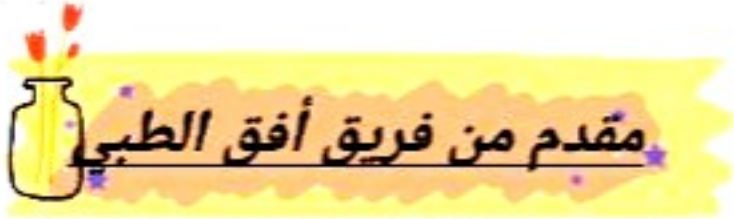

مقدم من فريق أفق الطبي


جامعة الأزهر - غزة
AL-AZHAR UNIVERSITY - GAZA
1412-1991


حركة الشبيبة الفتحاوية
جامعة الأزهر
Fateh Youth Movement

Genetic History

BY

DR. SAWSAN SHURRAB

تجميع: yousefabughali and Mohammad Salam

GREGOR MENDEL AND THE LAWS OF INHERITANCE

Early Beginnings

The history of genetics in relation to medicine is one of breathtaking discovery from which patients and families already benefit hugely but in today's world success will be measured by continuing progress in the treatment and prevention of disease.

Developments in genetics during the twentieth century have been truly spectacular.

In 1900 Mendel's principles were awaiting rediscovery,chromosomes were barely visible, and the science of molecular genetics did not exist.

By contrast, at this time over 14000 single-gene disorders or traits have been identified, chromosomes can be analyzed to a very high level of sophistication, and the draft sequence of the entire human genome has been reported .

This revolution in scientific knowledge and expertise has led to the realization that genetics is an area of major importance in almost every medical discipline.

Recent discoveries impinge not just on rare and esoteric 'small-print' diseases, but also on many of the common acquired disorders of adult life, such as cardiovascular disease, psychiatric illness and cancer.

Consequently genetics is now widely accepted as being at the forefront of medical science and has become an important and integral component of the undergraduate medical curriculum.

In order to put the exciting development and growth of genetic science into context we start with a brief overview of some of the most notable milestones in the history of medical genetics.

Early Greek philosophers and physicians such as Aristotle and Hippocrates concluded, with typical masculine modesty, that important human characteristics were determined by semen, utilizing menstrual blood as a culture medium and the uterus as an incubator.

Semen was thought to be produced by the whole body; hence baldheaded fathers would beget baldheaded sons.

These ideas prevailed until the seventeenth century, when Dutch scientists such as Leeuwenhoek and de Graaf recognized the existence of sperm and ova, thus explaining how the female could also transmit characteristics to her offspring.

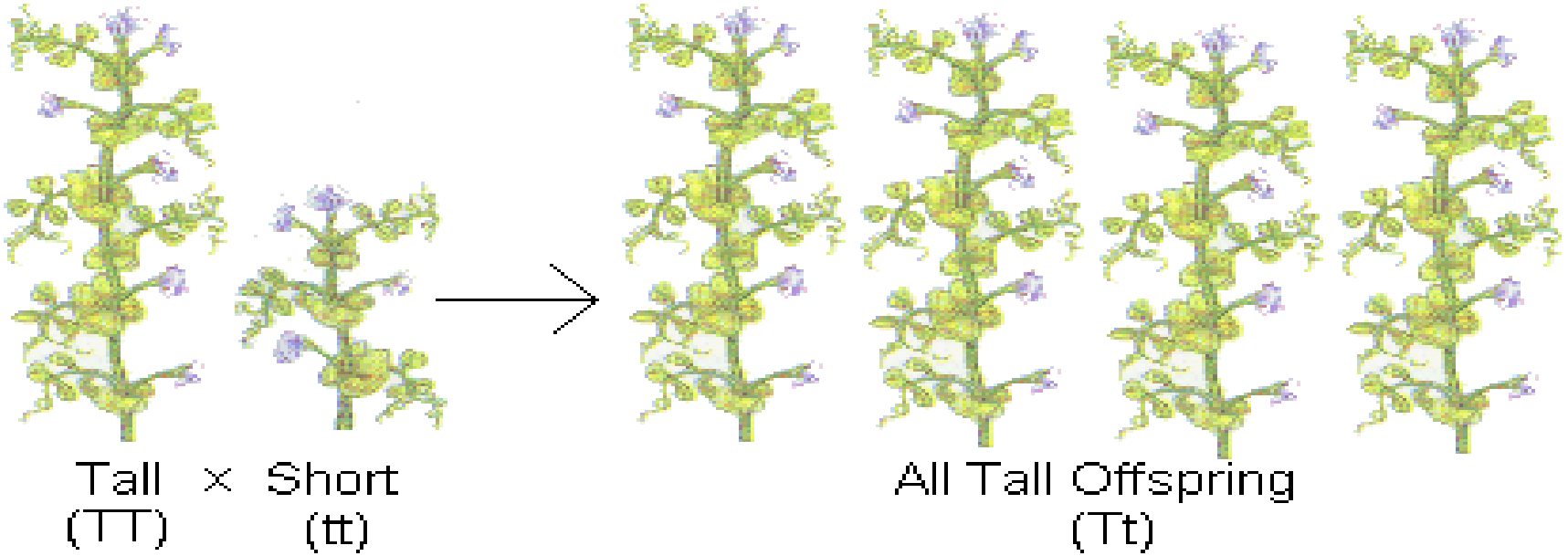
Man of Science, Man of God:

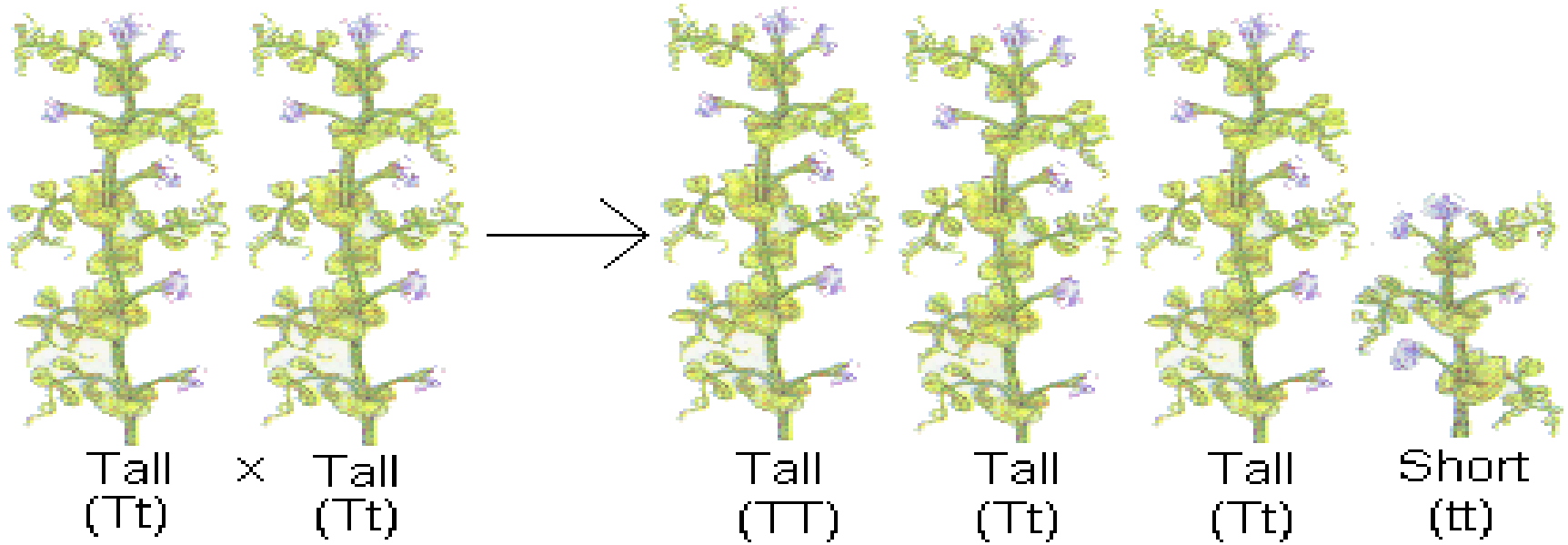
Gregor Johann Mendel

1865

In his breeding experiments Mendel studied contrasting characters in the garden pea, using for each experiment varieties that differed in only one characteristic.

For example, he noted that when strains that were bred for a feature such as tallness were crossed with plants bred to be short, all the offspring in the first *filial* or F1 generation were tall. If plants in this F1 generation were interbred, then this led to both tall and short plants in a ratio of three to one .


Tall
(TT)
×
Short
(tt)
All Tall Offspring
(Tt)


Tall
(Tt)
×
Tall
(Tt)
Tall
(TT)
Tall
(Tt)
Tall
(Tt)
Short
(tt)

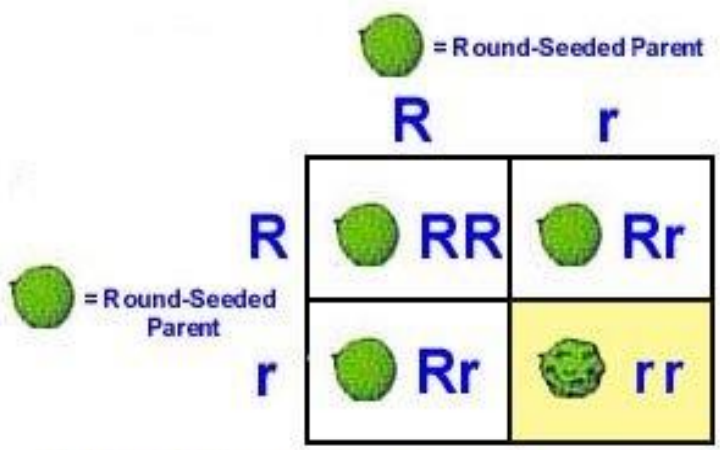
Mendel's proposal was that the plant characteristics being studied were each controlled by a pair of factors, one of which was inherited from each parent.

The pure-bred plants, with two identical genes, used in the initial cross would now be referred to as *homozygous*

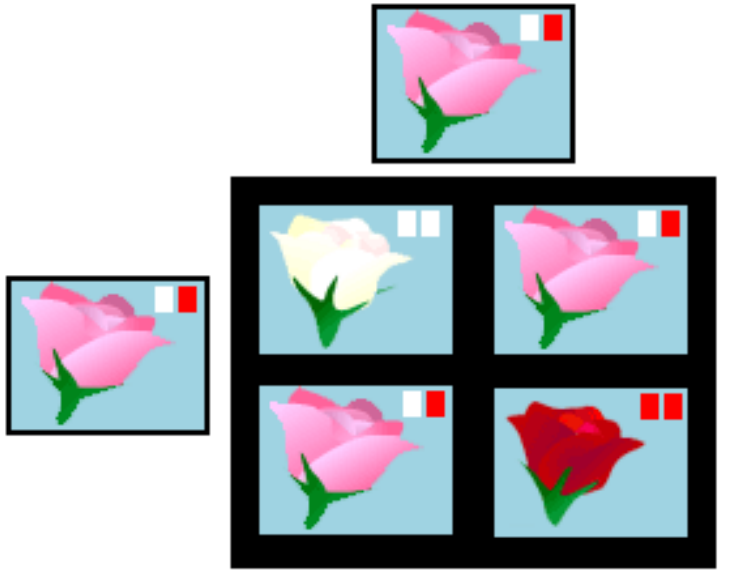
The hybrid F1 plants, each of which has one gene for tallness and one for shortness, would be referred to as *heterozygous* .The genes responsible for these contrasting characteristics are referred to as *allelomorphs* ‹or *alleles* .

An alternative method for determining *genotypes* in offspring involves the construction of what is known as a Punnett's square .

PUNNETT'S SQUARE



Punnett Square Showing a Cross of a Heterozygous Round-Seeded Pea with a Heterozygous Round-Seeded Pea Yielding 1/4 Wrinkled-Seeded Offspring



On the basis of Mendel's plant experiments, three main principles were established.

These are known as the laws of uniformity, segregation and independent assortment .

THE LAW OF UNIFORMITY :

The *law of uniformity* refers to the fact that when two homozygotes with different alleles are crossed, all the offspring in the F1 generation are identical and heterozygous.

In other words, the characteristics do not blend as had been believed previously, and can reappear in later generations .

THE LAW OF SEGREGATION :

The *law of segregation* refers to the observation that each individual possesses two genes for a particular characteristic, only one of which can be transmitted at any one time.

Rare exceptions to this rule can occur when two allelic genes fail to separate because of chromosome non-disjunction at the first meiotic division .

THE LAW OF INDEPENDENT ASSORTMENT :

Refers to the fact that members of different gene pairs segregate to offspring independently of one another.

In reality, this is not always true, as genes that are close together on the same chromosome tend to be inherited together, i.e. they are linked.

There are a number of other ways by which the laws of Mendelian inheritance are breached but, overall, they remain foundational to our understanding of the science.

THE CHROMOSOMAL BASIS OF INHERITANCE

As interest in Mendelian inheritance grew, there was much speculation as to how it actually occurred.

At that time it was also known that each cell contains a nucleus within which there are several thread-like structures known as *chromosomes* ‹so called because of their affinity for certain stains

chroma = color ‹*soma* = body.

In 1903, Walter Sutton, an American medical student, and Theodor B**overi**, a German biologist, independently proposed that chromosomes carry the hereditary factors, or genes.

These suggestions were prompted by the realization that the behaviour of chromosomes at cell division could provide an explanation for how genes could segregate.

When the connection between Mendelian inheritance and chromosomes was first made, it was thought that the normal chromosome number in humans was 48; the correct number of 46 was not established until 1956, 3 years after the correct structure of DNA was proposed.

Within a few years it was shown that some disorders in humans could be caused by loss or gain of a whole chromosome as well as an abnormality in a single gene.

DNA AS THE BASIS OF INHERITANCE

Whilst James Watson and Francis Crick are justifiably credited with discovering the structure of DNA in 1953, they were only attracted to working on it because its key role as the genetic material was established in the 1940.

Formerly many believed that hereditary characteristics were transmitted by proteins.

The genius of Watson and Crick, at Cambridge, was to hit on a structure for DNA that would explain the very fact of biological reproduction, and their elegant double helix has stood the test of time. Crucial to their discovery was the X-ray crystallography work of Maurice Wilkins and Rosalind Franklin at King's College, London .

The relationship between the sequence of bases in DNA and the sequence of amino acids in protein, i.e. the *genetic code* ‘was unravelled in some very elegant biochemical experiments in the 1960s. Thus it became possible to predict the base change in DNA that led to the amino acid change in the protein .

However, direct confirmation of that prediction had to wait until DNA sequencing methods became available after the advent of recombinant DNA techniques.
Interestingly, however, the first genetic trait to be characterized at the molecular level had already been identified in 1957 by laborious sequencing of the purified proteins.
This was sickle-cell anemia, in which the mutation affects the amino-acid sequence of the blood protein hemoglobin.

THE FRUIT FLY

Before returning to historical developments in human genetics, it is worth a brief diversion to consider the merits of this unlikely creature that has proved to be of great value in genetic research. The fruit fly ‹*Drosophila* possesses several distinct advantages for the study of genetics.

THESE ARE AS FOLLOWS :

- It can be bred easily in a laboratory .
- It reproduces rapidly and prolifically at a rate of 20-25 generations per annum .
- It has a number of easily recognized characteristics, such as *curly wings* and a *yellow body* ،which show Mendelian inheritance .

- *Drosophila melanogaster* ،the species most frequently studied, has only four pairs of chromosomes, each of which has a distinct appearance so that they can be identified easily .

- The chromosomes in the salivary glands of *Drosophila* larvae are among the largest known in nature, being at least 100 times bigger than those in other body cells .

In view of these unique properties, fruit flies were used extensively in early breeding experiments.
Today their study is still proving of great value in fields such as developmental biology, where knowledge of gene homology throughout the animal kingdom has enabled scientists to identify families of genes that are very important in human embryogenesis .

GENITICS 2

The Origin & Impacts Of Medical Genitics

By: Dr. Sawsan Shurrab

THE ORIGINS OF MEDICAL GENETICS

- John Dalton of atomic theory fame, observed that some conditions, notably color blindness and hemophilia, show what is now referred to as sex- or X-linked inheritance, and to this day color blindness is still sometimes referred to as daltonism .

- Inevitably, these founders of human and medical genetics could only speculate as to the nature of the mechanisms underlying their observations.

- In 1900 Mendel's work resurfaced.
- His papers were quoted almost simultaneously by three European botanists - De Vries (Holland), Correns (Germany) and Von Tschermak (Austria) - and this marked the real beginning of medical genetics, providing an enormous impetus for the study of inherited disease .

- During the course of the twentieth century it gradually became clear that hereditary factors are implicated in many conditions and that different genetic mechanisms are involved.

- Traditionally, hereditary conditions have been considered under the headings of *single gene* , *chromosomal* and *multifactorial* .Increasingly, it is becoming clear that the interplay of different genes (polygenic inheritance) is important in disease and a further category - *acquired* somatic genetic disease - should also be included .

SINGLE-GENE DISORDERS

- Credit for the first recognition of a single gene trait is shared by William Bateson and Archibald Garrod, who together proposed that alkaptonuria was a rare recessive disorder.

- In this relatively benign condition urine turns dark on standing or on exposure to alkali because of an inability on the part of the patient to metabolize homogentisic acid .

- Realizing that this was an inherited disorder involving a chemical process, Garrod coined the term inborn error of metabolism. Several hundred such disorders have now been identified, giving rise to the field of study known as biochemical genetics .

By 1966 almost 1500 single-gene disorders or traits had been identified, prompting the publication by an American physician, Victor McKusick of a catalog of all known single-gene conditions ..

- By late 2003 OMIM

(Online Mendelian Inheritance of Man) contained 11031 established gene loci and 8784 gene map loci (fewer than the established gene loci) because many genes are associated with more than one phenotype.

CHROMOSOME ABNORMALITIES

- Improved techniques for studying chromosomes led to the demonstration in 1959 that the presence of an additional number 21 chromosome results in Down syndrome.

  Other similar discoveries followed rapidly.

- The identification of chromosome abnormalities was further aided by the development of banding techniques.

- These enabled reliable identification of individual chromosomes and helped confirm that loss or gain of even a very small segment of a chromosome can have major effects.

- Most recently it has been shown that several rare conditions featuring learning difficulties and abnormal physical features are due to loss of such a tiny amount of chromosome material that no abnormality can be detected using even the most high-powered light microscope.
- These conditions are referred to as microdeletion syndrome.

- They are diagnosed using a technique known as FISH *fluorescent in-situ hybridization* which combines conventional chromosome analysis *cytogenetics* with much newer DNA diagnostic technology *molecular genetics*.

MULTIFACTORIAL DISORDERS

- This model of *quantitative inheritance* is now widely accepted and has been adapted to explain the pattern of inheritance observed for many relatively common conditions.

- These include congenital malformations such as cleft lip and palate, and late-onset conditions such as hypertension, diabetes mellitus and Alzheimer disease.

- The prevailing view is that genes at several loci interact to generate a susceptibility to the effects of adverse environmental trigger factors.

- Recent research has confirmed that many genes are involved in most of these adult-onset disorders, although progress in identifying specific susceptibility loci has been disappointingly slow.

- It has also emerged that in some conditions, such as type I diabetes mellitus, different genes can exert major or minor effects in determining susceptibility .

ACQUIRED SOMATIC GENETIC DISEASE

- Not all genetic errors are present from conception. Many billions of cell divisions (mitoses) occur in the course of an average human lifetime.
- During each *mitosis* there is an opportunity for both single-gene mutations to occur, because of DNA copy errors, and for numerical chromosome abnormalities to arise as a result of errors in chromosome separation.

- Accumulating somatic mutations and chromosome abnormalities are now known to play a major role in causing cancer.
- They probably also explain the rising incidence with age of many other serious illnesses, as well as the aging process itself.
- It is, therefore, necessary to appreciate that not all disease with a genetic basis is hereditary.

DEFINITIONS

Incidence

- *Incidence* refers to the rate at which new cases occur.
- Thus, if the birth incidence of a particular condition equals 1 in 1000, then on average 1 in every 1000 newborn infants is affected .

Prevalence

- This refers to the proportion of a population affected at any one time.
- The prevalence of a genetic disease will usually be less than its birth incidence, either because life expectancy is reduced or because the condition shows a delayed age of onset .

Frequency

- Is a general term that lacks scientific specificity, although the word is often taken as being synonymous with incidence when calculating gene frequencies .

Congenital

- *Congenital* means that a condition is present at birth. Thus, cleft palate represents an example of a congenital malformation. Not all genetic disorders are congenital in terms of age of onset (for example, Huntington disease), nor are all congenital abnormalities are genetic in origin .

THE IMPACT OF GENETIC DISEASE

- For some parameters, such as perinatal mortality, the actual numbers of cases with exclusively genetic causes have probably remained constant but their *relative* contribution to overall figures has increased as other causes, such as infection, have declined.

- During the twentieth century improvements in all areas of medicine, most notably public health and therapeutics, resulted in changing patterns of disease, with increasing recognition of the role of genetic factors in causing illness at all ages.

- For other conditions, such as the chronic diseases of adult life, the overall contribution of genetics has almost certainly increased as greater life expectancy has provided more opportunity for adverse genetic and environmental interaction to manifest itself, for example in coronary heart disease and diabetes .

Spontaneous miscarriages :

- Chromosome abnormality is present in 40-50% of all recognized first-trimester pregnancy loss.

- This figure would be much higher if unrecognized pregnancies could also be included and it is likely that a significant proportion of miscarriages with normal chromosomes do in fact have catastrophic submicroscopic genetic errors.

Newborn infants

- Of all neonates, 2-3% have at least one major congenital abnormality, at least 50% of which are caused exclusively or partially by genetic factors .

Childhood

Genetic disorders account for 50% of all childhood blindness, 50% of all childhood deafness and 50% of all cases of severe learning difficulty.

In developed countries genetic disorders and congenital malformations together also account for 30% of all childhood hospital admissions and 40-50% of all childhood deaths.

Adult life

- Approximately 1% of all malignancy is caused by single-gene inheritance, and between 5% and 10% of common cancers such as breast, colon and ovary have a strong hereditary component.

- By the age of 25, 5% of the population will have a disorder in which genetic factors play an important role.

- Taking into account the genetic contribution to cancer and cardiovascular diseases, such as coronary artery occlusion and hypertension, it has been estimated that over 50% of the older adult population in developed countries will have a genetically determined medical problem.

MAJOR NEW DEVELOPMENTS

- The study of genetics and its role in causing human disease is now widely acknowledged as being among the most exciting and influential areas of medical research.

- Since 1962 when Francis Crick, James Watson and Maurice Wilkins gained acclaim for their elucidation of the structure of DNA, the Nobel Prize for Medicine and/or Physiology has been won on 18 occasions by scientists working in human and molecular genetics or related fields .

1962: Nobel Prize in Physiology and Medicine

Watson, J.D. and F.H. Crick, "Molecular Structure of Nucleic Acids: A Structure for Deoxynucleic Acids". *Nature* 171 (1953), p. 738.

James D. Watson

Francis H. Crick

Maurice H. F. Wilkins

What about?
Rosalind Franklin

- With DNA technology rapidly progressing, a group of visionary scientists in the USA persuaded Congress in 1988 to fund a coordinated international program to sequence the entire human genome.

- The program would run from 1990 to 2005 and 3 billion US dollars were initially allocated to the project, including 5% allocated to the study of the ethical and social implications of the new knowledge.

- The draft DNA sequence of 3 billion base pairs was successfully completed in 2000.

- Prior to the closing stages of the project it was believed that there might be approximately 100000 coding genes that provide the blueprint for human life.

- It has come as a surprise to many that the number is much lower, with current estimates at around 30000 .

- However, many genes have the capacity to perform multiple functions, which in some cases is challenging traditional concepts of disease classification.

- The immediate benefits of the sequence data are being realized in research that is leading to better diagnosis and counseling for families with a genetic disease .

- In the longer term an improved understanding of how genes are expressed will hopefully lead to the development of new strategies for the prevention and treatment of both single-gene and polygenic disorders.
- Rapid DNA sequencing technologies currently under development will in due course greatly extend the range and ease of genetic testing, though the application has important ethical and social implications .

GENE THERAPY

- Most genetic disease is resistant to conventional treatment so that the prospect of successfully modifying the genetic code in a patient's cells is extremely attractive. Despite major investment and extensive research success in humans has so far been limited to a few very rare immunological disorders .

- For more common conditions, such as cystic fibrosis, major problems have been encountered, such as targeting the correct cell populations, overcoming the body's natural defense barriers and identifying suitably non-immunogenic vectors.

- There is universal optimism that in the short-to-medium term the prospects for successful gene therapy are good.

THE INTERNET

- The availability of information in genetics has been greatly enhanced by the development of several excellent online databases.

- These are updated regularly and provide instant access to a vast amount of contemporary information.

- For the basic scientist facilities such as the Genome Database and GenBank enable rapid determination of whether a particular gene has been cloned, together with details of its sequence, location and pattern of expression.

- For the clinician OMIM offers a full account of the important genetic aspects of all Mendelian disorders, together with pertinent clinical details and extensive references.
- It is clear that only electronic technology can hope to match the explosive pace of developments in all areas of genetic research .

GENITICS 3

The Cell & The Hereditary Material

By

Dr. Sawsan Salem Shurrab

- The hereditary material is present in the nucleus of the cell, while protein synthesis takes place in the cytoplasm. What is the chain of events that leads from the gene to the final product.

- To answer ,we should know basic cellular biology outlining the structure of DNA, the process of DNA replication, the types of DNA sequences, gene structure, the genetic code, the processes of transcription and translation.

THE CELL

- Within each cell of the body, visible with the light microscope, is the *cytoplasm* and a darkly staining body, the *nucleus* ,the latter containing the hereditary material in the form of *chromosomes.*

- The phospholipid bilayer of the plasma membrane protects the interior of the cell but remains selectively permeable and has integral proteins involved in recognition and signaling between cells.

- The nucleus has a darkly staining area, the *nucleolus.*

- The nucleus is surrounded by a membrane, the *nuclear envelope* , which separates it from the cytoplasm but still allows communication through *nuclear pores* .

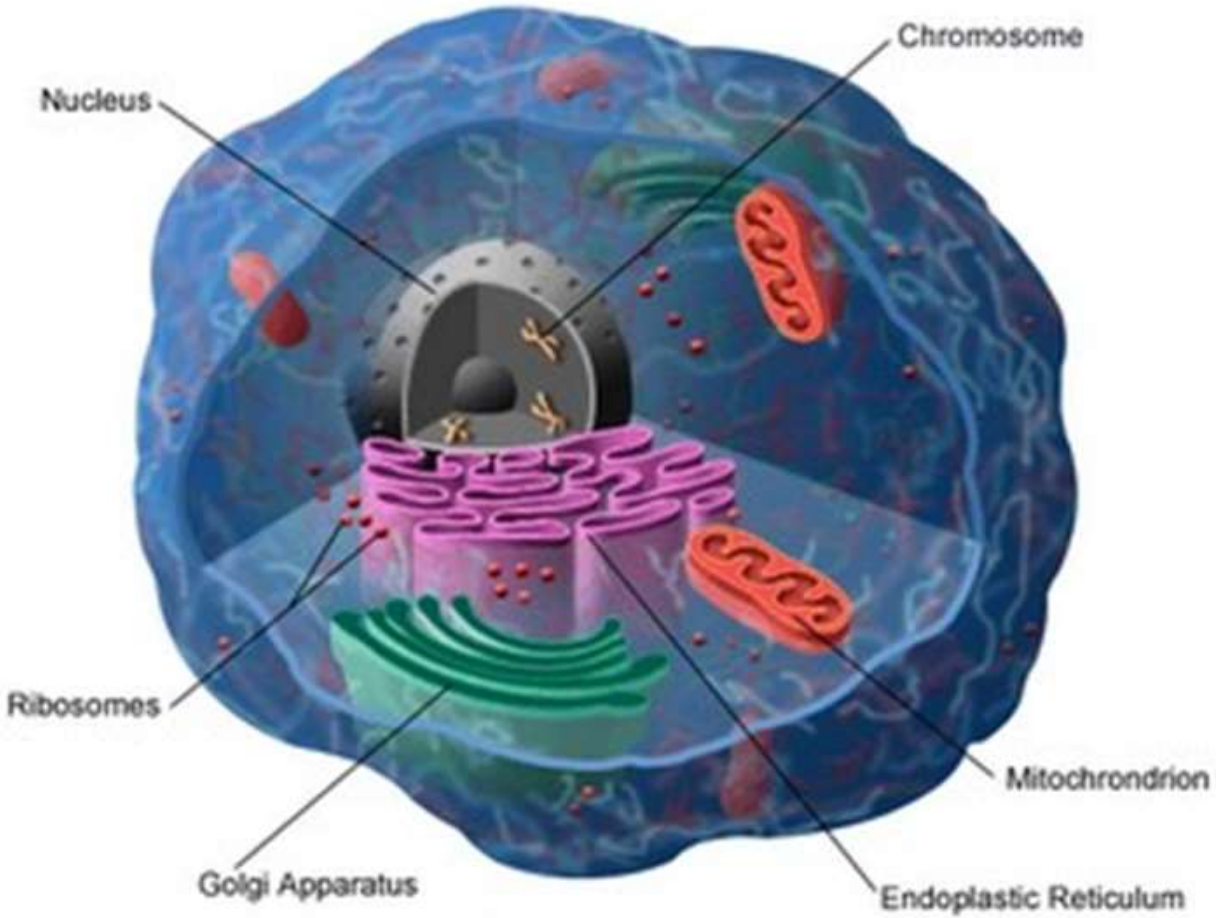
- The cytoplasm contains the *cytosol* ,which is semifluid in consistency containing both soluble elements and cytoskeletal structural elements.
- In addition, in the cytoplasm there is a complex arrangement of very fine, highly convoluted interconnecting channels, the *endoplasmic reticulum* .
- The endoplasmic reticulum, in association with the *ribosomes,*is involved in the biosynthesis of proteins and lipids.

- the Golgi apparatus, which is responsible for the secretion of cellular products.

- The *mitochondria* ,which are involved in energy production and metabolic pathways through the oxidative phosphorylation .

- *lysozomes* ,and peroxisomes both of which are involved in the degradation and disposal of cellular waste material and toxic molecule.

Anatomy of a Cell



DNA: THE HEREDITARY MATERIAL

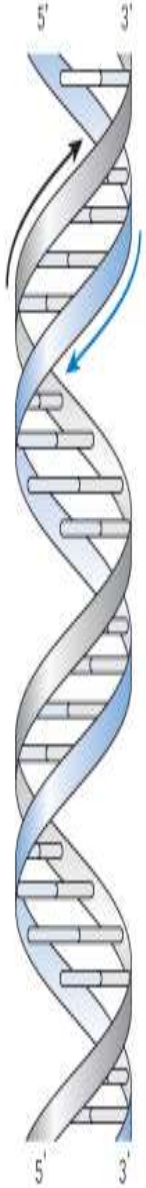
COMPOSITION

- Nucleic acid is composed of a long polymer of individual molecules called *nucleotides* .Each nucleotide is composed of a nitrogenous base, a sugar molecule and a phosphate molecule.

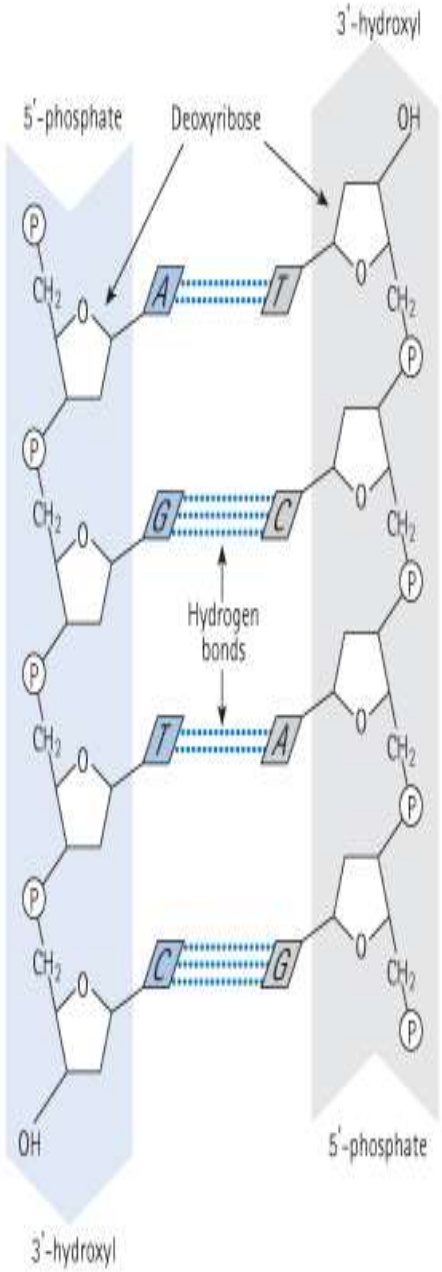
- The nitrogenous bases fall into two types , *purines* and *pyrimidines* .

- The purines include adenine and guanine; the pyrimidines include cytosine, thymine and uracil .

B



A



- RNA is present in the cytoplasm and in particularly high concentrations in the nucleolus of the nucleus.

- DNA, on the other hand, is found mainly in the chromosomes .

STRUCTURE

- For genes to be composed of DNA it is necessary that the latter should have a structure sufficiently versatile to account for the great variety of different genes and yet, at the same time, be able to reproduce itself in such a manner that an identical replica is formed at each cell division.

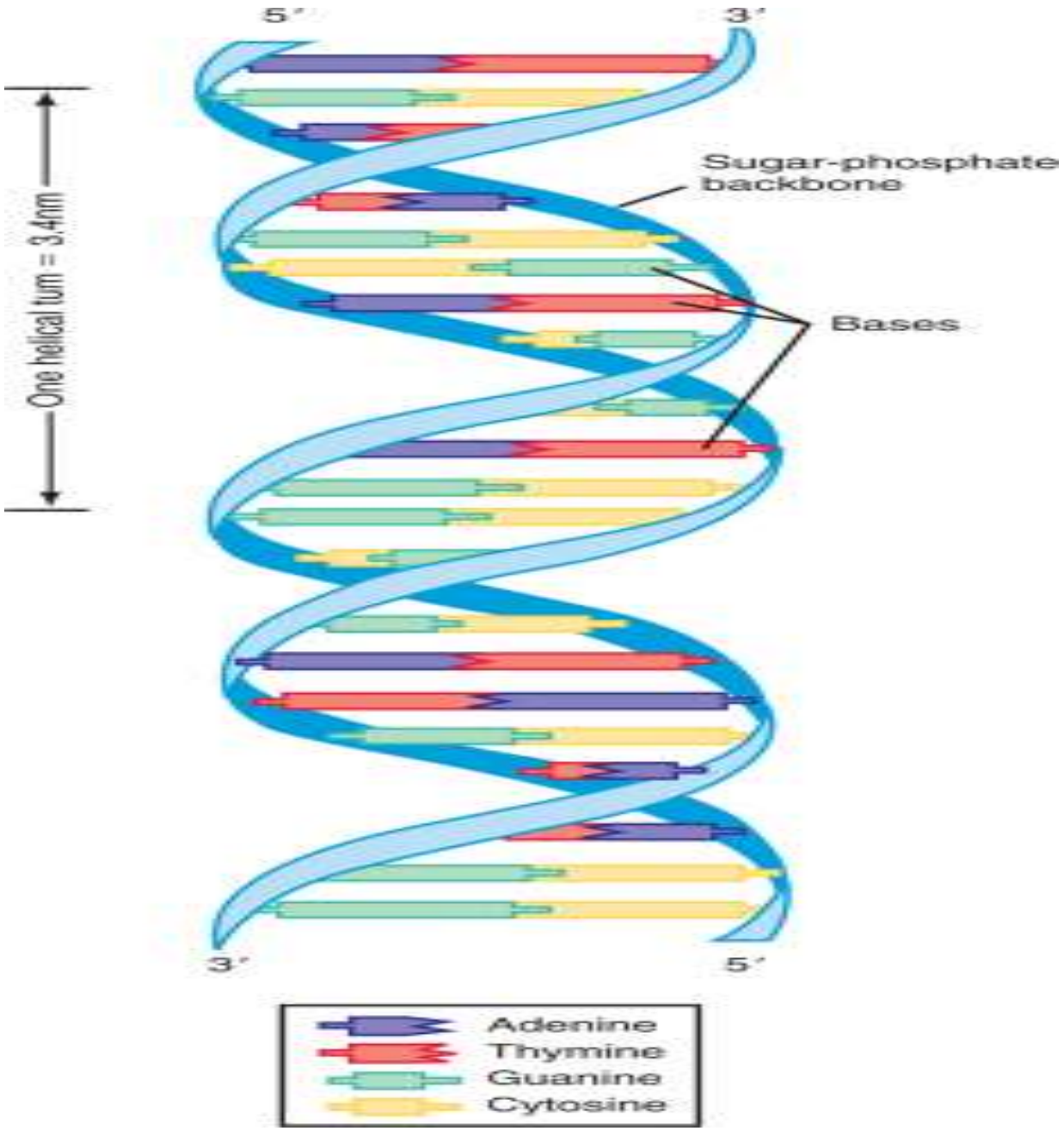
- DNA molecule is composed of two chains of nucleotides arranged in a double helix.
- The backbone of each chain is formed by phosphodiester bonds between the 3' and 5' carbons of adjacent sugars,
- The two chains being held together by hydrogen bonds between the nitrogenous bases which point in towards the centre of the helix.
- Each DNA chain has a polarity determined by the orientation of the sugar-phosphate backbone.

- The chain end terminated by the 5' carbon atom of the sugar molecule is referred to as the 5' end, and the end terminated by the 3' carbon atom is called the 3' end.

- In the DNA duplex the 5' end of one strand is opposite the 3' end of the other, i.e. they have opposite orientations and are said to be *antiparallel*.

- The arrangement of the bases in the DNA molecule is not random: a purine in one chain always pairs with a pyrimidine in the other chain, with specific pairing of the base pairs:

- Guanine in one chain always pairs with cytosine in the other chain and adenine always pairs with thymine, i.e. this base pairing forms complementary strands.


5′
3′
One helical turn = 3,4nm
Sugar-phosphate backbone
Bases
3′
5′
Adenine
Thymine
Guanine
Cytosine

REPLICATION

- The process of *DNA replication* provides an answer to the question of how genetic information is transmitted from one generation to the next.

- During nuclear division the two strands of the DNA double helix separate through the action of enzyme DNA helicase,

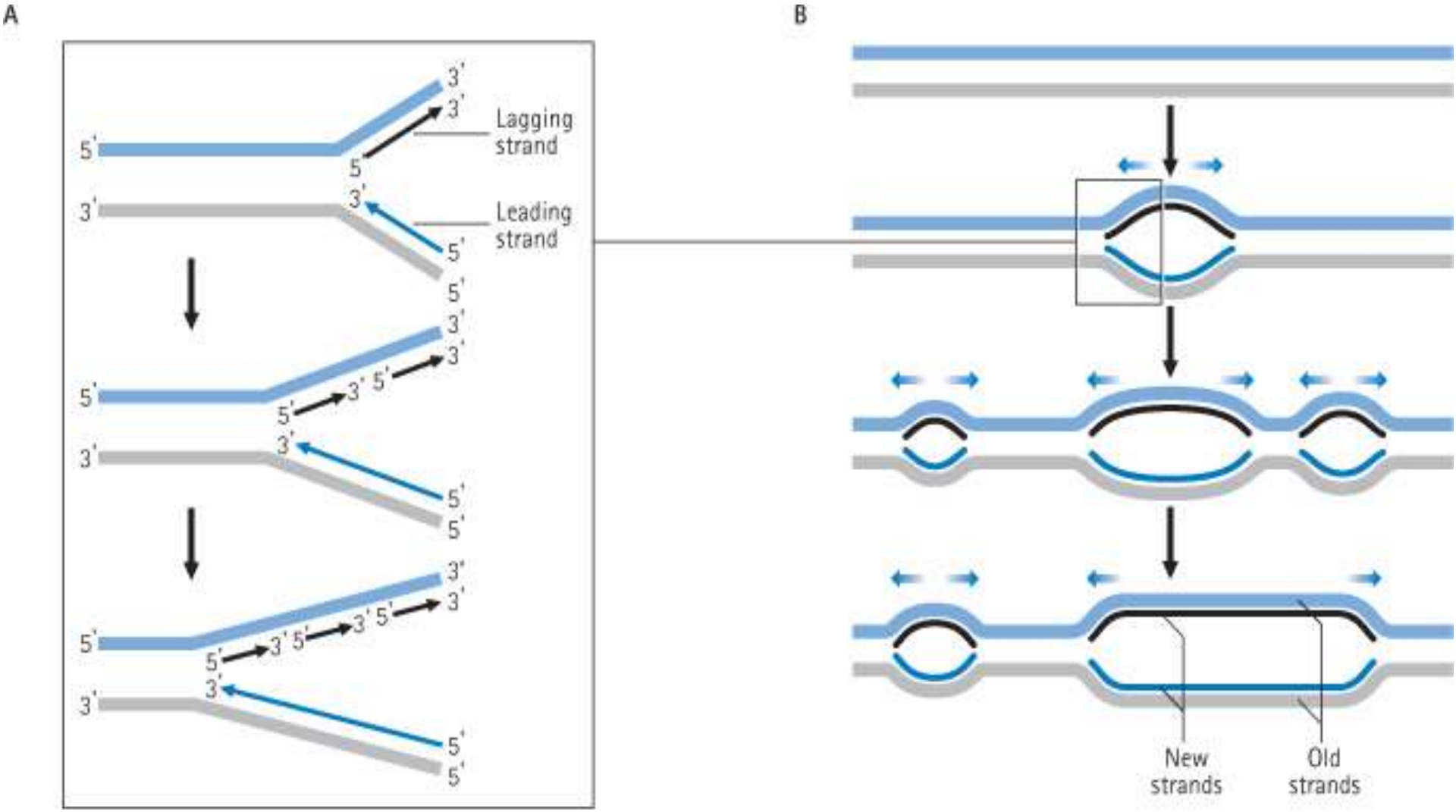
- Each DNA strand directing the synthesis of a complementary DNA strand through specific base pairing, resulting in two daughter DNA duplexes that are identical to the original parent molecule.

- In this way, when cells divide, the genetic information is conserved and transmitted unchanged to each daughter cell.

- The process of DNA replication is termed *semiconservative* ,as only one strand of each resultant daughter molecule is newly synthesized.

- DNA replication, through the action of the enzyme DNA polymerase, takes place at multiple points known as *origins of replication*, forming bifurcated Y-shaped structures known as *replication forks*.

- The synthesis of both complementary antiparallel DNA strands occurs in the 5' to 3' direction. One strand, known as the *leading strand*, is synthesized as a continuous process.

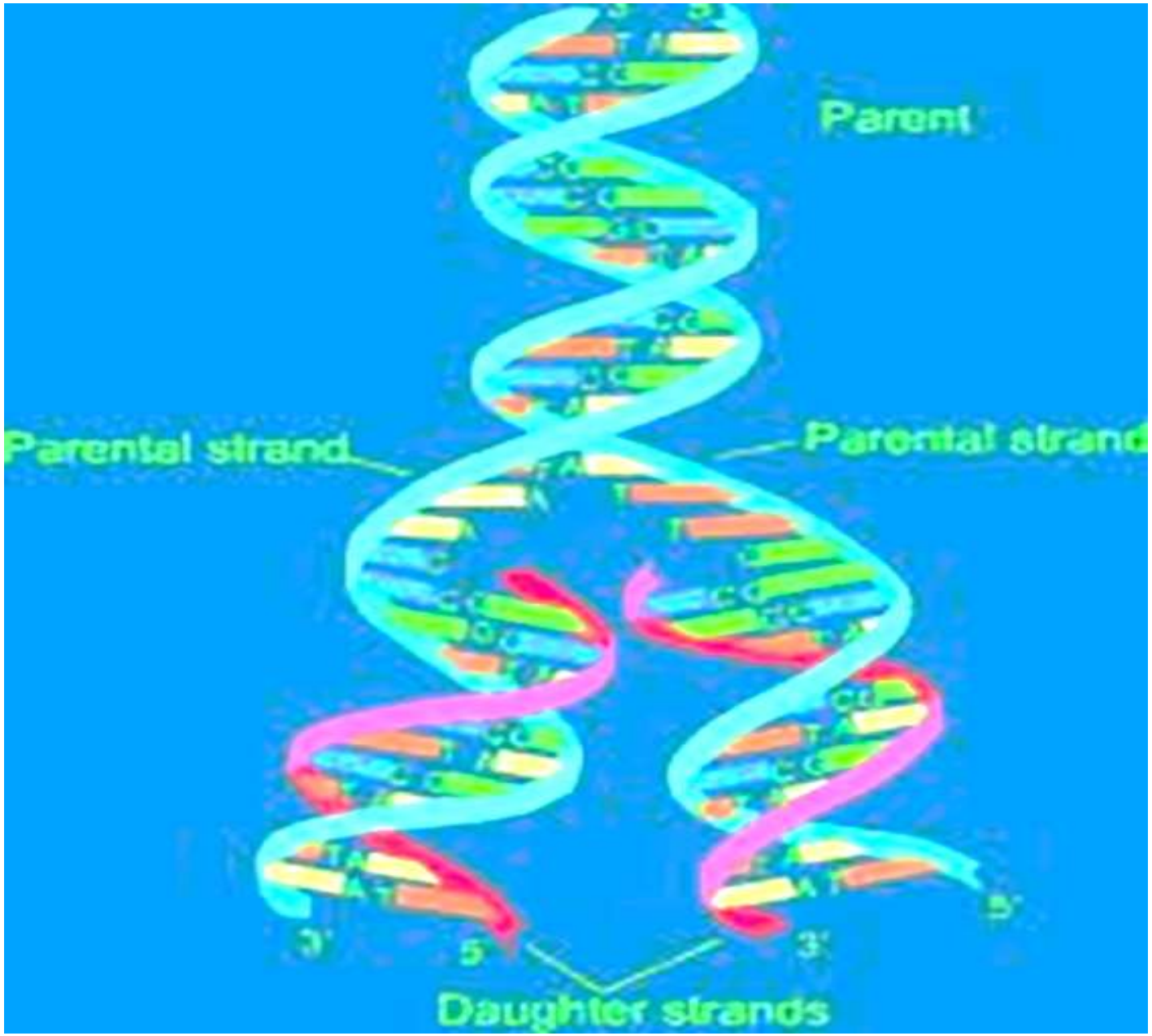


- The other strand, known as the *lagging strand*, is synthesized in pieces called Okazaki fragments, which are then joined together as a continuous strand by the enzyme DNA ligase .

- DNA replication progresses in both directions from these points of origin, forming bubble-shaped structures, or *replication bubbles*.

- DNA replication in individual replication units takes place at different times in the S phase of the cell cycle.

- Adjacent replication units fuse until all the DNA is copied, forming two complete identical daughter molecules.


Parent
Parental strand
Parental strand
3'
5'
5'
3'
Daughter strands

CHROMOSOME STRUCTURE

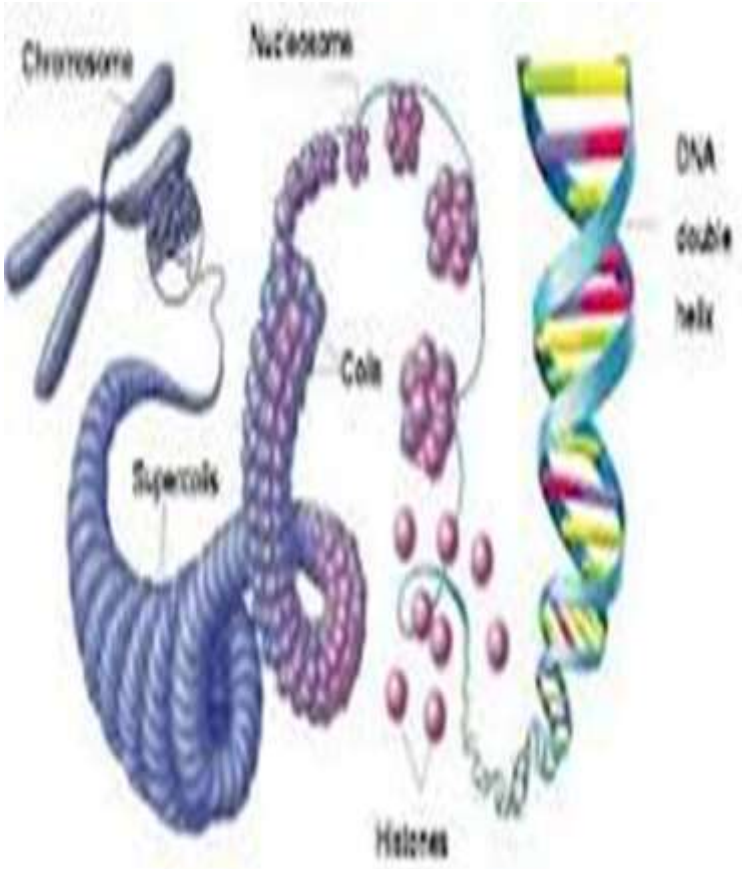
- The idea that each chromosome is composed of a single DNA double helix is an oversimplification.

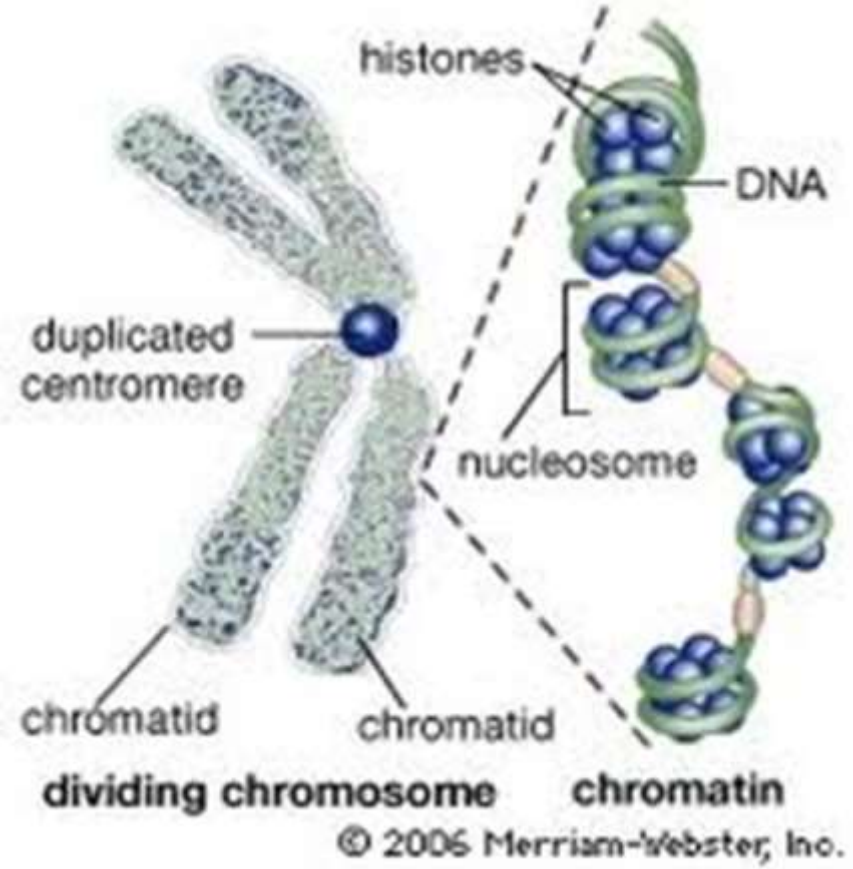
- A chromosome is very much wider than the diameter of a DNA double helix.

- In addition, the amount of DNA in the nucleus of each cell in humans means that the total length of DNA contained in the chromosomes, if fully extended, would be several meters long!
- In fact, the total length of the human chromosome complement is less than half a millimeter .

- The packaging of DNA into chromosomes involves several orders of DNA coiling and folding.

- In addition to the primary coiling of the DNA double helix, there is secondary coiling around spherical *histone*' beads', forming what are called *nucleosomes*.


Chromosome
Nucleosome
DNA
double
helix
Coils
Supercoils
Histones


histones
DNA
duplicated
centromere
nucleosome
chromatid
chromatid
dividing chromosome
chromatin
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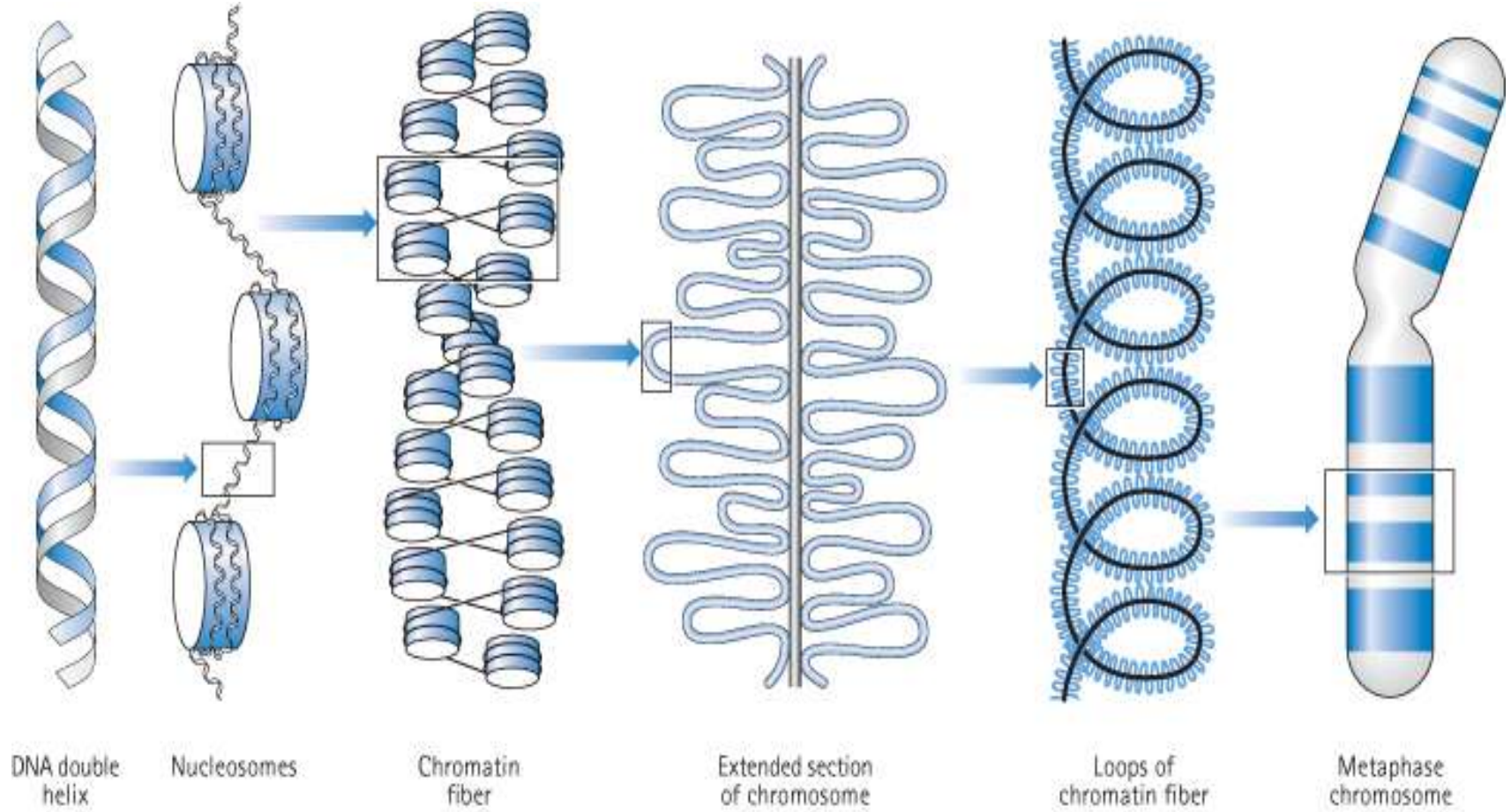
- There is a tertiary coiling of the nucleosomes to form the *chromatin fibres* that form long loops on a scaffold of non-histone acidic proteins, which are further wound in a tight coil to make up the chromosome as visualized under the light microscope .



GENITICS 4

Gene Structure & Classes Of DNA

Dr. Sawsan Salem Shurrab

TYPES OF DNA SEQUENCES

DNA, if denatured, will reassociate as a duplex at a rate that is dependent on the proportion of unique and repeat sequences present, the latter occurring more rapidly.

NUCLEAR GENES

It is estimated that there are approximately 30 000 genes in the nuclear genome.

The distribution of these genes varies greatly between chromosomal regions.

- For example, heterochromatic and centromeric regions are mostly non-coding, with the highest gene density observed in sub-telomeric regions .

Chromosomes 19 and 22 are gene rich, whereas 4 and 18 are relatively gene poor.

The size of genes also shows great variability: from small genes with single exons to genes with up to 79 exons e.g. dystrophin.

Unique single-copy genes

Most human genes are unique single-copy genes coding for polypeptides that are involved in or carry out a variety of cellular functions.

These include enzymes, hormones, receptors, and structural and regulatory proteins.

Multigene families

Many genes have similar functions.
They are known as *multigene families*.

Some are found physically close together in clusters, e.g. the α- and β-globin gene clusters on chromosomes 16 and 11 whereas others are widely dispersed throughout the genome occurring on different chromosomes.

Multigene families can be split into two types :

Classic gene families that show a high degree of sequence homology .

Gene superfamilies that have limited sequence homology but are functionally related, having similar structural domains.

Classic gene families

Examples of classic gene families include :

The numerous copies of genes coding for the various **ribosomal RNAs,** which are clustered as tandem arrays on the short arms of the five acrocentric chromosomes (13,14,15,21,22).

The different **transfer RNA** gene families, which are dispersed in numerous clusters throughout the human genome .

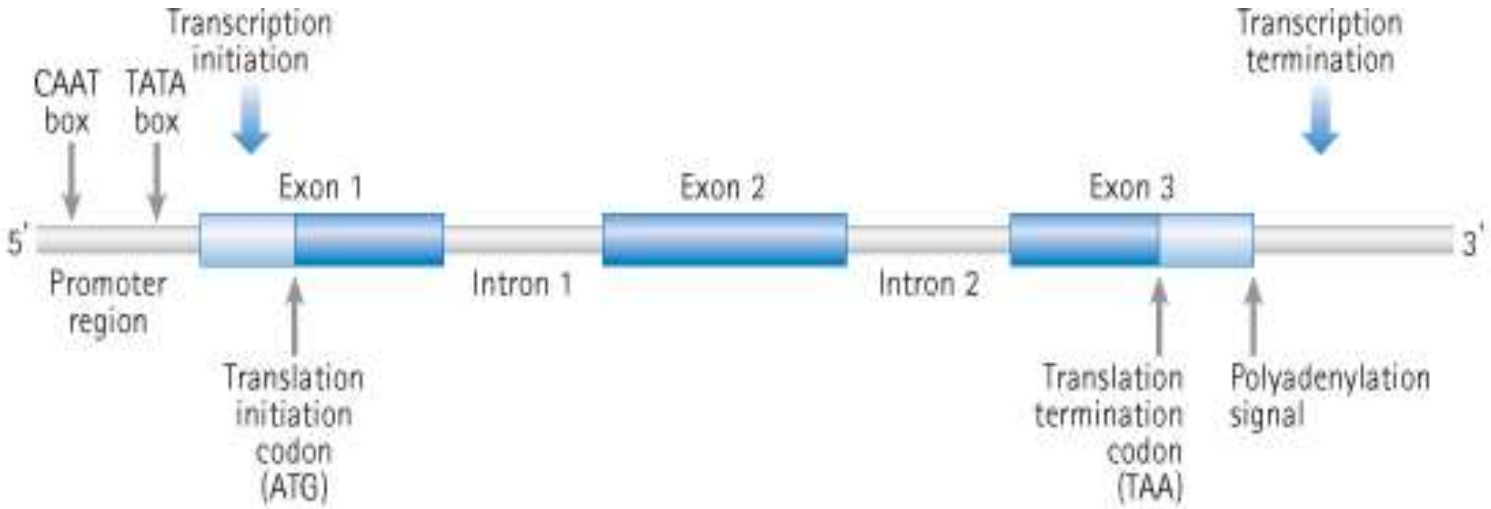
Gene superfamilies

Examples of gene superfamilies include the HLA genes on chromosome 6 and the T-cell receptor genes, which have structural homology with the immunoglobulin (Ig) genes.

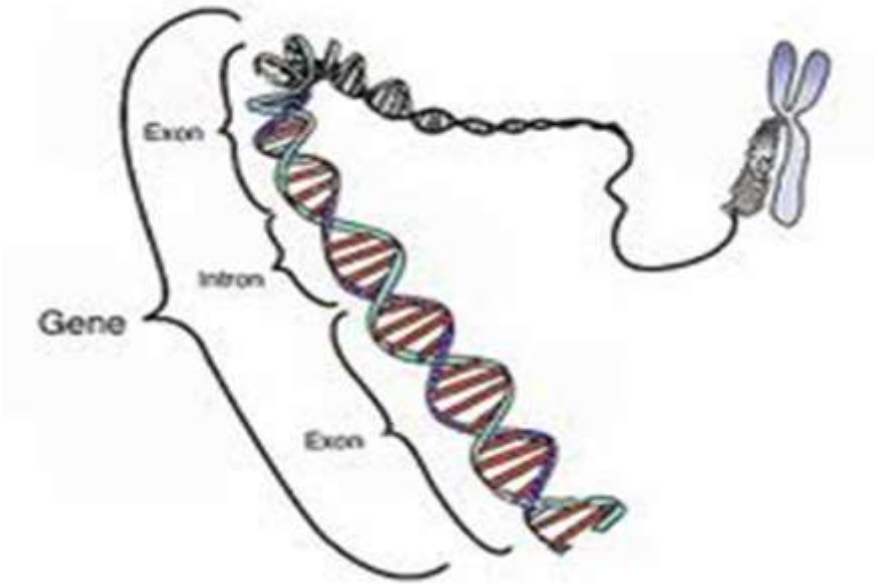
GENE STRUCTURE

The gene was much longer than necessary to code for the β-globin protein, containing non-coding intervening sequences or introns, which separate the coding sequences or exons.

It appears to be the exception for genes in humans to consist of uninterrupted coding sequences.



The number and size of introns in various genes in humans are extremely variable, although there is a general trend that the larger the gene, the greater the number and size of the exons.



Individual introns can be far larger than the coding sequences and some have been found to contain coding sequences for other genes, i.e. genes occurring within genes.

- Genes in humans do not usually overlap, being separated from each other although some of the genes in the HLA complex have been shown to be overlapping.

Pseudogenes

Particularly fascinating is the occurrence of genes that closely resemble known structural genes but which, in general, are not functionally expressed: so-called *pseudogen.*

These are thought to have arisen in two main ways:

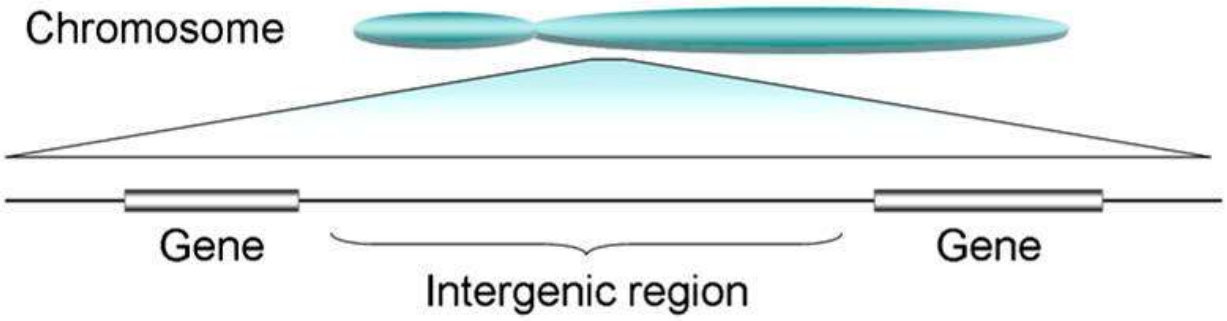
1. Genes undergoing duplication events that are rendered silent through the acquisition of mutations in coding or regulatory elements.

2. As the result of the insertion of complementary DNA sequences, produced by the action of the enzyme *reverse transcriptase* on a naturally occurring mRNA transcript, which lack the promoter sequences necessary for expression.

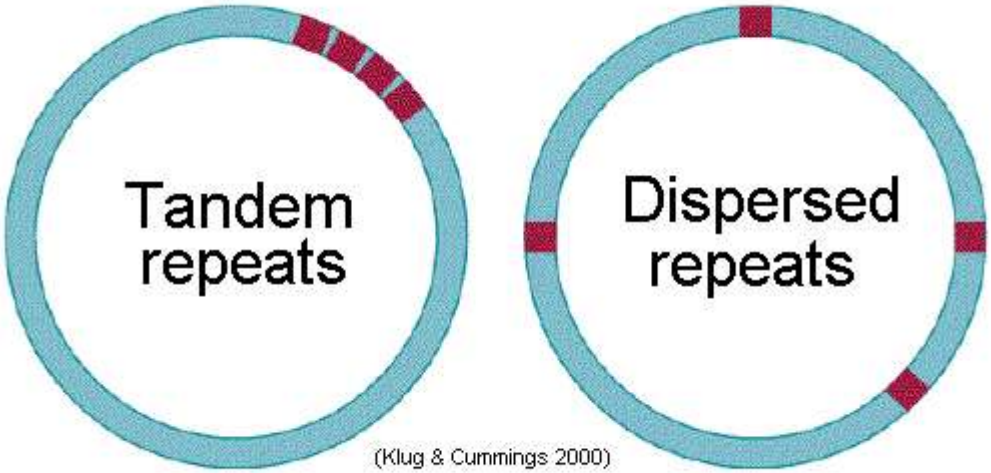
EXTRAGENIC DNA

The estimated 30 000 unique single-copy genes in humans represent less than 2% of the genome .

The remainder of the human genome is made up of repetitive DNA sequences that are predominantly transcriptionally inactive.


Chromosome
Gene
Gene
Intergenic region

It has been described as *junk* DNA, but some regions may play a role in the regulation of gene expression .



(Klug & Cummings 2000)

Tandemly repeated DNA sequences

Tandemly repeated DNA sequences consist of blocks of tandem repeats of non-coding DNA that can be either highly dispersed or restricted in their location in the genome.

Tandemly repeated DNA sequences can be divided into three subgroups: satellite, minisatellite and microsatellite DNA .

Satellite DNA

Accounts for approximately 10-15% of the repetitive DNA sequences of the human genome and consists of very large series of short tandemly repeated DNA sequences that are transcriptionally inactive and are clustered around the centromeres of certain chromosomes.

This class of DNA sequences can be separated on density-gradient centrifugation as a shoulder, or 'satellite', to the main peak of genomic DNA, and has therefore been referred to as satellite DNA.

Minisatellite DNA

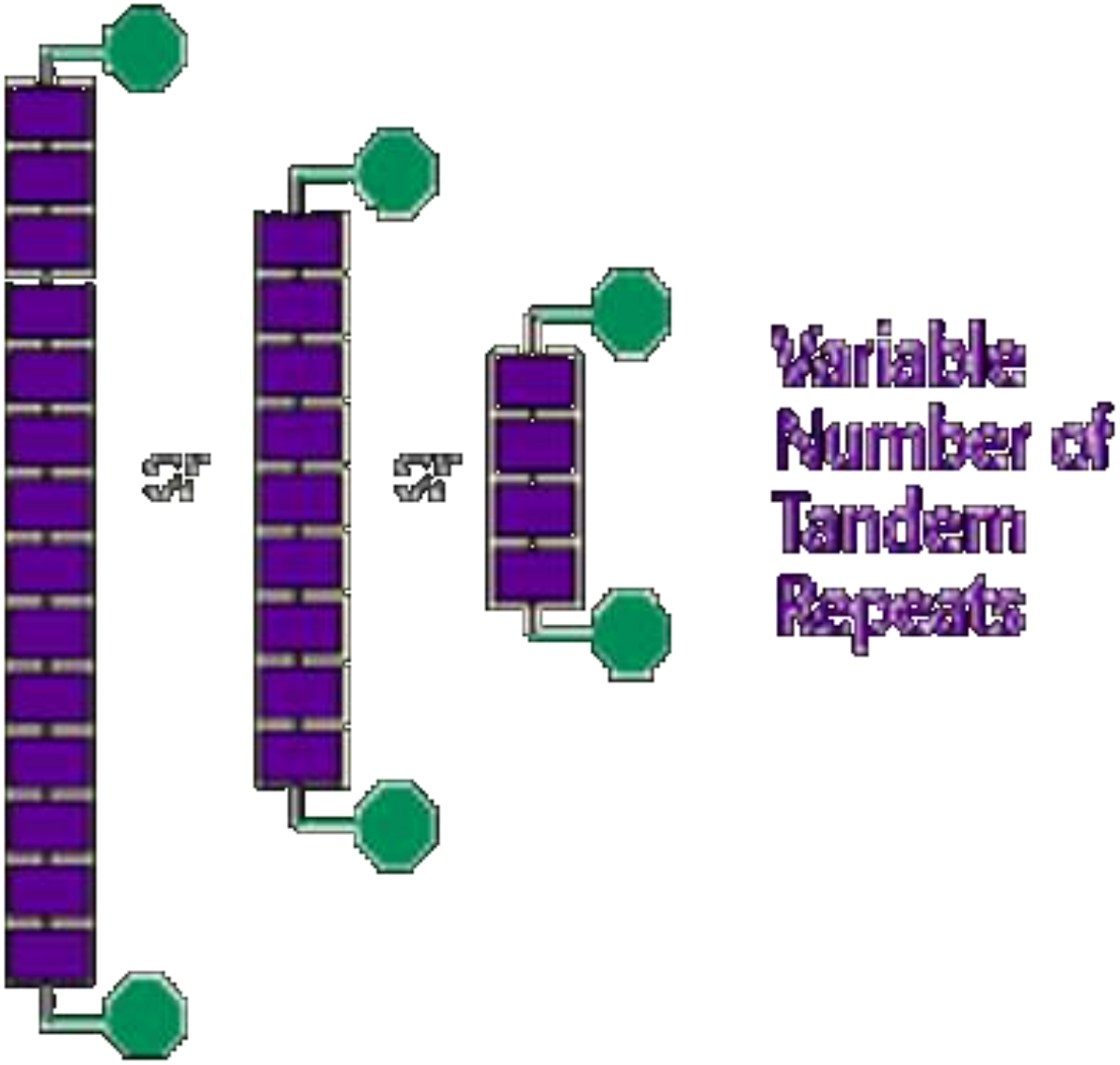
Minisatellite DNA consists of two families of tandemly repeated short DNA sequences, telomeric and hypervariable.

Telomeric DNA ; The terminal portions of the telomeres of the chromosomes are necessary for chromosomal integrity in replication and are added to the chromosome by a specialized enzyme known as telomerase .

Hypervariable minisatellite DNA

is made up of highly polymorphic DNA sequences consisting of short tandem repeat of a common core sequence forming the basis of DNA fingerprinting used in forensic and paternity testing .

Although often located near the telomeres, hypervariable minisatellite DNA also occurs at otherlocations in the chromosomes.


5'
3'
Variable
Number of
Tandem
Repeats

Microsatellite DNA

consists of tandem single, di-, tri- and tetranucleotide repeat base pair sequences located throughout the genome.

Microsatellite repeats rarely occur within coding sequences but trinucleotide repeats in or near genes are associated with certain inherited disorder .

DNA microsatellites are highly polymorphic.

Interspersed repetitive DNA sequences

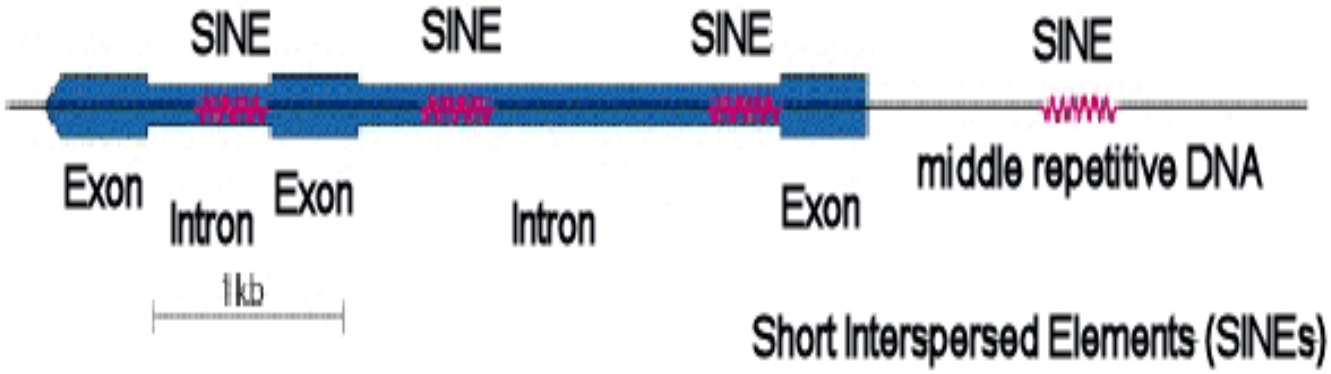
Approximately one-third of the human genome is made up of two main classes of short and long repetitive DNA sequences that are interspersed throughout the genome .

Short interspersed nuclear elements

About 5% of the human genome consists of some 750000 copies of *short interspersed nuclear elements* or *SINEs.*

The most common are DNA sequences that have sequence similarity to a signal recognition particle involved in protein synthesis.

They are called *Alu repeats* because they contain an *Alu* restriction enzyme recognition site .



(Klug & Cummings 2000)

Long interspersed nuclear elements

About 5% of the DNA of the human genome is made up of *long interspersed nuclear elements* or *LINEs* .

The most commonly occurring LINE, known as LINE-1 or an L1 element, consists of over 100 000 copies of a DNA sequence that encodes a reverse transcriptase.

The function of these interspersed repeat sequences is not clear at present.

Members of the *Alu* repeat family are called transposable elements or *transposons* .

Transposition can create significant mutations and alter the cell's genome size

Transposons are of two types according to their mechanism, which can be either "copy and paste“ (class I) or "cut and paste" (class II).

- Class I (Retrotransposons): They copy themselves in two stages, first from DNA to RNA by transcription, then from RNA back to DNA by reverse transcription.

- The DNA copy is then inserted into the genome in a new position.

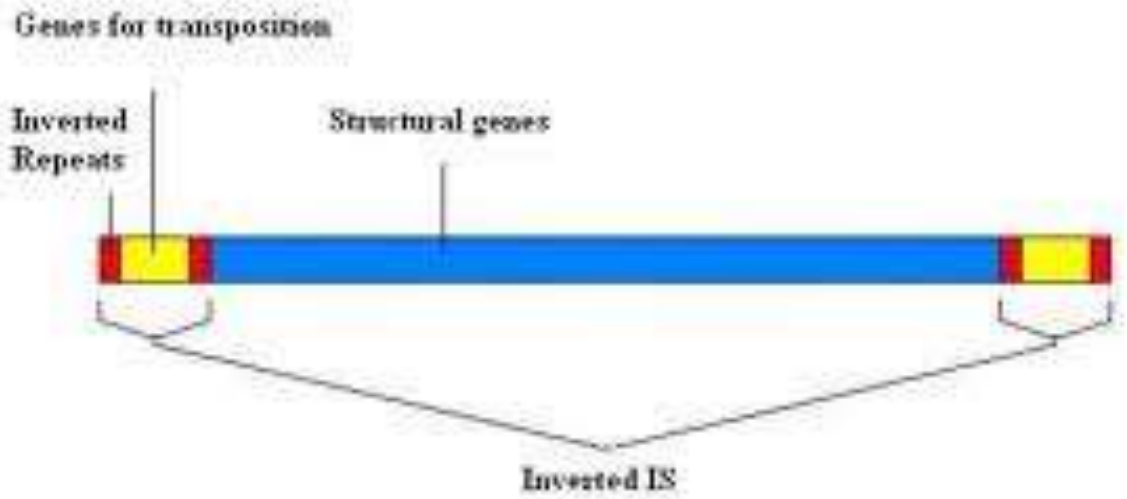
- Class II (DNA transposons):

  By contrast, the cut-and-paste transposition mechanisms of class II transposons do not involve an RNA intermediate.

They can damage the genome of their host cell in different ways:

- A transposon or a retroposon that inserts itself into a functional gene will most likely disable that gene.
- After a transposon leaves a gene, the resulting gap will probably not be repaired correctly.

Bacterial composite transposon

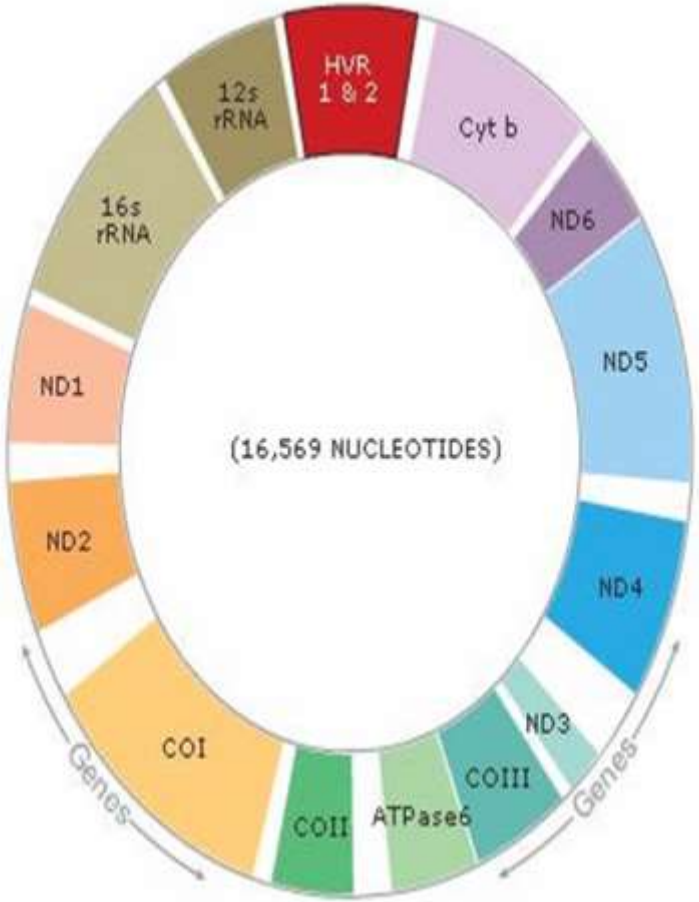


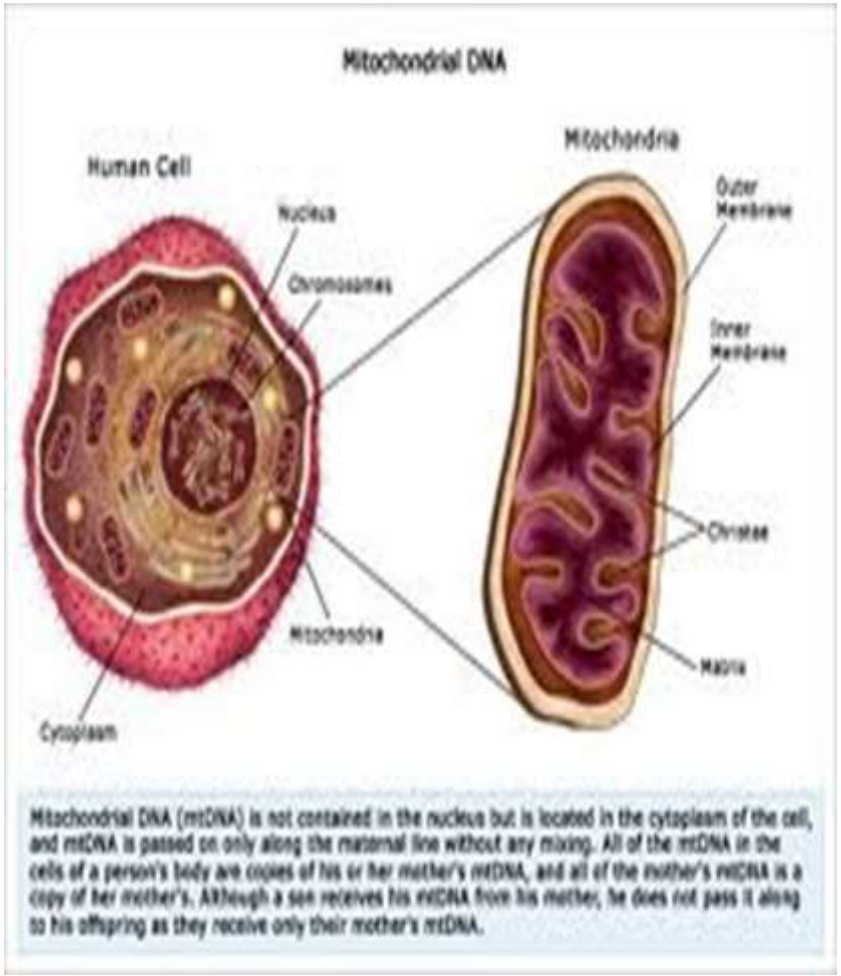
It is postulated that *Alu* repeats could promote unequal recombination, which could lead to pathogenic mutations or provide selective advantage in evolution by gene duplication.

Both *Alu* and LINE-1 repeat elements have been implicated as a cause of mutation in inherited human diseases.

MITOCHONDRIAL DNA

In addition to nuclear DNA, the several thousand mitochondria of each cell possess their own circular double-stranded DNA *mitochondrial DNA* or mtDNA .


HVR
1 & 2
Cyt b
ND6
ND5
ND4
ND3
COIII
ATPase6
COII
COI
ND2
ND1
16s
rRNA
12s
rRNA
(16,569 NUCLEOTIDES)
Genes
Genes


Mitochondrial DNA
Human Cell
Nucleus
Chromosomes
Mitochondria
Cytoplasm
Mitochondria
Outer Membrane
Inner Membrane
Cristae
Matrix
Mitochondrial DNA (mtDNA) is not contained in the nucleus but is located in the cytoplasm of the cell, and mtDNA is passed on only along the maternal line without any mixing. All of the mtDNA in the cells of a person's body are copies of his or her mother's mtDNA, and all of the mother's mtDNA is a copy of her mother's. Although a son receives his mtDNA from his mother, he does not pass it along to his offspring as they receive only their mother's mtDNA.

The mitochondrial DNA genome

is very compact, containing little repetitive DNA, and codes for 37 genes which include :

two types of ribosomal RNA.
22 transfer RNA .
13 protein subunits for enzymes, such as cytochrome b and cytochrome oxidase, which are involved in the energy-producing oxidative phosphorylation pathways.

The genetic code of the mtDNA differs slightly from that of nuclear DNA .

The mitochondria of the fertilized zygote are inherited almost exclusively from the oocyte, leading to the maternal pattern of inheritance of mitochondrially inherited disorders .

GENITICS 5

From Gene To Protein

Dr. Sawsan Salem Shurrab

The process whereby genetic information is transmitted from DNA to RNA is called

transcription.

The information stored in the genetic code is transmitted from the DNA of a gene to messenger RNA or mRNA.

Every base in the mRNA molecule is complementary to a corresponding base in the DNA of the gene but with uracil replacing thymine in mRNA.

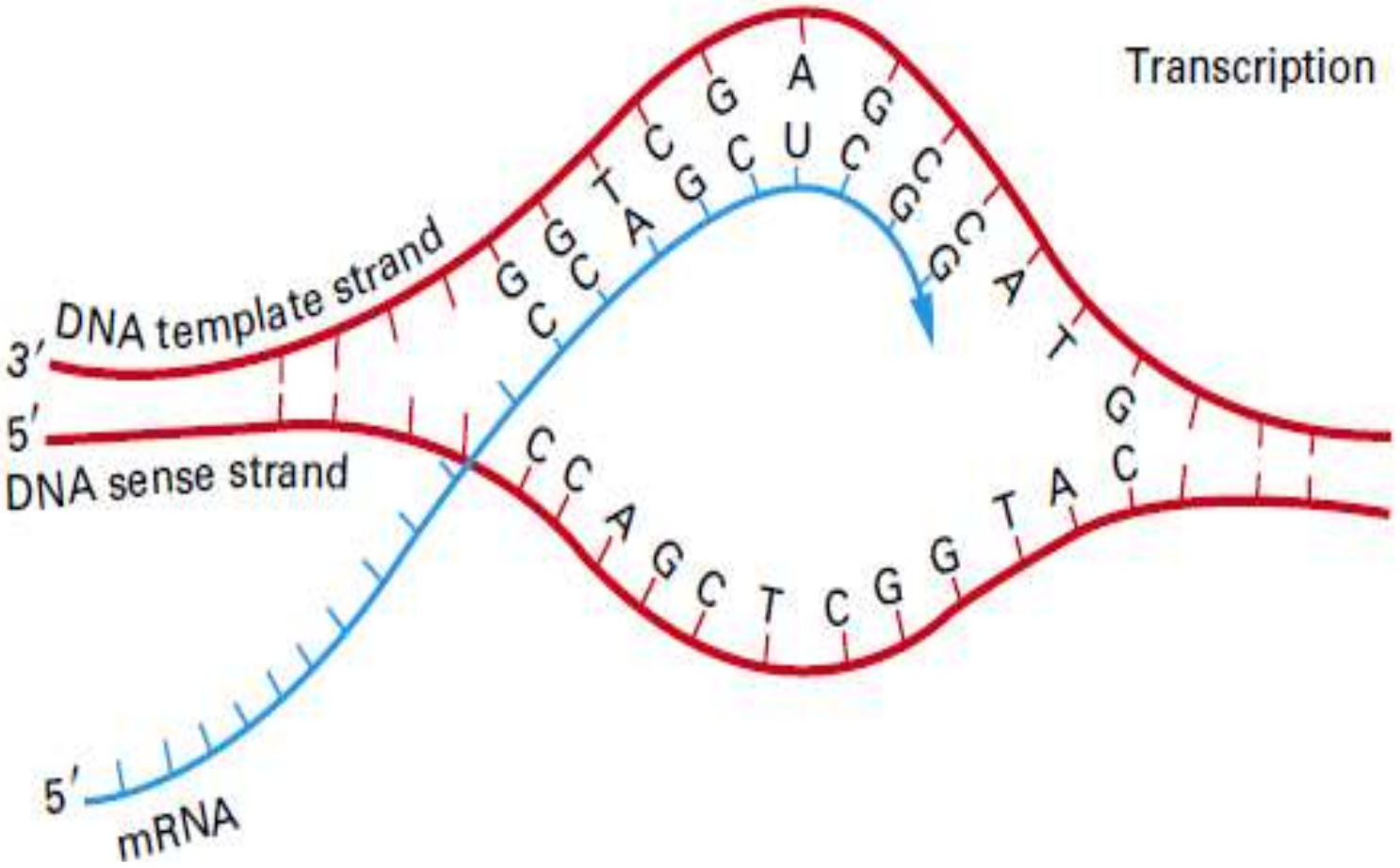
mRNA is single stranded, being synthesized by the enzyme RNA polymerase that adds the appropriate complementary ribonucleotide to the 3' end of the RNA chain.

In any particular gene only one DNA strand of the double helix acts as the so-called template strand .

The transcribed mRNA molecule is a copy of the complementary strand or what is called the sensestrand of the DNA double helix.

The template strand is sometimes called the antisense strand.

The particular strand of the DNA double helix used for RNA synthesis appears to differ throughout different regions of the genome.

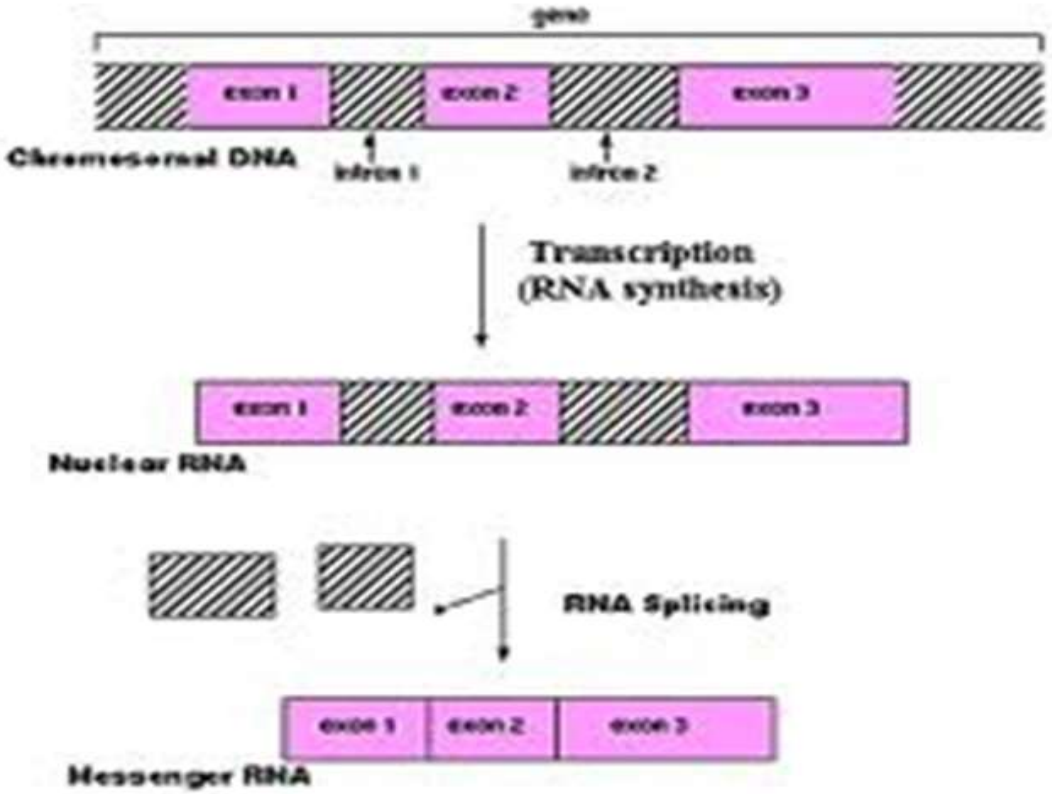

Transcription
3′
DNA template strand
5′
DNA sense strand
5′
mRNA
A
U
G
C
T
A
G
C
G
C
C
G
G
C
C
G
G
C
A
T
G
C
C
C
A
G
C
T
C
G
G
T
A
C

RNA PROCESSING

Before the primary mRNA molecule leaves the nucleus it undergoes a number of modifications, or what is known as RNA processing.
This involves splicing, capping and polyadenylation.

mRNA splicing

After transcription, the non-coding introns in the primary mRNA are excised, and the non-contiguous coding exons are spliced together to form a shorter mature mRNA before its transportation to the ribosomes in the cytoplasm for translation.



RNA synthesis and processing

5' capping

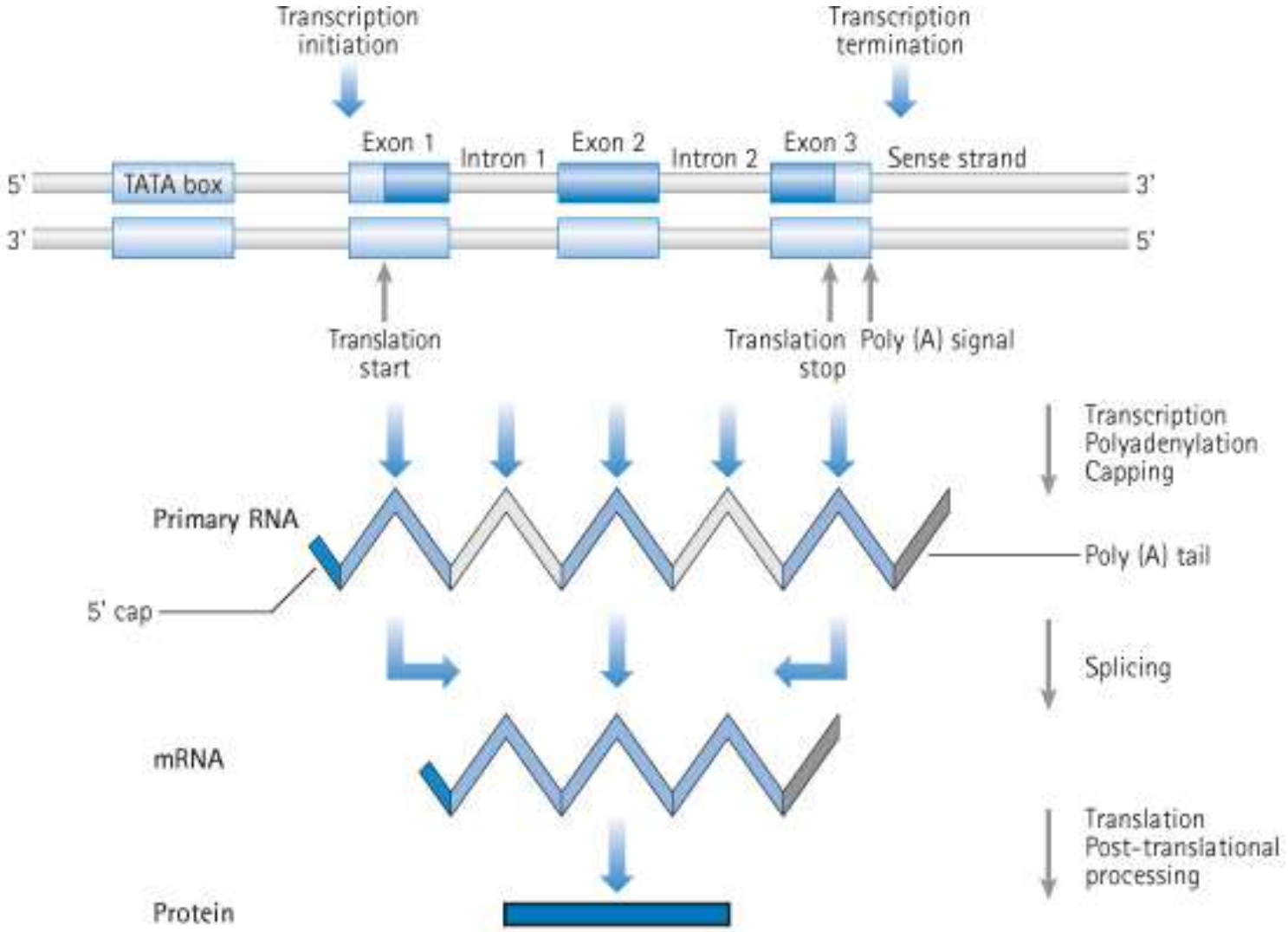
Shortly after transcription the nascent mRNA is modified by the addition of a methylated guanine nucleotide to the 5' end of the molecule by an unusual 5' to 5' phosphodiester bond, the so-called 5' cap.

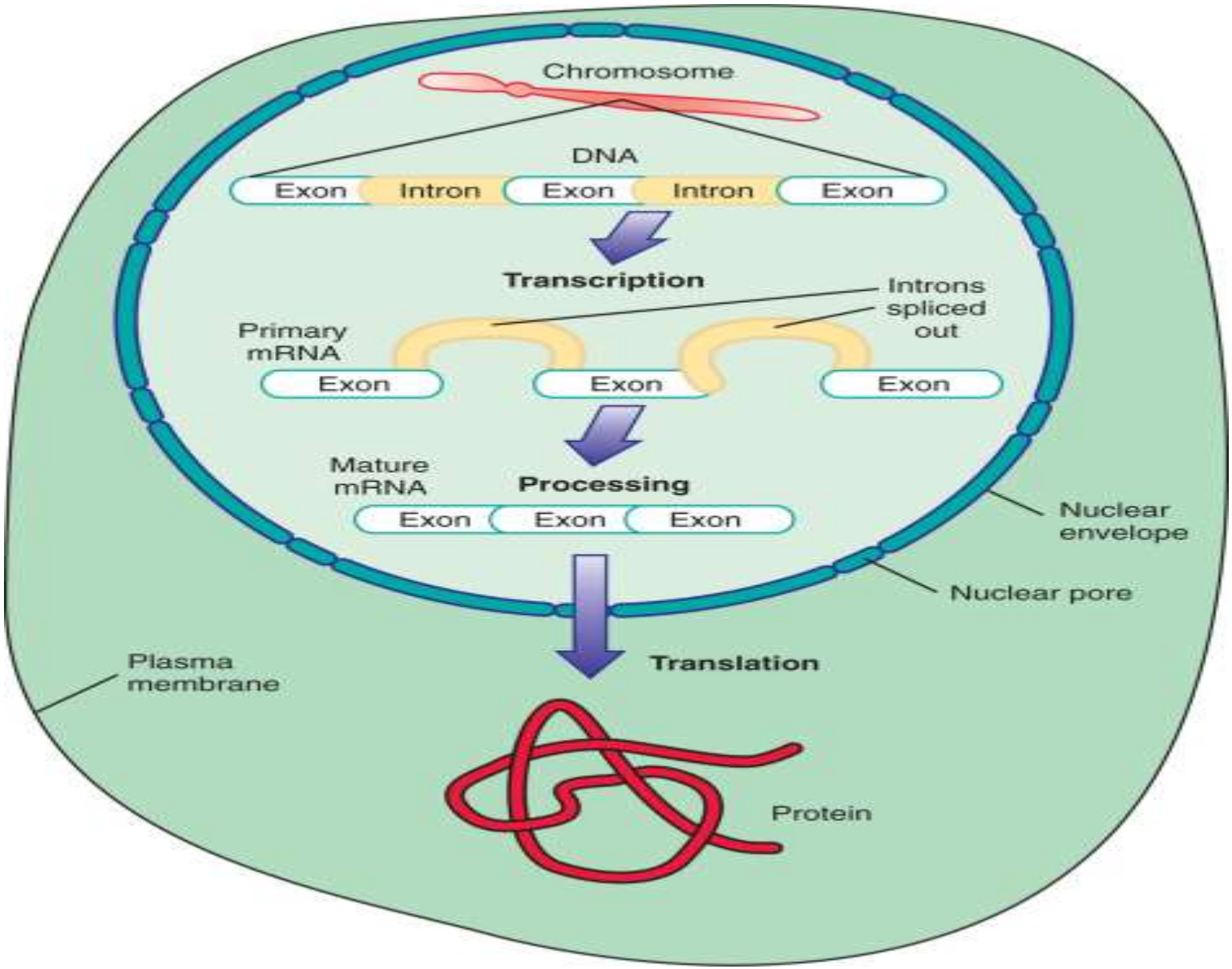
The 5' cap is thought to facilitate transport of the mRNA to the cytoplasm and attachment to the ribosomes, as well as to protect the RNA transcript from degradation by endogenous cellular exonucleases.

Polyadenylation

After cleavage of the 3' end of the mRNA molecule from the DNA ,approximately 200 adenylate residues, the so-called poly(A) tail will be added, after cleavage of the RNA transcript at a site downstream from a specific six-nucleotide sequence.

The addition of the poly(A) tail is thought to facilitate transport of the mRNA to the cytoplasm and translation.




Chromosome
DNA
Exon
Intron
Exon
Intron
Exon
Transcription
Introns spliced out
Primary mRNA
Exon
Exon
Exon
Mature mRNA
Processing
Exon
Exon
Exon
Nuclear envelope
Nuclear pore
Translation
Plasma membrane
Protein

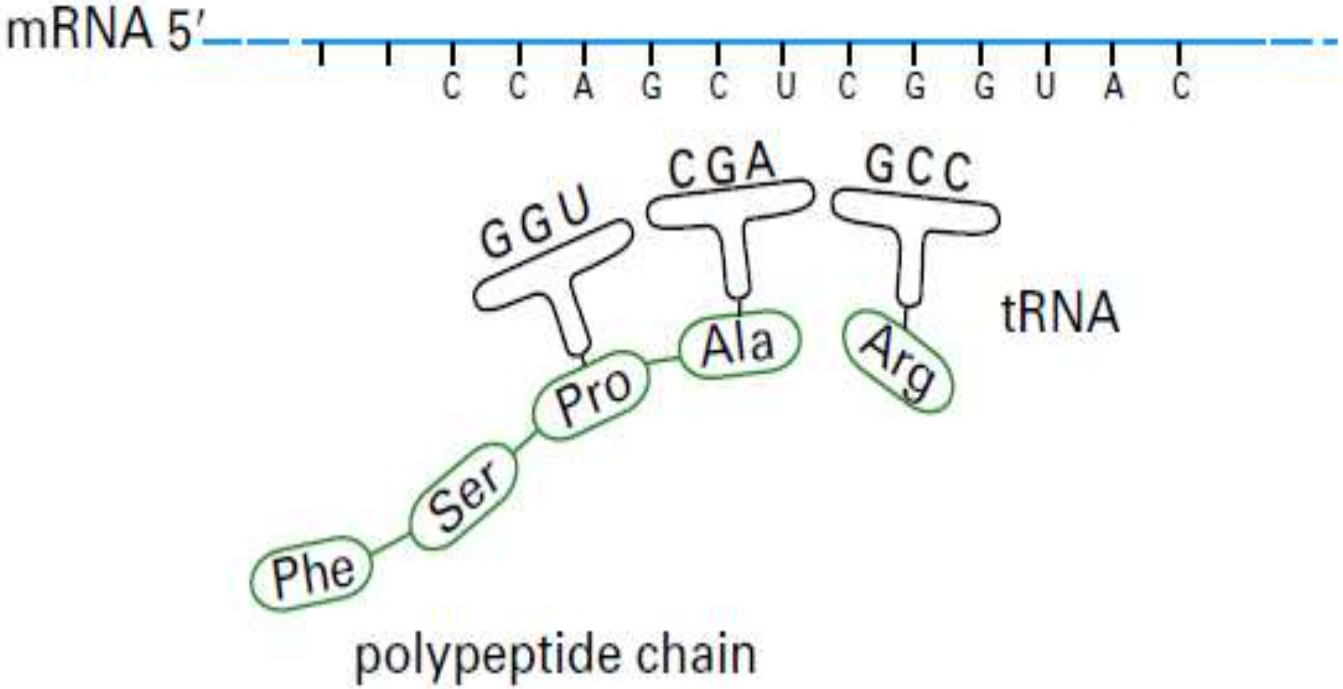
TRANSLATION

Translation is the transmission of the genetic information from mRNA to protein.
Newly processed mRNA is transported from the nucleus to the cytoaplasm, where it becomes associated with the ribosomes which are the site of protein synthesis.

Ribosomes are made up of two different sized subunits which consist of four different types of ribosomal RNA (rRNA) molecules, and a large number of ribosomal specific proteins.

Groups of ribosomes associated with the same molecule of mRNA are referred to as polyribosomes or polysomes .

In the ribosomes the mRNA forms the template for producing the specific sequence of amino acids of a particular polypepetide .

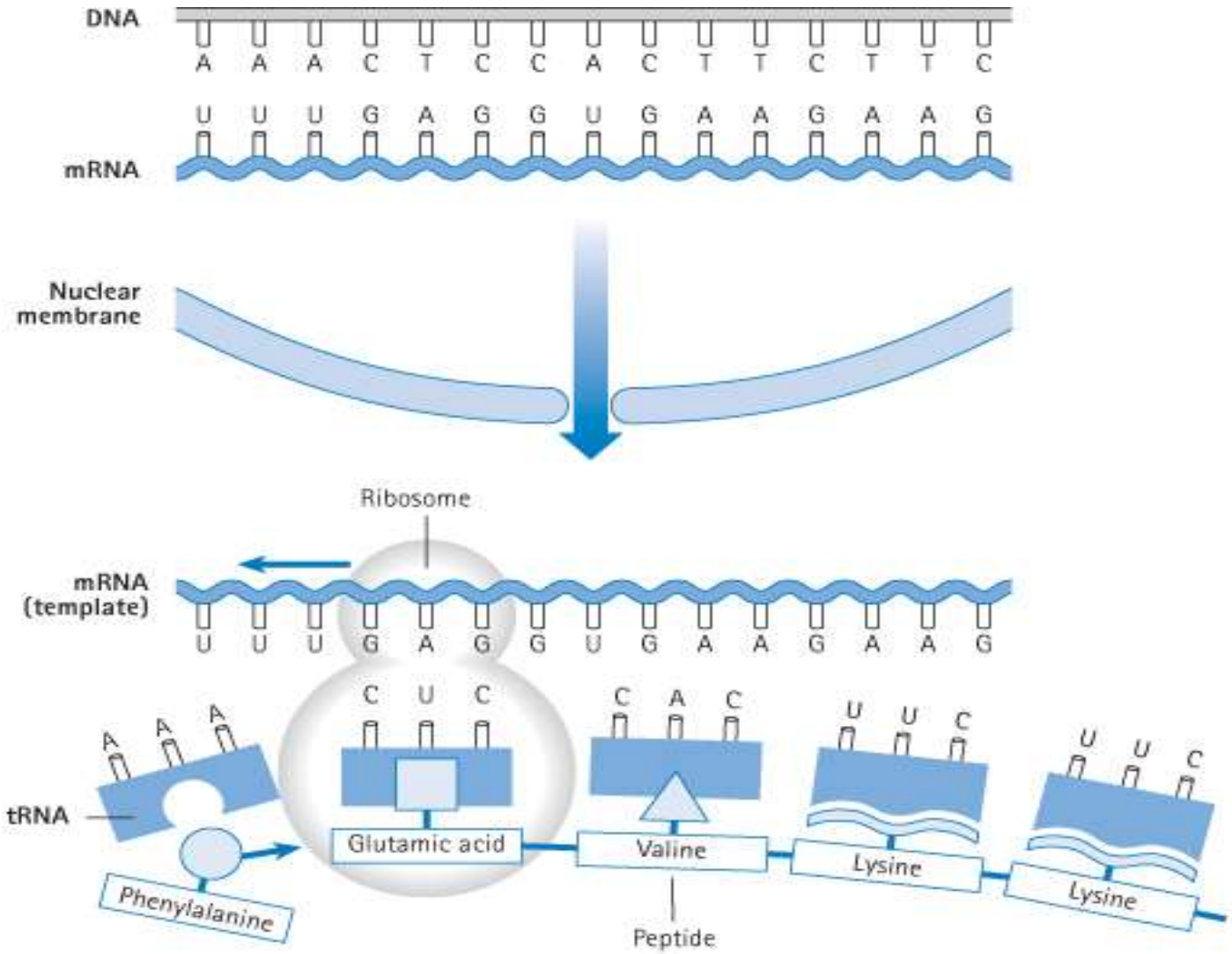

mRNA 5′
C C A G C U C G G U A C
GGU
CGA
GCC
tRNA
Pro
Ala
Arg
Ser
Phe
polypeptide chain

TRANSFER RNA

In the cytoplasm there is another form of RNA called transfer RNA or tRNA.

The incorporation of amino acids into a polypeptide chain requires the amino acids to be covalently bound by reacting with ATP to the specific tRNA molecule by the activity of the enzyme aminoacyl tRNA synthetase.

The ribosome, with its associated rRNAs, moves along the mRNA, the amino acids linking up by the formation of peptide bonds through the action of the enzyme peptidyl transferase to form a polypeptide chain .



Post Translational Modification

Many proteins, before they attain their normal structure or functional activity, undergo post-translational modification that can include:

- *modification of amino acid side-chains (e.g. hydroxylation, methylation etc.),*
- *The addition of carbohydrate or lipid moieties (e.g. glycosylation),*
- *Proteolytic cleavage of polypeptides (e.g. the conversion of proinsulin to insulin).*

This post-translational modification, along with certain short amino acid sequences known as localization sequences in the newly synthesized proteins, results in transport to specific cellular locations, e.g. the nucleus, or their secretion.

THE GENETIC CODE

Twenty different amino acids are found in proteins and, as DNA is composed of four different nitrogenous bases, then obviously a single base cannot specify one amino acid.
If two bases were to specify one amino acid, there would only be 4^2 or 16 possible combinations.

If, however, three bases specified one amino acid then the possible number of combinations of the four bases would be 4^3 or 64.
This is more than enough to account for all the 20 known amino acids and is known as the genetic code.

Table 15.1 Genetic code (RNA)*

First base (5′ end)	Second base				Third base (3′ end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

*Uracil (U) replaces thymine (T) in RNA.

The triplet of nucleotide bases in the mRNA that codes for a particular amino acid is called a codon.

Each triplet codon in sequence codes for a specific amino acid in sequence and so the genetic code is non-overlapping.

The order of the triplet codons in a gene is known as the translational reading frame.

However, some amino acids are coded for by more than one triplet and so the code is said to be degenerate.

Each tRNA species for a particular amino acid has a specific trinucleotide sequence called the anticodon, which is complementary to the codon of the mRNA.

Although there are 64 codons, there are only 30 cytoplasmic tRNAs, the anticodons of a number of the tRNAs recognizing codons that differ at the position of the third base, with guanine being able to pair with uracil as well as cytosine.

Termination of translation of the mRNA is signaled by the presence of one of the three stop or termination codons

The genetic code of mtDNA differs from that of the nuclear genome.

REGULATION OF GENE EXPRESSION

Many cellular processes, and therefore the genes that are expressed, are common to all cells, for example ribosomal, chromosomal and cytoskeleton proteins, constituting what are called the housekeeping genes.

Some cells express large quantities of a specific protein in certain tissues or at specific times in development, e.g. hemoglobin in red blood cells.

CONTROL OF TRANSCRIPTION

The control of transcription can be affected permanently or reversibly by a variety of factors, both environmental (e.g. hormones) and genetic (cell signaling).
This occurs through a number of different mechanisms which include :

- *Signaling molecules that bind to regulatory sequences in the DNA known as response elements.*

- *Intracellular receptors known as hormone nuclear receptors.*

- *Receptors for specific ligands on the cell surface involved in the process of signal transduction.*

All these mechanisms affect transcription through the binding of transcription factors to short specific DNA promoter elements the so-called promoter region that leads to activation of RNA polymerase . This includes the TATA (or Hogness), the GC (or GGGCGGG consensus sequence) and the CAAT boxes.

The TATA box, is involved in the initiation of transcription at a basal constitutive level and mutations in it can lead to alteration of the transcription start site.

The GC box, and the CAAT box increase the basal level of transriptional activity of the TATA box.

The regulatory elements in the promoter region are said to be cis-acting, that is, they only affect the expression of the adjacent gene on the same DNA duplex.

While the transcription factors are said to be trans-acting, acting on both copies of a gene on each chromosome being synthesized from genes that are located at a distance.

DNA sequences that increase transcriptional activity, such as the GC and CAAT boxes, are known as enhancers.

There are also negative regulatory elements or silencers that inhibit transcription.

In addition, there are short sequences of DNA, which are known as boundary elements, that block or inhibit the influence of regulatory elements of adjacent genes.

POST-TRANSCRIPTIONAL CONTROL OF GENE EXPRESSION

Regulation of expression of most genes occurs at the level of transcription but can also occur at the levels of RNA processing, RNA transport, mRNA degradation and translation.

For example, the G to A variant in the untranslated region of the prothrombin gene increases the stability of the mRNA transcript, resulting in higher plasma prothrombin levels.

ALTERNATIVE SPLICING

Many human genes undergo alternative splicing and therefore encode more than one protein.

Some genes have more than one promoter, and these alternative promoters may result in tissue-specific isoforms.

The extent of alternative splicing in humans may be inferred from the finding that the human genome includes only about 30000 genes, far fewer than the original prediction of over 100 000.

Mutation At DNA Level

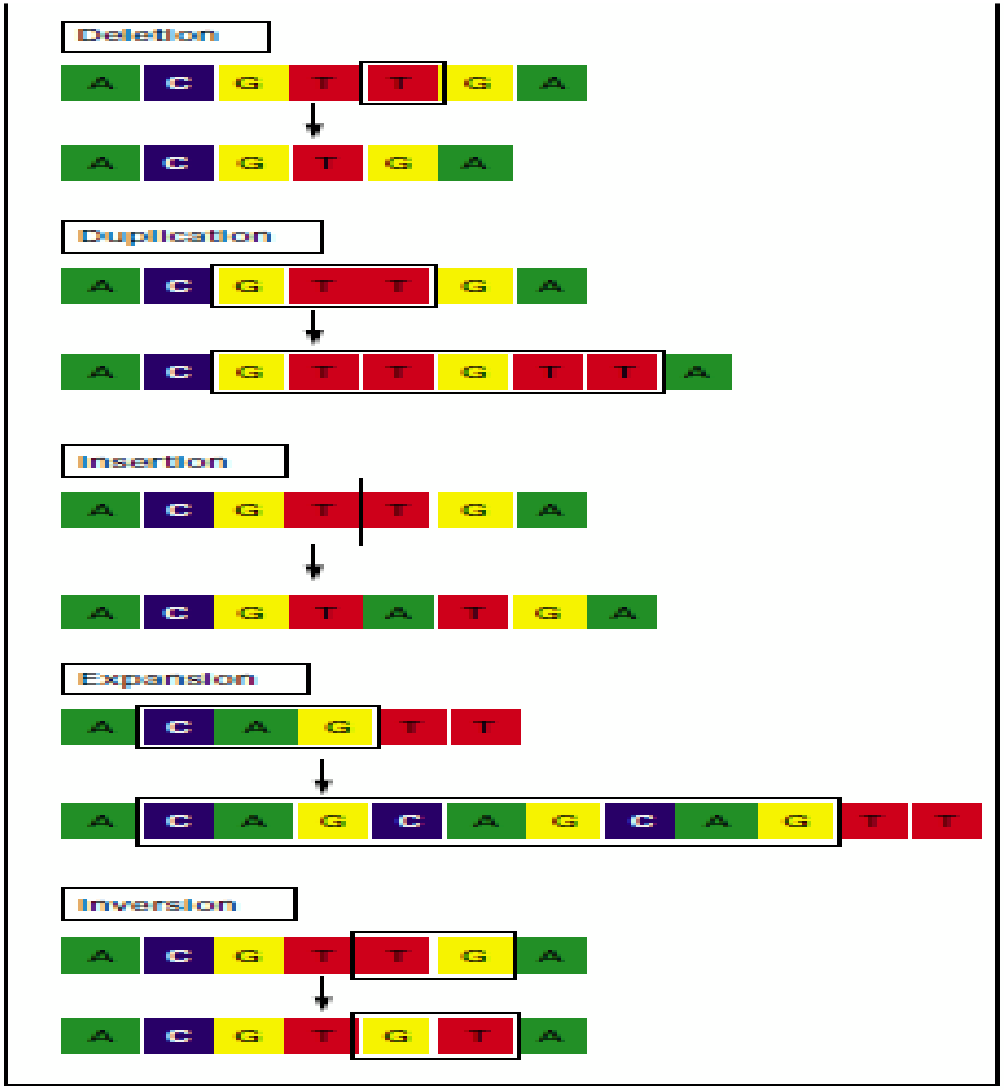


Figure 16.4 Mutation at the DNA level

STRUCTURAL EFFECTS OF MUTATIONS ON THE PROTEIN

Mutations can also be subdivided into two main groups according to the effect on the polypeptide sequence of the encoded protein, being either synonymous or non-synonymous.

Synonymous or Silent Mutations

If a mutation does not alter the polypeptide product of the gene, this is termed a synonymous or silent mutation.

A single base pair substitution, particularly if it occurs in the third position of a codon because of the degeneracy of the genetic code, will often result in another triplet that codes for the same amino acid with no alteration in the properties of the resulting protein.

Non–Synonymous Mutations

If a mutation leads to an alteration in the encoded polypeptide, it is known as a non-synonymous mutation.

Non-synonymous mutations are observed to occur less frequently than synonymous mutations.

Synonymous mutations will be selectively neutral, whereas alteration of the amino acid sequence of the protein product of a gene is likely to result in abnormal function that is usually associated with disease or lethality that has an obvious selective disadvantage.

Missence

A single base pair substitution can result in coding for a different amino acid and the synthesis of an altered protein, a so-called missense mutation.

If the mutation codes for an amino acid that is chemically dissimilar, e.g. a different charge, the structure of the protein will be altered.

Nonsense

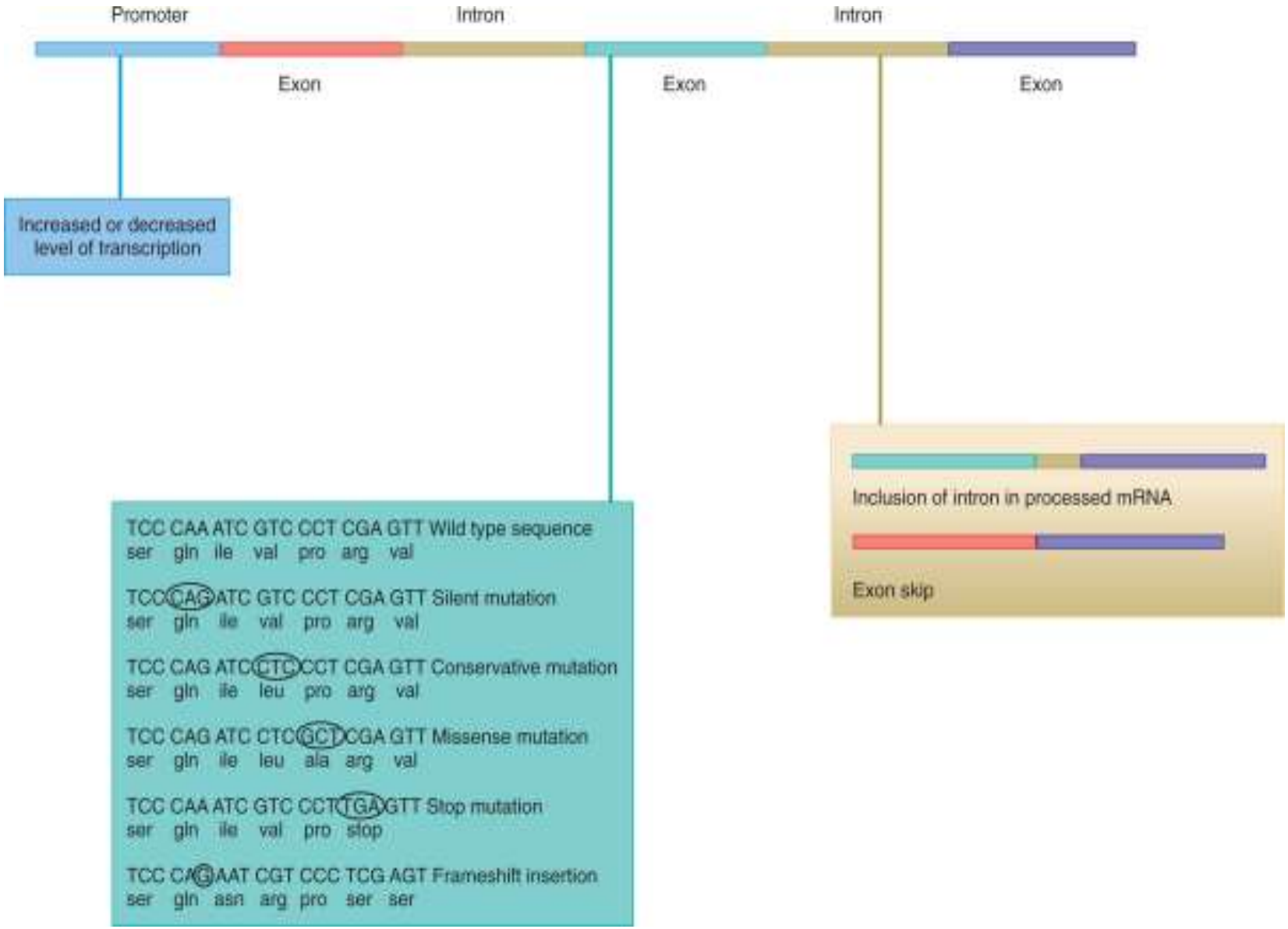
A substitution that leads to the generation of one of the stop codons will result in premature termination of translation of a peptide chain or what is termed a nonsense mutation.

In most cases the shortened chain is unlikely to retain normal biological activity, particularly if the termination codon results in the loss of an important functional domain(s) of the protein.

Frameshift

If a mutation involves the insertion or deletion of nucleotides that are not a multiple of three, it will disrupt the reading frame and constitute what is known as a frameshift mutation.

The amino acid sequence of the protein subsequent to the mutation bears no resemblance to the normal sequence and may have an adverse effect upon its function.


Promoter
Intron
Intron
Exon
Exon
Exon
Increased or decreased level of transcription
TCC CAA ATC GTC CCT CGA GTT Wild type sequence
ser gln ile val pro arg val
TCC CAG ATC GTC CCT CGA GTT Silent mutation
ser gln ile val pro arg val
TCC CAG ATC CTC CCT CGA GTT Conservative mutation
ser gln ile leu pro arg val
TCC CAG ATC CTC GCT CGA GTT Missense mutation
ser gln ile leu ala arg val
TCC CAA ATC GTC CCT TGA GTT Stop mutation
ser gln ile val pro stop
TCC CAG AAT CGT CCC TCG AGT Frameshift insertion
ser gln asn arg pro ser ser
Inclusion of intron in processed mRNA
Exon skip

GENITICS 6

Chromosomes

Dr.Sawsan Salem Shurrab

- At the molecular or submicroscopic level DNA can be regarded as the basic template that provides a blueprint for the formation and maintenance of an organism.

- DNA is packaged into chromosomes and at a very simple level these can be considered as being made up of tightly coiled long chains of genes.

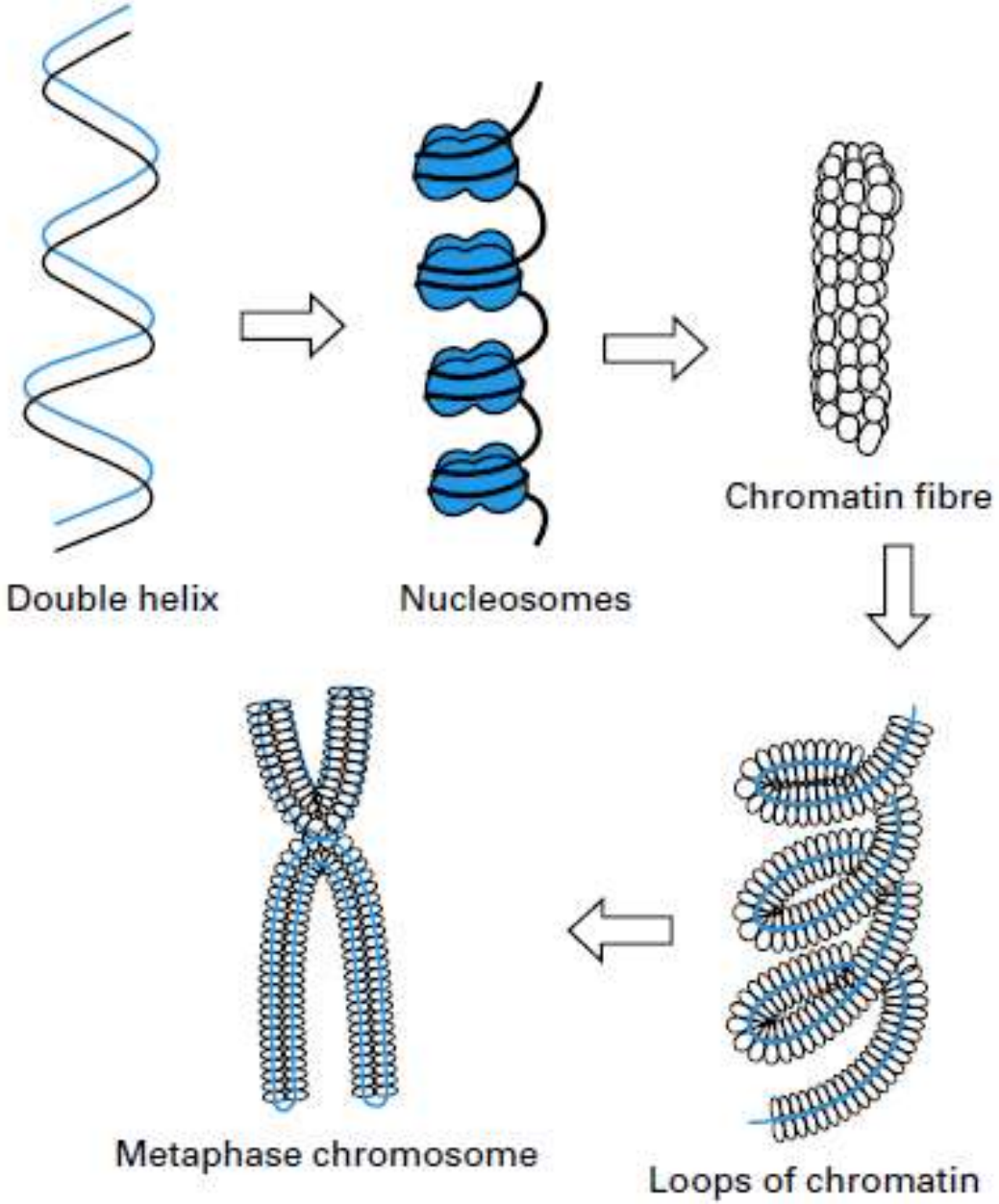


Figure 15.8 Packaging of DNA into chromosomes

- Unlike DNA, chromosomes can be visualized during cell division using a light microscope, under which they appear as thread-like structures or 'colored bodies'.

- The word chromosome is derived from the Greek chroma (= color) and soma (= body).

- Chromosomes are the factors that distinguish one species from another and that enable transmission of genetic information from one generation to the next.

- Their behavior at somatic cell division in *mitosis* provides a means of ensuring that each daughter cell retains its own complete genetic complement.

- Similarly, their behavior during gamete formation in *meiosis* enables each mature ovum and sperm to contain a unique single set of parental genes.

- Chromosomes are quite literally the vehicles that facilitate reproduction and the maintenance of a species.

- The study of chromosomes and cell division is referred to as *cytogenetics*.

- The correct chromosome number in humans is 46 and that maleness is determined by the presence of a Y chromosome regardless of the number of X chromosomes present in each cell.

- It was also realized that abnormalities of chromosome number and structure could seriously disrupt normal growth and development.

HUMAN CHROMOSOMES

MORPHOLOGY

- At the submicroscopic level chromosomes consist of an extremely elaborate complex, made up of supercoils of DNA.

- Under the electron microscope chromosomes can be seen to have a rounded and rather irregular morphology.

- However, most of our knowledge of chromosome structure has been gained using light microscopy. Special stains selectively taken up by DNA have enabled each individual chromosome to be identified.

- These are best seen during cell division,when the chromosomes are maximally contracted and the constituent genes can no longer be transcribed.

- At this time each chromosome can be seen to consist of two identical strands known as *chromatids*, or *sister chromatids*, which are the result of DNA replication having taken place during the S (synthesis) phase of the cell cycle

- These sister chromatids can be seen to be joined at a primary constriction known as the *centromere.*

- Centromeres consist of repetitive DNA and are responsible for the movement of chromosomes at cell division.

- Each centromere divides the chromosome into short and long arms, designated p (= petite) and q ('g' = grande), respectively.

- The tip of each chromosome arm is known as the telomere.
- Telomeres play a crucial role in sealing the ends of chromosomes and maintaining their structural integrity.
- Telomeres have been highly conserved throughout evolution and in humans they consist of many tandem repeats of a TTAGGG sequence.

- During DNA replication an enzyme known as *telomerase* replaces the 5' end of the long strand , which would otherwise become progressively shorter until a critical length is reached when the cell can no longer divide and thus becomes senescent.

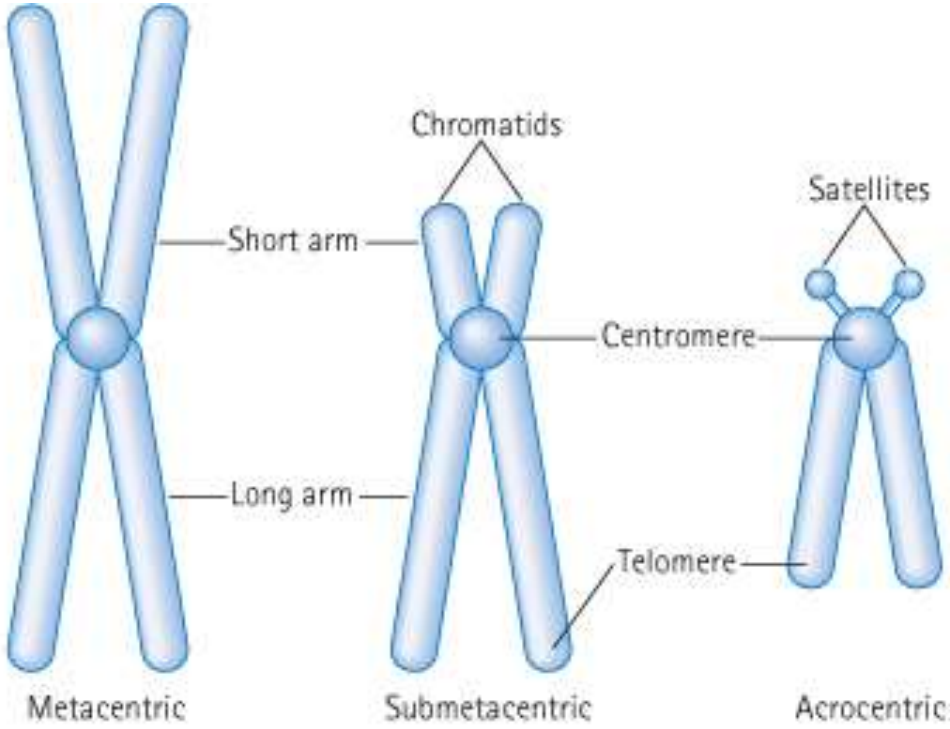
- This is in fact part of the normal cellular aging process, with most cells being unable to undergo more than 50-60 divisions.

- However, in some tumors increased telomerase activity has been implicated as a cause of abnormally prolonged cell survival.

- Morphologically chromosomes are classified according to the position of the centromere.

- If this is located centrally, the chromosome is *metacentric*, if terminal it is *acrocentric*, and if the centromere is in an intermediate position the chromosome is *submetacentric* .

- Acrocentric chromosomes sometimes have stalk-like appendages called *satellites* that form the nucleolus of the resting interphase cell and contain multiple repeat copies of the genes for ribosomal RNA.



CLASSIFICATION

- Individual chromosomes differ not only in the position of the centromere but also in their overall length.

- Based on the three parameters of length, position of the centromere and the presence or absence of satellites, early pioneers of cytogenetics were able to identify most individual chromosomes, or at least subdivide them into groups labelled A-G on the basis of overall morphology .

- In humans the normal cell nucleus contains 46 chromosomes, made up of 22 pairs of *autosomes* and a single pair of sex chromosomes - XX in the female and XY in the male.

- One member of each of these pairs is derived from each parent.

- Somatic cells are said to have a *diploid* complement of 46 chromosomes, whereas gametes (i.e. ova and sperm) have a *haploid* complement of 23 chromosomes.

- Members of a pair of chromosomes are known as *homologs.*

- The development of chromosome banding enabled very precise recognition of individual chromosomes and the detection of subtle chromosome abnormalities.

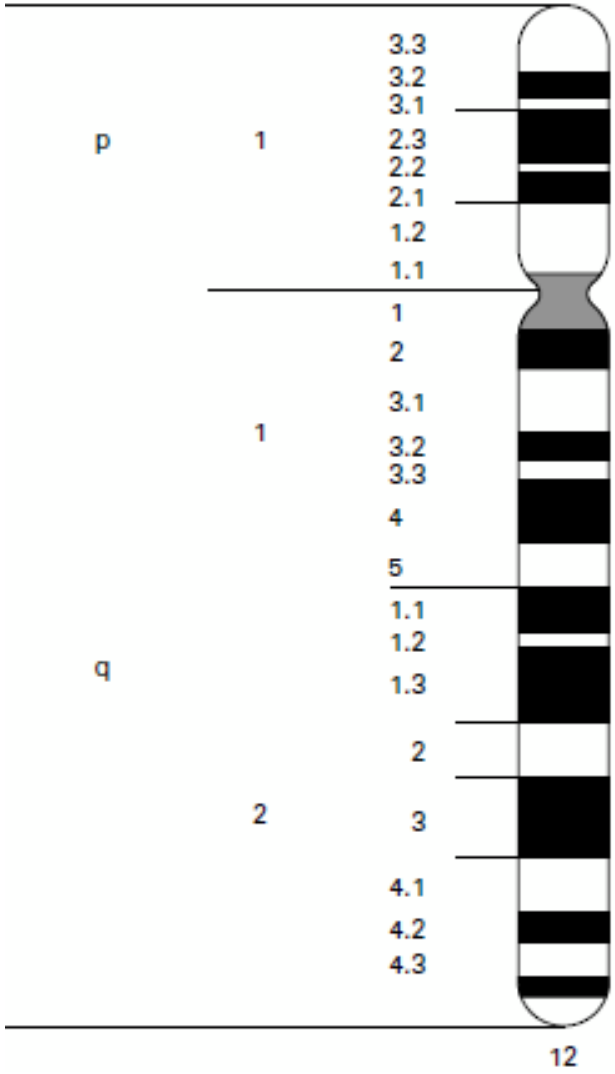


Figure 4.5 Simplified banding pattern of chromosome 12

- This technique also revealed that *chromatin*, the combination of DNA and histone proteins of which chromosomes are made, exists in two main forms.

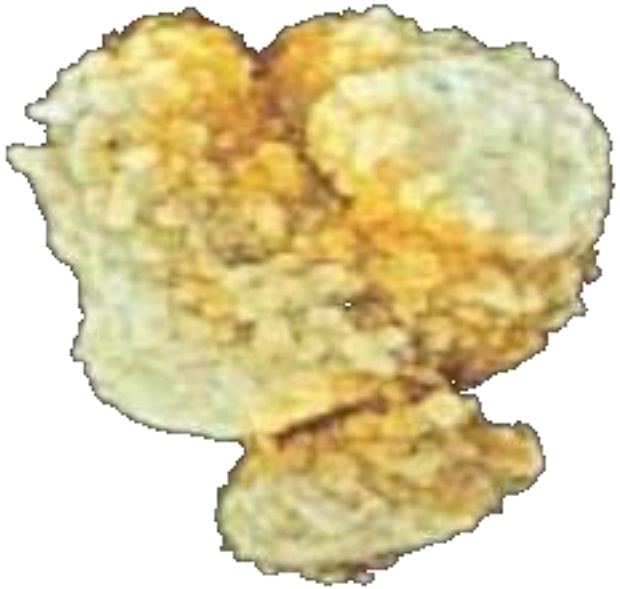
1. *Euchromatin* stains lightly and consists of genes that are actively expressed.
2. *Heterochromatin* stains darkly and is made up largely of inactive unexpressed repetitive DNA.

THE SEX CHROMOSOMES

- The X and Y chromosomes are known as the sex chromosomes because of their crucial role in sex determination.

- The X chromosome was originally labeled as such because of uncertainty as to its function .

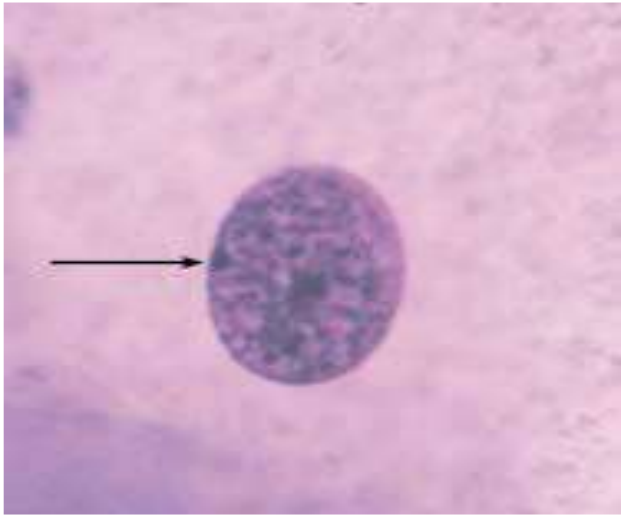
- In humans, and in most mammals, both the male and the female have two sex chromosomes - XX in the female and XY in the male .

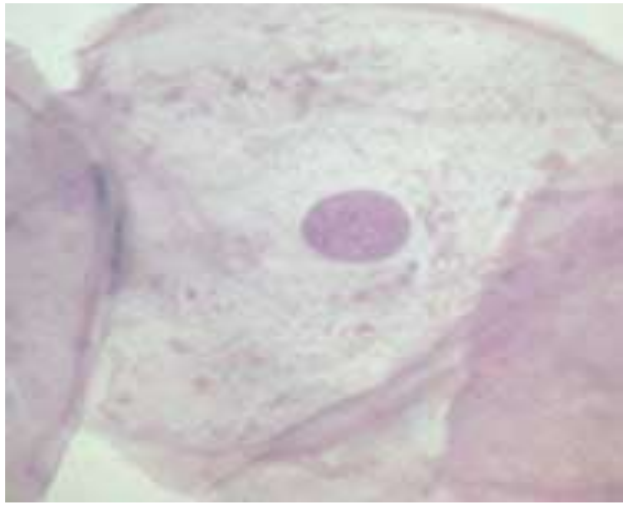


- The Y chromosome is much smaller than the X and carries only a few genes of functional importance, most notably the testis-determining factor, known as SRY .

- Other genes on the Y chromosome are known to be important in maintaining spermatogenesis.

- In the female each ovum carries an X chromosome, whereas in the male each sperm carries either an X or a Y chromosome.





A Barr body is an inactivated X chromosome.
(a) Female cell with a Barr body (indicated by arrow).
(b) Male cell without a Barr body.

Table 4.2 **Number of Barr bodies in human cells with different complements of sex chromosomes**

Sex Chromosomes	Syndrome	Number of Barr Bodies
XX	None	1
XY	None	0
XO	Turner	0
XXY	Klinefelter	1
XXYY	Klinefelter	1
XXXY	Klinefelter	2
XXXXY	Klinefelter	3
XXX	Triplo-X	2
XXXX	Poly-X female	3
XXXXX	Poly-X female	4

- As there is a roughly equal chance of either an X-bearing sperm or a Y-bearing sperm fertilizing an ovum, the numbers of male and female conceptions are approximately equal .

- In fact, slightly more male babies are born than females, although during childhood and adult life the sex ratio evens out at 1:1.

METHODS OF CHROMOSOME ANALYSIS

Genetic 7

By

Dr. Sawsan Salem Shurrab

CHROMOSOME PREPARATION

Any tissue with living nucleated cells that undergo division can be used for studying human chromosomes.

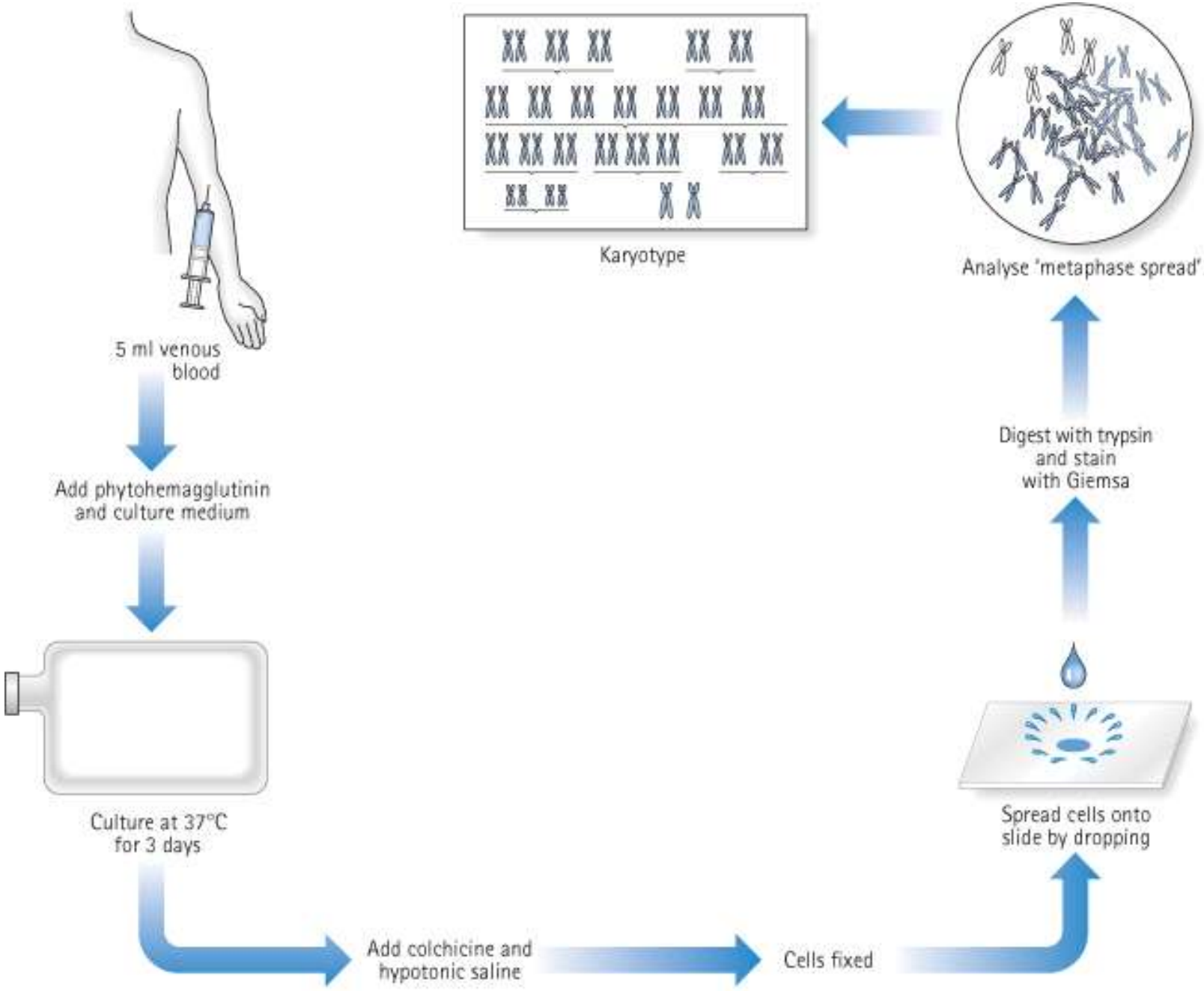
Most commonly circulating lymphocytes from peripheral blood are used, although *samples* for chromosomal analysis can be prepared relatively easily using skin, bone marrow, chorionic villi or cells from amniotic fluid (amniocytes).

- In the case of peripheral (venous) blood, a sample is added to a small volume of nutrient medium containing phytohemagglutinin, which stimulates T lymphocytes to divide.

- The cells are cultured under sterile conditions at 37°C for about 3 days, during which they divide, and colchicine is then added to each culture .

- This drug has the extremely useful property of preventing formation of the spindle, thereby arresting cell division during metaphase, the time when the chromosomes are maximally condensed and therefore most easily visible.

- Hypotonic saline is then added, which causes the red blood cells to lyze and results in spreading of the chromosomes, which are then fixed, mounted on a slide and stained ready for analysis .



CHROMOSOME BANDING

Several different staining methods can be utilized to identify individual chromosomes.

G (giemsa) banding

This is the method most commonly used.

The chromosomes are treated with trypsin, which denatures their protein content, and then stained with a DNA-binding dye known as Giemsa, which gives each chromosome a characteristic pattern of light and dark bands .

R (reverse) banding

- The chromosomes are heat-denatured before staining with Giemsa, yielding light and dark bands which are the reverse of those obtained using conventional G banding.

C (centromeric heterochromatin) banding

If the chromosomes are pretreated with acid followed by alkali prior to G banding, the centromeres and other heterochromatic regions containing highly repetitive DNA sequences are preferentially stained.

High-resolution banding

- G banding generally provides high-quality chromosome analysis .

- High-resolution banding of the chromosomes at an earlier stage of mitosis such as prophase or prometaphase, provides greater sensitivity , but is much more demanding technically.

- This involves first inhibiting cell division with an agent such as methotrexate or thymidine.

- Folic acid is added to the culture medium, which releases the cells into mitosis.

- Colchicine is then added at a specific time interval, when a higher proportion of cells will be in prometaphase and the chromosomes will not be fully contracted, giving a more detailed banding pattern.

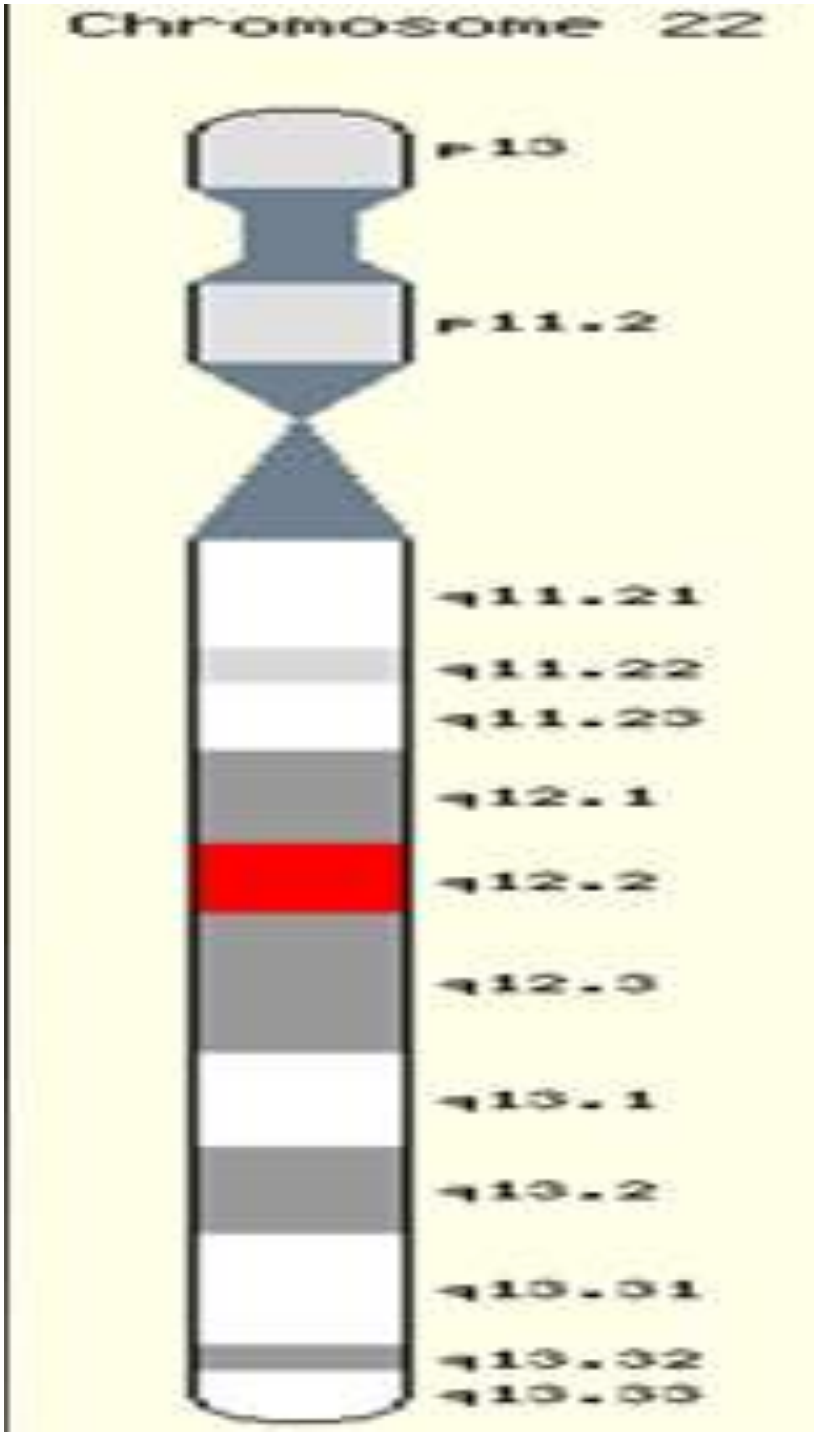
KARYOTYPE ANALYSIS

- The next stage in chromosome analysis involves first counting the number of chromosomes present in a specified number of cells, sometimes referred to as metaphase spreads.

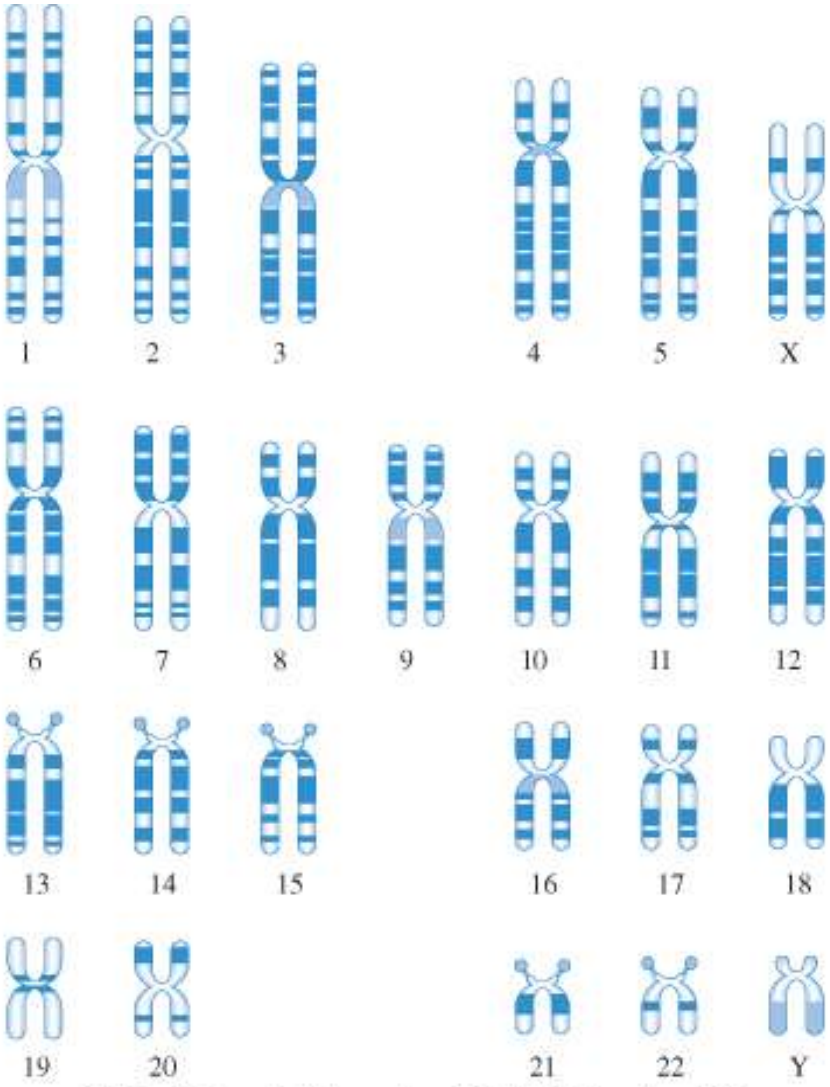
- Usually the total chromosome count is determined in 10-15 cells, but if mosaicism is suspected then 30 or more cell counts will be undertaken.

Detailed analysis of the banding pattern of the individual chromosomes is carried out on both members of each pair of homologs in approximately 3-5 metaphase spreads, which show high-quality banding.

- The banding pattern of each chromosome is specific and can be shown in the form of a stylized ideal karyotype known as an idiogram .


Chromosome 22
p13
p11.2
q11.21
q11.22
q11.23
q12.1
q12.2
q12.3
q13.1
q13.2
q13.31
q13.32
q13.33

- The cytogeneticist analyses each pair of homologous chromosomes, either while looking down the microscope or, increasingly, on a photograph of the metaphase spread, which can now be produced electronically .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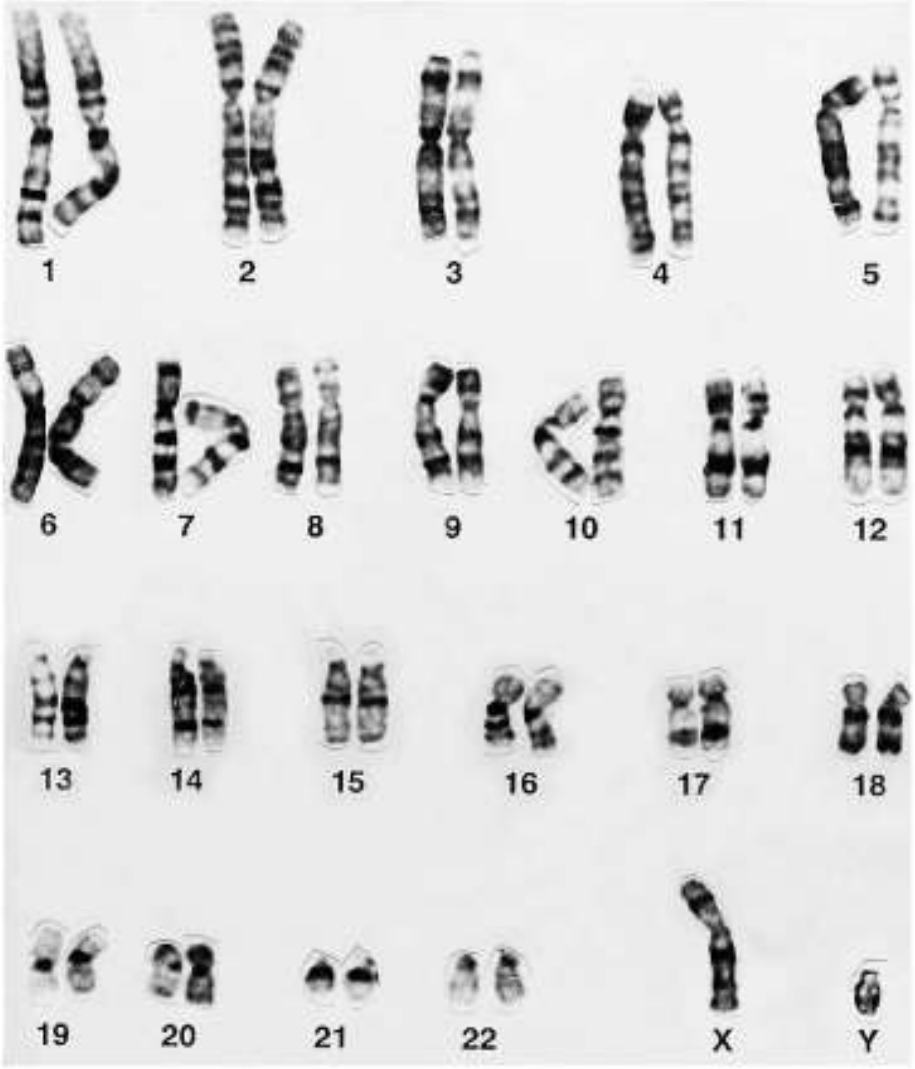
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MOLECULAR CYTOGENETICS

- FLUORESCENT IN-SITU HYBRIDIZATION

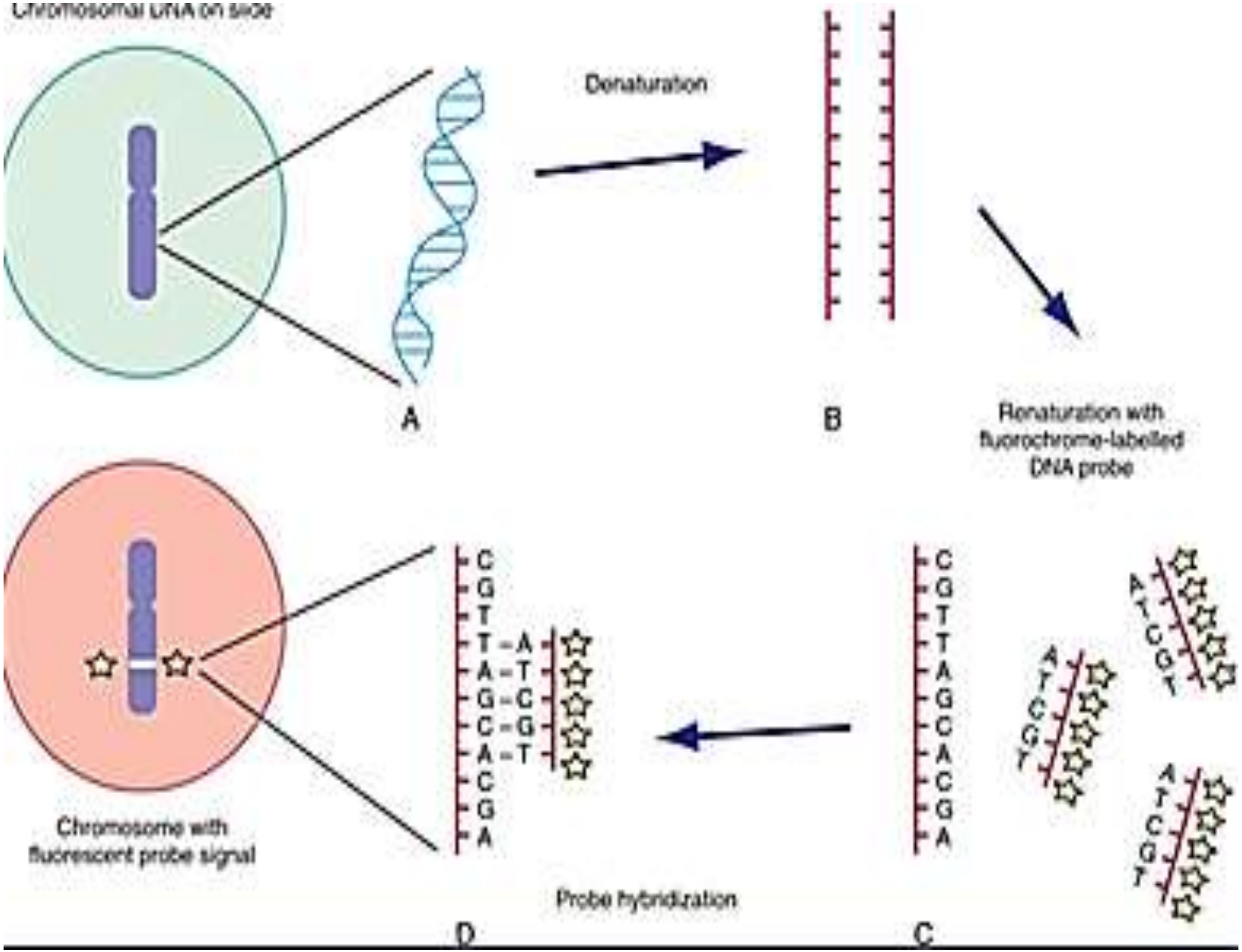
- This relatively new diagnostic tool combines conventional cytogenetics with molecular genetic technology.

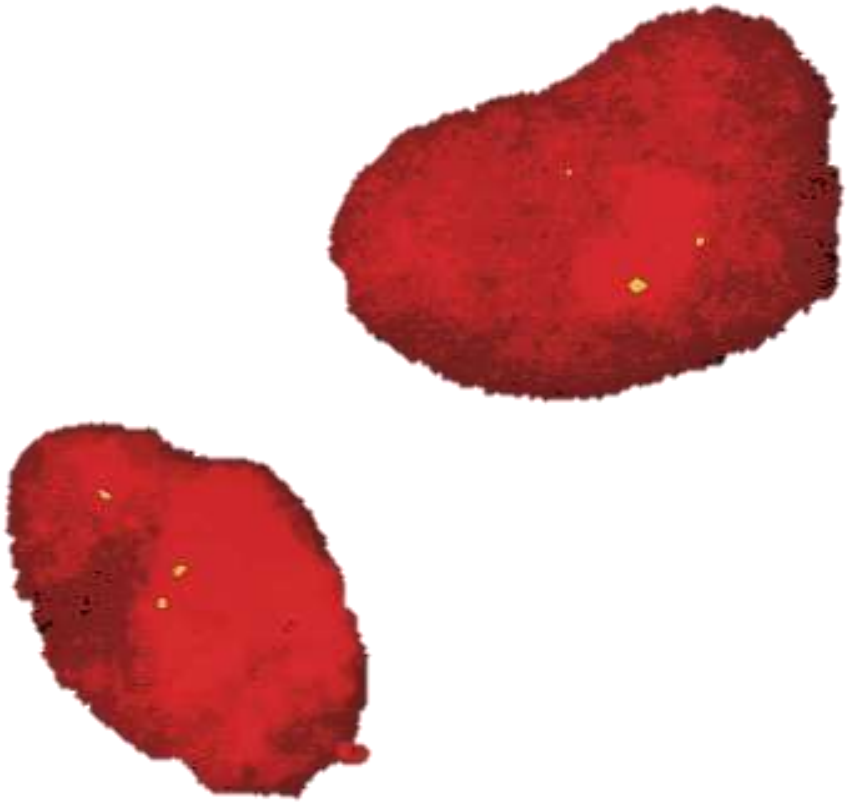
- It is based on the unique ability of a portion of single-stranded DNA, i.e. a probe , to anneal with its complementary target sequence wherever it is located on a metaphase spread.

- Unlike most other methods used for chromosome analysis, fluorescent in-situ hybridization (FISH) can also be used to study chromosomes in cells that are resting between cell division in the interval known as interphase.

- In this technique the DNA probe is conjugated with modified nucleotides which, after hybridization with the patient sample, allows the region where hybridization has occurred to be visualized using a fluorescent microscope.

- FISH is now used widely for clinical diagnostic purposes and one major advantage is that results are usually obtained very rapidly with interphase FISH.


Chromosomal DNA on slide
Denaturation
A
B
Renaturation with
fluorochrome-labelled
DNA probe
C
Probe hybridization
D
Chromosome with
fluorescent probe signal



- Fluorescent in situ hybridization (FISH) of interphase nuclei with a chromosome 21 centromeric probe (CAMBIO) showing three signals consistent with trisomy 21

Different types of fish probes

- Centromeric probes

  These consist of repetitive DNA sequences found in and around the centromere of a specific chromosome.

  They are suitable for making a rapid diagnosis of one of the common aneuploidy syndromes (using non-dividing cells in interphase obtained from a prenatal diagnostic sample of chorionic villi .

- Chromosome-specific unique sequence probes

  These are specific for a particular single locus.

  They are particularly useful for identifying tiny submicroscopic deletions and duplications .

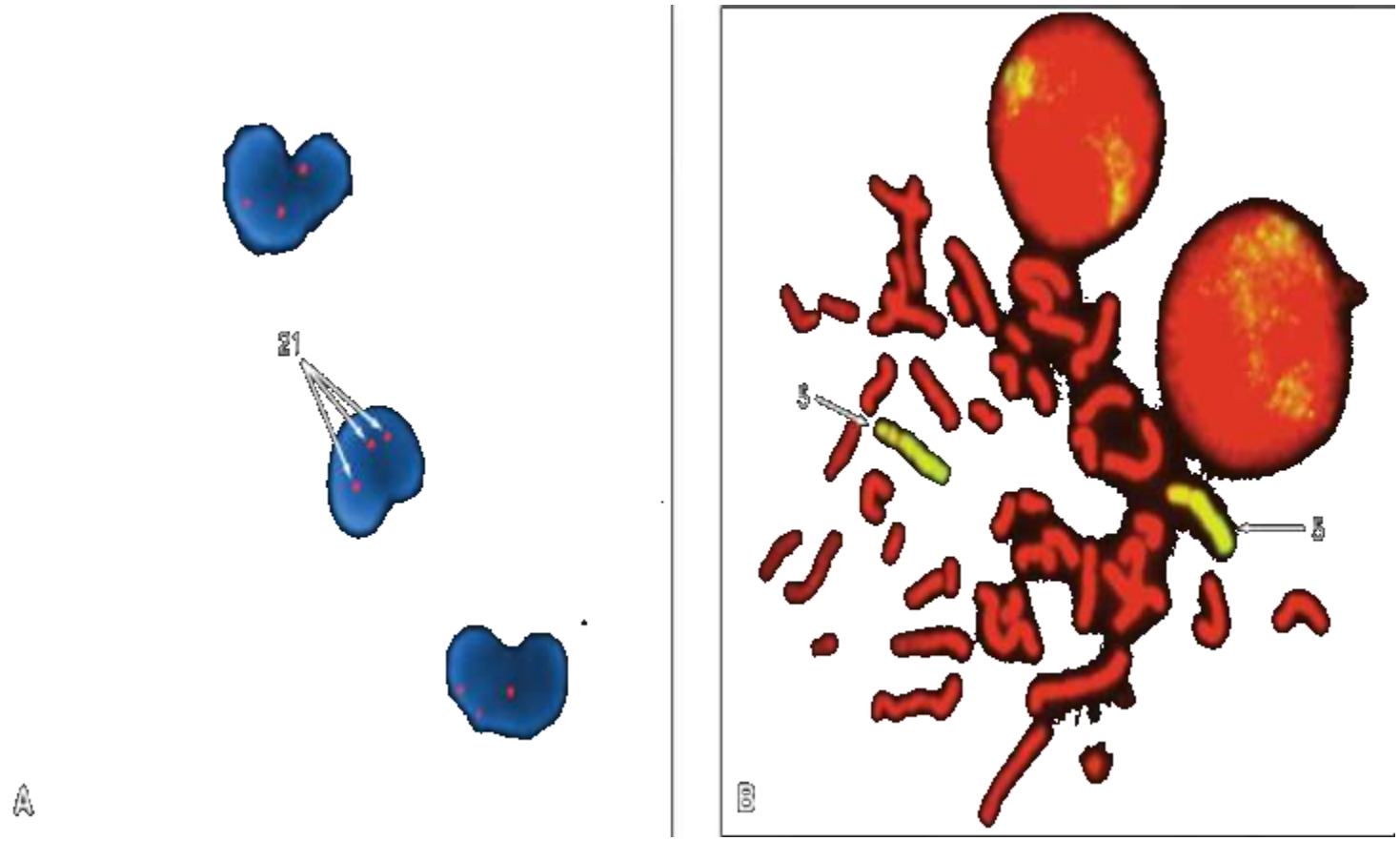
- Telomeric probes

  A complete set of telomeric probes has been developed for all 24 chromosomes (i.e. autosomes 1-22 plus X and Y).

- Whole chromosome paint probes

These consist of a cocktail of probes obtained from different parts of a particular chromosome.

When this mixture of probes is used together in a single hybridization, the entire relevant chromosome fluoresces, i.e. is 'painted'.



- ***A,*** **Fluorescence in situ hybridization (FISH) analysis of interphase peripheral blood cells from a patient with Down syndrome using a chromosome 21–specific probe. The 3 red signals mark the presence of 3 chromosomes 21.** ***B,*** **FISH analysis of a metaphase chromosome spread from a clinically normal individual using a whole-chromosome paint specific for chromosome 5. Both chromosomes 5 are completely labeled (yellow) along their entire length.**

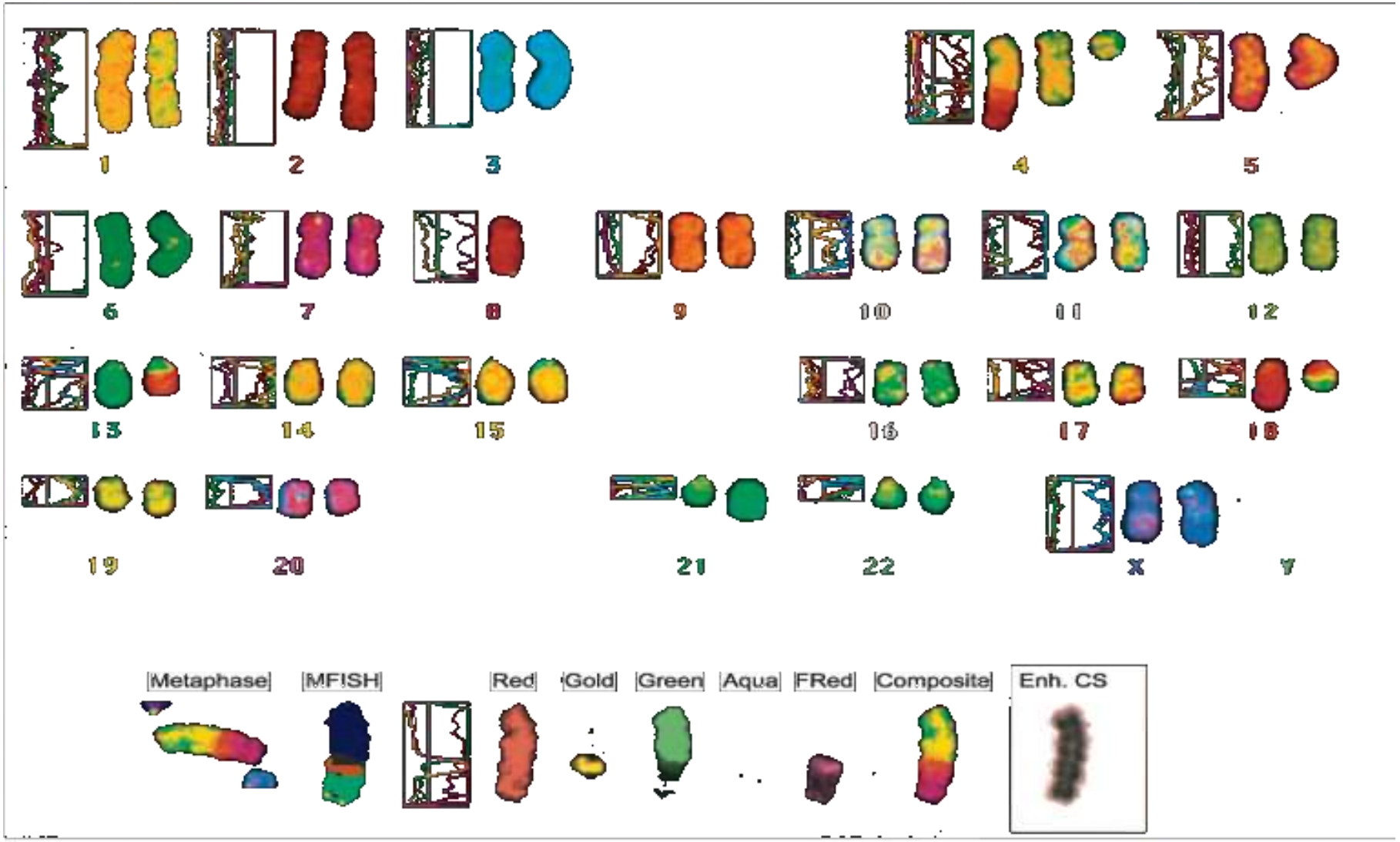
Reverse painting

- In this procedure a portion of unidentified chromosome material, such as a small supernumerary marker or ring, is used as a paint for hybridization to a normal metaphase spread.

- The origin of the unidentified chromosome segment is then revealed by identifying the chromosome(s) to which it hybridizes.

Multicolor spectral karyotyping (SKY) and multicolor fish (M-FISH)

These latest developments in FISH technology utilize pools of whole human chromosome paint probes to provide a multicolour human karyotype in which each pair of homologous chromosomes can be identified on the basis of its unique color when studied using computer-based image analysis.



- FLOW CYTOMETRY

- Because of their differing size and DNA composition chromosomes bind different amounts of fluorescent dyes, some of which bind specifically to GC ('gene-rich') sequences and others to AT ('gene-poor') sequence.

- Flow cytometry can be used to analyze an individual's chromosomes, but its clinical application is limited by its expense and by the relatively poor separation achieved for certain chromosomes.

- **COMPARATIVE GENOMIC HYBRIDIZATION**

- Tumor or 'test' DNA is labeled with a green paint and control normal DNA is labeled with a red paint.

- The two samples are mixed and hybridized competitively to normal metaphase chromosomes, and photographed using a fluorescence microscope .

- If the test sample contains more DNA from a particular chromosome region than the control sample, then that region is identified by an increase in the green to red fluorescence ratio .

CELL DIVISION

Genitics 8

MITOSIS

- At conception the human zygote consists of a single cell.

- This undergoes rapid division, leading ultimately to the mature human adult .

- In most organs and tissues, such as bone marrow and skin, cells continue to divide throughout life.

- This process of somatic cell division, during which the nucleus also divides, is known as mitosis.

- During mitosis each chromosome divides into two daughter chromosomes, one of which segregates into each daughter cell.

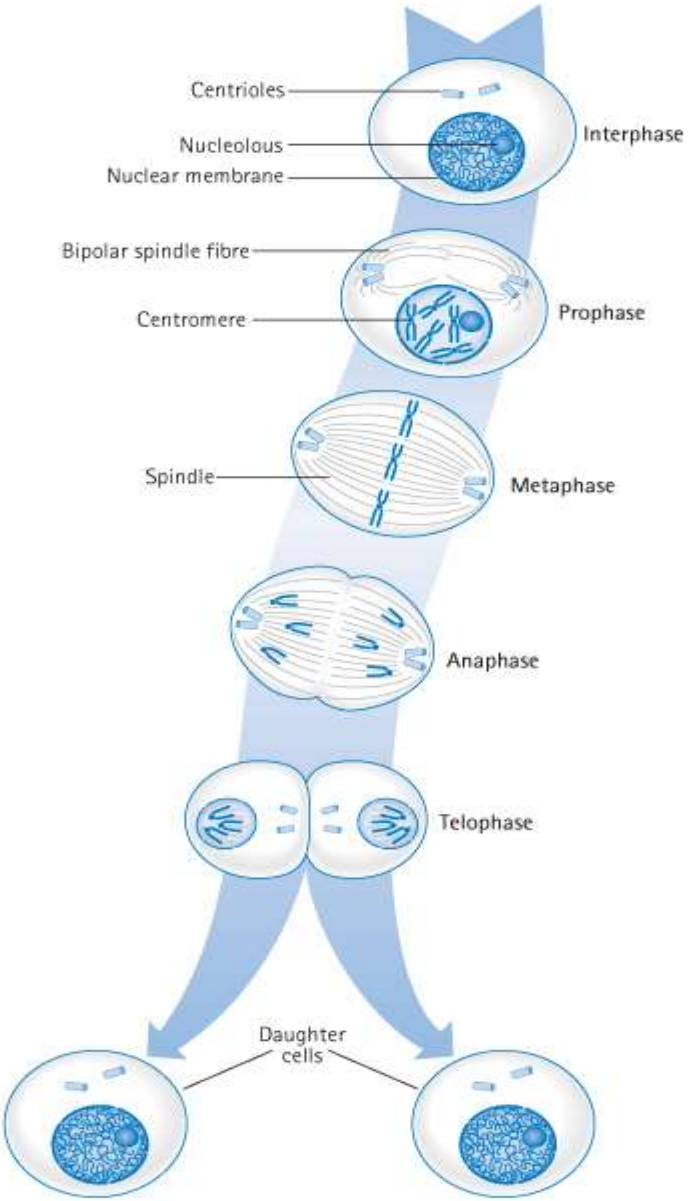
- Consequently the number of chromosomes per nucleus remains unchanged.

- Prior to a cell entering mitosis, each chromosome consists of two identical sister chromatids as a result of DNA replication having taken place during the S phase of the cell cycle .

- Mitosis is the process whereby each of these pairs of chromatids separates and disperses into separate daughter cells.

- Mitosis is a continuous process that usually lasts 1-2 h, but for descriptive purposes it is convenient to distinguish five distinct stages.

- These are prophase, prometaphase, metaphase, anaphase and telophase .



Prophase

During the initial stage of prophase the chromosomes condense and the mitotic spindle begins to form.

Two centrioles form in each cell, from which microtubules radiate as the centrioles move towards opposite poles of the cell.

Prometaphase

During *prometaphase* the nuclear membrane begins to disintegrate, allowing the chromosomes to spread around the cell. Each chromosome becomes attached at its centromere to a microtubule of the mitotic spindle.

Metaphase

- In *metaphase* the chromosomes become aligned along the equatorial plane or plate of the cell, where each chromosome is attached to the centriole by a microtubule forming the mature spindle.

- At this point the chromosomes are maximally contracted and, therefore, most easily visible.

- Each chromosome resembles the letter X in shape as the chromatids of each chromosome have separated longitudinally but remain attached at the centromere that has not yet undergone division.

Anaphase

In *anaphase* the centromere of each chromosome divides longitudinally and the two daughter chromatids separate to opposite poles of the cell.

Telophase

- By *telophase* the chromatids, which are now independent chromosomes consisting of a single double helix, have separated completely and the two groups of daughter chromosomes each become enveloped in a new nuclear membrane.

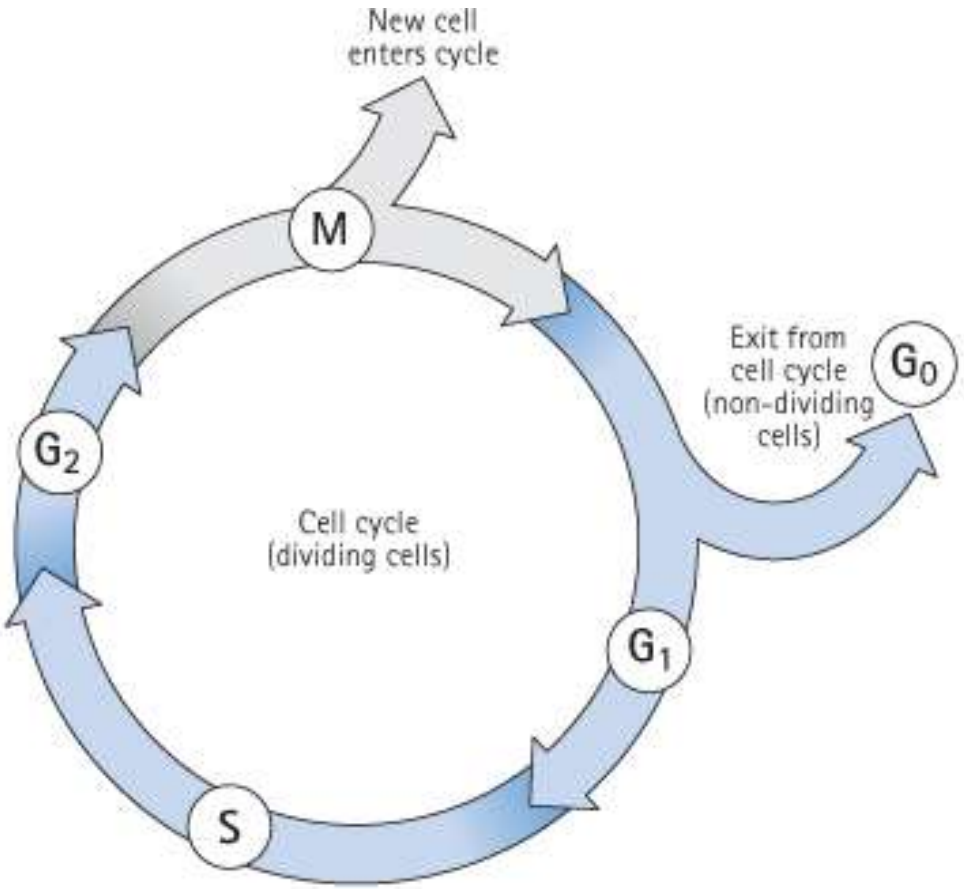
- The cell cytoplasm also separates (cytokinesis), resulting in the formation of two new daughter cells each of which contains a complete diploid chromosome complement.

THE CELL CYCLE

The period between successive mitoses is known as the *interphase* of the cell cycle

In rapidly dividing cells this lasts for between 16 and 24 h.

Interphase commences with the G_1 (G = gap) phase during which the chromosomes become thin and extended.



- This phase of the cycle is very variable in length and is responsible for the variation in generation time between different cell populations.

- Cells which have stopped dividing, such as neurons, usually arrest in this phase and are said to have entered a non-cyclic stage known as G0.

The G_1 phase is followed by the S phase (S = synthesis), when DNA replication occurs and the chromatin of each chromosome is replicated.

This results in the formation of two chromatids which give each chromosome its characteristic X-shaped configuration.

The process of DNA replication commences at multiple points on a chromosome .

Usually homologous pairs of chromosomes replicate in synchrony.

However, one of the X chromosomes is always late replicating.

This is the inactive X chromosome which forms the *sex chromatin*, or the so-called *Barr body*, which can be visualized in interphase in female somatic cells.

This used to be the basis of a rather unsatisfactory means of sex determination based on analysis of cells obtained by scraping the buccal mucosa - a 'buccal smear'.

Interphase is completed by a relatively short G_2 phase during which the chromosomes begin to condense in preparation for the next mitotic division.

Meiosis

Genetic 9

- Meiosis is the process of nuclear division that occurs during the final stage of gamete formation.

- Meiosis differs from mitosis in three fundamental ways:

1. Mitosis results in each daughter cell having a diploid chromosome complement .

   During meiosis the diploid count is halved so that each mature gamete receives a haploid complement of 23 chromosomes.

2. Mitosis takes place in somatic cells and during the early cell divisions in gamete formation.

Meiosis occurs only at the final division of gamete maturation.

3. Mitosis occurs as a single one-step process.

Meiosis can be considered as two cell divisions known as meiosis I and meiosis II, each of which can be considered as having prophase, metaphase, anaphase and telophase stages, as in mitosis .

Meiosis I

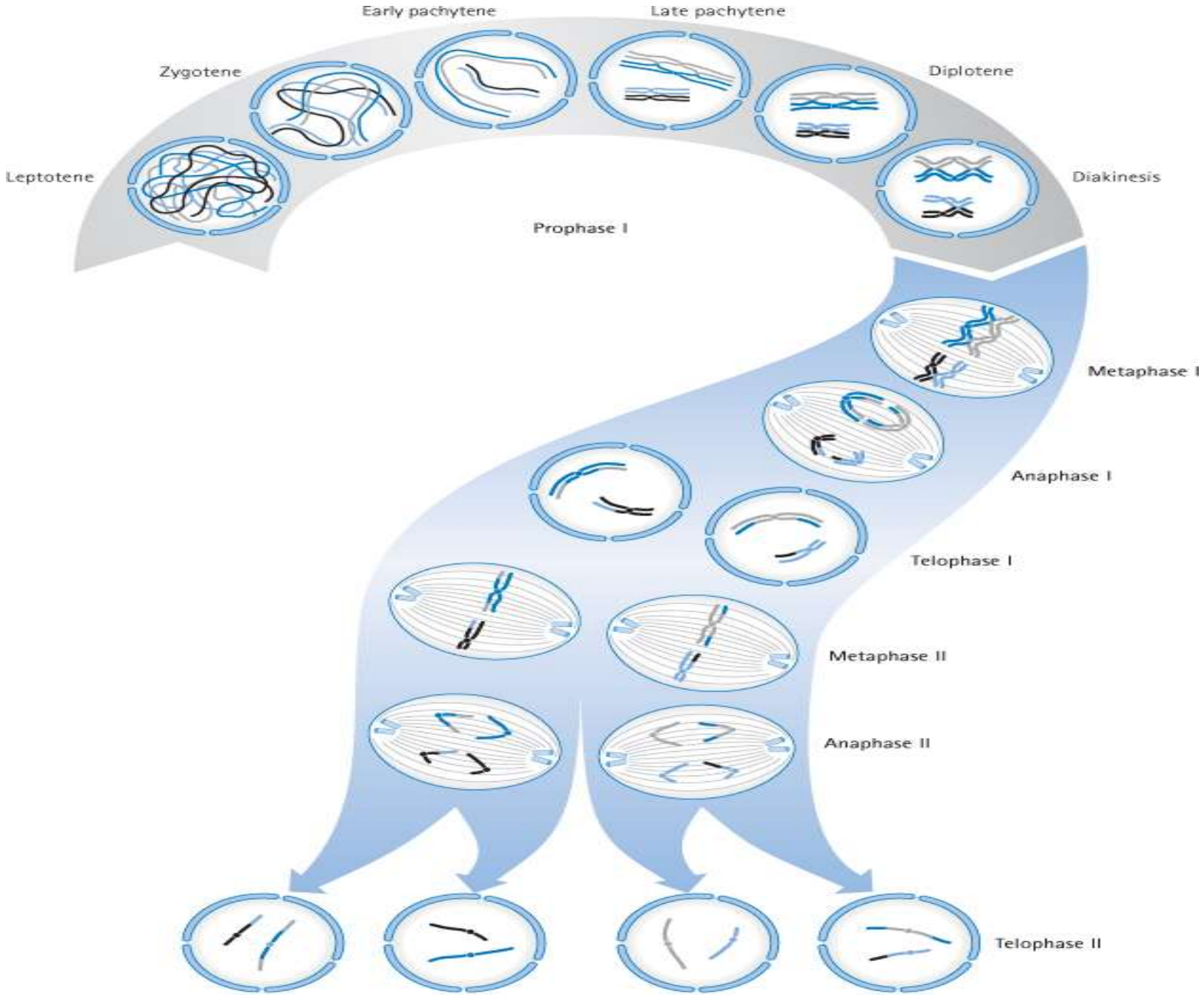
This is sometimes referred to as the reduction division because it is during the first meiotic division that the chromosome number is halved.

Prophase I

Chromosomes enter this stage already split longitudinally into two chromatids joined at the centromere.

Homologous chromosomes pair, and with the exception of the X and Y chromosomes in male meiosis, exchange of homologous segments occurs between non-sister chromatids, that is, chromatids from each of the pair of homologous chromosomes.

- This exchange of homologous segments between chromatids occurs as a result of a process known as *crossing over* or *recombination*.



During prophase I in the male pairing occurs between homologous segments of the X and Y chromosomes at the tip of their short arms, with this portion of each chromosome being known as the *pseudoautosomal* region .

The prophase stage of meiosis I is relatively lengthy and can be subdivided into five stages.

1. Leptotene

The chromosomes become visible as they start to condense.

2. Zygotene

Homologous chromosomes align directly opposite each other, a process known as synapsis, and are held together at several points along their length by filamentous structures known as *synaptonemal* complexes.

3. Pachytene

Each pair of homologous chromosomes, known as a *bivalent*, becomes tightly coiled.

Crossing over occurs, during which homologous regions of DNA are exchanged between chromatids.

4. Diplotene

The homologous recombinant chromosomes now begin to separate but remain attached at the points where crossing over has occurred.

- These are known as *chiasmata*.

- On average small, medium and large chromosomes have one, two and three chiasmata, respectively, giving an overall total of approximately 40 recombination events per meiosis per gamete.

5. Diakinesis

Separation of the homologous chromosome pairs proceeds as the chromosomes become maximally condensed.

Metaphase I

The nuclear membrane disappears and the chromosomes become aligned on the equatorial plane of the cell where they have become attached to the spindle, as in metaphase of mitosis.

Anaphase I

The chromosomes now separate to opposite poles of the cell as the spindle contracts.

Telophase I

Each set of haploid chromosomes has now separated completely to opposite ends of the cell, which cleaves into two new daughter gametes, so-called *secondary spermatocytes* or *oocytes*.

Meiosis II

This is essentially similar to an ordinary mitotic division.

Each chromosome, which exists as a pair of chromatids, becomes aligned along the equatorial plane and then splits longitudinally, leading to the formation of two new daughter gametes, known as spermatids or ova.

The consequences of meiosis

When considered in terms of reproduction and the maintenance of the species, meiosis achieves two major objectives.

1. It facilitates halving of the diploid number of chromosomes so that each child receives half of its chromosome complement from each parent.

2. It provides an extraordinary potential for generating genetic diversity.

- This is achieved in two ways:

1. When the bivalents separate during prophase of meiosis I, they do so independently of one another.

This is consistent with Mendel's third law .

Consequently each gamete receives a selection of parental chromosomes.

The likelihood that any two gametes from an individual will contain exactly the same chromosomes equals 1 in 8 million.

2. As a result of crossing over, each chromatid will usually contain portions of DNA derived from both parental homologous chromosomes.

- A large chromosome will typically consist of three or more segments of alternating parental origin. The ensuing probability that any two gametes will have an identical genome is therefore infinitesimally small.

- This dispersion of DNA into different gametes is sometimes referred to as 'gene shuffling'.

OOGENESIS

Mature ova develop from oogonia which originates from primordial germ cells .

By the completion of embryogenesis at 3 months of intrauterine life, the oogonia have begun to mature into primary oocytes that start to undergo meiosis.

At birth all of the primary oocytes have entered a phase of maturation arrest, known as *dictyotene*, in which they remain suspended until meiosis I is completed at the time of ovulation, when a single secondary oocyte is formed.

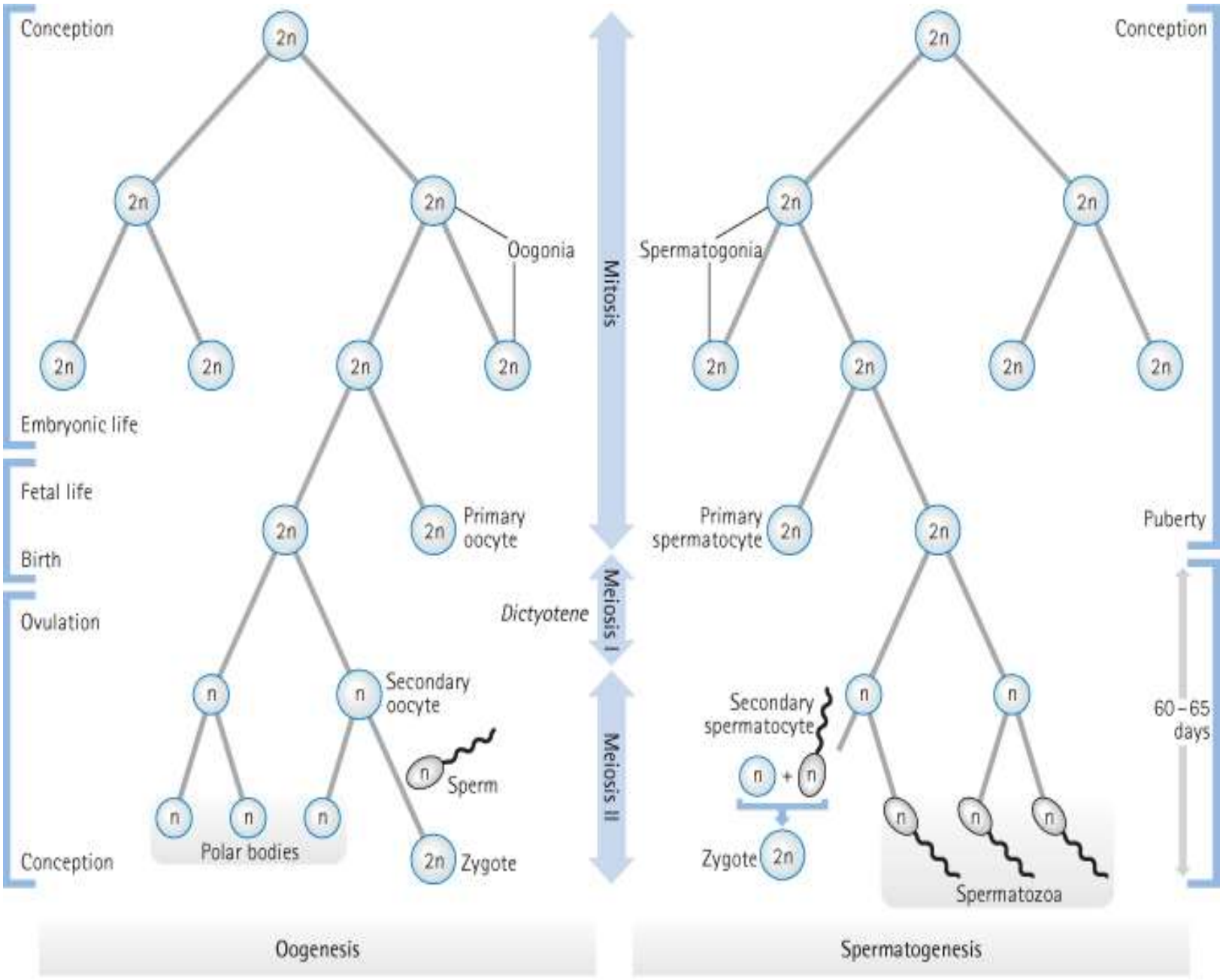
This receives most of the cytoplasm.
The other daughter cell from the first meiotic division consists largely of a nucleus and is known as a polar body.

Meiosis II then commences and results in the formation of a further polar body .

SPERMATOGENESIS

In contrast, spermatogenesis is a relatively rapid process with an average duration of 60-65 days.
At puberty spermatogonia, begin to mature into primary spermatocytes which enter meiosis I and emerge as haploid secondary spermatocytes.

These then undergo the second meiotic division to form spermatids, which in turn develop into mature spermatozoa.



CHROMOSOME ABNORMALITIES

GENITIC 10

Dr.SAWSAN S. SHURRAB

- These can be divided into numerical and structural, with a third category consisting of different chromosome constitutions in two or more cell lines .

Type of disorder	Example		Outcome
Numerical			
Polyploid	Triploidy	69 chromosomes	Lethal
Aneuploid	Trisomy of chromosome 21		Down syndrome
	Monosomy of X chromosome		Turner syndrome
	47 chromosomes (XXY)		Klinefelter syndrome
Structural			
Deletion	Terminal deletion 5p		Cri du chat syndrome
	Interstitial deletion 11p		Associated with Wilms tumour
Inversion	Pericentric inversion 9		Normal phenotype
Duplication	Isochromosome X (fusion of long arms with loss of short arms)	q q	Infertility in females
Ring chromosome	Ring chromosome 18		Mental retardation syndrome
Fragile site	Fragile X		Mental retardation syndrome
Translocation	Reciprocal		Balanced translocations cause no abnormality. Unbalanced translocations cause spontaneous abortions or syndromes of multiple physical and mental handicaps
	Robertsonian	14 13	

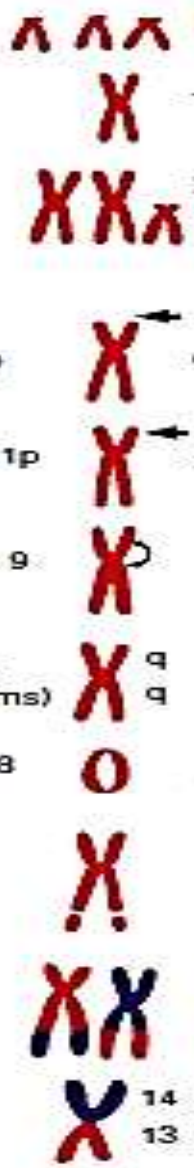


Figure 4.7 Types of chromosomal abnormality

NUMERICAL ABNORMALITIES

Numerical abnormalities involve the loss or gain of one or more chromosomes, which is referred to as *aneuploidy*, or the addition of one or more complete haploid complements, which is known as *polyploidy*.

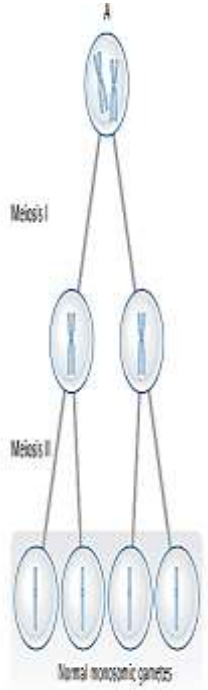
Loss of a single chromosome results in *monosomy*.
Gain of one or two homologous chromosomes is referred to as *trisomy* and *tetrasomy*, respectively.

Trisomy

The presence of an extra chromosome is referred to as trisomy.
Most cases of Down syndrome are due to the presence of an additional number 21 chromosome, hence Down syndrome is often known as trisomy 21.
Other autosomal trisomies that are compatible with survival to term are Patau syndrome (trisomy 13) and Edwards syndrome (trisomy 18) .

Most other autosomal trisomies result in early pregnancy loss, with trisomy 16 being a particularly common finding in first-trimester spontaneous miscarriages.

The presence of an additional sex chromosome (X or Y) has only mild phenotypic effects .



- Trisomy 21 is usually caused by failure of separation of one of the pairs of homologous chromosomes during anaphase of maternal meiosis I.

- This failure of the bivalent to separate is called *non-disjunction*.

- Less often, trisomy can be caused by non-disjunction occurring during meiosis II when a pair of sister chromatids fail to separate.

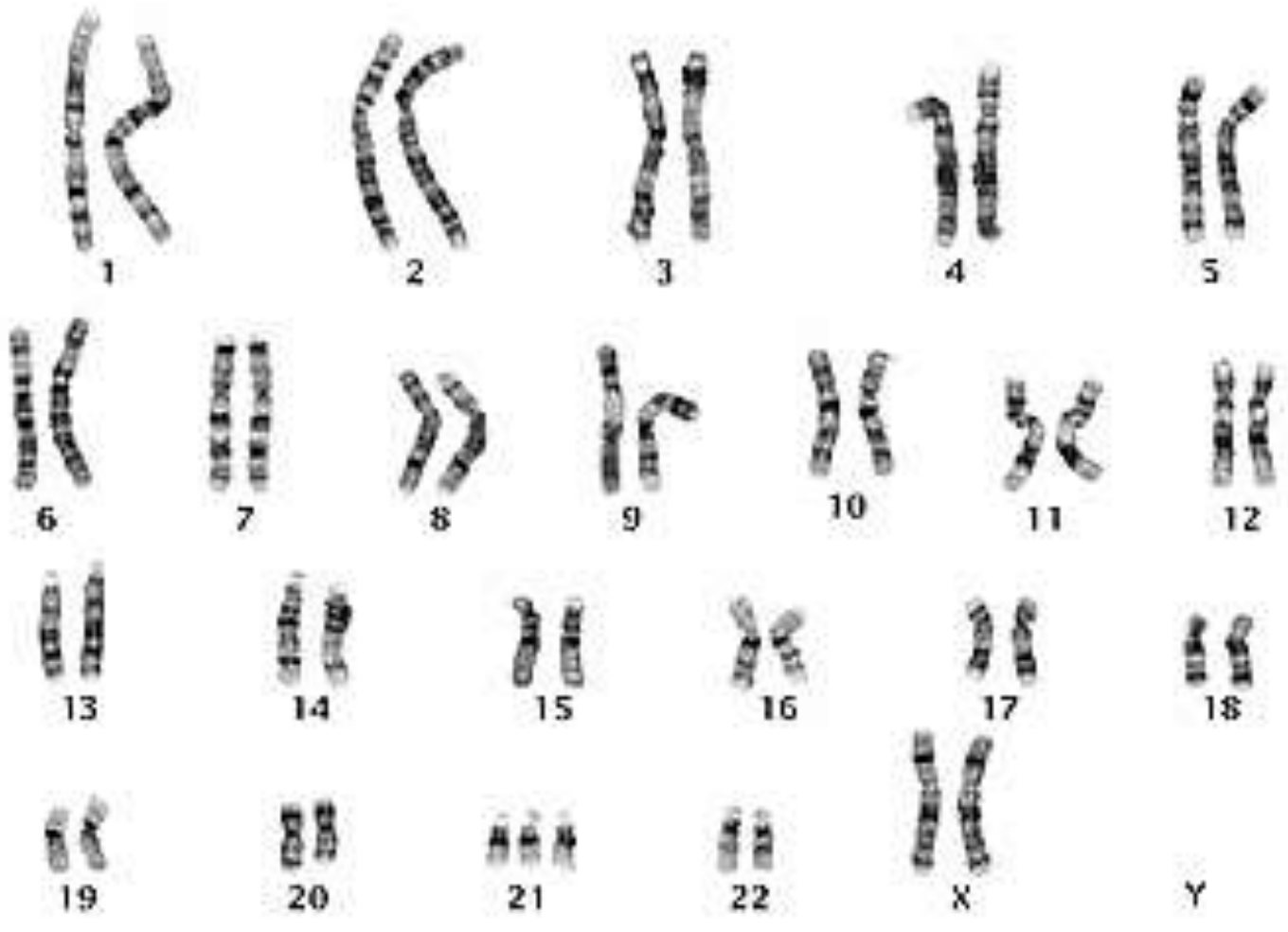
- Either way the gamete receives two homologous chromosomes (*disomy*), and if subsequent fertilization occurs a trisomic conceptus results.

The cause of non-disjunction

The cause of non-disjunction is uncertain.
The most favored explanation is that of an aging effect on the primary oocyte, which can remain in a state of suspended inactivity for up to 50 years .

This is based on the well-documented association between advancing maternal age and increased incidence of Down syndrome in offspring .

A maternal age effect has also been noted for trisomies 13 and 18.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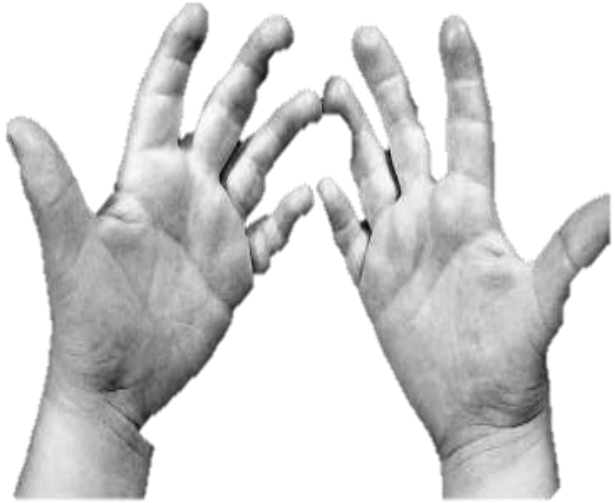
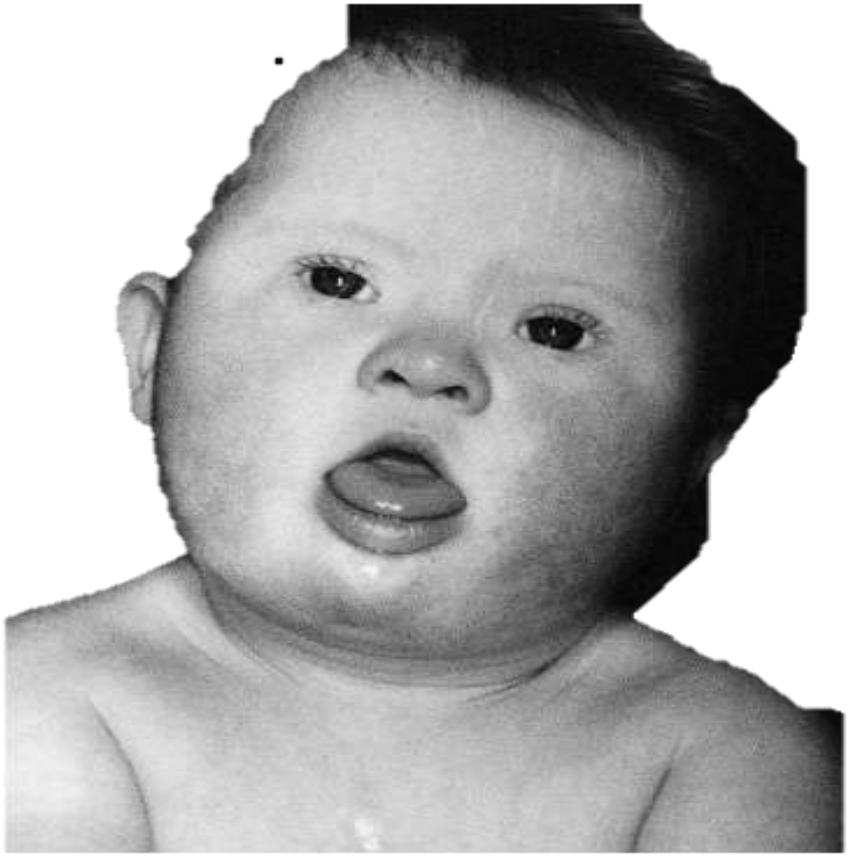
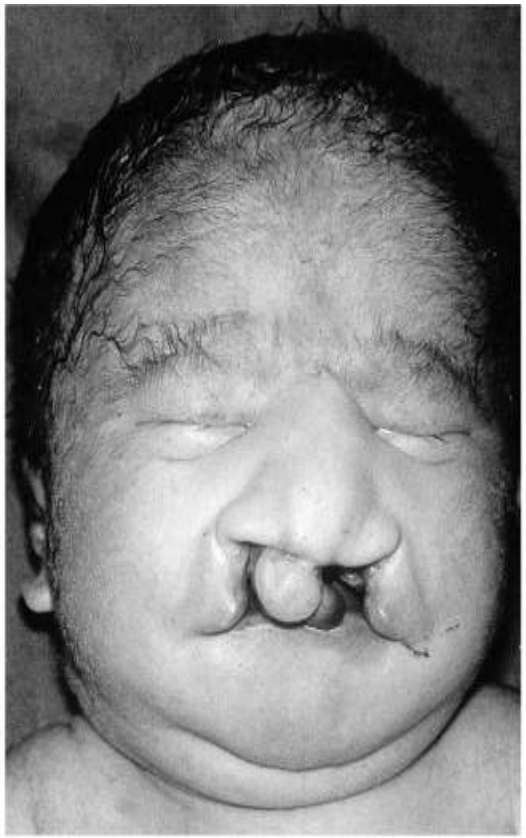




Trisomy 21
Major Clinical Features

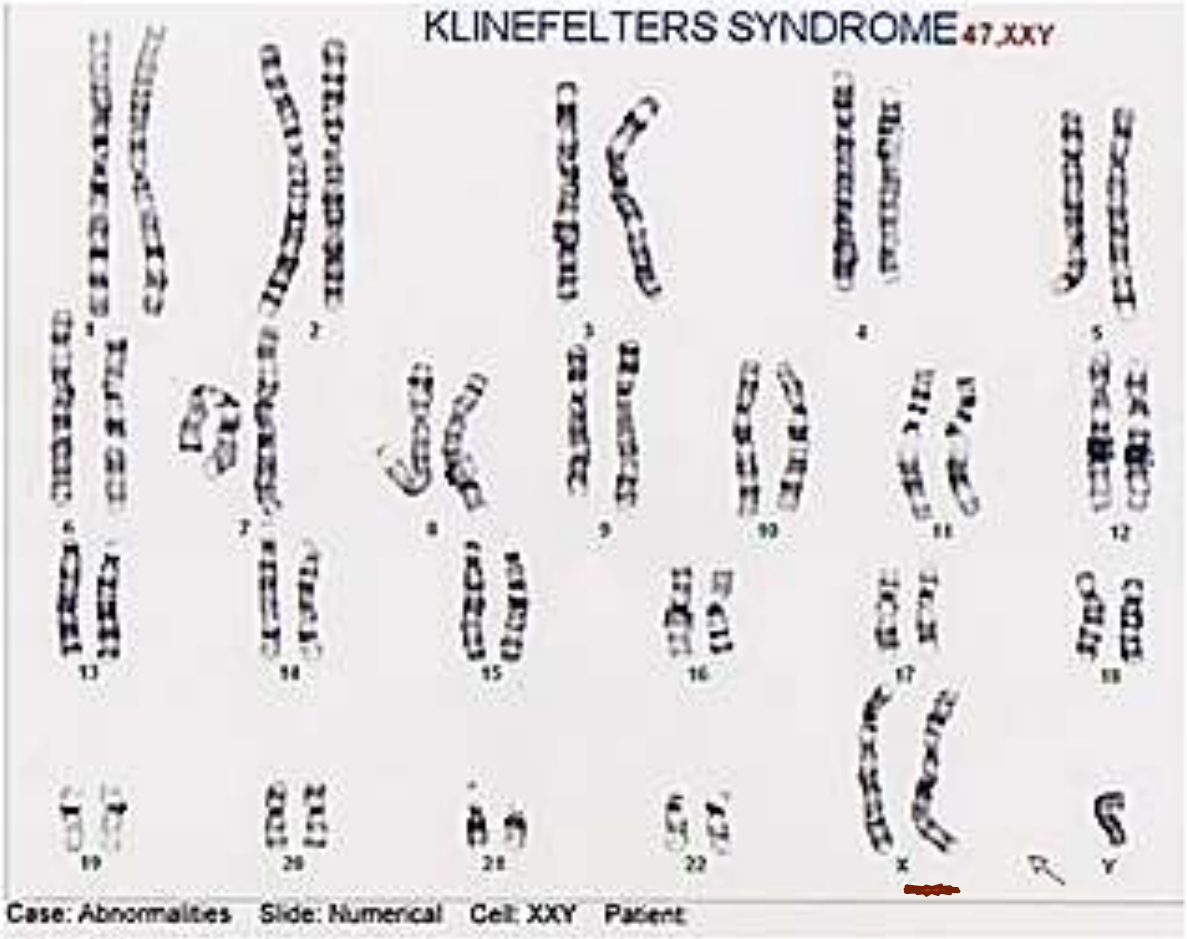
- mental retardation
- slanted palpebral fissures
- epicanthal folds
- small, round, flat face
- small mouth, protruding tongue
- congenital heart problems
- Brushfield spots (iris)
- small, hypoplastic ears
- simian creases
- hypotonia, lax joints, hyperextensive



Trisomy 18

Trisomy 13

Klinefelter's Syndrome 47,XXY



Klinefelter's Syndrome Major Clinical Features

- small testes
- aspermia (little to no sperm production)
- gynecomastia
- long limbs
- large hands & feet
- retardation in some
- fertility in some
- social limitations in some

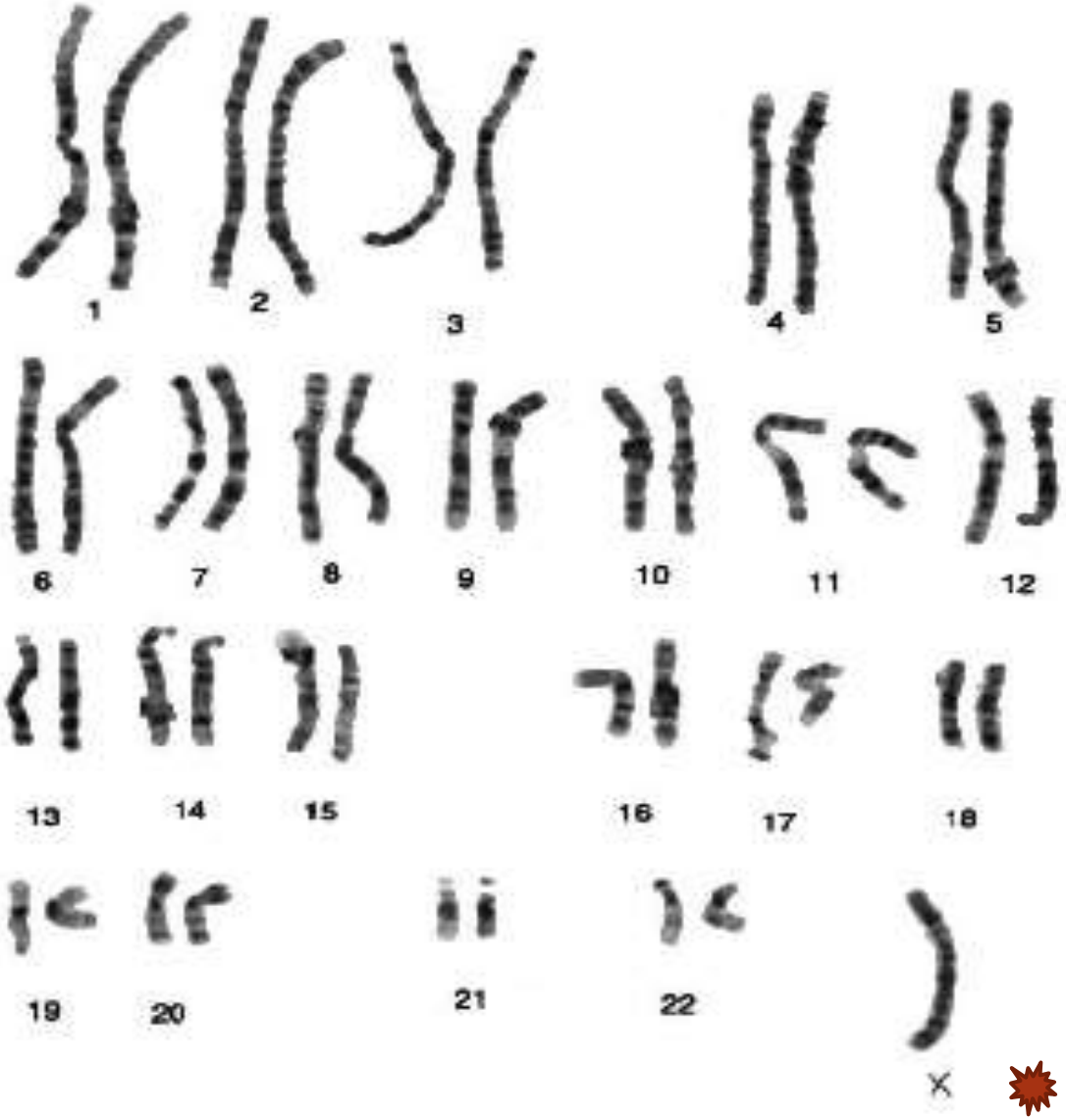
Monosomy

Monosomy for an autosome is almost always incompatible with survival to term.
Lack of contribution of an X or a Y chromosome results in a 45,X karyotype which causes the condition known as Turner syndrome .

As with trisomy, monosomy can result from non-disjunction in meiosis. '

If one gamete receives two copies of a homologous chromosome (*disomy*), the other corresponding daughter gamete will have no copy of the same chromosome (*nullisomy*).

Monosomy can also be caused by loss of a chromosome as it moves to the pole of the cell during anaphase, an event known as anaphase lag.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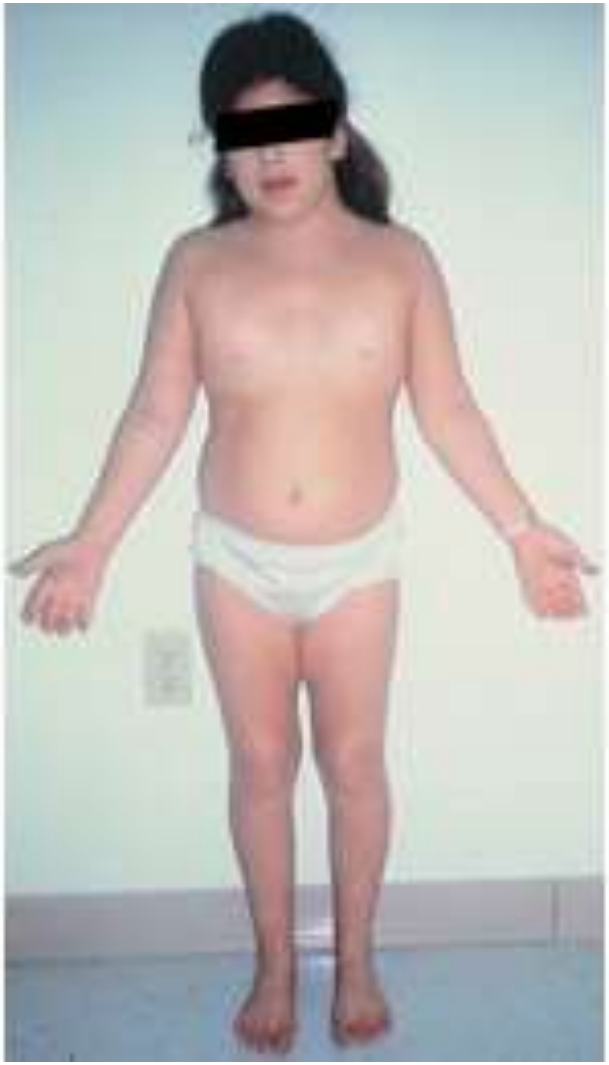
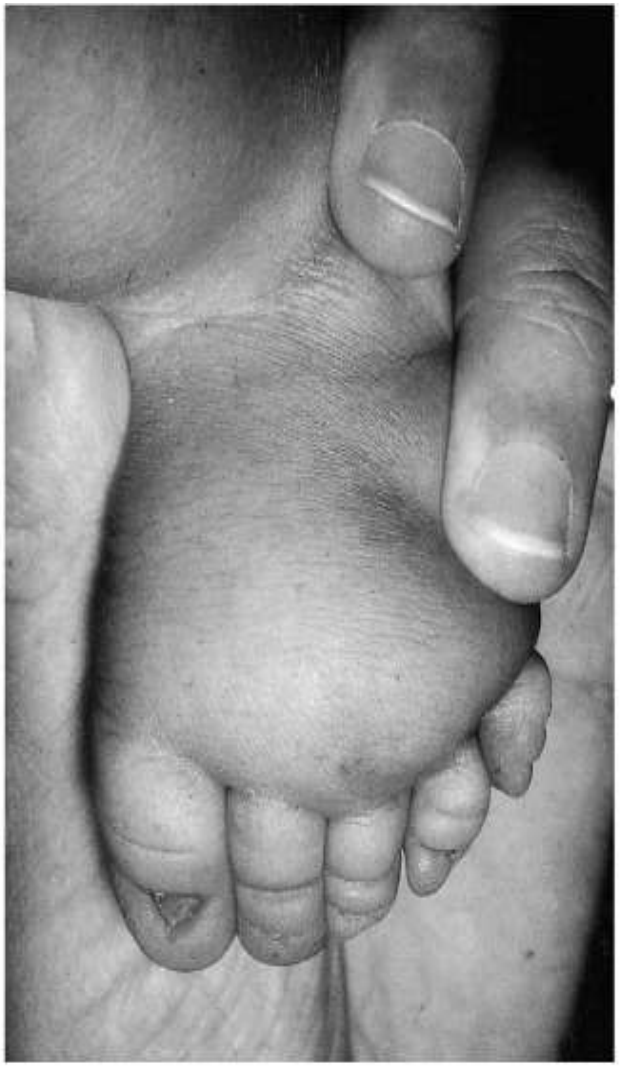




Turner's Syndrome
Major Clinical Features

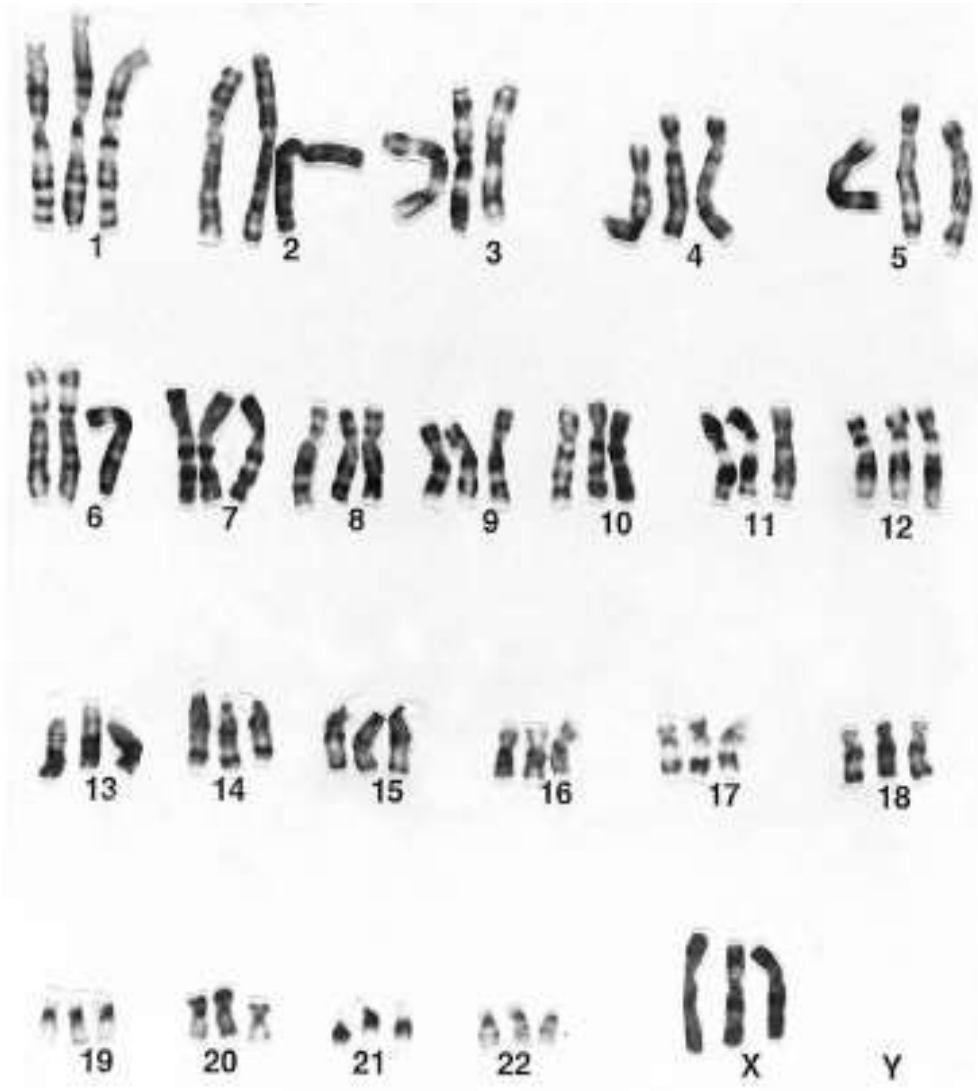
- female phenotype
- short (less than 5 feet)
- primary amenorrhea
- low estrogen levels
- maldevelopment of the ovaries
- sterility
- webbing of the skin of the neck
- wide-spaced nipples
- edema at birth
- cardiovascular problems

Polyploidy

Polyploid cells contain multiples of the haploid number of chromosomes such as 69, *triploidy*, or 92, *tetraploidy*.

In humans triploidy is found relatively often in material grown from spontaneous miscarriages, but survival beyond mid-pregnancy is rare.

Only a few triploid live births have been described and all have died soon after birth.



STRUCTURAL ABNORMAALITIES

Structural chromosome rearrangements result from chromosome breakage with subsequent reunion in a different configuration.
They can be balanced or unbalanced.

In balanced rearrangements the chromosome complement is complete, with no loss or gain of genetic material.
Consequently, balanced rearrangements are generally harmless .
However, carriers of balanced rearrangements are often at risk of producing children with an unbalanced chromosomal complement.

Translocations

A *translocation* refers to the transfer of genetic material from one chromosome to another.
A reciprocal translocation is formed when a break occurs in each of two chromosomes with the segments being exchanged to form two new derivative chromosomes.

A Robertsonian translocation is a particular type of reciprocal translocation in which the break-points are located at or close to the centromeres of two acrocentric chromosomes .

Reciprocal translocations

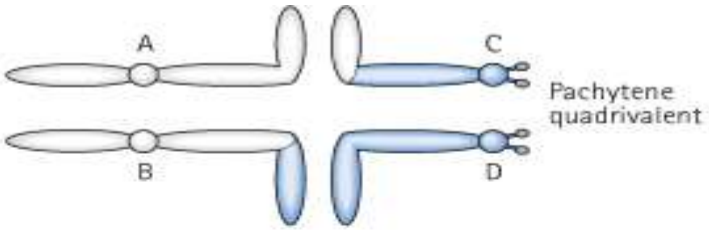
A reciprocal translocation involves breakage of at least two chromosomes with exchange of the fragments.
Usually the chromosome number remains at 46, and if the exchanged fragments are of roughly equal size, a reciprocal translocation can only be identified with detailed chromosomal banding studies or FISH .

Segregation at meiosis

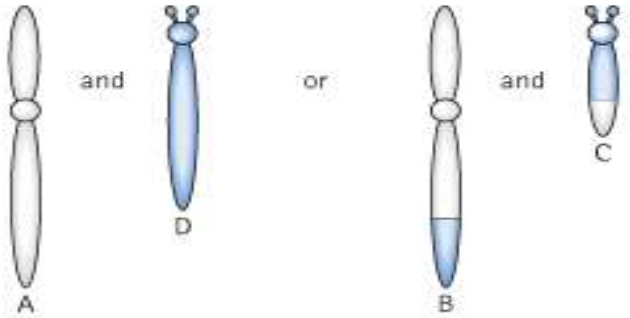
The importance of balanced reciprocal translocations lies in their behavior at meiosis, when they can segregate to generate significant chromosome imbalance.

This can lead to early pregnancy loss or to the birth of an infant with multiple abnormalities.

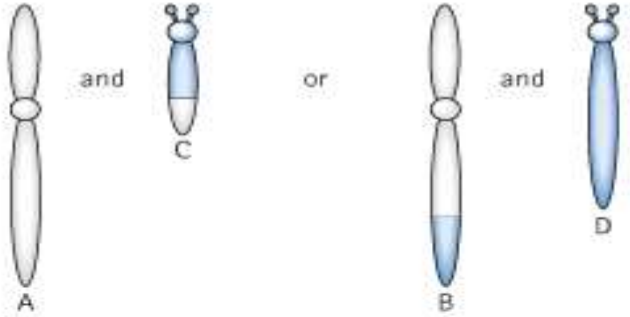
Problems arise at meiosis because the chromosomes involved in the translocation cannot pair normally to form bivalents.



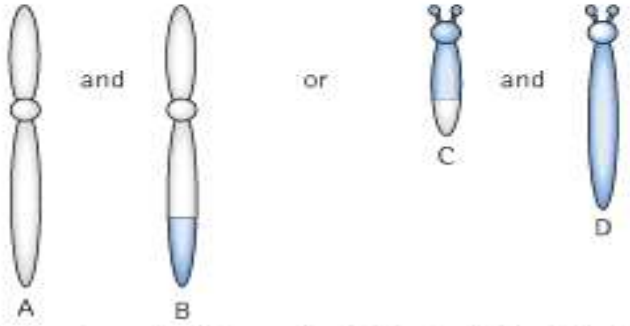
1 Alternate segregation yields normal or balanced haploid complement



2 Adjacent–1 segregation yields unbalanced haploid complement



3 Adjacent–2 segregation yields unbalanced haploid complement



Robertsonian translocations

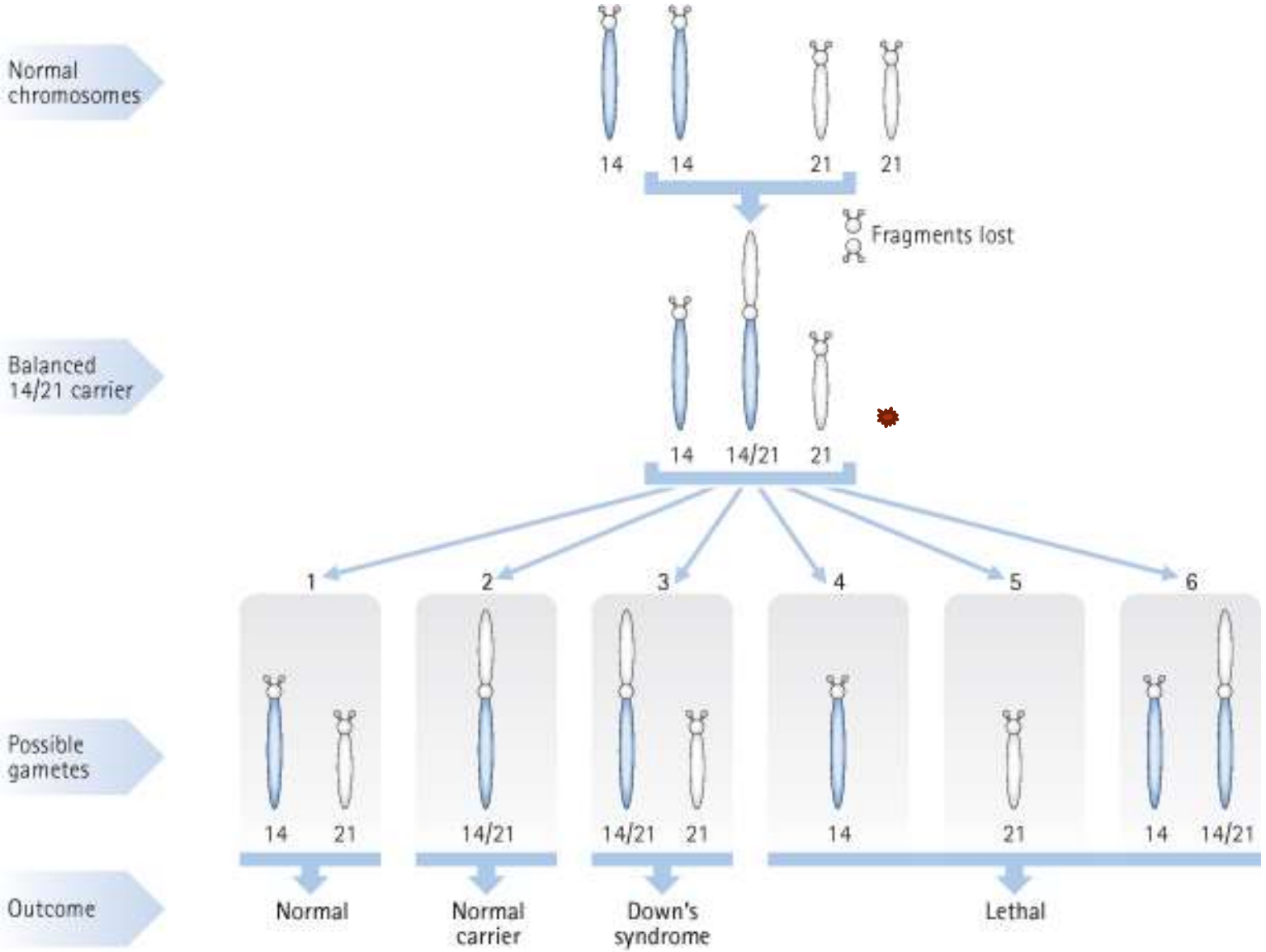
A Robertsonian translocation results from breakage of two acrocentric chromosomes (numbers 13, 14, 15, 21 and 22) at or close to their centromeres, with subsequent fusion of their long arms
This is also referred to as *centric fusion*.

The short arms of each chromosome are lost, this being of no clinical importance as they contain only genes for ribosomal RNA, for which there are multiple copies on the various other acrocentric chromosomes. The total chromosome number is reduced to 45.

Since there is no loss or gain of important genetic material this is a functionally balanced rearrangement.

Segregation at meiosis

As with reciprocal translocations, the importance of Robertsonian translocations lies in their behavior at meiosis.
For example, a carrier of a 14q21q translocation can produce gametes with :



1. A normal chromosome complement, i.e. a normal 14 and a normal 21.

2. A balanced chromosome complement, i.e. a 14q21q translocation chromosome.

3. An unbalanced chromosome complement possessing both the translocation chromosome and a normal 21. This will result in the fertilized embryo having Down syndrome.

4. An unbalanced chromosome complement with a normal 14 and a missing 21.

5. An unbalanced chromosome complement with a normal 21 and a missing 14.

6. An unbalanced chromosome complement with the translocation chromosome and a normal 14 chromosome.

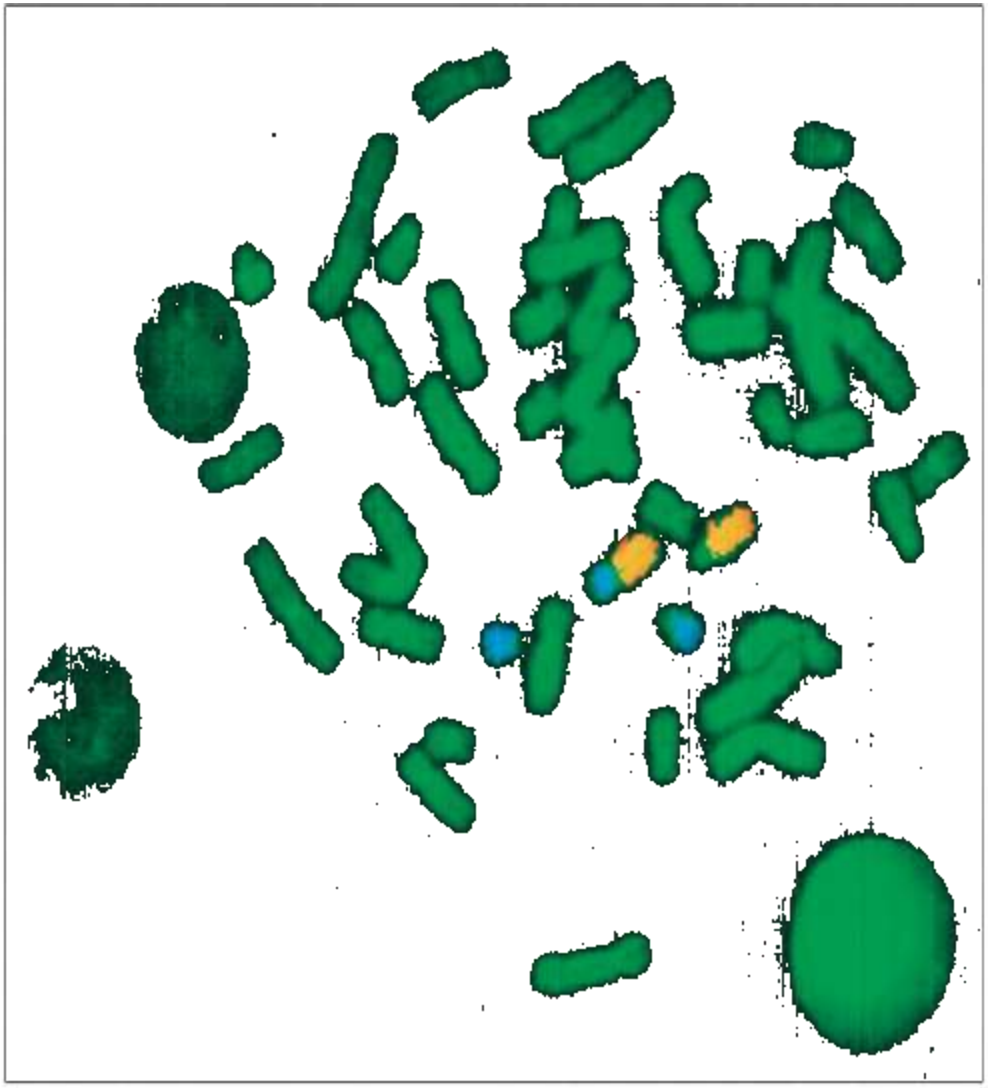
The last three combinations will result in zygotes with monosomy 21, monosomy 14 and trisomy 14, respectively. All of these combinations are incompatible with survival beyond early pregnancy.

Risks in Robertsonian translocations

- Studies have shown that the female carrier of either a 13q21q or a 14q21q Robertsonian translocation runs a risk of approximately 10% for having a baby with Down syndrome, whereas for male carriers the risk is 1-3%.

- For the unfortunate carrier of a 21q21q Robertsonian translocation. All gametes will be either nullisomic or disomic for chromosome 21.

- Consequently all pregnancies will end either in spontaneous miscarriage or in the birth of a child with Down syndrome.



Deletions

A *deletion* involves loss of part of a chromosome and results in monosomy for that segment of the chromosome. Usually a very large deletion will be incompatible with survival to term, and as a general rule any deletion resulting in loss of more than 2% of the total haploid genome will have a lethal outcome.

Deletions are now recognized as existing at two levels. A 'large' chromosomal deletion can be visualized under the microscope.

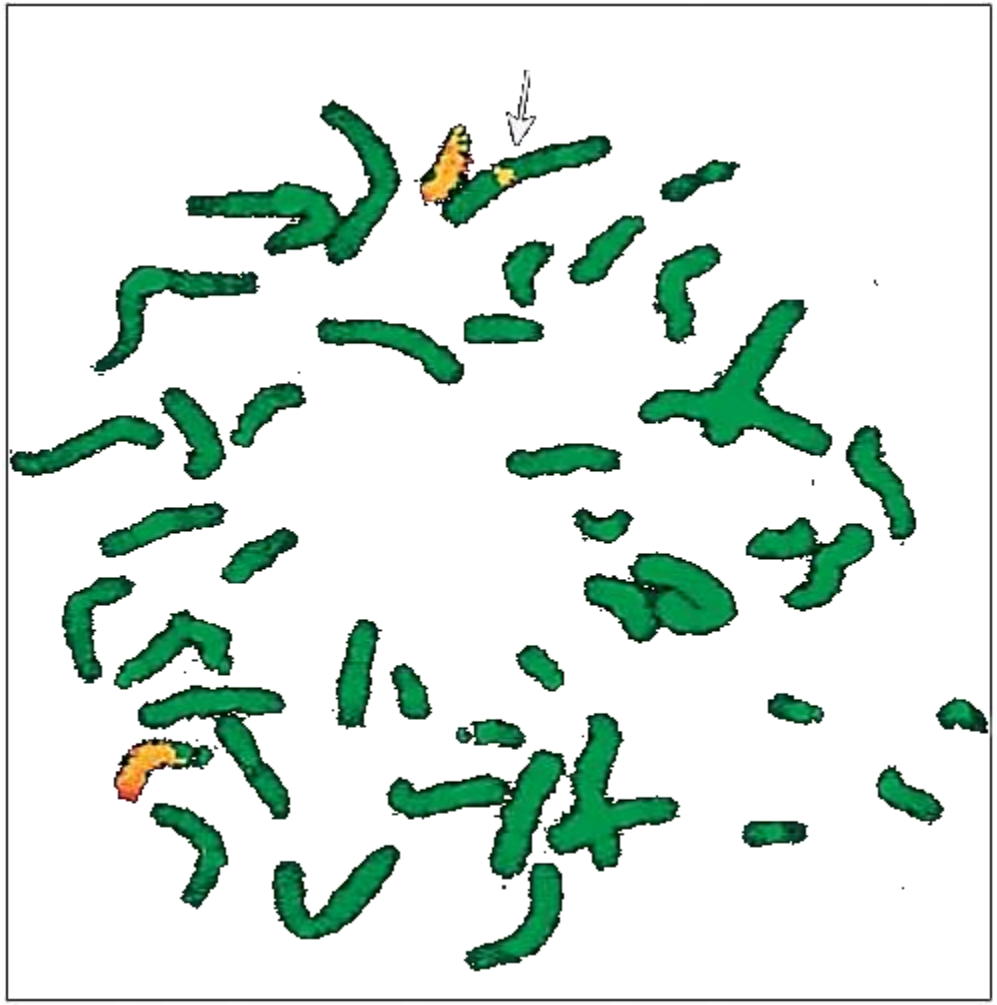
More recently submicroscopic microdeletions have been identified .

Insertions

An *insertion* occurs when a segment of one chromosome becomes inserted into another chromosome .
If the inserted material has moved from elsewhere in another chromosome then the karyotype is balanced.

Otherwise an insertion causes an unbalanced chromosome complement.

Carriers of a balanced deletion-insertion rearrangement are at a 50% risk of producing unbalanced gametes, as random chromosome segregation at meiosis will result in 50% of the gametes inheriting either the deletion or the insertion but not both.



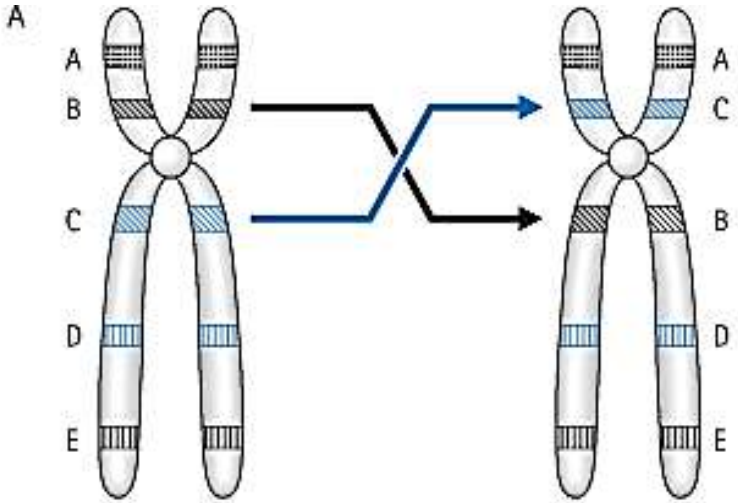
Chromosome painting using chromosome 13 paint showing material of chromosome 13 origin inserted into chromosome 5 (arrowed).

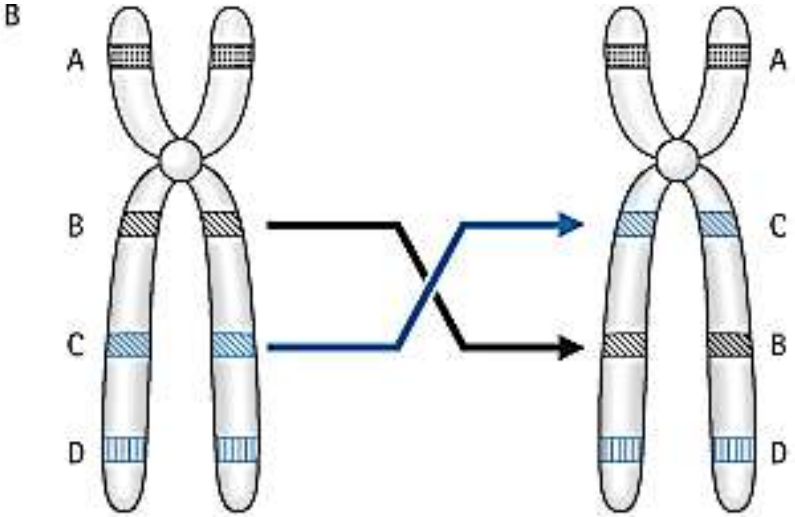
Inversions

An *inversion* is a two-break rearrangement involving a single chromosome in which a segment is reversed in position, i.e. inverted.

If the inversion segment involves the centromere it is termed a *pericentric inversion* .

If it involves only one arm of the chromosome it is known as a *paracentric inversion* .





- Inversions are balanced rearrangements that rarely cause problems in carriers unless one of the break-points has disrupted an important gene.

- However, inversions, while not causing any clinical problems in balanced carriers, can lead to significant chromosome imbalance in offspring, with important clinical consequences

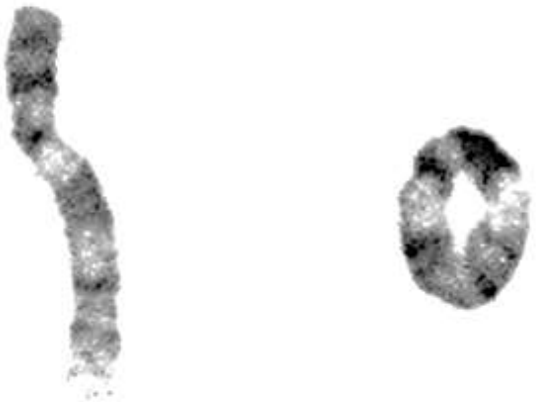
Ring chromosomes

A ring chromosome is formed when a break occurs on each arm of a chromosome leaving two 'sticky' ends on the central portion that reunite as a ring .

The two distal chromosomal fragments are lost so that, if the involved chromosome is an autosome, the effects are usually very serious.

Ring chromosomes are often unstable in mitosis so that it is common to find a ring chromosome in only a proportion of cells.

The other cells in the individual are usually monosomic because of the absence of the ring chromosome.



Isochromosomes

- An isochromosome shows loss of one arm with duplication of the other.

- The most probable explanation for the formation of an isochromosome is that the centromere has divided transversely rather than longitudinally.

- The most commonly encountered isochromosome is that which consists of two long arms of the X chromosome.
- This accounts for approximately 15% of all cases of Turner syndrome .

Table 4.2 Reporting of karyotypes

Total number of chromosomes given first followed by sex chromosome constitution	
46,XX	Normal female
47,XXY	Male with Klinefelter syndrome
47,XXX	Female with triple X syndrome
Additional or lost chromosomes indicated by + or −	
47,XY,+21	Male with trisomy 21 (Down syndrome)
46,XX,12p+	Additional unidentified material on short arm of chromosome 12
All cell lines present are shown for mosaics	
46,XX/47,XX,+21	Down syndrome mosaic
46,XX/47,XXX/45,X	Turner/triple X syndrome mosaic
Structural rearrangements are described, identifying p and q arms and location of abnormality	
46,XY,del11(p13)	Deletion of short arm of chromosome 11 at band 13
46,XX,t(X;7)(p21;q23)	Translocation between chromosomes X and 7 with break points in respective chromosomes

PATTERNS OF INHERITANCE 1

GENITIC 11

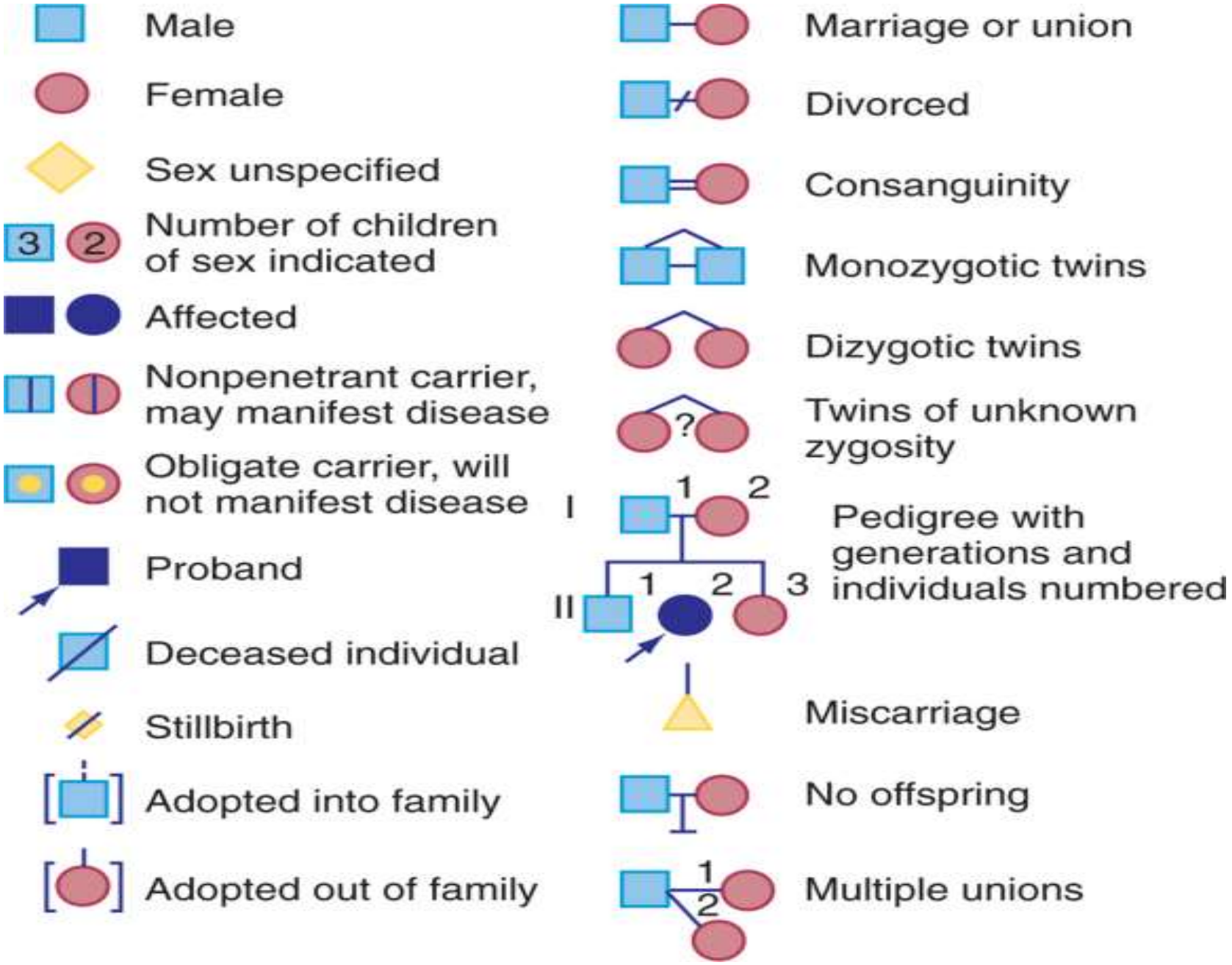
FAMILY STUDIES

If we wish to investigate whether a particular trait or disorder in humans is genetic and hereditary, we usually have to rely either on observation of the way in which it is transmitted from one generation to another, or on study of its frequency among relatives.

An important reason for studying the pattern of inheritance of disorders within families is to enable advice to be given to members of a family regarding the likelihood of their developing it or passing it on to their children, i.e. *genetic counseling* .

PEDIGREE DRAWING AND TERMINOLOGY

- **A family tree is a shorthand system of recording the pertinent information about a family.**
- **It usually begins with the person through whom the family came to the attention of the investigator.**
- **This person is referred to as the index case, proband or propositus, or if female, the proposita.**
- **The position of the proband in the family tree is indicated by an arrow.**


Male
Female
Sex unspecified
3
2
Number of children of sex indicated
Affected
Nonpenetrant carrier, may manifest disease
Obligate carrier, will not manifest disease
Proband
Deceased individual
Stillbirth
Adopted into family
Adopted out of family
Marriage or union
Divorced
Consanguinity
Monozygotic twins
Dizygotic twins
?
Twins of unknown zygosity
I
1
2
II
1
2
3
Pedigree with generations and individuals numbered
Miscarriage
No offspring
1
2
Multiple unions

MENDELIAN INHERITANCE

Over 11000 traits or disorders in humans exhibit single gene unifactorial or Mendelian inheritance.

However, characteristics such as height, and many common familial disorders, such as diabetes, hypertension, etc., do not usually follow a simple pattern of Mendelian inheritance .

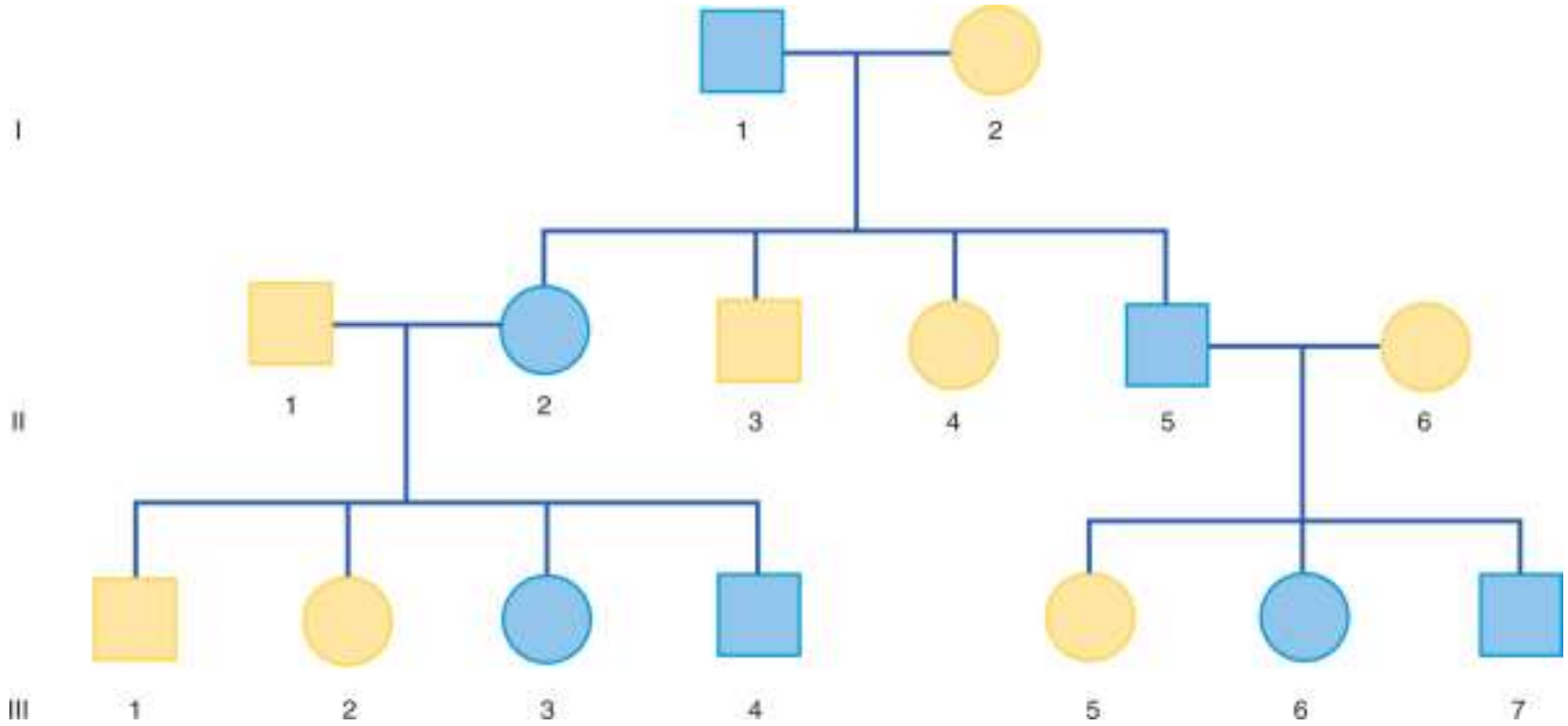
A trait or disorder that is determined by a gene on an autosome is said to show autosomal inheritance, whereas a trait or disorder determined by a gene on one of the sex chromosomes is said to show sex-linked inheritance.

AUTOSOMAL DOMINANT INHERITANCE

An autosomal dominant trait is one which manifests in the heterozygous state, i.e. in a person possessing both an abnormal or mutant allele and the normal allele.

It is often possible to trace a dominantly inherited trait or disorder through many generations of a family .

This pattern of inheritance is sometimes referred to as 'vertical' transmission and is confirmed when male-male (i.e. father to son) transmission is observed.



Autosomal dominant pedigree. Pedigree showing typical inheritance of a form of sensorineural deafness (DFNA8) inherited as an autosomal dominant trait.

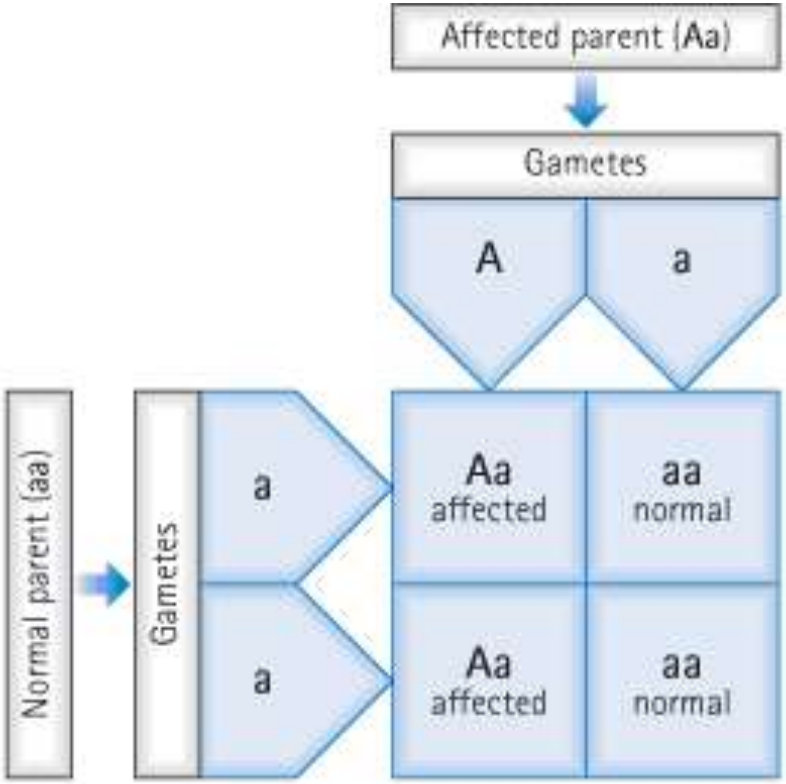
Blue, affected patients

Genetic risks

Each gamete from an individual with a dominant trait or disorder will contain either the normal allele or the mutant allele.

If we represent the dominant mutant allele as 'A' and the recessive normal allele as 'a', then the various possible combinations of the gametes can be represented in a Punnett's square .

Any child born to a person affected with a dominant trait or disorder has a 1 in 2 (50%) chance of inheriting it and being similarly affected.



Pleiotropy

Autosomal dominant traits may involve only one organ or part of the body, for example the eye in congenital cataracts.

It is common, however, for autosomal dominant disorders to manifest in different systems of the body in a variety of ways.

Pleiotropy : a single gene that may give rise to two or more apparently unrelated effects.

Variable expressivity

The clinical features in autosomal dominant disorders can show striking variation from person to person, even in the same family.
This difference between individuals is referred to as *variable expressivity*.

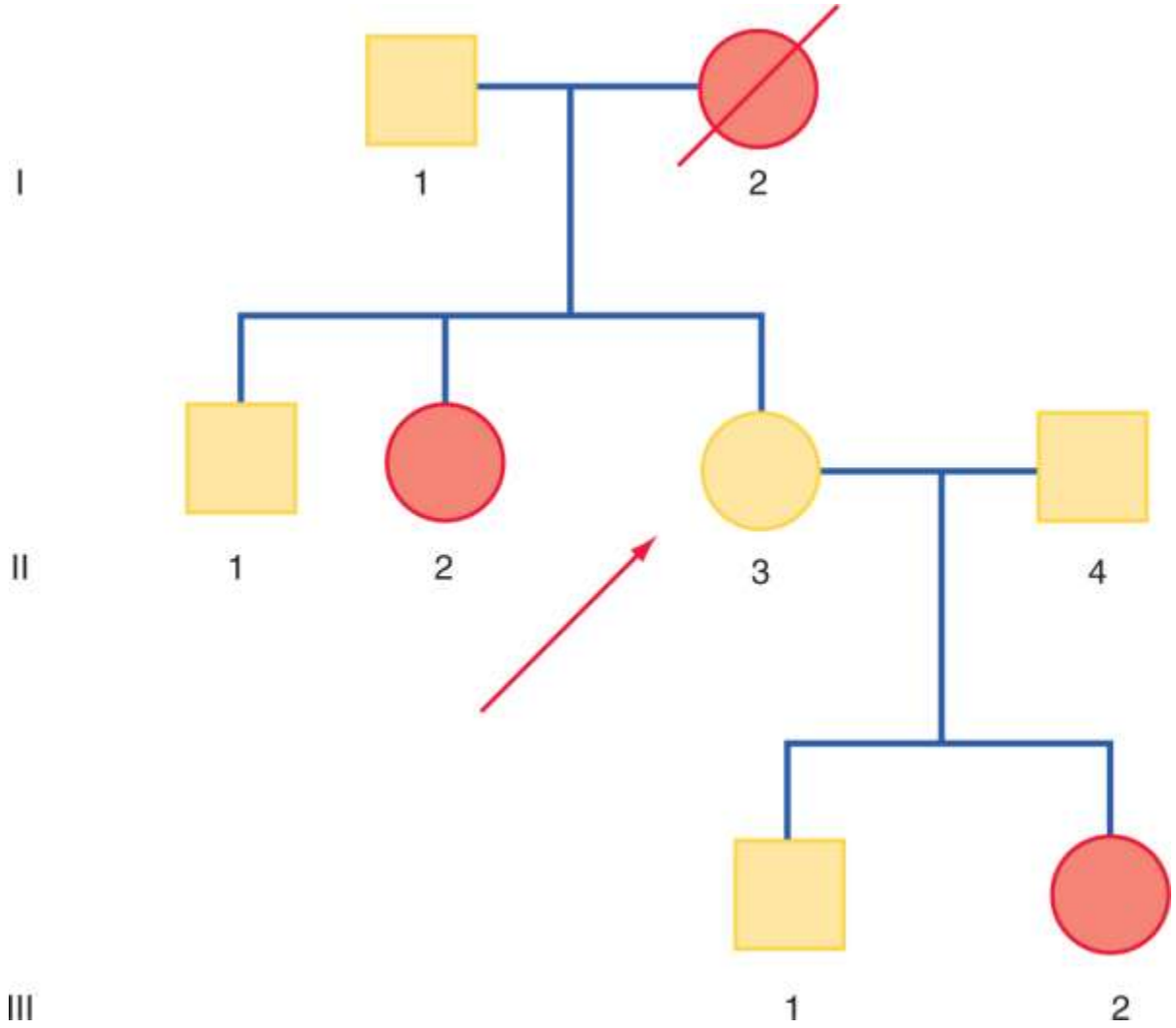
In autosomal dominant polycystic kidney disease, for example, some affected individuals develop renal failure in early adulthood whilst others have just a few renal cysts that do not significantly affect renal function.

Reduced penetrance

In some individuals heterozygous for gene mutations giving rise to certain autosomal dominant disorders there may be no abnormal clinical features, representing so-called reduced penetrance or what is commonly referred to in lay terms as 'skipping a generation'.

Reduced penetrance is thought to be the result of the modifying effects of other genes, as well as being due to interaction of the gene with environmental factors.

An individual who has no features of a disorder despite being heterozygous for a particular gene mutation is said to represent non-penetrance.



Individual II.3 is an obligate carrier, but there are no findings to suggest the disorder. She is termed nonpenetrant.

New mutations

In autosomal dominant disorders an affected person will usually have an affected parent. However, this is not always the case and it is not unusual for a trait to appear in an individual when there is no family history of the disorder.

A striking example is achondroplasia, a form of short-limbed dwarfism , in which the parents usually have normal stature.

The sudden unexpected appearance of a condition arising as a result of a mistake occurring in the transmission of a gene is called a new mutation.

New dominant mutations, in certain instances, have been associated with an increased age of the father.
It is believed that this is because of the large number of mitotic divisions that male gamete stem cells undergo during a man's reproductive lifetime

Codominance

Codominance is the term used for two allelic traits that are both expressed in the heterozygous state.

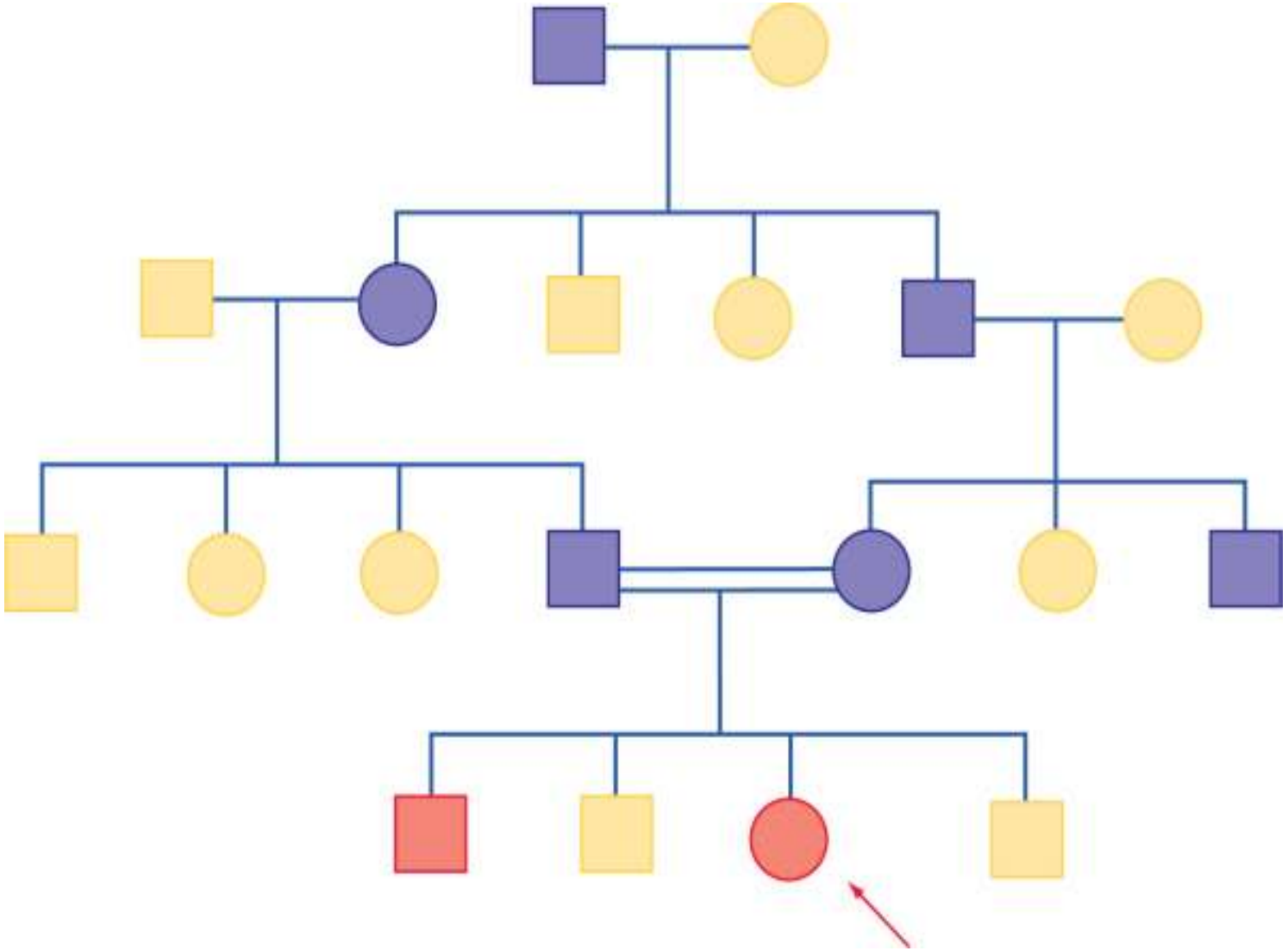
In persons with blood group AB it is possible to demonstrate both A and B blood group substances on the red blood cells, so that the A and B blood groups are therefore codominant.

AUTOSOMAL RECESSIVE INHERITANCE

Recessive traits and disorders are only manifest when the mutant allele is present in a double dose, i.e. homozygosity.

Individuals heterozygous for such mutant alleles show no features of the disorder and are perfectly healthy, i.e. they are carriers.

All the affected individuals in a family are usually in a single sibship, i.e. brothers and sisters. This is sometimes referred to as 'horizontal' transmission .



Autosomal recessive pedigree with parental consanguinity. Purple, carriers; Red, affected patients

CONSANGUINITY

Enquiry into the family history of individuals affected with rare recessive traits or disorders might reveal that their parents are related, i.e. consanguineous.

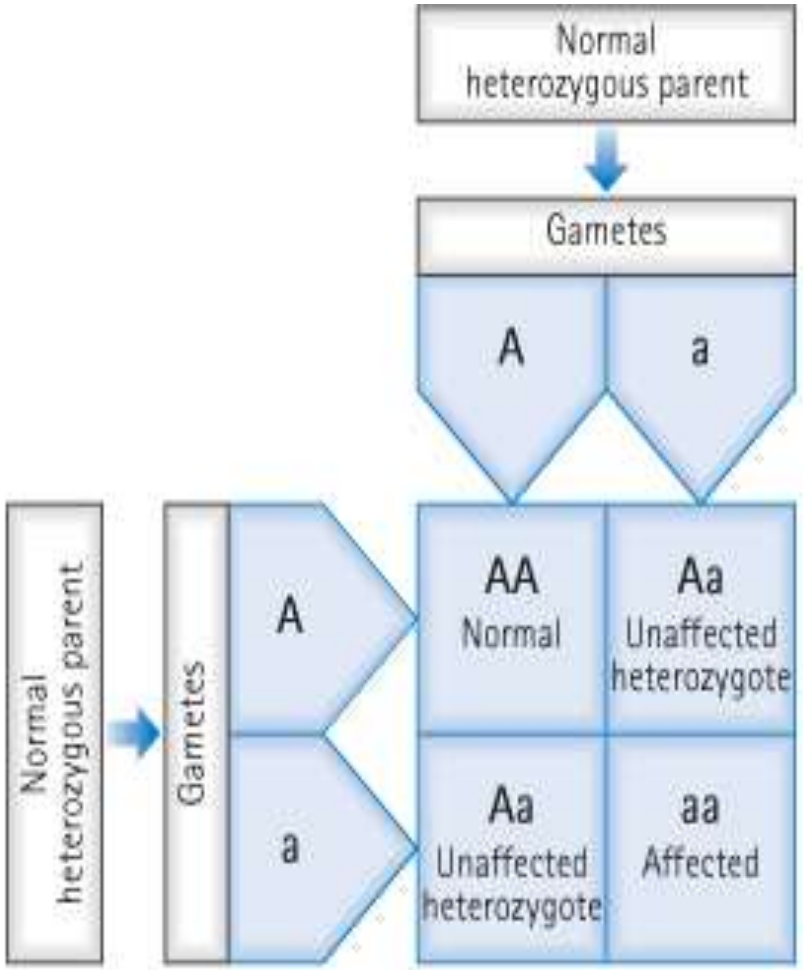
The rarer a recessive trait or disorder, the greater the frequency of consanguinity among the parents of affected individuals.

In cystic fibrosis, the commonest 'serious' autosomal recessive disorder in persons of Western European origin , the frequency of parental consanguinity is only slightly greater than that seen in the general population

Genetic risks

If we represent the normal dominant allele as 'A' and the recessive mutant allele as 'a', then each parental gamete carries either the mutant or the normal allele .

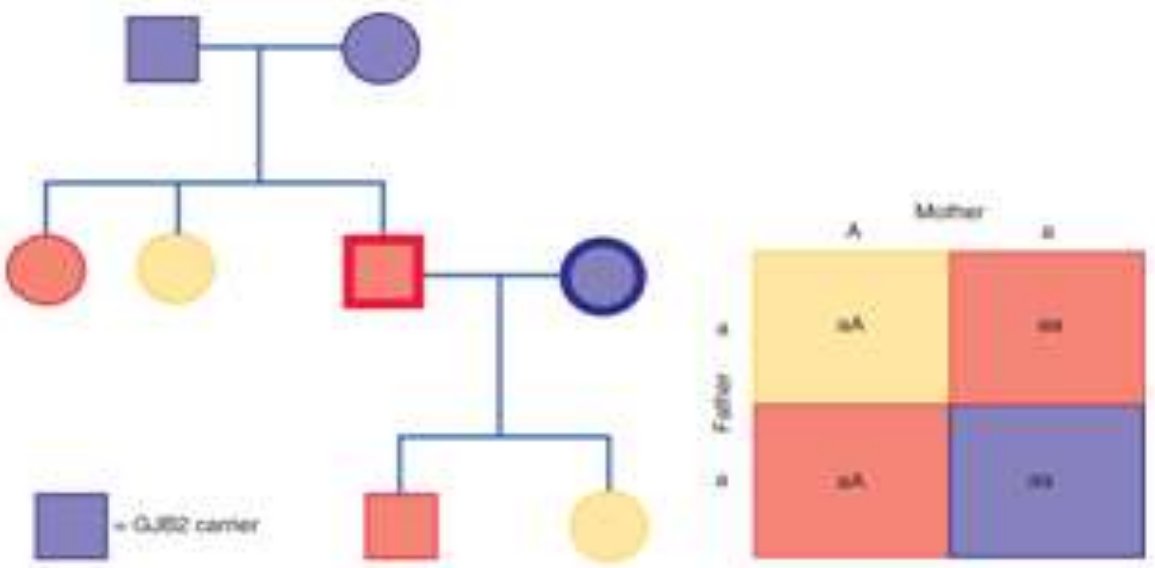
The various possible combinations of gametes mean that the offspring of two heterozygotes have a 1 in 4 (25%) chance of being homozygous affected, a 1 in 2 (50%) chance of being heterozygous unaffected, and a 1 in 4 (25%) chance of being homozygous unaffected .



Pseudodominance

If an individual who is homozygous for an autosomal recessive disorder marries a carrier of the same disorder, their children have a 1 in 2 (50%) chance of being affected.

Such a pedigree is said to exhibit pseudodominance



Pseudodominant inheritance. Red, affected deaf

Locus heterogeneity

It is recognized that sensorineural hearing impairment/deafness most commonly shows autosomal recessive inheritance.

It would be expected that if two deaf persons were homozygous for the same recessive gene, all of their children would be similarly affected.

Families have been described in which all the children born to parents deaf due to autosomal recessive genes have had perfectly normal hearing and are what is known as double heterozygotes.

The explanation for this must be that the parents were homozygous for mutant alleles at different loci.

Mutational heterogeneity

Heterogeneity can also occur at the allelic level. In the majority of single-gene disorders, e.g. β thalassemia, a large number of different mutations have been identified as being responsible

There are individuals who have two different mutations at the same locus and are known as compound heterozygotes, constituting what is known as allelic or mutational heterogeneity.

Most individuals affected with an autosomal recessive disorder are probably, in fact, compound heterozygotes rather than true homozygotes, unless their parents are related, when they are likely to be homozygous for the same mutation by descent, having inherited the same mutation from a common ancestor.

PATTERNS OF INHERITANCE 2

Genitics 12

SEX-LINKED INHERITANCE

- Sex-linked inheritance refers to the pattern of inheritance shown by genes that are located on either of the sex chromosomes.

- Genes carried on the X chromosome are referred to as being X-linked, while genes carried on the Y chromosome are referred to as exhibiting Y-linked or holandric inheritance .

X-linked recessive inheritance

- An X-linked recessive trait is one determined by a gene carried on the X chromosome and usually only manifests in males.

- A male with a mutant allele on his single X chromosome is said to be hemizygous for that allele.

- Diseases inherited in an X-linked manner are transmitted by healthy heterozygous female carriers to affected males, as well as by affected males to their obligate carrier daughters, with a consequent risk to male grandchildren through these daughters.

Genetic risks

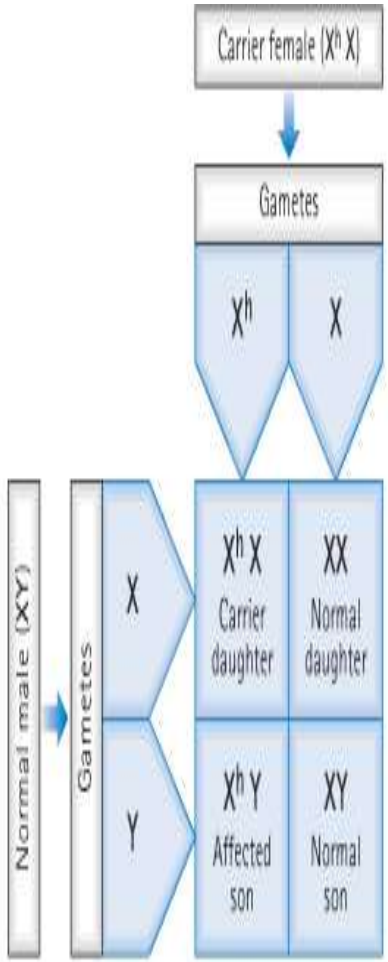
- A male transmits his X chromosome to each of his daughters and his Y chromosome to each of his sons.

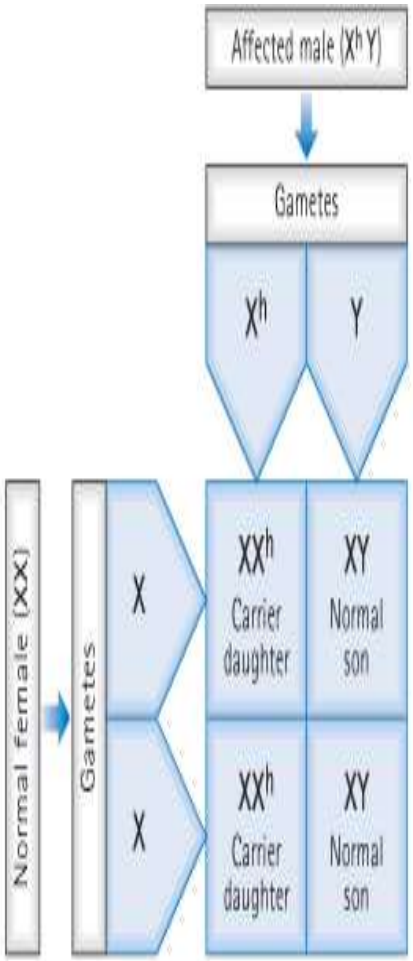
- If a male affected with hemophilia has children with a normal female, then all his daughters will be obligate carriers but none of his sons will be affected

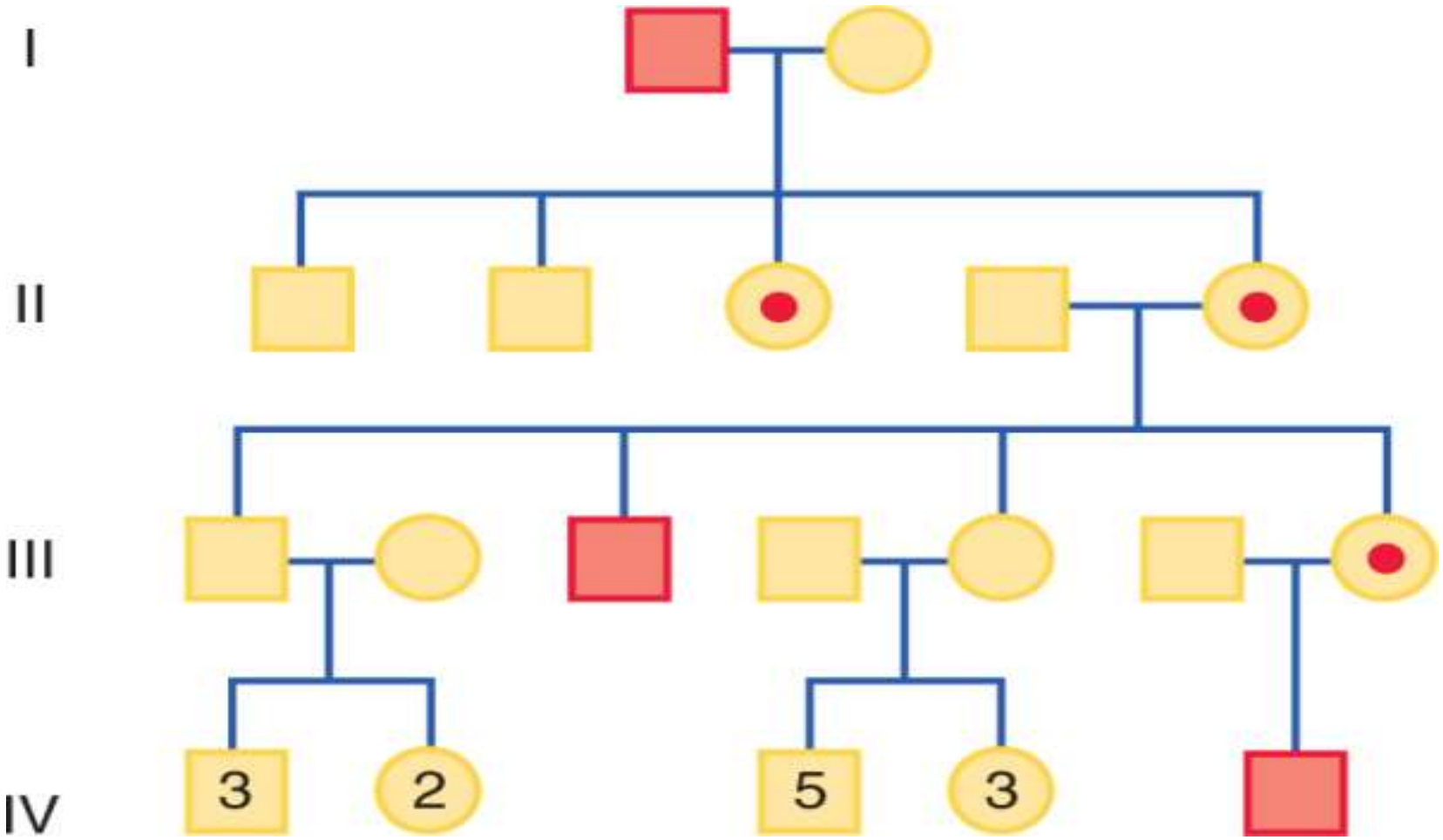
- For a carrier female of an X-linked recessive disorder having children with a normal male, each son has a 1 in 2 (50%) chance of being affected and each daughter has a 1 in 2 (50%) chance of being a carrier

- Some X-linked disorders are not compatible with survival to reproductive age and are not, therefore, transmitted by affected males.

- Duchenne muscular dystrophy is the commonest muscular dystrophy and is a severe disease .







Pedigree demonstrating X-linked recessive inheritance

- In humans, several X-linked disorders are known in which heterozygous females have a mosaic phenotype with a mixture of features of the normal and mutant alleles.

- In X-linked ocular albinism the iris and ocular fundus of affected males lack pigment.

- Careful examination of the ocular fundus in females heterozygous for ocular albinism reveals a mosaic pattern of pigmentation which can be explained through the random process of X-inactivation

- In the pigmented areas the normal gene is on the active X chromosome while in the depigmented areas the mutant allele is on the active X chromosome.

Females affected with X-linked recessive disorders

1. Homozygosity for X-linked recessive disorders

2. Numerical X chromosome abnormalities

A female could manifest an X-linked recessive disorder through being a carrier of an X-linked recessive mutation and having only a single X chromosome, i.e. Turner syndrome .

3. Skewed X-inactivation

The process of X-inactivation usually occurs randomly, there being an equal chance of either of the two X chromosomes in a heterozygous female being inactivated in any one cell.

Following X-inactivation in embryogenesis, therefore, in roughly half the cells one of the X chromosomes is active, whilst in the other half it is the other X which is active.

- Sometimes this process is not random, allowing for the possibility that the active X chromosome in most of the cells of a heterozygous female carrier is the one bearing the mutant allele.

- If this happens, a carrier female would exhibit some of the symptoms and signs of the disease and be a so-called manifesting heterozygote or carrier.

4. X-autosome translocations

Females with a translocation involving one of the X chromosomes and an autosome can be affected with an X-linked recessive disorder.
If the break-point of the translocation disrupts a gene on the X chromosome, then a female can be affected.

X-linked dominant inheritance

- Although uncommon, there are disorders that are manifest in the heterozygous female as well as in the male who has the mutant allele on his single X chromosome. This is known as X-linked dominant inheritance .

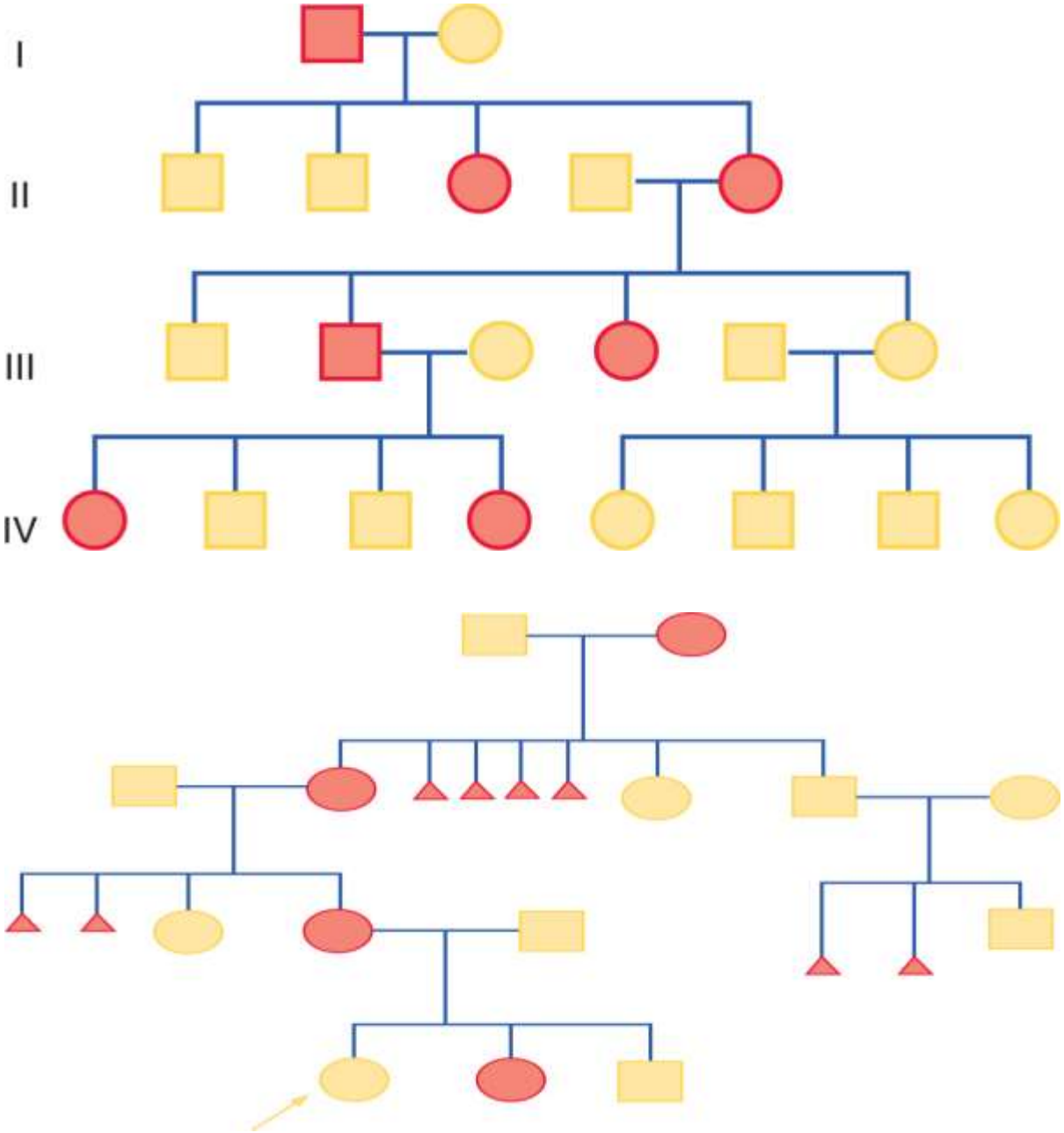
- X-linked dominant inheritance superficially resembles that of an autosomal dominant trait because both the daughters and sons of an affected female have a 1 in 2 (50%) chance of being affected

- There is, however, an important difference.

With an X-linked dominant trait an affected male transmits the trait to all his daughters but to none of his sons.
Therefore, in families with an X-linked dominant disorder there is an excess of affected females and direct male-to-male transmission cannot occur.

- An example of an X-linked dominant trait is vitamin D-resistant rickets.

- Rickets can be due to a dietary deficiency of vitamin D, but in vitamin D-resistant rickets the disorder occurs even when there is an adequate dietary intake of vitamin D.


I
II
III
IV

Y-linked inheritance

- Y-linked or holandric inheritance implies that only males are affected. An affected male transmits Y-linked traits to all his sons but to none of his daughters.

- Evidence clearly indicates, however, that the H-Y histocompatibility antigen and genes involved in spermatogenesis are carried on the Y chromosome and, therefore, manifest holandric inheritance.

Sex influence

- Some autosomal traits are expressed more frequently in one sex than another, so-called sex influence.
- Gout and presenile baldness are examples of sex-influenced autosomal dominant traits, males being predominantly affected in both cases.
- The influence of sex in these two examples is probably through the effect of male hormones.

Sex limitation

- Sex limitation refers to the appearance of certain features in individuals of only one sex.

- Examples include virilization of female infants affected with the autosomal recessive endocrine disorder, congenital adrenal hyperplasia

Features that support the Mendelian patterns of inheritance

Autosomal dominant

- Males and females affected in equal proportions
- Affected individuals in multiple generations
- Transmission by individuals of both sexes, i.e. male to male, female to female, male to female and female to male

Autosomal recessive

- Males and females affected in equal proportions
- Affected individuals usually only in a single generation
- Parents can be related, i.e. consanguineous

X-linked recessive

- Males usually only affected
- Transmitted through unaffected females
- Males cannot transmit the disorder to their sons, i.e. no male-to-male transmission

X-linked dominant

- Males and females affected but often with an excess of females
- Females less severely affected than males
- Affected males can transmit the disorder to their daughters but not sons

Y-linked inheritance

- Affected males only
- Affected males must transmit it to their sons

MULTIPLE ALLELES AND COMPLEX TRAITS

- So far, each of the traits we have considered has involved only two alleles, the normal and the mutant. However, some traits and diseases are neither monogenic nor polygenic.

- Some genes have more than two allelic forms, i.e. multiple alleles.

- Multiple alleles are the result of a normal gene having mutated to produce various different alleles, some of which can be dominant and others recessive to the normal allele. In the case of the ABO blood group system , there are at least four alleles (A1, A2, B and O).

ANTICIPATION

The disease occurs with increasing severity in subsequent generations

- It occurs as a result of the expansion of unstable triplet repeat sequences like CTG triplet repeat occurring predominantly in maternal meiosis, appears to be the explanation for the severe neonatal form of myotonic dystrophy .

- A similar expansion in the CAG expansion in the Huntington disease gene in paternal meiosis appears to account for the increased risk of juvenile Huntington disease when the gene is transmitted by the father.

- Fragile X syndrome and the inherited spinocerebellar ataxia group of conditions are other examples

MOSAICISM

- An individual, or a particular tissue of the body, can consist of more than one cell type or line, through an error occurring during mitosis at any stage after conception.

  It is either somatic or gonadal.

Somatic mosaicism

- The possibility of somatic mosaicism is suggested by the features of a single-gene disorder being less severe in an individual than usual or by being confined to a particular part of the body in a segmental distribution, e.g. as occasionally occurs in neurofibromatosis type I .

Gonadal mosaicism

- There have been many reports of families with autosomal dominant disorders, such as achondroplasia and X-linked recessive disorders, such as Duchenne muscular dystrophy and hemophilia, in which the parents are phenotypically normal and the results of investigations or genetic tests have also all been normal, but in which more than one of their children has been affected.

- The most favored explanation for these observations is gonadal or germ line mosaicism in one of the parents.

UNIPARENTAL DISOMY

- An individual normally inherits one of a pair of homologous chromosomes from each parent

Uniparental disomy has been shown to be the cause of a father with hemophilia having an affected son and of a child with cystic fibrosis being born to a couple in which only the mother was a carrier.

GENOMIC IMPRINTING

- It is now recognized that different clinical features can result, depending on whether a gene is inherited from the father or from the mother.

- This 'parent of origin' effect is referred to as genomic imprinting and methylation of DNA is thought to be the main mechanism by which expression is modified.

- Evidence of genomic imprinting has been observed in two dysmorphic syndromes associated with learning difficulties known as the Prader-Willi and Angelman syndromes.

The Prader-Willi syndrome

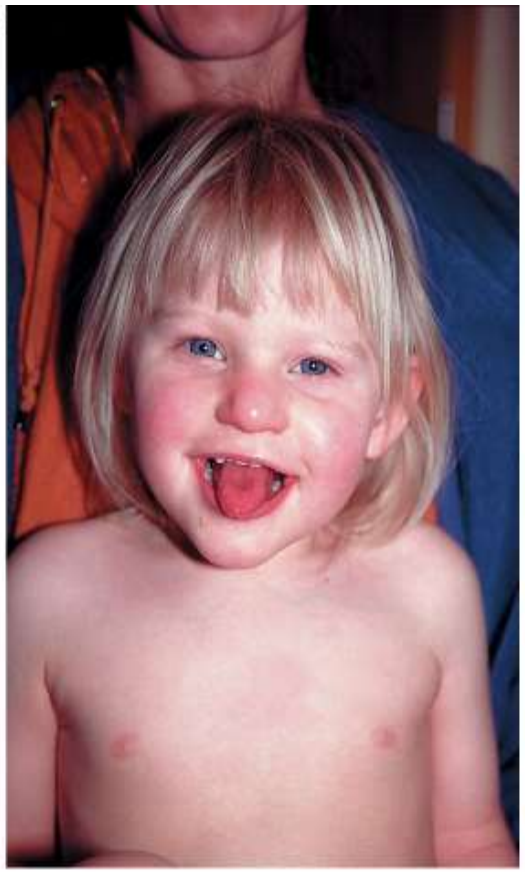
- Prader-Willi syndrome is characterized by short stature, obesity, hypogonadism and learning difficulty.

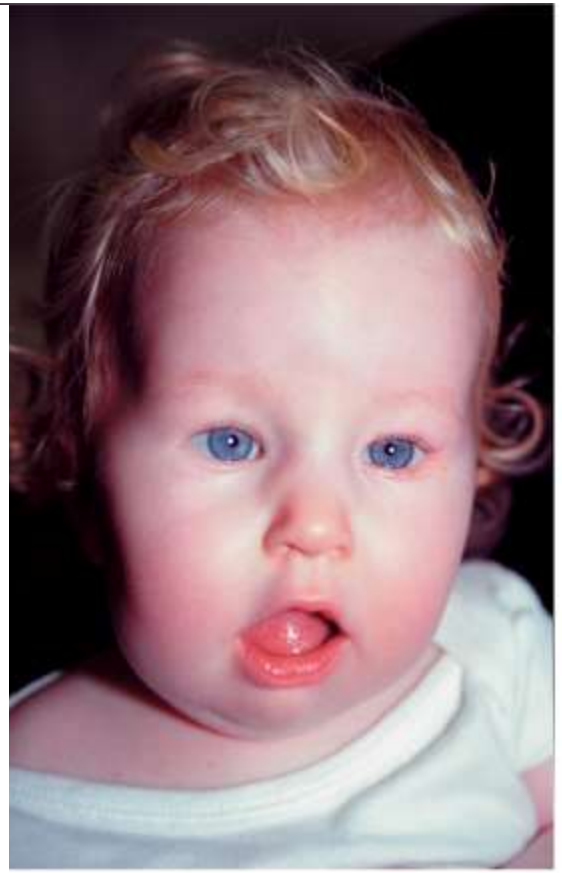
- Individuals with Prader-Willi syndrome can be shown to have an interstitial deletion of the proximal portion of the long arm o chromosome 15 .

- DNA analysis has revealed that the chromosome deleted is almost always the paternally derived homolog or have maternal uniparental disomy.

  This is functionally the equivalent of a deletion in the paternally derived chromosome 15.

A





Ellard: Emery's Elements of Medical Genetics 12E www.studentconsult.c

Angelman syndrome

Prader Willi syndrome

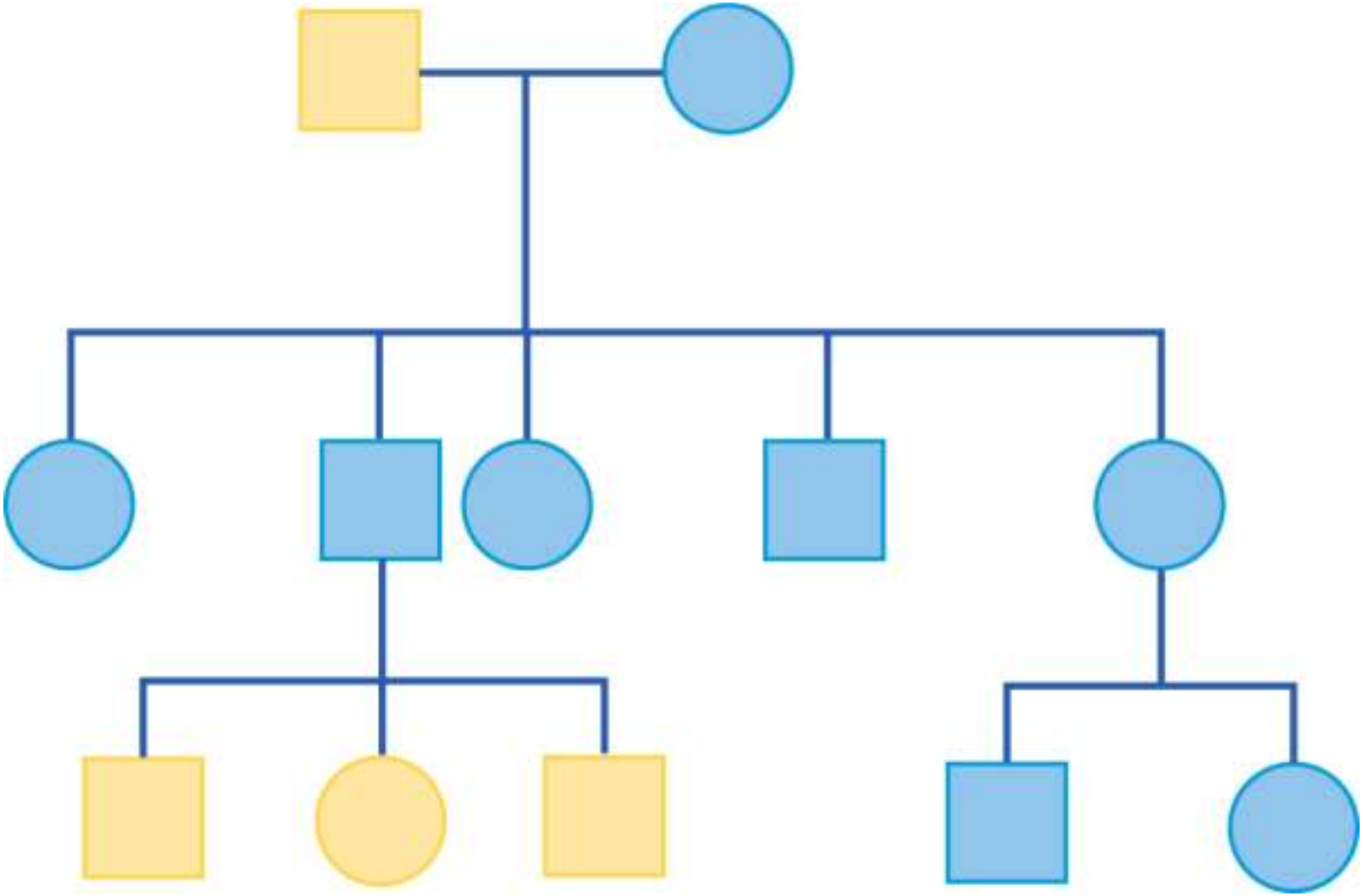
The Angelman syndrome

- Angelman syndrome is characterized by epilepsy, severe learning difficulties, an unsteady or ataxic gait and a happy affect .

- Individuals with Angelman syndrome have been shown to have an interstitial deletion of the same region of chromosome 15 as that involved in Prader-Willi syndrome, but in this case on the maternally derived homolog or can be shown to have arisen through paternal uniparental disomy.

MITOCHONDRIAL INHERITANCE

- Mitochondria, and therefore their DNA, are inherited almost exclusively from the mother through the oocyte .
- Mitochondrial DNA has a higher rate of spontaneous mutation than nuclear.
- A number of rare disorders with unusual combinations of neurological and myopathic features, sometimes occurring in association with other conditions such as cardiomyopathy and conduction defects, diabetes or deafness, have been characterized as being due to mutations in mitochondrial genes .



Mitochondrial Inheritance Blue is affected

TOOLS OF HUMAN MOLECULAR GENITICS

GENITICS 13

PRINCIPLES OF DNA TECHNOLOGY

- DNA technology can be split into two main areas:

  DNA cloning and methods of DNA analysis.

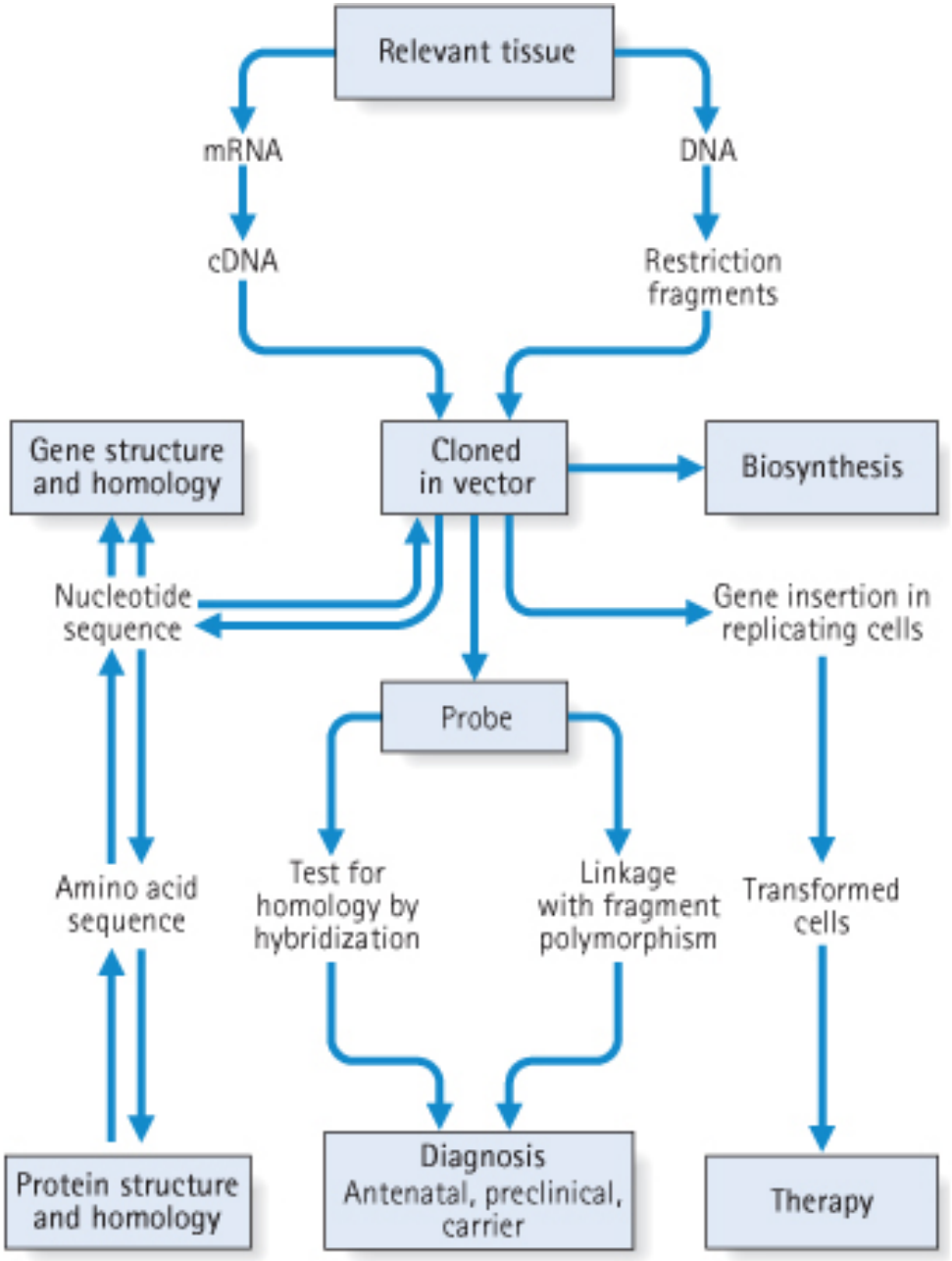
- DNA cloning falls into two main types:

1. Techniques that use natural in vivo cell-based mechanisms of DNA replication .

2. The more recently developed cell-free or in vitro polymerase chain reaction .

DNA CLONING

- DNA cloning is the selective amplification of a specific DNA fragment or sequence to produce relatively large amounts of a homogeneous DNA fragment that allows its structure and function to be analyzed in detail.



Applications of DNA technology

- Gene structure/mapping/function
- Clinical genetics :
  - Preimplantation genetic diagnosis
  - Prenatal diagnosis
  - Presymptomatic diagnosis
  - Carrier detection

- Diagnosis and pathogenesis of disease
  Genetic
  Acquired-infective, malignant

- Biosynthesis
  e.g. insulin, growth hormone, interferon, immunization

- Treatment of genetic disease
  Gene therapy

In vivo cell-based DNA cloning

- Although fragments of DNA can be produced by mechanical shearing techniques, this is a very haphazard process producing fragments which vary in size.

- In the early 1970s it was recognized that certain microbes contained enzymes that cleave double-stranded DNA in or near a particular sequence of nucleotides.

- These enzymes restrict the entry of foreign DNA into bacterial cells and were therefore called restriction enzymes.

- They recognize a nucleotide sequence of DNA between four and eight nucleotides in length, i.e. the same sequence of nucleotides occurring on the two complementary DNA strands when read in one direction of polarity, e.g. 5' to 3'

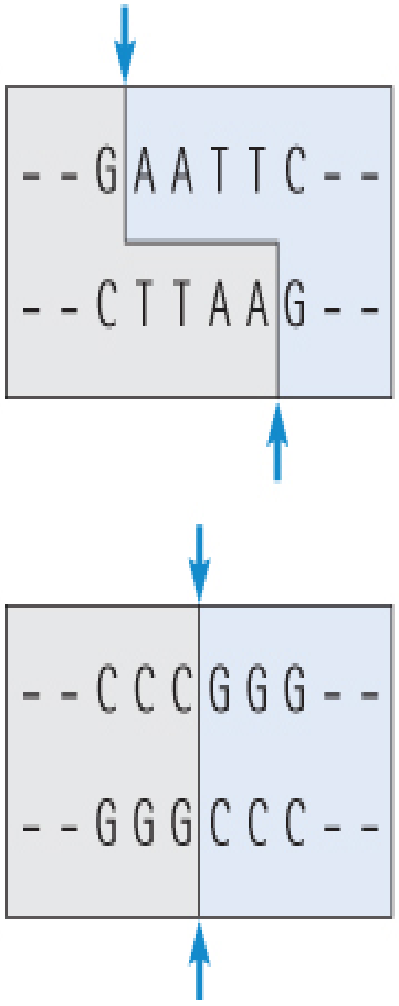
- The longer the nucleotide recognition sequence of the restriction enzyme, the larger the average size of the DNA fragments generated.

- Well over 300 different restriction enzymes have been isolated from various bacterial organisms.

- Restriction endonucleases are named according to the organism from which they are derived, e.g. Ecor 1 is from E. coli and was the first restriction enzyme isolated from that organism.

Cleavage site 5' 3'	**Organism**	**Enzyme**	
G • G AT C C	Bacillus amyloliquefaciens H	BamHI	
G • AAT T C	Escherichia coli RY 13	EcoRI	
G G • C C	Haemophilus aegyptius	HaeIII	

- The complementary pairing of bases in the DNA molecule means that cleavage of double-stranded DNA by a restriction endonuclease always creates double-stranded breaks, which, depending on the cleavage points of the particular restriction enzyme used, results in either a staggered or a blunt end .



- Digesting DNA from a specific source with a particular restriction enzyme will produce the same reproducible collection of DNA fragments each time the process is carried out.

Recombination of DNA fragments

- DNA from any source, when digested with the same restriction enzyme, will produce DNA fragments with identical complementary ends or termini.

- When DNA has been cleaved by a restriction enzyme which produces staggered termini, these are referred to as being 'sticky' or 'cohesive' since they will unite under appropriate conditions with complementary sequences produced by the same restriction enzyme on DNA from any source .

- Initially the cohesive termini are held together by hydrogen bonding but are covalently attached with the enzyme called DNA ligase.

- The union of two DNA fragments from different sources produces what is referred to as a recombinant DNA molecule.

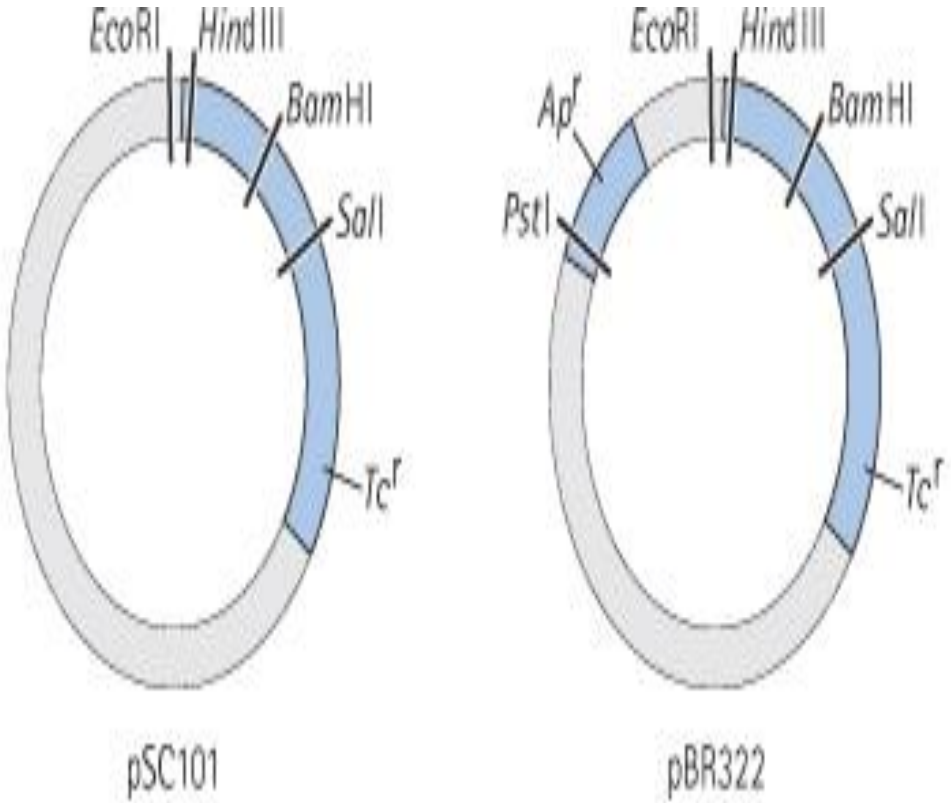
Vectors

A vector, or replicon, is the term for the carrier DNA molecule used in the cloning process which, through its own independent replication within a host organism, will allow the production of multiple copies of itself.

The incorporation of the foreign or target DNA into a vector allows the production of large amounts of that DNA fragment.

- In order for naturally occurring vectors to be used for DNA cloning, they need to be modified to ensure that the foreign DNA is inserted at a specific location and that recombinant vectors containing foreign inserted DNA can be detected.

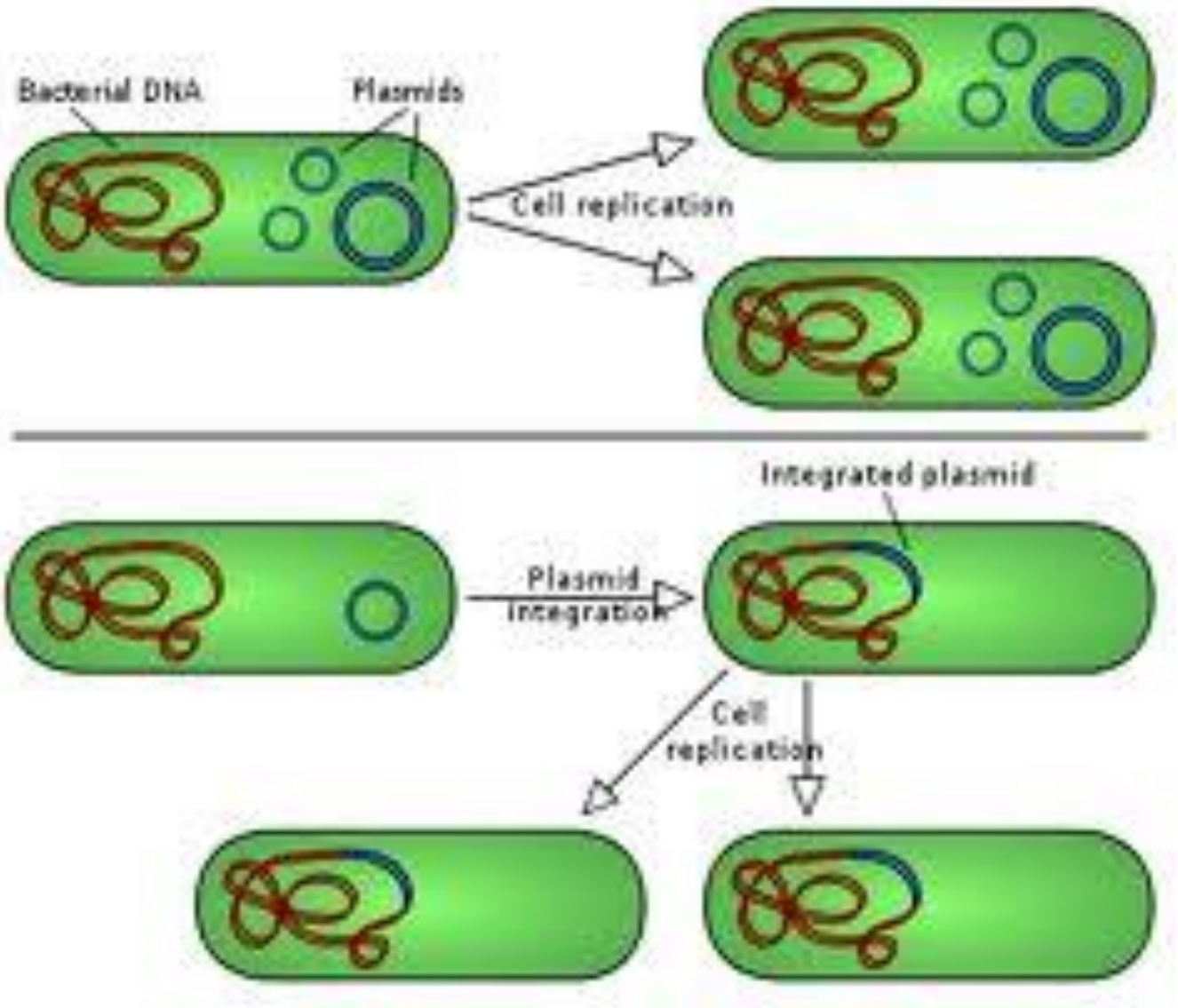
- Many of the early vectors were constructed so that insertion of the foreign DNA in a gene for antibiotic resistance resulted in loss of that function .



Two plasmids originally used in recombinant DNA technology showing drug resistance genes (Apr = ampicillin resistance; Tcr = tetracycline resistance) and cleavage sites of restriction endonucleases which are present in the DNA only once for use as a cloning site.

- There are five main types of vectors commonly used, which include plasmids, bacteriophages, cosmids, bacterial and yeast artificial chromosomes.

- The choice of vector used in cloning depends on a number of factors, such as the particular restriction enzyme being used and the size of the foreign DNA to be inserted.


Bacterial DNA
Plasmids
Cell replication
Integrated plasmid
Plasmid integration
Cell replication

- Some of the early vectors, such as plasmids and bacteriophages, were severely limited in the size of the foreign DNA fragment that could be inserted .

- A cosmid is essentially a plasmid which has had all but the minimum vector DNA necessary for propagation removed to enable insertion of the largest possible foreign DNA fragment and still allow replication.

- More recently, the development of bacterial and yeast artificial chromosomes (BACs and YACs) allows the possibility of cloning a longer DNA fragments .

- A yeast artificial chromosome consists of a plasmid which contains within it the minimum DNA sequences necessary for centromere and telomere formation plus DNA sequences known as autonomous replication sequences, all of which are necessary for accurate replication within yeast.

Transformation of the host organism

- After introduction of the foreign DNA fragment into the vector, the recombinant vector is introduced into specially modified bacterial or yeast host cells.

- The bacterial cell membrane is not normally permeable to large molecules such as DNA fragments but can be made permeable by a variety of different methods, including exposure to certain salts or high voltage - what is known as becoming competent.

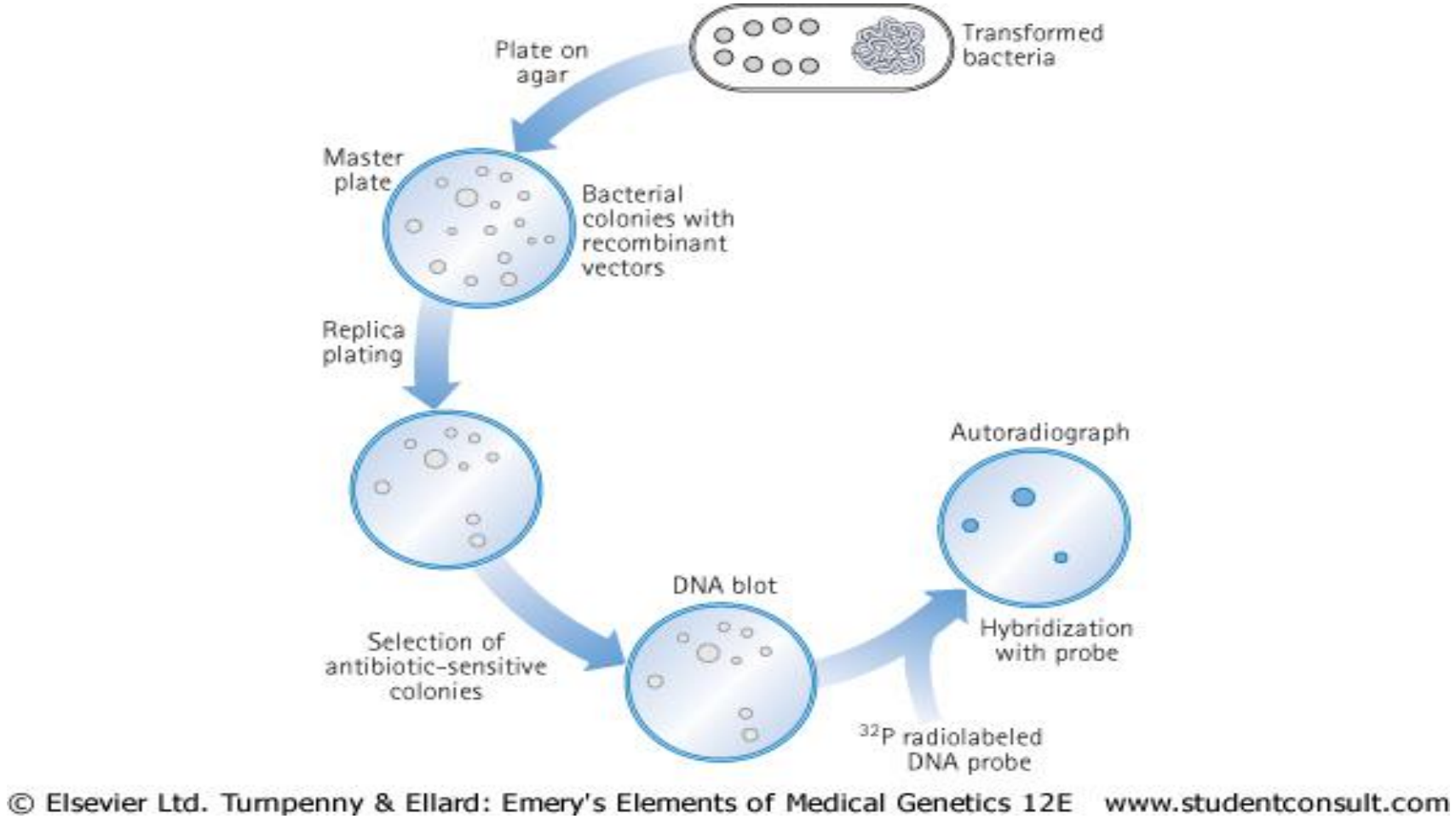
- Usually only a single DNA molecule will be taken up by a host cell undergoing the process known as transformation .

- If the transformed cells are allowed to multiply, large quantities of identical copies of the original single target DNA or clones will be produced .

Selection of specific clones

- A number of techniques have been developed to detect the presence of clones with specific DNA sequence inserts.
- The most widely used method is nucleic acid hybridization
- Colonies of transformed host bacteria with recombinant clones are used to make replica plates that are lyzed and then blotted on to a nitrocellulose filter to which nucleic acid binds.

- The DNA of the replica blot is then denatured to make the DNA single stranded, which will allow it to hybridize with single-stranded, radioactively labeled DNA or RNA probes that can then be detected by exposure to an X-ray film, or what is known as autoradiography.



- Identification of recombinant DNA clones with specific DNA inserts by loss of antibiotic resistance, nucleic acid hybridization and autoradiography

- In this way a transformed host bacterial colony containing a sequence complementary to the probe can be detected and, from its position on the replica plate, the colony containing that clone can be identified on the master plate, 'picked' and cultured separately .

DNA libraries

Different sources of DNA can be used to make recombinant DNA molecules.

- DNA from nucleated cells is termed total or genomic DNA.
- DNA made by the action of the enzyme reverse transcriptase on mRNA is called complementary DNA or cDNA.

- It is possible to enrich for DNA sequences of particular interest by using a specific tissue or cell type as a source of mRNA, e.g. immature red blood cells (reticulocytes) containing predominantly globin mRNA resulted in cloning of the genes for the globin chains of hemoglobin.

- The collection of recombinant DNA molecules generated from a specific source is referred to as a DNA library, e.g. a genomic or cDNA library.

- A DNA library of the human genome using plasmids as a vector would need to consist of several hundred thousand clones to be likely to contain the whole of the human genome.

- The use of YACs as cloning vectors with DNA digested by infrequently cutting restriction enzymes means that the whole of the human genome can be contained in a library of 13 000-14 000 clones.

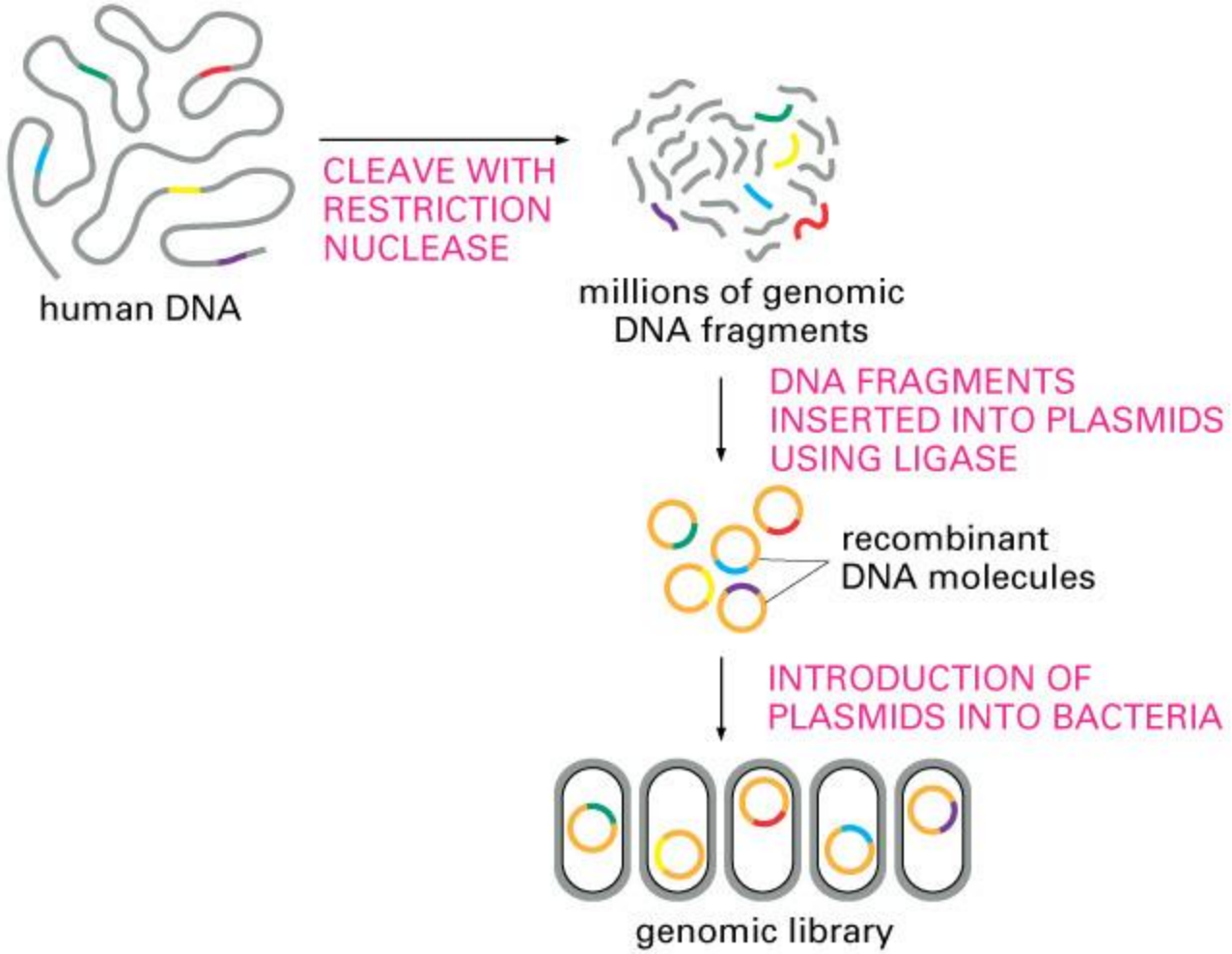
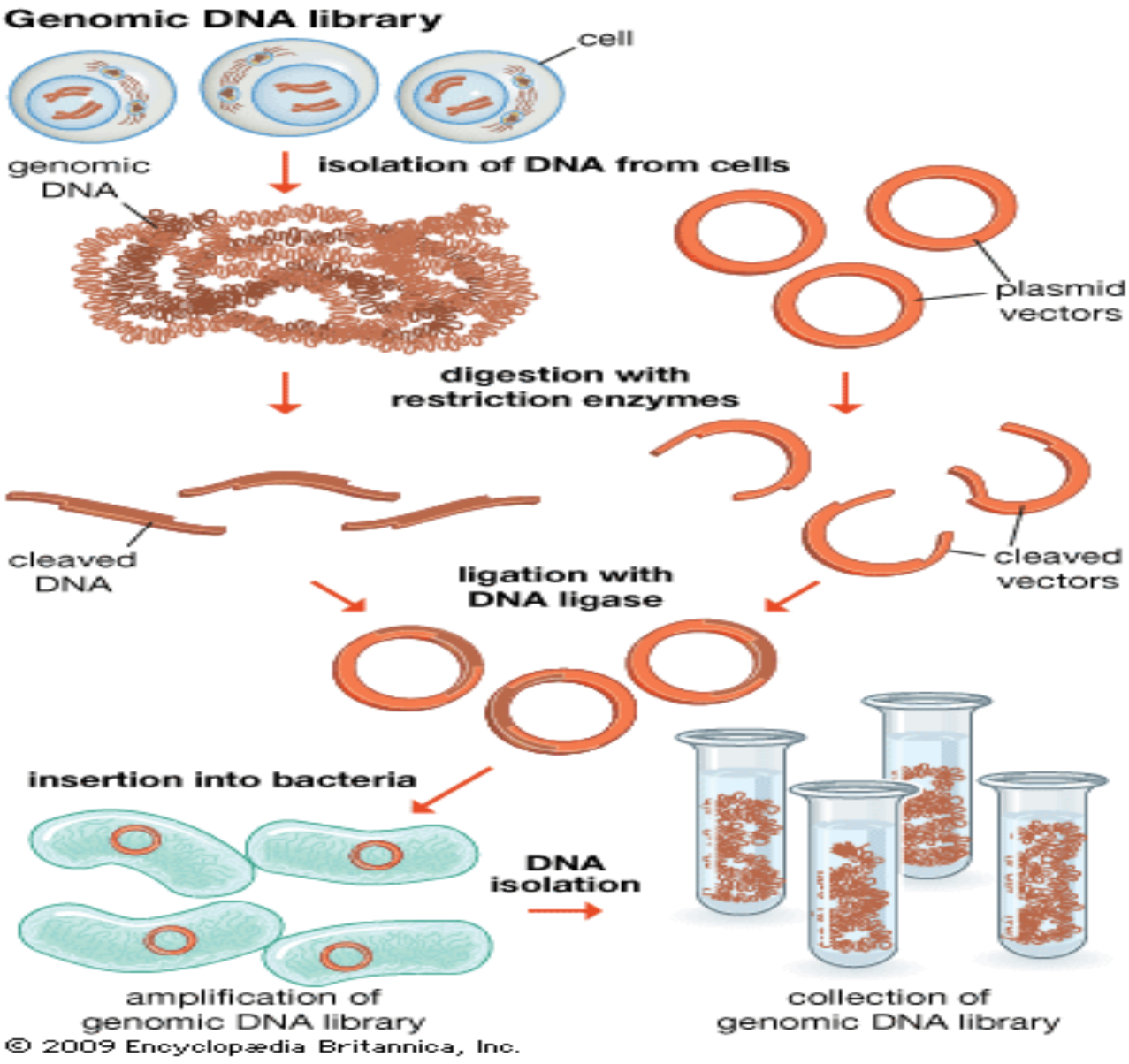


Figure 10-23 Essential Cell Biology, 2/e. (© 2004 Garland Science)


Genomic DNA library
cell
genomic DNA
isolation of DNA from cells
plasmid vectors
digestion with restriction enzymes
cleaved DNA
cleaved vectors
ligation with DNA ligase
insertion into bacteria
DNA isolation
amplification of genomic DNA library
collection of genomic DNA library
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METHODS OF NUCLEIC ACID ANALYSIS

GENITICS 14

The polymerase chain reaction

- DNA sequence information is used to design two oligonucleotide primers (amplimers) of approximately 20bp in length complementary to the DNA sequences flanking the target DNA fragment.

- The primers, when added to denatured DNA, will bind to the complementary DNA sequences that, with the action of DNA polymerase, will result in extension of the primer DNA on the single-stranded DNA template in the presence of the deoxynucleotide triphosphates (dATP, dCTP, dGTP and dTTP) to synthesize the complementary DNA sequence

- Subsequent heat denaturation of the double-stranded DNA, followed by annealing of the same primer sequences to the resulting single-stranded DNA, will result in synthesis of further copies of the target DNA.

- Thirty to 35 successive repeated cycles results in over 1 million copies (amplicons) of the DNA target, sufficient for direct visualization by ultraviolet fluorescence after ethidium bromide staining, without the need to use indirect detection techniques .

- The polymerase chain reaction (PCR) allows analysis of DNA from any cellular source containing nuclei and, in addition to blood, can include less invasive samples such as buccal scrapings or pathological archival material.

- It is also possible to start with quantities of DNA as small as that from a single cell, as is the case in preimplantation genetic diagnosis .

- Great care has to be taken with PCR, however, since DNA from a contaminating extraneous source, such as desquamated skin from a laboratory worker, will also be amplified.

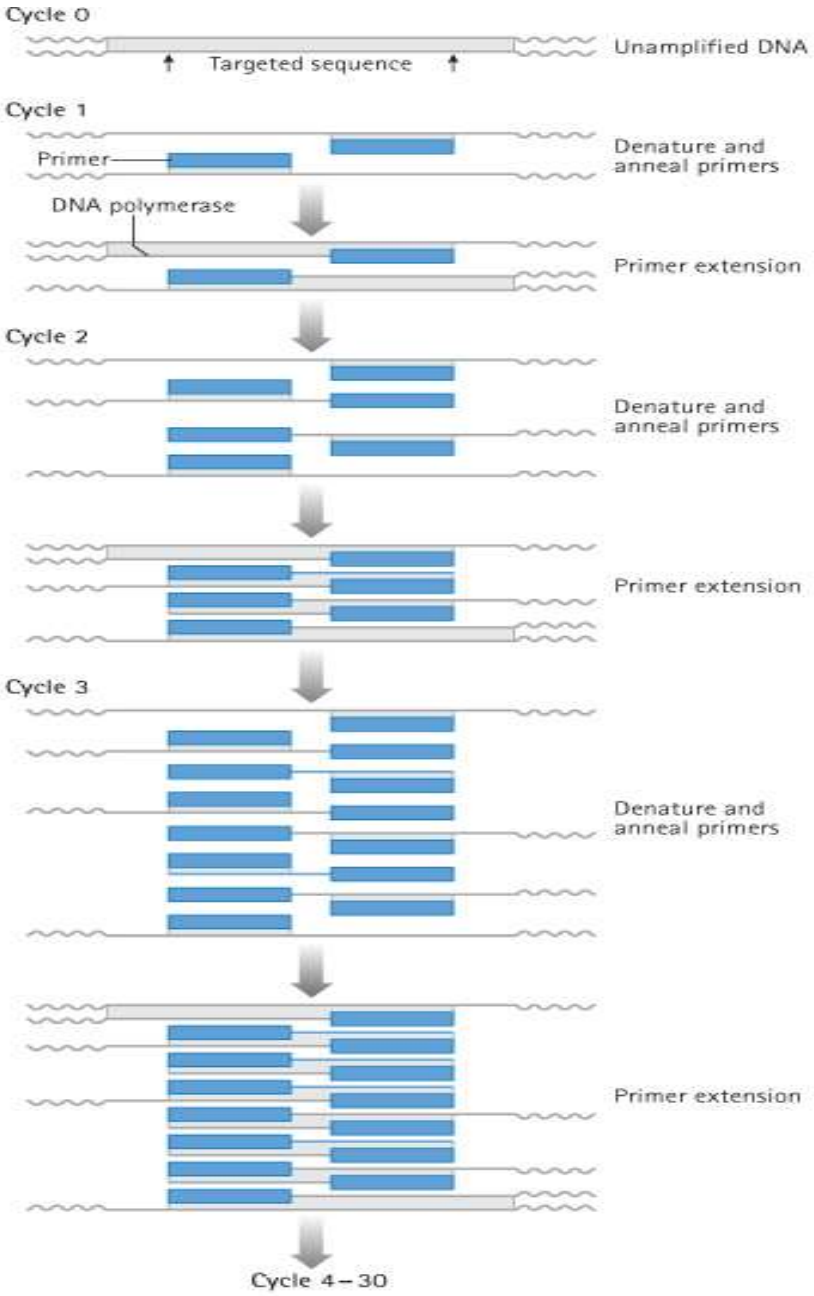
- This can lead to false-positive results unless the appropriate control studies are used to detect this possible source of error.

- Another advantage of PCR is the rapid turn-round time of samples for analysis.

- Use of the heat stable Taq DNA polymerase isolated from the bacterium Thermophilus aquaticus, which grows naturally in hot springs, generates PCR products in a matter of hours rather than the days or weeks required for cell-based in-vivo DNA cloning techniques

- Real-time PCR machines have reduced this time to less than 1 h, and fluorescent technology is used to monitor the generation of PCR products during each cycle, thus eliminating the need for gel electrophoresis.

- DNA cloning by PCR, in contrast to in-vivo cell-based techniques, has the disadvantage that it requires knowledge of the nucleotide sequence of the target DNA fragment and is best used to amplify DNA fragments up to 1kb, although long-range PCR allows the amplification of larger DNA fragments up to 20-30kb.


Cycle 0
Targeted sequence
Unamplified DNA
Cycle 1
Primer
DNA polymerase
Denature and anneal primers
Primer extension
Cycle 2
Denature and anneal primers
Primer extension
Cycle 3
Denature and anneal primers
Primer extension
Cycle 4 – 30

Southern blotting

- Southern blotting, named after Edwin Southern who developed the technique, involves digesting DNA by a restriction enzyme which is then subjected to electrophoresis on an agarose gel.

- This separates the DNA or restriction fragments by size, the smaller fragments migrating faster than the larger ones .

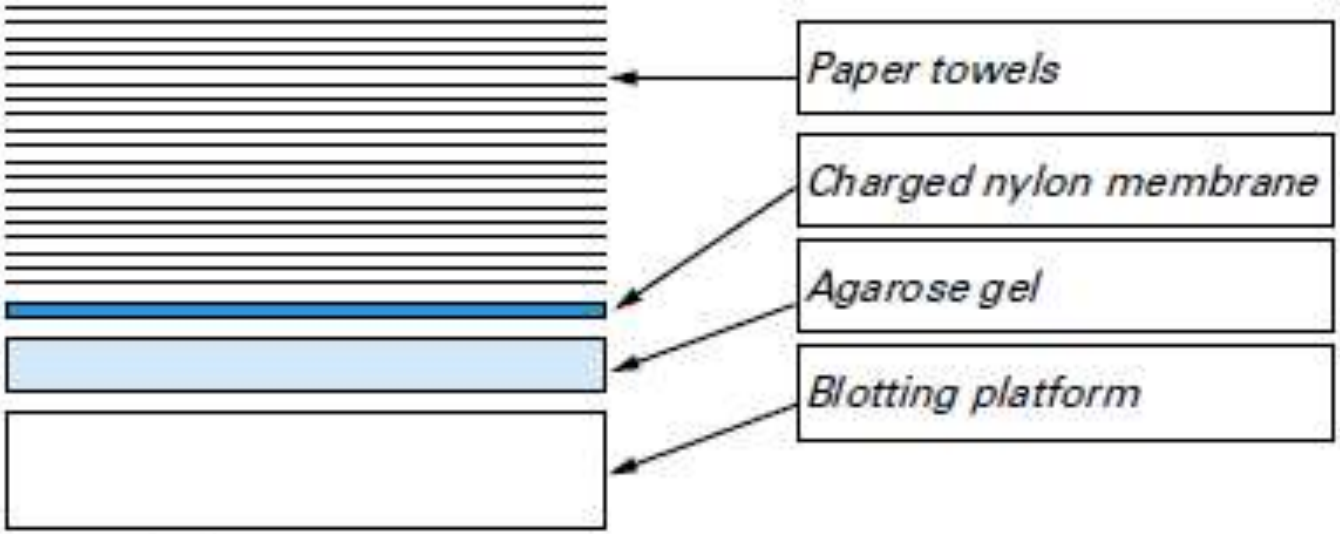
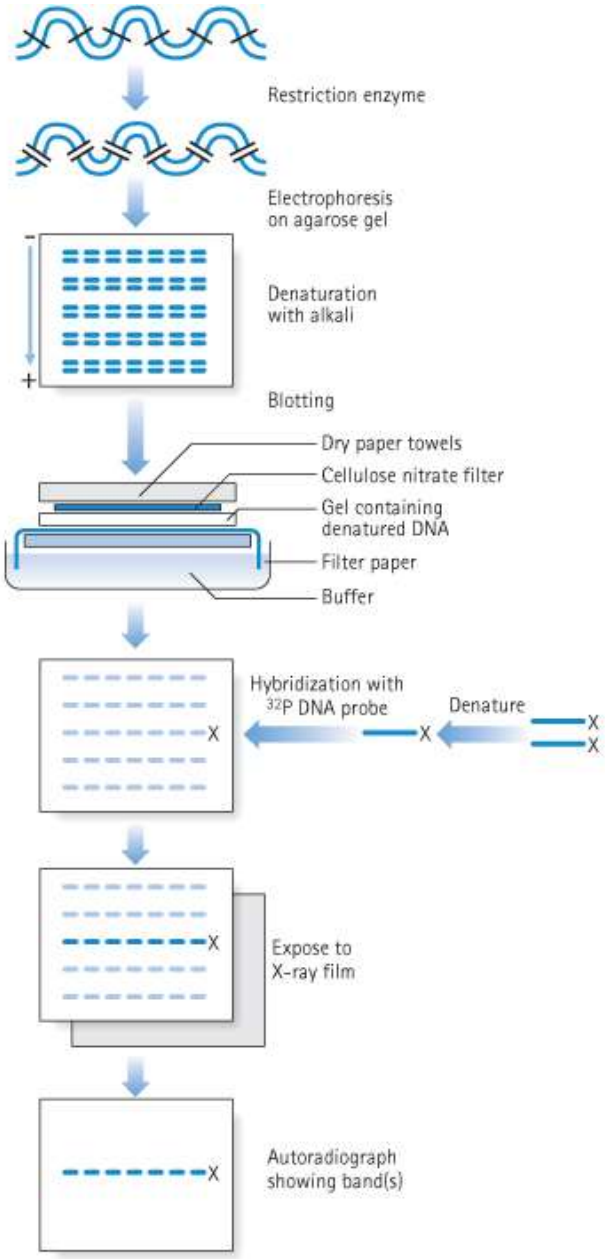


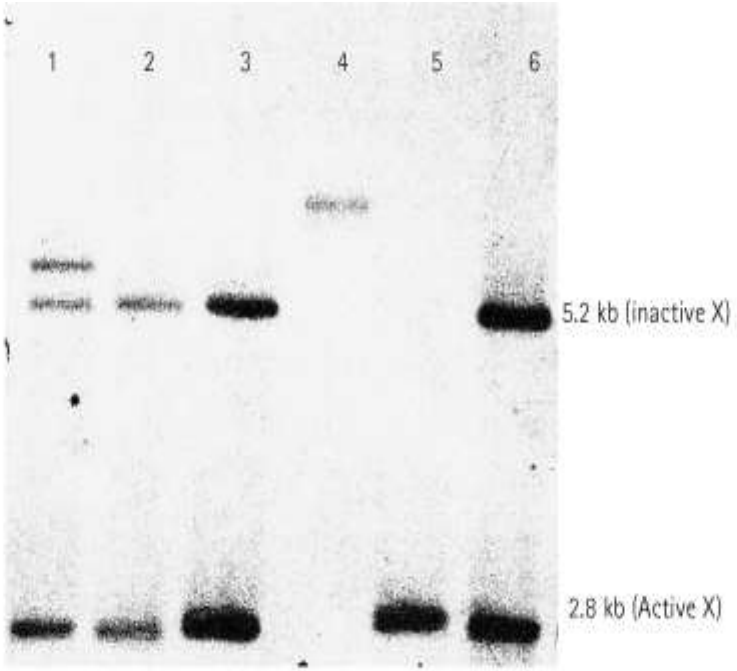
Figure 17.17 Setting-up a Southern blot (dry-blotting). Using a stack of paper towels to provide capillarity, the DNA in the agarose gel is transferred to the charged membrane before being hybridised with a radiolabelled DNA probe

- The DNA fragments in the gel are then denaturated with alkali, making them single stranded.

- A 'permanent' copy of these single-stranded fragments is made by transferring them on to a nitrocellulose filter which binds the single-stranded DNA, the so-called Southern blot.

- A particular target DNA fragment of interest from the collection on the filter can be visualized by adding a single-stranded 32P radioactively labeled DNA probe that will hybridize with homologous DNA fragments in the Southern blot, which can then be detected by autoradiography.


Restriction enzyme
Electrophoresis on agarose gel
Denaturation with alkali
Blotting
Dry paper towels
Cellulose nitrate filter
Gel containing denatured DNA
Filter paper
Buffer
Hybridization with ^{32}P DNA probe
Denature
Expose to X-ray film
Autoradiograph showing band(s)



- Southern blot to detect methylation of the FMR-1 promoter in patients with fragile X

  Patient 1 is a female with a methylated expansion, patients 2, 3 and 6 are normal females, patient 4 is an affected male and patient 5 is a normal male.

DNA sequencing

- This method originally employed radioactive labeling with manual interpretation of data.
- The use of fluorescent labels detected by computerized laser systems has improved ease of use and increased throughput and accuracy.
- Today's capillary sequencers can sequence around 1Mb (1000 000 bases) per day!

- Dideoxy sequencing involves using a single-stranded DNA template (e.g. denatured PCR products) to synthesize new complementary strands using a DNA polymerase and an appropriate oligonucleotide primer .

- In addition to the four normal deoxynucleotides (dNTPs), a proportion of each of the four respective dideoxynucleotides (ddNTPs) are included, each labeled with a different fluorescent dye.

- The dideoxynucleotides lack a hydroxyl group at the 3' carbon position that prevents phosphodiester bonding, resulting in each reaction container consisting of a mixture of DNA fragments of different lengths that terminate in their respective dideoxynucleotide.

- When the reaction products are separated by polyacrylamide gel or capillary electrophoresis, a ladder of DNA sequences of differing lengths is produced.

- The DNA sequence complementary to the single-stranded DNA template is generated by the computer software and the position of a mutation may be highlighted .

Allele Specific Oligonucleotide Probe Analysis

- An ASO is typically an oligonucleotide of 15–21 nucleotide bases in length.

- It is designed in a way that makes it specific for only allele, of the DNA being tested.

- These probes can usually be designed to detect a difference of as little as 1 base in the target's genetic sequence.

- To be detected after it has bound to its target, the ASO must be labeled with a radioactive or fluorescent tag.

Example

- The human disease sickle cell anemia is caused by a genetic mutation in the codon for the sixth amino acid of the blood protein beta-hemoglobin.

- The normal DNA sequence G-A-G codes for the amino acid glutamate, while the mutation changes the middle adenine to a thymine, leading to the sequence G-T-G (G-U-G in the mRNA).

- This altered sequence substitutes a valine into the final protein, distorting its structure.

- To test for the presence of the mutation in a DNA sample, an ASO probe would be synthesized to be complementary to the altered sequence labeled as "S".

- As a control, another ASO would be synthesized for the normal sequence "A".

- Each ASO is fully complementary to its target sequence (and will bind strongly), but has a single mismatch against its non-target allele (leading to weaker interaction).

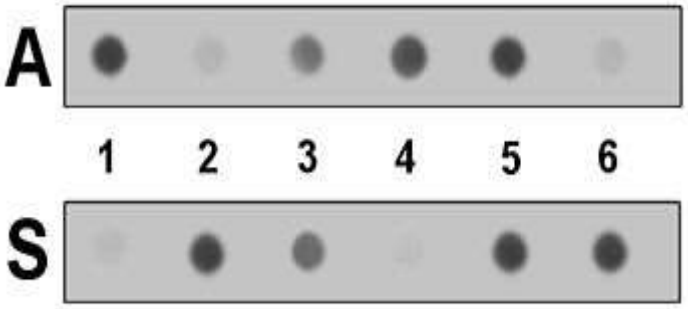
Binding of the "S" ASO probe to "S" DNA (top) or "A" DNA (bottom)

- A segment of the beta-hemoglobin genes in the sample DNA(s) would be amplified by PCR, and the resulting products applied to duplicate support membranes as Dot blots.

- The sample's DNA strands are separated with alkali, and each ASO probe is applied to a different blot.

- After hybridization, a washing protocol is used which can discriminate between the fully complementary and the mismatched hybrids.

- The mismatched ASOs are washed off of the blots, while the matched ASOs (and their labels) remain.



- six samples of amplified DNA have been applied to each of the two blots.

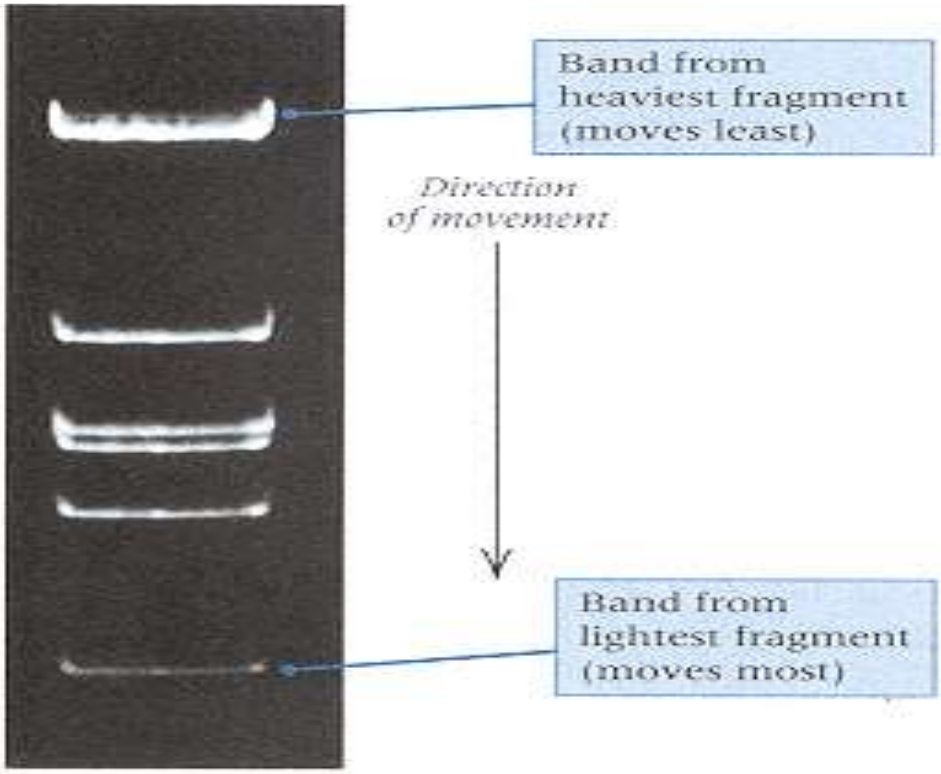
- Detection of the ASO label that remains after washing allows a direct reading of the genotype of the samples, each with two copies of the beta-hemoglobin gene. Samples 1 and 4 only have the normal "A" allele, while samples 3 and 5 have both the "A" and "S" alleles (and are therefore heterozygous carriers of this recessive mutation).

- Samples 2 and 6 have only the "S" allele, and would be affected by the disease. The small amount of 'cross hybridization' shown is typical, and is considered in the process of interpreting the final results.

Electrophoresis of Single Stranded DNA

This method follows digestion of genomic DNA with restriction endonucleases, denaturation in alkaline solution, and electrophoresis on a neutral polyacrylamide gel.

After transfer to a nylon membrane, the mobility shift due to a nucleotide substitution of a single-stranded DNA fragment could be detected by hybridization with RNA copies synthesized on each strand of the DNA fragment as probes.



Gel electrophoresis of DNA.
Molecules of different sizes were mixed and placed in a well.
Electrophoresis was in the vertical direction.
The DNA has been made visible by the addition of a dye (ethidium bromide) that binds only to DNA and that fluoresces when the gel is illuminated with short-wavelength ultraviolet light.