Post-Graduate Degree Programme (CBCS)
in
ZOOLOGY

SEMESTER-III

ELECTIVE THEORY PAPER

CYTOGENETICS AND MOLECULAR BIOLOGY
ZDSE(MJ)T302

SELF LEARNING MATERIAL

DIRECTORATE OFOPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI, NADIA,
W.B., INDIA

Content Writer:
Dr.Subhabrata Ghosh, Assistant Professor of Zoology, Directorate of Open and Distance Learning, University of Kalyani.

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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Manas Kumar Sanyal, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks is also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are self writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
University of Kalyani

Module	Unit	Content	
ZDSE(MJ)T302 Cytogenetics and Molecular Biology	**I**	Cancer: Clonal origin of Cancer; The nature of cancer; Warburg effect; Cancer stem cell concepts; Genetics and molecular basis of Cancer. Oncogenes, Tumour Suppressor genes, DNA repair genes and Apoptotic genes; Tumour microenvironment and promotion.	
	II	Tumour progression angiogenesis, invasion and metastasis; Cancer and environment-physical, chemical, biological carcinogens, Cancer therapy-radio-, chemo-and immunotherapy.	
	III	Mutations and mutagenesis: Types of mutation; biochemical basis of mutations; mutagenesis.	
	IV	Spontaneous and induced mutation; reversion as a means of detecting mutagens and carcinogens.	
	V	DNA repair and retrieval: Repair of spontaneous and induced mutations.	
	VI	Mechanism of DNA repair; repair by direct reversion; excision repair; SOS response.	
	VII	Karyotype and sex chromosomes; sex determination; role of Y-chromosome; sex mosaics.	
	VIII	Sex chromosome anomalies; sex influenced and sex limited genes.	
	IX	Behavioral genetics influence of single defects on behavior;	

	X	Genetic analysis of behavior in experimental animals.	
	XI	Chromosome anomalies and insight into human behavior.	
	XII	Environmental effects and gene expression.	
	XIII	Effects of external and internal environment; phenocopies.	
	XIV	Twin studies; concordance and discordance; identical and fraternal twins.	

UNIT-I

Clonal origin of Cancer; The nature of cancer; Warburg effect; Cancer stem cell concepts; Genetics and molecular basis of Cancer. Oncogenes, Tumour Suppressor genes, DNA repair genes and Apoptotic genes; Tumour microenvironment and promotion

Objective: In this unit we will discuss about Clonal origin of Cancer; The nature of cancer; Warburg effect; Cancer stem cell concepts; Genetics and molecular basis of Cancer. Oncogenes, Tumour Suppressor genes, DNA repair genes and Apoptotic genes; Tumour microenvironment and promotion

Introduction:

In multicellular organisms, cell division is a normal process. Cells divide for growth, for the development of organs, for healing of wounds and also for the replacement of older and damaged cells. Cell division is a very complex process which is controlled by a regulatory mechanism at both molecular and cellular level. Again, in higher multicellular organism, each and every cell belongs to a particular type of tissue like epithelial tissue, connective tissue muscular tissue etc.

Hence, when a cell of a specific tissue divides, it normally produces its own kinds of cell of the tissue to which it belongs. It never produces the cells of other tissues. Therefore, the process by which cells achieve this specification and specialization is known as cellular differentiation. Differentiation of cell begins during embryonic gastrulation stage and continues through tissue formation. Actually differentiation has a genetic basis and the process results from the interaction of the nucleus and the cytoplasm. After the cells become well- differentiated, they cannot go back normally to the undifferentiated stage unless disturbed internally or externally.

Therefore, in multicellular organism, the cell division, differentiation and survival of individual cells are carefully regulated to meet the needs of the organism as a whole. When this regulation is lost due to any reason, the cells behave unusually and defy their control mechanism. Then the cells grow and divide in an uncontrolled manner ultimately spreading throughout the body and interfering with the functions of normal tissues and organs. As a whole, this condition leads to cancer. Cancer develops from defects in fundamental regulatory mechanisms of the cell.

Meaning of Cancer:

Cancer is a non-infectious disease. It starts at the molecular level of the cell and, ultimately affects the cellular behaviour. Generally, it can be defined as uncontrolled proliferation of cells without any differentiation.

Differences of Normal cell and Cancer cells:

A malignant tumour, or cancer**,** is an aggregate of cells, all descended from an initial aberrant founder cell. In other words, the malignant cells are all members of a single clone, even in advanced cancers having multiple tumours at many sites in the body. Cancer cells typically differ from their normal neighbors by a host of phenotypic characters, such as rapid division rate, ability to invade new cellular territories (metastasis), high metabolic rate, and abnormal shape. For example, when cells from normal epithelial cell sheets are placed in cell culture, they can grow only when anchored to the culture dish itself. In addition, normal epithelial cells in culture divide only until they form a single continuous layer. At that point, they somehow recognize that they have formed a single epithelial sheet and stop dividing. In contrast, malignant cells derived from epithelial tissue continue to proliferate, piling up on one another. Clearly, the factors regulating normal cellular physiology have beenaltered.

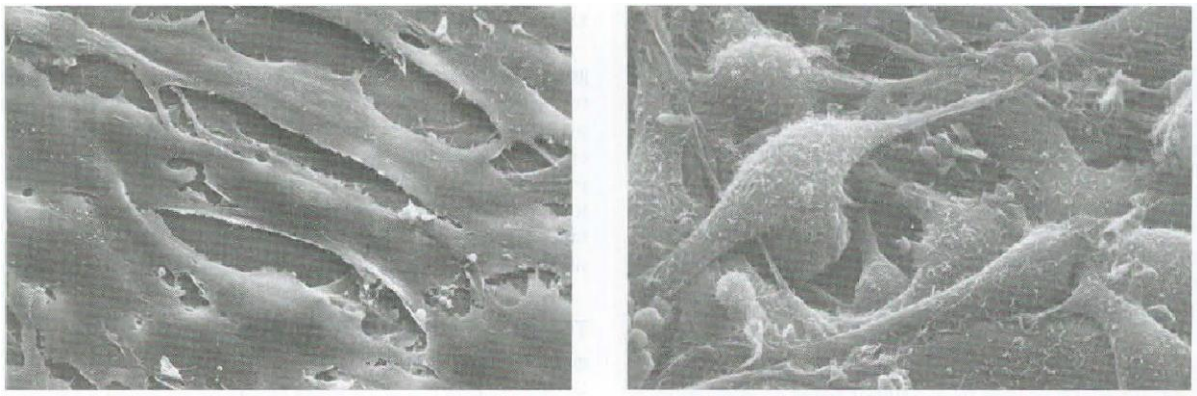


Fig 1: Electron microscopic picture of normal (left) and cancer (right) cells

The most striking feature of tumour cells is that their entire genetic makeup differs dramatically from that of normal cells. Characteristic of nearly all tumour cells is aneuploidy, the presence of an aberrant number of chromosomes generally too many. Normal differentiated cells rely on mitochondrial oxidative phosphorylation to satisfy their energy needs. Cells metabolize glucose to carbon dioxide by oxidation of pyruvate through the tricarboxylic acid (TCA) cycle in the mitochondria. Only under anaerobic conditions do cells undergo anerobic glycolysis and produce large amounts of lactate. In contrast to normal cells, most cancer cells relyon glycolysis forenergy production irrespective of whether oxygen levels arc high or low, producing large amounts of lactate. The use of glycolysis to produce energy even in the presence of oxygen, also called aerobic glycolysis, was first

discovered in cancer cells by the cell biologist Otto Warburg and is therefore called the Warburg effect.

Cell transformation and factors for cell proliferation:

The study of tumour induction by radiation, chemicals, or viruses requires experimental systems in which the effects of a carcinogenic agent can be reproducibly observed and quantitated. Although the activity of carcinogens can be assayed in intact animals, such experiments are difficult to quantitate and control. The development of *in vitro* assays to detect the conversion of normal cells to tumour cells in culture, a process called cell transformation, therefore represented a major advance in cancer research. Such assays are designed to detect transformed cells, which display the *in vitro* growth properties of tumour cells, following exposure of a culture of normal cells to a carcinogenic agent. Their application has allowed experimental analysis of cell transformation to reach a level of sophistication that could not have been attained by studies in whole animals alone.

The first and most widely used assay of cell transformation is the focus assay, which was developed by Howard Temin and Harry Rubin in 1958. The focus assay is based on the ability to recognize a group of transformed cells as a morphologically distinct "focus" against a background of normal cells on the surface of a culture dish. The focus assay takes advantage of three properties of transformed cells: altered morphology, loss of contact inhibition, and loss of density-dependent inhibition of growth. The result is the formation of a colony of morphologically altered transformed cells that overgrow the background of normal cells in the culture. Such foci of transformed cells can usually be detected and quantified within a week or two after exposure to a carcinogenic agent. In general, cells transformed *in vitro* are able to form tumours following inoculation into susceptible animals, supporting *in vitro* transformation as a valid indicator of the formation of cancer cells.

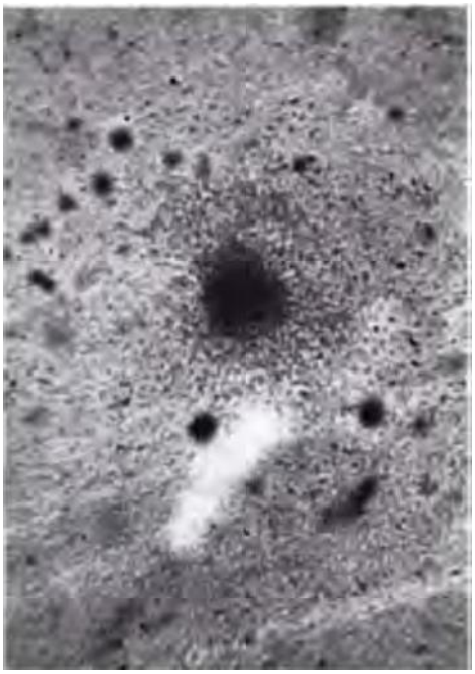


Fig 2 : A focus of chicken embryo fibroblast induced by Rous sarcoma virus

Types of Cancer:

Cancer is a large class of diverse disease. All types of cancer can result from uncontrolled cell growth and division of any of the different kinds of cells in the body. So there are more than a hundred distinct types of cancer which vary in their behaviour and response to treatment.The uncontrolled cell growth produces a mass of cells which are called tumours or neoplasm tumours may be benign or malignant. A benign tumour remains confined to its original location. They do not invade the surrounding normal tissues. They do not spread to distant body sites.

The most common example of tumour is the skin wart. A benign tumour consists of closely resembles normal cells and may function like normal cells. Generally benign tumours are harmless and can usually be removed surgically. However, these tumours may sometimes become quite harmful if they are located in organs like brain and liver.A malignant tumour does not remain confined to its original location. They are capable of both invading surrounding normal tissue and spreading throughout the body via the circulatory or lymphatic systems. Malignant tumours become life-threatening if, they spread throughout the body.

Only malignant tumours are properly designated as cancers. The cells of malignant tumour are derived from single cell, thus they are monoclonal in character. Malignant tumour is composed of aberrant cells. They behave like embryonic type, undifferentiated, having

irregular, large nucleus, and deficient of cytoplasm. Malignant tumours are generally classified into four main types on the basis of cell type from which they arise.

(i) Carcinomas:

It includes approximately 90% of human cancer. This type is principally derived from epithelial cells of ectoderm and endoderm. The solid tumours in nerve tissue and in tissues of body surfaces or their attached glands are example of carcinomas. Cervical, breast, skin and brain carcinomas are developed from malignant tumour.

(ii) Sarcomas:

Sarcomas are solid tumours of connective tissues such as muscle, bone, cartilage and fibrous tissue. These types of malignant tumours are rare in human (about 2% of human cancer).

(iii) Lymphomas:

It is a type of malignancy in which there is excessive production of lymphocytes by the lymph nodes and spleen. It accounts for approximately 8% of human cancers. Hodgkin's disease is an example of human lymphoma.

(iv) Leukemia's:

This type of malignancy arises from the blood forming cell. Leukemia's are commonly known as blood cancer. Leukemia's are neoplastic growth (uncontrolled cell growth at the cost of remaining cells) of leucocytes or WBC. They are characterised by excessive production of WBC of the blood. The name leukemia is derived from Greek leukos (white) + haima (blood) the massive proliferation of leukemia cells can cause a patient's blood to appear milky.

In addition to the types of cancer mentioned above, cancers are further classified according to tissue of origin, for example lung cancer, breast cancer, and the type of cells involved, for example fibro sarcoma arises from fibroblasts, erythromoidleukemia's from precursor of erythrocytes. Although there are many kinds of cancer, the four most common cancers are those of prostrate, breast, lung and colon/rectum.

Development of Cancer:

The development of cancer is a multistep process in which cells gradually become malignant through a progressive series of alternations. This process involves mutation and selection for cells with progressively increasing capacity for cell division, survival, invasion and metastasis (spread of cancer cells through the blood or lymphatic system to other organ sites).

The first step in the process is when a single cell within a tissue of the organ concerned is genetically modified. The modified cell divides rapidly, although surrounding cells do not— and a mass of tumour cells forms. These cells constitute a clone where cells are identical in terms

of structure, characteristics and function. Rapid cell proliferation leads to the tumourous outgrowth or adenoma or polyp. This tumour is still benign.

Tumour progression continues as additional mutationoccur within cells of tumour population. Some of these mutations give a selective advantage to the cell such as rapid growth and the descendants of a cell bearing such a mutation will consequently become dominant within the tumour population.This process is known as clonal selection. Clonal selection continues throughout tumour development and, consequently, tumour become more and more rapid, growing and increasingly malignant. The tumour cells by their rapid proliferation invade the basal lamina that surrounds the tissue.

Then tumour cells spread into blood vessels that will distribute them to other sites in the body. This is known as metastasis. If the tumour cells can exit from the blood vessels and grow at distant site, they are considered malignant (Fig. 23.1).

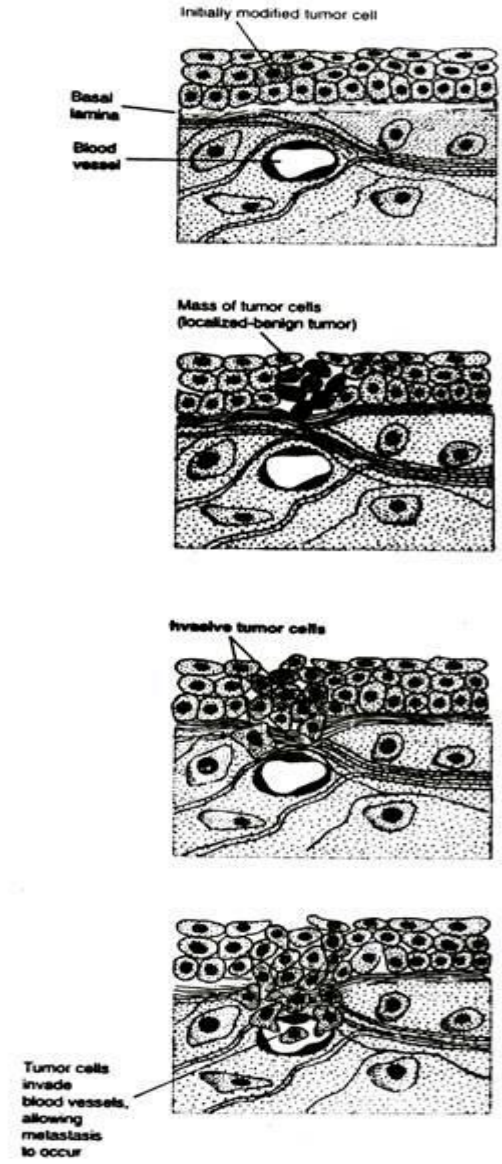


Fig. 23.1: Stages in tumour growth and metastasis.

Characteristics of Cancer Cells:

The uncontrolled growth of cancer cells results from accumulated abnormalities affecting many of the cell regulatory mechanisms. The process of cell change in which a normal cell loses its ability to control its rate of division and thus becomes a tumour cell is called cell transformation.

Cancer cells shows some typical characteristic properties that are absent in normal cells. Sometimes cancer cell properties are just opposite to the properties of normal cell. Cancer cells in vivo differ from their normal counterparts in several respects. Some characteristic properties of cancer cells can also be demonstrated by cell culture in vitro.

(i) Immortalization:

Normal cell culture do not survive indefinitely For example, human cell culture die after about 50 generations. On the other hand, transformed cell cultures can go on indefinitely and remain immortal if the nutrition is provided and overcrowding avoided.

(ii) Loss of Contact Inhibition:

Normal cells growing in tissue culture tend to make cell contacts by adhesion to neighbouring cells. At the points of adhesion some kind of electron-dense plaque is formed in both contacting cells. At the same time there is a slowing down of the amoeboid process which results in contact inhibition of movement. In contrast, cancer cells are unable to form adhesive junctions and do not show this type of contact inhibition.

Experimentally, it has been observed that when normal cells have become completely surrounded by other cells, their mobility stops and they form a monolayer. At the same time there is inhibition of growth and the number of cells in the Petridish remains practically constant. On the other hand, cancer cells continue to multiply and pile up forming irregular masses several layers deep. Cancerous cells undergo a change in property of their cell membranes and cell coat such as disappearance of gap junction, loss of coupling changes in glycolipid and glycoprotein and a reduction in gangliosides. In the cell coat fibronectin, a large glycoprotein found in footprints of moving cultured cells is reduced in cancerous cells. These changes enable the cells to dissociate from neighbouring cells and show loss of contact inhibition.

(iii) Reduced Cellular Adhesion:

Most cancer cells are less adhesive than the normal cells due to reduced expression of cell surface adhesive molecules. When normal cells are transformed into cancer cells, then a change of stickiness of their cell membrane results. Normal cells show stickiness or adhesiveness.

If normal cells are grown in a liquid nutrient medium kept in a glass vessel, the cells stick to glass wall rather than float in the medium. But when cancer cells are allowed to grow in nutrient medium, they stick to each other less than do normal cells. Adhesiveness shows considerable specificity. For example, a liver cell tends to stick with another liver cell and not to other types of cell such as kidney cell. Cancerous cells do not show this property. They are able to mix and stick to any type of normal cell. For example, a malignant liver cell can mix and stick to normal kidney cell. Hence this unusual behaviour of cancer cell explains that cancer cells can invade several normal organs.

(iv) Invasiveness:

One of the most important characteristics of cancer cells is their invasiveness. It is the ability to invade other tissues. Malignant cells generally secrete proteases that digest extracellular matrix components, allowing the cancer cells to invade adjacent normal tissues. For example, secretion of collagenase by the cancer cells helps to digest and penetrate through basal laminae to invade the underlying connective tissue.

Cancer cells also secrete growth factors that promote the formation of new blood vessels. This is known as angiogenesis. Angiogenesis is necessary to support the growth of tumour beyond the size of about a million cells at which point new blood vessels are needed to supply oxygen and nutrients to the multiplying tumour cells. Actually the growth factor secreted by the tumour cells stimulates the endothelial cells present in the wall of capillaries.

As a result, new outgrowth of the capillaries is formed into the tumour. These outgrowths of capillaries are also helpful for metastasis of malignant cells. Therefore, angiogenic stimulation induces the growth of new blood capillaries which penetrate easily in the tumour tissue and provide the opportunity for the cancer cells to enter the circulatory system. As a result, metastasis process begins.

(v) Failure to Differentiate:

Another general characteristic of most of the cancer cells is that they fail to differentiate. This property is closely related with the abnormal proliferation. Normal cells are fully differentiated. In most fully differentiated cells, cell division ceases. In case of cancer-cells, normal differentiation program is blocked at the early stages of differentiation. The relationship between defective differentiation and rapid proliferation is clearly noted in case of leukaemia.

All of the different types of blood cells develop from a common pluripotent stem cell in the bone marrow. Some of the descended cells develop erythrocytes but others differentiate to form lymphocytes, granulocytes and macrophages. Cells of each of these types become round as they differentiate but once they become fully differentiated cell division ceases But leukaemia cells fail to undergo terminal differentiation. Instead, they become blocked at early stage of maturation at which they retain their capacity for proliferation and continue to divide.

(vi) Auto stimulation of Cell Division:

Cancer cells produce growth factor that stimulates their own cell division. Such abnormal production of a growth factor by the cancer cell leads to continuous auto stimulation of cell division. This is known as autocrine growth stimulation. Hence the cancer cells are less dependent on general growth factor produced within the body physiologically from normal

source for inducing growth of all normal cells. It is also noted that the reduced growth factor dependence of cancer cell results from abnormalities in intracellular signalling system.

(vii) Apoptosis:

For every cell, there is a fixed span of life, i.e., time to live and time to die. This cell death is a very orderly process and so it is called Programmed Cell Death or PCD or Apoptosis. Apoptosis is a mechanism of programmed cell death or cell suicide which is essential for the survival of the organism, for the normal development of the organism as the programmed destruction of the organism as the programmed destruction of cells is found during embryo-genesis. It also protects the organism by removing damaged cells which may be due to viral infection or due to exposure to radiations. It also inhibits the tumour development and so any defect in the control of apoptosis may lead to cancer.

There are two methods by which cells may die such as:

1. Death by injury that is through mechanical damage or due to toxic chemicals.

2. By Apoptosis, i.e., through programmed cell death.

(a) Characteristic charges during apoptosis:

The following distinct morphological changes are found during apoptosis:

1. Shrinkage of cells.

2. Cell forms tight sphere.

3. Cell membrane forms bubble-like blebs on the outer surface.

4. Occurrence of nuclear membrane break.

5. Endonucleolytic clearance of DNA at inter-nucleosomal sites occurs leading to the degradation of chromatin.

6. Breakdown of mitochondria is found with the release of cytochrome C.

7. Breakage of cells into small fragments.

8. Engulfment of cells fragments by phagocytic cells:

(b) Genetic Control of Apoptosis:

Some apoptosis genes have already been identified which are responsible for switching on or off apoptosis. These genes include ICE (Interleukin-lb-Converting Enzyme) and P53. There are other factors that also regulate the process of apoptosis.

One of them is the signal protein which is released either due to some cell injury or through cytokine medicated pathways. There are some critical proteins or modulating factors which determine whether a cell will be repaired or undergo death.

These genes or factors may initiate some stimuli for cell death or induces cellular susceptibility to apoptosis or initiates some effector mechanisms for apoptosis. Some of the genes or factors responsible for apoptosis are listed in the Table 23.1.

Table 23.1: Gene/Factors

(a) Initiating Stimuli	**Function**
Tumour Necrosis Factor α receptor family (TNF)	Death signal
Ceramide	gives signal for apoptosis induction.
FAS/Apo–1	Death signal like TNF; For peripheral deletion of T lymphocytes.
Nur 77 (Zinc finger containing steroid receptor)	Death signal in thymocytes.
(b) Inducing Cellular succeptibility	
c-mye	produces myc protein which gives cell susceptibility for apoptosis
Rb–1	Deficiency of Rb–1 gives susceptibility,
	Rb protein may inhibit P 53 mediated apoptosis
E2F1	induces susceptibility
P 53	apoptosis in response to cell injury is dependent on P 53.
(c) Modulating factors	
DAD 1 gene	gives signal for cell death
BCl–2 gene family	Some members inhibit cell death, such as bcl–2, BCl–X. Members which promote death like bax, bid and bad.
(d) Effector mechanisms	
Caspases, ICE, Ich–1	Genes encoding cysteine proteases which are involved in the effector pathway of apoptosis.

(c) Mechanism of Apoptosis:

There are generally three different mechanisms for apoptosis. These are:

1. Triggered by internal signals, i.e., signals arising within the cell.

2. Triggered by external signals.

3. By Apoptosis-Inducing Factor (AIF).

1. By Internal Signals:

In a normal cell, the protein (BC1-2) produced from a gene BC1-2 remains on the outer surface of the mitochondria. The protein BC1-2 holds the apoptotic protease activating factor- 1 (Apaf-1). But when the damage occurs in the cell internally due to some reactive oxygen, the Apaf-1 factor is released from BC1- 2-Apaf-l complex.

This allows the protein Bax to penetrate the mitochondrial membrane causing a leakage of cytochrome C from the mitochondria.

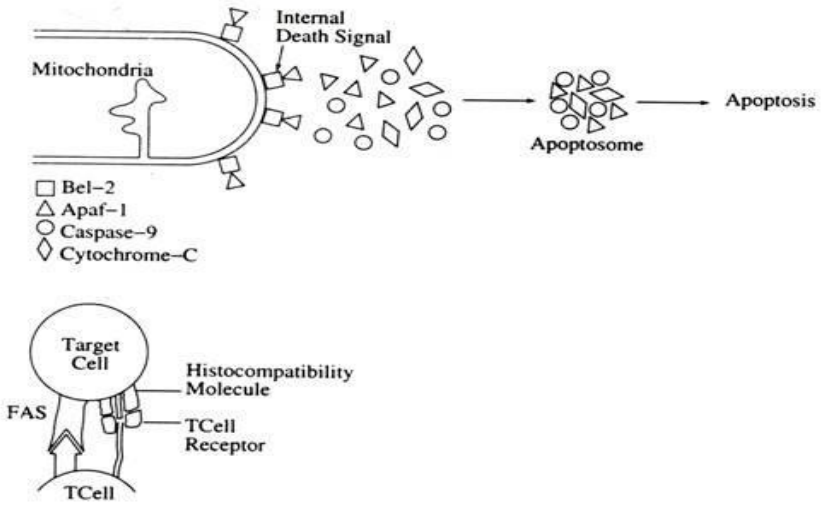
Then the released cytochrome C and Apaf-1 bind to molecules of caspase 9. The complex containing cytochrome C, Apaf- 1, caspase 9 and ATP is called Apoptosome. Caspase 9 is actually one form of protease which cleaves proteins at Aspartic acid residues. The caspase 9 activates other caspases creating a cascade of proteolytic activity which leads to the lysis of cell through digestion of structural proteins of the cytoplasm and degradation of chromosomal DNA.

2. External Signals:

Some receptor proteins (FAS and TNF) and other molecules residing on the surface of the cell are responsible for apoptosis. when cytotoxic T cells containing complementary factor FASL bind to the target cell, FASL binds with the FAS of the target cell leading to the death of the cell by apoptosis.

3. Apoptosis-Inducing Factor (AIF):

This AIF is a protein located in the inter-membrane space of mitochondria. When the cell receives the signal for its death, AIF is released from the mitochondria to the cytoplasm. AIF then goes to the nucleus and binds to DNA causing destruction of the DNA and finally the death of the cell.



In case of cancer, there are some virus like Human Papilloma Virus (HPV), Epstein-Barr Virus (EBV) produce a special type of protein E6 or BC1-2 which inactivate apoptosis promoter P 53, leading to the proliferation of cancer.

Again those cancer cells without the intervention of viruses also have some techniques to inactivate apoptosis. some B-cell leukemia's Melanoma (one type of skin cancer), lung cancer cells, colon cancer cells, etc. produce some proteins or factors like BC1-2 "decoy" molecule, Fas L can avoid apoptosis by inhibiting Apaf-1, or binding to Fas leading to proliferation of cancer.

(viii) Density-Dependent Inhibition:

One of the primary distinguishing characteristic features between cancer cell and normal cell is that normal cells show density-dependent inhibition of cell division in culture but cancer cells continue to proliferate independent of cell density.

Proliferation of normal cell continues until they reach a finite cell density. Normal cells are very sensitive to cell density. So when they reach a finite density they enter the G_0 state of the cell cycle. But cancer cells continue to divide to high cell density.

(ix) Cellular Characteristics:

Cancer cells can be distinguished from normal cells by microscopic examination. Cancer cells have a high nucleus to cytoplasm ratio, prominent nucleoli, many mitosis, and relatively little specialised structure. Normal cells have a cytoskeleton which consists of microtubules and microfilaments. But the cytoskeleton of cancer cells undergo de-polymerisation and the microtubules disaggregate.

(x) Chromosomal Change:

Normal cell contains normal chromosome number, e.g., normal cells of human beings contain 46 or 23 pairs chromosomes. But in cancer cell the chromosomes can undergo both structural and numerical changes. In human being the parent cell of any cancer has 46 chromosomes Later, after a series of abnormal divisions the cancer cells contain series of chromosome numbers and karyotype.

The chromosomes swell up and the number of chromosome sets increase owing to the growth of cancer cells. This condition is known as aneuploidy. Earlier workers have suggested that in different cancer cell populations there are chromosomal stem lines involving a particular spectrum of chromosome structure and number. An established cancer cell population will have a modal number in most of the cells over quite long periods and it is relatively stable. Generally speaking, no two karyotypes are identical in cancer cell and no typical chromosome group has been found to be involved. Therefore, the occurrence of any aneuploid cells in a particular tissue may have the possibility to become cancerous cell.

(xi) Interaction with Immune System:

A few normal cells may be transformed in pre-cancer cells every day in each of us in response to radiation, certain viruses or chemical carcinogens in the environment. Because they are abnormal cells, some of their surface proteins are different from those of normal body cells. Such proteins act as antigens and stimulate an immune response that generally destroys these abnormal pre-cancer cells.

If the pre-cancer cells are destroyed by the immune system, then how does cancer occur? Further investigation demonstrates that there are some transformed cancer cells whose surface proteins are not so changed. Hence such cancer cells may remain anti-genetically similar to normal cells. As a result, the immune system cells may fail to distinguish the cancer cell from normal cell. Some workers suggest that sometimes cells of the immune system do recognise cancer cells but are not able to destroy them.

In such case, cancer cells can stimulate B cells to produce IgG antibodies that combine with antigens on the surface of the cancer cells. These blocking antibodies may block the T cells so that they are unable to adhere to the surface of the cancer cells and destroy them. For some unknown reason, the blocking antibodies are not able to activate the complement system that would destroy the cancer cells.

The Warburg Effect is defined as an increase in the rate of glucose uptake and preferential production of lactate, even in the presence of oxygen. Each of these functions have been hypothesized to be the function of the Warburg Effect.

Causes of Cancer:

Many agents including radiation, chemicals and viruses have been found to induce cancer in both experimental animals and humans. Agents which cause cancers are called carcinogens. Radiation (Solar ultraviolet ray, X-ray) and chemical carcinogens act by damaging DNA and inducing somatic mutations. These carcinogens are generally called initiating agent because the induction of mutations in key target genes is supposed to be the initial event leading to cancer development.

Some of the initiating agents that cause human cancers include solar ultraviolet radiation—the major cause of skin cancer. The exposure of the thyroid gland to X-rays greatly increases the incidence of thyroid cancers. Varieties of chemical carcinogen including tobacco smoke (containing benzo(a)pyrene, dimethyl nitrosamine and nickel compound) and aflatoxin produced by some moulds are the major identified cause of human cancer. Other carcinogens induce the cancer development by stimulating cell proliferation rather than inducing mutations. Such compounds axe called tumour promoters.

The first suggestion that chemicals can cause cancer dates back to 1761, when a doctor noted that people who use snuff suffer from nasal cancer. A few years later a British physician observed a high incidence of cancer of the scrotum among the chimney-sweepers iii their youth. He explained the fact that the chimney soot became dissolved in the natural oil of the scrotum, irritating the skin and, consequently, initiates the development of cancer. On the basis of two separate observations it became evident that certain chemicals (Table 23.1) can cause cancer. Later, as the industrial revolution moved into twentieth century, more and more incidence of cancer were reported among the workers who were continuously exposed to industrial chemicals.

Table 23.1: Gene/Factors

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DAD 1 gene	gives signal for cell death
BCl–2 gene family	Some members inhibit cell death, such as bcl–2, BCl–X. Members which promote death like bax, bid and bad.
(d) Effector mechanisms	
Caspases, ICE, Ich–1	Genes encoding cysteine proteases which are involved in the effector pathway of apoptosis.

In the early 1940s Peyton Rous observed that repeated application of coal tar to rabbit skin causes tumour to develop, but the tumour disappears when application of the coal tar is stopped. It is also noted that when the skin is treated with turpentine, tumour again reappears. Normally turpentine does not cause cancer itself. Therefore the coal tar and turpentine are playing two different roles. Some carcinogens induce some normal cells to become irreversibly altered to a pre-neoplastic state.

This is known as initiation and the carcinogens are known as initiation agents. Here coal tar is an initiating agent. On the other hand, some carcinogens stimulate the pre-neoplastic cells to divide and form tumour. This is known as promotion and the carcinogens are termed promoting agents. Here turpentine behaves as promoting agents. Berenblum observed that painting the skin of a mouse a single time with methylcholanthrene rarely causes the development of tumours. But subsequently application of castor oil (an oil derived from seeds of *Croton tiglium*) triggers the formation of multiple tumours on the skin which has been exposed previously to methylcholanthren is acting as an initiator whereas castor oil acts as a promoter.

Initiation is a quick, irreversible process that causes a permanent change in a cell's DNA. The carcinogenic chemicals that act as initiating agent are capable to bind with DNA. Hence they

interfere with the normal function of DNA and induce somatic mutation and, consequently, bring about stable, inheritable changes in the cell's properties.

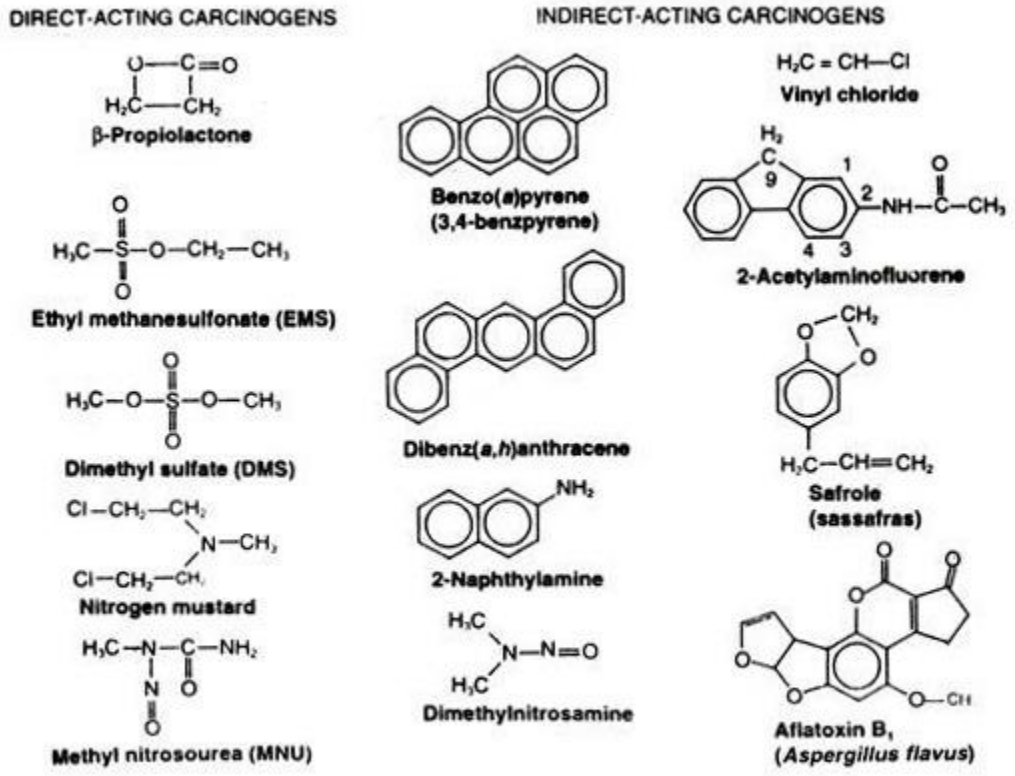

Fig. 23.2: Structure of some direct acting and indirect acting chemical carcinogens.

On the basis of action of chemical carcinogens on DNA, there are two broad categories of carcinogens—direct acting and indirect acting (Fig. 23.2). Direct acting carcinogens are highly electrophilic compounds that react with DNA. Indirect acting carcinogens are converted to ultimate carcinogens by introduction of electrophilic centres. In other words, indirect acting carcinogens must be metabolised before they can react with DNA.

The steps of metabolic activation of benzo(a)pyrene—a polycyclic aromatic hydrocarbon—are shown in Fig. 23.3.:

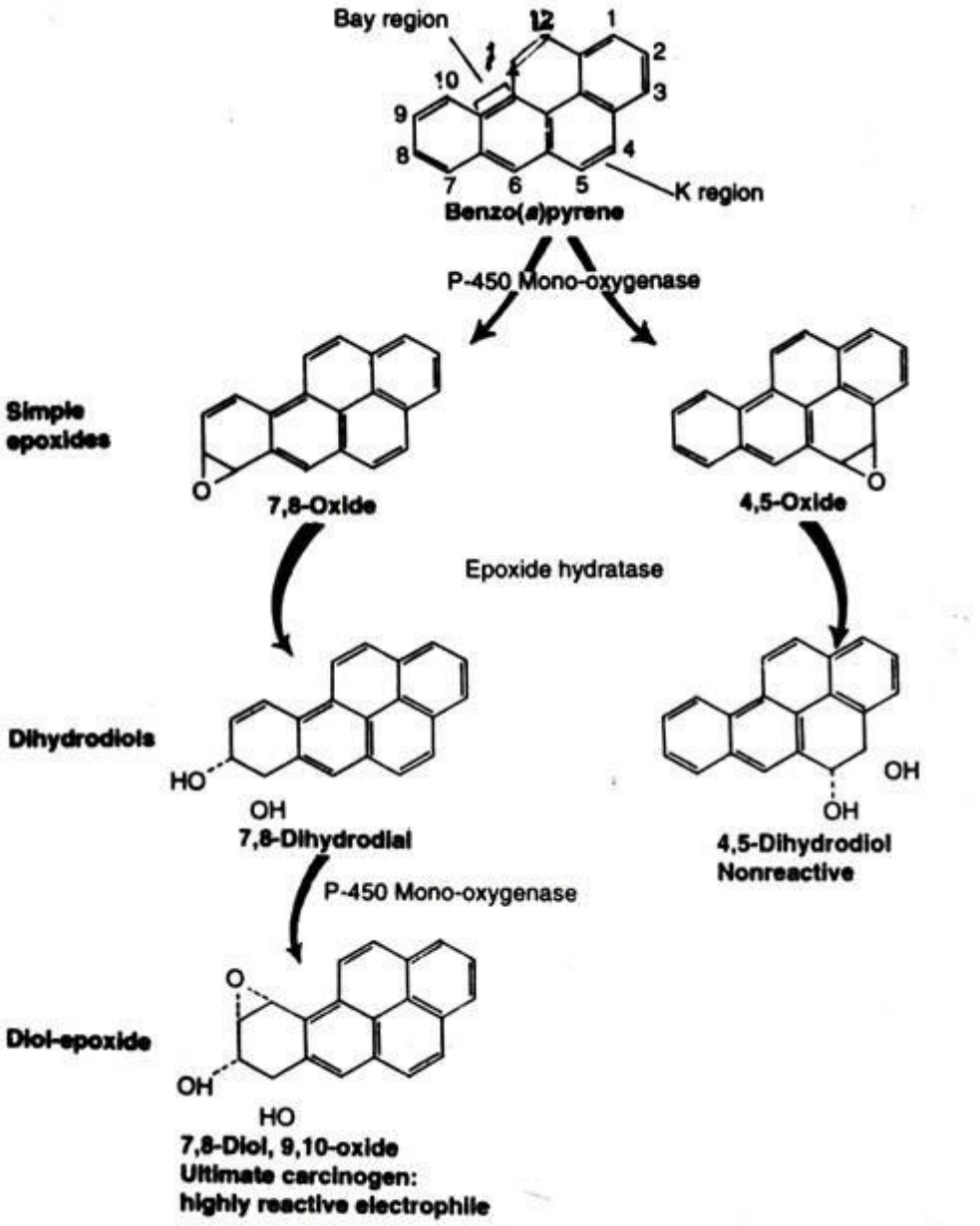

Fig. 23.3: Steps of metabolic activation of benzo(a)pyrene—a powerful carcinogen.

On the other hand, promotion is a gradual, partially reversible process that needs prolonged exposure to promoting agents. If a cell that has already undergone initiation is exposed to a promoting agent, the cell starts to divide and the number of genetically damaged cells goes up.

As the damaged cells continue to divide, a gradual selection for cells showing higher growth rate and invasive properties occurs—leading to the formation of malignant tumour. The

promotion phase continues for longer period. That is why cancer does not develop just after exposure to a carcinogenic agent. The mechanism of action of promoting agents have come from the studies of phorbol esters which are present in castor oil and act as tumour promoters. Phorbol esters bind to the plasma membrane and activate protein kinase C. Protein kinase C is a component of the phosphoinositide signalling pathway whose activity is normally controlled by the second messenger, diacylglycerol.

The activation of protein kinase C leads to phosphorylation of many target proteins and, consequently, activates the transcription factor API which switches on the transcription of genes involved in stimulating cell proliferation. Therefore, the mode of action of phorbol esters gives an insight into the possible mechanism of action of a promoting agent.

Energy that travel through space is known as radiation. Natural source of radiation to which humans are generally exposed are ultraviolet rays, cosmic rays and emission from radioactive elements. We are also exposed to another high- energy radiation like X-ray. Medical, industrial and military activities generally create the high-energy radiation.Sunlight has the ability to cause skin cancer in people who spend long hours in the sunlight. Sunlight contains ultraviolet rays which are also absorbed by normal skin pigmentation. Hence, for this reason, dark-stained or black people usually have lower rates of skin cancer than fair- skinned individual. Because ultraviolet radiation is very weak to pass through the skin, it does not induce any other type of cancer except skin cancer. It is more or less restricted superficially on skin because skin cancer rarely metastasizes.

This type of cancer can be cured by easily removing the affected site surgically. Xeroderma pigmentosum is a type of inherited malignant disease. Individuals with this malignant disease develop extensive skin tumours after exposure to sunlight. Homozygotes for the autosomal recessive mutation responsible for xeroderma pigmentosum are less efficient in the repair of DNA damaged by exposure to ultraviolet light. X-rays are high energy radiation. They are strong enough to penetrate the skin and reach internal organs. X-rays thus make a serious cancer hazard because they are able to induce gene mutation or DNA damage. Many radioactive elements emit radiation. It also acts as carcinogen and causes cancer.Marie Curie, the co-discoverer of the radioactive elements polonium and radium, died of a form of leukemia that appeared to be caused by her extensive exposure to radioactivity. Another example of radiation-induced cancer occurred in New Jersey in 1920. A group of women was employed by a factory that produced watch which glow in the dark. The luminescent point used to point the watch dial contained radium. The paint was applied with a fine-tipped brush that the employee frequently wetted with their tongue. During this process, minute quantities of radium were ingested through saliva in the digestive system from where they were readily abs, -bed and distributed in the different cells ana tissues thorough circulatory system.Several

years later these women suffered from bone cancer caused by radioactive radium that had gradually become concentrated in their bone.

The most well-known horrifying examples of radiation-induced cancer occurred in Japan and in Nevada of United States. In 1945 atomic bombs were exploded over Hiroshima and Nagasaki. The massive fallout of radioactive elements increased the incidence of leukaemia, lymphomas and cancers of the thyroid, breast,' uterus and gastrointestinal tract. Similarly, in Nevada, people suffered from cancer due to the radioactive fallout during nuclear bomb testing. It is suggested that radioactive carcinogen is thought to initiate malignant transformation by causing DNA damage. Alternatively, it is also explained that subsequent exposure of radiation damaged cells to promoting agents stimulates the cell to divide abnormally and form tumour.

Oncogenes:

Oncogene is a type of specific viral gene that is capable of inducing cancer or cell transformation—either in the body of host or in the tissue in culture. After the discovery of src oncogene in RSV, more than 40 different highly oncogenic retroviruses have been isolated (Table 23.3) from a variety of animals like mice, rat, cat, chickens, turkeys, monkeys etc.

All these viruses contain at least one (in some cases two) oncogene like RSV. These oncogene are not needed for viral replication but is responsible for cell transformation. In some cases different viruses contain the same oncogenes. Many of these genes encode protein which, in turn, acts as the key components of signalling pathways that induces cell transformation.

Table 23.3: Examples of Tumour Viruses

Class	Examples	Tumours induced	Organism
DNA viruses:			
Herpesviruses	Lucke virus	Kidney adenocarcinoma	Frogs
	Epstein-Barr virus (EBV)	Burkitt's lymphoma, nasopharyngeal carcinoma	Humans
	Marek's disease virus	Lymphoma	Chickens
Papovaviruses	Shope papilloma virus	Papillomas	Rabbits
	SV-40	Subcutaneous, kidney and lung sarcomas	Hamsters
	Polyoma	Liver, kidney, lung, bone, blood vessels, nervous tissue, connective tissues	Mice
	Human papillomaviruses	Cervical cancer	Humans
Hepatitis B virus		Liver cancer	Duck, Woodchucks, squirrels, human
Adenoviruses	Human adenoviruses	Subcutaneous, intraperitoneal, intracranial	Hamsters
RNA viruses:			
B-type viruses	Bittner mammary tumor virus	Mammary carcinoma	Mice
C-type viruses	Rous sarcoma virus	Sarcomas	Birds, mammals
	Murine leukemia viruses (Gross, Moloney, Friend, Rauscher, and others)	Leukemia	Mice
	Feline leukemia virus	Leukemia	Cats
	Murine sarcoma virus	Sarcoma	Mice
	Feline sarcoma virus	Sarcoma	Cats
	Avian leukemia viruses (avian myeloblastosis and others)	Leukemia	Chickens
	Human T-cell leukemia virus	Leukemias/Lymphomas	Humans
Plant viruses	Wound tumor virus	Roots and stems	Plants

Oncogene in Human Cancer:

Direct evidence for the involvement of cellular oncogenes (the term cellular oncogene is generally used to distinguish this group of cancer- causing genes from viral oncogenes) in human tumour was first derived from gene transfer experiment carried out in the laboratories of Robert Weinberg and Geoffrey Cooper in the early 1980s.

In this process, a DNA segment isolated from tumour cells is artificially introduced into normal cells to see its subsequent changes. DNA isolated from a human bladder carcinoma was found to efficiently induce malignant transformation of recipient mouse cells in culture. This experiment reveals that the human tumour contains a cellular oncogene.The first human oncogene identified in gene transfer experiment was the ras oncogene. The ras oncogenes are not present in normal cells, but they are generated in tumour cells as a consequence of point mutation of the ras proto-oncogene. This results in the change of a single amino acid at critical position of the ras protein molecule encoded by ras gene.

The first such mutation was the substitution of valine for glycine at position 12. A single nucleotide, change which alters codon 12 from GGC (Gly) to GTC(Val) is responsible for the

transforming activity. This is detected in bladder carcinoma DNA. The ras gene encodes membrane-bound guanine-nucleotide binding proteins (G- protein) that plays a central role in the transmission of singles from receptor-bound external growth factor to the cell interior.

During this process, GTP is hydrolysed into GDP. Therefore, Ras protein alternates between active (GTP bound) and inactive (GDP bound) states. But oncogenic ras proteins remain in the active GTP bound state and drive unregulated cell proliferation leading to the development of malignancy. In human tumour, point mutation is an important mechanism by which proto-oncogenes are converted into oncogenes. Besides this, the gene rearrangement—resulting mainly from chromosome translocation—sometimes lead to the conversion of proto-oncogene to oncogene. The classical example regarding the conversion of proto-oncogene to oncogene due to translocation of chromosome is the Burkitt's lymphoma. It produces the malignancy of the antibody producing B-lymphocytes.

In this case a piece of chromosome(s) 8 carrying c-myc proto-oncogene is trans-located to the immunoglobulin heavy chain locus on chromosome 14 (Fig. 23.6). Since the antibody genes are extremely active in lymphocytes, the transcriptional regulation of the adjacent myc proto-oncogene is disturbed, resulting in an abnormal pattern of synthesis of the myc protein product.Such abnormal pattern of expression of the c-myc gene—which encodes transcription factor normally induced in response to growth factor stimulation—is sufficient to drive cell proliferation and contribute to tumour development.

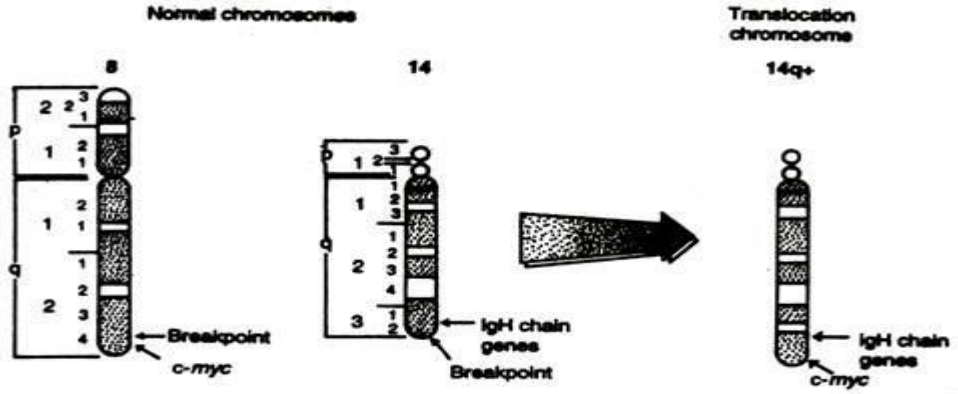

Fig. 23.6: Translocation of a c-myc protooncogene from chromosome 8 to 14.

Translocation of some proto-oncogene often causes the rearrangement of coding sequences which lead to the formation of abnormal gene products. In chronic myelegenousleukemia, the abl proto-oncogene is trans-located from chromosome 9 to chromosome 22 forming Philadelphia chromosome (Fig. 23.7).Theabl proto- oncogene which contains two alternative first exon (1A and IB) is joined to the middle to the bcr gene on chromosome 22. Exon IB is deleted as a result of the translocation. Transcription of the fused gene initiates at the bcr promotor

and continues through abl. Splicing then generates a fused bcr/abl mRNA, in which abl exon 1A sequences are also deleted and bcr sequences are joined to abl Exon 2.

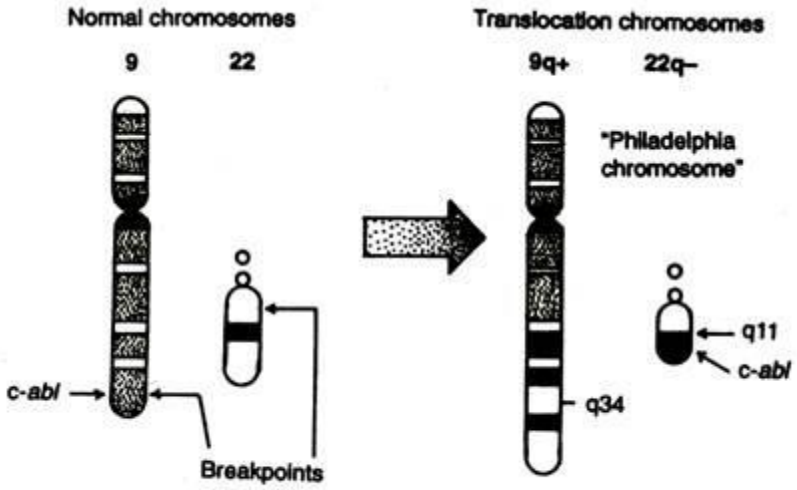

Fig. 23.7: Reciprocal translocation between chromosomes 9 and 22 that produce Philadelphia chromosome.

The bcr/abl mRNA is translated to yield a recombinant bcr/abl fusion protein in which the normal amino terminus of abl proto-oncogene has been replaced by bcr amino acid sequences. The fusion of bcr sequences results in aberrant activity and altered subcellular localisation of the abl protein tyrosine kinase, leading to cell transformation. Gene amplification occurring in the tumour cell is a common process by which proto- oncogenes are converted to oncogene. Gene amplification takes place due to an increase of the number of copies of a gene resulting from the repeated replication of a region of DNA.

Therefore, gene amplification leads to the overproduction of a particular protein or enzyme from the amplified gene. A prominent example of oncogene amplification is the involvement of the N-myc gene in neuroblastoma, a tumour of embryonal neuronal cells. Amplified copies of N-myc gene are frequently present in rapidly growing tumour. Hence it indicates that N-myc amplification is related with the development of neuroblastomas. Amplification of erb B-2 which encodes a receptor protein kinase is similarly associated to the development of breast and ovarian carcinomas.

Table 23.4: Retroviral Oncogenes

Oncogene	Virus	Species
abl	Abelson leukemia	Mouse
akt	AKT8 virus	Mouse
cbl	Cas NS-1	Mouse
crk	CT10 sarcoma	Chicken
erbA	Avian erythroblastosis-ES4	Chicken
erbB	Avian erythroblastosis-ES4	Chicken
ets	Avian erythroblastosis-E26	Chicken
fes	Gardner-Arnstein feline sarcoma	Cat
fgr	Gardner-Rasheed feline sarcoma	Cat
fms	McDonough feline sarcome	Cat
fos	FBJ murine osteogenic sarcome	Mouse
fps	Fujinami sarcome	Chicken
jun	Avian sarcoma-17	Chicken
kit	Hardy-Zuckerman feline sarcome	Cat
maf	Avian sarcoma-AS42	Chicken
mos	Moloney sarcoma	Mouse
mpl	Myeloproliferative leukemia	Mouse
myb	Avian myeloblastosis	Chicken
myc	Avian myelocytomatosis	Chicken
qin	Avian sarcoma 31	Chicken
raf	3611 murine sarcoma	Mouse
rasH	Harvey sarcoma	Rat
rasK	Kirsten sarcoma	Rat
rel	Reticuloendotheliosis	Turkey
ros	UR2 sarcoma	Chicken
sea	Avian erythroblastosis-S13	Chicken
sis	Simian sarcoma	Monkey
ski	Avian SK	Chicken
src	Rous sarcoma	Chicken
yes	Y73 sarcome	Chicken

Subsequent studies have discovered a number of oncogenes (Table 23.4) which are associated with human tumour. Among them chromosomal location of some oncogenes are shown in Fig. 23.8.

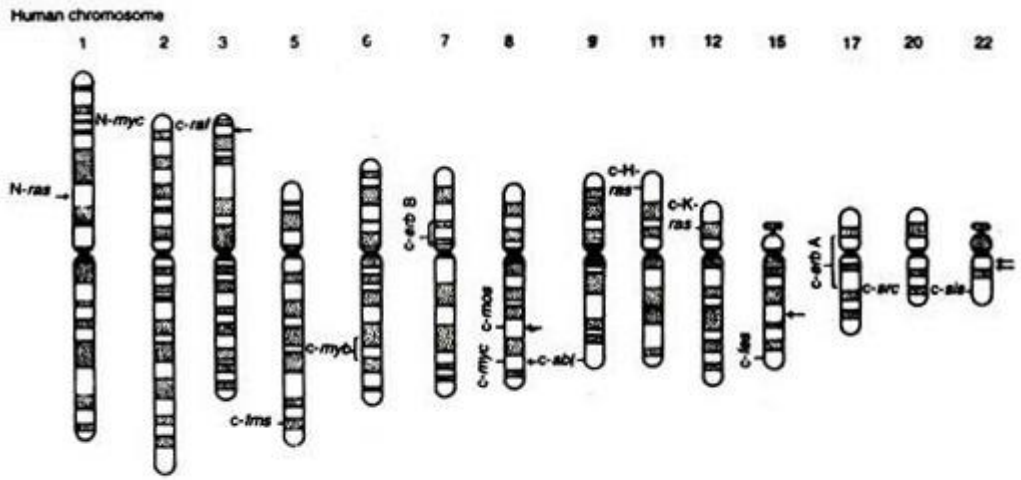

Fig. 23.8: Chromosomal location of some human protooncogenes.

Functions of Oncogene Products:

We have understood that alternation in normal genes, proto-oncogenes, can convert them into oncogenes that code for proteins that are abnormal in structure or are produced in inappropriate amounts. The proteins encoded by the normal genes regulate normal cell proliferation. But the protein encoded by the corresponding oncogene proteins drives the uncontrolled proliferation of the cancer cells.

In addition, some oncogene products involved in other aspects of the behaviour of cancer cells such as defective differentiation and failure to undergo programmed cell death. Besides this, majority of oncogene proteins function as elements of the signalling pathways that regulate cell proliferation in response to growth factor stimulation. These oncogene proteins include polypeptide growth factors, growth factor receptors, elements of intracellular signalling pathway and transcriptional factors (Table 23.5).

Table 23.5: Representative Oncogenes of Human Tumours

Oncogene	Type of cancer	Activation mechanism
abl	Chronic myelogenous leukemia, acute lymphocytic leukemia	Translocation
bcl-2	Follicular B-cell lymphoma	Translocation
*E2A/pbx*1	Acute lymphocytic leukemia	Translocation
erb B-2	Breast and overian carcinomas	Amplification
gip	Adrenal cortical and ovarian carcinomas	Point mutation
gli	Glioblastoma	Amplification
gsp	Pituitary and thyroid tumors	Point mutation
hox-11	Acute T-cell leukemia	Translocation
lyl	Acute T-cell leukemia	Translocation
c-*myc*	Burkitt's lymphoma	Translocation
c-*myc*	Breast and lung carcinomas	Amplification
L-*myc*	Lung carcinoma	Amplification
N-*myc*	Neuroblastoma, lung carcinoma	Amplification
PML/RA/Rα	Acute promyelocytic leukemia	Translocation
*PRAD*1	Parathyroid adenoma	Translocation
*PRAD*1	Breast carcinoma	Amplification
rasH	Thyroid carcinoma	Point mutation
rasK	Colon, lung, pancreatic, and thyroid carcinomas	Point mutation
rasN	Acute myelogenous and lymphocytic leukemias, thyroid carcinoma	Point mutation
ret	Thyroid carcinoma	DNA rearrangement

If the oncogenes induce uncontrolled cell growth that leads to cancer then it is obvious that the products of these genes would act by stimulating all division in some manner.For example, the product of the v-sis oncogene (the v stands for virus) of simian sarcoma virus is closely related to a polypeptide growth hormone called platelet-derived growth factor (PDGF). This factor produced by platelets promotes wound healing by stimulating growth of cells at wound site.

Simian sarcoma virus with v-sis gene in their genome when injected into the body of woolly monkey, induce sarcoma. They are also able to transform fibroblasts growing in culture to a tumourous state. This type of cellular transformation occurs by a mechanism which is possibly related to the effect of normal PDGF on cells at the wound site.Other oncogenes encode products that are identical to growth hormone as well as hormone receptors. For example, oncogene erb B and fms encode proteins that are closely related to the receptors for epidermal growth factor (EGF) and colony stimulating factor-1 (CSF-1).CSF-1 is a growth factor that stimulates growth and differentiation of macrophages. The receptor of this growth factor is a trans membrane-protein with growth factor domains on the outside of the cell and protein kinase domains on the inside of the cell (Fig. 23.9).

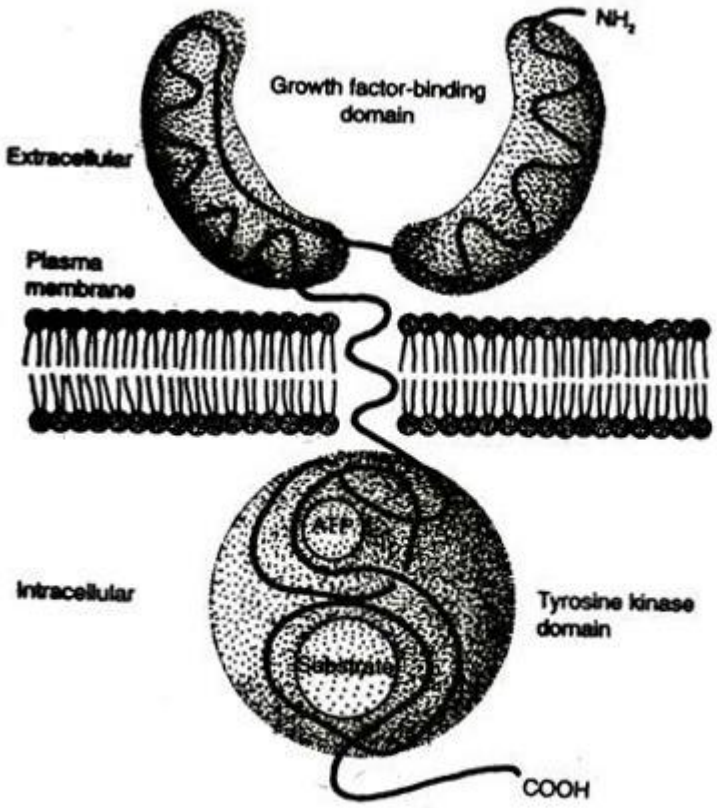

Fig. 23.9: Structure of transmembrane growth factor receptors.

These receptors are key components in trans-membrane signalling pathways. The erb gene product is an analog of the nuclear receptor for the thyroid hormone T_3. Therefore, all of the gene products are undoubtedly involved in intercellular communication circuit which control cell division during the growth and development of highly differentiated tissue. Protein tyrosine kinase is a trans-membrane receptor that is capable of transmitting a perfect signal instructing a cell to divide. Alternation in the structure and function of this enzyme will transmit a wrong signal instructing the cell to divide when it normally should not divide—the result will be tumour formation. Following the discovery that the src oncogene codes for a protein kinase, more than 20 other oncogenes have also been found to code for protein tyrosine kinases. These oncogene encoded tyrosine kinases can be subdivided into two main classes such as receptor protein tyrosine kinases and non-receptor protein tyrosine kinases. Receptor protein tyrosine kinases are trans-membrane proteins that contain a growth factor receptor domain which are exposed on the outer surface of the plasma membrane and a tyrosine kinase catalytic domain at the inner surface of the plasma membrane. In a normal receptor of this type, first appropriate growth such as PDGF, EGF, binds with receptors site and activates protein tyrosine kinase domain. Activation of protein tyrosine kinase stimulates cell proliferation through activation of the membrane associated G protein Ras (Fig. 23.10).

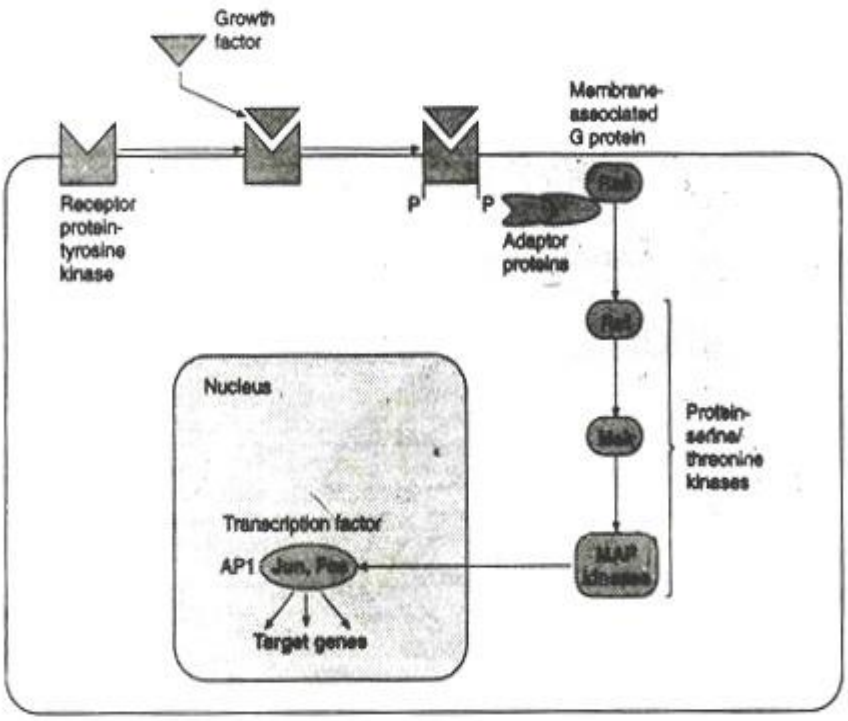

Fig. 23.10: Activation of protein tyrosine kinase that stimulates cell proliferation through activation of the membrane associated G protein Ras.

Activation of Ras triggers the phosphorylation of a series of cytoplasmic protein-serin/theronine kinase, thereby leading to phosphorylation of the nuclear API transcription factor which, in turn, activates genes involved in stimulating cell proliferation. Oncogenes can code for abnormal receptor protein- tyrosine kinases in which the growth factor binding site is disrupted leading to unregulated activity of the protein tyrosine kinase site.

Non-receptor protein tyrosine kinase are usually bound to the membrane's cytoplasm or free in the cytosol. The non-receptor protein tyrosine kinase is encoded by the src gene. Oncogene-encoded non-receptor kinases often show excessive unregulated protein-tyrosine kinase activity. Another group of oncogenes code for plasma membrane associated G proteins. In human cancer, ras oncogene shows almost resemblance with cellular ras gene of the host except that ras oncogene is the mutant form in contrast to cellular ras gene.

Hence mutant ras G proteins are produced. They retain bound GTP instead of hydrolyzing it to GDP. As a result mutant ras protein in its active form misleads the transmission of signal from external growth factors. Hence the host cells undergo abnormal cell division. Most of the protein kinase activity showed by mammalian cells catalyses the phosphorylation of the amino acids serine and threonine, not tyrosine. These protein-serine/threonine kinase like protein-tyrosine kinase can be encoded by oncogene.

The most important oncogene belonging to this group is the raf oncogene. It codes for a protein serine/threonine kinase that transmits signals from plasma membrane Ras protein to the cell interior. Some oncogenes code for proteins that function within the nucleus, particularly in the regulation of gene transcription. The examples of such oncogenes are the jun and fos oncogene which code for proteins that make up the AP! transcription factor.The AP_1 factor regulates the expression of a group of genes that are involved in stimulating cell proliferation. The myc oncogene, associated with several kinds of human cancer, also appears to code for a transcription factor.

Proto-Oncogene:

It is well-established that oncogenic virus contains a relatively small number of genes which has facilitated the identification of the viral genes that cause cell to become malignant. The first cancer-causing gene to be identified occurs in Rous sarcoma virus, a small retrovirus that produces sarcomas in chickens.An unexpected feature of retroviral oncogene is their lack of involvement in virus replication while other viral gene involves efficiently in the same process.

Again, the existence of viral oncogene is not an integral part of the virus life cycle. Therefore, the origin and existence of viral oncogene leads to a new line of investigation. Such investigations have led to the surprising discovery that the src gene is not present only in cancer cells. Using nucleic acid hybridisation techniques, it has been shown that DNA sequence that Eire homologous to— but not identical with—the Rous src gene can be detected in the genome of normal cells of a wide variety of organisms including salmon, mice, cows, birds and humans.

The unexpected discovery that cells contain DNA sequences that are closely related to viral oncogenes has been substantiated by studies on a variety of other tumour viruses and, in each case, they resemble genes present in the genome of normal cell. The term proto- oncogene has been introduced to refer to these normal cellular genes that closely resemble oncogenes. The resemblance of viral oncogenes to proto-oncogene suggests that viral oncogenes may have originally been derived from normal cellular genes. According to this concept, the first step in the creation of retro-viral oncogenes took place million years ago when the ancient virus infected cells and became integrated in the host chromosomal DNA adjacent to normal cellular proto-oncogenes. When the integrated pro-viral DNA was later transcribed to regenerate new viral RNA molecules, the adjacent proto-oncogene sequences might have been transcribed as well. In this way, a viral RNA molecule containing normal proto-oncogene sequences could have been created.

Since a proto-oncogene would initially serve no useful purpose for a virus, it would be free to mutate during subsequent cycles of viral infection. Such mutation would eventually convert proto-oncogene into an oncogene. Therefore, the realisation that oncogenic viruses contain genes that cause cell to become malignant raise the question of whether genetic alteration are also involved in non-virus induced cancers. The ability of many carcinogens to act as mutagens provides the reason to believe that genetic changes play a role in non-viral carcinogenesis.Besides this, recent research suggests that cellular oncogenes are derived from normal proto-oncogenes by at least five mechanisms:

(i) Point Mutation:

The simplest mechanism for converting a proto- oncogene into an oncogene, it involves a single base pair substitution or point mutation.

(ii) Local DNA Rearrangement:

The second mechanism for creating oncogenes is based on DNA rearrangements that cause either deletions or base sequence exchanges between proto-oncogene and surrounding genes.

(iii) Insertional Mutagenesis:

The evidence of third mechanism comes from the findings that some cancer-causing retrovirus lack oncogenes and these particular viruses cause cancer by integrating a DNA copy of their genetic information into a host chromosome in a region where a proto-oncogene is located and thus disrupt the structure of the host proto-oncogene and thereby convert it into an oncogene.

(iv) Gene Amplification:

The fourth mechanism for creating oncogenes uses gene amplification to increase the number of copies of a particular protogene. This overproduction of copies of a particular proto-oncogene leads to malignant transformation.

(v) Chromosomal Translocation:

The fifth mechanism for creating an oncogene involves chromosomal translocation. It is a process where a portion of one chromosome is physically broken and joined to another chromosome. As a result, the broken segment containing proto-oncogene is transferred from its normal location to a new location where it is converted as oncogene.

Tumour Suppressor Genes:

We have now seen how the presence of an oncogene can stimulate uncontrolled cell growth and division, thereby fostering the development of malignancy. Cancer can also be induced by the loss of tumour suppressor genes that normally inhibit cell proliferation. The term tumour suppressor gene implies that the normal function of gene of this type is to restrain cell growth and division. In other words, tumour suppressor genes act as brake on the process of cell proliferation and inhibit tumour development.

In many tumours these genes are lost or inactivated, thereby removing negative regulators of cell proliferation and contributing to the abnormal proliferation of tumour cells. Normally, the function of tumour suppressor gene is just opposite to oncogene. The first evidence of the activity of tumour suppressor gene came from somatic cell function experiment done by Henry Harris et al in 1969. The fusion of tumour cells with normal cell yields hybrids that contain chromosomes from both parents.

Such hybrids are usually non-tumerogenic. Suppression of tumourogenicity by cell fusion indicates that genes derived from the normal cell definitely suppress the tumour development. The first suppressor gene to be identified is involvement in hereditary retinoblastoma, a rare type of eye cancer that develops in children who have a family history of the disease. Such children inherit a chromosomal deletion in a specific region of one copy of chromosome 13.

Although the deletion occurs in all cells, only a few in the retina actually become malignant because the initial deletion in chromosome 13 does not cause cancer by itself; for cancer to develop, a subsequent mutation must also occur in the same region of the homologous chromosome 13.It has, therefore, been concluded that chromosome 13 contains a gene on homologous chromosome of a normal diploid cell where such gene normally functions to inhibit retinoblastomas. In inherited retinoblastoma one defected copy of gene is genetically transmitted. The loss of this single copy of gene is compensated by the identical second copy of the gene present on the same region of the second copy of chromosome 13.Therefore loss of a single copy of gene is not by itself sufl5cient to trigger tumour development, but retinoblastoma almost always develops in these individuals as a result of a second somatic mutation leading to further loss of the function of the remaining second copy of normal gene.

The gene lost in hereditary retinoblastoma is called RBI. It is a tumour suppressor gene that codes for the nuclear protein p^{RB} that inhibits expression of a group of genes whose products are needed for uncontrolled cell proliferation. In hereditary retinoblastoma a defective or copy of the RBI gene is inherited from the affected person. Hence a lack of p^{RB} resulting from loss of both copies of RBI (one due to deletion and other due to a second somatic mutation) can lead to uncontrolled proliferation which ultimately causes the development of

retinoblastoma. In nonhereditary cases, two normal RBI genes are inherited and retinoblastoma develops only if two somatic mutations in adult inactivate both copies of RBI in the same cell.

Table 23.6: Main classes of oncogenes categorised by nature of their Protein Products

Nature of Protein Product	Examples of Oncogenes	Comments
Growth factors	it sis	Platelet-derived growth factor (PDGF)
Protein-tyrosine kinases	*erb B*	Membrane receptor of epidermal growth factor (EGF)
	fms	Membrane receptor for colony-stimulating factor-1 (CSF-1)
	src, yes, fgr	Membrane nonreceptor proteintyrosine kinases
Membrane-associated G proteins	*ras*	Membrane-associated GTP-binding protein
	gsp	G_s(α sub-unit)
	gip	G_1(α sub-unit)
Protein-serine/threonine kinases	*raf, mos*	Cytoplasmic protein-serine/threonine kinases
Transcription factors	*jun, fos*	Components of AP1 transcription factor
	erb A	Thyroid hormone receptor

Following the discovery of the RBI gene several other tumour suppressor genes have been identified (Table 23.6). The second suppressor gene is p^{53} which is frequently inactivated in a wide variety of human cancer including leukemia's, lymphomas, sarcomas, bredn tumour and carcinomas of many tissues including breast, colon and lung. The p^{53} protein is a nuclear transcriptional factor that switches on the activity of genes that arrest cells in the G_1 phase of the cell cycle. Normally, the production of the p^{53} protein is stimulated when DNA is damaged due to exposure to ultraviolet ray or DNA damaging agents.

Hence p^{53} appears to act like a molecular policeman that checks the cell for DNA damage and prevents the cell from proliferation if damage is detected. The loss of p^{53} function allows the survival and reproduction of cells in which DNA damage has led to the production of oncogenes and/or the loss of other tumour suppressor genes. In addition to mediating cell cycle arrest P^{53} is required to apoptosis induced by DJNA damage. Unrepaired DNA damage normally induces apoptosis that eliminates cells which might develop into cancer. Cells lacking p fail to undergo apoptosis.

This failure contributes to the resistance of many tumours to chemotherapy. The failure of function of p is thought to account for the high frequency of p^{53} mutations that lead to

inactivation of p^{53}.Like p^{53}, the INK4 is a tumour suppressor gene that prevents lung cancer. Similarly, two other tumour suppressor genes such as APC and DCC prevent colon cancer. When these genes are deleted or mutated, such cancers develop. The product of RBI and INK4 tumour suppressor genes regulate cell cycle progression at the same point. These genes inhibit passage through the restriction point in G_1 by suppressing transcription of a number of genes involved in cell cycle progression and DNA synthesis.

A rare hereditary form of colon cancer, familial adenomatous polyposis, is produced due to inherited mutation of the APC gene. In this type of cancer hundreds of polyps or benign colon adenomas are produced within the colon of an individual. Some of these polyps are transformed into malignancy. Inactivated or mutated form of some additional tumour suppressor genes is also associated with the development of breast, ovarian and pancreatic carcinomas as well as in some rare inherited cancer syndromes such as Wilm's tumour (a childhood kidney tumour).The tumour suppressor gene of Wilm's tumour is WT1 which is frequently inactivated in Wilm s tumour. The product of WT1 gene appears to suppress transcription of a number of growth factor inducible genes.

Probable Questions:

1. Write down the differences between normal cells and cancerous cells.
2. Write down the types of cancers.
3. How cancer is developed?
4. Write down characteristics of cancerous cells.
5. What are the causes responsible for onset of cancer?

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7th Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwal.

UNIT-II

Tumour progression angiogenesis, invasion and metastasis; Cancer and environment-physical, chemical, biological carcinogens, Cancer therapy-radio-, chemo-and immunotherapy

Objective: In this unit we will discuss about Tumour progression angiogenesis, invasion and metastasis; Cancer and environment-physical, chemical, biological carcinogens, Cancer therapy-radio-, chemo-and immunotherapy

How Cancers Spread ?

When a doctor tells a person that he or she has cancer, one of the most important questions that need to be addressed is whether the cancer cells are still confined to their initial location. Once a tumour has invaded neighbouring tissues and begins to spread to other regions of the body, it becomes more difficult to treat.

The term metastasis refers to the spread of cancer cells via the bloodstream or lymphatic system to distant sites, where they form secondary tumours—called metastases—that are not physically connected to the primary tumour. Because they can arise in almost any vital organ, metastases rather than primary tumours are responsible for most cancer deaths.

Scientists have expended much effort in studying the cellular properties responsible for metastasis with the hope that a better understanding of the mechanisms involved will eventually lead to better treatments. Such studies have revealed that metastasis is a complex process that can be subdivided into several distinct stages, each involving a different set of cellular traits and interactions. Among the earliest steps in converting a tiny localized mass of cancer cells into an invasive, metastasizing tumour is the growth of blood vessels that penetrate into the tumour, supplying nutrients and oxygen to the cancer cells and removing waste products. In the absence of such a network of blood vessels, tumours cannot grow beyond a few millimeters in size and would not be a major health hazard.

Since the development of a sustaining network of blood vessels is therefore a crucial step in converting a tiny group of cancer cells into a larger tumour capable of spreading to distant sites.

Tumour Angiogenesis:

To survive and grow, all body tissues require a continual supply of oxygen and nutrients accompanied by the removal of carbon dioxide and other waste products. These needs are met by a system of blood vessels comprised of arteries that carry blood from the heart to the

rest of the body, veins that carry blood from the body back toward the heart, and tiny capillaries that connect the smallest arteries and veins.

The wall of a capillary is only a single cell layer thick, so oxygen and nutrients carried in the bloodstream can easily diffuse through capillary walls and nourish the surrounding tissues, and carbon dioxide and other waste products produced by tissues diffuse back into the capillaries for removal from the body.

Like the cells of any other tissue, tumour cells require a network of blood vessels to perform these same tasks. The vessels that feed and sustain tumours are produced by angiogenesis, a term that refers to the process by which new blood vessels sprout and grow from pre-existing vessels in the surrounding normal tissues.To understand how a tumour causes surrounding tissues to provide it with such a growing network of blood vessels, we first need to describe the process of normal angiogenesis and the factors that control it.

i. Angiogenesis is Prominent in Embryos but Relatively Infrequent in Adults:

Angiogenesis is a normal biological event that occurs at specific times for specific purposes. For example, a developing embryo in a mother's womb must create the vast network of arteries, veins, and capillaries that are needed for a mature circulatory system. To initiate blood vessel formation, the embryo first creates a primary population of cells, called endothelial cells that form the inner lining of blood vessels.

As part of this process of vasculogenesis, the newly created endothelial cells are organized into a primitive network of channels representing the major blood vessels of the circulatory system. Once the primordial network of vessels has been created, angiogenesis takes over.

Angiogenesis involves an extensive phase of growth and proliferation of pre-existing endothelial cells, which form buds that sprout from existing vessels and develop into an interconnected network of new vessels (Figure 1).

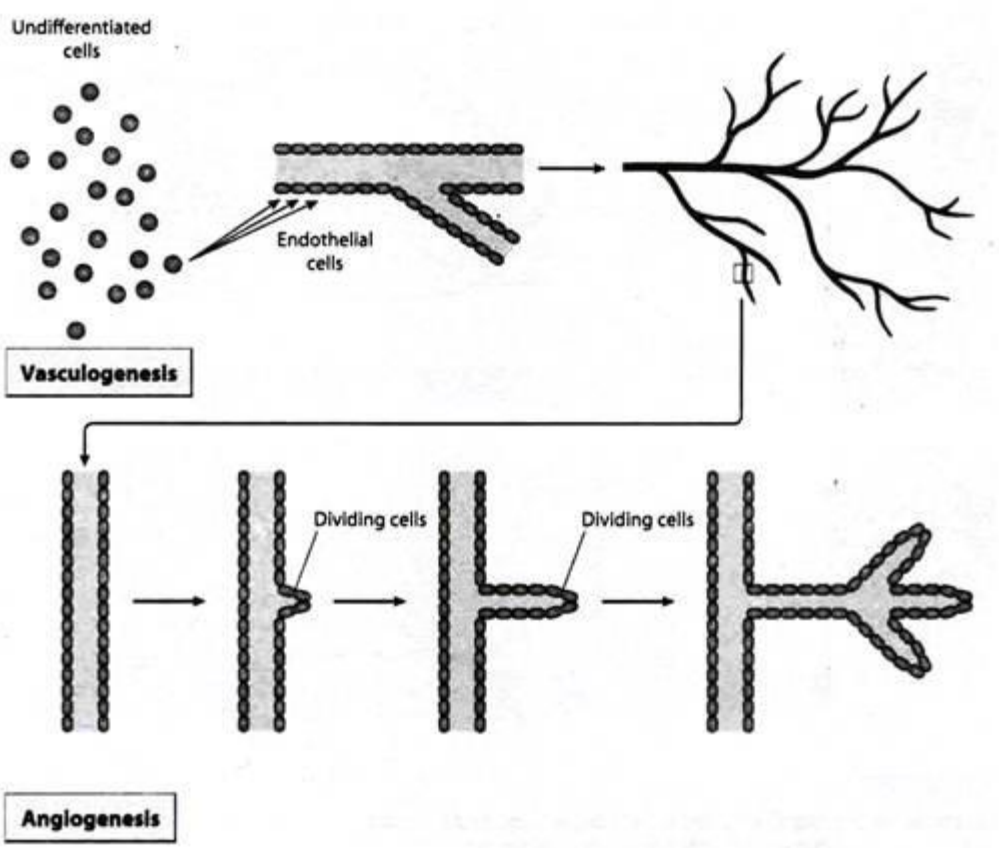

Figure 1 Vasculogenesis and Angiogenesis. (*Top*) During vasculogenesis, which occurs mainly during embryonic development, undifferentiated cells are converted into endothelial cells that organize themselves into a network of channels representing the major blood vessels. (*Bottom*) Angiogenesis refers to the growth and proliferation of the endothelial cells that line the inner surface of existing blood vessels, forming buds that sprout from the vessel wall and develop into new vessels.

Although vasculogenesis is restricted to early embryonic development, angiogenesis continues to occur after birth when additional blood vessels are required. In adults, who have a fully formed circulatory system, this need for new vessels is limited to a few special situations and endothelial cells rarely divide, doing so about once every three years on average. When new vessels are required, however, endothelial cell division can be stimulated and angiogenesis will take place.

For example, blood vessel growth is needed each month in the inner lining of the uterus as part of the normal menstrual cycle. Angiogenesis is therefore activated a few days each month in the uterine lining of women of reproductive age. In both males and females, angiogenesis is also called upon anytime an injury requires new blood vessels for wound healing and tissue repair.

Angiogenesis needs to be precisely regulated in such cases, turning on for a short time and then stopping. Regulation is accomplished through the use of both activator and inhibitor

molecules. Normally the inhibitors predominate, blocking angiogenesis. When a need for new blood vessels arises, angiogenesis activators increase in concentration and inhibitors decrease, triggering the proliferation of endothelial cells and the formation of new vessels. As we will see shortly, many of these regulatory molecules were first identified through the study of angiogenesis triggered by cancer cells.

ii. Angiogenesis is Required for Tumours to Grow beyond a Few Millimeters in Diameter:

For more than 100 years, scientists have known that tumours are supplied with a dense network of blood vessels. Some investigators initially believed that these blood vessels were pre-existing vessels that had expanded in response either to the increased metabolic activity of tumours or to toxic products that tumours release.

Others thought that the vessels were new structures formed as part of an inflammatory response designed to defend the host against the tumour. Then in 1971, Judah Folkman proposed a radical new idea regarding the significance of blood vessels in tumour development. He suggested that tumours release signalling molecules that trigger the growth of new blood vessels in the surrounding host tissues and that these new vessels are required to sustain tumour growth.

This concept was initially based on experiments in which cancer cells were grown in isolated organs under artificial laboratory conditions. In one such experiment, illustrated in Figure 2 (left), a normal thyroid gland was removed from a rabbit and placed in a glass chamber. A small number of cancer cells were then injected into the gland and a nutrient solution was pumped into the organ to keep it alive. The cancer cells divided for a few days but suddenly stopped when the tumour mass reached a diameter of 1 to 2 millimeters. Virtually every tumour stopped growing at exactly the same size, suggesting that some kind of limitation allowed them to grow only so far.

When tumour cells were removed from the thyroid gland and injected back into animals, cell proliferation resumed and massive tumours developed. Why did the tumours stop growing at a tiny size in the isolated thyroid gland and yet grow in an unrestrained fashion in live animals? On closer examination, a possible explanation became apparent.The tiny tumours, alive but dormant in the isolated thyroid gland, had failed to link up to the organs blood vessels; as a result, the tumours stopped growing when they reached a diameter of 1 to 2 mm. When injected into live animals, these same tumours became infiltrated with blood vessels and grew to an enormous size.

To test the theory that blood vessels are needed to sustain tumour growth, Folkman implanted tumour cells in the anterior chamber of a rabbit's eye, where there is no blood

supply. As shown in Figure 2 (right), cancer cells placed in this location survived and formed tiny tumours, but blood vessels from the nearby iris could not reach the cells and the tumours quickly stopped growing.

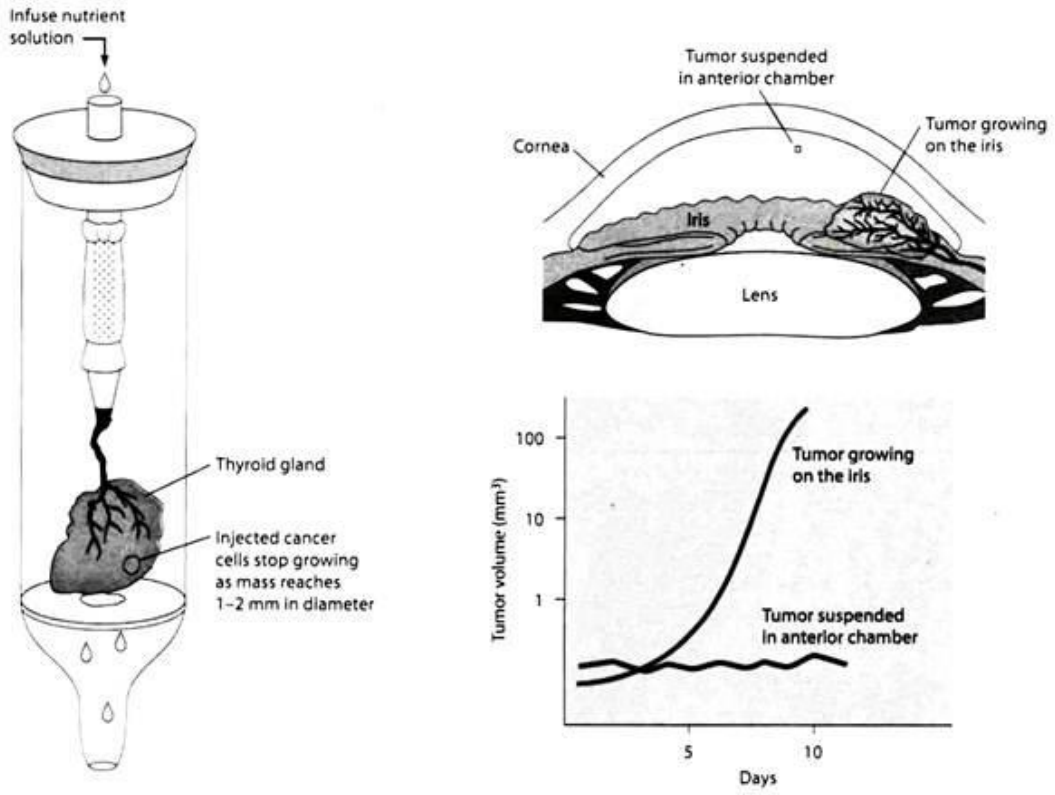

Figure 2 Two Experiments Showing That Tumor Growth Depends on Angiogenesis. (*Left*) Cancer cells were injected into an isolated rabbit thyroid gland that was kept alive by pumping a nutrient solution into its main blood vessel. The tumor cells fail to link up to the organ's blood vessels and the tumor mass stops growing when it reaches a diameter of roughly 1 to 2 millimeters. (*Right*) Cancer cells were either injected into the liquid-filled anterior chamber of a rabbit's eye, where there are no blood vessels, or were placed directly on the iris. Tumor cells in the anterior chamber, nourished solely by diffusion, remain alive but stop growing before the tumor mass reaches 1 millimeter in diameter. In contrast, blood vessels quickly infiltrate the cancer cells implanted on the iris, allowing the tumors to grow to thousands of times their original mass. [Adapted from J. Folkman, *Sci. Amer.* 234 (May 1976): 58.]

When such chambers are implanted into animals, new capillaries begin to proliferate in the surrounding host tissue. In contrast, normal cells placed in the same type of chamber do not stimulate blood vessel growth. The most straightforward interpretation is that cancer cells produce molecules that diffuse through the tiny pores in the filter and activate angiogenesis in the surrounding host tissue.

The next job was to identify the molecules responsible for stimulating angiogenesis, a task that occupied many investigators over a span of several decades. This intensive effort eventually led to the identification of more than a dozen proteins, as well as several smaller molecules, that can activate angiogenesis (Table 1).

Table 1 Some Natural Stimulators and Inhibitors of Angiogenesis

Stimulators
PROTEINS
Angiogenin
Epidermal growth factor
Fibroblast growth factor (FGF)
Granulocyte colony-stimulating factor
Hepatocyte growth factor
Interleukin 8
Placental growth factor
Platelet-derived endothelial growth factor
Transforming growth factor alpha
Tumor necrosis factor alpha
Vascular endothelial growth factor (VEGF)
SMALL MOLECULES
Adenosine
1-Butyryl glycerol
Nicotinamide
Prostaglandins E1 and E2
Inhibitors
PROTEINS
Angiostatin
Canstatin
Endostatin
Interferons
Platelet factor 4
Prolactin 16Kd fragment
Protamine
Thrombospondin
TIMP-1 (tissue inhibitor of metalloproteinase-1)
TIMP-2 (tissue inhibitor of metalloproteinase-2)
TIMP-3 (tissue inhibitor of metalloproteinase-3)
Tumstatin

Two of the proteins, vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), appear to be especially important for sustaining tumour growth. VEGF and FGF are produced by many kinds of cancer cells (and certain types of normal cells), and they trigger angiogenesis by binding to specific receptor proteins located on the surface of endothelial cells.

To see how this process works, let us briefly focus on VEGF (Figure 5). VEGF is produced by the majority of tumours and is secreted into the surrounding tissues. When VEGF molecules encounter an endothelial cell, they bind to and activate VEGF receptors located on the endothelial cell surface.

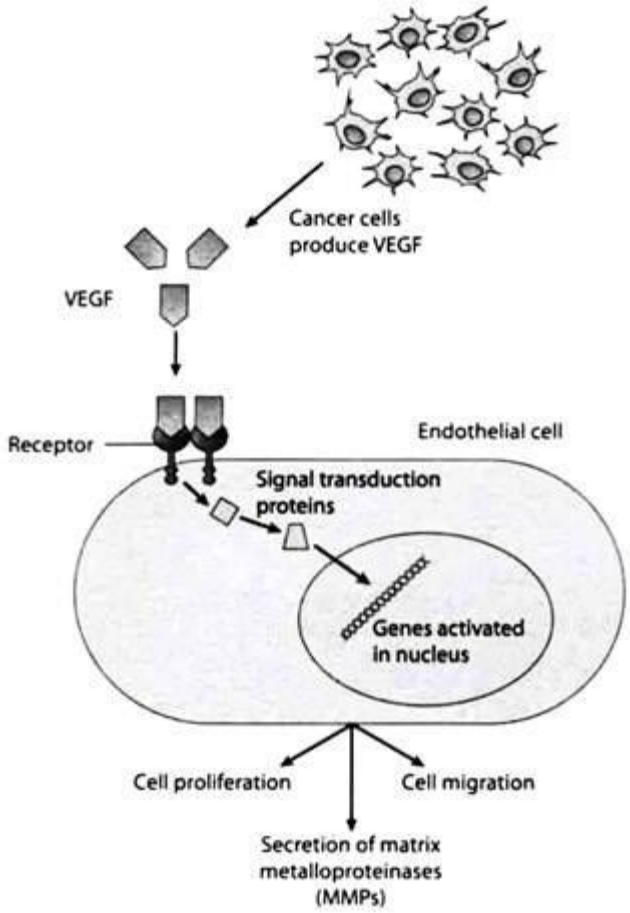

Figure 5 Main Steps Involved in Triggering Angiogenesis by VEGF. Cancer cells secrete VEGF molecules that bind to receptor proteins located on the surface of endothelial cells. The binding of VEGF to its receptor leads to the activation of a series of signal transduction proteins that trigger changes in gene expression and cell behavior. The net result is the stimulation of endothelial cell proliferation and migration as well as the secretion of MMPs that degrade components of the extracellular matrix. The proliferating endothelial cells are gradually organized into new networks of blood vessels. (The drawing of the endothelial cell is enlarged relative to the size of the cancer cells to illustrate the signaling pathway.)

The signal is then relayed from the activated receptors to a sequential pathway of signal transduction proteins that trigger changes in cell behavior and gene expression. As a result, endothelial cells begin to proliferate and to produce matrix metalloproteinases (MMPs), a group of protein-degrading enzymes that are released into the surrounding tissue.The MMPs break down components of the extracellular matrix that fills the spaces between neighbouring cells, thereby allowing the endothelial cells to migrate into the surrounding tissues. As they migrate, the proliferating endothelial cells become organized into hollow tubes that evolve into new networks of blood vessels.

Although many tumours produce VEGF or FGF, they are not the sole explanation for the activation of angiogenesis. For angiogenesis to proceed, these molecules must overcome the

effects of angiogenesis inhibitors that normally restrain the growth of blood vessels. More than a dozen naturally occurring inhibitors of angiogenesis have been identified (see Table 1), including the proteins angiostatin, endostatin, and thrombospondin.

A finely tuned balance between the concentration of these angiogenesis inhibitors and the concentration of activators (such as VEGF and FGF) determines whether a tumour will induce the growth of new blood vessels. When tumours trigger angiogenesis, it is usually accomplished by increasing the production of angiogenesis activators and, at the same time, decreasing the production of angiogenesis inhibitors.

iii. Inhibitors of Angiogenesis can Restrain Tumour Growth and Spread:
The discovery of angiogenesis inhibitors has caused scientists to speculate about the potential usefulness of such molecules. If sustained tumour development requires the proliferation of new blood vessels, using angiogenesis inhibitors to block vessel formation might be useful for slowing tumour growth.

This approach has been quite effective when tested in mice. In one striking study, mice with several types of cancer were injected with the angiogenesis inhibitor endostatin. After a few cycles of treatment, the primary tumours virtually disappeared (Figure 6).

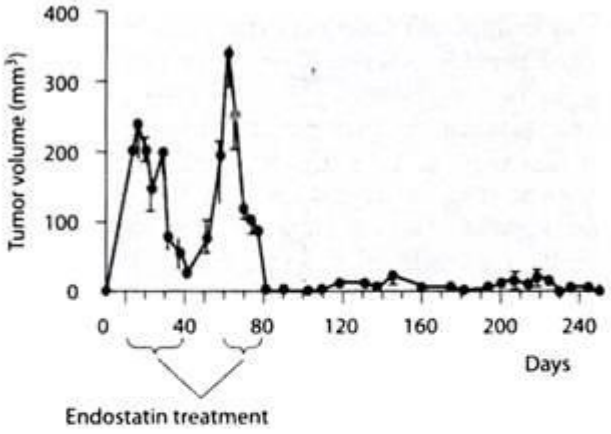

Figure 6 Treating Cancer by Inhibiting Angiogenesis. In this experiment, cancer cells were allowed to grow for about ten days to form a large tumor in mice. The mice were then injected with an angiogenesis inhibitor, endostatin, until the tumor regressed. After allowing the tumor to grow again in the absence of endostatin, a second treatment cycle was given. After the second treatment was stopped, the tumor no longer grew again. [Data from T. Boehm et al., *Nature* 390 (1997): 404.]

Studies involving mice with mutations that hinder angiogenesis have provided additional support for the idea that tumour growth can be restrained by inhibiting angiogenesis. As shown in Figure 7 (left), injecting breast cancer cells into such angiogenesis-deficient mutant

mice leads to the formation of tumours that grow for a short time and then completely regress.

In contrast, normal mice injected with the same breast cancer cells die of cancer within a few weeks. When lung cancer cells are injected into the same mutant mice, the results are slightly different. Unlike breast cancer cells, lung cancer cells do develop into tumours in the angiogenesis-deficient mice, but the tumours grow more slowly than in normal mice and fail to metastasize to other organs.

The failure of these lung cancer cells to metastasize in angiogenesis-deficient mice raises the possibility that angiogenesis-inhibiting drugs might be useful in preventing metastasis. In an experiment designed to address this issue, shown in Figure 7 (right), cancer cells were injected beneath the skin of laboratory mice and allowed to grow for two weeks.

The primary tumours were then removed and the animals were monitored for several weeks to see whether visible metastases would appear in other organs. Within a few weeks the average mouse developed almost 50 lung tumours, which arose from cancer cells that had spread to the lungs prior to removal of the primary tumour. In contrast, mice treated with angiostatin developed an average of only two or three tumours in their lungs, indicating that the angiogenesis inhibitor had reduced the rate of metastasis about 18-fold.Studies involving mice with mutations that hinder angiogenesis have provided additional support for the idea that tumour growth can be restrained by inhibiting angiogenesis. As shown in Figure 7 (left), injecting breast cancer cells into such angiogenesis-deficient mutant mice leads to the formation of tumours that grow for a short time and then completely regress.

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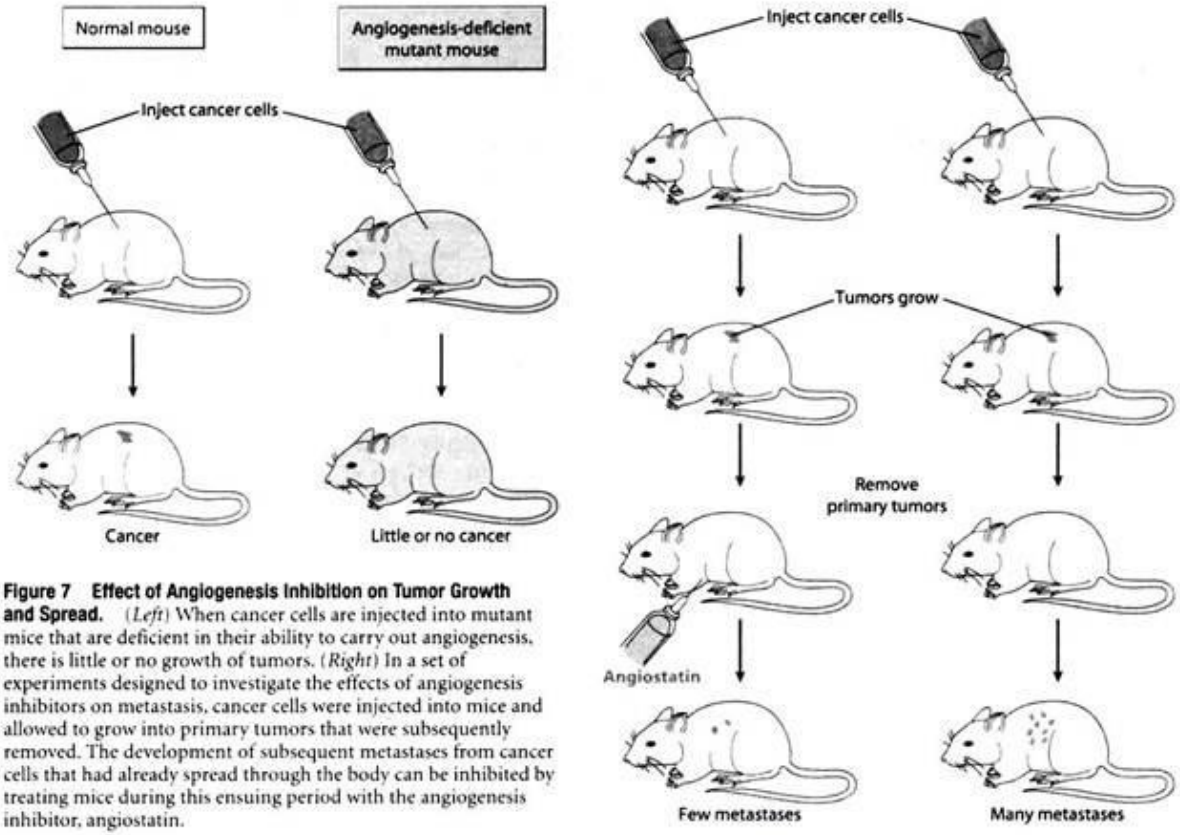


Figure 7 Effect of Angiogenesis Inhibition on Tumor Growth and Spread. (*Left*) When cancer cells are injected into mutant mice that are deficient in their ability to carry out angiogenesis, there is little or no growth of tumors. (*Right*) In a set of experiments designed to investigate the effects of angiogenesis inhibitors on metastasis, cancer cells were injected into mice and allowed to grow into primary tumors that were subsequently removed. The development of subsequent metastases from cancer cells that had already spread through the body can be inhibited by treating mice during this ensuing period with the angiogenesis inhibitor, angiostatin.

Such observations provide a possible explanation for the phenomenon of tumour dormancy, in which cancer cells spread from a primary tumour to another organ and form tiny clumps of cancer cells that remain dormant for prolonged periods of time. One factor that might contribute to tumour dormancy is that these tiny tumours, called micrometastases, may not have triggered the angiogenesis that is needed for tumour growth beyond a small size.

Animal studies suggest one possible reason- Some primary tumours produce large amounts of angiostain that spill over into the bloodstream and circulate throughout the body. It has been hypothesized that this circulating angiostatin can inhibit angiogenesis at other sites, thereby preventing micrometastases from growing into visible tumours.

Why wouldn't the angiostatin also prevent the angiogenesis that is needed for growth of the primary tumour? Most likely, the inhibitory effects of angiostatin on the primary tumour are overcome by the stimulatory effects of VEGF, which is also produced in large amounts by many primary tumours.Unlike angiostatin, however, VEGF is quickly destroyed when it enters the bloodstream. So angiostatin, but not VEGF, circulates in the bloodstream and might block angiogenesis at sites of micrometastases, which do not yet produce enough VEGF of their own to overcome the inhibitory effects of the circulating angiostatin.

Although the proposed role of angiogenesis inhibitors in tumour dormancy remains to be firmly established, the overall body of evidence strongly supports the idea that angiogenesis inhibitors can restrain the growth and spread of cancer cells in animals. Are such findings relevant to humans? To address this question, numerous angiogenesis- inhibiting drugs are being tested in cancer patients and one such drug, called Avastin, has already been approved for use in treating colon cancer.

2. Invasion and Metastasis:

Once angiogenesis has occurred at the site of a primary tumour, the stage is set for tumour cells to invade neighboring tissues and spread to distant sites. A few kinds of cancer, such as non-melanoma skin cancers, rarely invade and metastasize. About half the people who develop other forms of cancer, however, have tumours that have already begun to spread beyond the site of origin by the time the cancer is diagnosed.

Because cancer is much harder to treat after it has spread, this alarming statistic points to the importance of better procedures for early cancer detection. It might also be possible to develop improved treatments for cancer if we better understood the mechanisms that allow cancer cells to spread, the topic to which we now turn.

i. Spreading of Cancer Cells by Invasion and Metastasis is a Complex, Multistep Process:

Cancers spread through the body via two distinct mechanisms:

i. Invasion and

ii. Metastasis.

Invasion refers to the direct migration and penetration of cancer cells into neighboring tissues, whereas metastasis involves the ability of cancer cells to enter the bloodstream (or other body fluids) and travel to distant sites, where they form new tumours that are not physically contiguous with the primary tumour.

Metastasis involves a complex cascade of events, beginning with the process of angiogenesis that has already been described. The events following angiogenesis can be grouped into three main steps. First, cancer cells invade surrounding tissues and penetrate through the walls of lymphatic and blood vessels, thereby gaining access to the bloodstream. Second, these cancer cells are then transported by the circulatory system throughout the body. And third, the cancer cells leave the bloodstream and enter particular organs, where they establish new metastatic tumours (Figure 8).

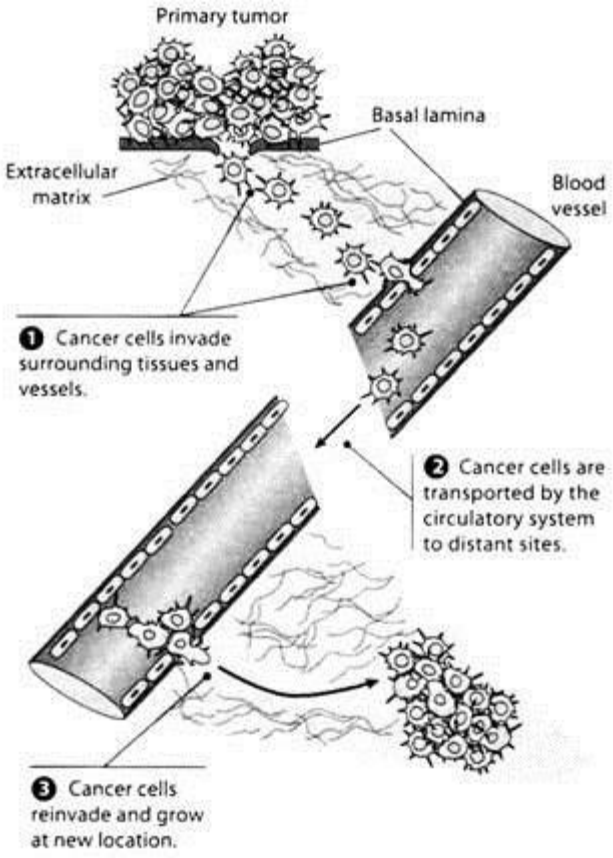


Figure 8 Steps Involved in the Process of Metastasis. Only a small fraction of the cells in a primary malignant tumor can successfully carry out all the steps required for metastasis. During step ①, cancer cells adhere to and invade through the basal lamina, degrade the extracellular matrix of the underlying tissue to open up a path through which the cells can move, and finally adhere to and invade through the basal lamina of a tiny vessel, thereby entering the circulatory system. During step ②, the cancer cells are transported via the bloodstream to distant sites. In step ③, the cancer cells become arrested in a capillary located in another organ, adhere to and invade through the basal lamina of the capillary wall, enter the surrounding tissue, and start to grow again.

If cells from the initial tumour fail to complete any of these steps, or if any of the steps can be prevented, metastasis will not occur. It is therefore crucial to understand how the properties of cancer cells make these three steps possible.

ii. Changes in Cell Adhesion, Motility, and Protease Production allow Cancer Cells to Invade Surrounding Tissues and Vessels:

The initial step leading to metastasis is the invasion of surrounding tissues and vessels by cancer cells. Thus, unlike the cells of benign tumours or most normal cells, which remain together in the location where they are formed, cancer cells are capable of leaving their original site and penetrating through surrounding tissue barriers, eventually entering the circulatory system.

Several mechanisms make this invasive behavior possible. The first involves changes in the adhesive forces between cells. In most tissues, adjoining cells are held together by binding interactions between cell-cell adhesion proteins found on the outer surface of each cell. These adhesion molecules, which normally function to keep cells in place, are often missing or deficient in cancer cells, thereby allowing cells to separate from the main tumour mass more readily.

The diminished adhesiveness of cancer cells can be readily demonstrated by a simple experiment: If you take a sample of cancer tissue, suspend it in a fluid-filled flask, and shake the flask vigorously, the cells will separate from one another more readily than cells obtained from a comparable sample of normal tissue.In many cases, the reduced adhesiveness of cancer cells can be traced to the loss of E-cadherin, a cell-cell adhesion protein that normally binds epithelial cells to one another. Highly invasive cancers usually have less E-cadherin than normal cells, suggesting a relationship between the loss of cadherins and the ability to invade.

Support for this idea has come from studies in which noninvasive populations of cancer cells were treated with antibodies that block the function of E-cadherin. Such treatment gives cancer cells the ability to invade. Conversely, restoring E-cadherin to cancer cells lacking this molecule inhibits their ability to form invasive tumours when the cells are injected back into animals. A second property involved in tumour invasion is cell motility, which is activated after the loss of cell-cell adhesion permits cancer cells to detach from one another. Cancer cells possess all the normal cytoplasmic machinery required for cell locomotion, but their actual movement needs to be stimulated by signalling molecules produced either by surrounding host tissues or by cancer cells themselves. Besides activating cell motility, some of these signalling molecules act as chemo attractants that guide cell movement by serving as attracting signals toward which the cancer cells will migrate.

In addition to decreased adhesiveness and activated motility, a third property involved in invasion is the production of proteases (protein-degrading enzymes). The purpose of these enzymes is to break down structures that would otherwise represent barriers to cancer cell movement. For example, epithelial cells, the source of about 90% of all human cancers, are separated from underlying tissues by a thin, dense layer of protein-containing material called the basal lamina. Before epithelial cancers can invade adjacent tissues, the basal lamina must first be breached. Cancer cells break through this barrier by producing proteases that facilitate degradation of the proteins that form the backbone of the basal lamina.

One such protease is plasminogen activator, an enzyme that converts the inactive precursor plasminogen into the active protease plasmin (Figure 9). Because high concentrations of plasminogen are present in almost all tissues, small amounts of plasminogen activator released by cancer cells can quickly catalyze the formation of large quantities of plasmin.

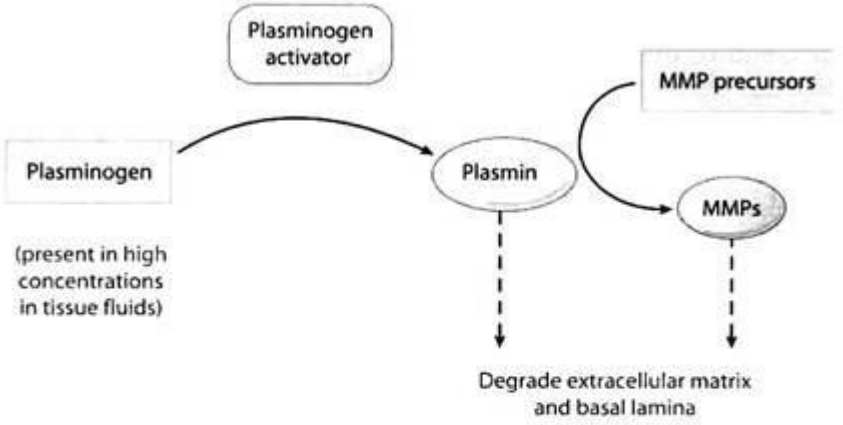


Figure 9 Proteases Involved in Degrading the Basal Lamina and Extracellular Matrix. Most cancer cells produce plasminogen activator, an enzyme that catalyzes the conversion of the inactive precursor, plasminogen, into the active protease, plasmin. Because plasminogen is present in high concentrations in almost all tissues, a small amount of plasminogen activator can produce large quantities of plasmin. The plasmin degrades components of the basal lamina and the extracellular matrix, thereby promoting invasion, and also converts inactive precursors of matrix metalloproteinases (MMPs) into active forms, thereby creating additional proteases that can degrade the basal lamina and extracellular matrix. The MMP precursors are produced to a large extent by surrounding host cells rather than by tumor cells.

The plasmin in turn performs two tasks:

(1) It degrades components of the basal lamina and the extracellular matrix, thereby facilitating tumour invasion; and

(2) It cleaves inactive precursors of matrix metalloproteinases (MMPs), produced mainly by surrounding host cells, into active enzymes that also degrade components of the basal lamina and extracellular matrix.

After proteases allow the basal lamina to be penetrated, they degrade the matrix of the underlying tissues to open up paths through which the cancer cells can move. The cancer cells migrate until they reach tiny blood or lymphatic vessels, which are also surrounded by a basal lamina. The proteases then digest holes in this second basal lamina, allowing cancer cells to pass through it and through the layer of endothelial cells that form the vessel's inner lining, at which point the cancer cells have finally gained entry into the circulatory system.

iii. Relatively Few Cancer Cells Survive the Voyage through the Bloodstream:

Cancer cells that penetrate through the walls of tiny blood vessels gain direct entry to the bloodstream, which then transports the cells to distant parts of the body (Figure 8, step ②). When cancer cells initially penetrate the walls of lymphatic vessels rather than blood capillaries, the cells are first carried to regional lymph nodes, where they may become lodged and grow.

For this reason, regional lymph nodes are a common site for the initial spread of cancer. Nonetheless, lymphatic vessels have numerous interconnections with blood vessels, so cancer cells that initially enter into the lymphatic system eventually arrive in the bloodstream and circulate throughout the body.

Regardless of their initial entry route, the net result is often large numbers of cancer cells in the bloodstream. Even a tiny malignant tumour weighing only a few grams can release several million cancer cells into the circulatory system each day. To produce metastases, however, the cells must survive the trip through the circulatory system, and most cancer cells do not thrive in such an environment.

Evidence for this conclusion has come from experiments in which cancer cells were radioactively labelled (to allow them to be identified) and then injected into the bloodstream of laboratory animals. After a few weeks, less than one in a thousand radioactive cells was found to be alive. Apparently the bloodstream is an inhospitable place for most cancer cells and only a tiny number survive the trip to potential sites of metastasis.

iv. The Ability to Metastasize Differ among Cancer Cells and Tumours:

Since only a tiny fraction of the cancer cells that enter the bloodstream survive and establish metastases, the question arises as to whether these cells are random members of the original tumour population or specialized cells better suited for metastasis. Figure 10 illustrates an experiment designed by Isaiah Fidler to address this question. In these studies, mouse melanoma cells were injected into the bloodstream of healthy mice to study the ability of the cells to metastasize.

A few weeks after the injections, metastases were detected in a variety of locations, but mainly the lungs. Cells from the lung metastases were removed and injected into another mouse, leading to the production of more lung metastases. By repeating the same procedure many times in succession, Fidler eventually obtained a population of cancer cells that formed greater numbers of lung metastases than did the original tumour cell population.

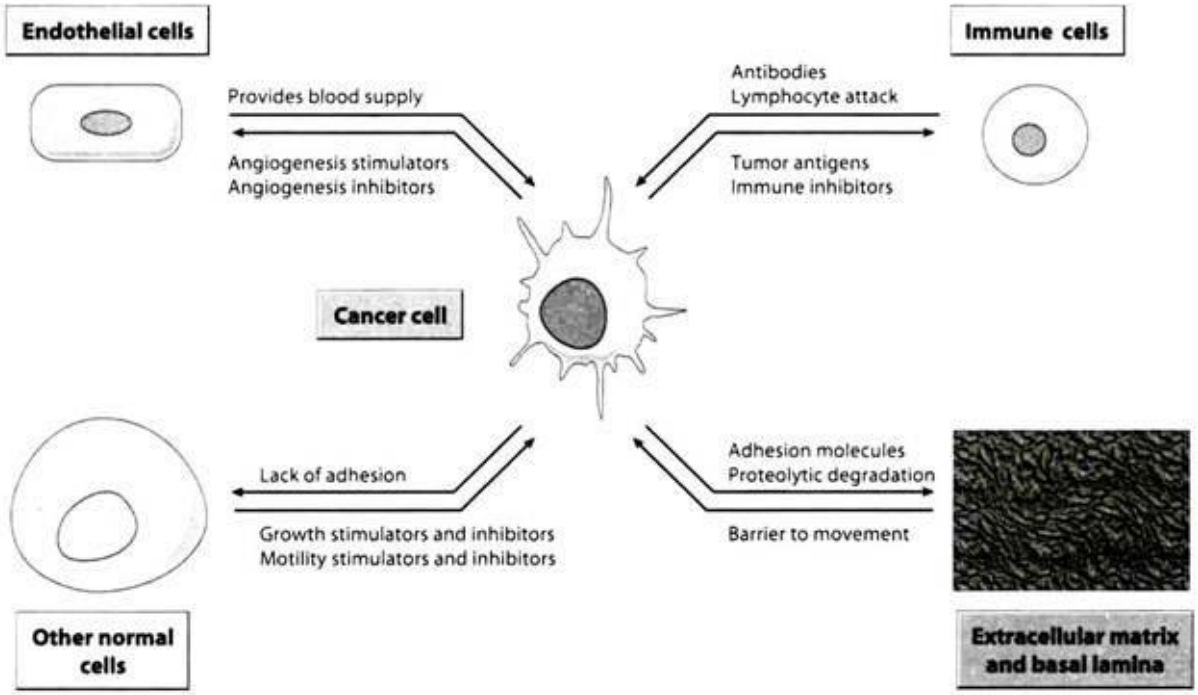


Figure 13 Tumor-Host Interactions. The behavior of malignant tumors depends not just on the traits of cancer cells, but also on interactions between cancer cells and normal cells of the host. This diagram summarizes a few of the many such interactions that affect the development of cancer.

The most straightforward interpretation was that the initial melanoma consisted of a heterogeneous population of cells with differing metastatic capabilities and that the successive experiments had gradually selected for those cells that are especially well suited for metastasizing. To test this hypothesis, studies were subsequently performed in which single cells were isolated from a primary melanoma and each isolated cell was allowed to grow in culture to form a separate population of cells.

Such cell populations, each derived from the proliferation of a single initial cell, are referred to as clones. When the various cloned populations of cells were injected into animals, some of the clones produced few metastases, some produced numerous metastases, and some fell in between. Since each clone was derived from a different cell in the original tumour, the results support the idea that the cells in a primary tumour differ in their ability to metastasize.It has been known for many years that human cancers of the same type can differ significantly in their ability to metastasize. In one set of experiments, investigators analyzed gene activity in lung cancers and discovered a pattern involving the expression of 17 genes that predicted whether a primary lung cancer was likely to metastasize.

This same gene-expression “signature” also predicts the metastatic behavior of other types of cancer. For example, prostate or breast cancer patients whose primary tumours exhibit the 17-gene signature are more likely to develop metastases than individuals whose tumours do

not exhibit the 17-gene signature. Thus, the likelihood that metastasis will occur appears to be genetically programmed into the cells of the primary tumour.

v. Blood-Flow Patterns often Dictate Where Cancer Cells will Metastasize:

After traveling through the circulatory system to distant parts of the body, cancer cells exit from the bloodstream and invade organs that may be located far from the primary tumour.

Although the bloodstream carries cancer cells everywhere in the body, the final distribution of metastases is not random, nor is it the same for every type of cancer, Instead, cancers arising in each organ preferentially metastasize to particular locations. For example, stomach and colon cancers frequently metastasize to the liver, prostate and breast cancers often metastasize to bone, and many forms of cancer tend to metastasize to the lungs.

One factor underlying these distinctive relationships is the pattern of blood flow in the circulatory system, which determines where cancer cells floating in the bloodstream are likely to become lodged. Based solely on size considerations, the most probable site for circulating cancer cells to become stuck is in capillaries—the tiny vessels whose diameter is generally no larger than that of a single blood cell. Because they are usually larger than blood cells, circulating cancer cells often become lodged in tiny capillaries as they move through the body. The arrested cancer cells may then adhere to and penetrate through the capillary walls and invade the surrounding organ, beginning the formation of a new metastatic tumour.

The preceding scenario suggests that after a cancer cell has entered the bloodstream, it is susceptible to becoming arrested in the first capillary bed it encounters. Figure 11 shows that for most primary tumours, the first such capillary bed will be in the lungs, which may help explain why the lungs are a relatively frequent site of metastasis for many kinds of cancer.

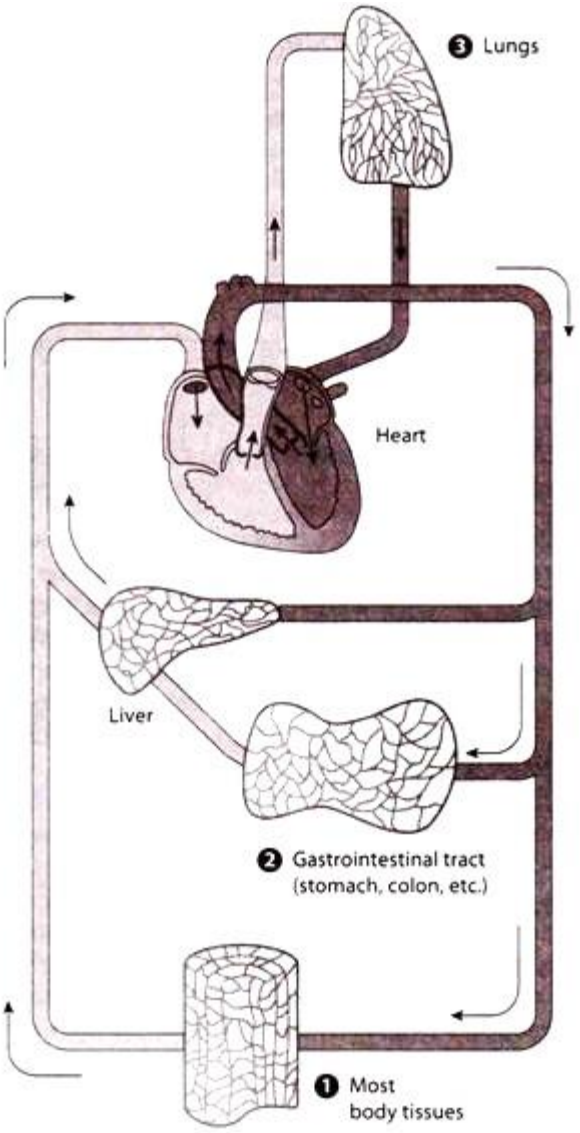


Figure 11 Blood-Flow Patterns in the Circulatory System. When cancer cells enter the bloodstream, they tend to get caught in the first downstream capillary bed they encounter. ① For primary tumors arising in most locations, the bloodstream first carries the cells to the heart and from there to the lungs, where the first capillary bed is encountered. ② For cancers arising in the gastrointestinal tract, the bloodstream first carries the cells to the liver, where the vessels break up into capillaries. This anatomy explains why the liver is a common site of metastases for stomach and colon cancers. ③ For cancers arising in the lung, the bloodstream carries the cells to the left side of the heart and from there to capillary beds that can be located anywhere in the body.

However, blood-flow patterns do not always point to the lungs. For cancers arising in the stomach and colon, cancer cells that enter the bloodstream are first carried to the liver, where the vessels break up into a bed of capillaries. As a result, the liver is a common site of

metastasis for stomach and colon cancers. Finally, when cancer arises in the lung, cancer cells entering the bloodstream will flow first to the left side of the heart and from there to capillary beds located throughout the body. It is therefore not surprising that lung cancers metastasize too many different organs, including the liver, bones, brain, kidney, adrenal gland, thyroid, and spleen.

vi. Organ-Specific Factors Play a Role in Determining where Cancer Cells will Metastasize:

Although blood-flow patterns are clearly important, they do not always explain the observed distribution of metastases. As early as 1889, Stephen Paget proposed that the nonrandom distribution of metastases arises in part because individual cancer cells have a special affinity for the environment provided by particular organs. Paget's idea is often referred to as the "seed and soil" hypothesis, based on his analogy that when a plant produces seeds, they are carried by the wind in all directions but they only grow if they fall on congenial soil.

According to this view, cancer cells are carried to a variety of organs by the bloodstream, but only a few sites provide an optimal environment for the growth of a particular type of cell. In other words, metastasis only takes place where the seed (a cancer cell) and the soil (a particular organ) are compatible. Support for the "seed and soil" concept has come from a systematic analysis of the sites to which various human cancers tend to metastasize. For roughly two-thirds of the cancer types examined, the rates of metastasis to each organ can be explained solely on the basis of blood-flow patterns. Of the remaining cases, some kinds of cancers metastasize to particular organs less frequently than would be expected and others metastasize to particular organs more frequently than would be expected.

Animal experiments have revealed that this non- random behavior can be traced to the properties of individual cancer cells. In one set of experiments, similar to those shown in Figure 10, mouse melanoma cells were injected into normal mice and metastases were isolated from the brain instead of the lung. The metastatic cells were then injected into another healthy mouse, and the same cycle was repeated again and again. Even though the melanoma cells employed in this study initially metastasized more often to the lung than they did to the brain, the repeated selection of metastatic cells from the brain eventually led to the isolation of cells that preferentially metastasize to the brain rather than to the lung.

Similar experiments involving the selection of cells derived from ovarian metastases yielded cells that preferentially metastasize to the ovary rather than to the lung or brain. Hence the initial tumour must have consisted of a heterogeneous population of cells that differ in the sites to which they tend to metastasize.Why do individual cancer cells grow best at particular sites? The general answer is that the ability to grow in different locations is affected by interactions between cancer cells and molecules present in the organs to which they are

delivered. An example of this principle is provided by prostate cancer cells, which preferentially metastasize to bone (a pattern that would not be predicted based on blood flow).

To investigate the reason for this preference, experiments have been performed in which prostate cancer cells were mixed together with cells from various locations—including bone, lung, and kidney—and the cell mixtures were then injected into animals. It was found that the ability of prostate cancer cells to develop into tumours was stimulated by the presence of cells derived from bone, but not from lung or kidney. Subsequent studies uncovered a possible explanation- Bone cells produce growth factors that stimulate the proliferation of prostate cancer cells. This example illustrates just one of several kinds of molecular interactions that influence the ability of cancer cells to grow in particular organs.

vii. Some of the Cellular Properties Involved in Invasion and Metastasis Arise during Tumour Progression:

After tumour cells have left the bloodstream and invaded an appropriate organ where conditions for their growth are favorable, the cells begin proliferating at the new site. Before a metastatic tumour can grow beyond a few millimeters in diameter, however, it must first trigger angiogenesis (as was the case for the primary tumour).Some metastatic tumours are slow to elicit angiogenesis and remain dormant for significant periods of time; whereas others are quick to trigger angiogenesis and may grow so rapidly that they soon exceed the size of the primary tumour from which they were derived.

In the latter case, the first sign that a person has cancer may arise from metastases rather than from the primary tumour. For example, the earliest symptom noticed by a person with lung cancer could be back pain triggered by cancer cells that have metastasized to the bones of the spinal column.Or a person might develop liver failure caused by cancer cells that metastasized to the liver from a smaller primary tumour located in the esophagus, stomach, or colon.

It may seem surprising that tumour metastases can be larger than the primary tumour from which they originated. Remember, however, that primary tumours consist of a heterogeneous population of cells that differ in their capacity to metastasize and in their ability to grow at particular sites. Those cells that successfully manage to metastasize to a particular organ may represent a subpopulation of the original cancer cells that are especially well suited for creating metastases.

Such cells may be faster growing, more invasive, better at triggering angiogenesis, and generally more aggressive than the average cell of the initial tumour population. Metastatic tumours may therefore end up growing and invading more efficiently than the original

primary tumour, shedding more cells into the bloodstream and generating further metastases that are even more aggressive. Cancer is thus a disease whose properties change with time. In the beginning stages of tumour formation, the properties required for malignant growth are not fully developed. Instead, as a tumour begins to grow, individual cells often acquire gene mutations and alter the genes they express, turning on some genes and turning off others.

Such alterations create a population of cells whose properties, including the ability to invade and metastasize, gradually change over time. Cells acquiring traits that confer a selective advantage—such as increased growth rate, increased invasiveness, ability to survive in the bloodstream, resistance to immune attack, ability to grow in other organs, resistance to drugs used in cancer treatment, and evasion of apoptosis—will be more successful than cells lacking these traits and so will gradually tend to predominate. This gradual change in the properties of a tumour cell population, as cells acquire more and more aberrant traits and become increasingly aggressive, is known as tumour progression.

The Immune System can Inhibit the Process of Metastasis:

Given the life-threatening nature of metastasis, the question arises as to whether the body has any defenses against it. One possibility is the immune system, which has the ability to attack and destroy foreign cells.

When cancer cells circulate in the bloodstream, where cells of the immune system travel in large numbers, they are especially vulnerable to attack. Of course, cancer cells are not literally of "foreign" origin, but they often exhibit molecular changes that allow the immune system to recognize the cells as being abnormal. Animal experiments suggest that in some cases, attack by the immune system does limit the process of metastasis. One such study involved two strains of mouse lung cancer cells; D122 cells that metastasize with high frequency and A9 cells that rarely metastasize.

In general, the ability of the immune system to recognize cells as being foreign or abnormal requires the involvement of cell surface proteins called major histocompatibility complex (MHC) molecules. The MHC molecules carried by the two lines of lung cancer cells exhibit a prominent difference- A9 cells carry two types of MHC (called H-2K and H-2D), whereas the D122 cells express only one form (H-2D).

The discovery that D122 and A9 cells carry different cell surface MHC molecules raises an important question; Is the differing metastatic behavior of the two cells related to the immune system's ability to recognize and attack the two cell types? This issue was investigated by injecting A9 and D122 cells into separate groups of animals and monitoring the production of cytotoxic T lymphocytes (CTLs), a class of immune cells specialized for attacking foreign and abnormal cells. The animals were found to produce numerous CTLs targeted against A9

cancer cells, but few CTLs targeted against D122 cells.Why do CTLs attack A9 cells more readily than D122 cells? The most obvious possibility is that the immune system recognizes the H-2K MHC molecules, which are carried by A9 cells but not by D122 cells. This hypothesis was tested by introducing purified DNA containing the H-2K gene into D122 cells, thereby causing the D122 cells to produce the H-2K form of MHC (Figure 12).

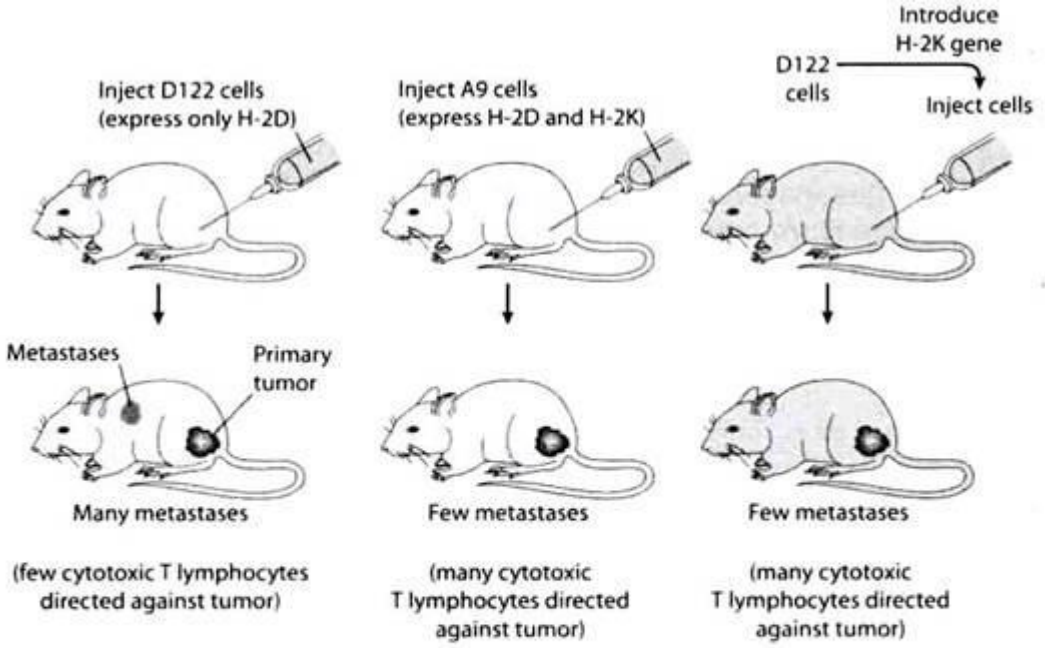


Figure 12 Effects of Immune Recognition on the Metastatic Competence of Cancer Cells. (*Left*) D122 lung cancer cells, which express cell surface H-2D molecules, produce numerous metastases. (*Middle*) A9 lung cancer cells, which express both H-2D and H-2K molecules, metastasize poorly. (*Right*) If the H-2K gene is introduced into D122 cells, the cells lose their ability to metastasize. Because H-2K is a cell surface MHC molecule recognized by the immune system, such studies suggest that immune recognition influences the ability of cancer cells to metastasize.

As predicted, the altered D122 cells expressing H-2K exhibited a reduced capacity to metastasize when injected into mice, suggesting that the presence of H-2K made the cells more susceptible to immune attack. However, the primary tumour at the site of injection grew normally, implying that tumour cells are more susceptible to immune attack when they are circulating in the bloodstream, where large numbers of immune cells reside.

viii. Invasion and Metastasis Involve a Variety of Tumour-Host Interactions:

The ability of the immune system to inhibit the process of metastasis illustrates an important principle: The behavior of malignant tumours depends not just on the traits of tumour cells, but also on interactions between tumour cells and normal cells of the surrounding host tissues.

As is summarized in Figure 13. For example, angiogenesis is triggered by growth factors released by tumour cells that act on normal endothelial cells of the surrounding host tissue, thereby stimulating the proliferation of new blood vessels.

Invasion is facilitated by both tumour- and host-derived proteases that degrade normal extracellular structures such as the basal lamina and the extracellular matrix. The motility of cancer cells and the direction in which they migrate is influenced by signaling molecules made by normal cells of the surrounding tissues.

Penetration through capillaries involves adhesion of cancer cells to molecules present in the basal lamina. And finally, the growth of metastases at distant sites is simulated by growth factors and other molecules produced by cells residing in the organs being invaded.Normal tissues also contain cells and molecules that are capable of hindering invasion and metastasis. For example, we have already seen that immune lymphocytes are capable of attacking and destroying cancer cells, thereby limiting their ability to metastasize. In addition, normal tissues produce protease inhibitors that reduce the activity of the proteases that cancer cells require for degrading the basal lamina and extracellular matrix.

The invasiveness of cancer cells therefore reflects a competition between proteases produced by tumour cells and protease inhibitors produced by surrounding normal cells. These are just a few of the numerous examples in which tumour-host interactions influence the ability of cancer cells to invade neighboring tissues and metastasize to distant sites.

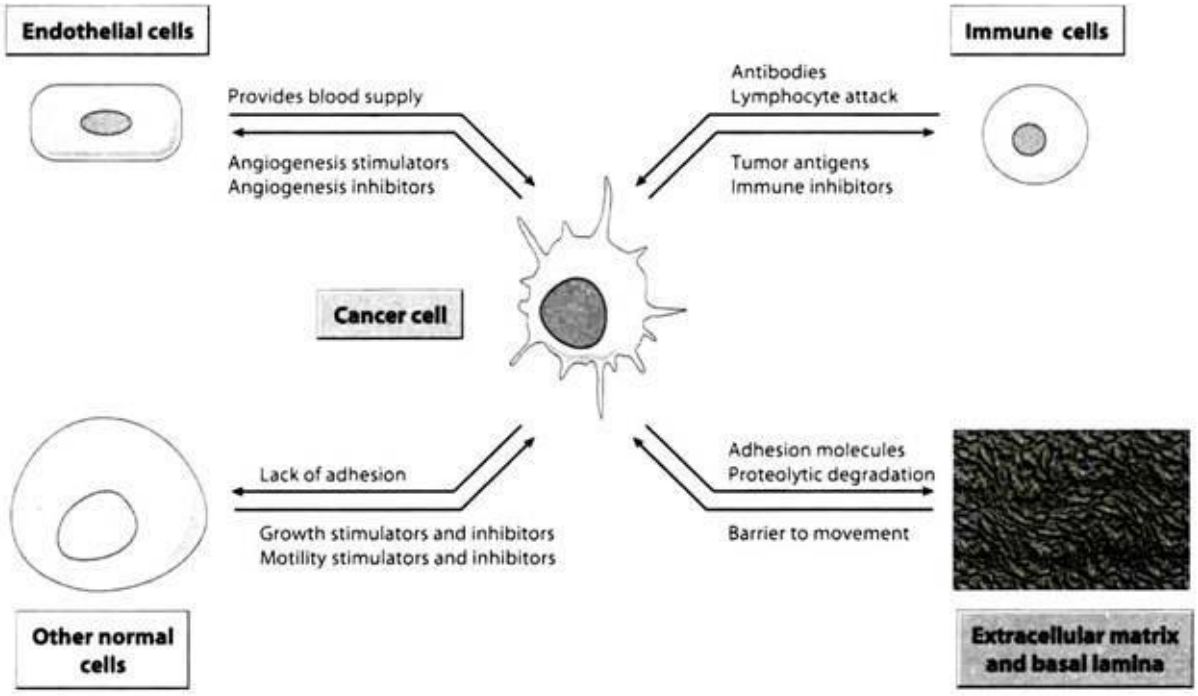


Figure 13 Tumor-Host Interactions. The behavior of malignant tumors depends not just on the traits of cancer cells, but also on interactions between cancer cells and normal cells of the host. This diagram summarizes a few of the many such interactions that affect the development of cancer.

ix. Specific Genes Promote or Suppress the Ability of Cancer Cells to Metastasize:

Because metastasis is the property that makes cancer so dangerous, scientists have been trying to identify some of the key molecules involved in metastasis that might serve as useful targets for new anticancer drugs. The matrix metalloproteinases (MMPs), which facilitate destruction of the basal lamina and extracellular matrix, are one possibility. MMPs are involved in angiogenesis as well as invasion and metastasis, so drugs that inhibit MMP activity could conceivably interfere with cancer progression in multiple ways.

MMP inhibitors are therefore being tested in human cancer patients to see if they can slow down or stop the spread of cancer. Another group of attractive targets are those molecules found on the cancer cell surface that help cancer cells adhere to capillary walls.If inhibitors could be found that block the activity of these cell surface molecules, it might be possible to hinder the interactions between cancer cells and capillaries that allow cancer cells to pass into and out of the bloodstream.

Attempts are also being made to target the growth factors that stimulate cancer cell proliferation in specific target organs, such as the growth factor produced in bone tissue that stimulates the proliferation of metastatic prostate cancer cells.Genetic analysis is beginning to

facilitate the identification of molecules that might be good targets for drugs designed to halt cancer spread. Through this approach, researchers have identified dozens of genes that influence the ability of cancer cells to metastasize, some exerting positive effects and others exerting negative effects.

The positively acting genes, called metastasis promoting genes, code for proteins that stimulate events associated with invasion and metastasis. While it might seem surprising that cells possess genes that promote invasion and metastasis, several traits exhibited by invasive cancer cells—for example, decreased adhesiveness and enhanced motility—are also important for certain kinds of normal cells, such as embryonic cells and cells of the immune system.The negatively acting genes, called metastasis suppressor genes, code for proteins that inhibit events associated with invasion and metastasis. Acquiring the ability to metastasize is usually associated with enhanced activity of metastasis promoting genes as well as diminished activity of metastasis suppressor genes.

Of the two classes of genes, metastasis suppressors are easier to identify because it often takes the action of only one of these genes to block metastasis. To determine whether a gene is a metastasis suppressor, a normal copy of the gene is simply introduced into a population of cancer cells that already possess the ability to metastasize. If activation of the newly introduced gene blocks the ability to metastasize without inhibiting the ability of the cells to form tumours, it is classified as a metastasis suppressor gene. Several metastasis suppressors have been identified using such approaches. One of the best understood is the CADI gene, which codes for E-cadherin.

E-cadherin is a cell-cell adhesion molecule whose loss from the cell surface contributes to tumour invasion by allowing cancer cells to detach from one another and move away from the primary tumour. A number of other metastasis suppressor genes have also been identified, including genes called NM23, KiSS1, KAI1, BRMS1, and MKK4. Some of these genes code for other proteins involved in cell adhesion and motility, but additional mechanisms appear to be involved as well.

Progress has also been made in identifying genes that promote rather than suppress metastasis. An especially interesting example is the gene coding for a protein called Twist, which regulates the activity of a specific group of genes during embryonic development. Genes activated by the Twist protein cause cells to lose their adhesive properties, become motile, and migrate from one part of the embryo to another.

After embryonic development is complete, the Twist protein is no longer needed and its production is shut down in most tissues. However, production of the Twist protein is reactivated in cancer cells, allowing them to reacquire the embryonic traits that allow cells to move throughout the body. Recent experiments have shown that introducing an inhibitor of

Twist production into mouse breast cancer cells reduces the ability of the cells to metastasize. It is therefore hoped that further study of metastasis promoting genes such as Twist will help identify those events and activities whose disruption by appropriate drugs would be most effective at preventing metastasis.

Application in medicine

Angiogenesis as a therapeutic target:

Angiogenesis may be a target for combating diseases such as heart disease characterized by either poor vascularisation or abnormal vasculature. Application of specific compounds that may inhibit or induce the creation of new blood vessels in the body may help combat such diseases. The presence of blood vessels where there should be none may affect the mechanical properties of a tissue, increasing the likelihood of failure. The absence of blood vessels in a repairing or otherwise metabolically active tissue may inhibit repair or other essential functions. Several diseases, such as ischemic chronic wounds, are the result of failure or insufficient blood vessel formation and may be treated by a local expansion of blood vessels, thus bringing new nutrients to the site, facilitating repair. Other diseases, such as age-related macular degeneration, may be created by a local expansion of blood vessels, interfering with normal physiological processes.

The modern clinical application of the principle of angiogenesis can be divided into two main areas: anti-angiogenic therapies, which angiogenic research began with, and pro-angiogenic therapies. Whereas anti-angiogenic therapies are being employed to fight cancer and malignancies, which require an abundance of oxygen and nutrients to proliferate, pro-angiogenic therapies are being explored as options to treat cardiovascular diseases, the number one cause of death in the Western world. One of the first applications of pro-angiogenic methods in humans was a German trial using fibroblast growth factor 1 (FGF-1) for the treatment of coronary artery disease.[19][37][38]

Regarding the mechanism of action, pro-angiogenic methods can be differentiated into three main categories: gene therapy, targeting genes of interest for amplification or inhibition; protein replacement therapy, which primarily manipulates angiogenic growth factors like FGF-1 or vascular endothelial growth factor, VEGF; and cell-based therapies, which involve the implantation of specific cell types.

There are still serious, unsolved problems related to gene therapy. Difficulties include effective integration of the therapeutic genes into the genome of target cells, reducing the risk of an undesired immune response, potential toxicity, immunogenicity, inflammatory responses, and oncogenesis related to the viral vectors used in implanting genes and the

sheer complexity of the genetic basis of angiogenesis. The most commonly occurring disorders in humans, such as heart disease, high blood pressure, diabetes and Alzheimer's disease, are most likely caused by the combined effects of variations in many genes, and, thus, injecting a single gene may not be significantly beneficial in such diseases.

By contrast, pro-angiogenic protein therapy uses well-defined, precisely structured proteins, with previously defined optimal doses of the individual protein for disease states, and with well-known biological effects. On the other hand, an obstacle of protein therapy is the mode of delivery. Oral, intravenous, intra-arterial, or intramuscular routes of protein administration are not always as effective, as the therapeutic protein may be metabolized or cleared before it can enter the target tissue. Cell-based pro-angiogenic therapies are still early stages of research, with many open questions regarding best cell types and dosages to use.

Prevention and Treatment of Cancer:

There is a general belief among the common people that cancer cannot be cured. Although this is partially true, it depends on several aspects of the patient and the time of detection. In many cases, when it is clinically detected then it is already late and it goes beyond the treatment.Actually, cancer is a disease that ultimately has to be understood at the molecular and cellular level. In fact many cancers can be cured if they are detected at the early stage of its development. In case of hereditary cancer, regular testing may allow early detection.

Therefore, whether cancer is curable or not is a debatable question. With the help of modern and sophisticated technology, cell biologists are always trying to improve the methods for prevention and treatment of cancer.

The first step in preventing cancer is to identify the agents that cause cancer. For example, it is already known that tobacco smoke causes cancer. So just to prevent the possibility of this type of lung cancer, it is advisable simply to avoid tobacco smoke. Similarly the discovery of carcinogenic properties of X-ray and sunlight suggests that individuals should avoid unnecessary medical X-ray and use protective lotions during long time exposure to sunlight.

Epidemiological data also allow potential carcinogens to be identified in exposed human population. The epidemiological approach is based on comparison of cancer rates among various groups of people exposed to different environmental conditions. For example, when Japanese individuals move to the United States heir susceptibility to developing stomach and lung cancer changes to reflect the rates for such cancers in the United States. Therefore, the comparison of the frequency of stomach and lung cancer in Japan, in the United States and in Japanese immigrants to the United States suggests that environmental factors play a prominent role in causing cancer. Epidemiological data have played an important role in identifying some of the environmental factors that may cause cancer. The Ames test is a rapid

screening method tor identifying potential carcinogens This method is based on the rationale that most carcinogens act as mutagens, it measures the ability of potential carcinogens to induce mutations in a strain of bacteria that lack the ability to synthesize the amino acids histidine.

Each bacterial cell that has mutated to a form in which it no longer needs histidine will grow into a colony that can be counted. The number of colonies indicates the mutagenic potency of the substance to be tested.Chemicals to be tested m the Ames test are first incubated with a liver homogenate because many of the chemicals to which humans are exposed only become carcinogenic after they have undergone biochemical modification in the liver (Fig. 23.11) Cancer can be prevented in several other ways A person can modify his life style in order to reduce the risk of developing cancer. Change of life style sometimes requires minimizing the exposure to carcinogens. Tobacco smoking and extensive meat consumption are the probable causative factors of cancer. If any person method of treatment is most effective when the cancer is detected at the early stage of development and when metastasis has not occurred. This method is not effective when the cancer has already been disseminated throughout the body by the process of metastasis. Therefore, early detection of cancer is very important for its treatment.

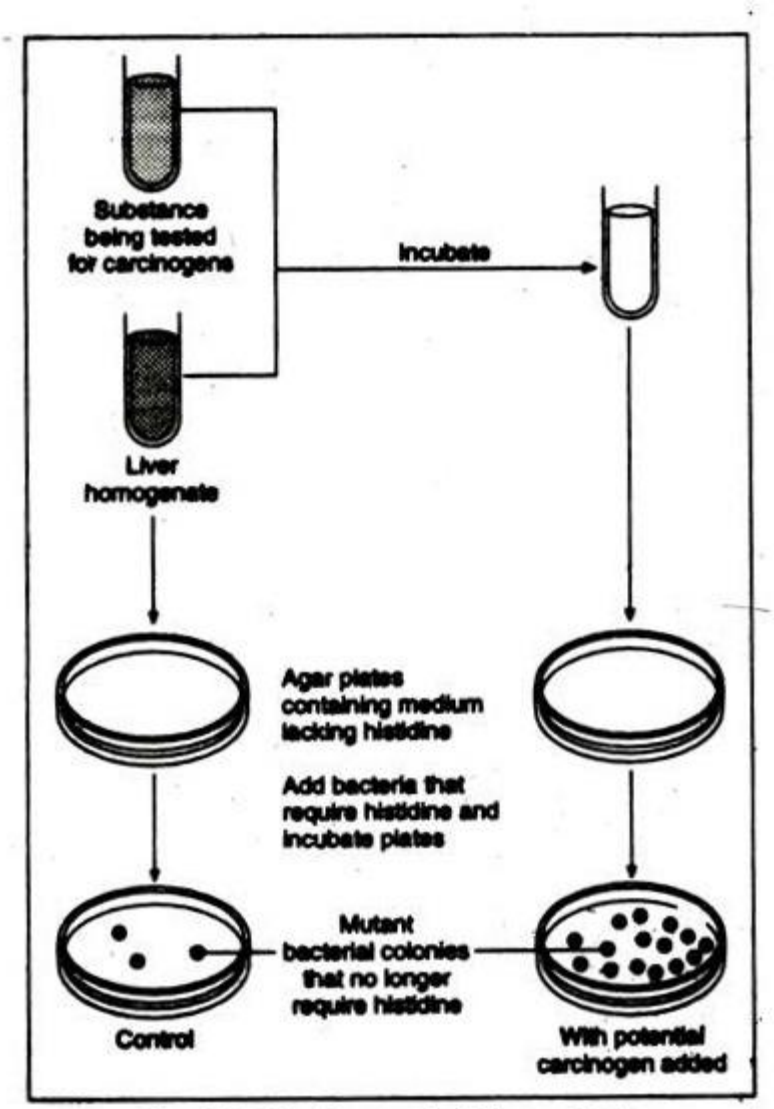


Fig. 23.11: Protocol of Ames Test.

Treatment of cancer-affected part of body with the help of X-radiation is another alternative method of curing cancer. X-ray is very effective for killing the cancer cells that are actively proliferating. The cells that are engaged in DNA synthesis prior to cell division, or are on the way of mitosis, are very sensitive to X-ray. But the main problem of using X- ray for the treatment of cancer is that normal and healthy dividing cells of the body-such as blood-forming cells in the bone marrow are also destroyed along with the cancer cells.

Moreover, X-radiation itself is carcinogenic. Hence there is always a chance of developing cancer after X-ray treatment. In spite of such risk posed by X-ray treatment, it is effective for the treatment of certain types of cancers like skin cancer, Hodgkin's disease and specific forms of testicular and bone cancer. Chemotherapy is another approach for treating cancer. This method is based on the use of certain drugs that are designed to kill the proliferating cells as in radiation treatment. This method is also effective when the cancerous cells have

already metastasized. The drugs are generally injected in the body and the circulatory system helps the drug to spread throughout the body. Some drugs used m cancer chemotherapy are given in the Table 23.7.

Table 23.7: Tumour Suppressor Genes

Gene	Type of cancer
APC	Colon/rectum carcinoma
BRCA1	Breast and ovarian carcinomas
BRCA2	Breast carcinoma
DCC	Colon/rectum carcinoma
DPC4	Pancreatic carcinoma
INK4	Melanoma, lung carcinoma, brain tumours, leukemias, lymphomas
NF1	Neurofibrosarcoma
NF2	Meningioma
P^{53}	Brain tumours, breast, colon/rectum, esophageal, liver, and lung carcinomas; leukemias and lymphomas
Rb	Retinoblastoma, sarcomas; bladder, breast, and lung carcinomas
VHL	Renal cell carcinoma
WT1	Wilm's tumour

Like radiation, chemotherapeutic drugs also kill the normal and healthy cells along with cancer cells. This type of treatment has also some toxic side-effect-like loss of hair (caused by destruction of hair follicle cells), diarrhoea (caused by destruction of cells of the intestinal lining) and susceptibility to infections (caused by destruction of blood cells).Sometimes two or more combination of drugs are also used for the treatment of cancer. Besides its side-effects and other disadvantages, it is true that, for certain types of cancer, chemotherapy is very successful for curing cancer like Burkitt s lymphoma, chorio carcinoma, acute lymphocytic leukaemia, Hodgkin's disease, lymphomas, mycosis fungoides, Wilm's tumour, Ewing s sarcoma, thabdomyosarcoma, retinoblastoma, and embryonal testicular tumours etc.

Table 23.8: Some drugs used in Cancer Chemotherapy

Class	Examples	Mechanism of Action
1. Antimetabolites	Methotrexate 5-Fluorouracil 6-Mercaptopurine	Inhibit enzymatic pathways for biosynthesis of nucleic acids by substituting for normal substrates
2. Antibiotics (sybstances produced by microorganisms)	Actinomycin D Adriamycin Daunorubicin	Bind to DNA
3. Alkylating agents	Nitrogen mustard Chlorambucil Cyclophosphamide Imidazole carboximides	Crosslink DNA
4. Mitotic inhibitors	Vincristine Vinblastine Taxol	Interfere with mitotic spindle
5. Hormones	Estrogen (for prostate cancer) Cortisone Progesterone Androgens	Inhibit growth of hormone-sensitive cells by interacting with hormone receptors
6. Miscellaneous agents	L-Asparaginase	Hydrolyzes asparagine

Although the use of surgery, radiation and chemotherapy has led to increased survival rates for certain kinds of cancer, many malignancies do not respond well to such treatment. Recent experimentation is attempting to exploit the ability of the immune system to recognize and kill tumour cells. This type of treatment is known as immunotherapy.

The basic principle of immunotherapy is to exploit the ability of the immune system to recognise and kill tumour cells. Tumour cells tend to show cell surface antigens which make them recognisable by the immune system. Initially, some scientists attempted to utilise a person's own lymphocytes to kill cancer cells. For this experiment, lymphocytes were isolated from the blood of cancer patients and grown in culture in presence of Interlaken 2 to stimulate the cancers destroying properties of the cell. The result was the isolation of a population of killer T-cells that were specifically targeted against the patient's tumour.

These cells, called tumour-infiltrating lymphocytes (TILs), were injected back into the patients from whom the blood was drawn. TILs axe more effective in inducing tumour regression. Recently TILs are made even more effective by using recombining DNA technique to insert some genes whose product enhances the additional potency of the TILs. A protein produced by march-panes called tumour necrosis factor (TNF) is effective in promoting the destruction of cancer cells if the TNF gene were inserted into the TILs. Obviously, the genetically engineered TILs would be more effective than normal TILs and would be more powerful to killing the tumour cells. Currently this technique is being tested in the hope of finding ways to promote immune destruction of cancer cells.

Chromosomal basis of human cancer:

Cancer is also regarded as a chromosomal disease. Accordingly carcinogenesis is initiated by random aneuploidies, which are induced by carcinogens or spontaneously. Since aneuploidy unbalances 1000s of genes, it corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is therefore a steady source of chromosomal variations from which, in classical Darwinian terms, selection encourages the evolution and malignant progression of cancer cells. The rates of specific chromosomal variations can exceed conventional mutations by 4-11 orders of magnitude, depending on the degrees of aneuploidy. Based on their chromosomal constitution cancer cells are new cell "species" with specific aneusomies, but unstable karyotypes. The cancer-specific aneusomies generate complex, malignant phenotypes through the abnormal dosages of 1000s of genes, just as trisomy 21 generates Downsyndrome.

Although chromosomal changes are highly variable, they can be grouped into two general categories. In balanced structural changes, the genetic material is exchanged evenly. An example of a balanced structural change is the Philadelphia chromosome translocation . In that case, although genetic information was rearranged into an abnormal gene, it resulted from an evenexchange of DNA. Conversely, in nonreciprocal or unbalanced structural changes, the exchange is not equal, and genetic material is added or lost. This can range from the loss or gain of a single base pair to the loss or gain of entire chromosomes. Scientists have hypothesized that the primary pathogenetic changes in cancer result from balanced rearrangements, while the secondary changes that occur during cancer progression are from unbalanced changes. Cancer is a multistep, progressive disease, and early chromosomal changes provide the cell with a proliferative advantage. Often, these changes hijack or interfere with the normal cellular control mechanisms by disrupting proto-oncogenes and tumour suppressor genes and allowing additional changes to occur in the genome. Cancer cells generally gain multiple types of chromosomal aberrations during tumour progression, including rearrangements, deletions, and duplications. As a result, the genome becomes progressively moreunstable.

Chromosomal rearrangements :

Chromosomal rearrangements can lead to cancer either by forming a hybrid gene or by causing disregulation of a gene. Since the discovery of a particular chromosomal rearrangement, thousands of other chromosomal aberrations have been determined to be associated with cancer. These chromosomal changes are the signature of gene deregulation in cancer and lead to instability of the genome. Chromosomal changes are highly variable in different cancers, and the resultant phenotypic effects are equally variable.

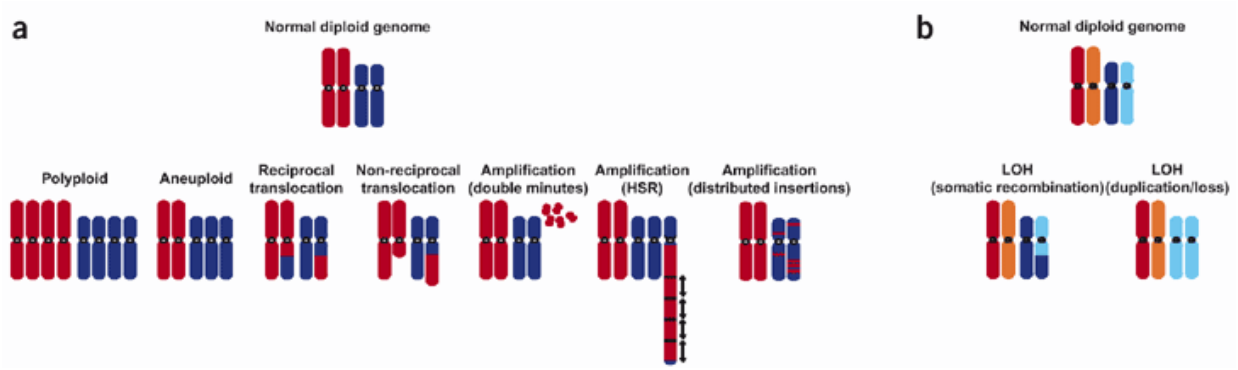


Fig 6: Different types of Chromosomal aberrations. (A) Chromosomal aberrations with altered gene number or sequence (B) Chromosomal aberrations with intact gene number or sequence The Philadelphia (Ph^1) chromosome, a small acrocentric chromosome seen in 90% of patients with chronic myeloid leukemia. The Ph^1 chromosome is one product of a balanced reciprocal 9;22 translocation. The breakpoint on chromosome 9 is within an intron of the *ABL1* oncogene. The translocation joins the 3' part of the *ABL1* genomic sequence onto the 5' part of the *BCR* (breakpoint cluster region) gene on chromosome 22, creating a novel fusion gene. This chimericgene is expressed to produce a tyrosine kinase related to the *ABL* product but with abnormal transforming properties.

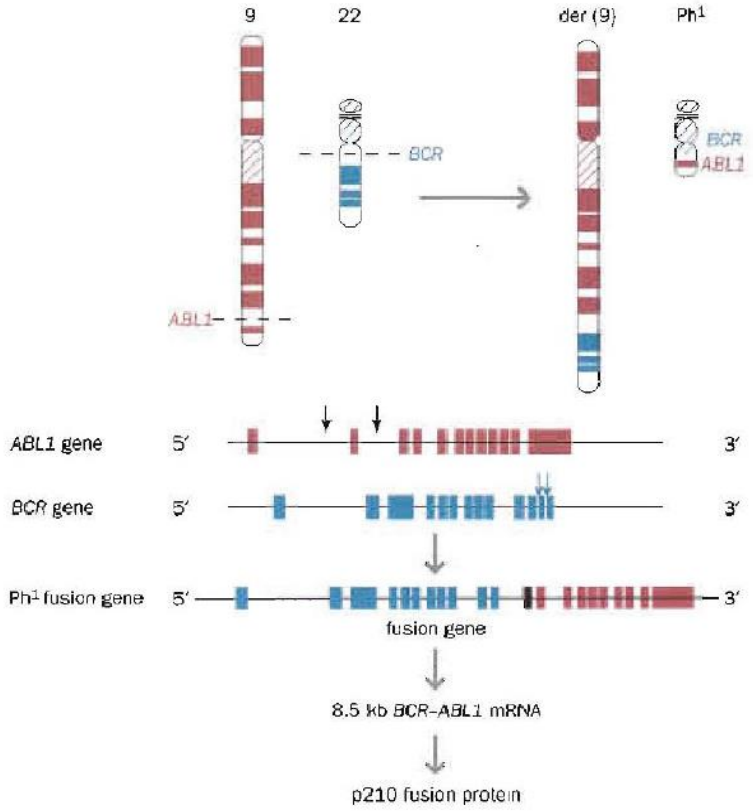


Fig 7: Chromosomal arrangement in Philadelphia chromosome.

Many other tumour-specific recurrent rearrangements that produce chimeric oncogenes have now been recognized**.** This mechanism is seen in 15-25% of leukemias, lymphomas, and sarcomas, but has been reported in only 1 % or less of the common solid epithelial tumours. Burkitt lymphoma is known to be associated with activation of the MYC oncogene A characteristic chromosomal translocation, t(8;14)(q24;q32), is seen in 75-85% of patients**.** The remainder have t(2;8)(p12;q24) or t(8;22)(q24;qll). Each of these translocations juxtaposes the MYC oncogene (normally located at 8q24) close to an immunoglobulin (IG) locus. This may be *IGH* at 14q32, *IGK* at 2p12, or *IGL* at 22qll. The translocation bring the oncogene under the influence of regulatory elements that normally ensure high expression of the immunoglobulin genes in antibody-producing B cells. In the 8; 14 translocation, the MYC and *IGH* genes are in opposite transcriptional orientations, head to head. Often, depending on the precise breakpoint, exon 1 of the MYC gene (which is noncoding) is not included in the translocated material. Deprived of its normal upstream controls and placed in an active chromatin domain, MYC is expressed at an inappropriately high level. Between 25% and 65% of all B-cell malignancies involve the activation of one or another oncogene by an immunoglobulin enhancer, and many T- cell malignancies involve a similar activation by an enhancer at a T-cell receptor locus.

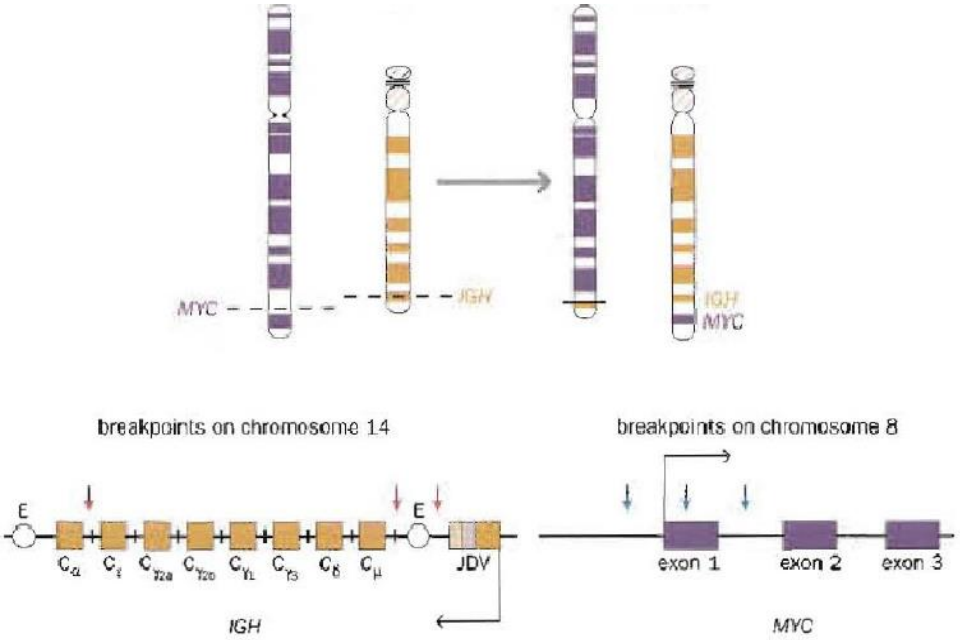


Fig 8: Chromosomal arrangement in Burkitt's lymphoma

Deletions and Duplications:

In cancers such as Wilms' tumour and retinoblastoma, gene deletions or inactivation are responsible for initiating cancer progression. In fact, inactivation of tumour suppressor genes is associated with many types of cancer, as chromosomal regions associated with tumour suppressors are commonly deleted or mutated. For example, deletions, inversions, and translocations are commonly detected in chromosome region 9p21 in gliomas, non-small-cell lung cancers, leukemias, and melanomas. These chromosomal changes inactivate a tumour suppressor called cyclin-dependent kinase inhibitor 2A. Along with these deletions of specific genes, large portions of chromosomes can also be lost. For instance, chromosomes 1p and 16q are commonly lost in solid tumourcells. Gene duplications and increases in gene copy numbers can also contribute to cancer and can be detected with transcriptional analysis or copy number variation arrays. For example, the chromosomal region 12q13-q14 is strikingly amplified in many sarcomas. This chromosomal region encodes a binding protein called MDM2, which is known to bind to a tumour suppressor called p53. When MDM2 is amplified, it prevents p53 from regulating cell growth, which can result in tumour formation.

Additionally, certain breast cancers are associated with overexpression and increases in copy number of the *ERBB2* gene, which codes for human epidermal growth factor receptor 2. Indeed, the presence of a high number of *ERBB2* copies has been found to be associated with aggressive forms of breast cancer. Therefore, measuring the *ERBB2* copy number can provide a diagnostic tool for breast cancer and other cancers. Along with these amplifications of specific genes, gains in chromosomal number, such as chromosomes 1q and 3q, are also associated with increased cancer risk.

Mutations in the genes necessary for DNA repair can additionally lead to rearrangements and duplications. For example, if a gene involved with chromosomal segregation is mutated, duplications and deletions are more likely. Furthermore, the accurate sorting and segregation of chromosomes during mitosis requires the activity of many gene products (proteins). Defects in the genes controlling the mitotic surveillance mechanisms necessary for chromosomal sorting can lead to chromosome instability and abnormalities in the number of chromosomes (polyploidy and aneuploidy), which can, in turn, lead to tumourigenesis. Another cause of genome instability and chromosomal aberrations in tumours is the presence of abnormal centromeres, which can lead to abnormal mitotic events with multiple spindles and result in the abnormal loss of chromosomes.

‘Chemicals and Cancer’ :

Identifying Chemicals that Cause Cancer:

We seem to be surrounded by a sea of chemical carcinogens. They are found in the air we breathe, the food we eat, the water and beverages we drink, the medications we take, the places where we work, and the homes in which we live. However, this assessment—while

technically correct—conveys the misimpression that we are faced with severe hazards everywhere we look and that these dangers cannot be avoided.

In fact, many of the carcinogens we normally encounter are only weakly carcinogenic, and most of the more potent ones can be easily avoided by the general public. So rather than lumping all chemical carcinogens together, we need to consider them as individual molecules and make informed judgments about the dangers posed by each one.

Discovery of Chemical Carcinogens:

The first indication that chemicals might cause cancer came from the observations of doctors who, in their struggle to understand the nature of the disease, asked cancer patients a variety of questions about their backgrounds, experiences, and habits. This allowed physicians to gain some impressions, if not firm evidence, about the possible causes of cancer.

Such an approach led a London doctor, John Hill, to point to chemicals as a probable cause of cancer more than two hundred years ago. In 1761, Hill reported that people who routinely use snuff— a powdered form of tobacco that is inhaled—suffered an abnormally high incidence of nasal cancer, suggesting the existence of one or more cancer-causing chemicals in tobacco. Several years later Percival Pott, another British physician, reported an unusual prevalence of oozing sores on the scrotums of men coming to his medical practice in London. While a less astute observer might have thought it was just one of the venereal diseases that were widespread at the time, Pott's close examination of the sores revealed that they were actually a form of skin cancer.

Careful questioning revealed that the men with this condition shared something in common. They had all served as chimney sweepers in their youth. It was common practice at the time to employ young boys to clean chimneys because they fit into narrow spaces more readily than adults.Pott therefore speculated that chimney soot chemicals had become dissolved in the natural oils of the scrotum, irritating the skin and eventually triggering the development of cancer. These ideas led to the first successful public health campaign for preventing a particular type of cancer- Scrotal cancer was virtually eliminated by promoting the use of protective clothing and regular bathing practices among chimney sweeps.

In the years since these pioneering observations, it has become increasingly evident that certain kinds of chemicals can cause cancer. Unfortunately, the ability of a particular chemical to cause cancer has often become apparent only after large numbers of cancers arise in people exposed to that chemical on a regular basis.For example, in the early 1900s elevated rates of skin cancer were noted among workers in the coal tar industry, and an increased incidence of bladder cancer was seen in factories that produced aniline dyes. The experience

in the aniline dye industry was especially dramatic and led to the discovery of several basic principles of chemical carcinogenesis, as will now be described.

Workers who Developed the First Cancer:

The late 1800s witnessed the birth of a series of new chemical industries that for the first time exposed large numbers of workers to high concentrations of toxic substances. A prominent example involved the industrial production of dyes used to color clothing and other fabrics.

Prior to the mid-1800s, most dyes were natural substances extracted from vegetable or animal sources. An accidental discovery made in 1856 by William Perkin, however, led to the birth of the synthetic dye industry and the first mass exposure of workers to potent carcinogenic chemicals.

Perkin was attempting to synthesize quinine, a drug for treating malaria, by carrying out chemical reactions on substances present in coal tar (a thick, black liquid formed during the distillation of coal). In one experiment, he extracted aniline from coal tar and oxidized it with potassium dichromate. The result was a dark brown precipitate. Most nineteenth-century chemists would have discarded any such dark masses of material because scientists were generally interested in clear, crystalline products. But Perkin was instinctively curious and decided to investigate further. To his pleasant surprise, dissolving the dark sludge in alcohol yielded an intense purple solution that exhibited strong dying properties. Perkin had discovered aniline purple, the first synthetic dye.

Within a few years, coal tar had yielded several other dyes and the aniline dye industry was born. Chemists quickly discovered that a compound related to aniline called 2-naphthylamine is an ideal starting material for the synthesis of many dyes, and large-scale production began in Germany around 1890. Unfortunately, factory employees working with 2-naphthylamine soon began developing bladder cancer in alarming numbers (Figure 1).

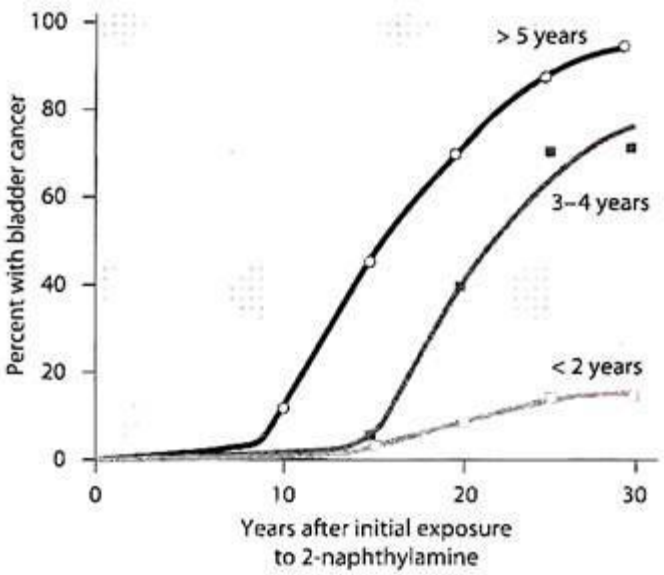


Figure 1 Relationship Between 2-Naphthylamine Exposure and Bladder Cancer in Factory Workers. These data show bladder cancer rates in three groups of men exposed for varying amounts of time to 2-naphthylamine in the workplace. Note that in the group of men with the longest exposure (more than 5 years), almost every person eventually developed bladder cancer. [Data from J. Cairns, *Cancer: Science and Society* (San Francisco: Freeman, 1978), p. 56.]

In one small factory, all 15 workers developed the disease. A vigorous and protracted debate ensued as to whether 2-naphthylamine was actually responsible because bladder cancer also occurs among the general public and there was little precedence for using epidemiological data to infer cause and effect. Eventually the cancer-causing ability of 2-naphthylamine was demonstrated in convincing fashion, using both epidemiological and animal data, but it took almost 50 years before the large-scale production of this highly potent carcinogen was stopped.

Bladder cancer triggered by occupational exposure to 2-naphthylamine was the first example of a human cancer known to be caused by a specific chemical compound. The introduction of 2-naphthylamine into the workplace was also a key event because it marked the beginning of a massive increase in industrial chemical production and illustrated some important principles that are widely relevant to chemical carcinogenesis.

One of these principles involves the long delay that is typically observed between exposure to a chemical carcinogen and the onset of cancer. Few cases of bladder cancer were seen in factory workers until 10 years after initial exposure to 2-naphthylamine, and most cases took between 15 and 30 years to appear. Such a long delay is typical of chemical carcinogenesis and reflects the multiple events that take place on the road to developing cancer.Another

principle illustrated by the experience with 2-naphthylamine is dose dependence- Workers who were exposed to the chemical over a longer period of time, and hence had a larger total exposure, exhibited higher bladder cancer rates than workers with shorter exposures.

Figure 1 reveals that almost every worker in the group with the longest exposure to 2-naphthylamine eventually developed bladder cancer. If virtually everyone develops cancer under such conditions, it means that hereditary differences did not play a significant role in determining risk.Finally, the experience with 2-naphthylamine illustrated the organ specificity that is a common feature of chemical carcinogenesis. Instead of causing cancer in general, chemical carcinogens tend to preferentially cause a few particular types of cancer. In the case of 2-naphthylamine, the bladder is the prime target.

We have already encountered other examples of organ specificity and will encounter additional examples later. Organ specificity is generally caused by the selective ways in which chemicals make contact with, or accumulate in, certain body tissues.For example, chemicals that become concentrated in the urine are likely to produce bladder cancer, and carcinogens that are inhaled tend to cause lung cancer. The ability of cigarette smoke to cause many kinds of cancer in addition to lung cancer may seem to violate this principle; tobacco smoke, however, contains more than 40 different carcinogens, and some of these accumulate in tissues other than the lung.

Asbestos as a Cause of Cancer Deaths:

The natural mineral asbestos is a particularly striking example of an organ-specific carcinogen. Commercial use of asbestos began in the late 1800s, when large deposits of asbestos rock were discovered in Canada and shipped to the United States and to the newly industrializing countries in Europe. Crushing the rock yields a mixture of fine fibers that can be woven into materials that exhibit excellent insulating and fire-retarding properties.

The most commonly used form of asbestos has the formula $(Mg,Fe)_3Si_2O_5(OH)_4$, but many chemical variations exist. Numerous fireproof products, ranging from oven mittens and fireproof clothing to various kinds of construction materials, have been manufactured with asbestos.

Unfortunately, the widespread use of asbestos has had severe health consequences. Asbestos readily breaks down into a fine dust containing numerous sharp, needle-like fibers that are so tiny that they can only be seen with an electron microscope. These “needles of death” are easily inhaled and become lodged in the lung, where they cause scarring that kills people through suffocation.

Shortly after this disease, called asbestosis, was first recognized among asbestos workers in the 1920s, the same workers began to develop lung cancer. Because cigarette smoking was not yet popular, lung cancer was still rare, and the connection between the cancer outbreak and exposure to asbestos was therefore easy to detect.Scientists eventually found that asbestos and cigarette smoke interact synergistically in causing lung cancer. As a result, smokers who have been heavily exposed to asbestos exhibit lung cancer rates that are 50 times higher than is observed in people who do not smoke or have significant exposure to asbestos.

An unusual property of asbestos is its ability to cause mesothelioma, a rare form of cancer derived from the mesothelial cells that cover the interior surfaces of the chest and abdominal cavities. This type of cancer was uncommon prior to the 1950s, when the first mesothelioma epidemic was reported in and around a group of asbestos mines in South Africa.Mesotheliomas were subsequently detected in many locations around the world; virtually everywhere that asbestos is used. An increased risk for mesothelioma is exhibited mainly by asbestos workers and by individuals who experience significant exposure to asbestos either by living in neighbourhoods surrounding asbestos factories or by working or living in asbestos- insulated buildings. At present, asbestos is the only clearly established cause of mesothelioma.

Microscopic examination of lung tissue obtained from asbestos workers has revealed that mesothelioma is caused by a rather unusual mechanism. Tiny, microscopic fibers of inhaled asbestos become embedded in the lung and gradually penetrate completely through the lung tissue, emerging into the chest cavity.

Here the asbestos fibers trigger a chronic irritation and inflammation that promotes the development of cancer in the mesothelial cells that cover the lungs and line the interior chest wall. In a similar fashion, asbestos fibers that have been inadvertently ingested can penetrate through the walls of the stomach and intestines, emerging into the abdominal cavity and triggering the development of abdominal mesotheliomas.

When the fatalities caused by asbestos- induced mesotheliomas and lung cancers are combined, asbestos ranks as the second most lethal commercial product (after tobacco) in terms of the number of cancer deaths caused. Governmental actions to regulate the production and use of asbestos began in earnest in the 1960s and have become progressively more restrictive in many countries, so the incidence of asbestos-induced cancers should eventually begin to decline. Nonetheless, mesothelioma deaths are still rising (Figure 2) and will probably continue to do so for several decades because a lag period of 30 or more years can intervene between asbestos exposure and developing cancer.

Workplace Exposure to Chemical Carcinogens as a Cause of Cancer:

As in the case of 2-naphthylamine and asbestos, carcinogenic chemicals are often identified only after a particular type of cancer starts to appear in people exposed to a specific substance in high doses. Once such observations point to a particular chemical as a potential carcinogen, follow-up animal testing is carried out to determine whether the substance really causes cancer.

Beginning around 1900 with 2-naphthylamine in the aniline dye industry, the list of known chemical carcinogens grew progressively as the Industrial Revolution proceeded throughout the twentieth century and unusual cancer patterns began to emerge among workers in various industries. Table 1 lists some of the main occupational carcinogens that were eventually discovered, including examples from the rubber, chemical, plastic, mining, fuel, and dye industries. Workplace exposure to occupational carcinogens was substantial in the first half of the twentieth century before the cancer risks from such agents came to be fully appreciated. In a few extreme cases, all the workers exposed to the chemicals present in certain factories eventually developed cancer.

However, most of the currently known occupational carcinogens were identified by the 1970s and relatively few new ones have been identified since then. In 1970, an act of the United States Congress created the Occupational Safety and Health Administration (OSHA) to formulate regulations designed to protect the safety and health of workers.

OSHA has worked to eliminate the most dangerous chemicals from the workplace and to limit worker exposure to other chemicals. As a result, many occupationally induced cancers that were once prevalent in the United States have declined in frequency, and workplace exposure to carcinogens now accounts for less than 5% of all cancer deaths.

While a similar pattern is evident in many other industrialized nations, progress has been far from uniform. To illustrate some of the disparities, the use of asbestos in Nordic countries has decreased dramatically in recent decades, falling to a negligible value of 4 grams per person in 1996; in that same year, asbestos use in the former Soviet Union was 600 times higher at a value of 2400 grams per person. In general, exposure to industrial carcinogens is a greater problem in developing countries, where less progress has been made in regulating the workplace use of toxic chemicals.

Environmental Pollution not a Major Source of Cancer Risk:

Cancers arising in the workplace are usually triggered by sustained high-dose exposures to specific chemical carcinogens. Small amounts of the same chemicals are also released inadvertently into the environment, where they contaminate the air we breathe, the water we

drink, and the food we eat. It has therefore become fashionable to blame industrial pollution for creating a growing cancer threat of epidemic proportions.

Table 1 Some Human Carcinogens for Which Exposure Is Mainly Occupational

Carcinogen	Industry	Type of Cancer
4-Aminobiphenyl	Rubber	Bladder
Arsenic	Glass, metals, pesticides	Lung, skin
Asbestos	Insulation, construction	Lung, pleura
Benzene	Solvent, fuel	Leukemia
Benzidine	Dye	Bladder
Bis(chloromethyl) ether*	Chemical	Lung
Chloromethyl methyl ether*	Chemical	Lung
Cadmium	Pigment, battery	Lung
Chromium	Metal plating, dye	Nasal cavity, lung
Coal-tar pitches	Construction, electrodes	Skin, lung, bladder
Coal-tars	Fuel	Skin, lung
Ethylene oxide	Chemical, sterilant	Leukemia
Mineral oils	Lubricant	Skin
Mustard gas*	Chemical weapon	Pharynx, lung
2-Naphthylamine*	Dye	Bladder
Silica	Construction, mining	Lung
Soots	Dye	Skin, lung
Sulfuric acid mist	Chemical	Larynx, lung
Talc	Paper, paint	Lung
Vinyl chloride	Plastic	Liver

*Mainly of historical interest.

However, there are reasons to question such a far-reaching conclusion. First, cancer risk is related to carcinogen dose, and the doses of industrial carcinogens to which the public is exposed are generally orders of magnitude lower than is encountered in the workplace. For example, consider the pesticide ethylene dibromide (EDB), which is designated by the U.S. government as a probable human carcinogen based on its ability to cause cancer in animals.

Workers who have experienced high-dose exposures to this suspected carcinogen encountered about 150 milligrams (mg) per day, whereas EDB residues in food (before EDB was banned in 1984) exposed the average person to a daily dose of 0.00042 mg, which is 300,000 times less than the workers' daily intake.

A similar situation exists for most of the other chemical contaminants in our food, air, or water, which do not represent a major cancer threat because they are present in concentrations thousands of times lower than typical industrial exposures. Another reason to question the assumption that pollution represents a major cancer hazard is based on his-

torical trends. If industrial pollution were a major cancer threat to the general public, one would have expected a significant increase in overall cancer rates during the twentieth century in response to the explosive growth in the use of industrial chemicals.

For example, the yearly production rates of plastics, pesticides, and synthetic rubber in the United States increased more than 100-fold between the 1940s and the 1970s. Any impact the chemicals used in these industries might have had in causing a cancer epidemic through environmental pollution should have been apparent by now. In fact, when the data are adjusted for the increasing average age of the population, it is clear that a significant growth in age-adjusted cancer rates has not occurred (Figure 3). Most of the cancers that are common today were also common one hundred years ago, and the main exception, lung cancer, is triggered by cigarette smoke and has little to do with industrial pollution.

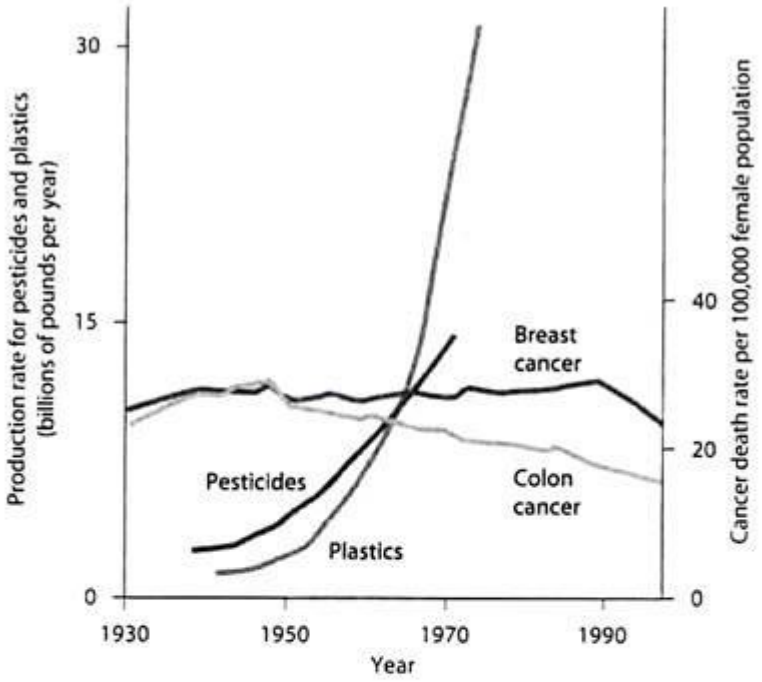


Figure 3 Patterns of Industrial Pollution and Cancer Death Rates in the United States. Industrial use of chemical carcinogens, as occurs in the plastics and pesticide industries, increased dramatically between the 1940s and 1970s. For most types of cancer, there was no corresponding increase in cancer incidence during the following decades. If industrial pollution had a major effect on cancer rates, it should have been evident by now. Mortality rates for breast and colon cancer in women are shown, but many cancers exhibit a similar pattern. The only cancer that has shown a major increase in incidence is lung cancer, which is caused mainly by tobacco smoke rather than industrial pollution. [Based on data from *Cancer Facts & Figures 2002* (Atlanta, GA: American Cancer Society, 2002), p. 3; and R. H. Harris et al. in *Origins of Human Cancers* (H. H. Hiatt et al., eds., Cold Spring Harbor, NY: CSHL Press, 1977), pp. 309–330 (Figure 2).]

Risks from Low-Dose Exposures to Chemical Carcinogens as a Cause of Cancer:

The preceding arguments suggest that for most people other than those who receive high-dose exposures by working with or living near a concentrated source of toxic chemicals, the cancer risks posed by environmental chemicals are relatively small. This does not mean, however, that the risk for the average citizen is zero.

If the public were being routinely exposed to low carcinogen doses that cause a tiny fraction of the population to develop cancer, such small effects would be difficult to detect using traditional epidemiological methods. In fact, a weak carcinogen present in the environment could theoretically cause hundreds or even thousands of cancer cases each year in a country of several hundred million people without being noticed. Assessing the actual risk, if any, from low-dose exposures to known or suspected carcinogens is a difficult task. To illustrate, let us briefly consider the dioxins, a family of chlorinated chemicals produced as a by-product during the burning of municipal wastes, the bleaching of paper, and the production of herbicides. Several epidemiological studies have demonstrated increased rates of cancer in workers exposed to large concentrations of dioxin, and high-dose animal studies have also shown increased cancer rates.Although the quantities released into the environment are quite small, dioxins are stable molecules that tend to persist for long periods of time, accumulating in the food chain. For humans the main source of exposure is through the food we eat especially fatty meats.

The ingested dioxin molecules are fat soluble and are metabolized slowly, so they tend to accumulate in our body fat. The net result is that even tiny exposures to dioxin can lead to significant concentrations inside the body. The large unanswered question is whether this accumulated dioxin represents a significant cancer risk. The most conservative approach has been to assume that even one molecule of a carcinogen can cause cancer—in other words, that there is no safe dose of dioxin. However, testing in rats has revealed that while high doses of dioxin cause cancer, low doses can sometimes decrease cancer rates compared to those observed in control animals.

Such data indicate that it is possible for low-dose carcinogen exposures to be safe, and perhaps even beneficial. Unfortunately, differences between humans and rodents in mode of exposure, metabolism, and genetics make it difficult to extrapolate such results to humans exposed to tiny amounts of dioxin. In other words, we don't really know whether the tiny amount of dioxin that we typically encounter is a small cancer risk, poses no cancer risk, or perhaps even decreases our risk of developing cancer. Another family of environmental contaminants that might represent a cancer hazard are the organochlorine pesticides, a group that includes the now-banned insecticide dichlorodiphenyltrichloroethane (DDT).

Compounds of this type can mimic the action of estrogen, which is known to promote the development of breast cancer.

Animals exposed to organochlorines exhibit increased cancer rates, and several widely quoted epidemiological studies have detected a correlation between exposure to organochlorine pesticides and breast cancer rates in women. However, most epidemiological studies have failed to detect such a relationship. Some especially interesting data emerged from a study of several thousand women in Long Island, an area in which organochlorines were extensively used and in which breast cancer rates are higher than the national average.To precisely quantify exposure to organochlorine pesticides, blood samples were obtained from breast cancer patients and from a group of comparable women without the disease. Measurements of the concentration of organochlorines in the blood failed to reveal any relationship between exposure to organochlorine compounds and the development of breast cancer.

Pollution of Outdoor and Indoor Air Creates Small Cancer Risks:

The general topic of air pollution provides yet another example of the difficulties encountered when trying to assess environmental cancer hazards involving low-dose exposures. In many cities, both large and small, the air is contaminated with fine particles of airborne soot emitted by cars and trucks, power plants, and factories.

A recent epidemiological study of 500,000 adults living in dozens of cities across the United States revealed that people located in cities with the largest amounts of this fine-particle soot have lung cancer death rates roughly 10% higher than in cities with minimal pollution.Of course, a 10% increase is not very much; for comparison purposes, cigarette smokers increase their risk of developing lung cancer by 2500% or more, a value that is 250 times higher than the small increase in lung cancer risk that might be associated with air pollution.

Although discussions of air pollution usually focus on outdoor air, our main exposure to polluted air may be indoors. In studies involving more than a dozen different cities, researchers equipped people with air-quality monitoring devices that were small enough to carry around as people performed their normal daily activities.For the average citizen, the greatest exposure to toxic airborne chemicals turned out to occur inside their homes (Figure 4). The sources of this indoor air pollution included ordinary consumer products such as cleaning compounds, paints, carpeting, gasoline, air fresheners, dry cleaning, and disinfectants

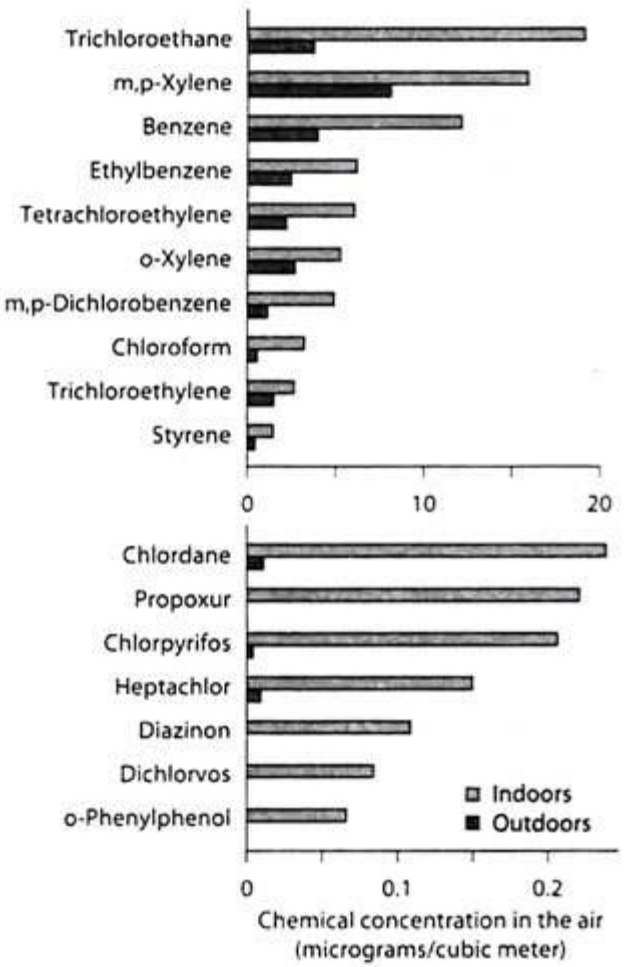


Figure 4 Comparison of Indoor and Outdoor Air Pollution. Pollution data were obtained from people equipped with portable air-quality monitoring devices designed to measure the concentration of toxic volatile organic chemicals (*top*) and pesticides (*bottom*) in indoor and outdoor air. Results from studies involving more than a dozen cities in the United States, including cities with chemical processing plants, have revealed that the air inside a person's home usually contains higher concentrations of potential carcinogens than are present in outdoor air. [Data from W. R. Ott and J. W. Roberts, *Sci. Amer.* 278 (February 1998): 88.]

Even in cities where the outdoor air was polluted by emissions from chemical processing plants, the concentration of many airborne carcinogens was higher inside homes than outdoors. However, these indoor concentrations were still much lower than those typically encountered in industrial workplaces, and it is difficult to know whether such low-dose exposures pose any cancer risks.

Thresholds can Cause Animal Studies to Overestimate Human Cancer Risks:

The difficulty in assessing the hazards of low-dose chemical exposures arises from limitations that are inherent to epidemiological and animal testing. The main problem with the epidemiological approach is that it is not sensitive enough to reliably detect small increases (less than a doubling) in cancer incidence, which is the type of effect that might be expected from low-dose carcinogen exposures. As a consequence, scientists often turn to animal testing.Animal testing also has shortcomings that limit the ability to assess risks from low-dose carcinogen exposures. One problem is the need to obtain a sufficient number of cancer cases to generate statistically reliable results.

For this reason, animals are often exposed for their entire lifetime to the maximum tolerated dose (MTD) of a suspected carcinogen, which is defined as the highest dose that can be administered without causing serious weight loss or signs of immediate life-threatening toxicity. At these high doses, many chemicals cause tissue destruction and cell death. The remaining cells proliferate to replace those cells that have been destroyed, and this enhanced cell proliferation creates conditions that are favourable for the development of cancer.

If the ability of a given chemical to cause cancer stems from this capacity to cause cell death at high doses, lower doses that do not kill cells may not cause cancer. The dose- response curve for such a carcinogen would exhibit a threshold—that is, a dose that must be exceeded before cancer rates begin to rise. Doses below the threshold would be safe in terms of cancer risk. Figure 5 shows how the existence of a threshold can cause the cancer risk of low-dose exposures to be overestimated.

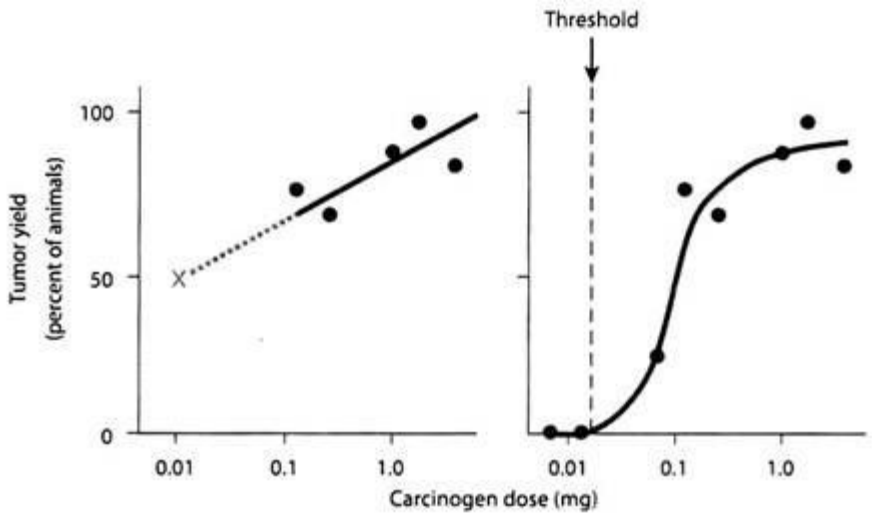


Figure 5 Possibility of Overestimating Cancer Risk When Extrapolating from High-Dose Data. Dose-response curves are illustrated for sarcomas arising in mice after a single injection of benzo[*a*]pyrene. (*Left*) The graph on the left is restricted to high doses of carcinogen (> 0.1 mg). The results appear to be roughly linear, and a straight line can be drawn through the data points to estimate the cancer risk for a lower dose (0.01 mg) of benzo[*a*]pyrene. This estimated cancer risk is indicated by the "X". (*Right*) When actual experiments are carried out that include lower doses of benzo[*a*]pyrene, the shape of the overall curve is seen to exhibit a threshold. Note that the actual cancer risk associated with a 0.01 mg dose of benzo[*a*]pyrene shown in the graph on the right is much lower than the risk estimated by the linear extrapolation derived from the high-dose data shown in the graph on the left. [Based on data from W. R. Bryan and M. B. Shimkin, *J. Natl. Cancer Inst.* 3 (1943): 503.]

The data in Figure 5 are for benzo[a]pyrene, a carcinogen present in gasoline exhaust fumes and in smoke generated by burning organic matter, including tobacco smoke. The graph on the left shows cancer rates for animals exposed to high doses of benzo[a]pyrene. If this data were the only information available, a straight line could be drawn through the data points and extrapolated to lower doses to estimate cancer risk for low-dose exposures to benzo[a]pyrene.The graph on the right, however, shows what happens when actual experiments are performed using lower doses of benzo[a]pyrene. The shape of the overall curve exhibits a threshold, and the actual cancer risk associated with low-dose benzo[a]pyrene exposure is much lower than predicted.

The preceding example highlights that cancer biologists have traditionally had two ways of viewing the relationship between high-dose and low-dose cancer risks: the linear model and the threshold model. As shown in Figure 6, the linear model assumes a linear dose-response relationship with no threshold, whereas the threshold model assumes no cancer risk at lower doses followed by a linear dose-response relationship at higher doses.

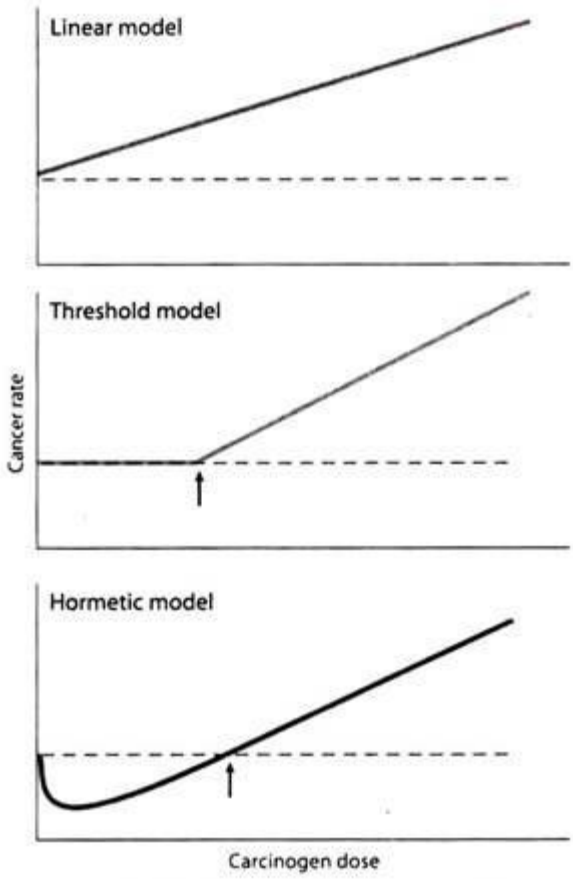


Figure 6 Three Models for the Relationship Between Carcinogen Dose and Cancer Risk. The dashed lines represent the background cancer rate in the absence of carcinogen. Note that the threshold and hormetic models both involve a threshold dose (*arrow*) that must be exceeded before cancer rates begin to rise. [Adapted from E. J. Calabrese, *Mutation Res.* 511 (2002): 181 (Figure 1).]

A third possibility, called the hormetic model, has also begun to receive some attention. The hormetic model proposes that dose-response curves can also be U-shaped. The U-shape, known as hormesis, reflects a situation in which cancer rates actually decline at very low doses of carcinogen and then begin to go up as the dose is further increased. While it is not clear how widely this model might apply to cancer risk, several carcinogenic agents have been reported to reduce cancer rates when administered to animals at low doses.

The hormetic and threshold models both include the concept of a "threshold"—that is, a dose that must be exceeded before cancer rates begin to rise. One possible explanation for the existence of thresholds is the ability of high-dose exposures to cause tissue destruction and cell death, which creates a unique set of conditions that do not exist at lower doses.Another possible reason for thresholds is that carcinogens often act by causing DNA damage. The presence of damaged DNA triggers a group of repair mechanisms that attempt to correct the problem. Such repair mechanisms might be able to fix small amounts of DNA damage caused

by low doses of carcinogens and may even help prevent cancer from arising in response to subsequent exposures to carcinogenic agents.

According to this view, carcinogen-induced mutations only begin to accumulate and initiate the development of cancer after a threshold dose has been exceeded and the capacity of these DNA repair pathways is overcome by massive DNA damage.

Humans and Animals Differ in their Susceptibilities to some Carcinogens:

Another problem that can complicate the extrapolation of animal data to humans is that animals often differ from one another, as well as from humans, in their susceptibility to different carcinogens. Consider the behaviour of 2-acetylaminofluorene (AAF), which is a potent carcinogen in rats but does not cause cancer in guinea pigs. Based on this information alone, it would be difficult to predict whether or not AAF is likely to be carcinogenic in humans.

The reason for the differing behavior of AAF in rats and guinea pigs became apparent when it was discovered that AAF is actually a "precarcinogen" that needs to be metabolically activated before it can cause cancer. Rats, but not guinea pigs, contain the enzyme that catalyzes this metabolic activation. Biochemical analysis of human tissues has revealed that we also contain the activating enzyme, so it is likely that AAF is carcinogenic in humans just as it is in rats. Of course, if AAF had only been tested in guinea pigs, it never would have been suspected of being a carcinogen in the first place.

The artificial sweetener saccharin provides another illustration of the difficulties that can arise when extrapolating data from animal studies to humans. At the peak of its use in the 1970s, Americans consumed more than five million pounds of saccharin per year in artificially sweetened foods and beverages. In 1981, the U.S. government labelled saccharin as a suspected human carcinogen and attempted to ban its use as a food additive because saccharin causes bladder cancer when fed to rats. Subsequent investigations, however, have revealed that saccharin causes bladder cancer in rats for reasons that do not apply to humans.

When rats ingest large amounts of any sodium salt, including sodium saccharin, a crystalline precipitate forms in the bladder that irritates the bladder lining, triggering cell proliferation and increasing the risk of developing cancer. But the precipitate only forms when there are large amounts of protein in the urine, and the urine protein concentration in rats is 100 to 1000 times greater than in humans. Subsequent studies have shown that other laboratory animals, such as hamsters, guinea pigs, and mice, do not develop bladder cancer when fed saccharin. As a consequence, saccharin was recently taken off the government's list of suspected human carcinogens. Because of the uncertainties involved in applying animal data

to humans, caution is needed in labeling substances as human carcinogens when the information has been derived largely from animal studies.

The U.S. government therefore publishes a list that subdivides carcinogens into two distinct categories:

(1) Known to be human carcinogens and

(2) Reasonably anticipated to be human carcinogens.

The list of "known" human carcinogens contains several dozen chemicals for which the data from animal studies have been supplemented with enough human data to clearly establish a cancer risk for humans. The list of "anticipated" human carcinogens includes more than 100 additional chemicals for which the potential cancer hazard has been extrapolated largely from animal studies.While many of these substances will almost certainly turn out to be human carcinogens, mistakes are possible because of the heavy reliance on animal data. For example, saccharin was listed as an "anticipated" human carcinogen for about 20 years before eventually being removed from the list.

Medications and Hormones can Cause Cancer:

We have now seen how difficult it can be to measure cancer risks associated with chemicals to which our exposures are small. Of course, this means that the hazards of such low-dose exposures must be rather small (or nonexistent) because larger risks would be readily detectable through epidemiological and animal testing.

The greatest cancer hazards are posed by chemical carcinogens that we encounter in high concentrations. Included in this category are several situations, including occupational exposure to industrial chemicals and inhalation of the carcinogenic chemicals present in tobacco smoke. Another type of high-dose exposure to specific chemicals comes from the use of prescription drugs for treating certain illnesses. Prescription drugs are often taken for prolonged periods, so it is important to know whether the resulting high-dose chemical exposures can cause cancer.

One tragic example involves diethylstilbestrol (DES), a synthetic estrogen that was prescribed to pregnant women starting in the 1940s as a way of preventing miscarriages. Several decades later, women whose mothers had taken DES during pregnancy began developing vaginal cancer at alarmingly high rates. By that time, roughly five million women in the United States had already taken DES.

This episode illustrates the difficulty in establishing the risks associated with ingesting any new chemical compound, even when it appears to be safe and is prescribed for a specific

medical purpose. In the case of DES, the drug's ability to cause cancer did not become apparent until several decades after women had used DES, and the cancer did not affect the person who took the drug, but rather her children.

Although DES has now been banned as a prescription drug, a number of other medications can also cause cancer (Table 2). Most are prescribed for serious medical problems where the potential benefits of the drug in question are thought to outweigh the risk that cancer might arise.

Table 2 Some Medications That Can Cause Cancer

Medication	Type of Cancer Caused
Analgesic:	
Phenacetin	Kidney
Cancer chemotherapy:	
Chlorambucil	Leukemia
Cyclophosphamide	Bladder, leukemia
Melphalan	Leukemia
Thiotepa	Leukemia
Hormones:	
Estrogens	Breast, uterus, vagina
Oxymetholone	Liver
Immunosuppressive drugs:	
Azathioprine	Lymphoma, skin, liver
Cyclosporin	Lymphoma, skin
Skin treatments:	
Arsenic compounds	Skin, liver, lung
Methoxypsoralen	Skin

For example, some drugs used for slowing or stopping tumour growth in cancer patients can themselves trigger development of a new cancer as a side effect. In a person who already has cancer, the small risk of causing another cancer (often many years in the future) is far outweighed by the possible benefits to be gained from a drug that might cure an existing cancer.A similar situation exists with immunosuppressive drugs, which inhibit immune function and are given to organ transplant patients to prevent rejection of a transplanted organ, such as a heart or kidney. Two of the most commonly used immunosuppressive drugs, azathioprine and cyclosporin, increase the risk of developing cancer, but organ transplant patients depend on the transplanted organ for survival and the benefits of these drugs are thought to outweigh the risk of developing cancer.

Nonetheless, cancer has turned out to be a major cause of death in organ transplant patients and a need therefore exists for better immunosuppressive drugs that do not increase cancer risk. One drug under current investigation is rapamycin (also called sirolimus), an antibiotic with immunosuppressive activity. Animal studies have shown that besides suppressing

immune function, rapamycin inhibits tumour growth under conditions in which another immunosuppressive drug, cyclosporin, stimulates tumour growth. A possible explanation for the antitumour effect of rapamycin has come from the discovery that it inhibits angiogenesis, both by depressing the production of VEGF and by inhibiting the ability of endothelial cells to respond to VEGF. Suppression of angiogenesis by rapamycin may therefore limit the ability of newly forming tumours to obtain the blood supply they require for growth beyond a tiny size.

Mechanisms of Chemical Carcinogenesis:

As the list of substances known to cause cancer has grown over the years, it has become increasingly apparent that carcinogens exhibit wide variations in structure and potency. At first this variability complicated our thinking about the origins of cancer because it was difficult to envision how such a diverse array of chemical substances could cause the same disease. Through an extensive series of studies, however, a common set of mechanisms and principles has begun to emerge that helps explain how the various kinds of carcinogens work.

Chemical Carcinogens can be Grouped into Several Distinct Categories:

Despite their structural diversity, chemical carcinogens can be grouped into a relatively small number of categories (Figure 7). Most are natural or synthetic organic chemicals—that is, carbon-containing compounds. They range from small organic molecules containing only a few carbon atoms to large, complex molecules constructed from multiple carbon-containing rings.

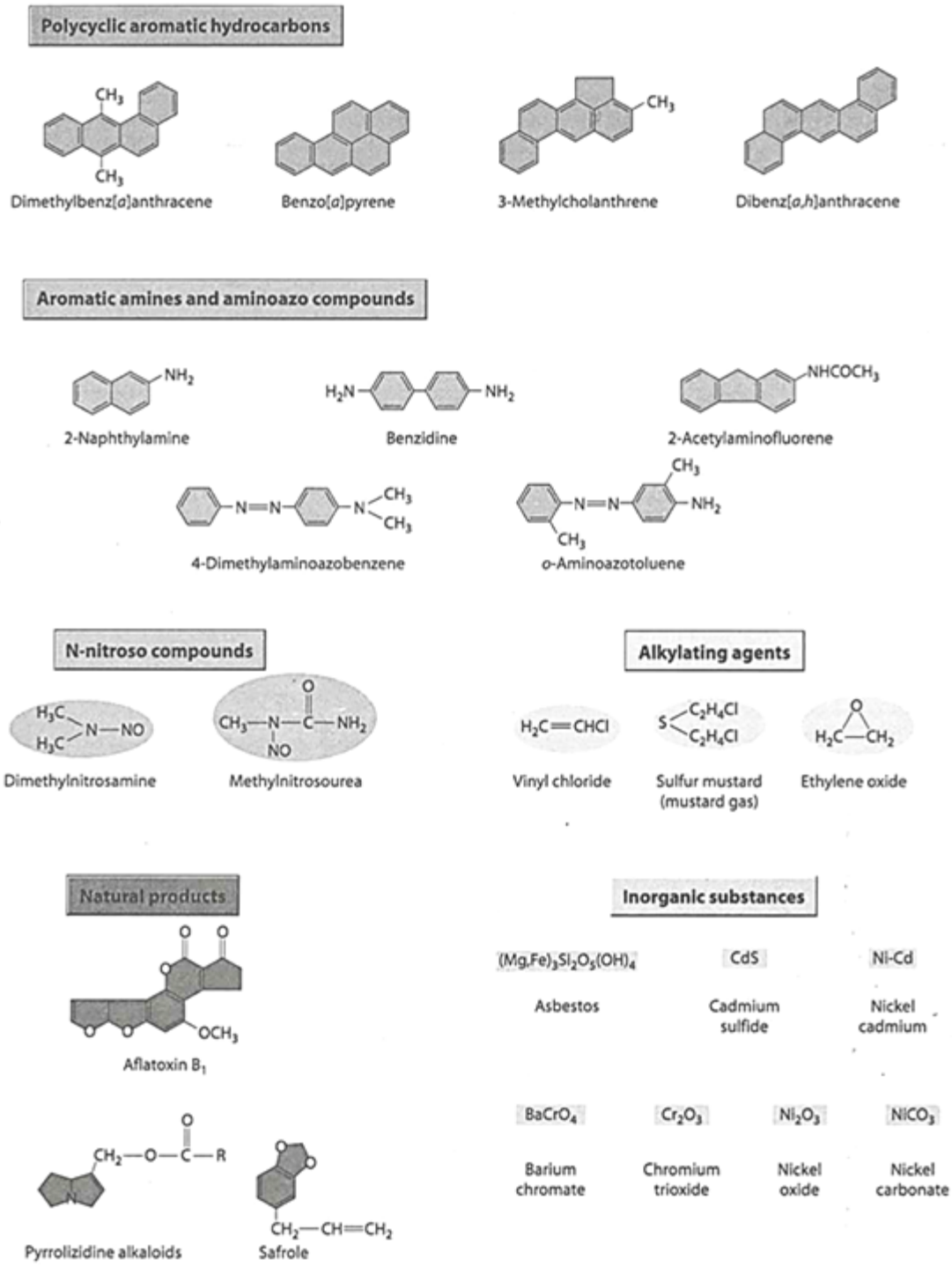


Figure 7 Main Classes of Carcinogenic Chemicals. Selected examples are illustrated for each of the main classes of cancer-causing chemicals. Some of these molecules are precarcinogens that need to be metabolically activated before they can cause cancer, whereas others are direct-acting carcinogens that do not require metabolic activation.

The vast majority fall into one of the following five categories:

1. Carcinogenic polycyclic aromatic hydrocarbons (or simply polycyclic hydrocarbons) are a diverse group of compounds constructed from multiple, fused benzene rings. Polycyclic hydrocarbons are natural components of coal tars, soots, and oils, and are also produced during the incomplete combustion of coal, oil, tobacco, meat, and just about any other organic material that can be burned.

The carcinogenic potency of polycyclic hydrocarbons varies widely, from weak or noncarcinogenic molecules to very potent carcinogens. The polycyclic hydrocarbons benzo[a]pyrene and dibenz[a,h]anthracene, isolated from coal tar in the 1930s, were the first purified chemical carcinogens of any kind to be identified.

2. Carcinogenic aromatic amines are organic molecules that possess an amino group (—NH_2) attached to a carbon backbone containing one or more benzene rings. Some aromatic amines are aminoazo compounds, which means that they contain an azo group (N=N) as well as an amino group. Among the carcinogens in these categories are the aromatic amines benzidine, 2-naphthylamine, 2-acetylaminofluorene, and 4-aminobiphenyl, and the aminoazo dyes 4-dimethylaminoazobenzene and o-aminoazotoluene.
Many of these compounds were once employed in the manufacturing of dyes, although most are no longer used in significant quantities because of their toxicity. Some aromatic amines, such as 2-naphthylamine and 4-aminobiphenyl, are components of tobacco smoke. As in the case of polycyclic hydrocarbons, the carcinogenic potency of aromatic amines and aminoazo dyes varies from substances that are strongly carcinogenic to substances that are not carcinogenic at all.

3. Carcinogenic N-nitroso compounds are organic chemicals that contain a nitroso group (N=O) joined to a nitrogen atom. Members of this group include the nitrosamines and nitrosoureas, which are potent carcinogens when tested in animals. Most of these compounds are industrial or research chemicals encountered mainly in the workplace, although a few are present in cigarette smoke.

Nitrates and nitrites used in the curing of meats, which are not carcinogenic in themselves, can be converted in the stomach into nitrosamines, but no consistent relationship between these compounds and human cancer has been established.

4. Carcinogenic alkylating agents are molecules that readily undergo reactions in which they attach a carbon-containing chemical group to some other molecule. Unlike the three preceding groups of carcinogens, which are defined by their chemical structures (i.e., the presence of multiple benzene rings, amino groups, or nitroso groups), alkylating agents are defined not by their structural features but by their chemical reactivity—that is, their ability

to join a chemical group to another molecule. The N-nitroso compounds, discussed in the preceding paragraph, are examples of carcinogens that function as alkylating agents.
Other examples include vinyl chloride (used in the production of plastics) and ethylene oxide (used in the production of antifreeze and other chemicals). Vinyl chloride and ethylene oxide are among the highest-volume chemicals produced in the United States. Other carcinogenic alkylating agents include sulfur mustard (a chemical warfare agent) and several drugs used in cancer chemotherapy.

5. Carcinogenic natural products are a structurally diverse group of cancer-causing molecules produced by biological organisms, mainly microorganisms and plants. Included in this category is aflatoxin, a carcinogenic chemical made by the mold Aspergillus. One of the most potent carcinogens known, aflatoxin sometimes contaminates grains and nuts that have been stored under humid conditions. Other carcinogenic natural products include plant-derived molecules such as safrole, a major component of sassafras root bark, and pyrrolizidine alkaloids, produced by a variety of different plants.

In addition to the preceding five classes of organic molecules, a small number of inorganic substances (compounds without carbon and hydrogen) are carcinogenic. Included in this group are compounds containing the metals cadmium, chromium, and nickel.

Some inorganic substances appear to be carcinogenic in the absence of chemical reactivity. For example, asbestos is a mineral composed of silicon, oxygen, magnesium, and iron, but its ability to cause cancer is related to the crystal structure and size of the microscopic fibers it forms rather than their precise chemical makeup.

Some Carcinogens need to be activated by Metabolic Reactions Occurring in the Liver:

The chemicals illustrated in Figure 7 are considered to be “carcinogens” because humans or animals develop cancer when exposed to them. This designation does not mean, however, that every carcinogen triggers cancer directly. For example, consider the behavior of 2-naphthylamine, whose ability to cause bladder cancer in industrial workers.

As might be expected, feeding 2-naphthylamine to laboratory animals induces a high incidence of bladder cancer, but cancer rarely arises when 2-naphthylamine is directly inserted into an animal’s bladder.

The reason for this discrepancy is that when 2-naphthylamine is ingested (by animals) or inhaled (by humans), it first passes through the liver and is metabolically converted into chemical compounds that are the actual causes of cancer. Inserting 2-naphthylamine directly into the bladder bypasses the liver and the molecule is never activated, so cancer does not

arise.Many carcinogens share a similar need for metabolic activation before they can cause cancer. Carcinogens exhibiting such behavior are more accurately called precarcinogens, a term referring to any substance that is capable of causing cancer only after it has been metabolically activated. The activation of precarcinogens is generally carried out by liver proteins that are members of the cytochrome P450 enzyme family.

One function of these liver enzymes is to catalyze the oxidation of ingested foreign chemicals, such as drugs and pollutants, with the aim of making molecules less toxic and easier to excrete from the body.

The hydroxylation reaction illustrated in Figure 8 is one of several ways in which cytochrome P450 oxidizes foreign chemicals to make them more water soluble, thereby facilitating their excretion in the urine. Occasionally, however, oxidation reactions catalyzed by cytochrome P450 accidentally convert substances into carcinogens, a phenomenon known as carcinogen activation. Evidence that cytochrome P450 is involved in carcinogen activation has come from numerous animal studies. One set of experiments involved a mutant strain of mice that produce abnormally large amounts of cytochrome P450 1A1, a form of cytochrome P450 that oxidizes polycyclic hydrocarbons. As would be expected if cytochrome P450 1A1 were involved in carcinogen activation, cancer rates are elevated in the mutant mice that produce large amounts of this enzyme. Cancer rates can be reduced in these same animals by using inhibitors that block the action of cytochrome P450 1A1.

Elevated amounts of cytochrome P450 1A1 are found in the livers of people who smoke cigarettes, apparently because tobacco smoke stimulates production of the enzyme by the liver. This means that in addition to containing dozens of chemicals that cause cancer, cigarette smoke also induces the production of liver enzymes that make the situation worse by activating carcinogenic activity in chemicals that might not otherwise cause cancer.

About one person in ten inherits a form of cytochrome P450 1A1 that is produced in especially large amounts in response to tobacco smoke. If such a person smokes cigarettes, he or she has an even higher risk of developing lung cancer than other smokers.The role played by liver enzymes in carcinogen activation explains why chemicals being assayed for mutagenic activity in the Ames test are first incubated with a liver homogenate to mimic any reactions that might take place in the body. The requirement for metabolic activation also helps explain why some chemicals only cause cancer in certain organisms.

For example, 2-acetylaminofluorene (AAF) is carcinogenic in rats but not in guinea pigs because guinea pigs lack the enzyme needed to convert AAF into an active carcinogen. Because of such differences in liver enzymes between organisms, it is important that suspected carcinogens be tested in more than one animal species.

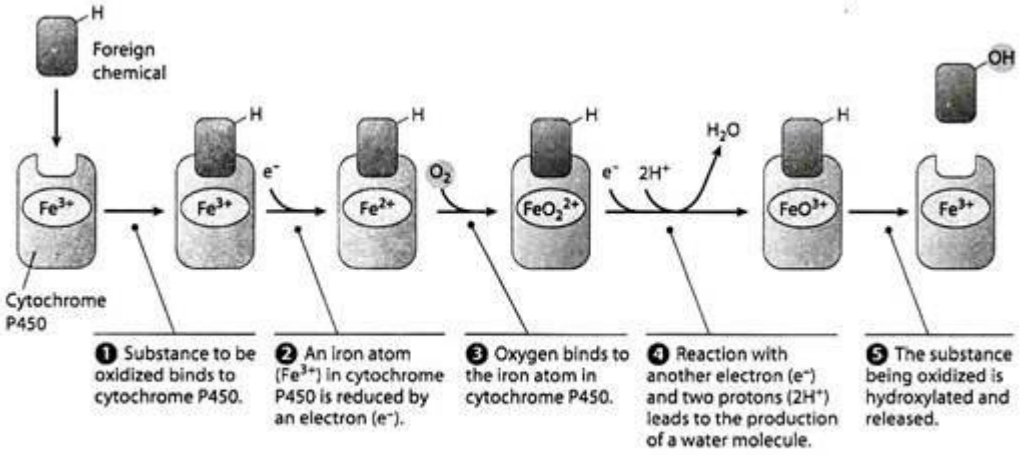


Figure 8 Hydroxylation Reaction Catalyzed by Cytochrome P450. Cytochrome P450 oxidizes chemicals by linking them to a hydroxyl group in a five-step oxidation reaction.

Many Carcinogens are Electrophilic Molecules that React Directly with DNA:

Despite the variations in molecular structure exhibited by the carcinogens illustrated in Figure 7, many share the same property: When metabolized in the liver, they are converted into highly unstable compounds with electron-deficient atoms. Such molecules are said to be electrophilic ("electron-loving") because they readily react with substances possessing atoms that are rich in electrons.

DNA, RNA, and proteins all have electron-rich atoms, making each a potential target for electrophilic carcinogens. Of the three, DNA is the prime candidate because the Ames test has shown that most carcinogens cause DNA mutations. An experiment designed to determine whether DNA is in fact the direct target of chemical carcinogens is summarized in Figure 9.

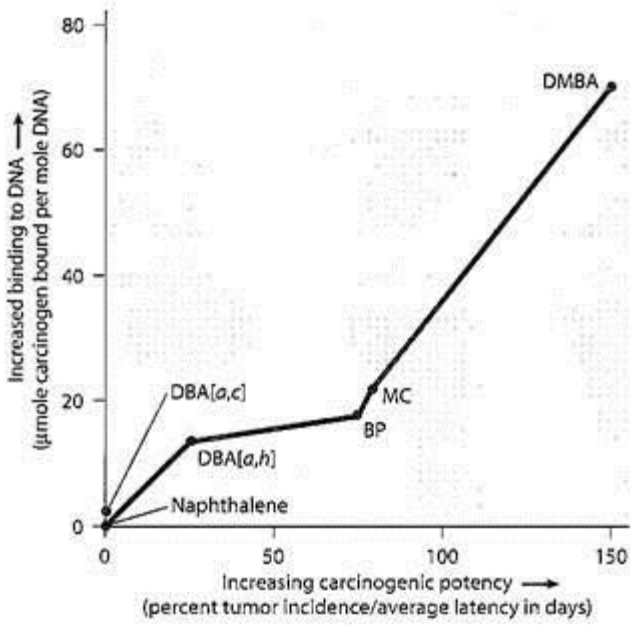


Figure 9 Relationship Between Carcinogenic Potency and DNA-Binding Ability. Six polycyclic hydrocarbons of varying carcinogenic potency were injected into animals and measurements were then made to identify the intracellular molecules to which they had become bound. The data show that the more potent the carcinogen, the more extensively it binds to DNA. *Abbreviations*: DBA[*a*,*c*] = dibenz[*a*,*c*]anthracene, DBA[*a*,*h*] = dibenz[*a*,*h*]anthracene, BP = benzo[*a*]pyrene, MC = 3-methylcholanthrene, DMBA = dimethylbenz[*a*]anthracene. [Data from P. Brookes and P. D. Lawley, *Nature* 202 (1964): 781 (Figure 5).]

In this study, animals were injected with various polycyclic hydrocarbons that differed in carcinogenic potency. Cells were then isolated from the treated animals and measurements were made to determine which intracellular molecules (if any) had become bound to the polycyclic hydrocarbons.

The data revealed a direct relationship between the carcinogenic potency of different polycyclic hydrocarbons and their ability to become covalently linked to DNA; in other words, those polycyclic hydrocarbons that became extensively bound to DNA were the most effective at causing cancer.Before a polycyclic hydrocarbon can interact with DNA, it must be activated. For example, consider the behavior of benzo[a]pyrene, which is normally a nonreactive, non-mutagenic compound. After entering the body, metabolic reactions catalyzed by cytochrome P450 in the liver convert benzo[a]pyrene into activated derivatives containing an epoxide group (Figure 10).

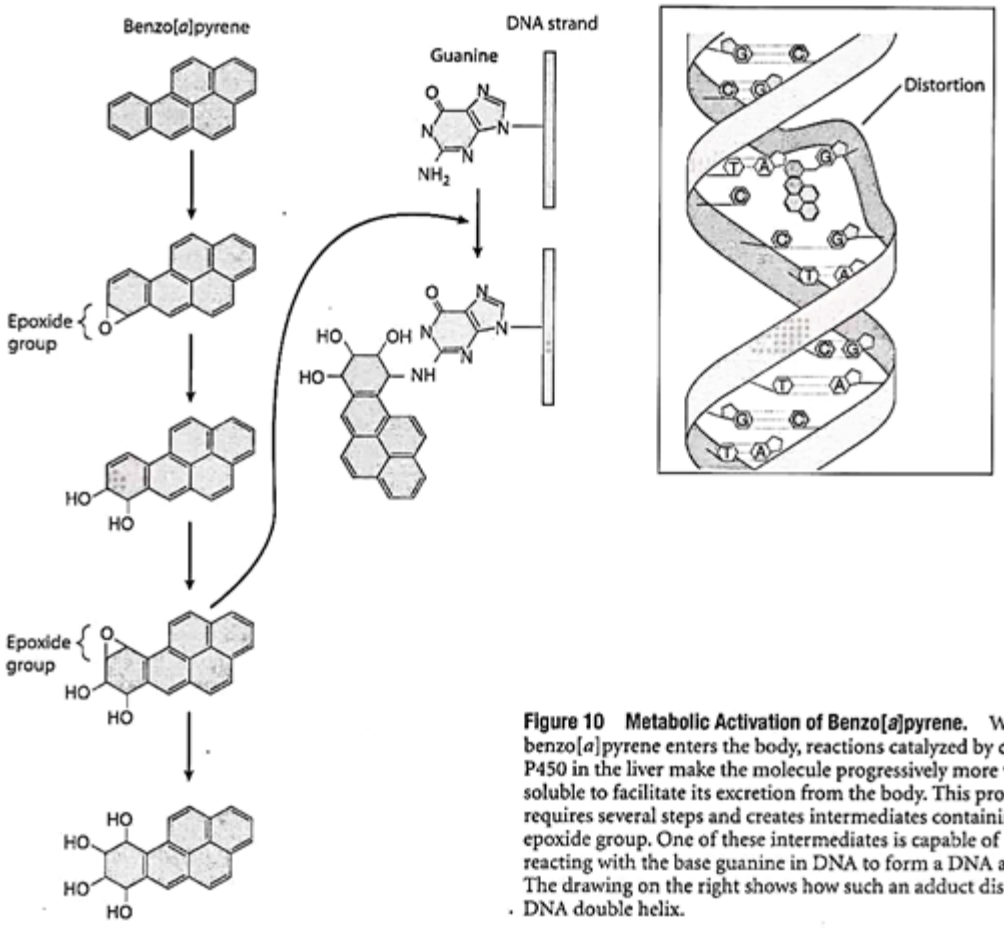
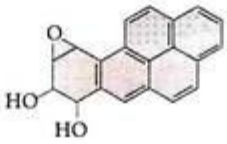
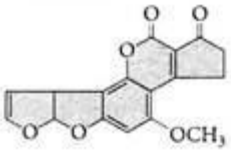
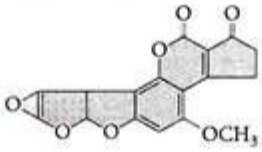
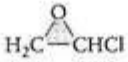


Figure 10 Metabolic Activation of Benzo[*a*]pyrene. When benzo[*a*]pyrene enters the body, reactions catalyzed by cytochrome P450 in the liver make the molecule progressively more water soluble to facilitate its excretion from the body. This process requires several steps and creates intermediates containing an epoxide group. One of these intermediates is capable of reacting with the base guanine in DNA to form a DNA adduct. The drawing on the right shows how such an adduct distorts the DNA double helix.

An epoxide is a three-membered ring containing an oxygen atom covalently bonded to two carbon atoms; these two carbons are electron deficient and therefore tend to react with atoms that are electron rich, such as the amino nitrogen found in the DNA base guanine. Reaction of the epoxide group with guanine causes the benzo[a]pyrene to become covalently bonded to DNA, thereby forming a DNA-carcinogen complex called a DNA adduct. The presence of the bound carcinogen distorts the DNA double helix and thereby causes errors in base sequence (mutations) to arise during DNA replication.

Epoxide formation is also involved in the activation of other classes of chemical carcinogens. For example, aflatoxin and vinyl chloride, which differ significantly from polycyclic hydrocarbons in chemical structure, are both oxidized by cytochrome P450 into epoxides that, like benzo[a]pyrene, react with DNA bases to form DNA adducts (Table 3).

Table 3 Examples of Several Carcinogens Activated by Epoxide Formation

Carcinogen	Major Active Metabolite*	Site of DNA Modification**
Benzo[*a*]pyrene (BP) 	BP 7,8-diol 9,10-epoxide 	N2 of guanine N6 of adenine
Aflatoxin B_1 	Aflatoxin B_1 8,9-epoxide 	N7 of guanine
Vinyl chloride	Chloroethylene oxide 	N3 and N4 of cytosine*** N1 and N6 of adenine N2 and N3 of guanine

*Green shading is used to highlight the epoxide group.
**The numbers in the third column refer to the numbered positions of nitrogen atoms illustrated in Figure 11.
***Vinyl chloride simultaneously attacks two positions on the same base, forming a cyclic adduct.

However, the various epoxides do not all react with the same DNA bases. In fact, depending on the carcinogen involved, almost every electron-rich site in the various DNA bases can serve as a target for carcinogen attachment (Figure 11). And epoxides are not the only electrophilic groups that react with DNA.

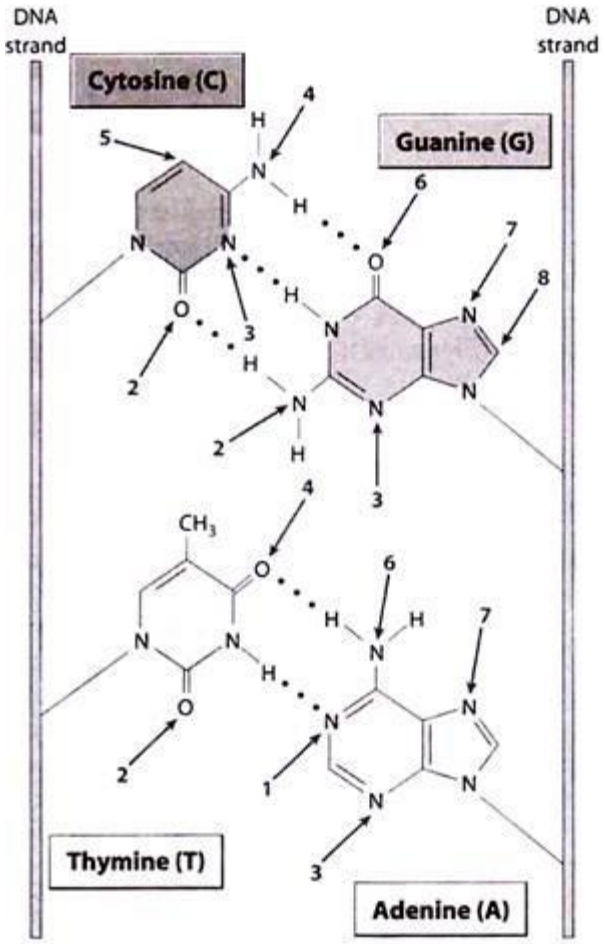


Figure 11 Sites of Carcinogen Attack in DNA Bases. The red arrows indicate the main sites in the four DNA bases that serve as targets for carcinogen attachment. The red numbers refer to the numbering system used to identify the atoms located at different positions within each base. The dotted blue lines represent the hydrogen bonds that normally hold the complementary base pairs together in the DNA double helix. Attachment of carcinogen molecules to the bases tends to distort the double helix and interfere with this hydrogen bonding, thereby leading to errors in DNA replication.

Some carcinogens are activated by reactions that create other types of electrophilic groups, such as positively charged nitrogen atoms (nitrenium ions) or carbon atoms (carbonium ions), or compounds containing an unpaired electron (free radicals). Like epoxides, these electrophilic groups also attack electron-rich atoms in DNA.

The preceding mechanisms illustrate that, despite their chemical diversity, many carcinogens share the property of being converted into electrophilic molecules that in turn become linked to DNA. This ability to form DNA adducts is one of the best predictors of a molecule's capacity to cause cancer.

In addition, carcinogens can inflict DNA damage in several other ways; for example, they may generate crosslinks between the two strands of the double helix, create chemical linkages between adjacent bases, hydroxylate or remove individual DNA bases, or cause breaks in one or both DNA strands (Figure 12).

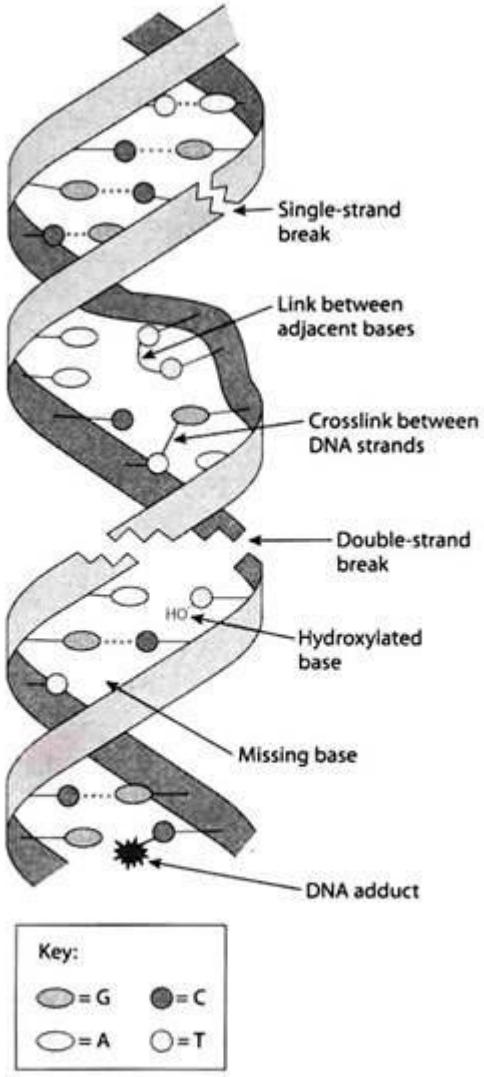


Figure 12 Summary of DNA Damage Caused by Chemical Carcinogens. Chemical carcinogens inflict DNA damage in a variety of ways, altering or removing individual bases and triggering breaks in one or both DNA strands.

Chemical Carcinogenesis is a Multistep Process:

An attack on DNA by an activated carcinogen is just the first of several steps involved in creating a cancer cell. The idea that cancer arises through a multistep process was first proposed in the early 1940s by Peyton Rous to explain a phenomenon he encountered when studying the ability of coal tar to cause cancer in rabbits.

Rous had observed that repeated application of coal tar to rabbit skin caused tumours to develop, but the tumours disappeared when application of the coal tar was stopped. Subsequent application of an irritant such as turpentine, which does not induce cancer by itself, caused the tumours to reappear. This pattern suggested to Rous that coal tar and turpentine play two different roles, which he called initiation and promotion. According to his theory, initiation converts normal cells to a precancerous state and promotion then stimulates the precancerous cells to divide and form tumours.

Because coal tar is a mixture of various chemicals, clarification of the initiation/promotion hypothesis required the isolation and study of individual coal tar components. One such chemical is the polycyclic hydrocarbon dimethylbenz[a]anthracene (DMBA). DMBA is a potent carcinogen, but feeding mice a single dose rarely causes tumours to arise. However, if the skin of a mouse fed a single dose of DMBA is later painted with a substance that causes skin irritation, cancer develops in the treated area (Figure 13).

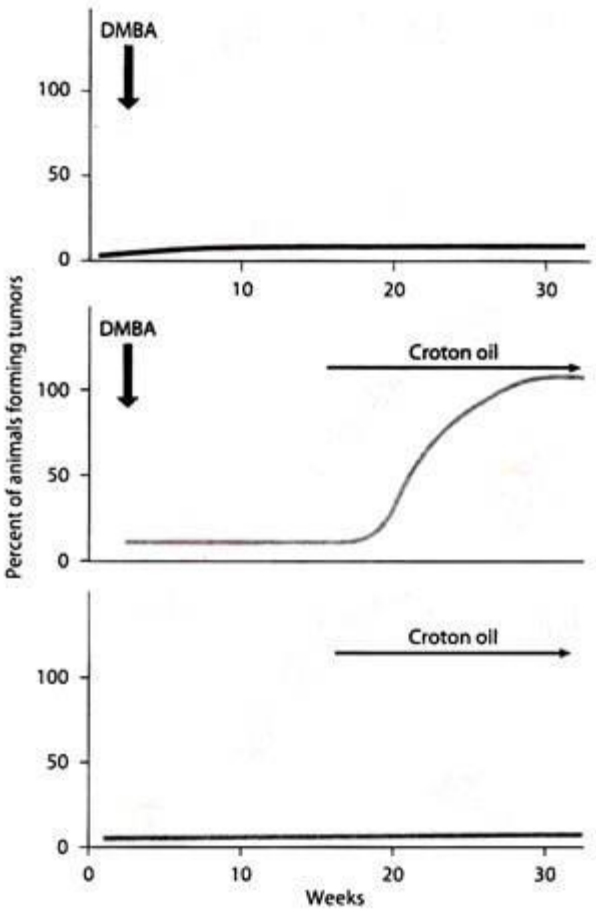


Figure 13 Evidence for the Existence of Initiation and Promotion Stages in Chemical Carcinogenesis. (*Top*) Mice treated with a single dose of DMBA (dimethylbenz[*a*]anthracene) do not form tumors. (*Middle*) Painting the skin of such animals twice a week with croton oil after the DMBA treatment leads to the appearance of skin tumors. If the croton oil application is stopped a few weeks into the treatment (data not shown), the tumors will regress. (*Bottom*) Croton oil alone does not produce skin tumors. These data are consistent with the conclusion that DMBA is an initiator and croton oil is a promoter. [Adapted from R. K. Boutwell, *Prog. Exp. Tumor Res.* 4 (1963): 207.]

Besides turpentine, the irritant most commonly used for triggering tumour formation in such experiments is croton oil, a substance derived from seeds of the tropical plant Croton tiglium. Croton oil does not cause cancer in the absence of prior exposure to a carcinogen such as DMBA, nor will cancer arise if DMBA is administered after the croton oil.

These observations support the concept that chemical carcinogenesis is a multistep process in which an initiator (in this case, DMBA) first creates an altered, precancerous state and then a promoting agent (in this case, croton oil) stimulates the development of tumours.

The Initiation Stage of Carcinogenesis is Based on DNA Mutation:

A year or more can transpire after feeding animals a single dose of DMBA and yet tumours will still arise if an animal's skin is then irritated with croton oil. Thus a single DMBA treatment creates a permanently altered, initiated state in cells located throughout the body, and a promoting agent (croton oil) can then act on these altered cells to promote tumour development.

Because the carcinogenic potency of most chemicals correlates with their ability to bind to DNA and cause mutations (see Figure 9), the permanent alteration is thought to be a mutation. Carcinogens that act in this way are said to be genotoxic because they cause gene damage. The ability to cause gene mutations explains how a single exposure to an initiating carcinogen can create a permanent, inheritable change in a cell's properties.

Referring to carcinogen-induced mutations as "permanent," however, implies that DNA damage cannot be repaired, which seems to contradict what we know about the existence of DNA repair mechanisms. Such mechanisms are in fact capable of repairing mutations created by initiating carcinogens as long as the damage is repaired in a timely fashion. Once a damaged DNA molecule has been replicated, as occurs each time a cell divides, mutations become very difficult, if not impossible, to repair and the initiated state therefore becomes permanent.

Figure 14 illustrates why this is the case, using the carcinogen methylnitrosourea as an example. Methylnitro- sourea attacks the base guanine (G) in DNA, creating a methylated guanine derivative. If the cell's DNA is replicated before repair mechanisms correct the defect, the methylated guanine tends to form an incorrect base pair with thymine (T) during DNA replication rather than pairing with its correct partner, cytosine (C).

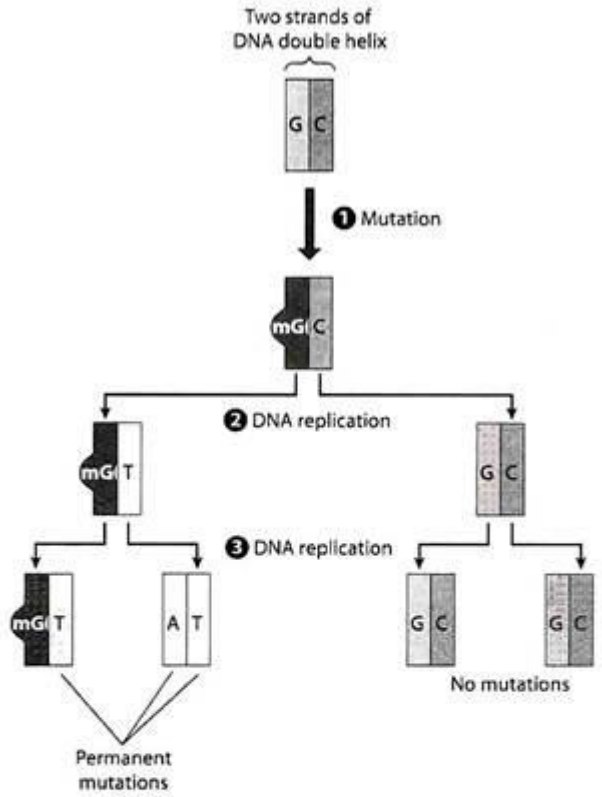


Figure 14 Process by Which a DNA Mutation Becomes Permanent. This hypothetical illustration involves a mutation triggered by exposure to the carcinogen *methylnitrosourea*, but a similar principle applies to many kinds of base mutations caused by chemical carcinogens. ① Methylnitrosourea causes a mutation by adding a methyl group to the base G (the methylated base is designated *mG*). ② If the DNA is replicated before the mG mutation can be repaired, the mG will form an incorrect base pair with T during DNA replication. ③ When the DNA is again replicated, the T forms a normal base pair with A. Hence, the DNA molecule now contains an AT base pair where a GC base pair had originally been located. Because the cell would not recognize anything abnormal about an AT base pair, the mutation is permanent.

During the next round of DNA replication, the incorrectly inserted T will form a base pair with its normal complementary base, adenine (A), creating an AT base pair. The DNA molecule now contains an AT base pair where a GC base pair had originally existed. Since DNA repair mechanisms would not recognize anything abnormal about an AT base pair, the error will persist.

The preceding scenario demonstrates an important principle that applies to many mutations- If DNA replication occurs before mutations are repaired, base-pair alterations tend to arise during replication that cannot be subsequently detected as mutations by cellular repair mechanisms. For this reason it is crucial that mutations be repaired swiftly, before subsequent rounds of DNA replication create a permanent mutation.

Tumour Promotion involves a Prolonged Period of Cell Proliferation:

In contrast to initiation, which requires only a single exposure to an initiating carcinogen, promotion is a gradual process that depends on prolonged or repeated exposure to a promoting agent. If the promoting agent is removed during the early stages of tumour formation, tumours stop growing and may even disappear.

How do we explain the ability of promoting agents to trigger an event that is potentially reversible, at least in its early stages? Studies of a wide variety of promoting agents have revealed that their main shared property is the ability to stimulate cell proliferation. When an initiated cell is exposed to a promoting agent, the cell starts dividing and the number of initiated cells increases.

In the early stages of this process, cell proliferation depends on the presence of the promoting agent, and the cells will stop dividing if the agent is removed. As cell division continues, however, natural selection favors those newly forming cells whose proliferation is faster and autonomous, eventually leading to the formation of a malignant tumour whose growth no longer depends on external promoting agents. The time required for promotion contributes to the long delay that often transpires between exposure to an initiating carcinogen and the development of cancer.

The way in which specific promoting agents stimulate cell proliferation was first established for phorbol esters, the class of tumour promoters found in croton oil. In terms of its tumour promoting activity, the most potent phorbol ester is tetradecanoyl phorbol acetate (TPA).TPA binds to and activates an enzyme called protein kinase C, which plays a key role in one of the cell's normal pathways for controlling cell proliferation (Figure 15). In the normal operation of this pathway, external signaling molecules bind to cell surface receptors whose activation leads to the production of an intracellular signaling molecule called diacylglycerol (DAG).

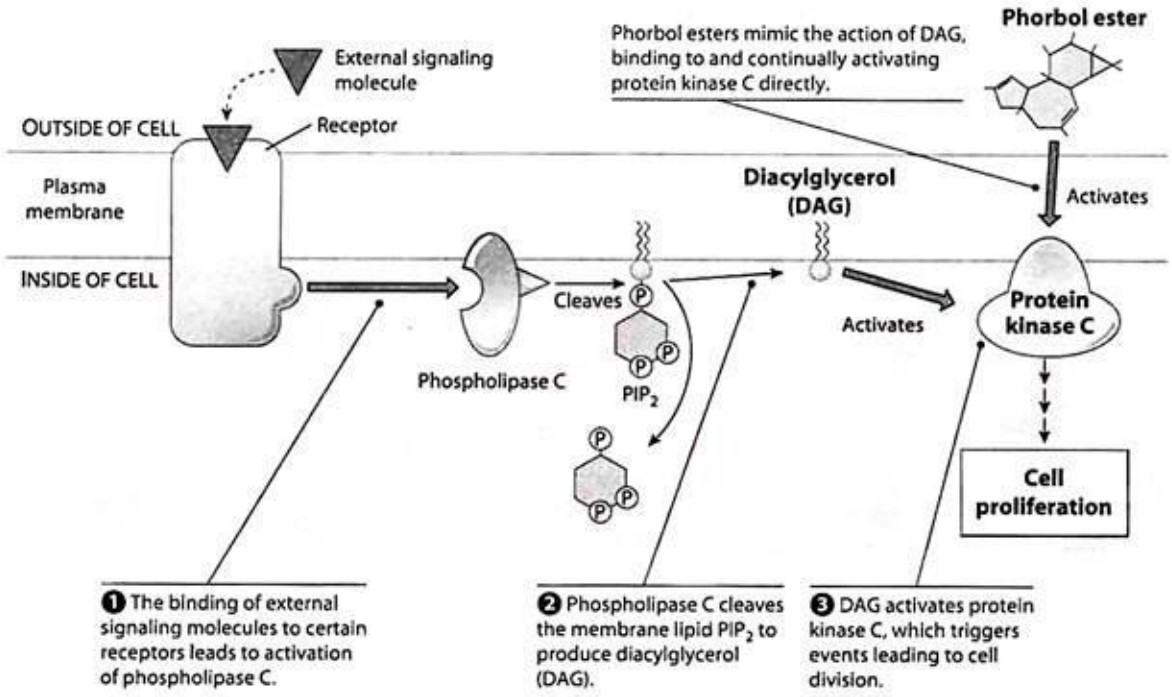


Figure 15 Mechanism of Action of Phorbol Esters. Phorbol esters activate protein kinase C, a component of a signaling pathway that stimulates cell proliferation. In the normal operation of this pathway, external signaling molecules bind to cell surface receptors whose activation leads to the production of diacylglycerol (DAG). The DAG then activates protein kinase C, which triggers events leading to cell division. Phorbol esters mimic the action of DAG, binding to and activating protein kinase C directly. (Green arrows represent activation reactions.)

DAG in turn activates protein kinase C, which triggers events leading to cell division. Phorbol esters mimic the action of DAG, binding to and activating protein kinase C. Unlike DAG, however, which is converted to inactive forms, phorbol esters continually activate the protein kinase C molecule. Activation of protein kinase C by TPA is a highly selective interaction; small changes in the chemical structure of TPA yield derivatives that exhibit diminished ability to bind to protein kinase C and, as a result, decreased ability to function as tumour promoters (Figure 16).

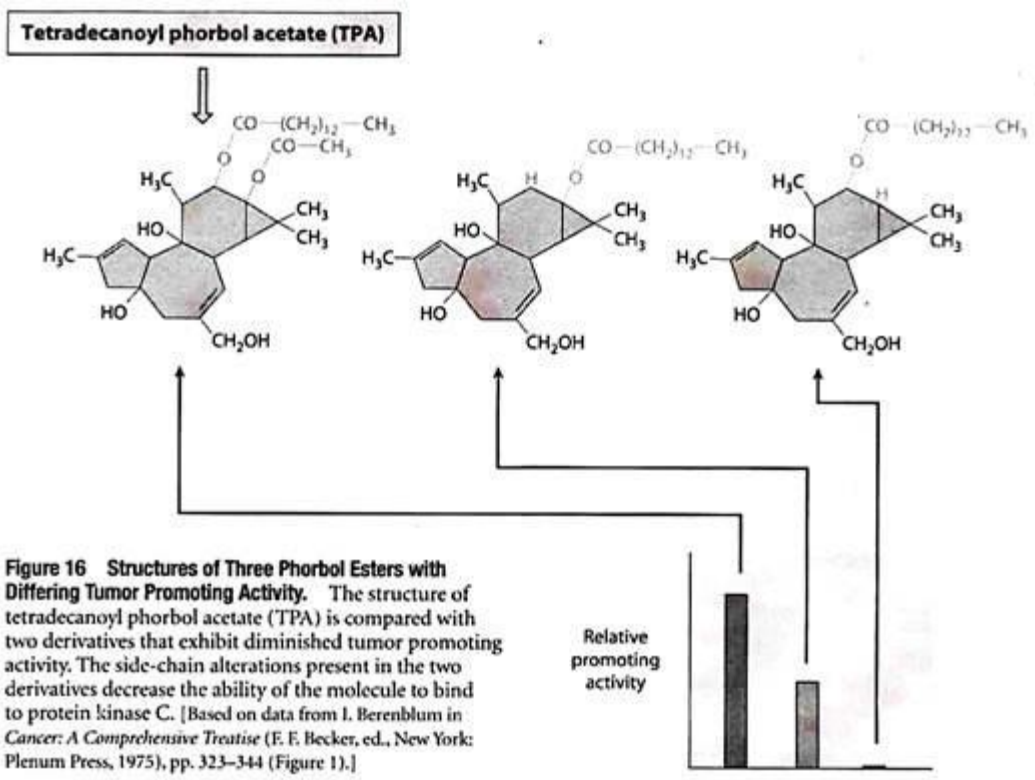


Figure 16 Structures of Three Phorbol Esters with Differing Tumor Promoting Activity. The structure of tetradecanoyl phorbol acetate (TPA) is compared with two derivatives that exhibit diminished tumor promoting activity. The side-chain alterations present in the two derivatives decrease the ability of the molecule to bind to protein kinase C. [Based on data from I. Berenblum in *Cancer: A Comprehensive Treatise* (F. F. Becker, ed., New York: Plenum Press, 1975), pp. 323–344 (Figure 1).]

In addition to phorbol esters, a variety of other foreign substances stimulate cell proliferation and thereby acts as promoting agents. Some of these molecules resemble phorbol esters in being able to interact with protein kinase C. The fungal toxin teleocidin and the algal toxin aplysiatoxin are two such agents that function by activating protein kinase C, even though their chemical structures differ significantly from those of phorbol esters.

Other promoting agents stimulate cell proliferation indirectly, causing tissue damage and cell destruction that make it necessary for the remaining cells of the affected tissue to proliferate to replace the cells that have been damaged and destroyed. Asbestos and alcohol are two previously discussed substances that function in this way.

Not all tumour promoters are foreign substances. Because cell proliferation occurs in normal cells as well as in tumour cells, molecules produced by the body for the purpose of stimulating normal cell division may also function inadvertently as tumour promoters. For example, estrogen is a naturally produced steroid hormone that can contribute to the development of breast and ovarian cancer by stimulating the proliferation of cells in these tissues. The hormone testosterone stimulates the proliferation of cells in the prostate gland and plays a comparable role in promoting the development of prostate cancer. Of course, the intended function of estrogen and testosterone is to stimulate the growth and division of normal cells, not cancer cells.

But if a breast or prostate cell has acquired an initiating mutation caused by a carcinogen or by an error in DNA replication, any normal hormone or growth factor that stimulates the proliferation of the mutant cell will act inadvertently as a tumour promoter.

In addition to foreign chemicals and natural hormones, certain components of the diet may also act as promoting agents—that is, agents that increase cancer risk by stimulating cell proliferation rather than by creating mutations. Dietary fat and alcohol are two examples that fit this category. In general, any chemical associated with an increased cancer risk that is found not to be genotoxic can be suspected of acting as a promoting agent.

Tumour Progression involves Repeated Cycles of Selection for Rapid Growth and Other Advantageous Properties:

When Rous first proposed that chemical carcinogenesis is a multistep process, he identified only two stages- initiation and promotion. It has gradually become apparent that a third stage, known as tumour progression, follows initiation and promotion (Figure 17). The concept of tumour progression refers to the gradual changes in the properties of proliferating tumour cell populations that occur over time as cells acquire more aberrant traits and become increasingly aggressive.

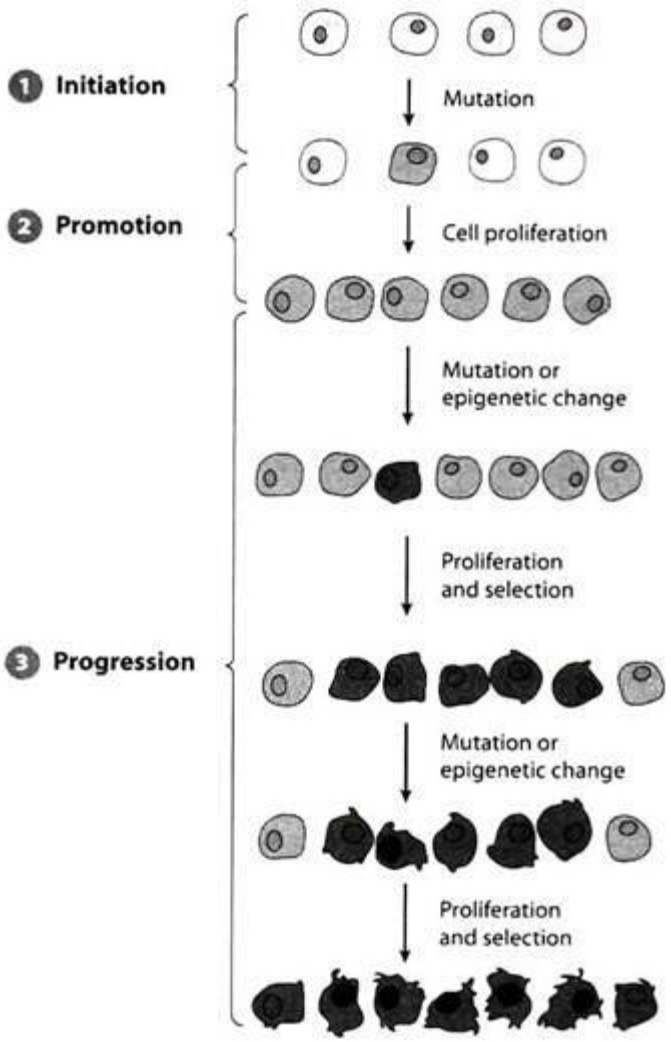


Figure 17 Main Stages of Carcinogenesis. Cancer arises by a complex process involving three main stages. ① The first stage, initiation, is based on DNA mutation. Initiation is followed by a ② promotion stage in which the initiated cell is stimulated to proliferate. ③ During tumor progression, further mutations and epigenetic changes in gene expression create variant cells exhibiting enhanced growth rates or other aggressive properties that give certain cells a selective advantage. Such cells tend to outgrow their companions and become the predominant cell population in the tumor. Repeated cycles of this process, called clonal selection, create a population of cells whose properties change over time.

The underlying explanation for tumour progression is that cells exhibiting traits that confer a selective advantage—for example, increased growth rate, increased invasiveness, ability to survive in the bloodstream, resistance to immune attack, ability to grow in other organs, resistance to drugs, and evasion of death-triggering mechanisms (apoptosis)—will be more successful than cells lacking these traits and will gradually come to predominate.

While it is easy to see why cells exhibiting such traits tend to prevail through natural selection, that does not explain how the aberrant traits originate in the first place. One way of creating new traits is through additional mutations. If a particular mutation causes a cell to divide more rapidly, cells produced by the proliferation of this mutant cell will outgrow their companions and become the predominant cell population in the tumour.

Such a process is called clonal selection because the cells that predominate represent a clone—that is, a population of cells derived from a single initial cell by successive rounds of cell division.One member of a clonal population may acquire another mutation that makes it grow even faster and the process repeats again, generating an even faster growing clone of cells. Multiple cycles of mutation and selection can occur in succession, each creating a population with enhanced growth rate or some other advantageous property. Although mutations play a central role in tumour progression, they are not the whole story. Cancer cell properties are also influenced by alterations in the expression of normal genes. The term epigenetic change is used to refer to any such alteration in gene expression that does not involve mutating the structure of a gene itself.

Cells possess a variety of mechanisms for altering gene expression. Among them, activating or inhibiting the transcription of individual genes into messenger RNA is especially important in cancer cells. For example, many of the traits required for cancer cell invasion and metastasis are produced by epigenetically turning on or turning off the transcription of normal genes rather than by gene mutation.Because the DNA base sequence is not being altered, epigenetic changes are easier to reverse than mutations. The question therefore arises as to whether a cancer cell can be epigenetically reprogrammed to reverse some of the changes responsible for malignant behavior. One way of addressing this question experimentally is to transfer the nucleus of a cancer cell into a different cytoplasmic environment to see if its gene expression patterns can be converted to a more normal state.

When nuclei are taken from mouse cancer cells and transplanted into mouse eggs whose own nuclei have been removed, the eggs divide and proceed through the early stages of embryonic development, even though the cells possess cancer cell nuclei. Especially striking results have been reported when mouse melanoma cells (a cancer of pigment cells) are used as a source of nuclei for transplantation.

Eggs receiving melanoma nuclei divide and produce embryonic cells that give rise to normal-appearing cells and tissues of adult mice (Figure 18). Nonetheless, mice containing such cells are not completely normal; the mice still exhibit an increased susceptibility to developing cancer.

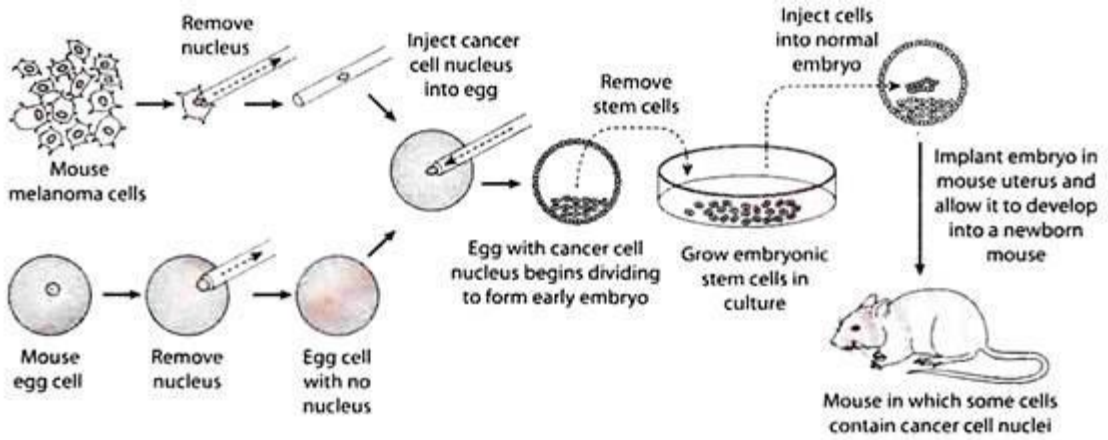


Figure 18 Transplanting a Cancer Cell Nucleus into an Egg Cell. When the nucleus of a mouse melanoma cell is transferred into a mouse egg whose own nucleus has been removed, the egg divides and proceeds through the early stages of embryonic development, although it cannot complete the process to form a new mouse. If *stem cells* (undifferentiated cells whose division gives rise to specialized cells) are removed at this stage and injected into a normal early embryo, these embryonic stem cells, with their cancer cell nuclei, will participate in the development of normal-appearing cells and tissues of a new mouse. Nonetheless, mice containing such cells are not completely normal; they still exhibit an increased susceptibility to developing cancer. [Based on experiments of K. Hochedlinger et al., *Genes Dev.* 18 (2004): 1875.]

Such results indicate that the DNA of a cancer cell nucleus can be reprogrammed to a more normal state, but a propensity for cancer to arise still remains. In other words, epigenetic and genetic changes both play important roles in tumour development.

To sum up, tumour progression is a phase of carcinogenesis that involves the gradual acquisition of DNA mutations and epigenetic changes in gene expression, accompanied by natural selection of cells that have acquired advantageous properties generated by these mechanisms.The net result is a population of cells whose properties, including growth rate and the ability to invade and metastasize, slowly change over time. The time required for tumour progression contributes to the lengthy delay commonly observed between exposure to carcinogenic chemicals and the development of cancer. These principles, derived largely from studies of chemical carcinogenesis, apply to cancers triggered by other cancer-causing agents as well.

Carcinogenesis is a Probabilistic Event that Depends on Carcinogen Dose and Potency:
The realization that chemical carcinogenesis is a multistep process involving several distinct stages and mechanisms can cause some confusion about the meaning of the term carcinogen. In common usage, any agent that increases the risk of developing cancer in animals or humans is considered to be a carcinogen. In this sense, either an initiating or a promoting agent would qualify as a carcinogen.

For clarification, the term incomplete carcinogen is sometimes employed when referring to a chemical that exerts only one of these two actions. Some chemicals possess both initiating and

promoting activities, and can therefore cause cancer by themselves; such chemicals are called complete carcinogens.

The ability to function as a complete carcinogen may be dose dependent. For example, certain polycyclic hydrocarbons act as initiating agents at lower doses but are complete carcinogens at higher doses. In normal human experience, people are exposed to chemical mixtures, such as tobacco smoke or coal tar that contain both initiating and promoting carcinogens. In such cases, the mixture acts as a complete carcinogen.

The multistep nature of chemical carcinogenesis also complicates the question of what scientists mean when they say that something "causes" cancer. For example, exposure to an incomplete carcinogen (i.e., an initiating or promoting agent) will not, by itself, cause cancer. Even a complete carcinogen rarely causes cancer in every exposed person or animal.When it is stated that a particular carcinogen causes cancer, what is really meant is that the agent in question increases the probability that cancer will arise. The magnitude of the increased risk depends on several factors, including the dose and potency of the agent involved and the issue of whether it is acting as an incomplete or complete carcinogen (complete carcinogens obviously carry a greater risk).

The reason for carcinogen dose dependence should now be more apparent. As the dose of an initiating carcinogen is increased, more DNA adducts and other types of DNA damage accumulate. To initiate the development of cancer, this damage must affect certain critical genes.The probability that one of these cancer-related genes will happen to undergo mutation is quite small because carcinogens trigger random DNA damage and the critical genes constitute only a tiny fraction of the total DNA. The higher the dose of carcinogen, however, the greater the overall DNA damage and hence the greater the chance that a critical gene will be affected by a random mutation. The likelihood that a particular carcinogen will cause cancer also depends on its potency. Carcinogen potency is generally assessed in animals by determining how large a dose is needed to cause cancer in 50% of the animals tested. Such testing has revealed that a ten-million-fold difference in strength separates the strongest carcinogens from the weakest (Figure 19).

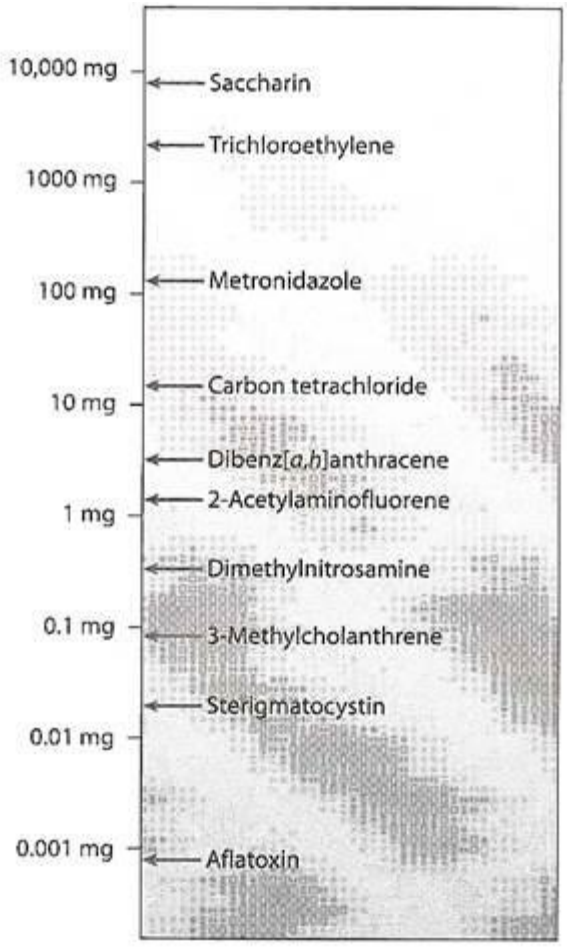


Figure 19 Differences in Carcinogenic Potency. The relative potency of several carcinogenic chemicals is compared on a scale that indicates how large a dose is needed to cause cancer in 50% of the animals tested. Note that the required dose of the weakest carcinogen shown (saccharin) is about ten-million-fold higher than the dose of the strongest carcinogen shown (aflatoxin). [Data from T. H. Maugh, *Science* 202 (1978): 38.]

Two properties are especially important in explaining this enormous variation in potency. The first involves the activation reactions catalyzed by cytochrome P450, which are more effective in converting certain types of chemicals into active carcinogens than they are for other chemicals. The second factor is related to the electrophilic strength of different carcinogens. Some substances are strongly electrophilic and are highly reactive with DNA, whereas others are weaker electrophiles and are less reactive with DNA. The probability that a carcinogen will happen to mutate a critical gene randomly is much greater for carcinogens that are stronger electrophiles because they trigger more mutations.

The random nature of mutation helps explain why everyone who is exposed to carcinogens does not develop cancer. For many years, tobacco companies tried to cast doubt on the relationship between cigarette smoking and lung cancer by pointing out that some cigarette smokers live long lives without ever developing cancer. The ability of carcinogens to trigger random DNA mutations provides a simple explanation for such observations: It is largely a matter of chance.

Tobacco smoke contains numerous carcinogens that cause random DNA damage, but for cancer to develop, a mutation must arise in a critical cancer-related gene. An apt metaphor is the game of Russian roulette, in which a single bullet is placed in a gun containing six chambers and the cylinder is then spun. When the trigger is pulled, the probability of firing a bullet that can kill is 1 in 6.A similar principle applies to smoking cigarettes, a potentially lethal practice governed by the laws of probability. Each cigarette has a small but finite probability of randomly creating a mutation that can cause cancer. Like Russian roulette, the more the game is played, the greater the chance that lethal damage will occur. So when it is stated that smoking cigarettes (or exposure to any other carcinogen) "causes" cancer, it simply means that a person's risk of developing cancer is increased. For this reason, agents exhibiting the potential to cause cancer are sometimes referred to as cancer risk factors.

In concluding, brief mention should be made of the fact that immunosuppressive drugs increase cancer risk in a fundamentally different way from the chemical carcinogens we have been discussing. Immunosuppressive drugs are given to organ transplant patients to inhibit the immune system and thereby minimize the possibility that a transplanted organ will be rejected.Because they inhibit immune function, some immunosuppressive drugs increase cancer risk by diminishing the likelihood that immune surveillance will destroy newly forming cancer cells. These immunosuppressive drugs differ from typical carcinogens in that they increase cancer risk indirectly, targeting the immune system rather than acting directly on the cells destined to become cancerous.

Role of Virus in Cancer formation:

There are many viruses which are capable of causing tumour in animals, human as well as plants (Table 23.2). These viruses are called tumour viruses or oncovirus. Some tumour viruses have RNA genome and are known as DNA tumour viruses.

Some tumour viruses have DNA genome and are known as retroviruses. Retrovirus replicates via synthesis of a DNA provirus in the infected cells. In addition, HIV is indirectly responsible for the cancer that develops in AIDS patient as a result of immunodeficiency.

Table 23.2: List of Chemical Carcinogens and Type of Cancer induced by such chemicals

Carcinogen	Type of cancer induced
Acrylonitrile	Colon, lung
4-Aminodiphenyl	Bladder
Aniline derivatives	Bladder
Arsenic compounds	Lung, skin
Asbestos	Lung, mesothelium
Benzene	Leukemia
Cadmium salts	Prostate, lung
Carbon tetrachloride	Liver
Chromium and chromates	Lung, nasal sinuses
Diethylstibestrol (DES)	Uterus, vagina
Lead	Kidney
Mustard gas	Lung, larynx
α-Naphthylamine	Bladder
Nickel	Lung, nose
Organochloride pesticides	Liver
Polychlorinated biphenyls	Liver
Radon	Lung
Soot and tars	Skin, lung, bladder
Vinyl chloride	Liver, lung, brain
Wood and leather dust	Nasal sinuses
Tobacco smoke, which contains the following:	Lung, oral cavity, larynx, esophagus, stomach, pancreas, others
Aminostilbene, arsenic, benz[*a*]anthracene, benz[*a*]pyrene, benzene, benzo[*b*]fluoranthene, benzo[*c*]phenanthrene, benzo[*j*]fluoranthene, cadmium, chrysene, dibenz[*a,c*]anthracene, dibenzo[*a,e*]fluoranthene, dibenz[*a,b*]acridine, dibenz[*a,j*]acridine,dibenzo[*c,g*]carbazone, N-dibutylnitrosamine, 2,3-dimethylchrysene, indeno[*1,2,3-c,d*]pyrene, S-methylchrysene, S-methylfluoranthene, α-naphthylamine, nickel compounds, N-nitrosodimethylamine, N-nitrosomethylethylamine, polonium-210, N-nitrosodiethylamine, N-nitrosonornicotiné, N-nitrosoanabasine, N-nitrosopiperidine	

The herpes viruses are the most complex animal viruses. The genome length of these viruses is 100-200 Kb. Many herpes viruses cause tumour in many animals such as frogs, chickens, monkeys etc. Epstein-Barr virus, a member of herpes virus, can trigger the development of

some human malignancies including Burkett's lymphoma in some region of Africa and nasopharyngeal carcinoma in China. It also causes B-cell lymphomas in AIDS patient and other immune suppressed persons. Cell transformation by herpes viruses is not fully understood because of the complexity of their genome. But it is evident that some viral genes are required to induce transformation of lymphocytes. Of the DNA tumour viruses, the papoviruses are the best studied DNA tumour viruses from the standpoint of molecular biology and have received particular attention because they have been critically important as models for understanding the molecular basis of cell transformation. The genome size of papoviruses is small (approximately 5 Kb). Simian virus 40 (SV_{40}) and polyomavirus are the important and commonly known member of papoviruses. Both these viruses are similar in size and general structure.

A virus usually multiplies in specific cells derived from animals in which the virus normally grows. Such cells are called permissive cells. Cells which do not allow the viruses to grow are called non-permissive cells.SV_{40} and polyoma viruses, on entering their respective host cells, undergo one of the two types of behaviour—they enter the permissive cell of the host, undergo the lytic phase, and multiply within host cell, ultimately killing them .Since a permissive cell is killed as a consequence of virus replication, it cannot become transformed. Sometimes viruses enter non-permissive cells and are not able to multiply, i.e., virus replication is blocked. In this case, the viral genome sometimes integrates into cellular DNA and expression of specific viral genes results in transformation of the infected cells. The SV_{40} and polyoma virus genes that trigger cell transformation have been identified, isolated and sequenced by molecular analysis. The genome of SV_{40} and polyomavirus are divided into early and late regions. The early region is expressed immediately after infection and is needed for synthesis of viral DNA.

The late region is not expressed until after viral DNA replication has begun. The early region of SV_{40} codes for two proteins which are known as small (17 Kd) and large (94 Kd) T-antigens. In addition to small and large regions, the genome of polyomavirus contains a third early region which is called as middle T region. It codes for a protein of about 55 Kd. Experimentally, it has been shown that large T of SV_{40} is sufficient to induce transformation and the middle T region of polyoma virus is primarily responsible for transformation. During lytic cycle, the early region proteins are needed to initiate viral DNA replication as well as to stimulate host cell gene expression and DNA synthesis. Since the replication viral DNA is dependent on host cell enzymes, therefore stimulation of gene expression of the host cell is a critical event in the viral life cycle. Most of the cells of adult animal cells become non-dividing. So the enzymes required for cell division are not available within the cell.

Therefore they must be stimulated to divide in order to induce the enzymes needed for viral DNA replication. This stimulation of cell division by the early gene products of virus can lead

to transformation if the viral DNA becomes stably integrated and expressed in nonpermissive cells. The early region proteins of SV_{40} and polyoma virus induce transformation by interacting with host proteins that regulate cell division.

The papilloma viruses are small DNA viruses. The genome length of such viruses is approximately 8 Kd. Some of these viruses induce only benign tumours such as warts. But some others cause malignant carcinomas— particularly cervical and anogenital cancers. Cell transformation by papilloma viruses occurs from the expression of two early region genes E_6 and E_7.The hepatitis B viruses are another group of DNA virus. They have the smallest genomes which is approximately 3 Kb. These viruses mainly infect the liver cells and cause liver damage. But how they induce cell-transformation is not clearly known. Possibly tumour results from expression of a viral gene. Alternatively, the chronic cell damage of liver simply induce the continuous cell division which, ultimately, causes the cell transformation.

The retroviruses, one family of RNA viruses, also cause human cancer. For example, human T-cell lymphotropic virus type-I (HTLV- I), a RNA virus, is the causative agent of T-cell leukemia. A related virus (HTLV-II) cause a rare form of leukaemia called hairy T- cell leukaemia.HIV (Human immunodeficiency virus) is the causative agent of AIDS. These viruses, i.e., HTLV-I, HTLV-II, HIV, actually does not cause cancer by directly converting a normal cell into a tumour cell. The AIDS patients become susceptible to high incidence of some malignancies like lymphomas and Kaposi's sarcoma due to immunosuppression of the patient. RNA viruses have an RNA genome which is extended at either end by a long terminal repeat (LTR). The LTR contains many of the signals that allow retrovirus to function (Fig. 23.4). Retroviruses use their genomic RNA as a template to make DNA with the help of reverse transcriptase.This DNA is then integrated into host's DNA as DNA the provirus. The DNA provirus is transcribed to yield genome length RNA provirus directed transcription involves a promoter—a sequence that directs the RNA polymerase to a specific initiation site and an enhancer—a sequence that facilitates transcription.

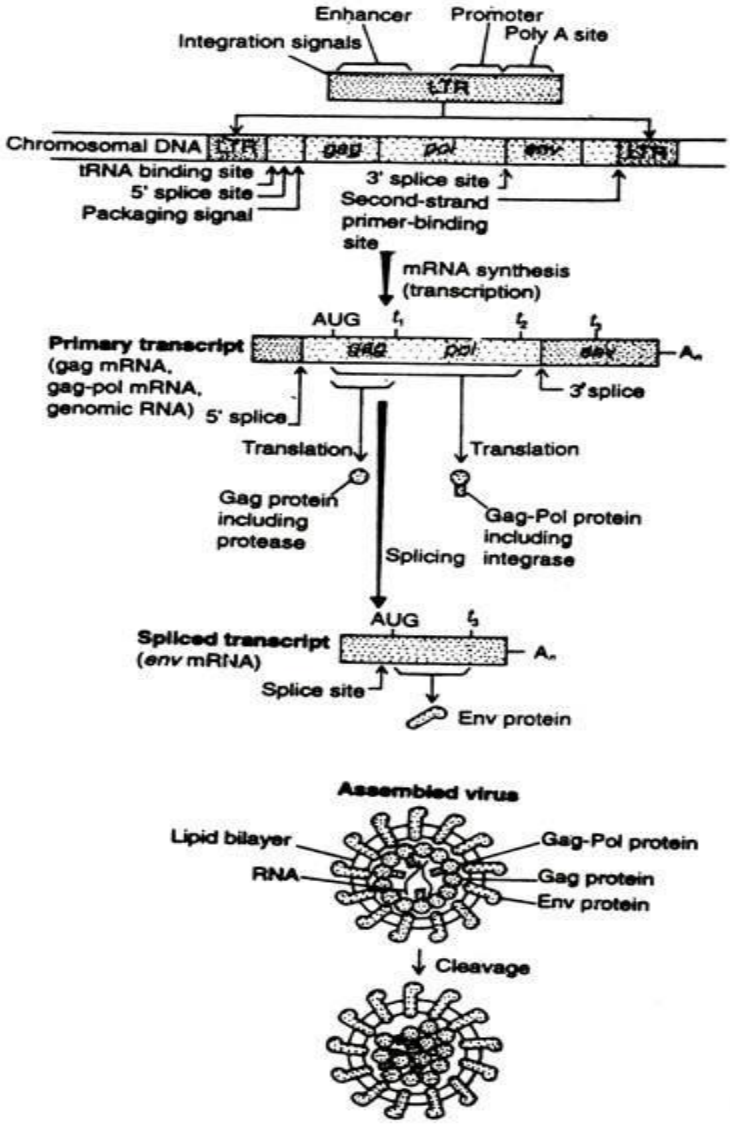


Fig. 23.4: Genetic elements of proviral DNA and the corresponding gene products.

The promoter and enhancer are located in the LTR. The primary transcript serves as the genomic RNA for progeny virus particles and as mRNA for the gag and pol genes. In addition the full length RNA is spliced to yield mRNA for env. The gag gene encodes the viral protease and structural proteins of the virus particle, pol encodes reverse transcriptase and integrase and env encodes envelope glycoproteins. These three genes are only required for viral replication but play no role in cell transformation.

This type of retrovirus causes tumour only when any mutation results at the time of integration of pro-viral DNA within or adjacent to host's genome. But there are some other retroviruses which contain specific genes which are responsible for the induction of cell

transformation and acts as potent carcinogens. The first cancer causing gene is f6und in the retrovirus called Rous Sarcoma virus (Fig. 23.5) that produces sarcomas in chicken. It was later named src gene. Genes like src which are capable of inducing malignant transformation, are referred to as oncogenes. The identification of the first viral oncogene has provided a model for understanding many aspects of cancer development at the molecular level.

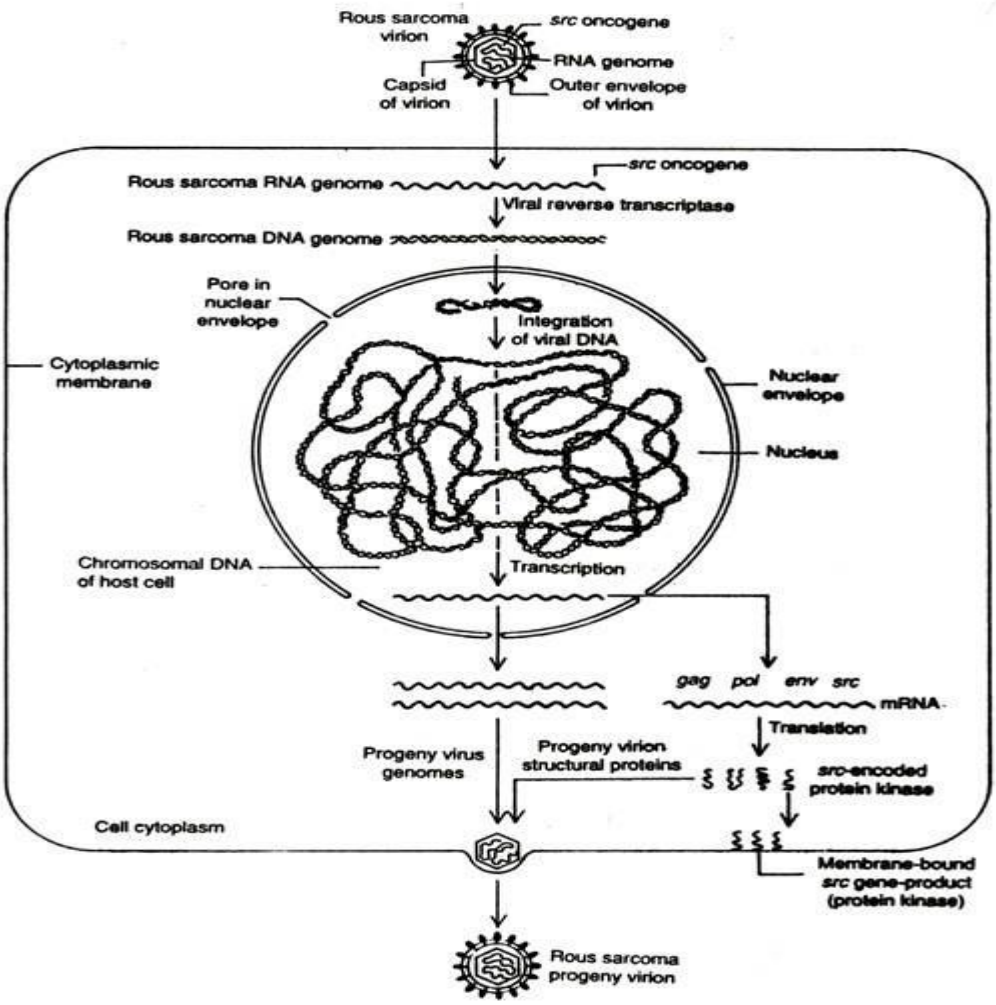


Fig. 23.5: Life cycle of Rous Sarcoma RNA tumour virus.

DNA Oncogenic Viruses:

Oncogenic viruses are distributed in several families of DNA viruses. These include Herpesviridae, Poxviridae, Papovaviridae and Hepadnaviridae. The Herpesviridae include the Epstein-Barr Virus (EBV) which has been found as the cause of two forms of human cancers — Burkitt's lymphoma and nasopharyngeal carcinoma. EBV has also been implicated with Hodgkin's disease, a cancer of lymphatic system. Other herpes-viruses have been associated with human cancers of lip and cervix.

The Papilloma viruses belonging to the Papovaviridae cause benign tumours as well as cancer in several species including human. In humans, papilloma viruses cause uterine (cervical) cancer. Another member of Papovaviridae, the Simian Virus 40 (SV40) is among the best studied DNA tumour viruses.Natural host of SV 40 is cultured fibroblast cells of monkey. Such a cell culture is called permissive, because it allows viral multiplication and release of progeny viruses by cell lysis. On the other hand, when SV 40 is inoculated into non-permissive cell cultures e.g. the fibroblast cells of mice, the virus cannot multiply, but in a small number cells the viral DNA is, integrated with the host DNA causing their transformation into cancer cells.

Due to integration into the host chromosome viral multiplication and cell lysis are absent. The phenomenon is comparable to lysogeny observed in temperate phage infection of bacteria. Integration of some DNA viruses is site-specific i.e. the viral DNA is inserted into a host chromosome at a specific site. But papova-viruses do not have such specificity and can be inserted at random. Hepatitis B virus (HBV) belonging to the Hepadnaviridae causes cancer of liver. Many animal experiments have yielded results which clearly indicate a connection of HBV and liver cancer. Although direct proof is lacking in case of human beings, a survey revealed that all people with liver cancer had a previous infection of HBV.

RNA Oncogenic Viruses:

Among the RNA viruses only some members of the family Retro-viridae can cause cancer. Other RNA viruses which replicate by RNA replicase are non-oncogenic. Retroviruses which have a single- stranded RNA genome replicate via a double-stranded DNA produced by an RNA-dependent DNA polymerase (reverse transcriptase) and they insert the DNA copy into the host chromosome as a provirus.

Rous Sarcoma Virus (RSV) is of historical importance, because it was the first tumour-inducing virus to be studied. RSV is a retrovirus with a single-stranded RNA genome and its DNA copy is integrated into a specific site of the host chromosome as a provirus. Research on RSV revealed identification of a cancer-inducing gene (an oncogene) in RSV genome. This gene, called src, is not essential for viral replication, as it does not code for any viral proteins. Later, it was discovered that a copy of the src gene is present in the host chromosome of normal cells and it was not oncogenic.

Thus, the viral src gene which is oncogenic is derived from the host. How the non-oncogenic chromosomal src gene is converted to an oncogene in RSV is not clearly understood. It may occur through a mutation. The entry of a chromosomal gene into the viral genome possibly occurs through a process similar to that which operates in restricted transduction in bacteria.It is thought that the RSV DNA produced through reverse transcription is inserted next to the chromosomal src gene and during transcription of the RSV RNA genome, src gene

might be included. In this way, src gene might enter into the viral genome. RSV causes cancer in chicken. Similar retroviruses are known to cause cancer in other animals including monkey. But definite evidence of retroviruses causing cancer in humans was not available until 1980. In that year Gallo isolated a virus that could transform normal T-lymphocytes into cancerous T-lymphocytes causing a disease, called T-cell leukaemia. The virus is known as Human T-cell Leukaemia Virus (HTLV). Later research during 1990s has confirmed the role of HTLV in causing human leukaemia. Another HTLV was later discovered causing hairy cell leukaemia in man. The malignant leucocytes develop hairy outgrowths on their surface. The second virus has been designated as HTLV-II. These retroviruses have been shown to transform normal T-cells by a regulatory protein which stimulates uncontrolled cell division. Besides leukaemia, HTLV is also known to cause neurological disorders, like spastic para-paresis. T-cell leukaemia is more or less restricted in several countries, like Japan, West Indies and some parts of West Africa.

Probable Questions:

1. Write down the differences between normal cells and cancerous cells.
2. Write down the types of cancers.
3. How cancer is developed?
4. Write down characteristics of cancerous cells.
5. What are the causes responsible for onset of cancer?

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7th Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwal.

UNIT-III

Mutations and mutagenesis: Types of mutation; biochemical basis of mutations; mutagenesis

Objective: In this unit we will discuss about mutations types, biochemical basis of mutations and also about the process of mutagenesis

Definition of Mutations:

Mutation refers to sudden heritable change in the phenotype of an individual. In the molecular term, mutation is defined as the permanent and relatively rare change in the number or sequence of nucleotides. Mutation was first discovered by Wright in 1791 in male lamb which had short legs. Later on mutation was reported by Hugo de Vries in 1900 in Oenothera, Morgan (1910) in Drosophila (white eye mutant) and several others in various organisms. The term mutation was coined by de Vries.

Characteristics of Mutations:

Mutations have several characteristic features.

Some of the important characteristics of mutations are briefly presented below:

i. Nature of Change:

Mutations are more or less permanent and heritable changes in the phenotype of an individual. Such changes occur due to alteration in number, kind or sequence of nucleotides of genetic material, i.e., DNA in most of the cases.

ii. Frequency:

Spontaneous mutations occur at a very low frequency. However, the mutation rate can be enhanced many fold by the use of physical and chemical mutagens.

The frequency of mutation for a gene is calculated as follows:

Frequency of gene mutation = M / M + N

where, M = number of individuals expressing mutation for a gene, and

N = number of normal individuals in a population.

iii. Mutation Rate:

Mutation rate varies from gene to gene. Some genes exhibit high mutation rate than others. Such genes are known as mutable genes, e.g., white eye in Drosophila. In some genomes, some genes enhance the natural mutation rate of other genes. Such genes are termed as mutator genes.

The example of mutator gene is dotted gene in maize. In some cases, some genes decrease the frequency of spontaneous mutations of other genes in the same genome, which are referred to as anti-mutator genes. Such gene has been reported in bacteria and bacteriophages.

iv. Direction of Change:

Mutations usually occur from dominant to recessive allele or wild type to mutant allele. However, reverse mutations are also known, e.g., notch wing and bar eye in Drosophila.

v. Effects:

Mutations are generally harmful to the organism. In other words, most of the mutations have deleterious effects. Only about 0.1% of the induced mutations are useful in crop improvement. In majority of cases, mutant alleles have pleiotropic effects. Mutations give rise to multiple alleles of a gene.

vi. Site of Mutation:

Muton which is a sub-division of gene is the site of mutation. An average gene contains 500 to 1000 mutational sites. Within a gene some sites are highly mutable than others. These are generally referred to as hot spots. Mutations may occur in any tissue of an organism, i.e., somatic or gametic.

vii. Type of Event:

Mutations are random events. They may occur in any gene (nuclear or cytoplasmic), in any cell (somatic or reproductive) and at any stage of development of an individual.

viii. Recurrence:

The same type of mutation may occur repeatedly or again and again in different individuals of the same population. Thus, mutations are of recurrent nature.

Classification of Mutations:

Mutations can be classified in various ways. A brief classification of mutations on the basis of:

(1) Source,(2) Direction,(3) Tissue,(4) Effects,(5) Site,(6) Character, and(7) Visibility

TABLE 14.1. Classification and brief description of mutations

Basis of classification and type of mutation	*Brief Description*
1. Based on Source	
Spontaneous	Mutations that occur in nature
Induced	Mutations which are produced by the use of mutagenic agents.
2. Based on Direction	
Forward mutation	Any change from wild type allele.
Reverse mutation	A change from mutant allele to wild type.
3. Based on Tissue	
Somatic mutation	A mutation in somatic tissue.
Germinal mutation	A mutation in germ line cell.
4. Based on Survival	
Lethal	A mutation which kills the individual that carries it.
Sub-lethal	When mortality is more than 50% of individuals that carry mutation.
Sub-vital	When mortality is less than 50% of individuals that carry mutation.
Vital	When all mutant individuals survive.
5. Based on Site	
Nuclear mutation	A mutation in nuclear gene.
Cytoplasmic mutation	A mutation in cytoplasmic gene.
6. Based on Character	
Morphological	A mutation that alters morphological character of an individual.
Biochemical	A mutation that alters biochemical function of an individual.
7. Based on Visibility	
Macro-mutations	Mutations with distinct morphological changes in phenotype. Generally found in qualitative characters.
Micro-mutations	Mutations with invisible phenotypic changes. Generally observed in quantitative characters.

Types of Mutants:

The product of a mutation is known as mutant. It may be a genotype or an individual or a cell or a polypeptide.

There are four main classes of identifiable mutants, viz:

(i) Morphological,

(ii) Lethal,

(iii) Conditional

(iv) Biochemical

These are briefly described below:

i. Morphological:

Morphological mutants refer to change in form, i.e., shape, size and colour. Albino spores in Neurospora, curly wings in Drosophila, dwarf peas, short legged sheep are some examples of morphological mutants.

ii. Lethal:

In this class, the new allele is recognized by its mortal or lethal effect on the organism. When the mutant allele is lethal all individuals carrying such allele will die; but when it is semi-lethal or sub-vital some of the individuals will survive.

iii. Conditional Lethal:

Some alleles produce a mutant phenotype under specific environmental conditions. Such mutants are called restrictive mutants. Under other conditions they produce normal phenotype and are called permissive. Such mutants can be grown under permissive conditions and then be shifted to restrictive conditions for evaluation.

iv. Biochemical Mutant:

Some mutants are identified by the loss of a biochemical function of the cell. The cell can assume normal function, if the medium is supplemented with appropriate nutrients. For example, adenine auxotroph's can be grown only if adenine is supplied, whereas wild type does not require adenine supplement.

Types of Mutation:

Mutations can be classified in various ways depending on the cause of the mutation, its effect on the function of the gene product or the kind of changes to the structure of the gene itself. Mutagenic agents such as carcinogens or high-energy radiation lead to changes to the genomic material. Some mutations occur as a natural byproduct of the error rate in DNA or RNA replication mechanisms. A mutation could be a loss-of-function or gain-of-function mutation, depending on whether the gene product is inactivated or has enhanced activity. In heterozygotes with two copies of every allele, some mutated gene products can suppress the effect of the wild- type allele. These are called dominant negative mutations. All these effects arise from a change to the structure of a gene or allied chromosomal material. These structural changes can be classified as substitutions, deletions, insertions, amplifications, ortranslocations. The term point mutation typically refers to the alteration of a single base pair of DNA or of a small number of adjacent base pairs. In this section, we will consider the effects of such changes at the phenotypic level. Point mutations are classified in molecular terms, which shows the main types of DNA changes and their effects on protein function when they occur within the protein- coding region of a gene

Substitution Mutations:

Substitution mutations are situations where a single nucleotide is changed into another. In organisms having double-stranded DNA or RNA, this usually means that the corresponding base pair is also altered. For example, an A:T base pair could be mutated into a G:C base pair or even a T:A base pair. Depending on the position of this change, it could have a variety of effects.

In highly conserved regions, both in the coding and regulatory stretches of DNA, mutations often lead to deleterious effects. Other, more variable stretches are more accommodating. In the promoter region or in other regulatory parts of the genome, a substitution mutation may change gene expression or the response of the gene to stimulus. Within the coding region, a substitution in the third or wobble position of a codon is called a silent mutation since there is no change to the amino acid sequence. When a substitution mutation results in a new amino acid but with similar properties-it is a neutral or a conserved mutation. For instance, if aspartic acid is substituted with glutamic acid, there is a reasonable chance that there would be very few changes to the biochemistry of the protein.

Insertions and Deletions:

Insertions and deletions refer to the addition or removal of short stretches of nucleotide sequences. These types of mutations are usually more deleterious than substitutions since they can cause frame shift mutations, altering the entire amino acid sequence downstream of the mutation site. They can lead to a change in polypeptide length, either creating

abnormally long proteins that cause aggregates or truncated polypeptides that are non-functional and can clog the translation machinery of the cell.

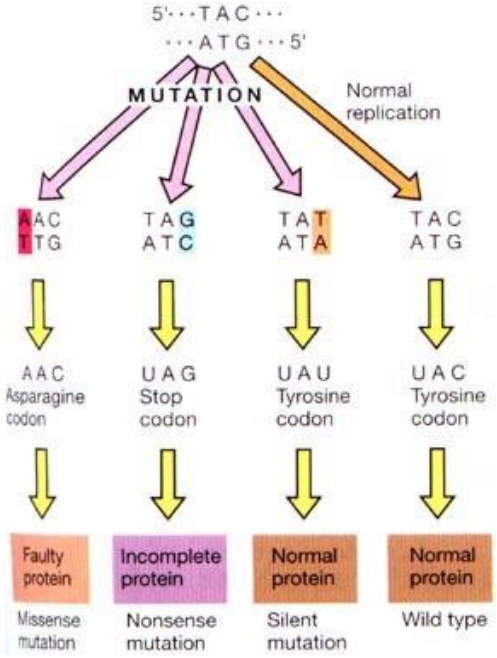


Fig 1: Types of Point mutation

Large-scale mutations:

Changes to the nucleotide sequence in genetic material can also occur on a large scale, sometimes involving thousands of base pairs and nucleotides. These kinds of mutations include amplifications, where segments of genetic material are present in multiple copies, and deletions, where a large chunk of genetic material is removed. Occasionally, some parts of the genome are translocated to a different chromosome, or reinserted into the same position, but in an inverted orientation. Translocations and deletions can bring together genes that are normally placed far apart from each other, either leading to the formation of mosaic polypeptides, or to the differential regulation of the genes within the segment.

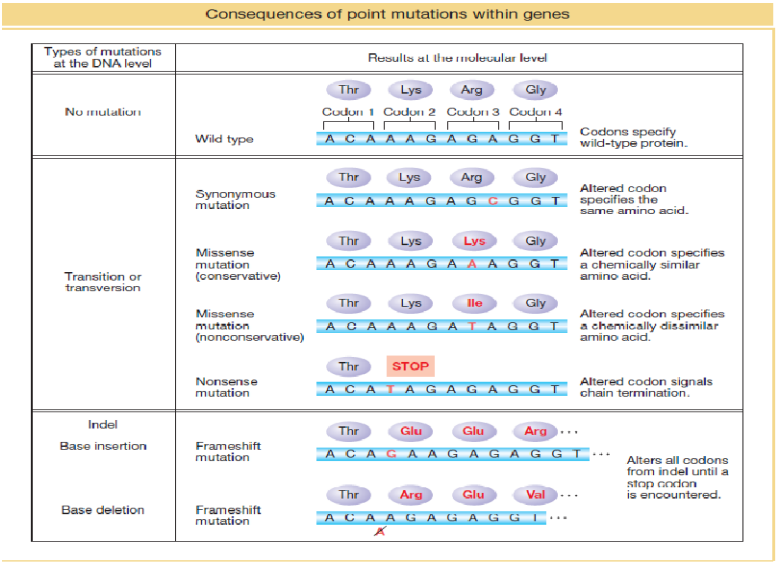


Table 1: Types of point mutation

Other Types of Mutation:

Based on change in genotype and phenotype, mutation are of two types : Pointmutation and Frameshiftmutation

1. Pointmutation

It occurs as a result of replacement of one nucleotide by other in specific nucleotide sequence of gene. Point mutation brings little phenotypic change as compared to frameshift mutation. Point mutation are two types based on the base pair substitution.

i) Transition:

It is the point mutation occur by substitution of one purine by another purine or one pyrimidine by another pyrimidine.

ii) Transversion:

It is the point mutation occur by substitution of purine by pyrimidine and vice versa. Based on transcriptional property point mutation are of **three types.**

a) Silentmutation

b) Missensemutation

c) Non-sensemutation

a) Silent mutation:

It is also known as neutral mutation. It is the mutation in which mutated codon codes same amino acids as the original codon. Since the amino acid is same as original one, it does not effects the structure and composition of protein. Silent mutation causes phenotype of bacteria remain similar to that of wild type.

b) Missense mutation:

In this mutation mutated codon codes different amino acid (other than original). Since new amino acid coded by mutated codon is altered, the protein formed from it is also altered. Such protein can be less active or completely inactive. If altered amino acids lie on active site of protein then such protein become completely non- functional. The missense mutation causes phenotypic change in organism.

c) Non sense mutation:

Mutation in which altered codon is stop codon or chain terminating codon, such mutation is called non-sense mutation. Non sense mutation causes incomplete synthesis. Such incomplete protein is always non-functional. Non-sense mutation bring greatest change in phenotype of an organism.

2. Frameshift mutation

It occurs as a result of addition or deletion of nucleotide in the sequence of DNA. Addition or deletion of nucleotide causes shift of the reading frame of mRNA. In a mRNA each codon is represented by three bases without punctuation and insertion or deletion of a nucleotide changes the entire frame. So frame shift mutation bring greater phenotypic change than point mutation.

Insertion or deletion of one or two base pair of nucleotide causes shift in frame. However, insertion or deletion of three base pair adds or remove a whole codon, this results in addition of removal of single amino acid from polypeptide chain.

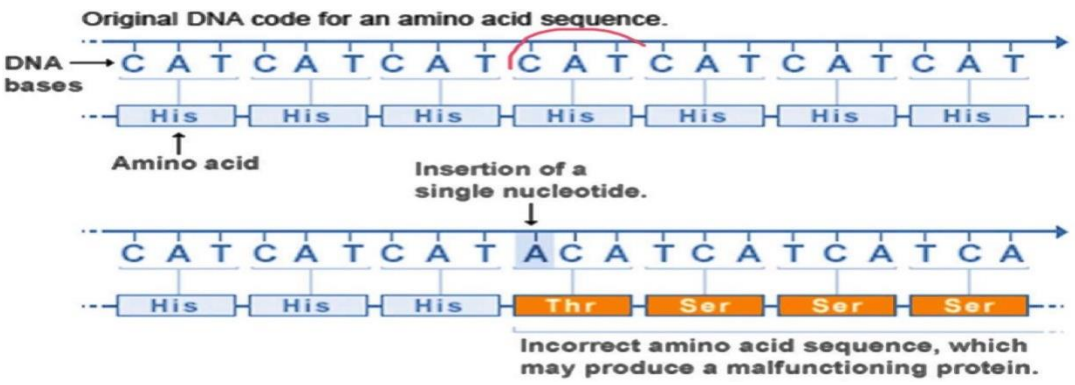


Fig 2: Frameshift Mutation

Agents of Mutations:

Mutagens:

Mutagens refer to physical or chemical agents which greatly enhance the frequency of mutations. Various radiations and chemicals are used as mutagens. Radiations come under physical mutagens. A brief description of various physical and chemical mutagens is presented below:

Physical Mutagens:

Physical mutagens include various types of radiations, viz. X-rays, gamma rays, alpha particles, beta particles, fast and thermal (slow) neutrons and ultra violet rays (Table 14.2)

A brief description of these mutagens is presented below:

TABLE 14.2. Commonly used physical mutagens (radiation), their properties and mode of action

Type of Radiation	*Main properties*	*Mode of action or changes caused*
1. X-rays	S.I., penetrating and non-particulate	Induce mutations by forming free radicals and ions. Cause addition, deletion, transitions and transversions.
2. Gamma rays	S.I., very penetrating and non-particulate	Induce mutations by ejecting atoms from the tissues. Cause all types of changes as above.
3. Alpha Particles	D.I., particulate, less penetrating and positively charged.	Act by ionization and excitation. Cause chromosomal and gene mutations.
4. Beta Rays Particles	S.I., particulate, more penetrating than alpha particles and negatively charged.	Act by ionization and excitation. Cause chromosomal and gene mutations.
5. Fast and Thermal Neutrons	D.I., particulate, neutral particles, highly penetrating	Cause chromosomal breakage and gene mutations.
6. Ultra Violet Rays	Non-ionizing, low penetrating	Cause chromosomal breakage and gene mutations.

ii. Gamma Rays:

Gamma rays are identical to X-rays in most of the physical properties and biological effects. But gamma rays have shorter wave length than X-rays and are more penetrating than X-rays. They are generated from radioactive decay of some elements like 14C, 60C, radium etc.

Of these, cobalt 60 is commonly used for the production of Gamma rays. Gamma rays cause chromosomal and gene mutations like X-rays by ejecting electrons from the atoms of tissues through which they pass. Nowadays, gamma rays are also widely used for induction of mutations in various crop plants.

iii. Alpha Particles:

Alpha rays are composed of alpha particles. They are made of two protons and two neutrons and thus have double positive charge. They are densely ionizing, but lesser penetrating than beta rays and neutrons. Alpha particles are emitted by the isotopes of heavier elements.They have positive charge and hence they are slowed down by negative charge of tissues resulting in low penetrating power. Alpha particles lead to both ionization and excitation resulting in chromosomal mutations.

iv. Beta Particles:

Beta rays are composed of beta particles. They are sparsely ionizing but more penetrating than alpha rays. Beta particles are generated from radioactive decay of heavier elements such as 3H, 32P, 35S etc. They are negatively charged, therefore, their action is reduced by positive charge of tissues. Beta particles also act by way of ionization and excitation like alpha particles and result in both chromosomal and gene mutations.

V. Ultraviolet (UV) Radiation:

UV radiation causes damage in the DNA duplex of the bacteria and phages. The UV rays are absorbed and cause excitation of macromolecules. The absorption maxima of nucleic acid = (280 nm) and protein (260 nm) are more or less similar. The DNA molecule is the target molecule for UV rays but not the proteins. However, absorption spectrum of RNA is quite similar to that of DNA.

The excited DNA leads to cross-linking, single strand breaks and base damage as minor lesion and generation of nucleotide dimer as a major one. Purines are generally more radio – resistant than the pyrimidine of the latter, thymine is more reactive than cytosine.

Hence, the ratio of thymine-thymine (TT), thymine-cytosine (TC), cytosine-cytosine (CC) dimer (Fig. 9.14) is 10:3:3, respectively. A few dimers of TU and UU also appear. The initial step in pyrimidine dimerization is known to be hydration of their 4: 5 bonds.

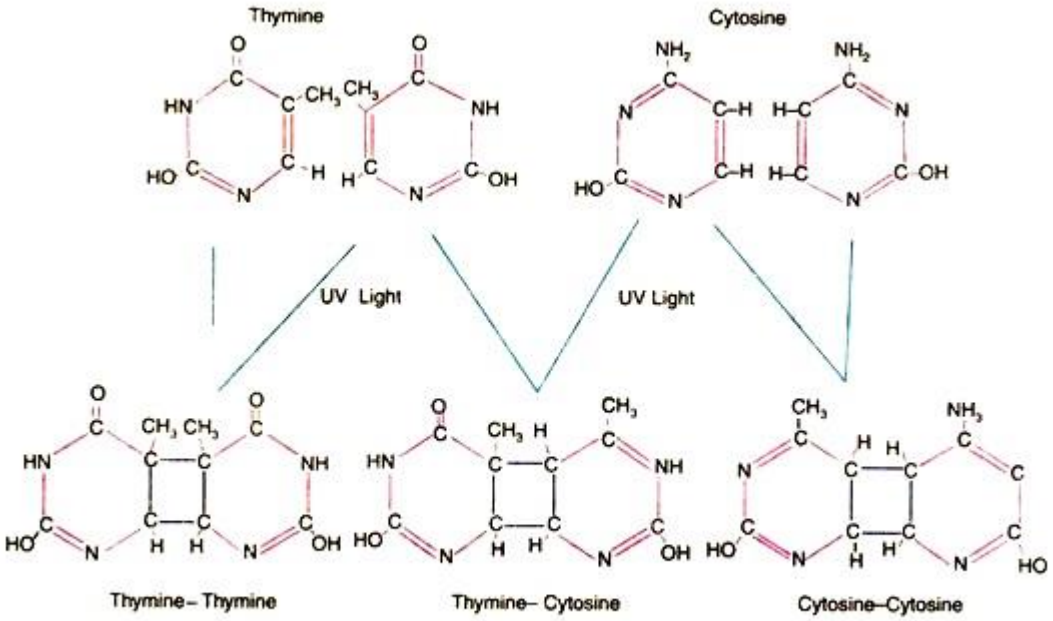


Fig. 9.14 : Formation of pyrimidine dimer induced by UV radiation.

Formation of thymine-thymine (TT) dimer causes distortion of DNA helix because the thymines are pulled towards one another. The distortion results in weakening of hydrogen-bonding to adenines in the opposing strand. This structural distortion inhibits the advance of replication fork.

vi. The X-Rays:

The X-rays cause breaking of phosphate ester linkages in the DNA. This breakage occurs at one or more points. Consequently, a large number of bases are deleted or rearranged in the DNA molecule.

The X-rays may break the DNA either in one or both strands. If breaks occur in both strands, it becomes lethal. The DNA segment between the two breaks is removed resulting in deletion. Since both the X-rays and UV rays bring about damage in DNA molecule, they are used in sterilization of bacteria and viruses.

Chemical Mutagens:

Singer and Kusmierek (1982) have published an excellent review on chemical mutagenesis.

Some of the chemical mutagens and mutagenesis are given in Table 9.3, and described below:

Table 9.3 : Different types of chemical mutagens

Class of Chemical	*Chemical Mutagens*
Acridines	Ethyleneimine (EI)
Mustard	Nitrogen mustard
	Sulphur mustard
Nitrosamines	Diethylnitrosamine (DMN)
	Diethylsulphonate (DES)
	Nitrosomethylurea (NMU)
Epoxide	Ethyleneoxide (EO)
	Diepoxybutane (DEB)
Alkyl sulphonates	Diethylsulphonate (DES)
	Methylmethanesulphonate (MMS)
	Ethylmethanesulphonate (EMS)
Others	Nitrous acid
	Maleic hydrazide
	Hydroxylamine

i. Base Analogues:

A base analogue is a chemical compound similar to one of the four bases of DNA. It can be incorporated into a growing polynucleotide chain when normal process of replication occurs.' These compounds have base pairing properties different from the bases. They replace the bases and cause stable mutation.

A very common and widely used base analogue is 5-bromouracil (5-BU) which is an analogue of thymine. The 5-BU functions like thymine and pairs with adenine (Fig. 9.6A).

The 5-BU undergoes tautomeric shift from keto form to enol form caused by bromine atom. The enol form can exist for a long time for 5-BU than for thymine (Fig. 9.6B). If 5-BU replaces a thymine, it generates a guanine during replication which in turn specifies cytosine causing G: C pair (Fig. 9.6A).

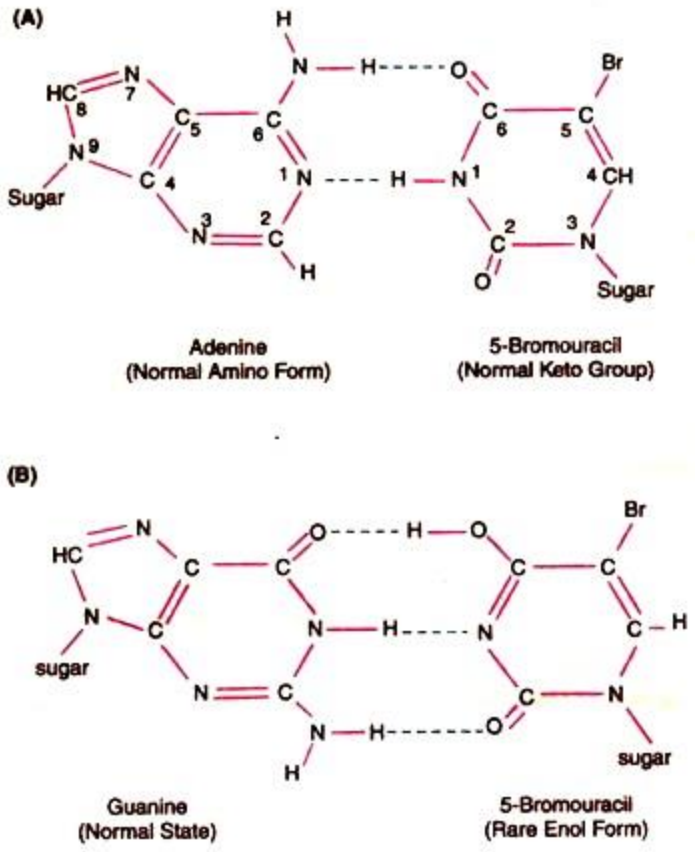


Fig. 9.6 : Mutagenesis by base analogue 5-bromouracil. A, the keto form of 5-BU pairs with adenine; B, 5-BU is tautomarised to enol form and pairs with guanine rather than adenine.

During the replication, keto form of 5-BU substitutes for T and the replication of an initial AT pair becomes an A: BU pair (Fig. 9.7A). The rare enol form of 5-BU that pairs with G is the first mutagenic step of replication. In the next round of replication G pairs with C. Thus, the transition is completed from AT→GC pair.

The 5-BU can also induce the conversion of GC to AT. The enol form infrequently acts as an analogue of cytosine rather than thymine. Due to error, GC pair is converted into a G: BU pair which in turn becomes an AT pair. Due to such pairing properties 5-BU is used in chemotherapy of viruses and cancer. Because of pairing with guanine it disturbs the normal replication process in microorganisms.

The 5-bromodeoxyuridine (5-BDU) can replace thymidine in DNA molecule. The 2-amino-purine (2-AP) and 2, 6-di-amino-purine (2, 6-DAP) are the purine analogues. The 2-AP normally pairs with thymine but it is able to form a single hydrogen bond with cytosine resulting in transition of AT to GC. The 2-AP and 2, 6-DAP are not as effective as 5-BU and 5-BDU.

ii. Chemicals Changing the Specificity of Hydrogen Bonding:

There are many chemicals that after incorporation into DNA change the specificity of hydrogen -bonding. Those which are used as mutagens are nitrous oxide (HNO_2), hydroxylamine (HA) and ethyl-methane-sulphonate (EMS).

(a) Nitrous Oxide (HNO_2):

Nitrous oxide converts the amino group of bases into keto group through oxidative deamination. The order of frequency of deamination (removal of amino group) is adenine > cytosine > guanine.

(b) Deamination of Adenine:

Deamination of adenine results in formation of hypoxanthine, the pairing behaviour of which is like guanine. Hence, it pairs with cytosine instead of thymine replacing AT pairing by GC pairing (Fig. 9.8A).

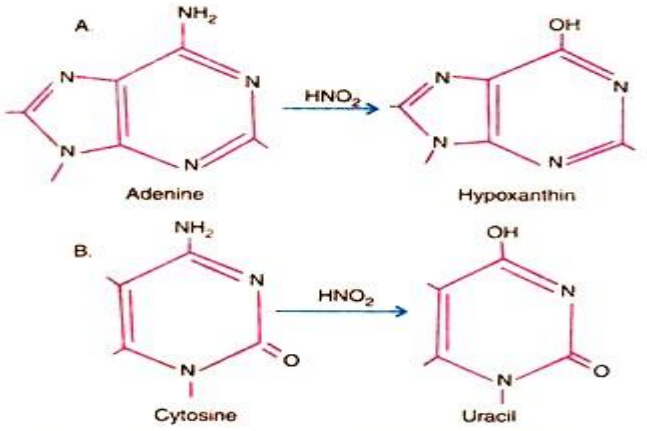


Fig. 9.8 : Deamination by nitrous oxide of adenine into hypoxanthin (A), and cytosine into uracil (B).

(c) Deamination of Cytosine:

Deamination of cytosine results in formation of uracil by replacing – NH_2 group with -OH group. The affinity for hydrogen bonding of uracil is like thymine; therefore, C-G pairing is replaced by U-A pairing (Fig. 9.8B).

(d) Deamination of Guanine:

Deamination of guanine results in formation of xanthine, the later is not mutagenic. Xanthine behaves like guanine because there is no change in pairing behaviour. Xanthine pairs with cytosine. Therefore, G-C pairing is replaced by X-C pairing.

(e) Hydroxylamine (NH_2OH):

It hydroxylates the C_4 nitrogen of cytosine and converts into a modified base via deamination which causes to base pairs like thyamine. Therefore, GC pairs are changed into AT pairs.

iii. Alkylating Agents:

Addition of an alkyl group to the hydrogen bonding oxygen of guanine (N_7 position) and adenine (at N_3 position) residues of DNA is done by alkylating agents. As a result of alkylation, possibility of ionization is increased with the introduction of pairing errors. Hydrolysis of linkage of base-sugar occurs resulting in gap in one chain.

This phenomenon of loss of alkylated base from the DNA molecule (by breakage of bond joining the nitrogen of purine and deoxyribose) is called depurination. Depurination is not always mutagenic. The gap created by loss of a purine can effectively be repaired.

Following are some of the important widely used alkylating agents:

(a) Dimethyl sulphate (DMS)

(b) Ethyl methane sulphonate (EMS) -$CH_3CH_2SO_3CH_3$

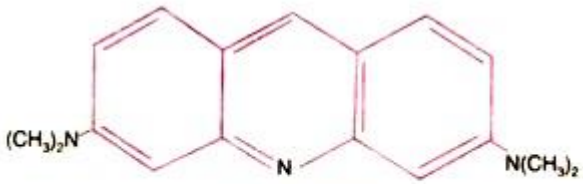
(c) Ethyl ethane sulphonate (EES) -$CH_3CH_2SO_3CH_2CH_3$

EMS has the specificity to remove guanine and cytosine from the chain and results in gap formation. Any base (A,T,G,C) may be inserted in the gap. During replication chain without gap will result in normal DNA. In the second round of replication gap is filled by suitable base.If the correct base is inserted, normal DNA sequence will be produced. Insertion of incorrect bases results in transversion or transition mutation. Another example is methyl nitrosoguanidine that adds methyl group to guanine causing it to mispair with thyamine. After subsequent replication, GC is converted into AT transition.

iv. Intercalating Agents:

There are certain dyes such as acridine orange, proflavine and acriflavin which are three ringed molecules of similar dimensions as those of purine pyrimidine pairs (Fig. 9.9). In aqueous solution these dyes can insert themselves in DNA (i.e. intercalate the DNA) between the bases in adjacent pairs by a process called intercalation.

Proflavin



Acridine Orange

Fig. 9.9 : Chemical structure of two mutagenic acridine derivatives.

Therefore, the dyes are called intercalating agents. The acridines are planer (flat) molecules which can be intercalated between the base pairs of DNA; distort the DNA and results deletion or insertion after replication of DNA molecule. Due to deletion or insertion of intercalating agents, there occur frameshift mutations (Fig. 9.10).

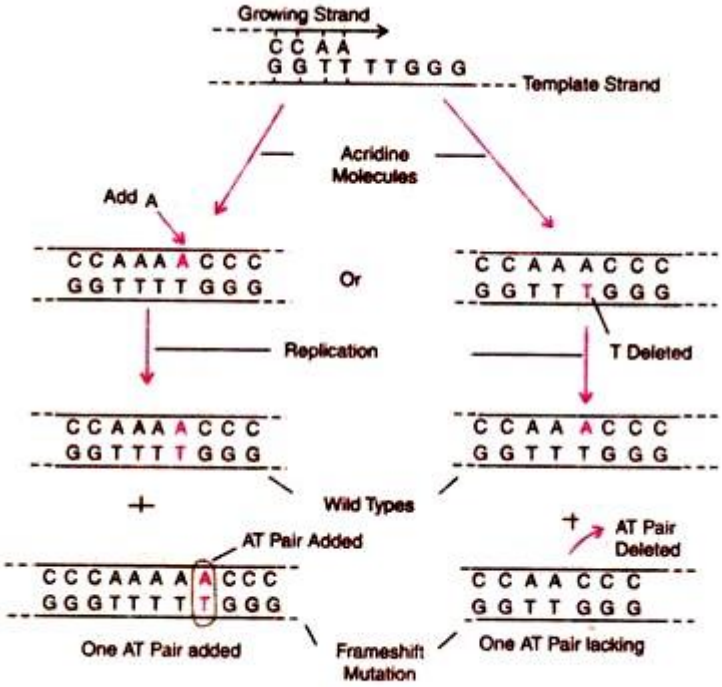


Fig. 9.10 : Mechanism of intercalation of an acridine molecule in the replication fork.

Probable Questions:

1. Define mutation. Sate the major characteristics of mutation.

2. Classify mutations.

3. Describe four main classes of identifiable mutants with examples.

4. Define mutagens. State about different types of physical mutagens.

5. State about different types of chemical mutagens.

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.

2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition

3. Principles of Genetics by Snustad and Simmons, Sixth Edition.

4. Molecular Biology of the Cell – by Bruce Alberts

5. Cell and Molecular Biology by Gerald Karp, 7th Edition.

6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.

7. Genetics . Verma and Agarwal.

UNIT-IV

Spontaneous and induced mutation; reversion as a means of detecting mutagens and carcinogens

Objective: In this unit we will discuss about Spontaneous and induced mutation; reversion as a means of detecting mutagens and carcinogens.

Detection of Mutation:

Detection of mutations depends on their types. Morphological mutations are detected either by change in the phenotype of an individual or by change in the segregation ratio in a cross between normal (with marker) and irradiated individuals. The molecular mutations are detected by a change in the nucleotide, and a biochemical mutation can be detected by alteration in a biochemical reaction. The methods of detection of morphological mutants have been developed mainly with Drosophila. Four methods, viz., (1) CIB method, (2) Muller's 5 method, (3) attached X-chromosome method, and (4) curly lobe plum method are in common use for detection of mutations in Drosophila.

A brief description of each method is presented below:

i. CIB Method:

This method was developed by Muller for detection of induced sex linked recessive lethal mutations in Drosophila male. In this technique, C represents a paracentric inversion in large part of X-chromosome which suppresses crossing over in the inverted portion. The I is a recessive lethal. Females with lethal gene can survive only in heterozygous condition. The B stands for bar eye which acts as a marker and helps in identification of flies. The I and B are inherited together because C does not allow crossing over to occur between them. The males with CIB chromosome do not survive because of lethal effect.

The important steps of this method are as follows:

(a) A cross is made between CIB female and mutagen treated male. In F_1 half of the males having normal X-chromosome will survive and those carrying CIB chromosome will die. Among the females, half have CIB chromosome and half normal chromosome (Fig. 14.2). From F_1, females with CIB chromosome and male with normal chromosome are selected for further crossing.

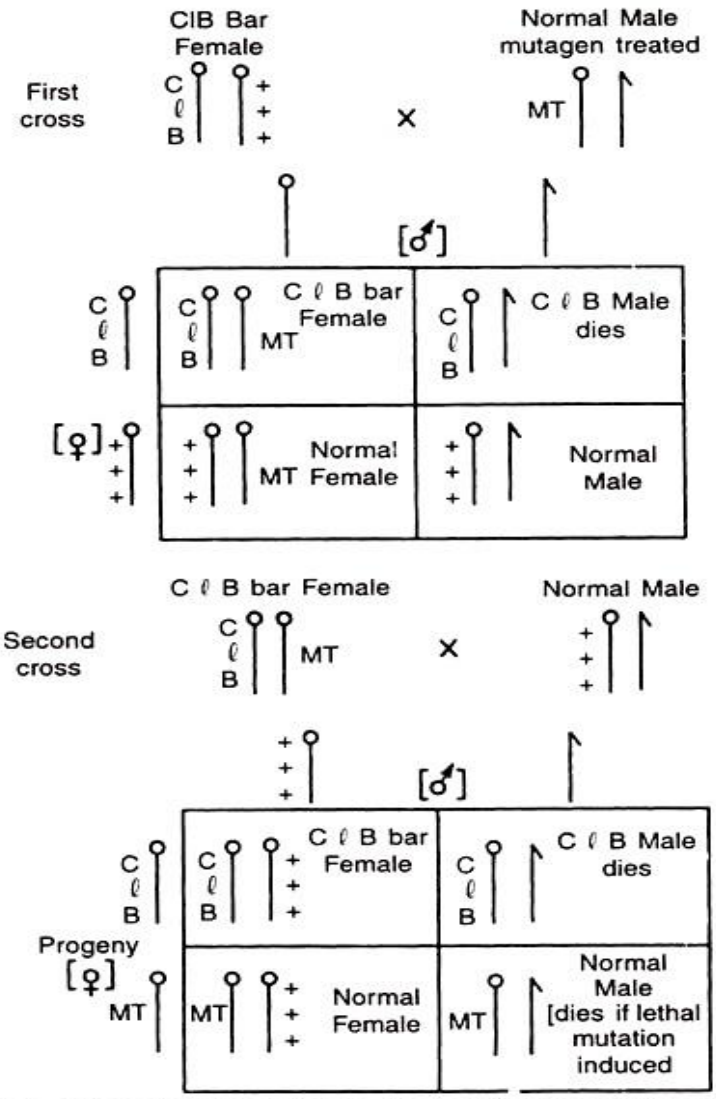


Fig. 14.2. Muller's CIB method for detection of lethal induced mutations in X-chromosome of **Drosophila**. Mt denotes mutagen treated X-chromsome.

(b) Now a cross is made between CIB female and normal male. This time the CIB female has one CIB chromosome and one mutagen treated chromosome received from the male in earlier cross.

This will produce two types of females, viz., half with CIB chromosome and half with mutagen treated chromosome (with normal phenotype). Both the progeny will survive. In case of males, half with CIB will die and other half have mutagen treated chromosome.

If a lethal mutation was induced in mutagen treated X-chromosome, the remaining half males will also die, resulting in absence of male progeny in the above cross. Absence of male

progeny in F_2 confirms the induction of sex linked recessive lethal mutation in the mutagen treated Drosophila male.

ii. Muller's Method:

This method was also developed by Muller to detect sex linked mutation in Drosophila. This method is an improved version of CIB method. This method differs from CIB method in two important aspects. First, this method utilizes apricot recessive gene in place of recessive lethal in CIB method. Second, the female is homozygous for bar apricot genes, whereas it is heterozygous for IB genes in CIB method.In this method, the mutation is detected by the absence of wild males in F_2 progeny. This method consists of following important steps (Fig. 14.3).

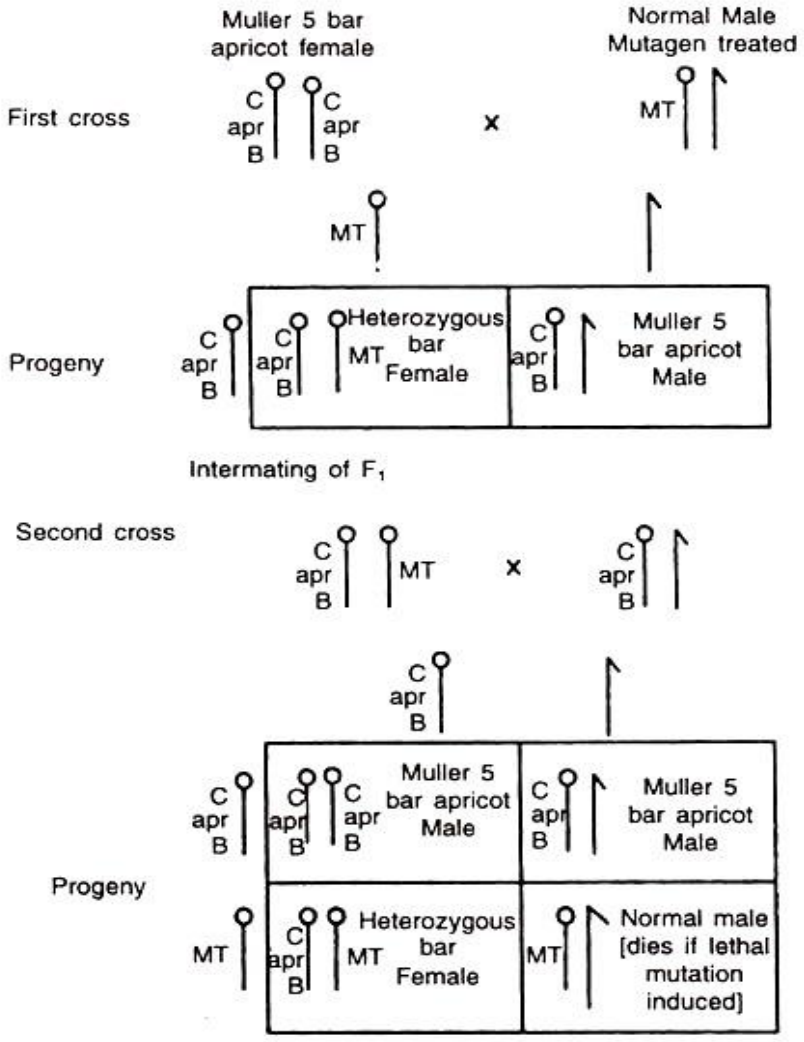


Fig. 14.3. Muller's 5 method of for detection of induced mutation in X-chromosome of **Drosophila.** Mt denotes mutagen treated X-chromosome.

a. A homozygous bar apricot female is crossed with mutagen treated male. In F_1 we get two types of progeny, viz., heterozygous bar females and bar apricot (Muller) males.
b. These F_1 are inter-mated. This produces four types of individuals. Half of the females are homozygous bar apricot, and half are bar heterozygous. Among the males, half are bar apricot (Muller 5) and half should be normal. If a lethal mutation is induced, the normal male will be absent in the progeny.

iii. Attached X-Method:

This method is used to detect sex linked visible mutations in Drosophila. In this method a female in which two X-chromosomes are united or attached together is used to study the mutation (Fig. 14.4). Therefore, this method is known as attached X-method. The attached X females (XXY) are crossed to mutagen treated male. This cross gives rise to super females (XX-X), attached female (XXY), mutant male (XY) and YY. The YY individuals die and super female also usually dies. The surviving male has received X-chromosome from mutagen treated male and Y chromosome from attached X-female. Since Y chromosome does not have corresponding allele of X-chromosome, even recessive mutation will express in such male which can be easily detected.

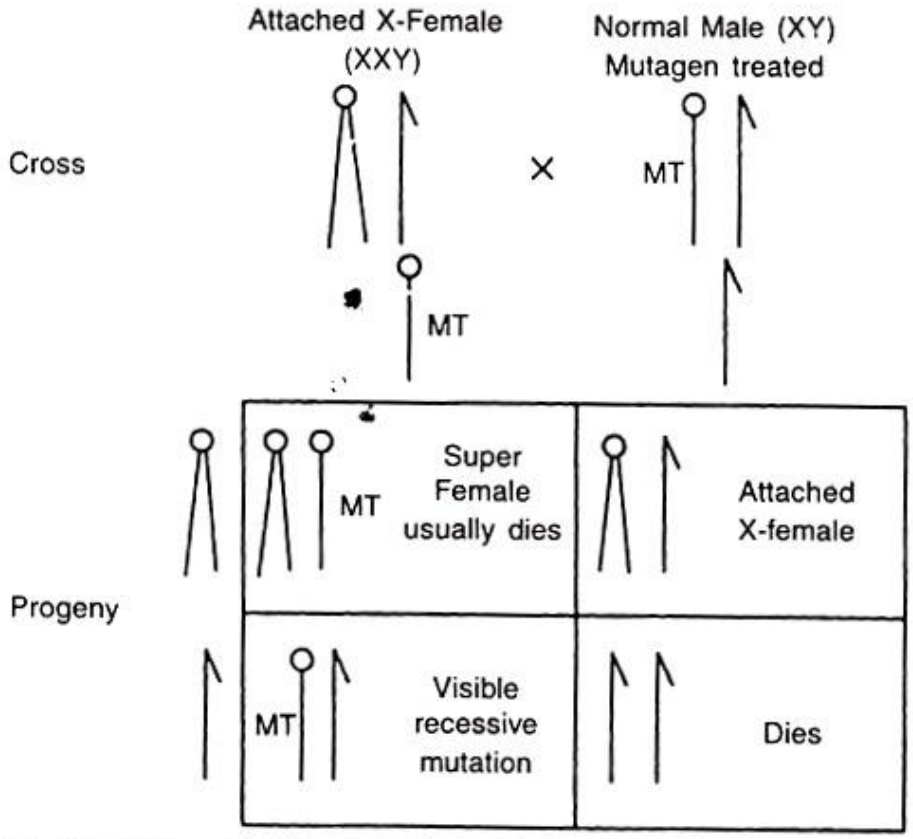


Fig. 14.4. Attach X-method for detection of induced visible mutation in chromosome of **Drosophila.** Mt. denotes mutagen treated X-chromosome.

iv. Curly Lobe-Plum Method:

This method is used for detection of mutation in autosomes. In this method curly refers to curly wings, lobe to lobed eye and plum to plum or brownish eye. All these three genes are recessive lethal. Curly (CY) and lobed (L) genes are located in one chromosome and plum (Pm) in another but homologous chromosome. Crossing over between these chromosomes cannot occur due to presence of inversion. Moreover, homozygous individuals for CYL or Pm cannot survive because of lethal effect. Only heterozygotes survive. Thus, this system is also known as balanced lethal system. This method consists of following steps (Fig. 14.5).

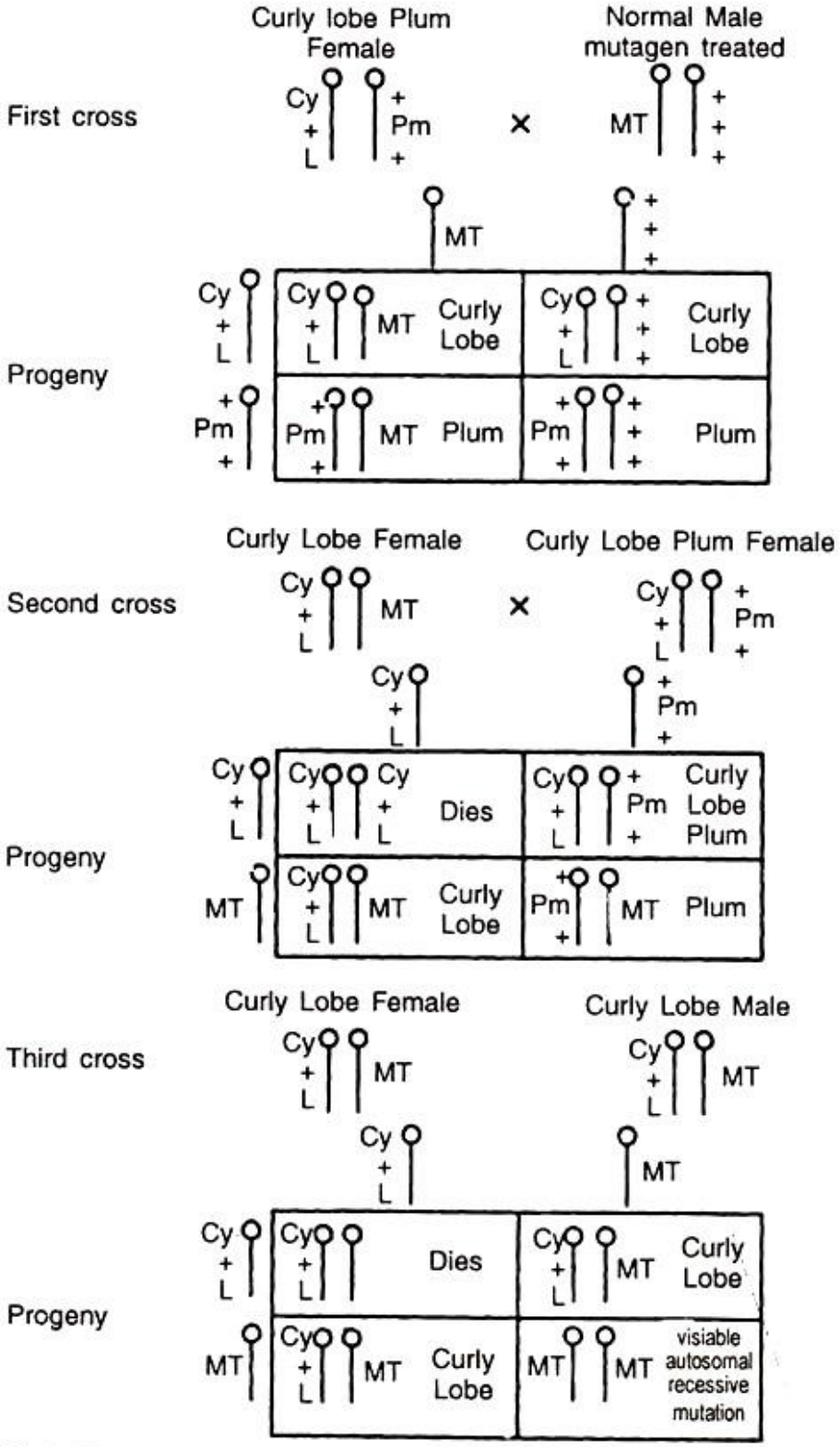


Fig. 14.5. Detection of induced autosomal recessive mutation in **Drosophila.**

a. A cross is made between curly lobe plum (CYL/Pm) female and mutagen treated male. This produces 50% progeny as curly lobe and 50% as plum.

TABLE 14.4. Comparison of Different Methods of Detection of Mutation in *Drosophila*

CIB Method	*Muller 5 Method*	*Attachad X Method*	*Curly lobe plum Method*
1. Used to detect sex linked lethal mutations.	Used to detect sex linked recessive lethal mutations.	Used to detect sex linked visible mutations.	Used to detect visible autosomes mutations.
2. Utilises heterozygous CIB females.	Uses homozygous bar apricot females.	Uses attached X female.	Used heterozygous curly lobe plum female.
3. CIB males die due to lethal effect.	No lethal effect in the parents.	YY individuals die to due to lethal effect.	Homozygous individuals for CYL/PM gene die.
4. Crossing over does not occur.	Crossing over does not occur.	Crossing over does not occur.	Crossing over does not occur.
5. Involves two crosses	Involves two crosses.	Involves one cross only.	Involves three crosses.
6. Mutation is detected by the absence of males in the progeny of second cross.	Mutation is detected by the absence of normal males in the progeny of second cross.	Males with visible mutations are found.	Individuals with treated autosomes show visible mutations.

b. In the second generation cross is made between curly lobe female and curly lobe plum male. This will give rise to curly lobe plum, curly lobe and plum individuals in 1 : 1 : 1 ratio and homozygous curly will die due to lethal effect. From this progeny, curly lobe females and males are selected for further mating.

c. In third generation, a cross is made between curly lobe female carrying one mutagen treated autosome and curly lobe male also carrying treated autosome. This results in production of 50% progeny as curly lobe, 25% homozygous curly lobe which die and 25% progeny homozygous for treated autosomes. This will express as autosomal recessive mutation and constitute one third of the surviving progeny. A comparison of different methods of detection of mutation in Drosophila is given in Table 14.4.

Nutritional Deficiency Method of Mutations:

This method of detection of induced mutations is used in micro-organisms like Neurospora. The normal strain is treated with a mutagen and then cultured on minimal medium. A minimal medium contains sugar, salt, inorganic acids, nitrogen and vitamin biotin. The normal strain of Neurospora grows well on the minimal medium, but a biochemical mutant fails to grow on such medium.

This confirms induction of mutation. Then minimal medium is supplemented with certain vitamins or amino acids, one by one and the growth is observed. The medium which results in normal growth of mutagen treated mould indicates that the mutant lacks synthesis of that particular vitamin or amino acid, addition of which to the minimal culture medium has resulted in normal growth of treated strain.

Spontaneous Mutations:

Naturally occurring mutations are known as spontaneous mutations. Such mutations are induced by chemical mutagens or radiations which are present in the external environment to which an organism is exposed. Temperature also affects the frequency of spontaneous mutations. A rise of 10°C in the temperature leads to fivefold increase in mutation rate in an organism exposed to such variation in temperature.

Drastic change of temperature in any direction produces still greater effect on mutation frequency. External environmental conditions of any type, i.e., either extremely high or low leads to increase in the mutation frequency.

Internal environment of an organism also plays an important role in the induction of spontaneous mutations. For example, spontaneous rearrangements of DNA bases result in base pair transitions. Similarly, errors in DNA repair or replication can cause spontaneous mutations.

Selection of Different Types of Mutants

1. Resistant Mutants:

From a wild-type bacterial population which is susceptible to agents like bacteriophage, various drugs, ultra-violet light etc., mutants which are resistant to any of these agents can be selected directly by using selective media. After induction of mutation with a suitable mutagen, the treated population is allowed to grow under permissive conditions for some time to allow the expression of the mutant gene.

The culture is then dilution-plated in a suitable selective medium which would be expected to completely suppress the growth of the wild type bacteria and would allow only the mutants to grow and form colonies. The mutant colonies can be picked up and purified by dilution plating to obtain pure clones of mutants.

For example, for isolation of resistant mutants against a specific lytic bacteriophage, the selective medium would be one containing the particular phage, so that the wild-type which is susceptible would be eliminated by the lytic phage and only the phage-resistant mutants

would form colonies. Purification is necessary to eliminate any wild-type cells that might be present associated with the resistant cells. Similar procedure can be adopted for selection of drug-resistant mutants, in which case the selective medium would be a growth-supporting one containing the drug at a concentration which is normally inhibitory for the wild-type cells.

Similar procedure can also be used for isolation of several other types of mutants. For example, mutations resulting in reversion of auxotroph's to prototroph can be detected. If it is desired to obtain a prototroph (his^+) from a population of histidine auxotroph (his^-), a mutagen-treated population of the auxotroph may be plated on a medium without histidine. Such a medium will allow growth of the prototroph only.Another type of mutants that can be selected by a similar procedure is those having a wider substrate range in comparison to that of the wild type. This means that a mutant is capable of utilizing a particular substrate which the wild-type is unable to use. The mutagen treated population of the wild- type may be initially grown in a liquid medium containing that substrate.

As this medium would allow only the mutants to grow, an increase in the number of mutants would be expected (enrichment). Plating on the same selective medium would allow the growth of mutants which are capable of utilizing the substrate, while the wild-type cells will fail to grow, because of their inability to utilize the substrate.

2. Regulation-Defective Mutants:

The metabolic pathways are controlled by regulatory mechanisms. One of the important mechanisms is feed-back control of biosynthetic pathways in which the end-product inhibits the first enzyme of the sequence. The first enzyme in such case is an allosteric protein which can bind both the substrate and the end-product at different sites of the molecule.

Mutation may result in the formation of a defective enzyme protein with loss of regulatory function. Such regulation-defective mutants may arise spontaneously, or may be induced by treatment with mutagens. A well-known example is a naturally occurring defective mutant of *Corynebacterium glutamicum* which produces large quantity of glutamic acid and has been used for commercial production of this amino acid.

Regulation-defective mutants of bacteria can be detected by using specific antimetabolites in selection medium. An antimetabolite is generally a non-biological chemical compound which is a structural analogue of a metabolite. They are able to inhibit growth by stopping the synthesis of the metabolite by reacting with the first enzyme of the biosynthetic pathway of the particular metabolite. This property of an antimetabolite can be made use of in detection of defective mutants which have the ability to escape the regulatory mechanism. For detection

of such regulation defective mutants, a mutagen-treated bacterial suspension containing 10^8 to 10^{10} cells/ml is spread over a medium containing an inhibitory concentration of the antimetabolite (specific for the metabolite). After incubation, only a few colonies develop in the plates, because most of the bacteria fail to grow in presence of the antimetabolite. The colonies develop only from the small number of antimetabolite-resistant mutants. However, all such resistant mutants may not be regulation-defective, because resistance may develop also by other means. The regulation-defective mutants can be identified in the plates by the presence of a halo of satellite colonies around the larger colonies of the mutants. The satellite colonies develop because the mutant colony secretes the metabolite into the medium which feeds the wild-type bacteria to make them grow. The satellite colonies are smaller in size, because they start developing later when the mutants secrete enough metabolite to overcome the effect of the antimetabolite (Fig. 9.67).

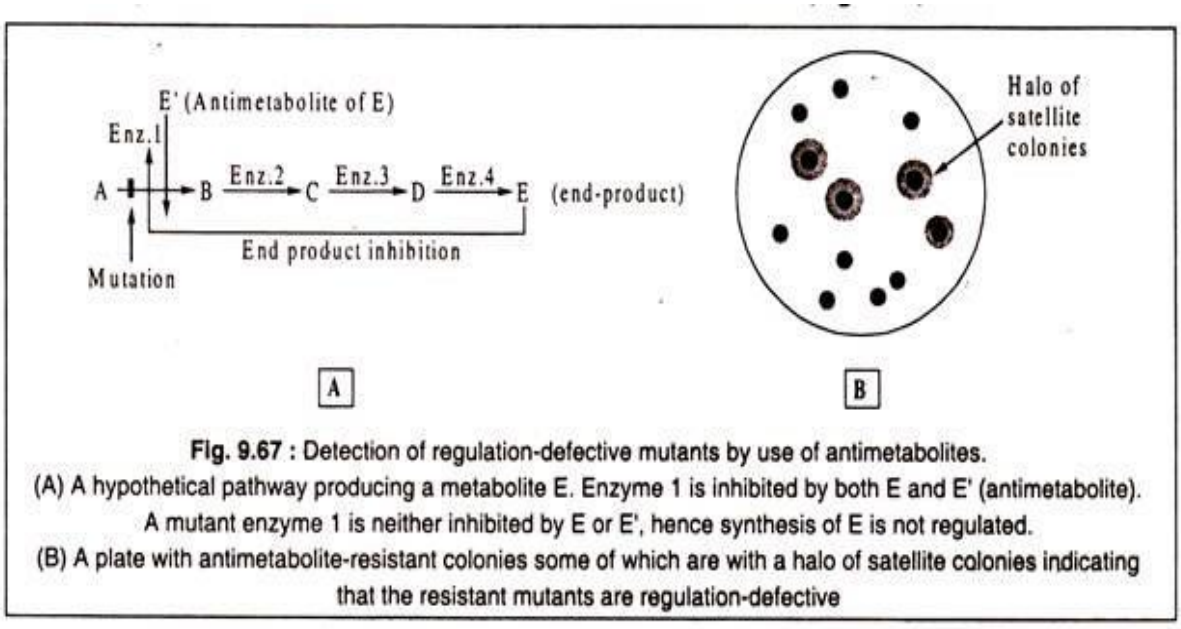


Fig. 9.67 : Detection of regulation-defective mutants by use of antimetabolites.
(A) A hypothetical pathway producing a metabolite E. Enzyme 1 is inhibited by both E and E' (antimetabolite). A mutant enzyme 1 is neither inhibited by E or E', hence synthesis of E is not regulated.
(B) A plate with antimetabolite-resistant colonies some of which are with a halo of satellite colonies indicating that the resistant mutants are regulation-defective

3. Auxotrophic Mutants:

An auxotrophic mutation differs from others in producing a loss of ability to synthesise an essential metabolite. It may be one of the protein amino acids, a nucleic acid base, a vitamin etc. As a result, the mutant loses ability to grow in a minimal medium which sustains growth of the wild-type, and it obligately requires supplementation of the metabolite which it no longer can synthesise.

Obviously, a straight-forward detection of auxotroph's is not possible in a selective medium. A laborious method would be to grow the mutagen-treated population in a medium which allows growth of both the wild-type and auxotroph's (complete medium) and then test each and every colony for its ability to grow in a complete medium and in a minimal medium plate.

Considering an average mutation rate of 10^{-6} to 10^{-7}, it can be realized that one million to 10 million colonies must be tested to find out a single auxotroph. However, treatment with mutagens can considerably increase the mutation rate. Even then, detection of auxotroph's remains a laborious task.

Two techniques which are used in conjunction to minimize this labour are the enrichment of the mutants in the total population by treatment with penicillin and the replica plating:

(a) Penicillin Enrichment Technique:

This method makes use of the bactericidal property of penicillin which is restricted to the actively growing organisms. The antibiotic cannot destroy the non-growing or resting bacteria. Thus, under conditions which support growth of the wild-type bacteria only, penicillin kills them preferentially and the mutants which do not grow survive.

As a result, the proportion of mutants in the total population increases substantially making their detection and isolation easier. For this purpose, the mutagen-treated population is grown in a minimal medium containing a lethal dose of penicillin for a period during which the wild-type bacteria grow and are killed by the antibiotic.The surviving bacteria having a higher proportion of mutants are centrifuged and washed to remove penicillin. They are suspended and plated on complete medium for detection of mutants by the replica plating technique. Penicillin treatment may have to be repeated to obtain the desired enrichment of the mutants.

(b) Replica-Plating Technique:

It is a simple but a very efficient method for detecting auxotrophic mutants. The technique developed by Lederberg makes it possible to test a reasonably large number of discrete colonies growing in a plate for the occurrence of mutants. Generally, 100 to 200 colonies growing in a single plate can be tested at a time, so that the labour and tediousness of examining each and every colony individually for detection of mutants are greatly minimized. The basic outfit (Fig. 9.68) consists of a wooden or metallic cylindrical block with a diameter slightly less than that of a standard Petri dish, a metallic collar and several pieces of velveteen cloth. The bristles of the cloth act as individual inoculating needles. The components are sterilized before use. The velveteen is spread tightly over the block and kept in place by the collar.

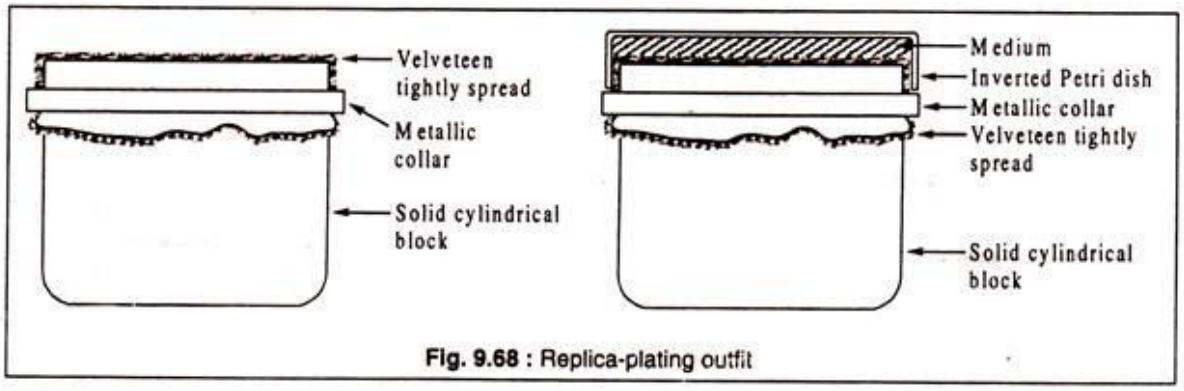


Fig. 9.68 : Replica-plating outfit

The master plate containing complete medium with 100-200 discrete colonies among which the majority colonies are wild type and few mutants is inverted over the velveteen covered cylindrical block and lightly pressed to transfer the impression of the colonies on to the bristles of velveteen.

After removing the master plate, a blank minimal agar plate is similarly placed on the velveteen surface to transfer the impression of the master plate to it. On incubation, colonies of only the wild-type grow on the minimal agar surface. When the two plates, i.e. the master plate and the minimal agar plate, are examined, it is observed that some colonies of the master plate are absent in the minimal agar plate indicating that they are probable mutants — because they grow in complete medium but fail to grow in the minimal agar.These colonies are picked up from the master plate and purified by dilution plates Individual colonies are picked up from the dilution plates and tested for their growth requirement. Detection of the mutants by comparison of the master plate and the minimal agar plates can be facilitated by transfer of the colony images of the master plate on a photographic film. By correct positioning of the minimal agar plate against the negative and by illumination from bottom, it becomes easier to locate the mutant colonies which are absent in the plate.

A schematic representation of the master plate and a replica plate of minimal agar is shown in Fig. 9.69:

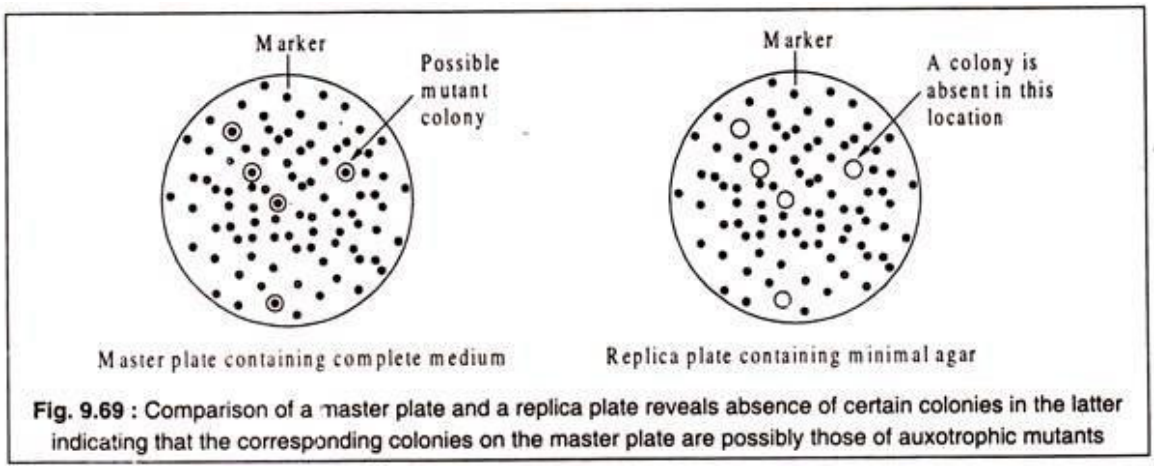


Fig. 9.69 : Comparison of a master plate and a replica plate reveals absence of certain colonies in the latter indicating that the corresponding colonies on the master plate are possibly those of auxotrophic mutants

Resistance Selection Method:

It is the other approach for isolation of mutants. Generally the wild type cells are not resistant either to antibiotics or bacteriophages. Therefore, it is possible to grow the bacterium in the presence of the agent (antibiotics or bacteriophage and look for survivors. This method is applied for isolation of mutants resistant to any chemical compounds that can be amended in agar, phage resistant mutants.

Substrate Utilization Method:

This method is employed in the selection of bacteria. Several bacteria utilize only a few primary carbon sources. The cultures are plated onto medium containing an alternate carbon sources. Any colony that grows on medium can use the substrate and are possibly mutants. These can be isolated.

Sugar utilization mutants are also isolated by means of colour indicator plates. A popular medium (EMB agar) is used for this purpose. The EMB agar contains two dyes eosin and methylene blue in the medium. Colour of these dyes is sensitive to pH. This medium also contains lactose sugar as carbon source and complete mixture of amino acids.Therefore, both lactose wild type (Lac^+) and lactose mutant (Lac^-) cells can grow and form colonies on EMB agar plates. The Lac^+ cells catabolize lactose and secrete acids, therefore, local pH of the medium decreases. This results in staining of colony to dark purple.On the other hand, Lac^- cells are unable to utilize lactose and use some of the amino acids as carbon source. After utilization of amino acid, possibly ammonia is produced that increases the local pH and decolorizes the dye resulting in white colon

Ames test

Principle

Ames test is developed by **Bruce N. Ames** in 1970s to test for determining if the chemical is mutagens. This test is based on the principle of **reverse mutation or back mutation**. So, the test is also known as **bacterial reverse mutation assay.**

Test organism: Ames test uses several strains of bacteria (*Salmonella, E.coli*) that carry mutation. Eg A particular strain of *Salmonella* Typhimurium carry mutation in gene that encodes histidine. So it is an auxotrophic mutant which loss the ability to synthesize histidine (an amino acid) utilizing the ingredients of culture media. Those strains are known as **His-** and require histidine in growth media.

Culturing His- salmonella is in a media containing certain chemicals, causes mutation in histidine encoding gene, such that they regain the ability to synthesize histidine **(His+**) This is the reverse mutation. Such chemicals responsible to revert the mutation are actually a mutagen. So, this Ames test is used to test mutagenic ability of varieties of chemicals.

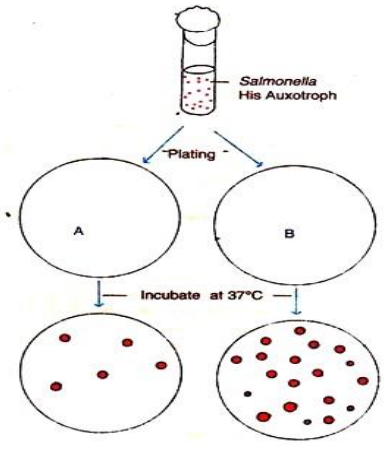


Fig. 9.16 : The Ames test for mutagenicity; A, complete medium containing a large amount of histidine; B, medium containing test mutagen and a small amount of histidine.

The Ames test follows the following steps

(i) Prepare the culture of Salmonella histidine auxotrophs (His^-).

(ii) Mix the bacterial cells and test substance (mutagen) in dilute molten top agar with a small amount of histidine in one set, and control with complete medium plus large amount of histidine.

(iii) Pour the molten mix on the top of minimal agar plates and incubate at 37°C for 2-3 days. Until histidine is depleted all the His^- cells will grow in the presence of test mutagens. When histidine is completely exhausted only the reverants (the mutants which have regained the original wild type characters) will grow on agar plate.The number of spontaneous reverants is low, whereas the number of reverants induced by the test mutagen is quite high. In order to estimate the relative mutagenicity of the mutagenic substance the visible colonies are counted and compared with control. The high number of colonies represents the greater mutagenicity.A mammalian liver extract is added to the above molten top agar before plating. The extract converts the carcinogens into electrophilic derivatives which will soon react with DNA molecule.

In natural way this process occurs in mammalian system when foreign substances are metabolized in the liver. Bacteria do not possess the metabolizing capacity as liver does; therefore, the liver extract is added to this test, just to promote the transformation.Therefore, several potential carcinogens that generally are not carcinogenic until modified in liver, for example aflatoxins viz., B_1, B_2, G_1, G_2, etc. The Ames test has now been used with thousands of substances and mixtures such as the industrial chemicals, food additives, pesticides, hair dyes and cosmetics.

Application:

1. The practical application of Ames test is to screen chemical mutagens that causes mutation and are carcinogenic to human and animals. Some of the chemicals used as food additive (AF-2), flavoring agent (Safrole) are mutagenic as well carcinogenic.
2. Isoniazid; an anti TB drug is also mutagens.
3. Ames test adopted to use eukaryotic cell culture, yeast cell, as well as animal model to test mutagens. Since, Salmonella is not a best test organism to test mutagens for Human. Certain chemicals initially are not mutagens to human but convert into mutagens when metabolized (acted upon by body enzymes). For example; sodium nitrate (NaNO3) is not mutagens until it is acted upon by HCL in stomach to form Nitrous oxide HNO2 (a potent mutagen).
4. Ames test can detects Suitable mutants in large population of bacteria with high sensitivity.
5. It is test for mutagenicity not carcinogenicity. However, most of the mutagens (more than 90%) detected by Ames test are responsible to cause cancer.
6. It is a bacterial reverse mutation assay. So the defective gene of bacteria can be mutated into functional gene.

Probable Questions:

1. Describe CIB method for detection of mutation.
2. Describe Muller's method for detection of mutation.
3. Describe attached X method for detection of mutation.
9. Describe Curly Lobe-Plum Method for detection of mutation.
4. What is auxotrophic mutation?
5. Describe Ames Test for detection of carcinogens.
6. Describe replica plating technique for detection of mutant.

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7th Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwal.

UNIT-V

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UNIT-VI

DNA repair and retrieval: Repair of spontaneous and induced mutations. Mechanism of DNA repair; repair by direct reversion; excision repair; SOS response.

Objective: In this unit we will discuss about DNA repair and retrieval system and also about repair of spontaneous and induced mutations. We will also discuss mechanism of DNA repair such as excision repair, SOS response and other mechanisms.

Introduction to DNA Damage and Repair:

DNA is a highly stable and versatile molecule. Though sometimes the damage is caused to it, it is able to maintain the integrity of information contained in it. The perpetuation of genetic material from generation to generation depends upon keeping the rates of mutation at low level. DNA has many elaborate mechanisms to repair any damage or distortion.

The most frequent sources of damage to DNA are the inaccuracy in DNA replication and chemical changes in DNA. Malfunction of the process of replication can lead to incorporation of wrong bases, which are mismatched with the complementary strand. The damage causing chemicals break the backbone of the strand and chemically alter the bases. Alkylation, oxidation and methylation cause damage to bases. X-rays and gamma radiations cause single or double stranded breaks in DNA.

A change in the sequence of bases if replicated and passed on to the next generation becomes permanent and leads to mutation. At the same time mutations are also necessary which provide raw material for evolution. Without evolution, the new species, even human beings would not have arisen. Therefore a balance between mutation and repair is necessary.

Types of Damage:

Damage to DNA includes any deviation from the usual double helix structure.

1. Simple Mutations:

Simplest mutations are switching of one base for another base. In transition one pyrimidine is substituted by another pyrimidine and purine with another purine. Trans-version involves

substitution of a pyrimidine by a purine and purine by a pyrimidine such as T by G or A and A by C or T. Other simple mutations are detection, insertion of a single nucleotide or a small number of nucleotides. Mutations which change a single nucleotide are called point mutations.

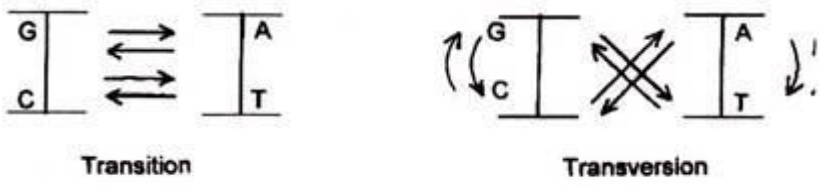


Fig. 5.1.

2. Deamination:

The common alteration of form or damage includes deamination of cytosine (C) to form uracil (u) which base pairs with adenine (A) in next replication instead of guanine (G) with which the original cytosine would have paired.

As uracil is not present in DNA, adenine base pairs with thymine (T). Therefore C-G pair is replaced by T-A in next replication cycle. Similarly, hypoxanthine results from adenine deamination.

3. Missing Bases:

Cleavage of N-glycosidic bond between purine and sugar causes loss of purine base from DNA. This is called depurination. This apurinic site becomes non-coding lesion.

4. Chemical Modification of Bases:

Chemical modification of any of the four bases of DNA leads to modified bases. Methyl groups are added to various bases. Guanine forms 7- methylguanine, 3-methylguanine. Adenine forms 3-methyladenine. Cytosine forms 5- Methylcytosine.

Replacement of amino group by a keto group converts 5-methylcytosine to thymine.

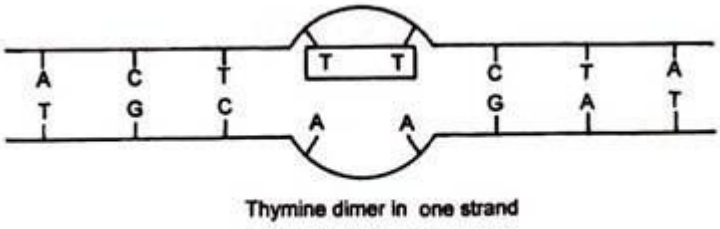


Fig. 5.2.

Figure: Formation of Pyrimidine Dimers (Thymine Dimers):

Formation of thymine dimers is very common in which a covalent bond (cyclobutyl ring) is formed between adjacent thymine bases. This leads to loss of base pairing with opposite stand. A bacteria may have thousands of dimers immediately after exposure to ultraviolet radiations.

6. Strand Breaks:

Sometimes phosphodiester bonds break in one strand of DNA helix. This is caused by various chemicals like peroxides, radiations and by enzymes like DNases. This leads to breaks in DNA backbone. Single strand breaks are more common than double strand breaks.

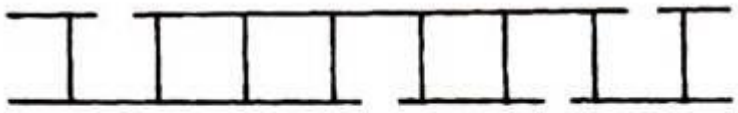


Fig. 5.3.

Sometimes X-rays, electronic beams and other radiations may cause phosphodiester bonds breaks in both strands which may not be directly opposite to each other. This leads to double strand breaks.

Some sites on DNA are more susceptible to damage. These are called hot-stops.

Repair Mechanisms:

A. Photo Reactivation:

We know that exposure of UV-irradiated bacteria immediately afterwards to visible light restores to a considerable degree the viability of the UV-inactivated bacteria. This phenomenon mown as photo reactivation, is based on enzymatic cleavage of the thymine dimers.The enzyme, photolyase, binds to the thymine dimer and catalyses photochemical cleavage of the cyclobutane ring of the dimer to make the thymine's free. The enzyme uses visible light for the reaction. Besides thymine-dimers, other pyrimidine-dimers—like cytosine-cytosine and cytosine-thymine dimer—are also attacked by the enzyme. The enzyme is devoid of any species-specificity. Photolyases have been detected in both prokaryotes and eukaryotes.

The action of photolyase on UV-irradiated DNA containing thymine dimers is schematically represented in Fig. 9.70:

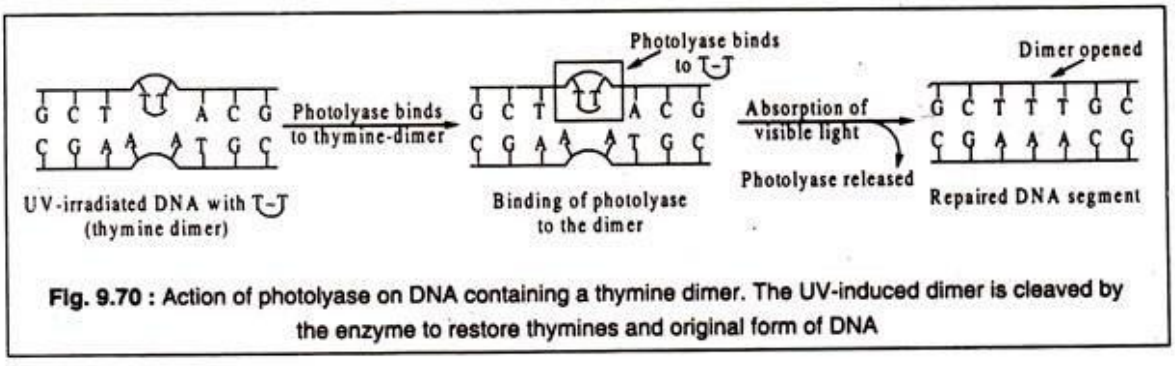


Fig. 9.70 : Action of photolyase on DNA containing a thymine dimer. The UV-induced dimer is cleaved by the enzyme to restore thymines and original form of DNA

It has been observed that UV-irradiated DNA containing 5-bromouracil — which is an analogue of thymine and is incorporated into replicating DNA replacing thymine — is resistant to photo reactivation. Such DNA binds the photolyase enzyme, but the enzyme neither dissociates from the dimer nor can liberate the free thymine molecules.

B. Excision Repair:

Apart from photo reactivation, there are also other mechanisms for repair of damaged DNA. One of these is excision repair which occurs in absence of light i.e. exposure to visible light is not required. It is also known as dark-repair.

The excision repair essentially consists of removal of a segment of DNA containing the damaged portion of one strand of DNA and new synthesis of the removed segment of the DNA strand using the undamaged strand as template. The first step, known as incision step, involves recognition of the damaged segment by an endonuclease.The enzyme cleaves the phosphodiester bond of the sugar- phosphate backbone at the 5′-end about eight nucleotides ahead of the damaged site producing a 3′-OH group free. The next step, known as excision step, involves a cut at a site 4 to 5 nucleotides downstream from the damaged site catalysed by a 5′-3′ exonuclease.

Thereby, a segment of DNA including the damaged site is removed. In the last step, known as the repair step, DNA polymerase I synthesizes a new stretch of DNA strand starting from the 3′-OH end using the intact complimentary strand as template. Finally, the newly synthesized strand is joined with the 5′-end by DNA ligase to complete the repair. The steps are diagrammatically shown in Fig. 9.71.Another variation of excision repair is catalysed by the enzyme glycosylase which cleaves the N-glycosidic bond between a thymine of the thymine dimer to the sugar-phosphate backbone of the DNA strand. At the next step, the phosphodiester bond is cleared by the endonuclease activity of the same enzyme which recognizes a blank deoxyribose without a base attached to it.

In the following step, DNA polymerase I initiates DNA synthesis at the free 3′-OH end displacing the thymine dimer along with a few more adjacent nucleotides as shown in Fig. 9.72. This type of excision repair occurs also in E. coli and several other bacteria like Micrococcus luteus.Excision repair is observed when UV-treated bacteria are stored in dark for a few hours in a medium which does not support growth before returning them to the normal growth supporting medium (liquid holding recovery). In excision repair mechanism DNA polymerase I seems to play an important role.It has been shown that E. coli mutants which are deficient in this enzyme show extensive DNA damage following UV-irradiation, presumably because such mutants are unable to repair the damaged DNA by excision repair mechanism. It may be reminded that in normal DNA replication, polymerase III (pol III) catalyses DNA synthesis.

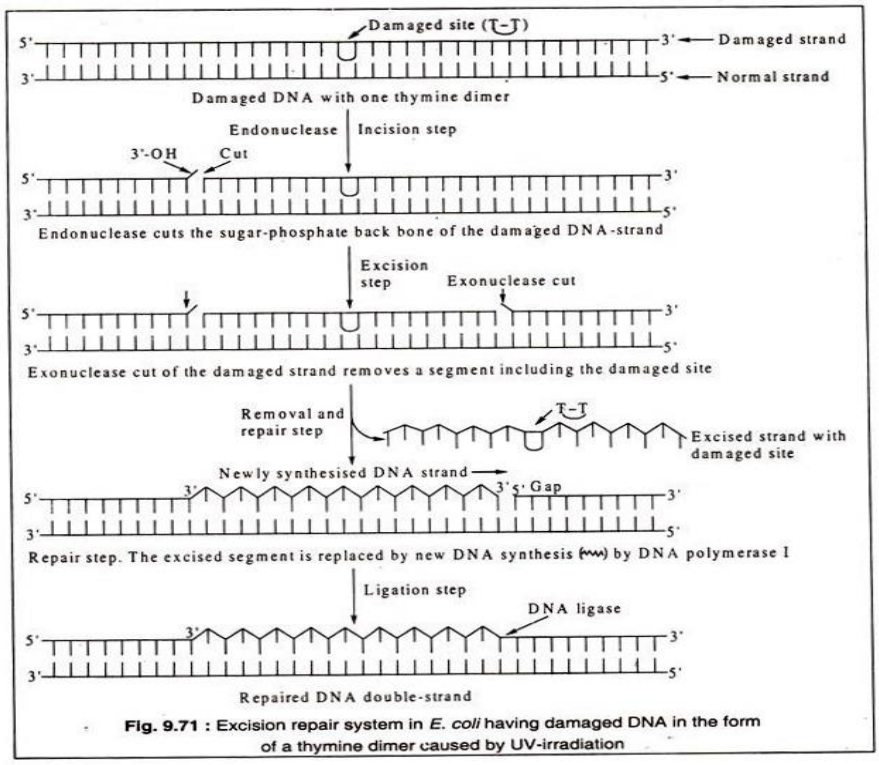


Fig. 9.71 : Excision repair system in *E. coli* having damaged DNA in the form of a thymine dimer caused by UV-irradiation

Excision repair are mainly two types:

(a) Base excision repair:

The lesions containing non-helix distortion (e.g. alkylating bases) are repaired by base excision repair. It involves at least six enzymes called DNA glycosylases.

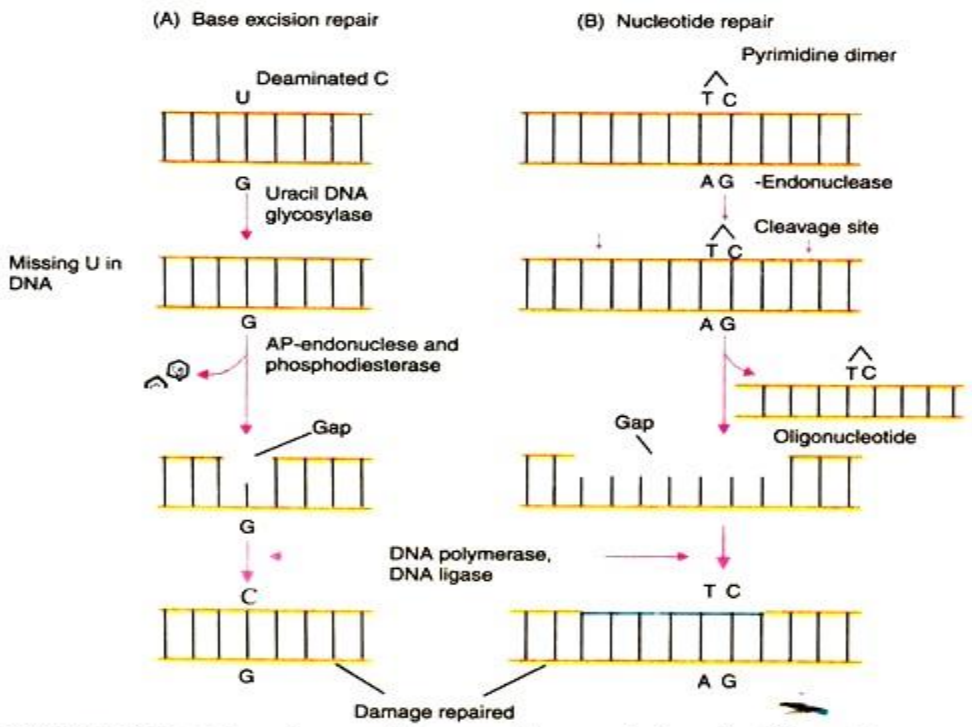


Fig. 9.18 : Excision repair pathways; A, base excision repair, B, nucleotide excision repair.

Each enzyme recognises at least bases and removes from DNA strand. The enzymes remove deaminated cytosine, deaminated adenine, alkylated or oxidised base. Base excision repair pathway starts with a DNA glycosylation. For example, the enzyme uracil DNA glycosylase removes the uracil that has wrongly joined with G which is really deaminated cytosine (Fig. 9.18A).

Then AP- endonuclease (apurinic or apyriminic site) and phosphodiesterase removes sugar-phosphate. AP- sites arise as a result of loss of a purine or a pyrimidine. A gap of single nucleotide develops on DNA which acts as template-primer for DNA polymerase to synthesise DNA and fill the gap by DNA ligase.

(b) Nucleotide excision repair:

Any type of damage having a large change in DNA helix causing helical changes in DNA structure is repaired by this pathway. Such damage may arise due to pyrimidine dimers (T-T, T-C and C-C) caused by sun light and covalently joins large hydrocarbon (e.g. the carcinogen benzopyrene).

In E. coli a repair endonuclease recognises the distortion produced by T-T dimer and makes two cuts in the sugar phosphate backbone on each side of the damage. The enzyme DNA helicases removes oligonucleotide from the double helix containing damage. DNA polymerase III and DNA ligase repair the gap produced in DNA helix (Fig. 9.18B).

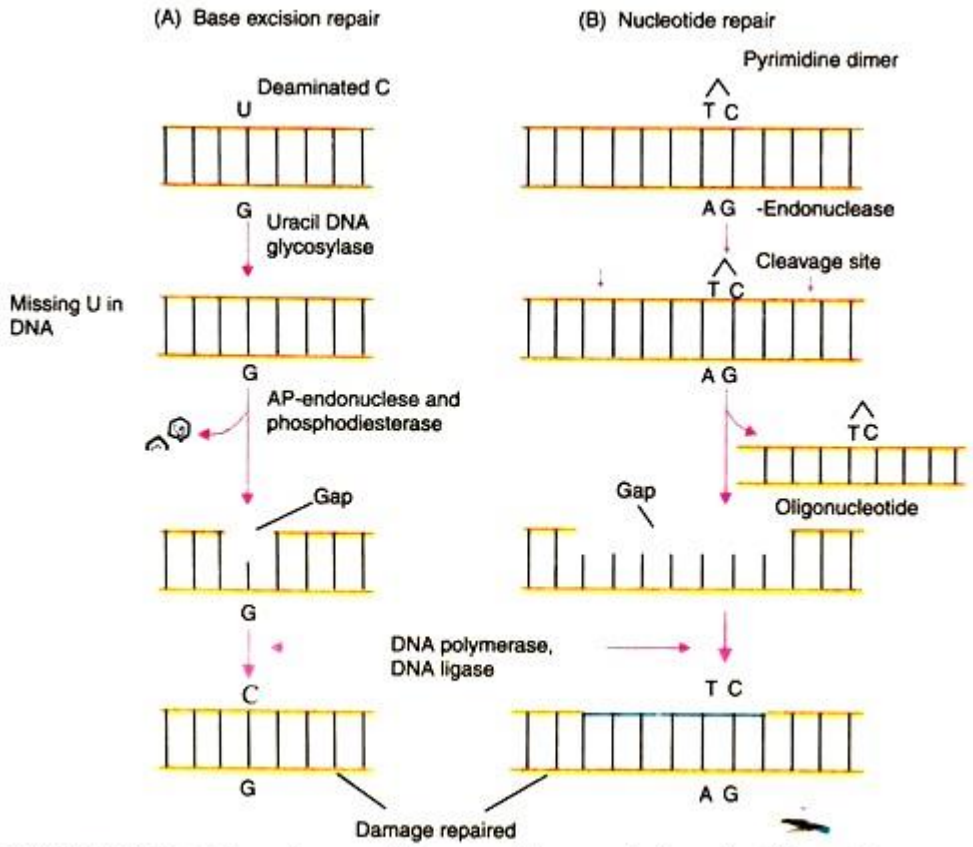


Fig. 9.18 : Excision repair pathways; A, base excision repair, B, nucleotide excision repair.

C. Re-Combinational Repair Mechanism:

This is another mode of repair of damaged DNA. It consists essentially of an exchange of a damaged segment of one DNA molecule by an undamaged segment of another one. As such exchange takes place only after replication of the damaged DNA has taken place it is also known as post- replication repair.

When a damaged DNA molecule — e.g. DNA containing thymine-dimers induced by UV-irradiation—begins replication with the help of DNA polymerase III, the enzyme stops synthesis as it reaches a dimer, because of the distortion caused by the dimer in the regular double helix.

As a result, the progress of the replication fork halts temporarily as it reaches a dimer. DNA synthesis is then reinitiated at a new site, few nucleotides past the thymine-dimer site. Thus, a gap is created opposite the dimer site and a few adjoining nucleotides. The newly synthesized

daughter strand is produced with several gaps i.e. in short pieces, if several dimers occur in the same template strand (Fig. 9.73).

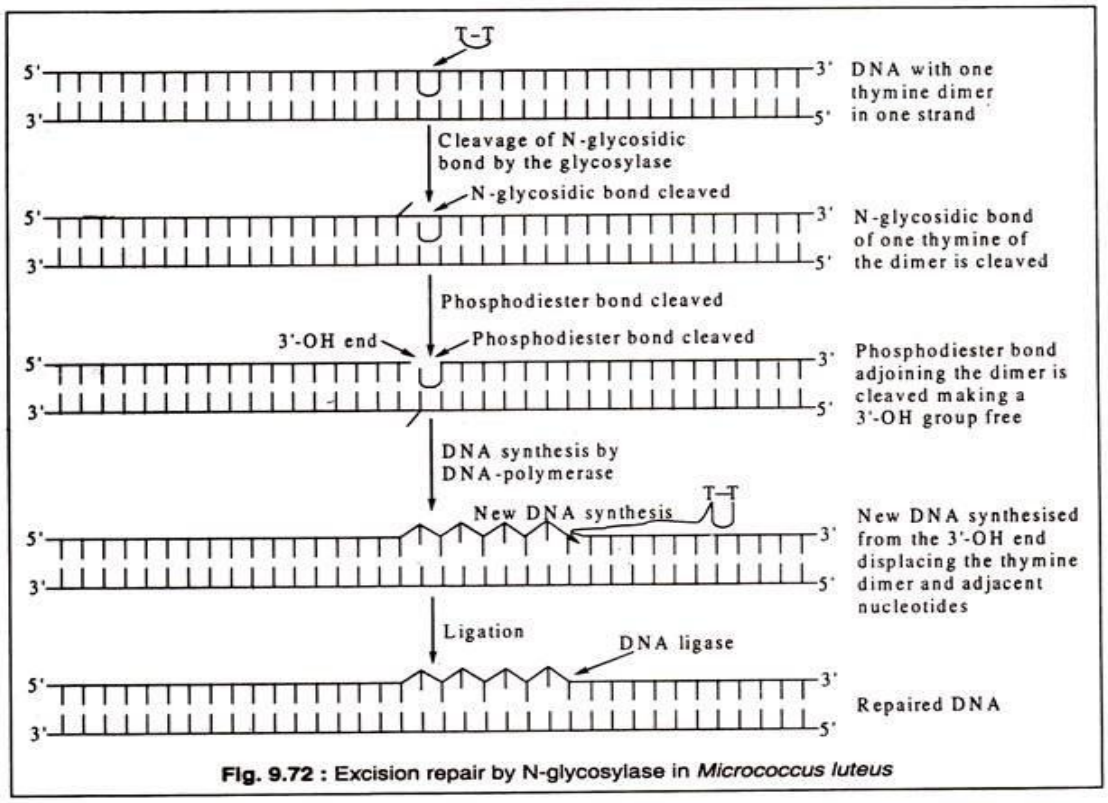


Fig. 9.72 : Excision repair by N-glycosylase in *Micrococcus luteus*

The gaps in the daughter strand are then filled up by exchanging undamaged homologous segments from a sister DNA double helix. The gaps produced in the donor strand are then filled up by new DNA synthesis with the help of DNA polymerase I and sealed by the DNA ligase as shown in Fig. 9.74. Thus, in re-combinational repair system, parts of DNA strand missing in one strand are retrieved from another normal DNA strand of a sister double helix.

The damaged DNA strand will continue to have the damaged site and will replicate to have gaps in the daughter strand which will be repaired by re-combinational repair mechanism. Ultimately, the damaged strand will be outnumbered by normal DNA and will be insignificant.In re-combinational repair mechanism, recA gene plays an important role. It has been observed that recA mutants are extremely sensitive to lethal effects of radiations and chemical mutagens. The recA gene is known to play a very important role in genetic recombination e.g. in conjugation where recA' recipient fails to show genetic recombination. The gene functions also in recombinational repair, where exchange of DNA strands is involved.

D. Repair of DNA by Homologous Recombination:

This type of repair is called for when both strands of DNA molecules are damaged at sites opposite each other. In such a case, the missing segments cannot be replaced from sister strands after replication by the usual recombinational repair mechanism. The lost portions have to be retrieved from another homologous DNA molecule.

In actively growing bacteria, each cell contains generally more than one copy of DNA. So, the lost portions of one damaged molecule can be repaired by crossing-over with a normal DNA molecule involving exchange of homologous segments. This type of DNA repair is known as homologous recombinational repair. Double-stranded breaks of DNA can be induced by exposure to X-rays. Rec A protein plays an important role also in this type of repair.

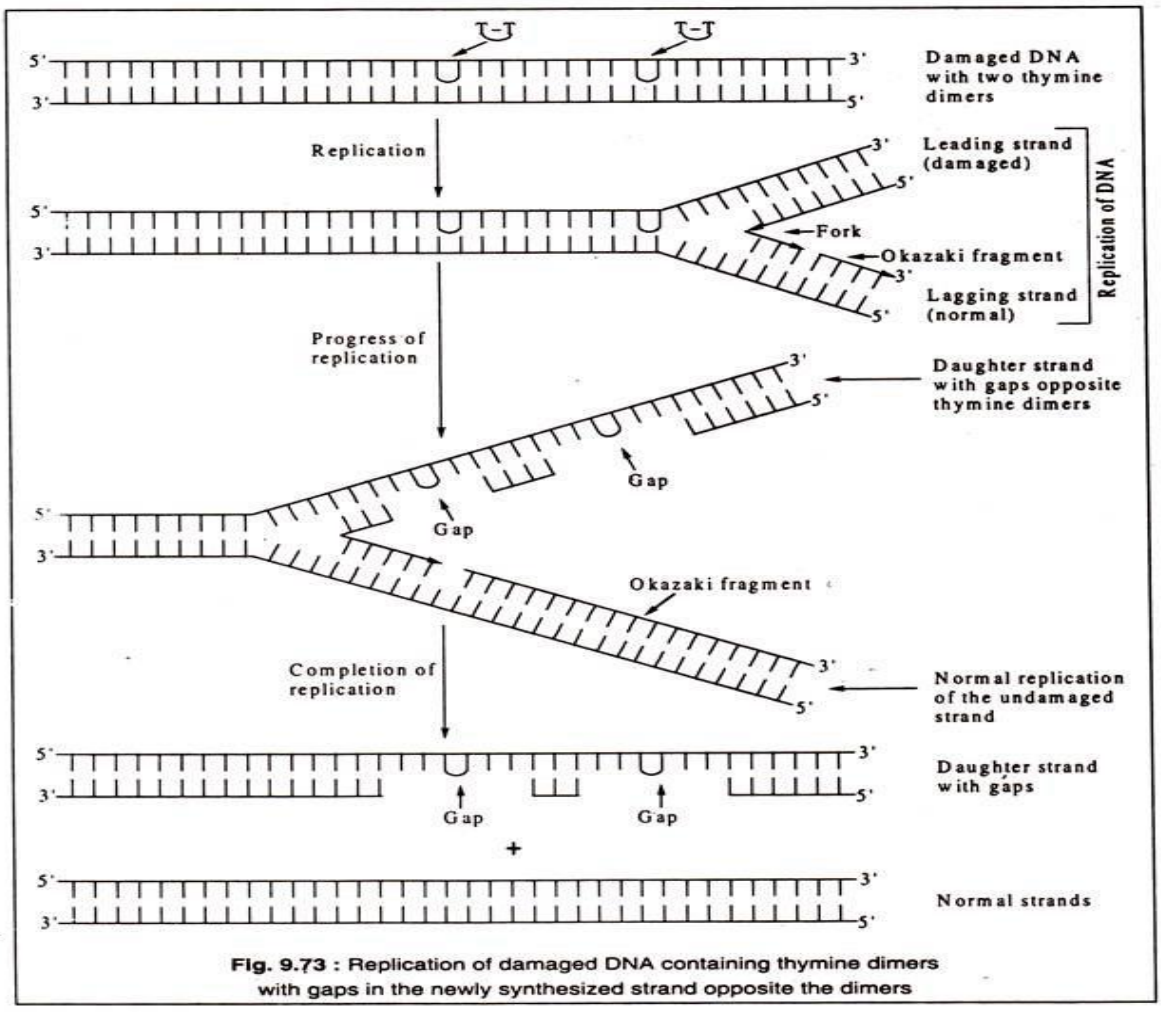


Fig. 9.73 : Replication of damaged DNA containing thymine dimers with gaps in the newly synthesized strand opposite the dimers

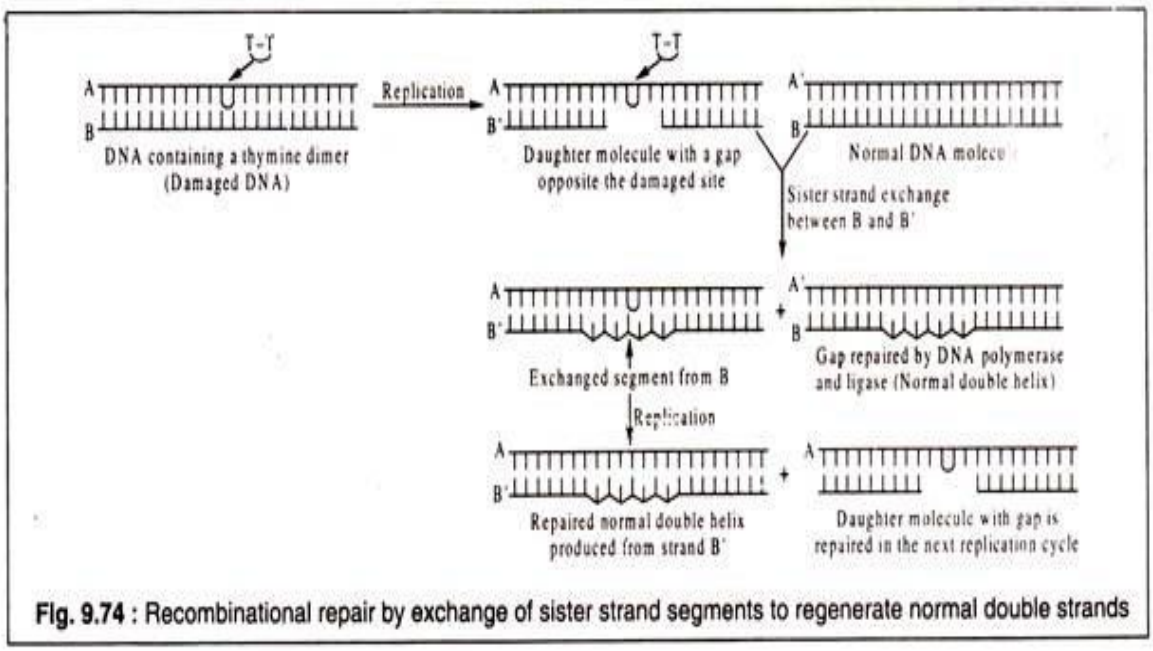


Fig. 9.74 : Recombinational repair by exchange of sister strand segments to regenerate normal double strands

The repair process begins with the production of single-stranded segments at the 3′-OH end of each strand through the action of a nuclease and binding of the Rec A protein to the single strands. The binding of Rec A protein initiates strand exchange between homologous segments of the normal DNA duplex and the damaged one. The crossing-over results in the formation of two DNA molecules, each having an intact strand and a strand with gaps.

The gaps are then filled up by new DNA synthesis catalysed by DNA polymerase and sealing by DNA ligase. The intact strand is used as template. Thus, crossing-over which is normally a mechanism for creating genetic diversity by mixing up genes located on homologous chromosomes, can also function as a means for repairing damaged DNA. Rec A protein plays vital roles in both the processes (Fig. 9.75).

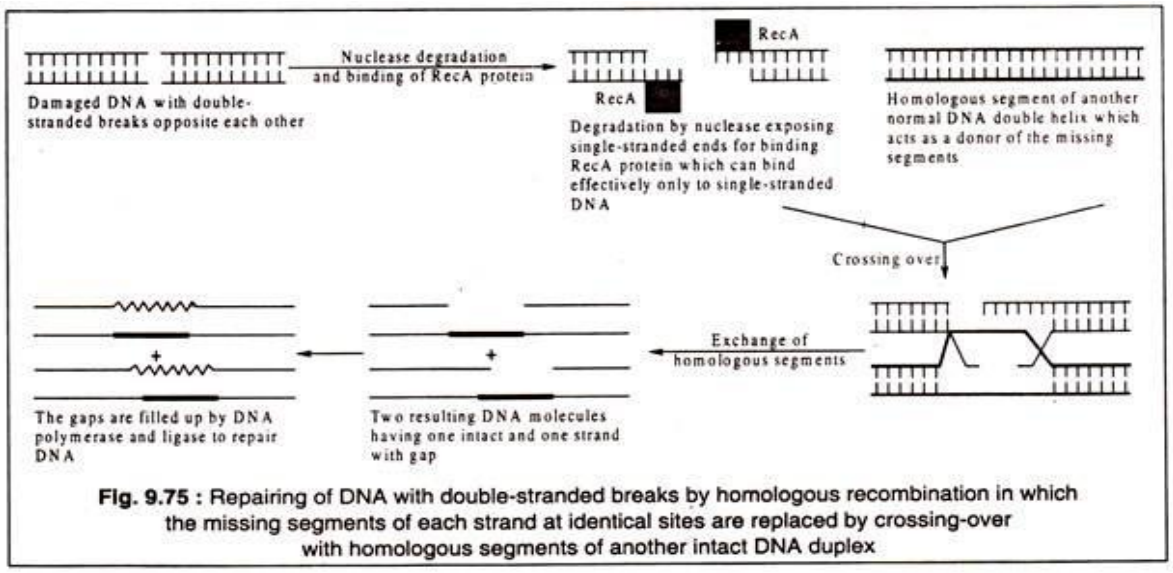


Fig. 9.75 : Repairing of DNA with double-stranded breaks by homologous recombination in which the missing segments of each strand at identical sites are replaced by crossing-over with homologous segments of another intact DNA duplex

E. SOS Repair of Damaged DNA:

The SOS repair mechanism functions in a more complicated way. Damages inflicted on DNA by mutagenic agents induce a complex series of changes which are collectively known as SOS response. The response leads to increased capacity to repair damaged DNA by excision repair and recombinational pathways.

The SOS response is set in action by the interaction of two proteins, — Rec A protein which is a product of the recA gene and Lex A protein, the product of lexA gene. The Rec A protein in addition to having a role in genetic recombination and recombinational repair also has a protease function. The Lex A protein acts as a repressor for a number of genes, known as SOS genes including the recA gene. Under normal conditions i.e. when the SOS response is not necessary, these genes remain repressed by the Lex A repressor.

The initial event in the SOS response is the activation of RecA protease activity induced by DNA damage. The activation of Rec A protease activity occurs within a few minutes of DNA damage. The protease activity catalyses cleavage of the Lex A repressor making it inactive. As a result, the SOS genes can now be expressed to produce the enzymes required for DNA repair

The events are shown in Fig. 9.76:

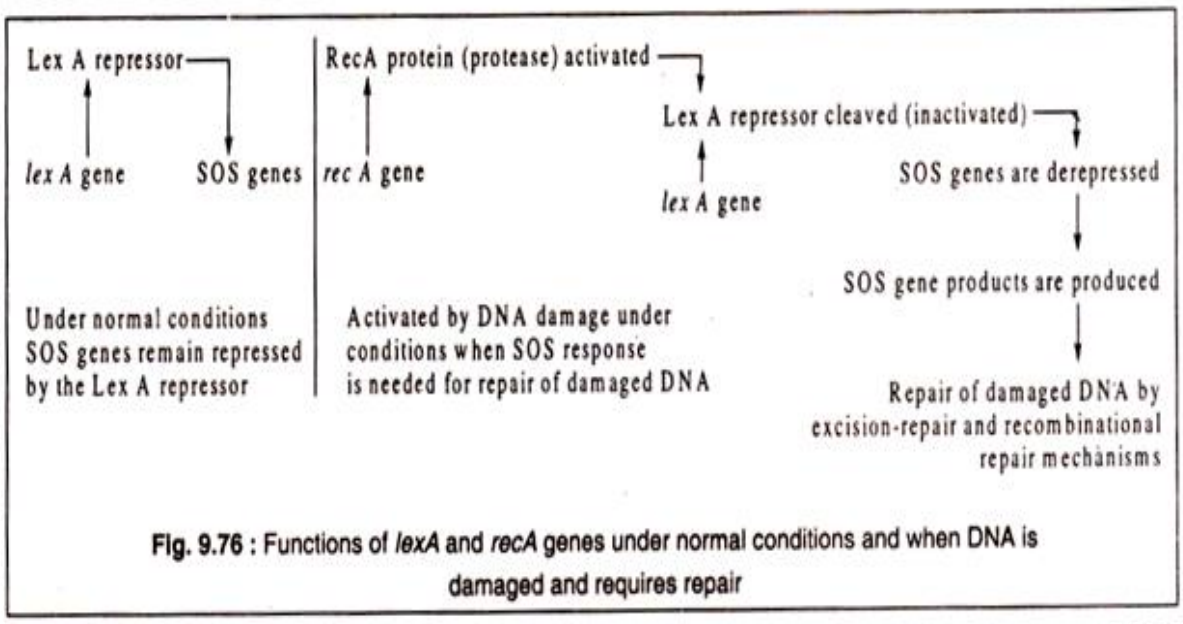


Fig. 9.76 : Functions of *lexA* and *recA* genes under normal conditions and when DNA is damaged and requires repair

The SOS response, as the name suggests, is an emergency measure to repair mutational damage. It makes it possible for the cell to survive under conditions which would have been otherwise lethal. However, the possibility of generating new mutations increases in the repair of DNA molecules. This is because the SOS repair system allows DNA synthesis bypassing the damaged site.

When the DNA polymerase III reaches a damaged site to which Rec A binds, the protein (Rec A) interacts with the epsilon subunit of the DNA polymerase molecule. This subunit is responsible for insertion of the correct base into the growing DNA strand. As a result, chain elongation continues bypassing the damaged site, but the chance of incorporation of a wrong base increases. SOS repair, therefore, enhances the chance of mutation due to mis-pairing of bases. This is known as error-prone bypass repair.

A more recent model based on SOS repair of UV-irradiated DNA in bacteriophages has been proposed. UV-irradiation is known to produce dimers of not only thymine, but also of thymine and cytosine and cytosine. During replication when T-C and C-C are reached, the SOS repair system is halted temporarily and cystosine is deaminated to uracil.
Uracil pairs with adenine, bringing about a transition mutation by changing C-G base pair to T-A. This is an error-free bypass repair thought it still causes a mutation. It is called error-free because the template DNA strand is faithfully copied in the newly synthesized strand. The change from C to U occurs in the template strand itself.

F. Methylation-directed very short patch repair:

Very short patch (VSP) repair is accomplished by involving methylation of bases especially cytosine and adenine. In E. coli methylation of adenine and in a sequence of -GATC- is done by the enzyme methylase (a product of dam gene) on both strands of DNA. After replication only A of -GATC- of one strand remains methylated, while the other remains un-methylated until methylase accomplishes methylation (Fig. 9.20 A-B).

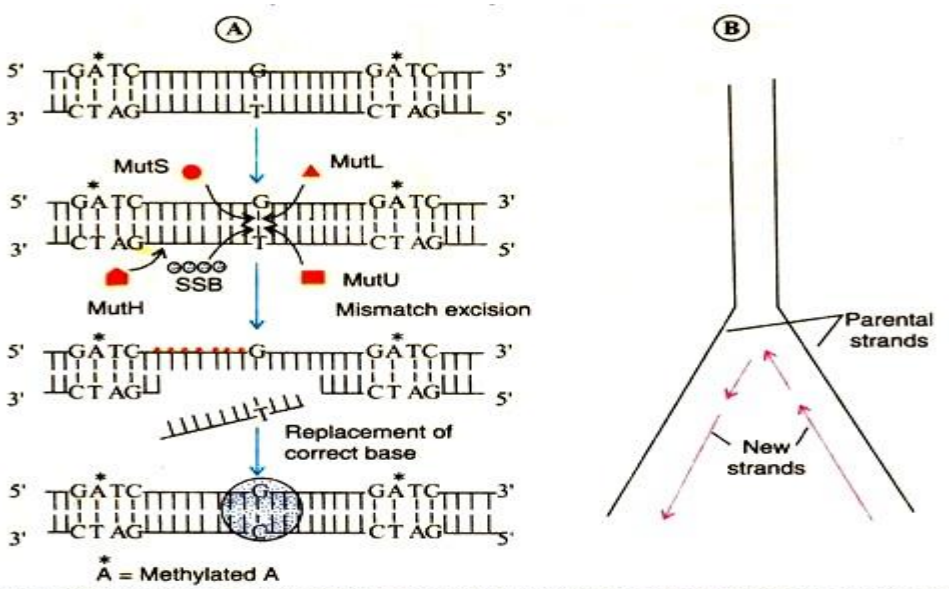


Fig. 9.20 : Mismatch repair. (A) excision of a newly synthesised strand and repair system; (B) arrows shows the region where methylation is not complete and dark region line shows the region where methylation is complete.

In E. coli repairing activity required four proteins viz., Mutl, MutS, MutU (UvrS) and MutH by the genes mutL, mutS, mutU, and mutH, respectively. The mut genes are the loci which increase the frequency of spontaneous mutation. The un-methylated -GATC allows the MutL to recognise the mismatch during transition period.

This helps MutS to bind to mismatch. MutU supports in unwinding the single strand and single strand DNA binding (SSB) proteins and maintain the structural topography of single strand. MutH cleaves the newly synthesised DNA strand and the protein MutU separates the mismatch strand (A).However, there is a gradient of methylation along the newly synthesised strand. Least methylation occurs at the replication fork. The parental strand is uniformly methylated.

The methylated bases direct the excision mechanisms to the newly synthesised strand containing the incorrect nucleotides (B). During this transition period, the repair system works and distinguishes the old and new strands and repairs only the new strands.

G. Mismatch Repair:

The rate of mutation varies usually in the range of 10^{-7} to 10^{-11} in bacteria. However, during normal DNA replication an error in inserting a correct base in the new strand occurs at a much higher rate, generally at a frequency the of 10^{-5}. This large difference indicates that bacteria possess an in-built mechanism to rectify most of errors during replication.
Most bacterial DNA polymerases, in addition to having the polymerase activity, have also an exonuclease activity which works in the opposite direction i.e. while polymerization proceeds in the 5′ —> 3′ direction, the exonuclease works in the 3′ —> 5′ direction. Whenever a wrong base is inserted into the polynucleotide chain by mistake, the DNA polymerase stops and goes one step backward and the incorrect base is removed by the exonuclease activity. The polymerase then resumes its normal activity by inserting a correct base. This is known as the proof-reading function of the DNA polymerase. Mutants with an altered epsilon subunit of the DNA polymerase fails to perform the proof-reading function.

Although proof-reading by DNA-polymerase is an efficient way of removing many mismatched bases, a number of such errors may still persist after replication. Such mismatched base-pairs require removal. These are corrected by another repair mechanism, known as mismatch repair.In this repair mechanism, three gene-products (proteins) are involved — Mut S, Mut L and Mut H. The first step of this repair process consists of binding of the Mut S protein to the mismatched base- pair. The second step involves the recognition of a specific sequence of the template which is -GATC- m E. coli in which A (adenine) is methylated in N-6 position.

The proteins Mut L and Mut H which bind to the unmethylated -GATC- sequence of the new strand form a complex with Mut S which is bound to the mismatch pair. Thereby the mismatch pair is brought close to the -GATC- sequences. The Mut H protein then nicks the unmethylated DNA strand at the GATC site and the mismatch is removed by an exonuclease.The resulting gap is then filled by DNA polymerase III and DNA ligase (Fig. 9.77):

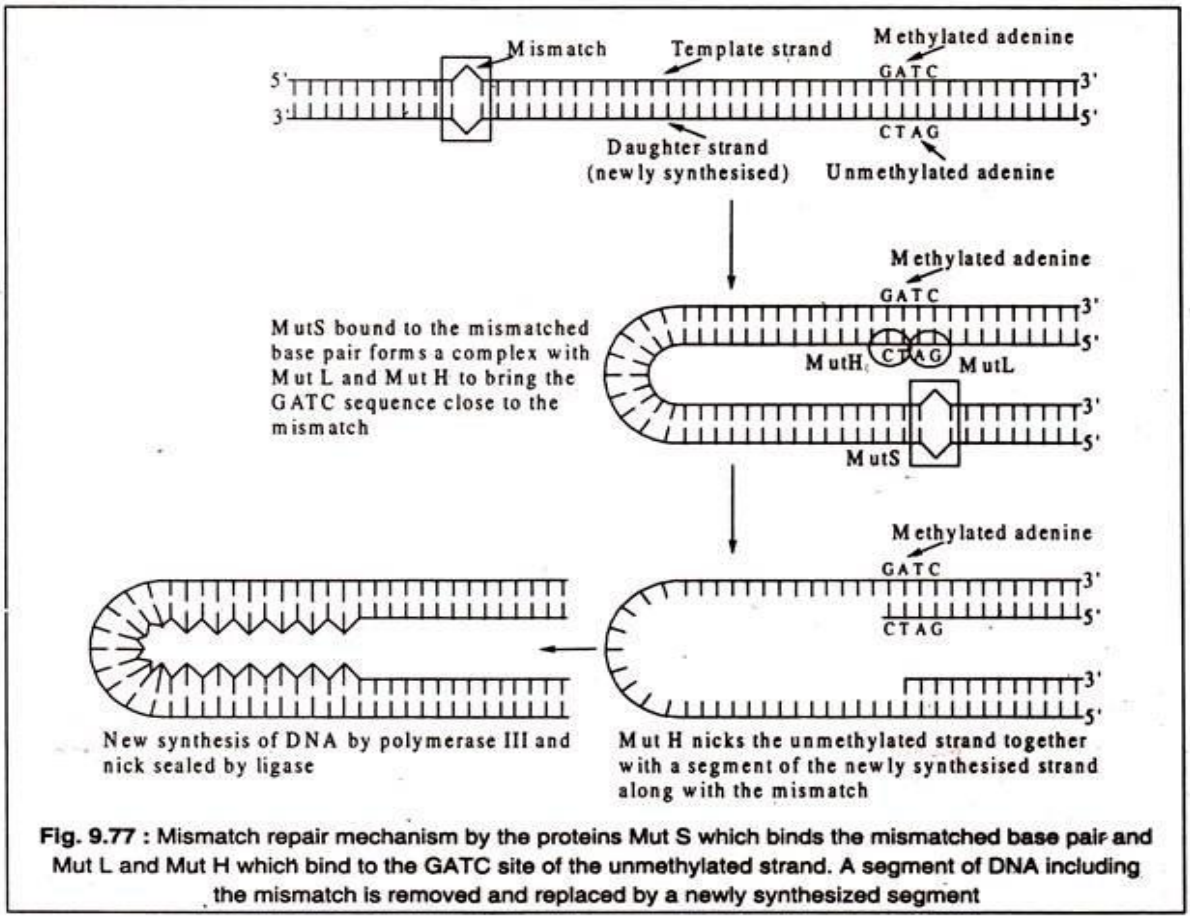


Fig. 9.77 : Mismatch repair mechanism by the proteins Mut S which binds the mismatched base pair and Mut L and Mut H which bind to the GATC site of the unmethylated strand. A segment of DNA including the mismatch is removed and replaced by a newly synthesized segment

Probable Questions:

1. State different types of DNA damage.
2. Describe photoactivation system of DNA repair with suitable diagram.
3. Describe mismatch system of DNA repair with suitable diagram.
4. Describe base excision repair system of DNA repair with suitable diagram.
5. Describe SOS repair system of DNA repair with suitable diagram.
6. What s the difference between base excision repair and nucleotide excision repair system?
7. Describe Re-Combinational Repair Mechanism of DNA with suitable diagram.
8. Describe homologous recombination DNA repair system with suitable diagram.
9. Describe methylation-directed very short patch repair of DNA with suitable diagram.
10. What are the importance of DNA repair ?

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7th Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics. Verma and Agarwal.

UNIT-VII

Karyotype and sex chromosomes; sex determination; role of Y-chromosome; sex mosaics

Objective: In this unit we will discuss about Karyotype and sex chromosomes and sex determination. We will also discuss about role of Y-chromosome and also about sex mosaics

Introduction to Chromosomes:

A chromosome can be considered a stainable threadlike nuclear component having special organisation, individuality and function. Their presence was first demonstrated in the eukaryotic cell by E. Strasburger in 1875 and these were first termed as chromosomes by W. Waldeyer in 1888.

This term is actually taken from Greek chromasoma which means "coloured bodies" (chroma = colour; soma = body) due to their marked affinity for basic dyes as a consequence of which they axe stained. This property is known as chromaticity.Staining the cell with certain types of stain (e.g., Aceto-orcein, Acetocamine, Feulgen's stain) shows that chromosomes are not visible in the interphase nucleus or metabolically active nucleus due to their high water content, but can be easily seen during cell division characteristics whether mitosis or meiosis.

During cell division, the chromosome undergoes dehydration, spinalisation and condensation. So they become progressively thicker and smaller and, accordingly, the satiability of chromosome also increases. Hence the chromosome becomes readily observable under microscope. Staining of chromosomes is generally carried out to make them visible under light microscope. Chromosomes are capable of duplication and maintaining their morphologic and physiologic properties through successive cell divisions. It has also been demonstrated that the chromosome contains DNA, which in turn, carries the genes and thus plays a major role in heredity.

When reproduction of organism takes place, they are passed on to the next generation through the gametes. Besides, they play an important role in variation, mutation and evolution, and in their control of morphogenesis, multiplication and equilibrium of vital processes. The term chromosome is mainly used to describe the chromosome of eukaryotic cell. The naked DNA of prokaryotes and DNA or RNA of viruses are sometimes broadly called prokaryotic chromosome and viral chromosome, respectively, due to their similarity in fundamental properties with eukaryotic chromosomes. But the morphology and the organisation of eukaryotic chromosome is much more complex. The morphology of

chromosomes in all eukaryotes is essentially similar—except some variations in number and size.

Most of the chromosomes in an eukaryotic cell are called autosomes which control all somatic characteristic of an organism [These are symbolized by 'A']. But, in addition, there are some other chromosomes which control some specialised characteristics of an organism and are called allosomes. Sex chromosome (X and Y) for determination of sex, B-chromosomes, L-chromosomes, M-chromosomes, S-chromosomes and E- chromosomes are examples of allosomes. Autosomes are universally present in all eukaryotic-organisms, but allosomes may or may not be present in all organisms.

Chromosome Number:

The number of chromosomes varies from species to species but it remains constant for a particular species. The number of chromosomes serves as an aid in the determination of phylogenetic status, such as taxonomic position of plant and animal species.In higher organisms, each somatic cell contains one set of chromosomes inherited from the maternal (female) parent or organ and a comparable set of chromosomes (homologous chromosomes or homologues) from the paternal (male) parent or organ.

The number of chromosomes in this dual set is called the diploid (2n) number. The suffix 'ploid' refers to chromosome "sets". Homologous chromosomes are two copies of a chromosome (one comes from the female and the other from the male parent or organ)—which are ordinarily identical in size and shape, gene content and gene order.Sex cells or gametes—which contain half the number of chromosome set found in somatic cell—are referred to as haploid cells (n). A genome is a set of chromosomes corresponding to the haploid set of a species. The number of chromosomes in each somatic cell is the same for all members of a given species.

Chromosome number varies widely and may be very low or high in both plant and animals. In animal kingdom *Ascaris megalocephala* var univalens shows a single pair of chromosomes in the cells of the germ line.But, since in the diploid soma the two chromosomes split into numerous small chromosomes, the single haploid chromosome has to be considered an aggregate chromosome or compound chromosome. It, for reasons unknown, maintains its unity under the conditions imposed by the cells of the germ line.

Again, the next lowest diploid chromosome number (2n) recorded so far in animals is four in *Mesotoma*(flat worm) and *Ophryotrochapuerilis* (Polychaeta). The highest diploid chromosome number (2n) in animals is 254 in *Eupagurusschotensis* (a hermit crab). Belar (1926) has, in fact, recorded that Aulacantha, a radiolarian has as a diploid number (2n) approximately 1,600 chromosomes. The somatic chromosome number generally remains

constant among individuals of the same species. But in many species, somatic cells of the same individual may exhibit different (2n, 4n, 8n etc.) chromosome numbers.

In such species, cellular differentiation is often accompanied in some cells with a phenomenon of endomitosis. Endomitosis means the duplication of chromosomes without division of the nucleus, resulting in increased chromosome number within a cell. Chromosome strands separate but the cell does not divide. Endomitosis leads to the production of endopolyploid cells having 2n, 4n, 8n etc. chromosomes. In natural polyploid individuals, it becomes necessary to find out the ancestral chromosome number which is represented by x and is called as the basic number.For example in common wheat *Triticum aestivum* 2n = 42; n = 21 and x = 7 showing that common wheat is a hexaploid (2n = 6x). The whole collection of chromosomes in the nucleus of an organism is referred to as chromosome complement.

Chromosome Size:

The size of chromosome of a cell shows a remarkable variation depending upon the stage of cell division. Chromosomes are longest and thinnest during interphase. But on the onset of prophase there is a progressive decrease in size associated with an increase in thickness. Chromosome are smallest during anaphase. But the measurement of chromosome size are practically taken during mitotic metaphase when they are very thick, quite short and well-spread.

The size of mitotic metaphase chromosome of various plants and animals varies from 0.5μ to 32μ in length and 0.2 μ to 3.0 μ in diameter. The giant chromosome found in the cells of salivary gland of Diptera are permanently in pre-metaphase stage and are easily visible in the interphase nucleus. These chromosomes are 300 μ in length and 10 μ in diameter.The size of each chromosome is 32 μ long. Again, chromosome size of Trillium is hundred times bigger than its closely related genus Medeola, size differences may be seen in the different species of a genus. For instance, the chromosomes of Allium Porum are half the size of the chromosome of Allium sativum.

The size of chromosome may vary in the different tissues within a single organism. For example, in plant Medeola, the root tip chromosomes are 50% bigger than the shoot tip chromosomes. Among animals, grasshopper, crickets, mantids, newts and salamanders have big chromosomes. In animals, size variation of chromosome has also been reported in different varieties within a species.

For example, the chromosome size of *chironomous thumiithumii* (fly) differs from its closely related varieties. During embryogenesis of certain marine insects the size of chromosomes of the early blastula are smaller than those of the later stage of development. The size, shape and

number of the metaphase chromosomes constitute the karyotype which is distinctive for each species. When all chromosomes of a species are more or less equal in size, the karyotype is called symmetrical karyotype.

Asymmetrical karyotype refers to the chromosome of different size. In most organisms, all cells have the same karyotype. However, species that appear quite similar can have very different karyotypes—indicating that similar genetic potential can be organised on chromosomes in very different ways.

Variation in the size of the chromosome can be induced by some factors:

i. When the cell divides at low temperature, the size of chromosomes become short and more compact.

ii. When the pre-treatment of cells is done with colchicine, the chromosomes become slightly shorter in size.

iii. Repeated and rapid cell divisions tend to result in smaller chromosomes.

Morphology of Chromosome:

It has been observed that the morphology of chromosome changes with the stage of cell division. During the prophase of meiosis, homologous chromosomes pair with each other at zygotene, the cell then enters the stage of pachytene where chromosomes become shortened and coiled.

Pachytene stages are very useful for the study of chromosome morphology because they are longer than the chromosomes in mitotic metaphase, so that the structural details of chromosomes can be easily resolved. But the meiotic cell division as well as the pachytene stages are not readily available at any time for experimental purpose. On the other hand, mitotic metaphase is easily available by arresting the divisional cycle with some chemical agents. Further, mitotic metaphase is also suitable as convenient stage for studies on chromosome morphology and some of the features are more clear during mitotic metaphase.At metaphase, each chromosome is made of two symmetrical structures, the chromatids. They are also called sister chromatids. Each chromatid contains a single DNA molecule. The chromatids are held together closely by the centromere (Fig. 13.1) and become

separated at the start of anaphase when the sister chromatids move to opposite poles.

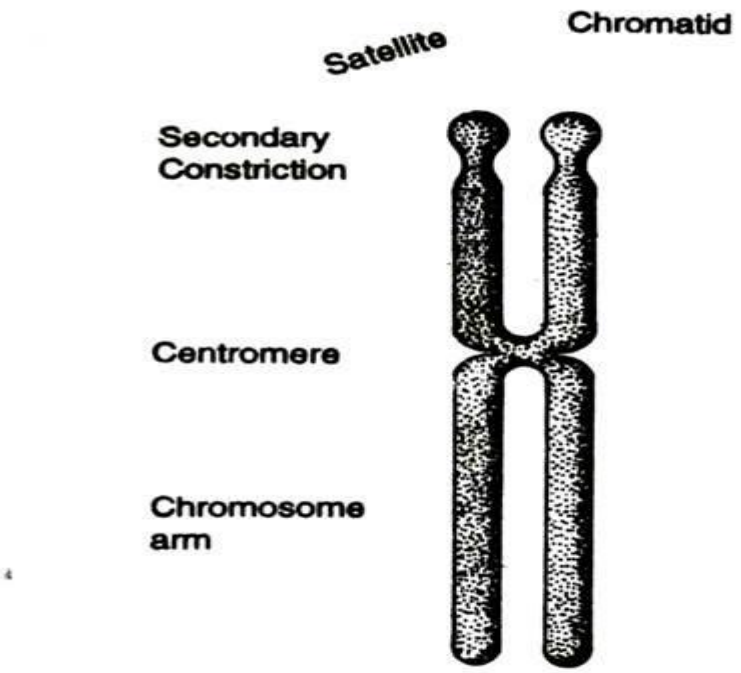


Fig. 13.1: Structure of a typical chromosome.

Therefore, until two sister chromatids share the common centromere, they are called chromatids. But as soon as they are separated at anaphase and possess their own individual centromere, they are called chromosome instead of chromatid. Hence, from anaphase to next G_1 phase, chromosomes have only one chromatid while from S phase to metaphase chromosomes have two (Fig. 13.2).

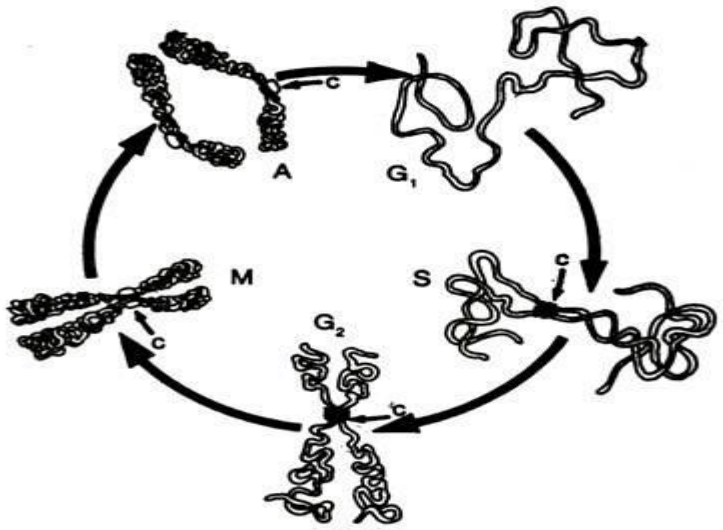


Fig. 13.2: The condensation and decondensation cycle of chromosomes.

The DNA present in each chromosome (made of a single chromatid) replicates during S phase to produce an identical copy of itself so that during G_2 prophase and metaphase each chromosome is composed of two chromatids. During prophase, and sometimes during interphase, the chromosomal material becomes visible as very thin threads which are called chromonemata and which represent chromatids in early stages of condensation. 'Chromatid' and 'chromonemata', therefore, are two names of the same structure. It is now accepted universally that chromatid is the structural and fundamental unit of chromosomes.

The region where two sister chromatids are held together is called the centromere. This region generally appears as a constricted or narrowed zone in the centromere, hence it is also known as primary constriction. Sometimes centromere appears as gap during metaphase because it does not take up any stain. Centromere has a clear zone in which the fibrils remained uncoiled or less coiled than those in the rest chromosomes. The reduced similarity of centromere" is understandable as the chromosome region in centrosome is less coiled or uncoiled and is composed of heterochromatin. At or near the centromere of each chromosome, the centromere is associated with a specialised structure called kinetochore. The structure of kinetochore is complex and is seen during late prophase. In ultra-thin sections of chromosomes, the kinetochore is seen as a stack of three-layered proteinaceous disc like plates (Fig. 13.3).

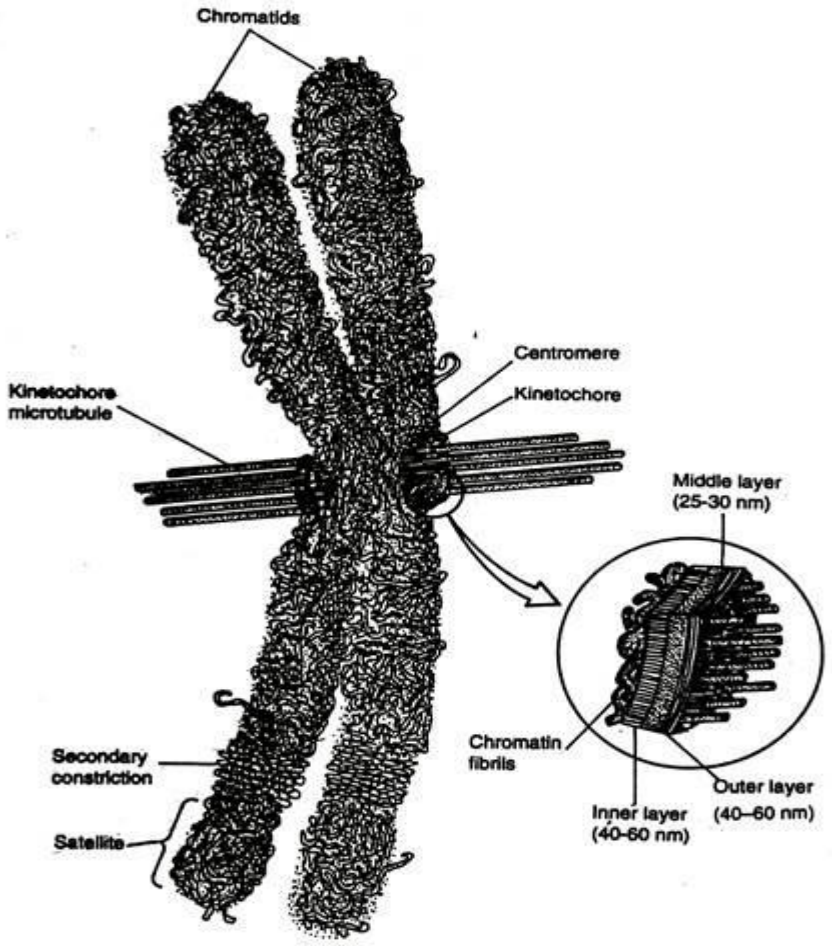


Fig. 13.3: Schematic diagram of metaphase chromosome showing the kinetochore.

The innermost disk (40-60 nm) probably consists of chromatin that is condensed differentially than the surrounding heterochromatic chromatin. The outer disk is a fibrous structure where kinetochore microtubules are attached by their ends. A 25 to 30 nm layer—the middle layer separates the inner and outer disk. A series of fine filaments which bridge the middle layer may help to hold the two disks together. The kinetochore microtubules—that extend toward the spindle pole of the cell play an active role in movement of chromosomes toward the poles during anaphase. The location of centromere and, hence, that of the kinetochore is directly controlled by a unique segment of chromosomal DNA termed centromeric DNA. Much of the information about the structure of auto mere has been obtained from genetic studies of the simple centromere in yeast.

Yeast centromere is very small and binds, a single microtubule. Sequence analysis of cloned centromeric DNAs (CEN DNAs) from yeast chromosomes shows that they are generally organ-

ised into three regions—centromeric DNA elements (CDEs) I, II and III (Fig. 13.4).Out of the three regions CDF II and CDF III appear to mediate interactions with microtubules through centromeric binding factors (CBF) 2 and 3, respectively, and through the kinesin-related protein Kar^{3}p. These proteins are an important link between kinetochore microtubules and the centromere CDE I is conserved in sequence but is not required.

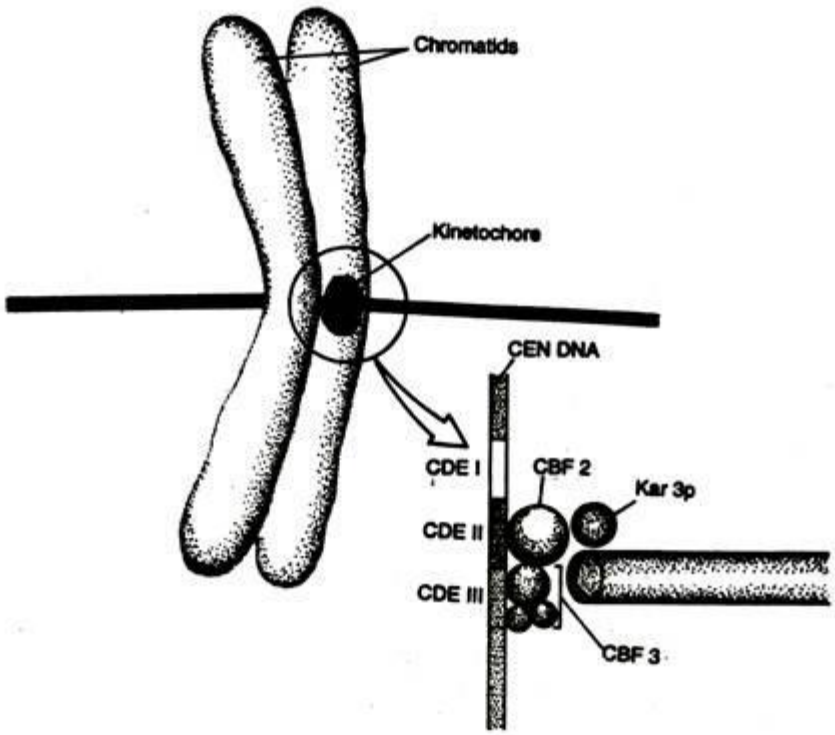


Fig. 13.4: The structure of a yeast centromere and kinetochore.

Each chromosome in a genome can be distinguished on the basis of the position of centromere which divides the chromosome into two arms of varying length. The portion of the chromosomes on either side of the centromere is called the arm of chromosomes—which may be equal or unequal. In case of unequal chromosome arms, one arm of a chromosome is longer than the other, hence they are termed long arm or q arm, and short arm or p arm, respectively.

Depending on the position of the centromere, chromosomes may be divided into four categories: metacentric, sub-metacentric, acrocentric and telocentric chromosome (Fig. 13.5).

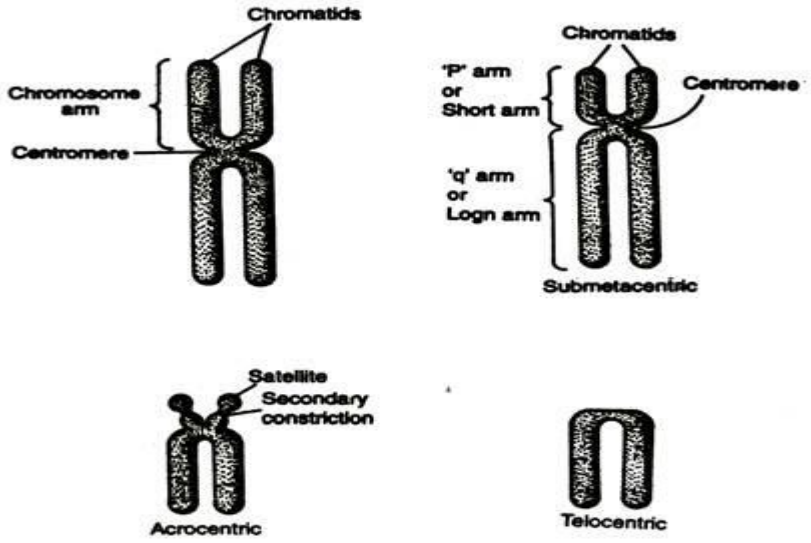


Fig. 13.5: Chromosomes are classified according to the position of the centromere.

In a metacentric chromosome it occurs at the centre, i.e., the centromere is median so that the two arms of such chromosomes are equal. The metacentric chromosomes look 'V'-shaped during anaphasic movement. In Trillium and Tradescantia, all the chromosomes are meta-centric. The sub-metacentric chromosomes look 'L'-shaped in anaphase and the centromere is located on one side of the centred point, i.e., the centromere is sub-median so that it divides the centromere into two unequal arms. In acrocentric or sub-telocentric or sub-terminad centromere, the centromere is situated almost near one end of the chromosome, i.e., centromere is sub-terminal in position and it gives two arms—one exceptionally short and the other long. Acrocentric chromosomes look 'J'-shaped in anaphase. Chromosomes of locust and some grasshoppers are acrocentric.

In some chromosomes, however, centromeres appear to be located at one end of the chromo-some, i.e., in the position normally occupied by one of the two telomeres. In this type, one arm is more or less equal to the length of the chromosome, and, other arm beyond the centromere is represented simply by dot. Acrocentric chromosome may appear 'rod- shaped' or T-shaped in anaphase. Telocentric chromosomes are of rare occurrence. Telocentric chromosome exist normally in certain species of holomastigote protozoa.

Telocentric chromosomes arising through a transverse fracturing of the centromere are believed to be unstable due to the centromere's irregular manner of division. This type of division is also known as misdivision of centromere—a process which leads to the formation of iso-chromosome (those in which the two arms are of equal length and are genetically homologous with each other). Misdivision of centromeres has been observed in Pea, Datura, Wheat and Fritillaria. Telocentric chromosomes have been experimentally produced in wheat,

maize etc. Usually, each chromosome has only one centromere but, in some species, each chromosome has more than one centromere. Again, in some cases the centromere is absent.

Hence, depending on the number of centromeres, chromosomes are classified as given:

(a) Acentric:

The chromosome is without any centromere. Acentric chromosome is very rare occurrence. It may arise due to unequal breaking of chromosome arm into two so that only one part has the centromere while, in the other part, centromere is absent.The part of chromosome having no centromere is called acentric fragment. Due to lack of centromere, acentric chromosomes are not able to attach with spindle fibres and they do not take part in cell division. Ultimately, the cell eliminates the acentric fragments.

(b) Mono-Centric:

Mono-centric chromosomes have only one centromere. It is a very common occurrence in most of the species.

(c) Dicentric:

A chromosome has two centromeres. Dicentric chromosome may be produced as a result of translocation, paracentric inversion etc. If the two centromeres tend to move to opposite poles during anaphase, the chromosome breaks. Rarely a new centromere may appear on the chromosome resulting in an abnormality. Such a centromere is called a neo-centromere. Di-centric chromosome is reported in the cells of wheat.

(d) Polycentric:

In addition to the shapely localised type of centromere described above, there exists a type of non-localised centromere where each chromosome has more than two centromeres. Such chromosomes are called polycentric.

Polycentric chromosomes are found in plant *Luzulapurpurea* (Fam Juncaceae), in the generative tissue of Ascaris *megalocephallaunivalens*, and in Thyanta. In both the above cases, the centromeric property is confined to one or more definite locus of the chromosome so that such centromere is Called localised centromere. However, in many insects—e.g., most homopteran and hemipteran insects—the centromere activity is non-localised and spread over the entire chromosome length. In such cases, the centromere is called a diffused centromere.

Polycentric chromosome often breaks into a number of smaller fragments. Each small segment functions independently. For instance, in case of *Ascaris megalocephalaunivalens*, the zygotic cell contains only two chromosomes. But during embryonic development these

chromosomes break in the somatic cells so that the cell may have up to 42 chromosomes. However, the cell that will give rise to the generative cell contains only two chromosomes.Non-staining gaps are seen in certain chromosomes in addition to the primary constriction regions. These regions are called secondary constrictions (Sc). Generally, secondary constrictions are located in the short arm of chromosomes near end but in many chromosomes they are located in the long arm and sometimes they may be present on both arms.

Secondary constrictions are constant in their position and extent. These constrictions are useful in identifying particular chromosomes in a set. The number of Sc in a genome varies from species to species. In some species, a somatic cell contains at least a pair of chromosomes with Sc while other chromosomes within the same cell are without Sc. In some other species, the number of chromosomes with Sc may be four (e.g., Viciahajastana), six, eight, ten (e.g., human somatic cell).

Secondary constrictions are distinguished from primary constriction:

i. Sc is without kinetochore.

ii. Sc is not able to attach with spindle fibre during anaphasic movement.

iii. Sc shows the absence of marked angular deviation of the chromosomal segments during anaphase.

Certain Sc contains the genes coding for 18S and 28S ribosomal RNA and that induce the formation of nucleoli. Since they are usually sites for the organisation of the nucleolus they are also called nucleolus organising regions or NOR.

The region of the chromosome separated from the rest of the chromosomes by NOR or Sc is a rounded body called satellite or trabant. If a fine thread is seen between satellite and the rest of chromosome, it is the satellite stalk and the chromosome is a SAT-chromosome.

The satellite and the constriction are constant in shape and size for each particular chromosome. In man, the nucleolar organizers are located in the secondary constrictions of chromosomes 13,14,15, 21 and 22—all of which are acrocentrics and have satellites.

The eukaryotic chromosome terminates at either end in a structure called the telomere. Telomeres have special properties when chromosomes are broken, the free ends without telomeres become “sticky” and tend to fuse with other broken chromosomes. However, the intact ends of unbroken or normal chromosomes are stable and show no tendency to fuse with other chromosomes or other ends.

The telomere differs in structure and composition from the rest of the chromosome. Telomere structure has been studied in a number of organisms. All telomeres so far studied have multiple (30-70) repeats of short species-specific sequences such as TTAGGG in humans, in *Tetrahymena thermophila*, TTTAGGG in plant Arabidopsis thaliana etc. Although these sequences are. somewhat variable in different species the basic repeat unit in all species has the pattern 5'Ti_{-4} $A_{0-1}G_{1-8}$3'Telomeres have either two strands of DNA covalently linked with a sub-teiminal nick or have a single- stranded 3′ end, i.e., the G-rich strand extends by 12-16 nucleotides beyond the C-rich strand. The protruding single-stranded DNA portion is also known as DNA primer. Telomere sequences are added by a special enzyme called a telomere terminal transferase or telomerases. Telomerase is a ribonucleoprotein. It contains a short RNA component, 159 bases long in Tetrahymena, 192 bases long in Euplotes. This RNA provides the template for synthesizing the G-rich repeating sequence to which it is complementary.

Centromeric Index

The position of centromere can be determined from microscopic studies by centromeric index. Centromeric index is the percentage of a quotient of the length of short arm of chromosome divided by the length of the chromosome. It is calculated from the following formula:

$$\text{Centromeric index or F\%} = \frac{\text{length of the short arm}}{\text{length of the chromosome}} \times 100.$$

The nature of primary constriction corresponding to the value of centromeric index is given in Table 13.1:

Table 13.1: Centromeric Index (F%)

Centromeric Index (F%)	Nature of constriction	Centromeric Index (F%)	Nature of constriction
50.00	median	18.74-12.51	nearly sub-terminal
49.99-37.51	nearly median	12.50	sub-terminal
37.50-25.01	nearly sub-median	12.49-6.25	nearly sub-terminal
25.00	sub-median	6.24-1.00	nearly terminal
24.99-18.75	nearly sub-median	1.00	terminal

Arm Ratio

Arm ratio is another useful numerical calculation for determining the position of centromere. It is the ratio of length of the long arm to the short arm of a chromosome. The position of centromere corresponding to the arm ratio is given in Table 13.2.

Table 13.2: Arm ratio value and centromere position

Arm ratio	Centromere Position
1.0	Median *sensu stricto*
1.7	Median
3.0	Submedian
7.0	Subterminal
0.0	Terminal

Three nucleotides (say … A AC …) of RNA template within the enzyme telomerase pairs with 3′ terminal three nucleotides (… TTG) of DNA primer, as shown in Fig. 13.6. RNA template directs and adds nucleotides to the 3′ end of DNA primer. It adds G and T bases one at a time to the primer as directed by the template and polymerisation continues to end of template region. The enzyme moves to new 3′ end of template before another round of addition takes place. The cycle starts again when one repeating unit has been added.

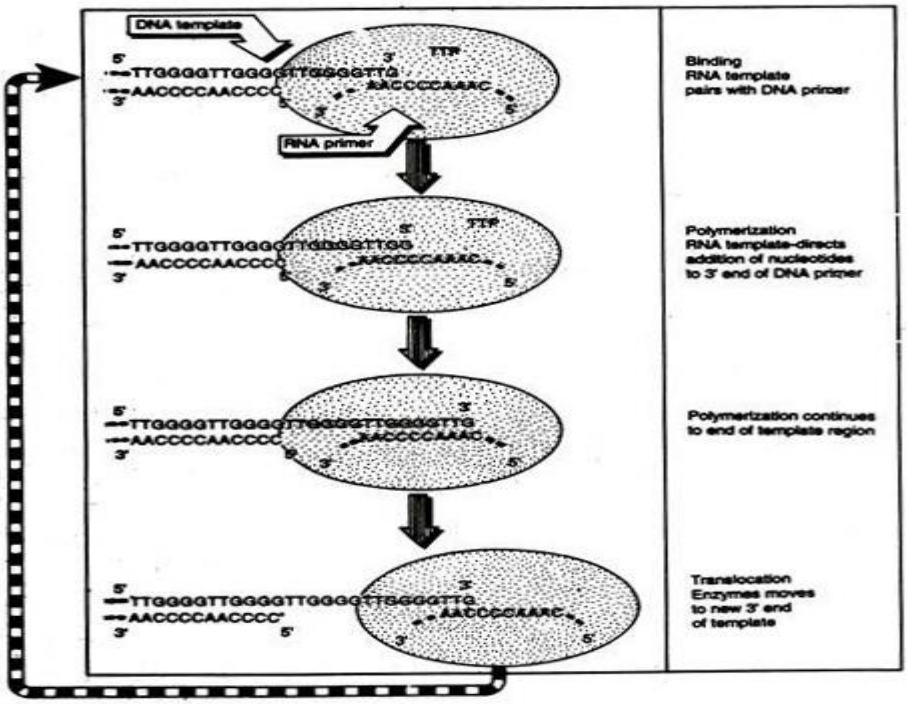


Fig. 13.6: Model for telomerase action in Tetrahymena.

The telomerase is a specialised example of a reverse transcriptase, an enzyme that synthesizes a DNA sequence using an RNA template. The added G-rich sequence can fold back on itself to form a novel hairpin or four stranded DNA loop. This involves G : G base pairing in which one or two of the G bases has the syn configuration (Fig. 13.7). Although such secondary structures may stabilise the ends of the chromosomes, they may also interfere with telomerase action.

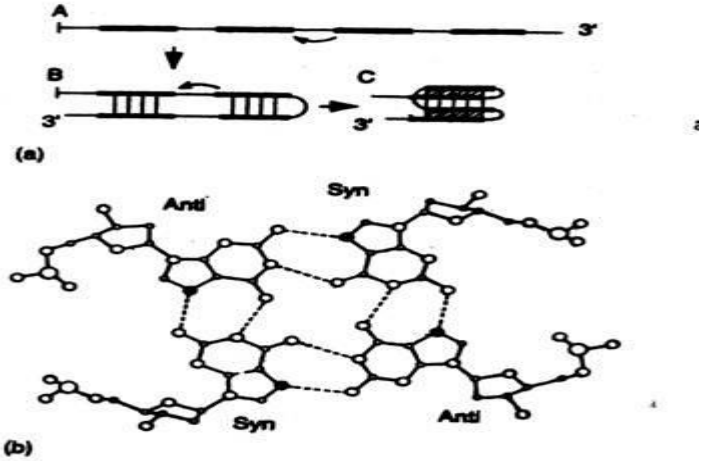


Fig. 13.7: (a) A model of how the G-rich strand could fold upon itself to form two- or four- stranded structure. (b) Shows a G tetrad involving in the Syn and anti configuration.

The unique structure of telomere of eukaryotic chromosomes performs three important functions:

i. It prevents exonucleases from degrading the ends of the linear DNA molecules.

ii. It facilitates replication of the ends of the DNA molecules without loss of termini.

The unusual behaviour of telomeric fractions are:

i. It consists of a basic repeat unit having the general pattern 5′ T_{1_4} A_{0_1} G_{1_8} 3′.
ii. In some species the telomeres terminate with a single-stranded G-rich region of the DNA strand with the 3′ end (a so-called 3′ overhang).

iii. The enzyme telomerase conditions the RNA component which provides the template for synthesizing the G-rich strand. This enzyme acts as a specialised Reverse transcriptase.

iv. The terminal DNA is folded and forms either a duplex hairpin by G-G pairing or a G quartet when one G is contributed by each of the 4 repeating units.

v. DNA polymerases are not able to replicate the terminal segment.

In the meiotic prophase and early mitotic prophase, the chromatin material is seen to have dense thickened areas at regular intervals giving the appearance of a string of beads. These bead-like areas are known as chromo- meres.

The distribution of chromomeres in a chromosome is highly characteristic and constant. Homologous chromosomes show an identical pattern. Chromomeres are specially obvious in polytene chromosomes where they become aligned laterally constituting the chromosome bands. Chromomeres of a single chromosome show a considerable variation in size. Once it was believed that genes were located within chromomeres and one chromosome may possibly represent a single gene.

But this idea has been a controversial one and available cytological evidence does not support this view. The chromomere can best be considered as a unit of functional coordination. Chromomere represents simply the tightly coiled or folded regions of DNA than in the neighbouring regions of chromosome called inter-chromomeric region. So they are visible in the phase of cell division. At metaphase, the chromosome is tightly coiled and chromomeres are no longer visible.

Chromosomes in Nucleoprotein:

(a) Chromatin:

Eukaryotic chromosomes in metaphase are generally known as chromosomes but in interphase the term chromatin is more generally used to describe the nucleoprotein fibres in the cell nucleus.

On the basis of stain-ability with basic dyes during various stages of cell cycle, chromatin is sub-divided into two main classes:(i) Euchromatin and(ii) Heterochromatin.

Interphase chromosome cannot be distinguished individually. The interphase nucleus is seen to contain scattered chromosome material and certain highly condensed bodies or chromocentre. The euchromatin region takes light stain in the interphase nucleus.It takes comparatively deep stain during cell division. On the other hand, heterochromatin takes deep stain during interphase and prophase while during metaphase it takes light stain (Fig. 13.8).

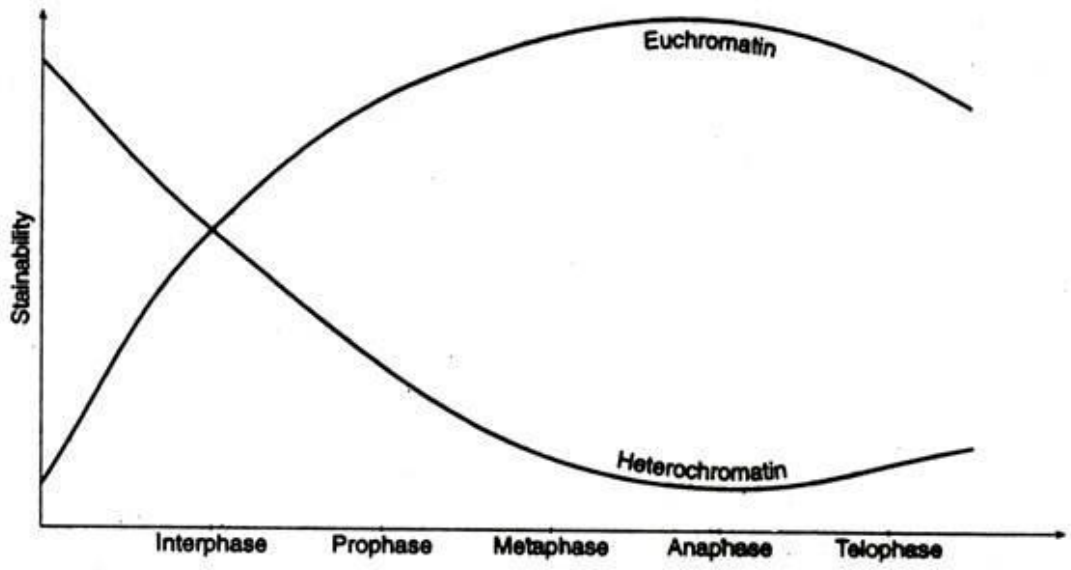


Fig. 13.8: Relationship between euchromatin and heterochromatin.

The changes of stain-ability can be correlated with the changes of condensation and decondensation property of the chromosome during various stages of cell division. It is obvious that condensed state takes deep stain while extended form takes light stain.

The distribution of heterochromatin of chromosomes has been studied extensively. Heterochromatin has been found to be located at some specific regions such as centromere, chro-

momere, nucleolar organising region, satellite etc. (Fig. 13.9). But besides these regions there are some other regions where heterochromatin may be present.

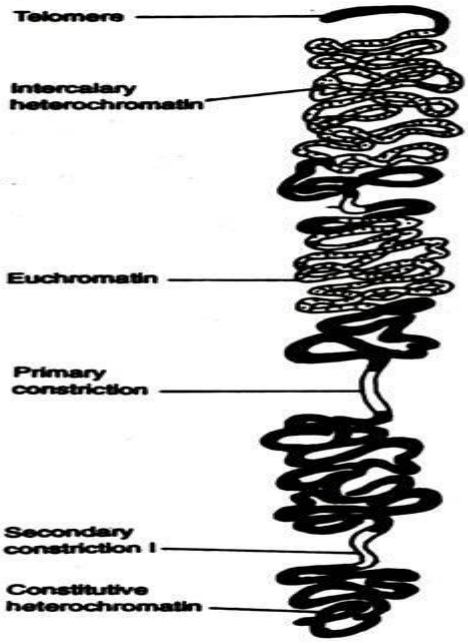


Fig. 13.9: Showing distribution of heterochromatin.

Heterochromatin may be classified into:(i) Constitutive, and(ii) Facultative.

Constitutive heterochromatin remains permanently in the heterochromatic state, i.e., it does not turn back to the euchromatic state. It tends to have a constant position on homologous chromosomes, e.g., centromere. In contrast, facultative heterochromatin is essentially euchromatin that has converted into heterochromatin (heterochromatinisation) whenever it needs and, again, it may be changed into euchromatin state (euchromatinisation).

Facultative heterochromatin is seen to develop during the development of an organism. Heterochromatinisation may involve a segment of a chromosome, a whole chromosomes (for example one X chromosome of human females) or one whole haploid set of chromosome (for example, full male set of chromosomes of mealy bug).

Within a chromosome, constitutive heterochromatin has been found to be located at certain regions:(i) Centromere,(ii) Telomere,(iii) Nucleolus organising regions and (iv) Intercalary segment of chromosome.

Entire chromosome may show heterochromatic behaviour, e.g., sex chromosomes in some plants, supernumerary chromosomes etc.

Heterochromatin shows different properties. All types of heterochromatin do not exhibit similar properties. Again, all such properties are not associated with a particular heterochromatin. Earlier workers considered that all heterochromatins were largely genetically inactive since addition or loss of heterochromatin did not have an appreciable phenotypic effect.It has been also suggested that highly condensed heterochromatin is generally not available for transcription and this condition leads to genetic inactivity. But these concepts are not of universal application. Heterochromatin may not be completely devoid of genes or genetic activity.

Muller demonstrated the presence of the "bobbed eye" gene on the Y-chromosome of Drosophila which was heterochromatic. Gates suggested the location of the gene for hairy earlobes in man on the presumed inert Y- chromosome. In tomato, a gene has been localised on heterochromatic segment. Again, the proportion of genes on heterochromatic and euchromatic regions in salivary gland chromosome was found to be same on a length to length basis.

When a heterochromatin segment is trans-located next to euchromatin segment, the activity of euchromatic genes is heterochromatinised. However, similar properties are seen when euchromatic part is shifted next to heterochromatin. This is called position effect.Temporary genetic inactivity has been observed in certain chromosome segment, which are facultatively heterochromatic. This property is very useful for explaining the process of differentiation. For example, in certain coccids (insects) a given chromosome becomes heterochromatic during embryonic development and, at the later stage, it turns back to the euchromatic state.

Heterochromatic regions are not able to synthesise RNA in vitro. It indicates that chromatin fibres in heterochromatic segments are more tightly packed than euchromatic regions.Experiment with the rate of incorporation of tritiated thymidine into different cells at different stages of development reveals that heterochromatic regions are seen to replicate their DNA in synthetic phase later and early than the euchromatic region. This phenomenon can be correlated with transition from euchromatin to heterochromatin.

In certain cases, heterochromatic region contains a high content of repetitive DNA. This explains the low phenotypic effect following the loss or gain of segment. Heterochromatic regions are easily broken by ionising agents and radiomimetic chemicals. Allocycly or heteropycnosis is another property of heterochromatin. Certain segments of the chromosome are more condensed than the rest of the chromosomes during various stages of the cell cycle. Such differences in thickening have been called heteropycnosis. It is exhibited principally by the heterochromatin on the two sides of the centromere. This property is not universal because many forms do not show complete allocycly.

Karyotype:

Karyotype represents the chromosome constitution of a cell or an individual. It deals with the length of chromosome, the position of centromere, presence of secondary constriction, and the size of satellite of the somatic chromosome complement. Generally, karyotype is prepared from well-scattered chromosomes of mitotic metaphase plate.

The information regarding chromosome constitutions are obtained from hand drawing of the microscopic view of chromosomes with the help of camera Lucida or drawing prism. Photomicrographs of metaphase plate are also used for the preparation of karyotype.Karyotypes are presented by arranging the chromosomes in a descending order of length in a straight line. The longest chromosome is always placed on the right side and the smallest one of the right. All chromosomes in a karyotype do not bear the centromere at the same position.

So, in a karyotype, chromosomes are grouped on the basis of the position of centromere and, in each group, descending order of length of chromosome is also maintained. Each chromosome of a karyotype is marked by a serial number from the extreme left to extreme right.

Broadly, karyotypes of different organisms may be classified into two categories:i. Symmetrical Karyotype and ii. Asymmetrical Karyotype.

A symmetrical karyotype has all metacentric chromosome of the same length.

In case of asymmetrical karyotype (Fig. 13.10) variation of length of chromosome complement is found and the position of centromere may or may not be identical.

In certain case, karyotype is asymmetrical but the length of chromosome is sharply two types—some chromosomes are very long and some are very short. This type of asymmetrical karyotype is known as bimodal karyotype.

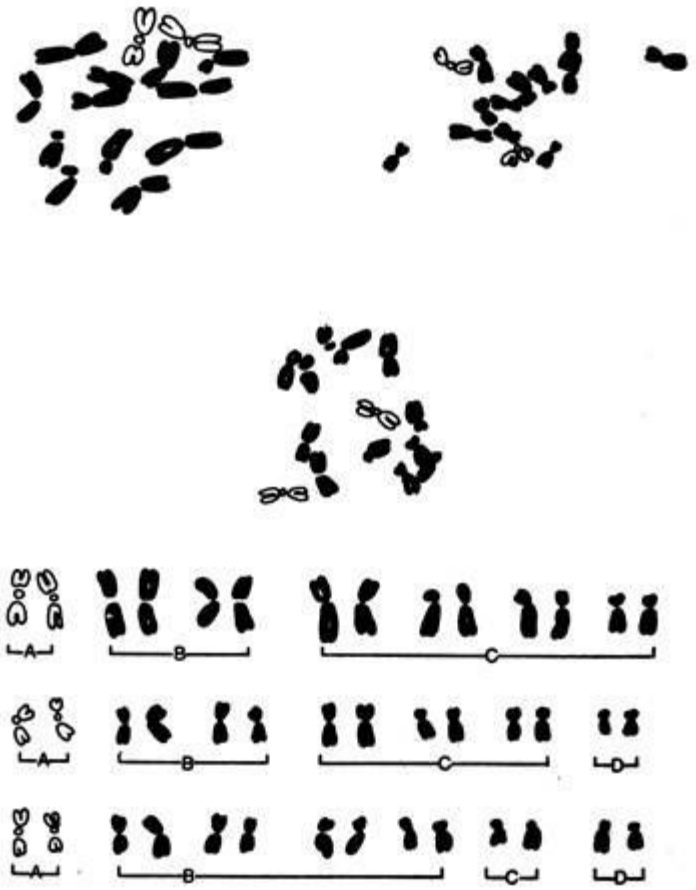


Fig. 13.10: Camera lucida drawing of chromosomes at metaphase and their Karyotype (asymmetric).

It is believed that symmetrical karyotype is the primitive form from which more advanced asymmetrical karyotype has been evolved. The karyotype of a species may be represented on graph or plain paper by bar diagram showing all morphological features of the chromosome. Such diagram is known as Idiotype or Idiogram (Fig. 13.11). Idiogram is prepared from haploid chromosome complement of an organism. An idiogram gives the identical information like that of karyotype.

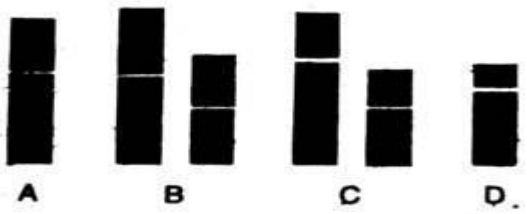


Fig. 13.11: Showing the idiogram of haploid set of chromosomes represented by bar.

Chromosomes Containing Single DNA Molecule:

All DNA viruses and bacterial cells contains a single chromosomal DNA molecules. The general belief is that all eukaryotic chromosomes also contain a single long linear DNA molecule. Extraction of longest DNA experimentally from lower and higher eukaryotes leads to hypothesis that each chromosome contains a single DNA molecule.

For example, physical analysis of the largest DNA molecules extracted from several genetically different species and stains of Drosophila exhibits that they are from 6×10^7 to 1×10^8 base pair long.These sizes match the DNA content of single stained metaphase chromosomes of Drosophila melanogaster, as measured by the amount of DNA specific stain absorbed. Hence, each chromosome possibly contains a single linear DNA.

The correspondence between the number of chromosomes and the number of DNA molecule per cell has been demonstrated in yeast cells. The length of yeast chromosomal DNA ranges from about 1.5×10^5 or 10^6 base pairs. The DNA of yeast chromosome can be separated and individually identified by pulse-field gel electrophoresis. The result of this technique shows that the number of separated DNA molecules equals to the number of chromosome in yeast.
The strongest evidence in the support of unineme (having a single DNA double helix per chromatid) concept is provided by studies on lamp brush chromosome. The loops of a lamp brush chromosome represent single chromatid in a fully extended state.In electron micrographs of RNase and protease treated lamp brush chromosomes, the loops have a diameter of 20A which is the diameter of a DNA double helix. Overwhelming evidence form a variety of studies supports the theory that each chromatid contains a single giant DNA molecule.

Types of Chromosomes:

In a vast majority of plants and animals, besides autosome, the cells of individual may contain one or more than one chromosome(s). These chromosomes are collectively referred to as allosomes. They differ from autosomes by their specialised functional role. Autosomes are

present in all cells of all organism but the existence of allosomes is not always universal. Allosomes are of different types. All types are not necessarily present in one organism.

(a) Sex Chromosomes:

Chromosomes that are connected with the determination of sex, are called sex chromosomes. There are two types of sex chromosomes; X and Y. X chromosome is found in both males and females although one sex has only one while the other sex have two X-chromosomes.

Y-chromosome occurs only in one of the two sexes of a species, e.g., male fruit fly, human, male mice, some male plants and female birds, reptiles. Y-chromosome contains mostly heterochromatin and only few genes are located in it. On the other hand, X-chromosome is made of euchromatin and many genes are located on it.

(b) B Chromosomes or Super Numerary Chromosome or Accessory Chromosome:

In some organisms, chromosomes in addition to normal autosomes are present as an extra chromosomes that are not genetically necessary for the individual and not homologous to any of the normal chromosomes. These chromosomes differ from the normal ones in their variable number, smaller size and greater degree of heterochromatinisation. They are found more commonly in plant than in animals (*Locustamigratoria, Camunla pellucida, Helix pomatia, Myrmeleo tettix maculatus*).

B-chromosome do not usually have any effect on the phenotype and, hence, they are not genetically desirable. In some plant their presence results some deleterious effect, i.e., loss of vigour. Though they are found to be deleterious, yet the occurrence of such chromosomes through generations indicates that they must have some positive adoptive role as well.B-chromosomes do not usually pair with normal chromosome during meiosis though they may pair with each other without the formation of chiasmata when present in even number.B-chromosomes may be eliminated from certain tissues or organs during embryogenesis.

The origin of B-chromosome is rather obscure. They may have been originated from the ordinary chromosome. It has been suggested that the centric heterochromatin part of an autosome is gradually converted into B-chromosome by the elimination of euchromatin part (Fig. 13.12).

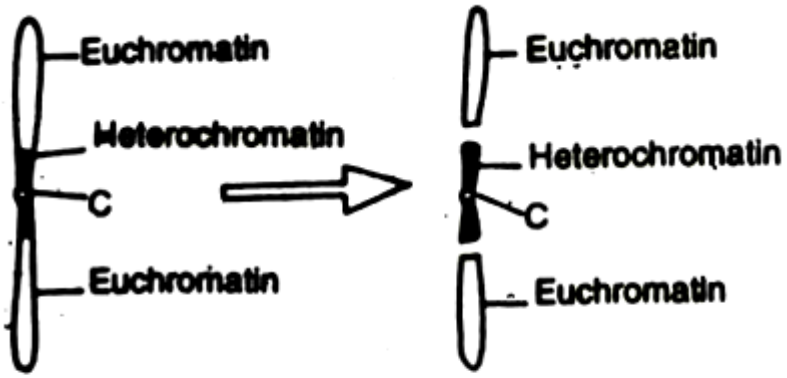


Fig. 13.12: Derivation of B-chromosome (C = centromere).

(c) Micro-Chromosome:

These chromosomes are also known as minute or m- chromosomes. They are so-called because of their extremely small, dot like size (about 0.5 μm). Micro-chromosomes are known both in plant (in many species of bryophyte) and animals [in insects of coreidae (Heteroptera), birds etc.].

They have been found mainly during meiosis and occasionally during mitosis. Micro-chromosomes are seen along with large chromosomes or bivalents. They contain DNA and undergo pairing into bivalents which are sometimes arranged in a rectangle like a quadripartite group. In a peat moss sphagnum there are 19 large bivalents and two m-chromosomes consisting of univalents and four m-chromosomes arranged in quadripartite fashion (Fig. 13.13).

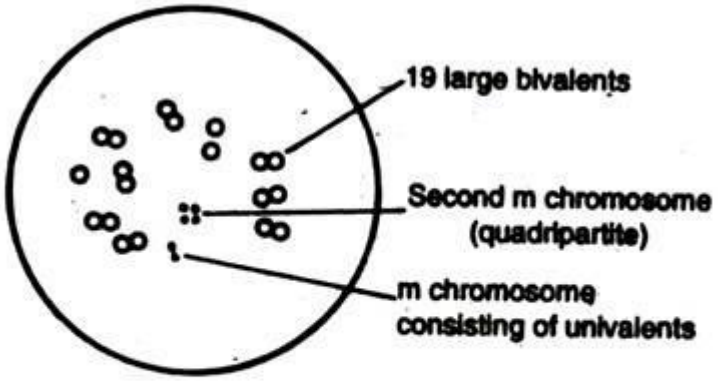


Fig. 13.13: Diagram of microchromosome (m chromosome).

In certain exceptions sat chromosomes are relatively rare and the property of organising nucleoli has been ascribed to specific micro-chromosomes as in bird.

(d) Mega-Chromosomes:

Mega-chromosomes are so called because they are non polytenic and many times longer than the length of normal chromosomes. They are not found in all cells and occur only in a small population of somatic cells. Generally, there is only one mega-chromosome per cell. Sometimes more than one mega-chromosomes have been reported.

Mega-chromosomes may be mono-centric, dicentric or acentric. They are found in the successive generations but they are not transmitted through the gametes. Hence mega-chromosomes are inheritable but the cells are able to produce them. Mega-chromosomes have been reported in a few species of Nicotiana hybrids.

(e) Limited Chromosomes:

Limited chromosomes are large in size and limited in distribution, i.e., they are found only in the germ cells. Limited chromosomes are also known as L-chromosome. They are found in insects of the family Sciaridae (Diptera).

During the embryonic developmental stage particularly the fifth and sixth cleavages limited chromosomes are eliminated from the somatic tissue but are retained in the germ line cells.In somatic cells of both male and female L-chromosomes are absent. Because L-chromosomes are present in all individuals of species in which they are found they are considered to be B-chromosomes.

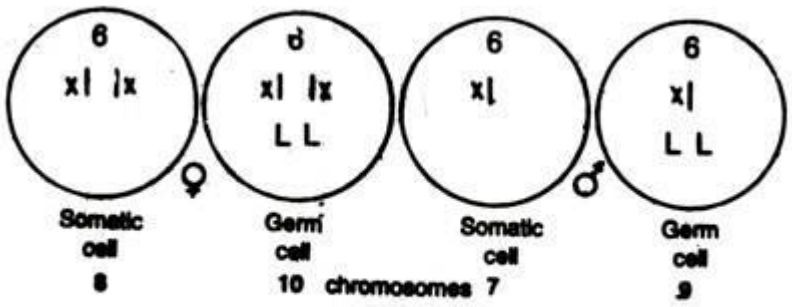


Fig. 13.14: Diagram of limited chromosome (L chromosome).

Fig. 13.14 shows the schematic representation of L-chromosome in the Sciaridae where the germ line cells of both male and female contain six autosome, sex chromosomes (two X-chromosomes in female and one X-chromosomes in male) and two L-chromosomes.

(f) Somatic Chromosome and Eliminated Chromosome:

Somatic chromosome or S-chromosome and eliminated chromosome or E-chromosome are so called because some chromosomes are retained in both somatic and germ line cells but other chromosomes are eliminated only in somatic cell during early cleavage stages of the embryo.

S and E chromosome have been found in gall insects (fam. Cecidomyiidae) and the insects belonging to the family chironomidiae (Fig. 13.15). In case of Maistor a gall insect both male and females have 48 chromosome in their germ cell and there is no loss of chromosomes. But in somatic cell, 36 chromosomes are lost in female and 42 chromosomes from male.Hence out of 48 chromosomes, 12 chromosome are present in somatic cell female and 6 chromosomes in male. Chromosomes which axe retained in both germ line cells as well somatic cells are referred to as S-chromosome. Those which are lost or eliminated from the somatic cells but Eire retained in germ cell are known as eliminated chromosome or E-chromosome.

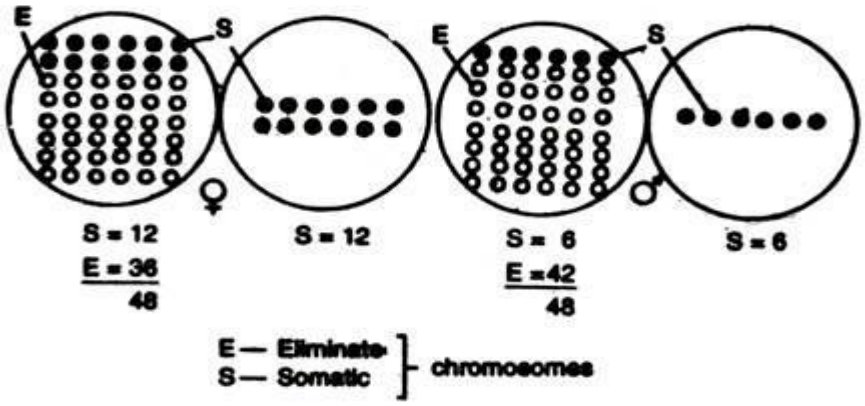


Fig. 13.15: Diagram of S and E chromosome.

(g) Special Type of Chromosomes/Giant Chromosomes:

In certain eukaryotic organisms there are special tissues where the chromosomes are of special structures not found in other cells of the same organism. These chromosomes attain their largest size in the nuclei of their respective cell. Hence these are also called giant chromosomes.

The giant chromosomes are found in the suspensors of the embryo of certain plant, cells of salivary glands of Drosophila and Chironomous, in the cells of Malpighian tubules, epithelium lining of gut of Drosophila, in the cells of fat bodies of larval stage of certain Diptera, oocyte nuclei of certain vertebrate, and invertebrate.

Special types of chromosome have been classified into two categories: (A) Polytene chromosome and(B) Lampbrush chromosome.

A. Polytene Chromosome:

Polytene chromosomes are those giant chromosomes in which DNA is replicated in such a way that the daughter chromatids do not separate. In more details, polytene is achieved by replication of the DNA many times without nuclear division (endomitosis) and the resulting daughter chromatids do not separate and remain aligned side by side to form a giant multi-stranded chromosome.

Polytene chromosome first provided the evidence that eukaryotic gene is regulated at the level of RNA synthesis. These chromosomes are the valuable material for the study of gene regulation because their gene transcription can be seen directly in the microscope. Polytene chromosome differs from polyploidy, in which there is also excess DNA per nucleus, but in which the new chromosomes are separated from each other.

(a) Polytene Chromosomes in Animal Cells:

Polytene chromosome in animal was first observed by E. G. Balbiani in 1881 in the salivary glands of chironomous (a dipteran fly) larva. That is why these chromosomes are also known salivary gland chromosome.

These chromosomes are easy to see in light microscope as large coiled bodies about 150- 200 times as large as gonad cell chromosome. Due to their enormous large size compared to that of normal chromosome they are also called giant chromosomes. In course of investigations of polytene chromosome in other animal cells it is observed that such chromosomes also exist frequently in the other tissues such as the living cells of the gut, Malpighian tubules muscles, fat cells in some other dipteran like flies, mosquitoes and midges.

The most prominent ones are located in the salivary gland larva of Drosophila melanogaster (fruit fly). These are easily and readily available for cytogenetical study. Hence the salivary gland chromosomes become an ideal material for the purpose of practical as well as research work.In Drosophila melanogaster these chromosomes—observed in the salivary glands to late larval (3rd instar) stages—are over 100 times the length of the somatic metaphase chromosomes which measure about 7.5 μ. According to Bridges (1938) the salivary gland chromosome of Drosophila measure up to 1180 μ or even up to 2,000 μ.

Those of a related genus *Rhyncosciara*are even larger and may reach even greater dimensions as a result of pathological disturbances. The salivary gland chromosomes show somatic pairing at interphase because of their multi-stranded giant nature. Hence the number of these chromosomes in the salivary gland cells always appear to be half of the normal somatic cells (2n = 8).Another characteristic feature of the polytene chromosome is that along the length of chromosome there is a series of dark bands alternating with other clear zones called inter-bands (Fig. 13.23).

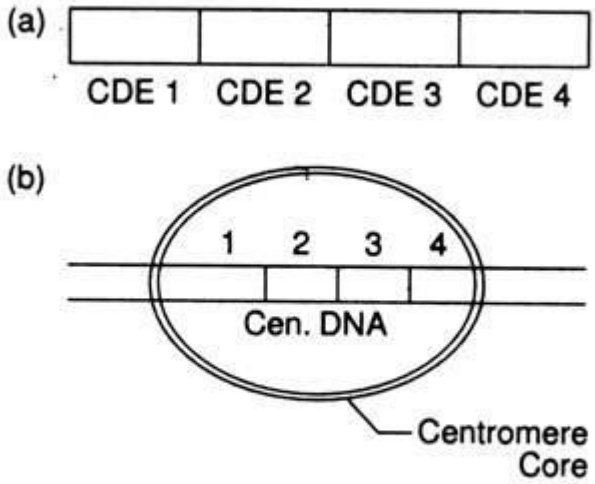


Fig. 13.23: (a) Diagram of Centromeric DNA in yeast; (b) Position of the Centromeric core particle.

The dark bands are heterochromatic in nature and stain intensely and are Feulgen positive. Furthermore they absorb ultraviolet light at 600A^0. There are about 5,000 bands in the Drosophila genome. About 85% of polytene chromosome is in bands and 15% is an inter-bands. Burke Judd et al have reported about 1,000 bands only on the X-chromosome. The bands of polytene chromosome are thought to represent a looped domain (loops of chromatin that extend at an angle from the main chromosome axis) that is highly folded as shown schematically in Fig. 13.24.

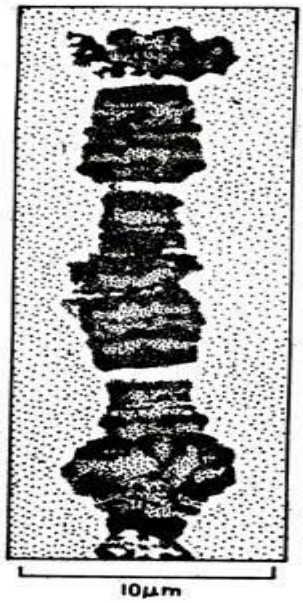


Fig. 13.24: Light micrograph of a portion of a polytene chromosome from Drosophila salivary glands showing the distinct patterns recognizable in different chromosome bands.

Depending on their size, individual bands are estimated to contain 3,000 to 300,000 nucleotide pairs per chromatin strand. Since the bands can be recognised by their different thickness and spacing's, each one has been given a number to generate a polytene chromosome "map". In polytene cell the chromosomes appear as five long strands and one short strand, attached to a central amorphous mass known as chromo Centre to which the single large nucleolus is attached (Fig. 13.25). The pericentromeric heterochromatin of all the Drosophila chromosomes coalesces in a chromo Centre. Of the six strands the short one represents chromosome 4 and the larger one represents the X-chromosome while the remaining four are the left and right arms of V shaped chromosome 2 and chromosome 3.

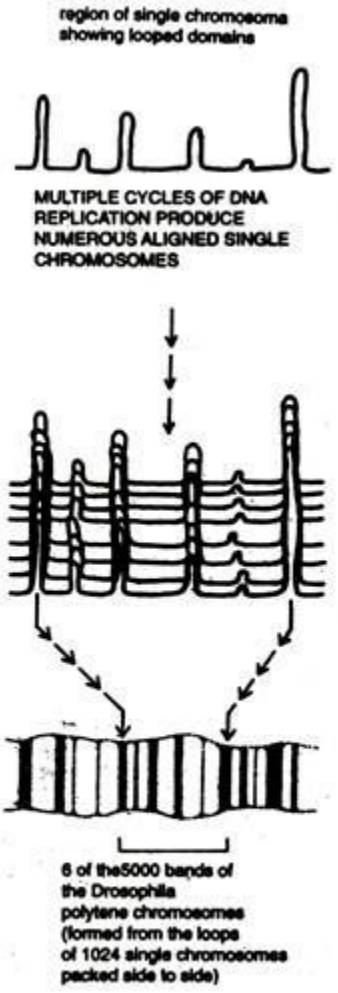


Fig. 13.25: Diagram showing how bands of polytene chromosome are thought to be generated by the side-to-side packing of homologous looped domains.

The 4th chromosome, being quite small, is almost completely inserted in chromo Centre and appears as a dot. In female flies, a pair of the X-chromosome appears as a single structure due to somatic pairing. But in male flies 'X' chromosome is single. The Y chromosome is fused

within the centromere. Hence Y chromosome is not seen as a separate strand.The DNA in each of the four Drosophila chromosomes has been replicated through 10 cycles without separation of the daughter chromosomes so that $2^{10} = 10^{24}$ identical strands of chromatin are lined up side-by-side (Fig. 13.26). Other Dipteran species have more DNA molecules per polytene chromosomes, for example, *Chironomus* has 16,000.

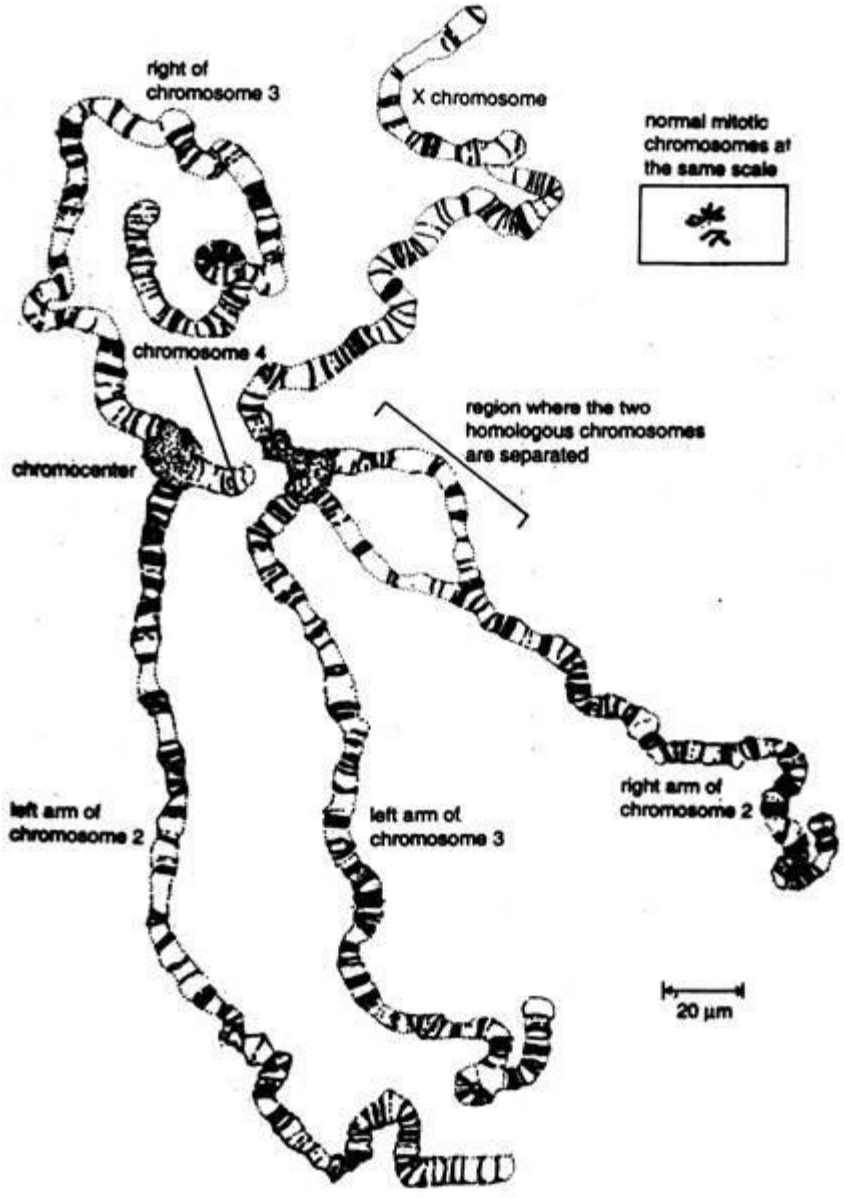


Fig. 13.26: A detailed sketch of the entire set of polytene chromosome of Drosophila.

One of the most important characteristics of polytene chromosome is that it is possible to see in them the genetic activity of particular chromosomal site at local enlargements. This is

known as puffs or chromosomal puffs or Balbiani rings which are associated with differential gene activation.

A puff is considered a band in which the DNA unfolds into open loops as a consequence of intense gene transcription, i.e., RNA synthesis. Puffing is a cyclic and reversible phenomenon at definite time and in different tissues of larvae. Puffs may appear, grow and disappear. Puff formation can be identified by labelling the cell briefly with the radioactive RNA precursor 3H uridine and locating the growing RNA transcripts by autoradiography. One of the main factors controlling the activity of genes in polytene chromosome is the insects' steroid hormone ecdysone. During larval development the level of ecdysone goes up and down—that induces the transcription of various gene coding for proteins that the larvae requires for each moult and for pupation. As the larvae progresses from one developmental stage to another new puff forms and old puff disappears as transcription units are activated and deactivated and different mRNA and proteins are made.

Electron microscopy of thin sections of a puff (Fig. 13.27) shows that the DNA is less condensed. This observations suggest that a looped domain can de-condense as a unit during transcription. It is also observed that puffing can be induced by heat shock when Drosophila larva, normally grown at 25°C, are transferred to a temperature at 37°C, a series of specific gene is activated while most other genes are deactivated.

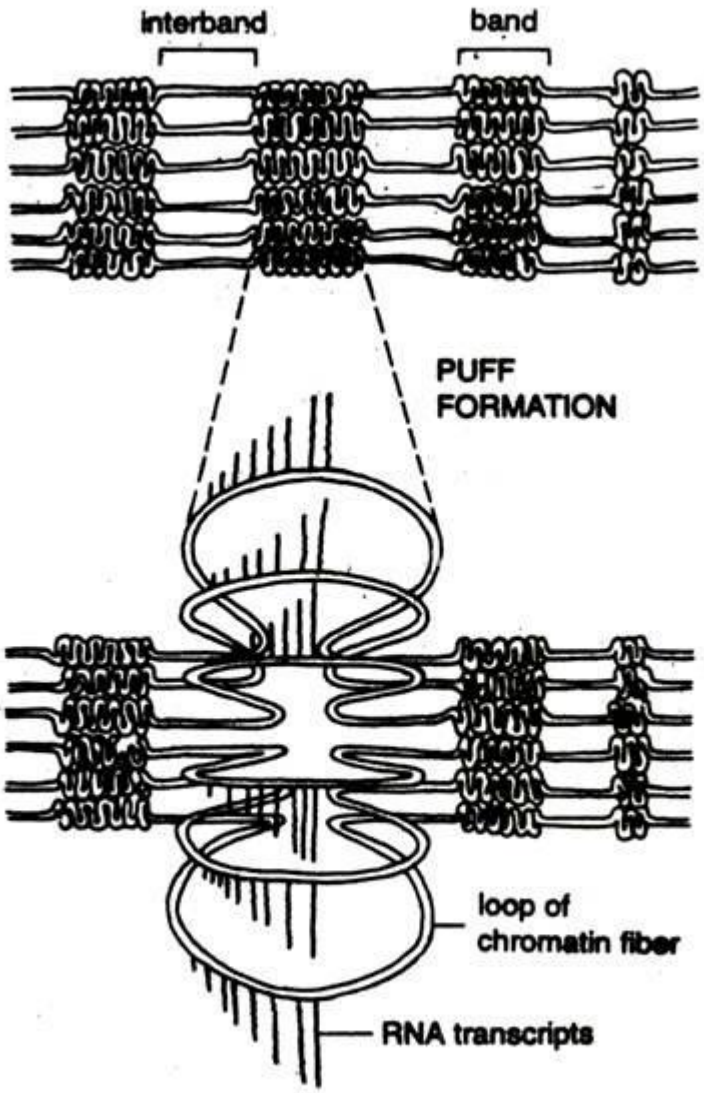


Fig. 13.27: Schematic diagram showing the process of puff formation in polytene chromosome.

B. Lampbrush Chromosomes in Animal Cell:

Lampbrush chromosome is another type of large diplotene chromosomes present in oocyte nuclei particularly conspicuous in urodele amphibians. These chromosomes have also been reported in the diplotene of oocytes of some fishes, sharks, molluscs, reptiles, birds and spermatocyte nuclei of Drosophila. Typical lampbrush chromosomes are made of a well-

defined chromosomal axis, chromomeres and numerous thin lateral loop extensions. The organisation of the lampbrush chromosome is shown schematically in Fig. 13.29.

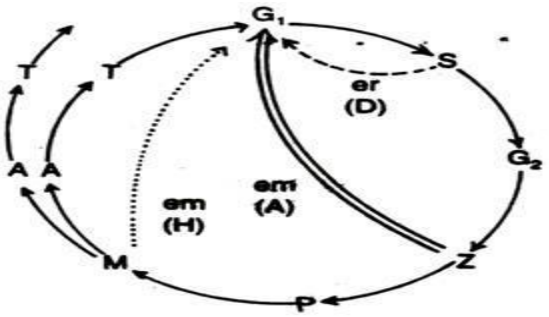


Fig. 13.29: Diagram of the mitotic cell cycle and its alteration (short-cut) in the course of endoreduplication (er) and endomitosis (em).

Lampbrush chromosomes were first observed by Fleming in 1882 and were described in detail by Ruckert in 1982. The name 'lamp- brush' was given because it is similar in appearance to the brushes used to clean the chimneys of oil lamp.

Lampbrush chromosomes have many fine lateral loops, giving them the characteristic 'hairy' appearance. Lampbrush chromosomes are found in meiotic prophase, they are present in the form of bivalents in which the homologous chromosomes axe held together by chiasmata. Each bivalent has four chromatids, two in each homologue.The axis of each homologue consists of a row of granules or chromomeres from each of which one to nine lateral loops may arise. The loops are always symmetrical, each chromosome having two of them—one for each chromatid. Lampbrush chromosome are up to 300μm long. There are about 10,000 per chromosome set. The size of loops varies from an average 9.5 μ to 200μ. An average sized loop can be estimated to contain roughly, 100,000 nucleotide pair of DNA.About 5 to 10% of the DNA is present in the lateral loop. The loops may vary in size, thickness and other morphological characteristics. Each loop has an axis formed by a single DNA molecule that is unfolded from the chromosome as a result of intense RNA synthesis.

Models of Chromosome Structure:

The chromosome of eukaryotic organism is basically made of two major components such as protein and nucleic acid like DNA. So chromosome is a nucleoprotein complex. But how the DNA protein complex builds up the chromosome structure is not clearly understood.So initially it was under speculation and several models have been proposed time to time to explain the association of proteins with DNA. After then various studies and experiments have

been done on chromosome structure to understand its biological architecture and again a new lots of model have been proposed. But all models are not universally accepted.

The various chromosome models may be grouped under three heads: a. Multi-stranded model;b. Single-stranded modelc. Nucleosome-Solenoid model.

(a) Multi-Stranded Model:

In the multiple strand model, the chromosome is supposed to be made of several nucleoprotein strands. Cytologists on the basis of their observation have proposed a number of multi-stranded models. According to some, each chromosome consists of two chromatids which is divisible into two half chromatids.

Each half chromatid is again composed of two quarter chromatids. Each quarter chromatid is composed of four chromatin fibres. Again each chromatin fibre is made of two strands. Each strand consists of a single DNA molecule plus the associated histone and non-histone proteins. DNA and proteins are held together by divalent cations like Ca^{++} and Mg^{++}.

Thus one chromatid is made of 4x2x2x2 = 32 DNA molecule. Hence a chromosome with two chromatids is composed of 32 x 2 = 64 DNA molecules. According to this model the chromosome consists of 64 double helices of DNA arranged in a parallel manner and twisted together like the strands of rope (Fig. 13.16).

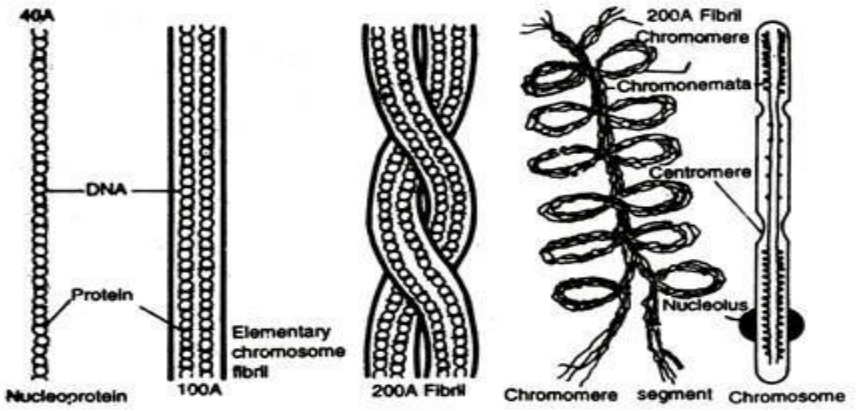


Fig. 13.16: Diagram of multistranded model.

According to Ris histone is associated with DNA in some regular but unspecific fashion to form a DNA-histone or nucleoprotein fibril. Two nucleoprotein fibrils make up the elementary

chromosome fibril. Two elementary chromosome fibrils wind spirally with each other to form a fibril.

Two fibrils constitute the chromonemata which forms several loops called chromomere. Each chromatid is made of four chromonemata. Hence each chromatid apparently has 16 elementary fibrils. Most of the evidence now indicate that chromosomes are not multi-stranded except giant polytene chromosome.

(b) Single-Stranded Model:

According to this model, chromosomes are single-stranded. Taylor (1957) proposed a single-stranded model according to which the chromosome is made of a long protein back bone from which DNA coils branch-off like the legs of a centipede.

Hence this model is known as Taylor's centipede model (Fig. 13.17). The protein backbone is composed of two parallel layers of proteins and these layers can be pulled apart during replication.

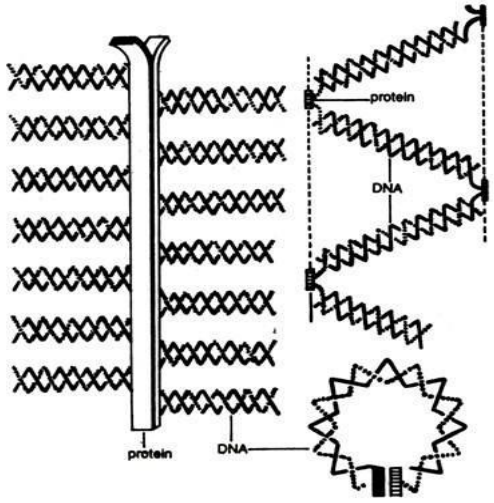


Fig. 13.17: Diagram of Taylor's centripede model.

It is thought that each layer has one strand of DNA helix on separation. On such a separated chromatid, a new chromatid could then be formed. The greatest demerit of this model is that it ignores the fact that genes are arranged in a linear fashion along the entire length of the chromosome. It is also inconsistent with genetic recombination data.

A second model was proposed jointly by Taylor and Freese. According to this model there are two protein spines instead of one. The DNA chains stretch between them like a Zigzag stair. In effect the DNA molecules are kept in position by the protein linkers (Fig. 13.14).If the linkers become closely put together they would form the axis of chromosome and the DNA would be

in the form of lateral loops. The only merit of this model that it can satisfy the concept that the genes are arranged in linear fashion.Ris (1967) postulated a modified single- stranded model. According to this model DNA double helix binds with histone protein to form nucleoprotein fibrils. Folding of this fibrils takes place because of Ca^{++} bridge to form basic fibrils. The basic fibrils undergoes still further folding to form the chromosome (Fig. 13.18).

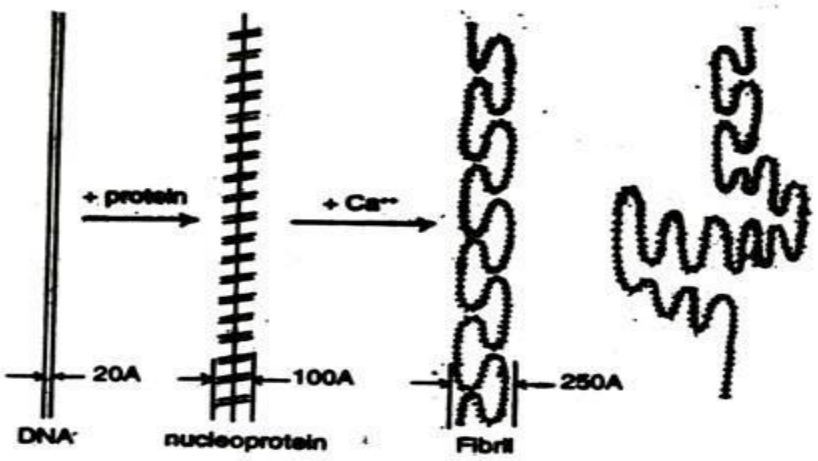


Fig. 13.18: Diagram of R is' modified model.

Du Praw, on the basis of his studies on human leucocytes under electron microscope, proposed a 'Folded-Fibre Model'—to describe the structure of chromosome. According to this model chromosomes are made of chromatin fibres. Each chromatin fibre contains only one DNA double helix which is spirally coiled and coated with histone and non-histone proteins.

The fibre then becomes folded back longitudinally and transversely and thus intertwined and forms the body of a chromatid. Two sister chromatids remain held at the centromere. The folded fibre model is applies to both the interphase and the metaphase chromosomes.During interphase folding is less and it is more at metaphase. This model is widely accepted and has been proved by various cytochemical, auto-radiographic and electron microscopic observation.

(c) The Nucleosome Model:

The nucleosome model is proposed by Roger Kornberg (1974). This model clearly explains the relationship of DNA and protein (Histone) as present within the chromosome. According to this model histones form core particle and DNA molecules coils around them so that the nucleoprotein fibre has beads on a string appearance.

(i) Histones:

There are five major types of histone molecules in the eukaryotic chromosome. These have been classified as histones H_1,H_2A,H_2B,H_3 and H_4. The histones Eire the basic proteins of low Mr (mobility rate on electrophoresis) and account for just about the same mass as the DNA.
Histones are readily isolated by salt or acid extraction of chromatin. Each histone molecule consists of a hydrophobic core region with one or two basic arms.

Histone Hi is a very lysine rich protein of about 215 amino acids. Histones H_2A and H_2B are highly conserved and are known as the slightly lysine-rich histones. The most conserved of all are the arginine rich histones H_3 and H_4.A special type of histone known as histone H_5 is found in the nucleated erythrocyte of fish, amphibians and birds. It bears many similarities to histone Hi and is thought to maintain the highly repressed state of the chromatin in these non-dividing cell. In non-dividing cells of mammals histones $H_1°$ and H_{1e} are present whereas histones H_{1a} and H_1b are present in large amounts only in dividing cells. Histones may be methylated, phosphorylated, acetylated or ADP-ribosylated and some of these modifications of histone may take place by altering the charge on the molecule which may affect the interactions of histones with each other or with DNA.
For example there are six subtypes of histone H_1 (H_{1a} – e and $H_1°$) giving rise to 14 different phosphorylated forms. Acetylation of histone H_4 in particular causes unfolding of the nucleosome core his tones and is associated with transcriptionally active segment of chromatin.
About 20% of H_2a histone is covalently linked with ubiquitin, a 76 residue polypeptide and forms a branched- chain protein known as UH2A which possibly control the gene expression.
In sperm cell histones are replaced by other small basic proteins known as protamine's.

(ii) Non-histone proteins:

Besides histones, some non-histone proteins are present in chromatin in an amount ap-proximately equal to the histone. About 100 types of different non-histone proteins have been isolated from the chromatin. Some of these are the enzymes involved in replication and transcription or to form part of the nuclear envelope.

Other non-histone proteins can be classified into two categories like low mobility group (LMG) and high mobility group (HMG) of protein on electrophoresis. Non-histone proteins are also basic protein like histone and they are present in multiple copies in the chromatin, i.e., they play a structural role. These proteins are not tightly associated with chromatin. The N-terminal and C-terminal part of non-histone proteins are separated by a short region which is rich in serine, glycine and proline. The most characterised are HMG_1, HMG_2, HMG_{14} and HMG_{17}.

(d) Experimental Evidence in Favour of Nucleosome Structure:

Several experimental studies have been made to prove the existence of nucleosome in chromatin structure.

The studies are:

(i) X-ray Diffraction Pattern Studies:

X-ray diffraction pattern of chromatin indicated the presence of a structure repeating every 10 nm.

(ii) Electron Microscopic Studies:

i. Electron microscopy of ruptured nuclei showed the presence of a series of spherical particles connected by a fine filament—the so called beads on a string. The beads have a diameter of 7-10 nm but the length of the filaments is variable.

Much work on the structure of sets nucleosome has been carried out with the virus SV40 (Simian Virus 40). DNA of Simian Virus 40 is a circular double- stranded molecule. When added to normal culture of cells, the DNA of SV40 may become integrated into the genome of the host. Normally viral DNA is devoid of nucleosomes. But under integrated condition viral DNA may form nucleosomal organisation.

Electron micrographs of SV40 infected cells also indicated the presence of nucleosome on viral DNA. The nucleosomal form of viral chromosome is known as minichromosome. Normally, the length of naked SV_{40} DNA is 1590 nm and that of the minichromosome is about 250 nm, it is clear that there has been six to seven fold packing of the DNA into the mini-chromosome.

(iii) Digestion of Chromatin with Micrococcal Nuclease:

It is already stated that the nucleosomes grossly appear as beads on string. It is obvious that beads are connected by non-beaded string or linker DNA which holds the nucleosomes. When a small fragments of DNA containing 4-5 nucleosomes are treated with micro-coccal nuclease, it gradually digests the linker DNA but nucleosome remains partially resistant to nuclease action.

An analysis of the size of the DNA showed that the spacing between successive nucleosomes was about 200 bp. On further digestion the size of the mono-nucleosome with about 200 bp DNA is reduced first to 166 bp and finally to 146 bp and $H_1$is lost.

(iv) Crosslinking Studies:

Cross linking studies using di-methyl-suberimidate have shown that in chromatin an octamer of histone composed of two molecules of H_2A, H_2B, H_3 and H_4 are present. Further studies have shown that one octamer is present per 200 bp DNA.

(v) Chromatin Digestion with Nuclease:

DNAse I (Nuclease) treatment makes nicks all along the length of DNA in chromatin. The nicks occur at ten base intervals. It means that the DNA is wrapped around a core of histones at a regular interval. Further studies involving the analysis of stoichemistry and X-ray crystallography have shown that one octamer is present per 200 bp DNA, i.e., per nucleosome. Each nucleosome is shallow, v-shaped structure around which a 146 bp core of DNA is wrapped making about one and three quarter turn.

(e) Nucleosome Structure:

All eukaryotic chromatin consists of nucleosomes. When interphase nuclei are ruptured by dipping them in a solution of low ionic strength, the chromatin fibres spill out of lysed nuclei. When isolated chromatin fibre is examined by electron microscope, it is seen that the chromatin fibre consists of a series of compactly organised ellipsoidal bead like particles.

The particles are joined by thin threads, a duplex of DNA. Actually a continuous duplex thread of DNA runs through the series of particles. The diameter of each particle is 110 A and the height is 60A. The beads or chromatin sub-unit is called nucleosome or Nu body. Individual nucleosome (Fig. 13.19) consists of a 146 nucleotide pair length of core DNA. Core DNA wraps round core histone by one and three quarter turns (1 3/4).

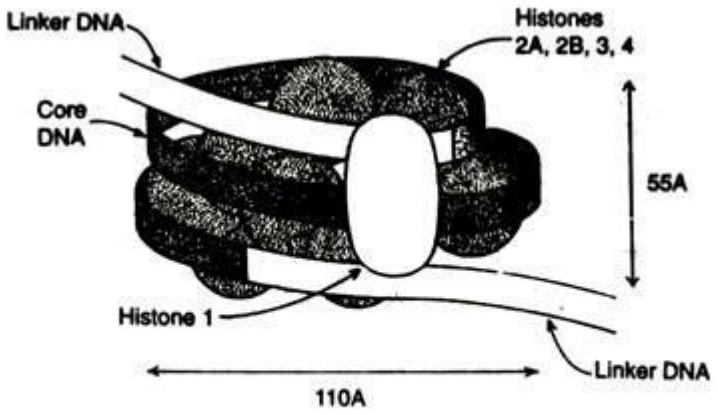


Fig. 13.19: Schematic diagram of a region of chromatin containing a nucleosome.

Each core histone is composed of octamer (Fig. 13.20) containing two copies of the four histones H_2A, H_2B, H_3 and H_4. Histone H_1 is present at one copy per nucleosome sealing the DNA entry/exit points to form a chromatosome of 166 bp and the remaining DNA forms the linker joining nucleosomes together to form oligonucleosomes. The length of the linker varies from species to species and even within tissue. Linkers as short as 8 nucleotide pairs and as long as 114 nucleotide pairs have been reported.

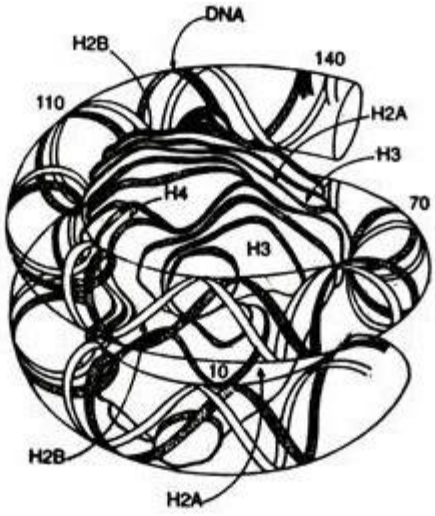


Fig. 13.20: Model of a nucleosome core showing DNA wound in a left-handed super-helix around the histone octamer.

A chromatin fibre is, therefore, made of a linear array of repeated nucleosome units plus a linker between every two nucleosomes (Fig. 13.21). Such a structural organisation constitutes a Poly-nucleosome. Under biological conditions, the nucleosome appear to be stable in position and to have little tendency to move along a length of DNA. The nucleosome play a

significant role in gene expression. Gene expression is related to the transcription which involves the unwinding of DNA and may require the fibre to unfold in restricted regions of chromatin that constitute a particular gene.

The linker DNA has no problem to unwind but the unwinding may be prevented where nucleosomes are present. It, therefore, seems inevitable that transcription of active gene must involve a structural change to unwind DNA.

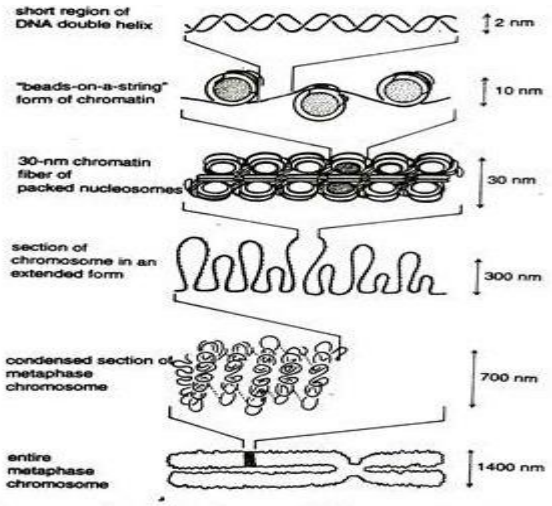


Fig. 13.21: Schematic representation showing the steps of DNA to metaphase chromosome organisation according to nucleosome model.

Again during transcription, the enzyme, RNA polymerase is essential to move along the length of template. On the basis of observation it is clear that an important structural change occurs when a gene is intensely transcribed. In case of the rRNA genes the nucleosomes are entirely displaced. Hence it seems that RNA polymerases displaces the nucleosome at the point of transcription but that the histone octamer immediately recaptures its position unless another RNA polymerase is present to prevent it from doing so. During replication the DNA is free of nucleosomes. Once DNA has been replicated, nucleosomes are quickly generated on both the duplicates.

The diameter of a double helix of DNA is 2 nm whereas the diameter of metaphase chromatid is much thicker. Hence it is obvious that DNA undergoes a higher order of supercoiling (Fig. 13.22). The diameter of a nucleosome is about 10 nm. Therefore, in the first state of condensation, the nucleosomes are packed into a spiral or solenoid arrangement with six nucleosome per turn.

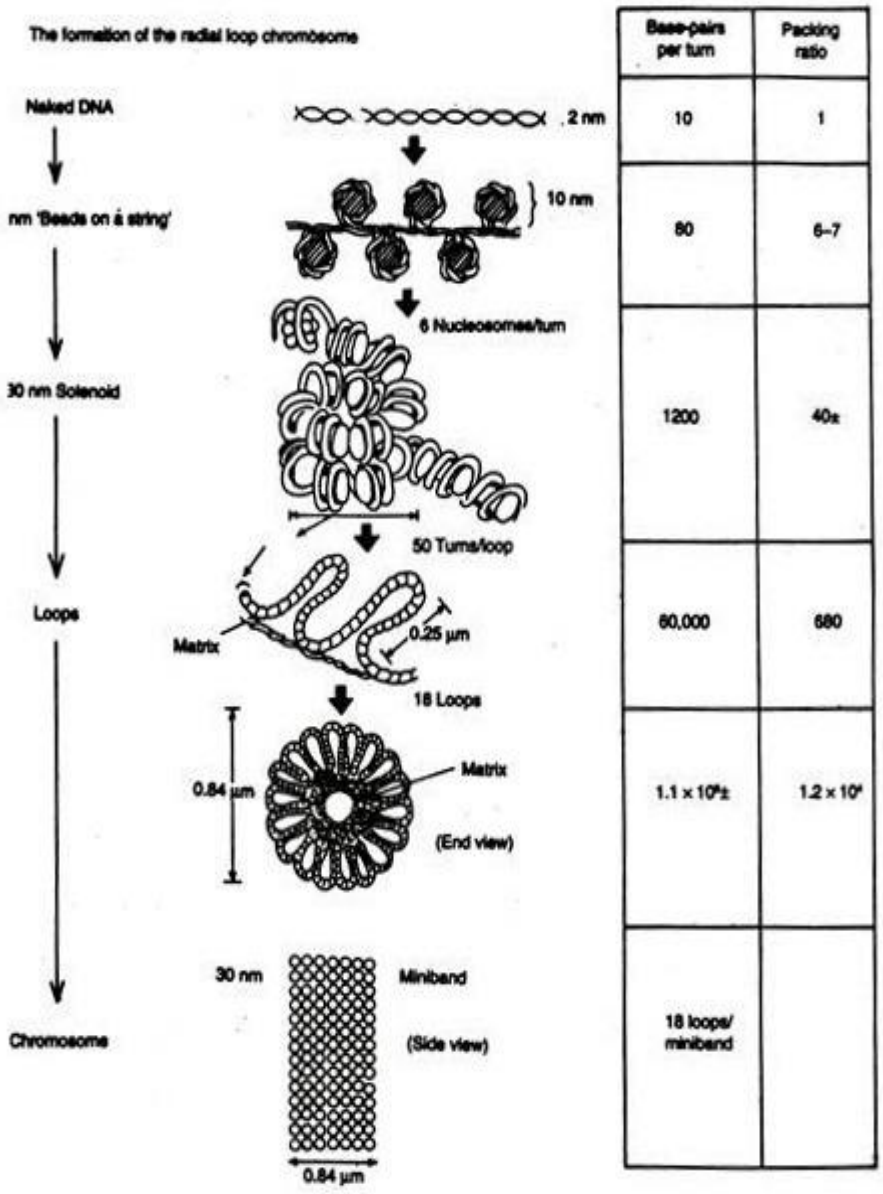


Fig. 13.22:. A schematic diagram showing the higher order organisation of a chromatid. (Courtesy of RLP Adams, J. T. Knowler and D. P. Leader)

The pitch of the solenoid is 11 nm and the faces of the nucleosome are approximately parallel to the solenoid axis. The fifth histone H_1, i.e., is bound to the DNA on the inside of the solenoid. The solenoid structure then forms a number of loops around a central core or scaffold or a matrix which is made of an ill-defined fibrous protein network. The scaffold proteins also include two abundant proteins of Mr (Mobility rate) 1,70,000 and 1,35,000. The larger is DNA topoisomerase II and the smaller binds MARs (Matrix attachment regions) in co-operative fashion.

Both initiation and continued replication of DNA occur in association with matrix proteins and topoisomerase II binding sites Eire found on matrix associated DNA. The binding sites for topoisomerase II are called Scaffold Associated Regions (SARs).

Chemical Structure of Chromosomes:

Chemical analysis of eukaryotic chromosomes has shown that they are composed of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) histone and non-histone proteins and certain metallic ions like Ca^{++}, Mg^{++}, etc. Primarily, chromosome contains about 90% DNA— basic protein forming a nucleoprotein complex and 10%. RNA non-histone protein, although it is variable according to the metabolic state of nucleus. Nucleoprotein complex constitutes the backbone of chromosome while RNA non-histone protein complex is sometimes regarded as residual chromosome. The ratio of DNA— basic, protein in chromatin—is nearly 1 : 1 and remains constant over a wide range of plants and animals. The histones axe joined with phosphate of DNA as salt linkage. The protamine's are bound to the DNA by ionic bonds. Besides this Mg^{++},Ca^{++} ions are supposed to maintain the chemical architecture of chromosome intact.

Biological Importance of Chromosome:

The chromosomes are considered the very important biological organisation because of the following reasons:

i. The genetic material DNA is localised in the chromosome and its contents are relatively constant from one generation to the next.

ii. The chromosomes retain their structure, individuality and continuity throughout the life-cycle of organism.

iii. The chromosomes maintain and replicate the genetic information contained in their DNA molecule and this information is transcribed at the right times in proper sequence into the specific types of RNA molecules which directs the synthesis of different types of proteins to form a body- form like the parents.

iv. The chromosomes form the only link between two generations and plays a significant role in the development of an organism from the zygote.

Sex Chromosomes:

Generally a genetic mechanism regulates the determination of sex in various organisms. There may be a single gene or gene complex that governs sex determination, e.g., in papaya, Asparagus and several fishes. One of the two sexes is homogametic in that it produces a single type of gametes, while the other sex is heterogametic and produces two types of gametes, while the other sex is heterogametic and produces two types of gametes. Thus the progeny in any generation consist of both the sexes in equal ratio.

In maize, male and female flowers (unisexual flowers) are produced on the same plant (monoecious condition). A simple system involving two pairs of genes (Ba ba and Ts ts) converts this monoecious plant into a dioecious one, i.e., the male and female flowers are produced on different plants.The dominant gene Ba produces normal female flowers in the cob, but its recessive allele ba in homozygous state interferes with the cob development and produces rudimentary female flowers. Thus baba plants are functionally male.

The dominant allele Ts of the other gene produces normal male flowers in the tassel, but its recessive allele ts (tassel seed) in homozygous condition causes seed setting in the tassel making the plant functionally female. When both the dominant genes Ba and Ts are present, the plant is monoecious. The double recessive plant (babatsts) is functionally female. Similarly, the plant of genotype baba Ts/Ts develops into male, while Ba Batsts plants develop into females. The plants of baba Ts ts genotype are heterogametic male. On crossing a babatsts (female) and baba Ts ts (male) plants, male and female plants are obtained in 1: 1 ratio in the progeny. Thus maize plant can be made dioecious by two genes ba and ts.

In many cases, there operates a chromosomal mechanism of sex determination. There are specific chromosomes which carry the genes responsible for sex determination. Such chromosomes are called allosomes or sex chromosomes, while the remaining chromosomes are called autosomes (symbolized by “A”). One of the sexes is homogametic while the other is heterogametic.The sex chromosome of the homogametic sex is designated as the X-chromosome. One sex chromosome of the heterogametic sex is the same as the X-chromosome, while the other (if present) is different from the X; it is designated as Y-chromosome.Several different chromosomal mechanisms of sex determination exist in the nature (Table 7.1). In certain plant species, such as, *Humulus lupulus* and *Rumexacetosa*, compound sex chromosomes are known to occur.

TABLE 7.1. Chromosomal mechanism of sex determination in different organisms

Mechanisms	Homogamatic sex	Gametes of homogametic sex	Heterogametic sex	Gametes of heterogametic sex	Example
Simple Sex Chromosomes					
XX–XO	Female (XX)	X	Male (OX)	X, O	Animals : *Protenor*, spiders, grasshopper, Orthoptera Plants : *Vallisneria spiralis*, *Dioscorea sinuata*
XX-XY	Female (XX)	X	Male (XY)	X, Y	Animals : Human, Diptera (*Drosophila*, house fly etc.), Hemiptera, Coleoptera, Some fishes and some amphibia Plants : *Melandrium (Silene)*, *Rumex*, *Humulus*, *Salix*, *Cannabis*, *Bryonia*
OX – XX (ZO – ZZ)	Male (XX)	X	Female (XO)	X, O	Animals : *Fumea* (a moth)
XY – XX (ZW – ZZ)	Male (XX)	X	Female (XY)	X, Y	Animals : Birds, reptiles, silkworm. Plants : *Frageria* elatior
Compound sex Chromosomes					
XX – XY_1Y_2	Female (XX)	X	Male (XY_1Y_2)	X,Y_1Y_2	Plants : *Rumex acetosa*, *Humulus japonicus*
$X_1X_1X_2X_2$ $X_1X_2Y_1Y_2$	Female ($X_1X_1X_2X_2$)	X_1X_2	Male ($X_1X_2Y_1Y_2$)	X_1X_2, Y_1Y_2	Plants : *Humulus lupulus* var. *cordiofolius*.

The X chromosome also carries genes that have no role in sex determination; they are called sex-linked genes. In some organisms, e.g., human, the Y- chromosome is smaller, while in others, such as, *Drosophila, Melandrium*, it is larger than the X-chromosome.Further, the Y chromosome is often more heterochromatic than the X. The Y chromosome carries genes for maleness in several organisms, e.g., human, *Melandrium* and *Cocciniaindica*. Genes located on the Y chromosome are inherited from father to son; such genes are called holandric genes.

In the system where female is heterogametic (XY) and male is homogametic (XX), the Y chromosome is inherited from mother to daughter, and the genes located on this chromosome are called hologynic genes. A few genes have been located on the human Y chromosome, e.g. histocompatibility gene (H-Y) and the testis determining factor (TDF).

Origin of sex chromosomes:

The accepted hypothesis of XY and ZW sex chromosome evolution is that they evolved at the same time, in two different branches. However, there is some evidence to suggest that there could have been transitions between ZW and XY, such as in *Xiphophorusmaculatus*, which have both ZW and XY systems in the same population, despite the fact that ZW and XY have

different gene locations. A recent theoretical model raises the possibility of both transitions between the XY/XX and ZZ/ZW system and environmental sex determination. The platypus' genes also back up the possible evolutionary link between XY and ZW, because they have the DMRT1 gene possessed by birds on their X chromosomes. Regardless, XY and ZW follow a similar route. All sex chromosomes started out as an original autosome of an original amniote that relied upon temperature to determine the sex of offspring. After the mammals separated, the branch further split into Lepidosauria and Archosauromorpha. These two groups both evolved the ZW system separately, as evidenced by the existence of different sex chromosomal locations. In mammals, one of the autosome pair, now Y, mutated its SOX3 gene into the SRY gene, causing that chromosome to designate sex. After this mutation, the SRY-containing chromosome inverted and was no longer completely homologous with its partner. The regions of the X and Y chromosomes that are still homologous to one another are known as the pseudoautosomal region. Once it inverted, the Y chromosome became unable to remedy deleterious mutations, and thus degenerated. There is some concern that the Y chromosome will shrink further and stop functioning in ten million years: but the Y chromosome has been strictly conserved after its initial rapid geneloss.

There are some species, such as the medaka fish, that evolved sex chromosomes separately; their Y chromosome never inverted and can still swap genes with the X. These species are still in an early phase of evolution with regard to their sex chromosomes. Because the Y does not have male-specific genes and can interact with the X, XY and YY females can be formed as well as XX males.

Meiotic behaviour:

A very small homologous segment is present at one end of the X and Y chromosomes in which pairing occurs; as a result, the X and Y chromosomes form a bivalent during meiosis and move to opposite poles during Al.

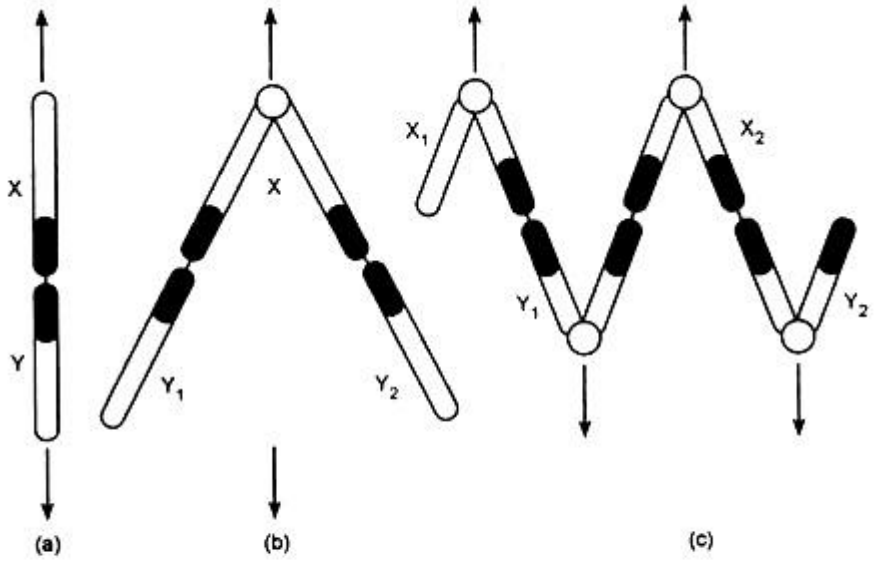


Fig. 7.6. Diagram showing orientation of the X and Y chromosomes at MI. **(a)** X and Y chromosomes oriented towards opposite poles. **(b)** The single X is oriented towards one pole, while the two Y chromosomes Y_1 and Y_2 are oriented towards the other pole. **(c)** X_1 and X_2 are oriented towards one pole, while Y_1 and Y_2 are oriented towards the other pole. The black regions denote the pairing regions in the X and Y chromosomes.

In case of compound sex chromosomes, say, XY_1Y_2 system, both the arms of the X chromosome possess pairing segments. The Y_1 and Y_2 chromosomes pair in such a way that the X chromosome moves to one pole, while both the Y chromosomes move to the opposite pole (Fig. 7.6). In the X, Y,X_2Y_2 system, both the X chromosomes move to one pole, while the Y chromosomes move to the opposite pole.

In the XX-XO mechanism, the XO individuals have a single X chromosome. Their X chromosome orients itself at the metaphase plate and moves to one pole at Al, leaving the other pole devoid of this chromosome. At All, the X chromosome divides and its chromatids move to the opposite poles. Thus only 50% of the gametes possess an X chromosome, while the remaining 50% of the gametes have no X chromosome.

Genie Balance Theory:

This theory was given by Bridges in 1921 based on his study of the progeny of crosses between triploid females and diploid males of D. melanogaster. According to this theory, the X chromosome of Drosophila carries the genes for femaleness, while the autosomes carry the genes for maleness. A balance between the number of X chromosomes and the number of sets of the autosomes determines the sex. If the ratio "X/autosomal set (A)" is equal to 1.0, the fly develops into a normal female, while if the ratio is 0.5, it develops into a normal male. The ratios falling between 1.0 and 0.5 lead to development of intersexes (Table 7.2).

Later, it was shown that the male determining factors are carried on the 2nd and 3rd chromosomes of Drosophila. The Y chromosome has no role in determination of sex but it is essential for male fertility; as a result, XO flies are phenotypically males but they are sterile.

TABLE 7.2. Chromosome constitutions and their sex expressions in *Drosophila melanogaster* and *Silene* (*Melandrium album*)

DROSOPHILA			SILENE (MELANDRIUM)		
Chromosome constitution	**X/A* ratio**	**Sex****	**Chromosome constitution**	**X/Y ratio**	**Sex****
2A+ XXX	1.5	super ♀	2A + XX	0.0 ♀	
2A + XX	1.0	normal ♀	2A + XYY	0.5 ♂	
2A + XXX	1.0	normal ♀	2A + XY	1.0 ♂	
3A + XXX	1.0	normal ♀	3A + XY	1.0 ♂	
4A + XXXX	1.0	normal ♀	4A + XY	1.0 ♂	
3A + XX	0.67	intersex	4A + XXYY	1.0 ♂	
3A + XXY	0.67	intersex	4A + XXXYY	1.5 ♂	
4A + XXX	0.75	intersex	2A + XXY	2.0 ♂	(occasional ☿ blossom)
2A + X	0.5	sterile ♂	3A + XXY	2.0 ♂	(occasional ☿ blossom)
2A + XY	0.5	normal ♂	4A + XXY	2.0 ♂	(occasional ☿ blossom)
2A + XYY	0.5	normal ♂	4A + XXXXYY	2.0 ♂	(occasional ☿ blossom)
4A + XX	0.5	sterile ♂	3A + XXXY	3.0 ♂	(occasional ☿ blossom)
3A + XY	0.33	super ♂	4A + XXXY	3.0 ♂	(occasional ☿ blossom)
			A4 + XXXXY	4.0 ♂	(occasional ♂ blossom)
			4A + XXXX	0.0 ♀	

*A = set of autosomes ; **♂ = male; ♀= female; ☿ = hermaphrodite (bisexual)

X-Y balance:

In several organisms male determining genes are present on the Y chromosome while the female determining genes are located on the X chromosome; sex is determined by the balance between the X and Y chromosomes.In human, Y chromosome is strongly male determining (Table 7.3). In the absence of Y chromosome, the phenotype of the individual is female. Thus a female phenotype develops in individuals of XO, XX, XXX, XXXX, XXXXX constitution. However, in presence of a single Y chromosome, they all (XY, XXY, XXXY, XXXXY, XXXXXY) develop into males.

TABLE 7.3. Sex determination in human

Chromosome complement	Sex phenotype
2A + X	female, sterile (Turner's syndrome)
2A + XX	female, normal
2A + XXX	female, fertile (metafemale)
2A + XXXX	female, fertile ? (metafemale)
3A + XXX	female, (triploid)
2A + XY	male, normal
2A + XYY	male, fertile
2A + XXY	male, sterile (Klinefelter's syndrome)
2A + XXXY	male, sterile (Klinefelter's syndrome)
2A + XXYY	male, sterile
3A + XXY	male, (triploid)

Y chromosome:

The Y chromosome is the sex chromosome confined to the heterogametic sex in the XX-XY system of sex determination (in contrast, X chromosome is found in both the sexes although the heterogametic sex has only one copy).

The size and function of the Y chromosome vary in different organisms. In Drosophila, the Y chromosome is slightly larger than the X chromosome; it is heterochromatic and does not carry the genes for maleness.However, it is necessary for the fertility of the males since XO male Drosophila are sterile. In contrast, in the case of humans and several plants like Melandrium, the Y chromosome possesses male determining genes. In human, the Y chromosome is very small, but it is strongly male determining so that individuals with XXXXY and mosaics with XXXY/XXXXY/XXXXXY constitution develop the male phenotype.

Genes for maleness are located on the short arm of human Y so that XY individuals with deleted short arm of the Y chromosomes develop the female phenotype. The segments bearing male determining genes in the short arm of Y may be trans-located to the X chromosome; in this case the XX individuals develop the male phenotype.In Silene (Melandrium), Y chromosome is much larger than the X chromosome. It possesses the following four distinct regions (Fig. 7.7).

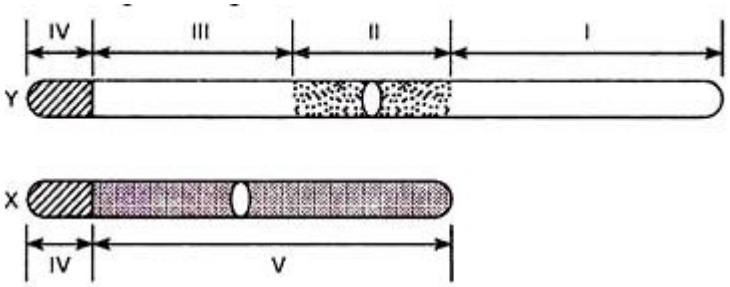


Fig. 7.7. Diagram of Y and X chromosomes of *Silene* (*Melandrium*) showing regions having different activities. Region I : female suppressor region (when absent, leads to bisexual development). Region II : essential male promoting region (when absent, leads absent, leads to female development). Region III : essential male fertility region (when absent, anthers abort). Region IV : Pairing region in both, X and Y. Region V (on X chromosome) : differential portion of the X (genes for femaleness). Segment lengths in this diagram are arbitrary).

(I) Female suppressor region:

It is located at the end of the Y chromosome and carries the genes for suppression of the female reproductive organs. In the absence of this region, hermaphrodite (bisexual) flowers are produced on the XY plant.

(II) Male promoting region:

This region lies next to the first (female suppressor) region and carries genes for initiation of anther development. Absence of this region causes the production of female flowers on the XY plant.

(III) Male fertility region:

It lies next to the second (male promoting) region and carries the genes for male fertility. When this region is absent, anthers abort and the plant is male sterile.

(IV) Pairing region:

It is located at one end of the Y chromosome and is homologous to a region in the X chromosome. During meiosis, the X and Y chromosomes pair in this region which ensures their proper separation during AI. The X chromosome of Silene possesses a differential region (V) which carries genes for the development of female reproductive organs (Fig. 7.7).

Dosage compensation:

In the sex determining system where XX individual is female and XY individual is male, females contain two X chromosomes, whereas males contain only one X chromosome. Apart from the genes governing the sex, other genes are also present in the X chromosome; they are called sex-linked genes.

These genes are in homozygous or heterozygous condition in the females (XX), but they are in hemizygous condition in the males (XO, XY) since they do not have corresponding alleles in the Y chromosome. But males and females are morphologically and physiologically similar in expression of these genes.The mechanism by which the effects of sex- linked genes in males (XO, XY) are equalized to their effects in females (XX) is known as dosage compensation.

In Drosophila, this mechanism operates by enhancing the activity of the X-linked genes in males, while the activity of these genes in the two X chromosomes of females is restrained. The mechanism of dosage compensation in man and other mammals differ from that in Drosophila. One of the two X chromosomes of the females is inactivated through heterochromatinization so that only one X chromosome remains active. Thus there is a balance of X-linked gene activity in the females and males.

Single active X-hypothesis or Lyon hypothesis of dosage compensation: The dosage compensation in human and other mammals is regulated by the inactivation of one X chromosome in the females. This is known as single active X hypothesis or Lyon hypothesis proposed by Lyon in 1961 and elaborated subsequently. The main genetic evidence for this hypothesis comes from the mosaic phenotype of female mice heterozygous for sex-linked recessive genes that affect coat colour.

According to this hypothesis:

(i) One of the two X chromosomes in the cells of normal female mammals is genetically inactive.

(ii) Inactivation occurs early in embryonic development.

(iii) The inactive X chromosome may be maternal paternal one in the different cells of the same animal.

(iv) The decision as to which X chromosome becomes inactive is taken at random. Once an X chromosome is inactivated in a cell, the same X chromosome will always be inactivated in all its progeny cells.

(v) The inactivation occurs due to heterochromatinization. The hetero-chromatinized X chromosome forms the sex chromatin observed during interphase, and is late replicating.

There occurs a preferential heterochromatinization of abnormal X chromosome (Table 7.4). If one X is normal and the other is an iso-X-chromosome, the iso-chromosome is always heterochromatic. In man, inactivation of sex-linked genes has been demonstrated at cellular level, for example, glucose-6-phosphate-dehydrogenase (G6PD), Hunter-hurler syndrome,

Juvenile hyperuricaemia.

TABLE 7.4. Chromosome constitution, sex chromatin and sex phenotype in human

Chromosome constitution**	Maximum number of sex chromatin bodies	Maximum number of late replicating X chromosomes*	Sex phenotype
45, X	0	0	♀ Turner's syndrome/ovarian dysgenesis
46, XX	1	1	♀ Normal
46, XXP–	1	1	♀ Turner's syndrome/ovarian dysgenesis
46, XXq–	1	1	♀ Turner's syndrome/ovarian dysgenesis
46, XXpi	1	1	♀ Turner's syndrome/ovarian dysgenesis
46, XXr	1	1	♀ Turner's syndrome/ovarian dysgenesis
47, XXX	2	2	♀ Normal, mentally deficient
48, XXXX	3	3	♀ Mental deficiency
49, XXXXX	4	4	♀ Mental deficiency
46, XY	0	0	♂ Normal
47, XYY	0	0	♂ Fertile, tall, aggressive
47, XXY	1	0	♂ Klinefelter's syndrome
48, XXXY	2	2	♂ Klinefelter's syndrome
48, XXYY	1	1	♂ Klinefelter's syndrome, tall, aggressive
49, XXXXY	3	3	♂ Infertile, somatic anomalies
46, XY#	0	0	♂ testicular feminization or intersex
46, XY##	1	1	♂ Klinefelter's syndrome or intersex (sex reversed females)

* Abnormal X chromosome is invariably the late replicating one.

** Xp– = deletion in the short arm of X ; Xq– = delection in the long arm of X ; Xpi = iso-chromosome for short arm of X; Xr = ring X chromosome.

Sex reversed genetic males produced due to some genes acting on the target organs making them insensitive to testosterone. It causes the development of female phenotype.

The XX males may be either (i) potential mixoploids (46, XX/47, XXY) from which the 47, XXY line was lost after sex determination, or (ii) XXY syndrome from which Y chromosome was lost, or (iii) male determining factors of Y were translocated to one X or to some autosome, or (iv) sex reversed females caused by some autosomal sex reversal gene.

♀= female; ♂ = male.

Sex chromatin (Barr body) Drum sticks:

Sex chromatin is the hetero-chromatinized X-chromosome observed as a condensed body in interphase nuclei of mammalian females. It was discovered by Barr and Bartram in 1949 in the neurons of cat and was called Barr body after M.L. Barr.

Generally the sex chromatin is observed as a planoconvex body lying adjacent to the inner

surface of the nuclear membrane. A detailed study shows that it has V or U-shaped structure and its apex points towards the centre of the nucleus. The size of sex chromatin ranges from 0.7 x 1.0 μm to 1.0 x 1.4 μm with an average of 0.8 x 1.1 μm in the different tissues and species. Sex chromatin is not visible in all the interphase nuclei of females; the frequency of cells showing sex chromatin varies in the different tissues of the same species.The frequency of **"sex chromatin positive"** nuclei is 85% in the nervous tissues, 96% in the whole mounts of amnion epithelium and from 20-25% to 60-70% in oral smears. It has been found that the number of haploid autosome complements influences the number of late replicating X chromosomes.

The relationship between numbers of sex chromatin, autosomal set and the X chromosome has been expressed by the following formula:

$B = X - (p/2)$ (7.1)

where, B = number of sex chromatin bodies

X = number of X chromosomes

P = number of autosomal sets

The above relationship holds for all even degrees of ploidy, viz., 2n, 4n, 6n, 8n, etc. Thus in human, a tetraploid cell (2n = Ax = 92, XXXX) has two sex chromatin bodies. But a tetraploid cell with XXYY constitution (92, XXYY) will not show any sex chromatin. In triploids (69, XXX), some cells have one and others have two sex chromatin bodies (average 1.5 per cell).

The abnormal males, such as, XXY (Klinefelter's syndrome) also show sex chromatin in their cells (Table 7.4). Abnormal X chromosome is, as a rule, always hetero-chromatinized. The Y chromosome has no role in sex chromatin formation; XYY males do not show any sex chromatin. Thus sex chromatin can be used as a direct clinical test to determine the number of X chromosomes in an individual.Davidson and Smith in 1954 found some bodies similar to sex chromatin in the circulating polymorphonuclear neutrophil leucocytes of human blood. This body is like a drum stick and is attached to one lobe of the polymorph nucleus.However, the frequency of drum sticks is very low, viz., 1 in 40 leucocytes in normal females and < 1 in 500 leucocytes in normal males. The drum sticks probably represent the hetero-chromatinized X chromosome.

Sex Determination:

A sex-determination system is a biological system that determines the development of sexual characteristics in an organism. Most organisms that create their offspring using sexual reproduction have two sexes. Occasionally, there are hermaphrodites in place of one or both sexes. There are also some species that are only one sex due to parthenogenesis, the act of a female reproducing without fertilization.

In many species, sex determination is genetic: males and females have different alleles or even different genes that specify their sexual morphology. In animals this is often accompanied by chromosomal differences, generally through combinations of XY, ZW, XO, ZO chromosomes, or haplodiploidy. The sexual differentiation is generally triggered by a main gene (a "sex locus"), with a multitude of other genes following in a dominoeffect.

In other cases, sex of a fetus is determined by environmental variables (such as temperature). The details of some sex-determination systems are not yet fully understood. Although they provide concrete analysis of complete biological sex-determinism. Hopes for future fetal biological system analysis include complete-reproduction-system initialized signals that can be measured during pregnancies to more accurately determine whether a determined sex of a fetus is male, or female. Such analysis of biological systems could also signal whether the fetus is hermaphrodite, which includes total or partial of both male and female reproductive organs.

Some species such as various flowers and fish do not have a fixed sex, and instead go through life cycles and change sex based on genetic cues during corresponding life stages of their type. This could be due to environmental factors such as seasons and temperature. Human fetus genitals can sometimes develop abnormalities during maternal pregnancies due to mutations in the fetuses sex-determinism system, resulting in the fetus becoming intersex. In nature a large number of diverse mechanisms exist for determination of sex in different species. The fruit fly Drosophila melanogaster and human beings are very important in the development of genetic concepts because in these two organisms, and in many others, individuals normally occur in one of two sex phenotypes, male or female.

In these species males produce male gametes, sperm, pollen or microspores while females produce female gametes namely, eggs, ovules or macrospores. In many species the two sexes are phenotypically indistinguishable except for the reproductive organs. Sex determination is aimed at identifying the factors responsible to make an organism a male or female or in some cases a hermaphrodite. So far the mechanism of sex determination has been related to the presence of sex chromosomes whose composition differs in male and female sexes.

However, in recent years sex determination has been differentiated from sex differentiation, and sex determination mechanism is explained more on the basis of the specific genes located on sex chromosomes and autosomes. Sex determination is recognized as a process in which signals are initiated for male or female developmental patterns. During sex differentiation, events occur in definite pathways leading to the development of male and female phenotypes and secondary sexual characters. Significant progress has been made in understanding the mechanism of sex in human beings and other mammals and new genes have been identified.

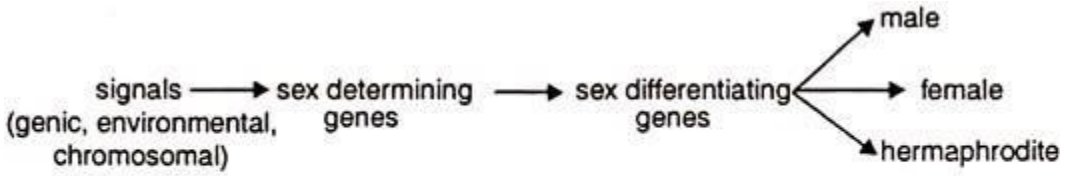


Fig. 1. Sex determination and sex differentiation mechanism involving two sets of genes and the signals.

Chromosome Theory of Sex Determination:

Sex determination in higher animals is controlled by the action of one or more genes. The testis determining factor (TDF) gene is the dominant sex determining factor in human beings. Hemking a German biologist identified a particular nuclear structure throughout the spermatogenesis in some insects. He named it as "X-body" and showed that sperm differed by its presence or absence. The X body was later found to be a chromosome that determined sex. It was identified in several insects and is known as the sex or Xchromosome. Thus, the chromosome theory of sex determination states that female and male individuals differ in their chromosomes. Chromosomes can be differentiated into two types, autosomes and sex chromosomes. Sex chromosomes carry genes for sex. In some animals, females have one more chromosome than males, thus they have two X chromosomes and males have only one. Females are therefore cytologically XX and males are XO, where 'O' denotes the absence of X chromosome. During meiosis in the female the 2X chromosome pairs and separates producing eggs that contain a single X chromosome. Thus all eggs are of the same type containing only one X chromosome. During meiosis in the male, the single X chromosome moves independently of all the other chromosomes and is incorporated into half of the sperm, the other half do not have any X chromosome. Thus, two types of sperms are produced, one with X chromosome and the other without the X chromosome or designated as 'O'.

When the sperm and eggs unite, two types of zygotes are produced; XX that develop into females and XO that develop into males. Because both of these types are equal in number, the reproductive mechanism preserves a 1:1 ratio of males tofemales. In many animals,

including human beings, males and females have the same number of chromosomes. This numerical equality is due to the presence of a chromosome in the male called the 'Y' chromosome, which pairs with the X. During meiosis in the male, the X and Y- chromosomes separate from each other producing two types of sperm, one type with X chromosome and the other type having Y chromosome.

The frequencies of the two types are approximately equal. Females with XX chromosomes produce only one type of eggs, all with X chromosome. In random fertilization, approximately half of the zygotes are with XX chromosomes and the other half with XY chromosomes leading to a sex ratio of 1:1.This mechanism is called XX – XY type of sex determination. The XY mechanism is more prevalent than the XO mechanism. The XY type is considered characteristic in higher animals and occurs in some plants. This mechanism is operative in Drosophila melanogaster and human beings. Both species exhibit the same pattern of transmission of X and Y chromosomes in normal individuals in natural populations. In human beings, the X chromosome is considerably longer than the Y chromosome. The total complement of human chromosomes includes 44 autosomes: XX in the female and XY in the male. Eggs produced by the female in oogenesis have a complement of 22 autosomes plus an X chromosome. Sperm from the male have the same autosomal number and either an X or a Y chromosome. Eggs fertilized with sperm containing a Y chromosome result in zygotes that develop into males; those fertilized with sperm containing an X chromosome develop into females.

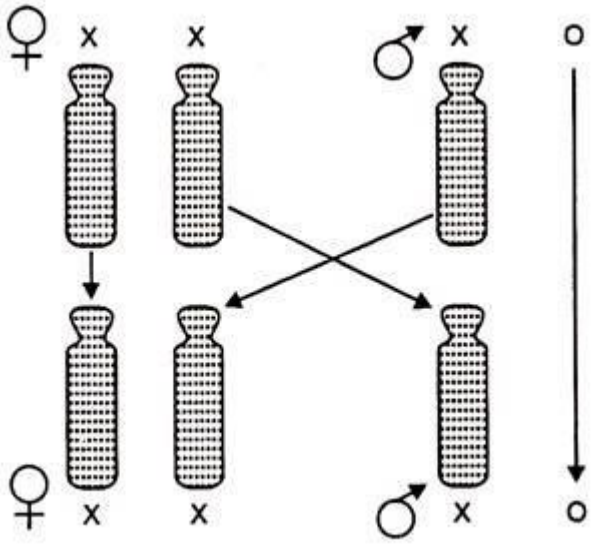


Fig. 2. Inheritance of sex chromosomes in animals with XX-XO mechanism.

In animals with XX-XY mechanism of sex determination, females (XX) produce gametes that have the same chromosome composition (one X plus one set of autosomes). These females are homogametic sex as all the gametes are the same. The males of these animals are heterogametic as they produce two types of gametes, one half containing one X chromosome

plus one set of autosomal chromosomes and the other one half contain one Y chromosome plus one set of autosomes.

Animals with Heterogametic Females:

In many birds, moths and some fish, the sex determination mechanism is identical to the XX-XY mechanism but the females are heterogametic (ZW) and males are homogametic (ZZ). This mechanism of sex determination is called ZZ-ZW.

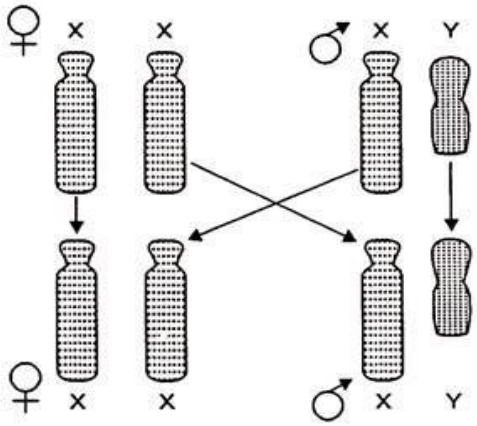


Fig. 3. Inheritance of sex chromosomes in animals with XX-XY mechanism.

n this mechanism the relationship between sex chromosomes and sex phenotypes is reversed. In birds the chromosome composition of the egg determines the sex of the offspring, whereas in humans and fruit flies, the chromosome composition of the sperm determines the sex of the offspring.

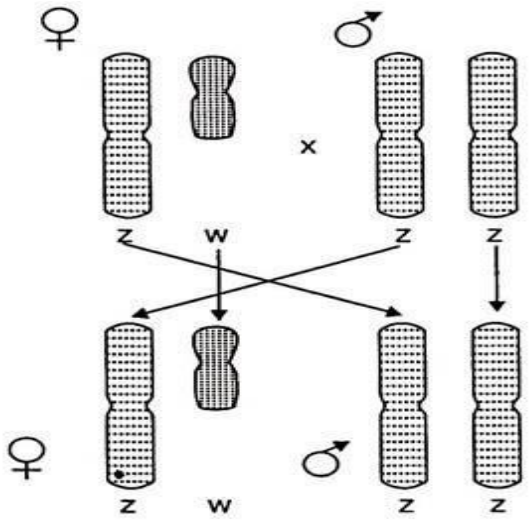


Fig. 4. Sex determination in birds with ZZ-ZW mechanism.

Process of Sex Determination in Human Beings:

In human beings, sex is determined by the number of X chromosomes or by the presence or absence of the Y chromosome. In human beings and other placental mammals, maleness is due to a dominant effect of the Y chromosome. The dominant effect of the Y chromosome is manifested early in development when it directs the primordial gonads to differentiate into testes. Once the testes are formed, they secrete testosterone that stimulates the development of male secondary sexual characteristics. Testis determining factor (TDF) is the product of a gene called SRY (Sex determining Region of Y), which is located in the short arm of the Y chromosome of the mouse. SRY was discovered in unusual individuals whose sex was not consistent with their chromosome constitution – males with XX chromosomes and females with XY chromosomes.

Some of the XX males carried a small piece of the Y chromosome inserted into one of the X chromosomes. It is evident that this small piece carried genes for maleness. Some of the XY females carried an incomplete Y chromosome. The part of the Y chromosome that was missing corresponded to the piece that was present in the XX males.

Its absence in the XY females prevented them from developing testes. These observations show that a particular segment of the Y chromosome was required for the development of the male. Further studies showed that the SRY gene is located in this male determining segment. Like that of the human SRY gene is present in the Y chromosome of the mouse and it specifies male development (Fig below).

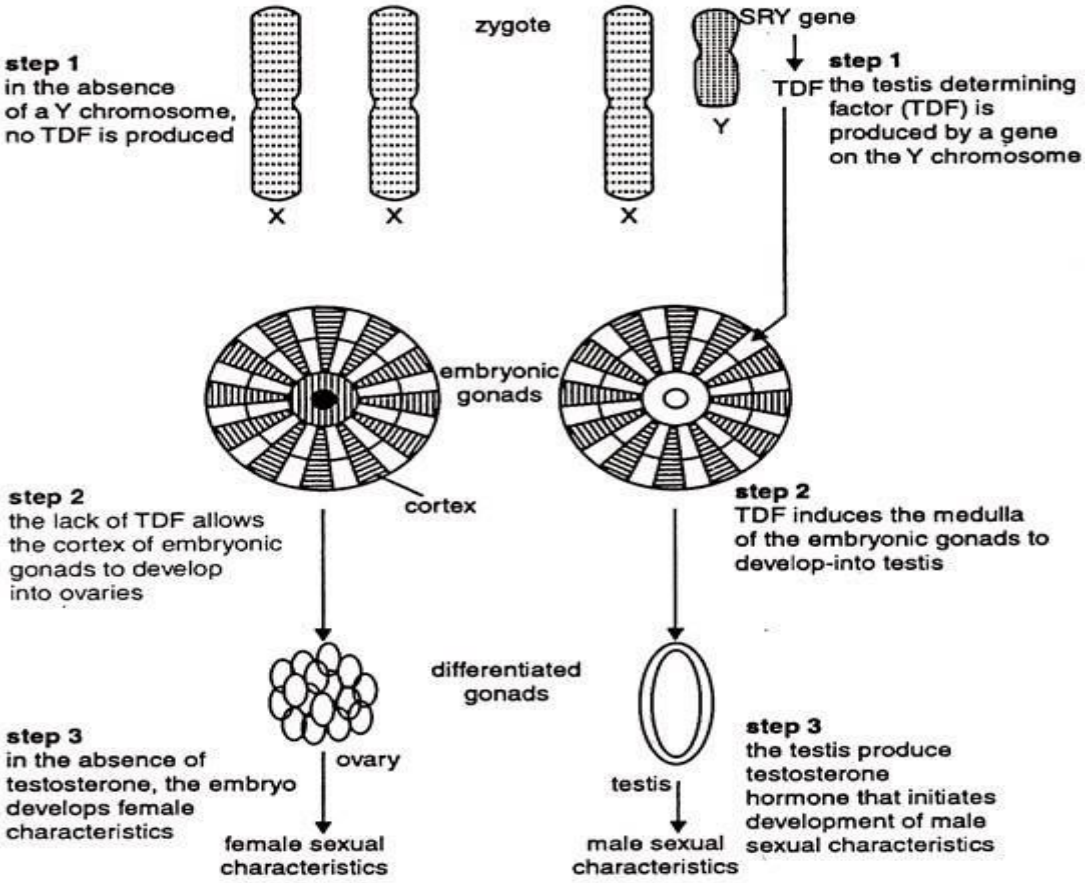
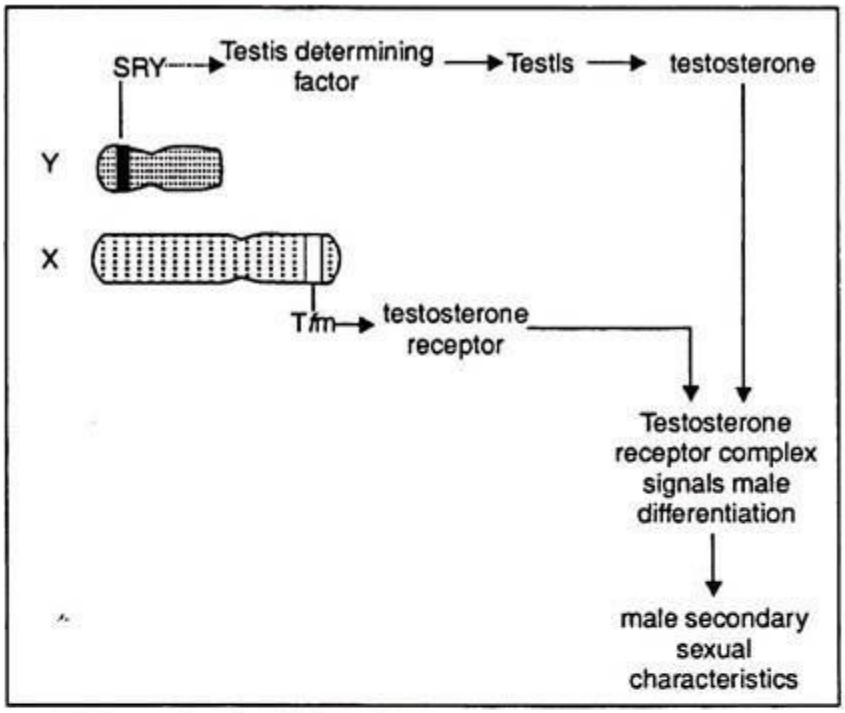


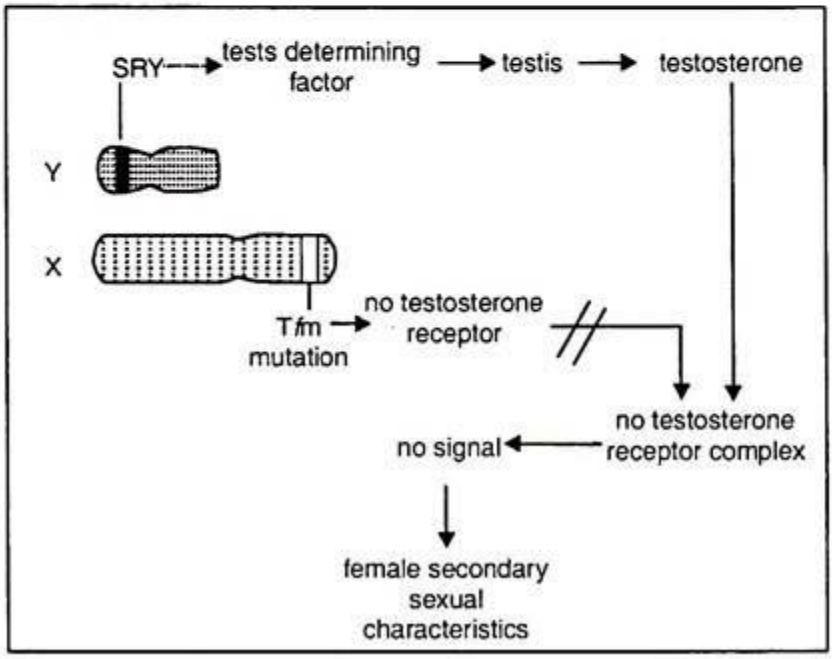
Fig. 5. Sex determination in human beings. Maleness is due to TDF by a gene on the Y chromosome.

After the formation of the testes, testosterone secretion initiates the development of male sexual characteristics. The hormone testosterone binds to receptors of several types of cells. This binding leads to the formation of a hormone – receptor complex that transmits signals to the cell instructing how to differentiate.

The combined differentiation of many types of cells leads to the development of male characteristic like beard, heavy musculature and deep voice. Failure of the testosterone signaling system leads to nonappearance of the male characters and the individual develops into a female. One of the reasons for failure is an inability to make the testosterone receptor.



(a) Normal male with the wild type Tfm gene.



(b) Male with the tfm mutation and testicular ferminizaton.

Fig. 6. Testicular feminization, a condition caused by an X-linked mutation. tfm that prevents the production of the testosterone receptor.

Individuals with XY chromosomal composition having this biochemical deficiency first develop into males. In such males, although testis is formed and testosterone secreted, it has

no effect because it cannot reach the target cell to transmit the developmental signal. Individuals lacking the testosterone receptor therefore can change sexes during embryological development and acquire female sexual characteristics. However, such individuals do not develop ovaries and remain sterile. This syndrome known as testicular feminization is due to a mutation in an X-linked gene, tfm that codes for the testosterone receptor. The tfm mutation is transmitted from mothers to sons who are actually phenotypically female in a typical X- linked manner.

Master Regulatory Gene:

In human beings irregular sex chromosome constitutions occur occasionally. Any number of X chromosomes (XXX or XXXX), in the absence of a Y chromosome give rise to a female. For maleness, the presence of a Y chromosome is essential and even if several X chromosomes are present (XXXXY), the presence of a single Y chromosome leads to maleness. The Y chromosome induces the development of the undifferentiated gonad medulla into testis, whereas an XX chromosomal set induces the undifferentiated gonadal cortex to develop into ovaries. The gene on the Y chromosome that induces the development of testes is called as Testis Determining Factor (TDF). It has been isolated, characterized and found to encode a protein that regulates the expression of other genes. Thus, the TDF gene is the master regulator gene that triggers the expression of large number of genes that produce male sex phenotype. In the absence of TDF gene, the genes that produce femaleness predominate and express to produce a female phenotype. The TDF exerts a very dominant effect on development of the sex phenotype.

Genic Balance Theory of Sex Determination in Drosophila:

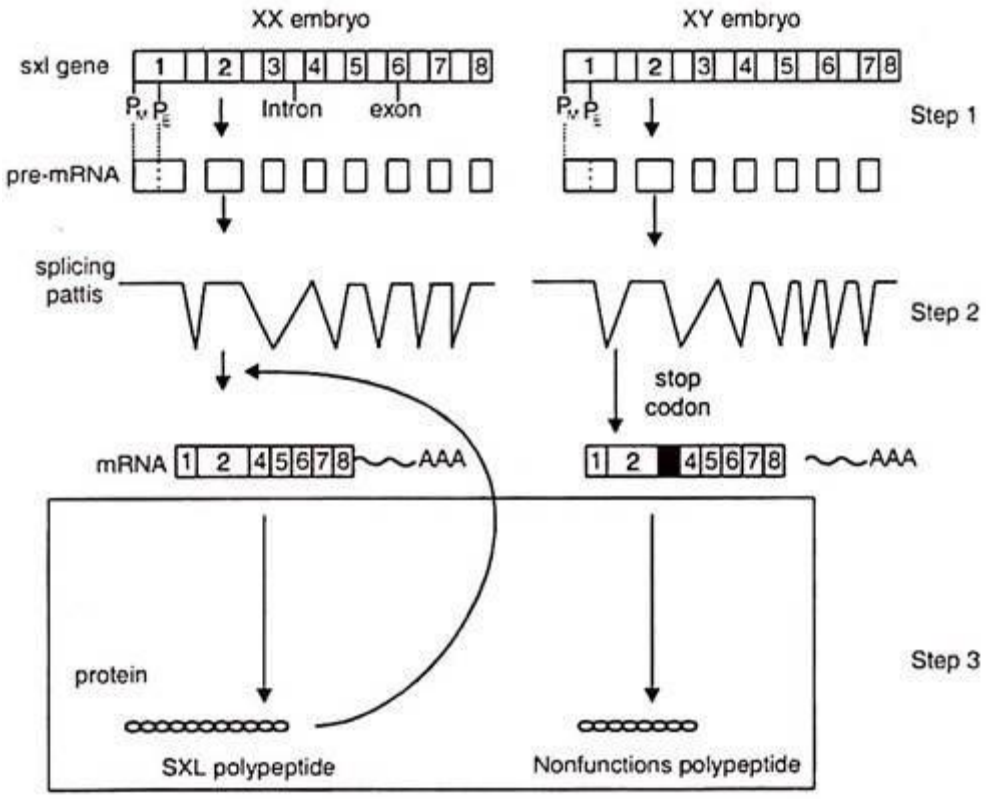
In Drosophila investigations by C.B. Bridges have shown that X chromosomes contain female determining genes and male determining genes are located on the autosomes and many chromosome segments are involved. The genie balance theory of sex determination in Drosophila explains the mechanism involved in sex determination in this fly.

The Y chromosome in Drosophila does not play any role in sex determination. Sex in this animal is determined by the ratio of X chromosomes to autosomes. Normal diploid insects have a pair of sex chromosomes, either XX or XY, and three pairs of autosomes. These are denoted by AA, each A representing one set of haploid autosomes. Flies with abnormal number of autosomes can be produced by genetic manipulation as shown in Table below.

Table 1. Ratio of X chromosomes to autosomes and the phenotype of *Drosophila.*

X chromosomes (X) and Sets of autosomes (A)	X : A ratio	Phenotype
1X 2A	0.5	Male
2X 2A	1.0	Female
3X 2A	1.5	Metafemale
4X 3A	1.33	Metafemale
4X 4A	1.0	Tetraploid female
3X 3A	1.0	Triploid female
3X 4A	0.75	Intersex
2X 3A	0.67	Intersex
2X 4A	0.5	Tetraploid male
1X 4A	0.33	Metamale

Whenever the ratio of X chromosomes to autosomes is 1.0 or above, the sex of the fly is female, and whenever it is 0.5 or less, the fly is male. If the ratio is between 0.5 and 1.0, it is an intersex with both male and female characters. In all these phenotypes, Y chromosome has no role to play but it is required for the fertility of the male. In Drosophila sex determination mechanism, an X- linked gene called Sex lethal (Sxl) plays an important role (Fig. below).



Step 1. Transcription in XX embryos, a molecular signal based on the X:A ratio initiates transcription of the Sxl gene from promoter P_E. Later transcription is initiated at promoter P_M in both XX and XY embryos.

Step 2. Splicing in XX embryos, the Sxl transcripts are spliced to contain all the exons except exon 3. In Xy embryos, the Sxl transcipts are splicied to contain all the exons including exon 3.

Step 3. Translation in XX embryos, the Sxl mRNA in translated into a polypeptide (SXL) that regulates splicing, including the splicing of SXL transcripts. In XY embyryos, a stop codon in exon 3 prevents the SXL mRNA from being translated into a functional polypeptide.

Fig. 7. Sex specific expression of the sex-lethal (Sxl) gene in *Drosophila*.

A number of X linked genes sets the level of Sxl activity in a zygote. If the ratio between X chromosomes and autosomes is 1.0 or above, the Sxl gene becomes activated and the zygote develops into a female. If the ratio is 0.5 or less, the Sxl gene is inactivated and the zygote develops into a male. A ratio between 0.5 and 1.0 leads to mixing of signals and the zygote develops into an intersex with a mixing of male and female characters.

The sex ratio of X chromosomes to autosomes and the phenotype of Drosophila determination pathway in Drosophila has three components:

(i) A system to ascertain the X : A ratio in the early embryo,

(ii) A system to convert this ratio into a developmental signal, and

(iii) A system to respond to this signal by producing either male or female structures.

The system to ascertain the X : A ratio involves interactions between maternally synthesized proteins that have been deposited in the eggs cytoplasm and embryologically synthesized proteins that are coded by several X-linked genes. These latter proteins are twice as abundant in XX embryos as in XY embryos and therefore provide a means for counting the number of X chromosomes present. Because the genes that encode these proteins effect the numerator of the X : A ratio, they are called numerator elements. Other genes located on the autosomes affect the denominator of X : A ratio and are therefore called as denominator elements. These encode proteins that antagonize the products of numerator elements (Fig.below).

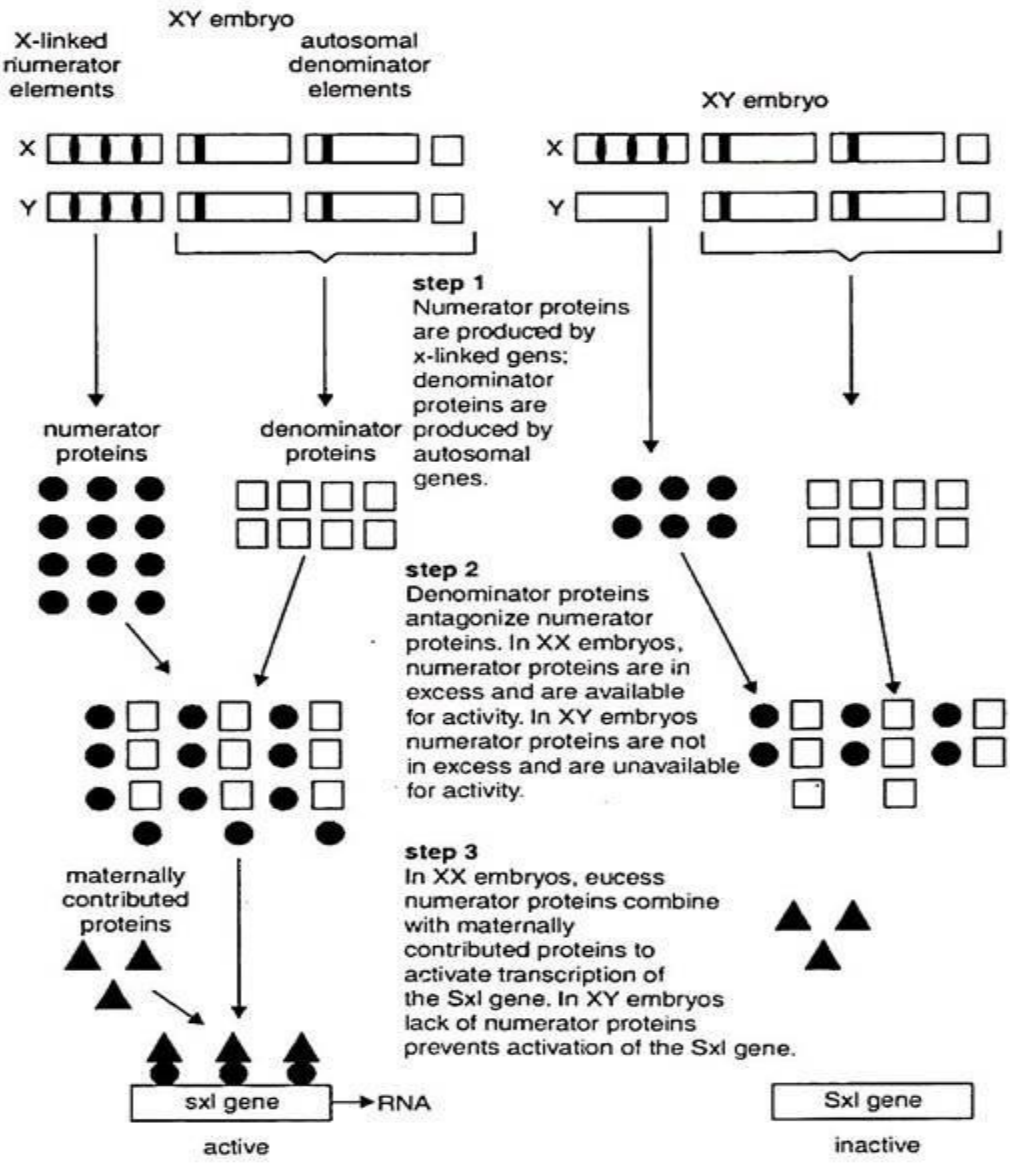


Fig. 8. Ascertainment of the X : A ratio by numerator and denominator elements in *Drosophila*.

The system for ascertainment of the X : A ratio in Drosophila is therefore based on antagonism between X-linked (numerator) and autosomal (denominator) gene products. Once the X : A ratio is ascertained, it is converted into a molecular signal that controls expression of the X-linked sex lethal gene (Sxl), the master regulator of the sex determination pathway.

Early in development, this signal activates transcription of the Sxl gene from PE' the gene's 'early' promoter, but only in XX embryos. The early transcripts from this promoter are processed and translated to produce functional sex-lethal proteins, denoted Sxl. After only a few cell divisions, transcription from the PE promoter is replaced by transcription from another promoter, PM. The so called maintenance promoter of the Sxl gene. Interestingly, transcription from the PM promoter is also initiated in XY embryo. However, the transcripts from PM are correctly processed only if Sxl protein is present. Consequently, in XY embryos,

where this protein is not synthesized, the Sxl transcripts are alternately spliced to include an exon with a stop codon, and when these alternately spliced transcripts are translated, they generate a short polypeptide without regulatory function.
Thus, alternate splicing of the Sxl transcripts in XY embryos does not lead to the production of functional Sxl protein and in the absence of this protein, these embryos develop as males. In XX embryos, where Sxl protein was initially made in response to X : A signal, Sxl transcripts from the PM promoter are spliced to produce more Sxl proteins. In XX embryos, this protein is therefore, a positive regulator of its own synthesis forming a feedback mechanism that maintains the expression of the Sxl proteins in XX embryos and prevents its expression in XY embryos. The Sxl protein also regulates the splicing of transcription from another gene in the sex determination pathways, transformers (tra). These transcripts can be processed in two different ways.
In chromosomal males, where the Sxl protein is absent, the splicing apparatus always leaves a stop codon in the second exon of the tra RNA. Thus, when spliced tra RNA is translated, it generates a truncated polypeptide. In females, where the Sxl protein is present, this premature stop codon is removed by alternate splicing in at least some of the transcripts. Thus, when they are translated, some functional transformer protein tra is produced. The Sxl protein therefore allows the synthesis of functional tra protein in XX embryos but not in XY embryos (Fig. 9).

The tra protein also turns out to be a regulator of RNA processing. Along with tra 2, a protein encoded by the transformer 2 (tra 2) gene, it encodes the expression of double sex (dsx) an autosomal gene that can produce two different proteins -through alternate splicing of its RNA. In XX embryos, where the tra protein is present, dsx transcripts are processed to encode a DSX protein that represses the genes required for male development. Therefore, such embryos develop into females. In XY embryos, where the TRA protein is absent, dsx transcripts are processes to encode a DSX protein that represses the gene required for female development. Consequently, such embryos develop into males. The dsx gene is therefore, the switch point at which a male or female developmental pathway is chosen. From this point, different sets of genes are specifically expressed in males and females to bring about sexual differentiation.

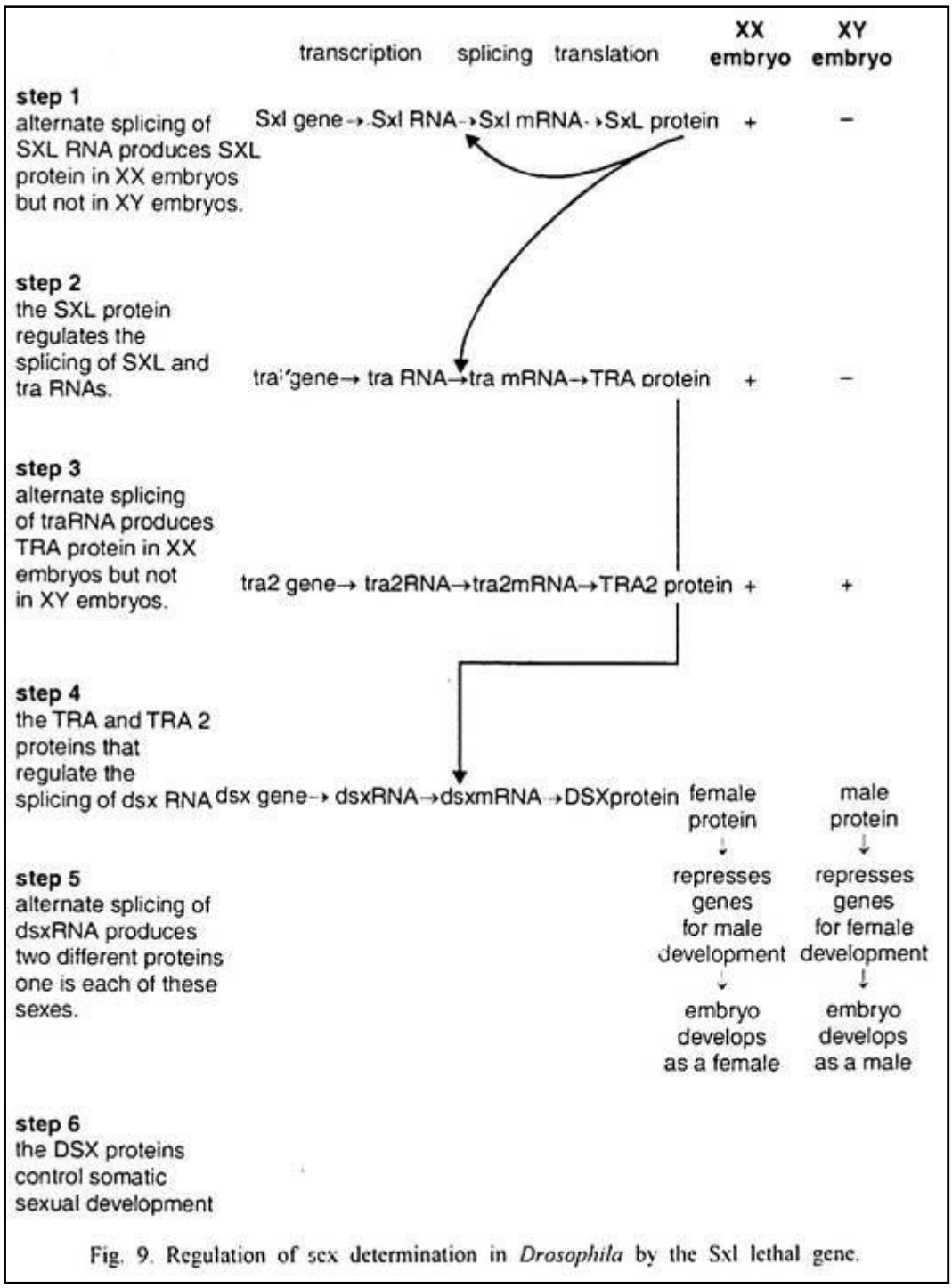


Fig. 9. Regulation of sex determination in *Drosophila* by the Sxl lethal gene.

Modern Theories of Sex Determination:

The Modern Theories Given For Sex Determination Are As Follows:

(1) Chromosomal theory or Theory of Heterogamy

(2) Genic balance theory

(3) Hormonal theory

(4) Theory of environmental factors.

(1) Chromosomal Theory or Theory of Heterogamy:

The complete account of chromosomal sex determination was at first worked out by Stevens (1905). This view was later supported by other scientists such as Wilson, Bridge, Goldschmidt and Whitings.

In majority of sexually reproducing animals two types of chromosomes are found:

(i) Autosomes:

They are found in all cells. The genes present on autosomes are responsible for determining the somatic characters but sometime influence the sex of the organism. The two members of each homologous pair are similar in shape and size (homomorphic).

(ii) Sex Chromosomes or Allosomes:

They carry genes for sex. A pair of them determines the sex. They are variously named as X and V chromosomes (Man and Drosophila), Z and W chromosomes (Birds and Moth), odd chromosomes, idiosoines, heterosomes or allosomes. The genes which determine the sex are being located on these chromosomes. The two members of this pair arc often dissimilar in male and are represented as X and Y chromosomes or as Z and W chromosomes.

(a) XX Female and XY Male Types:

This type of sex mechanism is found in Drosophila (fruitfly) and majority of mammals including man. In this type the female is homogametic (XX) and male is heterogametic (XY) consisting of two dissimilar chromosomes X and Y. The females produce ova all of one type having X chromosome. Males produce two types of sperms: -50% with X-chromosome and remaining 50% with Y-chromosome. Thus, the sex chromosomes in female are homomorphic and those of male are heteromorphic (Fig. 5.13).

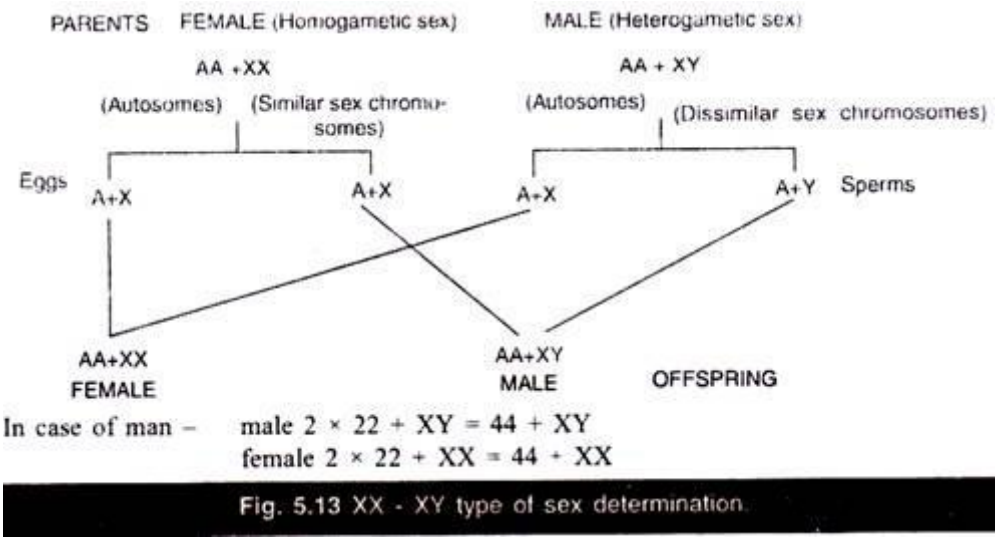


Fig. 5.13 XX - XY type of sex determination.

(b) ZW Female and ZZ Male Type:

In butterflies and birds, the female is heterogametic having dissimilar Z and W chromosomes whereas the male is homogametic having similar ZZ chromosomes (It is a convention to designate female as ZW instead of XY and male as ZZ instead of XX). The situation here is just reverse to first type.

(c) XX Female and XO Male Type:

Mc Clung and Wilson (1903) described this type of sex mechanism in insects especially in grasshopper. In male there is no mate for X chromosome, hence the name XO is given, there is no Y chromosome. They produce sperm of two types, 50% with X chromosome and 50% without X. In females there are two similar or homomorphic sex chromosomes XX.

2. Genic Balance Theory:

This theory was proposed by Calvin Bridges (1921). From his study on Drosophila he suggested that the X chromosomes carry factor for femaleness whereas autosomes "A" carry genes for maleness. Y chromosome does not take part in sex determination. From the Fig. 5.15 it is clear that the genic balance is governed by the ratio of the number of X-chromosomes to the number of sets of autosomes in the zygote at fertilization.

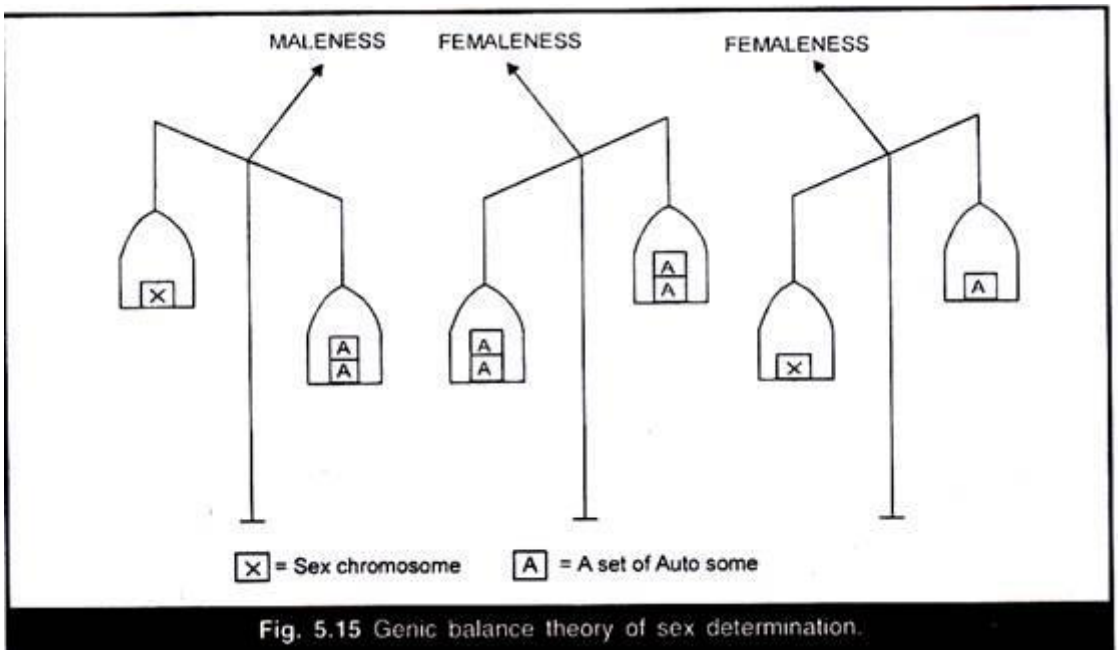


Fig. 5.15 Genic balance theory of sex determination.

II the ratio is 1.0 the offspring develops into female, but if it is 0.5, then the offspring develops into male. If the ratio is intermediate between 1.0 and 0.5, the resulting individual is neither a male or nor a female but an inter sex. Super females have a ratio of 1.5 and super males have a ratio 0.33.

(3.) Hormonal Theory:

Hormones are the secretion of the endocrine glands which in many instances modify the sex rather determining the sex. They are mainly responsible for the expression of secondary sexual characters. This theory is based upon the observation of Crew in chicks.

In course of his investigation he found a hen which laid fertile eggs, accidentally lost its ovary, stopped laying eggs, and developed male characters such as comb and male plumage and became a cock. The above case of sex reversal is explained by assuming that destruction or removal of the ovary led to stoppage of production of the ovarian hormones.But the rudiment of testis (present in all female birds) became functional following the loss of ovary and produced male hormone which is responsible for the appearance of male secondary sexual characters. Such a male produced sperms and became father of two chickens.Another classical example of sex reversal by the action of hormone is observed in free martin. In cattle, when twin calves of opposite sex are born, the female is usually somewhat abnormal and sterile. Such a calf is called freemartin. Since the male hormone appears earlier in the development, it passes into the body of the under developed female through the circulation and causes partial sex reversal of the female.

(IV) Theory of Environmental Factors:

Baltzer (1935) observed sex determination due to external environment in certain lower animals such as Bonellia. The adult female is several inches long but the male is very small of the size of large protozoa and lives in the reproductive tract of the female. The newly hatched young worm (Bonellia), when reared in isolation, develops into a female. But when, these are released into water containing mature females, some of them attach to the proboscis of female (to suck nourishment) develop into tiny males and come to lie in the oviduct of the female.

Sex Mosaics:

In genetics, a mosaic, or mosaicism, involves the presence of two or more populations of cells with different genotypes in one individual who has developed from a single fertilized egg. Mosaicism has been reported to be present in as high as 70% of cleavage stage embryos and 90% of blastocyst-stage embryos derived from in vitro fertilization.

Genetic mosaicism can result from many different mechanisms including chromosome non- disjunction, anaphase lag and endoreplication. Anaphase lagging is the most common way by which mosaicism arises in the preimplantation embryo. Mosaicism can also result from a mutation in one cell during development in which the mutation is passed on to only its daughter cells. Therefore, the mutation is only going to be present in a fraction of the adult cells.

Genetic mosaics may often be confused with chimerism, in which two or more genotypes arise in one individual similarly to mosaicism. However, the two genotypes arise from the fusion of more than one fertilized zygote in the early stages of embryonic development, rather than from a mutation or chromosome loss.Most people have 46 chromosomes in each of their cells, and two of those 46 chromosomes are sex chromosomes. Most girls and women have two X sex chromosomes (so we say their chromosomal component is “46,XX”). Most boys and men have an X sex chromosome and a Y sex chromosome (“46,XY”).

But some people have “mosaic” chromosomes, meaning that not all their cells have the same component of chromosomes. This is called “mosaicism” because it is sort of like the body is made up of a varied set of colored tiles, rather than a single-colored set of tiles. When a personhas more than one component of sex chromosomes, the person is said to have “sex chromosome mosaicism.”So, some people have 46,XX in some cells with 46,XY in other cells. Some may have 46,XY in some cells and 47,XXY in other cells. Some may have 45,X in some cells and 46,XX in others. Many other variations are possible.

In some insects sometimes one part of the body is male, the other part female producing sex mosaics known as gynandromorphs or gynanders. These have been studied most thoroughlyin Drosophila which has no sex hormones so that tissues develop autonomously. The sexual phenotype is determined by the number of X chromosomes against sets of autosomes. If during mitosis in early embryogenesis

there is nondisjunction between the two X chromosomes, some cells will have XX, others XO constitution. The descendants of XX containing cells will result in the development of female tissues while descendants of XO cells will produce male tissues. The resulting individual will be a spectacular mosaic. Gynandromorphs are usually bilateral with one side of the body male, the other side female.

There are irregular gynandromorphs also in which the proportions of male to female tissues are variable depending upon the time and stage of embryo development when XX nondisjunction took place. Sex mosaics are sometimes mistaken for intersexes. In a gynandromorph the boundary between male and female regions is always sharp and distinct, whereas in intersexes, all parts of the body may have a mixture of male and female characteristics showing a condition intermediate between maleness and femaleness. Sex mosaicism occurs in humans also. Chromosome preparations from peripheral blood may show XO/XX or XO/XXY cells frequently observed in Turner's and Klinefelter's syndromes.

Meaning of Gynandromorphs:

Gynander or gynandromorphs are the organisms in which the body consists of both male and female parts. Such organisms showing both female and male characteristics are called gynanders or gynandromorphs. The term is derived from the Greek words (gyne = woman; aner = man and morphe = form). Thus, in these animals one part of the body shows female and the other part male features. These occur in silkworms, bees and fruit flies. Gynandromorphs were first described in Drosophila by Morgan and Bridges.

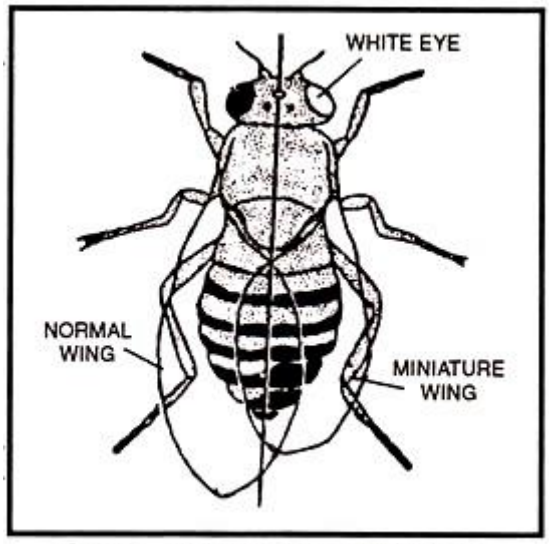


Fig. 73. Gynandromorph (*Drosophila*)

Types of Gynandromorphs:

Depending upon the position of sex tissue, the gynanders may be of the following types:

1. Bilateral Gynanders:

Some times one half of the body shows female characters while other half shows male characters. Sex intermediates of this type are called bilateral gynanders.

2. Anterior-Posterior Gynanders:

In such gynanders anterior region of the animal body has the characteristics of one sex and posterior half region has the characteristics of the other sex.

3. Sex Piebalds:

In these gynandromorphs the body consists of female tissue having spots of male tissue scattered irregularly. There are certain cases in which a few cells of the body show sex difference.

Origin and Occurrence of Gynandromorphs:

The gynandromorphs are supposed to have produced mainly by two or three methods:

(i) By Elimination of X-chromosome:

Generally, gynander begins its development with two X's. But in later stage of cell division one X gets disappeared or lost in daughter cells. Two X chromosomes in the mitosis become divided in to four X daughters chromosomes.One daughter cell receives two X's, and the other daughter cell gets only one X while fourth X becomes lost due to abnormal cell division. The daughter cells receiving two X's forms the female tissue while the other daughter cell receiving one X develops in to male tissue. The example is Drosophila.

(ii) By Retention (= holding) of Polar Nucleus in the Egg:

In silk worms female is XY. During Meiosis X and Y get separated, either of one X or Y going to egg and polocyte. But some times polar body nucleus remains in the egg along with egg nucleus. Thus, these eggs will be bi-nucleate (XY). As a result of fertilization two sperm cells which contain X, fuse with X and Y chromosome separately giving rise to male (XX) and female (XY) tissues respectively.

In bees also bi-nucleate eggs are found either due to fusion of polar body nucleus with egg nucleus or parthenogenetically producing gynanders. If this bi-nucleate egg was fertilized by single X carrying sperm then, only one of the two nuclei will be fertilized

and this one will give rise to female tissue. The unfertilized nucleus will give rise to male tissue.

Another possibility is that normal egg is fertilized by two X carrying sperm cells one of which combines with egg nucleus as usual, but the other one does not. The later then might give rise to haploid tissue, this would be male tissue.The fertilized nucleus would give rise to female tissue. Muller described another possibility of the gynander formation in a parasitic wasp-Habrobracon. In this wasp, female are heterozygous (XY) and males are either homozygous or haploid (XX).

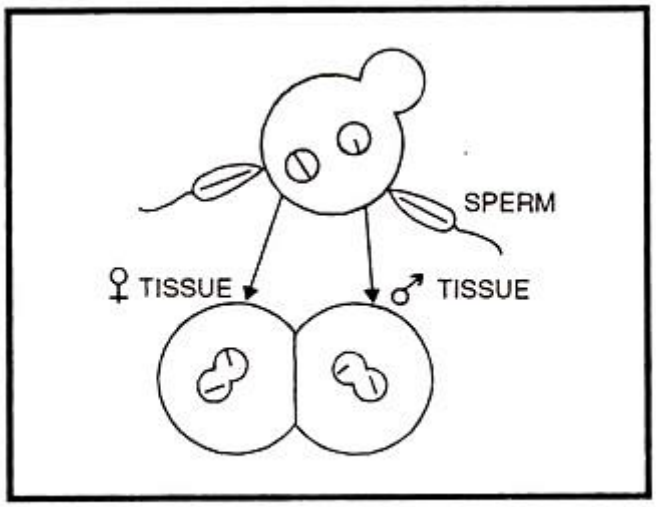


Fig. 74. Gynander's origin in silk worm.

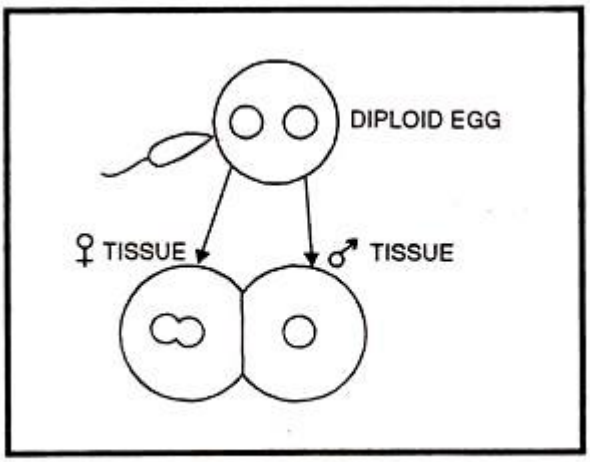


Fig. 75. Gynander's origin in bees.

The egg during oogenesis may come to contain polar nucleus as a result of fusion. Suppose egg nucleus is ‘a’ and polar nucleus is ‘b’, then both ‘a’ and ‘b’ will be inside the egg as it is bi-nucleate egg. If this bi-nucleate egg is fertilized by two sperms (suppose having ‘b’) the fertilized nuclei will be ‘ab’ and ‘bb’. The first ‘ab’ will form female tissue and ‘bb’ will develop in to male tissue.

Thusgynanders are produced mainly by the bi-nucleate eggs which are characteristic of certain bee races. These changes may also be brought about by sex hormones secreted by the primary sex glands. In rigid sense ‘gynandroniorph’ should be confined to individuals in which secondary sexual characters are not under the control of sex hormones.

Probable Questions:

1. What do you mean by Sex determination? What is its importance?
2. How sex is determined in XX-XY system?
3. How sex is determined in ZZ-ZW system.
4. What is Genic balance theory?
5. How sex is determined in human being?
6. Describe the role of Y chromosome in human sex determination.
7. Briefly describe morphology of a ideal chromosome.
8. Classify chromosome on the basis of position of centromere.
9. What are the differences between primary and secondary constrictions?
10. Define Karyotype and ideogram. What are the differences between asymmetric and symmetric karyotype.
11. Write a short notes on B chromosome.
12. Write a short notes on Lampbrush chromosome.
13. Write a short notes on Polytene chromosome.
14. Define mega, micro and limited chromosome.
15. Describe solenoid model of chromosome structure
16. Describe single stranded and double stranded model of chromosome structure.
17. Write down the chemical structure of chromosome.
18. What are the biological importance of chromosome?
19. Describe genic balance theory.
20 Describe the structure of Y chromosome.
21. Describe Dosage compensation.
22. What is Barr body?

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7th Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwal.

UNIT-VIII

Sex chromosome anomalies; sex influenced and sex limited genes

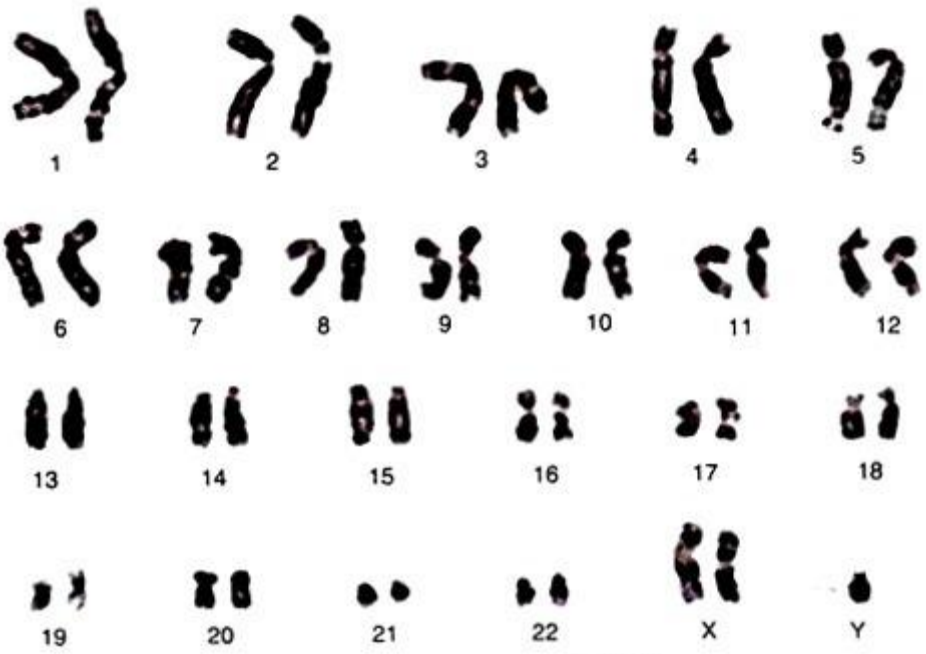
Objective: In this unit we will discuss sex chromosome anomalies and also discuss about sex influenced and sex limited genes

Sex Chromosome Anomalies:

i. Klinefelter's Syndrome:

In 1942 Klinefelter described a condition in phenotypic males which turned out to be due to an extra X chromosome (47, XXY). The affected individuals appear normal in childhood, the abnormalities becoming visible only in adult males. The syndrome is characterised by absence of spermatogenesis, gynaecomastia, and excessive secretion of gonadotropins in the urine.

Since buccal smears of Klinefelter's males show Barr bodies, they are referred to as chromatin-positive males. Most of the patients are mentally retarded and develop a variety of psychiatric problems. Although many have the karyotype 47, XXY, some may have 48, XXXY, 49, XXXXY, or 48, XXYY, or they may be cytogenetic mosaics.



Klinefelter syndrome—47,XXY

ii. Turner's Syndrome:

This is shown by females characterised by a short stature, gonadal dysgenesis, sexual infantilism, webbed neck, prominent ears, cubitus valgus (increased carrying angle of the arms) dystrophy of the nails and hypoplastic nipples.

Their sex chromosome constitution is XO and they have only 45 chromosomes. They are chromatin-negative females as they do not show Barr bodies. They are frequently mosaics with more than one cell line such as XO/XXX, XO/XX/XXX, and others. The incidence is one in about 5,000 births.

iii. The XYY Male:

"super-males" are usually tall (above 6 feet) and generally appear and act normal. However, they produce high levels of testosterone. During adolescence, they often are slender, have severe facial acne, and are poorly coordinated. They are usually fertile and lead ordinary lives as adults. Many, if no

In 1965 Jacobs et al found that many of the men kept in institutions for the retarded due to aggressive and antisocial behaviour have 47 chromosomes with XYY sex chromosomes. They are usually tall but not always mentally retarded, frequently show hypogonadism and are sterile. Males inherit an extra Y chromosome--their genotype is XYY. As adults, these t most, are unaware that they have a chromosomal abnormality. The frequency of XYY syndrome is not certain due to statistical differences between different studies. It may be as common as 1 in 900 male births to as rare as 1 in 1500 or even 1 in 2,000. XYY syndrome is also referred to as Jacobs syndrome. Early studies of XYY syndrome done in European prisons initially led to the erroneous conclusion that these men were genetically predisposed to antisocial, aggressive behaviour, below average intelligence, and homosexuality. Contributing to the early view that XYY syndrome men have serious personality disorders was the case of Richard Speck. In 1966, he coldly murdered 8 nurses in a Chicago dormitory. At his trial, his lawyer claimed that he was innocent due to uncontrollable urges caused by his XYY genotype. This novel appeal was akin to claiming insanity or severely diminished mental competence. The jury was not convinced and found him guilty of murder. He was sentenced to life in prison where he eventually died. In fact, Richard Speck did not have an XYY genotype. However, some researchers suggest that the high testosterone levels of XYY men can make them somewhat more prone to violence and that this may cause higher rates of wife beating. The presence of two Y chromosomes can be recognized as two brightly fluorescent bodies by proper staining. The discovery of this syndrome received publicity because of the possible association of a chromosome anomaly with human behaviour.

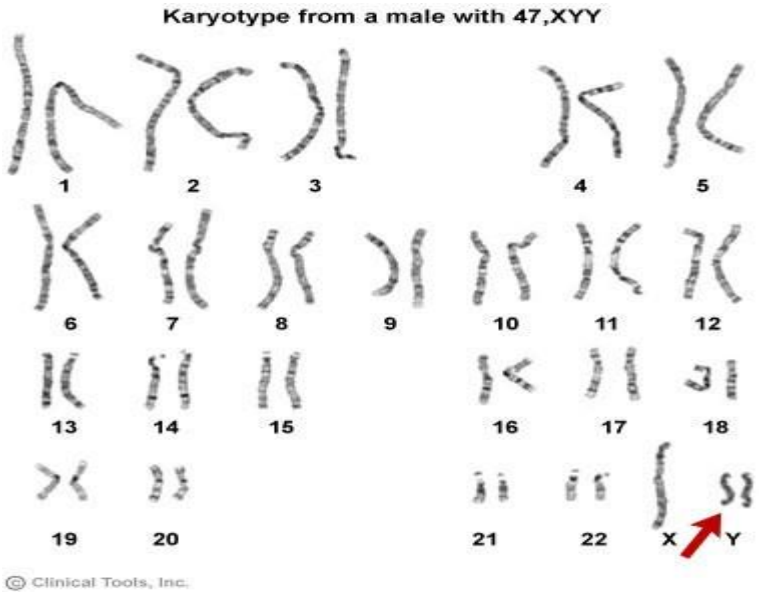


Fig : 47,XYY karyotype showing extra Y Chromosome at right side

iv. The Triple-X Syndrome:

These are individuals with 3X chromosomes designated super-females. They are mentally retarded, sexually normal and fertile. Although most triple-X females have 47, XXX karyotype, some may have 48, XXXX, 49, XXXX, and still others may be mosaics. They show 2, 3 or 4 Barr bodies in their buccal smears (always one Barr body less than the total number of X's).

It occurs in women who inherit three X chromosomes--their genotype is XXX or more rarely XXXX or XXXXX. As adults, these "super-females" or "metafemales", as they are sometimes known, generally are an inch or so taller than average with unusually long legs and slender torsos but otherwise appear normal. They usually have normal development of sexual characteristics and are fertile but tend to have some ovary abnormalities that can lead to premature ovarian failure. They may have slight learning difficulties, especially in speech and language skills, and are usually in the low range of normal intelligence (especially the XXXX and XXXXX individuals). They frequently are very tall in childhood and tend to be emotionally immature for their size. This sometimes results in teachers and other adults labeling them as troublemakers because they expect more maturity from bigger girls. However, they are usually as emotionally mature as other girls of their age. None of these traits prevent them from being socially accepted as ordinary adult women. Individuals who are genetic mosaics (XX/XXX)have less noticeable symptoms. Triple-X syndrome is less rare than Turner syndrome, but little is known about it. The frequency is approximately 1 in 1,000 female infants and it occurs more commonly when the

mother isolder.

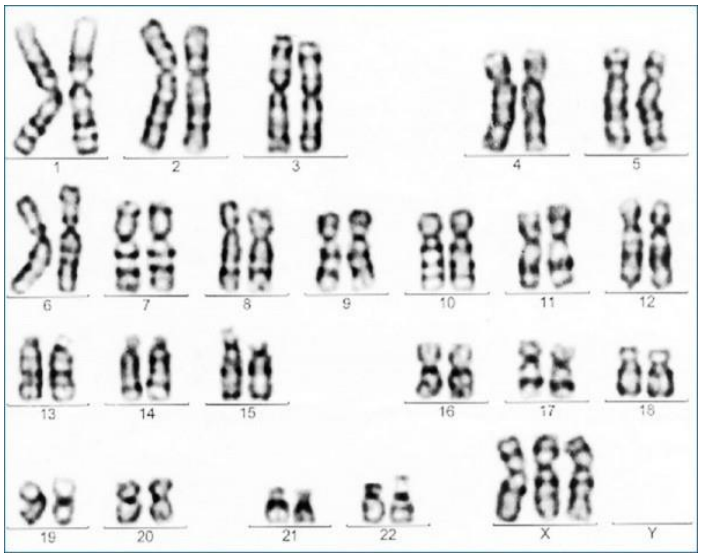


Fig : Karyotype of Triple XXX Syndrome

Individuals with both male and female gonadal tissues are called hermaphrodites (from Greek Hermaphrodites, the son of Hermes and Aphrodite). Their karyotype analysis shows that they are mosaics having both X and Y chromosomes in their cell lines. Their buccal smears may or may not show a Barr body. Their external genitalia are often ambiguous, and they are almost always sterile.

The condition of pseudo-hermaphroditism is also included among intersexes. Such individuals are cytogenetically normal with 46, XY (male pseudo-hermaphrodites) or 46, XX (female pseudo-hermaphrodites) chromosomes and normal buccal smears for one sex only. But phenotypically they show both male and female characters. There are two classes. Male pseudo-hermaphrodites that have testes and either ambiguous or female-like external genitalia. The female pseudo-hermaphrodites have ovaries and either ambiguous or male like external genitalia. The pseudo-hermaphrodites have some defect in the biosynthesis of testosterone in the testes or in the adrenal glands or in both.

Sex Limited Genes:

Sex limited genes are those which produce characteristics that are expressed in only one of the sexes. They are often confused with sex linked genes, but are entirely different in their mode of inheritance. Sex limited genes may be located in any of the chromosomes, while the sex linked genes are located only in the X or Z chromosome. Sex limited genes are responsible for secondary sexual characteristics as well as primary characters.

The beard in males is a good example in man. Both the males and the female carry all the genes necessary to produce a beard, but only man shows this trait. However, in rare cases, hormone imbalance in a woman results in a bearded lady. Similarly, breast development is normally limited to women, but hormone imbalance may cause breast development in men. In vertebrates, the sex limited characteristics depend upon the presence or absence of one of the sex hormones. For instance, the genes for masculine voice and masculine musculature depend on the presence of male hormones.

A castrated male will have female voice even though no female hormones are present. The genes for feminine voice and feminine musculature express themselves in the absence of the male hormone. They do not require the presence of female hormones. Thus, certain sex limited characteristics are expressed in the presence of sex hormones, while certain others are expressed in the absence of certain hormones. The sexual dimorphism in birds is another good example of sex limited inheritance. The bright plumage of the male peacock is a bold contrast to the dull plumage of the female.

Sex Influenced Traits:

The phenotypic expression of a number of autosomal and sex linked genes will be either dominant if the individual is a male or recessive if the individual is a female. These genes are known as sex influenced traits. A classic example is the pattern baldness in man.

A male shows this trait more than a female, because a male is bald if he has only one gene, whereas a woman must receive two genes to be bald. This is because a single gene can operate in the presence of a male hormone.

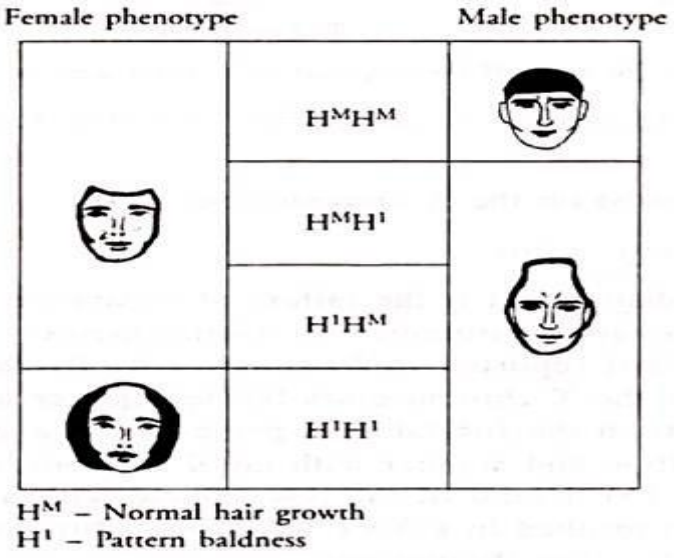


Fig. 16 Pattern baldness in man.

Another example is the length of the index finger. When the hand is placed so that the tip of the fourth finger touches the horizontal line, it will be noted that the index or second finger will not touch this line in many cases (Fig. 17). This short index finger is due to a gene which is dominant in the male and recessive in the female.

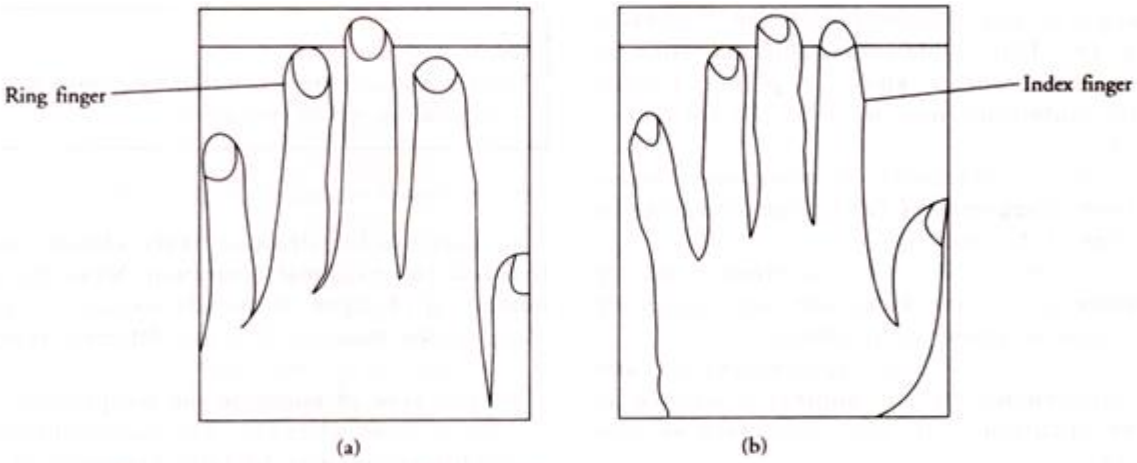


Fig. 17 Sex-influenced inheritance of length of index finger.

Y Chromosome Inheritance:

Y linked genes are genes located in the Y chromosomes. The inheritance of the Y linked genes, also known as holandric genes, is known as Y chromosome inheritance. An example is hypertrichosis, which is the growth of long hair in the ear.

Probable Questions:

1. What are the characteristics of Klinefelter syndrome?
2. What are the characteristics of Turner's syndrome?
3. What are the characteristics of XXX syndrome?
4. What is intersex. State its characteristics.
5. What is sex limited traits. Give examples.
6. what is sex influenced traits. Give examples.

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7th Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwa

UNIT-IX

Behavioral genetics influence of single defects on behavior.

UNIT-X

Genetic analysis of behavior in experimental animals.

UNIT-XI

Chromosome anomalies and insight into human behavior.

Objective: In these three units you will learn about Behavioral genetics influence of single defects on behavior; Genetic analysis of behavior in experimental animals, chromosome anomalies and insight into human behavior.

Behavioural Genetics:

Behavioural genetics, also referred to as behaviour genetics, is a field of scientific research that uses genetic methods to investigate the nature and origins of individual differences in behaviour. While the name "behavioural genetics" connotes a focus on genetic influences, the field broadly investigates genetic and environmental influences, using research designs that allow removal of the confounding of genes and environment. Behavioural genetics was founded as a scientific discipline by Francis Galton in the late 19th century, only to be discredited through association with eugenics movements before and during World War II. In the latter half of the 20th century, the field saw renewed prominence with research on inheritance of behaviour and mental illness in humans (typically using twin and family studies), as well as research on genetically informative model organisms through selective breeding and crosses. In the late 20th and early 21st centuries, technological advances in molecular genetics made it possible to measure and modify the genome directly. This led to major advances in model organism research (e.g., knockout mice) and in human studies (e.g., genome-wide association studies), leading to new scientific discoveries.

Findings from behavioural genetic research have broadly impacted modern understanding of the role of genetic and environmental influences on behaviour. These include evidence that nearly all researched behaviors are under a significant degree of genetic influence, and that influence tends to increase as individuals develop into adulthood. Further, most researched human behaviours are influenced by a very large number of genes and the individual effects of these genes are very small. Environmental influences also play a strong role, but they tend to make family members more different from one another, not more similar. Despite progress on these findings, the field continues to wrestle with unrealistic statistical and scientific assumptions.

Selective breeding and the domestication of animals is perhaps the earliest evidence that humans considered the idea that individual differences in behaviour could be due

to natural causes. Plato and Aristotle each speculated on the basis and mechanisms of inheritance of behavioural characteristics. Plato, for example, argued in The Republic that selective breeding among the citizenry to encourage the development of some traits and discourage others, what today might be called eugenics, was to be encouraged in the pursuit of an ideal society. Behavioural genetic concepts also existed during the English renaissance, where William Shakespeare perhaps first coined the terms "nature" versus "nurture" in The Tempest, where he wrote in Act IV, Scene I, that Caliban was "A devil, a born devil, on whose nature Nurture can neverstick".

Modern-day behavioural genetics began with Sir Francis Galton, a nineteenth-century intellectual and cousin of Charles Darwin. Galton was a polymath who studied many subjects, including the heritability of human abilities and mental characteristics. One of Galton's investigations involved a large pedigree study of social and intellectual achievement in the English upper class. In 1869, 10 years after Darwin's Origin of the species, Galton published his results in Hereditary Genius. In this work, Galton found that the rate of "eminence" was highest among close relatives of eminent individuals, and decreased as the degree of relationship to eminent individuals decreased. While Galton could not rule out the role of environmental influences on eminence, a fact which he acknowledged, the study served to initiate an important debate about the relative roles of genes and environment on behavioural characteristics. Through his work, Galton also "introduced multivariate analysis and paved the way towards modern Bayesian statistics" that are used throughout the sciences—launching what has been dubbed the "Statistical Enlightenment".

The field of behavioural genetics, as founded by Galton, was ultimately undermined by another of Galton's intellectual contributions, the founding of the eugenics movement in 20th century society. The primary idea behind eugenics was to use selective breeding combined with knowledge about the inheritance of behaviour to improve the human species. The eugenics movement was subsequently discredited by scientific corruption and genocidal actions in Nazi Germany. Behavioural genetics was thereby discredited through its association to eugenics. The field once again gained status as a distinct scientific discipline through the publication of early texts on behavioural genetics, such as Calvin S. Hall's 1951 book chapter on behavioural genetics, in which he introduced the term "psychogenetics", which enjoyed some limited popularity in the 1960s and 1970s. However, it eventually disappeared from usage in favor of "behaviour genetics".

The start of behavior genetics as a well-identified field was marked by the publication in 1960 of the book Behavior Genetics by John L. Fuller and William Robert (Bob) Thompson. It is widely accepted now that many if not most behaviours in animals and humans are under significant genetic influence, although the extent of genetic influence for any particular trait can differ widely. A decade later, in February 1970, the first issue of the journal Behavior Genetics was published and in 1972 the Behavior Genetics Association was formed with Theodosius Dobzhansky elected as the association's first president. The field has since grown and diversified, touching many

scientific disciplines.

Genetic analysis of behaviour in experimental animals:

Animal research can serve as models of gene-environment interactions and diseases identified in humans. In the case of social control of disease processes, the choice of species to be studied depends on the level of social interactions that needs to be examined. For example, rodent models can demonstrate how differences in social status, population density, or early experiences interact with genetic makeup to affect susceptibility to disease (e.g., examine effects of social factors in knockout or knock-in animals [or inbred strains] that differ in susceptibility to infection, cancer, autoimmunity). The advantages of rodent models include significant control over genetic, physiological, behavioral, and social factors and relatively short reproductive, developmental, and life cycles. They are amenable to studying a variety of important psychosocial variables, including social isolation, social relationships, attachment, parenting, temperament, and motivational states.

However, nonhuman primate models, which offer limited control over genetic factors and have a longer life span, may be best suited to examine the consequences of more complex social factors, such as those involving cooperation or trust. For example, after bouts of aggression, nonhuman primates demonstrate reconciliatory behavior that is thought to be important for maintaining cooperative social hierarchies (de Waal, 2000). Some aspects of human behavior (e.g., optimism, hope, guilt) may be studied in animals only when the investigator can demonstrate a robust animal model with multiple behavioral paradigms as well as shared neural mechanisms.

In addition, animal models developed for traditional biomedical research are also powerful models for studying the psychosocial modulation of known mechanisms of specific human diseases. There are many animal species, strains, and transgenic models developed through biomedical science, that have been well characterized in terms of the genetic, molecular, and cellular processes underlying human disease. Studying these animals in a variety of psychosocial paradigms, based on variables identified through survey, epidemiological, and human experimental research, can test hypothesized causal relations derived from correlational data in humans.

It is essential to study animals as evolved biological systems in which surviving and reproducing in particular social and physical environments have selected a constellation of interactions between social, behavioral, physiological systems, and gene function. Doing so reveals insights and principles that also underlie human health and disease but that are not salient in the modern world or in a typical biomedical approach. Moreover, ethology and evolutionary biology recognize that individual differences are not necessarily just “noise,” but represent different evolved strategies for survival in different contexts. Taking an ethological approach to variation in strategies reveals the range of gene-environment interactions that occur within

species as they have evolved in their natural ethological and ecological contexts.

Studies of deer mice (*Peromyscus maniculatus*), who live in highly seasonal environments, reveal that function of the immune system requires significant energy, so much so that during winter an animal trades off entering puberty and becoming reproductive in order to sustain the energetic requirements of fighting infectious disease (Prendergast and Nelson, 2001; Nelson, 2004). It is not the demands of the cold weather itself that signals this trade-off, but rather the shortened days that precede seasonal temperature change, allowing the animal to modulate relative balance of immune function and reproduction in anticipation of the energetic demands ofwinter.

In house sparrows, immune activity increases energy expenditure, illustrating the energetic costs of immune function that could otherwise be deployed to growth (Martin et al., 2003). Such animal research, set in an ecological context, provides a powerful animal model for such trade-offs in humans. When social structure restricts resources and results in a population living in an environment with a high pathogen load, slower growth can result, as is the case of children in the lowlands of Bolivia. This presumably happens because the allocation of energetic resources to immune function has been diverted from growth (McDade, 2005). This dynamic interaction between social access to energy stores, pathogen interaction, fat deposition, and growth likely involves leptin, a pleiotropic molecule with cytokine properties that is produced by fat cells during an inflammatory response (Faggioni et al., 2001; Fantuzzi, 2005).

Animal research has clarified concepts that are key to understanding the effects of social environment on health and disease and gene function, extending and moderating the conclusions based on epidemiological studies in humans. These concepts include genetics, immune and neuroendocrine function, causality, pleiotropy, and life-span fitness.

Genetics requires a broad conception that includes both functional genomics (intra-individual changes in gene expression over time) and the more traditional topic of structural polymorphism (inter-individual variations in DNA sequence or epigenetic characteristics). This broad conceptualization is essential because social influences on gene transcription are fairly well studied, while few studies have examined the relationships between social factors and genetic polymorphisms. That such effects exist is likely because structural polymorphisms generally exert their effects in the context of expressed genes.

An essential role of animal research is to test the relationship between presumptive genetic influences (e.g., inferred from studies of heritability) and defined genetic influences (e.g., effects attributable to the expression of specific genes or epigenetic characteristics). The immune system includes classical immune cells (e.g., leukocytes) as well as other cellular contexts relevant to disease pathogenesis or host defense, such as somatic cells responding to pathogens through innate immune responses (e.g., “danger signals” produced by Toll-like receptors, Type I interferon production). The

neuroendocrine system also is broadly defined to include not only true neurally driven hormone production (e.g., hypothalamic-pituitary-adrenal [HPA] axis), but also neuroeffector processes that do not necessarily involve systemic hormone distribution (e.g., local effects of neurotransmitter release from autonomic or sensory neurons or neuropeptides such as vasopressin and oxytocin).

Part of the reason so few genetic determinants of immune response currently are presently known may be an overly restrictive focus on “immune system” genes. Polymorphisms in many “nonimmune” genes, which are regulated by the psychosocial environment through physiological systems, may also influence leukocyte function and/or the pathogenesis of diseases involving immune or inflammatory components. For example, catecholamines are known to influence several aspects of leukocyte function (Sanders and Straub, 2002; Kavelaars, 2002), and polymorphisms in genes encoding their alpha—and beta—adrenergic receptors are associated with differential incidence of asthma, parasitic infections, and cardiovascular disease (Ramsay et al., 1999; Ulbrecht et al., 2000; Ukkola et al., 2001; Weiss, 2005; Thakkinstian et al., 2005; Lanfear et al., 2005). Glucocorticoids, another physiological system exquisitely sensitive to the psychosocial environment, play a key role in regulating inflammatory gene expression (Webster et al., 2002), and polymorphisms in the glucocorticoid receptor gene (NR3C1) have been linked to cardiovascular and autoimmune disease (Lin et al., 1999; Ukkola et al., 2001; Jiang et al., 2001; Dobson et al., 2001; van Rossum et al., 2002).

Chromosomal anomalies and insight into human behaviour:

The belief that demonic and supernatural forces caused deviant behavior was once widely held by human societies. In the western world it was reflected in such practices as witch burning and exorcism. Beginning with the Age of Enlightenment, the concept of the "bad seed," the idea that incorrigible antisocial behavior was inborn, gained acceptance. Many later attempts were made to correlate criminal behavior with certain physical characteristics of the individual,

and the hypothesis was advanced that criminal behavior stemmed from an atavistic level of biologic organization that was expressed in particular physical characteristics or anthropometric marks of inferiority. Efforts have continued to the present day to relate specific physical characteristics with sociopathic behavior. The studies of Dugdale and Goddard on the Jukes and Kallikak families, respectively, at the beginning of this century identified the role of heredity in the constellation of feeblemindedness, crime, and disease, and since then, much research has focused on heredity. Twins were studied in an attempt to demonstrate that genetic factors were involved in the origins of criminal or antisocial behavior, but the results were inconclusive because of the difficulty inherent in separating genetic from experiential factors. Discovery of the role of chromosome abnormalities in the etiology of human disease gave renewed impetus to the search for a genetic basis underlying behavioral disturbances. The report of Lejeune et al in 1959 linking Down's syndrome with trisomy 21 was quickly followed by discovery of the specific chromosome abnormalities in Turner's syndrome, Klinefelter's syndrome, and other conditions. These abnormalities have been

discussed in previous issues. In general, the autosomal defects, when not lethal, confer severe mental and physical handicaps; the effects of the sex chromosome anomalies appear to be more variable.

These are Turner's syndrome (45, X), the triple-X (4 7, XXX) female, Klinefelter's syndrome (4 7, XXY), and the 4 7, XYY male. The most common chromosomal abnormality seen in Turner's syndrome is the absence of the second X chromosome. Affected individuals tend to be short in stature (generally less than 5 feet tall), have webbing of the neck and nonfunctional, "streak" ovaries, and are sterile. Early reports of Turner's syndrome indicated that these individuals were mentally retarded, but most of the studies were carried out on institutionalized patients. What has since been learned about the behavioral effects of Turner's syndrome stems mainly from a series of studies carried out at Johns Hopkins University under the leadership of John Money, who obtained his cases from an outpatient endocrine clinic. Money and his coworkers found that I *Q* was not particularly depressed among Turner patients. In one study of 38 Turner patients, I *Q* was found to range from under 70 to above 130, with a mean of 96, which is close to normal. The most significant finding, however, was a difference of from 10 to 20 points between verbal and performance scores of the Turner's patients on the W eschsler I *Q* test, with the verbal score always higher. This result has been replicated by two other groups, one in Leyden and one in Boston. Methodologic criticism notwithstanding, a verbal-performance difference seems to be substantial. These findings are of interest because a verbal-performance I *Q* difference, favoring verbal, may be a cue to the existence of brain damage in the dominant or involved hemisphere. It is not known, however, whether Turner individuals actually have brain damage. Several investigators have reported an association between Turner's syndrome and abnormal EEG tracings, but the number of cases is too small to permit firm conclusions. Money and his group attributed the poorer performance I *Q* among Turner cases to what they called "space-form blindness." At least some persons with this syndrome have difficulty in telling left from right in others. Although they have a sense of their own left and right, when tested on a road map and asked whether a turn is being made to the left or to the right, Turner patients tend to confuse direction, and they display an inability to integrate the fact that objects rotate in space. They also do poorly on the Bender Gestalt test, a test of perceptual motor skills in which the individual is asked to copy designs while looking at them (see opposite page). Presumably, the Turner individuals' lower scores on the performance aspect of I *Q* tests is related to the fact that performance tasks call for motor skills. The deficit in cognitive and perceptual motor functioning is small and could be compensated for even if consistently present. It might not show up as a gross defect in behavioral functioning. The XXX female is usually physically normal and a considerable proportion of those studied have normal gonads. Interest has focused on the XXX abnormality recently because of findings by Arnold Kaplan in Cleveland and, independently, two investigators in the Soviet Union that the incidence of XXX females in mental institutions, particularly among schizophrenic populations, is higher than their incidence in the general population.

The observation is of interest because it suggests a distinct genotypic basis for a subgroup of the schizophrenias, but the evidence thus far is inconclusive. Many of the women studied have been of advanced age, introducing the possibility that the chromosome defect was not present at birth. It has been reported that a total of 16,000 cases of women institutionalized for mental abnormality or illness. Of this number, 42 were XXX and 46% of the sewereaged 60 or older. The incidence of x xx females in mental institutions is about 2.6 per 1,000, whereas the incidence among newborn females ranges from 0.3 to 1.4 per 1,000.

Thus, it would appear that there is a twofold or greater increase of x xx females in mental institutions over the newborn incidence. The inference is clear that x xx aneuploidy carries a heavy risk of behavioral abnormality, but one important statistic is missing from the calculation-: It is not known how many of the 42 institutionalized women were born with a 47, XXX karyotype. This is an important consideration since two laboratories have independently demonstrated that chromosome abnormalities, particularly those involving the C group of chromosomes, to which the X belongs, tend to increase with advancing age, especially in females. The large increase in x xx females in mental institutions may therefore be an age-related phenomenon. To find out, it would be necessary to compare the institutionalized population with an age-matched sample but, at present, information on the incidence of chromosomal disorders for age groups other than newborns is not availablein sufficient numbers. Almost invariably, it is pointed out that males with an extra X chromosome are mentally subnormal. Most of the evidence comes from anapparentfive- to sixfold increase over the newborn rate of Klinefelter individuals in institutions for the mentally subnormal. The x x \' anomaly is found among newborn males at a rate of about 1.2per I ,000, while the rate among institutionalized mentally subnormal males is 9.4 per 1,000. However, anybody examine the 1 Q scores of Klinefelter individuals detected in endocrine or fertility clinics, he will find that, like those with Turner's syndrome, they show a range from mild retardation to above average. Males with multiple X aneuploidy also show a wide variability in phenotype. Although they all tend to have more or less the same physical stigmata, with hypogonadism the most consistent feature, physical signs vary, depending on where the individual is detected. Those seen in endocrinology clinics tend to have more marked- gynecomastia, female pubic hair distribution, and absence of facial hair, whereas those seen in fertility clinics. Tend to have more normal physical characteristics. Aside from mental subnormality, the outstanding behavioral feature of Klinefelter's syndrome is a reported tendency toward aggressiveness that seems to carry a higher than average risk that the patients will end up in prisons and other penal institutions. A review of the literature that included more than 20 different surveys of prisons, special security institutions, and institutions for the criminally insane revealed that XYY individuals are to be found in such institutions at a rate of about 8 per 1,000. However, the meaning of the increase over the newborn rate is equivocal. Since other pertinent data on these individuals is missing, such as their ethnic, social, and family background, the conclusions that can be drawn from the institutional incidence are limited. It has been suggested that the institutionalization of some Klinefelter individuals may be the result of factors not directly related to the

chromosomal disorder. For example, a sentencing judge is more likely to institutionalize someone who does not have family responsibility than someone who is a parent and has dependents. Klinefelter individuals are less likely to be married, and certainly less likely to be parents because they are usually sterile. The decision-making factor involved in how people get into and out of institutions is one of the variables affecting incidence that has not been investigated. Another chromosomal abnormality possibly associated with criminality is the XYY karyotype. The first case, detected in 1961 in Buffalo, was a 44-year-old man, physically normal, of average intelligence, and the father of seven living children from two marriages. Most important in this context, he was not a criminal; ·rather, his chromosomal abnormality was discovered because of mongolism and other congenital anomalies among his children. By 1965, a total of 12 cases had been detected, most of them because they had some physical anomaly that brought them to medical attention and karyotyping. The association of an extra Y chromosome with criminality was not made until 1965, when M. D. Casey carried out a sex chromosome survey among individuals in penal institutions in England and discovered 21 with chromosomal abnormalities, of whom one third had an extra Y chromosome. Patricia Jacobs and her colleagues in Edinburgh postulated that the extra Y chromosome might carry the risk of personality defect predisposing the bearer to penal institutionalization. She carried out a chromosome survey of 315 men in a maximum security hospital for criminal psychopaths at Carstairs, Scotland, and found nine with the XYY karyotype, or almost 3% of the inmate population. Jacobs and her colleagues also observed that, on the average, these individuals were approximately six inches taller than the chromosomally normal inmates. It is from this study that the association of the XYY karyotype with criminality and tallness can be said to have originated. Subsequently, a series of surveys were carried out by different groups of investigators studying populations in penal institutions. In most of the studies, sampling focused on taller than- average individuals and, in fact, many XYY individuals were found. From these surveys, the following characteristics emerged as being potentially associated with the extra Y chromosome: the individual has one or more physical or physiologic abnormalities; he tends to be tall, mentally subnormal or of lower than average intelligence, impulsive and hyper aggressive; his criminal activity usually begins at an early age; often there is no predisposing family history to account for his criminal behavior, and therefore, inferentially, it is the extra Y chromosome that contributes to his 134 criminality; and lastly, his numbers in maximum security institutions exceed by as much as 20-fold the incidence of XYY males in the newborn population. Therefore, he has a considerably higher than average risk of behavioural dysfunction and conflict with authority. With respect to the physical and physiologic abnormalities, a large variety of abnormal characteristics has been found in at least one or more reported XYY cases. These include hypogonadism, webbing of the neck, vascular abnormalities, varicoseulceration, bone and joint abnormalities, and fingerprint and dermatoglyphic alterations. Some investigators have also noted acne and other skin disorders in these individuals. While all of these abnormalities have been found in a small proportion of XYY cases, it is important to note that the majority have appeared to be physically normal. Studies of urinary and plasma testosterone

levels in XYY men are of interest because of the known association between androgens and aggressive behavior. Elevated testosterone levels might be a mechanism through which the extra Y chromosome could influence or exacerbate behavior, particularly behavior arising at the time of puberty. Although individual cases of XYY men have been shown to have elevated levels of testosterone, when compared with chromosomally normal fellow inmates, no significantly different plasma or urinary testosterone levels have been found. Institutionalized XYY and XY males, however, both appear to have higher testosterone excretion levels than chromosomally normal noninstitutionalized controls. It is possible that institutionalization alone has an effect on testosterone levels. At any rate, the testosterone findings have at this point very dubious significance. Gonadotropin levels have also been studied, and here again, when the XYY males are matched with an adequate control group, no significant differences appear. Height is the one characteristic that has shown a strongly suggestive association with the XYY karyotype. Even when sampling is not confined to males 5'11" or taller, thosewiththe XYY karyotype turn out to have an average height greater than males with a single Y chromosome. For example, in Jacobs' survey at Carstairs, in which virtually every inmate was studied, the nine XYY men detected had a mean height of 181.2 cm, whereas the 305 inmates with a single Y chromosome had a mean height of 170.7 cm. Of 23 individuals detected by other investigators since 1961 for reasons other than height, 11 of them were six feet tall or taller, so the data do seem to show that the Y chromosome is related to height. The implication that the XYY karyotype carries a risk for sociopathic behavior, however, remains to be proved. Data imputing a higher risk for criminal behavior, including youthful age at first conviction, come mainly from the study by W. H. Price and P. B. Whatmore of the nine XYY males discovered at Carstairs. They compared these nine individuals, of whom seven were in a wing of the hospital for the mentally subnormal and two were in a wing for the mentally ill, with 18 inmates in the same institution selected at random from the entire chromosomally normal population of the hospital. Their findings indicated XYY's commit fewer crimes of violence against persons than the controls; XYY's manifest disturbed behavior at an earlier age than controls, as measured by age at first conviction - 13.1 years for the XYY's as against 18 years for the controls; siblings of the XYY's commit significantly fewer crimes than siblings of controls. Therefore the XYY’s criminal behavior is not influenced by familial environment and begins at an age too early to be influenced by factors outside the home; and XYY's are not amenable to rehabilitation. In other words, the x Y Y's behavior was thought to be incorrigible.

The investigators concluded that the XYY's behavior dysfunction is associated solely with the chromosomal abnormality, but this conclusion is premature for a number of reasons. First, it need not be argued that a familial predisposition to crime, determined by the number of convictions among the siblings, is present in the families of controls; 11 of the 18 controls came from families in which there was no history of criminal conviction among the siblings. The lack of criminal behavior in other family members does not rule out familial encouragement of aggressive behavior, however. Parental disciplinary and socialization practices have an influence on aggressive behaviour that

can be seen very early in a child's development. Influences on behavior from peers, school, and other sources outside the home also begin at an early age. Furthermore, it is doubtful whether age at first conviction is any indication that behavioural dysfunction begins at an earlier age in XYY's than in chromosomally normal individuals convicted of crimes. Indeed, the increased height of the XYY boy may be the critical factor. It is certainly conceivable that a court would be more likely to convict and punish a tall than a short adolescent. Subsequent studies have produced contradictory evidence on this point, some showing no difference in age at first conviction between x \' \' and x Y inmates. As for the behavioral characteristic of aggressiveness that has been linked with the x *Y* \' karyotype, the Price and Whatmore study made the point quite clearly that the x \' Y's at Carstairs were no more aggressive than their chromosomally normal controls. According to their penal records, the nine XYY inmates had a total of 92 convictions and the 18 controls 210. Only 9% of the XYY convictions were for crimes against persons, however, in contrast to 22% among the controls. On an observational basis, the x Y Y's also seemed to exhibit less aggressive behavior in the institution than the chromosomally normal inmates, who were more openly hostile and more likely to have violently aggressive outbursts. Other studies comparing criminal records have produced varying behavior, however. Parental disciplinary and socialization practices have an influence on aggressive behaviour that can be seen very early in a child's development. Influences on behavior from peers, school, and other sources outside the home also begin at an early age. Furthermore, it is doubtful whether age at first conviction is any indication that behavioural dysfunction begins at an earlier age in x YY's than in chromosomally normal individuals convicted of crimes. Indeed, the increased height of thex Y \' boy may be the critical factor. It is certainly conceivable that a court would be more likely to convict and punish a tall than a short adolescent. Subsequent studies have produced contradictory evidence on this point, some showing no difference in age at first conviction between x \' \' and x Y inmates.

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Since x Y v's have been identified who do not exhibit any abnormal or criminal behavior, it seems entirely possible that other genetic and/or environmental factors contribute to the antisocial behavior of the XYY's who have been studied in

institutions. The early findings from a prospective study of four XYY boys identified at birth are of interest in this regard. At three or four years of age, three of these boys were found to be functioning and developing normally, but the fourth child was described as hard to manage, hostile, and given to climbing to heights and breaking things. He was the one child out of the four who had been placed in a foster home, and by the time he was four years old, had already been in multiple foster homes.

Even if XYY's are more aggressive than normal individuals, which is' unclear from the studies done to date, one could not necessarily attribute it to a causal effect of the extra Y chromosome. One would have to look very carefully at the kinds of extra-institutional environments in which these individuals were reared and, specifically at how the expression of aggression was handled in those environments. If it can be proved that the extra Y chromosome is related to behavioral dysfunction -specifically, aggressiveness - the important issue will be how to treat these individuals to ameliorate the effects. Price and Whatmore concluded that institutionalized XYY's were rehabilitative failures, to be relegated to a life of crime or institutional confinement. As for rehabilitation failure, little is known about the techniques employed to help these individuals. In a maximum security institution, relatively ineffective methods may have been used. There is considerable evidence that the expression of aggression by an individual varies substantially from one environmental setting to another. It should be possible, through behavioural therapy or environmental construction in or out of institutions, to ameliorate or suppress the behavioural effects of an extra Y chromosome, assuming there are any. To summarize, then, the hypothesis that individuals with an extra Y chromosome have an increased risk for aggressive behavior or criminality appears attractive, but at this time there is essentially no good evidence to support it. In 1971, Jacobs carried out a chromosome survey of 2,500 men in penal institutions in Scotland and found no increased incidence of either XXY or XYY males over their respective newborn incidences. Fragmentary as our statistics are for the U.S., it can be assumed that at least 95% of males with the XYY karyotype are functioning at levels of behaviour adequate to keep them from coming into conflict with the law.

The available evidence of a risk for behavioral dysfunction must be interpreted very carefully because of the potential implications for individual families. For example, there seems to be no uniformity of opinion among geneticists or pediatricians as to whether to withhold the information from the parents of children detected with the x Y Y karyotype. Some centers have withheld the information from parents, but hiding information raises ethical problems and possibly legal ones as well. Some investigators have immediately told the parents that the child has a chromosomal abnormality; some have told the parents that the child has an increased risk for abnormal behavior. If the information is conveyed and the parents are told that there is a possibility, albeit small, that their child is going to behave abnormally, they are likely to have expectations of abnormal behavior. Where parents have been told, attempts have been made to convey the idea that the evidence for an increased risk of psychopathology is not very strong.

Several considerations need to be kept in mind in assessing the association between

chromosomes and behavior. Firstly, each of the chromosomal disorders discussed here shows considerable phenotypic variability. For each, some proportion of cases have E E G abnormalities or epilepsy or both, but the finding is not consistent and depends to a large extension where the individual is detected. Patients in an institution for the mentally subnormal tend to have a relatively higher rate of EFG abnormality, just as do the chromosomally normal individuals in the same institution. Move the sampling to outpatient clinics and the incidence of all types of abnormalities, physical and, presumably, behavioral, as well as FE G, decreases in frequency, which suggests that the findings are not specific for the chromosomal defect in question. There is also a broad range of phenotypic variability in each of the chromosome abnormalities, a case in point being Money's finding that the 1 Q's of Turner's syndrome patients range from 70 to 130. But while it is probable that persons with Turner's syndrome are not more likely to be retarded than others, the difference in verbal and performance IQ maybe specific for the syndrome.

Secondly, numerous assumptions need to be made when attempting to determine whether a given chromosomal disorder carries a risk for behavioural dysfunction. In general, such attempts are made by comparing the institutional incidence of the abnormality with its incidence among newborn infants. In doing so, it is assumed that the two groups being compared are similar, that is, that they come from the same social class, background, ethnic group, and so on. Another assumption is that there is no differential mortality for the individual carrying a chromosomal defect as compared with chromosomally normal individuals. If there is a differential postnatal mortality for the chromosomal defect, its incidence in the institutionalized population might be an understatement of the true risk for behavioral dysfunction. On the other hand, if there is an increase of aneuploidy with age, as may be the case with the x xx disorder, the institutional incidence rate may overstate the true risk for behavioral dysfunction. These and other variables have complicated the interpretations that can be drawn from the studies done to date on the association between behavioural dysfunction and chromosomal abnormality.

Thirdly, the conceptualization and assessment of the environments in which individuals with chromosomal disorders have been nurtured and in which they are functioning when detected has been inadequate. Environments simply cannot be ordered along a single harmonious- disharmonious or favorable-unfavorable continuum. An environment that may be favorable for the development of one set of behaviors, as, for example, the development of independence and self-reliance, may differ from an environment favorable for the development of other adaptive behaviors. Although techniques to assess social climates and to provide functional analyses of environments have been developed in recent years, these have not yet been applied in human behavior genetics research. Lastly, it is almost always assumed that chromosomal disorders must have abnormal consequences for psychologic development and functioning. However, possible adaptive consequences are conceivable and require more attention in future research. For example, there is evidence that taller males are more popular than average and have a greater likelihood

of becoming leaders with high self-esteem during their adolescence. If, as the evidence suggests, some x Y Y males are taller than their peers, they may, on the bases of compatibility or similarity be more likely to be attracted to and imitate other taller males. Such individuals may thus have a higher than average probability of becoming socially assertive and attaining economic success and of making outstanding social adjustments and contributions.

It is worth noting that about 1 in 200 babies is born with a chromosomal disorder. This means that about 12,000 to 18,000 children are born in the U.S. annually with an abnormality of either the autosomal or sex chromosomes. At any given time, more than a million persons are possibly carriers of chromosomal abnormalities, but relatively few of them are in institutions because of behavioral dysfunction. The majority of individuals with sex chromosome anomalies seem to be functioning in society reasonably well, indicating that the correlation between abnormal behavior and specific chromosomal disorders is not a strong one. To ascribe to a single chromosome particular personality characteristics or patterns of behaviour overlooks the fact that the behavioural phenotype of all human beings results from the interaction of genotype and environment.

Probable Questions:

1. Define behavioral genetics. What is its significance.
2. Describe some experiments on animals which depict behavioural genetics study.
3. How XYY genotype affects one's behaviour?
4. How XXX genotype affects one's behaviour?

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7th Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwal

UNIT-XII
Environmental effects and gene expression

UNIT-XIII
Effects of external and internal environment; phenocopies

Objective: In this unit you will learn about Environmental effects and gene expression and also the effects of external and internal environment including phenocopies

Introduction:

The gene expression is determined by two features called as penetrance and expressivity of the genes. Penetrance is the ratio of individuals exhibiting expected phenotype and expressivity is the extent of gene expression in an individual. The phenotype of an individual is determined by the genotype or the type of gene expressed. In general, phenotypic changes occur in individuals when exposed to various environmental factors. But the query, "Is the genotype of an individual is influenced by external environment?" lead to several researches throwing light on the effect of external or environmental factors like temperature, light, chemicals and nutrition in gene expression. Besides the effect of internal factors like hormones and metabolism on gene expression, external factors were also found to affect the gene expression and ultimately exhibiting phenotypic changes.

Penetrance and Expressivity:

The presence of a gene does not always bear an absolute relationship with the appearance or absence of a trait. In the ABO blood group system, the genes are expressed in an absolute way. But in many other instances the gene is expressed in a variable manner, i.e. the visible phenotype shows varying intensities.

The terms penetrance and expressivity are used to describe variable gene expression. Penetrance is the proportion of individuals that show an expected phenotype. When a gene is completely penetrant it is always expressed; when incompletely penetrant, the gene is expressed in some individuals, not in others, the proportions depending upon the degree of penetrance. For example in the recessive traits which Mendel studied, the phenotype was expressed fully when the gene was in homozygous condition; this is due to 100 per cent penetrance. Suppose instead that in a hypothetical cross, only 60 per cent of individuals show the expected trait when all 100 are carrying the gene; we say that in this case penetrance is 60 per cent. Expressivity is the degree to which a gene is expressed in the same or in different individuals. Thus the gene for lobe eye in Drosophila may show a complete range of phenotypic expression in different individuals. Some flies may have a normal sized eye, in others the eye is smaller, in still others the eye is absent.

Temperature:

The earliest studies related to the effect of temperature on genetic constitution were done on the Himalayan breed of rabbits and Siamese cats. Coat colour in rabbits is controlled by multiple alleles of a gene. When one of the recessive alleles c^h is present in the homozygous condition (c^hc^h), the Himalayan coat colour results. Such a rabbit is a mosaic with white fur all over the body except the nose, paws, ears and tail which are black (Fig. 5.1).

Fig. 5.1 The Himalayan rabbit.

The black extremities are the portions which have lower temperature (less than 34°C) than the rest of the body. If the extremities are exposed to higher temperature artificially, the new hair which starts growing is white.

Similarly, if some portion of the body bearing albino fur is artificially kept at a lower temperature, the new hair formed is black. These observations explain the temperature sensitive behaviour of the allele (c^h) which controls Himalayan trait in the homozygous state.The allele codes for an enzyme used in pigment formation which is temperature sensitive and is inactivated by temperatures above 34°C resulting in albino phenotype; if temperature is lower the same alleles promote synthesis of pigment and the phenotype is black.When rabbits of this genotype are grown at cold temperatures, they are completely black. The Siamese cat shows the same pigmentation pattern as the Himalayan rabbit due to the presence of similar type of temperature-sensitive allele. In Drosophila temperature changes the penetrance of the gene known as tetraptera which controls wing development. At 25°C the gene has 35 per cent penetrance so that the corresponding number of flies develops wings whereas 65 per cent do not. At 17°C penetrance is much reduced so that only one per cent of flies show the winged phenotype.

The recessive gene vg/vg which produces vestigial wings in Drosophila is also influenced by temperature. At 32°F the wings are feebly developed and extend very little from the body (Fig. 3.1). At 40°F the wings are better developed and have some venation. At 88°F wings are well developed with conspicuous venation.

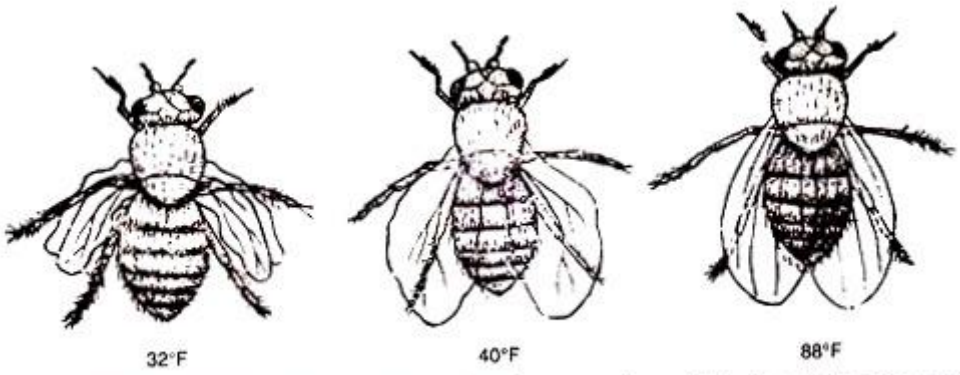

Fig. 3.1 Influence of temperature on the expression of the gene for vestigial wings (*vg*) in *Drosophila*.

Some temperature-sensitive mutations are exhibited in bacteriophages. In general the temperature at which normal phenotypes are produced is referred to as permissive temperature: that which produces mutant phenotypes is called restrictive temperature. Some lethal mutations in viruses and in Drosophila are temperature sensitive. Among plants, colour of flower in primrose changes from red to white when temperature is raised above 86°F.

Light:

There is a gene in maize plants which controls anthocyanin pigment formation. When ears of plants carrying the homozygous gene are exposed to sunlight by removing the green leafy coverings on the young cobs, the kernels become bright red in colour ("sunred").If however, the blue violet rays of the light spectrum are prevented from reaching ears of maize plants (by wrapping red cellophane paper around them so that only red rays penetrate the cells) the sunred phenotype is not visible.

In this case sunlight interferes with one or more chemical reactions leading to pigment formation. The reddish freckles on the sensitive skin of white skinned human races are also caused by sunlight in a similar way. In human beings a skin cancer known as xeroderma pigmentosum is caused by a homozygous recessive gene. The skin becomes extremely sensitive to sunlight so that even a minor exposure to faint light gives rise to pigmented spots on the facial skin. The spots can become cancerous and if they spread to other parts of the body, death results. If an individual homozygous for the recessive gene is not exposed to light, the gene is not able to express itself.

Environment and Sex Determination:

The marine worm *Bonellia* demonstrates the effect of environment on sex. In this sexually dimorphic organism the female is very large, about 10 cm in length; the male is 3 mm long and lives inside the cloaca of the female.

If the free swimming larvae that have arisen from fertilised eggs remain in the sea bed away from the females, they develop into female worms. But if females are available, the larva settles on the female proboscis, draws nourishment from it, and develops into a male.Of the many experiments performed with Bonellia, one is most interesting and

relevant here. If Bonellia are raised in the laboratory in a tank containing artificial sea water, the free-swimming larvae settle down at the bottom of the tank and develop into females. But if the artificial sea water is agitated by some mechanical device, the larvae develop into males.

Phenocopies:

Depending upon the extent to which the environment influences the genotype, the changes in the phenotype may be subtle or dramatic. Sometimes the phenotype becomes altered by the environment in such a way that the new phenotype resembles another phenotype produced by known genes. The induced phenotype is not inherited and is called a phenocopy.

In many instances phenocopies result from application of specific treatments like radiation, chemicals poisons, temperature shocks etc. The Himalayan rabbit described develops a coat that is all black if the rabbit is made to live in a cold environment.The Himalayan rabbit is a phenocopy of the genetically black rabbit. If both rabbits live together at moderately high temperature, the Himalayan rabbit has a phenotype very different from the genetically black rabbit.One of the most striking examples of phenocopies could be observed in what were known as thalidomide babies in the early 1960's. A number of deformed children were born in West Germany and Great Britain to mothers who had taken the tranquilizing drug thalidomide in their sixth week of pregnancy.The abnormal children showed deformities in limbs; some had one, two or three limbs, others had no limbs at all. The abnormalities showed a great resemblance to another phenotype known as phocomelia caused by a recessive gene. Diabetes mellitus is a heritable human trait associated with reduced amounts of the hormone insulin that is secreted by the pancreas. In the presence of insulin glucose is absorbed by the cell membranes. When the hormone is not produced in sufficient quantity, the unabsorbed glucose passes into the blood and urine. The exact mode of inheritance of diabetes is not properly understood. There are different types of diabetes arising from different causes; it therefore seems likely that there are several gene pairs controlling the trait. On the other hand the study of a pair of genetically identical twins, one of whom had diabetes the other not, indicates that the condition is due to a recessive gene with low penetrance.

If proper doses of insulin are administered to a diabetic person, he reverts to the normal phenotype. In other words, control of diabetes produces a phenocopy of the normal individual. There are many other examples in human beings where, by giving drugs, the mutant genotype produces a phenocopy of the normal phenotype. In haemophiliac patients, a specific protein required for blood clotting is either defective or deficient. If however, an anti-haemophiliac factor isolated from humans is injected into a patient, a phenocopy of the normal individual results. Similarly, if thyroxine is administered to a child whose thyroid gland does not secrete this substance in adequate quantities, the normal phenotype is produced.

The creeper trait in chickens is observed sometimes in domestic fowl when the newly hatched chickens have the legs drawn up under the body. The affected chicken is not able to walk but creeps along the ground.The creeper trait (Fig. 3.2) is expressed by the heterozygous condition of a dominant gene which is lethal when homozygous. Creeper chickens can also be produced if incubating eggs of normal fowls are treated with boric acid or insulin. Such induced creepers are phenocopies of the genetically controlled heterozygous creeper chickens.

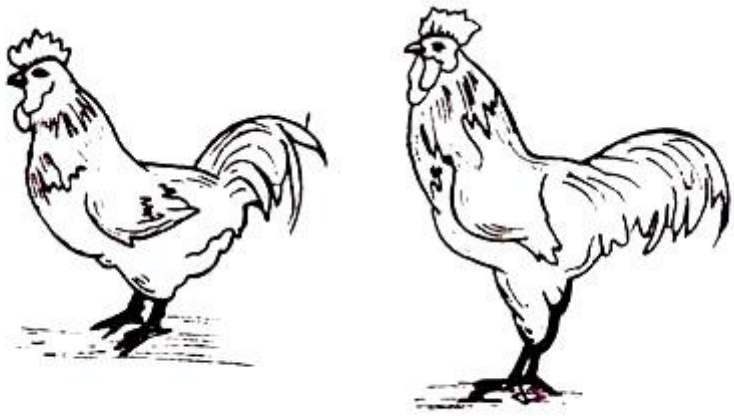

Fig. 3.2 Chicken expressing creeper trait (left) compared with a normal chicken (right).

Due to a recessive gene, maize plants become dwarfed, because they are deficient in the plant growth hormone known as gibberellic acid. But if the hormone is supplied to the dwarf plants they grow to normal height producing phenocopies of normal plants.

Environmental Effects and Twin Studies:

In human beings it is not possible to perform controlled breeding experiments. Twin studies are perhaps the best way of determining as to whether the observed differences between individuals are due to heredity. Twins are of two types—monozygotic or identical twins that arise from a single fertilised egg and have identical genotypes; dizygotic or fraternal twins which arise from two fertilised eggs and are therefore no more genetically alike than siblings (brothers and sisters).

The correct identification of twin types is difficult and unreliable unless done by a physician. For assessing the role of environment in heredity, the percentage of concordance (both twins showing identical phenotype) and discordance (different phenotypes) for a given trait must be determined for twins of both types.In general if concordance percentage for a trait is high in the case of monozygotic twins, and much less in dizygotic twins, one can conclude that heredity has played a role. If the concordance rate is similar in monozygotic and dizygotic twins, it suggests that the environment is determining the phenotype. From studies of a large number of twins it has been found that measles (caused by infection with Rubelia virus in early pregnancy) is largely controlled by the environment.

On the other hand conditions like diabetes, schizophrenia, Rickets and tuberculosis appear to be controlled by the genotype. Another useful aspect of twin studies is to determine the effects of different environments on identical genotypes by analysing those rare cases of monozygotic twins that have been separated from birth and reared apart. However in absence of adequate data it is not possible to conclude much on this aspect as yet.

Human Intelligence:

A number of studies have been done to determine how much of human intelligence and I.Q. are controlled by the genotype and how much by the environment. Both clarifications and complications have been revealed. The differences in intelligence among different racial groups have been extensively studied by Arthur Jensen in 1969. This work is highly controversial and has been much debated. Nevertheless, it is generally agreed that intelligence is under the control of several gene pairs interacting with the environment. From twin studies it has been further estimated that about one-half to three-fourths of human intelligence is determined genetically; the remainder is controlled by the environment.

Drug Resistance:

It is fairly well established that mosquitoes develop resistance to DDT and other insecticides used for eradicating malaria. The resistance develops due to change in the genotype in response to the environment, and is inherited. Similar resistance is reported also in insects which carry the causal agent for some other diseases like dengue fever, yellow fever, filariasis and river blindness. A number of pests which are harmful to major crops such as rice, maize, cotton, wheat and potato are also known to have become resistant to a wide range of insecticides.

Probable Questions:

1. What is penetrance and expressivity?
2. How temperature affect gene expression?
3. How light affect gene expression?
4. How sex is determined by environment?
5. Define and explain phenocopies.
6. How intelligence is controlled by genotype?
7. How drug resistance is controlled by genotype?
8 How twins are effected by environment?

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
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UNIT-XIV

Twin studies; concordance and discordance; identical and fraternal twins

Objective: In this unit you will learn about sex determination in humans and role of Y chromosome in the process. You will also learn about twin study, concordance and discordance.

Twin Studies:

The debate of nature versus nurture is known since antiquity. The close resemblance of twins has been the subject of many works of fiction as well. Means of distinguishing between the effects of tendencies received due to genes at birth and those imposed by the different environments they were exposed to during their lives after birth have always been the subject of interest to researchers. The objection to statistical evidence in proof of the inheritance of peculiar traits has always been blamed upon similar environmental conditions playing as a confounder. Twin studies provide a strong basis for exploring the importance of any potential risk factors on a trait or condition by controlling the genetic variations. It has been one of the favorite research tools of behavioral geneticists and psychologists since long, mainly utilized to estimate the heritability of traits and to quantify the effect of a person's shared environment (family) and unique environment (the individual events that shape a life) on a trait.Twins can be either dizygotic (fraternal) or monozygotic (identical). Dizygotic twins are the result of two different ova fertilized by two different sperm. Monozygotic twins are the result of one ovum fertilized by one sperm that divides to form two embryos. In the past, the only way of differentiating between monozygotic and dizygotic twins at birth was their sex and appearance. If the twins were of unlike sex, they are said to be dizygotic and if they were like-sexed and looked identical, they are said to be monozygotic. But this is not reliable. Today sex, placentation cord, blood type, HLA antigens and DNA fingerprinting are all used to differentiate between Monozygotic and Dizygotic twins.

However, DNA fingerprinting has become the only accurate method to differentiate between Monozygotic and Dizygotic twins. The monozygotic twins are genetically identical and any discordance between them is due to environmental influences whereas differences within dizygotic twin pairs are likely to be a combination of genetic and environmental factors.

Both monozygotic and dizygotic twins are known to have an increased risk of structural defects compared to the singletons. Structural defects in monozygotic twins however, are three times more frequent than among dizygotic twins and approximately 2-3 times more frequent than in single-tones. The incidence of

monozygotic twins is thought to be constant throughout the world. By contrast, the incidence of dizygotic twins varies from population to population with a higher prevalence in some areas like Nigeria, and lower prevalence in other areas, as in Japan.

The prevalence of monozygotic twins in remarkably constant and has not been observed to be affected by environmental or maternal factors. Ultrasound studies done early in pregnancy have shown that at least 10% of twin pregnancies are either lost early in pregnancy by miscarriage or are reduced to singletons. Several studies have confirmed that the number of twins at delivery is considerably less than the number of twins conceptions seen on ultrasound examinations in early pregnancy. Some of the mechanism that have been suggested for the vanishing twin include vascular compromise, life threatening malformations, or spontaneous mutations incompatible with life.

Dizygotic Twins:

Their genetic contribution is different since it comes from two different ova and two different sperm. Dizygotic twining is a common occurrence in animals. Mammals are known to have sizable litters, generally due to poly-ovulation, making every member of a litter a dizygotic twin. Dizygotic twins produced by the fertilization of multiple ova may be result of superfecundation and it occurs when two different ova are fertilized by two different sperm in more than one act of coitus, either during one ovarian cycle or in subsequent cycles. Dizygotic twins may also arise from superfetation. Superfetation occurs when a second fertilized ovum implants in a uterus already containing a pregnancy of at least one month.

Superfetation has been suggested in some cases in whom the twins are markedly discordant for birth weight supposedly due to different gestational ages. Polar body twins are other types of dizygotic twins, thought to arise from the simultaneous fertilization of the meiotic product of the same primary oocyte—the oocyte and the polar body—by two different spermatozoa. All most all dizygotic twins have two placentas, two chorions and two amnions, i.e., be diamniotic and di- chorionic. However these two may fuse and look like one. The highest dizygotic twining rate is seen in the black populations (Africans) and lowest in Asian populations. But dizygotic twining rate is closely related with maternal age, parity, height, weight and also on gonadotropin levels.
Tall and heavy women are more likely to give birth to dizygotic twins than are short and thin women: There are many reports of familial dizygotic twining and the female members of these families are thought to have an inherited predisposition to multiple ovulation and in turn have a higher number of dizygotic twin pairs when compared to general populations.

Monozygotic Twins:

These are also known as identical twins and are the result of the fertilization of one ovum by one sperm. The single fertilized ovum then divides into two embryos; both embryos are thought to have the same genetic contribution. The major cause for the monozygotic twining in human is still unknown, however several mechanisms have been proposed, which are:

(1) Lack of O_2 prior to implantation which caused developmental arrest and splitting in the zygote

(2) Delayed implantation

(3) Disturbances in the developmental clocks

(4) Delayed fertilization

(5) Rupture of zona pellucida

(6) Congenital anomaly or an abnormality in development

(7) Discordance in the expression of genetic information like X-inactivation's, imprinting, uniparental disomy, changes in the chromosome number and also mitochondrial mutations.

The incidence of monozygotic twins is constant throughout the world and it is about 3-4/1000 births. The rate of monozygotic twining appears to be unaffected by maternal age, parity, height or weight. But a few families have been reported in which monozygotic twining occurs more frequently than expected. This has been termed as "Familial monozygotic twining" and it is generally inherited from both the maternal and paternal side of the family.

It has also been suggested that this is due to a single gene effect which is unaffected by the sex of the parent transmitting gene. Monozygotic twins are known to have a higher incidence of all types of congenital anomalies and some of them are very unique to the monozygotic twining process itself. The sex ratio, i.e., the proportion of males to the combination of males and females—among monozygotic twins is lower than among dizygotic twins or singletons. Conjoined twins have an even lower sex ratio than that of monozygotic twins. Female conceptions may be at higher risk of late splitting of the embryo.

Anomaly	Descriptions
Fetus in fetu	Small parasitic dead twin attached to a normal twin which are often confused with a tumor. Generally located at the origin of the superior mesenteric vessels. Others sites have also been reported.
Fetus papyraceous	Mummified dead fetus usually attached to the placenta and present with a normal or more viable twin.
Acardia	Twin with an absent or rudimentary or non-functioning heart and whose circulation has been sustained by a normal twin. Associated with a higher rate of chromosomal anomalies
Conjointed Twins	Incomplete twins resulting from an abnormality of the twining process. They are derived from a single zygote and are always of the same sex. Incidence varies from 1-20,000 to 1-1,00,000. Females makes make up 80% of conjointed twins.

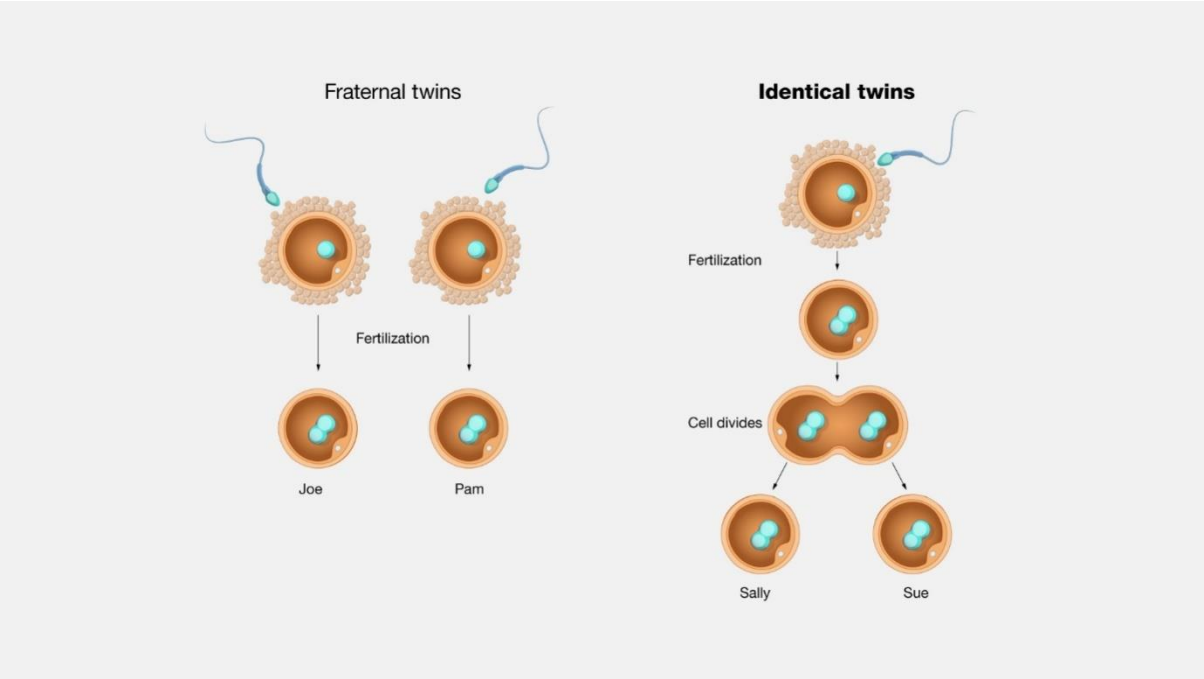

Fig: Monozygotic and Dizygotic twins

Twin Study and Multifactorial Inheritance:

Most quantitative traits behave in a more complex manner. The trait may be influenced not only by multiple genes but also by environmental factors. There may, in addition, be interactions among genes and between genes and the environment. How, then, can the contribution of genetic factors be dissected out? One of the most powerful tools for doing this is the twin study. Comparison of the concordance of a trait in identical twins with fraternal twins or full siblings is a powerful way to define the degree to which a trait is genetically determined.

For a single gene trait with complete penetrance, identical twins will, of course, be fully concordant. Siblings will be concordant less often, depending on whether they both inherit the mutant gene. For a non-genetic trait, concordance will be the same in identical twins or in full siblings and will depend on the degree of similarity of their exposure to environmental or other factors that determine the trait.

For a multifactorial trait concordance in identical twins will be greater than for siblings but not to the extent of a single gene trait with complete penetrance. The degree of concordance in monozygotic twins versus dizygotic twins or full siblings provides a measure of the contribution of genetic factors to the trait. Twin studies have helped to identify genetic contributions not only to congenital anomalies but also to common disorders such as hypertension, asthma and diabetes. Though powerful, twin studies are limited by the relative scarcity of identical twins who have a trait of interest, a problem that has been partly addressed through the development of twin registries. Another limitation is that twins share not only genetic identity but also some common environmental exposures, beginning with having developed in the same of identical twins. These offspring are the uterine environment. Studies of twins separated at birth control for post-natal environmental effects but not for prenatal effects. Another environment, approach has been to compare the offspring Identical twins. These offspring are the equivalent of half siblings and share half their genes, yet they are born and raised in different environment.

Evolution of Twin Studies

The similarity between twins has been a source of curiosity since time immemorial. The idea of using twins to study the heritability of traits can be traced back to the British researcher Sir Francis Galton. His pioneering work *The History of Twins* in 1875 inspired much debate by suggesting that England's "chief men of genius" were the product more of good breeding (nature) than of good rearing (nurture). Based on the similarities he found between twins from 80 questionnaires, Galton proudly announced his conclusion to the world that nature soundly beats nurture, though his sample was too small and consisted of all upper-class individuals, without any control group. After nearly five

decades, in the 1920s researchers “perfected' Galton's methods by comparing identical and fraternal twins and inferring heritability from the differences between the two.

The first reported classical twin study was a study performed by Walter Jablonski in 1922, investigating the contribution of heredity to refraction in human eyes. Jablonski examined the eyes of 52 twin pairs and by comparing the size of within-pair differences between identical and nonidentical twins was able to infer the heritability of a trait.

Even later, in 1990, Thomas J. Bouchard, Jr. and his colleagues (including esteemed twin researcher Nancy L. Segal) at the University of Minnesota conducted one of the most famous research studies on genetic influence in humans. They studied identical twins separated since birth and raised by different families (adoption studies), and so assumed that similarities, if found any, must be those that are heavily influenced by a person's genetic heritage. The study was invoked by the sensational news reports of two identical twins reunited after a lifetime apart. James Lewis and James Springer were separated 4 weeks after birth and each infant was taken in by a different adoptive family. When they were reunited at the age of 39, an extraordinary collection of coincidences emerged. Both of the “Jim twins” had married and divorced women named Linda. Both had second marriages with women named Betty. Both had police training and worked part-time with law enforcement agencies. Both had childhood pets named Toy. They had identical drinking and smoking patterns, and both chewed their fingernails to the nub. Their firstborn sons were named James Alan Lewis and James Allan Springer. Bouchard and Segal reported that about 70% of the variance in intelligence quotient (IQ) found in their particular sample of identical twins was found to be associated with genetic variation. Furthermore, identical twins reared apart were eerily similar to identical twins reared together in various measures of personality, personal mannerisms, expressive social behavior, and occupational and leisure-time interests. However, they did not find outstanding similarities between identical twins on measures such as standardized personality tests. Still, Bouchard's findings can be interpreted as strong support for genetic influences on personality. Bouchard's data set was unique and probably a one-time event in history because modern adoption agencies no longer break up sets of identical twins.

The modern-day classical twin study design relies on studying twins raised in the same family environments, which provides control not only for genetic background but also for shared environment in early life. As monozygotic (identical) twins develop from a single egg fertilized by a single sperm, which splits after the egg starts to develop, they are expected to share all of their genes, whereas dizygotic (fraternal) twins share only about 50% of them, which is the same as non twin siblings. Thus, if any excess similarity is seen between the identical twins when a researcher compares the similarity between sets of identical twins to the similarity between sets of fraternal twins for a trait or condition, then most probably the reason behind this similarity is due to genes rather than environment.

Some assumptions are also made in twin studies; one of them is the assumption of random mating, which assumes that people are as likely to choose partners who are different from themselves as they are to choose partners who are similar for a particular trait. If, instead, people tend to choose mates like themselves, then fraternal twins could share a greater percentage of their genes than expected. In the case of nonrandom mating, fraternal twins would have more genetically influenced traits in common than expected because the genes they receive from their mothers and fathers would be similar to each other. Similarly, the assumption of equal environments is also made, which assumes that fraternal and identical twins raised in the same homes experience similar environments. It is assumed that genes and the environment typically make only separate and distinct contributions to a trait. In general, it is also assumed that only one type of genetic mechanism—usually additive—operates for a particular trait. However, traits can be inherited through different genetic mechanisms. Additive genetic mechanisms mix together the effects of each allele. For example, if genes for curly hair were additive, a curly-haired father and a straight-haired mother might have a child who has wavy hair.

There can be variations in the classical model, which may sometimes provide an added advantage, for example if twins are followed up over longer duration of time in longitudinal manner to assess the development of adult-onset traits and conditions. This slight deviation will allow for a more complete and accurate assessment of environmental factors over time. Similarly, on combining with molecular genetics, information about the presence or absence of specific genetic variants to determine the impact on the trait of interest can be explored. The advances in molecular genetics have substantiated hypotheses generated by the traditional twin research design by pinpointing the effects of a particular gene. Depending on the objectives of the study, one may need only monozygotic or dizygotic twins, or a combination of the two.

Methods Used in Twin Research

The large pool of data related to twins gathered can be analyzed in various ways with the help of new, innovative as well as complex statistical softwares. Twin studies intend to measure the heritability of a trait, which can be determined by concordance rates.

Concordance rate (CR) for a disease or trait among identical and fraternal twin pairs is actually a statistical measure of probability: If one twin has a specific trait or condition, what is the probability that the other twin has (or will develop) that same trait or disease? Historically, CRs are computed separately for monozygotic (MZ) and dizygotic (DZ) pairs. When MZ concordances are greater than DZ concordances, genetic influences are indicated.

Quantitative genetic analyses and heritability estimation, including comparisons of concordances or intraclass correlations and structural equation modelling, can also be used to investigate the relative importance of genetic and environmental influences on a particular trait or condition. Linear structural equations and fit models over all types of twins can be used to describe the causes of variation in a phenotype. Structural equation modelling of data can provide further refinement in the results. The total variance in the trait can be partitioned into genetic variance, common environmental variance including shared (familial) environmental variance, and unique environmental variance. In order to estimate the parameters of interest, the equation for the twins is written and the parameters studied. Heritability, the relative importance of genetic influences for variation in a trait, is defined as genetic variance divided by the total phenotypic variance.

Tetrachoric correlations:

It is calculated for two normally distributed phenotypic variables that are both expressed as a dichotomy (disease or no disease) and reflect the similarity of twin pairs. Thus, differences in correlations between various groups provide information about the presence of genetic effects. Multivariate analyses of twin data can additionally offer estimates of the extent to which allelic variants and environment may influence different traits and conditions.

The co-twin control analyses method is applied *in* situ*a*tions where one wants to investigate the importance of an expected risk factor after controlling for genetic and shared environmental effects. It should be noted that the co-twin control method may entail control of factors in the biological pathway between exposure and disease, which may cause an underestimation of the exposure studied.

Co-twin control analyses: Disease-discordant twins

In studies of disease-discordant twins, two control groups usually are used: External controls and internal or co-twin controls. The analysis classically is conducted in three steps.

Step 1*:* Association between exposure and outcome (comparison with external controls). The first step, which is essentially a classic case-control study, is to compare twins diagnosed as cases with external controls (other twins not related to the index probands), and to evaluate the risk for disease given an exposure. This approach facilitates comparisons with results from ordinary case-control studies on singletons.

Step 2*:* Controlling for confounding from unmeasured early environment (healthy co-twin as control). In the second step, the healthy co-twin (in both MZ and DZ twin pairs)

can be used as a control for the diseased twin. Because twins share the same intrauterine environment and typically are reared together, the co-twin control method provides a very effective tool to minimize confounding by differences in an (unmeasured) childhood or adolescent environment.

If analyses with external controls show associations between exposure and disease and the relative risk remains similarly high in the within-pair (co-twin) analyses, it speaks in favour of a causal effect of the exposure on the disease. On the other hand, if the relative risk is not increased in the within-pair comparisons (but only in the first-step analyses with external comparisons), this indicates that environmental factors early in life (for example, foetal environment, maternal smoking, or childhood socioeconomic status (SES)) are responsible for the initially observed findings. If the relative risks from steps 1 and 2 differ, a direct test of significance of difference in risks can be performed by applying regression: The exposure on control status (external versus internal control).

Step 3*:* Controlling for unmeasured genetic background (healthy monozygotic co-twin as control). In the third step, analyses are applied only to disease-discordant MZ pairs. This design is ideal in controlling for potential confounding from genetic factors, as the cases and controls are genetically identical. Thus, one is confident that an observed effect is not confounded by genetic predisposition. If the twin with the exposure in MZ pairs more often has a specific chronic disease, this will provide strong support for the likelihood that the exposure contributes to the causation of the disease. On the other hand, if an association exists in analyses of external controls among disease-discordant DZ pairs but not among MZ pairs, genetic effects have probably confounded the results.

Co-twin control analyses: Exposure-discordant twins

As mentioned above, one can also focus on exposure-discordant pairs that are followed longitudinally for a disease outcome. In this case, *t*-tests or proportional hazard regressions can be utilized for estimating the relative risk between exposed and unexposed individuals, whereas matched analyses should be used in within-pair analyses, similar to the disease-discordant pairs.

Finally, as the twin registries contain longitudinal data on large samples, they can therefore be used for conventional epidemiological analyses disregarding twinship status. Several studies have been performed on the association between exposure and outcomes using the registries as a population-based cohort or as the basis for nested case-control studies. When using twin data for these types of studies, the dependency between the twins in a pair should be taken into account by using generalized linear models or other techniques.

Statistical methods and analysis

Various complex software packages such as Statistical Analysis System (SAS), Mx (Mx is a software developed by (Michale Neale, Department of Psychiatry and School of Medicine, Virginia Common Wealth University, Richmond, VA-23298-0126, USA. Mx is a matrix algebra interpreter and numerical optimizer for structural equation modelling and other types of statistical modelling of data.)) are used for statistical analysis for the twin studies: For applying logistic regression, SAS PROC GENMOD using generalized estimating equation (GEE) model can be used, while for conditional logistic regression, SAS PROC PHREG can be used.

Advantages of twin studies

- Twin studies allow disentanglement of the shared genetic and environmental factors for the trait of interest.
- Researchers can estimate the proportion of variance in a trait attributable to genetic variation versus the proportion that is due to shared environment or unshared environment.
- The use of twins can improve the statistical power of a genetic study by reducing the amount of genetic and/or environmental variability; the extent to which different assumptions matter may depend on which trait is being studied.

Limitations of twin studies

- Results from twin studies cannot be directly generalized to the general population, due to lack of randomization; in addition, they are different with regard to their developmental environment, as two fetuses growing simultaneously.
- Some researchers also suggest that genetic factors may lead to a higher incidence of twin births in some women.
- Though lot of changes happened in the field of genetics over time, twin studies today are also based on the same assumptions that were made back in 1920s. Many of these are deeply flawed.
- Findings from twin studies are often misunderstood, misinterpreted, and blown out of proportion, not just by the media, but even by serious scientists who get their work published.
- Many twin registries depend on the voluntary participation of twins. This leads to volunteer bias or recruitment bias, a special type of selection bias, which may lead to overinclusion of identical and female twins, resulting in overestimation of the heritability of the trait or condition under study.
- The use of twins does not allow the researcher to consider the effects of both shared-environment and gene/environment interaction simultaneously. This can be addressed by including additional siblings in the design.

Conclusion

Scholars have long studied twins to address the “nature and nurture” question; however, opposing “nature” to “nurture” is misleading. Genes combine with the environment to produce complex human traits. The importance of genes suggested by earlier twin studies has often been confirmed by later molecular genetic studies. Therefore, twin studies will continue to inform mankind about the relative importance of genes and the environment on traits in ways that no other type of research ever can. Though they have received much criticism, the advancement of statistical techniques (such as structural equation modelling) and the implementation of additional controls have allayed some of the concerns, if not all. The original twin study design has expanded to include studies of twins' extended families, longitudinal studies, and other variations. Some of these variations may allow researchers to address previous limitations also. Many molecular genetic studies have shown the usefulness of twin studies as an exploratory tool, whether or not the assumptions of equal environments and assortative mating are exactly met.

Therefore, twin studies will continue to be an important tool along with emerging genome and molecular research methods in shedding light on various aspects of human genetics and on how environmental factors and genetics combine to create human traits and behaviors.

Probable Questions:

1. Why twin study is important?
2. write down the characteristics of monozygotic twins.
3. 8. write down the characteristics of dizygotic twins.
4. Write a short note on twin study and multifactorial inheritance.
5. What are the limitations of twin studies?
6. What are the advantages of twin studies?
7. Discuss Co-twin control analyses

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7th Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwal.

Disclaimer:

The study materials of this book have been collected from various books, e-books, journals and other e sources.