequence of cell division.

The events occuring prior to prophase, although not visible under the microscope directly, are of primary importance because they control and prepare the cell for the more dramatic events of prophase, metaphase, anaphase and telophase. If a cell is making another product (for example hemoglobin or insulin) unrelated to division and is induced to divide, its manufacturing machinery must be turned off and the divisional machinery must be turned on. This is necessary because a cell prepares to divide long before the actual division takes place and if a division does occur, the incident is determined by the metabolic activities of the cell; that is, the division process itself makes a heavy metabolic demand on the cell and if the cell's metabolism is shunted in another direction it is unlikely to be able to prepare itself for division.

Although centrioles replicate in the duration of cell cycle in advance, this act does not determine whether a cell divides or not. The replication of chromosomes, however, appears to be the first crucial step. The cell cannot replicate its DNA and form the necessary histones of the chromosomes if it is engaged in any other significant metabolic activity.

Table 2.1 The sequence of events taking place in preperation for, and during cell division:



2.2. SEXUAL REPRODUCTION, MEIOSIS

In sexsual reproduction a new individual is produced by the coming together and fusion of two different sex cells, or *gametes*, usually derived from two parents of different sex or mating type. Gametes are produced in the *gonads*. The fusion of gametes is called *fertilization* or *syngamy* and the cell produced is called the *zygote*.

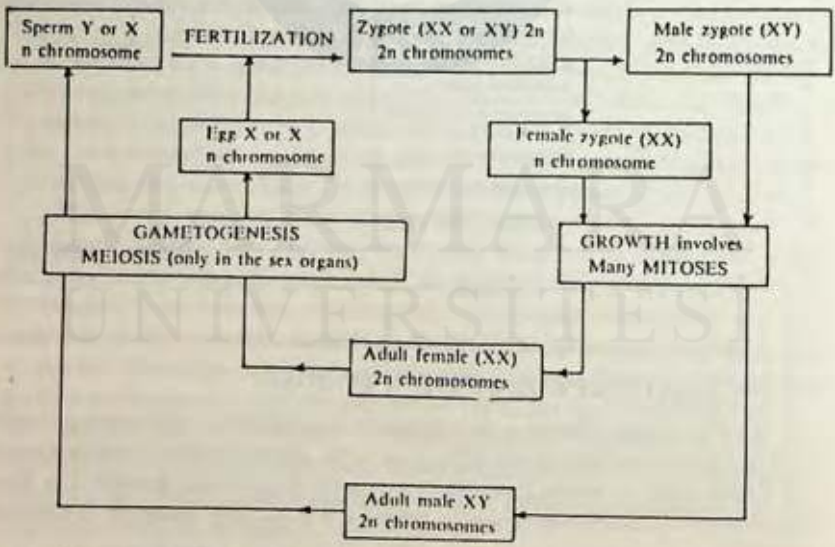
The zygote that results from fertilization contains a mixture of DNA, and therefore of genetic programs, from the two parents that contribute the gametes. The new organism grows by means of mitotic divisions of the zygote.

Gametes differ from all other body cells *(somatic cells)* in their DNA content.

If they had the same amount of DNA as body cells, designated by (2n) number, the zygote would have twice the amount of DNA as the body cells of each parent, and so would all the cells of the new individual. In every succeeding generation the amount of DNA per cell would double. If this were to go on indefinitely, there would eventually be so many chromosomes packed inside each cell that cell division (and gamete production) would be impossible. This chaos is prevented by a unique process called *meiosis*, which occurs only in the production of gametes. Meiosis involves the halving of the genetic material; each gamete designated by (n) number, emerges from meiosis with half as many chromosomes as present in all other cells of the body.

Somatic cells are almost all *diploid (2n);* that is, they contain pairs of matched chromosomes. As a result of meiosis, gametes are *haploid (n)*- they contain only the chromosome from each pair. When two gametes fuse, each contributes one set of chromosomes to the zygote, producing a diploid cell. Thus, each cell of the new individual that develops from the zygote has two copies of the genetic program-one from the female parent (X or X) and one from the male parent (X or Y) as shown in Table 2.2.

Table 2.2. The Cycle of Growth and Reproduction in Sexual Organisms.



We can summarize by saying that MEIOSIS is the process of two successive nuclear divisions (karyokinesis), which involves only one replication of the chromosomes. At the same time the process involves the two succesive divisions of the cytoplasm into four daughter cells (cytokinesis).

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While the basic pattern of chromosome behaviour is usually the same in both the male and female of a species, the two sexes often differ in the timing of the different stages of meiosis. In man, for example, the entire process is completed in a postpubertal male in about 2 months, whereas in the female the first meiotic division commences in the foetal ovary and is not completed until ovulation, which may take place fifty years later.

Another sex difference to evolve in the higher animal is the production of four functional gametes following meiosis in the male, but only one ovum in the female; the other nuclear products of female meiosis become polar bodies and degenerate.

A human egg, prior to fertilization, posseses 23 chomosomes, in contrast to the 46 in the zygote. The 46 choromosomes are not all individually different, they exist as 23 pairs, the members of each pair are HOMOLOGOUS to each other and NONHOMOLOGOUS with respect to the other chromosomes. In a zygote every pair of homologous chromosomes, or *homologous*, consists of one member contributed by the sperm and one by the egg.

One exception to the similarity of paired homologous in shape and size is the pair of chromosomes characterizing the two sexes. The human female is XX and consequently her paired chromosomes are similar and homologous, the male is XY, and the two chromosomes are essentially nonhomologous.

Meiosis is a rather complicated type of cell division yet the remarkable thing about it is that, like mitosis, the crucial nuclear events and end results are essentially the same whenever encountered. Consequently, a single account of it applies equally well to a fungus, an insect, a flowering plant or a man. Except for the type of cell resulting from meiosis, the process is basically similar in both sexes.

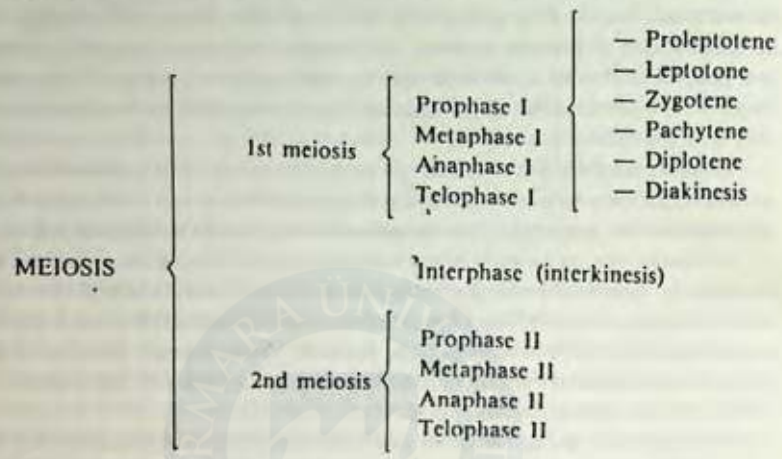
In a typical meiosis, the first division separates the members of each homologous pair of chromosomes after they have paired intimately with each other *(synapsis)* and have exchanged genetic material *(crossing-over);* two haploid nuclei result from this division. The second meiotic division separates the two longitudinal haves of the chromosomes *(i.e. chromatids)* in each of these nuclei, producing a total of four haploid nuclei. The first division is characterized by a long prophase which is subdivided into 6 stages: proleptotene, Leptotene, zygotene, Pachytene, Diplotene and Diakinesis. These are followed by the first meiotic Metaphase. The sequential stages of meiosis are shown in Table 2.3.

PROLEPTOTENE: This is the earliest stage in Prophase 1, It is very difficult to see the chromosomes because they are very thin, thread like structures at this stage.

LEPTOTONE: The chromosomes appear as an apparently confused network of fine threads bearing numerous dark staining granules, the *chromomeres*, which are thought to be differentially coiled segments of the basic chromosome thread.

Close inspection of certain areas of the nucleus reveals an association between matching pairs of chromomeres on homologous chromosomes , suggesting that the leptotene chromosomes have a definite, organization. Although no split is visible in

Table 2.3. Diagrammatic Representation of Sequential Stages of Meiosis.



the threads and chromomeres of individual chromosomes, it is well established that the bulk of chromosome replication has already occured during the pre-meiotic interphase and that by the time of their appearance at leptotene the chromosomes have doubled.

Nuclei at the leptotene stage often contain chromosomes in which small homologous segments appear to be undergoing synapsis, the definitive feature of zygotene. During the zygotene stage some paired homologues begin to condense in a form which is characteristic of the pachytene stage. Such observations emphasize the continuous and dynamic nature of the meiotic process and the diffuculty of separating one stage from another. It is often convenient, therefore, to consider the terms leptotone, zygotone etc. as adjectives describing the condition of chromosomes, rather than strictly definable and mutually exclusive stages.

ZYGOTENE: It is characterized by the synapsis of homologous chromosomes. When the *synapsis* is complete, the paired chromosomes are referred to as *bivalents*. As with other mammalian chromosomes, the human X and Y chromosomes behave differently from the autosomes by condensing into a dark-stained heterochromatic body called a *sex-vesicle* in which they are assumed to be paired by a small terminal region of homology.

PACHYTENE: This is the longest stage of the meiotic prophase during which the bivalents condense by developing a complex spiral structure. The sex vesicle remains clearly visible until the later stages when the sex chromosomes revert to the same state of condensation as the bivalents begin to separate.

DIPLOTENE: Contraction of the bivalents and the repulsion of the homologous partners are the main features of diplotene. The integrity of each

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bivalent is maintained only by *chiasmata* which are structures formed between homologous chromatids as a result of earlier genetic crossing-over between the synapsed homologues. Each chiasma reflects the accurance of a single cross-over event. As the bivalents continue to contract and repulsion between the chromosomes increases, the chiasmata appear to move towards the ends of the chromosomes. The process is called *terminalization* and is responsible for a decrease in the total number of chiasmata from the beginning of diplotene to the end of the following stage, diakinesis.

DIAKINESIS: This stage is similar to diplotene and is characterized by the bivalents and reduction in the number of chiasmata by terminalization.

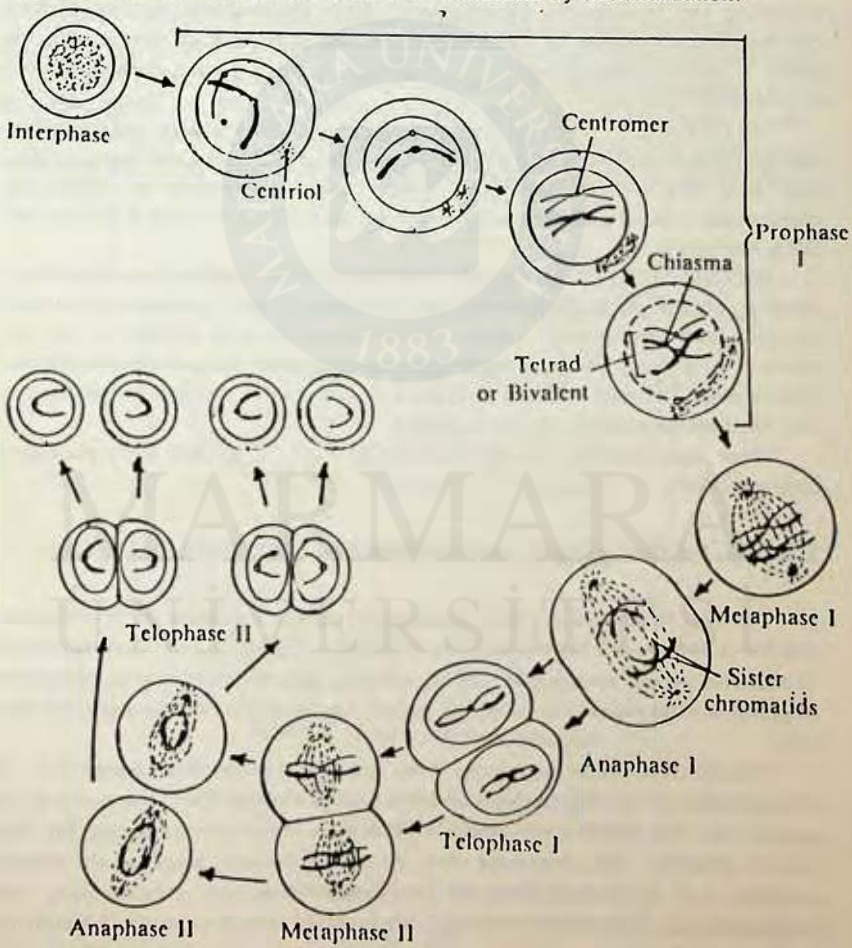

Fig. 2.2. Diagram of Meiosis

METAPHASE I: The bivalents are at their maximum state of condensation when they line up along the equator of the cell. The centromeres of each pair of chromosomes forming a bivalent become attached to the spindle fibres on opposite sides of the equatorial plate. The shape of each bivalent is determined by the number and location of the chiasmata. In the human male, at the first meiotic metaphase, the number of chiasmata per bivalent is usually between one and five, the XY bivalent being rod shaped as a consequence of its single, terminally placed chiasma.

ANAPHASE I: Opposing centromeres move to opposite poles of the cell, thus separating the homologous chromosomes, Each chromosome consists of two chromatids held together by the centromere which, in contrast to mitosis, remains intact. It should be remembered that as a result of crossing-over, each chromatid is genetically different.

TELOPHASE I: When the two groups of chromosomes arrive at the poles, they may pass immediately into the prophase of the second meiotic division, or they may pass into a brief interphase period called *interkinesis* in which the chromosomes uncoil and become very diffuse. In neither case there is any further DNA replication.

SECOND MEIOTIC DIVISION: The second meiotic division consists of Prophase II, Metaphase II, Anaphase II and Telophase II and is similar to a mitotic division. Each chromosome consists of two chromatids held together at the centromere. At division the centromere splits and separates the two chromatids one to each pole. The only essential difference from a mitotic division is that there is only the haploid number of chromosomes.

These stages can be followed through in Fig. 2.2 as they are represented diagramatically.

2.3. CROSSING-OVER AND GENETIC RECOMBINATION

The two significant features of meiosis are: the reduction of chromosome number to compensate for the doubling effect of fertilization, and the reassortment of maternal genetic material so that the resulting gametes carry new combinations of grandparental genes. The reduction process has been described in a previous section.

Genetic recombination results from (a) the independent segregation of chromosomes at the first meiotic division and b) crossing-over, the exchange of genetic material between the paired homologous chromosomes during the first meiotic prophase. The chiasmata observed during the later stages of the meiotic prophase are cytological evidence of earlier cross-over events. Only two chromatids, one from each homologue, are involved in each chiasma. A simple interpretation of a chiasma and its genetic consequences can be explained by the two

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commonly accepted crossing-over hypotheses.

The mechanism of crossing-over is neither well understood nor is the prerequisite of chromosome synapsis. A number of hypotheses have been put forward to explain each of these events but none has been verified experimentally.

i- Crossing-Over

The first theory of crossing-over, which was most widely accepted during the classical period of the 1930's, involved physical breakage and exchange of genetic material at the chromatid level (1)

It was postulated that tension created in the paired chromosomes by their coiling was relieved at different points by breakage of non-sister chromatids followed by a crossways reunion of their broken ends, resulting in a reciprocal exchange between chromatids of different parental origin. The later appreciation of the molecular nature of genes and the precision with which crossing-over must occur at the molecular level made this "torsion hypothesis" difficult to accept. (Fig. 2.3.A)



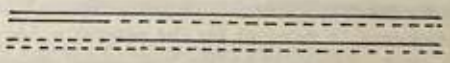
Homologues paired and coiled



Torsion pressured relived by breakage



Reunion of non-sister chromatids



Recombination of parental genes

Fig. 2.3A. Breakage und reunion hypothesis of crossing-over;

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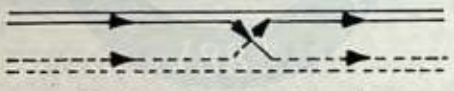
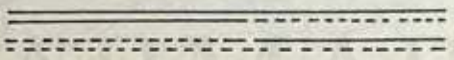
An alternative mechanism, known as "copy choice", was proposed in the 1950's (2). The hypothesis related crossing-over to chromosome replication and was a modification of a concept originally proposed by Belling in 1933 (3). It suggested that if chromosome replication occured while homologous chromosomes were closely paired, then the new DNA strand being formed along the template of one chromosome could switch to copying the template of the partner chromosome. A reciprocal switch at the same point by the partner would result in reciprocally recombinant chromatids. The apparent association of homologous chromosomes during interphase makes this hypothesis attractive, but there is an abundance of evidence against it, including the requirement of a conservative method of chromosome replication, which conflicts with the generally accepted semi-conservative replication of both DNA and chromosomes (4.5). As seen in Fig. 2.3 B.



Homologues closely associated during chromosome (DNA) replication



Reciprocal switch of template



Recombination of parental genes

Fig. 2.3B. Copy choice hypothesis of crossing-over:

In the mid-1960's it was clearly demonstrated that material from the parental chromosomes was physically incorporated into the recombinant chromosomes indicating that crossing-over must involve some form of breakage and reunion. At about the same time a number of complex hypotheses for crossing-over were proposed which attempted to account for the process at the molecular level such as Whitehouse, Holliday, (6) and Uhl. (7) The model proposed by Whitehouse and Hastings in 1965 (8) reconciles the requirment for breakage and reunion with evidence that low levels of DNA (and protein) synthesis occuring at the zygotene and pachytene stage of meiosis may be necessary for crossing-over which is shown by Hotta et al in 1966 (9) and Lima-de-Faria et al in 1966 (10) in their publications.

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In summary we can say that the breakage hypothesis consists of a break occuring at the same level in a pair of non-sister chromatids and following the break, the non-sisters join together. The result is two reciprocally recombinant chromosomes. The copy-choice hypothesis assumes that recombination occurs during the process of chromosome duplication, the process of "copying" or duplication can proceed along either chromosome when this occurs a cross-over is the result.

You will note that according to the last hypothesis only the two newly formed strands can participate in a copy-choice type of recombination. The two original strands remain intact. In fact, tetrad analysis has demonstrated that all four chromatids can participate in crossing-over as shown by the fact that multiple-strand double cross-overs occur.

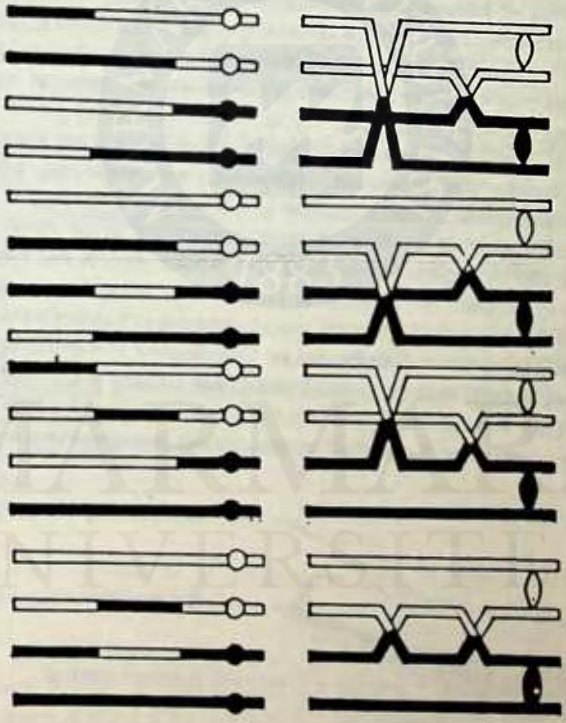

Fig. 2.3C. Types of double chiasmata and their genetic consequences. In some experiments with Neurospora, all four types of double chiasmata are found to occur with equal frequency. There seems to be no chromatid interference in chiasma formation. In other words, the strands forming one chiasma are not effected by those which form the other.

Furthermore, crossing-over occurs at a four-strand stage and it is reciprocal. The fact that all four chromatids participate in crossing-over cannot be reconciled with the copy-choice hypothesis unless the assumption is made that a mechanism exists allowing crossing-over to occur between sister strands. The recombination in phage and bacteria can occur by the breaking and joining of double-stranded DNA molecules. There is as yet, however, no direct evidence to show that recombination can occur by the breaking and joining of double-stranded DNA in nucleated organisms. Some fraction of recombination in all organisms may possibly occur by a copychoice mechanism, but there is no evidence of it at a molecular level (11).

ii- Chromosome Pairing

The pairing of homologous chromosomes can occur at a superficial level or at a very intimate one. The intimate form of pairing or synapsis is restricted to the first meiotic prophase. Observations done by various researchers suggest that there is some degree of homologous association before the onset of spermatogonial mitosis. Since there is little scope for chromosome movement in an interphase nucleus, it is probable that homologous chromosomes form some sort of close alignment during the preceding telophase and maintain their association throughout interphase. Close alignment of homologues during the premeiotic interphase would account for their apparent association at the onset of the meiotic prophase noted in the leptotone phase and would obviate the need for complex hypotheses to account for long range forces which bring homologues together in preparation for synapsis. The numerous observations of somatic chromosome pairing and the non-random distribution of homologous chromosomes in metaphase preparations suggest that an ordered interphase nucleus is the rule rather than the exception (12). A model in which this order is maintained by attachment of interphase chromosomes to the nuclear membrane has been proposed by Comings, 1968 (13).

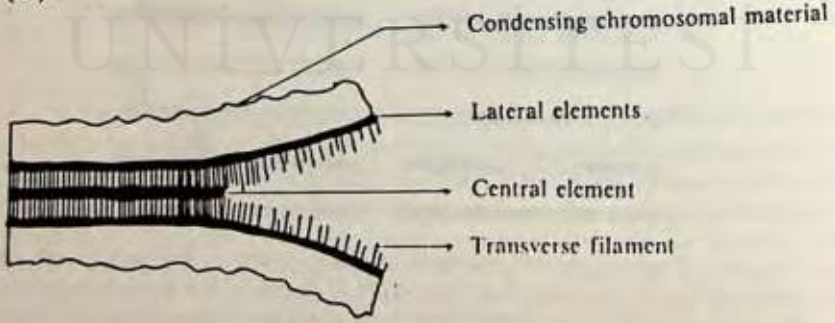

Fig. 2.4. Synaptonemal Complex.

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Synapsis is limited to the first meiotic prophase, and is distinguished from superficial pairing by the formation of a tripartite structure called *synaptonemal complex* (14) which lies between the paired chromosomes as seen in Fig. 2.4. It shows that the synaptonemal complex is protein in nature and consists of a central element connected to a lateral element on the pairing face of each chromosome by transverse filaments. the dimensions and complexity of the structure vary considerably in different organisms but these basic features of the complex are constant throughout the wide range of plants and animal species studied. There has been much speculation on its properties and function. Since enzyme studies indicate that all the elements and filaments of the complex protein and do not appear to involve DNA, it is unlikely that the complex is itself the manifestation of crossing-over between the genetic material of the chromosomes. It presents, however, an ideal structure for bringing the genetic elements together at the molecular level in a zipper-like manner (15 and 16), although evidence for this must be regarded as inconclusive. As a general rule, it appears that synapsis and the formation of the synaptonemal complex are necessary for crossing-over and the formation of chiasmata.

2.4. DIFFERENCES BETWEEN MITOTIC AND MEIOTIC CELL DIVISION

MITOSIS	MEIOSIS
1) It is observed in somatic cells.	1) It is observed only in sexually reproducing cells.i.e. gametogenetic cells.
2) It continues throughout the lifetime of the organism.	2) It takes place only when the organism reaches sexual maturity.
3) In the life cycle of a mammalian cell, interphase, which consists of G_1, S, G_2, is followed by only one mitotic cell division.	3) In gametogenetic cells, interphase is followed by two mitotic cell divisions.
4) It takes a short time.	4) It continues for a considerably longer time.
5) There is no occurence of crossing over between the chromatids.	5) There is usually genetic material crossing-over observed in the chromatids in meiotic division.
6) There is no change in the chromosome number. It always contains the same diploid (2n) somatic cell number.	6) Chromosome number is reduced to haploid (n) gametic number.
7) It is almost the same in all living organisms.	7) In each individual living organism, it shows specific details.
8) Mitotic division enables the continuation of cells.	8) Meiotic division enables the continuation of species.

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2.5. SEXUAL REPRODUCTION IN MAMMALS

In respect to cell division, the reproductive cycles of men and mice are no different in principle than in *drosophila* (friut fly). Differing from plants, animals generally produce their haploid gametes by meiosis directly from diploid tissue (Primordial germ cell) rather than through the intervention of haploid gametophyte generation. As in most animals, the haploid phase of mammals is reduced to a single cell stage, i.e., the sperm or the ovum. The process that gives rise to gametes is called *gametogenesis* and takes place in *gonads*. The gametes of male animals, *sperm*, are formed in the *testes* (sing.; testis) of diploid adults. This process is called *spermatogenesis*. The gametes of female animals, ova (sing.; ovum), are produced in the ovaries by the process of *oogenesis*.

In mammals, fertilization is internal, like in reptiles and birds, but there is a great difference. In mammals, after fertilization, the eggs (ova) are retained in the female's body until the young animals are more or less fully formed. After birth the young are fed on milk secreted by the *mammary glands* of the female adult and they are protected by their parents until they become fully independent.

i- Spermatogenesis

In spermatogenesis, primordial germ cells divide repeatedly by mitosis to form diploid *spermatogonia*. There then follows a brief period of growth, during which each spermatogonium increases in size to form a *primary spermatocyte*. This then divides meiotically, the first meiotic division giving two *secondary spermatocyte*.

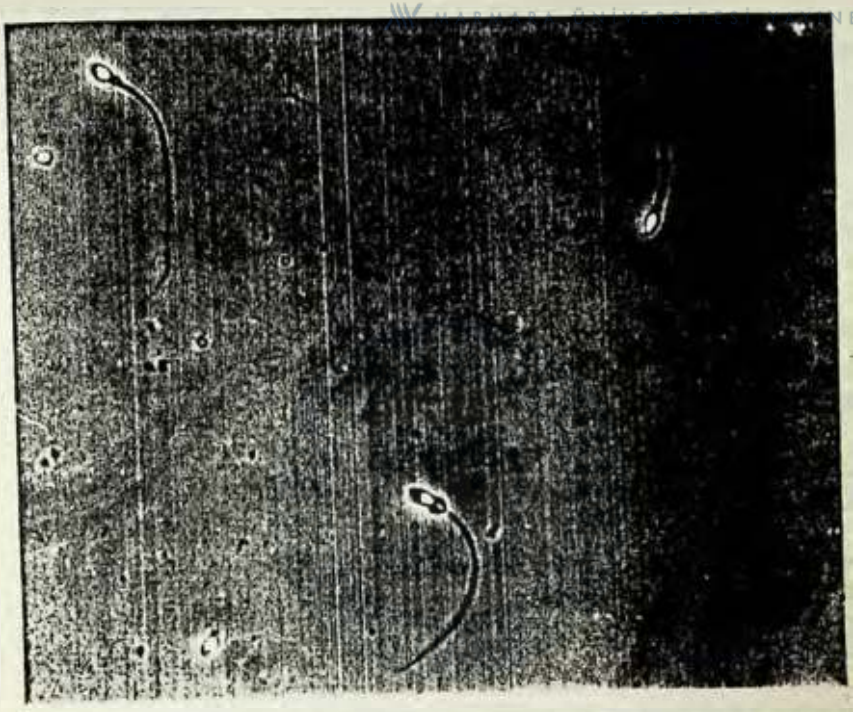

Fig. 2.5. Phase contrast photograph of normal and abnormal mobile human sperms in the suitable culture medium.

and the second a group of four *spermatids*. Finally each spermatid, at this stage a seemingly unspecialized cell, differentiates into a *spermatozoon* with characteristic head and tail (Fig. 2.5). Clearly a large number of spermatozoa are formed from a single primordial germ cell. Sperm are approximately uniform in size. A sexually mature human male produces about 50,000 sperm per minute (Fig. 2.5).

A microscopical section of a mature testis shows sperm at different stages of development as described above. The testis is composed of numerous *seminiferous tubules* in whose walls spermatogenesis takes place (Fig. 2.6). The primordial germ cells are formed from the *germinal epitelium* lining the outside of the tubule, and as cell divisions proceed the daughter cells move towards the lumen of the tubule. The final transformation of spermatids to spermatozoa takes place in the part of the wall immediately adjacent to the lumen. At this stage the heads of the developing sperm are enveloped by large *sertoli cells*, from which they derive their nourishment. The sperm tails project into the fluid-filled lumen of the tubule. Eventually,

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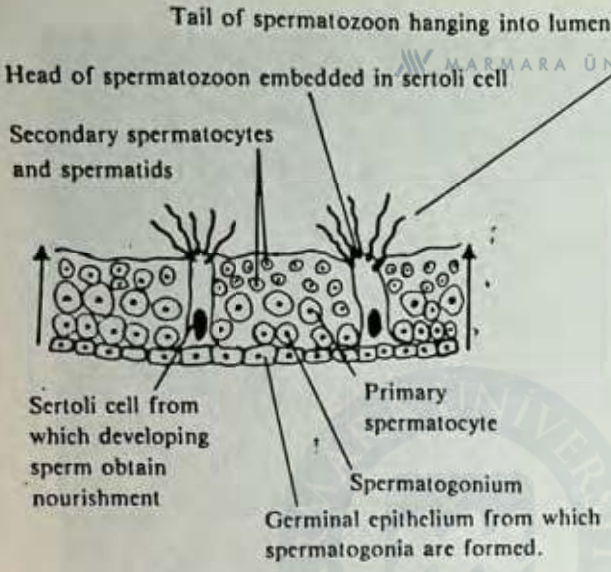

Fig. 2.6. Section through the wall of semiferous tubule showing the formation of spermatozoa.

the mature spermatozoa become detached and are released into the lumen. Spermatogenesis is particularly sensitive to temperature. If the temperature is too high, the sperm fails to develop, or develop abnormally. This is why the testes are held in a *scrotal sac* outside the abdominal cavity, and it is also why insulated, or moved back into the abdomen by surgical means, a low sperm-count results.

ii- Oogenesis

Oogenesis is basically similar to spermatogenesis, but different in detail. A primordial germ cell proliferates mitotically to form *oogonia*, but only one of these grow into a *primary oocyte*. The others degenerate. the amount of growth that takes place at this stage is much greater than in spermatogenesis; this is what makes the egg so much larger than the spermatozoa. The primary oocyte now undergoes meiosis, but the divisions are unequal resulting in the products differing greatly in size. The first meiotic division produces a *secondary oocyte* and a very much smaller *polar body* which may be seen adhering to it. The second meiotic division results in production of another polar body. The secondary oocyte now becomes the functional ovum, with the haploid number of chromosomes. Meanwhile the first polar body may undergo a second meiotic division resulting in a total of three polar bodies, all haploid of course (Fig. 2.8). The events described are essentially about the formation of spermatozoa, but whereas in spermatogenesis all four pro-

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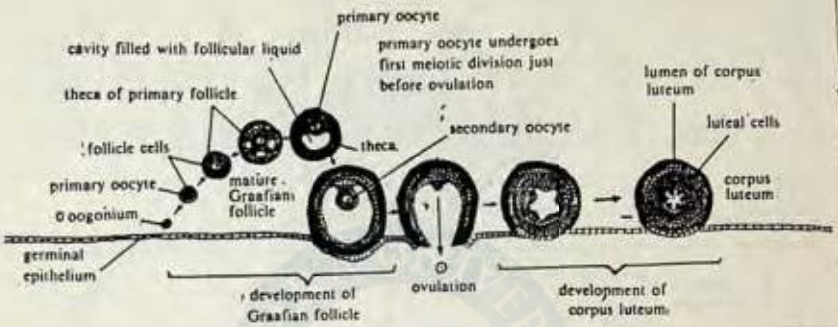

Fig. 2.7. Formation of an egg in a mammalian ovary.

ducts of meiosis develop into functional gametes, in oogenesis only one of the four becomes a functional ovum. The rest are extruded as non-functional polar bodies which eventually degenerate. The polar bodies are the inevitable result of the fact that meiosis involves two successive divisions; their function is simply to receive half the chromosomes, thereby making the ovum haploid.

A histological overview of an ovary shows comparable events taking place in the formation of ovum. Primordial germ cells, whose precise origin is uncertain but possibly derived from the germinal epithelium lining the outside of the ovary, divide to form oogonia which migrate inwards towards the centre. Each oogonium becomes enveloped by a layer of *follicle cells*. The whole structure, about 50 μm in diameter, is called a *primary follicle* (Fig. 2.7). At birth there are between 200,000 - 400,000 primary follicles in each ovary, but only 200 to 400 complete their development. The rest degenerate into *atretic follicles* which are visible in the ovary as small cyst-like bodies that never produce ova.

As the oogonium grows into a primary oocyte, the follicle cells surrounding it proliferate to form a wall many cells thick. As this occurs, a fluid, called *follicular liquid*, collects between the cells, forming little pools. As more and more fluid accumulates, the pools come together, eventually forming one large cavity filled with follicular liquid. The oocyte is embedded in a little hillock of follicle cells projecting into this cavity. Meanwhile, connective tissue inside the ovary forms a protective sheath surrounding the follicle. This is divided into two layers a highly vascular *theca interna*, and a less vascular, more fibrous, *theca externa*. The whole thing is now called a *graafian follicle*. The follicle moves slowly back towards the

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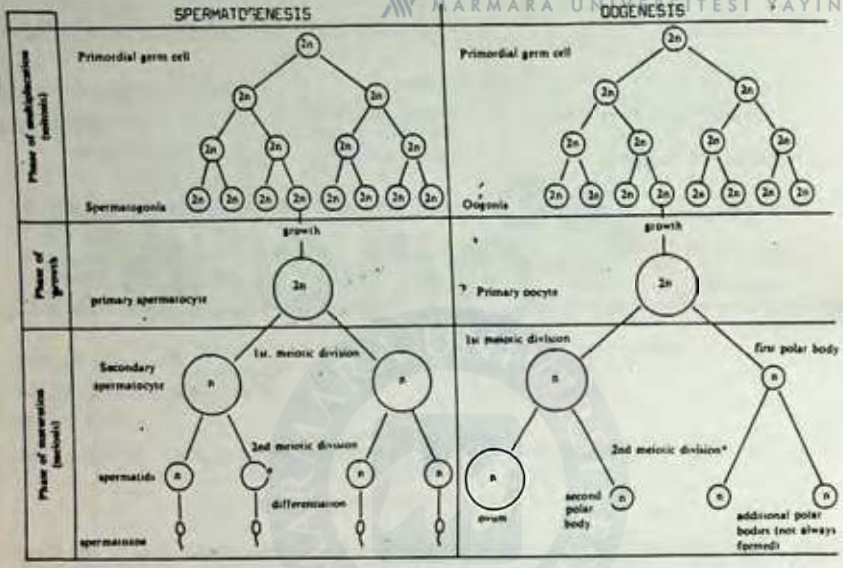

Fig. 2.8 Summary of events involved in the formation of sperm and ova in a mammal.

surface of the ovary, increasing in size as it does so. From a mere 50 μm in diameter, it eventually reaches a diameter of about 12-16 mm and causes a distinct bulge on the surface of ovary. When it is time, this bulge ruptures and the oocyte, pinched off from its attachment to the wall of the follicle, is extruded from the ovary (see Fig. 2.7). This is known as *ovulation*, and in a human female who has reached puberty, it occurs in one of her two ovaries approximately once every 28 days. Shortly before ovulation, ovum, which you will remember is a primary oocyte at this stage, undergoes its first meiotic division (Fig. 2.9) The second meiotic division does not normally take place until fertilization. After ovulation the role of the follicle in protecting and nourishing the ovum comes to an end.

When there is a successful union of sperm and ovum, *fertilization* occurs, and the resulting diploid cell is called a *zygote* (Fig. 2.10). By the time the zygote reaches the uterus, it has already divided mitotically to form a hollow sphere of cells, the blastocyst. After several days the blastocyst becomes embedded in the lining of the uterus, a process called *implantation*, and there it continues its development.

* usually occurs immediately after fertilization.

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Fig. 2.9 A typical immature human oocyte displaying the absence of an expanding cumulus mass, a compact layer of coronal cells adherent to the zona pellucida, slightly granular appearing ooplasm, and an intact germinal vesicle with nucleolus.

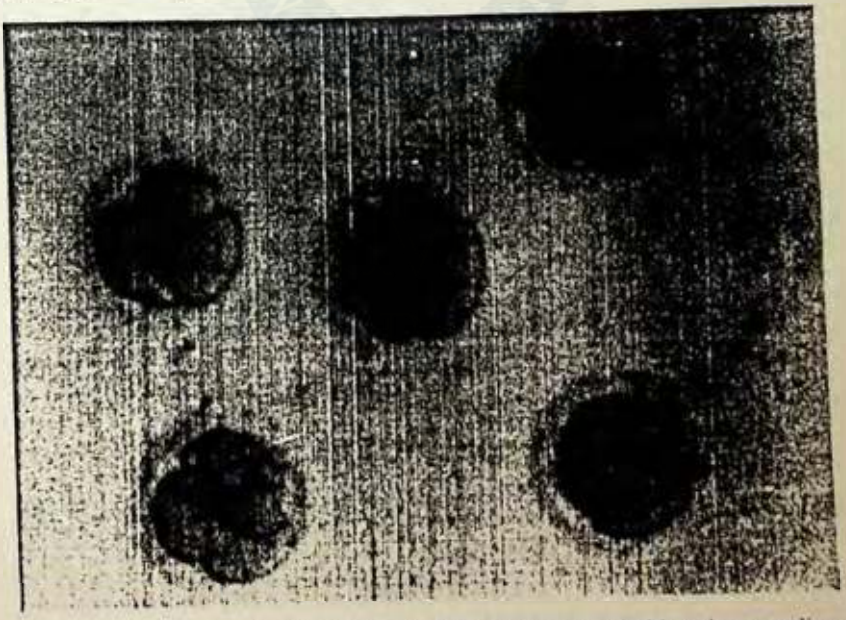

Fig. 2.10. After an incubation period of 72-90 hours in a suitable culture medium, normally fertilized human oocytes at different stages of their development.

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3

MENDELIAN PRINCIPLES

The discovery of the basic rules of heredity by the Austrian monk Gregor Mendel is an often-repeated tale. However, it is hard to overemphasize the methodological as well as the conceptual leaps that Mendel made. First, he chose a simple organism, the garden pea, which could be easily grown and fertilized. The flowers normally self-pollinate, but they can be opened up and artificially pollinated without damaging them or their ability to form seed (Fig. 3.1). Thus Mendel was able to make experimental genetic crosses by fertilizing the ovules of

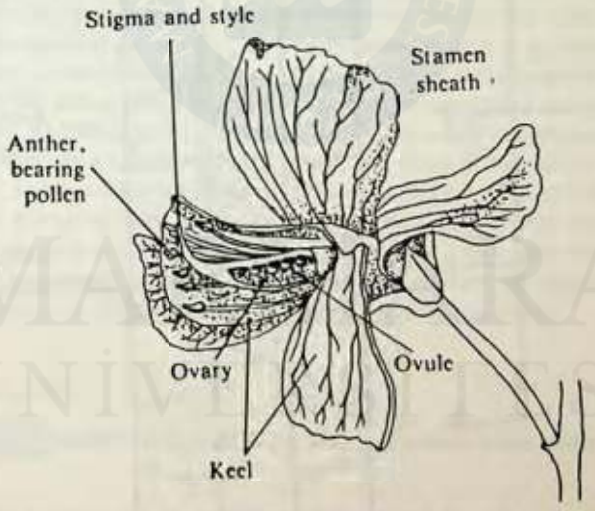

Fig. 3.1 Cutaway view of reproductive parts of a pea flower. (After J.B. Hill, H.W. Popp, and A.R. Grove, Jr., Botany, McGraw Hill, 1967).

one strain with the pollen from another. Second, he was careful to select a particular variety of pea *(Pisum sativum)* that had been purified by repeated self-pollination so that each pure strain always "bred-true"; that is, all plants produced

had identical characteristics. Third, the "characters" or traits, that Mendel chose to study-such as the colour and texture of seeds and seed coats, the position of flowers on the stems, and the length of the stems-could be determined unambigously so that no confusion arose in counting the number of offspring of each type after each cross. Finally, and most important, he reached his conclusions with the aid of vigorous mathematical analyses of large numbers of descendants of his experimental crosses.

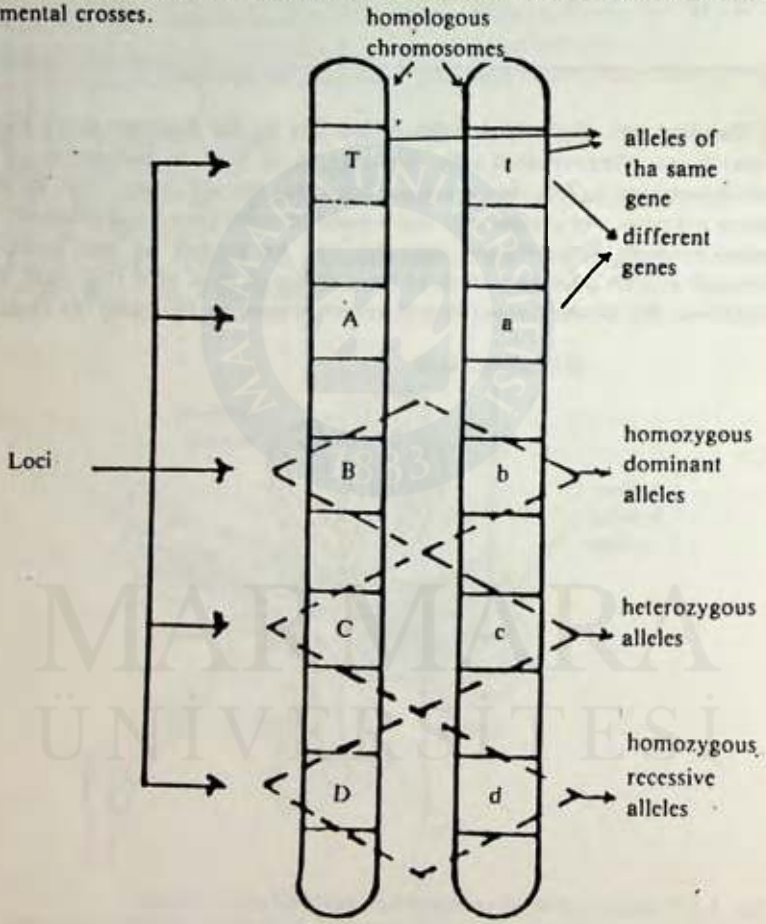

Fig. 3.2. Diagram summarizing the relationship between the genes and chromosomes of a diploid organism, and the terms used to describe them. The loci (singular: locus) are the positions along the length of the chromosomes where the genes occur. Alleles of the same gene always occur at one particular locus.

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Mendel used his pure strains of peas to demostrate that if certain traits are expressed in either parent, all off-spring will express those traits. Such traits are called *dominant*. Other traits are expressed in offspring only if they are expressed in both parents (each a pure strain). These *recessive* traits are alternate forms of the dominant traits.

When a gene pair in a organism contains two identical alleles, e.g, R and R, the organism is considered *homozygous* for that gene pair and is called a *homozygote*. When two different alleles are present in a gene pair, e.g. R and r, the organism is *heterozygous* for that gene pair and is called a *heterozygote*. The effect of a recessive gene does not appear in a heterozygote that also contains its dominant allele. Recessive characteristics are therefore only apparent in recessive homozygotes. Of course, an individual pea that is a recessive homozygote at one pair need not necesarrily be so at another, e.g., rrYy (Fig. 3.2).

As an important aid in distinguishing the appearance of an organism from the genetic factors that influence it, the term *phenotype* is used for the former and *genotype* for the latter. In their broad definitions, phenotype refers to all the manifold biological appearances, including chemical structural and behavioral attributes, that we can observe about an organism but excludes its genetic constitution. Genotype defines only the complement of genetic material (or genome) that an organism inherits from its parents. The developmental transformation of an organism from one stage of its life to another is a result of the unique interaction of its genes and its environment at each moment of its life history. Organisms are determined neither by their genes nor by their environment, they are the consequence of the interaction of genes and environment. Although the phenotype changes with time as the appearance of the organism changes, the genotype remains constant except for the rare genetic changes known as mutations.

3.1. INDEPENDENT SEGREGATION (CROSSES WITH SINGLE FACTORS)

Mendel concentrated on seven "unit characters" or types of traits. For each unit character he had plants with two alternative traits; tall versus short stem, axial flowers versus terminal flowers, round versus wrinkled, yellow versus green, inflated versus pinched pods and so on. In a typical test cross he had in the first filial generation all round seeds.

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parental generation (P) ♀ RR X rr ♂

gametes R r

First filial generation (F_1) Rr

Phenotypes : all round seeds

r: represents recessive and wrinkled trait R: represents dominant and round traits

The *reciprocal cross;* that is, a round male crossed with a wrinkled female plant, produced the same result in F_1. Mendel concluded that it makes no difference which way the cross is made.

When F_1 self-pollenated, in the resulting second Filial F_2, he found that 3/4 were round seeds and 1/4 were wrinkled seeds.

F_1 Rr X Rr

gametes ½R ½r ½R ½r

F_2 RR Rr Rr rr

3/4 1/4

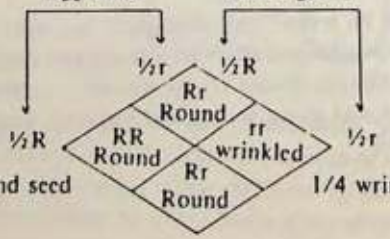
phenotypes: 3/4 round seeds
1/4 wrinkled seed (alternative trait absent in F_1)

The combination of factors for the F_1 cross are presented in a Punnett square, in which all possible combinations of segregated factors (genes) are shown.

F_1 plant Rr X Rr (All F_1 seeds are round because R dominates over r)

F_1 gametes ½R ½r ½R ½r

Egg cells Pollen grains



F_2 plant 3/4 round seed 1/4 wrinkled seeds

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The reapperance of the recessive character in F_2 generation indicates that recessive genes are neither modified nor lost in the hybrid (Rr) generation, but that the dominant and recessive genes are independently transmitted and so are able to segragate independently during the formation of sex cells. This rule of independent segregation is frequently referred as *Mendel's first law.*

In crosses like in F_1 and F_2, individuals homozygous for the dominant factor (RR) cannot be distinguished from heterozygous individuals (Rr) by inspection because they both have the dominant round-seed phenotype. To distinguish between homozygous dominant plants and heterozygous plants with only one dominant factor, one can perform an experiment called a *testcross.* In a testcross the individual to be tested is crossed with a homozygous recessive individual. In such a cross, all progeny of homozygous dominant display the dominant phenotype, whereas half the progeny of a heterozygote have the dominant phenotype and half have the recessive trait.

Parent (P) ♀ Rr X rr ♂

Testcross progeny gametes (G) ½R ½r ½r ½r

F_1 Rr rr Rr rr

Phenotypes: 1/2 round 1/2 wrinkled

3.2. INDEPENDENT ASSORTMENT (CROSSES WITH MULTIPLE FACTORS)

The experiments described thus far dealt with a single gene pair (a mono hybrid system), where only one gene is effecting one character. The next obvious question is what happens when a dihybrid cross is made, involving two pairs of genes affecting two different characters.

Mendel's experiments with parents differing in two pairs of traits such as Round and yellow seeds (RRYY) versus wrinkled and green seeds (rryy) showed that according to the rules of chance, the four possible combinations (Ry, RY, rY, ry) would be present in equal numbers. If, for each characteristics one trait showed dominance, the over all ratio of individuals in the F_2 generation should be nine with round yellow seeds to three with round green seeds, three with wrinkled yellow seeds and one with wrinkled green seeds or simply 9:3:3:1.

A dihybrid cross represented in the Punnet square for the F_2 generation

shows that phenotypes in the 16 boxes are in a ratio of 9:3:3:1 because of the independent assortment of alleles from the F_1 generation.

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P generation ♀ R R Y Y X rr yy ♂
Round-Yellow Wrinkled-Green

Gametes R Y r y

F_1 generation Rr Yy
Round-Yellow

RrYy X RrYy

Gametes
(Pollen grains ♂) ¼RY, ¼Ry, ¼rY, ¼ry (Egg cells ♀) ¼RY, ¼Ry, ¼rY, ¼ry

F_2 generation

♂/♀	RY	Ry	rY	ry
RY	RRYY	RRYy	RrYY	RrYy
Ry	RRYy	RRyy	RrYy	Rryy
rY	RrYY	RrYy	rrYY	rrYy
ry	RrYy	Rryy	rrYy	rryy

F_2 generation phenotypes

round yellow seed in a 9:16 ratio
round green seed in a 3:16 ratio
wrinkled-yellow seed in a 3:16 ratio
wrinkled-green seed in a 1:16 ratio

The main conclusion emerging from this experiment is that two pairs of alleles are transmitted independently from parents to offspring and assort freely. This idea is embodied in *Mendel's second law of independent assortments* which says that each of a pair of alleles may combine randomly with either of another pair.

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3.3. THE CHROMOSOMAL THEORY OF HEREDITY

The regularity with which chromosomes duplicate, separate, and are restored in number after fertilization suggests that chromosomes must be biologically important structures. A large number of individuals *within* a given species demostrate a constant number of chromosomes in every individual (with very rare exceptions). Chromosome numbers vary remarkably between different species. The entire chromosome complement of a single somatic cell is called a *genome*.

Soon after the rediscovery of Mendel's laws in 1900, Sutton and Boveri (1) recognized that there is a parallel behavior of Mendelian genes and chromosomes with respect to segregation and independent assortment. This connection led to the formulation of the *Chromosomal theory of inheritance*, according to which the chromosomes, which become visible to the microscope only during meiosis or mitosis, actually have a continous existance and are made up of genes. In today's terms we can summarize that an organism's phenotype is determined by the kind of genes it has in its cells (Fig. 3.3).

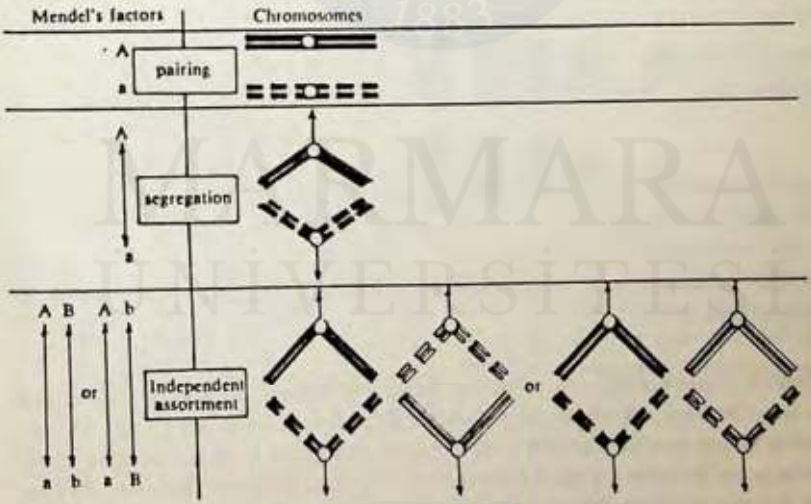

Fig. 3.3 Parallels in Behavior of Mendel's hypothetical particles (genes) and chromosomes at meiosis. Solid and dashed lines are used to distinguish one homologue from another.

3.4. SEX LİNKAGE

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In the crosses discussed so far it did not matter which sex of parent was selected from the strain under study; that is, reciprocal crosses yield similar progeny. The first exception to this pattern was discoved in 1910 by Morgan (2) who studied inheretence in *Drosophila melonagaster*. The normal eye colour of Drosophila is bright red. However, he had disçovered a male with white eyes. When he crossed this male with red-eyed females, all F_1 progeny had red eyes, showing that the allele for white is recessive. Crossing red-eyed F_1 males and females, Morgan obtained a 3:1 ratio, but all white-eyed flies were males. Among the red-eyed flies, the ratio of females to males was 2:1. Furthermore, when he crossed white-eyed males with red-eyed female progeny of white males and red females, he obtained red males, red females, and white females in equal numbers. Finally, in a cross of white females and red males (which is a reciprocal cross of the original white male with a red female), all females were red and the males were white. This is a criss-cross inheritance in which the female progeny of this cross are phenotypically similar to their fathers, and males to their mothers. Morgan constructed an interperation of this genetic data. First, it appears that X and Y chromosomes determine the sex of the fly. Females have 4 chromosome pairs whereas males have 3 normal pairs plus a heteromorphic pair (Fig 3.4). Thus,

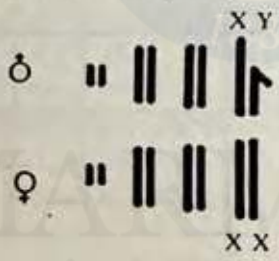

Fig. 3.4. Diagrammatic representation of the chromosomal constitution of Drosophila.

meiosis in males produces two types of sperm, one bearing an X chromosome and the other bearing a Y chromosome. According to this, union of an egg with X-bearing sperm produces an XX female zygote and union with a Y-bearing sperm produces an XY male zygote. Furthermore, both have an equal chance so they appear in equal numbers. Morgan then turned to the problem of eye colour. Assume that the alleles for eye colour are present on the X chromosome with no counterpart on the Y. Thus, females have two copies of this gene, whereas males have only one. A similar explanation also agreed with the results of crosses made with chickens

and moths. In summary, the special inheritance pattern of some genes makes it extremely likely that they are borne on the chromosomes associated with sex, which show parallel pattern of inheritance.

Humans and all mammals also show an X-Y sex-determining mechanism, with males having XY and females having XX. The sex chromosomes can be tentatively divided into pairing and differential regions (Fig.3.5). These regions are based on

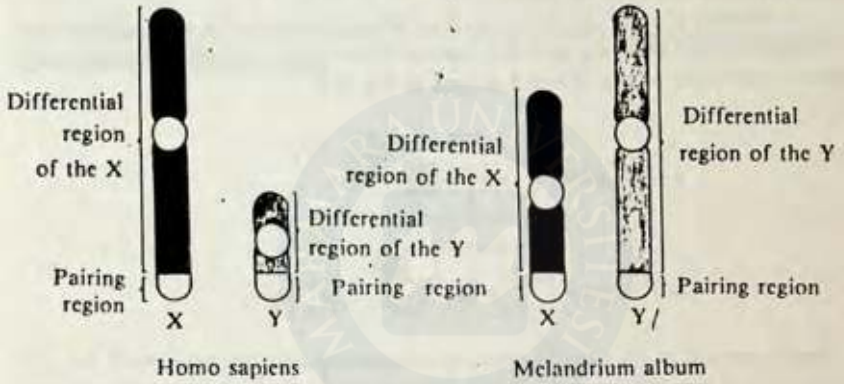

Fig. 3.5. Differential pairing of regions of sex chromosomes of humans and of Melandrium album plant. The pairing regions have been located chiefly through cytological examination.

studies of meiosis in males. The pairing regions of the X and Y chromosomes are thought to be homologous. The differential regions of each chromosome appear to hold genes that have no counterparts on the other kind of sex chromosome. These genes, whether dominant or recessive, show their effects in the male phenotype. Genes in the differential regions are called *hemizygous*. Hemizygous genes in the differential region of the X chromosome show an inheritance pattern called *X-Linkage;* those in the differential region of the Y show *Y-Linkage*. In general, genes on the sex chromosomes show *sex-linkage*

i- X-Linked Inheritance

We have already seen an example of X-linked inheritance in *Drosophila*. The same is true in humans, where many X-linked genes have been discovered through pedigree analysis of which hardly any could be construed to have any connection to sexual function.

Recessive genes showing X-linked inheritance can be detected in human pedigrees from the following clues:

1- Typically, many more males than females show the recessive phenotype. This is because an affected male can result only when both mother and father bear the gene (for example, X^AX^a versus X^aY), however an affected male can result when only the mother carries the gene. If the recessive gene is very rare, almost all observed cases will occur in males.

2- Usually, none of the offspring of an affected male will be affected, but all his daughters will carry the gene in a masked heterozygous condition, so that one half of their sons will be affected as seen in Fig. 3.6.

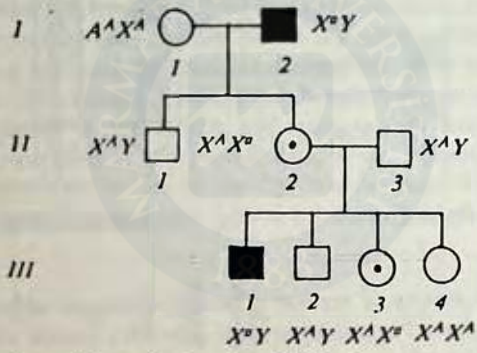

Fig. 3.6. Illustrative pedigree showing how X-linked recessives are expressed in first generation males, but unexpressed in second generation females who act as carriers, later to be expressed in their sons (Note that III-3 and III-4 ca not be distinguished phenotypically.)

3- None of the sons of an affected male will inherit the gene, so not only will they be free of the phenotype, but they will not pass the gene along to their offspring.

Some good examples of X-linked recessive genes in humans are hemophilia, red-colour blindness, and Duchenne's muscular dystrophy.

Dominant genes showing X-linked dominant inheritance can be detected in human pedigrees from the following clues:

1- The most important clue here is that affected males pass the condition on to all of their daughters but to none of their sons as seen in Fig. 3.7.

2- Females, on the other hand, usually pass the condition on to one-half of their sons and daughters as seen in Fig. 3.8.

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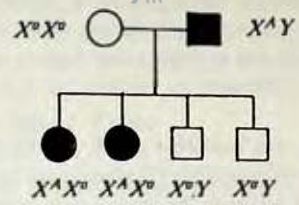

Fig. 3.7. Illustrative pedigree showing how X-linked dominants are expressed in all the daughters of affected males.

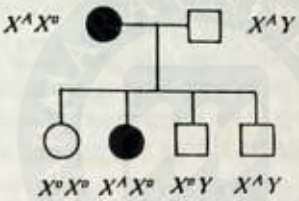

Fig. 3.8. Illustrative pedigree showing how females affected by an X-linked dominant condition usually are heterozygous and pass the condition to one-half of their progeny.

ii- Y-Linked Inheritance

In humans, genes on the differential region of the Y would be inherited only by males, with transmission from father to son. Few examples of such inheritance have been confirmed, however hairy ear rims have been suggested as a likely possibility. It is now believed that in humans, the presence of the Y and its unique regions determines maleness. Therefore, it seems safe to speculate that "maleness" genes of some kind must exist of the differential region of the Y.

3.5. VARIATIONS ON DOMINANCE RELATIONS

Although the genes that were first discovered by Mendel were the ones that exhibited dominance or recessiveness, these characteristics are not found in all sets of alleles. One of these exceptions arose from a study of the inheritance of traits which vary quantitatively among different individuals. Mendel had restricted his studies to traits which varied in a clear-cut, qualitative way. But men are neither

tall nor short; nor are they either heavy or light. Many traits differ in a continuous, quantitative way throughout a population. Some of the variations can be explained by differences in diet and perhaps other factors in the environment. Environment is not, however, sufficient to explain the full range of heights and weights.

By 1909 the information was available which provided a solution to the problem. The Swedish geneticist Nilsson-Ehle (3) studied the inheritance of kernel colour in wheat. Using Mendel's methods, he mated pure-breeding red-kernel strains (RR R'R') with pure-breeding white-kernel (rr r'r') strains. F_1 offspring were all red (Rr R'r'), but the intensity of the colour was much less than in the red parent F_1 generation as it had a pink colour. It seemed as though the effect of the red allele in the F_1 generation was being modified by the presence of the white-allele. This phenomenon is called *incomplete dominance.*

To identify an Incomplete Dominant gene, one should look for a heterozygote whose phenotype is intermediate between those of the homozygotes. The behavior of a heterozygote is also the key in the phenomenon of *codominance,* in which the heterozygote shows the phenotypes of both the homozyotes as was seen in further experiments of Nilsson-Ehle.

When he mated two F_1 plants, he produced an F_2 generation in which red-kerneled plants outnumbered white-kerneled plants 15:1. Close examination revealed that the red kernels were not all alike. They could be categoriesed in four groups, 1:15 of them were deep red, like the parent type. 4:15 of them were medium-dark red; 6:15 of them were medium red (like the F_1 generation, pink colour) and 4:15 of them were light red. These results could be explained by assuming that kernel colour in wheat is controlled by not one, but two pairs of genes, the effects of which add up without distinct dominance.

The occurence of continous variation of a trait in a population can thus be explained by assuming it is controlled by several pairs of genes, the effects of which are added together. This hypothesis is called the *multiple-factor hypothesis.*

A good sample for codominance in humans is found in sickle-cell anemia.The gene pair concerned effects the oxygen-transport molecule hemoglobin, the major constituent of red blood cells. The three genotypes have different phenotypes, as follows:

Genotype	: Phenotype
$Hb^A\ Hb^A$	: normal
$Hb^S\ Hb^S$	: sickle-cell anemia (a severe anemia)
$Hb^A\ Hb^S$	: sickle-cell trait (a mild anemia)

One might be tempted to call this incomplete dominance at first sight, but further electrophoretic studies revealed that it also shows the characteristics of codominance. If we summarize briefly, codominance is observed if both alleles are

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active in some way and incomplete dominance is observed in cases where one allele is completely inactive a *(null allele)* and cannot be compensated by the one active allele. With full dominance, such compensation is made.

i- Codominance and Blood Types

Another good example of codominance is found in the M-N blood-group gene pair in humans. Aside from its quantitative expression, a gene also has a qualitative expression in the sense that it usually affects the production of a particular substance having a special structure or function as seen in codominance.

One technique that has gained considerable favour in recognizing qualitative differences depends upon utilizing the sensitivity of an organism to material introduced from different organisms. When a foreign material, an *antigen*, enters the bloodstrem of an organism, it will sometimes elicit the production of substances in the host, called *antibodies*, which react with this material and there by reduce harmful effects. These antibodies are specific for a particular antigen and each different antigen causes the development of a different antibody (4). Thus the boodstream (especially, the bood serum) of an individual may contain many different kinds of antigens. Human erythrocytes are one type of material that can be used to elicit the production of antibodies. If such cells are washed and injected into a rabbit, the rabbit's bood serum (antiserum) soon contains antibodies that can be extracted by special methods and will react with that particular type of red blood cell. The type of reaction that usually occurs is a clumping, or *agglutination*, of cells into groups (5).

When Lendsteiner and Levine (6) tested the red blood cells of various people, they found, at first, three general types, called M, N, and MN, respectively. The M type elicited antibodies (anti-M serum) specific for M which could not agglutinate N, while the N red blood cells caused the production of antibodies specific for N (anti-N serum). Both types of antibodies, however, could agglutinate the MN red blood cells. By analyzing the relationship between people carrying the various blood types, it was found that the genes for M and N appeared to be alleles to each other. The gene was designated L and the alleles for M and N were named L^M and L^N respectively. L^M L^M individuals had M phenotype and produced only L^M gametes; L^N L^N individuals had the N phenotype and produced only L^N gametes; L^M L^N individuals had the MN phenotype and produced both L^M and L^N gametes. Since there are 3 different MN genotypes, there are 6 different possible matings, each of which (as shown in Table 3.1) produced its own particular combination offspring. Note that among different mating combinations, some exclude the appearance of particular classes of offspring; i.e., N × N matings cannot give rise to M and MN offspring. These exclusions are occasionally important in helping to decide doubtful family relations in paternity cases; that is, if the blood types of off-

spring and suspected parent can be determined, particular individuals may, at times, be excluded as possible biological parents.

Table 3.1. Phenotypes of offspring produced by different mating combinations MN blood types.

Parents	Offspring Rations		
	M	MN	N
$L^ML^M \times L^ML^M$	all	—	—
$L^ML^M \times L^ML^N$	1	1	—
$L^ML^M \times L^NL^N$	—	all	—
$L^ML^N \times L^ML^N$	1	2	1
$L^ML^N \times L^NL^N$	—	1	1
$L^NL^N \times L^NL^N$	—	—	all

ii- Multiple Alleles and A, B, O Blood Groups

Alleles can be defined as genes that are members of the same gene pair, each kind of allele effecting a particular character somewhat differently than the other. A particular gene pair in a diploid organism contains, by definition, only two alleles at a time, one for each member of the pair. Actually, there are more than only two possible kinds of alleles in a gene pair; in some instances, hundreds of possibilities exist, i.e., A_1, A_2, A_3 and B_1, B_2, B_3. The grouping off all the different possible alleles that may be present in a gene pair is defined as a system of *multiple alleles.*

A good example of such multiple alleles is provided by the alleles controlling the ABO blood group system in man. This system was shown by Bernstein in 1926 (7) to consist of three alleles of a single gene, I^A, I^B and I^O, forming our different phenotypic groups: A ($I^A I^A$ or $I^A I^O$), B ($I^B I^B$ or $I^B I^O$), AB ($I^A I^B$) and O ($I^O I^O$). The I^O allele is also symbolized as lower case (i), so that the O blood genotype is represented as (ii). The three genes can also be symbolized without the I, that is, A, B and O. In this case the blood serum of man himself manufactures the antibodies that can reach the blood-cell antigens of other individuals. As shown in Fig. 3.9, transfusions of blood between individuals of different ABO types may result in an agglutination reaction, especially if large quantities of a different blood type are being introduced; either the antibodies of the recipient will destroy (hemolyze) the red cells of the donor, or the antibodies of the donor will hemolyze the red cells of the recipient. If small quantities of blood are being introduced, however, transfusion can be considered safe as long as the serum of the recipient does not contain antibodies for the blood-cell antigens of the donor. In small

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transfusions, even if the donor's serum transmits antibodies to the recepient's blood cells, the antibodies are usually quickly absorbed by other tissues or greatly diluted in the recepient's bloodstream. On this basis, people of O blood type have been described as "universal donors" (absence of A and B antigens) and people of AB blood type as "universal recepients" (absence of A and B antibodies).

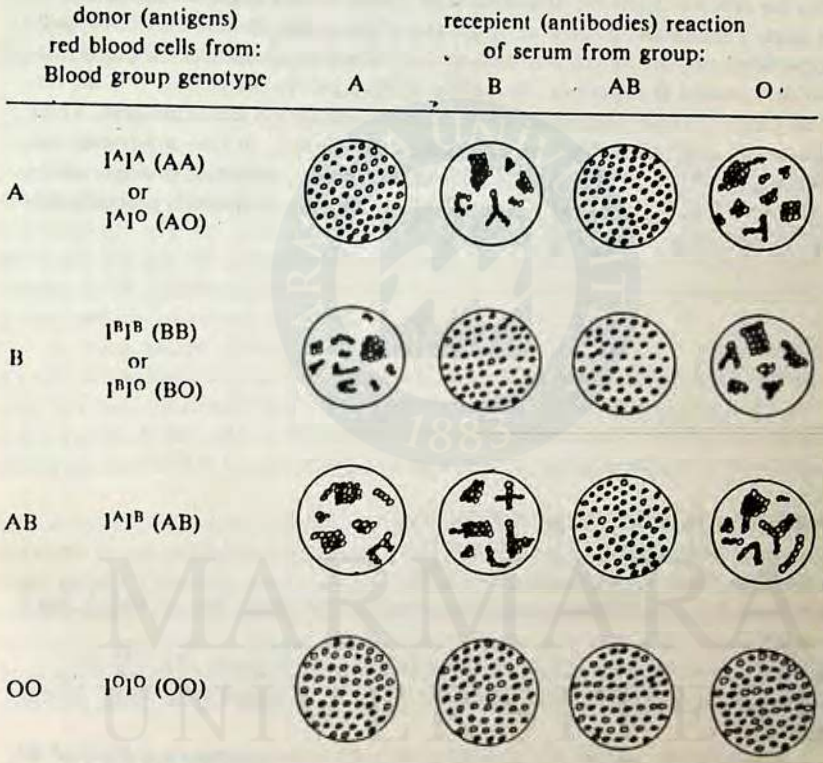

Fig. 3.9. Action of serum from persons of various blood types (recepients) with the different kinds of red blood cells (donor). Clumping of cells indicates reaction of serum antibodies with cell antigens. The A serum carries antibodies for B and AB cells, and the B serum has antibodies for A and AB cells. AB serum has no antibodies for any of the cells, whereas the O serum carries antibodies for all types of cells except its own. (After Strickberger, M.W., 1976 "Genetics" Mac Millian Pub.Co.).

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A and B antigens are found not only in red cells but often in the body fluids as well. Individuals who possess such antigens in their fluids, such as the saliva, are called *"secretors"*, and can be shown to be either homozygous (SeSe) or heterozygous (Sese) for the dominant secretor allele (controlled by a different gene pair than the ABO blood group genes.) either secreted or attached to red blood cells, the ABO antigens are now known to represent only slight modifications of the sugar (saccharide) portion of either the protein-sugar compounds (mucopolysaccharides) present in the red cells or the fat-sugar compounds (lipopolysaccharides) present in secretions. According to Watkins (4) and others, it is the terminal sugars of these compounds which differ between the A and B antigens. These differences, although small, are nevertheless significant, so that antibodies can discriminate between one antigen and the other. In fact, additional multiple alleles at the ABO locus have recently been fonud (A_2, A_3, A_x, A_m) which probably differ in even more minor respects.

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4

CYTOGENETIC TECHNIQUES

For most studies on mitotic chromosomes, the cytogeneticist requires chromosomes at the metaphase stage which necessitates the provision of large numbers of cells in active division. In higher animals, mitotic metaphase chromosomes can be prepared either directly from tissues which undergo rapid division *in vivo*, such cells from the bone marrow or spleen, or from tissues which grown and are stimulated to divide rapidly in *in vitro* culture.

A more recent development in the tissue culture field (of considerable significance in human cytogenetics) has been the culture of foetal cells derived from amniotic fluid, enabling the prenatal detection of chromosome abnormalities, and other genetic defects. Also, other tissue such as bone marrow or peripharal blood and specimens of skin are the common ones used in studying human chromosomes.

Advances in tissue culture techniques were supplemented by improved methods for spreading chromosomes. In particular, the use of colchicine, to prevent spindle formation-which stops meiosis in the metaphase stage when the chromosomes are maximally contracted and most clearly defined-, the use of hypotonic solutions to induce swelling of the cells before fixation, and the introduction of the air-dry spreading technique which greatly facilitated the counting and karyotping to the large chromosome complements of mammals.

4.1. KARYOTYPING

The advance in cytogenetic techniques of the 1950's resulted in a dramatic increase in studies of mammalian chromosomes (1). In gross structure (Fig. 4.1) the mitotic chromosome is usually a rod like body with one constriction at the centromere, called the *primary constriction*. In some chromosomes, further constrictions can be seen which may include the pinching off of a small chromosomal section called the *satellite*. The *secondary constrictions* are often associated with regions where the nucleolus is formed or attached (nucleolar organizers) on chromosomes. When a mitotic chromosome is stretched out, as in prophase, the

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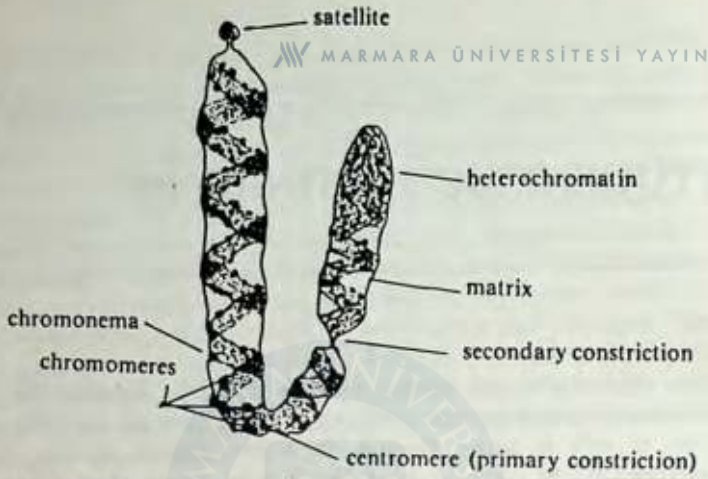

Fig. 4.1. A generalized mitotic chromosome with structures often described under the light microscope.

knoblike *chromomere* regions along its length often show a distinct size and occupy specific positions there by giving each nonhomologous chromosome a morphologically distinct appearance. The method of identifying chromosomes was largely based on the relative size, the position of the centromere, and to a lesser extent the presence and absence of structures such as satellites or secondary constrictions. According to their positions of the centromere chromosomes are classified as: a) Metacentric, when the two arms are nearly identical in length (i.e. the centromere is in the middle of the chromosome); b) Submetacentric, when the two arms of the chromosome are unequal (i.e., the centromere is more toward one end of the chromosome than the other end); c) Acrocentric, when the two arms are unequal (i.e., the centromere is very near to one end of the chromosome than to the other); d) Telocentric, when there is only one clearly distinguishable arm (i.e., the centromere is at one end of the chromosome) as seen in Fig. 4.2.

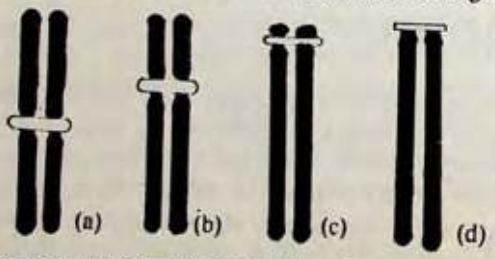

Fig. 4.2. Chromosome types. a) metacentric b) submetacentric c) acrocentric d) telocentric.

Following the demostration of the correct number of chromosomes in man by Tjoi and Levan in 1956 (2) and subsequent publication of numerous studies on the human karyotype (3, 4), it became necessary to adopt an international system of nomenclature for identifying human chromosomes. The Denver system (5), in which the chromosomes were numbered and arranged in the karyotype in descending order of size, was widely accepted and proved invaluable once the literature began to fill with reports of numerical and structural chromosome abnormalities associated with clinical defects (Chapter 6). The autosomes were numbered 1 to 22 and the sex chromosomes were designated X and Y (XX + 22 in female; XY + 22 in male). As was the case for many mammals, however, many chromosomes in the human karyotype could not be individually identified and had to be allocated to groups. Many investigators divide chromosomes into seven groups A to G depending on size and the position of the centromere (A = 1-3 large metacentric chromosomes, B = 4-5 middle range submetacentric chromosomes, C= 6-12 submetacentric chromosomes, D= 13-15 and E= 19-20 small metacentric chromosomes, G= 21-22, the smallest chromosomes are in this group). The resulting arrangement of the chromosome is referred to as the *karyotype*.

The karyotyping methods use a variety of tissues in studying human chromosomes, but most commonly used are specimens of skin, bone marrow or

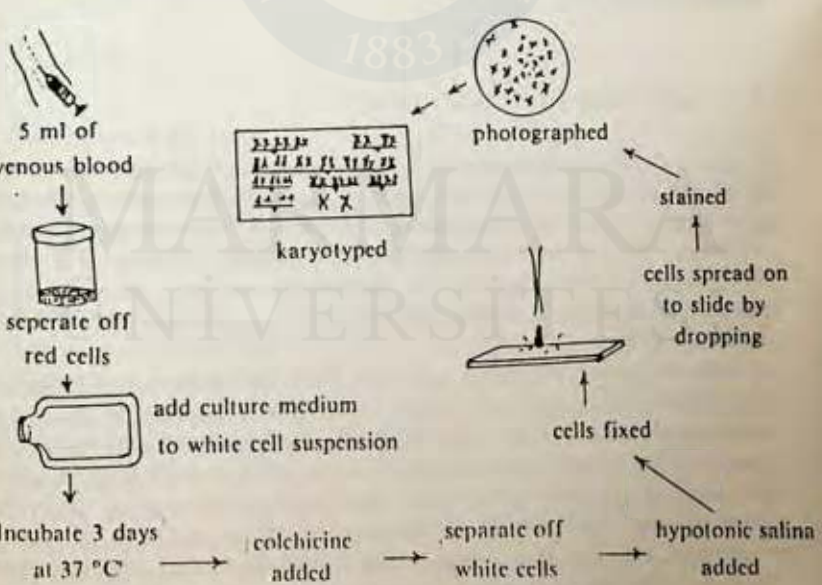

Fig. 4.3. Preparation of Karyotype.

peripheral blood. In the case of blood, the leucocytes are separated off and added to a small volume of nutrient medium containing phytohaemagglutinin. The addition of phytohaemagglutinin, a substance extracted from the French bean *Phaseolus vulgaris,* is very important because it stimulates the leucocytes to divide. The cells are cultured under sterile conditions at body temperature (37 °C) for about three days. During this time the cells divide and then a small amount of colchicine is added to each culture. Colchicine is a complex organic compound which is extracted from the *autumn crocus* and had been used for several years before the advent of human cytogenetics in the treatment of Gout. colchicine has the unique property of preventing the formation of the spindle and stops mitosis at the stage of metaphase when the chromosomes are maximally contracted and most clearly defined. After an hour or so a hpotonic saline solution is added. This makes the cells swell and has the effect of spreading the chromosomes which otherwise would remain clumped together and make the accurate counting of chromosomes very diffucult.

The next step is to photograph the resulting chromosome "spread" with the aid of a high-power microscope with a camera attachment. Later, individual chromosomes are cut out from the photograph, arranged in pairs and in decreasing order of size and numbered from 1 to 22 with the two sex chromosomes indicated separately (Fig. 4.3).

4.2. CHROMOSOME BANDING

The specific identification of chromosomes has been possible only since 1970 when Caspersson et al.(6) demonstrated that chromosomes stained with quinacrine dyes produce characteristic and reproducible patterns of fluorescence in the human karyotype. The discovery, made on plant chromosomes, has resulted in rapid advances in mammalian cytogenetics in the last few years, and a wide range of mammalian karyotypes have now been elucidated by the Q-banding (Q = quinacrine) technique (7-9).

The technical difficulties and the need for expensive equipment for fluorescence microscopy prompted the subsequent development of simpler, alternative techniques for producing banding patterns in chromosomes. The most commonly used is the G-banding technique, so called because the bands, which have the same distributions as Q-bands, are produced by staining with Giemsa pretreatments. These include heat and alkali (10,11), digestion with proteolytic enzymes (12, 13), urea and detergents (14) and potassium permenganate (15). An alternative banding method is the controlled heat denaturation of chromosomes to produce reverse bands, or R-bands (16). With this technique the Giemsa stain produces a banding pattern which is reciprocal of the Q and G banding pattern; i.e.,

heavily stained Q and G-bands become lightly stained R-bands and vice versa. Table 4.1 shows chromosome structure as inferred from banding patterns.

Table 4.1 Chromosome structure as inferred from banding patterns.

Banding method	Essentials of the technique	Inferred structure of bands
C	Treatment with dilute acid followed by strong alkali and warm saline prior to Giemsa staining	Complexes of highly repetitious DNA sequences (e.g. satellite DNA)
G	Proteolytic digestion or warm saline prior to Giemsa staining	Chromatin compacted by nonhistone protein
Q	Quinacrine (fluorescent) staining	Chromatin compacted by protein, and rich in A-T bases
R	Saline heat treatment prior to Giemsa staninig	Differential response of chromosomal proteins to denaturation. Reverse of G method

These technical developments in chromosome identification made it desirable to establish a standardized system of nomenclature to describe individual chromosomes and chromosome regions. Such a system, based on the Q, G and R banding techniques, was agreed upon at the 4th International Congress of Human Genetics in Paris in 1971 (17) as seen in Fig. 4.4.

A forth type of banding technique, C-banding, demostrates certain types of constitutive (= C) heterochromatin (18). In many mammals, including mice and man, C-bands are generally located in the centromeric regions (19), but distribution can vary. In the ferret, for example, there are no centromeric C-bands, and in the weasel only a few, but both species have C-bands in the arms of some autosomes. Clearly, there are different types of constitutive heterochromatin, not all of which can be identified by existing C-banding techniques. On the other hand, differential staining of the secondary constriction of human chromosome 9 by the GII (alkaline Giemsa) technique (20) enables the identification of a specific type of constitutive heterochromatin. The variable distribution of this "9 chromatin" in man and the hominoid apes has proved useful in phylogenetic studies of the primates (9).

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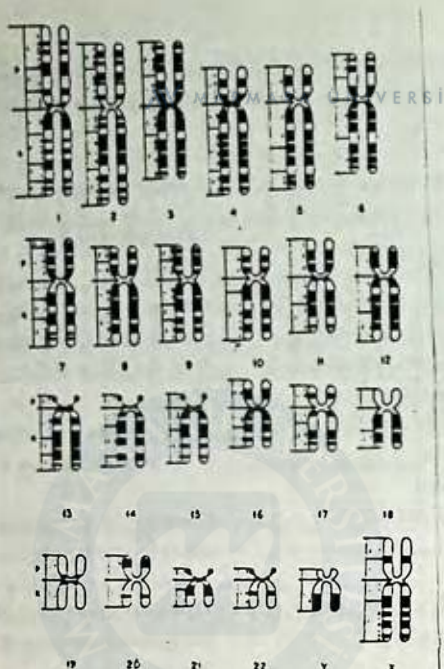

Fig. 4.4 Identification of chromosome bands of the human karyotype according to the Paris Conference system (1971). (After Mc Dermott A., 1975, Churchill Livingstone, London.)

4.3. LINKAGE MAPPING BY RECOMBINATION AND CHROMOSOME MAPPING BY CELL HYBRIDIZATION

Linkage studies occupy a large proportion of the routine day-to-day activity of geneticists. When a new variant gene is discovered, one of the first questions to be asked is, "Where does it map"? Not only is this a necessary component in the engineering and maintenance of genetic stocks for research use, but it is also fundamental importance in the piecing together of an overall view of the architecture of the chromosome, and in fact, of the entire genome. The operational key for the detection of linkage is the same in studies from viruses to humans and that key is the nonindependence of genes in their transmission from generation to generation.

Using pedigree studies, the total frequency of recombinants can be estimated and a map is based upon this. For example; the percentage of recombinant gametes formed by F_1 individuals studied in *zea mays* showed that less than 3.6 per cent of the individuals were recombinant. In others the percentage of recombinant genes

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was higher. Assuming for a moment that the gene loci are in a linear order from one end of a chromosome to the other, we may deduce that the higher the percentage of recombinant gametes formed for a given pair of traits, the greater the distance separating the two loci. The opposite is also true. Using this knowledge one can plot a linkage map for as many of the gene loci on a given chromosome as can be discovered. Let us see linkage in corn as another example; it has been seen that about 3 per cent of recombinant gametes are produced a result of crossing over between gene loci C (kernel colour, a dominant trait) and S (smooth texture a dominant trait). A dihybrid can then be developed for another pair of linked traits, say kernel colour (C) and waxy endosprem (WX). This dihybrid produces 33 per cent recombinant gametes, indicating that the loci C and WX are farther apart then the loci C and S. However, one does not yet know whether the locus WX is on the same side of C as S or the opposite side (Fig. 4.5). The answer can be determined by

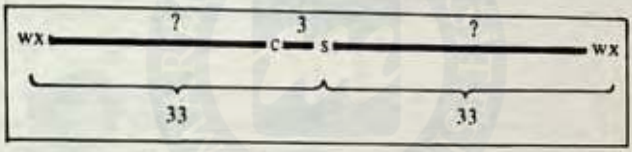

Fig. 4.5 Plotting a linkage map. The production of 30 per cent recombinant gametes as a result of crossing over between the S and WX loci tells us that locus WX is on the same side of locus C as is locus S.

developing a dihybrid for S and WX. If the percentage of recombinant gametes produced by this individual is less than 33 per cent, then the gene locus WX must be on the same side of the locus as locus S is. The opposite is true if the percentage of recombinant gametes turns out to be greater than 33 per cent. Actually the number of recombinant gametes formed is about 30 per cent. Thus we know that the sequence of gene loci on this chromosome is C-S-WX. Furthermore, the fact that the sum of the percents of recombinants between C and S and WX is so close to the percentage between C and WX lends strong support to idea that the gene loci are arranged in a line along the length of the chromosome (Fig. 4.5). A straight line is the only geometric arrangement in which this simple numerical relationship can exist. By pursuing this method with as many linked genes on a given chromosome as can be discovered, it is possible to plot chromosome maps.

Human beings do not readily submit themselves to traditional genetic analysis. Until recently, geneticists have had to rely on the study of family pedigrees in order to deduce linkage of various traits by inference from these hopelessly inadequate

data. Most of the analyses are converned with the X chromosome; sex-linkage is relatively easily shown is these cases, and testcrosses are not necessary because the male's phenotype reflects his X-chromosome genotype (chapter 5). Recently, however, a technique has been developed that has revolutionized the mapping of both sex-linked and autosomal genes in humans. This technique uses human cells grown in culture. This field of study began with the demostration that two different

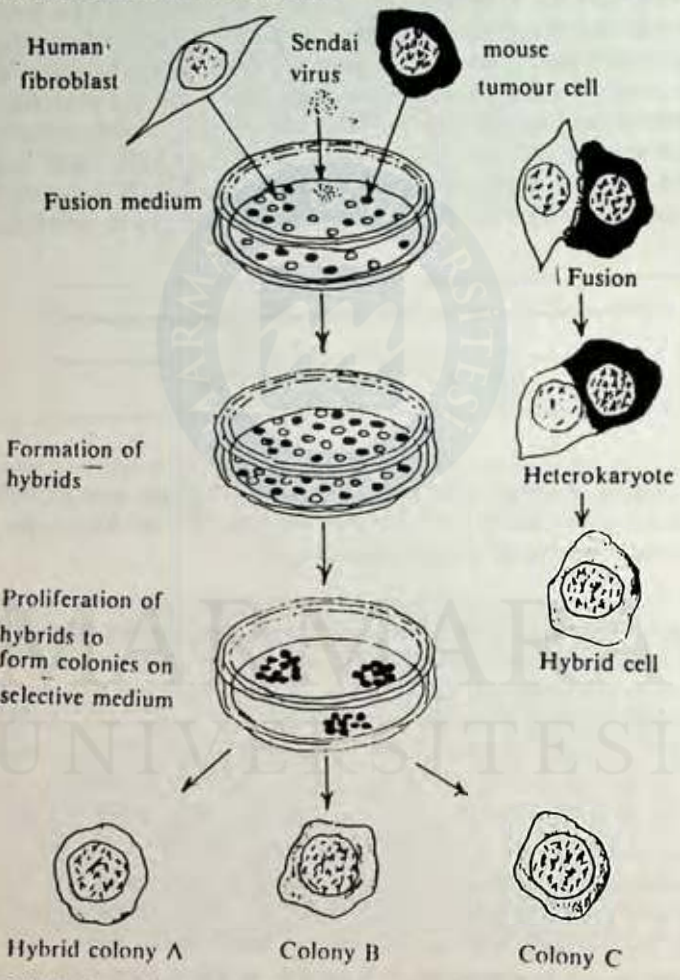

Fig. 4.6 Cell-fusion techniques applied to human and mouse cells, producing colonies each containing a full mouse genome plus a few human chromosomes (lighter shades). A fibroblast is a cell from fibrous connective tissue (After from Suzuki D.V. A.J.F. Griffiths and R.C. Lewontin "An Indroduction to Genetic Analysis" 1981 Freeman and Comp. Lond.

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lines of mouse cell could fuse in culture to produce a viable hybrid cell line in which the chromosome complements of both parental lines were contained within a single nucleus (21). The fusion of cells from animals of the same or different species may occur spontaneously, but can be induced at will by introducing certain viruses into cultures of mixed cells. Since Harris and Watkins (22) demostrated that inactivated Sendai virus could induce fusion between cells from widely different species, this member of the par-influenza group of myxoviruses has become the standard reagent for inducing cell fusion. It is a convenient agent to use as its ability to effect cell fusion rests in the lipoprotein viral envelope and is not impaired by the destruction of the infectivity of the virus (23). Normally, a virus has a specific point for attachment to and penetration of a host cell. Each Sendai virus has several points of attachment, so that it can simultaneously attach to two different cells if they happen to be close together. A virus, though, is very small in comparasion with a cell, so that the two cells to which it is attached are held very close together indeed. In fact, in many cases, the membranes of the two cells fuse together, and the two cells become one, a binucleate *heterokaryon* (Fig. 4.6).

If a suspension of human and mouse cells are mixed together in the presence of Sendai virus (which has been inactivated by ultraviolet light), the virus can mediate fusion of the two kinds of cells (Fig. 4.6). Once the cells fuse, the nuclei can fuse to form a uninucleate cell line. Because the mouse and human chromosomes are very different in number and shape, the hybrid cells can be readily recognized. However, for some unknown reason, the human chromosomes are gradually eliminated from the hybrid as the cells divide. This process can be arrested to encourage the formation of a stable partial hybrid in the following way. The mouse cells that are used can be made genetically deficient in some function (usually a nutrient one) so that, for growth of cells to occur, the function must be supplied by a human genome. This selective technique usually results in the maintenance of hybrid cells that have a complete set of mouse chromosomes and a small number of human chromosomes, which vary in number and type from hybrid to hybrid but always include the nutritionally sufficient human chromosome.

Luckily, this process can be followed under the microscope because mouse chromosomes can easily be distinguished from human chromosomes. Recently, this procedure has been made a lot easier by the development of fluorescent stains (such as quinacrine and giemsa) that reveal a pattern of "banding" within the chromosomes. The size and position of these bands vary from chromosome to chromosome, but the banding patterns are highly specific and constant for each chromosome. Thus, for any hybrid, it is relatively easy to indentify the human chromosomes that are present.

The mapping technique as follow: If the human chromosome set contains a genetic marker (such as a gene that controls a specific cell-surface antigen, drug resistance, a nutritional requirement, or a protein variant), then the presence or absence of the genetic marker in each line of hybrid cell can be correlated with the presence or absence of certain human chromosomes in each line (Table 4.2). We can see that, in the different hybrid cell lines, gene 1 and 3 are always present or absent together. We conclude, then, that they are linked. Furthermore, the presence or absence of genes 1 and 3 is directly correlated with the presence and absence of chromosome 2,so we assume these genes are located on chromosome 2. By the same reasoning, gene 2 must be on chromosome 1, but the location of gene 4 can not be assigned.

Large numbers of human genes have now been localized to specific chromosomes in this way, but of course we cannot derive a linkage map showing the order and distance between genes. Other tricks are needed, for example, the loss or gain of variously sized bits a specific chromosome might be correlated with the presence or absence of genetic markers.

Table 4.2 Comparison of five hybrid lines.

		Hybrid cell lines				
		A	B	C	D	E
Human genes	1	+	—	—	+	—
	2	—	+	—	+	—
	3	+	—	—	+	—
	4	+	+	+	—	—
Human chromosomes	1	—	+	—	+	—
	2	+	—	—	+	—
	3	—	—	—	+	+

4.4 GENETIC ENGINEERING AND HUMAN GENES

A major goal of human genetics, although still a distant one, is to correct genetic defects by gene manipulation-by replacing or supplementing defective genes with good, normal ones. Such "genetic surgery" aims first at reaching somatic cells in order to improve their preformance; it may also attempt to correct defective genes in germ cells, a goal that still appears to be well beyond reach.

How is the technology of genetic engineering utilized for this goal? Two main techniques of molecular biology is used for this purpose; the isolation or

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reconstruction of single-gene DNA, and the introduction of this DNA into cells where it can multiply and function.

The advent of techniques for the synthesis of DNA molecules in which a eucaryotic gene is "implanted" in a procaryotic chromosome has revolutionized molecular biology for the second time in many generations. The hybrid DNA molecules are somewhat poetically known as *chimeras*. The potential uses of recombinant DNA technology is virtually limitless, ranging from the synthesis of rare and expensive hormones to the invention of plants that contain the genes for nitrogenase. Pure science also profits from recombinant DNA techniques. For example, such techniques provide a convenient way of studying eukaryotic genes, which are more complex and for more difficult to study than prokaryotic genes. As with any new technology, the potential benefits of synthetic recombinant DNA are in some sense "obvious", but the potential hazards are unknown. These hazards have been much discussed and appear, at present, to be minimal.

The synthesis of a DNA molecule in which a eukaryotic gene is inserted into a prokaryotic DNA molecule is a multistep process. First, the DNA molecule to be inserted is isolated. Then, it is structurally modified and incorporated into a carrier molecule, such as a bacterial plasmid or a bacteriophage such as lambda. The recombinant DNA molecule is then introduced into the host bacterium. Since not all bacteria will take up the plasmid, some experimental method, such as resistance to an antibiotic (conferred by a gene which is also conveniently located in a plasmid), is used to identify the bacteria that actually possess the recombinant DNA molecule. These selected bacteria are separated and allowed to reproduce. Progeny descended from the same ancestor are known as *clones*. The clones of the selected bacteria possess the recombinant DNA molecule, now *amplified* thousands of times. The clones are each little factories that produce copious quantities of the protein whose gene was inserted in the original bacterial plasmid or bacteriophage. Fig. 4.7 summarizes the genetic engineering of a cloned gene.

i- Gene Cloning

Gene cloning refers to the operation of isolating and amplifying single genes in recipient prokaryatic or eukaryotic cells. Those cells containing the required gene can be used a) to produce large amounts of the protein coded for by the gene or b) to produce large amounts of the gene itself in a very purified form. Two strategies of gene cloning are currently employed. In strategy 1, the genomic DNA of an organism is first randomly split into small gene-sized pieces by restriction endonucleases (see 4.7). Each fragment is then inserted via a vector into a recipient cell which then multiplies and amplifies the gene or genes contained in the fragment. This method will not be discussed further. In strategy 2, the messenger RNA

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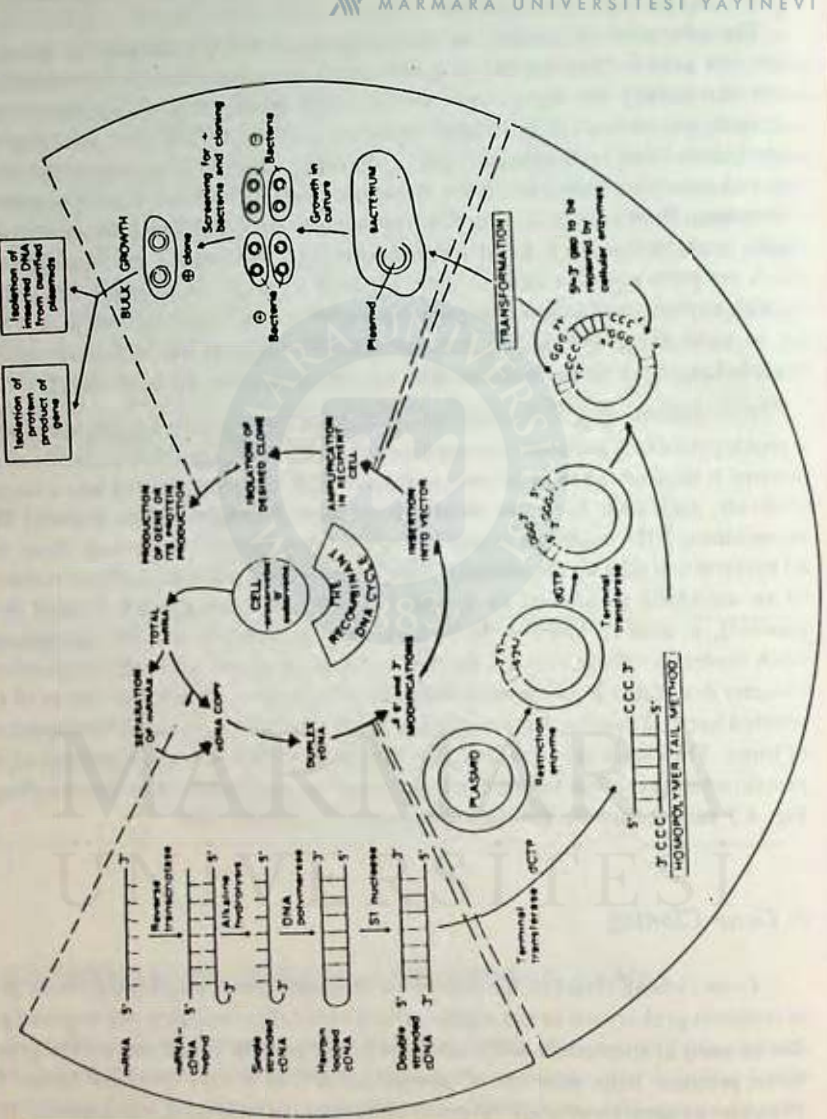

Fig. 4.7. Genetic Engineering—the Clonning of Genes.

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of a particular type of cell in the organism is turned into DNA copies (cDNA) which are then inserted into recipient cells, at an average of one gene per cell and amplified as in method 1.

Recipient cells for gene cloning may be either prokaryotic or eukaryotic. The most commonly used are bacteria since they are easily grown in large numbers. However, if the gene is obtained from a mammalian cell by method 1 above, it must be cloned in a eukoryotic cell such as yeast because of the inability of prokaryotic cells to process DNA containing insertion sequences (introns). Method 2 now will be discussed in detail.

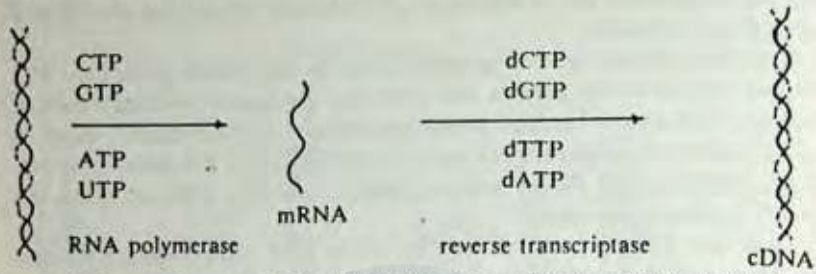
Messenger RNA is obtained from the cell or tissue expressing the required gene. Thus to clone the proinsulin gene, the pancreatic B cell would be chosen since it normally expresses high levels of the mRNA for proinsulin. The total cellular mRNA can be crudely fractionated by centrifugation on a sucrose gradient. This partial purification reduces the number of unwanted mRNAs in the required mRNA preparation. The mRNA molecules within the desired size range (for a protein the size of proinsulin will be a molecule containing about 25 kilobases) are then copied into DNA. DNA copying is carried out by a reverse transcriptase (or RNA-dependent DNA polymerase) usually obtained from suitable RNA viruses. The enzyme requires a short (10 bases) DNA primer to start the copying and often oligo-(T) is used. This primer will spontaneously base pair to the poly-(dA) track present at the 3'-terminus of eukaryotic mRNA molecules. When copying is complete, the RNA strand is degraded.

Degradation of the RNA strand is accomplished by alkaline hydrolysis. DNA chains are stable to this treatment but RNA is completely hydrolysed. The DNA remaining is single-stranded (ss) with a short hairpin loop at one end. This loop is present because most mRNAs possess a self-complementary sequence at the 5'-end which, after copying, will be present in the cDNA. Thus the end of the cDNA molecule is able to loop around itself for as far as this sequence extends.

ii- Synthesis of Complementary DNA (cDNA)

The genetic information of RNA tumour viruses is contained in RNA rather than in DNA. The viruses contain a gene that is translated by the host cell to produce *reverse transcriptase*. This enzyme uses RNA as template to synthesize a DNA molecule, cDNA, which is complementary to the RNA of the virus. In the translation of the genetic message DNA is first transcribed to an RNA copy known as mRNA. If a mRNA copy of a gene is isolated, then a complementary DNA molecule can be synthesized in vitro by incubation of deoxyribonucleoside triphosphates with reverse transcriptase.The cDNA molecule that is synthesized in this way contains all of the information of the original gene in its single strand. In-

cubation of the cDNA with DNA polymerase and deoxyribonucleoside triphosphate pool results in the synthesis of double stranded DNA.



Synthesis of complementary DNA (cDNA) from an mRNA molecule catalyzed by reverse transcriptase.

Double-stranded (ds) DNA is produced when ss cDNA is copied by an enzyme such as DNA polymerase I. This enzyme usually requires a DNA primer but the short length of DNA already present in the hairpin loop is able to fulfill this function. The ds-cDNA still retains a closed loop at the 5'-end and this is removed by the enzyme S1 endonuclease. This enzyme cleaves the loop and also truncates the chains so as to remove any residual ssDNA. The ds-cDNA can now be inserted into a vector.

A *vector* is a sort of molecule which is able to carry extraneous DNA into the bacterial cell in such a way that it well be replicated. There are two major types of vectors, bacterial plasmids and bacteriophages. Here, only the former will be considered. Plasmids are naturally occurring extrachromosomal elements consisting of closed circles of ds DNA. They are able to replicate idependently of the bacterial genomic-DNA. Often, plasmids will contain genes whose protein products confer resistance to certain antibiotics. This is useful in distinguishing plasmid-containing from plasmid-minus bacteria.In order to insert the cDNA into a plasmid, the closed circle of the plasmid must be "opened up". This is accomplished by incubating the plasmids with a restriction enzyme.

iii- Cleavage of DNA by Restriction Endonucleases

A restriction enzyme cleaves dsDNA at specific nucleotide sequences called restriction sites. These are usually short stretches of palindromic sequences and different enzymes recognise different palindromes. (A palindrome is a word/series of words, or number that reads the same either forward or back wards such as *"Madam'I'madaM"*

the most important restriction endonucleases in recombinant DNA research are those that cleave DNA symmetrically, such as the enzyme *EcoR I* which recognizes the sequence, and cleaves at the points indicated by arrows.

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3' - C TTAA G - 5'
5' - G AATT C - 3'

If the sequences of the two strands are read in the direction 5' ■ 3', they are the same. Suppose that an *E. coli* plasmid possesses a sequence that is recognized by the restriction endonuclease that was used to cleave the eukaryotic gene. Cleavage of the plasmid by this restriction endonuclease gives single-standed ends whose base sequences are precisely complementary to the exposed ends of the eukaryotic DNA. Mixing the plasmid with eukaryotic DNA leads to spontaneous annealing. The nicks are sealed, and now the bacterial plasmid contains the eukaryotic gene. The same technique is employed to incorporate DNA produced by chemical synthesis or to incorporate cDNA in a plasmid. In these cases complementary strands are introduced enzymatically into both the cDNA and the plasmid. Annealing then follows.

If the sequence of a protein whose gene is to be cloned is known, then the genetic code can be used to deduce a primary structure for the gene. (The sequence is not unique because the genetic code is degenerated, that is, some amino acids are represented by more than one code word). This gene can then be synthesized by chemical methods. This method has been used to synthesize the genes for somatostatin and insulin. The technique by which the chemically synthesized gene for somatostatin was introduced in an *E. coli* plasmid pBR322 (*) is shown in Fig. 4.8. In this case the 14-residue polypeptide of somatostatin was covalently bound to Beta-galactosidase. Cleavage with cyanogen bromide released free somatostatin. The product possessed, by now, full biological activity.

Ligation is the procedure during which the foreign DNA is inserted into (or ligated to) the two ends of the plasmid DNA with the enzyme DNA ligase. Before this can be done, the ends of both cDNA and plasmid DNA must be made "sticky". That is, sequences must be present which will enable the two sets of ends to base-pair with each other thus forming a recombinant plasmid. The method shown for doing this is called *homopolymer tailing*. A second method is called *restriction linking* but will not be described further here.

iv- Homoplymer Tailing

Homopolymer tailing refers to the addition of short lengths of DNA with a

(*) Naturally occuring plasmids often have many retriction sites for a particular retriction endonuclease. Since ideally only a single cleavage point is desirable, plasmids may be specially selected or "engineered" for this purpose. For example, the plasmid pBR322, a commonly used vector, possessing a single restriction site for many enzymes, has genes which confer resistance to both ampicillin and penicillin and has had some of its "unimportant" genes removed. The genes retained are those essential for bacterial transformation and for its replication.

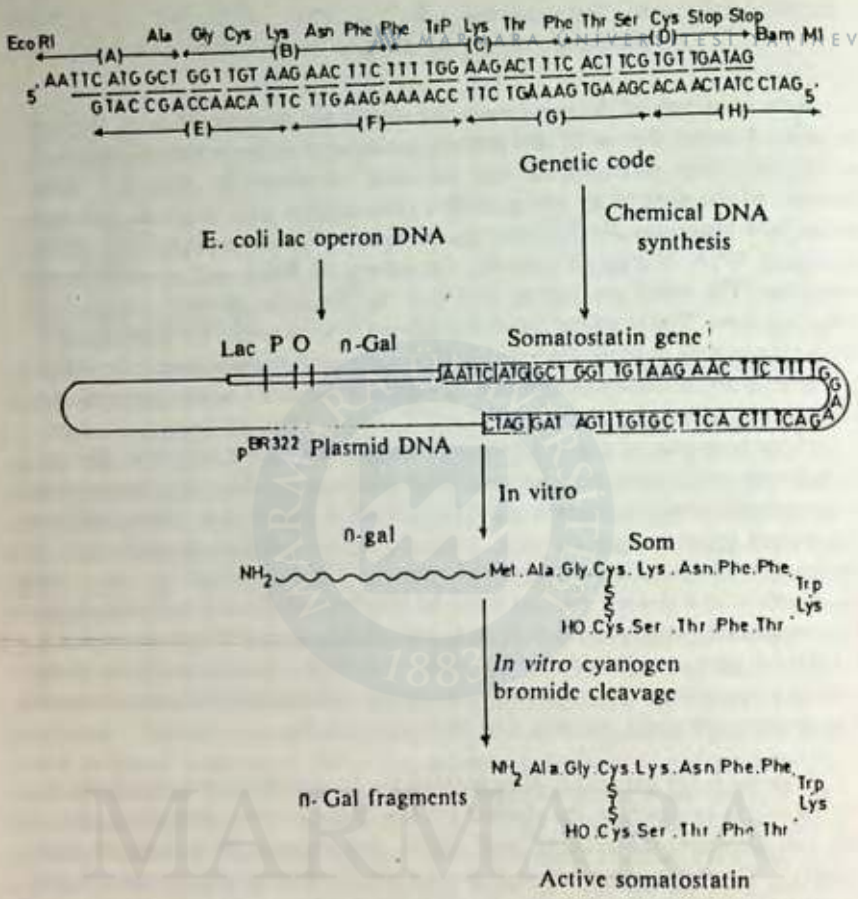

Fig. 4.8. Schematic diagram of the incorporation of somatostatin gene into an E. coli plasmid (designated pBR322) Adapted from Itakura, Kand Riggs A.D. Science 209, 1404 (1980).

repeating sequence (a homopolymer) to the *3'-ends* of the double-strands. If two different bases which are complementary are added to the plasmid and cDNA respectively, on mixing, the two component will base-pair to each other.

Transformation occurs when the recombinant plasmid is added to the recipient bacteria and after entry becomes part of the bacterial life cycle. Since not all cells will have been transformed, a selection process is desirable so that only recombinant-positive bacteria are allowed to grow. Selection is accomplished by exploiting the fact that the plasmid will have conferred resistance to certain an-

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tibiotics. Thus, only plasmid-containing bacteria will grow in the presence of these antibiotics.

Screening is required since in the original cDNA preparation many mRNA molecules will have been represented and only a proportion of the plasmids will contain the desired gene. The methods used are too involved to be described here and are dealt with in detail in the references given. Once identified, the appropriate bacteria can be cloned and grown in bulk culture. This is the amplification step.

Amplification is achieved both because many plasmid copies may be found in a single bacterium and because a large number of cells can be obtained. Once purified, the plasmids can be treated with the appropriate restriction enzyme to excise the inserted gene copies. This amplified gene may then be used for further genetic engineering experiments of the type described in the reading list. Alternatively, the bacteria can be used to produce the protein product of the inserted gene provided (a) it is transcribed, and (b) the protein is secreted by the bacterium as it is seen in Fig. 4.7.

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5

CHROMOSOMAL ABNORMALITIES

5.1. ABNORMALITIES OF CHROMOSOME NUMBER

As we know from the previous chapters, the basic set of chromosomes in a species is the haploid set (n), usually found in the gametes. the two parental haploid sets contributed to the zygote at fertilization constitute the diploid set (2n) and is the chromosome number usually referred to in a species.

Chromosome numbers which deviate from the normal number of chromosomes for a species are said to be *"heteroploid"*. They result from the distribution of chromosomes or chromatids at meiotic or mitotic cell division and are of two types: *Euploid and Aneuploid.*

i- Euploidy

Euploidy describes chromosome number which are multiples of the haploid number (n), i.e, individuals with n, 2n, 3n, 4n etc. are euploid. Multiples of the haploid number which are higher than diploid are referred to as *polyploid*, or may be specifically designated *triploid* (3n), *tetraploid* (4n) and so on. Polyploidy usually results from the occurrence of karyokinesis (nuclear division) without simultaneous cytokinesis (cell division). This may be effected by a number of mechanisms, each of which enables the number of chromosomes in a cell to be doubled, e.g., diploid to tetraploid, tetraploid to octoploid etc. One mechanism is the *"C-mitosis"* or *"C-mitotic duplication"* in which the spindle apparatus fails to function,and the duplicated chromosomes remain in the same cell.This process resembles the *"colchicine arrested"* mitosis from which it takes its name. In an alternative mechanism, *endomitosis*, the process of chromosome replication, condensation, and chromatid seperation occur within the nuclear envelope, and there is apparently no involvement with any spindle apparatus.This absence of spindle formation is also characteristic of *endoreduplication*, in which chromosomes go through a replication cycle without contracting or undergoing chromatid sepera-

tion. When the chromosomes contract during a subsequent cell division, they are seen as diplo-chromosomes or quadruple-chromosomes, in which four or eight (or more) chromatids are held together at the centromere.

Failure of the regular division processes to occur in either the mitotic or meiotic stages of gametogenesis may result in the production of diploid gametes. Such gametes produce triploid zygotes, following fusion with a normal haploid gamete at fertilization. Alternatively, triploidy may result from the fertilization of a normal egg by two spermatozoa.

ii- Aneuploidy

Aneuploidy describes chromosome numbers which are not multiples of the haploid number (n), for example, 2n-1, 2n + 1 etc. are aneuploid chromosome complements. These are more specifically designated *"monosomic"* (2n-1), where one member of the pair of chromosomes is missing from the diploid set *"trisomic"* (2n + 1), where there is one extra chromosome which is usually homologous with one pair of chromosomes in the diploid set; and so on. Aneuploid cells result from non-disjunction, which is the term used to describe the failure of paired chromosomes or sister-chromatids to pass to opposite poles of the spindle at the anaphase of meiosis or mitosis. The process usually involves only one chromosome. Thus, in mitosis, non-disjunction will most commonly result in one trisomic daughter cell and one monosomic daughter cell. Occasionally, non-disjunction results from anaphase lagging in which chromosomes separate normally in the early stages of cell division, but one chromosome (or sometimes more) moves noticeably slowly to the spindle pole, and subsequently fail(s) to be included in the nucleus of either daughter cell. Thus, in mitosis this would result in one daughter cell being a normal diploid and the other monosomic.

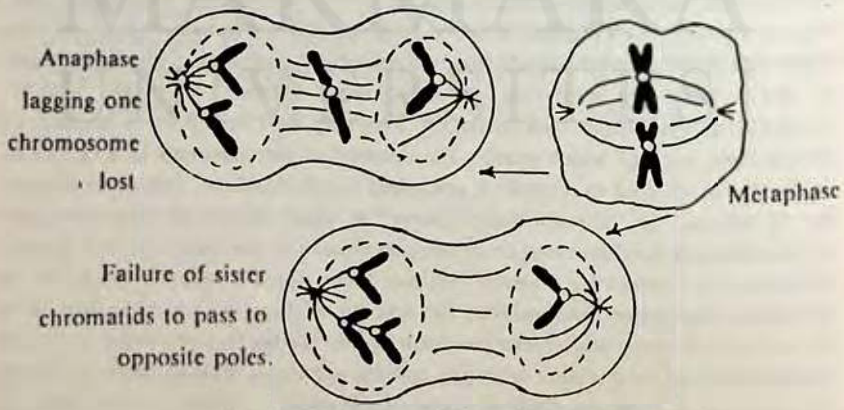

Fig. 5.1 Two types of non-disjunctional event.

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iii- Mosaicism

The term mosaic describes an organism which consists of two or more cell types, each genetically or structurally different yet each having a common zygotic origin. In cytogenetics, mosaicism, which is also sometimes referred as "mixoploidy" is usually recognized by cells having chromosome complements which differ in number or structure. Numerical differences are more common and are usually due to non-disjunction in mitosis (1).

Mosaicism which is initiated during the early cleavage divisions of the zygote is referred to as *"developmental mosaicism"* (2). When it occurs at stages following embryonic development, it is referred to as *"proliferative mosaicism"*. The latter may be associated with increasing age (3) or neoplastic changes (4).

The detection of mosaicism is often difficult if investigations are limited to one or a few specific tissues, as is frequently the case in investigations on human subjects. In addition, since cytogenetic researches on the higher animals and man are usually performed on cell cultures, there is no guarantee that the different cell types will grow *in vitro* as well as *in vivo*, even when the correct tissues are available for study.

For these reasons in the field of human cytogenetics, the existance of mosaicism can never be excluded in cases where phenotypic appearance and clinical observations do not correlate with the apparent karyotype.

iv- Chimaerism

The term *chimaera* refers to an organism which, like a mosaic, has more than one cell type. In contrast to mosaic, however, the differenet cell populations are of different zygotic origins and their admixture may be the result of artificial or natural mechanisms. The occurence of chimaerism in man is well established (5).

Chimaerism may be a result of various mechanisms (1). They fall into two categories. The first entails two separate acts of syngamy and involves either dispermy or the fusion of the zygote nucleus with the nucleus of the second polar body, the latter giving rise to a diploid/triploid (2n/3n) chimaera (6). The second category consists of mechanisms which involve a transfer or exchange of cells between individuals of different zygotic origins. These include the early fusion of embryos, placental cross-circulation between dizygotic twins, maternal-foetal transplacental exchange or the artificial introduction of differenet cell types by transfusion or grafting. Human chimaeras are most commonly the result of cross-circulation between dizygotic twins and are usually detected in the first instance by diffuculties in the determination of blood groups. They are of particular

cytogenetic interest when the different cell lines can be distinguished by karyotypic differences such as the possession of different sex-chromosome complements (7).

5.2. ABNORMALITIES OF CHROMOSOME STRUCTURE

Structural rearrangements in chromosomes are the result of transverse breakage in one or more chromosomes with the subsequent reunion of the broken ends in such a way that the linear order of the genetic material is disturbed. (For details of the mechanism see Chapter 2.)

Breakage may occur spontaneously or may be directly attributable to a wide range of physical, chemical, or biological agents. The aberrations can be divided into two basic types: the *chromosome-type*, in which the rearrangement takes place in the G1 phase, prior to chromosome replications, and *chromatid-type*, in which breakage and reunion occur during the S or G_2 phase, when the chromosomes are functionally double. The two types are basically similar, although there is slightly greater variety in the chromatid-type arrangements (8, 9). The important types of chromosome rearrangement are as follows:

i- Deletions

A deleted chromosome is one which has lost a portion of its original material. The deleted segment may be terminal or interstitial, although stability of the terminal regions of chromosomes (telomeres) suggest that most deletions are likely to be the result of two breaks with loss of the chromosomal material between them as seen in Fig. 5.2.

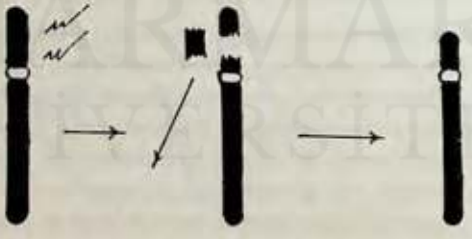

Fig. 5.2 Formation of a deletion.

ii- Ring Chromosomes

A ring chromosome is another type of deletion. It results from breaks in both arms of a chromosome followed by the loss of the terminal segments and reunion

of the two broken ends carying the centromere as seen in Fig. 5.3.

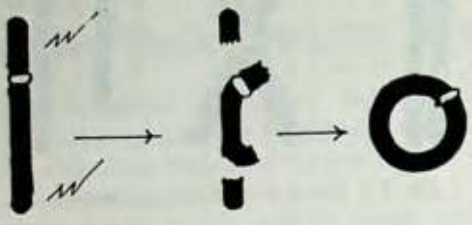

Fig. 5.3 Formation of a ring chromosome.

iii- Inversion

The formation of an inverted chromosome requires two breaks; they may occur on the same side or on opposite sides of the centromere. The broken ends rejoin in such a way that the chromosome segment between the breaks becomes inverted as shown in Fig. 5.4.

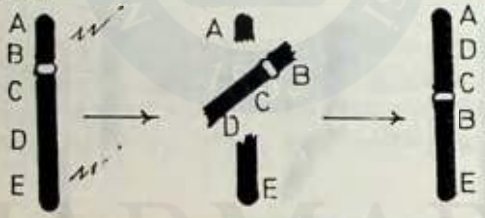

Fig. 5.4 Formation of a pericentric inversion (in a paracentric inversion the relative position of the centromere remains unchanged).

When the inverted segment includes the centromere, the inversion is referred to as *pericentric;* when confined to one arm of the chromosome it is *paracentric.* Pericentric inversions frequently cause a change in the arm ratio of a chromosome, enabling the structual alteration to be readily recognized in mitotic chromosomes. This is not the case in paracentric inversions, which require the application of chromosome banding techniques to reveal their presence in mitotic chromosomes.

iv- Isochromosomes

An isochromosome is a metacentric chromosome in which the two arms are structually and genetically identical. It is the result of a transverse break at the centromere followed by the reunion of the centromeric elements of the sister chromatids as shown in Fig. 5.5.

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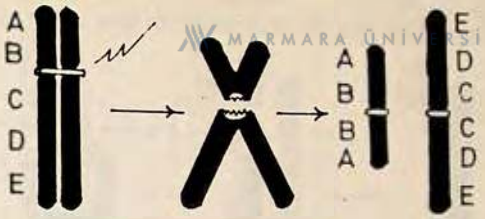

Fig. 5.5 Formation of isochromosomes.

v- Translocations

A translocation occurs when breakage in a chromosome is followed by the transfer of a chromosome segment from its usual position to a new position, usually on a different chromosome. When there is a mutual exchange of segments between two chromosomes, the resulting balanced rearrangement is descibed as a *"reciprocal translocation"*, or *"inter-change"*. This can be accomplished with only two breaks, one in each chromosome (Fig. 5.6). The less common *"non-reciprocal*

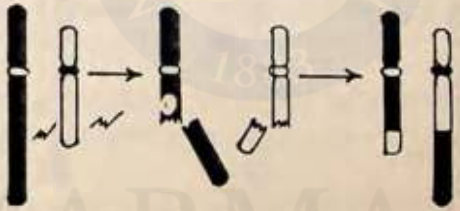

Fig. 5.6 Formation of reciprocal translocation.

translocation", sometimes referred to as an *"insertion"* or *"transposition"*, requires three breaks. Another type of translocation is the *"shift"*, in which a segment from one part of a chromosome is inserted into a differenet part of the same chromosome. The rearrangement also requires three breaks.

An exceptional type of reciprocal translocation is the *Robertsonian Translocation*, or centric fusion, which occurs between two acrocentric chromosomes. The breaks occur very close to the centromere and the broken ends rejoin to form one large metacentric chromosome and one minute centric fragment (Fig 5.7). The centric fragment is usually lost, causing a reduction in chromosome number, but no change in phenotype. Robertsonian translocations are of particular importance in human cytogenetics since they are the most common form of rearrangement in man (Chapter 6), they are also of significance in the evolution of karyotypes.

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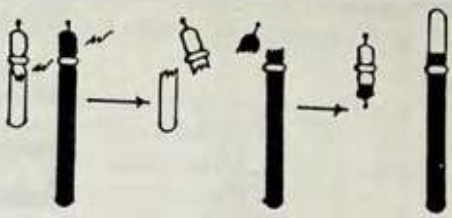


Fig. 5.7. Formation of Robertsonian type (centricfusion) of translocation.

In general, providing that, there is no subsequent loss or gain of genetic material, an interchange does not usually have any phenotypic effect. The only well established exceptions to this are "the position affect" in *Drosophila* (10) and "the V-type position effects" in mice (11-12). In this respect, however, it may be relevant to note two recent observations in the field of human genetics: first, the deleted human chromosome 22 (Philadelphia chromosome), long associated with chronic myeloid leukemia (13), is apparently a part of a balanced interchange between chromosome 22 and chromosome 9 (14), and second, there may be an increased incidence of mental retardation in some individuals with apparently balanced chromosome interchanges (15).

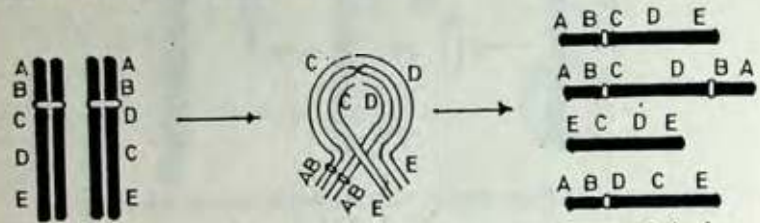
5.3. MEIOTIC CONSEQUENCES OF STRUCTURAL REARRANGEMENTS IN MEIOSIS

Meiotic pairing of the homologous regions of structually altered chromosomes may be achieved by the formation of abnormal bivalent or multivalent configurations of varying complexity, according to the type of rearrangement involved. Those of greatest genetic consequences are the inversion and reciprocal translocation (16).

i- Inversions in Meiosis

Complete synapsis of a pair of homologues, one of which contains an inverted segment, requires the formation of an *inversion loop*. The consequences of crossing-over and chiasma formation inside the loop vary according to the number of chiasmata formed, and whether the inversion is paracentric or pericentric. A single cross-over in a paracentric inversion loop followed by the regular segregation of the bivalent components will produce four different gametes: one with the normal chromosome, one with the inverted chromosome, one with a duplicated segment, and two centromeres (dicentric), and the fourth with a deleted chromosome

without a centromere (acentric) Fig 5.8(a). Dicentric chromosomes are usually unstable as a result of the problems they create in cell division, and acentric



(a) Paracentric inversion Crossing-over in loop

Product of single cross-over

(b) Pericentric inversion Crossing-over in loop

Product of single cross-over

Fig. 5.8 Crossing-over in (a) paracentric inversion loop and (b) pericentric inversion loop.

fragments are invariably lost. For practical purposes, gametes receiving either of these two chromosomes are likely to be inviable, and so the expected consequence of a paracentric inversion is the production of gametes which have either the normal chromosome or the original inverted chromosome, in approximately equal proportions.

Crossing-over within the loop of a pericentric inversion has quite different consequences. A single-cross over will produce deleted and duplicated segments in each of the two chromatids involved in the exchange; both retain a single centromere, and may be considerably lengthened or shortened, depending on the size of the inverted segment and the position of the cross-over (Fig 5.8-b). Thus, two of the four resulting gametes will receive stable but genetically unbalanced chromosomes. As before, the other two will be genetically balanced; one receiving the normal chromosome and one the original inverted chromosome. The genetically unbalanced gametes may be viable and take part in fertilization leading to phenotypic abnormalities in the resulting offsprings. Several such instances have been recorded in man (see Chapter 6).

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ii- Reciprocal Translocations in Meiosis

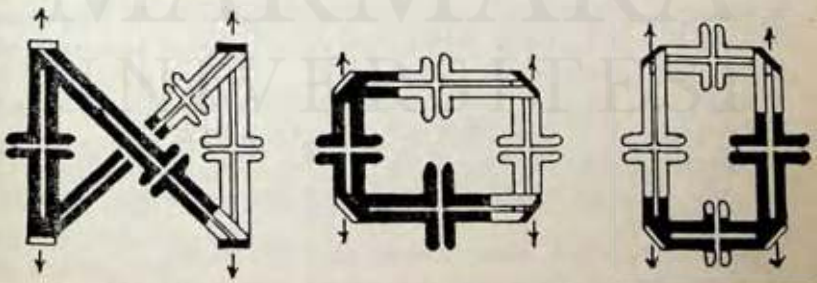
Each of the four chromosomes involved in a typical reciprocal translocation has regions which are homologous with two of the other chromosomes. Consequently, complete synapsis of all homologous regions during meiotic prophase can be achieved only by the formation of a cross-shaped quadrivalent (Fig. 5.9). The ex-



Fig. 5.9 Diagramatic representation of homologous pairing leading to quadrivalent formation at pachytene in a balanced reciprocal translocation.

tent to which gametes receive balanced or unbalanced chromosome complements is influenced by both the manner in which the quadrivalent chromosomes disjoin, and the location of chiasmata in the quadrivalent structure.

There are three ways in which the chromosomes of a quadrivalent can segregate in pairs to opposite poles of the spindle. These are designated *alternate segregation, adjacent 1 segreation* and *adjacent 2 segregation* (Fig. 5.10). In alternate segregation, the centromeres of alternate chromosomes in quadrivalent pass to



(a) Alternate segregation (b) Adjacent 1 segregation (c) Adjacent 2 segregation

Fig. 5.10 Types of segregation in a balanced reciprocal translocation, following quadrivalent formation of type in Fig 5.9 Only alternative segregation (a) results in balanced chromosome complements.

the same pole. Two types of gametes are produced, both with balanced chromosome complements; one has normal chromosomes, the other has the reciprocal translocation. In adjent I segregation, chromosomes with homologous centromeres pass to the opposite poles of the cell. In adjacent II segregation, chromosomes with homologous centromeres pass to the same pole.All gametes produced as a result of adjacent 1 or adjacent 2 segregation types have unbalanced chromosome complements.

The usual outcome of these segregation patterns may be altered if crossing-over occurs in the interstitial regions of a quadrivalent, i.e., between the points of translocation and the centromere. Such a cross-over produces equal proportions of balanced and unbalanced gametes when followed by either alternate or adjacent I segregation, and can increase the variety of unbalanced types of gamete when followed by adjacent 2 segregation.

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6

GENETIC ASPECTS OF HUMAN DISEASES

More than one-fifth of the proteins (and hence genes) in each human being exist in a form that differs from the ones present in the majority of the population. This remarkable genetic variability, or polymorphism, among *"normal"* people accounts for much of the normal variations in body traits such as height, intelligence, and blood pressure. These genetic differences, including those that produce disease. All human diseases can be considered to result from an interaction between an individual's unique genetic make-up and the environment. In certain diseases, the genetic component is so overwhelming that it expresses itself in a predictable manner without a requirement for extraordinary environmental challenges. Such diseases are termed *genetic disorders* which generally fall into one of three categories:

1) *Chromosomal disorders* involve tha lack, excess, or abnormal arrangement of one or more chromosomes, producing excessive or deficient genetic material.

2) *Mendelian* or *simply inherited disorders* are determined primarily by a single mutant gene. These disorders display inheritance patterns which can be classified into Autosomal Dominant; Autosomal Recessive and X-linked types.

3) *Multifactorial disorders* are caused by an interaction of multiple genes and multiple exogenous or environmental factors. Although many of these multifactorial disorders, such as essential hypertension and cleft lip and palate, are said to run in families; the inheritance pattern is complex and the risk to relatives is less than in the single-gene (Mendelian) disorders. Each of these categories present different problems with respect to causation, prevention, diagnosis, genetic counseling, and treatment.

6.1. CHROMOSOMAL DİSORDERS

I would like to give a brief consideration to some of the chromosome aberrations of clinical significance in the following sections.

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6.1.1. Abnormalities of Chromosome Number

6.1.1.1 Euploidy

Triploidy and tetralploidy in man are incompatible with life unless they occur in mosaic form with a normal diploid cell line. A small number of liveborn infants with presumptively pure triploidy have been reported, but they have survived for only a matter of hours (1). Cytogenetic research on spontaneous abortions indicate that triploidy is relatively common in man (2), perhaps as high as 1 per cent of all conceptions, but the embryonic mortality probably exceeds 99 per cent (3). A variety of congenital malformations associated with the condititon have been described: common ones are anomalies of the genitalia, fusion of the digits (syndactyly) and in those cases (mosaics) which have survived long enough for assessment, mental retardation (4, 5).

6.1.1.2. Aneuploidy: (autosomal abnormalities)

Many types of autosomal aneuploidy have been found in spontaneously aborted foetuses, but only trisomic conditions of some of the smaller autosomes are compatible with post-natal-life, unless the abnormal cell line occurs in mosaic form with a normal cell line. In cancer cells (leukemias, colonic cancer, meningiomas etc.) all manner of numerical and structural chromosome abnormalities occur. Certain chromosomes, however, seem to be especially involved (7, 8, 9, 14, 21 and 22) and it may be that these chromosomes carry genes of primary importance in malignant transformation. Studies have shown that variations in the number and structure of chromosomes can be found in pre-malignant tissues taken from the cervix. Premalignant tissues are tissues in which there are certain microscopic changes which suggest to the pathologist that the tissue is beginning to undergo malignant transformation but true carcinomatous change has not yet occurred. It is still too early to assess the true significance of these findings, but perhaps the causative agent evokes alterations in the chromosomes which are then responsible for all the subsequent pathological changes we associate with malignancy. Three autosomal trisomies are well established in man: *Trisomy 21; Trisomy 18, Trisomy 13.* All three types show an increased mean maternal age at birth.

i- Trisomy 21 (47, XX + 21 or 47, XY + 21; Down's Syndrome)*

This is the most cammon autosomal anomaly in man occuring with a frequen-

* There is a short hand notation for chromosome abnormalities. Some of the abbreviations used by cytogeteeists include: *p* and *q* for the short and long arms of chromosomes respectively, *t* for a translocation, *inv* for an inversion, i for an isochromosome and r for a ring chromosome. The + or - sings are placed *before* the appropriate symbol where they mean additional or missing whole chromosomes, but *after* a symbol where an increase or decrease in length is meant. For example, a male with Down's syndrome due to an additional chromosome 21 is represented as 47, XY, + 21 where as a male with 46 chromosomes and a deletion of the short arm of chromosome 5 is represented as 46, XY, 5p - .A woman with 45 chromosomes and a balanced translocation involving the long arms of chromosomes 15 and 21 is represented as 45, XX, - 15, - 21, + t (15 q 21 q).

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cy of about 1 per 650 births. The chromosomal abnormality gives rise to the clinical syndrome of *"Mongolism"* or *"Down's sydrome"* which has been well recognized as a clinical entity for over a century (6, 7). Banding studies indicate that this is a trisomy of the distal part of the long arm of chromosome 21 which causes the syndrome. The caharacteristic facial features of children with Down's syndrome are familiar to most people, and are remarkable for their similarity from one afflicted individual to another. As with all chromosomal diseases, the syndrome is caharacterized by a combination of several morphological features. The more common ones are a flat broad face, epicanthus (a skin fold in the medial angle of the eye), convergent strabismus (squint), a furrowed and protruding tongue, single palmar crease (Simian crease), short crooked fifth finger, hyperflexibility of the joints, small ears with an angular overhanging helix, irregular and abnormal sets of teeth and a flattened occiput. Severe mental retardaditon is always present with I.Q's generally in the 25 to 50 range. They have increased susceptibility to infections, often have congenital heart diseases and an increased frequency of acute leukemia. Data suggests that over half of the conceptions with trisomy-21 fails to survive to full-term (8), which indicates that the extra chromosome also interferes with intrauterine development. Down's syndrome children are credited with being good natured and affectionate and with having a faculty of mimicry and a sence of rhythm. They tend to have a shorter life exceptancy than average, due largely to their increased susceptibility to chest infections or bronchial pneumonia. The increased effectiveness of modern drugs, however, has increased their average life expectancy considerably over recent years, from an estimated 9 years in 1929 (9) to over 18 years in 1963 (10), a figure which is almost certainly an underestimate for the present day in United Kingdom.

If, during meiosis, an accident happens or occasionally two homologous chromosomes fail to separate, and thus migrate together into the same gamete; This event is called *non-disjunction* which can be observed in trisomy 21. Clearly, if a gamete containing two chromosomes 21 unites with a normal gamet containing a single chromosome 21, the result will be a zygote which is trisomic for this chromosome. Non-disjunction may occur not only during meiosis in gamete formation but also in the early mitotic division of a developing zygote, though the former is more frequent. In the latter case, the corresponding monosomic cells with only one chromosome 21 probably die. The cause of non-disjunction and why certain women have children with Down's syndrome while others do not, is probably the result of an ageing effect on the ova. It has been known for many years that the frequency of Down's syndrome is closely related to the age of the mother.

The increased incidence of Down's syndrome with increasing maternal age is quite dramatic. The risk to a mother aged 20 is approximately 1/2000 and at age

30 about 1/1000; by the age of 40, it rises rapidly to 1/100 and after the age of 45, may be as high as 1/50 (11). There is no significant paternal age effect. It is possible that differences in the pattern of meiosis in the two sexes may account for the restriction of the age-related effect on women. As indicated in the previous sections, meiosis in the post-pubertal male is a continuous process in which all the different stages up to the production of mature spermatozoa takes place within a period of about two months. In contrast, in the female, the first meiotic division commences in the foetal ovary and is not completed in any given oocyte until ovulation. In the intervening years, the paired meiotic chromosomes enter a resting stage *(dictyotene)* in which they are held together rather tenuously by chiasmata, which are ultimately essential for co-orientation of the bivalents at the first meiotic metaphase. It seems reasonable to suppose that longer the bivalents remain in this form, the greater is the risk that their integrity will be disrupted, either a result of an ageing process or from environmental influences such as irradiation, drugs, or infections. Since it is likely that only one chiasma is involved in maintaining bivalent 21 (12), it would be surprising if this pair of chromosomes were particularly susceptible to non-disjunction in an ageing oocytes. An alternative suggestion relates the increase in non-disjunction to the production of fewer chiasmata in oocytes which are formed towards the end of the development of the foetal ovary and which are though to be shed late in reproductive life (13, 14, 15). The hypothesis is primarily based on observations on mouse oocytes in which a decrease in the number of chiasmata in ageing oocytes results in termination of the chiasmata and an increase in the presence of the univalents. Similar anomalies have been observed in human oocytes in culture, but it has not been possible to establish a relationship between chiasma frequency and maternal age.

Clearly, not all cases of Down's syndrome can be related to a maternal age effect; some will be the result of non-disjunction in the father, a genetic predisposition for non-disjunction, environmental influences causing non-disjunction (e.g. X-rays), or unbalanced translocations involving chromosome 21. Same may also result from secondary non-disjunction in parents who may be mosaic for trisomy 21, but who show no phenotypic features because the mosaicism affects only a few tissues or is limited to the gonads. Turning now to structural abnormalities of the autosomes, those that have been most clearly defined in man are *Translocations* in which there is an exchange of segments between non-homologous chromosomes, and *Deletions* in which a segment of a chromosome has been lost. Although the majority of children with Down's syndrome have 47 chromosomes being trisomic for chromosome 21, a few (3 per cent) have the normal number of 46 chromosomes. If the chromosomes of one of these children are carefully studied, it is found that although the actual number of chromosomes is normal, the chromosomes themselves are not normal. The usual finding is that a chromosome in the D (13 - 15) group is missing but there is an additional chromosome in the C (6 - 12) group, and in the mother one of the chromosomes 21 is missing as well.

The explanation is that there has been an exchange of chromosome material between a D group chromosome and chromosome 21. A large part of the long arm of chromosome 21 is exchanged with a part of the short arm of a D group chromosome. The *"fragment"* formed by the fusion of the short arms of the two chromosomes is lost and this is sometimes referred to as *Centric Fusion*, and this type of translocation as a *Robetsonian Translocation*.

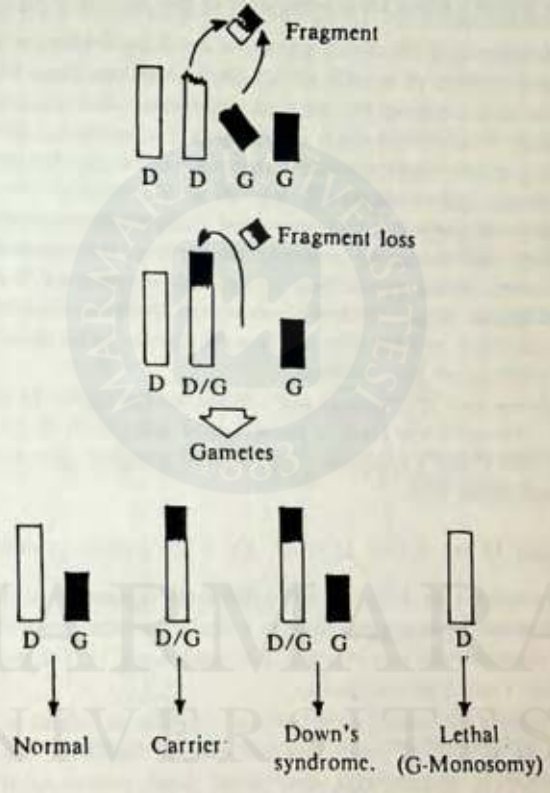

Fig. 6.1 Translocation mechanism in Down's syndrome.

Over 90 per cent of all individuals with Down's syndrome are of the pure trisomy 21 type; about 5 per cent is the mosaic type, although it is probable that many cases of mosaicism remain undetected; and about 2-5 per cent is the result of translocation of the greater part of chromosome 21 onto another chromosome. These chromosomally different types cannot be distinguished on clinical grounds alone, since a wide range of phenotypic expression, from mild to severe, occurs in all three forms of the conditions. As might be expected, however, mosaic forms are sometimes less affected.

ii- Trisomy 18 (47, XX + 18 or 47, XY + 18; Edward's syndrome)

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Unlike Down's syndrome, was not recognized as a clinical entity until the chromosomal condition was first described by Edward in 1960 (16). It was not possible at that time to distinguish between chromosomes 17 and 18, and confirmation that it was indeed trisomy for chromosome 18 was made later by Yunis in 1964 (17).

The frequency of trisomy 18 si in the order of 1 per 5000 births, although there have been a number of reports of *"clustering"* of the disease (18, 19, 20), suggesting that environmental factors, such as epidemic viral infections may be causally related to sudden, localized increases in the incidence of this trisomy. A significantly greater proportion of females are born with the disease than males, the ratio being approximately 3:1.

Some of the more common congenital malformations associated with trisomy 18 includes an elongated occiput, small jaw *(Microgenia)*, small mouth *(Microstomia)*, widely spaced eyes *(Ocular Hypertelorism)*, low-set and pointed ears, and flexed fingers with a characteristic grasp in which the index finger overlaps the third, and the fifth overlaps the fourth. Heart defects and malformations of kidneys are also common.

Apparent mental deficiency and a failure of the infants to thrive are constant findings. Few survive beyond six months, and only about 10 per cent beyond one year. As with Down's syndrome, trisomy 18 is found in pure form, mosaic form, and translocation form.

iii- Trisomy 13 (47, XX + 13 or 47, XY + 13; Patau's syndrome)

It is possible that the gross phenotypic malformations associated with trisomy of chromosome 13 were recognized as a distinct clinical syndrome long before it was first demonstrated as a chromosomal condition (21). The incidence of trisomy 13 is about 1 per 5000 live births.

The grossly malformed appearance of the affected infants is largely due to the common occurence of cleft lip and palate, small or absent eye (micro or anophthalmia respectively), and small head (microcephaly) with sloping forehead. Other conspicuous features may be extra digits (polydactyly) and abnormalities of the external genitalia, particularly in males. Features such as wide set eyes, epicanthic folds, a small chin, low-set malformed ears, rockerbottom heels, short big toes, and heart defects are common to both this syndrome and Trisomy 18. As may be expected, there is severe mental defect, although few individuals survive long enough for assessment. The majority die within one year although few survive into later childhood. Most cases are pure trisomies for chromosome 13, but mosaic and translocation forms have also been described.

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6.1.1.3. Aneuploidy (Sex chromosome abnormalities)

Sex chromosome abnormalities arise in the same way as in Down's syndrome with trisomy-21; that is, they are the results of non-disjunction having occurred during gametogenesis (Fig. 6.2.) Non-disjunction may also occur during early embryogenesis and we shall disscuss this later. If non-dusjunction occurred during oogenesis and the ovum containing no sex chromosomes was fertilized by an X-bearing sperm, the result would be a zygote with an XO sex chromosome constitution. If the ovum were fertilized by a Y-bearing sperm, then the result would be a zygote with a YO sex chromosome constitution. This is probably lethal because no individual has ever been found with this sex chromosome pattern. The X chromosome is of maternal origin in about 75 per cent of individuals with Turner's syndrome, and the two X chromosomes are of maternal origin in about 75 per cent of individuals with Klinefelter's syndrome. Cases with four or more sex chromosomes cannot be explained by a single-non-disjunctional event and we have to postulate that two such events have occurred, one during meiosis I, and the other during meiosis II. XYY individuals can only result during gametogenesis if non-disjunction occurs during meiosis II, i.e. the Y chromatids fail to separate.

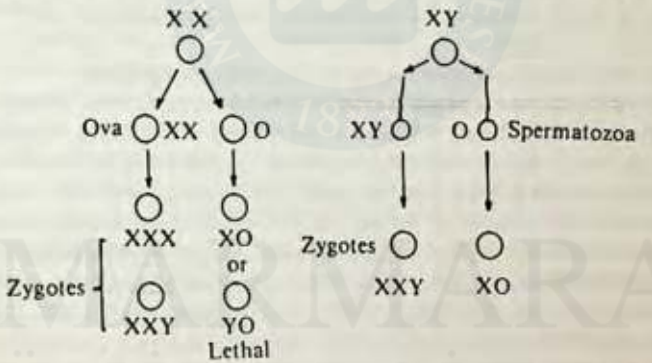

Fig. 6.2 Non-disjunction during oogenesis and spermatogenesis.

i- The 47, XXY syndrome (Klinefelter's syndrome)

The presence of an extra X chromosome in males in the condition referred as Klinefelter's syndrome (21), was first demonstrated by Jacobs and Strong in 1959 (22). It has subsequently been shown to be the most common sex chromosome abnormality,occurring with a frequency of about 1 per 500 live male births. The phenotypic effects of their genetic unbalance are remarkably slight, the only consistent feature being small testes which are unable to produce spermatozoa. A proportion of 47, XXY men, however, have additional features such as breast

development (gynaecomastia) and underdeveloped secondary sexual characters. In addition, a number of surveys have shown an increased frequency of males with this chromosomal abnormality in mentally defective and certain prison populations (23, 24).

ii- The 47, XXX syndrome (The triple X female)

The first 47, XXX syndrome to be described (25) was a 35 year-old female with malformations which included underdeveloped breasts, infantile genitalia, and a lower than average intelligence. It has become apparent in later investigations that the majority of triple X females have quite normal development. They are usually fertile and produce offsprings wtih normal chromosome complements. This is remarkable since it would be expected that half of the gametes would contain two X chromosomes giving rise to either 47, XXX females or 47, XXY males and suggests that there may be some form of preferential segregation with the 24, XX secondary oocytes always becoming the polar body, and the 23, X oocyte becoming the functional ovum.

The incidence of the 47, XXX chromosome constitution in the normal female population appears to be less than 1 per 1000, but is considerably high in the populations of mental defective institutions and mental hospitals (26, 27).

iii- The 45, XO syndrome (Turner's syndrome: gonadal dysgenesis)

The 45, XO syndrome is unique among the human chromosome anomalies in being the only pure monosomic condition to be compatable with life. The clinical syndrome was recognized in 1962 (27) long before the chromosome abnormality was first demonstrated in 1965 (28). The most constant and significant feature is the replacement of the ovaries by *"streak gonads"* which are completely devoid of germ cells. As a consequence, there is amenorrhoea (absence of menstruation), immature sexual development, and sterility. Other common physical abnormalities are short stature (less than 1.53 cm.); dwarfing is almost universal, webbing of the neck, and a variety of skeletal abnormalities. Intellectually, they are slightly subnormal. Deafness often occurs. The syndrome may be found in mosaic form, the most common being XO/XX or XO/XY, or in association with a variety of structurally altered X chromosomes, including ring chromosomes, isochromosomes, and deletions of the long and short arms. The incidence of 45, XO females is low, about one per 2500 female births, but the karyotype is found frequently in spontaneous abortions.

iv- The 47, XYY syndrome

The first individual with 47, XYY karyotype to be described in 1962 was a phenotypically normal male, but subsequent chromosome studies on the inmates of high security hospitals in 1965 and 1966, USA (28, 29) suggested that the posses-

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sion of two Y chromosomes as a part of 47, XYY or 48, XXYY complement could be associated with tall stature (over 1.83 cm.) mental subnormality, and a predisposition to aggressive and antisocial behaviour.Surveys of new born males, however, indicate that the incidence of the 47, XYY karyotype may be quite high, about 1 per 600 males (30, 31), suggesting that the majority of individuals with this chromosomal constitution are developmentally normal males, and are inconspicuous in the general population.

6.1.2. Abnormalities of Chromosome Structure

We have talked about the various structural abnormalities in the previous chapters, with the exception of the *"shift"* that have all been observed in human chromosomes. Some structural abnormalities such as specific deletions of certain chromosomes, may be associated with distinctive clinical syndromes and are of diagnostic value; other types of rearrangement, such as pericentric inversions and reciprocal translocation, may be of additional clinical significance in being the basis for genetic counselling. Now we will deal with the clinical significance of deletions, inversions, and reciprocal translocations.

i- Deletions

A number of clinical syndromes have been associated with specific chromosome deletions, of which the best known and most clearly defined is the *"cri du chat"* (cat cry) syndrome associated with a deletion of the short arm of chromosome 5. The cardinal feature of the syndrome is the high pitched, mewing cry of affected babies which disappears after the first few months of the infancy. The other features include wide-set eyes, epicanthic folds, low-set or malformed ears, and severe mental retardation, a group of congenital malformations commonly found in less specific groups of mental defectives. Although the babies are usually small at birth and fail to thrive, the condition appears generally compatible with survival. The incidence of the defect is low, probably less than 1 per 50000 births. In the majority, the 5p deletion has arisen *de novo*, but a number of cases have been the result of the unbalanced segregation of a reciprocal translocation in one of the parents. This illustrates the importance of investigating parental chromosomes whenever a structurally altered chromosome is formed in a child with congenital malformations and mental retardation.

ii- Inversions

Paracentric inversions have not been described in man, but a number of pericentric inversions, confirmed by banding techniques. It is evident from chapter 5 that the phenotype of an individual who carries a pericentric inversion should not be influenced by the chromosome rearrangement since there is no loss or gain of genetic material. The danger to the carrier is the possibility of producing unbalanced gametes as a consequence of cross-over within the inversion loop formed during meiosis.

Iii- Reciprocal translocations

The nature of reciprocal translocations and their meiotic consequences have been considered in Chapter 5. As with a pericentric inversion, the main problem is not the effect of the reciprocal translocation on the phenotype of the carrier, but on the high risk of producing gametes which are genetically unbalanced as a consequence of events at meiosis.

Examples of situations which may be encountered in human cytogenetics are as follows:

The first one is an example of a Robertsonian translocation, i.e., the centric fusion of two acrocentric chromosomes, in this case no 13 with no 14. Typically, the centric fragment has been lost in the carrier parent who has a karyotype of only 45 chromosomes. As a result of either adjacent -1 or adjacent- 2 segregation (depending on which centromere is çarried by the translocation chromosome) at first meiotic division, the offspring has inherited both the 13/14 translocation chromosome and the normal 13 chromosome from the carrier parent. Thus the child has a karyotype of 46 chromosomes but is effectively trisomic for most of chromosome 13 and as a result has the phenotype of *Patau's syndrome.* In the second example the reciprocal translocation involves chromosomes 1 and 6; the rearrangement is not associated with a recognized clinical syndrome. The possibilites for producing balanced or unbalanced gametes exists as before, and in this case adjacent-1 segregation at meiosis has resulted in the child receiving an unbalanced form of the translocation in which the terminal portion of chromosome 1 is present in triplicate.

6.1.3 Incidence of chromosomal abnormalities (Frequency and clinical spectrum)

Chromosome abnormalities are among the best defined causes of fetal loss or congenital diseases. The frequency of spontaneous abortion is estimated to be between 15 and 20 per cent of all pregnancies, and of these approximately50 per cent are associated with chromosome abnormalities. Overall, it is probable that about 6 per cent or more of all yearly human conceptions which lead to an identifiable pregnancy are chromosomally abnormal.

The frequency of chromosome abnormalities at birth is approximately 5.6 per 1000 (Table 6.1). Of these, about 2 per 1000 are due to a variation in the number of sex chromosomes, 1.7 per 1000 are due to a variation in the number of autosomal chromosomes, and 1.9 per 1000 are major chromosomal rearrangements.

The frequency of chromosome aberrations in spontaneous abortions is very high; the average incidence in the large surveys is over 20 per cent in 1970 (34, 35). Autosomal trisomies constitute the largest group of anomalies, about 40 per cent of the total, with trisomies of E (mostly 16), and group G being the most common. About 20 per cent of the total are 45 (XO), and about the same proportion are triploid.

Combining the data from the newborn and abortion surveys leads to the conclusion that a minimum of approximately 4 per cent of human conceptions must

Table 6.1 Common chromosome disorders.

Condition	Frequency/1000 live births
Numerical	
Sex chromosome	
45 X	0,07
47 XXX	0,54
47 XXY	0,61
47 XYY	0,49
Autosomal	
47 + D	0.12
47 + E	0.19
47 + G	1.14
Structural	
Balanced D/D centric fusions	0,79
Balanced D/G centric fusions	0,21
Translocations/inversions	0.28

be chromosomally abnormal and over 90 per cent of these abort spontaneously as reported in a survey in 1973 (36) in United Kingdom.

6.1.4. Acquired chromosome abnormalities

Chromosomes are subject to damage as a result of exposure to a wide range of physical, chemical, and biological mutagens. The same agents can induce neoplasia, the development of new cell lines, which have a proliferative advantage over the normal cell lines, as in cancers. It is not surprising to find, therefore, that diverse chromosome abnormalities are found in most types of cancerous growths. It is difficult to evaluate the significance of these observations, particularly in assessing the relationship of chromosome abnormalities to the malignant changes. It is not known whether the chromosome abnormalities cause the malignant changes, or are result from them; or whether they are both the result of some common factor. The study of the subject is further complicated by the failure, with one notable exception, to associate any particular form of neoplastic growth with a specific chromosome abnormality. The exception is the usual occurence of the Philadelphia (Ph) chromosome in the bone marrow, and often the leucocyte cultures from individuals with chronic myeloid leukaemia (37). Because of the predisposition of children with Down's syndrome to develop leukaemia, the Ph

chromosome was long thought to be chromosome 21 with a deletion of the long arm (q); but following the indroduction of the fluorescent technique it was clearly demostrated that the anomaly may not be a simple deletion, but a reciprocal translocation between the long arm of chromosome 22 and the long arm of chromosome 9 (39).

6.2 MENDELIAN OR SIMPLY INHERITED DISORDERS

These are determined primarily by a single mutant gene. These disorders display inheritance patterns which can be classified into a) Autosomal dominant b) Autosomal recessive c) X-linked types. The distinction between *"dominant"* and *"recessive"* is of convenience in pedigree analysis and does not simply a fundamental difference in genetic mechanism. The term *dominant* implies that a mutation is clinically manifested when an individual has a single dose of this mutation *(or is heterozygous for it)* while *recessive* implies that a double dose *(or homozygous)* is required for clinical detection. Genes are never dominant or recessive; their effects, however, produce clinical patterns that are classified as dominant or recessive. Despite their overall clinical "normality", individuals who are heterozygous for "recessive" genes often have biochemical abnormalities that are demonstrable in the laboratory on the other hand, those who are homozygous for "dominant" genes are usually more severely affected than are the heterozygotes.

The genes for more than 100 simply inherited diseases have been assigned to specific chromosomes. Disease producing genes assigned to the X chromosome out numbers those, so for assigned to any single autosome. This is because assignment to the X-chromosome requires only pedigree studies showing X-linked inheritance, Fig. 6.3 shows the tentive map of the X-chromosome. Assignment to an autosome

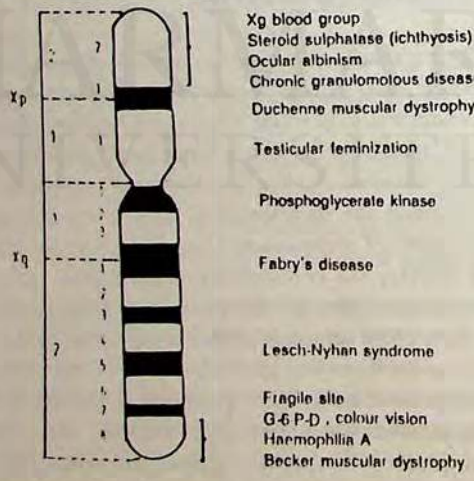

Fig. 6.3. A tentive map of the X-chromosome.

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is more complicated, requiring sophisticated techniques of somatic cell hybridization or pedigree studies showing linkage between a disease-producing gene and a "marker" gene that is known to be on a certain chromosome.

The demostration that a particular disease or syndrome shows one of the three Mendelian patterns of inheritance implies that its pathogenesis, no matter how complex, is due to an abnormality in a single protein molecule. For example, in sickle cell anemia, the entire clinical syndrome, including such seemingly unrelated disturbances as anemia, pain crises, nephropathy, and predisposition to pneumococcal infections, are all the physiologic consequences of having thymine instead of adenine at a specific locus on the gene that codes for the beta chain of hemoglobin, producing a substitution of a valine for a glutamic acid in the sixth amino acid position in the protein sequence.

In many Mendelian disorders, especially in those with dominant inheritance, it is not possible to demostrate directly the protein that is primarily altered by the mutation. In such cases (e.g., adult polycystic kidney diseases and tuberous sclerosis) only the distal physiologic effects of the mutation are recognizable. Nevertheless, it is safe to assume that a single primary defect exists whenever a disease is transmitted by a single gene mechanism and a various manifestations of the disease all can be related to the mutational events by a more or less complicated "pedigree of causes".

i- Autosomal Dominant Disorders

A dominant trait is one which shows itself clearly in the heterozygote. That is, a person with an autosomal dominant trait possesses the abnormal (mutant) gene which causes the disorder as well as the normal gene or allele. This is true for *rare* genes, but if the mutant gene is common then it is possible that some affected individuals might be homozygous for the mutant gene. The gene responsible for an autosomal dominant disorder is located on one of the 22 autosomes, and both males, and females can be affected. Since alleles segregate independently at meiosis, there is a 50 per cent chance that the offsprings of an affected heterozygote will inherit the mutant allele and, similarly, a 50 per cent chance of the offsprings inheriting the normal allele. In autosomal traits, if an affected individual marries a normal person, on *average* half their children will be affected. The reason is that in an affected person, each gamete carries either the gene for the trait or the normal gene. All the gametes of the unaffected partner will carry the normal gene. If we represent the dominant (mutant) gene as "A" and the recessive (normal) gene as "a" then the various gametic combinations can be represented as:

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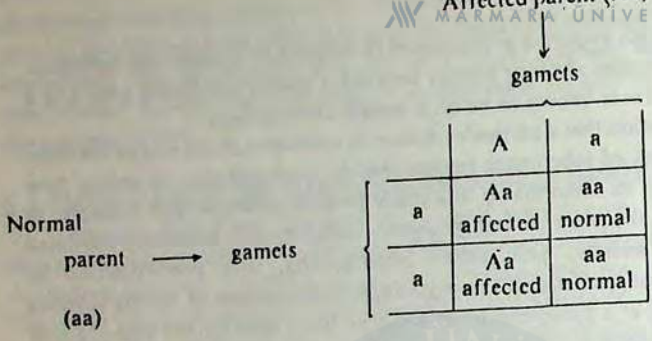

Fig. 6.4 shows a typical pedigree involving an autosomal dominant trait. The following features are characteristic: (1) Each affected individual has an affected parent (unless the condition arose by a new mutation or is mildly expressed in the affected parent); (2) An affected individual will bear, on avarage, both normal and affected offsprings in equal proportions; (3) Normal children of an affected individual will have only normal offsprings; (4) Males and females are affected in equal proportions; (5) Each sex is equally likely to transmit the condition to male and female offsprings, with male to male transmission occurring;(6) Vertical transmission of the condition through successive generations occurs, especially when the trait does not impair reproductive capacity.

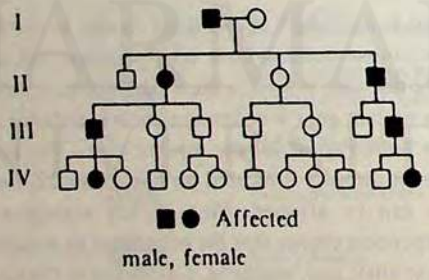

Fig. 6.4 Pedigree pattern of an autosomal dominant trait.

While half of the offsprings of an individual with an autosomal dominant condition will inherit the disease, it is not necessarily true that each affected person must have an affected parent. In every autosomal dominant disease a certain proportion of affected persons owe their disorder to a new mutation rather than an inherited mutation. Since the estimated frequency of mutation is 5×10^{-6} mutations per gene per generation, and since a dominant trait, by defination, requires a mutation in only one of a pair of alleles, one would expect that about 1 in 100000 newborn persons would possess a new mutation at any given genetic locus.

Table 6.2 The most commonly encountered Mendelian autosomal dominant disorders.

System	Disorder	Frequency/ 1000 births	Main clinical features
Nervous	Huntington's chorea	0,5	Involuntary movement and dementia from middle life
Kidney	Polycystic disease	0,8	Progressive kidney failure
Eyes	Dominant blindness	0,1	Blindness
Ears	Otosclerosis	1,0	Reduced hearing from adolescence or later
Blood	Hypercholesterolaemia	2,0	Early-onset coronary artery disease
Teeth	Dentinogenesis imperfecta	0,1	Maldevelopment of teeth
Skeleton	Osteogenesis imperfecta	0,04	Fragile bones. Fractures. Deformity
	Cleft lip/palate	0,01	Cleft lip with/without cleft palate
Metabolism	$Alfa_1$-antitrypsin deficiency	0,3-0,8	Liver failure in infancy chronic lung disease-emphysema

Autosomal dominant traits tend to be extremely variable in expression. This variability in clinical manifestations is sometimes referred to as *expressivity*. In Osteogenesis imperfecta, for example, some individuals may be so mildy affected that the condition hardly affects their every day life, but others may be so severely affected that they may become complete invalids with gross skeletal deformaties. most affected individuals lie somewhere between the two extremes. Sometimes a gene may not express itself at all, in which case it is said to be *nonpenetrant*, and

this explains apparent *"skipped"* generations in certain pedigrees. Such variations in the expression of a mutant gene are the result of the modifying effects of other genes as well as environmental factors on the expression of the mutant gene.

Some autosomal genes are expressed more frequently in one sex than the other. This is called *sex influence*. In the extreme, when only one sex is affected, this is referred to as *sex limitation*. Gout and presenile baldness are examples of sex-influenced autosomal dominant traits; males being predominantly affected in both cases. Hydro-metrocolpos (a recessive disorder) which only occurs in females is a sample for sex limitation cases.

ii- Autosomal recessive inheritance

Autosomal recessive traits affects both sexes. However, recessive traits are only manifest when the gene is present in a double dose; that is, in persons homozygous for that particular mutant gene. Usually heterozygotes are perfectly healthy and all the offsprings of an affected person are normal, unless the affected person marries a heterozygote, but because of the rarity of most recessive traits this would be very unlikely. Of course, if two affected persons, homozygous for the same gene mary, then all their children would be affected, but again such an event would be unlikely. In general, both the parents and the offsprings of persons homozygous for a rare recessive gene are perfectly healthy. About one-quarter of the offsprings of two heterozygotes will be affected. the reason why one-quarter should be affected is because each parental gamete carries either the gene for the trait or the normal allele. If we represent the normal gene (which in this case is dominant) as "A", and the recessive (mutant) gene as "a", then the various gametic combinations can be represented as:

Normal heterozygous parent (Aa)

↓

gametes

Normal heterozygous parent (Aa) → gametes	A	a
A	AA normal	Aa unaffected heterozygous
a	Aa unaffected heterozygote	aa affected

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The pedigree pattern is shown in Fig. 6.5.

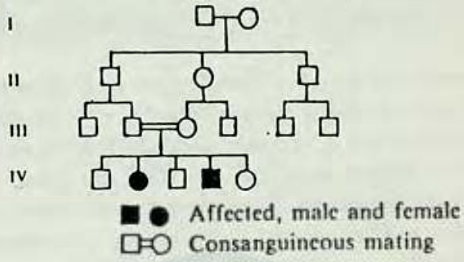

Fig. 6.5 Pedigree pattern of an autosomal recessive trait.

The pedigree pattern is shown in Fig. 6.5. Indicates that it is not possible to trace the disease through several generations; all the affected individuals in a family are in one *sibship*, that is, they are borthers and sisters.

Table 6.3. Shows some of the autosomal recessive conditions more common in north Europeans, together with their approximate frequency per 1000 UK births.

System	Disorder	Frequency/ 1000 births	Main Clinical features
Metabolism	Cystic fibrosis	0,5	Viscid secretions chest infection
	Phenylketonuria	0,1	Mental retardation
	Galactosaemia	0,02	Progressive liver failure from early infancy.
Nervous	Non-specific mental retardation	0,5	Mental retardation
Blood*	Sickle cell anemia	0,1	Severe anemia. Bone deformity Splenomegaly
	Beta - thalassaemia	0,05	Several syndromes. Include virilism in females, abnormal development of male genitalia, Addisonian crises etc.

* Represents an approximate estimate based on gene frequencies in immigrant populations (Mousouras, M., et al., (1980) Lancet II, 806).

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iii- X-Linked Disorders

It was stated in Chapter 3 that the behaviour of one pair of chromosomes, the sex-chromosomes, would require a more detailed description than the general account then given.

The genes possible for X-linked disorders are located on the X-chromosome; therefore, the clinical risk and severity of the diseases are different for the two sexes. Since a female has two X chromosomes, she may be either heterozygous or homozygous for the mutant gene and the trait, therefore, results in either a recessive or dominant expression. Males, on the otherhand, have only one X-chromosome, so they can be expected to display the full syndrome whenever they inherit the gene regardless of whether the gene behaves as a recessive or as a dominant trait in the female. Thus, the terms X-linked dominant or X-linked recessive refer only to the expression of the gene in women.

An important feature of all X-linked inheritance is the absence of male-to-male (i.e. father to son) transmission of the trait. This follows because a male must always contribute his Y-chromosome to his sons, hence, he can never contribute his X-chromosome. On the other hand, a male contributes his one X-chromosome to all his daughters (40). Fig. 6.6. illustrates the characteristic features of X-linked inheritance. For further explanations see Chapter 3.

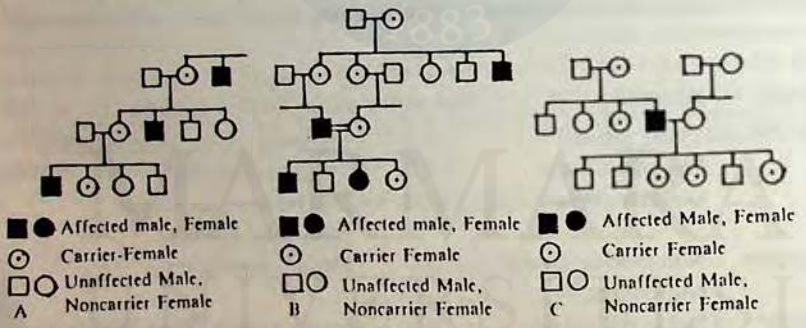

Fig. 6.6 Pedigree patterns of on X-linked recessive trait. A. Note the oblique pattern of inheritance. B. An affected female can result from the mating of an affected male and a carrier female, as in the consanguineous marriage shown here. C. An affected male mating with a normal noncarrier female has all normal sons and all carrier daughters.

Examples of X-linked recessive disorders in humans include hemophilia A, nephrogenic diabetes insipidus, The Lesch-Nyhan syndrome, Duchenne form of Muscular dystrophy, Glucose-6- phospate dehydrogenesa (G6PD) deficiency, Testicular feminization and Farby's disease. Colour blindness is also inherited as an X-linked recessive trait,but it is sufficiently frequent (occurring in about 8 per

cent of white males)so that the occurrence of homozygous colour-blind females in no rarity (41).

X-Linked dominant inheritance is shown by the pedigree in Fig. 6.7. Its characteristics are as follows: 1) Females are affected about twice as often as

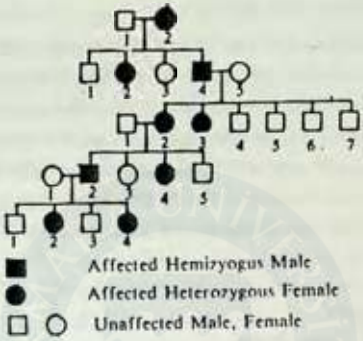

Fig. 6.7 Pedigree pattern of an X-linked dominant trait.

males 2) an affected female transmits the disorder to half of her sons and half of her daughters 3) an affected male transmits the disorder to all his daughters and to none of his sons, and 4) the syndrome is more variable and less severe in heterozygous affected females than in hemizygous affected males.

Some rare conditions may be inherited as X-linked dominant traits in which there is lethality in the hemizygous male. The characteristics of this form of inheritance are shown by the pedigree in Fig. 6.8. 1) The disorder occurs only in

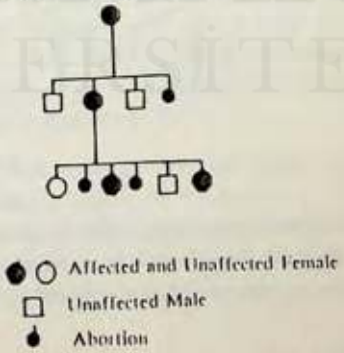

Fig. 6.8 Pedigree pattern of an X-linked dominant trait lethal in the hemizygous male.

females who are heterozygous for the mutant gene 2) an affected mother transmits the trait to half of her daughters 3) an increased frequency of abortions occurs in affected women, the abortions representing affected male foetuses. Conditions that appear to be transmitted by this mode of inheritance include incontinentia pigmenti, focal dermal hypoplasia, orofaciodigital syndrome and hyperammonemia due to ornithine transcarbamylase deficiency.

Expression of X-linked traits in females tends to be variable because of the phenomenon of X-chromosome inactivation. Early in embryonic development one of the X-chromosomes in each somatic cell of a female is inactivated. The inactivation process is random, so that for each cell there is an equal probability that the paternally or maternally derived X-chromosome will be inactivated. The inactivated X-chromosome is rendered permanently nonfunctional, so that all progeny of the initial cell inherit the same active and inactive X-chromosome (Fig. 6.9).

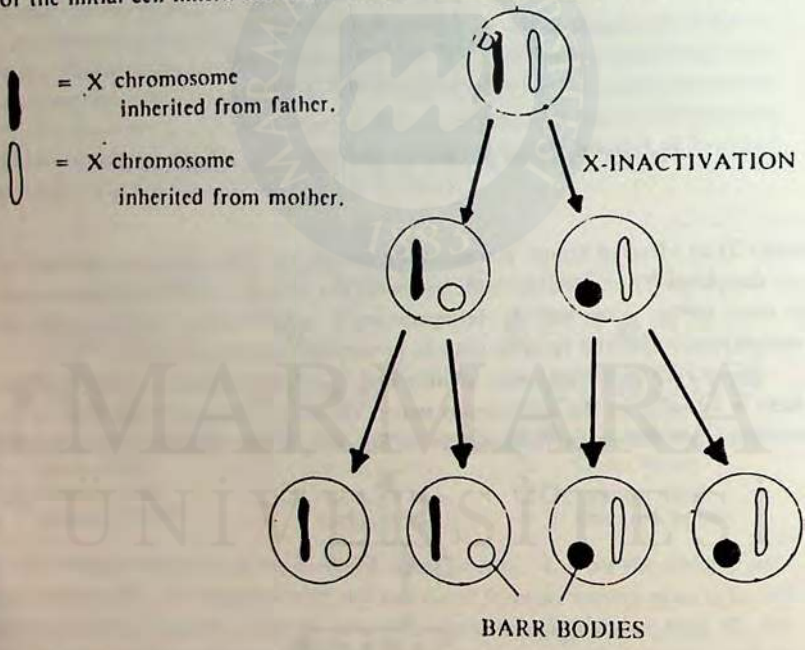

Fig. 6.9 X-chromosome inactivation with the formation of a Barr body. Which X-chromosomes becomes inactivated in a particular cell is a matter of chance. However, all the descendants of that cell will have the same X-chromosome inactivated.

Thus each female is a mosaic; on the average half of her cells express the chromosome of the father, and half express the X-chromosome of the mother. If a mutation in a gene is carried on one of her X-chromosomes, about one-half of the cells in each tissue will be normal and the other half will manifest the mutant phenotype. However, chance or selection of one or the other set of clones of cells may disturb these proportions in any given individual. Depending on the proportions of mutant and normal X-chromosomes in each tissue, a genetically heterozygous female may either be clinically normal or have mild or severe manifestations of the disease. To illustrate, mothers of boys with the X-linked recessive Duchenne form of muscular dystrophy may occasionally show mild manifestation of the disease, such limb girdle weakness or hypertrophied calves.

In each female cell the nonfunctional X-chromosome can be visualized by several techniques. By ordinary staining, the inactivated X-chromosome in metaphase appears as a condensed body and it replicates late in the mitotic cycle (late-labeling with tritiated thymidine). In nondividing cells the inactivated X-chromosome can be observed as a clump of cromatin at the periphery of the nucleus, the so called *barry body*. In abnormal states with more than two X-chromosomes such as 47, all but one of the X-chromosomes are inactivated, so female cells may have multiple X-cromatin bodies (Fig. 6.9).

Since a single mutant allele is sufficient for the expression of X-linked recessive orders, consanguinity does not increase the likelihood of expression in males, unlike the case in the rare autosomal recessive disorders, On the other hand, just as in the dominantly inherited disorders, new mutations can be a factor. In general, if an X-linked recessive condition reduces biologic fitness to nil, one-third of affected males will be a result of new mutations, and a additional one-third will be born to mothers who themselves are carriers as a result of new mutation. Thus, only one-third will come from a classic pedigree manifesting oblique transmission. An example of such a disease is the Duchenne form of muscular dystrophy in which affected hemizygous males are so severely disabled that they never reproduce (42). In hemophilia A, in which biologic fitness is greater than nil, about 20 per cent of affected males represent new mutations (43).

In families in which only one male is affected with an X-linked recessive disease and there is no other family history of the trait it is essential for proper genetic counseling that the mother undergo biochemical tests or other relevant studies to determine whether she is a carrier. If she is a carrier, half of her daughters will be carriers and half of her sons will be affected as can be illustrate;

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Carrier female (X^h X)

X^h = represent haemophilia gene

gametes

Normal male (XY) → gametes		X^h	X
	X	X^hX Carrier daughter	XX normal daughter
	Y	X^hY affected son	XY normal son

On the otherhand, if her affected son represents a new mutation only her daughter will inherit the gene. At present, biochemical tests can identify female carriers for several X-linked diseases including the Lesch-Nyhan syndrome, Fabry's disease, Hunter's syndrome, hemophilia and the Duchenne form of muscular dystrophy.

The distinction between X-linked inheritance and sex-influenced autosomal inheritance is important. Baldness is probably inherited as an autosomal dominant trait, yet it is manifested mainly in men and rarely in women. Heterozygous females express the baldness gene only when a source of testosterone becomes available as occurs with a masculinizing tumour of the ovary.

6.3. MULTIFACTORIAL DISORDERS

The common chronic diseases of adults (such as essential hypertension, cronary heart disease, diabetes mellitus, peptic ulcer disease, and schizophrenia) as well as the common birth defects (such as cleft lip and palate, spina bifida, and congenital heart disease) have been long known to *"run in families"*. They best fit into the category of multifactorial genetic diseases. The genetic element in these disorders rearly manifests itself in an all-or-none fashion as it does in the simply inherited disorders and in chromosomal aberrations. Instead, it is the interaction of multiple genes with multiple environmental factors that produces the familial aggregation.

In the multifactorial disorders, there is a *polygenic component* consisting of a series of genes that interact in a cumulative fashion. An individual who inherits the right combination of these genes passes beyond a "threshold of risk", at which point an *environmental component* determines whether and to what extent that person is clinically affected. In order for another individual in the same family to express the same syndrome, the same or similar combination of genes must be inherited. Since the first-degree relatives of an affected individual (i.e. parents, sibl-

ings and offspring) each share half of that person's genes, they are all at increased risk of exhibiting the same polygenic syndrome. Second-degree relatives (uncles, aunts and grandparents) share on the average one-fourth of an individual's gene, and third-degree relatives (causins) share one-eight. Thus, as the degree of relation becomes more distant, the likelihood of a relative inheriting the same combination of genes comes less. Moreover, the chances of any relative inheriting the right combination of risk genes decrease as the number of genes required for the expression of a given trait increases (44).

Multifactorial disorders are heterogenous in the sense that the relative contribution of the polygenic factors (risk genes) and environmental factors to the etiology vary greatly from patient to patient. However, it is important to remember that among common phenotypes which are largely multifactorial, often a small proportion will be created by major mutant genes. For example, although coronary heart disease is usually of multifactorial etiology, about 5 per cent of subjects with premature myocardial infarctions are heterozygotes for familial hypercholesterolaemia which is a single-gene disorder that produces atherosclerosis in the absence of any other predisposing factor. Similarly, in a small proportion of patients with other common diseases such as peptic ulcer disease or "essential" hypertension, the condition is not multifactorial but determined by a single-gene, as in the multiple endocrine adenome-peptic ulcer syndrome or the medullary thyroid carcinoma-pheochromocytoma syndrome, respectively.

6.4. THE INBORN ERRORS OF METABOLISM

The complexities that arise in understanding the behaviour of gene defects in patients are often compounded by our lack of knowledge of the precise mechanism involved. It therefore, seemed useful to begin with a group of inherited disorders where the biochemical basis is known. Inherited disorders of the metabolism are of great practical importance in clinical genetics; an increasing number can be effectively treated or at least managed in such a way as to minimize the deleterious effects, provided they are diagnosed early, even more are amenable to prenatal diaginosis, and detection of the heterozygous state can allow every precise genetic counselling for the family. Our purpose here is to extract from the great number of examples available, a few conditions that illustrate the principles involved. First, it should be noted that on the whole these disorders are inherited in a straight forward Mendelian fashion; nearly all are autosomal recessive, with a few X-linked or autosomal dominant. It is not the pattern of inheritance that is of special interest, but the mechanism by which the gene defect produces its clinical effect.

The story started at the beginning of the century with the first demostration of Mendelian inheritance in man.At first Garrod (45) had studied Alkaptonuria. Later Beadle and Tatum (46) developed the idea that metobolic processess, whether

in man or in any other organism, proceed by steps. Each step is controlled by a particular enzyme and this in turn is the product of a particular gene. This is referred to as the "one gene - one enzyme" concept, which is illustrated by such conditions as *phenylketonuria, alkaptonuria, albinism* and *galactosaemia.*

Children with *phenylketonuria* are severely mentally retarded, frequently have convulsions, and often have to be institutionalized. In these children a particular enzyme necessary for the conversion of phenylalanine to tyrosine (phenylalanine hydroxylase) is deficient; that is, there is a genetic block at (A) in the metabolic pathway as it can be seen in Fig. 6.10. As a result, Phenylalanine cannot be converted into tyrosine; instead it accumulates in the blood and is converted into phenylpyruvic acid, and this is turns into phenyllactic acid and phenylacetic acid. One or more of these substances probably has toxic effects and produces phenylketonuria (PKU). Some phenylalanine converted into pheylpyruvic acid is excreted in urine. Because of a deficiency of tyrosine, which results from the enzyme block at (A), there is a reduction in melanin formation (Fig. 6.10). Affected children therefore often have blond hair and blue eyes and areas of the brain which are normally pigmented (e.g the substantia nigra) may also lack pigment. It is interesting to speculate on the possibility that, it may not be so much the rise in phenylalanine, but the deficiency of tyrosine which causes the mental retardation. Bessman (47) has suggested, in his "justification hypothesis" that the reduced ability of a mother (because she is heterozygous) to deliver an appropriate amount of tyrosine to her foetus, which in turn is unable to correct for this deficiency because of its own genetic constitution, may result in reduced foetal brain growth. Such a mechanism might be extended to other aminoacidopathies, and conceivably explain a significant proportion of "non-specific" mental retardation.

An obvious method for treating phenylketonuria would be to give the patient the enzyme he lacks but this is not practicable for many reasons. Alternatively it would be expected that if phenylalaine were removed from the diet this might be an effective treatment. In fact this is so. If an affected child is detected early enough, the disease can be prevented by giving a special diet containing little phenylalanine (this is an essential amino acid and therefore can not be entirely removed from the diet). The condition can be diagnosed by tests which detect phenylpyruvic acid in the urine (ferric chloride test) or excess phenyalaline in the blood (e.g. Guthrie test).

In *Alkaptonuria,* which is also inherited as an autosomal recessive trait following Mendelian rules, patients having this disease suffer from severe arthiritis, but their condition can be diagnosed prior to the onset of arthritic pain because they excrete a large amount of homogentisic acid in their urine as, can be seen in Fig. 6.10 where there is a block at (C) the breakdown of homogentisic acid. Therefore it accumulates and is excreted in the urine, to which it imparts a dark colour. Dark pigment is also deposited in certain tissues such as the cartilage of the ears and joints.

In *Albinism*, there is a block at (E) as shown in Fig. 6.10 due to the deficiency of Tyrosinase preventing the conversion of tyrosine into melanin. In generalized albinism there is lack of pigment in the skin, hair and ocular fundus.

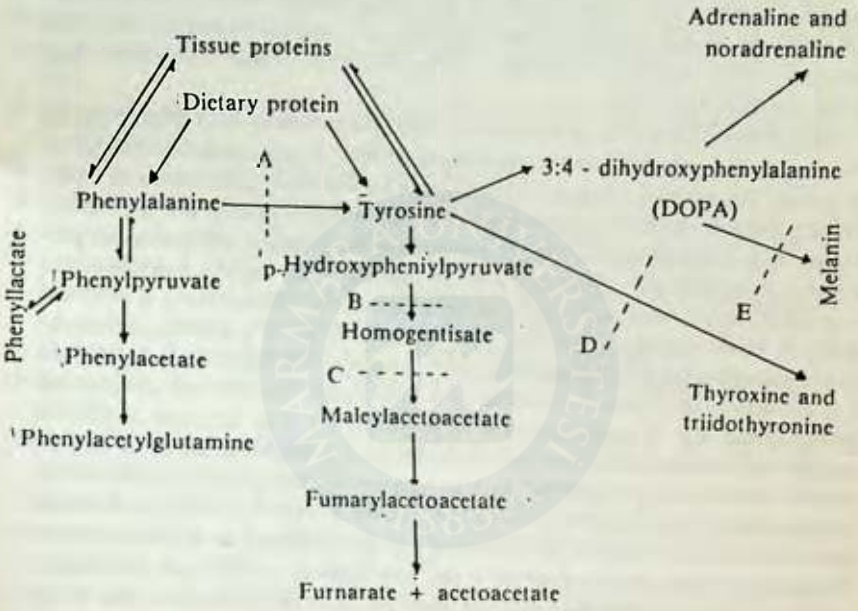

Fig. 6.10 Possible blocks in the metabolism of phenylalanine and tyrosine. A, phenylketonuria; B, tyrosinosis; C, alkaptonuria; D. goitrous cretinism, E, albinism.

In *tyrosinosis*, there is a block at B as seen in Fig. 6.10 due to the deficiency of the enzyme p-hydoxyphenylpyruvic acid oxidase, thus leading to a high concentration in the urine of p-hydroxyphenylpyruvate and of tyrosine.

In *Goitrous cretinism* which is another recessive defect, there is a block at D as seen in Fig. 6.10 due to the deficiency of dehalogenase, interfering with the production of the thyroid hormones.

Another condition in man in which a genetic-block has been identified is *Galactosaemia*. The disease manifests itself early in life and is characterized by vomiting and diarrhoea and failure to thrive. Later complications include severe mental retardation, Cataracts, and Cirrhosis of the liver. The disease is due to a deficiency of the enzyme necessary for the metabolism of the sugar, galactose (galactose-1-phosphate uridyl transferase). The disease can be prevented by feeding a diet which does not contain galactose (or lactose which, in the body, is broken

down into galactose). Early diagnosis and treatment are essential if the severe complications of galactosaemia are to be prevented.

Approaching 200, inborn erros of metabolism are known in which the specific enzyme defect has been identified. These are autosomal recessive or X-linked recessive disorders. However, in only a few autosomal dominant disorders (excluding the hemoglobinopathies) has a specific biochemical defect so far been identified. For example, familial hypercholesterolaemia, porphria and angioneuroticoedema (48).

Not all genetically determined disorders are due to a deficiency of an enzyme, but enzymes are proteins and the currently held view of gene action is that genes control the synthesis of proteins. When there is an abnormal gene (mutant), a particular protein is either not synthesized at all, or, it is synthesized but is defective in some way. Normal blood coagulation requires the presence of a particular protein known as antihaemophilic globulin. In haemophilia, there is a deficiency of functionally active antihaemophilic globulin and in this disease there is little difficulty in visualising the primary cause as being a defect of a specific protein. In fact in most patients with haemophilia antihaemophilic globulin is synthesized but it is not active: the factor is replaced by a functionally inactive but antigenically similar factor. But in such conditions as polydactyly it is at fist sight difficult to imagine the primary cause to be an abnormality of a single protein. It is possible that in "structural abnormalities" such as polydactyly, the primary defect involves a more extensive piece of chromosome material than in those conditions which appear more directly related to the effects of a single abnormal or deficient protein. Yet another factor to be considered is that the chain of events from the primary action of the gene to the final outward manifestations of the disorder may be extremely complex because of interactions between various tissues and hormonal influences.

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7

FUTURE POSSIBILITIES

It is only fair to mention some future possibilities in medical genetics before ending the subject. Apart from the obvious problem of finding effective treatments for genetic disorders, there is an important need to ensure that genetic advice is made as widely available as possible. There is evidence that there are many people in our population who are at risk of having a child with a serious hereditary disorder but are unaware of this,increasing the knowledge of genetics and appreciation of the risks by physicians will help to reduce this problem.

At some future time when we are able to recognise persons predisposed to such a disease as schizophrenia it may be possible to prevent disease either by suitable drugs or even by special psychotherapy. It is often assumed that once the diagnosis of genetic disease has been made, little more can be done for the patient. This is not true. The recognition of the basic biochemical defect can lead to the development of effective methods of treatment, though cure, in the sense of replacing a mutant gene by a normal gene, seems a very remote possibility at present. One day there may even be a place for treating the child with a hereditary disorder before birth. Recent work with mice makes this suggestion appear a little less remote than it at first seems. In mice there is a particular eye defect which leads to blindness and is caused by an autosomal recessive gene. Of pregnant female mice are treated with cortisone, this eye anomaly in their offspring can be prevented (1). Similarly in certain strains of mice thyroxine administered to pregnant females reduces the incidence of spontaneously occurring hare lip and dietary supplements of manganese given to the mother during pregnancy prevents hereditary ataxia in certain other strains. This is not meant to implay that these findings suggest cures for analogous diseases in man but they do indicate another way in which the prevention of genetic disease might be approached in the future. There are already reports (2) that it may be possible to prevent congenital non-haemolytic jaundice by giving mothers small doses of phenobarbitone before delivery.

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In the future the physician may become more involved in treating people who appear perfectly healthy but are genetically predisposed to a particular disease. One may find one-self treating mothers during pregnancy in order to prevent certain hereditary diseases in their offspring. Medical cytogenetics seems likely to become the preventive medicine of the future.

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