A CONCISE TEXTBOOK FOR
THE STUDENTS OF B.SC.

BOTANY

PART- III

(AS PER NATIONAL EDUCATION POLICY 2020)

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DR. (MRS.) PRABHA CHOUDHARY

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About the Authors

Prof. S.S. Choudhary has been heading the Department of Botany T.N.B. College, Bhagalpur, a Constituent College, of T.M. Bhagalpur University, Bhagalpur since 1985. He received his M.Sc. and Ph.D. degree from Patna University, Patna under the noble guidance of Prof. R.P. Roy, F.N.A., Jawahar Lal Nehru Fellow, Emeritus Scientist, CSIR, New Delhi. Prof. Choudhary has successfully guided several research students leading to Ph.D. degree. He has published more than forty research papers in the journal of national and international repute covering varied topics like cytogenetics, cytotaxonomy, interspecific hybridizations, embryology, phytochemistry, biosystematics, biochemical affinities and breeding experiments in pulses. He has been teaching undergraduate and postgraduate students at Ranchi and Bhagalpur Universities. He is a member of several prestigious scientific societies. He has been regularly invited to attend various seminars, symposia and All India Conferences.

In 2001 UGC sponsored three years degree course in Biotechnology was introduced at TNB College, Bhagalpur. The Council of the Heads of the College opted for Prof. Choudhary to act as course coordinator of this newly introduced course (B.Sc. Hons. in Biotechnology). Today the Department of Biotechnology at TNB College has emerged as a premier Institute in the state of Bihar. Prof. Choudhary superannuated from active service in 2003.

Dr. (Mrs.) Prabha Choudhary obtained her M.Sc. and Ph.D. degree from Bhagalpur University, Bhagalpur. She has been teaching graduate and post graduate students since 1980 and 1985 respectively at S.M. College, Bhagalpur a Constituent Unit of T.M. Bhagalpur University, Bhagalpur. She has successfully guided research works leading to Ph.D. degree. Several research papers in journals of national and international repute are to her credit. She is the member of several scientific societies, has attended seminars, symposia and national level conferences. She was actively engaged in teaching and research in the fields of cytogenetics, biosystematics and plant pathology. She superannuated from active service in the year 2005. The authors have 9 books of university level to their credit published from Ludhiana, Delhi and Agra.

Other books published by authors are as listed below:

Sr No	Book Title	Publisher	Year	ISBN
1	Laboratory Technique in cytogenetics and Plant Breeding	Kalyani Publisher, Ludhiana	1989	81-7096-228-5
2	Laboratory Technique in Cell Biology, Plant Physiology and environmental Science	Kalyani Publisher, Ludhiana	1992	81-7096-631-0
3	A Concise Text Book of Botany	CBS Publishers, New Delhi	1997	81-239-0494-0
4	Practical Botany	CBS Publishers, New Delhi	2001	81-239-0602-1
5	A Comprehensive Textbook of Botany	S R Scientific Publications, Agra	2006	81-88805-26-2 81-88805-27-0
6	Essentials of Biotechnology	S R Scientific Publications, Agra	2008	81-88805-63-7
7	Phytopathology and Innovations in Agriculture	Kalyani Publisher, Ludhiana	2019	978-93-88842-42-6
8	College Botany- Part-I	S R Scientific Publications, Agra	2023	978-9383774-708
9	College Botany – Part -II	S R Scientific Publications, Agra	2023	978-9383774-715

Preface

The present book “Concise Textbook for Students of B.Sc. Botany- Part III” is in accordance of NATIONAL EDUCATION POLICY 2020. The text has been organized in four major sections and each of these sections has 8 subsections. Major sections are:

1. Plant Physiology, Metabolism and Biochemistry.

2. Molecular Biology and Informatics.

3. Cytogenetics, Plant Breeding, Nanotechnology

4. Ecology, Environment Management.

It is our hope that chosen contents are sufficient and useful for the purpose in mind. We have freely consulted books and other available information, and we are indebted to all of them for this venture. If we have inadvertently failed to cite any published works or other sources from which we have benefitted in compiling this book, then we apologise to them in advance.

We have drawn upon our extensive teaching experience to develop this comprehensive resource.

It is believed the book will serve as an invaluable resource for the student and educators alike. A comprehensive question bank and matters for exercise has been depicted in the appendix to help the students in understanding the subject matters easily.

Valuable suggestions for improvement of the text will be thankfully acknowledged.

We are thankful to Authors Press. And the staff of Publishing House for being patient enough.

December 2025 **Authors**

Acknowledgement

The book in the present form cannot be presented without the constructive suggestions from our sons Praveen Shankar, Naveen Shankar and their respective spouses Rashmi Shankar and Neha Shankar.

We cannot forget to mention the names of our grandchildren Varnit, Manasi and Kartik for rendering help in the preparation of the book.

Authors.

Contents

BACHELOR OF SCIENCE (BOTANY)

Programme/Class: ***Bachelor of Science***	Year: **III**	Semester: **V** **Paper-I**
Subject: BOTANY		
Course Code: B040501T	Course Title: **Plant Physiology, Metabolism & Biochemistry**	

Course outcomes:

After the completion of the course the students will be able to:

1. Understand the role of Physiological and metabolic processes for plant growth and development.
2. Learn the symptoms of Mineral Deficiency in crops and their management.
3. Assimilate Knowledge about Biochemical constitution of plant diversity.
4. Know the role of plants in development of natural products, nutraceuticals, dietary supplements, antioxidants

Credits: **4**	**Core Compulsory**
Max. Marks: **25+75**	Min. Passing Marks:

Total No. of Lectures-Tutorials-Practical (in hours per week) **4-0-0**

Unit	Topic	No. of Lectures(60hrs)
I	**Plant water relation, Mineral Nutrition, Transpiration and translocation in phloem** Importance of water, water potential and its components; Transpiration and its significance; Factors affecting transpiration; Root pressure and guttation. Criteria of essentiality of elements; Role of essential elements; Symptoms of mineral deficiency in major crops, Transport of ions across cell membrane, active and passive transport. Composition of phloem sap, girdling experiment; Pressure flow model.	7
II	**Carbon Oxidation** Krebs cycle, Glycolysis, fate of pyruvate- aerobic and anaerobic respiration and fermentation, regulation of glycolysis, oxidative pentose phosphate pathway, oxidative decarboxylation of pyruvate, regulation of Kerbs cycle, mitochondrial electron transport, oxidative phosphorylation, ATP-Synthetase,Chemiosmotic mechanism, P/O ratio , cyanide-resistant respiration, factors affecting respiration.	7
III	**Nitrogen Metabolism** Nitrate assimilation, biological nitrogen fixation (examples of legumes and non-legumes), Physiology and biochemistry of nitrogen fixation, Ammonia assimilation (GS-GOGAT), reductive amination and transamination, amino acid synthesis.	8
IV	**Lipid Metabolism & Photosynthesis** Lipid Metabolism : Synthesis and breakdown of triglycerides, -oxidation, glyoxylate cycle, gluconeogenesis and its role in mobilization of lipids during seed germination, -oxidation. ; Photosynthesis: Pigments, Action spectra and Enhancement effect, Electron transport system and Photophosphorylation, C3 & C4 photosynthesis, CAM- Reaction and Significance	7
V	**Plant Development, Movements, Dormancy & Responses** Developmental roles of Phytohormones (auxins, gibberellins, cytokinins, ABA, ethylene.) autonomic & paratonic movements, Control and Coordination in plants, Photoperiodism (SDP, LDP, Day neutral plants); Phytochrome (discovery and structure), red and far red-light responses on photomorphogenesis, Seed physiology & Dormancy, Vernalization & Senescence	8
VI	**Biomolecules** *Carbohydrates:* Nomenclature and classification; Role of monosaccharides (glucose, fructose, sugar alcohols – mannitol and sorbitol); Disaccharides (sucrose, maltose, lactose), Oligosaccharides and polysaccharides (structural-cellulose, hemicelluloses, pectin, chitin, mucilage; storage – starch, inulin). *Lipids*: Storage lipids: Fatty acids structure and functions, Structural lipids: Phosphoglycerides; Lipid functions: cell signals, cofactors, prostaglandins, Introduction of lipid micelles, monolayers, bilayers	8
VII	**Proteins**: Structure of amino acids; Peptide bonds; Levels of protein structure-primary, secondary, Ramchandran plot,tertiary and quarternary; Isoelectric point; Protein denaturation and biological roles of proteins **Nucleic acids:** Structure of nitrogenous bases; Structure and function of nucleic acids,Nucleic acid denaturation &Re-naturation ,MiRNA	7
VIII	**Enzymes:** Structure of enzyme: holoenzyme, apoenzyme, cofactors, coenzymes and prosthetic group; mechanism of action (activation energy, lock and key hypothesis, induced - fit theory), enzyme inhibition and factors affecting enzyme activity.Allosteric enzymes & Abzymes. Phytonutrients, Nutraceuticals, dietary supplements and antioxidants.	8

Suggested Continuous Evaluation Methods: Continuous Internal Evaluation shall be based on allotted Assignment and Class Tests

Internal Assessment	Marks
Class Interaction	5
Quiz	5
Seminar	7
Assignment (Charts/ Flora/ Rural Service/ Technology Dissemination)	8
	25

Programme/Class: ***Bachelor of Science***	Year: **III**	Semester: **V** **Paper-II**
Subject: BOTANY		
Course Code: B040502T	Course Title: **Molecular Biology & Bioinformatics**	

Course outcomes:

After the completion of the course the students will be able to:

1. Understand nucleic acids, organization of DNA in prokaryotes and Eukaryotes, DNA replication mechanism, genetic code and transcription process.
2. Know about Processing and modification of RNA and translation process, function and regulation of expression.
3. Gain working knowledge of the practical and theoretical concepts of bioinformatics

Credits: **4**	**CC / Elective**
Max. Marks: **25+75**	Min. Passing Marks:

Total No. of Lectures-Tutorials-Practical (in hours per week) **4-0-0**

Unit	Topic	No. of Lectures(60hrs)
I	**Genetic material** Miescher to Watson and Crick- historic perspective, Griffith's and Avery's transformation experiments, Hershey-Chase, bacteriophage experiment, DNA structure, types of DNA, types of genetic material. DNA replication (Prokaryotes and eukaryotes): semi-conservative. DNA replication (Prokaryotes and eukaryotes): bidirectional replication, semi-conservative, semi discontinuous RNA priming, Ø (theta) mode of replication, replication of linear, dsDNA, replicating the 5 end of linear chromosome including replication enzymes.	7
II	**Transcription & Regulation of gene expression** Types of structures of RNA (mRNA, tRNA, rRNA), RNA polymerase- various types; Translation, (Prokaryotes and eukaryotes), genetic code. Regulation of gene expression in Prokaryotes: Lac operon and Tryptophan operon; and in Eukaryotes	7
III	**Principles &Techniques of genetic engineering** Blotting techniques: Northern, Southern and Western Blotting, DNA Fingerprinting; Molecular DNA markers i.e. RAPD, RFLP, SNPs; DNA sequencing, PCR and Reverse Transcriptase-PCR. Hybridoma and monoclonal antibodies, ELISA and Immunodetection. Antibody Engineering.	8
IV	**Applications of Genetic engineering** Pest resistant (Bt-cotton); herbicide resistant plants (RoundUp Ready soybean); Transgenic crops with improved quality traits (Flavr Savr tomato, Golden rice); Improved horticultural varieties (Moondust carnations); Role of transgenics in bioremediation (Superbug); Industrial enzymes (Aspergillase, Protease, Lipase); Genetically Engineered Products, Biosafety concerns..	7

V	**Bioinformatics & its applications** Computer fundamentals - programming languages in bioinformatics, role of supercomputers in biology. Historical background. Scope of bioinformatics - Genomics, Transcriptomics, Proteomics, Metabolomics, Molecular Phylogeny, computer aided Drug Design (structure based and ligand based approaches), Systems Biology and Functional Biology. Applications and Limitations of bioinformatics.	8
VI	**Biological databases :** Introduction to biological databases - primary, secondary and composite databases, NCBI, nucleic acid databases (GenBank, EMBL, DDBJ, NDB), protein databases (PIR, Swiss-Prot, TrEMBL, PDB), metabolic pathway database (KEGG, EcoCyc, and MetaCyc), small molecule databases (PubChem,)	8
VII	**Data Generation and Data Retrieval** Generation of data (Gene sequencing, Protein sequencing, Mass spectrometry, Microarray), Sequence submission tools (BankIt, Sequin, Webin); Sequence file format (flat file, FASTA, GCG, EMBL, Clustal, Phylip, Swiss-Prot); Sequence annotation; Data retrieval systems (SRS, Entrez)	7
VIII	**Phylogenetic analysis** Similarity, identity and homology, Alignment – local and global alignment, pairwise and multiple sequence alignments, alignment algorithms. Methods of Alignment (Dot matrix, Dynamic Programming, BLAST and FASTA); Phylogenetic analysis: Construction of phylogenetic tree, dendrograms, methods of construction of phylogenetic trees.	8

Programme/Class: *Bachelor of Science*	Year: **III**	Semester: **VI** **Paper-I**
Subject: **Botany**		
Course Code: B040601T	Course Title: **Cytogenetics, Plant Breeding & Nanotechnology**	

Course outcomes: After the completion of the course the students will be able:

1. Acquire knowledge on ultrastructure of cell.
2. Understand the structure and chemical composition of chromatin and concept of cell division.
3. Interpret the Mendel's principles, acquire knowledge on cytoplasmic inheritance and sex linked inheritance.
4. Understand the concept of 'one gene one enzyme hypothesis' along with molecular mechanism of mutation.
5. Interpret the concept of Lemarkism, Neo Lamarkism, Darwinism and also understand the concept of natural selection.

Credits: **4**	**Core Compulsory**
Max. Marks: **25+75**	Min. Passing Marks:

Total No. of Lectures-Tutorials-Practical (in hours per week): **4-0-0**

Unit	Topic	No. of Lectures (60hrs)
I	**Cell biology** Structure and function of cell wall, plasma membrane, ribosomes, Endoplasmic reticulum, golgi apparatus, mitochondria, chloroplast, lysosomes, peroxisomes and cell inclusions - Organization of nucleus: nuclear envelope, nucleoplasm and nucleolus. Chromosomal nomenclature- chromatids, centromere, telomere, satellite, secondary constriction.Organization of chromosomes- Nucleic acid and histones- types and classification. Lampbrush chromosomes and polytene chromosomes- Karyotype and idiogram.Cell cycle: G0, G1, S and G2 phases – mitosis: open and closed mitosis – amitosis - meiosis. Variation in Chromosome number (Numerical aberrations)- anueploidy and Euploidy-haploidy , polyploidy- significance (Structural aberrations) - deletion, duplication, inversion and translocation.	8
II	**Genetics** **Ch**romosome theory of inheritance, crossing over and linkage; Incomplete dominance and codominance; Interaction of Genes; Multiple alleles, Lethal alleles, Epistasis, Pleiotropy, Polygenic inheritance; Extra-nuclear Inheritance, Linkage, crossing over , Concept of sex determination and Sex chromosomes; Patterns of Sex determination in plants	7

III	**Plant breeding** Plant introduction. Agencies of plant introduction in India, Procedure of introduction - Acclimatization – Achievements, Selection - mass selection, pure line selection and clonal selection. Genetic basis of selection methods , Hybridization: Procedure of hybridization, inter generic, inter specific, inter varietal hybridization with examples. Composite and synthetic varieties, Male sterility , Heterosis and its exploitation in plant breeding, Mutation, Molecular Breeding (use of DNA markers in plant breeding) , achievements in India, Breeding for pest, pathogenic diseases and stress resistance.	8
IV	**Biostatistics:** Definition, statistical methods, basic principles, variables- measurements, functions, limitations and uses of statistics. Biometry: Data, Sample, Population, random sampling, Frequency distribution- definition only, Central tendency– Arithmetic Mean, Mode and Median; Measurement of dispersion–Coefficient of variation, Standard Deviation, Standard error of Mean; Test of significance: chi- square test for goodness of fit. Computer application in biostatistics - MS Excel and SPSS	7
V	**Plant tissue culture** Principles,components and techniques of in vitro plant cultures, Callus cultures, Cell culture, cell suspension cultures, Embryogenesis and organogenesis , Protoplast- isolation and culturing of protoplast- principle and application, regeneration of protoplasts, protoplast fusion and somatic hybridization- selection of hybrid cells, Somaclonal variation, , Plant secondary metabolites production.	8
VI	**Nanotechnology** Fundamentals of nanoscale self-assembly process involved in important functional biomolecules such as Nucleic acid (DNA and RNA), Proteins, Enzymes. Cell structure and organelles,nanoscale assembly of cellular components (cell membrane and liposomes). Nanoscale assembly of microorganisms (virus). Nano-particles synthesis, Biological synthesis of Nanoparticles, Advantages and applications of biologically synthesized nanomaterials. Introduction to biological nanomaterials.,Biomineralization, Magnetosomes, nano-pesticides, nano-fertilizers, nano-sensors.	7
VII	**Artificial Intelligence in Plant Sciences** Big Data Analytics, Blockchain Technology, 3-D Printing, Machine learning , Algorithms of Machine Learning, Expert systems and Fuzzy logic , Artificial Neural Networks and Genetic algorithms, Predictive Analytics, Agents and Robotics, IoT Sensors, Object Image capture & analysis ; Applications of Artificial Neural Networks in Plant Science.	8
VIII	**Introduction to use of Digital technologies – AI, IoT & ICT in Botany** Educational software- INFLIBNET, NICNET, BRNET, internet as a knowledge repository- google scholar, science direct. resource management, weather forecasting. IoT Database management ,IoT platforms , IoT Graphical user interface • IoT application development for Android Mobile phones, ICT Applications for different crops and horticulture	7

Programme/Class: **Bachelor of Science**	Year: **III**	Semester: **VI** **Paper-II**
Subject: **Botany**		
Course Code: B040602T	Course Title: **Ecology & Environment**	

Course outcomes:

1. acquaint the students with complex interrelationship between organisms and environment;
2. make them understand methods for studying vegetation, community patterns and processes, ecosystem functions, and principles of phytogeography.
3. This knowledge is critical in evolving strategies for sustainable natural resource management and biodiversity conservation.

Credits: **4**	**Core Compulsory/Elective**
Max. Marks: **25+75**	Min. Passing Marks:

Total No. of Lectures-Tutorials-Practical (in hours per week): **4-0-0**

Unit	Topic	No. of Lectures (60 hrs)
I	**Natural resources & Sustainable utilization** : Land Utilization , Soil degradation and management strategies; Restoration of degraded lands. Water , Wetlands; Threats and management strategies, Ramsar sites ,Forests: Major and minor forest products; Depletion, Biological Invasion, Energy: Renewable and non-renewable sources of energy , Contemporary practices in resource management : EIA, GIS, Participatory Resource Appraisal, Ecological Footprint with emphasis on carbon footprint, Resource Accounting.	7

II	**Ecology & Ecosystem** Definition of Ecology, Ecological Factors, Positive and negative interactions. Ecosystem – Concept of an ecosystem-structure and function of an ecosystem. Abiotic and biotic com-Energy flow in an ecosystem Ecological Succession-Definition &types. Processes and types (autogenic,allogenic,autotrophic,heterotrophic,primary &secondary). Hydrosere and Xerosere. Food chains and food webs , Ecological pyramids, production and productivity; And components. Types of ecosystems: Forest Ecosystem, Grass land ,Crop land, aquatic Ecosystems Ecological Adaptations – Hydrophytes, Xerophytes, Halophytes, Epiphytes and Parasites.	8
III	**Soil Formation, Properties & Conservation** Soil: Origin, Formation, composition, Soil types, Soil Profile, Soil Microorganisms, soil processes, Soil Erosion, Biogeochemical cycles, Soil Conservation: Biological– Contour farming, Mulching, Strip cropping, Terracing and Crop rotation. Mechanical–Basin Listing, Construction of dams, Water Shed Management, Soil reclamation	7
IV	**Biodiversity and its conservation:** Definition -genetic, species,and ecosystem diversity. Value of biodiversity: : social,ethical, aesthetic and option values hot spots of Biodiversity &threats to biodiversity, Biotic communities and populations, their characteristics and dynamics. Endemic and endangered species of plants in India.Ecological niche,ecotypes,ecological indicators. *Conservation of Biodiversity:* Ex-situ and in-situ conservation, Red data book, botanical gardens, National park, Sanctuaries, hot & hottest spots and Bioreserves. Role of Seed Bank and Gene Bank Valuing plant resources,ecotourism, Role of NBPGR, FAO, BSI..	7
V	**Phytogeography:** Biogeographic regions of India & world. Agroecological & Floristic zones of India . Natural vegetation of India, static and dynamic plant geography, basic principles governing geographical distribution of plants, Phytogeographical regions of India, Vegetational types in Uttar Pradesh.	7
VI	**Environmental audit & Sustainability** Concept of environmental audit; Guidelines of environmental audit; Methodologies adopted along with some industrial case studies; Environmental standards: ISO 14000 series; Scheme of labelling of environment friendly products (Ecomark); Life cycle analysis; Concept of energy and green audit, Sustainability indices; Strategies and debates on sustainable development; Concept of Sustainable Agriculture; India's environment action programme: issues, approaches and initiatives towards Sustainability; Sustainable development in practice; Urbanization; Concept and characteristics of smart city; Urban resources and environmental problems; Carrying capacity analysis; Concept of ecological footprints.	8
VII	**Pollution ,Waste management & Circular Economy** Environmental pollution, Environmental protection laws, Bioremediation, Activated Sludge Process (ASP) – Trickling Filters – oxidation ponds, fluidized bed reactors, membrane bioreactor, neutralization, ETP sludge management; digesters, up flow anaerobic sludge blanket reactor, fixed film reactors, sequencing batch reactors, hybrid reactors, bioscrubbers, biotrickling filters; regulatory framework for pollution monitoring and control; case study: Ganga Action Plan; Yamuna Action Plan; implementation of CNG ;Waste- Types , collection and disposal, Recycling of solid wastes (hazardous & non-hazardous) - classification, collection and segregation , Incineration, Pyrolysis and gasification , Sanitary landfilling ; composting, Biogas production ,Circular Economy & sustainability.	8
VIII	**Environmental ethics, Carbon Credits &Role of GIS** Carbon credit: concept, exchange of carbon credits. Carbon sequestration, importance, meaning and ways. Climate change, global warming, acid rain, ozone layer depletion, nuclear accidents and holocaust. Wasteland reclamation. Consumerism and waste products. Clean development mechanism. Geographical Information Systems: definitions and components; spatial and non-spatial data; GIS software packages; GPS survey, data import, processing, and mapping. Applications and case studies of remote sensing and GIS in land use planning, forest resources& agriculture studies.	8

SECTION 1
Plant Physiology, Metabolism, Biochemistry

Chapter 1
Water Molecule; Water Potential

Water Molecule

A unique property of a water molecule is that a large amount of energy is required to raise its temperature. Water is an excellent solvent for molecules that have electric charges. Two categories of such hydrophilic (water loving) are ions and polar molecules. (Figure 1).

Water is a polar molecule because it has a symmetrical distribution of electric charge. The eight protons in an oxygen molecule attract electrons more strongly than single proton in each of the hydrogen nuclei. The side of the molecule with two hydrogen atoms has a slightly positive charge and the side without the hydrogen atoms has a slightly negative charge. The two sides of the molecule resemble the two poles of a magnet, which is why the molecule is said to be polar. Hydrogen bonds are formed between water molecules because slightly positive end of one molecule is attracted to the slightly negative end of another molecule. Each water molecule can form maximum four hydrogen bonds as shown in Figure 2.

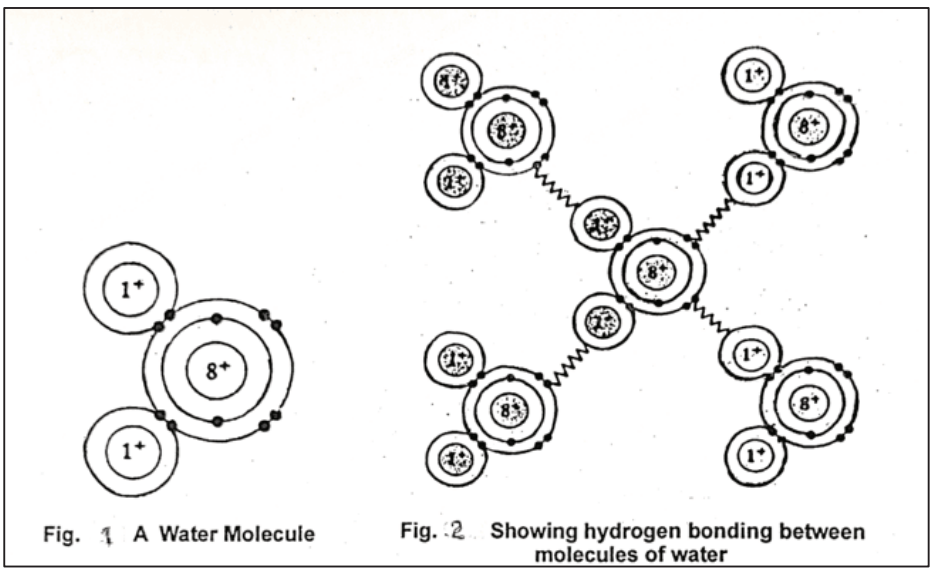


Fig. 1 A Water Molecule

Fig. 2 Showing hydrogen bonding between molecules of water

Electrolysis of water

The chemical reaction on passing an electric current through water to decompose hydrogen and oxygen gases is termed as electrolysis of water.

$$2H_2O \rightarrow \text{Electric Current} \rightarrow O_2\,(g) + 2H_2\,(g=\text{Gas})$$

Hydrogen is used in making nylon, hydrogen peroxide and ammonia.

Water Potential

Water Potential is the potential energy of water per unit volume relative to pure water. Water potential quantifies the tendency of water to move from one area to another due to osmosis, gravity, mechanical pressure, and matrix effects like capillary action.

Water potential in biology is the tendency of water to diffuse from one area to another, from the areas of highwater potential to areas of low water potential by osmosis. Water potential is directly proportional to the solute concentration. One great example is the movement of water in a tree from the root tips to the stem.

Most of the water uptake in plants is governed by water potential of the cell and environment. Pressure potential can be measured by osmometer. It is calculated by the equation.

$$\Psi = \Psi \varpi + \Psi p^*$$

Ψ = psi; (Water Potential)

$\Psi \varpi$ = psi pi; (Osmotic Potential)

Ψp^* = psi p; (Pressure Potential)

Importance of water: Water maintains temperature and protects our spinal cord and other sensitive tissues, gets rid of waste through urine, perspiration and bowel movement. It moistens tissues in eyes, nose and mouth and carries nutrients and oxygen to cells.

Water is also important to plants. It is necessary in photosynthesis. It maintains turgidity for structure and growth period. It helps in transport of nutrients and serves as raw material for various chemical processes. Water is required for germination of seeds. All living things need water to stay alive.

Water potential of pure water at atmospheric pressure is zero, the water potential of water in cells and solutions (containing dissolved solutes) is less than zero i.e. negative.

Water potential, osmotic potential and pressure potential are interrelated, and they can be regarded as different components of water potential. Some scientists consider an additional potential- ‘Matrix potential’ which is also an important contributor to water potential. Matrix potential is on account of colloids and surfaces in the cell and its surfaces. Tension Forces in the micro capillaries of cell walls.

Chapter 2
Transpiration, Guttation, Ringing Experiment

Transpiration

Transpiration is a process in which water is lost as water vapor from the aerial parts of the plants through stomata. It is a passive process that requires no energy expense by the plant. Transpiration also cools plants, changes osmotic pressure of cells and facilitates mass flow of mineral nutrients.

Types of transpiration:

It is of literal three types.

1. Stomatal transpiration,
2. Cuticular transpiration and
3. Lenticular transpiration.

Stomatal transpiration: 3% area of the leaf surface occupies transpiration. When stomata are open, CO_2 enters into it. It is necessary for photosynthesis & water in form of vapor comes out.

Cuticular transpiration. Leaf surface has a waxy cuticle through which water vapor can evaporate. It happens when stomata are closed.

Lenticular transpiration: In bark of some plants there are small openings called Lenticels. Where some water loss occurs.

Factors affecting transpiration.

1. Solar radiation is most important factor as stomata are open only in daylight.
2. Water vapor pressure deficit in the surrounding area must be lower than the water potential of the leaves.
3. Humidity - Relative humidity also has a factor. At higher relative humidity, there is less transpiration.
4. Carbon dioxide concentration in the air that controls the stomata opening will also influence transpiration rate.
5. Various Biochemicals and morphological features of plant affects transpiration rate.
6. Besides density of plants, species composition will also play a role in determining large scale transpiration rates.

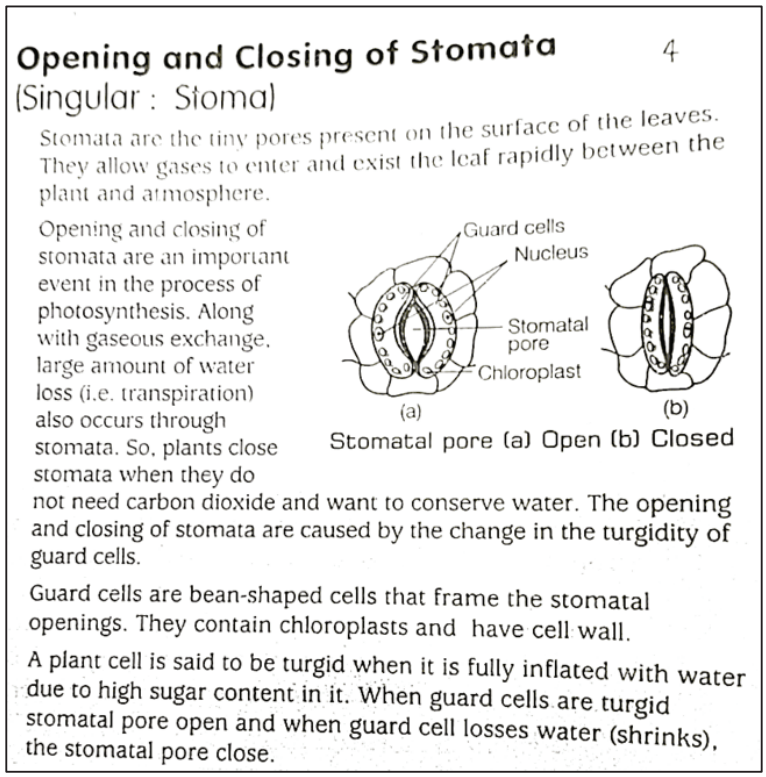
Mechanism of stomatal movement:

Stomata open in the light and closes in the dark. There are two theories for this.

i. During day time, CO_2 in the guard cell is used up in photosynthesis. pH of guard cells increases. Starch is converted into glucose. Water rushes into the guard cells from epidermal cells. It increases the turgor of the guard cells and stomata opens.

During darkness, Photosynthesis is stopped. CO_2 concentration increases, pH decreases, glucose is converted into starch, guard cells lose water due to exosmosis and stomata are closed.

ii. Potassium pump and proton secretion theory was proposed by S. Mumamura and M. Fujino in 1959. They proposed that K^+ in large amount moves into the guard cells. In dark, K^+ moves out of guard cells. Osmotic potential becomes less negative, water osmoses out of guard cells their turgidity decreases and stomata closes.



Mechanism:

During day light, guard cells vapor comes out. At night, guard cells are closed. Photosynthesis stops, carbon dioxide concentration increases., pH decreases., sugar converts into a starch. Come on, guard cells lose water. And causes exhaust masses of water and stomata is closed.

S. Mumamura and M. Fujino (1959) said that large amount of K+ moves into guard cells in daylight. In dark K+ moves out of guard cells causing closing of stomata.

Significance of transpiration:

1. Helps in conducting of water and minerals in different parts of plant.
2. Keeps balance of water movement in plants
3. Maintains osmosis and keeps cells rigid
4. It creates suction force which helps in upward movement of water.
5. Maintains turgidity.
6. Cooling effect of tree is due to transpiration.

Osmosis: Osmosis is a process of diffusion of water molecules across a semi permeable membrane.

Chemiosmosis: It is a process of pumping protons across the membrane to generate the proton gradient. Chemiosmosis occurs in mitochondria during respiration and in the chloroplast during photosynthesis.

Peter Mitchell, (1961) postulated the chemiosmosis hypothesis. The process of ATP synthesis using free energy obtained when electrons are passed to several carriers. (Electron transport carrier) is called chemiosmosis. When electrons pass the inner mitochondrial membrane; Synthesis of ATP takes place.

Guttation

It is a natural process in which plants release excess water from the tips, edges and surface of their leaves. It occurs at night when the stomata on the leaves are closed. It is a sign of healthy plants. Guttation is also known as teardrops of leaves. Guttation word comes from the Latin word *'Gutta'* means drop. Guttation is the process of removal of water from the hydathodes whereas transpiration is removal of water from the stomata. Dew is formed on the plant's surface from condensation of moisture in the air, while guttation is moisture emitted from the plant itself. (Figure 3).

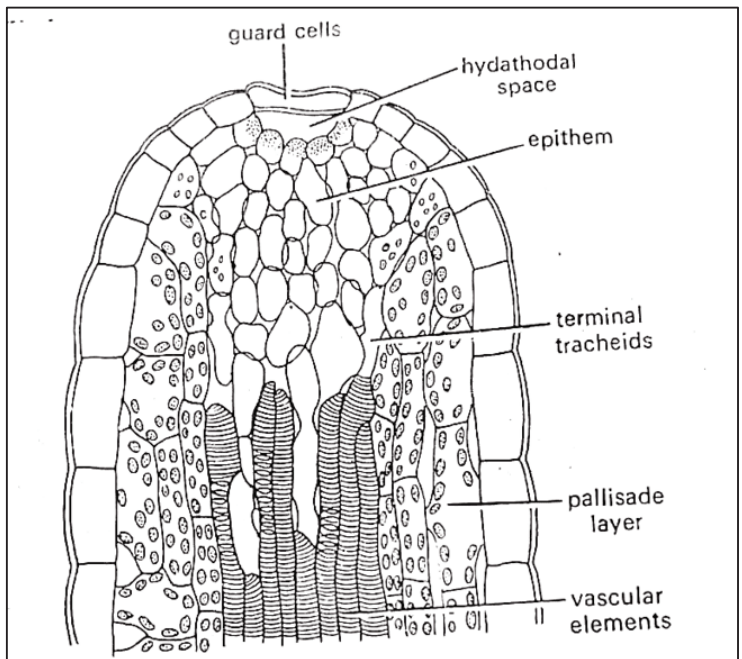


Fig. 3, Longitudinal section of *Primula sinensis* leaf through hydathode (Redrawn from G. Haberlandt; Physiological Plant Anatomy 1914).

Root Pressure: The 'Root Pressure' term was given by Stephen Hales. However, the Root Pressure theory was given by Priestley. The root pressure is a type of transverse osmotic pressure in the root system that helps in the development of ascent of SAP in the leaves. It develops in the Xylem SAP of the root. It is a manifestation of active water absorption. The common example of root pressure is the exudation of droplets of liquid from the margins and tips of leaves. Root pressure is maximum when transpiration is very low and absorption is high. The root pressure is a force that helps move fluids upward into the Xylem. It is generated by osmotic pressure in the root cells.

Ringing and Girdling Experiment

This experiment in which the continuity of vascular tissues is disturbed by their removal are known as ringing or girdling experiments. Girdling usually employed for the removal of all the tissues outer to bark cortex. And flowing from the woody stem.

In woody stem it is easily possible to remove a broadband of bark, including Phloem and cambium under such condition, the upper part of the plant is attached to the lower part by the central Xylem cylinder and pith.

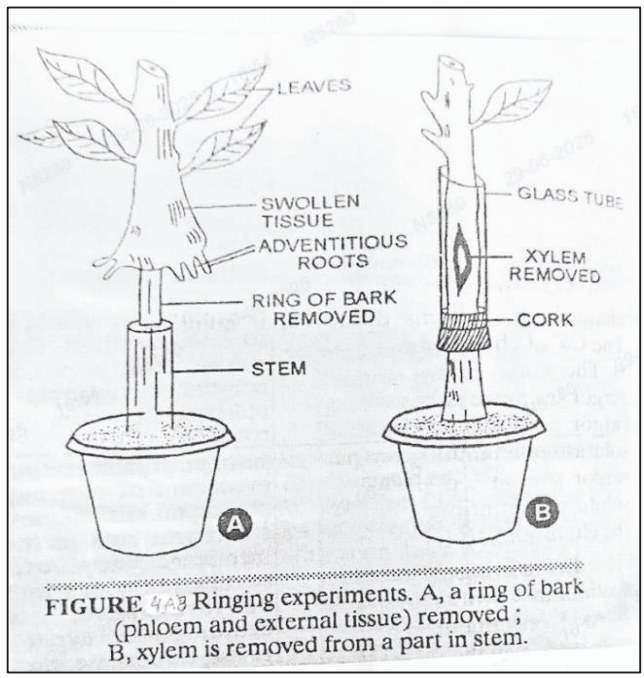


FIGURE 4A3 Ringing experiments. A, a ring of bark (phloem and external tissue) removed ; B, xylem is removed from a part in stem.

It results in the accumulation of food material just above the ring, which is evident by swelling and sometimes formation of adventitious roots. (Figure 4A). Furthermore, it can be observed that the roots die first in a girdled plant. This can be explained because the transport of water and minerals occur through Xylem. The upper part gets ample amount of water supply, minerals and all the important factors for metabolism. Therefore, the upper part continues to grow. This girdling experiment proves that food is transported in the stem through Phloem.

In ringing experiment, it is proved that the path of water in the stem is through Xylem vessels. (Figure 4b).

These experiments were performed by Malpighi in 1672 A.D., by Stephen in 1727 A.D, and by Hartig in 1837 A.D.

Chapter 3
Translocation

Transport or movement of soluble products of photosynthesis from leaves to other parts of plant is termed as translocation. It occurs in Phloem. Phloem also transports amino acids and hormones. These substances are delivered to storage organs of roots, fruits, seeds, and growing organs.

Translocations of substances take place in the sieve tubes with the help of adjacent companion cells, both in upward and downward directions. Translocation in phloem, is mainly achieved by utilizing energy. Sucrose is transferred into Phloem using energy from ATP, which increases the osmotic pressure of the tissue, causing water to move into it. The pressure then moves the material in the Phloem. For example, sugar is stored in roots or stem tissues would be transported to the bud which require more energy to grow. It is also called as mass flow hypothesis. It was proposed by Ernst Munch in 1930. Phloem is composed of sieve elements, parenchyma and sclerenchyma. (Figure 5). Second name of Phloem is bast.

The role of Phloem parenchyma is in the storage of water, non-structural carbohydrates and storage proteins. Phloem vessels contain cytoplasm which goes through the holes in the sieve plates from one cell to another. Translocation plays a pivotal role in plant growth and development. It also plays a key role in the plant's response to environmental changes and stress conditions.

Passive transport: When transport protein, move the solute without a boost from cellular energy, it is called passive transport. There is a continuous movement of ions and molecules across biologic membrane. Some substances move through transport protein embedded in the lipid layer. Movement of solute requires such protein, called passive transport, in contrast with simple diffusion in which no such proteins are involved.

In Active transport, the cell must expend energy to move a solute against a concentration gradient or electrostatic gradient. Here a solute and an ATP molecule bind to a transport protein. The ATP is hydrolyzed and its terminal phosphate group is transferred to the protein. Addition of this phosphate group stimulate the changes in protein shape that moves the solute across the membrane. (Figure 5).

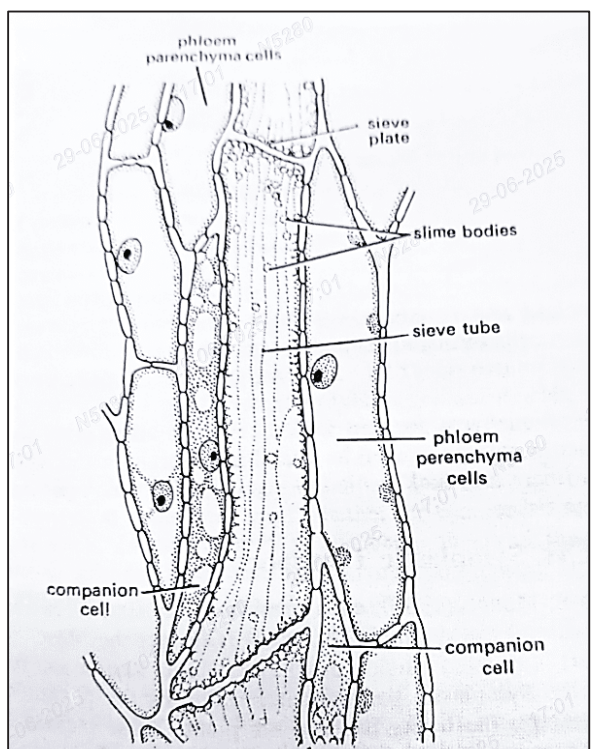


Fig. 5. A longitudinal view of phloem cells (Redrawn from F.B. Salisbury and C.W. Ross; Plant Physiology 1978).

Pressure flow model of phloem: A high concentration of sugar at source creates a low solute potential which draws water in the phloem from adjacent xylem. This creates a high turgor pressure in the phloem. It is also called as mass flow hypothesis proposed by Munch in 1930.

In this process, glucose generated by photosynthesis at source is converted to sucrose which move to the sieve tubes of phloem cells through companion cells by active transport. The food is translocated from source (Leaves) to consumption end or storage regions. It is bi-directional. Food is translocated from Phloem up.

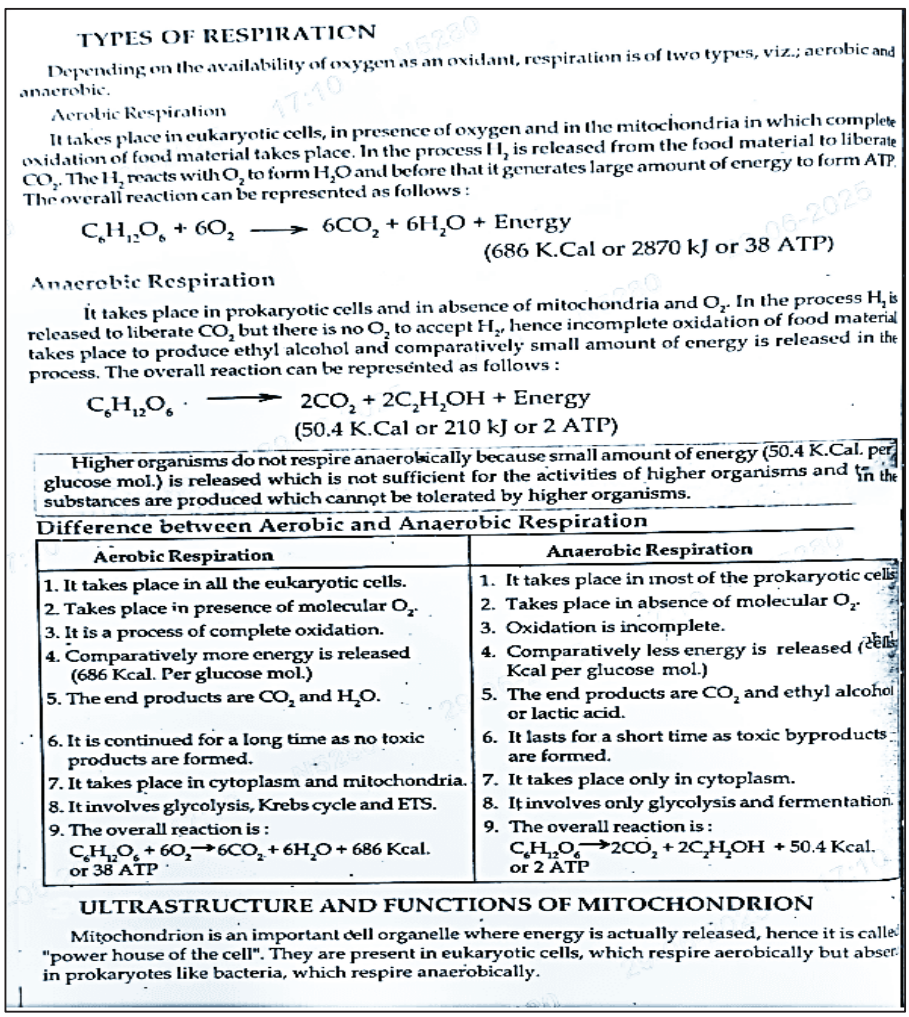
Translocation velocity may be 55cm/hour (for sucrose), 270 cm / hour in sugarcane and 297 cm/hour in squash. Removal of sugar from sieve cells is called Phloem Up and loading and gain of Sugar is called Phloem Loading.

Chapter 4
Carbon Oxidation (Respiration)

Respiration is an energy releasing biological process in which organic materials are oxidized to simpler compound. The reaction may be denoted by the chemical formula:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 674{,}000 \text{ Cal} / 674\text{K Cal.}$$

Plants do not possess any organ comparable with the respiratory organs in animals. Inner stomata, common lenticels, and intracellular spaces help in gaseous diffusion.



TYPES OF RESPIRATION

Depending on the availability of oxygen as an oxidant, respiration is of two types, viz.; aerobic and anaerobic.

Aerobic Respiration

It takes place in eukaryotic cells, in presence of oxygen and in the mitochondria in which complete oxidation of food material takes place. In the process H_2 is released from the food material to liberate CO_2. The H_2 reacts with O_2 to form H_2O and before that it generates large amount of energy to form ATP. The overall reaction can be represented as follows :

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy}$$

(686 K.Cal or 2870 kJ or 38 ATP)

Anaerobic Respiration

It takes place in prokaryotic cells and in absence of mitochondria and O_2. In the process H_2 is released to liberate CO_2 but there is no O_2 to accept H_2, hence incomplete oxidation of food material takes place to produce ethyl alcohol and comparatively small amount of energy is released in the process. The overall reaction can be represented as follows :

$$C_6H_{12}O_6 \longrightarrow 2CO_2 + 2C_2H_5OH + \text{Energy}$$

(50.4 K.Cal or 210 kJ or 2 ATP)

Higher organisms do not respire anaerobically because small amount of energy (50.4 K.Cal. per glucose mol.) is released which is not sufficient for the activities of higher organisms and toxic substances are produced which cannot be tolerated by higher organisms.

Difference between Aerobic and Anaerobic Respiration

Aerobic Respiration	Anaerobic Respiration
1. It takes place in all the eukaryotic cells.	1. It takes place in most of the prokaryotic cells.
2. Takes place in presence of molecular O_2.	2. Takes place in absence of molecular O_2.
3. It is a process of complete oxidation.	3. Oxidation is incomplete.
4. Comparatively more energy is released (686 Kcal. Per glucose mol.)	4. Comparatively less energy is released (2 Kcal per glucose mol.)
5. The end products are CO_2 and H_2O.	5. The end products are CO_2 and ethyl alcohol or lactic acid.
6. It is continued for a long time as no toxic products are formed.	6. It lasts for a short time as toxic byproducts are formed.
7. It takes place in cytoplasm and mitochondria.	7. It takes place only in cytoplasm.
8. It involves glycolysis, Krebs cycle and ETS.	8. It involves only glycolysis and fermentation.
9. The overall reaction is : $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686$ Kcal. or 38 ATP	9. The overall reaction is : $C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH + 50.4$ Kcal. or 2 ATP

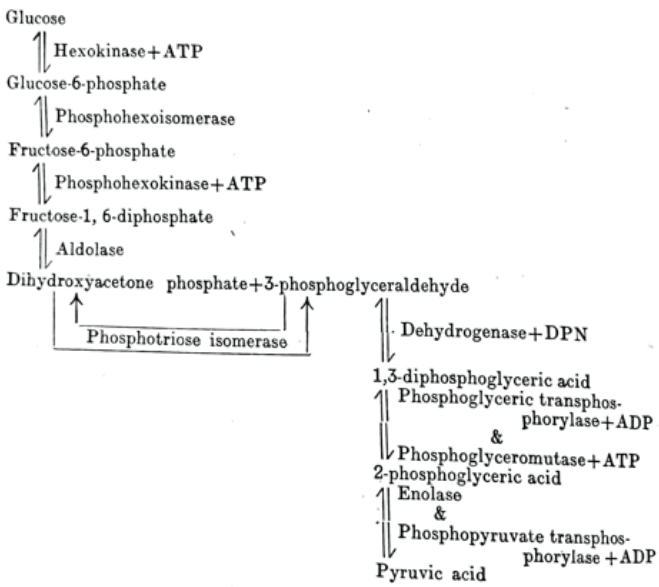
ULTRASTRUCTURE AND FUNCTIONS OF MITOCHONDRION

Mitochondrion is an important cell organelle where energy is actually released, hence it is called "power house of the cell". They are present in eukaryotic cells, which respire aerobically but absent in prokaryotes like bacteria, which respire anaerobically.

Glycolysis occurs in cytoplasm. It is also known as E M P pathway i.e. Embden Meyerhoff and Parnas.

Carbon assimilation means, the formation of carbon containing compounds by the plants from CO_2 and H_2O, Carbon Oxidation is a heterogenous reaction at the solid surface. Carbon surface absorbs oxygen and de-absorbs CO or CO_2.

A schematic representation of the chemistry of respiration is as follows:—



The chemical reactions involved in the entire process starting from the hexose (glucose) upto the formation of the pyruvic acid is given below:

(*i*) $C_6H_{12}O_6$ + Phosphates $\xrightleftharpoons{\text{Hexokinase+ATP}}$ $C_6H_{11}O_5OPO_3H_2$
(Glucose) (Glucose-6-phosphate)

(*ii*) $C_6H_{11}O_5OPO_3H_2 \xrightleftharpoons{\text{Phosphohexoisomerase}} C_6H_{11}O_5OPO_3H_2$
(Fructose-6-phosphate)

(*iii*) $C_6H_{11}O_5OPO_3H_2 \xrightleftharpoons{\text{Phosphohexokinase+ATP}} C_6H_{10}O_4.(PO_4H_2)_2$
(Fructose-diphosphate)

(iv) $C_6H_{10}O_4.(PO_4H_2)_2 \overset{\text{Aldolase}}{\rightleftharpoons} CH_2.(PO_4H_2).CHOH.CHO$ (Phosphoglyceraldehyde) $+ CH_2.(PO_4H_2).CO.CH_2OH$ (Dihydroxyacetone phosphate)

(v) $CH_2.(PO_4H_2).CHOH.CHO + H_3PO_4 \overset{\text{Triosephosphate dehydrogenase}}{\rightleftharpoons} CH_2O.(PO_3H_2)_2.CHOH.COOH$ (Diphosphoglyceric acid)

(vi) $CH_2O.(PO_3H_2)_2.CHOH.COOH \overset{\text{Phosphoglyceric transphosphorylase + ADP}}{\rightleftharpoons} CH_2.(PO_4H_2).CHOH.COOH$ (Phosphoglyceric acid)

(vii) $CH_2.(PO_4H_2).CHOH.COOH \overset{\text{Enolase}}{\rightleftharpoons} CH_2.CO.PO_3H_2.COOH + H_2O$ (Phosphopyruvic acid)

(viii) $CH_2.CO.PO_3H_2.COOH \overset{\text{Phosphopyruvate transphosphorylase + ADP}}{\rightleftharpoons} CH_3.CO.COOH$ (Pyruvic acid)

The chemical reactions taking place may be represented as follows:

(i) $CH_3.CO.COOH$ (Pyruvic acid) $\overset{\text{Carboxylase}}{\rightleftharpoons} CH_3.CHO + CO_2$ (Acetaldehyde)

(ii) $CH_3.CHO + DPN.H_2 \overset{\text{Alcohol dehydrogenase}}{\rightleftharpoons} CH_3CH_2OH + DPN$ (Ethyl alcohol)

In some cases of bacterial fermentation, the pyruvic acid, by the action of $DPN.H_2$, is converted into lactic acid and DPN as follows:

$CH_3.CO.COOH + DPN.H_2 \rightleftharpoons CH_3.CHOH.COOH + DPN$ (Lactic acid)

It is to be noted, however, that although he majority of the fermentation reactions are anaerobic, a few of these may be aerobic also, and the amount of energy liberated is far less than that of aerobic respiration such as in case of alcoholic fermentation, the energy liberated is about 28 calories.

On the other hand, in the aerobic oxidation of pyruvic acid, in which the end products are carbon dioxide and water, the mechanism involved in the formation of a 6-carbon acid (e.g. cis-aconitic acid, etc) by the reactions of oxalacetic acid (4-carbon acid) and pyruvic acid (3-carbon acid) accompanied by the evolution of carbon dioxide and the subsequent conversion of this 6-carbon acid into 5-carbon acid (α-ketoglutaric acid) which in its turn give rise to 4-carbon acids (e.g. Succinic acid, axalacetic acid, etc) which again reform the 6-carbon acid as mentioned above. The chemical reactions involved are given below in a cyclic fashion, the cycle being known as *Krebs cycle, tricarboxylic acid cycle, citric acid cycle, or cyclophorase system.*

From the diagram, it is evident that one molecule of water is liberated during each of the four stages of the reactions:

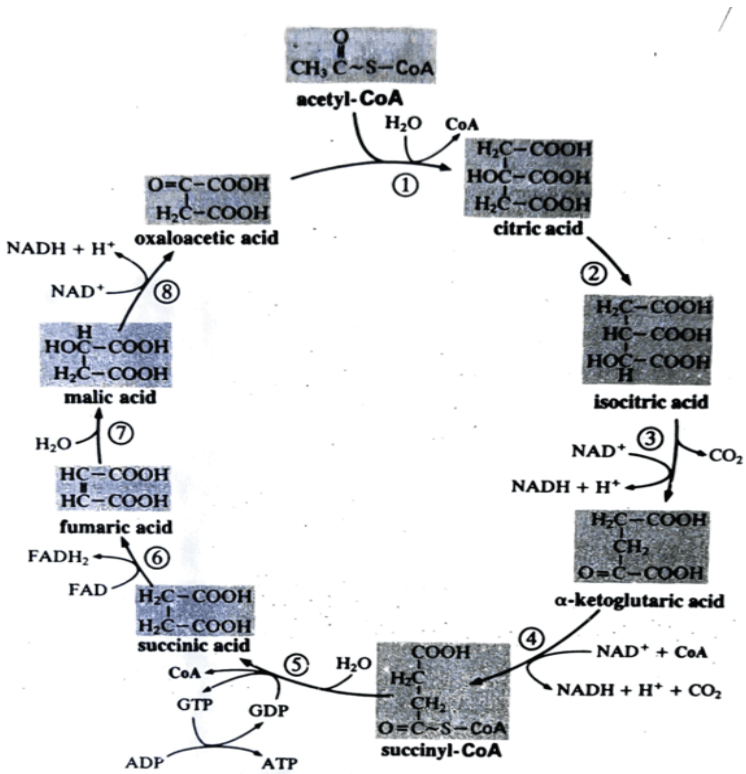
i. during the formation of cis-aconitic acid from pyruvic acid
ii. in the formation of alpha-ketoglutaric acid from cis-aconitic acid via iso-citric acid
iii. in the formation of fumaric acid from alpha-ketoglutaric acid via succinic acid, and
iv. during the formation of oxal-acetic acid from fumaric acid.

The pyruvic acid thus produced undergoes further changes both in the presence as well in the absence of outside supply of oxygen. The former process constituting the terminal phase of aerobic respiration and the later one that of anaerobic respiration.

During the anaerobic oxidation of pyruvic acid which may take place in the fungi and micro-organism amongst the lower plants (where it is known as fermentation) as well as in the living tissues of higher plants, in complete breakdown of the intermediate substrate gives rise to various types of organic acids, alcohol etc. with the subsequent evolution of carbon dioxide.

They pyruvic acid under the influence of the enzyme carboxylase, is converted into acetaldehyde and carbon dioxide, the latter being liberated.

The acetaldehyde then, being acted upon by the coenzyme DPNH2 gives rise to ethyl-alcohol and DPN. Pyruvate enters in mitochondria where it is oxidized by Kreb's cycle or TCS (Tri carboxylic acid cycle). (As shown below).



The Krebs citric acid cycle.

Respiration is the process of oxidation-reduction in which organic substances are step wise degraded to release, energy incorporated into ATP.

Respiration is of two types i.e. **aerobic** - which takes place in presence of free oxygen, releasing comparatively more energy (38 ATP) and **un-aerobic** taking place in absence of free oxygen, releasing comparatively less energy (2 ATP).

Aerobic respiration takes place in three steps. Glycolysis cycle and electron transfer and terminal oxidation.

Glycolysis takes place in cytoplasm in absence of free oxygen in which glucose is broken down into two molecules of pyruvic acid and generating 2 ATP.

Krebs cycle takes place in the matrix of mitochondria in presence of Oxygen and yields 12 ATP per molecule of pyruvic acid. Electron transfer is the last step during which hydrogen combines with free oxygen to form

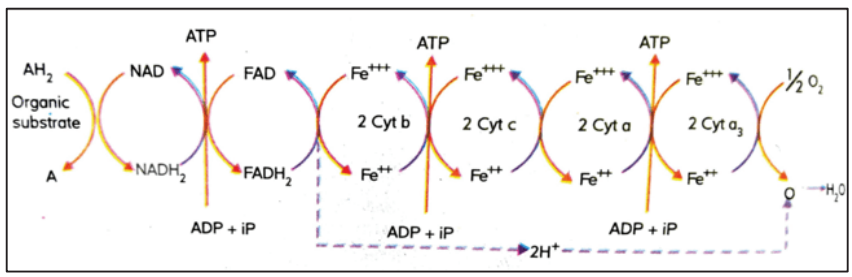
water molecule and energy is actually released due to cytochromes and enzyme systems located on the F_1 particles of aristae.

Fermentation is a process in which chemical changes are brought about In an organic substrate by the action of enzyme secreted by organisms it is of two types alcoholic fermentation and lactic acid fermentation.

Electron Transfer System (ETS) and terminal Oxidation

Electron Transfer System (ETS)

Electron Transfer is the last step of aerobic respiration in which energy is released to form ATP that takes place in mitochondria. The cristae of mitochondria are studded with F1 particles. Each F1 particle has a respiratory assembly called electron transfer system. It is composed of hydrogen acceptor like NAD, SAD and Electron carriers such as cytochromes b, c, a and a_3. The entire electron transfer system is arranged in order of decreasing energy levels in f1 particle. At each step, a small amount of energy is given out which combines 1 phosphate with ADP to form ATP. If all the energy is liberated at a time, plant cells are unable to utilize it.



Electron Transfer System (ETS) and terminal Oxidation

Terminal Oxidation

The H2 liberated during oxidation is first accepted by NAD which gets reduced to form $NADH_2$. When H_2 leaves $NADH_2$ and goes to FAD, the NADH2 is oxidized back to NAD. While FAD is reduced to FADH2. During this transfer energy is released to form 1 ATP Molecule. The H2 leaves FADH2 and split up into 2 H+ and 2e-. Of these, the electron pair passes through cytochromes b, c, a and a3. The cytochromes are Ferric (Fe+++) compounds. Each cytochrome accepts electron and gets reduced to ferrous (Fe++). When it gives out electron, it is oxidized back to Ferric (Fe^{+++}). Then the electron goes to near cytochrome.

Thus, when electron pair passes through cytochrome b, c, a and a_3, the cytochromes, are alternately reduced and oxidized and generate energy to form 2 more molecules of ATP, one during the transfer of electrons from cytochrome, b to c and other during the transfer of electron from cytochrome a to a_3. Thus totally 3 ATP Molecules are formed per H_2 liberated during oxidation of the substrate. Hence this process is called **Oxidative Phosphorylation**. As Hydrogen acceptance and cytochromes are alternately reduced and oxidized during the process, respiration is called **Redox Reaction.**

Significance of Terminal Oxidation

i) The process of oxidation is completed due to terminal Oxidation.

ii) As oxidation is complete, poisonous substances are not produced.

iii) Hydrogen does not remain free to reduce aldehyde or any other useful substances of the cell.

In aerobic respiration, 38 ATP molecules are produced per glucose molecule, as shown below.

Sr. No	Sequence of Reactions			ATP generated
1	Glycolysis		=	2 ATP
2	Oxidation of $NADH_2$ (Formed in glycolysis)	(3X2)	=	6 ATP
3	Acetyl Co enzyme Formation	(3X2)	=	6 ATP
4	Krebs Cycle	(12X2)	=	24 ATP
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$			**38 ATP**	

Energy efficiency of Aerobic Respiration

By complete oxidation of 1 molecule of glucose, 686 Kcal or 2870 KJ energy is generated. However, this entire energy is not conserved as you have seen that per glucose molecule only 38 ATP are formed. Since each terminal, high energy bond of ATP is equal to 7.3 K Cal or 36.6 KJ, the total energy conserved is 277.4 Kcal (38 X 7.3 Kcal) or 11,628 KJ (38 X 30.6 KJ). Thus, out of 686 Kcal, only 11628 KJ energy is conserved which is approximately 40.5%. Still, it is much more than any man-made fuel machine.

Cyanide Resistant Respiration.

Cyanide resistant respiration occurs in yeast, that involves a mitochondrial alternative, oxidases, sensitive to Salicylhydroxamin acid (SHAM). In this cyanide resistant respiration, most of the energy liberated is lost as heat and only little of it is consumed in the production of ATP. Most cyanide produced in plants is detoxified by B-Cyanoalanin synthase. Cyanide binds with iron in cytochrome a3 preventing electron transport in the cytochrome. In *Spinacia* plant, cyanide resistant respiration is found.

Gluconeogenesis

Gluconeogenesis is a process that transforms non carbohydrate substances (like lactate, amino acids and glycerol) into glucose.

Lactate and Alanine are converted into pyruvate and then enters the mitochondria and is carboxylated to Oxalo Acetic acid by Pyruvate Carboxylase. It is essential in the regulation of acid -base balance, amino acid metabolism and synthesis of carbohydrates derived structure components.

Final product of gluconeogenesis is the formation of new glucose molecule. Major source of gluconeogenesis is carbohydrate and then amino acid.

Glycolysis occurs in the cytoplasm and gluconeogenesis is the reverse reaction of glycolysis where two pyruvate molecules come together to form glucose molecule. Gluconeogenesis is a biochemical pathway in which glycogen breaks down into glucose and glucose 1-phosphate. It is a group of metabolic reactions in cytosol and mitochondria to maintain the blood glucose level.

Chapter 5
Nitrate Assimilation

Nitrate assimilation is one of the two major biological processes in which inorganic nitrogen is converted to ammonia and hence to organic nitrogen. Nitrate reductase converts nitrate to ammonia. Nitrate (NO_2) is highly reactive, potentially toxic ion. Plant cells immediately transport the nitrate generated by nitrate reduction from the Cytosol into Chloroplast in leaves and plastids in roots. The enzyme nitrite reductase reduces nitrite to ammonia. Nitrate assimilation is a key process for nitrogen acquisition in Chlorophycean algae.

NITROGEN FIXATION

Biological nitrogen fixation may be defined as "Reduction of atmospheric nitrogen into ammonia under anaerobic or more correctly in micro-aerophilic condition by a variety of micro-organisms such as Bacteria and blue green algae".

Reduction of nitrogen to ammonia is an exothermic process. Nitrogenase enzyme system, for fixation of nitrogen, requires energy which is accomplished through the hydrolysis of ATP.

Process of Nitrogen fixation: Winogradsky (1891-94) observed that Nitrogen fixation is found in prokaryotes like bacteria and blue green algae. Some fungi may also have this property.

There are three categories of organisms concerned with nitrogen fixation and those are:

(a) Symbiotic fixers. (b) Non-symbiotic fixers (c) Blue green algae.

Symbiotic nitrogen fixation Bond (1968) has listed nine main types of symbiosis involved in nitrogen fixation. Nodulated legumes are the best known.

1. Nodulated roots of legumes.
2. Nodulated roots of non-legumes.
3. Leaf nodules.
4. Corolloid root nodules of cycas.
5. Phyllosphere association.
6. Mycorrhiza.
7. Lichen having blue green algae.
8. Liver worts with blue green algae.
9. *Azolla-Anabaena* association.

Nitrogen fixation in nodulated Legumes

Members of family papilionaceae bear small tubercle like outgrowth called nodules. The process of Nitrogen fixation by nodulated legumes can be studied under the following heads :

(a) Process of nodulation.

(b) The leg-haemoglobin and its role.

(c) The role of enzyme nitrogenase.

(d) Reduction of molecular nitrogen.

(e) The genetical aspects in the light of recent works.

Process of Nodulation

(1) The nodules are formed by the local proliferation of host cells.

(2) Infection spreads from the variety of bacteria present in the soil. They are *Rhizobium leguminosarum, R. radicicola, Pseudomonas radicicola, Bacillus radicicola* etc.

(3) They enter the root through root hair or superficial lesion. Inside the root they form branched infection threads.

(4) The infection threads advance in the root cortex and sheaths of infection thread burst. Bacteria are liberated inside the host cells. Then they multiply and give rise to tiny branched structure called bacteroid.

(5) The infected cells are stimulated to grow and divide. The nearby cells also divide rapidly and produce a nodule.

(6) The stimulation of nodule formation seems to be provided by Indole-3- acetic acid which is secreted by invading Rhizobium. (Thimann, 1939, Turner & Anderson, 1964).

(7) *Rhizobia* are parasitic at least in initial stages. As soon as Nitrogen is fixed, most of it is passed out of bacteroid into the host cells which are connected with a vascular strand for quick translocation to other parts of the plant.

Root nodulated Non-legume Angiosperms: Root nodules are present in several non-legume angiosperms e.g. *Almus, Casuarina, Hippophae, Elaeaghus* etc.

Dommergues (1963) has estimated that *Casuarina equisetifolia* accumulates about 58 kg. of nitrogen per hectare annually.

Schede (1962), Silver (1964) and Gardner (1965) has reported actinomycetes responsible for Nitrogen fixation in non-legume roots.

Leaf nodules About 100-200 nodules may be present on a single leaf. The Nitrogen fixation is carried out under anaerobic condition by *Klebsiella* and *Xanthomonas*. Leaf nodules are found in tropical and subtropical species belonging to the family *Rubiaceae* and *Myrsinaceae.*

Cycad corolloid root nodule: Cycads corolloid negatively geotropic roots possess deep green zone of blue green algae. These fixes Nitrogen, Parts of its organic nitrogen is lost to the cycad host by excretion.

$$2NO_2 + 7NADH_2 / NADPH_2 \xrightarrow[\text{Ferredoxin}]{\text{Nitrate reductase}} 2NH_3 + 4H_2O + 7NAD / NADP$$

Genetical aspect: Infectiveness (Inf^{+}) and effectiveness (Eff^{e+}) are the two characteristics of Rhizobium, which permit the organism to enter into the roots in a symbiotic fashion and form nodules which fix nitrogen.

The *Rhizobium* lacking both (Inf and Eff) cannot form nodules, strains with Inf character for nodules but cannot fix nitrogen.

Now it is possible to introduce bacterial genes of "nif" genes called by geneticists. Such genetic engineering will be helpful to fix nitrogen without the association of bacteria and other nitrogen fixing organisms. The "nif" gene from *Rhizobium trifolii* has been successfully transferred to *Klebsiella aerogenes* a bacterium which does not naturally fix nitrogen with the help of restriction endonuclease. It is now possible to cut and join the DNA carrying "nif" gene from one source to another DNA molecule (Plasmid) and reconstruct a viable hybrid DNA molecule carrying the "nif" gene.

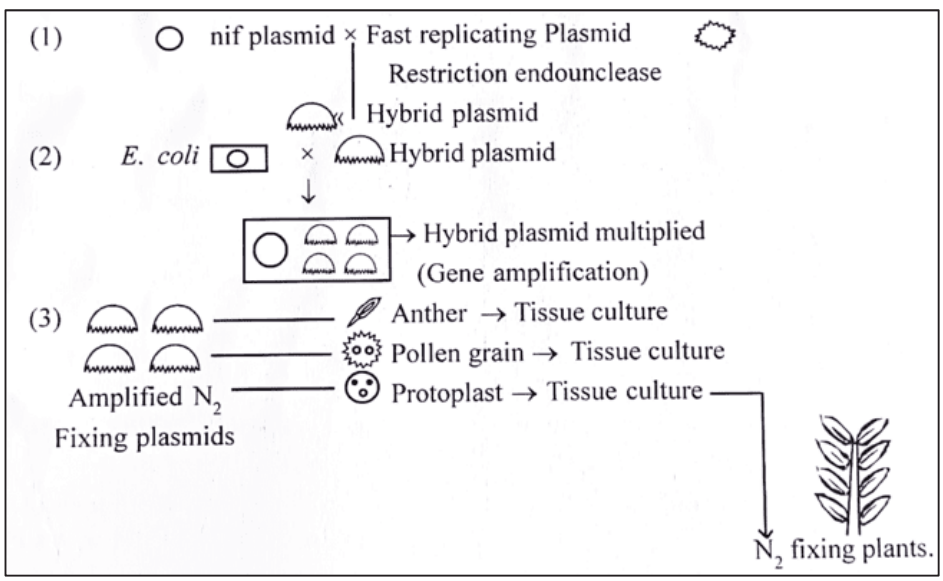
There are two methods to introduce this "nif" DNA molecule to any crop.

(1) Forced incorporation of this "nif" DNA into the host plant and subsequent integration

into its genetic system.

(2) The "nif" carrying DNA can be hooked on a plasmid and transfer to plant protoplast cultures to produce crop plants.

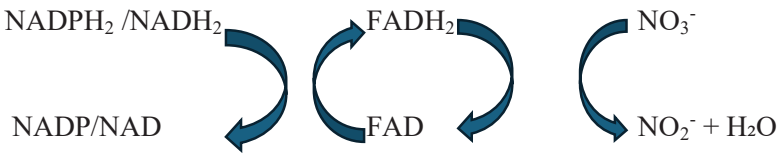
When this 'nif' gene is introduced into a crop plant, the new host may not fix nitrogen if there is no leghaemoglobin to regulate the cellular oxygen pressure. This is an active area of research. Following are the methods of genetic engineering to obtain nitrogen fixing variety of crop plant.



(1) Reduction of nitrate to nitrite: It takes place in presence of enzyme nitrate reductase:

$$NO3^- + 2H^+ \xrightarrow[reductase]{Nitrate} NO_2^- + H_2O$$

H+ required for reduction of nitrate comes from $NADPH_2$ or $NADH_2$



(2) Reduction of nitrite to Ammonia: Bussey (1968), Sims, Folkes and Bussey (1968) has shown in yeast that it occurs by a group of enzyme complex, but Ingle et al (1966) had suggested a separate nitrate reductase in higher plants.

Nitrite combines with enzyme. It reduces stepwise to NH, but none of the intermediate occurs in the free state.

The leg-haemoglobin and its role: The leguminous nodule has red/pink pigment which resemble haemoglobin. It is found in the cells possessing bacteroid. The leg-haemoglobin has two parts (1) globin (2) haeme (iron). Host cells contribute the protein globin part and the bacteroid contribute haem as suggested by Cutting and Schulman (1968). Thus, leg- haemoglobin is true molecule of symbiosis.

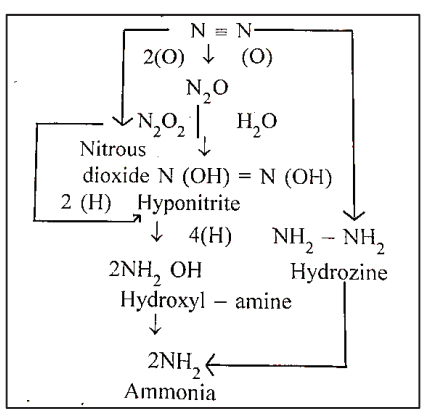
The work with N^{15} has shown that the power of Nitrogen fixation resides inside the bacteroids. The leg haemoglobin picks up the oxygen which is in short supply in soil and it also protects the nitrogenase from free O_2 as O_2 inactivates the enzyme. In this way leg haemoglobin keeps the respiratory activity at optimum levels for forming ATP required for N, fixation without harming nitrogenase.

The role of nitrogenase: Nitrogenase found in bacteroid and considered as Central Dogme of Nitrogen fixation. It has power to reduce a wide array of substrate.

The nitrogenase has two components (a) Mo-Fe Protein and (b) Fe-protein. The nitrogenase is Oxygen sensitive. Therefore, N_2 fixation occurs in anaerotic condition because of inactivated nitrogenase.

The mechanism for protecting the nitrogenase from O_2 is not clear. Nitrogenase activity is associated with the availability of carbon skeleton. The possible role of carbon skeleton appears to be in the incorporation and metabolism of the fixed N_2. External supply of this skeleton is essential for N, fixation by obligatory and associative symbiolic nitrogen fixers. In the nodules carbohydrates are transported from the host. The synthesis of nitrogenase requires m-RNA from legume host as reported by Dilworth and Parker (1969).

1. **Reduction of molecular Nitrogen**: It requires a dark or light generated reducing power, source of energy like ATP and some enzymes. It involves oxidation, reduction, and hydration.



Reduction of molecular nitrogen takes place in presence of enzyme nitrogenase which weakens or activate the nitrogen molecule so that it can be reduced easily.

Another type of enzyme required is hydrogenase which acts directly on nitrogen to form nitrogenase complex or after hydration of N_2. Hydroxylamine (NH_2OH) is a key product of N_2 reduction. It can combine with some organic acids to form oximes or further reduced to

Ammonia. Hydroxylamine or ammonia do not occur in free state. They probably remain attached to a proteinaceous compound till the final product combines with some organic acids

derived from Kreb's cycle to give rise to organic compounds of Nitrogen.

Phyllosphere Association: Ruinen (1965,67) found two types of Bacteria namely Azotobacter and Beijerinckia growing on the leaves of a number of trees and shrubs. Some of the nitrogenous compounds are absorbed by the foliar surface from the bacterial excretion. Leaching of bacterial excretions by rain and dew can also enrich the soil.

Mycorrhiza: A large number of higher plants like *Pinus radiata*, *Ammophilia* species bear mycorrhiza as reported by Stevenson (1959), Hassouna and Wadeing (1964). The fungus partner absorbs organic nitrogen from the soil and hand over to the higher plants.

(2) **Non-Symbiotic N_2 fixation**: Non-symbiotic bacteria responsible for N_2 fixation are of four types. (a) *Azotobacter* species, (b) *Clostridium* species, (c) photosynthetic sulphur bacteria, (d) Chemosynthetic sulphur bacteria. *Azotobacter* is obligatory aerobic while Clostridium is strictly anaerobic.

The N_2 fixation is done by a group of enzyme system present in the protoplasm of Bacteria called "Azotase". According to Miettinen (1963) all photosynthetic bacteria possess the property of nitrogen fixation. e.g. *Rhodopseudomonas capsulatus* is a common photosynthetic nitrogen fixing bacterium of the rice field.

(3) Nitrogen fixation by blue green Algae. As many as 20 heterocyst bearing species belonging to *Anabaena, Oscillatoria* and *Nostoc* have been shown to capable of N_2 fixation. These organisms can fix nitrogen aerobic and anaerobic both conditions. In heterocyst photosynthesis occurs without oxygen generation and N_2 fixation occurs similarly in these cells. Singh (1958) reported that blue green algae increase the N_2 contents of the Paddy fields. They also fix nitrogen in anaerobic conditions. It was considered that direct photoreduction of the molecular N_2 may occurs as follows.

$$\mathbf{N_2 + 3H_2O \longrightarrow 2NH_3 + 1.5\ O_2}$$

Later on it was suggested that this O_2 evolution takes place in photosynthesis and not in Photoreduction of molecular N_2. (The reducing power generated by the decarboxylation of pyruvate considered to be passed on to the enzyme complex nitrogenase via ferrodoxin). The presence of enzyme complex nitrogenase) in the heterocyst has been established and the role of

heterocysts as sites of nitrogen fixation has been proved. Blue green heterocystaceous algae always contain nitrogenous compound in their mucilage sheaths. These compounds are liberated along with mucilage from the pores present in the cell wall, in the form of peptide compounds or amino acids or rarely nitrates and nitrites.

Chapter 6
Carbon Assimilation

Carbon assimilation means the formation of carbon containing comprends by the plants from carbon dioxide and water. Green Plants with the help of Chlorophyll in presence of sunlight are

carry on the process known as Photosynthesis- Photosynthesis is oxidation reduction process in between CO_2 and H_2O in presence of sunlight with help of chlorophyll to synthesize carbohydrates and O_2 is liberated from CO_2 as bi- -product. This can be written as:

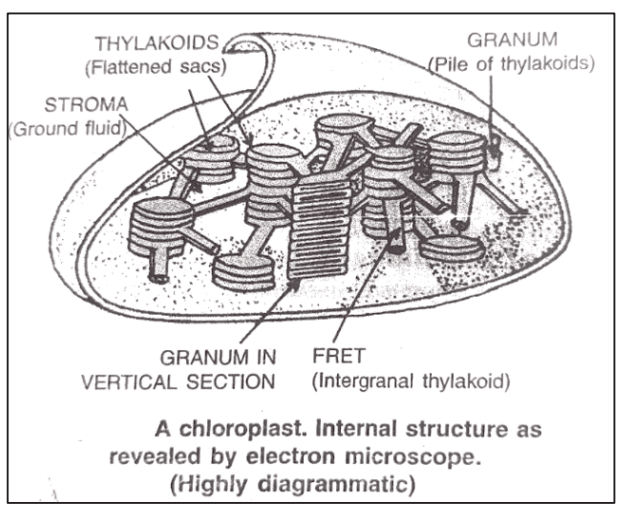
$$6\ CO_2 + 12\ H_2O \xrightarrow[Chlorophyll]{sunlight} C_6H_{12}O_6 + 6H_2O + 6O_2$$

Van Niel (1930) observed that O_2 is liberated from CO_2 and not from H_2O. Robert Hill (1939) experimentally proved that O_2 is not evolved from CO_2 but by splitting of H_2O molecule in Sunlight. This in called **photolysis of water** or **Hill reaction**. In this process water is processed that uses light energy to split water molecules into oxygen and hydrogen.

It occurs in the granum of chloroplast where chloroplast absorbs light and converts it to chemical energy. This energy splits water molecules into H+ and O^-. Oxygen is released into the atmosphere.

Hydrogen bonded to NADP to form NADPH. It helps to produce ATP during the light phase and acts as reducing agent that provides H^- ions. Reuben and Kamen (1941) confirmed that H_2O was source of O_2.

Photophosphorylation: In this process of Photosynthesis. the photophosphorylation of ADP to form ATP using the energy of sunlight. In other words, light energy is converted into Chemical energy in the form of ATP molecule.



A chloroplast. Internal structure as revealed by electron microscope. (Highly diagrammatic)

Photophosphorylation is of two types: (1) cyclic and (2) non-cyclic.

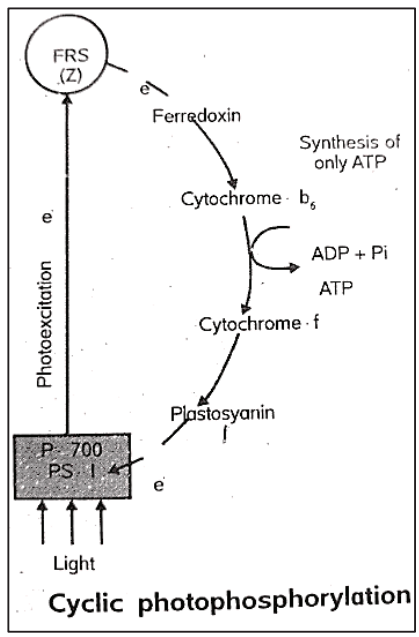
Cyclic Photophosphorylation involves synthesis of ATP activated by Photo- System I and proceed in long wavelength (700nen) In non-cyclic Photophosphorylation both NADPH and ATP are produced as shown in the following diagrams.

a) Cyclic photophosphorylation: This process involves photosystem I only. It consists of reaction centre formed by a molecule of chlorophyll-a called P-700 which has maximum absorption at 700nm wavelength of light. Besides, it consists of molecules of essential pigment chlorophyll-a and those of accessory pigments like chlorophyll-b, carotene and xanthophyll. All these molecules act as "antennae" to absorb and transfer the light energy to the reaction centre i.e. P-700. Due to this, the reaction centre (P-700) gets excited and emits out an energy rich electron. It is first accepted by primary electron acceptor Z which is ferredoxin reducing substance (FRS). From FRS the electron passes to

Ferredoxin, Cytochrome by Cytochrome f and then to plastocyanin. During this journey the energy is stepped down from energy rich electron and is utilized in the synthesis of ATP from ADP and iP. ATP synthesis takes place when electron passes from cytochrome b, to cytochrome f. The deenergised electron then passes from plastocyanin to P-700. As P-700 receives its own electron back, the process is said to be

cyclic and as light energy is converted into ATP, it is called photophosphorylation.

b) Non-cyclic photophosphorylation: This process involves both the photosystems i.e. photosystem I and photosystem II. In photosystem I the reaction centre is formed by a molecule of chlorophyll-a called P-700 which has maximum absorption at 700nm wavelength of light while in photosystem II the reaction centre is formed by a molecule of chlorophyll-a called P- 680 which has maximum absorption at 680nm wavelength of light. Both the photosystems also have molecules of pigment chlorophyll-a and those of accessory pigments like chlorophyll-b, carotene and xanthophyll.



Significance: Light energy is converted into chemical energy in the form of ATP.

When photosystem II is illuminated by light, P-680 is excited and emits out energy rich electron which is first accepted by primary electron acceptor Q which is Plastoquinone Reducing Substance (PQRS). From Q the electron passes to plastoquinone and then to cytochrome by, cytochrome f and plastocyanin. During this journey the energy is stepped down from energy rich electron and is utilized in the synthesis of ATP by adding iP to ADP. ATP synthesis takes place when electron passes from cytochrome b, to cytochrome f. From plastcyanin the electron of PS-II passes to PS-I i.e. to P-700. When photosystem I is illuminated by lig ht, P-700 is excited and emits out energy rich electron which is first accepted by primary electron acceptor Z which is ferredoxin reducing substance (FRS) From Z, the electron is transferred to ferredoxin and then to NADP. NADP gets ionised when it accepts the electron. Thus, the electron does not go back to P-680. At the same time, water molecules undergo photolysis to form H+ and OH ions. H+ ions go to ionised NADP to form NADPH, while OH ions lose their electrons to form OH which then form H_2O and O_2. The electrons of OH ions go to P-680 of PS-II to replace the electrons donated by it. Thus P-680 receives electrons of OH and not of its own as they are donated to P-700. Since, PS-I receives electrons of PS-II, NADP receives electrons of PS I and PS-II receives electrons of OH, the process is non-cyclic, and as light energy is converted into ATP, it is called photophosphorylation.

Significance of non-cyclic photophosphorylation

1. In the light reaction, light energy is absorbed by pigments of grana, but plants cannot utilize it

therefore, light energy is converted into chemical energy to form ATP.

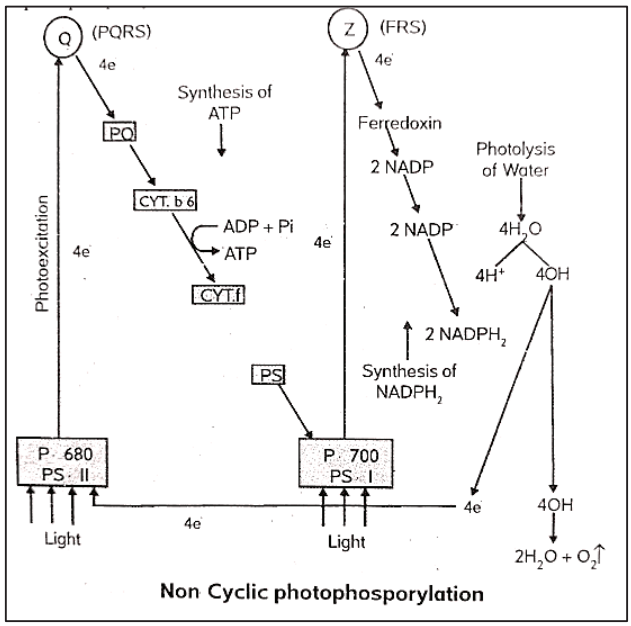
2. In addition, a strong reducing agent NADPH, is formed which reduces CO_2 in the dark reaction with the help of ATP.

3. During the process O_2 is released as byproduct.

4. Non-cyclic photophosphorylation is more efficient than cyclic, as it produces both ATP and

Light reaction takes place in grana and dark reaction in Stroma of Mitochondria. Light energy is absorbed by pigments of grana and stored by adding inorganic phosphate and hence

it is called photophosphorylation.



$NADPH_2$. Arnon (1957) called it assimilatory power as both ATP and $NADPH_2$ are required for CO_2 assimilation. Comparison between Cyclic and Non-cyclic photophosphorylation.

Cyclic photophosphorylation	Non-cyclic photophosphorylation
1. Involves photosystem I only.	1. Involves both photosystems I and II.
2. Chlorophyll-a of PS-1 receives its own electron back.	2. PS-I receives electrons of Ps-II, NADP receives electrons of PS-I and PS-II receives electron of OH formed due to photolysis of water.
3. Movement of electrons is in cyclic manner.	3. Movement of electron is unidirectional.
4. Only ATP is generated. Does not involve photolysis of water.	4. Both ATP and $NADPH_2$ are formed.
6. Free O_2 is not evolved.	5. Involves photolysis of water.
7. Less efficient.	6. Free O_2 is evolved.
8. Water molecules are not required.	7. More efficient.
9. Water molecules are not regenerated.	8. Water molecules are required to donate electrons.
	9. Water molecules are regenerated.

DARK REACTION OR BIOCHEMICAL PHASE OR SECONDARY PROCESSES

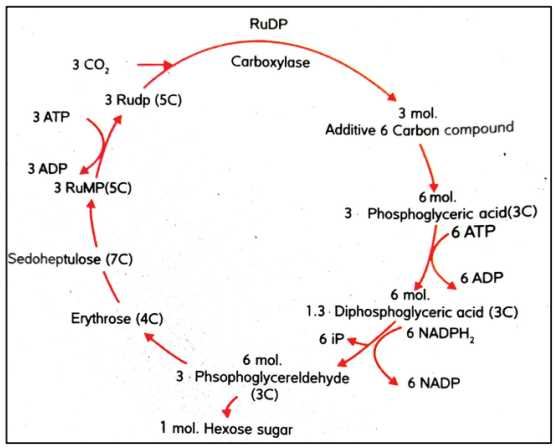
This reaction takes place in stroma for which light is not essential. It is a biochemical reaction in which CO_2 is fixed with the help of ATP and $NADPH_2$ formed in light reaction, to form carbohydrates. The path of carbon during the reaction was studied by Melvin Calvin in 1946 by using the culture of unicellular alga *chlorella* and the radioactive isotope ^{14}C. He separated the products by paper chromatography and identified them by

autoradiography. The entire reaction is cyclic and is called Calvin - cycle. Calvin was awarded Nobel prize in 1961.

The main steps of Calvin cycle are described below:

1. Three molecules of Ribulose monophosphate (RuMP), 5C compound, are phosphorylated by ATP to form three molecules of Ribulose diphosphate (RuDP).
2. RuDP is the first CO_2 acceptor. It absorbs CO_2 in presence of RuDP- carboxylase for form transitory or additive 6C compound, which is unstable.
3. Three molecules of additive 6C compound immediately split up by hydrolysis into six molecules of 3C compound called 3-phosphoglyceric acid (3-PGA). As first stable product is 3C compound the plants performing Calvin cycle are called C3 plants and the process is also called C3 pathway.
4. Six molecules of 3-PGA are further phosphorylated by ATP and converted into six molecules of 1, 3-diphosphoglyceric acid (DPGA).
5. Six molecules of 1,3-DPGA are now reduced to six molecules of 3-phosphoglyceraldehyde (PGAL) by utilizing H_2 from $NADPH_2$ and releasing one phosphate each.
6. One molecule of 3-PGAL, a triose sugar can be directly used for metabolism of the plant or it can be converted into Hexose sugar and is ultimately stored in the form of starch.
7. Five molecules of 3-PGAL (3C) are converted into 3 molecules of RuMP (5C) through intermediate products like erythrose and sedoheptulose to continue the cycle.

To form one molecule of glucose, 18 mols. of ATP and 12 mols. of $NADPH_2$ are required.



Significance of Calvin - Cycle

Calvin Cycle

i. By using ATP and NADPH, formed in light reaction, CO_2 is reduced to form organic food in the form of 3-phospho-glyceraldehyde (3C) or glucose (6C) which can be utilized directly in plant metabolism or can be stored in the form of starch.

ii. ATP and $NADPH_2$ are utilized as energy donor and H_2 donor and H_2 donor to form ADP and NADP respectively for reuse in the light reaction.

Differences between light and dark reactions

Light reaction	Dark reaction
1. It is photochemical reaction.	1. It is biochemical reaction.
2. It takes place only in presence of light.	2. It is independent of light.
3. It takes place in grana of chloroplast.	3. It takes place in stroma of chloroplast.
4. It synthesizes ATP and NADPH.	4. It utilizes ATP and NADPH.
5. O_2 is released.	5. CO_2 is fixed or reduced to produce simple sugars.
6. Involves photolysis of water.	6. Does not involve photolysis of water.
7. Water molecules are regenerated.	7. Water molecules are not regenerated.
8. It takes place in both cyclic and non-cyclic manner.	8. It is only cyclic.

Bacterial Photosynthesis- Photosynthetic Bacteria can convert light energy into chemical energy, fix CO_2 into organic compounds and are used in agriculture, waste water treatment and other industries. They are of two types. 1) Oxygenic and 2) Anoxygenic.

Oxygenic bacteria are like cyanobacteria and anoxygenic bacteria are clostridium etc. It does not release oxygen.

It is of three categories:

1) Green Sulphur Bacteria e.g. *Chlorobium*

$$6CO_2 + 12H_2S \xrightarrow[Chlorophyll]{energy} C_6H_{12}O_6 + 6H_2O + 12\,S$$

2) Purple Sulphur Bacteria e.g. *Chromatium*

$$3NO_2S_2O_3 + 6CO_2 + 15H_2O \xrightarrow[Bacterio\ Chlorophyll]{energy} C_6H_{12}O_6 + 6H_2O + 6NaHSO_4$$

3) Purple Non-sulphur Bacteria e.g. *Rhodospirillum*

$$6CO_2 + 12\,CH_3CHOHCH_3 \rightarrow C_6H_{12}O_6 + 12\,CH_3COCH_3 + 6H_2O$$

It utilizes wavelength longer than 700nm /B-890. Electron donor is not water, No evolution of oxygen.

Photorespiration:

It is a process occurs in Calvin cycle during Plant metabolism. Enzyme RUBISCO is responsible for the fixing of CO_2, reacts with oxygen rather than CO_2. Why Photorespiration takes place? In too hot or day, Plants often close their stomata to prevent water loss. This prevents CO_2 from entering the leaf. O_2 builds up inside the leaf and Photo- respiration occurs in stead of Calvin cycle.

The products are one molecule of Phosphoglycerate and another 2 phosphorylate, where the carboxylase activity yields two molecules of Phosphoglyceric acid.

Photorespiration was discovered by Decker when he observed the rate of respiration of green leaves much higher in light than in dark. It occurs when there is more O_2 in the environment than CO_2. It is also known as oxidative photosynthetic carbon cycle. RUBISCO (Ribulose 1,5 phosphate carboxylase oxygenase enzyme).

It occurs across three different organelles in the plant cell, the chloroplast, the peroxisome, and the mitochondria. It is a wasteful process that occurs in 3C plants under high oxygen and low carbon dioxide.

In chloroplast glycolate is formed. It changes in glyoxylate in presence of enzymes, in peroxisome. The glyoxylate changes to glycine. The glycine changes to serine in mitochondria. It is not involved in ATP production and lacks bundle sheath.

Respiratory quotient (R.Q): It is represented as CO_2 evolved / O_2 consumed

R.Q of carbohydrate: 6 /6 =1

R.Q of fats: $2C_{51}H_{98}O_6 + 145O_2 = 102\ CO_2 + 98\ H_2O = 102/145 = 0.7$ (<1)

R.Q of Oxalic Acid: $2C_2H_2O_4 + O_2 = 2H_2O\ 4\ CO_2 = 4/1$ (>1)

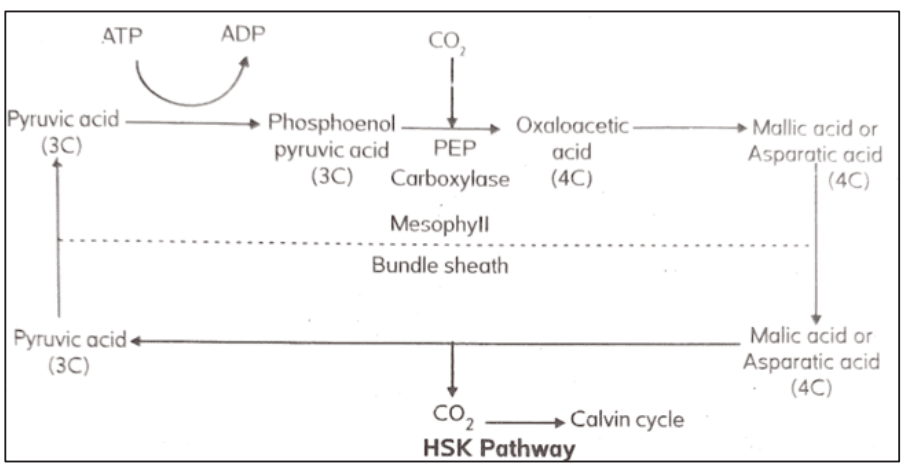
R.Q of Anaerobic oxidation of carbohydrate:

$$C_6H_{12}O_6 + \text{Zymase} \longrightarrow 2C_2H_5OH + 2\ CO_2 = 2/0$$

Hatch Slack and Kortschak (HSK) Pathway or C_4 Pathway

A major new pathway for carbort during photosynthesis, other than Calvin-cycle, was established in some tropical plants like sugarcane, maize, jowar, millet, and dicotyledonous plants like Amaranthus.

Initial studies by Kortschak et al (1965) in Hawaii on sugarcane revealed that the major early products of phtotosynthesis are C_4 acids like malate and aspartate. The Australian botanists Hatch and Slack (1966) confirmed this and proposed C_4 pathway. Hence, this pathway is also called Hatch, Slack and Kortschalk (HSK) pathway.



HSK Pathway

The plants following C4 pathway show Kranz anatomy (Kranz = wreath). i.e.

i) The vascular bundles are surrounded by bundle sheath cells, forming wreath-like layer.

ii) Around the bundle sheath cells are the loosely arranged mesophyll cells.

iii) Chloroplasts of bundle sheath cells are agranal i.e. without grana and chloroplasts of mesophyll cells are granal i.e. with well-developed grana. Thus, chloroplasts show dimorphism.

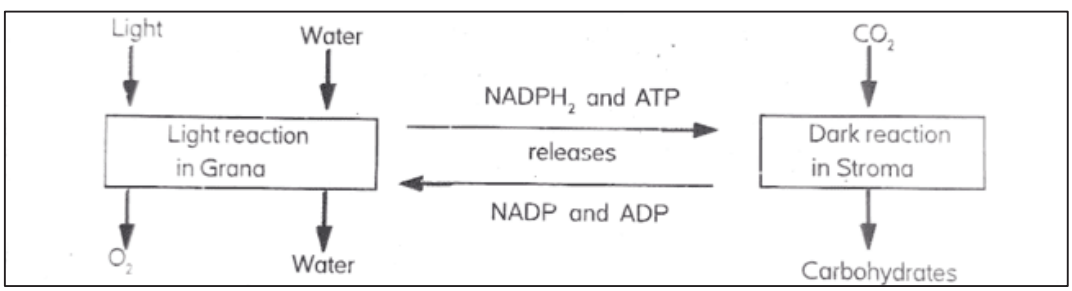
Differences between C_3 plants and C_4 plants

C_3 plants	C_4 plants
1. Usually temperature plants.	1. Usually tropical plants.
2. Do not show Kranz anatomy.	2. Show Kranz anatomy.
3. Both light and dark reactions take place in mesophyll cells.	3. Light reaction takes place in mesophyll cells and dark reaction in bundle sheath cells.
4.Only one type of chloroplasts and they are only in mesophyll cells. Can do photosynthesis only at high concentration.	4. Chloroplast dimorphism is seen. Granal chloroplasts are in mesophyll cells and agranal chloroplasts in bundle sheath cells.
	5. Can do photosynthesis at low concentration of CO_2 of CO2 (50 to 60 ppm) (1 to 2 ppm).
6. Can do photosynthesis at moderate light intensity and at moderate temperature.	6. Can do photosynthesis at high light intensity and at high temperature.
7. Can grow only in high amount of water.	7. Can grow well in low amount of water.
8. First stable product is C_3 compound i.e. PGA.	8. First stable product is C_4 compound i.e. malate or aspartate.
9 Moderately productive.	9. Highly productive.
10. Examples-maize, jowar, millet, sugarcane.	10. Sunflower, shoe-flower, rice.

Pentose phosphate pathway: It was discovered by Horecker et a. (1951) and Racker (1954). It operates when NADP is available instead of NAD. In this pathway 36 ATP are yielded.

Interdependence of Light and Dark Reactions: The two phases of photosynthesis, viz., light reaction and dark reaction are dependent on each other. Light reaction produces ATP and $NADPH_2$ which donate energy and H, respectively essential for CO_2 fixation in dark reaction. Dark reaction converts ATP and $NADPH_2$ into ADP and NADP and furnish these to light reaction for recharging into ATP and $NADPH_2$ to continue the process. Hence, light reaction and dark reaction are interdependent, neither of them alone can continue photosynthesis.

It can be summarised as follows.



SIGNIFICANCE OF PHOTOSYNTHESIS

1. Photosynthesis is energy trapping process through which light energy enters the biosphere.
2. It provides food directly or indirectly to all the organisms. There is no ecosystem without photosynthesis.
3. It releases O_2 which is indispensable for life.
4. Coupled with respiration it maintains balance of CO_2 and O_2 in the atmosphere and helps to control air pollution.
5. It provides fossil fuel such as coal, petroleum and natural gas, which are incompletely decomposed remains of photosynthetic capital of ancient plants buried deep into the earth's crust.
6. Molecules of O_2 evolved during photosynthesis are converted into ozone (O_3) by high energy solar radiations or by electric discharge. It forms a layer between 15 and 35 km above the earth surface and absorbs harmful ultraviolet radiations which would otherwise reach the earth surface and bring about mutations in organisms causing various skin diseases including cancer and increase in the temperature on the earth.

Difference between Photosynthesis and Respiration

Photosynthesis	**Respiration**
1. Energy trapping process.	1. Energy releasing process.
2. Water and CO_2 are utilised as raw materials	2. Water and CO_2 are given out as by products.
3. O_2 is released.	3. O_2 is utilised.
4. Takes place only in green cells.	4. Takes place in all living cells.
5. Takes place only in chloroplast.	5. Takes place in mitochondria and cytoplasm.
6. It takes place in presence of sunlight.	6. It is a continuous process taking place day and night.
7. It builds up complex organic molecules hence anabolic process.	7. It breaks down complex organic molecules hence catabolic process.
8. Overall reaction is: $6CO_2 + 12H_2O$ Light -------→ Chlorophyll $C_6H_{12}O_6 + 6H_2O + 6O_2$	8. Overall reaction is: $C_6H_{12}O_6 + 6H_2O$ ---------- $6O_2+6H_2O$+ Energy (38 ATP)

Chapter 7
Carbohydrates

Carbohydrates are made up of carbon, hydrogen and oxygen. They are synthesized in chlorophyll containing cells of plant kingdom with the help of CO_2 and H_2O in presence of sunlight and hence the process is called Photosynthesis.

Chemically carbohydrates are polyhydroxy aldehydes and ketones. Types: Generally, carbohydrates are grouped in three categories:

(1) Monosaccharides

(2) Oligosaccharides and

(3) Polysaccharides.

(1) **Monosaccharides**: It is the simplest carbohydrates. It cannot be hydrolysed into smaller units. It has usually 3 to 7 carbon atoms per molecule and have their names as triose (=$C_3H_6O_3$) e.g., glyceraldehyde and dihyroxyacetone, tetrose (=$C_4H_8O_4$) e.g., erythrose, pentose (=$C_5H_{10}O_5$) e.g., ribose, ribulose, xylose etc, Hexose ($C_6H_{12}O_6$) e.g., glucose, fructose galactose etc and heptose (=$C_7H_{14}O_7$) e.g., sedoheptulose etc. Most fruits have glucose, fructose, and dextrose. Ribose is constituent of RNA (Ribonucleic acid) and deoxyribose occurs in DNA (Deoxyribonucleic acid).

(2) **Oligosaccharides**: It is formed by the union of 2-6 monosaccharide molecules through a process called condensation. The bond formed between two monosaccharides are called residues, e.g., maltose has two glucose residues.

- **Classification**: Oligosaccharides are classified on the basis of their monosaccharide units or monomers as follows:
- **Disaccharide**: It includes maltose, sucrose, lactose etc. Sucrose has glucose & fructose units; lactose has glucose and galactose. On hydrolysis, disaccharide splits into monomers.
- **Trisaccharides**: e.g., Raffinose found in sugar beet & cotton seed. It is made up of galactose, glucose & fructose.
- **Tetrasaccharide**: e.g., Stachyose found in Stachys tubifera. It is made up of two galactose, one glucose and one fructose units.

(3) **Polysaccharides**: It is composed of several monosaccharide units joined together by glycosidic bonds. polysaccharides are tasteless and colourless amorphous powder which are little soluble in water. Some of these are as follows:

Starch: It is storage product of plants. It is made up of amylase and amyl pectin.

Glycogen: It is storage carbohydrate of animals found in liver and muscles. It is water soluble.

Cellulose: It is made up of 1600 to 1700 glucose units. It is long thread like molecule. It forms the cell wall of plants.

Inulin: It consists of 30-35 fructose units. It is found in rhizome.

Peptidoglycan: It is Polysaccharide and structural macromolecule of bacterial cell wall. The biosynthesis of peptidoglycan in general occurs at three locations viz;

(1) the precursor biosynthesis leading to the formation of n-acetylglucosamine and n-actylmuramic acid takes place in cytoplasm.

(2) Second step occurs on the membrane involving biosynthesis of disaccharide pentapeptide and

(3) takes place on the pre-existing cell-wall involving the transfer of these molecules to the growing peptidoglycan.

The final reactions in the biosynthesis of peptidoglycan occur outside the cell membrane involving two reactions;

(i) n-acetyl glucosamine and n-acetylmuramic acid pentapeptide is transferred from the lipid pyrophosphate to a pre-existing polysaccharide chain and

(ii) cross linking occurs between tetrapeptide and the cross bridge.

The walls of gram-negative bacteria contain 5-10% peptidoglycan which constitute the inner layer in the cell wall.

Difference between the Gram-positive bacteria and Gram-Negative bacterial cell wall has been tabled below:

Hanschristian Gram (1884) developed Gram Staining Technique. Gram-Positive stains purple colour and Gram-Negative stains Reddish colour.

Sr No	Gram Positive Bacteria	Gram Negative Bacteria
1	Cell wall has thick peptide glycan layer No outer lipid membrane	Single layered Smooth cell wall & another membrane
2	Thickness of wall is 20-80 nm	Thickness of wall is 8-10 nm
3	Teichoic acid present	Teichoic acid absent
4	Mesosome are prominent	Mesosome are less prominent
5	Spore forming rods	None Spore forming rods
6	Two rings in basal body	Four rings in basal body
7	More susceptible to antibiotic	Less susceptible to antibiotic like Penicillin.
8	Produces exotoxins	Produces endotoxins

Biological importance of Carbohydrates: It is involved in structural organization of cells, tissues of both plants and animals. They are also stored as food in seeds, tubers and rhizomes. It has a great role in the production of energy in the form of ATP.

Lipids: Lipids are stored in the form of Chemical energy in the seed. As soon as seed germinates it enters a phase of rapid growth and development culminating in the formation of photosynthetically active seedlings.

Lipids provide the energy for metabolic processes Lipids are structural components for membranes and intracellular signals.

The transition from embryo dormancy to germination combines the activation of developmental programmes and catabolic metabolism and mobilisation of storage seed reserves, which results in radicle emergence, seedlings establishments and finally photoautotrophic growth. A saturated fat like butter Ghee is solid at room temperature. An unsaturated fat like olive oil and corn oil are liquid at room temperature. Olive oil is monosaturated and corn oil is Polysaturated.

Some common lipids are oil, ghee, waxes, cholesterol, carotene, lycopene, menthol, vitamins A, E and K and eucalyptus oil. Lipids are non-polar and hence insoluble in water. It dissolve in non-polar organic solvents like ether, chloroform, acetone & benzene. It disposes in water forming emulsion. It constitutes 3-5% of cell contents. They are found in seeds and fruits, adipose tissue, bone marrow and nervous tissue.

Classification of Lipids: It can be classified as simple lipids, compound lipids and derived lipids.

Simple lipids: They are esters of fatty acids with various alcohols e.g. fats and waxes. Fats are dry glycerides in which three molecules of fatty acids condensed with 1 molecule of glycerol to give rise fat. Cholesterol is a steroid.

Whether a fat is saturated or unsaturated depends on the kinds of covalent bonds in the fatty acid parts of the molecule. A saturated fatty acid has only single bonds between its carbon atoms. Saturated fatty acid has some double bonds between its carbon atoms.

Saturated fats with saturated fatty acids are solid at room temperature. And unsaturated fats with unsaturated fatty acids are liquid at room temperature.

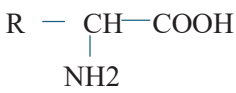
Compound lipids it contains some addition group beside fatty acid and alcohol. Additional group may be nitrogen phosphorus protein or sulphur. Phospholipids include Lecithins etc. Lecithins are widely distributed in biological systems. Plasmogens are found in brain and muscles. Phospholipids form the framework of cell, nuclear mitochondria, chloroplast and endoplasmic membrane.

Derived lipids are those which do not contain any ester linkage but may be considered to have been derived from naturally occurring esterified materials. In other words, derived lipids are substances formed on the hydrolysis of simple or compound lipids which still retain the properties of this class of compounds. Derived lipids may be of the following types:

1. **Fatty acids**: It may be saturated or unsaturated
2. **Alcohols**: Alcohol of high molecular weight but not glycerol e.g. aliphatic alcohols; esterols like cholesterols, ergosterol and stigmasterol; and alcohols having β-ionone ring like vitamin A.

Amino Acids: Amino acids contain Amino ($-NH_2$) and carboxyl (-COOH) functional groups. Depending up on the relative position of amino group with respect to carboxyl group, the amino acids can be classified as α, β, γ, Q and so on.

Only α-amino acids are obtained on hydrolysis of proteins. They may contain another functional group also.



α -amino acid (R=side Chain)

All amino acids have trivial names which usually reflects Glycine the property of that compound or its source so named because it has sweet taste (Glykos-Sweet in Greek) and tyrosine was first obtained from Cheese (Tyros-Cheese). Amino acids are generally represented by a three letter Symbol, sometimes one letter symbol is also used. Structure of some commonly occurring amino acids along with their 3-letter and 1-letter symbols are depicted in the following table.

Methionine is the first amino acid Synthesized. 10 amino acids which we can produce are Alanine, Asparagine, Aspartid acid Cysteine, glutamic acid, glutamine, Glycine, Proline, Serine and tyrosine (Table-1).

Function of Amino acids: It breaks down food, grow and repair body tissue, make hormones and brain chemicals i.e. neurotransmitters. Amino acids provide an energy source maintain healthy skin, hair and nails, build muscles boost immune System.

First Amino acid was isolated from **Asparagus Plant**, so its name was asparagine. There are 64 codons, out of them 61 codons specify amino acids and three codons are used as stop codon viz; UAG, UAA and UGA.

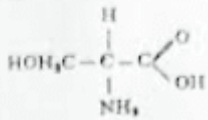
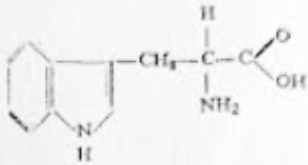
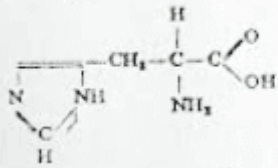
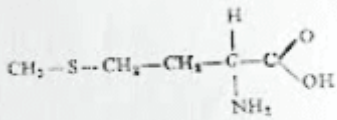
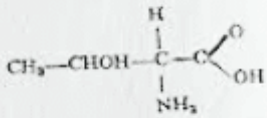
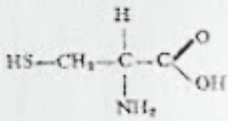
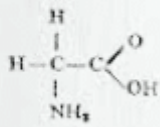
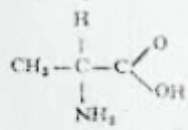
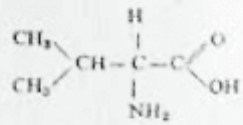
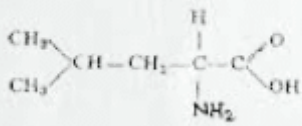
There are 20 common amino acids. Essential amino acids: Arginine, Histidine Isoleucine, Leucine, Lysine, methionine, Phenylalanine, Threonine, Tryptophane and valine. Non-essential amino acids: Alamine, Asparagine, Aspartate, Cysteine, Glutamate, Glutamine, Glycine, Proline, Serine and Tyrosine.

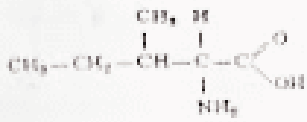
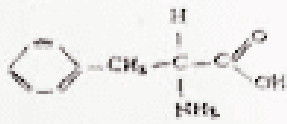
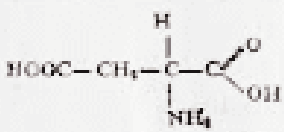
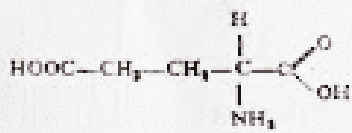
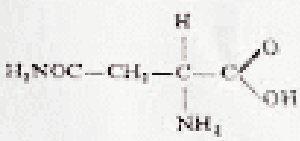
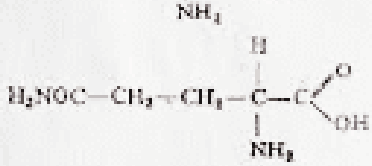
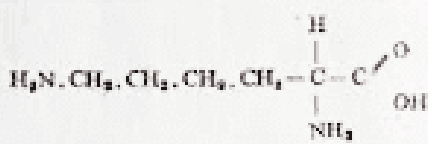
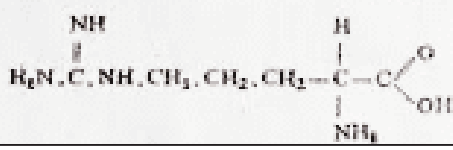
Selenocysteine is 21st Amino acid - Sec.

Pyrrolysine is 22nd amino acid - Pyl

Table 1

The Common Amino Acids, RCHNH2-COOH

Common Name	Abbreviation	Structure
Neutral amino acids (Monoamino Monocarboxylic acids)		
Serine	Ser	
Tryptopnan	Trp	
Histidine	His	
Methionine	Met	
Threonine	Thr	
Cysteine	Cys or Cys—SH	
Glycine	Gly	
Alanine	Ala	
Valine	Val	
Leucine	Leu	

Isoleucine	Ile	
Proline	Pro	
Phenylalanine	Phe	
Tyrosine	Tyr	
Acidic amino acids (Monoamino dicarboxylic acids		
Aspartic acid	Asp or Asp—NH_2	
Glutamic acid	Gle or Glu—NH_2	
Amides of the acidic amino acids		
Asparagine	Asn	
Glutamine	Gln	
Basic amino acids (Diamino monocarboxylic acids)		
Lysine	Lys	
Arginine	Arg	

Biomolecules: PROTEINS

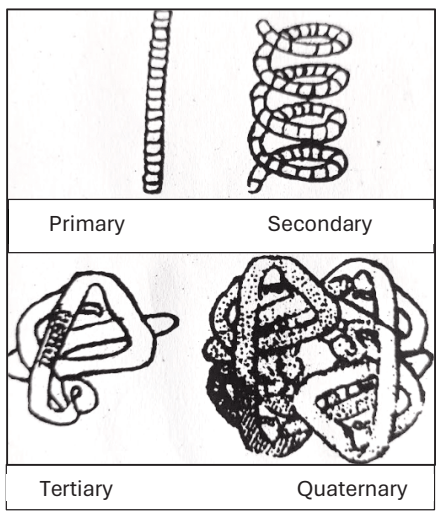
Proteins are macromolecules executing mediator and catalytic function. The word protein was coined by Berzelius and Geradus Johannes Mulder mentioned in their book, the proteins are big molecules made up of several small molecules. They are unbranched linear polymers of amino acids. In natural proteins there are 20 amino acids linked with each other by Peptide bonds. So, it is polypeptide chain of amino acids. Proteins are of unlimited variety because a polypeptide may have any number of 20 amino acids present in any proportion and arranged in any sequence.

Structure: The primary structure of a protein is a linear sequence of about 40-1000 amino acids in which amino acids are joined by co-valent bonds between peptide bonds. These peptides bonds are strong, so the primary structure of protein is most stable part. The sequence of amino acids is determined by the sequence of nucleotide triplets in DNA of the nucleus or nucieoid. Ribonuclease (enzyme) and myoglobin are the only proteins with primary structure.

Secondary structure: It is bonding or coiling of the primary structure called alpha helix. The side chain of amino acids extends outward from the spiral (Fig. 6)

Two or more polypeptides' chains may join together by means of intermolecular hydrogen bonds and may bond into parallel folds to form a pleated sheet. Alpha helix, random coil and pleated sheet are in secondary structure. e.g. Silk fibre protein-fibroin has pleated structure; myosin of muscle and keratin of hair have helical structure.

Tertiary structure: The helical secondary structure may fold on itself taking spherical form or rod like or any other to a geometrical shape known as tertiary structure. This structure of protein brings distant amino acid side chains nearer to form active site of enzyme protein. Globular protein has this structure.



(Fig. 6)

Quaternary structure: Some proteins with two or more polypeptide chains, each having primary secondary and tertiary structure, have quaternary structure e.g. insulin and haemoglobin.

Insulin has two polypeptide chains joined by sulphur bridge. Haemoglobin has two alpha & two beta chains.

Pyruvate dehydratogenase enzyme has 72 polypeptides chains. Ferritin-a liver protein has 20 polypeptides as shown above figs.

Properties: Proteins are macromolecules with 4500-4,600,000 molecular weights. It forms colloidal systems and does not diffuse through cell membrane. It goes or comes out by endocytosis or exocytosis.

Classification:

On the basis of shape proteins are grouped as such:

- **Fibrous protein** e.g. collagen of connective tissue, keratin of scales etc
- **Globular proteins** e.g. egg albumen, glutens of wheat etc

On the basis of components proteins are of following types:

- **Simple protein**: It is made up of only amino acids e.g. gluten, egg albumen etc.
- **Conjugated proteins**: They are made up of amino acids and other non protein group (Prosthetic group) e.g. nucleoproteins, glycoproteins, lipoproteins etc.
- **Derived protein:** They are formed by partial breakdown of natural proteins in digestion e.g.- peptones, proteases etc.

Chapter 8
Enzymes and Enzyme Immobilization

Enzymes are protein molecules. Some enzymes are simple protein (containing only amino acids where as others are conjugated proteins (containing amino acids and non-proteinaceous part or prosthetic group). The prosthetic group in also known as co-enzyme and the protein part of a conjugated protein is termed as apoenzyme and the intact molecule (conjugated protein + co-enzyme) is termed as holoenzyme.

Some enzymes are not protein, like ribozymes. Ribozymes are RNA molecules having catalytic properties of enzymes. It lowers the energy of activation of the substrate during action. Ribozymes are made up of RNA and protein. The active site of enzyme is on RNA subunit, e.g. RNAse P in prokaryotes.

Some useful Information about an enzyme:

A substance that accelerates a chemical reaction, but itself is not changed when the reaction is over, is known as catalyst. In living things these substances are proteins known as enzymes. Since enzymes are secreted by living things and they speed up chemical reactions, hence, they are also called biocatalysts. The word enzyme was coined by Eduard Buchner (1887). Thomas Cech et.al.

(1980) discovered RNA molecules that act as biocatalyst and called them Ribozymes. It catalyses synthesis and splicing of RNA as shown below:

1. RNA precursor with introns and exons → Spliced exons - 26s r-RNA
   Introns of 414 nucleotides
2. Introns of 414 nucleotides → circular chain of 399 nucleotides + chain of 15 nucleotides
3. Circular chain of 399 nucleotides → Linear chain of 399 nucleotides.
4. Linear chain of 399 nucleotides → Circular chain of 395 nucleotides + Chain of 4-nucleotides.

↓

Linear (Ribozyme) L19 RNA with 3' & 5' ends

L_{19} RNA (Ribozyme) is found in ciliated protozoan *Tetrahynema thermophila*.

Carbonic anhydrase is fastest known enzyme found in red blood cells. It hydrates 36- million CO_2 molecules to carbonic acid and H+ just in a minute as shown below:

$$CO_2 + H_2O \xrightarrow[Anhydrase]{Carbonic} HCO_3^- + H^+$$

An enzyme promotes a chemical reaction by lowering the energy of activation. The active site of an enzyme is formed by a few of the enzymes R Groups of the amino acids. The shape of an enzyme and consequently its activity can be reversibly altered from moment to moment by allosteric

sub units. The function of an enzyme is to change the rates of chemical reaction. Active site of an enzyme holds the substrate during a reaction. Allosteric inhibitor inactivates an enzyme by changing the shape of an enzyme. Tertiary structure produces the active site of an enzyme. Irreversible inhibitor inactivates an enzyme by denaturing it. Competitive inhibitor inactivates an enzyme by occupying its active site.

A high fever is dangerous to a human because enzymes are denatured by heat, without their tertiary and quaternary structure enzymes can no longer function.

Isoenzymes or Isozymes: It is an enzyme with slightly different molecule but with similar catalytic function, e.g. lactic dehydrogenase.

Sub-unit of enzymes: Some enzymes act is association with certain-non-protein substance- called co factors which may be metal ions, e.g., Mg++ & K+ or complex organic compounds called co-enzyme. The enzyme which acts in presence of co-factor is called apoenzyme. Non-protein organic co-factor tightly bound to the enzyme is known as prosthetic group e.g., haem of haemoglobin.

Apoenzyme and co-enzyme or co-factor is known as holoenzyme of enzyme system.

Enzyme Action: There are two views for mode of action of an enzyme.

(1) Lock and Key hypothesis and (2) Induced fit hypothesis.

Lock and Key hypothesis: This hypothesis was suggested by Emil Fisher (1894). In this mode enzyme molecule reacts by chemically uniting with the substrate molecule called enzyme substrate complex. The catalytic property of the enzyme is situated on its surface at specific region. These regions are active sites. It is small pockets or grooves formed during the formation of three-dimensional structure of the enzyme. The substrate has reactive sites on its surface to precisely fit the active site of the enzyme. The active and reactive sites fit like a lock and key as shown in Fig.7

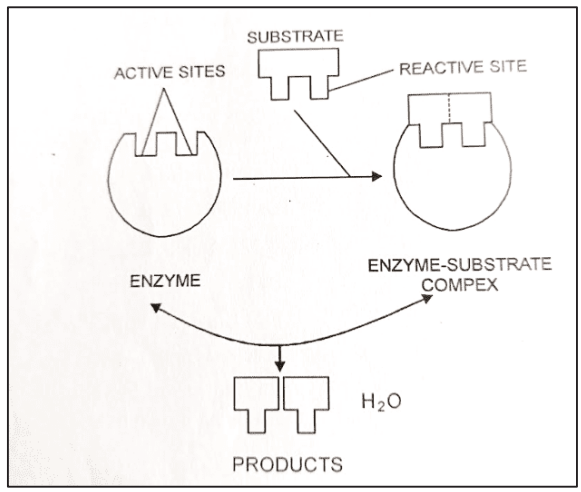


Fig. 7

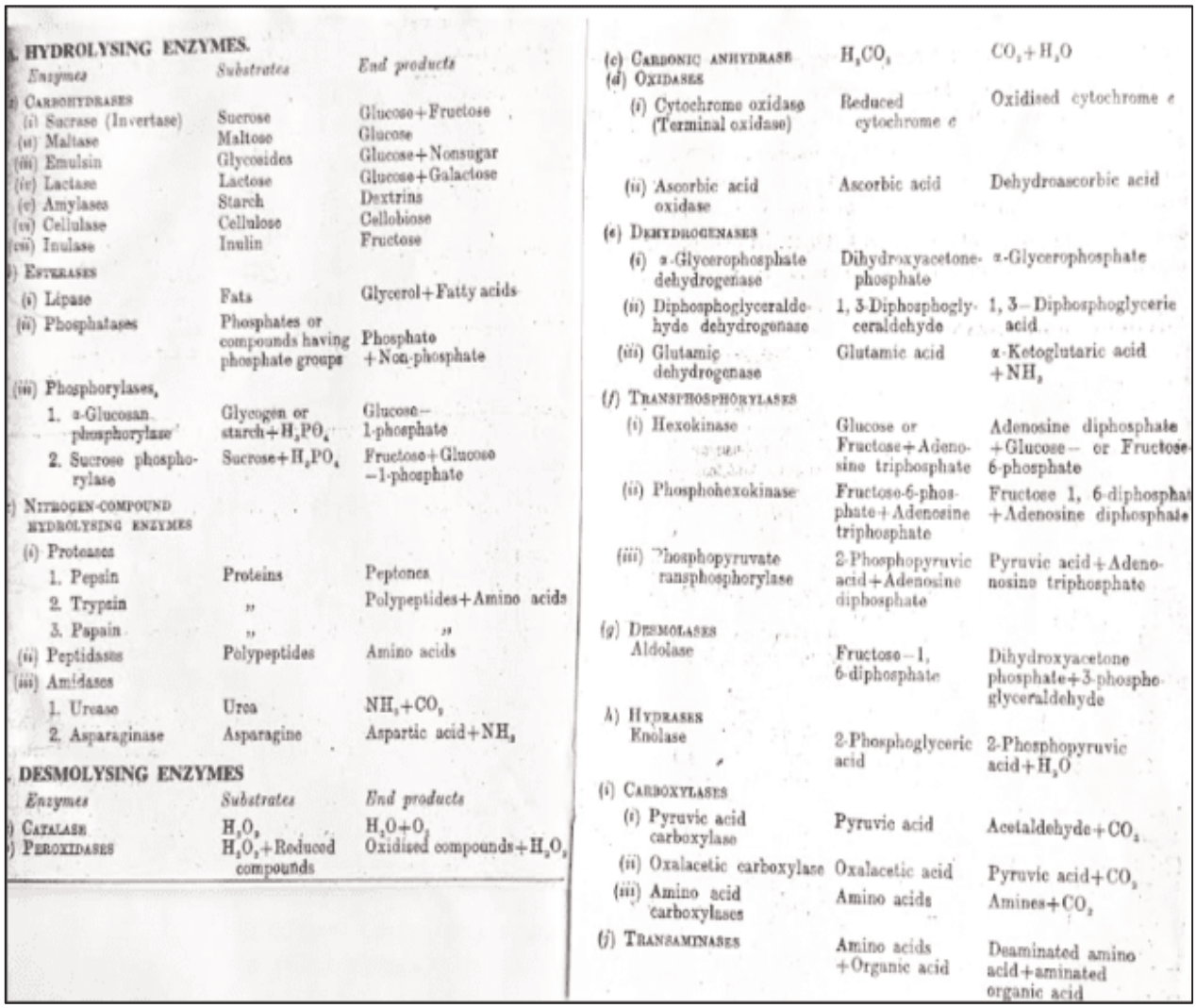
Classification of Plant Enzymes:

Enzymes are broadly classified under three heads;

(1) the hydrol, which causes hydrolysis of Substrate by removal of two hydrogen atoms from it; (2) desmolases which bring about a break down in the carbon linkage or add or remove atoms or groups of atoms to or from a molecule,

(3) oxidoreductase which causes Simultaneous oxidation and reduction in Plants.

Following are a list of Some important enzymes



A. HYDROLYSING ENZYMES.

Enzymes	*Substrates*	*End products*
a) Carbohydrases		
(i) Sucrase (Invertase)	Sucrose	Glucose+Fructose
(ii) Maltase	Maltose	Glucose
(iii) Emulsin	Glycosides	Glucose+Nonsugar
(iv) Lactase	Lactose	Glucose+Galactose
(v) Amylases	Starch	Dextrins
(vi) Cellulase	Cellulose	Cellobiose
(vii) Inulase	Inulin	Fructose
b) Esterases		
(i) Lipase	Fats	Glycerol+Fatty acids
(ii) Phosphatases	Phosphates or compounds having phosphate groups	Phosphate +Non-phosphate
(iii) Phosphorylases,		
1. α-Glucosan phosphorylase	Glycogen or starch+H_3PO_4	Glucose-1-phosphate
2. Sucrose phosphorylase	Sucrose+H_3PO_4	Fructose+Glucose-1-phosphate
c) Nitrogen-compound hydrolysing enzymes		
(i) Proteases		
1. Pepsin	Proteins	Peptones
2. Trypsin	"	Polypeptides+Amino acids
3. Papain	"	"
(ii) Peptidases	Polypeptides	Amino acids
(iii) Amidases		
1. Urease	Urea	NH_3+CO_2
2. Asparaginase	Asparagine	Aspartic acid+NH_3

B. DESMOLYSING ENZYMES

Enzymes	*Substrates*	*End products*
a) Catalase	H_2O_2	H_2O+O_2
b) Peroxidases	H_2O_2+Reduced compounds	Oxidised compounds+H_2O_2
c) Carbonic anhydrase	H_2CO_3	CO_2+H_2O
d) Oxidases		
(i) Cytochrome oxidase (Terminal oxidase)	Reduced cytochrome c	Oxidised cytochrome c
(ii) Ascorbic acid oxidase	Ascorbic acid	Dehydroascorbic acid
e) Dehydrogenases		
(i) α-Glycerophosphate dehydrogenase	Dihydroxyacetone-phosphate	α-Glycerophosphate
(ii) Diphosphoglyceraldehyde dehydrogenase	1, 3-Diphosphoglyceraldehyde	1, 3-Diphosphoglyceric acid
(iii) Glutamic dehydrogenase	Glutamic acid	α-Ketoglutaric acid +NH_3
f) Transphosphorylases		
(i) Hexokinase	Glucose or Fructose+Adenosine triphosphate	Adenosine diphosphate +Glucose- or Fructose 6-phosphate
(ii) Phosphohexokinase	Fructose-6-phosphate+Adenosine triphosphate	Fructose 1, 6-diphosphate +Adenosine diphosphate
(iii) Phosphopyruvate transphosphorylase	2-Phosphopyruvic acid+Adenosine diphosphate	Pyruvic acid+Adenosine triphosphate
g) Desmolases Aldolase	Fructose-1, 6-diphosphate	Dihydroxyacetone phosphate+3-phosphoglyceraldehyde
h) Hydrases Enolase	2-Phosphoglyceric acid	2-Phosphopyruvic acid+H_2O
i) Carboxylases		
(i) Pyruvic acid carboxylase	Pyruvic acid	Acetaldehyde+CO_2
(ii) Oxalacetic carboxylase	Oxalacetic acid	Pyruvic acid+CO_2
(iii) Amino acid carboxylases	Amino acids	Amines+CO_2
j) Transaminases	Amino acids +Organic acid	Deaminated amino acid+aminated organic acid

It was Pasteur who first discovered the presence of the enzyme Zymase in living yeast cells. Later on, Harden and Young have demonstrated the presence of Various enzymes in Zymase. consisting of enzymes hexokinase Phosphohexolso- mirase, Phosphohexokinase, aldolase, enolase, carboxylase and other dehydrogenases.

An immobilized enzyme is an enzyme which is attached to an inert, insoluble material such as calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride). This can provide increased resistance to changes in conditions such as pH or temperature. It also allows enzymes to be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for enzyme catalysed reactions.

In 1916, Nelson and Griffin discovered that invertase "exhibited the same activity when absorbed on a solid (charcoal or aluminium hydroxide) at the bottom of the reaction vessel as when uniformly distributed throughout the solution". This discovery was the first of various enzyme immobilization techniques currently available.

Besides adsorption, different covalent methods of enzyme immobilization were developed in the 1950s and 1960s. Up to now, more than 5000 publications and patents have been published on enzyme immobilization

techniques. Several hundred enzymes have been immobilized in different forms and approximately a dozen immobilized enzymes, for example penicillin G acylase, lipases, proteases, invertase, etc. have been used as catalysts in various large-scale processes.

Immobilisation of an Enzyme

There are three different ways in which one can immobilise an enzyme which are the following, and are listed in order of effectiveness:

- **Adsorption on glass, alginate beads or matrix**: Enzyme is attached to the outside of an inert material. Generally speaking, this method is the slowest among those listed here. As adsorption is not a [[chemical of the immobilized enzyme may be blocked by the matrix or bead, greatly reducing the activity of the enzyme.
- **Entrapment**: The enzyme is trapped in insoluble beads or microspheres, such as calcium alginate beads. However, this insoluble substance hinders the arrival of the substrate, and the exit of products.
- **Cross-linkage**: The enzyme is covalently bonded to a matrix through a chemical reaction. This method is by far the most effective method among those listed here. As the chemical reaction ensures that the binding site does not cover the enzyme's active site, the activity of the enzyme is only affected by immobility. However, the inflexibility of the covalent bonds precludes the self-healing properties exhibited by chemo adsorbed self-assembled monolayers. Use of a spacer molecule like poly (ethylene glycol) helps reduce the steric hindrance by the substrate in this case.

Commercial use

Immobilised enzymes are very important for commercial uses as they possess many benefits to the expenses and processes of the reaction of which include:

- Convenience: Minuscule amounts of protein dissolve in the reaction, so workup can be much easier. Upon completion, reaction mixtures typically contain only solvent and reaction products.
- Economical: The immobilized enzyme is easily removed from the reaction making it easy to recycle the biocatalyst.
- Stability: Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme.

Chapter 9
Essential Elements Required by Plants

Organic substances synthesized by plants from simpler elements like carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, calcium potassium, magnesium, Loon etc derived from various inorganic Sources. It has been found that certain elements are indispensable for building up the normal body of the plant. These are known as essential elements. The essential elements fall onto two classes:

(1) Macro metabolic elements. Which are required in considerable amount and

(2) Micro metabolic elements-which are required in traces and these are known as trace elements or micronutrients.

Role of a few important essential elements.

1. **Phosphorus**. It has got both direct and indirect effects in the metabolism of the plant. The number of tillers increases es with an increased supply of phosphorus up to a certain 11mit in the absence of phosphorus the leaves become purple-co- ploured, the absorption of potassium is directly hampered, and the normal respiration is upset, thereby adversely affecting the process of protein synthesis.

2. **Calcium**. It is necessary for the general development of the plant, translocation of carbohydrates, and response of roots It neutralises the harmful effects of some organic acids.

calcium reduces the toxic effect of single salt of sodium, potassium 1 LLTH and magnesium, which is known as antagonism of salts. 9n the absence of calcium the mitosis in the apical meristems is badly affected. Calcium is also known to play a role in the nitrogen metabolism of plants.

3. Iron. It is of the greatest importance in the formation chlorophyll. In its absence leaves and stems become yellow in colour (chlorosis or chlorotic condition). It also helps in re spiration in the form of iron compounds present in the enzymes and carriers operating the process.

4. Potassium. It has different influences upon different types of plants. It gives resistive power to the water-content of the plant is dependent upon the amount of potassium present in it. It is essential to plant-life and presumably active during the processes of carbohydrate metabolism, formation of protoplasm and vigorous cell-division. Its deficiency causes a general reduction in the yield: Potassium so acts as a catalyst in the chemistry of the plant. Some the authors hold that it has got a great influence on some of enzyme actions. It is further suggested that potassium is essential for the synthesis of proteins.

5. Sulphur. It generally helps in the building up of materials within the plant body. It causes an increase in the absorbing system or root-system in plants. The colour of the chlorophyll is also dependent on it; its absence produces a pale green colour in the chloroplasts. The nodules of leguminosae plants increase in number with its proper supply. Some symptoms like those of N-deficiency develop with S-deficiency. It also helps in the translocation of starch and sugars.

6. Magnesium. It acts as a carrier of phosphorus and helps in its absorption, and thereby influences respiration. It is very important in the synthetic process of plant and is also a constituent of the chlorophyll. The number of tillers in increases with higher concentration accompanied by the production of numerous adventitious roots. Its deficiency causa Seswilting, drying off, dropping off of the tips of plants, and and chlorosis.

7. Boron. Recently the effect of boron has been studied ed thoroughly. It influences the growth and development of plants, but a higher concentration produces a retarding effect et. Its effects vary with different types of plants, e.g., in leguminous plants the nodule formation is dependent upon it. Boron is also connected with the metabolisms of calcium and potassium and synthesis of proteins. Distortion of leaves, breaking down n of the conducting system, brittleness of stems and petioles, and extremely poor development of roots are all caused by boron deficiency.

8. Manganese. It influences the growth of plants and acts as a catalyst in respiration. Its deficiency causes dis disturbed carbohydrate metabolism, chlorosis, retardation in growth, a decrease in the ash-content and failure in reproduction.

9. Aluminium. It influences the growth and production, the mutual relationship existing between the plant and the soil, and the functions of cells. Its deficiency causes the dis-appearance of starch, an increase in the permeability of protoplasm with the loss of sugar, an increase in the enzymatic action and a lowering in the rate of photosynthesis. The colours of flowers are also influenced by its concentration.

10. Sodium. Its action is dependent upon the environ- me mental condition. Its presence retards the absorption of potassium. Sometimes it replaces the function of potassium and also also acts as an antidote against the toxic effects of certain Salts.

11. Zinc. It is essential for the development of corn in Plants, e.g., Ry, Pea, Onion, etc., and is a powerful stimulant for the development of micro-organisms, e.g., Aspergillus. It is an important constituent of the enzyme, carbonic anhydrase, an and of the hormone, indole acetic acid. Its deficiency causes diseases known as "little leaf" or "mottled leaf" in Citrus.

12. Copper. It is one of the important constituents of the oxidising-reducing enzymes influencing respiration. Its de deficiency causes the "die back" disease of Citrus and other Plants.

13. Molybdenum. The application of molybdenum in very minute quantity to the soil increases the yield of crop.

14. Silicon. It influences the intake and metabolism of P phosphorus compounds. Some workers believe that it also ¡ imparts rigidity and stiffness to cell-walls to resist insect and fungus attacks.

15. Chlorine. It does not play any direct role in the metabolism of plants but brings about changes in the ionic inter-relationship in the nutrients and thus influences different g groups of plants in different ways.

Chapter 10
Plant Hormones/Growth Regulators

A plant growth regulator may be defined as "a naturally occurring organic substance other thana nutrient, synthesized in certain plant parts and translocated to other site where it evokes specific morphological, physiological and biochemical response even in low concentration.

Originally, the term "growth hormone" was used (In Greek, hormone means to set in motion or to stimulate) as it was thought that all the plant hormones are formed in certain plant parts and were translocated to other sites where they have controlling and regulating effect. However, it is now clear that transport is not the essential property of all the plant hormones. e.g. ethylene brings about the changes in the same tissue where it is synthesized. So, Trewavas (1981) favoured the idea of replacing the term plant hormone with plant growth regulator or substance. The plant growth regulator exhibits a variety of responses depending upon the type of organ on which they act. This is in contrast to animal hormones which are specific action.

The main naturally occurring plant growth regulators are auxins gibberellins cytokinins ethylene and abscisic acid.

Auxins (auxein = to grow)

Auxins is a group of hormones produced by apices of the stem and natural plant root which migrate from the apex to the zone of cell elongation.

The fist auxin Indole-3- Acetic Acid (IAA) was discovered by W. E. went in 1928 on the basis of Avena curvature test

There are several uses of auxins in agriculture as well as horticulture.

a) For cell elongation: Auxins induce rapid cell elongation.

b) For root initiation: Auxins like NAA (Naphyhalene Acetic Acid) cause enhancement of root formation.

c) Apical dominance: It is the domination of the apical region of shoot axis over the lateral buds i.e. the growth of lateral buds is inhibited by auxin (IAA) produced in the shoot apex. If the shoot apex is removed, the lateral buds formation is stimulated.

d) Flowering: Auxins like IAA and NAA promote flowering.

e) Leaf and fruit abscission: Natural auxin IAA prevents premature leaf abscission while artificial auxins like NAA; 2,4-D (2,4-Dichlorophenoxy Acetic Acid) prevent premature fruit drop in apple, pear, lemon, grapes etc.

f) Development of parthenocarpic fruits: Auxins play importance role in the development seedless fruits formed without fertilization.

g) As a weedicide: 2, 1-D is selective weedicide. It destroys broad leaved dicotyledonous weeds. It does not affect monocotyledonous plants.

h) For potato storage: NAA prevents sprouting of potato tubers and therefore, it's used in potato storage.

Gibberellins (GA):

These are naturally produced plant hormones. In 1898, a disease of rice plants was being studied Japan. The rice seedlings were infected by the Ascomycetous fungus fajikuroi. The infected plants were growing excessively tall and the Japanese farmers called this the "bakane Kurosaw disease" (foolishing seedling disease). In 1926, a Japanese plant pathologist in Taiwan cultured the fungus in a nutrient medium and showed that the fungus free medium itself could induce the abnormal elongation of rice seedlings. In 1938, Yabuta and Sumiki isolated the active factor from the funga and named it "Gibberellin". Since then several types of Gibberellins such as GA, GA, GA, to up GA, of which GA, (Gibberellic acid) is most common in plants and well studied.

Uses of Gibberellins: These are also widely used in agriculture and horticulture.

a) Stem elongation : Use of GAs, in stem elongation is based in cell elongation. Ita tried in several plants like pea, bean, tomato, sweet corn, cucumber, lettuce, cabbage etc.

b) Flower formation in long day plants Of all the plant hormones, only GA's have been shown to effectively cause flower formation in long day plants and cold requiring plants.

c) Induction of seed germination and enzyme production: GAs are effectively used in germination of seeds of cereals. It is also reported that GAS stimulate the formation of enzyme a amylase which hydrolyzes starch present in endosperrn to form simple sugars. However, site of production of GAs is embryo.

d) Breaking dormancy of buds: Buds of evergreen trees remain dormant in autumn undergo resting period in winter and grow in spring as they require cold period, long day and red light. GAs can act as a substitute for all these conditions and break the dormancy of buds.

e) Parthenocarpy: GAs also induce Parthenocarpic fruits i.e. seedless fruits formed without fertilization.

f) Bolting of Cabbage: GAs influence stem elongation or quick growth (bolting) cabbage and other plants.

Cytokinins

Skoog, Miller and others in 1950 discovered this growth regular responsible for cell division and found out that it was purine derivative. Since, nucleic acids contain purine, Miller used old sampla of Herring sperm DNA (Herring is silvery edible fish) which was capable of tobacco cell divisions. Th conclusion is that the cell division factor is the breakdown product of nucleic acid which was late recognised as cytokinin.

Cytokinins are widespread in plants, embryos, developing fruits and seeds etc. In intact plant, rod are the site of synthesis of cytokinins. Since the cy tokinin level in xylem sap is very high, the logia conclusion is that the cytokinin synthesized and supplied by roots can regulate the developmenti changes in the plant. Cytokinin has antagonistic properly with auxin. It promotes the growth of later buds by neutralizing the effect of natural auxin. Thimann (1960) reported that cytokinin activats protein synthesis in buds. Letham (1963) isolated ancther type of cytokinin from sweet corn extra and termed it as Zeatin.

Abscisic acid (ABA) :

In contrast to all the growth hormones described earlier, ABA retards or suppresses the growths it is a growth inhibitor. It was first reported by Osborne (1955). Ohkuma and others in (1965) isolat two fractions from

cotton fruits and called them Abscisin I and II. The new name Abscisic acid wa approved which gives indication of the compounds chemical nature (Addicott and others, 19685)

Uses of Abscisic acid: ABA has following uses:

a) Growth inhibition: ABA acts as grow th inhibitor at high concentration. However, at very loN concentrations it promotes parthenocarpy, rootings of cuttings etc.

b) Water stress: When water stress is developed, ABA reduces the stomatal aperture for wata conservations. Thus it plays role in draught resistance and desiccation tolerance.

c) Dormancy: ABA increases seed dormancy by inhibiting seed germination. It also stimulates by dormancy. It inhibits formation of amylase in the seeds.

d) Abscission: ABA promotes leaf abscission. It decreases the synthesis of RNA and proteins in the leaves.

Ehylene: This hormone in found in the form of gas. Ghane 1934, could identity chemically that ehylene is the normal outcome of plant metabolism. Ethylene accelerates ripening of all edible fruits led to the idea that the gas is a plant horm0ne (Burg,1962). Ethylene as ripening hormone for bananas tomatoes, melons etc. Ethylene stimulates germination of dormant seeds and prolonged seed longevity. Ethlene involves in reduction in elongation of stems and roots. Root growth is stimulated by Low concentration of ethylene and inhibit a higher concentration Higher concentration of ethylene initiates adventitious root formation. Ethylene accelerates the abscission of plant organs. It also effect the flowering in Mango pine apple and apple. Reid and others (1983) revealed that ethylene causes rapid pollination.

Phytochrome: It is photo morphogenic pigment and chlorophyll is a green pigment and Cytochrome is present as a protein that binds a tetra pyrole chromophore allowing them to absorb light. Phytochrome developed chlorophyll and not its synthesis.

VERNALIZATION

Not all the plants will flower when subjected to correct photoperiod. In many plants low temperature has a profound effect on flowering.

In annual plants flowering takes place in summer so effect of low temperature on flowering is secondary to that of light for 'annuals. Biennials, on the other hand, remain vegetative in first growing season and, after prolonged exposure to the cold temperature of winter, flower in the following season. Without exposure to cold treatment, these plants remain vegetative indefinitely. The importance of low temperature in the induction of flowering was established as early as in 1918 by Gassner. The phenomenon is known as vernalization. Chourad (1960) defined vernalization as "the acquisition or acceleration of the ability to flower by a chilling treatment."

It must be emphasized that plant exhibit inductive type of response to vernalization by showing actual initiation of flower primordial at high temperatures and not at vernalizing temperatures, thus the action of chilling is inductive and it is called thermo induction.

As a rule, winter annuals can be vernalized as imbibed seeds whereas biennials and perennials plant should attain particular size before giving treatment.

Generally, vernalization is the slow process Usually1 -3 months exposure to low temperature is required to induce flowering; but celery flowers by low temperature treatment of of 1 -2 days.

Vernalization is the quantitative process i.e. longer the chilling treatment, the more effective it is usually 1^0 C to 7^0 C temperature is found to be more effective. However, as low as -6° C temperature is effective in certain cereals.

Presence of oxygen and water is prerequisites for seed vernalization. Similarly, only imbibed seeds are sensitive while dry seeds cannot be vernalized.

Vernalization of *Secale cereale (Petkus winter rye)*: Petkus rye has two varieties, spring variety and winter variety. The spring variety is a typical annual rosette plant, flowing in one growing season. The winter variety is a typical biennial rosette plant, remaining vegetative in first growing season and then flowering in next growing season after a prolonged exposure to the cold temperature of winter. The winter variety, when vernalized, resembles the spring variety.

Chapter 11
Vernalization, Photoperiodism, Movements in Plants

In 1920, of U.S. Department of Agriculture, observed that a tobacco variety Maryland mammot flowered when the relative length of day was shorter than the length of dark period. The photo period that promoted early flowering of this tobacco variety was termed as short day and the plant wa referred to as short day plant. The terms "photoperiod" and "photoperiodism" were coined by them.

Photoperiodism can be defined as "the response given by the plant to relative length of light and dark period with regard to the initiation of flowering." However, it was observed that the duration dark period is much more important than duration of light period and total quantity of light received can have influencing effect. Thus, it is generally accepted that any response, by a plant to the duration and order of sequence of light and dark periods, is called photoperiodic response.

Accordingly, Garner and Allard classified the plants into three basic response types, viz., Shor day plants (SDP), long day plants (LDP) and day neutral plants (DNP).

Short day plants: These plants flower only when they get light period less than their critical day length. Critical day length can be defined as the light period required to induce flowering. If the ligh period exceeds the critical day length, then the plants remain vegetative. e.g. Nicotiana (tobacco Xanthium, Chrysanthemum, Dahlia, potato, Sugarcane etc.

Short day plants are actually long night plants. Hamner and Banner (1938) worked on Xanthium a short day plant, and established the crucial role of the night period. The plant has critical day length of 15½ hours and it requires at least 8½ hours of night period. This plant will flower only when ligh period less than a critical day length is given followed by at least 8½ hours of continuous dark period The plant will flower even if the light period is interrupted by a brief darkness followed by at least 8 ours of continuous dark period; but it will not flower if dark period is interrupted by a brief flashd light. Thus all that is important is continuous dark periods for flowering of shor day plants.

Long Day Plants: These plants flower only when they get lighted more than their critical day length. The critical day length differs from species to species eg. radish, spinach, lettuce, beet, maize, oat, etc.

These plants will flower even if a dark period is interrupted by a brief light provided the total light period exceeds the critical day length.

Day Neutral Plants: These plants flower after a period of vegetative growth regardless of the light. eg. cucumber, tomato, pea, cellon, sum flower etc.

Sachs in 19th century supported the idea that flower forming substance are present in the flowering plants. He observed that leaf cuttings from flowering Bignonia plants produced adventitious shoots which quickly flower, whereas leaf cuttings prepared from Vegetative plants regenerate only vegetative Shoots. It was concluded that leaves are receptors of photoperiod and the site & synthesis of flower forming Substance from where it is

transferred to meristems where flowering is induced. Chailakhyam (1937) proposed that there in the leaf is substance & hormonal nature and named it **Florigen** whose exact Chemical nature of **Florigen** is unknown.

Movement in Plants

Plants move in response to stimuli through a variety of mechanism. There are five types of movement in plants as such:

(1) Phototropism (2) geotropism (3) Chemotropism (4) Hydrotropism and (5) Thigmotropism based on stimuli.

Movement of Plants can be divided into tropic movement and nastic movement:

Tropic movements are-Phototropism, Gravitropism, Chemotropism, Thigmotropism, hydrotropism.

Turgor movement may be exemplified in bending movement of flowers of Sunflower plant. The sun and sleep movement of leaves of certain plants at night are also turgid movement.

Touch me not or sensitive plants are example of sleep / touch movement sleep movement is folding of leaves afts Sunset and unfolding of leaves after sunrise-observed in pinnate leaves.

In-Phototropism plants grow in light due to auxin reaction to light. Hydropism are movement of plants towards water. Here stimulus is water. In chemotropism- movements of plant is in response to chemical stimulus. It is seen in growth & pollen tube towards the ovule during fertilization in flower.

Nastic movements are in response to environmental Stimuli not reliant on the direction of the stimuli.

Nastic movements are of five types. (1) seis monastic (2) Photonastic (3) Thermonastic (4) Nyctinastic and (5) Thigmonastic.

Autonomic movement is a spontaneous movement and paratonic movement is induced movement. Tropism depends on the direction of the touch Stimuli, Nastic movement is due to Change in turgor Changes.

Thigmonasty or seismonasty is influenced by a plant or fungus or touch Vibration.

Epinasty is when a flat organ begin to bend downward due to greater growth on the upper side. Flowers open at maturity due to epinasty.

Chapter 12
Seed Physiology, Seed Dormancy

Seed is a living plant in a quiescent state. The nascent plant contained within the seeds. It is an embryo surrounded by nutritive tissue and a protective covering. Seeds are produced to propagate the next generation to generate genetically viable offsprings that can be stored to survive harsh condition and disperse into new environment.

Seed physiology is one area that is critical to the successful operation of seed gene banks requiring understanding of seed quality during development and maturation, beed dormancy and germination as well as seed longevity in the storage.

Process of seed germination starts with the imbibition of wates by seed coat and the emergence of growing root tip of the embryo and development of seedling and it has its own photosynthetic system-

Seed Dormancy

Seeds of many plants fail to germinate inspite of favourable environmental factors Embry growth is arrested by the condition within the seed itself. Seed dormancy may be defined as a state of Inhibited growth of seed due to endogenous factors. Following are the factors responsible for seed Dormancy.

(1) Impermeability of seed coat to water as legume seed; *Xanthium.* seeds

(2) Rudimentary embryos in *Gingo Biloba*

(3) Mechanical resistance of the seed coat to expansion embryo as in *Amaranthus*, mustard et

(4) Germination inhibitors like phenolic and abscic acid

(5) Due to specific light requirement.

Method to break Dormancy. It is of two types (1) mechanical and (2) chemical.

In mechanical method seeds are machine thrashed to rupture seed coat. In chemical method seeds are treated with ethylene, Potassium nitrate, mineral acids etc. When dormancy period is over, seeds germinate.

Chapter 13
Magnetosomes, P/O ratio, Gluconeogenesis

Magnetosomes: Magnetosomes are intracellular organelles found in Magnetotactic bacteria that allow them to sense and allign themselves along a magnetic field lines. magnetosomes are prokaryotic organellels that serve as navigational device in magnetotactic bacteria. It consists of membrane enclosed intracellular crystals of magnetic iron mineral. These iron rich magnetic particles are enclosed within a lipid bilayer membrane.

P/O ratio: It is phosphate/oxygen ratio refers to the amount of ATP produced from the movement of two electrons through a defined election transport chain, terminated by reduction of an oxygen atom. P/O ratio is difficult to measure. Determination of oxygen consumed is easies but calibration is difficult, because the dissolved oxygen Concentration depends on temperature, ionic Strength, history of the Solution and atmospheric pressure. Determining ATP made by mitochondria is also error prone. Furthermore, mitochondria may damage and thus provide underestimates of the P/O ratio, because oxygen consumption can proceed without full coupling to ATP Synthesis.

Gluconeogenesis: It is syntheses of new glucose from non-carbohydrate precursors. It provides glucose when dietary intake is insufficient or absent. It occurs in hypatocytes and myocites. It is metabolic process to maintain blood glucose level. In germinating seeds stored protein or protein are converted to sugars by gluconeogenesis. Glyoxysomes and Peroxysomes are present in germinating cotyledons. In oil seeds, mobilization of storage lipids via peroxisomal oxydation and glyoxylate cycle provide energy and carbon Skeletons for germination and seedling establishments. Gluconeogenesis has its role in mobilization of Lipid during germination of seeds.

Relative Afferent Pupillary Defect (RAPD) : It is a condition in which Pupils respond differently to light stimuli shown in one eye at a time due to unilateral or assymetrical diseases of the Retina or optic nerve.

Technique: Random amplified polymorphic DNA is a PCR based technique for identifying genetic variation. It involves the use of a single arbitrary primer in a PCR reaction, resulting in the amplification of many discrete DNA products.

Restriction Fragment Length Polymorphism (RFLP): It is used for evaluating genetic relationship of crop germ plasm. RAPD analysis is quick, simple and useful for the comparison of small number of isolates. RFLP is more reproducible and therefore better suited for the accumulation. RFLP fingerprints, helps for long term local surveillance and large epidemiological studies.

Single Nucleotide polymorphism (SNP): They are most common type of genetic variation among people. Each SNP represents a difference in the single DNA building block called a nucleotide. An example of an SNP is the substitution of AC for AG in a nucleotide sequence AACGAT. Thereby producing the sequence AACCAT. The DNA of human may contain many SNPs, since these variations occur at a rate of one in every 100-300 nucleotides in the human genome.

European Molecular Biology Laboratory (EMBL): Here basic research are performed in molecular biology and molecular medicines as well as train scientists, students and visitors.

DNA Data Base of Japan (DDBJ): It is comprehensive data base that Contains publicly available nucleotide sequences.

Dendrogram is a diagram representing a tree. It is used in hierarchical clustering. It illustrates the arrangement of the clusters produced by the Corresponding analyses. They are often used in computational biology to illustrate the clustering of genes or samples.

Federal Assets Sale Transport Act (FASTA): This act was passed In December 2016 for the federal government to reduce its inventory of civilian real property. FASTA project team will review and evaluate the property for consideration as a submission to the public building reform board.

Genetics Computer Group (GCG): it is a software package for the analysis of genes and proteins sequences on Unix machine. It enables scientists to analyse DNA and protein sequences by editing, mapping, comparing and aligning them.

SECTION 2
Molecular Biology, Bioinformatics

Chapter 14
Nucleic Acids (DNA and RNA), Mechanism of DNA Replication

DNA: Friedrich Miescher in 1869 isolated DNA from puss cells and called it nuclein by virtue of its presence in the nuclei of the cell.

Albrecht Kossel provided chemical name Deoxyribonucleic acid.

Richard Altman (1889) renames nuclein to nucleic acid.

Wilhelm Johannson in 1909 used the word 'gene' to name unit of heredity.

Phoebus Leven identified the building blocks of DNA including the four bases: Adenine (A), cytosine (c), Guanine (G) and Thymine(T).

Arthur Kornberg (1956) discovered DNA polymerase- an enzyme that replicates DNA.

Francis Crick (1957) said that 3 bases in DNA always specify one amino acid in a protein.

Forms of DNA: There are seven forms of DNA as noted below:

1. B-form: Proposed by Watson & Crick.

2. A-form: (A-DNA)

3.C form: (C-DNA)

4. D form: (D-DNA)

5. Z form: Left-handed DNA

6. Single stranded DNA (ss DNA)

7. Circular or supercoiled DNA

DNA is read from 5'-3', 5' is upstream and 3' is downstream. 3'prime end of DNA is called tail end.

Diploid cell contains 6 Picogram (pg) of DNA. Semen contains 3 Picogram (pg) of DNA.

Levine showed that nucleic acid could be broken into smaller parts celled nucleotides. Each nucleotide consists of a ribose sugar, a phosphate group and nitrogenous base (Fig. 8).

The nucleic acids (DNA & RNA) are made up of two nitrogenous bases, (1) Purines and (2) Pyrimidines. In DNA, two purines, namely adenine and guinine and two pyrimidines, viz., Cytosine and Thymine are found. In RNA, pyrimidine thymine is not found, instead pyrimidine uracil is found (Fig 9). Purines and pyrimidines form chemical linkage with ribose (5-carbon) sugars resulting as nucleosides which is an elementary precursor for DNA or RNA synthesis. In DNA, the sugar is deoxyribose and in RNA, it is ribose.

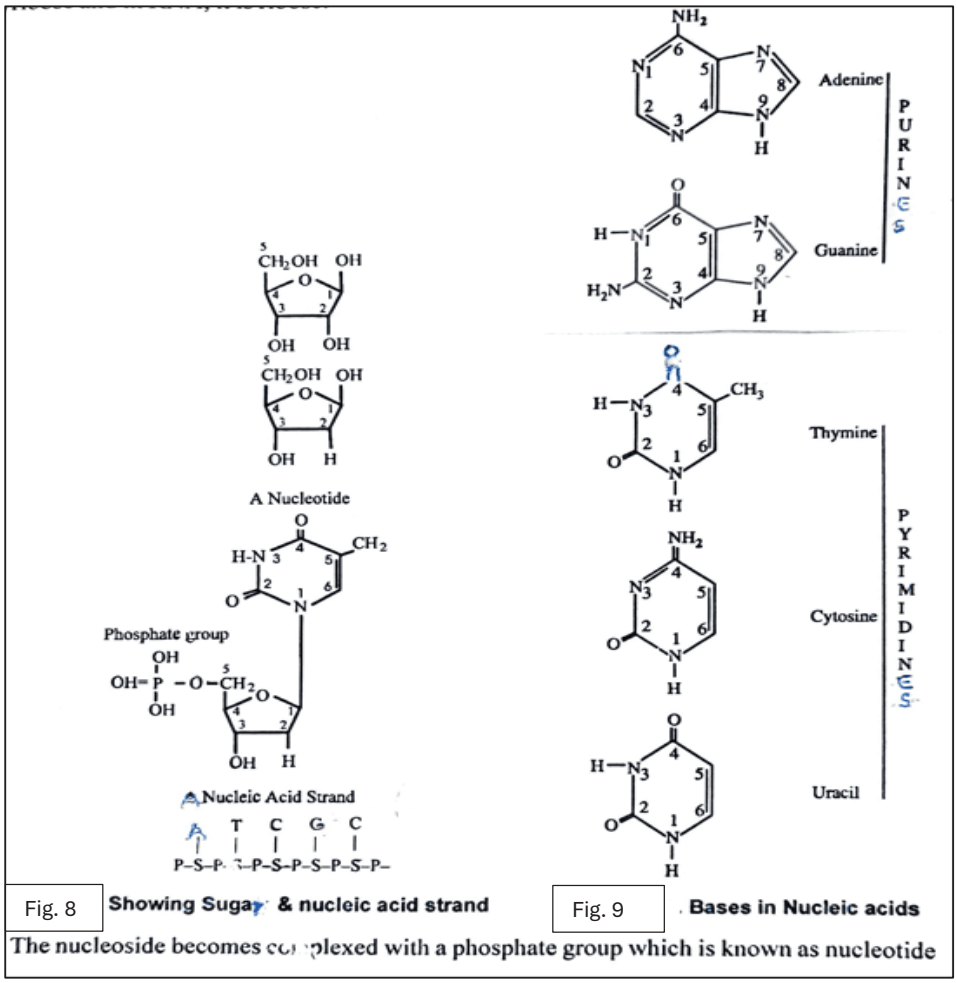


Fig. 8 Showing Sugar & nucleic acid strand

Fig. 9 Bases in Nucleic acids

The nucleoside becomes complexed with a phosphate group which is known as nucleotide

The nucleoside becomes complexed with a phosphate group which is known as nucleotide

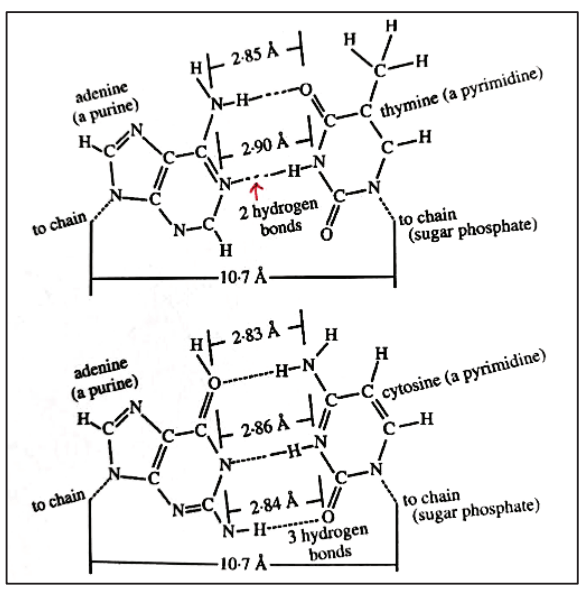


Fig. 10 Base pairing in DNA

DNA and RNA are Simply polymers of nucleotides. Many nucleotides are joined together to form polynucleotides. The Phosphate group is bound to 5th carbon of pentose sugar (ribose or deoxyribose) on one nucleotide and 3rd carbon of the pentose sugar on second nucleotide. Thus, polymer of nucleotides held together with 5-3 phosphate linkages are formed.

Polynucleotides are structurally polarized in the nucleic acids. The 5th phosphate and the 3rd hydroxyl (3-OH) ends can be observed in any chain (Fig. 10,11,12).

Types of DNA in different organisms

1. DNA-SS linear -Parvo virus.

   SS Circular-OX174.

   DS linear-Adenovirus, Herpes

   DS linear with closed ends Vaccine virus.

   DS circular e.g. Bacteriophage, cauliflower Vitus.

1. Bacterial DNA-E. coli DS 2µm thick 1360µm long nucleotide pairs, circle.
2. Eukaryotic Nuclear DNA-DS linear with histone.
3. Mitochondrial DNA: DS, circular not associated with histone, plant mt DNA contains introns.
4. Chloroplast DNA (CP-DNA) 40-60 nm long.
5. Yolk spherule DNA-linear DNA
6. Circular DNA-e.g. *Paramoecium*,

Formation of several H-bonds, provides high degree of stability and rigidity to the DNA molecule.

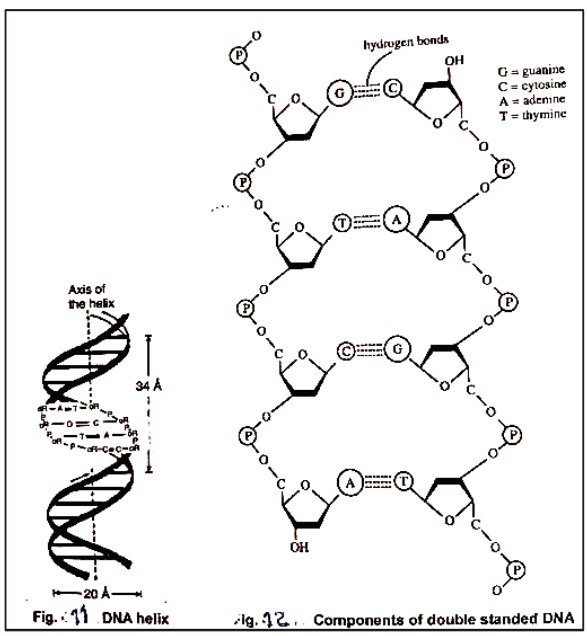


Fig. 11 DNA helix

Fig. 12. Components of double standed DNA

There are quantitative relationships between bases of two stranded DNA in which A+ T/G+C=1 or A=T and C= G are one or A+ T= C+G. Animals and higher plants have more A+T than C+G. In viruses, bacteria and lower plants there are variations in the base ratios.

Different Forms of DNA

Repetitious DNA: The non-coding DNA has many base sequences repeated several times. The repeated sequences are known as Repetitious DNA

Bonding: Long chains of DNA are more flexible than the short pieces of DNA of about 100 base pairs (bp). Short pieces are stiff and difficult to bend. However, certain sequence in DNA generates an intrinsic bond, known as bendable DNA segment. Each mini circle of neoplasm DNA (K-DNA) of Trypanosomes contains bendable DNA sequence. Olyacrylamide gel electrophoresis is helpful to identify such sequence.

There are some non-intrinsic bend which are formed by a bound protein. Many non-histone proteins also induce bending of DNA e.g. Catabolic activator protein CAP, DNA gyrase restriction enzymes.

Intermolecular triplex or H-DNA: DNA may contain repeated segments of purines & pyrimidines. These segments can form a three stranded helix known as H-DNA. A triplex is formed when half of the normal duplex is disrupted and the polypyrimidine strand folds back and insert itself into the major groove of the remaining duplex. (Fig 13).

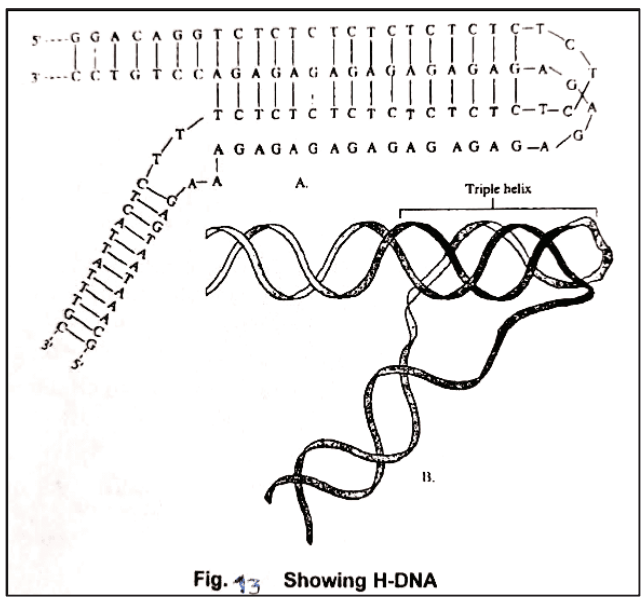


Fig. 13 Showing H-DNA

Cruciform DNA: It means a DNA with cross or plus sign like cruciform DNA may be formed at the site of Palindrome DNA sequence. (AND=DNA). Which is an inverted repeats with a two fold axis of symmetry.

R-loop & D-loop: A RNA-DNA hybrid duplex and a displaced stretch of single stranded DNA is R-loop It results from the hybridization of 18s and 25s ribosomal RNA form yeast with a region of bacteriophage DNA that contains an inserted stretch of yeast ribosomal genes.

D-Loop is formed when additional single strands of DNA are taken up by the duplex. It is observed in mammalian mitochondria (Fig.14,15,16). The structure of DNA as described earlier are commonly found in living systems. It shows right-handed helical coiling and this type of DNA is known as B-DNA or B- Form.

B- form is found at 92% relative humidity and low ionic strength. It has been noticed that DNA may remain in other forms differing in features such as spacing of residues nucleotide along the helical axis or number of residues per turn or vertical rise per base pair (bp) or helical diameter. Following are the different forms of DNA.

A-Form or A-DNA" - It is found at 75% relative humidity in presence of Na^+ K^+ Ca^+ ions. It has 11 bp per turn, 2.5 Å vertical rise per bp & 23Å helical diameter.

C-Form or C-DNA -It is found at 66% relative humidity in presence of lithium ions. It consists of 9.33bp per turn, 3.32Å, vertical rise per bp and 19Å helical diameter.

D-Form-It has 8 bp per turn and it is found in some DNA molecules lacking guanine.

E-Form-It consists of 7 bp per turn and it is found in guanine lacking DNA molecules.

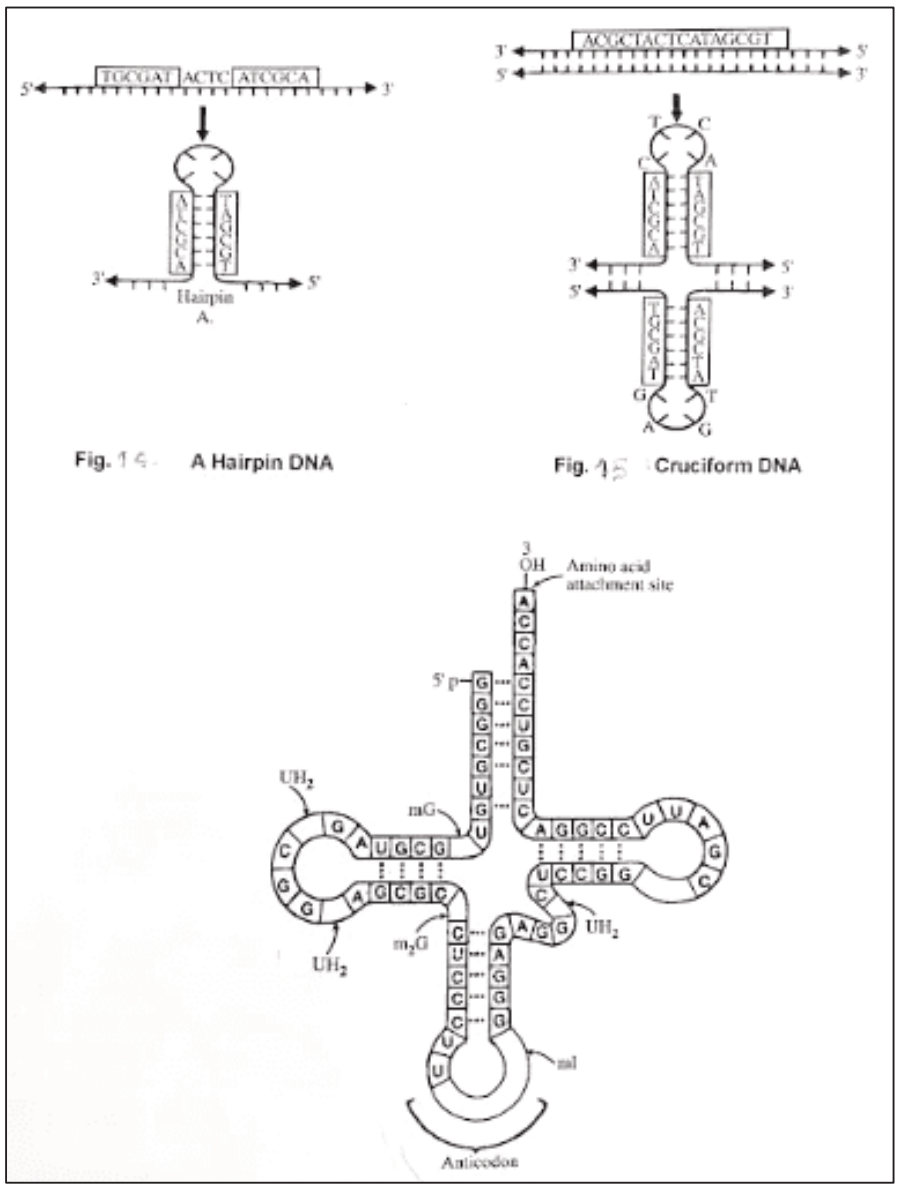


Fig. 1 A Hairpin DNA

Fig. 1 Cruciform DNA

Fig. 16 Cloverleaf model of the base sequence of yeast Alanine-specific **tRNA.**

Left handed or Z-DNA -This form of DNA was reported by Wang (1979), He synthesized double helical fragment of DNA- having 6bp in alternating sequence i.e., d (C-G)3.

This DNA anti-parelled strands showing left-handed helical sense. The sugar-phosphate backbone follows a zig-zag course. The sugar molecules attached to guanine has '0' ring pointing upwards whereas '0' ring of deoxyribose sugar attached to cytosine is pointed downwards. Thus, due to this alternating configuration of the sugar molecule, the repeat unit in the hexamer DNA fragment is dinucleotide and not a mononucleotide as found in Watson - Crick model. The helix in Z-DNA has 12 base pairs per turn. The bases are tilted 7° from the axis of the helix due to which bases are not stacked directly one above the other. The guanine residues are situated away from the centre and are stacked upon the oxygen alone of the deoxyribose sugar. Z-DNA appears as a cylinder & less grooved. It has a diameter of 18A. One complete turn of helix is covered by 12 bp within a length of 44.6 Å.

Comparison between B-DNA and Z-DNA: The comparative studies in between B-DNA and Z-DNA are summarized in the following table.

Features	**Z-DNA**	**B-DNA**
Natures of helix-	1. Double helical 2. Double helices are antiparallel 3. Helical sense Left-handed 4. Helix pitch is 18Å	1. Double helical 2. Double helix is antiparallel 3. Helical sense-right handed 4. Helix pitch is 20Å
Diameter of polymer	18°	20°
Base pair tilt	7°	6°
Rise per base pair	3.7	3.4
Angle of twist per	60°	36° Repeating unit
Base pair per turn	12	10
Position of guanine	Away from the Centre	Closer to the centre
Residues Groove formation	Minor grooves	Minor grooves above
Course followed by sugar phosphate back bone	Major groove not prominent	Major groove prominent
Nature of repeat unit	Zig-Zag - Dinucleotide	Normal Mononucleotide

Genetic Material and its Function

Genetic material must have the following properties:
1. It must have all the biologically useful information in a stable form.
2. The information must be able to replicate and transmitted faithfully to the next generation.
3. It must have the ability to express itself.
4. It should have the capability to induce variations. This criterion is contradictory to the 1st criteria, but for evolution the genetic material must be capable of mutations and recombination causing stable and heritable changes.

F Griffith (1928), Avery, Mcleod and McCarthy (1944) and in 1952 Hershey and Chase have experimentally proved that DNA is the genetic material. Similarly, Gierer and Schramm (1956) and H. F. Conrat (1957) have proved that RNA present in TMV is genetic material.

Chapter 15
Function of DNA, RNA, Genetic Code

Function of DNA: It has two major functions namely (1) Autocatalytic and (2) Hetero-catalytic.

Autocatalytic function of DNA: Here DNA synthesizes another DNA molecule identical to it and this is also known as replication of DNA.

Watson and Crick model of DNA suggests that the mode of replication of DNA is semi-conservative which was experimentally proved by Meselson and Stahl in 1958. However, Delbruck (1961) advocated three types of replication viz.,

(1) Conservative (2) dispersive and (3) Semi conservative.

Semi-conservative mode of replication was also demonstrated by J.H.Taylor and P. Woods (1957) in *Vicia faba* with the help of radioactive tritium i.e.,^{3}H. Hence, semi-conservative mode of replication is universal. (Fig. 17).

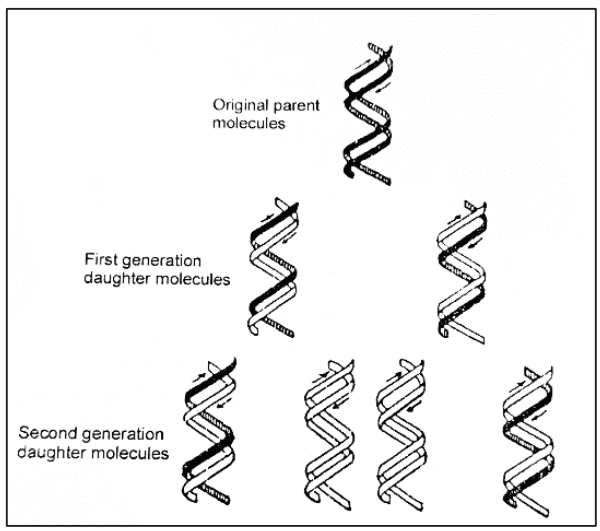


Fig. 17 Diagram interpreting the experiment of Messesion and Stahl (1958) to show the semiconservative method of DNA replication

Requirement for DNA replication: Following are requirements for replication of DNA:

(1) DNA template

(2) Deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxythymidine triphosphate and deoxycytidine triphosphate.

(3) Unwinding enzyme i.e., topoisomerase (replicative protein) discovered by Johnston et. al. (1979).

(4) Helix destabilizing protein and ATP (adenosine triphosphate).

(5) DNA gyrase.

(6) Single strand binding protein (SSB protein).

(7) RNA primer.

(8) Pre-priming proteins like dna B, dna C, dna n, dna n1, dna n11 and dna i.

(9) Primosome (primase in combination with other enzymes).

(10) DNA dependent RNA polymerase.

(11) DNA polymerase I and III.

(12) Endonuclease- it cuts a single nucleotide chain into pieces and

(13) Polynucleotide ligase- It joins broken ends of the two chains by the synthesis of phosphodiester bond.

(14) Exonuclease- It attacks nucleic acid at terminal nucleotide.

Mechanism of DNA Replication in Prokaryotes

The process of replication involves following steps:

1. DNA strand is cut by endonuclease. DNA replication starts at a definite site called as 'O' site or replication origin site-. This site is only one in bacteria and virus where as eukaryotes have many linearly arranged replication origin sites or replicons.
2. Two strands of DNA unwind with the help of unwinding enzymes, helix destabilizing protein ATP and DNA gyrase. It results in the formation of Y shaped structure called replication fork.
3. Exposed single-strands of DNA stabilizes under the influence of SSB protein or helix destabilizing protein. It holds open the two separated strands.
4. Replication begins with the formation of RNA primer with the help of pre-priming intermediate pre-priming proteins and primosome.
5. Leading strand is synthesized as the primer strand elongates under the influence of DNA polymerase III. DNA replication occurs in 5- 3' direction i.e. addition of nucleotides takes place to 3'-OH end. Thus, the nucleotide at 3'end of sugar is newly added one.
6. RNA primer strand is removed with the help of exonuclease and the gaps due to excision of RNA primer are filled by DNA polymerase I.
7. In the process of DNA replication on one template strand of the two parental strands, new strand is synthesized continuously known as leading daughter strand.
8. Synthesis of another new daughter strand on another remaining template strand occurs in the form of short pieces of DNA segment know as Okazaki fragments with the help of

   DNA dependent RNA polymerase. This is known as lagging daughter strand.
9. Discontinuous pieces or *okajaki* fragments of lagging strand are joined together with the help of polynucleotide ligase or DNA ligase.

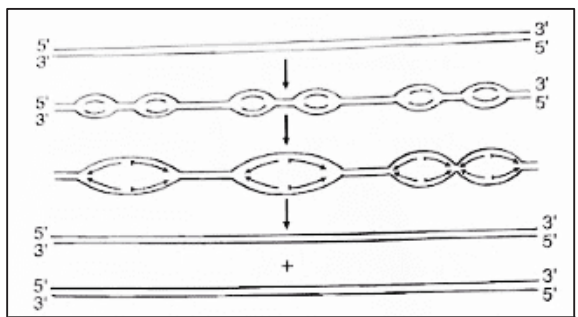


Fig. 18 : Multiple origins of replication

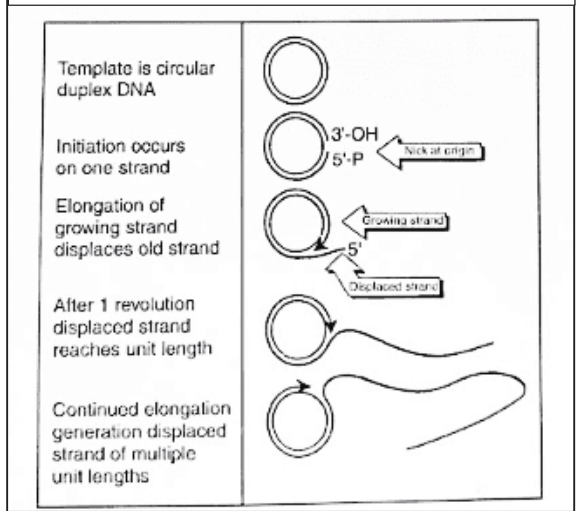


Fig. 19 Rolling circle model of DNA replication

DNA Replication in Eukaryotes

In this way two DNA molecules are produced from one molecule. Each of them is made up of one old and another new strand (Fig. 18,19)

Replication in Eukaryotes: DNA in eukaryotes is in the form of chromatin i.e., combination of DNA and protein. Chromatin is organized in nucleosome. And 200 pairs of nucleotides of DNA are wound around an octomer (H2A, H2B, H3 and H4) x 2 of histones (basic proteins). The long chromosomal DNA molecules (108 base pairs) probably replicate from multiple origins of replication. There are numerous origins of replication and DNA synthesis is bidirectional from these origins.

The Okazaki fragments are smaller than in prokaryotes (about 200 nucleotides). Filling of gaps is done by DNA polymerase (I) or DNA polymerase (H). The fragments are bound to one another by polynucleotide ligase. The replication of continuous strand is carried out by RNA polymerase (III). DNA polymerase is localized in mitochondria and has an exonuclease activity 3'→5'. It can be summarised as such:

1. In eukaryotes DNA replication starts at may origin of replication sites-called autonomously replicating sequences (ARSS).
2. It has two regions-A has 3x11 Mers, binds transcriptional factors E2F, topoisomerase, helices and an RNA polymerase.
3. B regions bind transcriptional activators -Oct. 1& AP-1, Fos &Jun or CTF &NF-I depending upon ARS.
4. Transcriptional activators are induced by Growth factors.
5. Transcriptional factors in A-region & Transcriptional activators in B-region resulting in bending of DNA and melting (Separation) of DNA paired strand starts. Then RNA synthesis occurs which serve as primer for leading strand.
6. Many origin sites located nearest at distance of every 1.0,000 Kbp. Yeast chromosomes have 400 origin sites, Chinese hamster cells have 1000 sites.
7. DNA polymerase II relieves positive super coils. Helicase unwinds the two strands.
8. Telomeres protect the ends of chromosomes from exonuclease degradation.
9. DNA repair during proof reading done by pol E hams 3-5' direction.

Genetic code

Genetic information contained in the DNA is expressed in the form of proteins. Proteins are made of twenty different types of amino acids. The information regarding number and sequence of these amino acids forming protein is present in DNA and this information is, passed on to m-RNA during transcription. 5 Carbon Sugar and phosphate of DNA can not form the message for amino acids because sugar and phosphate both only one type. Thus, only nucleotides are left to form the message. for 20 amino acids. The nuceotides are of four types which are less for 20 amino acids.

Thus, four bases are alphabets of the language of nucleic acids and 20 amino acids are alphabets of the language of proteins. In order to prepare a dictionary for translating the language of m-RNA into language of protein (one is four alphabet language. Other is 20 alphabet language). Singlet code will give 4 codons (4^1), a doublet code would give 16 codons (4^2) and a triplet code will give 64 codons (4^3) in the dictionary. (Table 2 A, B). This was reported by Marshal W. Nirenberg, J.H. Matthaei and H.G. Khorana (1965); Nirenberg and Khorana was awarded nobel prize for this.

Wobble hypothesis: Third base in a triplet code is not important as some t-RNA may recognise more than one codon differing at 3rd position. This is called wobbling which allows economy in the number of t-RNA. R.W. Holley (1964) gave the details of t-RNA.

Wobble in the anticodon of t-RNA

Base in anticodon	Base in codon
G	U OR C
C	G
A	U
I (INSOSINE)	A, U or C

Properties of genetic code: Following are the important properties of genetic code.

(1) Codes is triplet consisting of three nucleotides i.e. AUG, UGG etc. for each amino acids.

(2) Some codes are particulate e.g. AUG and UGG codes for methionine and tryptophan respectively.

(3) Code is degenerate: There are 20 amino acids and so 20 codons are required. However there are 64 codons, hence same amino acid can be coded by more than one codon e.g. UCU, UCC, UCA, UCG, code for scrine. This is called degeneracy of code.

(4) Some codes are non sense but brings about termination of polypeptide chain and hence act as stop single e.g. UAA, UAG and UGA known as ochre, amber and opal respectively.

(5) Code is non overlapping

(6) Code is commaless: There is no gap of nucleotide between two consecutive codons.

(7) Code is non ambiguous. Each code always recognises the same amino acid.

(8) Code is Universal. The same code is applicable to all organisms.

(9) Chain initiation and chain terminator codons are definite. The initiation Condon for methionine on m-RNA is AUG. Initiating methionine is in formylated state but at other position methionine is not formylated. Both these methionine are recognised by different t-RNA.

Table (2 A, B) The genetic dictionary of RNA (the trinucleotide codons for corresponding amino acids are written in 5'-3' direction).

Single code	Doublet code				Triplet code			
A	AA	AG	AC	AT	AAA	AAG	AAC	AAT
G	GA	GG	GC	GT	AGA	AGG	AGC	AGT
C	CA	CG	CC	CT	ACA	ACG	ACC	ACT
T	TA	TG	TC	TT	ATA	ATG	ATC	ATT
					GAA	GAG	GAC	GAT
					GGA	GGG	GGC	GGT
					GCA	GCG	GCC	GCT
					GTA	GTG	GTC	GTT
					CAA	CAG	CAC	CAT
					CGA	CGG	CGC	CGT
					CCA	CCG	CCC	CCT
					CTA	CTG	CTC	CTT
					TAA	TAG	TAC	TAT
					TGA	TGG	TGC	TGT
					TCA	TCG	TCC	TCT
					TTA	TTG	TTC	TTT

Table 2 A

Table 2B Showing genetic code.

First base (5' end)	*Second base* U	C	A	G	*Third base (3' end)*
U	UUU Phen	UGU Ser	UAU Tyr	UGU Cys	U
	UUC Phen	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA**STOP	UGA**STOP	A
	UUG Leu	UCG Ser	UAG**STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU As	AGU Ser	U
	AUC Ile	ACC Thr	AAC As	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG* Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG* Val	GCG Ala	GAG Glu	GGG Alu	G

* Start codons, **stop codons.

Table 2 B

Heterocatalytic Function of DNA

Since DNA is genetic material it carries all the information essential to programme the functions of a cell by controlling the synthesis of proteins or enzymes. Heterocatalytic function of DNA involves coding and decoding of these information and formation of enzymes (proteins) required. Enzymes are proteinaceous in nature and each enzyme is produced by a single gene as has been suggested by Beadle and Tatum (1948) in their one gene-one enzyme hypothesis. Since some enzymes are made of more than one polypeptide and many proteins are not enzymes. This one gene one enzyme hypothesis has now been modified as one gene one polypeptide.

All the hereditary characters are coded on DNA. So, decoding of these characters governed by genes (discrete units of DNA) is done in two steps viz., (1) transcription and (2) translation resulting in the formation of mRNA and protein respectively. Thus, when a particular gene controls a function or a reaction its information is copied on to another nucleic acid i.e. messenger RNA (m-RNA) which in turn directs the synthesis of specific protein. There are three major steps in the flow of genetic information contained in DNA. These are:

1. Replication: In this process the genetic information present in the DNA is duplicated by a template mechanism.
2. Transcription: In this step transfer of genetic information occurs from DNA to m-RNA. This occurs in nucleus.
3. Translation: In this step m-RNA is translated into a sequence of amino acids that make the polypeptide (protein). This process occurs in cytoplasm.

This is one way flow of information i.e., from DNA-transcription-m-RNA-translation-proteins. H. Taming and D. Baltimore (1970) discovered independently reverse transcription in some virus for which they were awarded Nobel Prize along with R. Dulbecco in 1975. The flow of genetic information can now be shown as such:

Replication→DNA transcription→m-RNA translation →Protein.

Proteins are aggregates of amino acids. Amino acids are joined together with peptide bonds between them. Therefore, proteins are polypeptide chains of amino acids. Biological protein synthesis takes place on ribosomes present in cytoplasm. Genetic information for specific protein synthesis is coded on m-RNA formed on DNA template in the nucleus. The overall processes of biological synthesis of protein is described below.

There are two major steps for protein synthesis under the control of genes. They are (1) Transcription and (2) Translation.

1. Transcription: Synthesis of m-RNA on DNA-template is called transcription. DNA is double stranded structure. Only one of them acts as a template for m-RNA synthesis. From genetic evidence it is concluded that one-gene controls the formation of one polypeptide chain of protein. Hence, one of the two strands of DNA forms m-RNA strand in which information for protein synthesis is transcribed. If both the DNA strands act as template for m-RNA synthesis, then only one mRNA strand remains functional. Now it is experimentally proved that only one m-RNA is formed during transcription for which only one strand of DNA duplex acts as a template.

The step wise reactions under transcription are as follows:

Formation of mRNA on DNA template:

1. First of all RNA polymerase attaches to promoter region (a segment) of DNA. RNApolymerase moves along DNA and DNA strands open by unwinding of complementary strands. The promoter region is AT rich region of about 10 bases, earlier than the starting point of mRNA synthesis. It has a common sequence of TATAATG known as TATA box. RNA polymerase is made up of 5 types of polypeptide chains, two alpha chains, two beta chains and one sigma chain.

   Alpha and Beta chains form core enzyme responsible for formation of phosphodiester linkage between nucleotides of growing mRNA. The sigma is responsible for recognition of start signal along DNA molecule. After initiation occurs, sigma dissociates from DNA -RNA core complex and becomes free to attach to another core.

2. Free bases pair with ribonucleotide precursor on one of the DNA strand. RNA-polymerase moves on along DNA template and m- RNA strand formation goes on.

3. Synthesis of m- RNA chain occurs in a fixed direction from 5' to 3'at a speed of 30 to 40 nucleotides per second.

4. Termination of m- RNA chain formation takes place when RNA polymerase reaches the terminator sequence like UAA, UGA or UAG m- RNA chain formation stops by stopping of unwinding of DNA strand in presence of rho factor. This may allow m-RNA to form a hairpin.

Terminator region is rich in GC (in prokaryotes) or AT (in eukaryotes). Complementary m-RNA

strand is not bonded to template DNA strand and hence easily separate. Diagramatic representation of transcription of an RNA molecule on DNA template (Fig 20). One the function of mRNA is over, it is degraded by cellular ribonuclease.

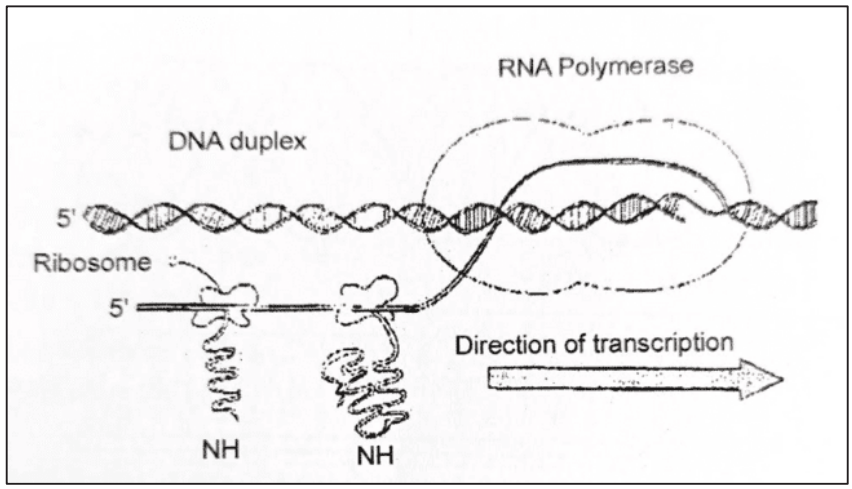


Fig. 20 Showing formation of m RNA

2. Translation: It is second major step in which coded information for protein synthesis on m-RNA is translated on ribosomes It has several stepwise reactions as follows:

(A) **Activation of amino acid**: Amino acid is activated with ATP in presence of ligase (amino acylsynthetase) to from Amino acyladenylate.

Amino acid + ATP + ligase amino acyladenylate.

(B) Formation of amino acid + t-RNA complex"

Now amino acyladenlylate (activated amino acid) attaches itself with t-RNA under the

influence of ligase resulting in the formation of Amino acyladenlylate t-RNA complex.

(C) **Chain initiation**: Chain initiation occurs in the following manner:

1. 30 S subunit of ribosome binds with m-RNA in presence of initiation Factor 3 to form 30 S-m-RNA complex.

2. Formyl methionine t-RNA complex (initiating amino acid t-RNA complex) attaches to 30 S-m-RNA complex in presence of initiation Factor 1 (IF,).

3. Now 50 s subunit of ribosome joins with 30 S-mRNA f-meth-tRNA complex. Thus, ribosome particle of 70 S is completed. Each ribosome particle has two sites (1) 'A site (Amino acyl site) and (2) 'P' site (Peptidyl site):

F-meth-tRNA complex is at 'P' site of ribosome along its initiating codon AUG or GUG on mRNA.

(D) **Chain elongation:** It occurs as follows:

1. Another amino acid t-RNA complex moves to 'A' site with the help of GTP (Guanosine Triphosphate) and Initiation factor TS and Elongation Factor TU along n+I codon on m- RNA.
2. Peptide bond is formed in between amino acids in presence of peptidyl transferase bound to 50 s subunit of ribosome.
3. Deacylated tRNA is ejected from P site with the help of GTP and Release Factor I

(RF_1).

4. Growing polypeptide chain with amino acid t-RNA complex moves from 'A' to 'P' site in presence of GTP and RF,. At the same time m-RNA moves on ribosome to place n +2 codon at 'A' site.

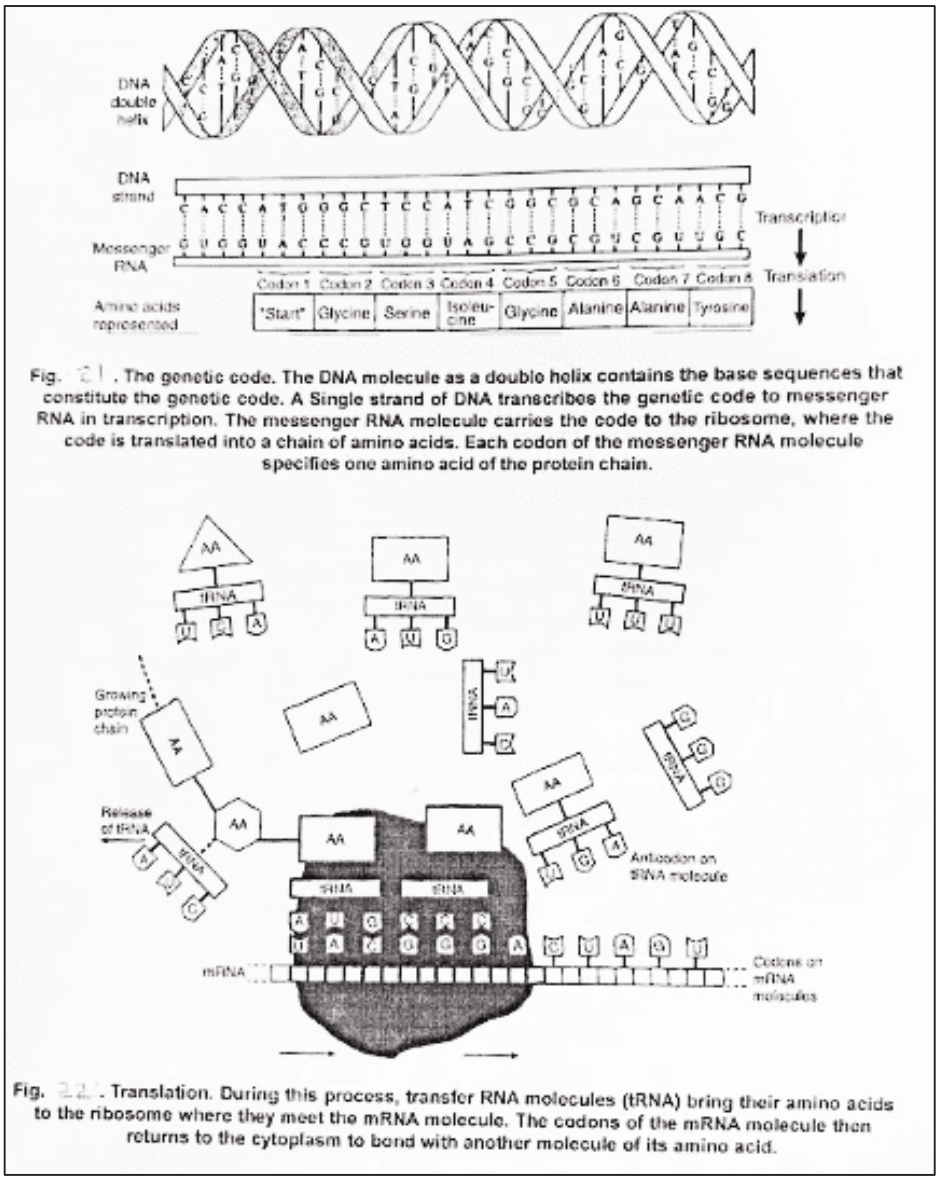


Fig. 21. The genetic code. The DNA molecule as a double helix contains the base sequences that constitute the genetic code. A Single strand of DNA transcribes the genetic code to messenger RNA in transcription. The messenger RNA molecule carries the code to the ribosome, where the code is translated into a chain of amino acids. Each codon of the messenger RNA molecule specifies one amino acid of the protein chain.

Fig. 22. Translation. During this process, transfer RNA molecules (tRNA) bring their amino acids to the ribosome where they meet the mRNA molecule. The codons of the mRNA molecule then returns to the cytoplasm to bond with another molecule of its amino acid.

The whole process of chain elongation is repeated and polypeptide chain of amino acid is formed. In E.coli, protein of 300-400 amino acids are formed within 10-20 seconds with tertiary structure of protein.

(E) Chain termination: When chain termination codon UAA or UGA or UAG moves on ribosome, polypeptide chain with t-RNA is detached from ribosome. The release factor breaks tRNA polypeptide complex releasing t-RNA and, polypeptide chain folds up simultaneously. One ribosome requires 80 nucleotides of m-RNA. m-RNA simultaneously work on many ribosomes. It is also thought that ribosome moves along m-RNA (Fig. 21,22).

Control of Transcription

Generally there are two types of control at the transcription initiation level viz., (1) negative control and (2) positive control.

Negative control: Here transcription by polymerase alone is prevented by the presence of repressor (a protein factor).

Positive control: Here transcription can not take place in presence of RNA polymerase alone, but other protein factors (activator of maltose system or CRP-protein) are required to permit this transcription.

Control of transcription in Lactose operon: Jacob and Monad (1961) proposed a model for the control of the expression of genes in *E.coli*. This operon contains three structural genes X, Y and Z and regulator gene for the synthesis of enzymes involved in the metabolism of lactose, i.e. B-galactosidase (coded by Z gene), permease (coded by Y gene) and transacetylase (coded by X gene). Following diagram demonstrates the control mechanism enabling the 3 structural genes of lactose operon to be expressed only when required and not the rest of the time.

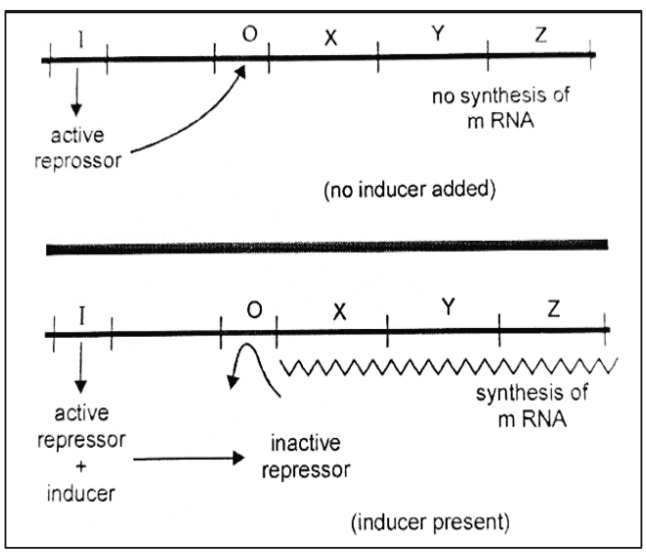


Fig. 23 shows the lactose operon repressed which exists in absence of lactose. The regulatory gene I controls the synthesis of repressor which has an affinity for O (operator gene). The binding of repressor to the operator prevents the transcription of structural genes (X, Y and Z) by RNA polymerase resulting in the absence of mRNA, the 3 enzymes are not synthesized. Repressor has347 amino acids and it prevents movement of RNA polymerase towards structural

Fig. 23 'lac' operon showing induction by inducer.

It has been shown that in presence of inducer (lactose), the repressor forms with the inducer a complex whose structure is modified in such a manner that it can no longer bind to the operator.

Genes X, Y and Z can then be transcribed into a polycistronic mRNA which permits the coordinated synthesis of the 3 corresponding enzymes. And metabolism of lactose takes place.

RNA

E. Miescher (1869) discovered nucleic acid in W.B.C. and gave its name "nuclein". Altman (1.889) called it nucleic acid.

P. A. Levin (1931.) differentiated nucleic acid into (1) DNA =Deoxyribose nucleic acid and (2) Ribose nucleic acid= RNA. Khorana (1963) synthesized RNA in laboratory, Holley (1964) analysed the sequence of nucleotides in tRNA for alanine. Sinsheimer (1959) proved that RNA is also genetic material in TMV.

Structure: RNA is found in the nucleus as well as cytoplasm. RNA is a polymer made of 4 types of monomers or nucleotides. These are (1) Adenosine monophosphate, (2) Guanosine monophosphate (3) Cytidine monophosphate and (4) Uridine monophosphate (Fig 24 A).

Walter Gilbret used the term RNA

Leslie Elezar Orget was known as father of RNA. He suggested that early molecules was much simples than RNA and that could copied itself in the test tube. This molecule was named as peptide nucleic acid.

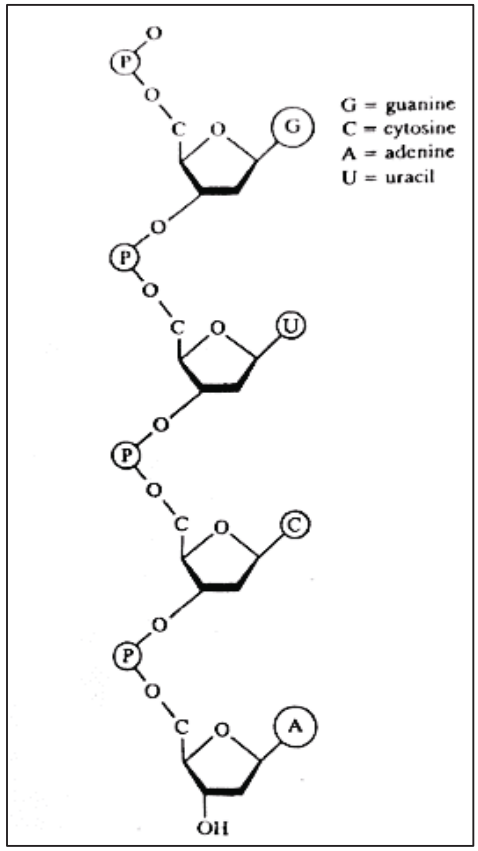


Fig 24 A. Nucleotides in RNA

Types of RNA:

1 Ribosomal RNA (rRNA)

2. Transfer RNA (ERNA)

3.Small nuclear RNA (SnRNA)

4. Regulatory RNA

5. Transfer messenger RNA (mRNA)

6. Double Stranded RNA (ds RNA)

7. Messenger RNA (mRNA)

8 Ribozymes = RNA enzymes

9. Mitochondrial RNA = mt. RNA.

miRNA = micro–RNA is in human serum. These small single strands non-coding RNA play an important role in gene expression and regulation and have been linked to tumour progression and meta stages. Detecting miRNA is a challenge because of their Low concentration in serum.

Photonic Resonator Absorption microscopy (PRAM) helps to capture miRNA biomarkers. PRAM uses nano gold particles which capture and bind to miRNA in a liquid sample. The bound particles then diffuse around in the sample and float to the bottom of the test well where they are detected with a photonic crystal bio-sensor after around 1-2 hours.

It is a point of care test for cancer detection. miRNA are small non-coding RNAs with an average 22 nucleotides in length. It plays an important role in regulating gene expression.

Types: On the basis of function, RNA is of two types namely (1) Genetic RNA and (2) Non- genetic RNA.

GENETIC RNA: H.F. Conrat has shown in 1957 that RNA of TMV is genetic material. Later this idea was strengthened by Sinsheimer in 1959, because it replicates and mutates.

NON-GENETIC RNA: This type of RNA is found in the cells where RNA acts as genetic material These non-genetic RNA is synthesized on DNA template. Non-genetic RNA is of nine types and they are as noted above.

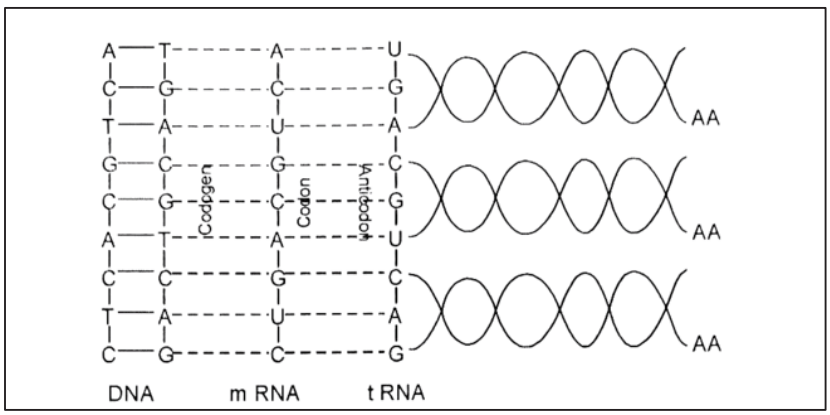
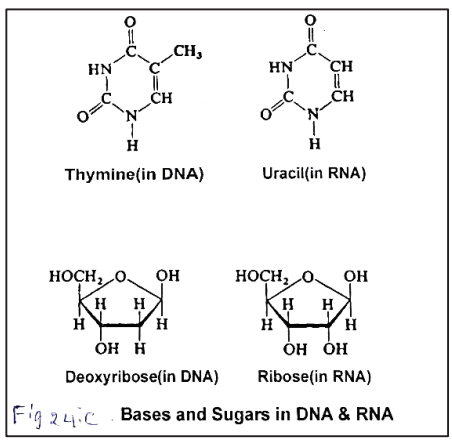


Fig. 24 B Showing relationship between Codogen, Codon and Anticodon loop.

2nd loop has 7 bases and known as ribosome binding loop and 3rd loop is also of 7 bases act as anti codon for a particular Condon present on mRNA. Besides, there is a lump in tRNA. (Fig 16).



Following are the comparative features of RNA and DNA.

RNA	DNA
1. Generally it is single stranded but in some viruses like reovirus-it is double stranded.	1. Usually, it is double stranded except in Q (QX) 174 and other viruses.
2. Nitrogenous bases are adenine, guanine, cytosine and uracil.	2. Nitrogenous bases are adenine, guanine, cytosine and thymine.
3. Pentose sugar is ribose. (C_5 H_{10} O_5).	3. Pentose sugar is deoxyribose (CH1004).
4. Complementary base pairings are adenine uracil and cytosine- guanine.	4. Complementary bases are adenine-thymine and guanine- cytosine.
5. Helical region shows base pairing	5. Whole length of DNA (double stranded) shows base pairing.
6. It is of three types, messenger RNA (or m-RNA), ribosomal RNA (or r-DNA) and transfer or soluble RNA (1-RNA or s-RNA)	6.DNA is of one type. However, some other forms of DNA like A DNA, B-DNA, Z DNA etc. are also known having specific configurations.
7. Generally, it is found in cytoplasm.	7. Mostly it is in the nucleus except mitochondria) and plastid DNA.
8. It exhibits complete and instantaneous reversibility of penetration (melting).	8. In DNA, penetration is partially reversible only under certain condition of naturation (Slow cooling) DNA replicates and forms RNA on transcription.
9. Usually, it does not replicate or transcribe except in some viruses like TMV (RNA forms RNA).	9. DNA replicates and forms RNA on transcription.
10. RNA contains comparatively less number of nucleotides (up to 12,000).	10. DNA contains comparatively more number of nucleotides (up to 4-3 million).

Insertion Elements and Transposons

Generally the genome of an organism is not variable and has a definite structure. It has been noticed that conservation of genetic structure in certain region of the genome is not always rigid, rather there are stretch of DNA which can move from one place to another within the genome.

Sequence of base pair

These movable elements cannot duplicate themselves and may be present in variable copy in different cells in an organism. Such stretch of DNA (genes) is referred as transposons or jumping genes or nomadic genes or transposable elements. These are discrete sequences in the genome

which are mobile. Transposons are found in eukaryotes as well as prokaryotes. In maize, these elements were known as controlling elements as it exerts control over the expression of genes.

Hedges and Jacob (1974) used the term transposon for a DNA segment of bacteria which could move from one molecule to another and carried resistance for ampicillin - an antibiotic. This transfer of transposon involve

normal recombination. Transposable elements usually code for a gene which is flanked by Short Direct (DR) or inverted repeats (IR) and jointly known as terminal repeats (Fig. 25) These elements are of following types:

(1) Insertion sequence (IS): It is found in plasmids and bacterial genomes. They do not code for any other protein which is beneficial to the host. Therefore, it is also called as "selfish gene".

(2) Transposon: It is larger than IS elements.

(3) Composite transposon: It is relatively still larger units with a complex structure. Entire transposon moves, only bordering IS sequences move.

(4) Controlling elements in maize: These elements can be helpful in providing the variegation by somatic development. It also has inverted repeats and short direct repeats at the target site.

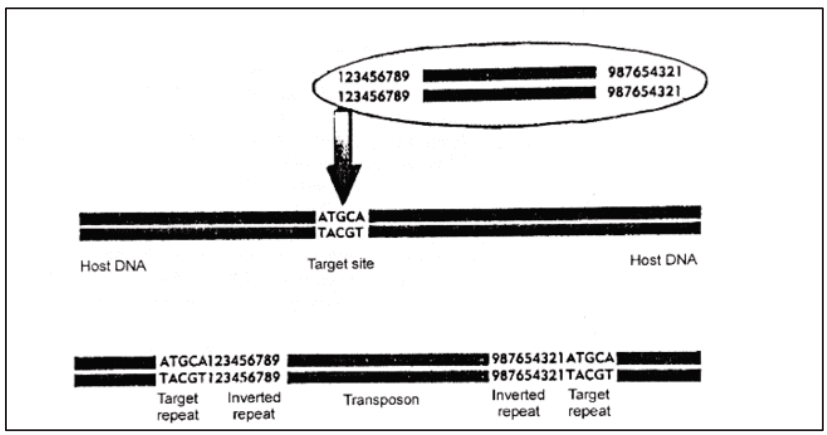


Fig. 25. Transposons have inverted terminal repeats and generate direct repeats of flanking DNA at the target site. The ends of the transposon consist of inverted repeals of 9 hp, where the numbers 1 through 9 indicate a target repeat.

(5) Ty elements in Yeast: It is transposable elements in yeast. It is of two types (1) Ty 1 and Ty 917 of 6.3 kb long with 330 bp DR (direct repeat) on both ends. Ty elements are transposed through RNA intermediate. They do not form infection particles.

Uses of transposons: It is used as genetic marker to construct linkage maps.

Chapter 16
Structure of Prokaryotic Gene and its expression

Traditionally, the gene has been defined as a piece of DNA which has necessary information for the biosynthesis of a protein. The gene as a basic unit of heredity has a complex structure. The eukaryotic and prokaryotic gene differ in their structures. However, there are several common features between them. The prokaryotic gene is polycistronic means it code for more than one protein where as the eukaryotic genes are monocistronic i.e., code for a single protein. A typical prokaryotic gene has two regions viz; (1) regulatory region and (2) transcriptmional region.

The regulatory region: Possess the sequences essential for the transcription of the gene but are not transcribed themselves, where as transcription region is transcribed to form a RNA molecule. The transcriptional region starts from a transcription starts site". This is the position where RNA polymerase initiates RNA synthesis during transcription and this molecule is designated as the 1^{st} nucleotide. The regulatory region has the nucleotides which are upstreams of the "transcription start site."

Regulatory Unit: The regulatory region has several different elements, out of them, promoter is the most essential. Binding of RNA polymerase to the gene to initiate transcription is done by the promoter region. Promoter has two regions of conserve nucleotide sequences. First region in recognition site which recognize RNA polymerise which binds to the template. The second region is polymerase, RNA polymerase binding site and are nearer to the transcription initiation site and helps to initiate RNA synthesis by opening of the ds (double stranded) DNA.

Promoter: It is the first region of promoter consisted of hexanucleotide i.e.. TTGACA, situated between 30-40 nucleotides upstream of transcription initiation site and is shown as "-35 region." The second region of promoter is also with TATAAT sequence of nucleotides. It is called as "-10 region." Or" Prebnow box" and its location is from-5 to-14 or from -9 to-18. The "-10" region is AT rich as shown in (Fig. 26).

Transcriptional region: This region starts from the transcription initiation site and ends at the terminator of transcription. It forms a single unit of RNA. It is 6-9 nucleotides down stream of the prebnow box. There is a spacer region just after transcription start site. The base of this region pairs with 16S rRNA which helps to bind small subunit of ribosome to mRNA during the start of translation. Therefore, this region is called as RBS (Ribosome binding site) or SD (Shine Dargarno) sequences. Change in RBS site can result in no translation or, poor translation. After spacer region of 7-1.3 bases, the initiation codon ATG is present. The initiation codon is followed by coding region of the gene which is continuous structure which ends in one of the three stop codons.

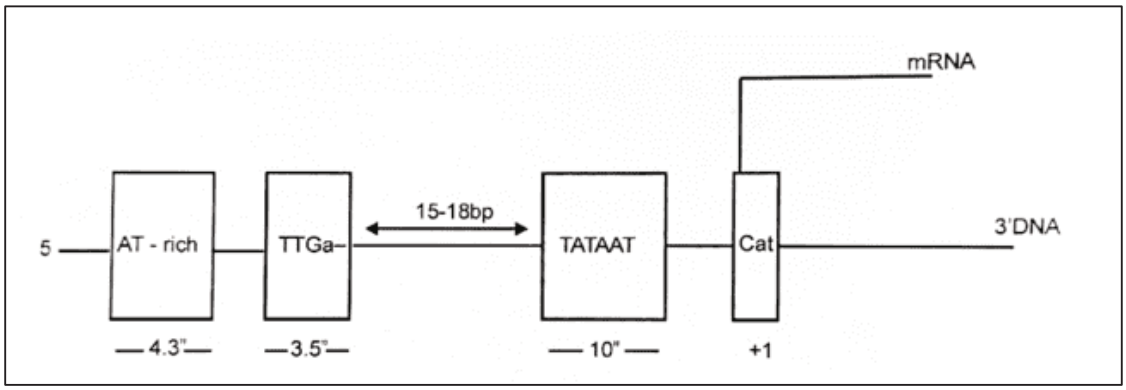


Fig. 26 Structure of a Prokaryotic promotor

Structure of Eukaryotic genes: Monocistronic eukaryotic genes have two regions;

(1) regulatory region and (2) transcriptional region. The regulatory region has promoter which facilitates the binding of RNA polymerase to the genes and directs it to start transcription. The first region of promoter is recognition region, similar to 35 region of prokaryotes and known as "CART box".

Its nucleotide sequence is CAATCT and located between-70 to 80. The second region is "TATA" box or "Hogness box" having TATA sequence and located between -19 to -27 similar to prebnow box of prokaryotes. The TATA box is surrounded by G-C rich region. Many of eukaryotic promoters have G: C box situated at -120 positions as shown in the fig. 27 A, B, C. Eukaryotic genes also possess other regulatory units like activators, silencers and enhancers. These are "cis" acting elements located on the gene. These enhance the level of expression. They may be situated at a distance of thousands of nucleotides from the site of their action.

Transcriptional region is similar to prokaryotic transcriptional region. Majority of eukaryotic genes have several intervening sequences interspersed in between the coding region. These sequences do not code for any polypeptide known as "introns". Initially these sequences are copied into RNA called primary transcript but later they are removed from them RNA by process known as splicing. The coding region ends in one of the three stop codons followed by 3- non coding region. There is a downstream regulatory system sequences located 10-20 nucleotides upstream of the transcription terminator. This sequence has AAATAA known as polyadenylation site. It direct poly (A) polymerase.

to add a stretch of A residues of 100-200 nucleotides at 3' end of m RNA. It also provides signal for end of the transcription.

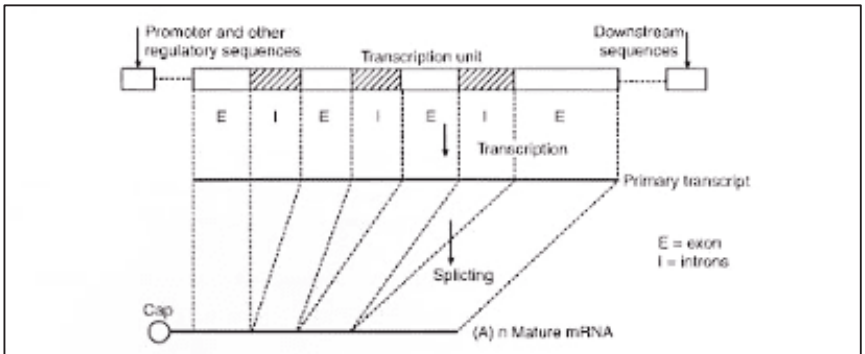


Fig 27 a. Eukaryotic Genes

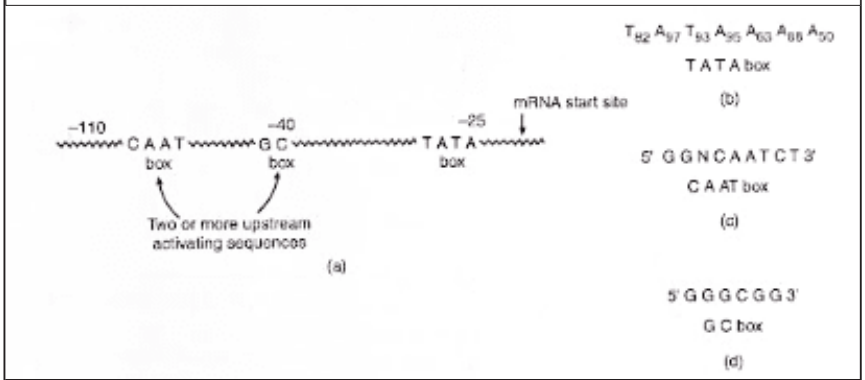


Fig. 27 b. Structure of eucaryotic promoter

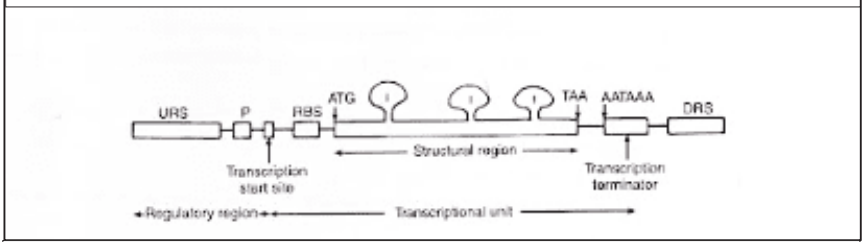


Fig 27 c General structure of a gene. URS-Upstream regulator sequences such as activator, silencer, enhancers etc., present only in eukaryotes. P-Promoter. RBS-Ribosome binding site.

Prokaryotic Transcription & Translation

Expression of gene is performed through transcription and translation. Transcription is synthesis of m RNA which carries the genetic information present in DNA. Translation is the transfer of genetic information from the DNA to its ultimate produce i.e. protein - a functional molecule as enzymes) for metabolic activities in the cells. Following are the stepwise events related with prokaryotic transcription and translation:

Mechanism of Prokaryotic transcription

Transcription are carried out as such:

(a) Template recognition: Promotor (-35 bp) directs, RNA Polymerase to recognise sigma factor of RNA which helps binding of RNA polymerase to DNA. RNA polymerase has 5 subunits (2-alpha, 2-beta and 1-gamma) & Core enzyme '! alpha, alpha , beta and beta responsible for phosphodister bond formation. Holoenzyme alpha, alpha, beta, beta and gamma.

(b) **Initiation**: DNA + RNA polymerase complex'! melting of -10 region 1 opening of ds DNA-SSDNA. First two nucleotides are incorporated & phosphodister bond is formed, followed by complementary nucleotides addition upto-9, then RNA polymerase moves. Sigma dissociates for other core.

(c) **Elongation**: At any given time about 17 bp of DNA remain open. During movement of enzyme-30 bp of DNA are covered. So, entire length of m RNA, except 17+30 = 47 bp, remain hanging from the (DNA + Enzyme) complex.

(d) **Termination**: Once enzyme hits the terminator, it (RNA polymerase) falls off the template & transcription stops. Terminator codons - UAA, UGA or UAG (rich in GC or AT (in eukaryotes)

1. Rho factor binds to growing RNA chain and moves along.
2. Once rho catches RNA polymerase - Chain terminates, RNA polymerase is detached, mRNA chain also comes off & transcription stops.
3. Complementary m RNA is not bonded to template DNA strand and hence easily separated. Once the function of m-RNA is over, it is degraded by cellular ribounclease.

Prokaryotic- translation

Translation: Here coded information for protein syntheses is translated on ribosome.

1. Activation of amino acid (a.a) & formation of aminoacyl t-RNA with the help of aminoacyl t-RNA synthetase, and ATP aminoacyladenylate.
2. Formation of Aminoacyladenylate t RNA complex
   (a) Protein synthesis is initiated by AUG coden and IF3 which helps in binding 30s to m- RNA 30s. m-RNA complex is formed.
   (b) Formyle methionine t RNA attaches to 30s-m-RNA complex in presence of IF1 30s-mRNA. f.meth t-RNA complex.
   (c) 50s subunits of Ribosome join with 30s-m-RNA f-meth t-RNA complex. Ribosome is completed as initiation complex. Ribosome has two sites (1) Amino acyl site (2) Peptidyl site.
   (d) F. meth-t RNA complex is at 'P site along its initiating colon- AUG/GUG on in- RNA.
3. **Chain elongation**
   a) Another aatRNA complex moves to A site with the help of GTP and elongation factor EF-TU and TS.
   b) Peptide bond is formed in between amino acids in presence of peptidyl transferase bound to 50s subunits of ribosome.
   c) Deacylated t RNA is released (ejected) from 'P site with the help of GTP and release factor 1 (RF).
   d) Growing polypeptide chain with amino acid tRNA complex moves from A to P site in presence of GTP & RF.
   e) At the same times RNA moves on ribosome to place n + 2 codon at A site. The whole process of chain elongation is repeated & polypeptide chain of amino acid is formed (40 aa/second).

4. **Chain termination**: Chain terminating codon UAA, UGA or UAG moves on ribosome. Polypeptide chain is detached. One ribosome requires 80 nucleotides and moves along mRNA.

Gene Expression in Prokaryotes:

Jacob and Monad (1961) grew wild *E.coli* on medium containing lactose and glucose. It was found that E.coli did not utilize lactose and so the product - galactosidase was very less.

When this wild E.coli was grown on lactose medium without glucose, the synthesis of galactosidase occurred & hence-galactosidase was considered to be lac enzyme.

Lac operon regulates the metabolism of lactose as follows:

1. *E.coli* → $\xrightarrow[\square]{\text{grown on lactose medium}}$ →Lac operon becomes functional.

2. *E.coli* → $\xrightarrow[\square]{\text{Grown on medium with lactose + Glucose}}$ → tetrameric enzyme -galactosidase

hydrolysis

3. α-galactosidase + Lactose. → $\xrightarrow[\square]{\text{Hydrolysis}}$ → galactose + glucose

Lactose operon in *E.coli*

Britten and Davidson (1969) summarised the operon model of Jacob and Monad. Gene

expression is performed by the following elements:

(A) **Operon or structural genes:** There are 3 structural genes, z, y and a. The product of z gene is polypeptide chains of 134,000 Dalton which combines with other like chains to form tetrameric enzyme i.e., galactosidase which converts lactose into galactose and glucose. Y gene forms. M-protein/galactose permease located on bacterial cell membrane which permits lactose from external medium into the cells. A gene produces thiogalactoside transacetylase which transfer acetyl group from Acetyl co- enzyme A.

(B) **Promotor gene**: It consists of sequence of bases and recognized by DNA dependent RNA polymerase. Promotors are regulated by regulator protein or repressor. Repressor prevents movement of RNA polymerase towards the structural genes.

(C) **Operator gene:** It also consists of sequence of 35 nucleotide base pairs. It lies between promotor and beginning of the structural genes. Operator + repressor prevents RNA polymerase to form initiation complex.

(D) **Regulatory gene**: It controls the operator and promoter. Regulatory gene produces repressor or regulator protein of 347 amino acids.

Repressor+ operator/Promotor → __ Grown on medium with lactose + Glucose →>Regulate genetic transcription

Inducer or Effectors molecule-They are a sugar, an amino acid or a nucleotide molecule which combines with regulator protein to interact with operator or promoter which inhibits operon expression or allows operon expression and regulator is pulled out (Fig. 28)

Trp operon: It is a polycistronic gene which codes for 5 enzymes involved in tryptophane synthesis. These genes are trpE, trpD,trp C,trpBtrp A as depicted in the following diagram (Fig. 29).

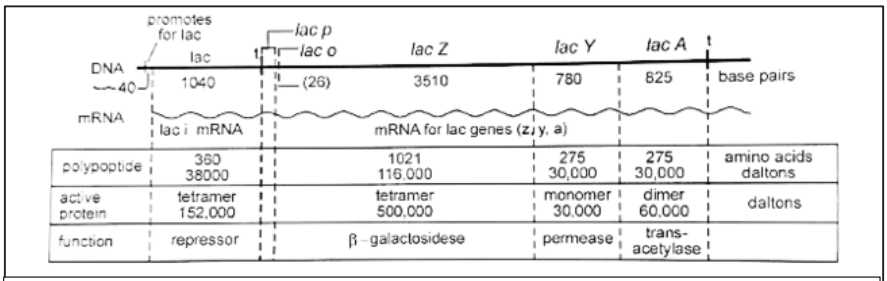


Fig. 28 The lactose operon of E. coli and its regulatory gene (redrawn from Lewin's Genes

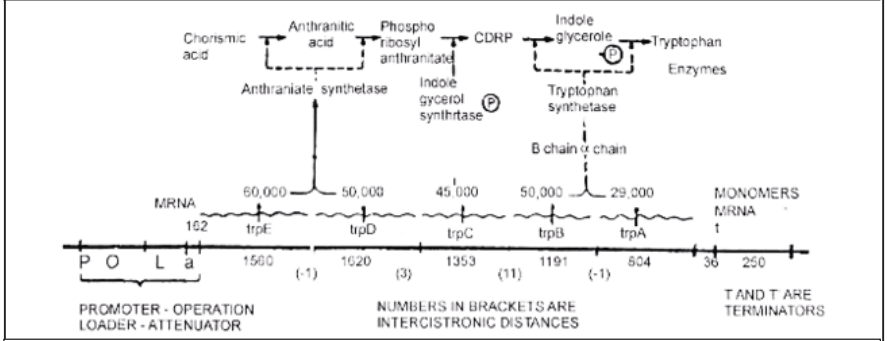


Fig. 29 Tryptophan operon with its genes, the promoter-operator leader-attenuator region and

Gene Expression in Yeast

I. $Yeast \xrightarrow[\textbf{Lactase}]{\textbf{Lactose}} > Glucose + Galactose$

II. $Yeast \xrightarrow[\textbf{without Lactose}]{\textbf{Medium}} > No\ Breakdown\ of\ Lactose$

III. $Yeast \xrightarrow[\textbf{Lactase (14 hrs)}]{\textbf{Lactose}} > Glucose\ and\ Galactose$

Presence of lactase in the medium induces cells to produce lactose. Therefore, Lactase is an inducible enzyme.

Substrate ***Substrate***

Substrate A → Enzyme A → B, Enzyme B → C, Enzyme C → end product. Inhibition

Enzyme activity is repressed by inhibition and no protein synthesis (negative feedback).

Split genes: This was discovered by Phillip A. Sharp and Richard J. Roberts in 1977 for which they were awarded Nobel prize in 1993.

There is co-linearity between sequence of nucleotides on DNA and sequence of amino acids in corresponding proteins. Later in 1977 it was observed by sharp and Roberts that a gene may or may not be represented by a continuous sequence of nucleotides but may be interrupted by some intervening sequences of nucleotides which are not present on mRNA after transcription for protein synthesis (Fig.30).

Such genes with intervening sequence of nucleotides were called split genes.

Split genes: It may include

(1) Nuclear genes for r RNA.

(2) Nuclear genes for proteins

(3) Nuclear genes for t RNA

(4) Mitochondrial genes in yeast

(5) Chloroplast gene

(6) Genes in bacteriophages of *E.coli*

(7) Genes in Archae bacteria

(8) Introns were also reported in eubacteria

Intron of one gene may be the exon of another gene. Exon sequences are conserved, but introns sequences vary.

Overlapping genes: If we know the length of DNA, we can determine the length of protein. Suppose there are 1200 nucleotides in a DNA, it will give rise a protein of=1200/3 = 400 amino acids (3 nucleotides code for one amino acid). φx 174 bacteriophage has about 5400 nucleotides and it contains 9 citrons (A.B.C.D.E.EG.H and I)

Barrell et.al (1976). The proteins formed by 5400 nucleotides were calculated and it was observed that these proteins of 9 cistrons require at least 6000 nucleotides. How? It was explained that sequences in one cistron are utilized by two different citrons for different proteins. Thus, cistron or gene overlaps and they function at different times and nucleotide sequence are translated at two different reading frames.

9 cistrons (genes) in **ϕX 174** bacteriophage of *E. Coli* are:

A B C D I F G H

= =

E

Thus, cistron E overlaps cistron D I

This work done by F. Sanger et al and got' nobel prize in 1980.

Pseudogenes. It represents DNA sequences obtained from m-RNA through reverse transcriptase. It differs from true genes (spilt genes) by the absence of intron sequences. Examples are human globin and globin pseudogenes, histone pseudogenes in Drosophila etc.

RNA polymerase II (meant for m RNA)

RNA polymerase III (meant for t RNA & 5s rRNA)

Promiscus DNA. DNA which moves in between nucleus, chloroplast and mitochondria are promiscus DNA. In yeast.

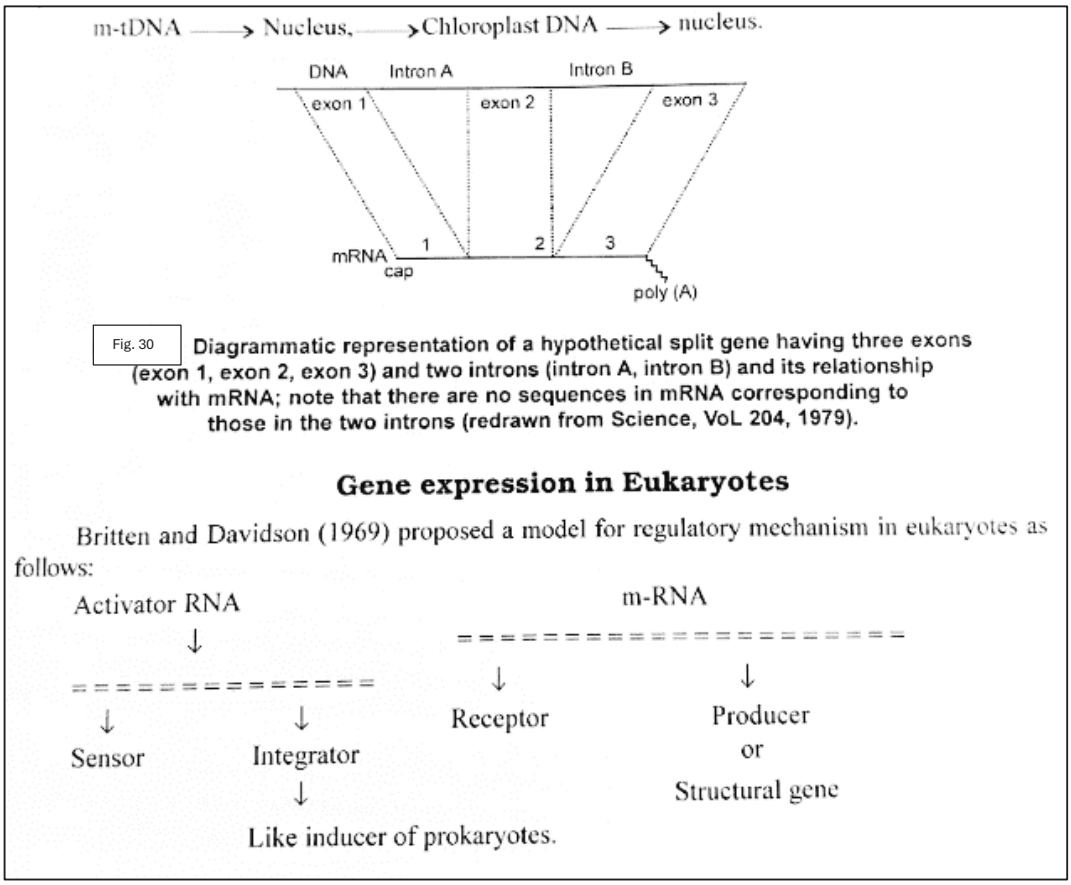


Fig. 30 **Diagrammatic representation of a hypothetical split gene having three exons (exon 1, exon 2, exon 3) and two introns (intron A, intron B) and its relationship with mRNA; note that there are no sequences in mRNA corresponding to those in the two introns (redrawn from Science, VoL 204, 1979).**

Gene expression in Eukaryotes

Britten and Davidson (1969) proposed a model for regulatory mechanism in eukaryotes as follows:

Producer gene: It can be compared with structural genes of prokaryotes.

Receptor site: It is present adjacent to each producer site. It can be compared to operator gene of prokaryotes.

Integrator gene: Comparable to regulator gene of prokaryotes. They are responsible for the synthesis of activator RNA.

Integrator and receptor sites may be repeated several times to control a large number of genes active in the same cells.

Catabolic repression: Bacteria are capable to adopt themselves very quickly with the change in the environment. *E.coli* growing in presence of glucose do not synthesize enzymes involved in catabolism of other sugars. This repression of enzyme activity is known as catabolic repression. c-AMP (adenosine 3 phosphate cyclic monophosphate) plays an important role in catabolic regulation of gene expression.

In eukaryotes-cAMP action is different when blood glucose level is low i.e. hypoglycemic, the alpha cells of pancreas are stimulated and the blood glucose level goes up. The increased glucose level leads to increased c-AMP level which stimulates CAMP dependent kinase. The kinase regulates the activity of enzymes by phosphorylation of the proteins. As a result glycogen synthesis is decreased and there in an increase in blood sugar level.

Chapter 17
Gene Organization and Expression in Mitochondria and Chloroplast

Mitochondria also possess their own genome. It contain dual system of genetic information one set is coded by the mitochondria itself and second set is coded by the nucleus. It defers from nuclear genome in their sensitivity to metabolic inhibitors. For example, nuclear transcription is inhibited by alpha aminitin whereas that of mitochondria is sensitive to acridines and ethidium salts. Similarly, nuclear translation is blocked by cyclohexamide while mitochondrial translation is sensitive to erythromycin, tetracycline and chloramphenicol. There is no transport of biomolecules from mitochondria to cytoplasm. There is no de novo genesis of mitochondria. During the formation of new mitochondria, the mitochondrial genome replicates through out cell cycle resulting in doubling of its mass. After DNA replication (in mitochondria) the two DNA molecules of mitochondria (m-t - DNA) move apart and septum formation starts which divides the mitochondria into two unequal compartments. Both the compartments are then separated and two mitochondria are formed.

Number of DNA molecules in a mitochondrion is always constant. The regulatory mechanism of mitochondrial genesis is not well known. A large number of nuclear genes is required to maintain

the mitochondria genome.

Structure: Mitochondrial DNA is circular except in *Paramecium* and *Chlamydomonas* in which it is linear. The size of mt DNA varies from 16,6 kb to 250 kb. The number of DNA molecules in mitochondria also varies.

Function: Mitochondria Produce 95% of ATP and hence they are known as power house. Mitochondria have its own protein synthesizing mechanism. There is no introns in human mt DNA, but it is present in mitochondria of yeast. These introns may move in and out of a gene like transposable elements of nuclear genome. Mitochondrial genome has 22 t RNAs against 40 different t-RNAs in cytoplasm. The ribosome of mitochondria also differs from the cytoplasmic ribosomes. Mitochondrial ribosome is of 60s (in HeLa Cells of human) 75s (in yeast) and 73s (in *Neurospora*) where as their cytoplasmic ribosomes are of 74s, 80s and 77s respectively.

Protein synthesis: The mechanism is similar to that of bacteria. However, the initiating codon is AUG in m-RNA of prokaryotic & eukaryotic codons, where as it is AUA and AUU in mitochondria. Mitochondrial protein synthesis mechanism requires t-RNA-formylmethionine similar to bacterial mechanism. Following table indicates the features of mitochondria), and nuclear genome comparable to that of chloroplast genome also.

Table 3: Showing Features of mt-DNA, cp-DNA and Nuclear DNA

Nuclear DNA	Mitochondrial DNA	chloroplast DNA
1. Histone associated Structure nucleoside 2. Slow resuscitation 3. Large size genome contain many genes 4. It in linear 5. Repetitive sequence are common 6. UGA is stop code 7. Methionine is starting	1. No histone associated DNA 2. Rapid Resuscitation 3. Small size genome with less genes 4. It is circular except in *paramoecium*. 5. Repetitive sequences are rare 6. UGA Code for Tryptophane 7. N. formyl-methionine is starting amino acid.	1. No histone associated with DNA 2. Rapid Resuscitation 3. Small size of genome varies from 12 Lacs bp to 15 lacs bp. 4. It is circular or double stranded 5. Absent 6. As Mitochondrial DNA 7. N. formyl-methionine is starting amino acid.

N.B.in *Mycoplasma capricolum* UAA and UAG code of Glutamine instead of stop signals.

Transcription: mtDNA transcription starts at a site within the displacement loop (D-loop). In mouse mt DNA there are two promoters for transcription within D loop separated from each other by 150 bp (Fig 31). Two promoters are HSP (H-strand promoter) and LSP (L- strand promoter). Each of them has (1) a short sequence and (2) an upstream sequence i.e., -12 to -39 for initiation. Transcription factor (mt TF-1) binds to the sequence upstream to start site.mt.TF-1 activates transcription by unwinding and bending of DNA at promoter A base sequence of 1.3 nucleotides 5' TGGCAGAGCCCGG-3' is responsible for termination of transcription.

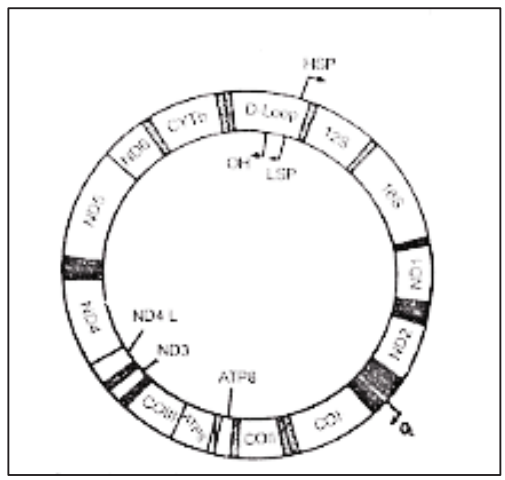


Fig. 31 A map of vertebrate mtDNA, showing location ct D loop, origins for DNA replication (On and OL), promoters for transcription (HSP and LSP), genes for 22 tRNAs (shaded areas) and ther protein coding genes (CO-cytochrome oxideed ATP6, ATP8-ATPase subunits; cytb= cylochrome b; ND NADH dehydrogenase); arrows indicate direction of replication or transcription.

Translation: The components of translation are synthesized outside of mitochondria and transported into the organelle. The ribosomes are smaller, tRNA is species specific and its number is 22 against 55 in nuclear DNA. Initiation of translation occurs by formylemethionyl tRNA. Rest of the steps are similar to prokaryotic mechanism. Products of nuclear genes are essential for translation of specific gene in mitochondria.

Chloroplast genome: Chloroplast also has its own genome known as cpDNA (Chloroplast DNA) which resembles with prokaryotic genomes. cpDNA has three regions (1) two inverted repeats (IR), (2) short single copy sequence (SSC) and (3) long single copy sequence (LSC). Two inverted repeats of 10-24 kb carry ribosomal genes, SSC has 18-20 kb and about 1.10 genes are encoded in cp-DNA (Fig. 32).

Functional mechanism: Chroplast DNA has four components for its autonomy viz, DNA polymerase, RNA polymerase, DNA and protein synthesizing mechanism. There are 20-60 copies of circular double stranded DNA. Ribosomes are of 70s type. 5-methyl cytosine is absent in cpDNA. Presence of DNA in chloroplast was shown by Ris and plaut (1962). The genetic code used by cpDNA is similar to that of nuclear DNA. Promotor sequences for transcription has been observed in cpDNA similar to that of E.coli.

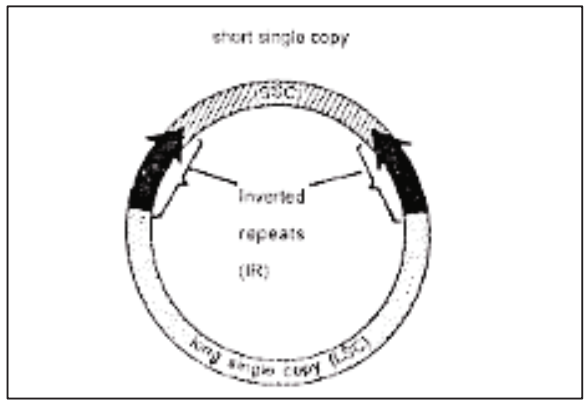


Fig. 32 Three characteristic regions in acp DNA molecule

Chloroplast Gene expression: Gene expression or protein synthesis takes place on ribosomes with the help of t-RNA and amino acids besides several factors and enzymes. There are about 120 genes in chloroplast DNA. Out of them 30 genes code plastid protein, 30 genes code photosystem II, LATP synthetase and cytochrom b6, and rest code other protein Transcription and translation constitute the mechanism of protein synthesis. In transcription-promoter sequences are at-10 and -35, terminator is of short inverted repeat sequences and ep m-RNA is devoid of a cap at 5' end and a long poly A tail at 3' end. Introns are present in ep-DNA. Most genes in plant mt DNA is monocistronic. Transcription initiation site has a conserved CRTA (R-A or G) motif.

Post Translation Regulation of Gene Expression.

There is a sequence of 162 bases between initiation codon AUG and the promoter-operator region of trp E gene for anthranilate synthetase of E.coli. This is (sequence of .162 bases) known as "leader" sequence and it has post transcriptional control. The leader sequences have a function of alternating (reducing) transcription in a controlled manner. Such transcriptional regulation is not observed in eukaryotes because in eukaryotes transcription and translation cannot be coupled due to separation by nuclear membrane. Besides, there are several proteins (e.g., p32 protein) work as repressor for repression of their own products due to binding of repressor protein to ribosome binding site which is called as Shine-Delgarno sequence (initiating codon AUG of mRNA).

RNA-m RNA hybrids prevents m-RNA from being' translated. This RNA in hybrid will interfere with translation of m RNA-it is called anti sense RNA or mic RNA.

Translation of some genes is influenced by translation of neighbouring genes and this is translational coupling. Sometimes, the end product of metabolic pathway under control of enzyme is accumulated which may stop further synthesis of that product. Thus, enzyme regulated action mechanism is called as feed back inhabitation as illustrated below.

Threonine →deaminase→alpha keto butyric acid →alpha keto Isoleucine→isoleucine

Feedback inhibition in the synthesis of isoleucine is due to binding of isoleucine to threonine deaminase in *E.coli*.

Chapter 18
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B. Events in Molecular Biology

1869 Friedrich Miescher discovered DNA.

1941 Beadle and Tatum Demonstrated that a gene codes for a single protein.

1944 Avery proved that DNA is the genetic material.

1953 Watson and Crick proposed the double helical structure for DNA.

1958 Masselson and Stahl demonstrated that DNA replicates semi-conservatively.

1961 The triplet nature of the genetic code was discovered.

1961 Messenger RNA was uncovered.

1961 Jacob and Monod proposed the operon model for gene regulation.

1970 Temin and Baltimore reported the discovery of reverse transcriptase in retroviruses.

1973 Type II restriction endonucleases were discovered.

1974 Eukaryotic genes were cloned in bacterial plasmids.

1976 Retroviral oncogenes were identified as the causative agents of cellular transformation.

1977 Interrupted genes were discovered and splicing mechanism for their removal from the primary transcripts was deciphered.

1981 Catalytic activity of RNA was discovered.

1981 Transgenic mice and files were obtained by introducing new DNA into the germline.

1997 A sheep was cloned from somatic cell genome, establishing the possible totipotency in animal cells.

Multiple Choice Questions

Tick the correct alternative in the following:

1. Synthesis of a gene in a test tube and its transfer to a cell is called:

(a) Genetic engineering

(c) Mutation

(b) Cloning

(d) Hybridization.

2. The enzyme that cleaves DNA at specific sites, producing sticky ends is called :

(a) Lysing enzyme (c) Exonuclease

(b) Cleaving enzyme (d) Restriction endonuclease.

3. Structure involved in genetic engineering is :

(a) Plasmid (b) Codon

(c) Plastid (d) Scissors.

4. Which enzymes is useful in genetic engineering?

(a) DNA ase (b) Amylase

(c) Lipase (d) Restriction endonuclease.

5. Restriction endonucleases are used in genetic engineering, because :

(a) They can degrade harmful proteins (b) They can join DNA at specific base sequences

(c) They can cut DNA at variable sites. (d) They can cut DNA at variable sites.

6. The Transgenic animals are those which have:

(a) Foreign DNA in some of their cells (b) Foreign DNA in all of their cells

(c) Foreign RNA in all of their cells (d) Both a and c.

7. First artificial gene was synthesized by:

(a) Nirenberg (b) Mendel

(c) Morgan (d) Khorana.

8. Genetic engineerings is

(a) Plastic surgery (b) Addition or removel of genes

(c) Study of extranuclear genes (d) All the above.

9.Advancement in genetic engineering has been possible due to the discovery of:

(a) Oncogenes (b) Transposons

(c) Restriction endonuclease (d) Exonucleases.

10. Which of the following is a genetic vector?

(a) Plasmid (b) Phage

(c) Cosmid (d) Exonucleases.

11. Genetic engineering would not have been possible if which of the following were not known?

(a) DNA polymerase (b) RNA synthetase

(c) DNA ligase (d) Reverse transcriptase.

12. Genetic engineering is possible because :

(a) Phenomenon of transduction in bacteria is well understood

(b) We can cut DNA at specific sites by endonucleases like DNAase 1

(c) We can see DNA by electron microscope

(d) Restriction endonucleases purified from bacteria can be used in vitro.

13. Restriction endonucleases are:

(a) Synthesized by bacteria degradation of DNA

(b) Present in mammalian cells for

(c) Used for in vitro DNA synthesis

(d) Used in genetic engineering.

14. The chemical knives of DNA are:

(a) Ligases (b) Polymerases

(c) Endonucleases (d) Transcriptase.

15. When the genotype of an organism is the process is called:

(a) Biotechnology (b) Tissue culture

(c) Genetic engineering (d) Genetic diversity.

16. "Molecular scissors" used in genetic

(a) DNA polymerase (b) DNA ligase

(c) Restriction endonuclease (d) Helicase.

17. Plasmids occur in:

(a) Viruses (b) Chromosomes

(c) Bacteria (d) Chloroplasts.

improved by the addition of foreign genes,

engineering is :

18. One of the following is used to join the segments of DNA during genetic engineering:

(a) Lipase (b) Ligase

(c) Gyrase (d) Helicase.

19. Restriction endonuclease enzymes are used in genetics and they:

(a) Cut DNA at specific sites (b) Cut DNA at variable sites

(c) Join DNA segments (d) Cut RNA at specific sites.

20. The tool of genetic engineering which makes it successful is :

(a) DNA polymerase (b) Permease

(c) Restriction endonuclease (d) Topoisomerase.

Answer

1.(a) 2.(b) 3.(a) 4.(a) 5.(c) 6.(b) 7.(d)

8.(b) 9.(c) 10.(a) 11.(c) 12.(a) 13.(d) 14.(c)

15.(a) 16.(c) 17.(c) 18.(b) 19.(a) 20.(c).

Additional Information on DNA Replication

Cairn (1963) by using H^3 Thymidine also confirmed semi-conservative mode of DNA replication in bacteria. The DNA of Chromosome consists of several replicating units called replicons. Endonuclease makes a cut or nick thus identifying the origin of replication. The molecule is unwind by unwinding proteins called Helicase. Helicase II & III get attached to lagging strand and rep protein to the leading strand. The formation of bands or kinks is avoided by single stranded DNA binding protein (SSB). The DNA molecule replicates in pieces called okajaki pieces. While one strand replicates continuously in 5' →3' direction (leading strand), others replicates discontinuously in 3'→5' direction. (Lagging Strand).

The short & long strands are joined by DNA ligase. Okajaki fragments are joined by Polyneucleotide ligase.

The DNA Synthesis requires a short sequence of RNA primer which is synthesized by RNA polymerase called primase. The circular DNA of *E. coli* replicates by a bubble at the origin. The topoisomerase act and 'θ' shaped intermediate is formed. Replication may be unidirectional but generally it is bidirectional. Thus, Polymerase, Helicase are involved in replication of DNA in *E.coli.* The DNA polymerase occurs in *E-coli* are Polymerase I, II and III. In eukaryotes α, β and γ polymerase exist.

Mechanism of replication in prokaryotes: Following steps are involved

1. DNA strand is cut by endonuclease. DNA Replication starts at site called "O" site. This site is only one in prokaryotes.
2. Two strands of DNA unwind with the help of unwinding enzyme helix destabilizing protein ATP, and DNAgyrase. It results in Y-shaped structure called **replication fork**.
3. Exposed single strand of DNA stabilizes under the influence of SSB protein or helix destabilizing protein. It holds open the two separated strand.
4. Replication begin with the formation of RNA primer with the help of pre priming intermediates, pre priming proteins and primosomes.
5. Leading strand is synthesized as the primary strands elongates under the influence of DNA polymerase-III. DNA replication occurs in 5' →3' direction i.e. addition of nucleotides take place to 3'OH ends. Thus the nucleotide at 3' end of sugar is newly added one.

Chapter 19
Genetic Material and its function

Autocatalytic function of DNA

Here DNA synthesizes another DNA molecule identical to it and this is also known as replication of DNA. Watson and Crick model of DNA suggests that the mode of replication of DNA is semi-conservative which was experimentally proved by Meselson and Stahl in 1958,

However, Delbruck (1956) advocated three types of replication viz. (1) Conservative. (2) dispersive and (3) Semi-conservative. Semi-conservative mode of replication was also demonstrated by J.H. Taylor and P. Woods (1957) in Vicia faba with the help of radioactive tritium i.e. 3H. Hence, semi-conservative mode of replication is Universal. (Fig. 33).

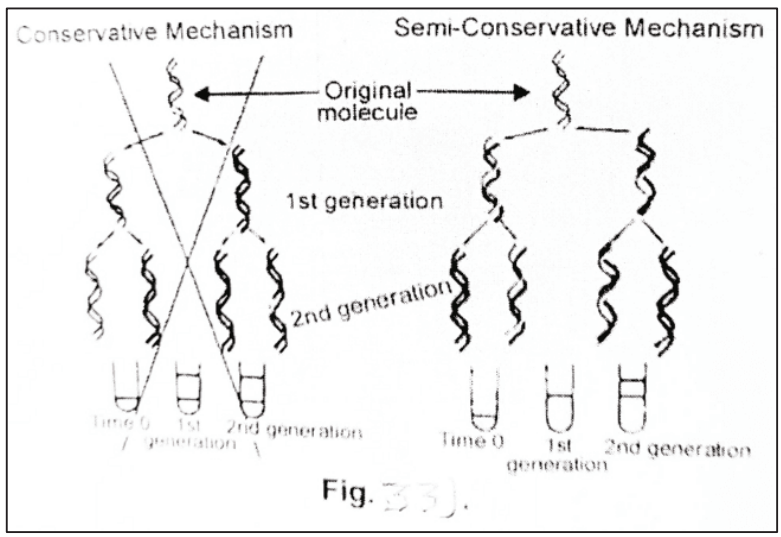


Fig. 33).

(6) RNA primer strand is removed with the help of exonuclease and the gaps due to excision of RNA primer are filled by DNA polymerase 1.

(7) In the process of DNA replication on one template strand of the two parental strand, new strand is synthesized continously known as leading daughter strand.

(8) Synthesis of another new daughter strand on another remaining template strand occurs in the form of short pieces of RNA segment known as okazaki fragments with the help of DNA dependent RNA polymerase. This is known as lagging daughter strand.

(9) Discontinuous pieces or okajaki fragments of lagging strand are joined together with the help of polynucleotide ligase or DNA ligase. (Fig. 34, 35)

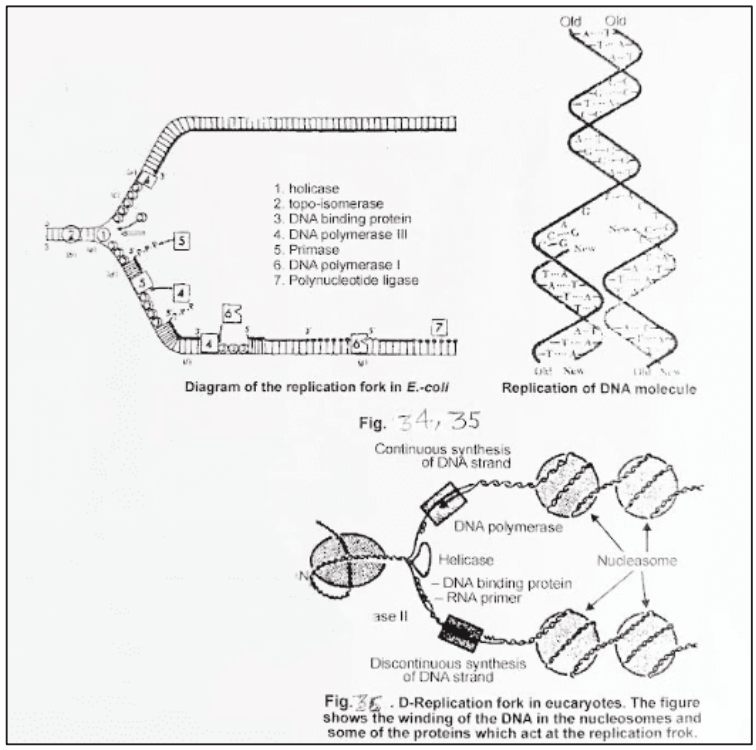


Fig.35 D-Replication fork in eucaryotes. The figure shows the winding of the DNA in the nucleosomes and some of the proteins which act at the replication fork.

In this way two DNA molecules are produced from one molecule. Each of them is made up

of one old and another new strand.

Replication in Eukaryotes: DNA in eukaryotes is in the form of chromatin i.e. combination of DNA and protein. Chromatin is organised in nucleosome. About 200 pairs of nucleotides of DNA are wound around an octamer [(H, A, H, B, H, and H) x 2] of histones (basic proteins). The long chromosomal DNA molecules (10% base pairs) probably replicate from multiple origins of replication. There are numerous origins of replication and DNA synthesis is bidirectional from these origins. The okazaki fragments are smaller than in prokaryotes (about 200 nucleotides).

Filling of gape is done by DNA polymerase a (1) or DNA polymerase ß (II). The fragments are bound to one another by polynucleotide ligase. The replication of continuous strand is carried out by DNA polymerase (III). DNA polymerase is localised in mitochondria and has an exonuclease activity 3'5' (Fig. 36).

Hetrocatalytic function of DNA

Since DNA is genetic material, it carries all the information essential to programme the functions of a cell by controlling the synthesis of proteins or enzymes. Hetro-catalytic function of DNA involves coding and decoding of these information and formation of enzymes (proteins) required. Enzymes are proteinaceous in nature and each enzyme is produced by a single gene as has been suggested by Beadle and Tatum (1948) in their one gene-one enzyme hypothesis. Since some enzymes are made of more than one polypeptide and many proteins are not enzymes, this one gene one enzyme hypothesis has now been modified as one gene one polypeptide.

All the hereditary characters are coded on DNA. So, decoding of these characters governed by genes (discrete units of DNA) is done in two steps viz. (1) transcription and (2) translation resulting in the formation of m-RNA and protein respectively. Thus, when a particular gene controls a function or a reaction its information is copied on to another nucleic acid i.e. messenger RNA (m-RNA) which in turn directs the synthesis of specific protein. There are three major steps in the flow of genetic information contained in DNA. These are:

1. **Replication**: In this process the genetic information present in the DNA is duplicated by a template mechanism.

2. **Transcription**: In this step transfer of genetic information occurs from DNA to m-RNA. This occurs in nucleus.

3. **Translation**: In this step m-RNA is translated into a sequence of amino acids that make the polypeptide chain of amino acids (protein). This process occurs in cytoplasm.

DNA →transcription → →m-RNA →translation →proteins.

This is one way flow of information i.e. from DNA → transcription →m-RNA →translation →proteins.

H. Temin and D. Baltimore (1970) discovered independently reverse transcription in some virus for which they were awarded Nobel prize along with R. Dulbecco in 1975.

Biosynthesis of Protein: (Heterocatalytic function)

Proteins are aggregates of amino acids. Amino acids are joined together with peptide bonds between them. Therefore, proteins are polypeptide chains of amino acids.

Biological protein synthesis takes place on ribosomes present in cytoplasm. Genetic information for specific protein synthesis is coded on m-RNA formed on DNA template in the nucleus. The overall processes of biologically synthesis of proteins is described below.

There are two major steps for protein synthesis under the control of genes.

They are (1) Transcription and (2) Translation.

1. **Transcription:** Synthesis of m-RNA on DNA-template is called transcription. DNA is double stranded structure. Only one of them acts as a template for m-RNA synthesis. From genetic evidence it is concluded that one-gene controls the formation of one polypeptide chain of protein. Hence, one of the two strands of DNA forms m-RNA strand in which informations for protein synthesis transcribed. If both the DNA strands act as template for m-RNA synthesis, then only one m-RNA strand remains functional. Now it is experimentally proved that only one m-RNA is formed during transcription for which only one strand of DNA duplex acts as a template. The stepwise reactions under transcription are as follows:

   Formation of m-RNA on DNA template

   a) RNA polymerase attaches to promotor region (a segment) of DNA. RNA polymerase moves along DNA and DNA strands open by unwinding of complementary strands. The promotor region is AT rich region of about 10 bases, earlier than the starting point of m-RNA synthesis. It has a common sequence of TATAATG known as TATA box. RNA polymerase is made up of 5 types of polypeptide chains, two alpha chains, two beta chains and one sigma chain. Alpha and Beta chains form core enzyme responsible for formation of phosphodiester linkage between nucleotides of

growing m-RNA. The sigma is responsible for recognition of start signal along DNA molecule. After initiation occurs, sigma dissociates from DNA-RNA core complex and become free to attach to another core.

b) Free bases pair with ribonucleotide precursor on one of the DNA strand. RNA-polymerase moves on along DNA template and m-RNA strand formation goes on.

c) Synthesis of m-RNA chain occurs in a fixed direction from 5' to 3' at a speed of 30 to 40 nucleotides per second.

d) Termination of m-RNA chain formation takes place when RNA polymerase reaches the terminator sequence like UAA, UGA or UAG m-RNA chain formation stops by stopping of unwinding of DNA strand in presence of rho factor. This may allow m-RNA to form a hairpin. Terminator region is rich in

GC (in prokaryotes) or AT (in eukaryotes). Complementary m-RNA strand is not bonded to template DNA strand and hence easily separated.

Diagrammatic representation of transcription of an RNA molecule on DNA template is shown in Fig. 37.

Once the function of m-RNA is over, it is degraded by cellular ribonuclease.

2. **Translation:** It is second major step in which coded information for protein synthesis on m-RNA is translated on ribosomes. It has several step wise reactions as follows:

a) **Activation of amino acid**: Amino acid is activated with ATP in presence of amino acyl synthetase to from Amino acyladenylate.

Ligase/amino acyl synthetase

Amino acid + ATP ---------- > Amino acyl adenylate.

b) **Formation of amino acid +RNA complex**:

Now amino acyl adenylate (activated amino acid) attaches itself with t-RNA under the influence of ligase resulting in the formation of Amino acyladenylate t-RNA complex.

c) **Chain initiation**: Chain initiation occurs in the following manner:

(i) 30S subunit of ribosome binds with m-RNA in presence of Initiation Factor 3 to form 30S-m-RNA complex

(ii) Formyl methionine t-RNA complex (initiating amino acid t-RNA complex) attaches to 30S-m-RNA complex in presence of Initiation factor 1 (IF).

(iii) Now 50S subunit of ribosome joins with 30S-m-RNA f-meth-t-RNA complex. Thus ribosome particle of 70S is complicated. Each ribosome particle has two sites :

(iv) 'A' site (Amino acyl site) and (2) 'P' site (Peptidyl site).

a. F-meth-t-RNA complex is at 'P' site of ribosome along its initiating codon AUG or GUG on m-RNA.

(d) **Chain elongation**: It occurs as follows:

(1) Another amino acid t-RNA complex moves to 'A' site with the help of GTP (Guanosine Triphosphate) and Initiation factor TS and Elongation Factor and Tu along n + 1 codon on m-RNA.

(2) Peptide bond is formed in between amino acids in presence of peptidyl transferase bound to 50S subunit of ribosome.

(3) Deacylated t-RNA is ejected form 'P' site with the help of GTP and Release Factor I (RF).

(4) Growing polypeptide chain with amino acid t-RNA complex moves from 'A' to 'P' site in presence of GTP and RF, A the same time m-RNA moves on ribosome to place n+2 codon at 'A' site.

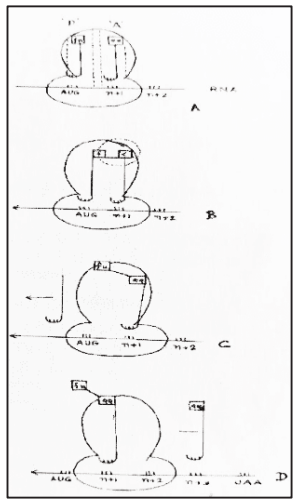


Fig. 37c

The whole process of chain elongation is repeated and polypeptide chain of amino acid are formed.

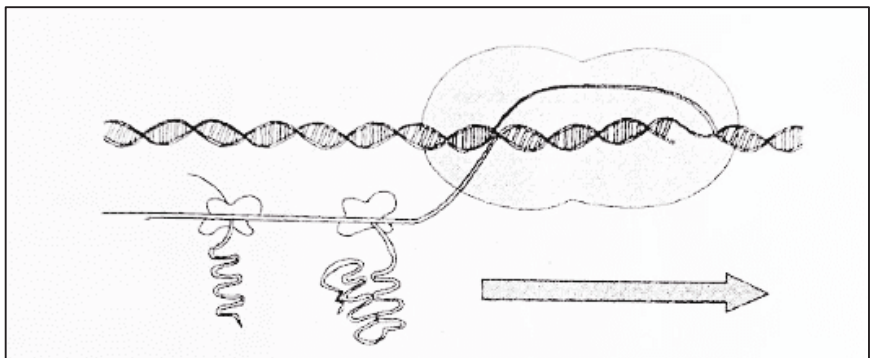


Fig. 38. Showing formation of m RNA

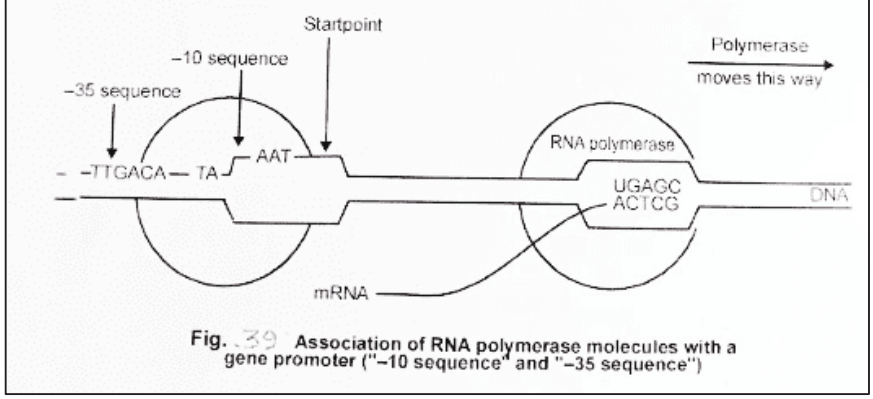


Fig. 39 Association of RNA polymerase molecules with a gene promoter ("–10 sequence" and "–35 sequence")

In *E. coli*, protein of 300-400 amino acids are formed within 10-20 seconds with tertiary structure of protein. mRNA Hairpin (Fig.40)

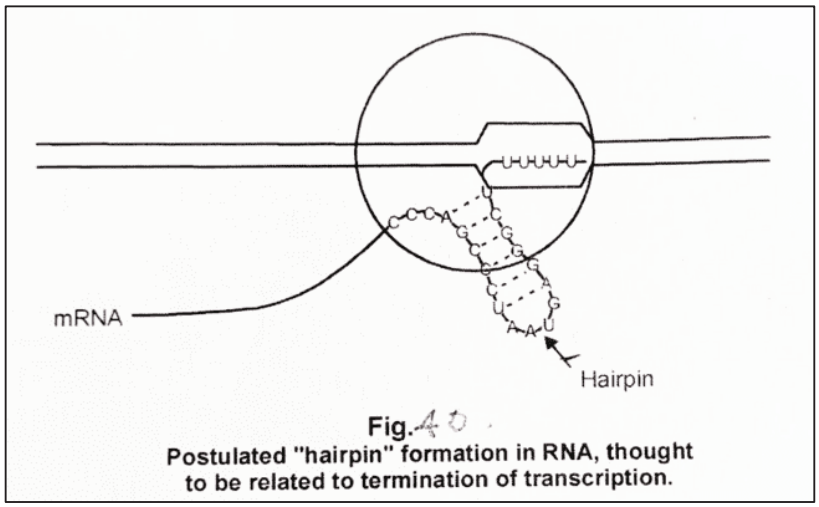


Fig. 40

Postulated "hairpin" formation in RNA, thought to be related to termination of transcription.

Postulated "hairpin" formation in RNA, thought to be related to termination of transcription.

(e) Chain termination: When chain terminating codon UAA or UGA or UAG moves on ribosome, polypeptide chain with t-RNA is detached from ribosome. The release factor breaks t-RNA polypeptide complex releasing t-RNA and, polypeptide chain folds up simultaneously.

One ribosome requires 80 nucleotides of m-RNA. m-RNA simultaneously work on many ribosome. It is also thought that ribosome moves along m-RNA. (Fig.38,39,40).

Control of Transcription

Generally there are two types of control at the transcription initiation level viz. (1) negative control and (2) positive control.

Negative control: Here transcription by polymerase alone is prevented by the presence of repressor (a protein factor).

Positive control: Here transcription cannot take place in presence of RNA polymerase alone, but other protein factors (activator of maltose system or CRP-protein) are required to permit this transcription.

Control of transcription in Lactose operon: Jacob and Monod (1961) proposed a model for the control of the expression of genes in E. Coli. This operon contains three structural genes X, Y and Z and regulator gene for the synthesis of enzymes involved in the metabolism of lactose i.e. B-galactosidase (coded by Z gene), permease (coded by Y gene) and transacetylase (coded by X gene). Following diagram demonstrates the control mechanism enabling the 3 structural genes of lactose operon to be expressed only when required and not the rest of the time.

Fig. 41 shows the lactose operon repressed which exists in absense of lactose. The regulatory gene R controls the synthesis of repressor which has an affinity for O (operator gene). The binding of repressor to the operator prevents the transcription of structural genes (X,Y and Z) by RNA polymerase resulting in the absence of m-RNA, the 3 enzymes are not synthesized.

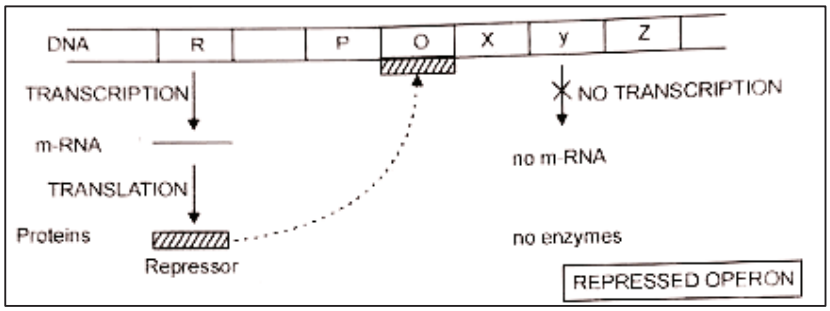
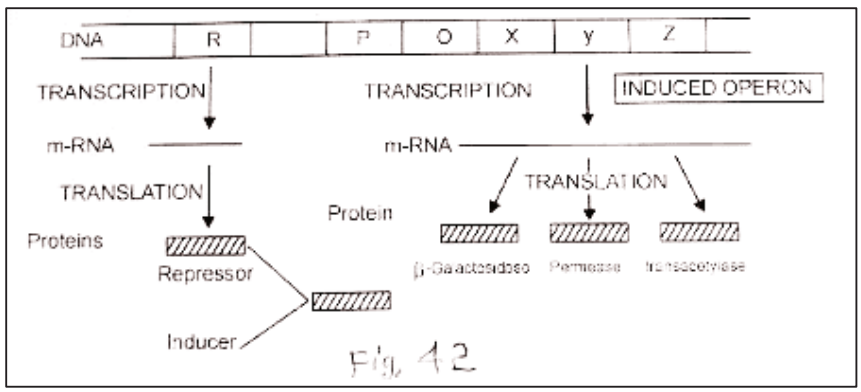


Fig. 41

Fig 42 shows that in presence of inducer (lactose), the repressor forms with the inducer a complex whose structure is modified in such a manner that it can no longer bind to the operator. Genes X, Y and Z can be transcribed into a polycistronic m-RNA which permits the coordinated synthesis of the 3 corresponding enzymes. The operon is induced and the initiation site recongnised by RNA polymerase is (P) promoter site.



Fig, 42

Genetic code

Genetic information contained in the DNA is expressed in the form of proteins. Proteins are made of twenty different types of amino acids. The information regarding number and sequence of these amino acids forming protein is present in DNA and this information is passed on to m-RNA during transcription. 5 Carbon sugar and phosphate of DNA can not form the message for amino acids because sugar and phosphate both are only one type. Thus, only nucleotides are left to form the message for 20 amino acids. The nucleotides are of four types which are less for 20 amino acids. Thus, four bases are alphabets of the language of nucleic acids and 20 amino acids are alphabets of the language of proteins. In order to prepare a dictionary for translating the language of m-RNA into language of protein (one is four alphabet language. Other is 20 alphabets

language) a singlet code will give 4 codons (4') a double code would give 16 codons (42) and a triplet code will give 64 codons (43) in the dictionary. This was reported by Marshal W. Nirenberg, J.H. Matthaei and H.G. Khorana (1965). Nirenberg and Khorana was awarded Nobel prize for this (Fig. 43)

Wobble hypothesis:

Third base in a triplet code is not important as some t-RNA may recognise more than one codon differing at 3rd position. This is called wobbling which allows economy in the number of t-RNA. R.W. Holley (1964) gave the details of t-RNA.

Wobble in the anticodon of t-RNA

Base in Anticodon	Base in Codon
G	U or C
C	G
A	U
U	A or G
I (INSOSINE)	A, U or C

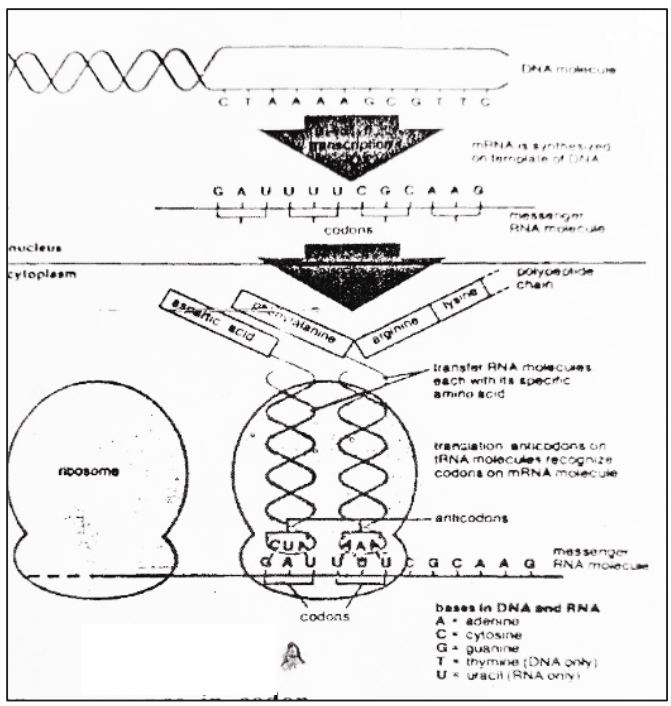


Fig. 43

Properties of genetic code: Following are the important properties of genetic

1) Code is triplet consisting of three nucleotides i.e. AUG UGG etc. for each amino acid.
2) Some codes are particulate e.g. AUG and UGG codes for methionine and tryptophan respectively.
3) Code is degenerate There are 20 amino acids and so 20 codons are required. However, there are 64 codons, hence same amino acid can be coded by more than one codon e.g. UCU, UCC, UCA, UCG, code for serine. This is called degeneracy of code.
4) Some codes are non sense but brings about termination of polypeptide chain and hence act as stop signal e.g. UAA, UAG and UGA known as ochre, amber and opal respectively.
5) Code is non-overlapping.

6) Code is commaless: There is no gap of nucleotide between two consecutive codons.
7) Code is non ambiguous. Each code always recognize the same amino
8) Code is Universal. The same code is applicable to all organisms.
9) Chain initiation and chain terminator codons are definite. The initiation condon for methionine on m-RNA is AUG Initiating methionine is in formylated state but at other position methionine is not formylated. Both these methionines are recognised by different t-RNA.

Theta 'θ' mode of DNA Replication: It is common type of replication that occurs in circular DNA found in E-coli, and other bacteria. It generates a structure which resembles the greek letter 'θ'. The unwinding of the double helix generates a loop, known as replication bubble.

It is unidirectional process. The single stranded replication produces a daughter ssDNA strand which separates the two strands of DNA duplex and allows hybridization with one of them. Creating a D-loop and hence its name of this DNA replication is called 'θ' mode of replication.

Rolling Circle mode of replication is a process of Unidirectional nucleic acid replication that can rapidly Synthesize multiple copies of circular molecules of DNA or RNA, as in plasmids, the circular RNA of viroids.

Abzyme or catalytic antibody: It is also called as monoclonal antibody with catalytic activity. It is found in normal individuals, such as *antivasoactive* intestinal peptide autoantibodies and individuals with autoimmune problem. The first catalytic abzyme was reported in 1986.

Superbug: It is an informal term of a bacterium that has become resistant to antibiotics, e.g. methicilline resistant *Staphylococcus aureus* (MRSA).

Bioremediation of super bug: It can degrade hydrocarbon found in petroleum wastes. It is a multiplasmid strain developed by genetic engineering technique. It was developed by Prof Anand Mohan Chakrabarty in 1971 using Pseudomonas-an oil eating bacteria called Superbug.

DNA marker: It is random amplified Polymorphic DNA (RAPD) It is a type of PCR (Polymerase Chain Reaction) but the segment of DNA that are amplified are random. It is generated using short Synthetic primer (generally of 10 bp) of random segment. They can also be generated by length differences in the amplified Sequence between primer annealing sites. PCR leads to the amplification of a particular segment of DNA. RAPD is suitable for work on nonemous genome where only limited quantity of DNA are available. It is efficient and low expensive.

RFLP: It is restriction fragment length polymerase. It is a molecular method of genetic analysis which allows individuals to be identified based on thinque patterns of restriction enzyme cutting in specific segment.

Restriction enzyme digest the DNA sample producing restriction segment that separated by gel electrophoresis.

Thus, Random Amplified Polymorphic DNA (RAPD) requires a small amount of DNA for the assay While RFLP requires a large amount of DNA. RAPD is rapid process while RLFP is slower process which can only detect 1-3 loci. RAPD is unable to detect allelic variant white RFLP can detect allelic variant.

These two techniques are to detect genetic markers. Steps involved in RAPD:

(1) DNA extraction,

(2) amplification by random primers,

(3) Gel Electrophores and finally visualisation of markers.

Three steps for RLFP:

(1) Restriction digestion of DNA,

(2) Gel electrophoresis,

(3) Southern blotting by specific probes and detection.

SNPs (Single nucleotide Polymorphism). There are most common type of genetic variation among people. Each SNP represents a different, in a single DNA building block called mucleotide. The variations are found in the DNA between genes. SNP in the coding regions are of two types; (1) Synonymous, (2) non-synonymous SNPs.

Synonymous do not affect the protein sequence

Non-Synonymous SNPs change the amino acid sequence in protein. It is used to identify disease causing genes in humans and to understand the individual variation in drug response. These areas of research have major medical benefits. SNP can be detected by RFLP.

Molecular phylogeny: It involves the comparative analysis of mucleotide sequence of genes and the amino acid sequence and structural features of proteins from which evolutionary histories and relationships can be ascertained. This impossible to determine the process by which diversity among species has occured-

Golden Rice: It is genetically modified to produce beta carotene which is not normally present in normal rice. Beta carotene is converted into vitamin A when metabolized by human body. This ice can be used in areas where Vit. A deficiency is common. Thus it can help prevent blindness.

Golden rice is produced through genetic engineering. It was engineered by Ingo Potrycus and Peter Beyer in 1990 to help improve human health.

VAD= (Vitamin A Deficiency). In golden rice has golden colour. It is to produce and accumulate carotene in the endosperm

Flavr Savr Tomato: This tomato was brought to the market in 1994. Grown in Mexico. It is sweeter, juicier and flavour full. It is beneficial to the farmers and not consumer. It is the first

genetically engineered vegetable. Scientists turn off the Pesky Little gene that makes tomate soft and could stay fresh & firm over a month.

Moon Dust Carnations: They are first genetically modified flowers sold in the world. Traditionally breeding techniques cannot produce flowers with colours. Pigments imparts colent to flower and fruits.

Dianthus carnation is first genetically improved flower in 1996. Florigen Company (1996) used genetic engineering to extract certain genes for *Petunia* and Snapdragon flowers to produce blue mauve.

Chapter 20
Genetic Engineering and Recombinant DNA Technology

The technology which involves the breakage of a DNA molecule at two desired places to isolate

a specific DNA segment for its insertion in another DNA molecule, the product of this technology is known as Recombinant DNA (r DNA). With the use of recombinant DNA technology' one can isolate and clone single copy of a DNA segment, or a gene into an indefinite number of identical copies. This technique to obtain multiple copies of inserted segment of DNA or gene in the viral or bacterial chromosome is called gene cloning. Now-a-days- polymerase Chain Reaction (PCR) is being used to obtain millions of copies of a DNA segment of desired length (gene).

Need to clone a gene / segment of a DNA: It (cloning) is a very powerful tool in the hands

of molecular biologists for various applications as such:

1. To understand the structure, function and relationship of genes.

2. To produce biomolecules by a cell of one's choice or by an organism which is not

produced by them in normal condition.

3. To cure a genetic disease.

4. Diagnostic probes of disease based on DNA is possible.

5. Early detection of disease like cancer and so on.

Tools and techniques: There are several enzymes used for gene cloning as given below:

1. Restriction endonuclease: It digest double stranded DNA molecule at specific sites. It is of 3 types viz, type 1, type 2 and type 3. e.g., Eco RI is the first enzyme isolated from R strain of *E. coli*.
2. DNA polymerase: It is used to synthesize a small strech of DNA molecule. It requires a template which is DNA molecule except for RNA dependent DNA polymerase which uses RNA as its template. DNA polymerase can add more nucleotides to a pre-existing DNA that is primer. The primer may be either DNA or RNA molecule. It also requires Mg^{++} for its action as is required by restriction endonuclease.
3. Enzymes like DNA ligase, alkaline phosphatase, polynucleotide kinase, Dnase, Rnase, nuclease methylases, RNA polymerase etc are required.
4. The first step in gene cloning is to collect all the genes of a genome so that these can easily be studied. This is done by making a gene library. Two types of genes are used in recombinant DNA technology: (1) Natural genes and complementary DNA = c DNA. c- DNA is obtained with the help of reverse transcriptase. It is DNA copy of m RNA (messenger RNA) having entire coding system lacking introns.

5. Cloning vectors: Genomic DNA or c DNA are inserted in a specific vehicle which allows the transfer of foreign DNA segment or gene to desired place of host. Those vehicles are cloning vectors. Three different types of cloning vectors are used such as: (1) Bacteriophages (2) Cosmids and (3) Plasmids.

Cosmids are made as a hybrid molecule between a plasmid and a phage (from cos sites of lamda phage pBR322 and a mid from plasmid).

Techniques used to obtain Recombinant DNA (r DNA): Extracted DNA is digested with

restriction endonuclease resulting in segments of DNA. Segments of various sizes are separated through polyacrylamide gel electrophoresis. (PAGE). DNA fragments of different sizes are seen on the gel which is isolated for further study. Pulsed field Gel Electrophoresis (PFGE) is a technique for separation of chromosomes or large segments of DNA. In this technique agarose plugs are used in which DNA is embeded to prevent fragmentation of large DNA molecules like that of chromosomes.

Transfer of separated bands on gel to a sheet of nitro cellulose membrance is done by (1) southern blotting (on the name of E.M. southern), (2) Northern bloting and (3) Western blotting technique. In southern blotting tehcnique m RNA bands from the gel are transferred in place of DNA bands. Western blotting technique is used for protein (a translated product)- The proteins are separated by PAGE, then transferred on to nitrocellulose membrane.

Recombinant DNA has made to achieve expression of foreign DNA into microbial host with

the help of (i) Messenger DNA besides vector and enzymes.

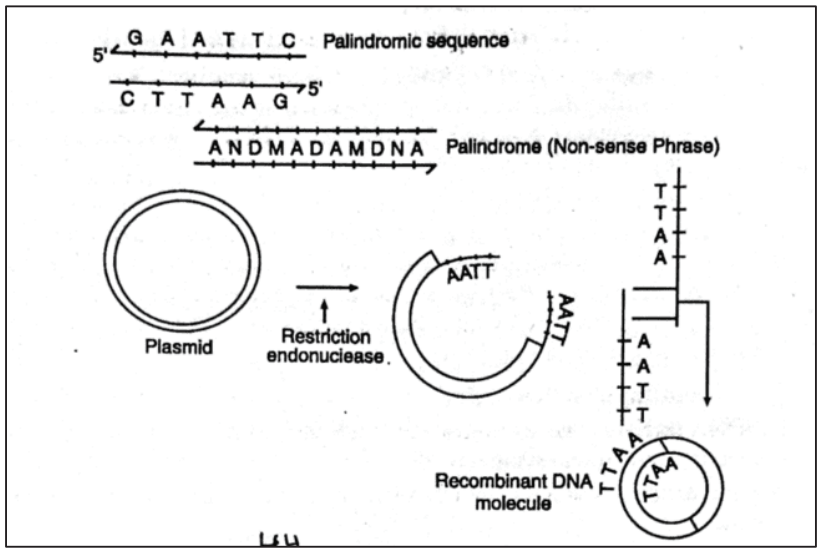
Messenger DNA: It is the DNA which is transferred from one organism into another by combining it with vehicle/vector DNA. Messenger DNA are of three types namely- (a) Complementary DNA (c-DNA), (b) Synthetic, s-DNA and (c) Recombinant DNA (r-DNA), c-DNA is obtained on RNA template with the help of reverse transcriptase and nucleotides. The DNA strand is isolated from RNA-DNA hybrid by using alkaline phosphatase enzymes-

DNA strand is then synthesized on isolated single stranded DNA template using DNA polymerase. This c-DNA can be joined with vector DNA for incorporation into a host cell. Synthetic DNA polymerase was synthesized from free deoxyribonucleotides without a template using DNA polymerase. It was synthesized by Kornberg (1961) and H.Khorana (1965 and 1979). Genes for insuline and somatotrophin have also been synthesized (Fig.44)

Schedule for the synthesis of r-DNA: It involves integration of messenger DNA to vector

DNA. These DNAs are taken out from the cells by using lysosome. The DNA is isolated from other cell contents by ultracentrifugation and then it is purified. Both the DNAs are cleaved with the help of the same restriction endonuclease so that they have complementary sticky strands. Then they are mixed under suitable laboratory conditions. Their ends are sealed with ligase to obtain r-DNA (Fig. 45)

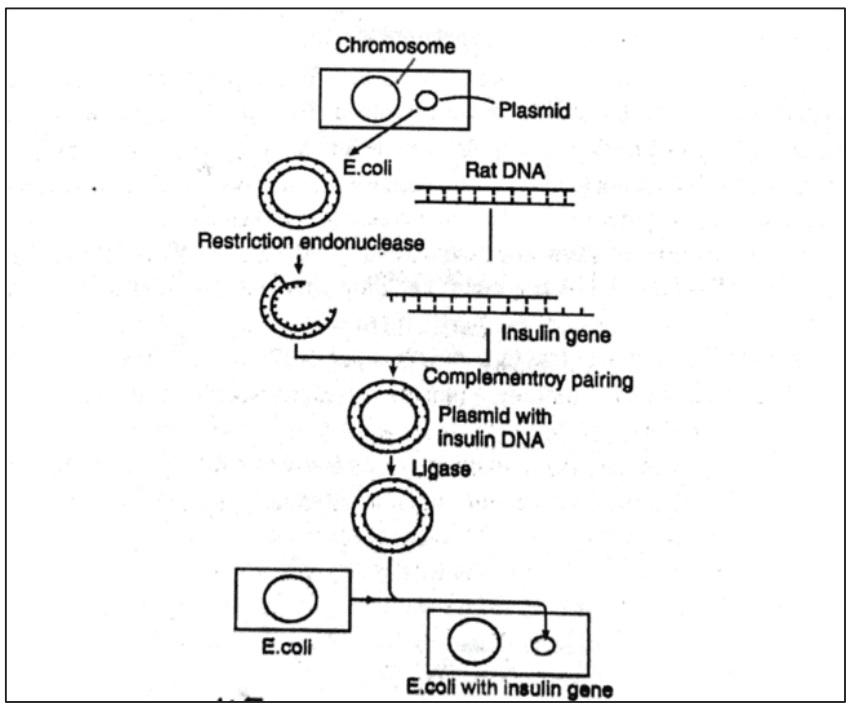
Recombinant DNA is then introduced into a host cell like *E.coli* and other bacteria having no plasmids. The technique employed for this be transformation or vehicle less transduction. Vectorless or vehicle less gene transfer can be done with electroporation or with the use of chemicals like polyethylene glycol. In electroporation, temporary pores are created in the plasma membrane of host



44 Fig. Showing synthesis of r-DNA

cell from where foreign DNA makes entry. Besides Tungsten particles coated with foreign DNA are bombarded into the host cell. Foreign genes are also directly introduced with a micro injection needle.

Before injecting, the host cell is immobilized by applying a mild suction (Fig. 36.1)



Recombinant DNA (rDNA) and its Uses

It is first obtained by Cohen et al (1970) in *E.coli* strain in two steps viz. (1) Cutting of DNA agments and (2) inserting the foreign DNA into a vector at restriction endonuclease site leading to the formation of Recombinant DNA. The Recombinant DNA (rDNA) are introduced. into a host to replicate-This process is gene/DNA cloning.

Desired DNA fragments are obtained with the help of endonuclease enzyme. Hamilton QSmith (1970) isolated this enzyme from Hemophilus influenzae bacterium which would cut both the strands of DNA. This endonculease enzyme is known as Hind II endonculease. For this discovery HO. Smith got nobel prize in 1978. Restriction endonuclease enzyme obtained from E.coli (RI) cuts DNA to produce single stranded DNA with cohesive/sticky ends. The cut ends have nucleotides in Palindromic sequence as shown in Fig. 35. 1.

Useful tips for recombinant DNA techniques

1. In r DNA technique genes from a donor cell can be implanted into a bacterium for DNA replication and protein synthesis.
2. A gene carried by recombinant DNA is cloned when its host bacterium divides by binary fission.
3. A restriction enzyme breaks bonds between sugar and phosphate components of a nucleic acid molecule.
4. Restriction enzymes are synthesized by bacteria only.
5. The natural function of a restriction enzyme is to cut up foreign DNA.
6. A bacterium adds methyl group to its DNA, by a process known as modification, in order to protect its DNA form its own restriction enzymes.
7. An advantage of using yeast rather than bacteria as recipient cells for recombination of eukaryotic DNA is that yeast can excise introns from the RNA transcript,
8. The sticky ends of a fragmented DNA molecule are made of unpaired bases
9. A fragment of DNA cut by a restriction enzyme, forms bonds with other DNA molecule that have been fragmented by the same restriction enzyme.
10. The formation of covalent bonds between deoxyribose of one DNA fragment and phosphate of another DNA fragment (i.e., phosphodiester bonds) is facilitated by DNA ligase.
11. Each restriction enzyme cleaves a molecule at a particular nucleotide sequence.
12. In recombinant DNA technology, a plasmid vector must be cleaned by the same enzyme that cleaves the donor gene.
13. Recombinant DNA is a DNA molecule carrying a new combination of genes.
14. The two main vectors used in genetic engineering to carry genes into bacteria are plasmids & bacteriophage.
15. Recombinant DNA research uses mostly prokaryotic bacteria and eukaryotic yeast as recipient cells because they reproduce rapidly under laboratory condition.

Some beneficial achievements are listed below obtained by rDNA technology:

1. DNA sequencing, discovery of somatostatin and split gene became possible by rDNA technology.
2. Commercial production of genetically engineered human insulin from *E.coli*.
3. Viral antigen for foot and mounth disease was cloned
4. Insertion of cloned genes into plants to make resistance to pathogens and herbicides.
5. Production of transgenic pigs, goats, Dolly from non-reproductive cells of an adult animal
6. Human monoclonal antibodies produced in genetically engineered mice.
7. Development of engineered Ti- plasmid to transform crop for desired characters.

Introduction of Purified DNA into Living Cell

This has been achieved by electroporation In this method, cells are exposed to high voltage electric pulse to make reversible permeability in the cell membrane. An artificial lipid vesicles-Liposomes are used to transfer DNA into cells. Tobacco protoplast have been delivered by electroporation.

DNA can be delivered by micro- injection. Another technique involves preparation of metallic pellets coated with DNA using 0.22 calibre gun (microprojectiles), In this method DNA is directly delivered into cells. Bacterial and viral genes were directly introduced into onion plants and the plant started producing proteins under the control of delivered genes.

(Fig 46

There are seven major steps in plasmid mediated genetic transfer.

Step 1. Bacteria are placed in a test tube containing a detergent. It dissolves the outer membrane of the cell, thus spilling out the DNA strands.

Step 2. The plasmids are separated from chromosomal DNA in a centrifuge and are put in a

solution with particular restriction endonuclease. The restriction enzyme opens up the circular plasmid DNA into a linear strand with sticky ends.

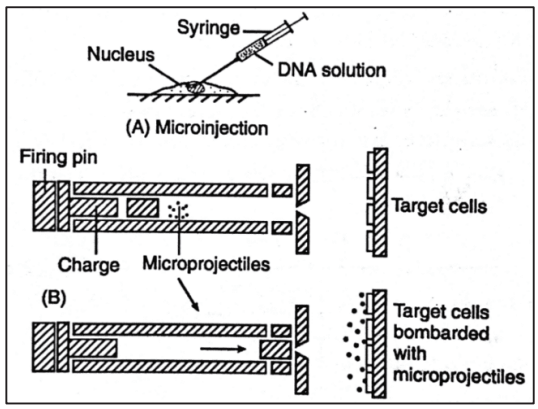


Fig. (46). Two methods of physically introducing DNA into host cell

Step 3. In the mean while the desirable foreign DNA segment (from plants, animals or; other

bacteria) is isolated or synthesized and treated with the same restriction endonuclease to generate sticky ends.

Steps 4. Linear plasmid DNA is mixed up with the desirable DNA in presence of another enzyme ligase. The plasmid and the foreign DNA join together and thus recombinant DNA is obtained.

Step 5. The recombinant DNA is placed in a solution of cold calcium chloride containing normal

E.coli cells.

Step 6. The solution is suddenly heated, making the bacterial cells permeable to the entry of hybrid plasmids.

Step7. The new plasmid duplicataes inside the host cell and treats a new form of life inspired by the characters of the host as well as from an entirely different organism.

Chemical Synthesis of Gene

Before starting this type of gene synthesis, one should know the structure of gene. The structure of gene could be inferred only from its products. For example if a gene is responsible for giving rise a polypeptide chain of amino acid and the structure of this chain is known, then from the genetic code dictionary, structure of the gene could be easily inferred.

Gene synthesis machines: Several versions of gene machines are now available. These machines, under the control of microprocessor, synthesize specific short sequence of single stranded DNA automatically. The desired sequence is entered on a key board and the microprocessor automatically opens the valaves of the

container of sucessive nucleotides, reagents and solvent needed at each step, into a synthesizer column which is packed with tiny silica beads. These beads provide support on which DNA molecules are assembled.

Synthesis of gene from m-RNA: After the discovery of "RNA directed DNA polymerase"

enzyme by Temin & Baltimore in 1970, it becomes possible to synthesize complementary DNA (=c DNA) using m-RNA as template. By copying eukaryotic purified m-RNA several genes were synthesized artificially (Fig. 47) But this type of gene lacks intron sequences found in eukaryotic split gene (exon intron).

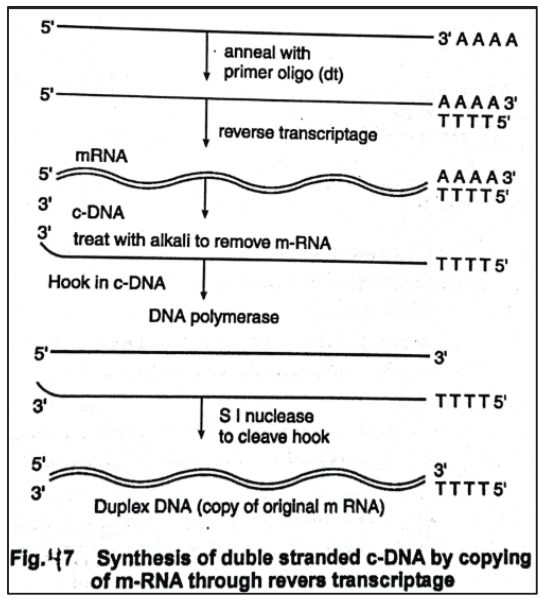


Fig. 47 Synthesis of double stranded c-DNA by copying of m-RNA through reverse

Gene Synthesizing Machines

Gene synthesizing Machine: Synthesis of gene (DNA molecule of known base sequence)

is now possible rapidly with the help of polymerase chain reaction (PCR).

The machine for synthesis of gene become possible due to innovation and development of silica based support which are insoluble and provide support for solid phase synthesis of DNA chains and development of stable deoxyribonucleoside phosphoramidites as synthans, which are stable towards hydrolysis and oxidation. an ideal for synthesis of DNA.

Gene machines are under the control of microprocessor and synthesize specific sequence of

single stranded DNA automatically. The desired sequence of nucleotides are entered on a key board and the microprocessor automatically opens the valves of the containers of successive nucleotides, reagents and solvents required at each step, into a synthesizer column which is packed with tiny silica beads. These beads give support on which DNA molecules are assembled. The gene synthesizing machine and its working has been shown in Fig. 48

Isolation of DNA from Plant cells

Isolation of DNA from plant cells is difficult due to its rigid cell wall, pressence of vacuoles

which releases some organic acids in presence of nuclease. The procedure adopted by Muthu krishnan in 1990 is summarised below.

Plant leaves are grinded. The ground leaves are added to a solution of EDTA and proteinase K.

Then the solution is agitated for three hours at 50°C. A mixture of chloroform and isoamyl alcohol in the ratio of 24: 1 is added and again the flask in agitated at 150 rpm for 5 minutes. The sample thus obtained is spinned for 10 minutes at 7000 rpm at 10°C. Top layer is transferred to a dialysis tube having dialysis buffer (mixture of 1M Tris, 0.5M EDTA, 2M NaCl in distilled water) over night in a cold room with slow stirring. Dialysed samples are then transferred to a siliconised cylinder to measure the volume and then to a siliconised erlenmeyer flask. Cesium gradient is established for centrifugation. 27gm of CsCl (cesium chlride) and 1.2ml of ethidium bromide (10mg/ml) is added to every 30ml of sample. Flasks are wrapped with almumium foil to prevent light from nicking the DNA. Now the sample is divided into two 40ml Beckmann quick seal tubes. These are loaded with VTi-50 rotor. It is ultracentrifused at 60,000 rpm at 20°C for eight hours. DNA bands are observed is ultraviolet light and it is collected. One mg of DNA of high molecular weight can be obtained by this process. The extracted DNA fragment varies from 45-60 kilobase pairs.t

Cloning of DNA

It was developed by Cohen et al (1970) in E.coli strain in two steps namely (1) Cutting of DNA into fragments and (2) inserting the foreign DNA into a vector at restriction endonuclease site leading to the formation of recombinant DNA. The recombinant DNA are introduced into a host to replicate.

This process is gene/DNA cloning. Desired DNA fragments are obtained with the help of endonuclease enzyme. Hamilton O. Smith (1970) isolated this enzyme from Hemophilus influenzae bacterium which would cut both the strands of DNA. The endonuclease enzyme is known as Hind II endonuclease. For this discovery. H.O. Smith got Nobel prize in 1978. Restriction endonuclease enzyme obtained from *E.coli* RI, cuts DNA to produce single stranded DNA with cohesive/sticky ends. The cut ends have nucleotides in palindromic sequence.

Bacteriophage is E. coli phage. This phage was used in cloning purposes. It has double stranded DNA with about 50 genes. The DNA of bacteriophage has two short complementary sequence of 12 nucleotides at two ends. known as cos-cohesive ends. The sequence of nucleotides are 5-GGGCGGGCGACT-3.

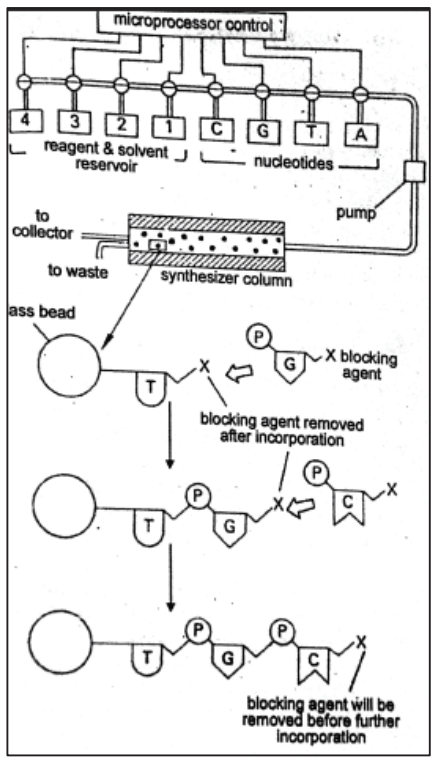


Fig. 48 An outline of a gene synthesis machine

Cloning vectors for plants: T_1 plasmid of *Agrobacterium tumefacies* and R_1 plasmid of *Agrobacterium rhizogenes* are used as vector to carry genes resistance to herbicides and antibiotics.

Types of DNA as Genes carrier or cloning vector: Following types of DNA are used as vector or vehicle namely, (a) plasmid DNA (b) bacteriophage DNA, (c) DNA of plant or animal virus, (d) jumping genes (transposones or nomadic genes and (c) artificial gene, plasmids and bacteriophage

DNA Hybridization Technique

If two different DNA pieces are mixed together and hydrogen bends are broken on heating, the strands of both the types of DNA will separate out and upon lowering the temperature, hydrogen bonds are formed again. Some of the resultant double stranded DNA will be hybrids (composed of one strand of one type and other complementary strand of another type. This is DNA hybridization and this concept has been exploited for utilizing the DNA molecules as probes.

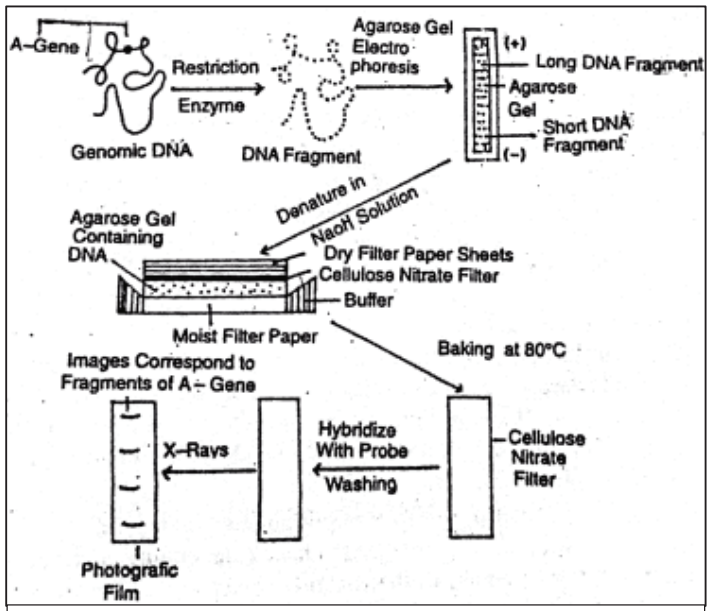


Fig. 49 The Southern blotting technique

Edwards M. Southern of Edinburgh gave a procedure for detecting specific DNA fragments so that a particular gene could be isolated from a complex DNA mixture known as "Blot Hybridization Technique". This technique is for transferring and hybridizing a specific sequence of nucleic acids that have been separated by a gel electrophoresis. The original report dealing with transfer of DNA pieces was authored by Edward M. Southern, this technique is known as "Southern blot". Since DNA transfer was a southern blot, it looked natural to give the nick name "Northern blot" to the transfer of RNA. Transfer and detection of proteins separated by electrophoresis are often called "western blots". Following is the method of southern blot technique.

It starts with digestion of DNA population with the help of one or more restriction enzymes.

This yield fragments of unequal length of DNA. It is passed through agarose gel electrophoresis leading to separation of DNA/molecules according to their respective size. Short DNA fragments are at the bottom (-) and large DNA segments are at the top (+) of the electrophoresis as shown in Fig. 49. The DNA fragments in gel are denatured by NaOH (alkali) solution. Thereafter gel is kept on top of buffer saturated fitter paper. Upper surface of gel is covered with ribocellulose filter, then cover with dry filter paper. The dry filter paper draws the buffer through gel. Buffer contains single stranded DNA. Nitrocellulose filter paper binds DNA fragments firmly when comes in contact of it.

After baking at 65°C-80°C for about 2 hours DNA fragments are permanently fixed to the ribocellulose filter. Now the filter is incubated with a solution (0.1 ml per square cm of filter) containing radio labelled RNA of denatured prob of known sequence for about 20 minutes. These are complementary in sequence to the blot transferred DNA. The radio labelled nucleic acid prob hybridizes the complementary DNA on nitrocellulose. The filter is thoroughly washed to remove the prob. The hybridized regions are detected auto radiographically by placing the nitrocellulose filter in contact with photographic film. The image of hybridized DNA molecules are seen. Thus, the sequence of DNA is recognised following the sequence of nucleic acid prob.

Chapter 21
DNA sequencing, DNA Finger Printing, Blotting technique

Sequencing of DNA is to determine the nucleotide sequence (base sequence) of a length of DNA. Sanger et. al. (1977) determined the nucleotide sequence of entire DNA length φ * 174 virus consisting of 5387 nucleotides. The DNA of lambda (y) phage has also been sequenced which contains 48502 nucleotide pairs by the same author in 1982. Now the nucleotide sequence of entire eukaryotic chromosomes of human being has been sequenced and stored in computer data banks for future use and reference.

There are two principal methods of DNA sequencing devised by Sanger et. at. (1977) called chain termination method and another by Maxam and Gilbert (1977) Known as chemical method.

Chain termination method requires:

(1) single stranded DNA of the sequence to be determined, which was obtained by cloning M_1, bacteriophage. This M_13 bacteriophage is E. coli bacteriophage having single stranded DNA. This single stranded DNA is used as templete. The nucleotide sequence of this template strand is 3'-TGATTACG AA-5'.

(2) A primer strand with a free 3' hydroxyl end i.e. 5' OH',

(3) Four DNA precursors i.e. dGTP, dATP, dCTP and dTTP, out of which one or more labelled with radio active isotope, i.e. 23p dCTP and (4) DNA polymerase I lacking endonuclease activity having only 3-5' exonuclease activity. This is large half of E.coli DNA polymerase I known as "Klenow fragment".

With the help of above noted four requirements, four reactions are carried out in parallel. Each of the four reactions contains one of the four 2',3' dideoxyribonucleoside triphosphate (chain terminator)

The four chain terminators are ddGTP, ddATP, ddCTP and ddTTP. The amount of chain terminators are kept low in the ratio of 100: 1 and are adjusted so that they are incorporated randomly into different places during synthesis. Whenever a chain terminator is incorporated into the nascent DNA, the synthesis stops and DNA fragments are released. The size of DNA fragment depends on the point at which the incorporation occured. In this way each reaction mixture produces a population of nascent DNA of different sizes and are released from the template strand on denaturation. They are separated by polyacrylmide gel electrophoresis. The shortest fragment migrates to the greatest distance developing a band nearest the opposite electrode i.e. anode. Each successive band contains chain with one nucleotide longer than the chain in the preceding band on the electrophoregram. By reading the bands, the complete nucleotide sequence of a DNA chain can be determined. The results are visualised by autoradiogrphy.

In reaction 1, ddGTP is added in the four requirements reaction mixture mentioned earlier which generates polynucleotide chain of-ACTAATGdd size depending on the base sequence of the template. In reaction 2, ddATP in added to the reaction mixture which generates polynucleotide chains of-Add, -ACTAdd and -ACTAA dd sizes. In reaction 3, ddCTP is added to the reaction mixture which generates polynucleotide chains of-ACdd and -ACTAATGCd d sizes. In reaction 4,

ddTTP is added to the reactiion mixture which generates polynucleotide chains of-ACTdd, -ACTAAT dd,-ACTAATGCTdd and -ACTAATGCTTdd sizes.

Polyacrylamide gel electrophoresis and autoradiography of the all the products of each reactions are shown in fig. 50.

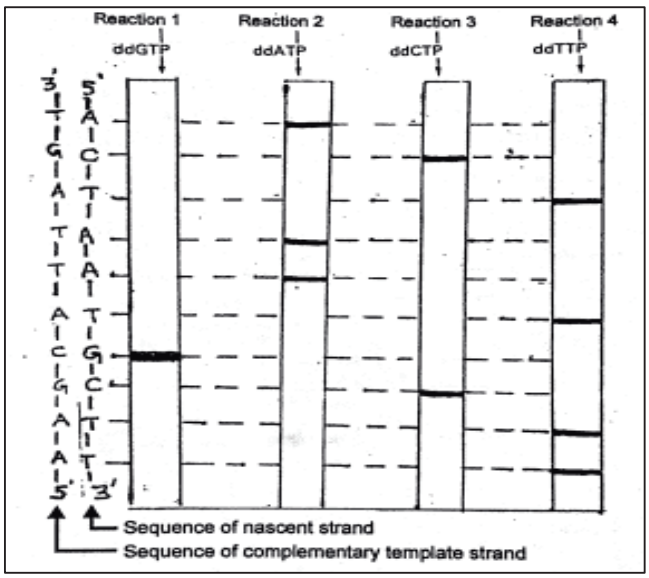


Fig.50 To show the DNA sequencing technique using 2'-3' dideoxy-ribonucleoside triphosphate chain terminators Four reactions are carried out simulataneously, each of them contains one of the four terminators. (dd GTP, dd ATP, dd CTP and dd TTP). All reaction mixtures have (1) template strand, (2) a primer strand with 3'-OH and (3) four DNA precursors (dGTP, dATP, dCTP and dTTP) in which at least one of them is radioactive 32pdCTP and (4) Klenow fragment.

References: Sanger, F.S., Nichlen and A. R. Clouson 1977. DNA sequencing with chain terminating inhibitors. Proc. Nat. Aca. Sci. U.S. 74: 5463-5467.

Maxam, A.M. and W. Gilbert. 1977. A new method for sequencing DNA. Proc. Nat. Aca. Sci. US. 74:560-564.

Chemical method of DNA sequencing: Maxam and Gilbert (1977) devised the sequencing of DNA by chemical method. Here DNA molecule to be sequenced has been isolated by cloning so that many identical copies remain in the solution. A fragment of DNA is labelled with 32p-phosphate at 5' end using T4 polynucleotide kinase and gamma 32p ATP substrate. The DNA is cut with an appropriate restriction enzyme. One of the two labelled pieces is isolated by electrophoresis, meaning there by only one of the two complementary strands has radioactive label with 32p. The sample is divided into four parts. One part of DNA sample is treated with DMS (Dimethly sulphate) and heated. This breaks DNA chains preferable at-G base. The reaction is adjusted so that there are few breaks per chain. For each G in the sequence there are some chains which extend from that base to the labelled end. Another part is treated with DMS and acid. This breaks chain preferably at A base.

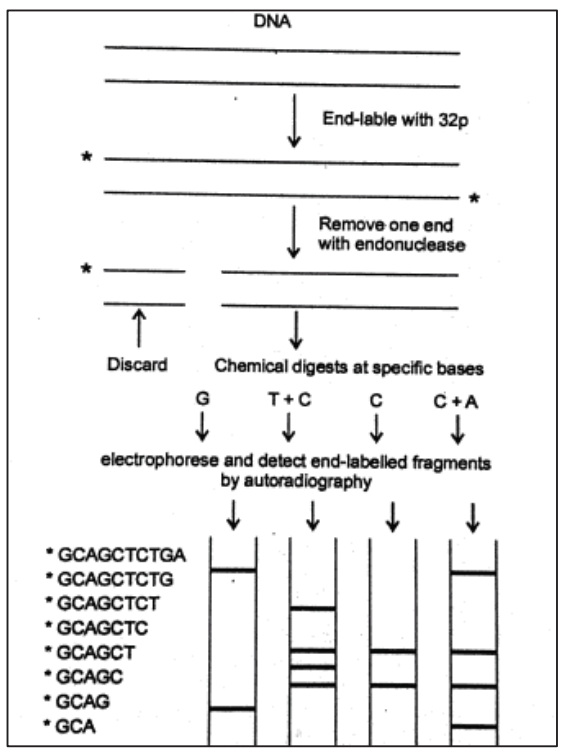


Fig. 51 DNA sequencing by the Maxam-Gilbert, method

Third part is treated with hydrazine in presence of high salt. This breaks chain at C base. Finally, fourth part in treated with hydrazine in presence of low concentration of salt. It breaks chain at both C and T bases.

The chain broken at T bases are those not broken with hydrazine and high salt. These four parts are separated on polyacrylamide gel and autoradiographed. The fragments having labelled terminus are read from the autoradiogram as in the Sangar method described earlier. Fig. 51.

DNA finger printing

Alee. J. Jeffrey discovered the basic technique of genetic finger printing in 1984. Following are the stepwise processes in making DNA finger printing.

1. DNA is recovered from the body cells-may be skin, blood, saliva, Hair follicle etc.
2. DNA is cut at specific site with the help of Eco RI enzyme. This enzyme (restriction endonuclease) will cut DNA on GAATTC sequence of nucleotides in the DNA (Fig. 51). The fragments of DNA are applied to one end of a thin agarose gel and an electric current is passed through the gel. The smaller fragment of DNA moves faster towards bottom of the gel. The larger fragments of DNA travel comparatively shorter distance and remain nearer to the top of the gel in electrophoresis.
3. A thin nylon membrane is spread over its surface and covered by a layer of paper towels (blotting paper). The paper towel draws moisture from the gel. This facilitate the transfer of DNA fragments onto the surface of nylon membrane. This process is called blotting.
4. In order to make DNA bands visible a solution of radioactive probes made from non- coding fragments of DNA (called minisatellites) is passed over the surface of the nylon membranes. If any of the probes have similar composition as that of DNA fragment, it will bind to it.
5. Development of Bands on Film: A photographic film is placed over the membrane, the radioactive labels will expose the film, producing a thick and thin dark bands as shown in Fig. 52 this is DNA finger printing.

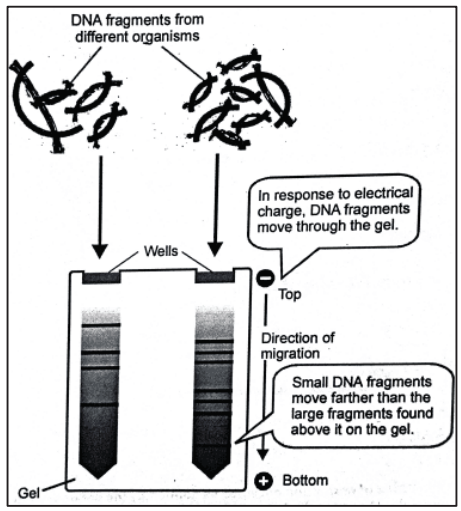


Fig. 52 Making DNA fingerprints

Significance of DNA finger printing:

1. It is useful in determining the nature of crime and criminal by police or detective agents.
2. It is useful technique to establish maternity or paternity of a child.
3. It is used to establish the inherited disease in newborn or pre-natal babies. Genetic counsellors apply DNA finger printing technique to help prospective parents, understand the risk of inherited disorders in affected child.
4. It is also useful is protecting the endangered species through the study of breeding system.
5. The identity of a lost persons, soldiers in war can be established by the use of finger printing technique if their DNA finger prints are stored or collected earlier.

Eco R_1 (enzyme from *Escherichia coli*):

5'...GAATTC...3' -----→ 5'...G AATTC...3'

3'...CTTAAG...5' 3'...CTTA G...5'

Bam H1 (enzyme from *Bacillus amylolique faciens* H)

5'...GGATCC...3'

3'...CCTAGG....5'

Hind III (enzyme from *Haemophilus influenzae*)

5'...AAGCTT...3'

3'...TTCGAA...5'

Pst. I (enzyme from *Providencia stuartii*).

5'...CTGCAG...3'

3'...GACGTC...5'

Hpa 1 (enzyme from *Haemophilus parainfluenzae*)

5'...GTTAAC...3'

3'...CAATTG....5'

Some examples of restriction enzymes as defined by the sequences of DNA that they cut. Dots indicate unspecified bases in the DNA chains. Arrows show the cutting points. Note that each sequence is a palindrome; has a 2-fold rotational axis of symmetry.

“Blotting Technique”: Blotting is a technique by which a micro- molecule like DNA, RNA, Protein is resolved in aget matrix, transferred to a solid support and detected with a specific probe. This technique allow the researcher to identify and characterise specific molecule in a complex mixture of related molecule.

“Southern blotting method” and northern blotting method can be used to identify specific DNA and RNA sequences. The western blotting techniques are used to identify specific protein. Southern blotting technique is used to detect in a given sample.

“Northern blotting techniques”: This technique was developed by James Alwine, David Kemp and George Stank in 1977.

Procedure: Collected sample is homogenized. RNA sequence is separated In electrophoresis unit. Agarose gelin used for nuclelcada separation. Separated RNA sequence is transferred to the nylon membrane. Agarose gel is placed on the bottom of the stack followed by blotting men on the top. A mild weight (glass plate) is placed. The entire setup is kept in a beaker containing transfer buffer.

RNA transferred to the nylon membrane is then fixed using UV radiation. The fixed nylon membrane is fixed with RNA sequences on the blot corresponding to the sequence of interest. The blot membrane is washed to remove unwanted probe. The Labelled probe is detected by

autoradiography showing dark bands in x-ray film.

“Southern blotting”: Southern blotting is based on the principles of separation of DNA fragments by gel electrophoresis and identified by labelled probe hybridization. DNA fragments are separated based on size via electrophores then transferring them to membrane, hybridize with labelled sequence. It was developed by Dr. Edwin Southern developed this technique to identify specific

DNA sequence in 1975. It is based on the following steps

(1) Restriction digest by RE enzyme and amplifying by PCR/Polymerase Chain reaction),

(2) Gel electrophoresis,

(3) Denaturation by transpiring with HCL and NaOH.

(4) Blotting

(5) Baking and blocking with casein in BSA (Bovine Serum Albumin).

(6) hybridization by labelled prob.

(7) visualisation by autoradiogram-

“Western Blotting”: It is often used to separate and identify protein. A mixture of protein is separated based on molecular weight through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein-Five steps are involved in Western blotting procedure and detection assay namely transfer, blocking primary antibody, incubation,

Secondary antibody, incubation and protein detection.

Chapter 22
Gene Transfer in Micro Organisms

Gene is unit of heredity. Physically a gene is that much part of DNA molecule which codes for a meaningful protein molecule. Genetic materials include gene present in DNA molecule. There are several genes in an organism ranging from about 600 to many thousands. Generally, gene transfer involves sexual reproduction. In microorganisms like bacteria gene transfer occurs by the following methods (i) Transmission (Conjugation), (2) Transformation (3) Transduction and F- duction- or F-medicated sexduction.

Transmission

It was reported by Lederberg and Tatum (1946) in Escheritia coli and named it conjugation. Similar phenomenon was also observed by Jacob and Wollman (1961) in which donor bacterium (+ or male) sends out a conjugation tube of cytoplasm origin into the recipient (- or female) bacterium through which genetic material (DNA) passes into recipient bacterium.

Here the hereditary characters enter on direct contact. A male bacterial cell can transfer a part or whole of its chromosome to a female bacterial cell. This method is termed as conjugation which can be interrupted and a correlation is observed between the quantity of DNA having entered the receptor (female) cell and the number of characters transferred. Conjugation causes recombination by sexual union in bacteria. It is one way transfer of genetic material from a male type donor to a female type receptor and the process was known as conjugation.

There is no conjugation between two F strains or between two F strains. The bacteria having fertility factor known as F+ strain and which lacks F factor termed as F strain. The F element contains about 2 per cent of cell's total DNA. It is circular, double stranded DNA. It contains about 15 genes, 8 of which control the formation of conjugation tube extending from the surface of F+ cells.

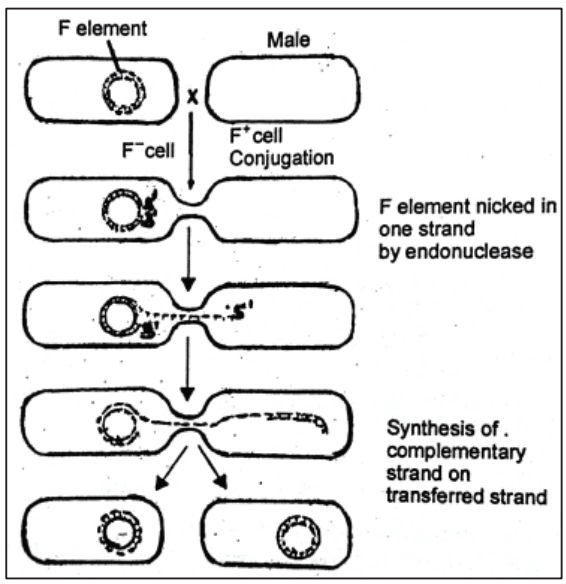


Fig. 53

Transfer of F element from F^+ to F^- cell during conjugation in E.coli has been shown in Fig 53

Process: In a mixture of F^+ and F^- strains a donor cell F^+ establishes contact with a recipient cell (F^-) by F^+ (protoplasmic connection between two cells i.e., tube. Transfer occurs when DNA replicates by rolling circle method.

A nick is produced in one strand of plasmid DNA duplex in presence of endonuclease resulting in free 5' and a 3' end. The strand moves across the tube with 5' end first. into F cell. The second inner strand of plasmid DNA duplex is retained in the F+cell and synthesize its complementary strand. After mating, the two cells separate that are known as exconjugant. Integration of sex factor (F plasmid) takes place at a specific site in a host chromosome

having homologous sequences. Such an integrated plasmid is termed as episome and helps the transfer of the main bacterial chromosome from F^+ to F^- cells during conjugation. This is an event followed by recombination.

Transformation

This phenomenon was discovered by Griffiths (1928) in Diplococcus pneumoniae. In this experiment a suspension containing a mixture of heat killed virulent, encapsulated cells and live, non- virulent, non-encapsulated cell was injected into a mouse. A small number of live bacteria become transformed into the virulent encapsulated type. The transferring ability was inherited by descendants of the newly transformed live strain. It was concluded that when the cells of the virulent strain was killed by heat, their chromosome (genetic material) liberated from heat killed cells can pass through the cell wall of the living cells and become integrated in the host chromosome. The DNA of donor cells is transferred to recipient cells where it undergoes genetic exchange with recipient DNA to produce recombinant progeny.

Success of transformation depends on several factors like (1) size of donor DNA fragments, (2) molecular configuration of donor DNA which must be double stranded. (3) Physiologically competent state of recipient cells, and (4) the amount of DNA added per recipient cell. Competence factor (a protein) is produced by cells in culture. This factor changes the cell surface either by formation of receptor site or increasing permeability to donor molecule. Uptake of DNA: The double stranded donor DNA molecules bind, to the receptor site on the recipient cell surface. The donor DNA is cleaved by endonuclease on the surface of the recipient cell to a size which varies in different bacterial species.

After attachment to the recipient cell wall, the donor DNA is passed inside the cell. One strand of donor DNA is degraded. Immediately there is no transforming activity and so it is eclips period. Now the single stranded fragment pairs with homologous region of recipient DNA. Genetic exchange takes place and a single strand of donor DNA carrying one or more genes becomes integrated in the homologous portion of recipient DNA. The single stranded segment which breaks from the recipient DNA is degraded in the cell and lost. (Fig. 54).

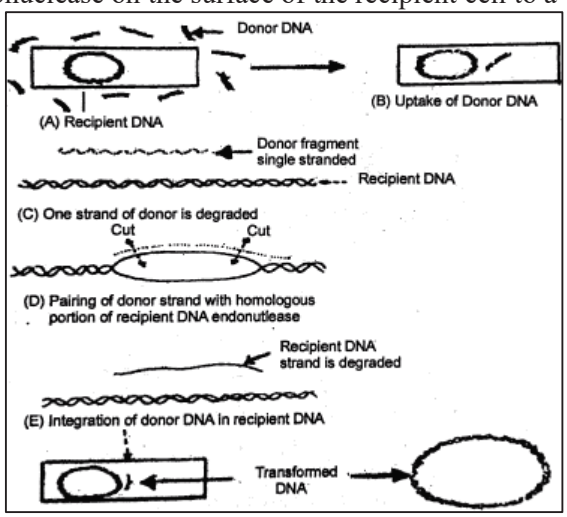


Fig. 54

Transduction

Zinder and Lederberg (1952) have shown that bacteriophage of Salmonella typhimurium could transfer a part of the bacterial chromosome from one bacterial' strain to another. The phages involved in transduction are temperate (which either lyse the cell at once or remain in the host cell without killing it) and the DNA fragment is termed as prophage. They can multiply inside the bacterial cell and finally cause lysis of the host cell. A bacterium which harbours temperate phages (non- replicating phase) is called lysogenic and the phenomenon is called lysogeny.

There are three types of transduction *viz*., (1) Generalized, (2) Specialized and (3) F-mediated.

Generalised Transduction: In generalized transduction a piece of donor bacterial chromosome becomes incorporated into a phase head and is carried over (transduced) to recipient bacterium where it may insert into the recipient genome like a process during transformation. Towards the end of lytic cycle of phage in a donor

host cell, an occasional piece of host DNA, usually of the same length as a phage chromosome, becomes mistakenly packaged in a phage coat and is liberated along with the other phage progeny in the host. The bacterial DNA in this transducing particle can be efficiently injected into a second bacterium (recipient) and it occasionally inserts into the host chromosome recipient chromosome resulting in recombinant bacterium (Fig. 55).

Specialised transduction: Occasionally, the phage does not cause the lyses of bacterium. The nucleic acid of phage codes for repressor protein which prevents the viral gene for the production of materials necessary for replication and subsequently new virus particles. The DNA may remain in the host cytoplasm as prophage either in the form of a plasmid or may attach itself to the bacterial chromosome as F factor. However, at certain point in future the phage may stop coding for repressor protein and the lytic cycle will begin. The viral DNA which was attached to the bacterial chromosome will now break free and directs the production of coat proteins for the new viruses. In the process of detachment, the viral DNA may carry with it a few bacterial genes from the bacterial chromosome.

(c)

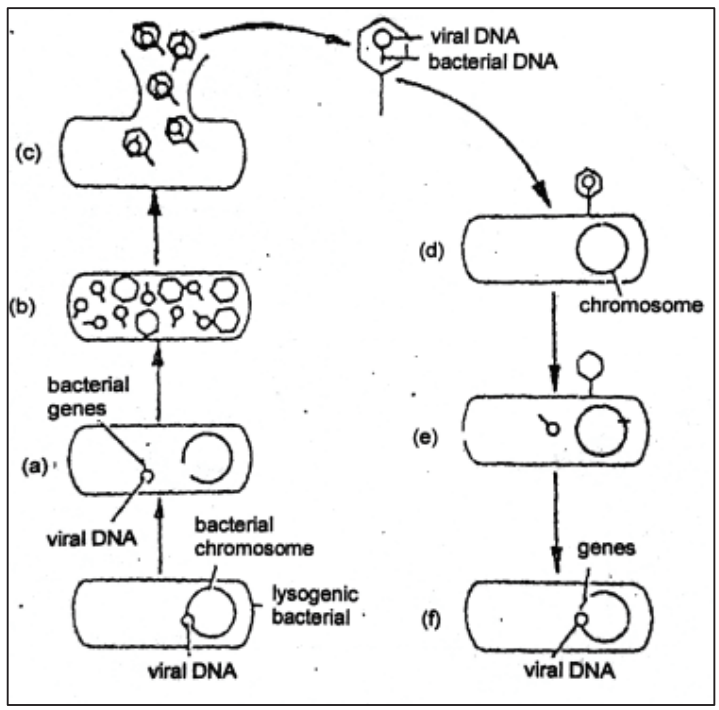


Fig. 55.

The viral DNA then replicated along with the bacterial gene and may become part of the new phage particles. During the next infection, the phage DNA enters the new bacterial cells and inserts onto a new bacterial chromosome and the bacterium becomes transduced and recombinant. Since specific genes are removed from the bacterial chromosome depending on the attachment point of viral DNA, this type of transduction is known as specialized transduction. (Fig.55)

The phenomenon of lysogeny may be observed in Herpes simplex virus which remains for several years as prophage in the cytoplasm of the body cells. It expresses at long intervals.

F-Mediated Sexduction

The F element of E.coli integrates into the main bacterial chromosome. Rarely an integrated F can undergo excision and become detached, carrying with it some bacterial genes which remain

attached to it. Such an F element is known as (F) factor. It behaves like F factor and can be transferred to F carrier bacterial genes. It is able to pair with the corresponding region in the bacterial chromosome. A bacterium receiving an F factor becomes partial diploid for the bacterial genes introduced by the F factor. The bacterial genes in the F factor will not take part in exchange with the recipient's chromosomal genes to produce true recombinants, a phenomenon commonly known as sexduction or F- duction or F-mediated sexduction.

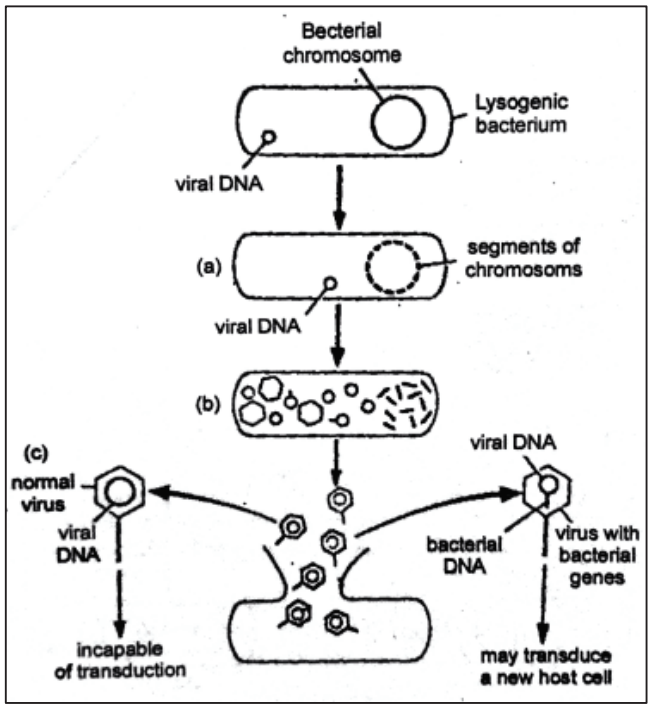


Fig. 56

Nature of Microbial Cell Surface

Microbes include unicellular organisms of both eukaryotes and prokaryotes. Here we consider prokaryotic unicellular microbes in which bacteria are prominent. Bacterial cell surface is featured as such.

A typical bacterial cell has a rigid cell wall, below which there is a delicate inner membrane consists of mycoprotein and outer rigid layer containing muramic acid and glucosamine. The other chemical compound in the cell wall (surface) are polysaccharides, lipids and proteins. The cytoplasmic membrane contains permease enzyme which helps in the movement of specific protein.

The cell wall of gram positive bacteria are 20-80 μm thick and constitutes 85-90% mucopeptides

and teichoic acid where as the cell wall of gram negative bacteria are 10-30μm thick and has 12-20% mucopeptides phospholipids, lipopolysccharides and lipoproteins. It lacks teichoic acids. Some bacterial cell has an envelope outside the cell wall known as “capsule” which is gelatinous, viscous & transparent. It is made up of polysaccharides (Fig. 56).

Structure of Bacterial Cell Wall.

Bacteria are considered to be the plants due to presence of a rigid cell wall. The cell wall is made-up of peptidoglycan, known as mucopeptide or murein. Peptidoglycan has acetyl muramic acid. L-alanine, D-glutamic acid, Diaminopimelic acid and D-alanine.

The cell wall has permeases, which helps in transportation of organic and inorganic minerals from one part to another part. The cell walls of Acetobacter xylimum, A. acetigenum, Zymosarcina venticuli etc. are made-up of cellulose.

Chapter 23
Mutation

The word Mutation was derived from Latin "Mutare" meaning to change (De Varies, 1900).

Types of Mutation: (1) Chromosomal mutation (ploidy) (2) chromosomal aberration. (3) Gene mutation(4) cytoplasmic mutation Gene mutation are of considerable importance in plant breeding for economic benefits of man because they indeed provide the raw material for evolution as well as for selections and recombination. The creation of mutaion at will and their utilization for the production of improved new varieties of crop, animals and microbes are need of the present civilization On the basis of origin mutation are of two types (1) spontaneous and (2) induced Artificially mutations are induced by mutagenic substances or mutagens. Two types of mutagens (1) Radiations (2) Chemicals. Radiations are of two types (1) ionising (2) non-ionising An Atom has Nucleus consist Proton & Electron (-) in the orbit and Nucleus (+) in the centre. Protoni (+) weight is of 1/1800 of electron. Neutron's (-) weight is equivelent to proton's weight. When neutron number is altered, unstable conditons arise in which an atom tends to split or to give off particles or energy there by achieving stability. Such unstable, atoms are known as radio active isotopes or Radioisotopes and definned as atom of same element having different weight due to unbalanced nuclei. In C14 there are 6 protons 8 neutrons and 6 electrons. Therefore atomic weight is 14. The extra 2 neutrons treat a state of instability and heavy C atom tends to give off radiations. The movement of energy which is given off by radioactive isotopes in either particle form or wave form, through a space is considered as radiations. The radiations in the form of high energy atomic particles which can transfer their kinetic energy to any matter through which they pass is known as Particulate or corpuscular radiations.

The radiations in the form of high energy short waves which cause electric and magnetic disturbances affecting the internal structure, of matter is known as ‘electromagnetic radiation’. The treatment of substance with radiation is called irradiation. An ion is an atom which has either a positive or a negative charge and the ionization is the process in which ions are produced.

The particulate radiation can produce ionizaiton when a fast moving charged particle passes through a matter and pulls an electron out of the orbit of an atom. This atom becomes a positiveion. The ejected electrons gets attached to the another atom of the substance and form a negative ion.

The neutrons are with no charge, do not cause expulsions of orbital electrons but they can cause ionization by striking the nucleus of an atom in the substance which result in excitation and emission of the charged particles, the emission in turn produces ionization by affecting orbital electron.

Electromagnetic radiation: The energy absorbed from waves treats the state of instability and this energy in dissipated by throwing off one orbital electrons. This electron produces additionalionization since it is a charged particle moving through the matter. Such a radiation which cause ionization also known as inonizing radiations.

Units of measurement: Units of measurement for ionizing radiations are many such as:

Roentgen 'r'-one roentgen or r is the amount of radiation which produces 2 billion ($2x\ 10^9$) ionpairs per cubic centimetre of air. It is for X rays or gamma-rays.

Roentgen equivalent physical ('rep',) radiation absorbed dose ('rad'), roentgen equivalent man (rem) are the unit of radiation doses. The effect of all radiations is measured by comparison with that of a roentgen of X-rays.

Alpha rays: It is radioactive rays obtained from radon and polonium made up of two protons and two neutrons with positive charge. They are emitted by isotopes and when pass through a matter create strong ionization. They have less penetrating power in living tissue because of being positively charged which are slowed down by negative charged in the water and cause chromosomal aberrations.

Beta rays: It is radioactive rays with negative charge. They are high speed electron emitted from the nucleus of an unstable atom. They have less ionizing power but more penetrating power than alpha rays. Being negatively charged, they are slowed down lay positive charge and lose energy readily. Thus, they are not very penetrating and cause chromosomal aberrations and gene mutations.

P^{32} and S^{35}, H^3, C^{14} are used as its source and are available in the form of chemicals out of which

solution is prepared and desired treatment is given.

X-rays: They are electromagnetic radiation and produced in a broad range of energies. Soft X-rays of longer wavelength (10-angstrom) are less penetrating but more densely ionizing than the shorter wavelength (0.1-0.05 angstrom). Hard rays of average wavelength of X ray from X-rays machine is generally 0.5 angstsrom. X-rays transfer their energy to the atom of tissue through which they pass, which causes an ejection of planetary electron causing ionization and excitation of atoms or molecules and consequently giving rise to chromosomal and gene mutations.

Gamma rays: It is electromagnetic radiation similar to X-rays in their physical characteristic and action on the organism. It is of very short wave length (0.01 angstrom) by virtue of which they are more penetrating. The ionizing effect are due to high electron they eject form the atom of tissue through which they pass. Its source is Co or radium. These radiation are counted and detected by Geiger Muller counters or scintillation counters.

Gamma garden: 1st gamma garden of the world was installed near New York in 1958. In India 1st was at Bose Research Institute Calcutta in 1959 and 2nd at I.A.R.I. New Delhi on 25th August 1960, largest in Asia.

It has an area of 3 acres encircled by a wall 3' thick, 12' high, built with bricks on either side and earth compact in the middle. Outside the brick wall, barbed wire fencing has been erected. The radioactive cobalt source is in the form of small pellets weighting about 6 grams. Those pellets are inside an aluminium capsule and the capsule is welded to the lead container in which the radioactive cobalt is kept. The strength source is 200 curies. The radioactive cobalt source can be raised form the container by lifting the lid of container. The lid travels, through guide rods inside a thin aluminium tube and is lifted electromagnetically by pushing a button in the central panel installed in a room 205 feet away from the ground and as soon as it is taken out of the lead container, the whole garden receives gamma radiation. Plants are grown in concentric circles. Irrigation water is pumped through hydrants fixed at suitable intervals. Two radiation monitors are fixed to the circular wall and the other which is portable and can be moved radially help to measure accurately the radiation doses received by the plants at different distances from the source. Radioisotope Laboratory at I.A.R.I: Work started in July 1968, will help to study how to produce new crop varieties by artificial transmutation of gene.

Neutrons: They are electrically neutral particles.It may be highly penetrating due to lacking of any electric charge and they are not slowed by charged particles of matter and thus tend to move in a straight line until they have a collision. The source of treatment are nuclear reactors and generators available with atomic energy department.

Ultraviolet rays: It is non-ionizing with wave length between 1000 to 4000 angstsrom i.e., long wavelength and therefore, do not penetrate the tissue appreciably but simply cause the excitation of planetary electrons which result in increased chemical reactivity inside the tissue . Thus, their biological effects are only due to excitation and photochemical reaction .Because of very long penetrating power, it is not efficient in producing chromosomal gene mutation. Ultraviolet rays are produced in mercury vapour lamps and tubes and treatment is carried out by exposing to them.

Chemical mutagens: Following are the chemical mutagens in practice.

(1) Mustard compounds: Mustard gas, sulphur mustard, N, mustard. Mustard oil, chloro acetone. Dichloro acetone is weak mutagens.
(2) Alkylating agents: Ethylene oxide, N-Butyl methane sulphonate, cis-1:4-D methane sulphonoxybutan, P-N-di (chloroethyl)-phenyl propionic acid, L.-4-Di-methane sulphonoxy butane, P-N-dichloroethyl phenyl amino butaric acid.
(3) Purins and purine derivatives: decreasing order-Caffeine, Theophyline, Paraxanthine, Thobromine, Tetra methyl uric acid, 8-Methoxy caffeine, Adenine, Nebularine.
(4) Others: Potassium thiocyanate, Ethyl carbonate, Formalin, Phenols Malice hydrazide Manganous chloride, colchicine.

TYPES OF MUTATIONS

Common mutations are classified under three main categories:

1. **Chromosomal Mutation**: It may be:
   a. Intra-chromosomal like deletion, duplication and inversion or
   b. Inter-chromosomal e.g., Translocation. Translocation may be simple or reciprocal. All these cause mutations.
2. **Genomic Mutations**: In this category, whole genome is increased leading to polyploidy. Besides, one or two chromosomes may be added or deleted a phenomenon called Aneuploidy. It may be monosomic (2n-1), trisomic (2n+1) and so on. Aneuploidy and polyploidy cause mutations.
3. **Point Mutations:** It refers to substitution of one nucleotide for another or deletion or addition of one or many bases bring about mutations. It can be shown in the following ways. (Fig.57.)

Spontaneous mutations occur at low rate presumably due to error in replication and transmission of genome.

Mutagens increase mutation rates by stimulating the occurrence of such errors.

Clastogens are sub-class of mutagens that stimulate chromosomes wreckage.

Carcinogens are agents that cause normal cells to become malignant that is they divide uncontrollably and possess the ability to spread and establish cell lines in inappropriate parts of the body called metastasis.

Mechanism of Mutagenesis: Here modes of induction of mutations by chemical and physical mutagens have been shown in Fig. 57. It involves substitution, addition, deletion reversion and suppression of nucleotides during replication of DNA. This results in mismatch pairing.

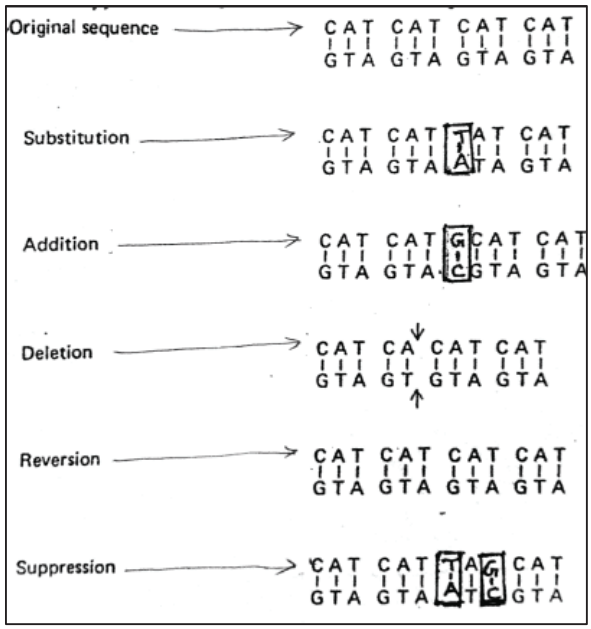


Fig. 57

POINT MUTATIONS AND THEIR CORRECTION

Chemical Mutagens like nitrous acid, ethylnitrosourea and 5-BUdr aminopurine etc induces point mutations. It transforms the chemical structure of nucleotide giving it the base pairing properties of other nucleotides. The resultant miss-pairing will give rise to two types of mutations.

1. Transition- in which purine is replaced by another purine or pyrimidine is replaced by

another pyrimidine. e.g., A->G, GA, T-C, and C→T.

2. Transversion-in which a purine is replaced by Pyrimidine or vice versa. E.g. A->C,

G➞C.

CG can be replaced by GC and vice versa, Similarly AT can be replaced by TA and vice versa. (Fig. 58).

In transition, a particular adenine 'A', on one strand of DNA duplex is transformed into modified adenine 'A* under the influence of mutagen which has base pairing properties of GUANINE. At replication, this modified adenine will miss-pair with cytosine and the complimentary daughter strand will carry a cytosine at this position. After one round of replication, the cytosine will pair with guanine thus the grand daughter strand will carry purine guanine instead of purine adenine. This is an A→G transition.

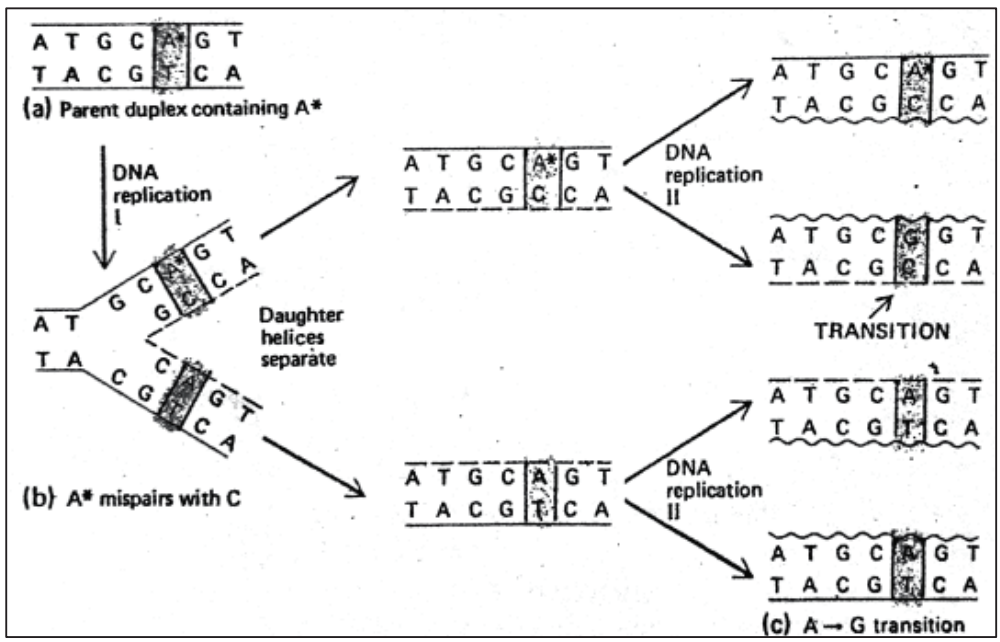


Fig. 58 Transition induced by chemical modification.

The mispairing of a modified adenine (A*) with cytosine produces an AG transition. Each of the four normal bases of DNA can exist in two alternative forms due to single porton shifts called tautomer. Thus - NH_2 form of adenine" -changed to inano (-NH) of Guanine and so on.

In Transversion, nitrous acid deaminates cytosine resulting in uracil which can produce a C→T transition.

Cytosine pairs with Uracil under the influence of HNO2, which has pairing-partner Adenine.

Adenine pairs with Hypoxanthine under the influence of HNO2, which has pairing-partner- Cytosine.

Similarly, Cytosine is converted into Hydroxylimine under the influence of Hydroxylamine which pairs with Adenine to produce a C→T transition.

And Guanine is converted into O Ethylguanine in presence of Ethylmethane sulphonate, which pairs with Thymine to generate G →A transition. (Fig. 58)

Base Analogues have chemical structures analogous to naturally occurring bases with critical modifications. They are mutagenic during replication.

5-bromodeoxyuridine (5- BUdr) is an analogue to thymine and usually pairs with adenine which generates A→G transition. (Fig.59).

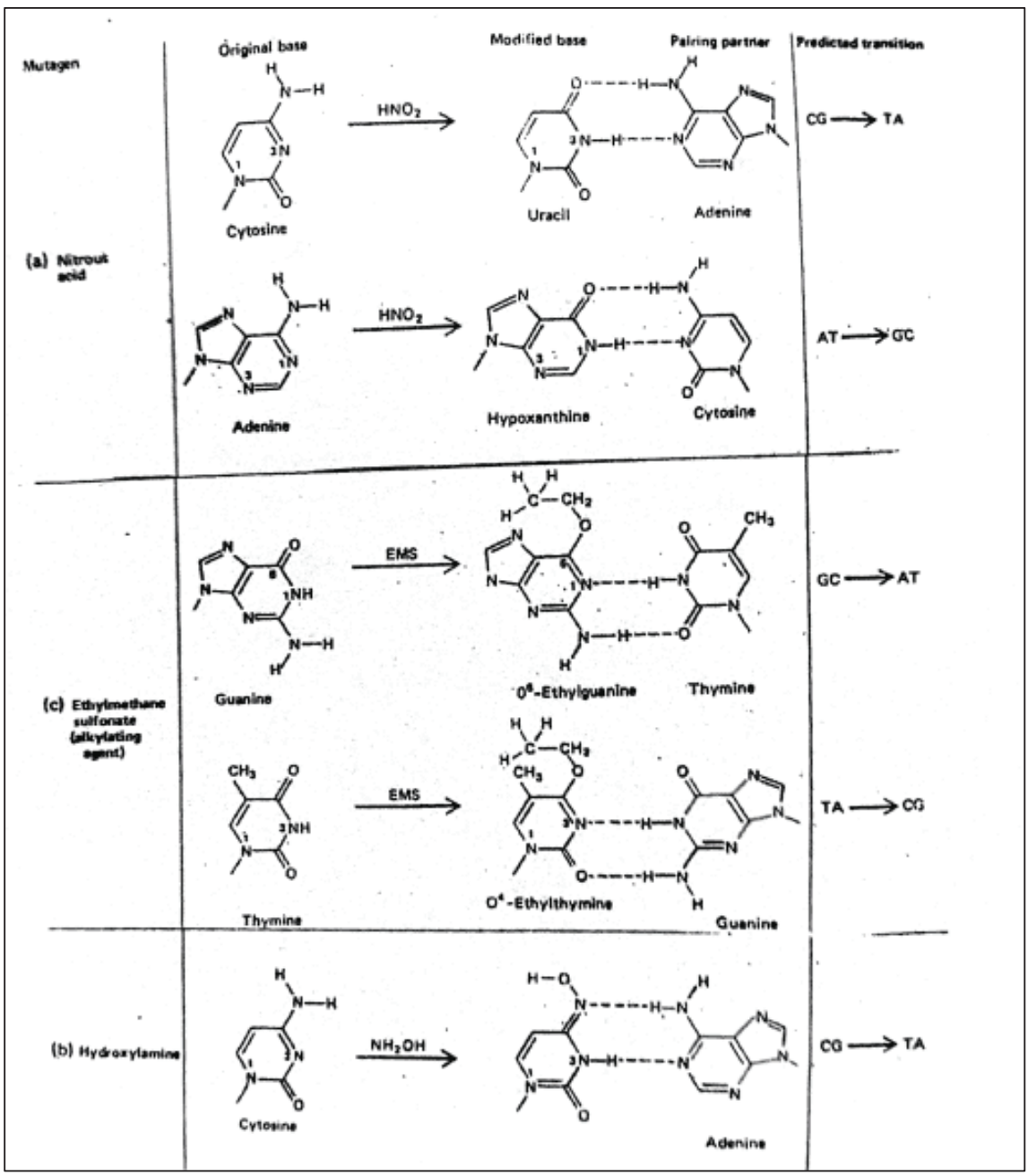
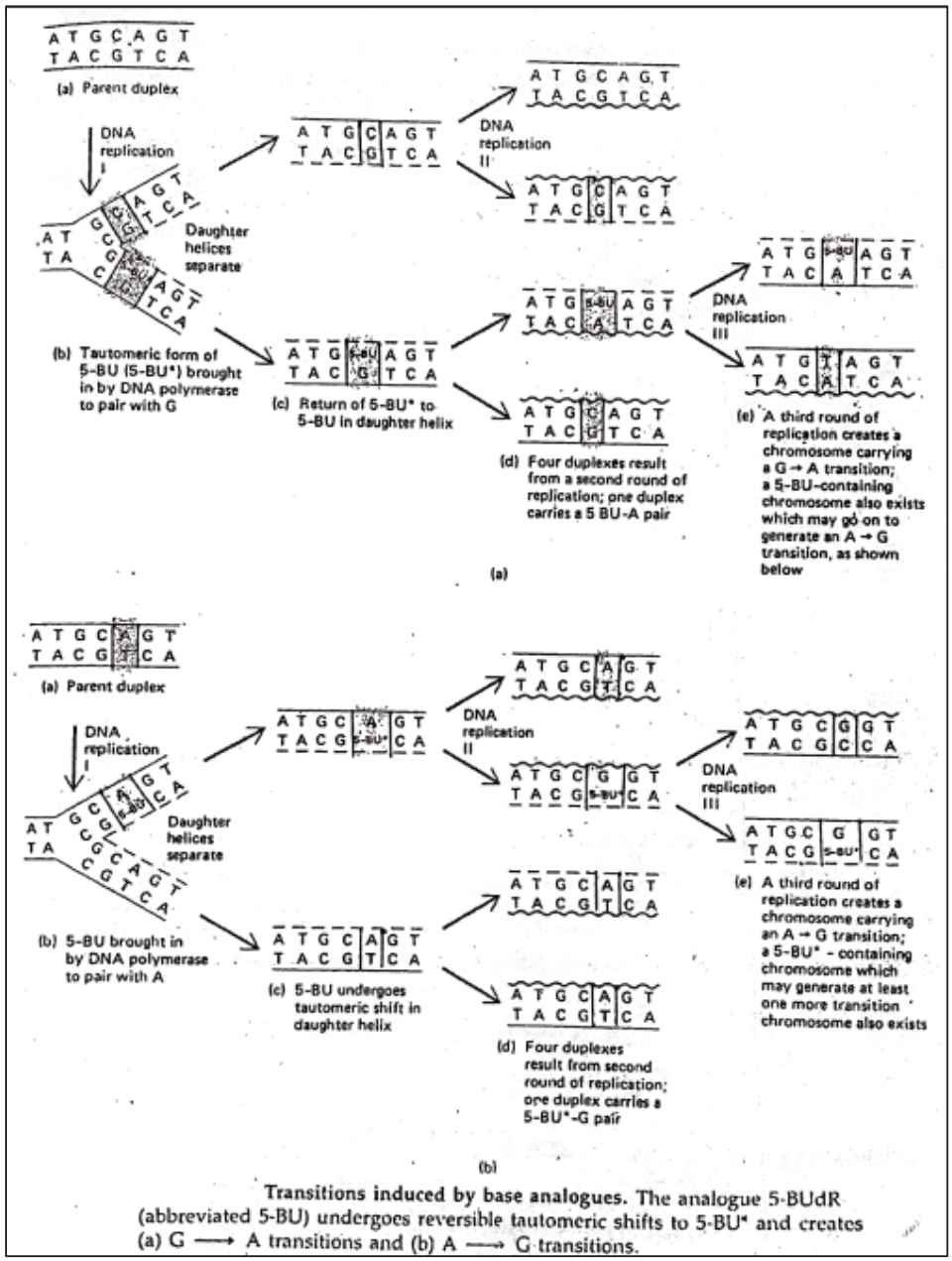


Fig58. Guanine Adenine Mutagens that modify the chemical structure of bases.



Transitions induced by base analogues. The analogue 5-BUdR (abbreviated 5-BU) undergoes reversible tautomeric shifts to 5-BU* and creates (a) G ⟶ A transitions and (b) A ⟶ G transitions.

Fig.59

Procedure of Mutation Breeding

Plant material for irradiation: The plant can be treated in seed, seedlings, or cuttings with radiation of different kinds.

Treatment: For X-irradiation the material in taken inside the X-ray room. The seeds are kept in petridishes while seedings at pot. For gamma irradiation the pot grown plants are taken very near

the source in a circle. For seed treatment a tray is fixed to the aluminium tube through which the radioactive cobalt travel. Neutron treatment is done by exposing the seeds, as well as seedlings to the neutron flash from the generator. Beta rays treatment is done with the radioisotopes P32 and S35 available in the form of

phosphoric acid and sulphuric acid. The seeds and seedlings to be treated are immersed in solution of desired strength for specific duration. In some cases the solution is applied to the soil.

Dosage: The proper dose will be one which give an appreciable frequency of beneficial mutants on selection and makes the heritability value of yield and other desirable characters highest. This dose varies from crop to crop depending upon the nature and genetical constitution of the plant material.

The amount of radiation required to kill 50% of the exposed individuals is termed as lethal dose (LD 50). The radiations are detected by the counter.

First irradiated generation: After irradiation, the storage of treated material increases the injuries in R, and hence it is removed immediately and sown into the field surrounded by the control material of mother strain to (1) isolate the treated population and to (2) compare the treated generation with control.

Effect of treatment in R_1: The low doses do not show any severe effect and the plants do not reveal any disturbance. High doses produce gross visible disturbance such as: (1) Death (2) Growth inhibition (3) Morphological and developmental abnormality. (4) Genetic changes. Selection: Almost every suspected plant is picked in R, and kept separate for further investigation.

R_2 generation: In R_2 the seed, of individual ears from the R, are grown in progeny rows, because the R, ears are affected independently. Sometimes one or 3 grains from R_1 plants are picked up and sown in R_2 rows and they are known one plant one grain or one plant 3 grains method.

Screening and selection in R_3:

R_3 to R_5 generation: In R3 plant to progeny rows are raised with original variety as check. If all the plants in R3 line are true to type as in R_2 they are called mutants. The true beading lines are carried up in R4 in progeny rows with original strain as control. In R, all the inferior lines are eliminated completely.

Yield trials: It is done in moderate rate in R, generation. If the mutants give very less yield, but is superior in desired character, it may be carried in hybridization. This brings all the desirable characters in mutants.

Precautions: Be sure that the mutants isolated are really due to mutation.

Application of mutation breeding: Mutation breeding can be used in all -the three types of crops viz self-pollinated (useful easily) cross pollinated (not easy and less useful) and vegetatively propagated plants (useful).

The mutation breeding is unavoidable:

(1) When whole of the naturally occurring variability in exhausted.

(2) When a gene for desirable improvement is known to exist but unattainable.

(3) When a desirable gene is to know to exist and attainable but not desirable to attain it.

(4) To break close linkage and transferring the little segment of a chromosome to non-homologous chromosome.

Limitation:

(1) Without full information about genotype, mutagen, frequency of mutation etc.

(2) Continue to a few institution.

(3) Does not produce ready made varieties but; treats inexhaustible variation from where for vehicle desirable character are picked up.

Advantage:

(1) It produces inexhaustible variation.

(2) When no improvement by conventional method then only this is done

(1) Maximum utility to crop improvement.

(2) Time, labour, land expenses are less comparatively.

Achievement: Following improved varieties of crop were developed through mutation breeding:

Crop	Varieties	Crop Treatment Place
(Hordeum vulgare L)	Pallas	Sweden from Variety Bonus (Borg 1958)
		High yielding and shift strawed
	Marij	By Gustafsson (1960)
	Jutta	Central Germany
Bean	Sanilae	U.S.A.(Down and Anderson 1956) Early maturing.
		(*Phaseolus Vulgarisy L*)
Cotton	Indore-2	India-Malwa Upland -4 at plant (*Gossypium hirsutum*)
		Industries Indore-India
Jute	JRO-514	From JRO-632 at Jute Agric Research Station
(*Corchoru olitodus L*)	JRO-412	Barackpore -India.
Mustard	APM	Bose Research- Institute,Culcutta India
(*B. juncea*)	(Appressed Pod Mutant)	
Rice	P500-28	From T-1145-Bose Res Institute. Calcutta.
(Oryza Sativa)		
Wheat	N.P-836 ←	For awnless N.P799.at I.A.R.I.New Delhi
(Triticum aestivum L) ←	Fully awned and high yielding.	
Sharbati sonara ←	From Sonora 64 by gamma radiation, at I.A.R.I Delhi. It is dwarf amber grained and high Yielding	

There are several mutant varieties in vegetables, pulses, potatoes, being developed as per requirements. Quantitative as well as. qualitative traits are being induced in these crops through mutation.

Mutations in microbes: Beadle and Tatum (1941) induced mutations in Neurospora crasa for the study of biochemical genetics which formed the base for one gene enzyme or one gene-one peptide-theory. *E.coli* was mutated from susceptible strain to resistant strain (Luria and Delbruck -1943).

Mutation in Animal: Induced mutation in domestic animal yields more milk, protein meat etc. Genetically improved cotton (BT Cotton), lady finger and other food crops are being developed through mutation breeding programme.

SECTION 3
Cytogenetics, Plant Breeding & Nano Biotechnology

Chapter 24
Cell Biology, Cell Organelles, Chromosomes

Cell as a basic unit of living system: The cell Theory

The cell is a structural and functional unit of all living organisms capable of carrying out all the

activities necessary for life.

1. Cell theory deals that all living matter is made up of cells or cell is the unit of life.
2. Rene Joachim Henri Dulrochet (1824) was proponents of cell theory.
3. Malthias Jacob Schleiden and Theoder Schwann of Germany (1847) said that all tissues are made of cells and formulated the cell theory as such: Each cell has two functions, pertaining to its : (a) development and (b) intermediary.
4. Rudolph Virchow (1858) formulated the cell theory as such: Each living organism appears as a sum of vital unit, each of which bears the complete characteristics of life.
5. The cell theory of Schwann and Scheiden (1847) states:
    a. All living things are made up of one or more cells.
    b. New cells are formed from pre existing cells through division.
    c. There are basic similarities in chemical composition and metabolic functions of all cells.
    d. The activities of organism is the collective activities and interactions of its cellular structure.

It was experimentally proved by Francesco Redi (1668) and confirmed that life arise from previous life and prevailing belief in the spontaneous generation of life was refuted.

Pre-Cellular Evolution and artificial creation of cell

1. Earth was formed 5 billion years ago from clouds of gas and dust.
2. First sea formed was from water that came from the atmosphere.
3. Most biologists agree that the first cell on earth developed in the oceans.
4. A.I. Oparin (1920, 24) and J.B.S. Haldane (1929) developed a hypothesis that early atmosphere combined with an energy source - developed into organic monomers and it was tested by S.Miller (1953) and H.Urey using water vapour, methane, ammonia and hydrogen gas. The energy used in the experiment was an electric spark. (Fig 60)

Oparin's Hypothesis: The conditions favouring a biotic formation of organic monomers existed in primitive atmosphere-which was rich in Hydrogen gas, deficient in oxygen gas, chiefly methane, ammonia, water vapour, nitrogen gas and some carbon mono-oxide and carbon dioxide.

Energy Source as such solar radiation, lightning, hot volcanic ash would have interacted with these gases to from organic monomers. It was carried to the earth's surface by rain water, accumulated in the ocean as a warm dilute soup. Thus the stage was set for origin of cells. This hypothesis was tested by Miller and Urey (1953) at Chicago University.

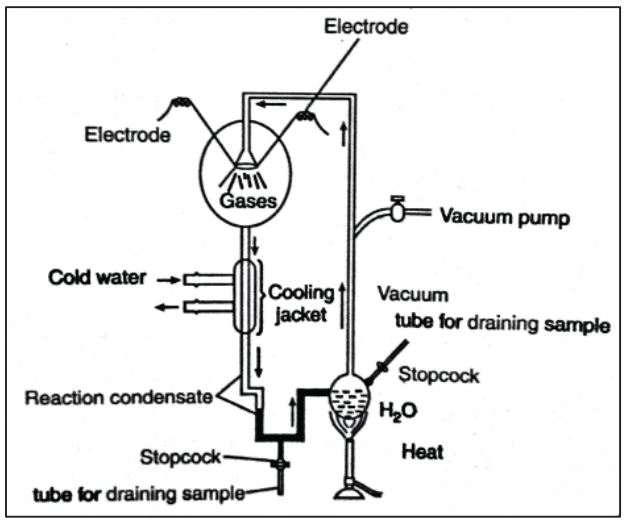


Fig.60 The apparatus used in the Miller-Urey Experiments

1. Created atmosphere of methane, ammonia, hydrogen gas and water vapour in a closed environment (glass container).
2. Placed this atmosphere over hot water (existing on the cooling earth).
3. The atmosphere was subjected to electric discharge as from lightning
4. Many of organic monomers molecules, formed in all living organisms, were formed.

   Polymers have formed from monomers prior to existence of cells. Monomers combine together by removing water molecule to from polymers.

There are several ways in which monomers could have become concentrated in the primitive

ocean. An energy source such as lightning or ultraviolet radiations could have driven the removal of water molecule from the monomers.

Once some polymers formed, they may have self-assembled into spherical structure called Protobionts. Such structures are formed readily in the laboratory.

Protobionts made up of polypeptides are called microspheres, those of lipids called Liposomes and those of combination of polypeptides, nucleic acids and polysaccharides, are called coacervates.

Gradual changes in such protobionts led to the formation of cells.

Coacervate droplets had certain life like activities therefore Oparin presented them as the model of Protocells.

Synthesis of coacervate droplets: By combining the solutions of gelatin + gum, serum albumin

+ gum, histone + gelatin, histone + RNA, gelatin + gum +DNA.

When two solutions are bought together, they interact to form swarms or cluster. They are separated out from solutions by acquiring certain size in the form of coacervate droplets.

Oparin et al (1968): Gum + Histon + 6.2pH+ Phosphorylase

Coacervate droplets + Enzyme-= Coacervate droplets (Chemical evolution).

Polynucleotides

S. Fox (1969) mixed all 20 amino acids & heated them on volcanic rocks in an oven at 160-210°C for 2 hours. Two surfaces of rock were sprinkled with water & allowed to cool. The residue was found to have millions of small spheres (0.5-0.7 mm dia), called microspheres or proteinoids which resembled coccoid bacteria.

Polypeptides in proteinoids consisted of 18 amino acids.

Properties of proteinoid microspheres: Electron microscopic observations show:

(1) Double layered boundaries through which diffusions of materials takes place.

(2) Motility

(3) Growth

(4) Binary fission

(5) Budding and fragmentation

(6) Stainable by gram's stains

(7) Catalytic activities like enzymes.

The basic structure of first cell must have included:

1. An outer membrane to control movement of materials into and out of cell to promote high concentration of energy & molecule.
2. ATP to store and transport energy.
3. Energy to regulate chemical reactions.
4. Ribosome to assemble protein from amino acids.
5. Nucleic acid to provide instructions for protein synthesis and system of self-replication. Oldest fossil cells resemble heterotrophic bacteria from the rock 3.5 billion years ago. Evidence of earlier form of cell may never be seen since rocks were too fluid to form fossils at that time.

Evolution of Prokaryotes

The earliest prokaryotes must have been chemo heterotrophs and have acquired energy from

fermentation of organic molecules. Oxygen releasing prokaryotes first appeared at 2.5 billion years ago.

The first organism to give off oxygen gas was probably the Cyanobacteria. Heterotrophic prokaryote benefited from the evolution of cyanobacteria mostly because these new forms provided materials for aerobic cellular respiration.

Aerobic cellular respiration probably appeared first in photosynthetic prokaryotes since they already had electron transport chain for photosynthesis.

The oldest fossil cells were prokaryotic cells, they resemble contemporary heterotrophic bacteria.

Heterotrophs and methogens had a common ancestor and we do not know what the first cells were like.

Origin of the Eukaryotic Cell

The oldest eukaryotic fossils is 1.5 billion years old.

According to autogenons hypothesis eukaryotic cells evolved by specialization of internal membranes derived from the plasma membrane of prokaryotes.

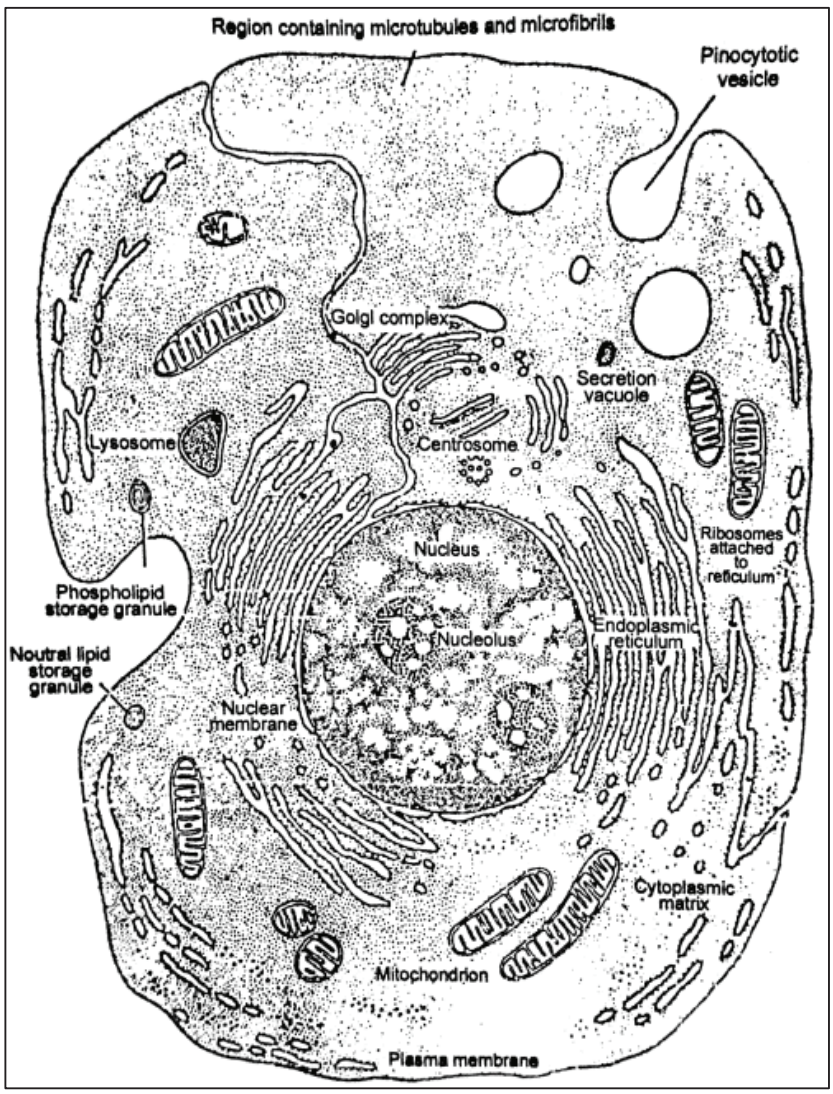


Fig. 61 A generalised animal cell as seen under electron microscope

According to endosymbiotic hypothesis eukaryotic cells evolved from the symbiosis of several prokaryotes. In this view, chloroplast originated from pre-existing chloroplast. Sexually reproductive organisms inherit its mitochondria and chloroplast from its mothers.

Hypothesis explaining the Origin of Eukaryotic Cells

The hypotheses are (1) autogenous hypothesis and (2) endosymbiotic hypothesis.

Autogenous hypothesis: Eukaryotic cells evolved as prokaryotic cells, gradually increased in complexity. Complex structure e.g. mitochondria, chloroplast etc. evolved as indentation & modification of plasma membrane.

Endosymbiotic hypothesis: Structure within eukaryotic cells evolved suddenly from the union of different kinds of prokaryotes. Larger prokaryotic cells engulfed but did not digest smaller prokaryotic cells and an endosymbiotic relationship developed in which the smaller cells became specialised in some functions and the larger cells in others. In time, smaller cells became organelles incapable of existing any longer on their own. Evidence in its support that mitochondria and chloroplast contain a regular DNA like those formed in prokaryotes. Ribosomes & RNA are of same fate.

Organelles within eukaryotic cells appear to be remnants of prokaryotic cells that once existed as independent organisms. Both the hypothesis provides valid explanations (Fig.62).

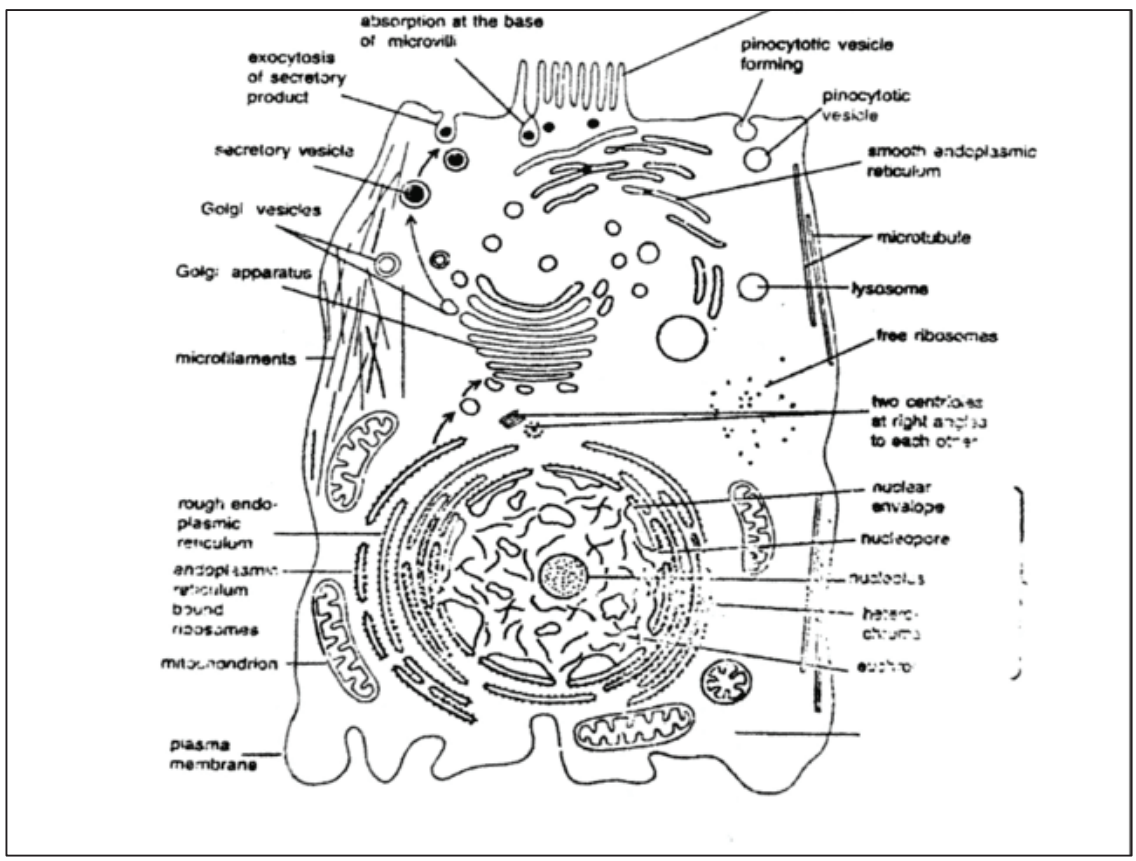


Fig. 62 . Ultrastructure of a generalised animal cell as seen with electron microscope

Broad classification of cell types

Aristotle (4th century BC) divided living organism (kingdom) into (1) animals and (2) Plantae.

E. Haeckel: (1886) added one more i.e., protists for Algae, Fungi, Protozoa & Bacteria. Radically

there are two different kinds of cell.

(1) Prokaryotic cell

(2) Eukaryotic Cell

R.H. Whittaker (1969): Classified the living world into 5 groups on the basis of cells as such:

1. Monera: Prokaryotic & unicellular (Bacteria)

2. Protista: Eukaryotic & unicellular

3. Fungi:

4. Plantae Bryophytes and Trachaeophyta.

5. Animalia: Vertebrates & invertebrates.

Main Group of micro-organisms

1. Bacteria: Eubacteria, archaebacteria, cyanobactria or Oxyphotobactria, Mycoplasma, rickettsiae and chlamydiac.
2. Archaebacteria: Ancient lineage diverged from eubacteria and survived in specialised ecological niches. It has following features:

   (1) No peptidoglycan present in wall

   (2) Cell Membrane is single layered of glycero-hydro carbon glycerol chains

   (3) Ribosome insensitive to chloramphenicol

   (4) Inhibit extreme environment

      (a) Methan generating bacteria in anaerobic-muds

      (b) Extreme red halophiles

      (c) Salt loving bacteria of saturated brine and salted fish and

      (d) Thermoacedophiles - found in hot sulphur spring or smouldering coal wastes.

Now, Living cell are classified into:

1. Bacteria

2. Archaea

3. Eukarya

During an expedition to deep sea Methanococcus was discovered from 3km beneath the pacific

ocean-to be a *Methanococeus Jannaschi* after the name of expedition leader Holger Jannasch in the year 1982.

Genome of this bacteria was fully sequenced in 1996,besides the genome of *Haemophilus inflnenzae*, *Mycoplasmia genetalinum*, *Saccharomyces cerevisiae* & *E.coli* in 1997.

Archaea has specific features common to Eukaryote e.g. DNA, replication, transcription, translation, histone etc. and to prokaryote e.g., Transport of Na+, K+, across the cell and the unique gene such as:

1. only one DNA polymerase

2. several tRNA synthetase missing
3. 18 inteins inserted in protein & then removed
4. lives at 48°c- 95°c (Themophile- heat loving)
5. at-200 atmospheric pressure
6. grow at 10 times more salt concentration than sea
7. killed by oxygen and
8. Autotroph using Carbon dioxide, Nitrogen and Hydrogen to produce methane so it may be a common ancestor present 3.6 billion years ago.

Rickettsie:

1. Tiny rods like organism transmitted by arthropods
2. Multiply only in living cells
3. Intracellular parasites of eukaryotes

Mycoplasmas:

1. Smallest organisms
2. can be cultivated outside living tissue
3. involved in lung disorders.
4. Parasites of prokaryotes & in soil
5. Penetrate through periplasm where they replicate causing lysis
6. Also called bdello vibrios

Actinomycetes:

1. Majority are mycelial
2. Gram-positive bacteria eg. Streptomyces

Viroids:

1. Single naked RNA molecule
2. Causes disease in plants only
3. CEV Citrus exocortis viroid - 371 nucleotides

Prions:

1. Proteinaceous infective particles
2. Associated with degenerative disease of central nervous system, by few tribes of New Guinea.
3. Transmitted by eating uncooked brain
4. Scrapie: disease in sheep & goat. Crutzfeldt Jacob discovered in animals.

Cell membrane and cell organelles

All cells and subcellular organelles are bounded by thin membrane. It could he identified with the advent of electron microscope. The cell membrane interact with other adjacent cells forming

tissues and organs. Cell membrane/plasma membrane acts as a receptor site for hormones, immune protein, neurotransmitter etc. It prevents the loss of different macromolecules. It also protects the cell from the uptake of some harmful materials.

Structure: Cell membrane is made up of protein and lipids. According to Fluid mosaic model of S.J.Singer and G Nicholson (1972) the principle of membrane organisation is as follows:

1. Lipids are present in two layers.
2. Proteins are arranged in two ways called integral proteins and peripheral proteins.

Lipid exists in the membrane as a bilayer. Phospholipid is most predominant. In myelin and

Chloroplast, glycolipids are predominant. One common property is found in both the types of lipids i.e., they are amphipathic means it has hydrophobic and hydrophilic proteins in a single molecule.

Cholesterol is also present in some membrane. The plasma membrane of plant cell has sitosterol and stigmasterol.

Membrane proteins may be intrinsic or integral protein which are embedded in the lipid layer and

the peripheral proteins attached to the membrane surface. The integral proteins are attached to the membrane by hydrophobic interactions with the tails of the fatty acid chains of the lipid layer (Fig 4.1). This makes the integral protein buried inside the membrane. Integral proteins also possess hydrophilic amino acid which are exposed to the surface of the membrane. Peripheral proteins do not form a continuous layer. This fluid mosaic model of membrane is thermodynamically stable because hydrophilic area of lipid and membrane proteins are away from the water surface.

Fluid phase of the cytoplasm is known as cytosol.

Golgi Complex: It was discovered by C. Golgi (1898). Electron Microscopic structure was

given by Dalton & Felix (1954). They are found in eukaryotic cell except, RBC, sperm cells of

bryophyta & pteridophyta and sieve tube of plants. Golgi complexes are present within the cell as bryophyta.

nm nanometer

1 nm 10 Å (Angstrom)

1 micron= 1000 nanometer

Nucleus

Robert Brown (1831) first described the nucleus in Tradecantia though it was first observed by Fontana (1781), Nucleus is cell organelle and controlling centre of cell. Nucleus is membrane bound structure and hence eukaryon (Eu = true, karyon = nucleus). Cells without true nucleus arc prokaryotic cells eg. Bacteria, pleuro pneumonia like organism (PPLO) and blue green algae.

Number: Generally there is only one nucleus in a cell, but many nuclei are also found in a cell eg. Paramoecium has two nuclei, Liver cells have two nuclei bone narrow cells are multinucleate. Multinucleate cells are of Vaucheria, endosperms and they are called coenocytes/syncytium. Red blood corpuscles (RBC) are devoid of nucleus.

Shape: Nucleus is generally spherical. However C shaped nucleus is found in Vorticella, disc

like in epithelium cells, kidney shaped in Paramoecium, bibbed in white blood corpuscles (WBC),

moniliform in Spirostomum and branched nucleus in Platyphylax (insect). Size of the nucleus is

directly proportional to that of the cytoplasm and it is determined by Hert wig's formula as such:

Volume of nucleus (Vn)

Nucleo Cytoplasmic index (NP) = Volume of the cell (Vc)

Sometimes, number of chromosomes is related with the size of nucleus, (= e.g.,) polyploid cells.

Active cell has larger nucleus than that of resting cell.

Nuclear membrane (Karyotheca): It disappears in metaphase of eukaryotes, reappear at telophase. No membrane is found in prokaryotes. Electronmicroscopic structure reveals that there are two concentric membranes viz (1) inner nuclear membrane and outer nuclear membrane. Each membrane is 75-90A thick, madeup of lipoproteins separated by perinuclear space of 100-170Å Perinuclear space is known as perinuclear cisternae. Outer member is extended to from endoplasmic reticulum membrane form

Nuclear pore: Nuclear membrane is perforated by nuclear pore. At the margin of each pore, outer and inner membrane is fused. The number of pores varies ranging from several thousand to millions. The diameter of nuclear pore is about 100 nm. The pores are enclosed by annuli which are cylindrical and circular. The central hole is plugged by central granule (inactive ribosome) attached to cytoplasmic side. Nuclear membranes protect the inner contents of nucleus and prevent the entry of active ribosomes and other cytoplasmic materials.

Nuclear lamina: Inner nuclear membrane is lined by nuclear lamina of 30-100 nm thickness and connects the inner membrane with chromatin. Nuclear lamina is made up of extrinsic proteins, 12-(La Lamm A, B and C which forms a fibrous network. Laming regulate the assembly and disassembly of nuclear membrane during cell division. It helps to form micronuclei when the cells are left for long time in colchicine. It is attached with chromatin and nuclear membrane.

Nucleoplasm: The contents of nucleus is called nucleoplasm. It is composed mainly of nucleoproteins besides organic and inorganic substances, enzymes necessary for synthesis of DNA and RNA.

Nucleolus: It is the only organized body found in the nucleus. During nuclear division it is disorganized in late prophase and re-organize in telophase. In some cases it may persists till metaphase as in pea, *Spirogyra* etc. It was noticed by Fontana (1784). It is formed in the nuclear organising region of the chromosome. It is the site for the synthesis and biogenesis of ribosomal nucleic acid. According to Me Gill (1906) nucleous has two regions: (1) Central Oxyphilic and (2) Peripheral basophilic area. Nucleolus chromosome association was established by Heitz (1931). Casperson (1940) said the presence of RNA in the nucleolus. So felo (1951) reported the presence of nucleonema (a filamentous component) and structure less pars amorpha within the nucleolus. Nucleolus has no membrane. Nucleolus is absent in the cells of prokaryotes,

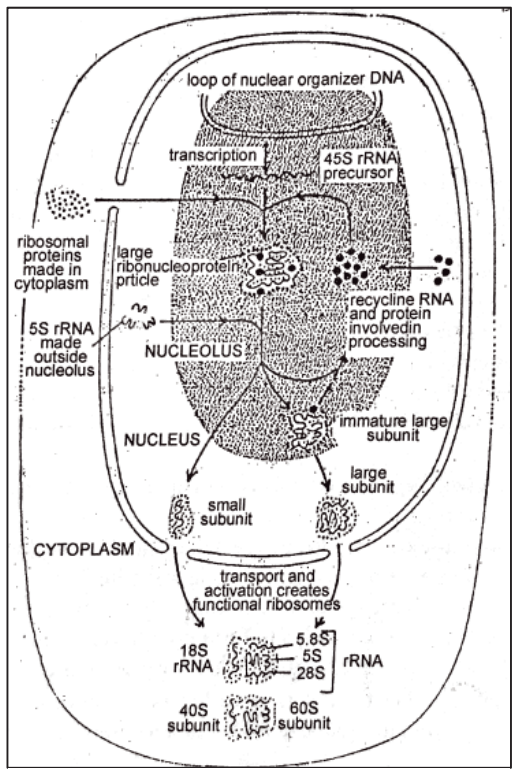


Fig. 63. The function of the nucleolus in ribosome synthesis

blue green algae, spermatozoa, reticulocytes, undifferentiated embryonic cells etc. One or more nucleoli are found in a cell.

Origin: Nucleolus first appear as a droplets which increases in size as the cell synthesizes more RNA and accumulates more proteins.

The DNA axis isolated from molecules or nucleolar orgainser contains multiple copies of r-

RNA genes that codes for r RNA.

Function: Nucleolus is a site for rRNA synthesis. 28S r-RNA and 18S-rRNA can be selectively

hybridised with nucleolar DNA which indicated that r-RNA is complementary to the nucleolar DNA. Accumulation of cytoplasmic r-RNA can be prevented by UV rays. Inhibition of nucleolar RNA synthesis by actinomycin D or inactivation of nucleolus by UV rays can prevents the accumulation of r-RNA in the cytoplasm. Nucleotide composition of molecular RNA is identical with cytoplasmic RNA. Nucleolus may be linked with the transport of nuclear RNA (Fig. 63).

Chapter 25
Cell Cycle, Mitosis, Meiosis

As shown in the diagram, the M stage of the cycle occurs when the cell is dividing to form two cells. It includes both karyokinesis and cytokinesis. This stage is the shortest stage of the cell cycle. The G_1, S and G_2 stages are all parts of interphase. The G, (first Gap) stage is the period after cytokinesis but before DNA synthesis. It is a time of cell growth, high metabolic activity and synthesis of enzymes required for DNA replication. The length of this stage is 8-10 hours in a 24 hr cycle. The S or synthesis stage is the period for DNA synthesis. The single long molecule of DNA is a chromosome or Sister chromatids. Latter are held together at centromeric point which consists of DNA that was not replicated along with the rest of the molecule.

The G_2 or second gap stage begins after chromosomal replication. Proteins are Synthesized required for mitosis (Karyokinesis). The G_2 stage is followed by mitosis (M) stage and complete the cycle (Fig. 11.1).

Cell Cycle control system: There are two theories for regulatory mechanism to enter the cell into S phase and M phase as in Fig. 64

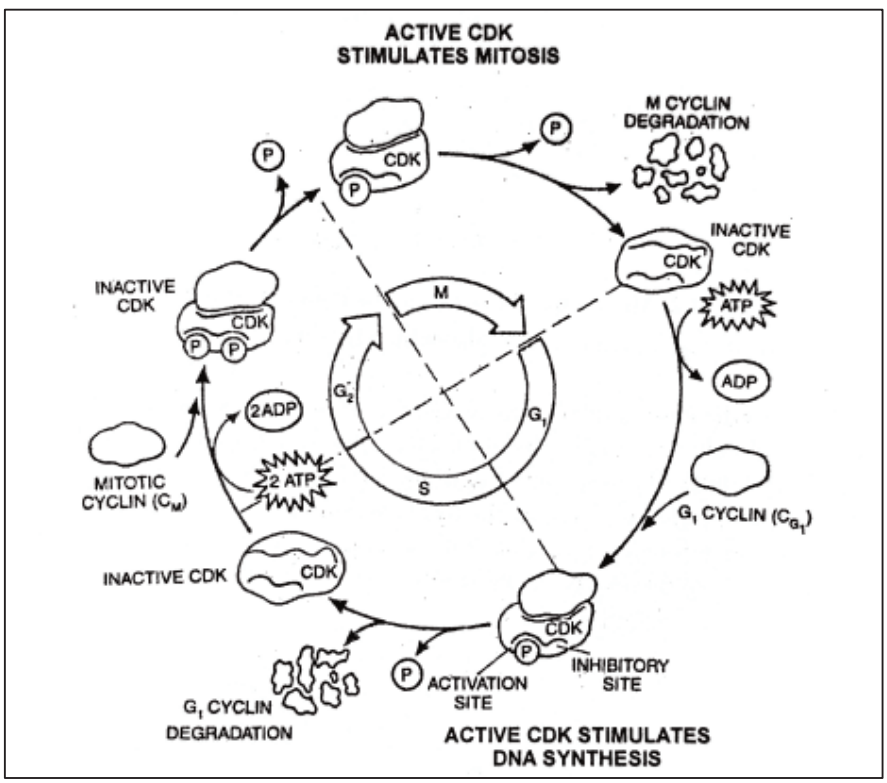


Fig. 64 Diagram of the cell cycle showing regulation of the cell cycle by cyclin dependent protein kinase (CDK).

In G_1, CDK becomes active by G_1 Cyclin and ATP at its activation site. It causes transition of G1 to S phase. G_1 cyclinis destroyed at the end of S phase and CDK again becomes inactive. Cell enters G_2. In G_2 Inactive CDK binds to cyclin CM. It gets phosphorylated at its both activation and inhibitory sites and remain inactive.

On removal of PO from the inhibitory site, it gets active and cause transition from G_2 to M phase. At the end of M phase, cyclin CM is degraded and PO4 at activation site is removed and the cell enters G_1 again.

(1) Domino theory: According to this theory cell cycle is a set of dependent reactions having several check points as such: A. Cell halts at S phase if DNA synthesis is blocked by chemicals like Hydroxy urea, amphidocolin etc. B. In yeast rad 9 gene delay mitotic entry of G_2 cell. PS_3 protein causes delay in the entry of cells into S phase and cell stays at G,. Mutation in PS, causes cancer.

(2) Clock theory: Events of cell cycle occurs in succession regulated by a control system that operates independently (Fig.65).

Exp: Role of cyclin dependent kinase (Cdk) which facilitate passage through different phases of cell cycle.

Miotic cell cycle indicates interphase having G_1, S and G2 stages, which is marked by preparatory

stages for going into mitosis i.e., nuclear division. Two main functions are essential for preparations: (1) Preparation of substances of mitotic apparatus and (2) Energy reservoirs. (Fig.69).

Point of no return: Point of no return can be identified as such. If a cell can be prevented from making a given transition before a certain time but can no longer be blocked after that time, the point of no return is identified. Following are the points of no return in mitotic cycle.

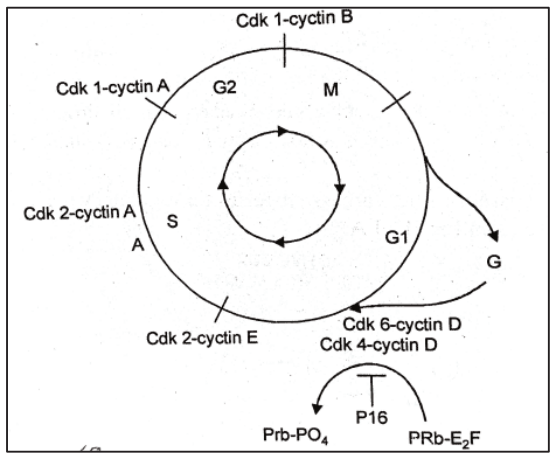


Fig.65 An outline of cell cycle in a mammalian cell

1. Chromosome reproduction
2. Energy supply
3. Entrance into anaphase
4. Entrance into cytokinesis and
5. Dependence of mitosis on nucleolus.

Cell division (Mitosis)

If we ask why cell must divide, the answer can be given in terms of what happens it they do not. The answer is that they die. Division is a necessity for survival, growth and dilution of cell substances. Mazia (1952) and Brachet (1957) proposed replacement hypothesis. It indicates the functional synthetic units in the cytoplasm have a limited life time after which they have to be replaced or have to receive fresh information from the nucleus. Nuclear mass and cytoplasmic mass are in a state of optimum equilibrium, the so called nucleocytoplasmic ratio or index. In a cell nucleus is controlling master and cytoplasm is the field of reaction. A definite nuclear mass is capable to control the activity of a definite mass of cytoplasm. If the cytoplasmic mass exceeds then it becomes difficult for the nucleus to control it. In order to keep all the increased activity of increased cytoplasmic mass, extra nucleus is needed and for that nuclear division takes place followed by cytoplasmic division in order to form two identical cells.

$$\frac{Mn}{Mc} = \frac{2Mn}{2Mc} = \frac{4\mathrm{Mn}}{4Mc}$$

(Mn = Nuclear Mass; Mc = Cytoplasmic Mass

Mitotic division can be divided into following stages.

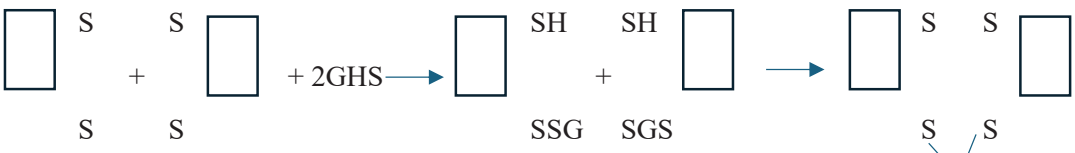
Interphase: Nuclear membrane, acidophilic nuclear mass, flakes of twisted filements of chromatin and nucleoli are seen at this stage. The volume of nucleus becomes larger.

Prophase: Chromosomes appear double stranded. RNA accumulates due to chromosome RNA

cycle. At the end of this stage, nuclear membrane and nucleolus disappear. The exact reason is not known about the breakdown of this substance, like nuclear membrane & nucleolus.

Spindle zones are formed by dividing centrosome in animal cells and in plant cells nuclear zone

is established at two opposite poles of the cell from where spindle fibres radiate. Spindle fibres are made of protein fibre with RNA and lipids. Rapkin (1931) found that glutathione cycle operates during cell division. Mazia (1954) used strong urea solution on isolated mitotic apparatus (spindle) and observed that S-S bonds are involved in the linkage of the protein molecules to form structural fibres of the spindle as such:



S= Sulphur bond, GSH-Glutathione.

Metaphase: In this stage, nuclear membrane and nucleolus disappears, chromosomes come to

lie on equatorial plane. Spindles are established and chromosomes are attached with spindle fibre.

Anaphase: The parting of sister chromatids is a distinct event marking the transition form metaphase to anaphase. It depends on a signal that affect all the chromosomes within a cell as well as miotic apparatus. The reported speed of Daughter chromosome movement in anaphase is variable and ranges from 0.2µ per minute to 5µ minute. Anaphase events are (1) elongation of central spindle and (2) Movement of Daughter chromosomes towards the pole. Several theories were proposed by workers like Metz (1936), Darlington (1937) oestergren (1950) etc. Oestergren and Bajor (1960) suggested loss of material for spindle fibre is the main feature for the anaphasic movements of D-chromosomes attached fibre. (D-chromosome = Daughter Chromosome).

Telophase: Completion of movement of D-chromosomes to the poles in anaphase is accompanied by chromosomal RNA cycle. Chromosome pickup ribonucleoprotein at prophase and discard it in an equatorial direction at anaphase. The conspicuous events at telophase are (1) reversion of the chromosomes to their interphase condition by a process resembles a swelling rather than despiralization. (2) reformation of nucleoli (3) re-establishment of nuclear membrane and (4) dismantling of mitotic apparatus Formation of nucleoli is described by the appearance of small masses of nucleolar substances in the vicinity of the chromosome and their assembly at the organizer region. (Fig.66).

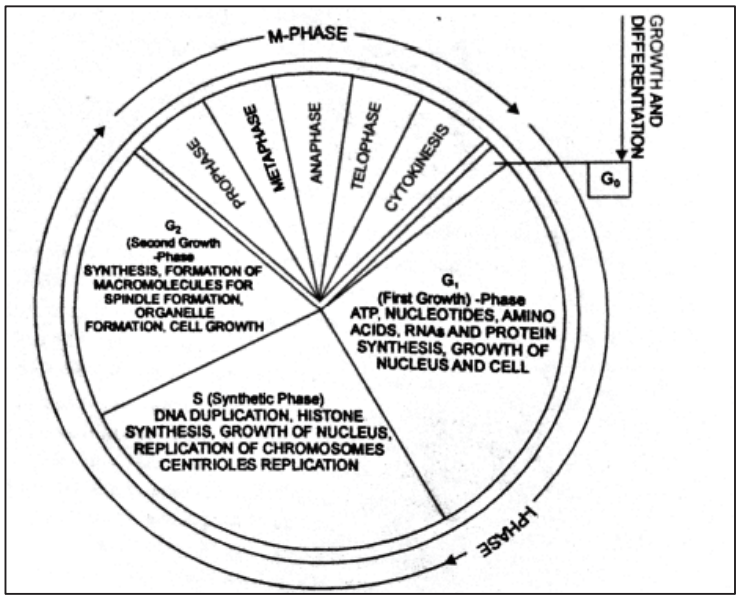


Fig. 66. Cell Cycle

Cytokinesis: It refers to all schemes of cytoplasmic division. Animal cells do not possess rigid cell wall and its cytoplasm divide by furrowing. In plant, essential feature of cytokinesis is cell plate formation, a membrane crossing the equator which develops into a definite wall between the daughter cells. During cell plate formation interzonal region of the spindle differentiates as a phragmoplast and then plate is formed across its equator. Cell plates become the middle lamella of the cell. (Fig. 67).

Duration of Mitotic Stages in Minutes.

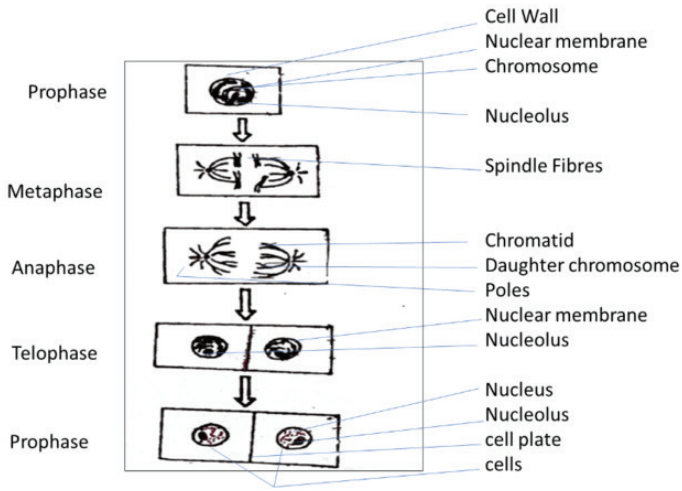
Cells	Prophase	Metaphase	Anaphase	Telophase	Total
Pea	78	14.4	4.2	13.2	109.8
Onion	71	6.5	2.4	3.8	83.7
Spleen cells of Mouse	21	13	9.0	57	100.00

Duration of Interphase i.e., G., S and G, and M in 24 hr cycle (in hours)

Cells	Prophase	Metaphase	Anaphase	Telophase	Total
G_1	-	8	-	10 hours	General
S	-	6	-	8 hours	
G_2 M-1-2 hours	-	2	-	4 hours	

Duration of interphase stages in Mouse and Vicia faba

Stage	Mouse Cell	*Vicia faba*
G_1	12	12
S	6-8	6
G_2	3-6	12
M	1	1



Meiosis

Meiosis was first discovered by Strasberger in 1888. It is a complicated process of nuclear division where by chromosome number is reduced to half in four nuclei fromed by two divisions of nucleus accompanied by one division of chromosome. Farmer and Moore (1905) named it as meiosis.

Transition from somatic mitosis to meiosis remains mystery. However Darlington (1937) said that leptotene chromosomes pair because of singleness which satisfy the mitotic affinity for doubleness by pairing. It is to be noted that at mitosis chromosomes appear as double stranded. This view of Darlington was termed as precocity theory of meiosis.

S. K. Sinha (1960) suggested that RNA : DNA ratio determines wethere a mitotic or meiotic division will occur. Higher RNA: DNA ratio resuts in mitosis and lower one in meiosis. The onset of meiotic prophase is marked by an increase in nuclear volume follwed by gradual modification in nuclear structure. Since, there are two nuclear divisions in meiosis, hence meiosis is divided as meiosis I or Division I & Meiosis II or division II. The stage of meiosis I is (1) Interphase I (2) Prophase 1 (3) Metaphase I (4) Anaphase I and (5) Telophase I.

Stages of meiosis 11 are:

(1) Prophase II (2) Metaphase II (3) Anaphase II and (4) Telophase 11: Interphase II may or may not occur. Interphase I is divided into G1,S and G2 and it takes more than 80% of time in the cell cycle.

Prophase I has been divided into 5 Substages like (a) leptotene (b) zygotene (c) pachytene

(d) diplotene and (e) diakinesis.

(a) Leptotene: (Leptops = Single, Tene = thread). Chromosomes appear as single stranded, long and uncoiled. Entire length of chromosome has beads called chromomeres which are congregation of DNA. Nebel and Ruttle (1936) noticed chromosome at leptolene a bipartite structure in Tradescantia and Trillium.

(b) Zygotene: Leptotene homologus chromosomes pair or synapse due to attractive force between them. The Phenomenon is called synapsis. Pairing is always two by two at one region. There are several views regarding nature of synaptic force as follows:

Lindegren and Bridges (1938) proposed an agglutination hypothesis. Each chromosome was considered to act as antigen forming a specific antibody on its surface. Adsorption occur when two allelic chromosomes happen to come in contact. Each side of agglutinated chromosome would fuse and a zipper like pairing would result.

Mc Clintock discovered a gene in maize for synapsis. Moses (1956) observed tripartite structure

between two paired homologus structure known as Synaptonemal complex It has a central element in ladder like configuration in the centre of the complex. Two lateral elements composed of fibres called synaptomeres lie parallel to the central element. Transverse elements are electron dense filaments which connect the central element with the lateral elements. The distance between central & lateral elements ranges from 20 nm to 100 nm. These elements are rich in RNA, DNA and protein (Fig. 68). King (1970) proposed synaptomere zygosome hypothesis for the formation of synaptonemal complex. It is assumed that paired pachytene chromosomes are recombinational nodules which are involved in meiotic recombination (Fig. 69) by changes in the interzonal region i.e., equatorial plane. There is accumulation of RNA related to it.

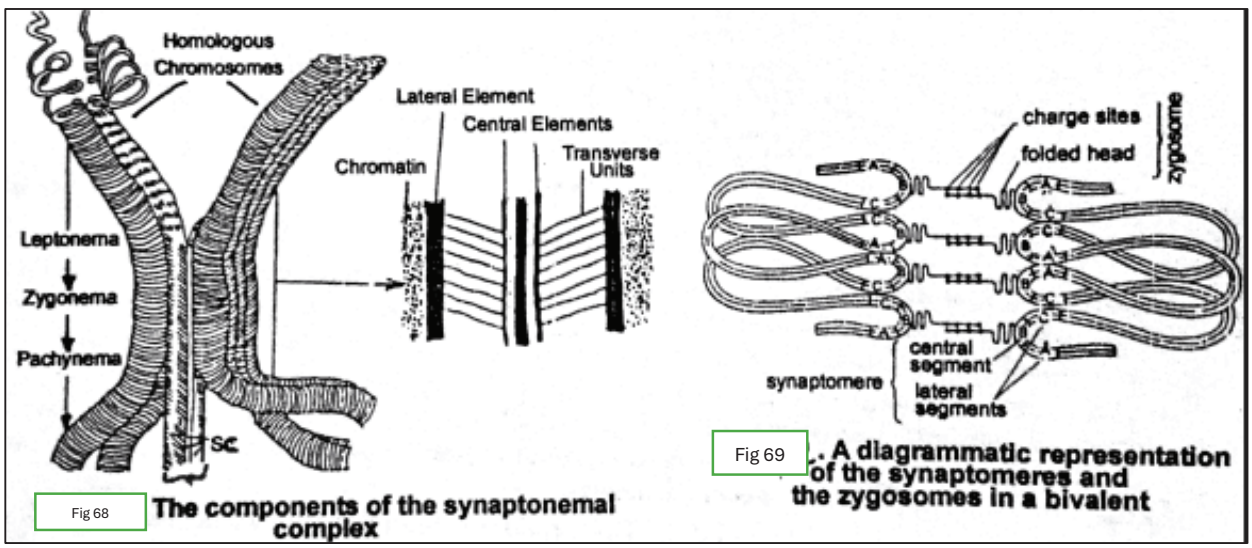


Fig 68 The components of the synaptonemal complex

Fig 69 . A diagrammatic representation of the synaptomeres and the zygosomes in a bivalent

(c) Pachytene: When zygotene pairing is ceases, the nucleus is at pachytene. The paired homologous chromosome has 4 chromatids. Deeply stained segment of this structure is *heterochromatin* and lightly stained finer chromomeric structure is called *euchromatin*.

Mechanism of Crossing over at Pachytene chromosome: According to Belling (1931) chromosome duplication at pachytene consists of two stages (1) formation of new genes (chromomeres) and (2) formation of new connections between these genes (chromomeres) as shown in Fig.70

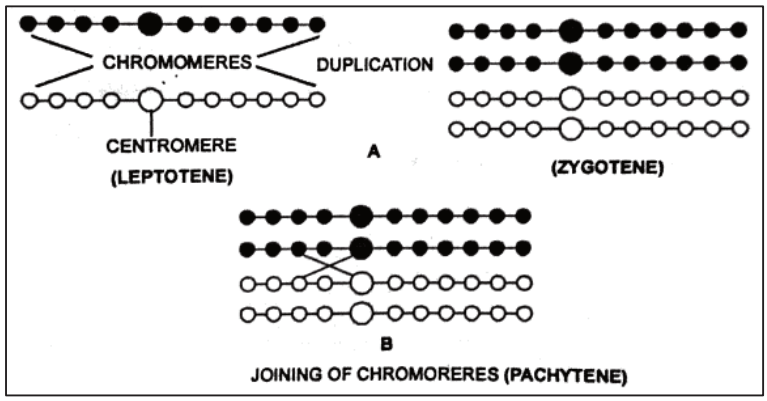


Fig. 70 Belling's copy choice model of crossing over

Darlington's torsion hypothesis: Each chromosome possesses an internal molecular coiling. The paired but undivided chromosome coil relationally around one another so that the torsion of their internal coiling is in equilibrium with the opposite torsion of their relational coiling. i.e., direction of relational coiling opposite to that of internal coiling. Division into chromatids takes place which upsets this equilibrium and subject the chromatids to torsion. A break in one chromatid is followed by a break in the second (non-sistser) chromatid at exactly the same point. The broken ends rotate so that fusion can occur giving rise crossover chromatid as shown in (Fig. 71)

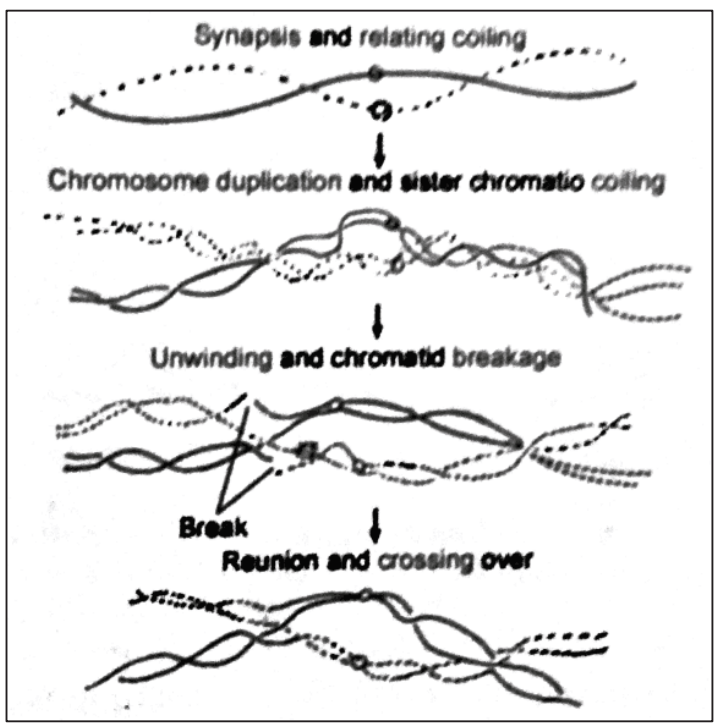


Fig. 71. Diagram showing Darlington's torsion theory of chromosomal crossing over due to breaks caused by unwinding of chromosomes and chromatids.

Crossing over is due to breakage and rejoining of intact, DNA molecule under the influence of endonuclease and ligage. Benzer (1955) found that crossing over occurred within the gene to be a part of DNA. A copy choice hypothesis was proposed by Holliday (1964). According to copy choice mechanism, new strand is

formed along the paternal strand during replication of paired chromosome and switches to the maternal one. When the complementary replica of the maternal strand also switches templates, two reciprocally recombinant strands would be formed and the structure is bivalent. The point at which crossing over has occurred is known as chiasma (Fig.72)

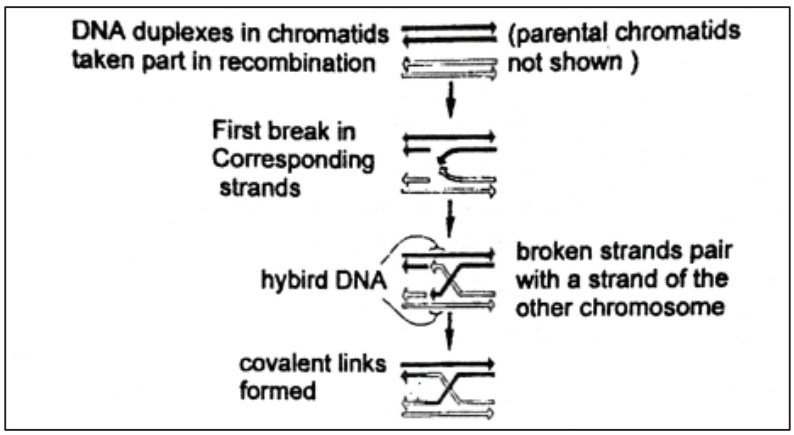


Fig. 72 Holiday model of crossing over

The relationship between chiasmata to crossing over: Two main theories have been proposed : (1) Classical theory or Two plane theory proposed by Me Clung (1927 b) and Sax (1932). This indicates crossing over is caused by chiasma., (2) Partial chiasma type or one plane theory proposed by Janssen (1909, 1924) and Darlington (1932). This says that each chiasma represents one genetic crossover and there is always reductional separation on both sides of a chiasma as shown in Fig. 73

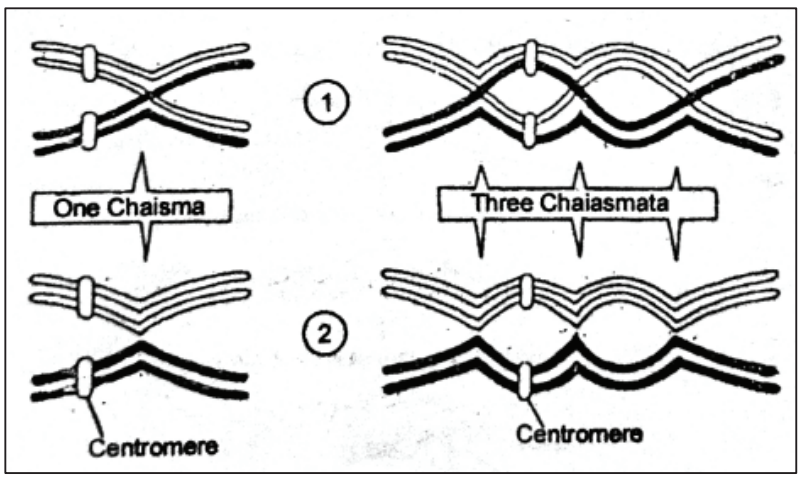


Fig. 73 Sketches as per theory of chaismata: (1) Classical theory and (2) Partial-chaisma type theory.

Diplotene: At this stage homologes repel and open into loops which are held as cross like configuration called chiasma (PI= chiasmata) Diakinesis: There is a progressive shift in the distribution of chiasmata between diplotene and metaphase I. This shift is terminalisation as per Darlington. At the end of diakinesis nuclear membrane disappears.

Metaphase I: After disappearance of nuclear membrane, a bipolar spindle is formed onto which bivalents move and become oriented.

Reappearance and disappearance of nuclear membrane: It is correlated with assembly and disassembly of nuclear lamina caused by depolymerisation which in turn is due to phosphorytion catalysed by enzyme lamin kinase. Nuclear envelop breaks into several small residues that disperse in the cytoplasm. Lamin B remains attached with these vesicles while Lamin A & C depolymerise into small oligomer and dispersed into the cytoplasms (Fig. 74).

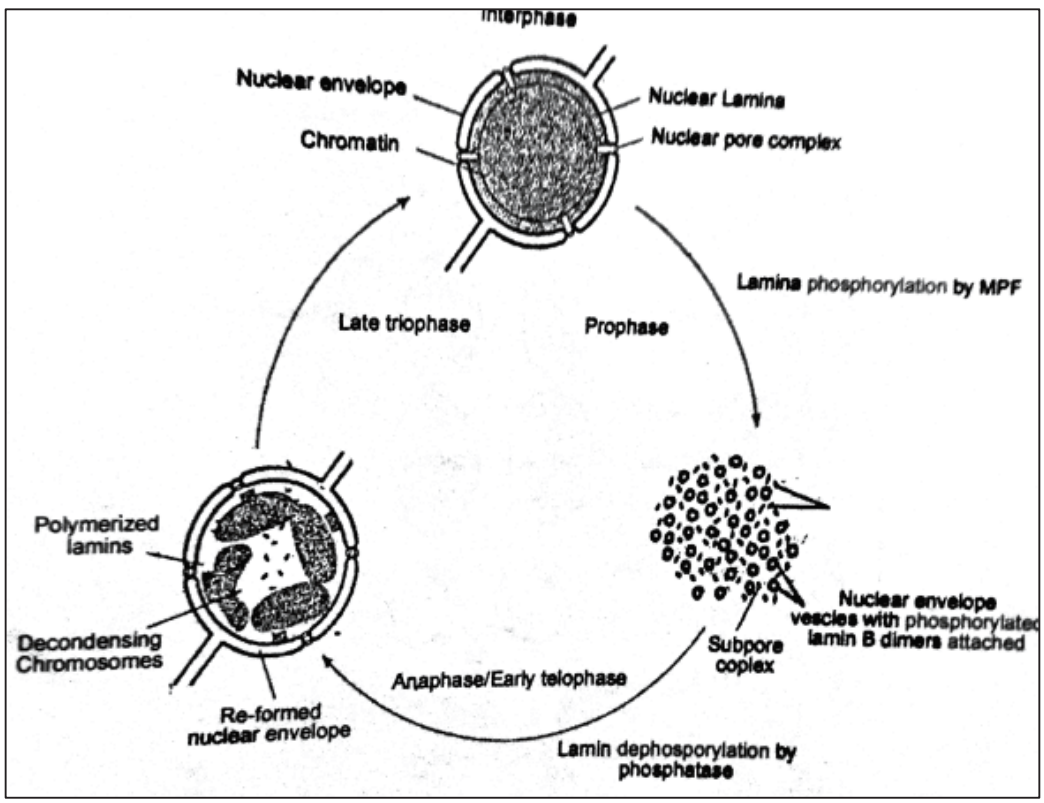


Fig. 74. Overview of depolymerisation and polymerisation of nuclear lamins during the cell cycle.

Nuclear assembly is induced by dephosphorylation and polymerisation of lamins into fibrous network. Small nuclear vesicle fuse with each others. All polymerised lamin A, B & C make a bridge between the chromatin and fused small vesicle of nuclear envelop.

Anaphase I: Separation of each tetrad into dyad chromosome takes place. A dyad chromosome with two chromatids appears at anaphase I as a double V (in metacentric) or double (in acrocentric) II or single (in telocentric chromosome).

Telophase I and Interphase II: After all the dyads reach the poles they form at each pole a telophasic nucleus with a nuclear membrane and they move into a short interphase passing through the second meiotic division. In Trillium the telophase I and interphase II is omitted. In maize cell plate is formed between the two telophasic nuclei but in others cytokinesis is deferred until the end of both meiotic division.

Prophase II: The chromosome appear as x since they are joined by common centric region and the four arms are widely separated.

Metaphase II: The nuclear membrane disappears prior to metaphase II and the spindle develops upon which the chromosomes orients with their centromeres lying on the equatorial plane.

Anaphase II: The centromere of each chromosome now becomes functionally double. It is fastened by chromosomal fibre & spindle fibre and the two chromatids move to the opposite poles. Anaphase II chromosome is called monad. On reaching the poles, chromosomes become organized into a new nucleus. Meiosis has run its course and both reduction in number and recombination has achieved (Fig. 75).

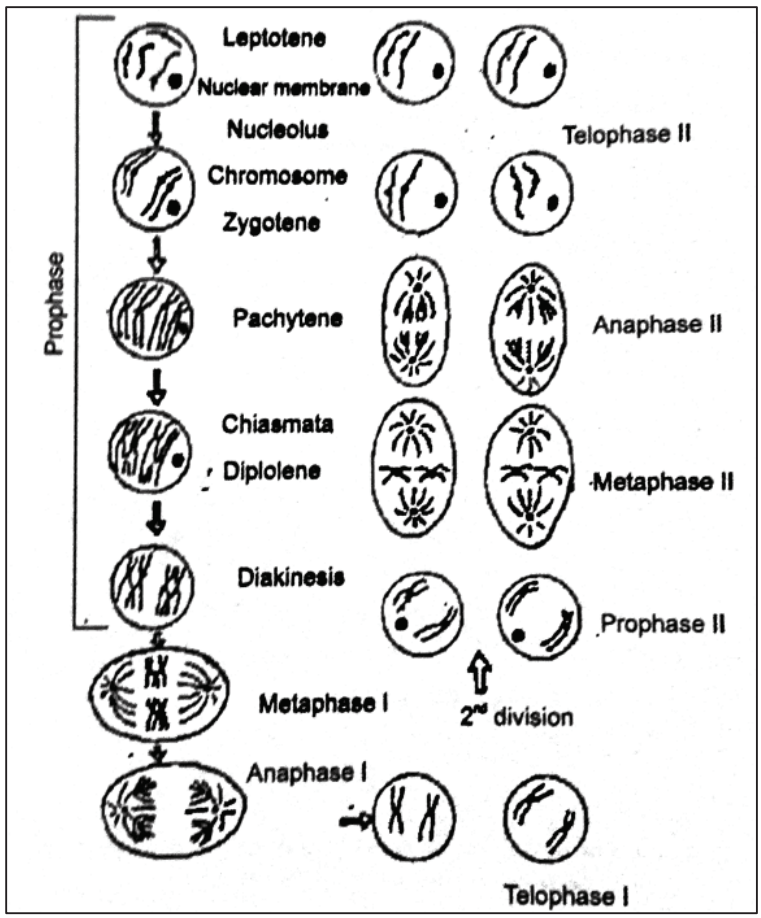


Fig. 75 Stages of meiosis.

Meiosis in ring chromosome: It was observed by Schwartz (1953, 1955) in x-rays induced ring chromosome 6 in zea mays and Michaellis (1958) observed in Antirrhinum -spontaneous its origin.

Several possible anaphase configuration can be produced by different patterns of crossing over in a ring and rod chromosome association as shown in Fig 76

Fig 76 shows origin of x-rays induced ring Chromosome 6 of *Zea mays*. The nucleus is stippled and its organiser cross hatched the centromere is clear circle.

All living cells of organism retain the full genetic potentialities of the original zygote, but certain genes are active at certain phases of development and other genes at other times. The control of genetic activity is of intense interest to biologists and biochemists.

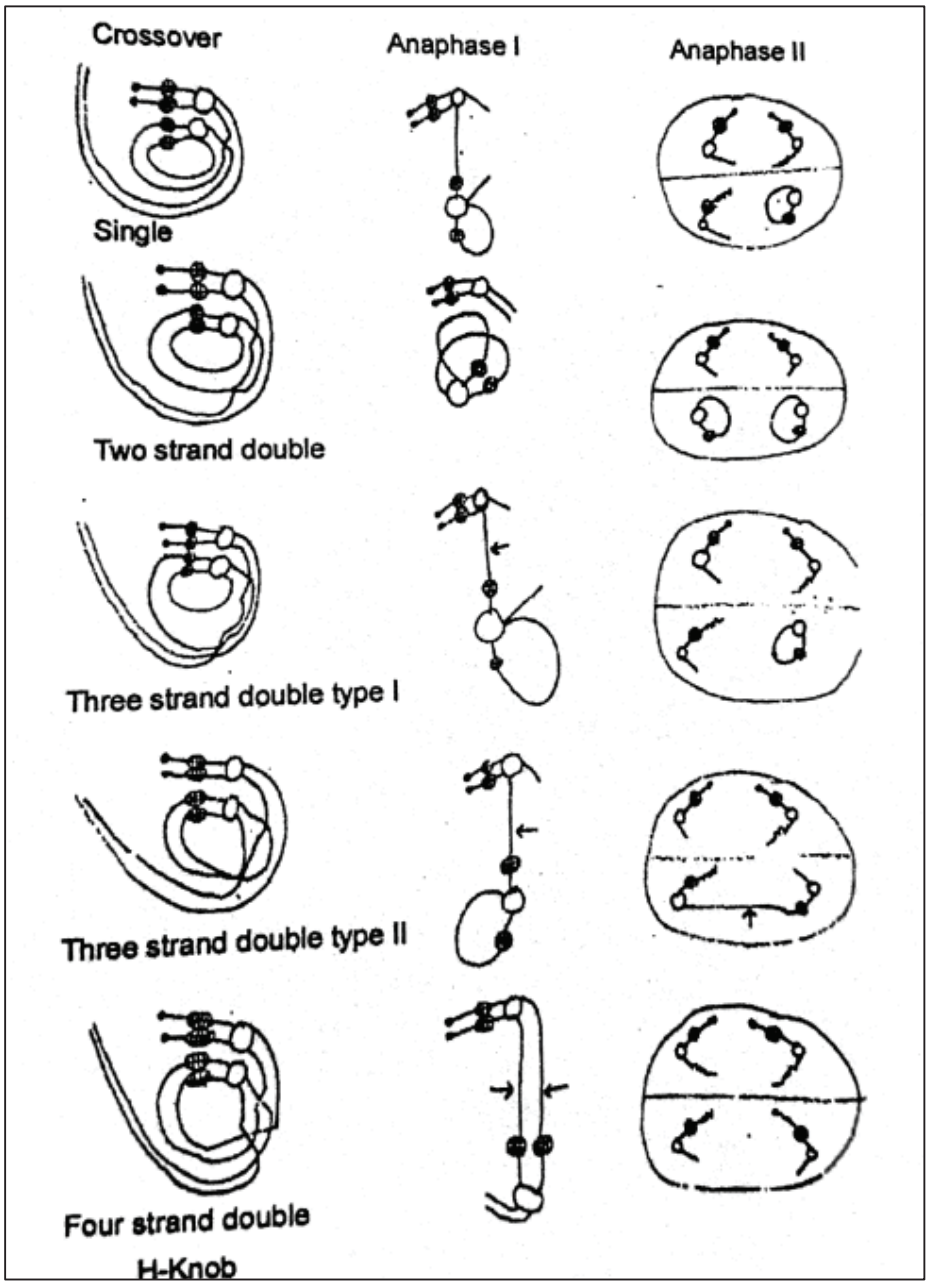


Fig. 76. Anaphaseic configuration from crossing over between ring chromosome 6 and its normal homologues in Zea mays

Chapter 26
Glossary, Cell Biology & Genetics

A. Units of measurement

1 million	=	1,000,000, (10^6)
1 billion	=	1,000,000,000, (10^9)
1 trillion	=	10^{12}
1 quadrillion	=	10^{15}
1 kilometre (km)	=	1000 metres
1 mile	=	1.6093 km
1 acre	=	4940 square yards = 4046.86m^2
1 metre (m)	=	100 cm, 39.37 inches
1 inch	=	2.54 cm
1 centimetre (cm)	=	10^{-2} m; 10mm
1 millimetre (mm)	=	10^{-3} m
1 micron (μ)	=	10^{-6} or 1,000,000 (One Million)
1 micro meter (mμ)	=	10^6m, 10^4 cm, 10^3 mm = 1000 nm
1 nano meter (nm)	=	10^9m, 10^8cm, 10^7mm, 10^4mμ, 10^{-1}nm
1 angstrom (Å)	=	10^{-10}m, 10^{-8cm}, 10^{-7}mm, 10^{-4}mμ, 10^{-1}nm
1 Picometre (pm)	=	10^{-12}m, 10^{-3}nm
1 femtometre (fm)	=	10^{-15}m, 10^{-6}nm
1 attometre (am)	=	10^{-18}m, 10^{-9}nm
1 tonne (t), metric tonne	=	1000 kg
1 ton (long ton)	=	1016.05 kg (=2240 lbs)
1 quintal	=	100 kg
1 kilogram (kg)	=	1000 g = 2.2 lb
1 pound (lb)	=	0.4536 kg = 453.6 g
1 gram (gm, g)	=	10^{-3} kg
1 milligram (mg)	=	10^{-6} kg, 10^{-3} g
1 microgram (g)	=	10^{-9} kg, 10^{-6} g
1 gallon (gal)	=	4.546 litres
1 U.S. gallon	=	3.785 litres
1 litre (l)	=	10^{-3} l, = 1cubic decimetre
I millilitre (ml)	=	1^{-3} cubic decimetre
1 Dalton	=	1.66 x 10^{-24} g

Test Questions (Cell Biology)

One Marks Questions (with answers)

1. Who stated first of all that new cells are formed from division of pre-existing cells?

   Schwann and Schleiden (1847).

2. At what stage a grown up cell divides?

   A cell divides when it has grown to a certain maximum size which disturbs the karyocytoplasmic or kern-plasma ratio.

3. Define cell division?

   The process of formation of new or daughter cells from a pre-existing cell is known as

   cell division.

4. Which type of mitosis is known as direct cell division?

   Amitosis is called direct cell division.

5. What is spireme stage?

   It is the name of early prophase when elongated chromosomes occur in overlapped condition like a ball of wool without their ends being visible.

Multiple Choice Questions

1. In meiosis I, centromere:

(a) Divides between prophase and metaphase
(b) Divide between and anaphase
(c) Does not divide
(d) Divides but the two daughters remain attached.

2. Meiosis II is meant for:

(a) Separation of chromatids
(c) Separation of sex chromosomes
(b) Separation of homologous chromosomes
(d) Synthesis of DNA.

3. Cell from pre-existing cells' is the famous saying of:

(a) Lamarck
(c) Mitochondria
(b) Schwann
(d) Chromosomes

4. Best stage to observe shape, size and number of chromosomes is:

(a) Prophase
(b) Metaphase
(c) Anaphase

5. Phragmoplast is precursor of:

(a) Cell plate
(b) Chromosomes
(c) Leucoplast
(d) Centriole.

Cell Biology and Genetics

GLOSSARY

Acentric chromosome. Chromosome fragment lacking a centromere.

Acquired character. A modification impressed on an organism by environmental influences during development.

Adaptation. Adjustment of an organism or a population to an environment.

Adenine. A purine base found in RNA and DNA.

Agglutinin. An antibody in blood plasma which brings about clumping (agglutination) of blood cells carrying an incompatible agglutinogen.

Agglutinogen. An antigen carried in red blood cells which reacts with a specific agglutinin in the plasma to cause clumping of the cells a specific antigen when injected an animal body stimulates the production of a corresponding antibody.

Agouti. A grizzled color of the fur of animals resulting form alternating light and dark bands on

the individual hairs plants.

Albinism. Absence of pigment in skin hair, and eyes of an animal. Absence of chlorophyll in plants

Alcaptonuria. An inherited metabolic disorder. Alcaptonuries excrete excessive amounts of

homogentisic acid (alcapton) in the urine.

Aleurone. The protein matter occurring in the form of minute grains in the endosperm of ripe seeds.

Allele (allelomorph), Adj, allelic (allelomorphic). One of a pair, or series of alternative genes

that can occur at a given locus in homologous chromosomes; one contrasting form of a gene. Alleles are symbolized with the same basic symbol (e.g., D for tall peas and d for dwarf), see multiple alleles.

Allopolyploid. A polyploid having chromosomes sets from different sources, such as different genetically. A polyploid containing sets derived from two or more species.

Allotetraploid. An organism with four genomes derived from hybridization of different species. Usually, in forms that become established, two of the four genomes are from one species and two are from another species.

Alu family. It is a set of dispensed and related sequences eact; 300hp. long in the human genome.

Amber codon is the nucleotide triplet UAG that causes termination of protein synthesis.

Amber mutation. It includes any change in DNA that creates an amber codon.

Amber suppressors are genes that code for tRNAs whose anticodons have been altered so that they can respond to UAG codons as well as to their previous codons.

Amino acid. Any one of a class of organic compounds containing the amino (NH2) group and the carboxyl (COON) group. Amino acids are building blocks of proteins. Alanine, proline, threonine,

histidine, lysine, glutamine, phenylalanine, tryptophan, valine, arginine, and leucine are among the common amino acids.

Amphidiploid. A species or type of plant derived from doubling the chromosomes in the F hybrid of two species, an allopolyploid capital in an amphidiploid the two species are definitely known whereas in other alloplyploids they may not be known.

Anaphase. The stage of nuclear division during which the daughter chromosomes pass from the equatorial plate toward opposite poles of the cell (towards the spindle). Anaphase follows metaphase and precedes telophase.

Androgen. A substance with male sex hormone active in vertebrate animals.

Aneuploid or heteroploid. An organism or cell having a chromosome number which is not an exact multiple of the monoploid (n) or basic number, hyperploid, higher (e.g., 4n+ 1), hypoploid, lower (e.g., 4n-1).

Anther. Male part of a plant flower in which pollen in produced.

Anthocyanin. Glucoside pigment in plants. Anthocyanins range in color from pink to blue.

Anthoxanthine. Any of a group of yellow pigments found especially in plants.

Antibody. Substance in a tissue or fluid of the body which acts in antagonism to a foreign

substance (antigen).

Antigen. A substance usually a protein, Which stimulates the production of antibodies when introduced into a living organism.

Antihemophilia globulin. Blood globulin which reduces the clotting time of hemophilic blood.

Apomixis. Development of an individual plant from an egg without fertilization or fusion with pollen nucleus. The egg may be normally reduced (haploid) or, more commonly, through failure of reduction division may remain diploid.

Asexual reproduction. A process of reproduction which does not involve the formation and union of gametes from the two sexes.

Asynapsis. The failure or partial failure of pairing of homologous chromosomes during the meiotic prophase, failure of chiasma formation resulting in a high frequency of univalent cell.

ATP. Adenosine triphosphate; an energy-rich compound that promotes certain activities in the

Atropy. Decrease in size or wasting away of an organ or tissue.

Autopolyploid. A polyploid that has multiple and identical or nearly identical sets of chromosomes (genomes). A polyploid species with genomes derived from the same original species.

Autoradiograph. A record or photograph prepared by labeling a substance as DNA with a radioactive material such as tritiated to develop on a film over a period of time. Autosome. Any chromosome that is not a sex chromosome.

Auxotroph. A mutant organism (bacterium) that will not grow on a minimal medium but requires the addition of some growth factor.

B

Backcross. The cross of a hybrid to one of the parental types. The offspring of such a cross are referred to as the back cross generation or backcross progeny (see Test cross).

Bacteriophage. Virus that attacks bacteria. Such viruses are called bacteriophages because they destroy their bacterial host.

Balanced lethal. Lethal genes on the same pair of chromosomes that remain in repulsion because of close linkage or crossover suppression. Only heterozygotes survive.

Binomial expansion. Exponential multiplication of an expression consisting of two terms connected a + or - such as (a + b)".

Bipartite structure (chromosome). One having two corresponding parts.

Biometry. Application of statistical methods to the study of biological problems.

Biotype. Distinct physiological race or strain within morphological species. A population of individuals with identical genetic constitution. A biotype may be made up of homozygotes or of heterozygotes, of which only the forimer would be expected to breed true.

Bivalent. A pair of synapsed or associated homologous chromosomes which may or may not have undergone the duplication process to form a group of four chromatids.

Blended inheritance. Inheritance in which the characters of two dissimilar parents appear to be blended in the offspring, and segregation fairs to occur in later generation.

Bud sport or chimera. A branch, flower, or fruit which differs genetically from the remainder of the plant.

C

Carcinogen. A substance capable of inducing cancer in an organism.

Carotenoid. Any of a group of yellow and red pigments found in plants and in the fat of animals.

Carrier. An individual which carries a recessive gene that is not expressed (i.e., obscured by a dominant allele).

Centriole. Central granule in many animal cells which appears to be the active principle of the centrosome and which undergoes duplication preceding the division of the centrosome proper.

Centromere or kinetochore. Spindle fibre attachment region of a chromosome.

Centrosome. A self-propagating cytoplasmic body usually present in animal cells and those of some lower plants, but not present in flowering plants; consisting of a centriole and sometime a centrophere (when active); located at each pole of the spindle during the process of nuclear division (mitosis).

Character (contraction of the word characteristic). One of the many details of structure, from, substance, or function. Which make up an individual organism. The Medelian characters represent the end products of development, during which the entire complex of genes interacts within itself and with the environment.

Chaiasma, (pl chalamata). A visible change of partners or crossover in two of a group of four chromatids during the first meiotic prophase. In the diplotene satge of meiosis the 4 chromatids of a bivalent are associated in pairs but in such a way that in one part or their. length two chromatids are associated, but in the remainder of their length each is associated with one of the other two chromatids. This point of "change of partner" is the chaiasma.

Chimera. A mixture of tissues of genetically different constitution in the same part of an organism. It may result from mutation, irregular mitosis, somatic crossing over, or artifical with parallel layers of genetically different tissues; or sectorial.

Chloroplastid. Green structure in plant cytoplasm.which contains chlorophyll and in which starch is synthesized. A mode of cytoplasmic inheritance independent of cytoplasmic inheritance independent of nuclear genes has been associated with these cytoplasmic structures.

Chondrisomes. See Mitochondria.

Chromatid. One of the two identical strands resulting from self-duplication of a chromosome during mitosis or meiosis. One of the four strands making up a bivalent during the later meiotic prophase.

Chromatin. The nuclear substance which takes basic stain and becomes incorporated in the chromosomes, so called because of the readiness with which it becomes stained with certain dyes (chromaticity).

Chromatography. A method of analyzing and comparing chemicals.

Chromocenter. Body produced by fusion of the heterochromatin regions of the autosomes and Y chromosome in salivary gland preparation of certain Diptera.

Chromomeres. Small bodies described by belling which he indetified by their characterstic size and linear arrangement on the chromosome thread.

Chromonema, chromonemata. An optically single thread within the chromosome.

Chromosome aberration. Abnormal arrangement of the chromosome complement caused by chromosomal breakage and reunion.

Chromosomes. Microsopically observable nucleprotein bodies, darkstaining within basic dyes, in the cell during cell division. They carry the genes which are arranged in linear of order. Each species has a characteristic chromosome number.

Cilium/pl. cilia; adj. Ciliate, Hair-like locomotor structure on certain cells; a locomotor structure on a cilate protozoan.

Cis. See Coupling.

Cistron. A unit of function; a working difinition of a gene. One cistron in the DNA specifies one polypeptide in protein synthesis.

Clone. All the individuals derived by vegetative propagation from a single original individual.

Codominant genes. Alleles, each of which produces an independent effect in heterozygotes.

Codon. A set of bases in DNA which will code one amino acid.

Coenzyme. A substance necessary for the activity of an enzyme.

Coincidence. The ration of observed double crossovers to expected doubles calculated on the basis of independent occurrence and expressed as a decimal fraction.

Colchicine. An alkaloid derived from the autumn crocus which is used as an agent to arrest spindle formation and interrupt mitosis.

Complementary genes. Genes which are similar in phenotypic effect when present separately,

but which together interact to produce a different character. If two such genes are complementary for a dominant effect, a 9.7 ration results in F2; if two complementary for recessive effect, a 15:1 ratio result in F2.

Conidium, (pl. conidia). An asexual spore produced by a specialized hypha in certain fungi.

Conjugation. Side-by-side association or synapsis of homologous chromosomes, as in meiosis; joining of paramecia or other protozoans as a part of the fertilization process.

Continuous variation. Variation not represented by distinct classes. Individuals grade into each other and measurement data are required for analysis, c.f. discontinuous variation. Multiple genes or polygenes are usually responsible for this type of variation.

Coordinate repression. Control of structural genes in an operon by a single operator gene.

Copolymers. Mixtures consisting of more than one polymer. Example: polymers of two kinds of organic bases such as uracil; and cytosine (poly-UC) have been combined for studies of genetic coding.

Copy choice'. An explanation for crossing over first suggested by J. Bell-ing in 1930, 'which assumes that crossing over occurs during the process of chromosome duplication. Duplication or "copying" proceeds partially along one homologue partially along the other.

Coupling or cis-arrangement. The condition in linked inheritance in which an individual heterozygous for two pairs of genes received the two dominant members from one parent and the two recessives from the other parent, (e.g., AABB x aabb). Compare repulsion.

Crossing over. A process inferred genetically by new associations of linked factors and demonstrated cytologically from new demonstrated associations of parts of chromosomes. It results in an exchange of genes and therefore produces combinations differing from those characteristics of the parents. The term 'genetic cross over' may be applied to the new gene combinations (see Recombination).

Crossover unit. A frequency of exchange of 1 percent of crossing over is equal to 1 unit on a linkage map.

Cytogenetics. Area of biology concerned with chromosomes and their implications in genetics.

Cytokinesis. Cytoplasmic division and other changes exclusive of nuclear division which are a part of mitosis or meiosis.

Cytology. The study of the structure and function of the cell.

Cytoplasm. The protoplasm of a cell outside the nucleus in which cell organelles (mitochondria, plastids, etc.) are embedded. All living parts of the cell expect the nucleus.

Cytoplasmic inheritance. Hereditary transmission dependent on the cytoplasm or stucture in the cytoplasm rather than the nuclear genes is extranuclear inheritance. Example: Plastic characteristics in plants may be inherited by a mechanism independent of nuclear genes.

Cytosine. A pyrimidine base found in RNA and DNA.

D

D-loop. It is a region within mitochondria) DNA in which a short stetch of RNA is paired with one strand of DNA displacing the orginal partner DNA strand in this region.

Dauermodification. A characteristic-induced by the environment which persists and appears to be inherited, sometimes for several successive generations. A type of acquired characteristic.

Deficiency (deletion). Absence of a segment of a chromosome involving one or more genes.

Determination. Process by which embryonic parts become capable of developing into only one kind of adult tissue or organ.

Deviation. As used in statistics, a variation from an expected number.

Diaknesis. A stage of meiosis just before metaphase I in which the bivalents are shortened and thickened.

Dicentric chromosome. One having two centromeres.

Differentiation. Modification of different parts of the body for particular functions during development of the organism.

Dihybrid. An individual that is heterozygous with respect to two pairs of alleles. The product of a cross between homozygous parents differing in two respects.

Dimer. A compound having the same percentage composition as another but twice polymerization.

Dimorphism. Two different forms in a group as determined by such characteristic as sex, size, or coloration.

Dioecious plant. A unisexual: each plant is either a male or a female (see Monoecious).

Diploid. An organism or cell with two sets of chromosomes (2n) or two genomes. Somatic tissues of higher plants and animals are ordinarily diploid in chromosomes constitution in contrast with the haploid (monoploid) gamets.

Diplonema, adj. Diplotene. That stage in prophase of meiosis following the pachytene stage, but preceding diakinesis, in which the chromosomes are visiblely double; stage characterized by centromere repulsion of bivalents resulting in the formation of loops.

Discontinuous variation. Distinct classes such as red vs white, tall vs dwarf e. f. continuous variation.

Disome. See Monosomic.

Distinguishable hybrid. A hybrid in which intermediate inheritance is expressed (i.e., the heterozygous combination is distinguishable by a phenotype).

Dizygous twins. Two-eggs or fraternal twins.

DNA. Deoxyribonucleic acid, the chemical material of which the information-carrying material or gene is composed.

Dominance. Applied to one member of an allelic pair of genes which has the ability to manifest itself wholly or largely at the exclusion of the expression of the other member. An inherited trait expressed when the controlling gene is either homozygous or heterozygous.

Drift or random genetic drift. Changes in gene frequency in small breeding populations due to chance fluctuations. Appropriately called the "Sewall Wright Effect" because of the basic contributions of professor wright in this area.

Duplication. The occurance of a segment more man once in the same chromosome or genome.

E

Egg (ovum). A germ cell produced by a female organism.

Electrophoresis. The migration of suspended particles in an electric field.

Embryo sac. A large thin-walled space within the ovule of the seed plant in which the egg and, after fertilization, the embryo develop, the mature female gametophyte in higher plants tract.

Endoderm. Inner layer of an early animal embryo which gives rise to the lining of the digestive

Endomitosis. Duplication of chromosomes without division of the chromosomes resulting in increased chromosome number within cells or endopolyploidy. Chromosome stands separate but the cells do not divide.

Endoplasmic reticulum. Network in the cytoplasm to which ribosomes adhere.

Endosperm. Nutritive tissue in the embryo sac of most angiosperms. It usually follow the fertilization of the two fused primary endosperm nuclei of the embryo sac by one of the two male garrets the endosperms is triploid (3n) but in some, for example lily, the endosperm is (5n).

Environment. The aggregate of all the external conditions and influences affecting the life and development of the organism.

Enzyme. A protein that accelerates a specific chemical reaction in a living system.

Epigenesis. The concept that embryo develops a new from undifferentiated material in each

generation, in contrast to preformation.

Epigenesist. One who visualizes embryological development as a step-by-step process from a relatively undifferentiated zygote to a complex adult.

Episome. A genetic element that may be present or absent in different cells, associated with chromosome or independent in the cytoplasm, Example: Fertility factor (F) in E.coli.

Epistasis. The suppression of the action of a gene by a gene or genes not allelomorphic to those

suppressed. Those suppressed are said to the sypostatic distinguished from dominance which refers to the members of one allelomorphic pair.

Equational or homotypic division. Mitotic-type division which is usually the second division

in the meiotic sequence; somatic mitosis and the non-reductional division of meiosis.

Equatorial plate. The figure formed by the chromosomes in the center (equatorial plane) of the spindle in mitiosis.

Estrogen. Female hormone or estrus-producing compound.

Euchromatin. Parts of chromosomes which are genetically active and have characteristic staining properties. Most of the arm-like structures of Drosophila salivary preparations are euchromatin. (Compare heterochromatin).

Eugenic. The situation that tends towards improvements in the hereditary qualities of future generations of mankind. C.f. dysgenic.

Eugenics. The sciences of improving the qualities of the human race; the application of the principles of genetics to the improvements of mankind.

Euploid. An organism or cell having a chromosome number that is an exact multiple of the monoploid (n) or haploid number. Terms used to identify different levels in an euploid, tetraploid, etc. see Heteroploid.

Exon. It is any segment of interrupted gene that is represented in the mature RNA product.

Expressivity. Degree of expression of a trait controlled by a gene. A particular gene may produce varying degrees of expression in different individuals.

Extrachromosomal. Structures that are not a part of the chromosomes; DNA units that control cytoplasmic inheritance.

F

F_1. The first filial generation. The first generation of descent from a given mating.

F_2. The second filial generation, produced by crossing inter se or by self-pollinating the F_1. The inbred "grandchildren" of a given mating. The term is loosely used to indicate any second generation progeny from a given mating, but controlled genetic experimentation, inbreeding of the F_1 (or equivalent) is implied.

Fertillity. Ability to produce offspring.

Fertilization. The fusion of a male gamete (sperm) with a female gamete (egg) to form a zygote.

Foetus. Parental stage of a viviparous animal between the embryonic stage and the time of birth. In man, the final seven months before birth.

Filial. See F_1 and F_2.

Fitness. The number of offspring left by an individual as compared with the average of the population or compared to individuals of different genotype.

Flagellum; pl. flagella; adj Flagellate. A whiplike organaelle or locomotion in certain cells; locomotor structures in flagellate protozoa.

Freemartin. A sexually underdeveloped female calf born twined with a male.

G

Gamete. A mature male or female reproductive cell (sperm or egg).

Gametogenesis. The formation of the gametes.

Gametophyte. That phase of the plant life cycle that bear the gamets; cells have n chromosomes.

Gene. A particulate hereditary determiner, a unit of inheritance; a unit of DNA; located in a fixed location in the chromosome.

Gene frequency. The proportion of one allele as represented in a breeding population. Gene pool. Sum total of all genes in a breeding population.-

Genetic equilibrium. Condition in a group of interbreeding organisms in which particular gene

frequencies remain constant through succeeding generations.

Genetics. The science of heredity and variation.

Genegold. DNA carrying cytoplasmic particle.

Genome. A complete set of chromosomes (hence of genes), inherited as a unit from one parent.

Genotype. The genetic constitution (gene make-up) expressed and latent, of an organism (i.e., Dd or dd). Individuals of the same genotype breed alike. (compare with phenotype)

Germ cell. A reproductive cell capable when mature of being fertilized and reproducing an entire organism.

Germ plasm. The germinal material or physical basis of heredity. The sum total of the genes. Guanin. A purine base found in DNA and RNA.

Gynandromorph. An individual of which one part of the body is female and another part is male; a sex mosaic.

Haemoglobin. Conjugated protein compound containing iron, located in eryhrocytes of vertebrates; important in the transportation of oxygen to the cells of the body.

Haemolymph. The mixture of blood and other fluids in the body cavity of an invertebrate.

Haemophillia. A bleeder's disease; tendency to bleed f-ely from even a slight wound; hereditary condition dependent on a sex-linked recessive gene.

Haploid or monoploid. An organism or cell having only one'complete set (n) of chromosomes or one genome.

Haptoglobin. A serum protein, alphaglobulin in the blood.

Helix. Any structure with a spiral shape. The Watson and Crick model of DNA is in the form of a double helix.

Hemizygous. The condition in which only one allele of a pair is present, as in deletion or sex linkage.

Heredity. Resemblance among individuals related by descent; transmission of traits from parents to offspring plant.

Heritability. Degree to which a given trait is controlled by inheritance.

Hermaphrodite. An individual with both male and female reproductive organs. See Monoecious

Heterocaryon. A fungus hypha with two nuclei of different genotypes; the nuclei do not fuse but divide independently and simultaneously as new cells are formed.

Heterochromatin. Chromatin staining differently and functioning differently than euchromatin which contains most of the genes. In drosphila salivary preparations the heterochromatin is mostly in the chromocenter.

Heterogametic sex. Producing unlike gametes, particularly with regard to the sex chromosome In species in which the male is "XY", the male is heterogametic, the female (XX). Homogametic.

Heteroplid or aneuploid. An organism characterized by a chromosome number other than the true haploid (monoploid) or diploid number (2n+1 or 2n-1) see Euploid.

Heteropyknosis, adj. Heteropyknotic. Property of certain chromosomes or of their parts remain more dense and stain more intensely than other chromosomes or parts during the nuclear cycle.

"Holandric" gene. A gene carried on the Y chromosome and therefore transmitted from father to son.

Homogametic sex. Producing like gametes (see Heterogametic sex.)

Homologous Chromosomes. Chromosomes which occur in pairs and are generally similar in size and shape, one having come from the male and one from the female parent.

Homozygote. Adj. Homozygous. An organism whose chromosomes carry identical members of any given pair of genes. The gametes are therefore all alike with respect to this locus and the individual will breed true.

Homunculus. Miniature individual imagined by early biologists to be present in the sperm cell.

Hormone or internal secretion. An organic product of cells of part of the body which is transported by the body fluids to another part where it influences activity or serves as a coordinating agent.

Hybrid. An offspring of homozygous parents differing in one or more genes.

Hybrid vigor or heterosis. Unusual growth, strength, and health of Hybrids from two less vigorus parents.

Hybridization. Interbreeding of species laces, varieties, etc. among plants or animals. A process of forming a Hybrid by cross pollination of plants or by mating animals of different type.

Hypha, (pl-hyphae). A branched filament of a fungus.

Idiogram. A diagrammatic representation of the chromosomes of an individuals illustrating their relative sizes and appearance.

Immunize. To induce a resistance to parasite or foreign substance Noun: immunization.

Immunoglobulin. see globulin.

Inbreeding. Matings among related individuals.

Incomplete dominance. Expression of heterozygous alleles different from those of the parents producing distinguishable hybrids.

Independent combination or independent assortment. The random behaviour of genes in different chromosomes. The distribution of one pair of genes is not controlled by other nonhomologous chromosomes.

Inhibitor. Any substance or object that retards a chemical reaction; a major or modifier gene

that interferes with a reaction.

Interference. Crossing over at one point reduces the chance of another cross-over in adjacent regions. Detected by studying crossovers of three or more linked genes.

Intermediate inheritance. An alternative to dominance in which the heterozygotes are distinguishable form both homozygotes.

Interphase. The stage in the cell cycle when the cell is not dividing; the metabolic stage; the stage following telophase of one division and extending to the beginning of prophase in the next

division.

Intersex. An organism displaying secondary sexual characters intermediate between male and female; a type that shows some phenotypic characteristics of both males and females.

Intron. It is a segment of DNA that is transcribed but is removed from within the transcript by splicing together the sequences (exons) on either side of it.

Inversion. A rearrangement of a group of genes in a chromosome in such a way that their order in the chromosome is reversed.

Isoagglutinogen. An antigen such as A or B blood- type factor that occurs normally in an

individual, that is without artificial stimulation.

K

Karyotype. The appearance of the metaphase. Chromosomes of an individual or species; comparative size, shape, and morphology of the different chromosomes.

Kb. It is abbreviation for 1000 base pairs of DNA or 1000 bases or RNA.

Kinetochore. See Centromere.

Kinetosome. Granular body at the base of a flagellum or a cilium.

L

Lampbrush chromosomes. Greatly enlarged chromosomes in the oocytes of amphibians. They have a main axis and side loops suggesting the name "lampbrush."

Leptonema, adj. Leptotene. Stage in meiosis immediately preceding synapsis in which the chromosomes appear as single fine threadlike structures.

Lethal gene. A gene that renders inviable an organism or a cell possessing it.

Linkage. Association of genes that are physically located in the same chromosome, such a group of linked genes is called a linkage group.

Locus, (pl. Loci). A fixed position on a chromosome occupied by a given gene or one of its alleles.

Lysis. Destruction of bacteria as the breaking of cells following infection by bacteriophage.

Lysogenic bacteria. Those harbouring temperate bacteriophages.

M

Macromolecule. A large molecule; term used to identify such structures as ribosomes in living cells.

Map units. One percent of crossing over represents one unit on a linkage map.

Maternal Inheritance (maternal effect). Inheritance from nother to offspring unaffected by inheritance from the father.

Maturation. The formation of gametes or spores.

Mean. The arithmetic average; the sum of all measurements or values of a group of objects divided by the number of object.

Megaspore (macrospore). A spore having the property of giving rise to a gametophyte (embryosac) bearing only a female gamete. One of the four cells produced by two meiotic divisions of the megaspore-mother-cell called a megasporocyte. Megaspore-mother-cell or megasporocyte. The cell which undergoes two meiotic divisions to produce four megaspores.

Megasporogenesis. Process of production of megaspores. See megaspore.

Meiosis. The process by which the chromosome number of a reproductive cell becomes reduced to half the diploid (2n)-or somatic number, results in the formation of gametes in animals, or of spores in plants; important source of variability through recombination.

Melanin. Brown or back pigment of animal origin.

Mendelian population. A natural interbreeding unit of sexually reproducing plants or animals.

Merozygote. Partial zygote produced by a process of partial genetic exchange such a transformation in bacteria.

Messenger- RNA. A particular kind of RNA that carries information necessary for protein synthesis form the DNA to the ribosome.

Metabolism. Sum total of all chemical processes in living cells by which energy is provided and used.

Metamorphosis. Change of form, structure, or substance.

Metaphase. That stage of cell division in which the chromosomes are most discrete and arranged in an equatorial plate.

Microspore. One of the four cells produced by the two meiotic divisions of the microspore - divisions of the microsporocyte. A spore having the property of giving rise to a gametophyte bearing only male gamete.

Microspore-mother-cell. See Pollen mother cell.

Microsporogenesis. Process of production of microspores. See Microspore.

Mitochondria. Small bodies in the cytoplasm of most plant and animal cells.

Mitosis. Cell division in which there is first a duplication of chromosomes followed by migration of chromosomes to the ends of the spindle and dividing of the cytoplasm.

Modifier or modifying gene. A gene that affects the expression of another nonallelic gene.

Monoecious plant. Plant with separate staminate (male) and pistillate (female) flowers on the same plant.

Monohybrid. An offspring of two homozygous parents differing from one another in only one

gene locus.

Monohybrid cross. A cross between parents differing in only one trait or in which only one

trait is being considered.

Monomer. A simple molecule of a compound of relatively low molecular weight. Monoploid or

haploid. Individual having a single set of chromosomes or one genome(n).

Monosomic. A diploid organism lacking one chromosome, disome to two chromosomes of a kind, trisome to three chromosomes of a kind.

Monozygous twins. One-egg or identical twins.

Morphology. Study of the form of an organism. Development history of visible structures and the comparative relation of similar structures in different organisms.

Mosaic. An organism part of which is made up of tissue genetically different form the remaining part.

Mosaic egg. A fertilized egg (zygote) in which a high degree of organization has already occurred. Parts develop according to a predetermined plan.

Multiple genes. Those with an unusually high mutation rate (unstable genes).

Mutant. A cell or individual organism that shows a change brought about by a mutation. A changed gene.

Mutation. A sudden change in the genotype of an organism. The term is used loosely to included point mutations involoving a single gene, and chromosomal changes.

Mutation pressure. A constant mutation rate that adds mutant genes to a population. Mutator

genes. Those which increase the frequency of mutation in other genes

Mutot. The smallest unit of DNA that can undergo change resulting in a mutation.

Mycelium, (pl. mycelia). Threadlike filament making up the vegetative portion of thallus in fungi.

Natural Selection. Natural process favouring individual that are better auapted and tending to eliminate those unfitted to their environment.

Non-disjunction. Failure of disjunction or separation of homologous chromosomes in meiosis

resulting in too many chromosomes in some daughter cells and too few in others.

Nucleic acid. An acid composed of phosphoric acid, pentose sugar, and organic bases. DNA and RNA are nucleic acids.

Nucleoprotein. Conjugated protein composed of nucleic acid and protein, and making up the chromosomes.

Nucleotide. A unit of the DNA molecule containing a phosphate, a sugar, and an organic base.

Nucleus. Part of a cell containing genes and surrounded by cytoplasm.

Nullisomic. An otherwise diploid cell or organism lacking both members of a chromosomes pair (chromosome formula: 2n-2).

O

Octaploid. Cell or organism with eight genomes or monoploid sets of chromosomes; a polyploid.

Ontogeny. The complete development history of an organism form egg. Spore, bud, etc, to the adult individual.

Oocyte. The egg-mother cell; the cell that undergoes two meiotic divisions (oogenesis) to form the egg cell. Primary oocyte-before completion of the first meiotic division; secondary oocyte-after completion of the first meiotic division.

Oogenesis. The formation of the egg or ovum in animals.

Oogonium. Pl. oogonia. A germ cell of the female animal before meiosis begins.

Operator gene. A gene making up a part of an operon which controls the activity of one or more structural genes.

Organelle. Specialized part of a cell with a particular function or consists of an operator gene and structural genes.

Organizer. An inductor, a chemical substance in a living system that determines the fate in

development of certain cells or groups of cells.

Outbreeding. Mating of unrelated individuals or of those not closely related.

Ovary. The swollen part of the pistil of a plant flower that contains the ovules. The female reproductive gland or gonad in animals.

Ovule. The macrosporangium of a flowering plant that becomes the seed. It includes the nucellus and the integuments.

P

P. Symbolizes the parental generation or parents of a given individual.

Pachynema, adj. Pachytene. A midprophase stage in meiosis immediately following zygonema and preceding diplonema. In favourable microscope preparations, the chromosomes are visible as long, paired threads. Sometimes four chromatids are observed.

Paracentric inversion. An inversion that is entirely within one arm of a chromosome, does not include the centromere.

Parameter. Value or constant based on an entire population, c.f. statistic.

Parthenogenesis. The development of a new individual from an egg without fertilization.

Paternal. Pertaining to the father, set of chromosomes derived from the sperm in animals or pollen in plants.

Pathogen. An organism or virus that causes a disease.

Pedigree. A table, chart or diagram representing the ancestral history of an individual.

Penetrance. The proportion (in percent) of individuals with particular gene combination that express the corresponding trait.

Peptide. A compound containing amino acids. A breakdown or buildup unit in protein metabolism.

Peptide bond. A chemical bond holding amino acid subunits together.

Pericentric inversion. An inversion including the centromere, hence involving both arms of a chromosome.

Phage. See Bacteriophage.

Phenocopy. An organism whose phenotype (but not genotype) has been changed by the environment to resemble the phenotype of a different (mutant) organism.

Phenotype. Characteristic of an individual observed or discernable by other means (i.e., tallness in garden peas; color blindness or blood-type in man). Individuals of the same phenotype may appear alike but may not breed alike.

Pistil. The female part of the flower consisting of ovary, style, and stigma.

Plaque. Clear area on an otherwise opaque culture plate of bacteria where the bacteria have been killed by a virus.

Plasmagenes. Self-replicating cytoplasmic particles capable of transmitting traits in inheritance. Unit believed to be responsible for extranuclear inheritance.

Plastid. A cytoplasmic body found in the cells of plants and some protozoans. Chloroplastids produce chlorophyll which is involved in photosynthesis.

Pleiotropy, adj. Pleiotropic. Condition in which a single gene influence more than one trait.

Polar bodies. In female animals, the smaller cells produced at meiosis that do not develop into egg cells. The first polar body is produced at division I and may or may not go through division II. The second polar body is produced at division II.

Pollen-mother-cell, microsporocyte or microspore mother-cell. The plant-cell that undergoes the meiotic sequence and produces four microspores.

Pollen parent. The male plant that produces pollen. The term is used to designate the pollen-producing parent in a cross.

Polydactyly. The occurrence of more than the usual number of fingers or toes. Polygene. One of a series of multiple genes involved in quantitative inheritance.

Polymer. A compound composed of two or more units of the same substance; results from a process of polymerization.

Polymerization. Chemical union of two or more molecules of the same kind to form a new compound having the same elements in the same proportions, but a higher molecular weight and different physical properties.

Polymorphism. Two or more kinds of individuals in a breeding population where the rarer form is maintained by some mechanism other then recurrent mutation.

Polynucleotide. A unit of DNA consisting of four nucleotides.

Polyeptide. A compound containing two or more amino acids and one or more peptide groups. They are called dipeptides, tripeptides, etc. according to the number of amino acids contained.

Polyploid. An organism with more than two sets of chromosomes or genomes (e.g, triploid (3n), tetraploid (4n), pentaploid (5n), hexaploid (6n), heptaploid (7n), octoploid (8n).

Population. Entire group of organisms of one kind; an interbreeding group of plants or animals. The infinite gourp form which a sample might be taken.

Population genetics. The branch of genetics which deals with frequencies of alleles in breeding groups of individuals.

Position effect. A difference in phenotype that is dependent on the position of a gene or group of genes in relation to other genes.

Prepotency. Ablity of an individual to transmit particular characterstics to the offspring.

Probability. Likelihood of occurrence.

Progeny. Offspring of animal or plants; individuals resulting from a particular mating. Prophage.

Non-infectious stage of a temperate phage in a bacterial cell.

Prophase. The states of mitosis or meiosis from the appearance of the chromosomes following interphase to metaphase.

Prosthetic group. An organic component of conjugated proteins. Conjugated proteins are a combination of simple protein and another substance called the prosthetic group. Example: Nucleoproteins have as prothetic groups, nucleic acids.

Protoplast. A unit body of protoplasm; the mass of living material within a single cell, including cytoplasm and nucleus.

Prototroph. A wild-type organism (bacterium) that will grow on a minimal medium.

Pseudoalleles, closely liked genes that behave ordinarily as if they were alleles but which have

been shown by extensive experiments to be separable by crossing over.

Pseudodominance. Apparent dominance of a recessive gene in the area opposite a chromosome deficiency. A recessive gene may come to expression to be separable by crossing over.

Pseudodominance. Apparent dominance of a recessive gene in the area opposite a chromosome deficiency. A recessive tgene may come to expression to because its dominant allele is absent.

Pure line. A strain of organisms that is comparatively pure genetically (homozygous) because of continued inbreeding or through other means.

Q

Quadripartite structure (chromosome ring), One having hour corresponding parts.

Quadrivalent. A group of 4 chromosomes of the same kind in a cell. They may be united by chiasmata in the first division prophase of meiosis. Quadrivalent result from chromosome. Translocations.

R

Recessive. Applied to one member of an allelic pair lacking the ability to manifest itself when the other or dominant member is present. An inherited trait expressed only when the controlling gene is homozygous.

Reciprocal crosses. A second cross involving the same strains but carrid by sexes opposite to those in the first cross, e.g., a female from strain A x a male from stain B and a male from A x female from B are reciprocal crosses.

Recombination. The observed new combinations of traits different from those combination exhibited by the parents. Percentage of recombination equals percentage of crossing over only when the genes are relatively close together. Cytological chiasma refers to the observed change of partners among chromatids whereas recombination or genetic crossing over refers to the observed genetic result. Random recombination occurs in meiosis.

Recon. The smallest unit of DNA capable of recombination, in becteria, the smallest unit capable of being integrated or replaced in host chromosome subjected to the transformation process. Reduction division or heterotypic division. Phaso of meiosis in which the maternal paternal elements of the bivalent separate, c.f. equational or homotypic division.

Regulator gene. A gene that controls the rate of production of another gene or genes. Example:

The operon involved in lactose production in E. soli has a regulator, an operator and structural genes.

Repulsion or trans arrangements. The condition in linked inheritance in which an individual heterozygous for two pairs of linked genes received the dominant member of one pair from one parent and the reverse arrangement (from the other parent, (e.g., Aabb x aaBB). Compare coupling.

Reticulocyte. A young red blood cell.

Reversion. Appearance of a trait expressed by a remote ancestor, a throwback atavism.

Rihousox. Cytoplasmic structure in which proteins are synthesized.

Roentgenr (r) Unit of ionizing radiation.

RNA-Ribonucleic acid. The information carrying material in plant viruses. Certain kinds of RNA are involved in the transcription of genetic information from DNA (i.e., m-RNA); transfer of amino acids to the ribosomes for incorporation into proteins (i.e., t-RNA); and the makeup of the ribosome (i.e., r-RNA).

S

Segregation. The separation of the paternal from maternal chromosomes at meiosis and the consequent separation of alleles and the their phenotypic differences as observed in the offspring. Mendels's first principle of inheritance.

Selection. Any natural or artificial process favouring the survival and propogation of certain individuals.

Selection pressure. Effectiveness of the environment in changing the frequency of alleles in a population of individuals.

Self-fertilization. Pollen of a given plant fertilizes the ovules of the same plant. Plants fertilized in this way are said to be selfed.

Serology, adj. Serological. The study of interactions between antigens and antibodies.

Sex chromosomes. Chromosomes that are particularly connected with the determination of sex

Sex-influenced traits. Those traits in which the dominant expression depends on the sex of the individual (e.g., horns in sheep are dominant in males and recessive in females).

Sex-limited. Expression of a trait in only one sex. Examples: milk production, horns in Rambouillet sheep, egg production.

Sex-linkage, Association o linkage of a hereditary character with sex, the gene is in a sex chromosome.

Sex mosaic. See Gynandromorph.

Sex reversal. A change in the characteristics of an individual from male to (Female and vice versa).

Sexual reproduction. Reproduction involving the formation of mature germ cells (i.e., eggs and sperm).

Sib mating, crossing of siblings. Matings involving two or more individuals of the same parentage brother-sister mating.

Sickle-cell anaemia, An inherited blood abnormality produced by defective haemoglobin. Red blood cells become sickle shaped because of reduced oxygen tension.

Soma cells. The cells that make up the body in contrast with germ cells that are capable of reproducing the organism.

Somatic cells. Referring to body tissues; having two sets of chromosomes, one set normally coming from the female parent and one from the female parent and one from the male, as contrasted with germinal tissue that will give rise two germ cells.

Somatoplasm. The non-productive material making up the body of the organism in contrast to germ plasm.

Species. A basic category in the classification of plant or animals below the genus level and above the variety or subspecies level. A group of plants or animals that interbreed among themselves but ordinarily not with members of other species.

Sperm, abb. Of spermatozoon, pl. spermatozoa. A mature male germ cell.

Spermatids. The four cells formed by the meiotic divisions in spermatogenesis. Spermatids become mature spermatozoa or sperm.

Spermatocyte or sperm-mother-cell. The cell that undergoes two meiotic divisions (spermatogenesis) to form four spermatids; primary spermatocyte, before completion of the first meiotic division; secondary spermatocyte after completion of the first meiotic division.

Spermatogenesis. The process by which maturation of the gametes (sperm) or the male takes.

Spermatagonium, pl. spermatogonia. Primordial male germ cell that may divide by mitosis to produce more spermatogonia. A spermatogonium may enter a grow phase and give rise to a primary spermatocyte.

Spore. A unit of Protoplasm capable of developing asexually into a new individual; in higher plants the haploid product of meiosis that gives rise to male or female garrets.

Sporocyte. The spore mother cell of a plant.

Sporogenesis. Formation of the spore or reproductive element in plants.

Sporophyte. The spore-forming generation in the life cycle of plants, normally diploid.

Stamen. Male part of the flower which includes the pollen-producing another and the filament.

Standard deviation. A measure of variability in a population of individuals.

Standard error. A measure of variation of a population of means.

Statistic. A value based on a sample or samples of a population from which estimates of a population value or parameter may be obtained.

Sterility. Inability to produce offspring.

Stigma. Female part of the flower which receives pollen.

Structural gene. A gene that controls actual protein production by determining the amino acid sequence (c.f. operator and regulator genes).

Style. Part of the pistil between the stigma and the ovary in a flower through which the pollen tube grows.

Sublethal gene. A lethal gene with delayed effect. The gene in proper combination kills its possessor in infancy, childhood, or adulthood.

Symbiont. An organism living in intimate association with another dissimilar organism.

Synapsis. The conjugation or pairing of homologous chromosomes.

Syndrome. A group of symptoms that occur together and represent a particular disease.

Syngamy. Union of the gametes in fertilization.

Telophase. The last stage of mitosis in which the chromosomes are assembled at the poles of the division spindle.

Temperate phage. A phage (virus) that invades but does not destroy the host (bacteria) cell (c.f. virulent phage.)

Template. A pattern or mold. DNA stores coded information and acts as a model or template from which information is taken by messenger-RNA.

Terminalization. Repelling movement of the centromeres of bivalents in the diplotene stages of the meiotic prophase, that tends to move the visible chiasmata toward the ends of the bivalents. The point where the exchange of chromatids or the chiasma occurs is believed to be the point of a genetic cross-over, but the chiasmata appear to slip toward the ends of the bivalents, and therefore after the diplotene looping begins there is no longer a relation between the chiasma and the point of crossing over.

Test cross. Backcross to the recessive parental type or a cross between genetically unknown

individuals with a fully recessive tester to determine whether an individual in question is heterozygous or homozygous for a certaing allele. Also used as a test for linkage.

Tetrad. The four cells arising from the second meiotic division in plants (i.e., pollen tetrads). The term is also used to identify the quadruple chromatids group of chromatids formed by the association of split homologous chromosomes during meiosis, but the term bivalent is preferred in this usage.

Tetraploid. An organism whose cells contain four haploid (4n) sets of chromosomes or genomes.

Tetrasomic, noun: Tetrasome. Pertaining to a nucleus or an organism having four members of one of its chromosomes, the remainder of the chromosomes being normally diploid. (Chromosome formula: 2n +2).

Thymine. A pyrimidine base found in DNA. The other three organic bases, adenine, cytosine and guanine are found in both RNA and DNA but in RNA thymine is replaced by uracil.

Totipotent cell. An undifferentiated cell such as as blastomere which when isolated develops into a complete embryo.

Transduction. Genetic recombination in bacteria mediated by acteriophage.

Transferrin. Blood serum protein, beta globulin (see Globulin).

Transfer-RNA. A particular kind of RNA that transports amino acids to the ribosome where they are assembled in proteins.

Transformation. Genetic recombination in bacteria brought about by adding foreign DNA to a culture.

Transgressive variation. The appearance in the F2 (or later) generations of individuals showing

a more extreme development of a character than either parent. Assumed to be due to cumulative and complementary effects of genes contributed by the parents of the original hybrid. Adequate testing of variation in the parents is required to establish its occurrence.

Translocation. Change in position of a segment of a chromosome to another part of the same

chromosome or to a different chromosome.

Trihybrid. The offspring from homozygous parents differing in three pairs of genes. Triploid. An organism with three genomes or sets of chromosomes (3n).

Trisomic. An otherwise diploid cell or organism which has an extra chromosome of one pair (chromosome formula: 2n+1).

Twins. To individuals from the same birth. Identical twins- from the same fertilized egg. Fraternal twins-from different fertilized eggs.

U

Unipartite structures (chromosomes). Single units.

Univalent, Aa chromosome unpaired at meiosis.

Uracil. A pyrimidine base found in RNA but not in DNA. In DNA Uracil is replaced by thymine.

V

Variance. The square of the standard deviation.

Variation. In biology, the occurrence of differences among the individuals of the same species.

Viability. Degree of capability to live and develop normally.

Virulent phage. A phage (virus) that destroys the host (bacterial) cell (see temperate phage).

W

Wild type. The customary phenotype or standard for comparison.

X

X chromosome. A chromosome associated with sex determination. In most animals k the female has two and the male one.

Xenia. Immediate effect of pollen on the endosperm, due to the phenomenon of double fertilization in the seed plants.

Y

Y chromosome. The male to the X chromosome in the male of most animal species, usually carries few genes; in Drosophila composed mostly of heterochromatin. In man the Y chromosome carries genes which influence maleness.

Z

Zygonema, adj. Zygotene. Stage in meiosis during which synapsis occurs; after the leptotene stage and before the pachytene stage in the mewiotic prophase.

Zygote. The cell produced by the union of two mature sex cells (gemetes) in reproduction; also used in genetics to designate the individual developing from such a cell.

Ethno Botany

Ethnobotany is the study of plants utilised by the aboriginal and Primitive tribes. The term ethno-Botany was suggested by Hursh-Herger (1896). Tribals worship plants and they are superstitious. Their custom and traditions are associated with plants. Faulks (1958), Richard (1978), K.S. Manilal (1988) have drawn convincing Conclusion in their publications-Small about relationship between the Plants and societies of primitive people.

We know *Ocimum sanctum* (Tulsi) *Ficus religiosa* (Peepal) are sacred for Hindus and others. Tribals in Kerala use *Terminelia belerica* against allergy caused by *Senecarpus anacardium* (Chiru vernacular name) in their language.

Economy of Sri Lanka is based on Tea and coffee similarly. economy of Brazil is based on rubber plant. In India politics is influenced by sugar lobby of north India, rubber lobby of kerala and so on. We can say that plants have a role in human Civilization. The subject of ethnobotany is in its in fancy and there is need of more research work in this field. The ancient record of medicinal use of plants is mentioned in Reg Veda and Atharva veda. Ayurvedic System of medicine was at its top in Buddhist Period (800 BC-1000AD).

Susruta and Charak had depicted medicine system which formed the basis of Ayurvedic system of medicines in modern time. Chark samhita and susurta Samhita are compilation of this system of medicines. Gupta (1919) and Sarma(1931) have given excellent information about ayurvedic system of medicine.

Hippocrates, Theophrastus and Discorides have mentioned Indian plants in their writings. Ancient Roman authors and Greek used to consider that the tubers of orchids resembles to testicles, hence these are meant for giving sexual power According to doctrine of signature herbals were prepared in Rome and Greek and it was revived in Europe by Paracelsus and others in sixteenth century.

American nation is trying to control the plant research of different countries through a treaty called GATT. According to this treaty there was a general agreement by 23 countries like Geneva, Switzerland etc on tariff and trade (GATT), signed on 30th October 1947. It be effective from January 1, 1948 and W.T.O. approved it on January 1, 1995.

Folk remedies: V.K. sarin (1990) has worked on folk remedies. Names of disease and

plants to remedy them are as such:

Insomnia disease: The leaves of Ericaceae are used to cure it.

Sexual disease: various sexual diseases are cured by roots of *Polygonum verticillatum* plant.

Rhizomes and fronds of *Adiantum caudatum* (fern plant) are used to cure the herpes disease.

Urticaria: *Thymus serphyllum* and *Urtica dioica* are used to cure urticaria.

Diarrhoea and vomiting: Fresh leaves juice of mint (*Mentha sylvestris*) is used to treat the ailment.

Tribals are very expert in herbal medicines. They know the various plants to treat the diseases. some examples are given below:

Acacia catechu: The heart wood of this plant is used to control excessive bleeding during

childbirth.

Ricinus communis: Its leaves are warmed and tied on abdomen' to cure abdominal pain.

Calotropis Procera: Ash of the leaves is placed on the tongue to cure cold and cough.

Azadirachta indica: Leaves of this plants are poultice and applied at night, on eyelids to

cure night blindness.

Achyranthes aspera: Ash of whole plant mixed with maize flour to make cake. Baking of ake is made and is given before sleeping to cure cough and cold

Chloroplasts

Chloroplasts are green plastids found in plant cells. It is oval or spherical in appearance. Its diameter varies from 4-6 μ and thickness varies from 1-3 μ. Its number varies from 20-40. Chloroplasts have double membrane, chlorophyllous, lamellar structure. Lamellae are protein rich compound known as stroma. Some small granules are present within the homogenous stroma known as grana. The diameter of each granum varies from 0.5-2.0 micrometer. The membrane consists of two layers, outer and inner with inter membrane space. Grana are aggregation of flat membranous sacs or vesicles known as thylakoids. Thylakoids are stacked one after another. Space between thylakoids is termed as thylakoid space. Some spherical particles protrude from the thylakoid membrane resembling mitochondrial cristae. These spherical particle contain protein complex which has significant role in the formation of ATP during photosynthesis. Separate thylakoids without any stacking arc observed in red algae. In red algae and blue green algae granules containing pigments are known as phycobilisomes present on the membranes of thylakoid. Structural alternation takes place in presence of light.

Enzymes for carbon dioxide fixation and other dark reactions are present in the stroma and the

enzymes for light reactions are present in thylakoids. There are two types of carbon dioxide fixation in higher plants: (1) C_3 and (2) C_4 plants. C_3 plants (wheat, rice, soyabean etc) have chloroplast with starch grains show large number of grana. In C_3 plants special type of protein or enzyme known as Rubulose 1, 5 biphosphate carboxylase-oxygenase (RUBISCO).

(RUBISCO) are present which helps in the addition of CO2 to ribulose biphosphate to form 3-phosphoglyceric acid in the stroma.

In C4 plants (Sugarcane, Maize etc) CO_2 fixation takes place in mesophyll cells containing chloroplast which do not store starch. It lacks, RUBISCO. Here one molecule of CO2 is added to one molecule of PEP (Phosphoenol pyruvate carboxylase). This path way forms 4-carbon compound so it is called C_4 type. (Fig 77 A, B).

Chloroplast DNA: It is circular & double stranded, synthesizes all t RNA, all r RNA and a few proteins for organellels. In Acetabularia chloroplast DNA is linear. Chloroplast chromosome, has

600-800 kbp which specify 75-120 proteins (for ribosomal protein and pseudo genes), Pseudogenes lack promoter regions. It encode the r RNA and t RNA.

Replication in chloroplast DNA: "Ori 1" and "Ori 2" act simultaneously. It is unidirectional but appear bidirectional when new strand pass each other.

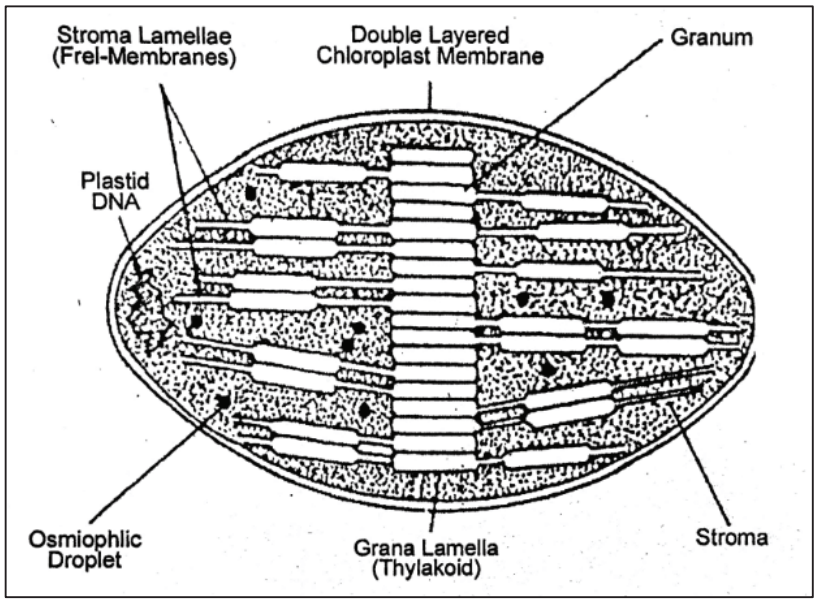


Fig. 77A. Structure of chloroplast (Sectional view) as seen under electron microscope

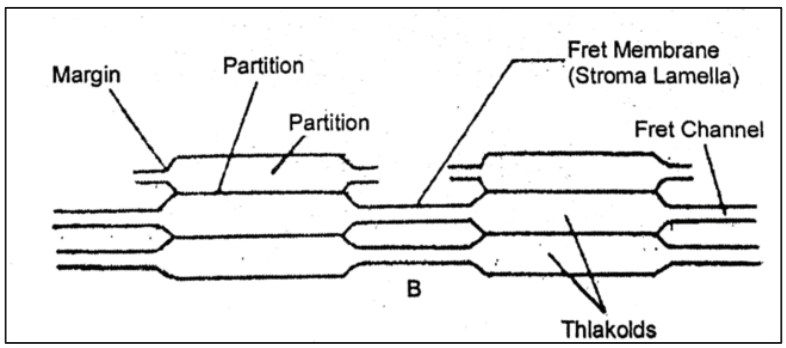


Fig. 77B. Structure of Grana

Mitochondria

Mitochondria are cytoplasmic organelles found in all eukaryotic cells. Its number varies from 1-1000. They are said to be power house of the cell. Its size varies from 0.2-many microns. Kolliker

(1880) observed it in muscle cells of insect. Benda (1887) observed similar substance and named it Mitochondria. Buchholz (1947) gave its electron microscopic structure. They are stained by Janus green. It is bounded by two membranes of lipo-protein. Membranes are 50-70 Å thick. The outer membrane is smooth and inner membrane is infolded to form cristae of variable forms and number.

Two membranes are separated by intermembrane space which is 20-60 Å wide.

The inner mitochondrial membrane has two faces: The outer C-face or cytosol and the inner M-face or matrix. There are two types of cristae: (1) Plate like (true cristae) and tubular. Inner membrane contains 80% proteins and 20% lipids. Knob like elementary particles with short stalks are scattered all along the inner membrane. They vary in diameter from 8 to 9 nm (Fig. 78). Inter (81) membrane space contains a fluid and some enzymes. Matrix is enclosed in the inner membrane.

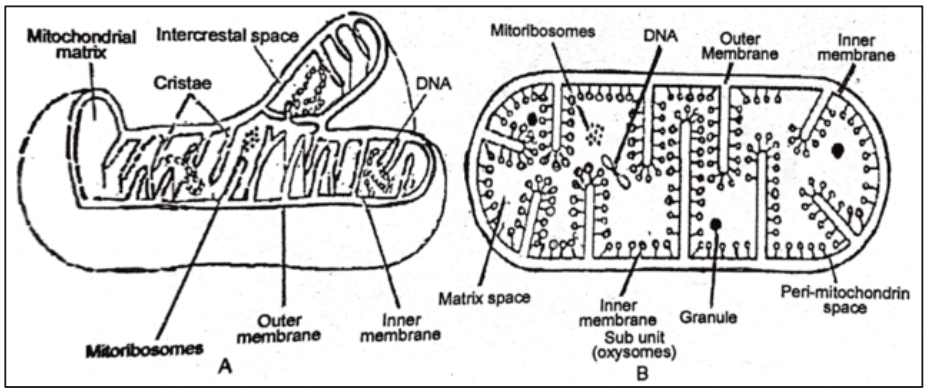


Fig. 78 Mitochondria

Role of mitochondria in Respiration: FADH2 and NADH formed in glycolysis, citric acid cycle and fatty acid oxidation are oxidised in mitochondria to generate energy for the synthesis of ATP The electrons are transferred through several electron carriers and finally to oxygen. This chain is generally referred to electron transport chain / respiratory assembly. The electron transport chain consists of four enzymic complexes as such: (Fig. 79)

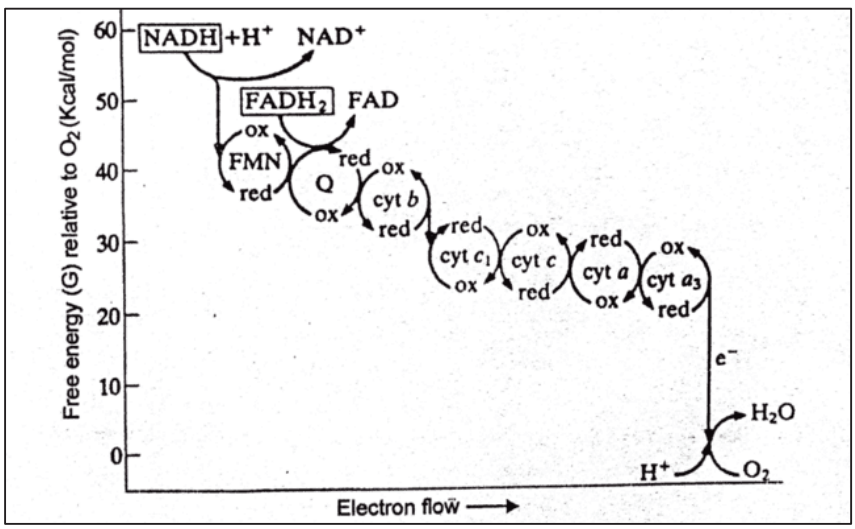


Fig. 79. Controlled release of energy in the electron transport system

1. NADH-ubiquinone reductase
2. Succinate ubiquinone reductase
3. Reduced ubiquinone cytochrome c-reductase and
4. Cytochrome oxidase.

Chapter 27
Mendelism-Mendel's Law of Inheritance

Gregor John Mendel was a monk in 1843 in the monastery of Brenn now known as Czechoslovakia. He studied natural history and mathematics at the University of Vienna from 1861 to 1853. There he developed interest in hybridization. He returned to monastery and conducted-breeding experiment on pea from 1856-1865. He collected true breeding seeds for seven pairs of contrasting characters in pea as such:

(1) height of plant (tall & dwarf) (2) position of flower (axillary & terminal) (3) colour of unripe pod (green & yellow) (4) form of unripe pod (smooth & constricted (5) colour of seed coat or flower (coloured and white) (6) colour of cotyledons (yellow & green) and) (7) shape of seed (round and wrinkled).

True breeding seeds means the seed will develope only the characters of parent plant. eg. seeds obtained from tall plant will give rise only tall plant on germination for generations after genrations and so on. He performed two types of experiments involving plants having contrasting characters, one is monohybrid cross and second is dihybrid cross.

A. Monohybrid cross: He selected Tall & Dwarf plants developed from true breeding seed obtained from tall & dwarf plants. After crossing between male tall plant & female dwarf plant the seeds obtained by this cross gave rise only tall plants in Fl, generation (F1 filial 1). The F1 tall plants were sown which gave rise F2 plants. The Fl tall plants were allowed to self-pollinate (as the pea plant is bisexual & cleistogamous) and finally seeds of F2 generation was obtained. Now these F2 seeds were sown to raise plants of F2 generation which were found to be in the ratio of 3:1, for tall & dwarf characters respectively. This is F2 ration as has been shown below Fig. (80), EPB).

From the above experiment Mendel concluded some principles or laws as follows:

1. **Law of unit characters:** It states that various hereditary characters are controlled by factors and there is a pair of factors for each character.

2. **Law of dominance**: This states that there are factors in a pair express itself in F1 generation which mask the appearance of other. The factor or trait which appeared in Fl generation is dominant and the masked factor or trait is recessive which masker its appearance in F2 generation.

3. Law of segregation: The factors of each character segregate during gametogenesis so that each gamete receives only one factor and the gametes are always pure. This law is also known as law of purity of gametes.

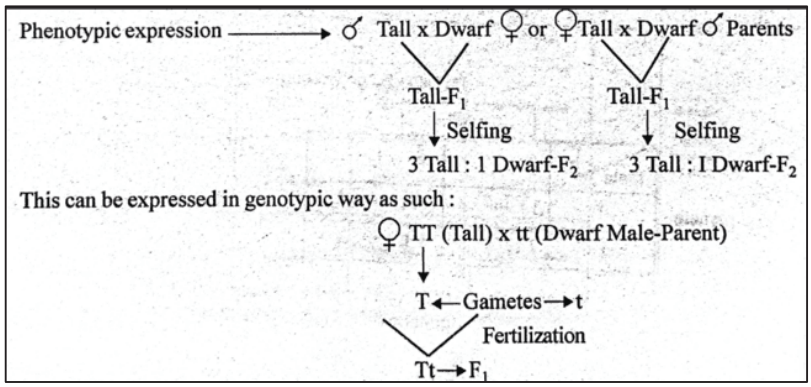
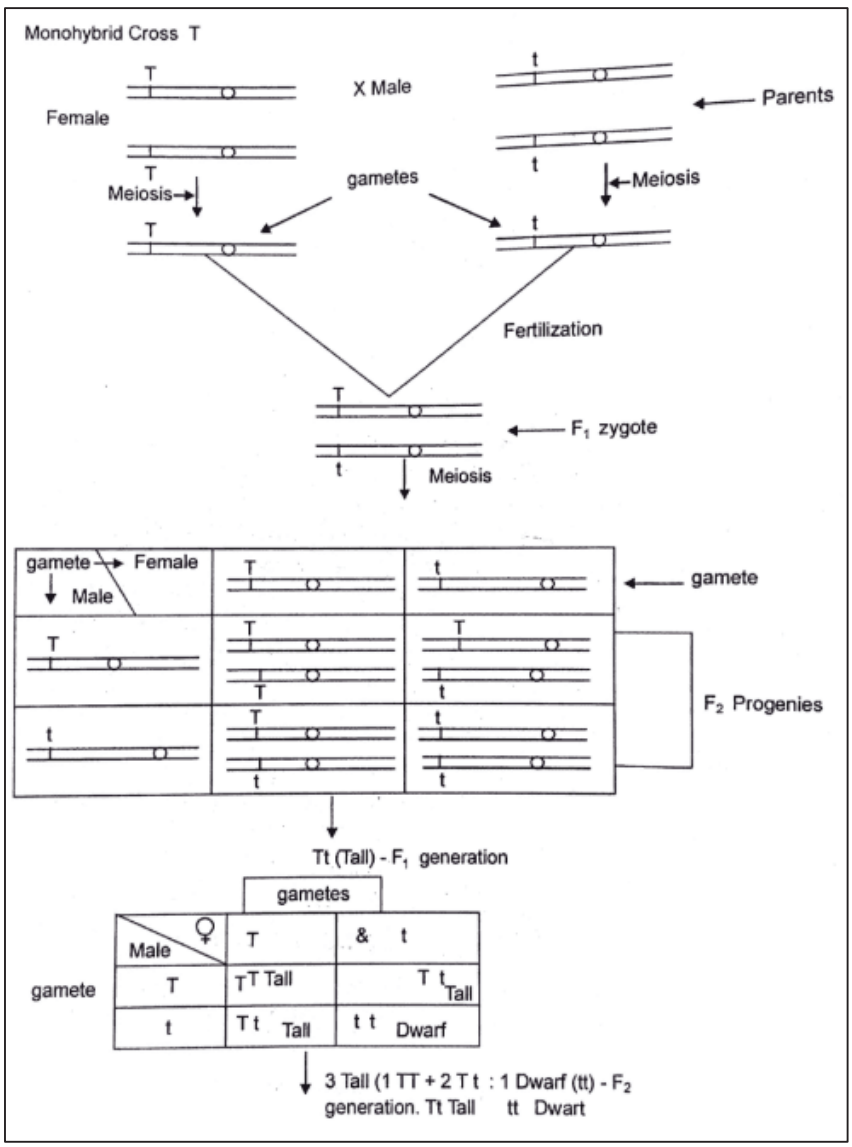


Fig. 80 Showing monohybrid cross up to 12 generation (genotypic expression)



Dihybrid Cross: In this cross two characters were considered in a parent and another two contrasting characters in second parent for hybridization as follows: (Fig. 81 A, B)

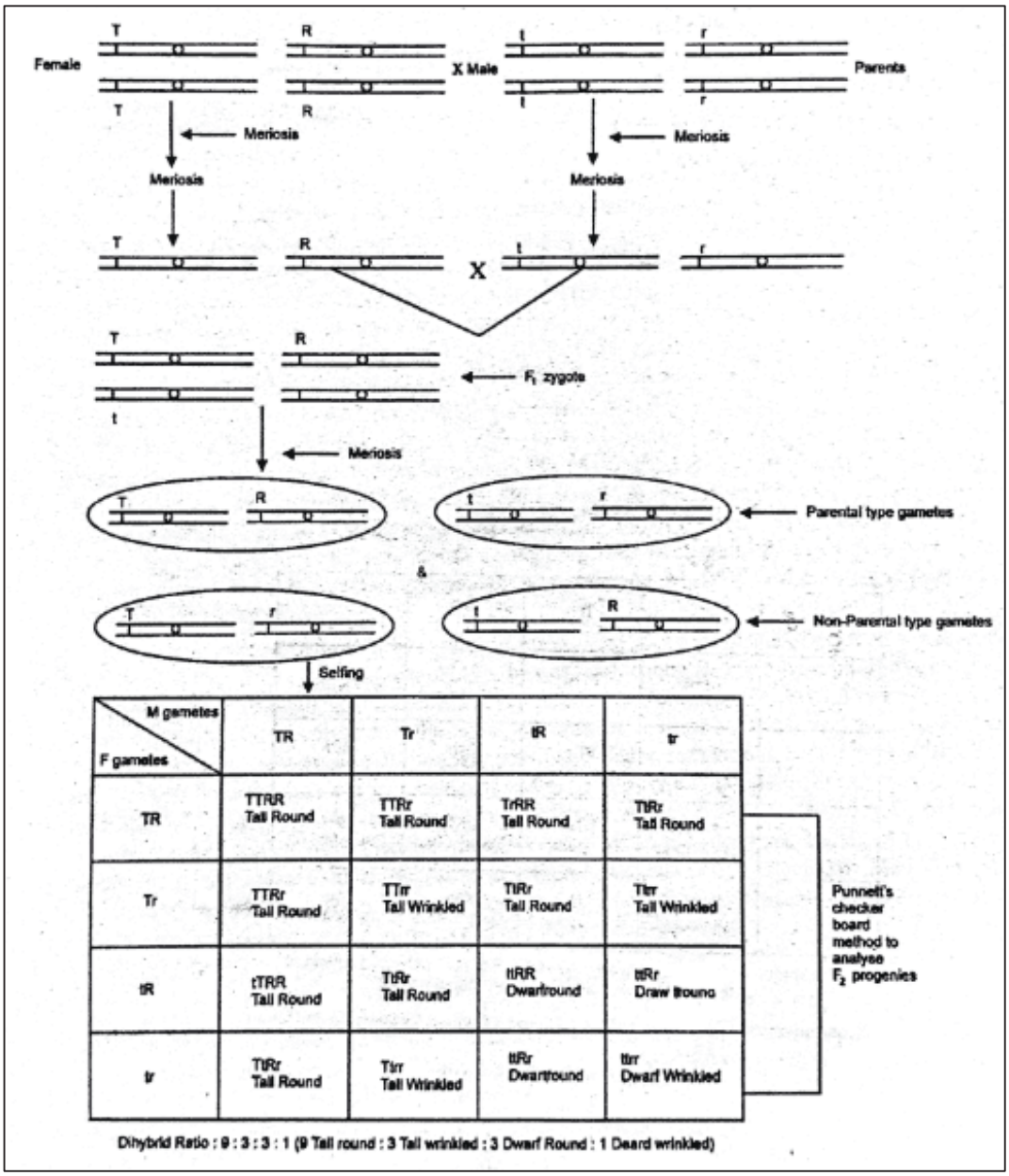


Fig. 81 A.

Mendel concluded the principle from dihybrid cross known as Law of independent assortment or law of recombination. This states that factors for each characters are distributed into the gametes independent of the factors of any other characters. In F_2 generation two different classes of progenies were also obtained besides two parental classes. The appearance of non- parental or recombinant classes in F, was also termed as law of recombination.

In this way altogether four Laws of inheritance was proposed by Mendel in 1866 as such :

1. Law of unit characters

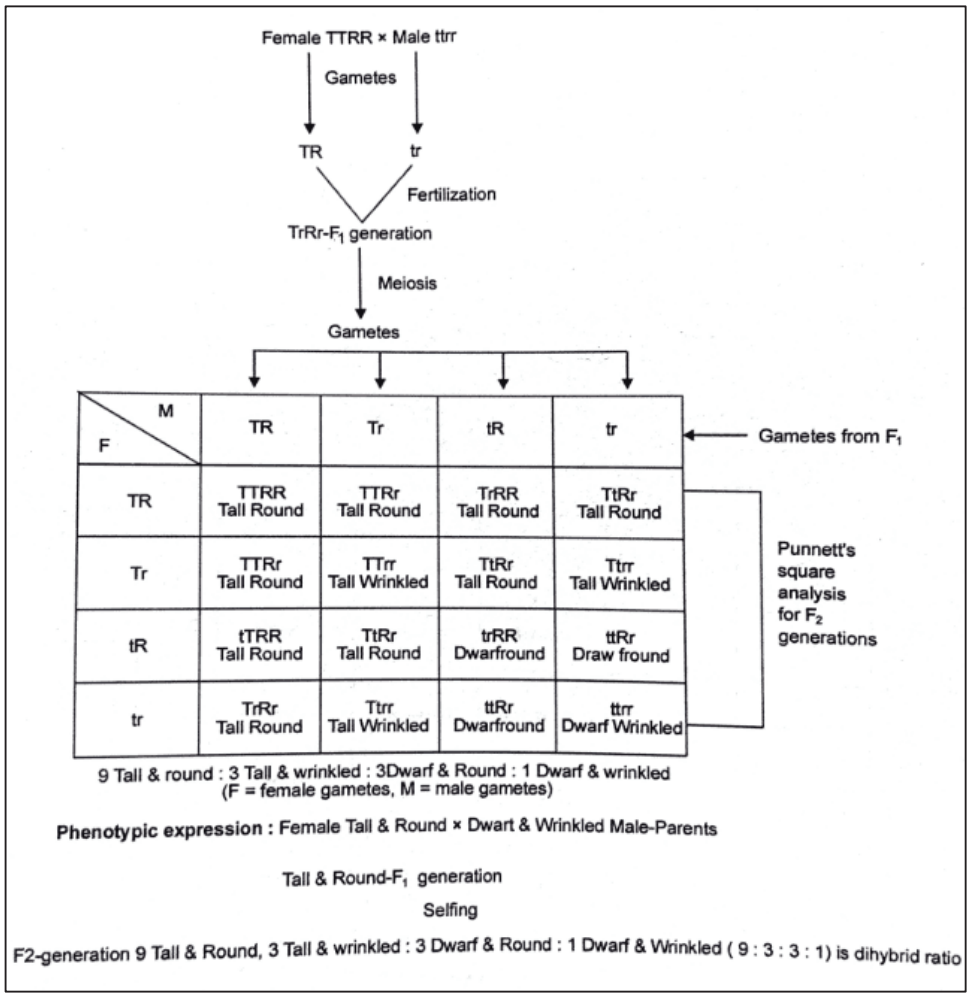


Fig.81 B. Phenotypic expression of the same dihybrid Cross

2. Law of dominance

3. Law of segregation or purity of gametes and

4. Law of independent assortment or Law of recombination.

These laws of inheritance are Mendelism. Mendels work & principles remained uncared till 1900 when De Vries of Dutch, Carl Correns of German and Tshermak of Austria came to the same conclusion independently which was already formulated by Mendel as early as in 1866. This was rediscovery of Mendelism.

Mendelism in the light of meiosis: Mendel did not know about chromosomes or meiosis because it was not discovered at that time. Even then, the law of purity of gametes and law of recombination obeys the meiotic segregation, during gametogenesis. The diploid parents with factors for tallness is symbolised by T T and factors for dwarfness as t t. During gametogenesis, meiosis occurs and haploid gametes are produced with reduced (haploid) number of chromosomes or factor.

TT gives T gamete and t t gives t gametes. After fertilization, the zygote becomes diploid having Tt factors or chromosome. When this heterozygous individuals produced by heterozygous zygote (Tt) forms gamete after meiosis, it is segregated like T and t in the gametes and hence they are pure either with only T or with t. This is law of segregation or law of purity of gametes.

Similarly, in dihybrid cross TTR R and t t r r parents with two pairs of contrasting factors produce gametes with TR & tr factors separately. On fertilization F_1, zygote contains Tt Rr all the factors of both parents but in single dose each. During meiosis, bivalents of T t and Rr segregate at anaphase I forming TR and t r containing gametes. Formation of recombinant gametes Tr & t R is possible only when bivalent rotates randomly giving rise different orientation at metaphase I and thus anaphasic I separation will give rise recombinant types of gametes with Tr & tR factors as shown in the following diagram (Fig. 84A, B).

Despite lack of information regarding meiosis Mendel's experiment obeyed the meiotic separation of factors during gametogenesis and so he was lucky enough to choose the pairs of contrasting characters / factors situated each on all the seven pairs of chromosomes present in pea plants. Had there been even two factors present on the same chromosome, the result observed by him, would have become different and hence no conclusion was possible. Selection of pea plant was also beneficial for Mendel's experiment, because it was bisexual and male and female plants were easy to isolate for hybridisation. By removing stamens from flower, the flower will act as female with having gynoecium only. The matured pollen from bisexual flower are carried to the receptive stigma of emasculated (stamens removed) flower to make hybridisation. Thirdly, the characters selected by Mendel did not show interaction, linkage, incomplete dominance etc. All these favourable features are the pillars of Mendel's successful breeding experiments and to formulate Mendelism.

Interaction of Gene

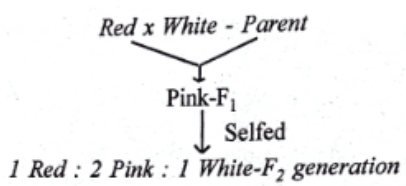
Phenotypic expression of a mendelian factor or Johannsen's gene (1909) may be altered by the influence of other genes or factors. This modification of normal phenotypic expression of certain gene by other gene is termed as interaction of genes.

Types: Interaction of genes may be considered of two types:

(1) Intragenic interaction e.g. co-dominance, incomplete dominance, multiple alleles etc. and (2) intergenic interactions e.g. epistatic gene, complementary gene, supplementary gene, duplicate gene etc.

These interactions are exception to mendelian inheritance and hence it is also known as neo-mendelism.

Incomplete dominance: Cross between red colour of flower bearing plants of *Mirabilis jalapa* and white coloured flower bearing plant of *M. Jalapa* give rise pink coloured flower bearing plants in F_1 generation as shown below. This is an example of incomplete dominance.



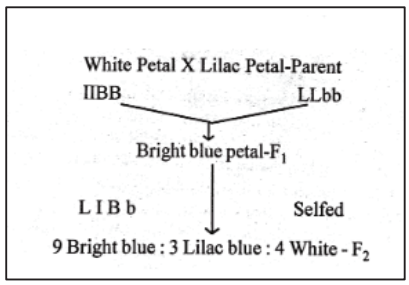
Codominance: It is independent expression of two alleles in an individual eg.. The alleles I^ and 1B of human blood groups are codominant because both/none of them prevents the expression of others.

Multiple alleles: According to mendelism there are only two alleles of a gene or factor. But multiple alleles of a gene have been noticed e.g., eye colour gene in Drosophila have several alternative eye colours indicating there by multiple alleles of the eye colour.

Intergenic interaction of genes: There are several examples of intergenic interaction of genes which have modified the mendelian dihybrid ratio showing 9:3:4 ratio, 9:6:1 ratio, 12:3:

1 ratio, 9:7 ratio, 13:3 ratio, 15:1 ratio and so on.

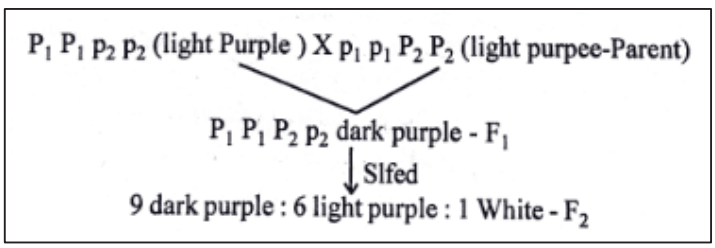
9:3:4 ratio is obtained due to supplementary action of gene as shown below:



In the above example L stands for lilac colour, B act as supplementary factor. Therefore, LL bb is lilac and II B B is white in which I has epistatic effect on B. II b b genotype will express white phenotype.

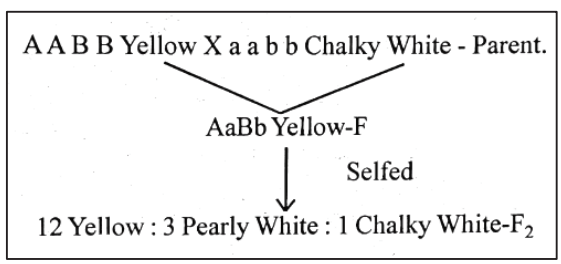
9:6:1 ratio: It is due to additive or polymeric genes. When two plants of light purple colour of flower are crossed, F, shows dark purple colour of the flower.

On selfing F_2 gives 9 dark purple: 6 light purple and I white as shown below:

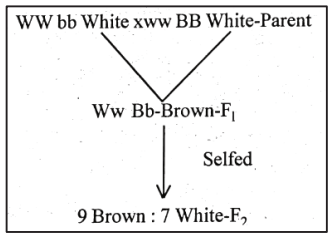


Here light purple colour is due to presence of dominant gene P1 & P2. The two non-allelic dominant genes P_1 & P2. exerts an additive effect so that colour becomes dark purple. When both the dominant genes come together it produces dark purple. The recessive of both genes give white colour.

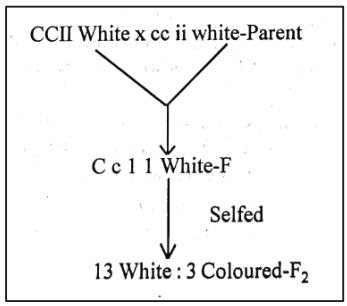
12:3: 1: It is due to epistatic action of gene. There are two independent genes. In epistatic action one gene masks the expression of other gene which is not its allele. The gene which masks is called epistatic as shown here.



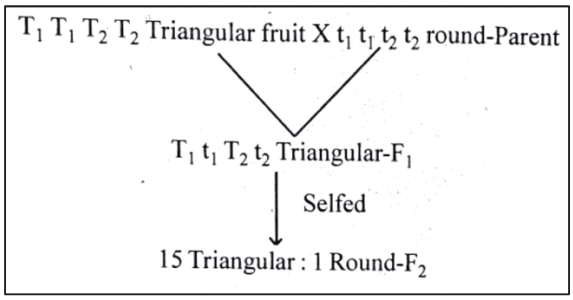
9:7 ratio: Complementary action of gene: Here grain colour depends on interaction of two complementary factors so that either of them alone has no effect. When both the factors come together they are capable of producing an effect due to their complementary action as shown below.



13:3 ratio: It is due to inhibitory action of gene. Two plants having white colour gave rise white progeny in F. On selfing 13 white and 3 coloured characters appear as shown below. C stands for coloured, I stands for inhibitory gene.

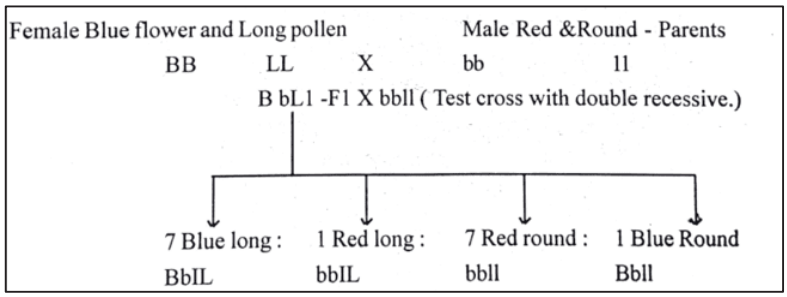


15:1 ratio: It is due to duplicate genes situated on different loci expressing no cumulative



Linkage

Absence of crossing over is linkage. Bateson and Punnett(1906) observed in *Lathyrus odoratus* that two pairs of genes did nor assort independency of each other. They pointed out that when two or more dominant genes entered form the same parent, they tended to remain together so that recombinant class were less than the parental class. Similar was the case with recessive genes (alleles) This phenomenon was termed as "coupling". When two dominant genes introduced in the cross by different parents tended to remain apart in subsequent generation -this is "repulsion".



Or 7:1:1:7 ratio showing tendency of dominant alleles to remain together. Similarly, there is a tendency of recessive alleles to remain together. This is termed as coupling.

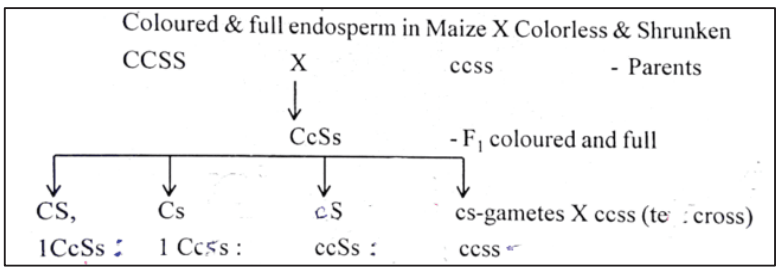
When two such dominant or recessive alleles come from different parents, they tend to remain separate. This was termed as repulsion. Bateson and punnett could not explain the reasons of coupling and repulsion. TH. Morgan (1910) said coupling or repulsion was not complete. When two genes are present on the same chromosome it is coupling or if they are present on different chromosome, it is repulsion. These genes are often termed as linked genes and phenomenon of inheritance of linked genes is known as linkage. Morgan (1910) postulated the Chromosome theory of linkage as follows.

(1) Linked genes are situated on the same chromosome, and they cannot be separated 'during the process of inheritance.

(2) Strength of linkage is determined by distance, between the linked genes.

(3) Genes are linearly arranged in the chromosomes. Linkage may be complete or incomplete.

Incomplete linkage in maize was determined by the following test cross methods.



But actually coloured & full was 4032 or 48%, colour less shrunken was 4035 or 48 coloured shrunken was 149 or 2% and colourless full was 152 or 2%. Thus crossing over has occurred only in 2+2=4% between linked genes. So, it is incomplete.

Interference: It states that crossing over in one region interferes with the crossing over in the adjacent region. Interference becomes 100% if double cross overs are absent. The value of double cross over is always less than the expected, For example if three genes are arranged in order A -B-C and the recombination values of double crossover will be predicted IfA-B = 10% and B-C=20%, the double crossover in A-C region would be 10% of 20 % i.e., 2%.

Coincidence: It is used as synonym of interference. If double cross overs equal the expected value, there is 100% coincidence and if there is no double cross over, coincidence becomes zero. The coefficient of coincidence can be calculated as such:

$$\text{Coefficient of coincidence} = \frac{\%\ \text{actual double cross overs}}{\%\ \text{expected double crossovers}}$$

The greater the coincidence lesser will be interference and greater the interference, lesser would-be coincidence.

Mapping Genes (loci) on Chromosome

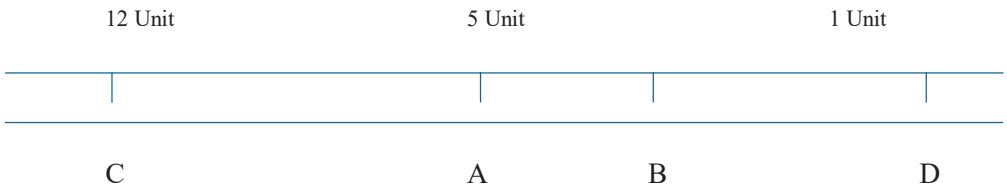
It is done with the help of cross over between two loci in a chromosome. A .H. Sturtevant (1913) discovered the relationship between cross over frequency and distance between loci. The frequency of recombination is always the same between two particular loci, but different for other pairs of loci. He developed the hypothesis

and said that (a) crossovers occur less often between genes that are close together than between genes that are far apart in the linear array within a chromosome, (b) genes have fixed position in linear array; and (c) the sequence of genes on a chromosome can be determined by knowing recombination frequencies. From this hypothesis we can map the loci (genes) in a linear array within a chromosome. For example.

Following are the percentage of recombinant forms among genes A, B, C and D

Recombinants	Gene Loci
5%	A & B
18%	C & D
17%	B & C
12%	A & C
6%	A & D
1%	B & D

From the above data, genes (loci) C and D are farthest apart, B and D are closest together, A is closer to B than to C. Their position must be like this. Thus, the two distant loci (genes) can be mapped only by knowing recombination frequencies between them. Maximum distance would be 50 units l.e., 50% recombination



Sex Determination in Plants

In majority of dioecious organisms, the chromosomes play an important role in sex determination. Mc Clung (1902) observed in grasshopper that there were eleven pairs of chromosomes and an odd chromosome with no mate. He considered this odd chromosome an accessory chromosome associated with sex determination. Wilson (1905) working on bugs observed 22chromosomes in somatic cells of female bug. On the other hand male bug had only 21 chromosomes in its somatic cells and at meiosis only ten pairs of chromosomes and an odd accessory chromosome, Wilson suggested the name of odd chromosome to be called as X-chromosome. The male bug has XO sex chromosome while female bug has XX chromosome, and this is known as XO method of sex determination. Later Wilson (1909) and Stevens found another sex chromosome to be known as Y chromosome. There after chromosomal method of sex determination was established in various ways as follows:

Allen (1917) observed in haploid *Spherocarpus* plant dot like X chromosome in female plant and rod like Y chromosome in male plants.

XY male and XX female in diploid plants: Warmke (1946) reported in *Melandrium album* different doses of X and Y chromosomes in diploid, triploid and tetraploid plants as such:

Diploid - XX Female

XY - Male

Triploid - XXY Male

Tetraploid - XXXY Male

In *Coccinia indica* mechanism of sex determination was studied by Prof R. P. Roy (1973) in which diploid plant with XX as female, with XY as male sex was observed. Likewise triploid with XXY (male) and with XXX as female and tetraploid with XXXX (female) and with XXXY as male plants were reported by him and other.

Various types of sex expression were observed in higher plants as summarized below:

Types of flowers		**Types of phenotypic expression of sex**
1. All perfect bisexual flowers	-	1. Hermaphrodites
2. Separate male and female flowers on the same plant	-	2. Monoecious
3. Separate male and female	-	3. Dioecious
4. Perfect bisexual and male flowers on the same plant	-	4. Andromonoecious
5. Perfect bisexual and female flowers on the same plant	-	5. Gynomonoecious

Chapter 28
Structural Aberrations in Chromosome

Genes are linearly arranged in chromosome. Sometimes genes occasionally mutate and chromosomes produce aberrations. Two types of chromosomal aberrations are established (1) structural and (2) numerical.

Structural changes in chromosomes: Variations in the structure of chromosomes have been observed in natural populations and it can also be produced artificially in the organisms.

Structural changes within chromosomes do not change the amount of genetic material or chromosome number. These are visible alterations in the shape of chromosomes. It causes changes in phenotypes and in the expected genetic ratios.

Structural changes in the chromosomes are of following types:

1. Deletions
2. Duplications
3. Inversions
4. Translocations and
5. Fragmentation

Deletion or Deficiency: It was discovered and worked out by Bridges (1917; and Mohr (1923). It is loss of a part of a chromosome. Smaller deletion, in heterozygous condition, will form a loop in a bivalent which is seen at pachytene/diplotene stage. Due to heterozygous deletion, a recessive allele will behave like a dominant allele (Pseudo dominance). McClintoc (1941) has shown origin of variegated aleurone colour in maize through breakage fusion bride cycle resulting in deletion. Fig. 82 A

Duplication: Duplication involves attachment of a chromosomal fragment resulting in addition of one or more genes to a chromosome. Whenever there is a duplication in a chromosome, there is corresponding deletion in another chromosome. Several types of duplication can be seen.

Bar eye in Drosophila is due to a duplication in region 16 A of x-chromosome observed by Bridge (1919). Cytologically, duplication may produce similar configurations in bivalent as a deletion does. Fig. 82 B.

Inversions: This result from two breaks in a chromosome and the detached portion become united in the reversed order. Inversion does not necessarily supresses cytological crossing over.

However genetic recombination are suppressed. Inversion may be of two types depending on the inclusion or exclusion of centromere within the inverted segment and these are

(1) Paracentric inversion and

(2) Pericentric inversion

Paracentric inversion does not include centromere.

It was discovered by Sturtevant (1926) in Drosophila melanogaster.

Pericentric inversion includes centromere in the inverted segment.

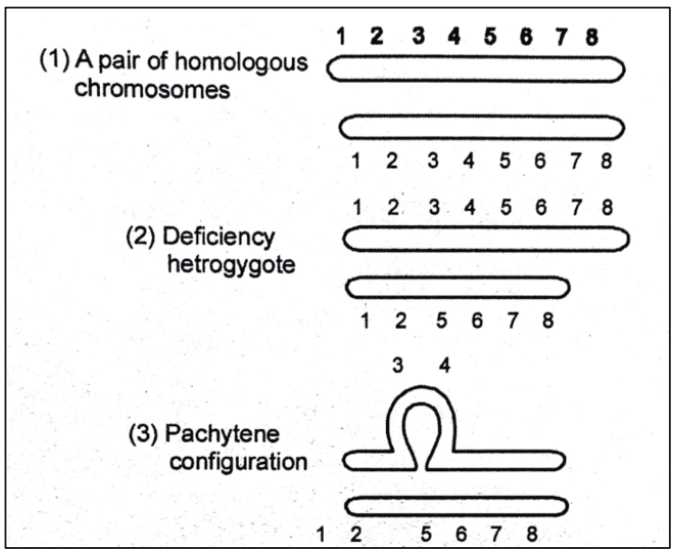


Fig 82 A Chromosome pairing in a deficient heterozygote.

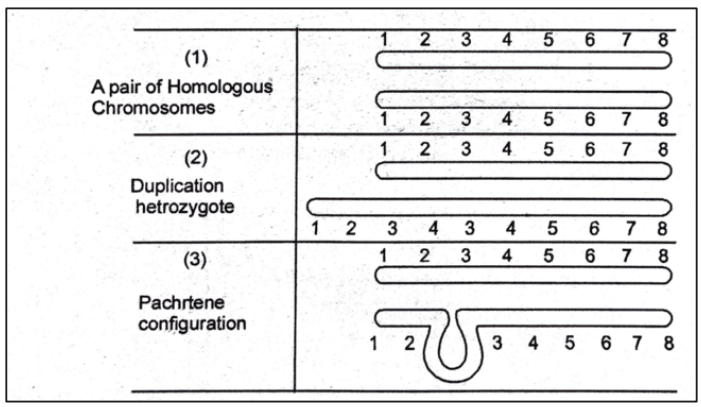


Fig. 82 B. Chromosome pairing in a duplicate heterozygote.

Meiosis in inversion heterozygotes: The meiotic behaviour in paracentric inversion will be as follows.

At zygotene, pairing of homologous chromosomes leads to the formation of a loop which includes the inverted segment. Depending on the number of crossing over, various configurations at metaphase I and metaphase II would appear as shown in the following diagrams (Fig. 83) In pericentric inversion a single crossover will result in the formation of 50% gametes with deficient chromosomes (Fig. 83)- Genetic consequences: In homozygous inversion it is normal. A high degree of sterility is produced in inversion heterozygotes. It act as crossover suppressor and used to detect lethal mutation.

Translocation: It was discovered by Bridges (1923) in Drosophila melanogaster. Sometimes a segment of a chromosome becomes detached and unites with another chromosome. This is called translocation. It may be (1) Simple (2) shift translocation and (3) Reciprocal.

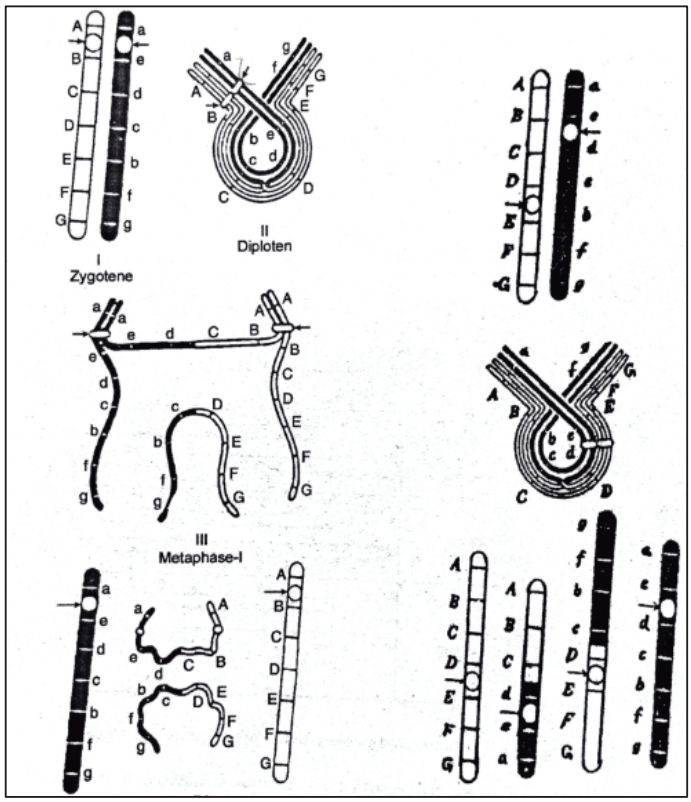


Fig. 83 It shows behaviour of chromosomes in pericentric inversion heterozygotes. (After Sinnot, Dunn and Dhobzansky)

(1) Simple translocation: It involves a simple break in the chromosome, the broken piece of chromosome is translocated onto, the end of another chromosome. It is rare in the nature.

(2) Shift translocation: It involves 3 breaks in the chromosome, there is insertion of a intercalary piece of chromosome into a different non-homologous chromosome. This type of translocation is common (Fig. 83).

(3) Reciprocal translocation: It is also known as interchange. It occurs when single break takes place in two non-homologous chromosome and produce a exchange of

Metaphase I

Metaphase II

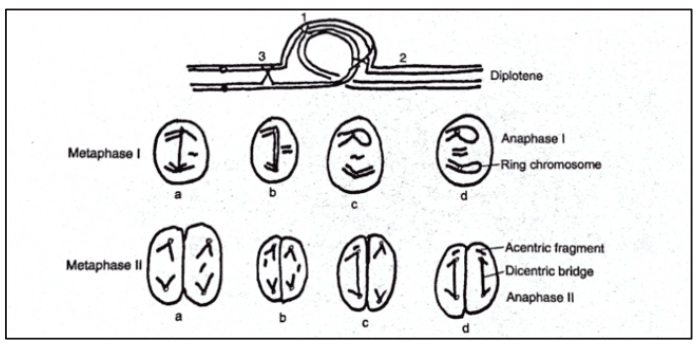


Fig. 84, Showing meiosis in inversion Configuration following a crossover at I or 2 in the inversion (b) Configuration following a crossover at 1 or 2 in both the inversion (c) Configuration following a crossover at 2 or 3 in both the inversion

(d) Configuration following a crossover at 1, 2 or 3 in the inversion chromosome segment between them, or in other words - In reciprocal translocation there is an exchange of segments between non homologous chromosome

Meiosis in translocation heterozygotes:

Translocation heterozygotes bear translocated chromosome with their normal counterpart or homologous chromosome as shown in fig. 85

The result of pairing in meiosis are different. At the pachytene stage a cross shaped 4 chromosome complex is formed, in which the position of cross indicates the breakage points. There are 3 possible arrangements of the chromosome in Meta I forming different types of gametes. Three segregation patterns occur at anaphase I (Fig. 85).

(1) Alternate Segregation: Non-homologous chromosome go to the same pole. Each gamete contains a complete balanced gene compliment.

(2) Adjacent I: Non homologue adjacent chromosome go to the same pole. In this separation each gamete contains non-balanced gene due to deficiencies and duplication, which causes sterility.

(3) Adjacent II: In this case normal and translocated chromosome go to the pole. Hence due

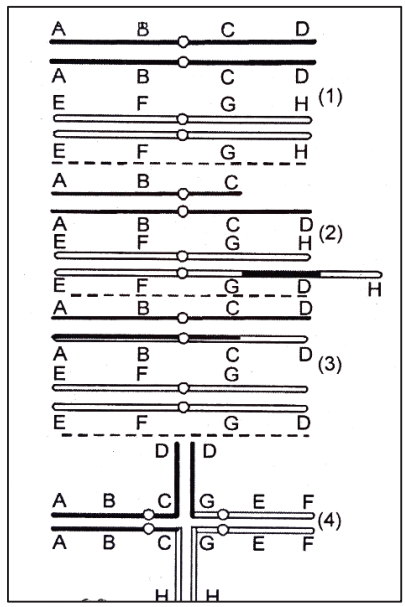


Fig.85 (1) Two normal homologues (2) Simple translocation (3) Reciprocal translocation (4) Tetravalent at zygotene in reciprocal translocation heterozygote

to duplication and deficiency unbalanced sterile gametes are produced. The overall process of Meiosis 1. is shown in Fig 86.

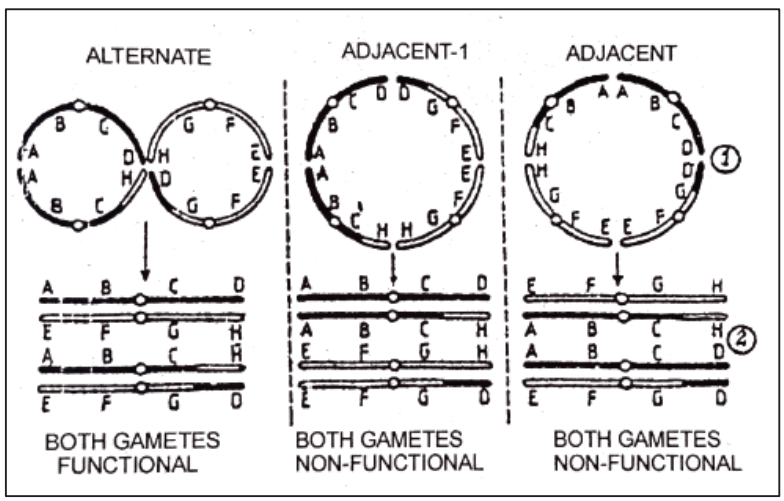


Fig. 86.

Genetic consequence: Meiosis in translocation heterozygote leads to the formation of unbalanced gamete in adjacent segregation pattern, causing sterility. Translocation heterozygotes maintains the population of balanced lethal system. In *Oenothera Lamarckiana* Renner (1921) observed in *O. lamarckiana* two groups of genes namely Gauden and Velan. This group of gene is called Renner complex. At the time of gamete formation, this complex segregates, certain gametes receive only Gauden complex while other receive the

Velan complex. During gametic fusion three types offspring can be expected namely-(1) homozygous gauden, (2) homozygote velan and (3) heterozygous velan and gauden (fig. 87)

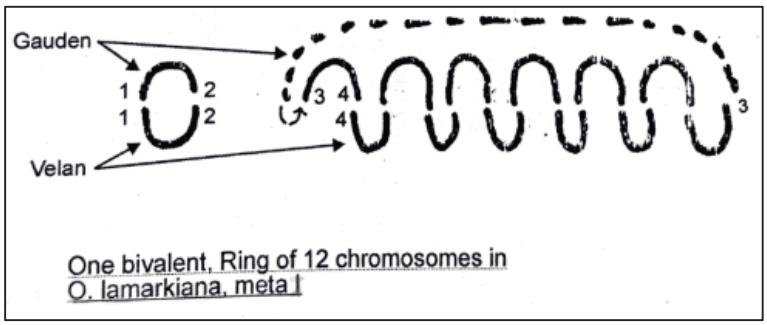


Fig. 87. Gauden velan complex in *Oenothera lamarkiana*

In homozygones condition lethal genes express and offspring die. Only heterozygones individual carrying Gauden & Velan complex persists.

Numerical Aberration in Chromosome

Numerical changes in chromosomes are increase or decrease in chromosome number above the normal zygotic (sporophytic / diploid) number. It is of two types: (1) Euploidy and (2) aneuploidy. In euploid, the organism will have one or more entire (full) set of chromosomes. (a set means all the chromosomes in haploid complement). Euploid has chromosome complement consisting of whole set of genome.

Aneuploid means organism having a chromosome number which is not an exact multiple of genome (set of chromosomes). It may be hyperploid i.e. 2n +1 or, hypoploid i.e. 2n-1.

Aneuploid may be:

(1) Nullisomic 2n-1

(2) Monosomic 2n-1

(3) Trisomic 2n+1

(4) Tetrasomic 2n+2

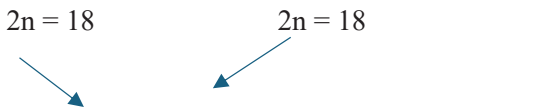
Aneuploids are individual with an uneven number of chromosomes.

It has one or more chromosomes less or more than the normal diploid number. It results from non-disjunction during meiosis.

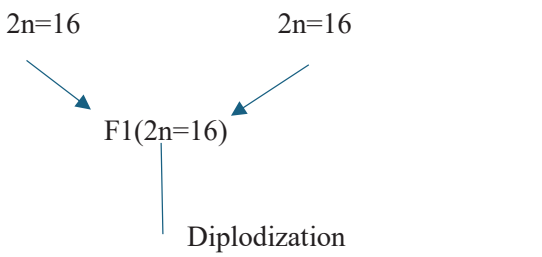
Polyploidy: Euploids are individual with complete set of Chromosomes (genome). Individual with one set - it is monoploid. Individual with two sets of chromosomes- it is diploid. Individual with three sets of chromosomes -it is triploid, with 4 sets it is tetraploid and so on. Thus polyploid is an organism whose somatic number of Chromosomes is multiple of basic number of Chromosome.

Types of Polyploid: It is classified as autopolyploid and allopolyploid. Autopolyploid is multiple of similar genomes. Allopolyploids are multiple of dissimilar genomes from different organisms e.g.

(1) *Raphanus sativus* x *Brassica oleracea*



(2) *Galeopsis speciosa* x *Galeopsis pubescens*



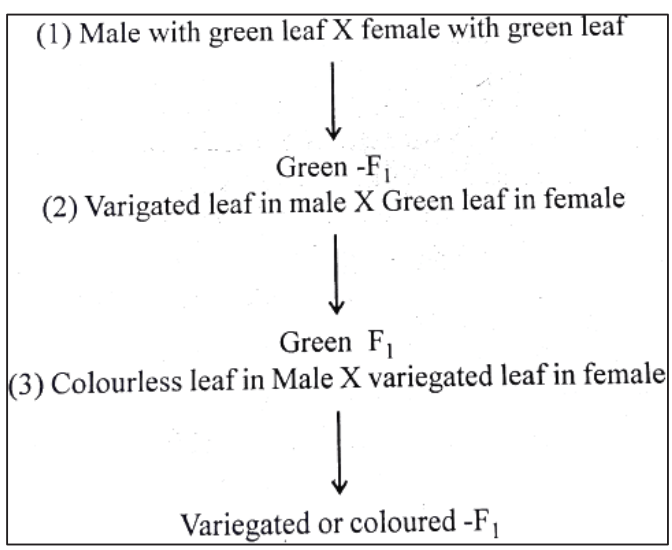
Polyploidy can be induced with the application of colchicine etc. origin of bread wheat is the result of Polyploidization and so on.

Chapter 29
Extra Chromosomal Inheritance

The genes of nuclear DNA can not be considered as a role vehicle of inheritance, because some experiments revealed the occurrence of extra nuclear DNA (gene) in the cytoplasm or organelles of prokaryotes and eukaryotes. These extra nuclear genes of mitochondria, plastids, plasmid and cellular surfaces have a characteristic pattern of inheritance which does not resemble with that of genes of nuclear origin and is termed as extrachromosomal, maternal, non mendelian, uniparental, non-chromosomal, extra nuclear or cytoplasmic inheritance. The inheritance of nuclear genes (chromosomal) contribute equally to the genetic constitution of the progenies and therefore reciprocal crosses between parents of different homozygous genotypes will yield offsprings of identical phenotypes except for sex linked genes, while in extrachromosomal inheritance male and female parents do not make equal contribution of extra nuclear genes to the progeny because sperms or pollen carry no extra chromosomal genes while egg contributes large amount of cytoplasm. There are many extra nuclear genes in the form of DNA of mitochondria, chloroplast etc. Therefore, reciprocal crosses in extra chromosomal inheritance yield nonmendiean or different results.

Following are the examples of extrachromosomal inheritance:

1. Plastid inheritance in Mirabilis: Correns (1908) described in Mirabilis jalapa inheritance of plastid characters due to plasma genes located in plastids. Crosses between the parents of different characters result in different fashion as such:



In the above experiment, it is obvious that inheritance is from female parent.

Maternal inheritance in snails: It was studied by Boycott *et al* (1930). Coiling in snail is of two types: (1) clockwise or sinistral and (2) anticlockwise or dextral

(1) *Dextral female* X *Sinistral Male*

↓

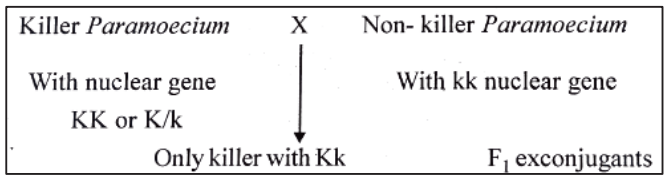
Dextral -F_1

(2) *Sinistral female* X *Dextral Male*

↓

Sinistral-F_1

Inheritance of Kappa particles in *Paramoecium*: It was studied by Sonneborn (1938) Paramoecium produces poisonous substance called paramecin which is lethal to other, called sensitives. The production of paramecin is dependent on Kappa particle present in the cytoplasm of Paramoecium called killer. Kappa particles do not harm their host. Kappa particles in killer Paramoecium is maitained and replicated by chromosomal dominant gene K. Paramoecium with recessive kk gene are unable to produce Kappa particles.



But if the conjugation is allowed only for short period so that cytoplasmic exchange has not taken palace between the two conjugating Paramoecium, F, will have both killer and non-killer exconjugants. (1938)

Milk factor in Mice: Bittner (1938) observed in female mice to be highly susceptible to mammary cancer and this trait is maternally transmitted through milk. The presence of milk factor also depends on nuclear gene. This factor is virus called Mouse mammary cancer Virus (MTV).

Extra nuclear inheritance in Bacteria: It is performed by episomes and plasmids. These are extra genetic material. The F or sex factor element of E. coli is a plasmid which is self replicating and autonomous, Episomes & plasmids carry some genes which are transmitted independently.

There are several examples indicating presence of cytoplasmic genes which follow the mode of inheritance in non-mendelian fashion.

Chapter 30
Plant Breeding & Bio Statistics

Plant breeding may be defined as the artificial manipulation of the variability -the alteration in the composition of experimental Population-in a chosen direction as they go from one generation to next through sexual reproduction. The Primary purpose of plant breeding is to obtain or develop varieties or hybrids that give the greater return of higher quality product per unit area in relation to cost. The practice of plant breeding is as old as civilization. However, after the birth of Cytology and genetics, it developed into a new science. A plant breeder must improve his technique and knowledge in order to keep pace with increasing demand of better form of flower, fruits and vegetables and also to maintain its individuality. The breeder wishes to create artificially a variable population for the reaction of types with desired combination of characters. Valuable characteristics are observed in different races, varieties or species and therefore by repeated hybridization, a single type may be evolved having all the good characters. The artificial hybridization process consists of following steps:

1. Study of floral morphology and emasculation.

2. Bagging and protection of flowers of the selected male and female parents.

3. Pollination of the emasculated flower.

4. Records and labelling.

5. Harvesting and observation.

Study of floral morphology and emasculation. It is essential for the plant breeder to know the morphology of flowers of the plant on which he is going to perform breeding experiment. It includes presence or absence of perianth, number of the stamens, nature of stigma etc. The majority of the flowers are bisexual, therefore, emasculation i.e. removal of stamens from the parent acting as female, becomes imperative, otherwise chances of self-pollination will exist.

Removal of stamens from a bisexual flower is called emasculation. Success of crossing largely depends upon emasculation. If emasculation is done without having proper knowledge of floral morphology, there is chance for injury to the plants and flower may wither away (Fig 88).

Emasculation is generally done with the help of forceps and needle. The forceps, fingers and needle should be sterilized, with rectified spirit or absolute ethanol before starting emasculation. Only those flowers/flower buds should be in which anthesis (dehiscence of anther) has not taken place. If there is any suspicion of pollen having been shed during emasculation, the flower should be discarded, Sometimes, simply longitudinal splits are made in perianth and the anthers are taken out through them.

During emasculation least injury should be done to the flower (Fig.89).

Bagging and protection of the selected flower. It is necessary to protect the female flower from unwanted pollen and insects.

Single flower or whole inflorescence may be covered with oil/ butter paper bag specially designed to suit it. It will not allow rain, or dew drops to reach the flower and spoil it. It is tied with stem of the plant by means of thread. It is supported with bamboo stick where support is required. Paper bag may become filled with CO_2 coming out of respiration causing harm to the flower. Hence, a small hole is made in the lower end of the bag to prevent entry of insects as well. There should be plenty of room for the flower inside the bag, otherwise bag may rupture, and flower may get injured. Sometimes whole plant or group of plants are covered by cags made up of wooden frame and muslin cloth. The cags are weighted with bricks to prevent from being blown off by wind.

Sometimes green houses are made insect-proof by fixing either muslin cloth or wire gauge over all ventilators inside the house.

Adequate protection may often be secured by isolating plants. It is applied in those cases where true breeding stains are to be raised strains for seeds.

Pollination of the Emasculated Flower. The next process after emasculation is of pollination. As stamens from female parent have been removed, the gynoecium gets more food and develop more vigorously. Therefore, within one or two days the gynoecium gets ready for fertilization. It becomes duty of the breeder to bring desired pollen in contact with the gynoecium. This process is called pollination.

Mature and ripe anthers from the desired male parent are taken and pollen grains from it are shed over the stigma of emasculated plants and flower is again covered inside the bag. After 12 or

24 h pollination is again made to ensure the success of the process.

After two or three days it is noticed that fruit is developing from each flower if the fertilization has occurred and whole process of breeding were carried out successfully.

Before making pollination, it is necessary to know the actual time of receptivity of stigma. For successful germination of pollen grains receptivity of stigma play a great role. High percentage of viable pollen grains to be dusted are also needed for effective hybridization programme.

Records and Labelling. After crossing the female flower, they are properly labelled and tagged with the help of threads. A label of hard paper with the following information is immediately tagged for future reference (a) Field record number i.e. reference number,

(b) Date of emasculation, (c) Date and time of 1st cross, (d) Date and time of second cross, (e) Date of harvesting, (f) Details of male and female parents.

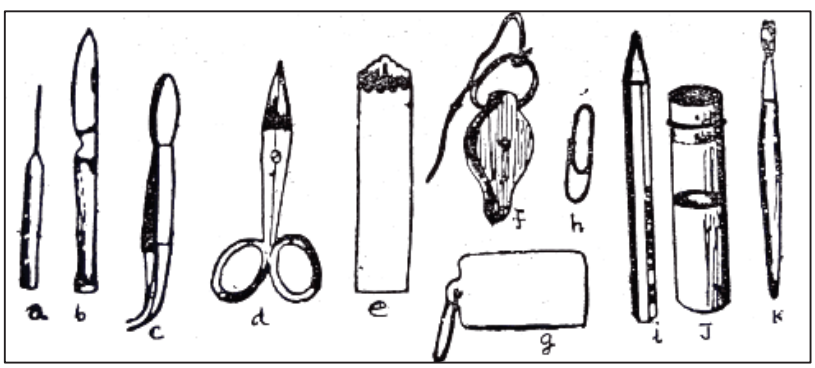


Fig.88(a-k). The instruments commonly used in emasculation and crossing. (a) needle, (b) scalpel, (c) forcep, (d) scissor, (e) bag, (f) pocket lens, (g) tag and label, (h) u-pin, (i) pencil, (j) spirit, and (k) brush.

The other details should also be recorded in field notebook along with specific observations made from time to time.

On maturity of fruit, they are collected along with their labels in envelopes. In the next season, seeds from these fruits are sown separately to raise F, generation. The plants of Fi generation are known as hybrids as they are the progenies of crossed plants. If the desired characters are not found in the F, hybrids, they are again crossed with the desired parent to get F, hybrids and the process is repeated till the required characters are incorporated in the hybrids.

Then the seeds are bulked and distributed among farmers after successful field trials.

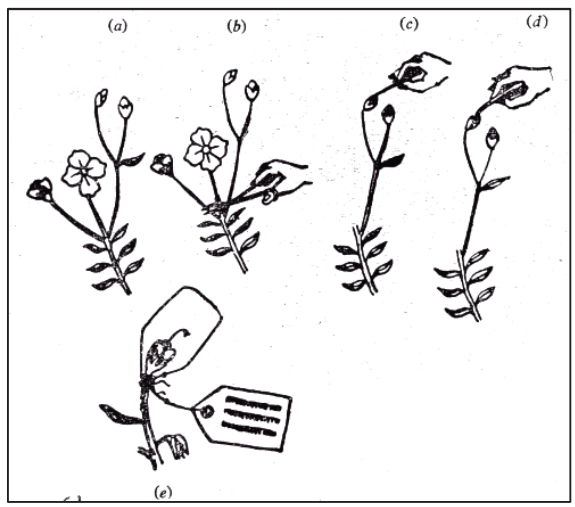


Fig 89.Fig. Hybridization technique (a) A twig with buds and flower,(b) Removal of unwanted flowers, (c) Removal of calyx and corolla for emasculation, (d) Emasculation, (e) Emasculated flower bud is enclosed inside a bag and a label is tagged to the bag.

Schedule for Mutation Breeding

Induction of mutation and their utilization for production of improved crop varieties is known as mutation breeding. Mutation breeding is a new departure from the conventional breeding proce- dure in agriculture and it started after the mutational researches by Muller (1927) and Stadler (1928). Now-a-days it is commonly used for crop improvement.

Mutation is defined as sudden heritable change in an organism other than those due to mendelian recombination and segregation.

Mutation can be induced by the applications of (1) radiations such as Alpha rays, X-rays, Gamma rays, Beta rays, Ultraviolet rays etc. and (2) Chemical compounds viz. Mustard gas, Ethylene oxide, Caffeine, Theophyline, Ethyl carbamate, Magnus chloride etc. The substance causing mutations are termed as mutagens or mutagenic agents.

The first step of mutation breeding is treatment of plants/seeds or seedlings with mutagen. Seeds or seedlings are exposed to radiations or mutagenic chemicals. Sometimes cuttings are also exposed to mutagenic substances. Seeds are soaked in desired concentrations of mutagenic chemical or treated with desired dose of radiation for 30 mt to 1 hour before sowing. Similarly seedlings or cuttings are also given radiation dose for the induction of mutation. Aqueous solutions of mutagenic chemical compounds are applied to the growing tips of seedings or cuttings for the purpose.

After irradiation or mutagenic chemical treatment seeds /seedlings /cutting are sown immediately in the field surrounded by control mother material. The M, (mutation 1) generations raised, and the seeds are collected for raising M, generations. Mutational effects, like death, growth inhibition morphological and developmental abnormalities may be due to chromosomal, cytoplasmic or genic changes.

Every suspected plant is picked from among the plants and is kept for raising M, generation of plants.

The seeds of individual plant/ear from M_1 are grown in M2 progeny rows. 14 to 15 seeds are sown in each M, progeny row to eliminate error due to too lesser/too many seeds per row. A row of original variety is sown after every 10^{th} M_2 progeny row for comparison and detection of mutation in M,. Seeds of M_2 plants are

collected the process is repeated to raise M, generation. If all the plants in M, rows are true to the M, types, they are called mutant plants.

True breeding strains are carried in M_4 generation. All the inferior stains are eliminated in M_4 generation where the best desired ones are selected for full yield comparison. If the mutant thus raised gives comparatively less yield, but is superior in desired character, it may be hybridised further with other variety. This may bring the desired character of high yield into the mutant variety. All the steps and techniques of hybridization are the same as described for conventional hybridization schedule.

Micrometry

In biological studies, it is often required to measure the length, breadth or height. In case of a cell or cell organelle, the diameter is to be recorded. For these micro-measurements ocular and stage micro- meters are used. Measurements with the help of microscope is called micrometry. Microscopic objects are measured with the help of a stage and an ocular micrometre. The ocular micrometre is a circular plastic or glass disc. In the centre of the disc a line is drawn which is further sub-divided into 100 parts. The value of each part is not mentioned. The calibration is done by comprising the scale of ocular micrometre with that of stage micrometre. A stage micrometre is a microscopic slide in which a line of 1 mm is drawn in the centre.

This line is again divided into 10 parts and each part into 10 smaller parts. Thus I mm is divided into 100 parts each measuring 0.01 mm or 10 (1 mm-1000 μ or microns).

The ocular micrometre is placed on the metal diaphragm in the eye piece of the microscope. Now stage micrometre slide is placed on the stage of the microscope and its scale is brought into focus. Both the lines- one on the ocular micrometre and the other on the stage micrometer are visible. By moving the stage micrometre and rotating the eye piece, both the lines are made to coincide i.e. overlapping each other at the starting point. The exact overlapping point of other ends of both the slides are observed. For example, the scale readings indicate 50 division of ocular micrometre scale equal to 70 division of stage micro meter scale i.e. 70×10 =700 μ. Therefore 1 div. of ocular micrometre will measure 700/50 μ or 14 μ. (Fig.90).

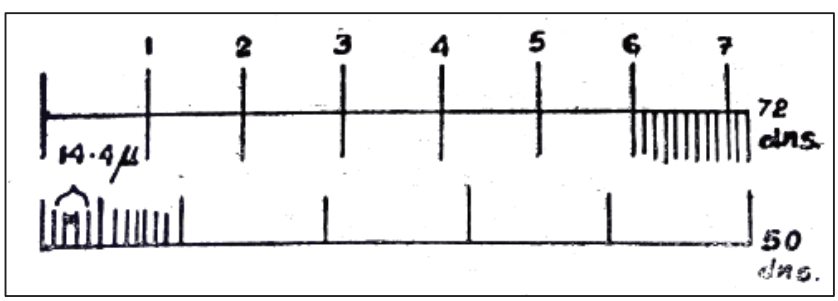


Fig 90 Stage micro meter scale 72 divisions or 0.72 mm (72μ equals to 50 division of ocular micro meter).

It is to be noted that this value of each ocular division will vary whenever a new combination of objective and eye piece is used.

Therefore, it is advisable that at each new combination of eye piece and objective, the ocular micrometre should be calibrated afresh for references.

After calculating the value of one division of ocular scale at a particular magnification, the microscopic preparations are placed on the stage of microscope. The organelle on cell or organ to be studied is focused. The length or breadth or diameter of the object can be noted as being equal to the number of ocular divisions.

As the value of each ocular division is known at that combination of objective and eye piece, the exact diameter or breadth can be recorded.

To measure the length of Chromosome with the help of Camera Lucide

Mitotic chromosomes are depicted on drawing sheet with the help of Camera lucida using 10x eye piece and 100X oil immersion objective. Camera lucida diagram of one division of stage micrometre is also drawn on the drawing sheet using the same eye piece and objective. Suppose one division of stage micrometre line (=10 μ) depicted on drawing sheet measures 12.5 mm. The depicted chromosomes measure 15 mm which will be equal to 10/12.5 X 15 μ or 12 μ. In this way length of chromosome and its arms can be measured.

Biostatistics

The involvement of statistics in biological studies is Biostatistics. The study of genetics incorporates a statistical analysis because every genetic principle is predicted upon numerical data and systematic study of relationships of numerical data is statistics. Statistical methods are required in almost all genetic reasoning as chance is directly involved in the act of Mendelian segregation. In genetics, the ratios of different phenotypes and genotypes arises from probability relationship *i.e.* the chance segregation of genes in gametes and their chance combination into zygotes. Probability means "on an average" of an event that has a certain chance of occurring. In such cases, statistical tests are done to decide whether the observed deviations support or contradict the proposed explanation. Some of these are described in the next chapter.

A. Chi-Square Test

It is a method to determine how close an obtained ratio fits into the expected ratio. It can be determined by the following formula:

$$\text{Chi-square } (\chi^2) = \frac{(\text{Observed-Expected})^2}{\text{Expected}}$$

When the X2 value is 3.841 or more its means the observed ratio is probably not an illustration of the ratio for which it was determined and something other than chance is operating. In order to know about goodness of fit table of Chi-square values is consulted. In the given table on column indicates degree of freedom. The degree of freedom is one less than the total number of classes involved. Chi-square values are mentioned in horizontal line adjacent to a degree of freedom Above the Chi-square value probability of chance is given. From this chart we find the possibility of occurrence of chance which has a range from 001 to 9 i.e. 1% to 90%. If the obtained Chi-square value 1.5 falls between 1.074 and 1'642 in the table it indicates probabilities in between 3 and 2 i.e. 30% and 20% respectively. This means, the deviation obtained would be expected in more than 20% of the cases provided that only chance is operating. (Table 5)

Chi-Square (χ^2) Values Arranged According to Chance Occurrence in Percentage

Degree of Freedom	Probability of Chance Occurrence									
	95% or 0·95	90% or 0·9	80% or 0·8	70% or 0·7	50% or 0·5	30% or 0·3	20% or 0·2	10% or 0·1	5% or 0·05	1% or 0·01
1	0·0039	0·016	0·064	0·148	0·455	1·074	1·642	2·706	3·841	6·635
2	0·103	0·211	0·446	0·713	1·386	2·408	3·219	4·605	5·991	9·210
3	0·352	0·584	1·005	1·424	2·366	3·665	4·642	6·251	7·815	11·341
4	0·711	1·064	1·649	2·195	3·357	4·878	5·989	7·779	9·488	13·277
5	1·635	1·610	2·343	3·000	4·351	6·064	7·289	9·236	11·070	15·086
6	1·635	2·204	3·070	3·828	5·348	7·231	8·558	10·645	12·592	16·812
7	2·167	2·833	3·822	4·671	6·346	8·383	9·803	12·017	14·067	18·475
8	2·733	3·490	4·594	5·527	7·344	9·524	11·030	13·362	15·507	20 090

Table -5

B. Standard Deviation and Standard Error

Standard Deviation. It is an indication of the variation of the data from the mean. Mean is the arithmetic average. It can be obtained by adding of all the values and dividing by the total number i.e. x

Standard deviation can be determined by the formula

$$\text{Standard deviation or } S = \frac{\Sigma (x-\bar{x})^2}{n}$$

$$\text{If } n \text{ is below 30 then } S = \frac{\Sigma (x-\bar{x})^2}{n-1}$$

Where S or 6-Standard deviation, x=a single measurement =the mean, the number of measurement and Σ=the sum of.

Standard Error. It is a measure of the variation of the means or standard error is the means of the mean. It can be determined as such S.E. Z= S.E. x=1

In an example the number of bivalents observed were 8, 7, 8, 8 and

7. The mean (x) of these observations would be 38/5=7.6.

$$\text{Now } S=\sqrt{\frac{(x-x)^2}{n-1}}. \text{ So.}$$

$$S=\sqrt{\frac{(8-7)^2+(7-7.6)^2+(8-7\cdot6)^2+(8-7.6)^2+(7-7.6)^2}{5-1}}$$

$$\text{or} \quad \sqrt{\frac{(0.16+0\cdot36+0.16+0.16+0\,36}{4}} \text{ or } \sqrt{\frac{1.2}{4}}=\frac{0\cdot11}{4}$$

Hence standard error of the mean *i.e.*

$$\text{SEX} = \frac{0\cdot11}{4} = 0\cdot0275$$

Sometimes probable error is used which is equal to 0'6745 times the standard error. If the deviation is more than three times of the probable error, the observed ratio is considered not a true example of the theoretical ratio.

C. 'T' Test

Sometimes the measurements of two different samples vary. 't' test is a method of testing whether the difference significant enough to enable one to assert that the two samples are really different or the difference is merely due to some cause of fluctuation or some error in the experiment. It can be determined as per the following formula:

$$t = \frac{\text{mean difference}}{\text{Standard error of difference}}$$

$$\text{Mean difference} = \frac{\text{Sum of differences}}{\text{Number of trials } (n)}$$

$$\text{Standard error of differences} = \sqrt{\frac{\text{Sum of square of differences}}{(n-1).\ n}} = \sqrt{\frac{(\text{Sum of difference})^2}{n}}$$

Determination of 't' test is illustrated in the following example.

There are two varieties of wheat: Variety A and Variety B. They were collected from four different localities which showed difference in the yield per acre as mentioned in the table. Let it be devided by means of 't' test whether the yield of variety B is significantly superior to the yield of variety A.

TABLE: 6

Data showing wheat yield in kilogram/acre collected from different localities.

Their yield differences and square of difference are also indicated

Locality	Wheat yield in Kg /acre		Differences	Square of differences
	Variety A	Variety B		
1	800	810	10	100
2	825	855	30	900
3	750	750	20	400
4	780	800	20	400
			Sum=80	1800

$$\text{Mean differences} = \frac{80}{4} = 20.$$

$$\text{Standard error of differences} = \sqrt{\frac{1800}{3\,4} - \frac{6400}{4}} = \sqrt{\frac{1800-1600}{12}} = \sqrt{\frac{200}{12}} = \sqrt{16.66} = \pm 4\,07.$$

In making the statement on some measurement, the average should be mentioned as ± standard error. (Table-6]

Therefore t=- 4.93

If the value of t is more than 6, it means it is not true to the expectation.

The value of 't' is also determined as :

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(\text{S.E. } \bar{x}_1) + (\text{S.E. } \bar{x}_2)^2}}$$

Chapter 31
Tissue Culture

Haber Landt (1902) developed the concept of cell culture in vitro. He was the first to culture isolated and differentiated cells in nutrient medium containing Knop's salt solutions glucose and peptone. Thus, he is regarded as the father of tissue culture. Following are the basic requirements for tissue culture laboratory.

For Culture room:

(a) Temperature control between 17°C - 27°C.

(b) Shelves for culture racks.

(c) Fluorescent tubes for lighting.

(d) Rotary shaker.

For Culture media:44

(a) Washing and storage facilities.

(b) Media preparation, sterilization and storage room.

(c) Incubators for maintaining cultures under controlled conditions of temperature, humidity and light.

(d) Transfer area/plot for aseptic manipulation.

For Isolation of culture:

(a) Laminar air flow hood.

(b) Spirit lamp/Bunsen burner.

(c) Ethyl alcohol 90% and Hypochlorite solution.

Equipment and Glassware:

(a) Gas, water and Electricity supply.

(b) Store/water heater.

(c) Hot plate with magnetic stirrer.

(d) Pipette washers, measuring cylinders, flasks, beakers, Petri dishes, pipettes with teats, culture tubes etc.

(e) Autoclave or pressure steam sterilizer.

(f) pH meter.

(g) Balances (centigram).

(h) Spatulas, scalpels, forceps, dissecting needles, culture racks.

(i) Micro oven/Hot air oven.

(j) Distillation plant and water de-ioniser.

(k) Filter-membrane.

(l) Dis infectants /détergent etc.

(m) Various chemicals for preparing culture media.

(n) Refrigerator/Deep Freeze.

(o) Glass atomiser.

(p) Wiremess basket, trolley with tray, racks for holding test tubes or culture vials in the autoclave.

(q) Microscopes and microphotographic equipment.

Technique of Plant Tissue Culture: It is growing of plant tissues in nutrient medium in vitro. The growing plant tissues include parts of plants i.e. explants like ovule, ovary, pollen, cell, tissue, Protoplast etc. Under this technique the explants are separated and cultured aseptically on a culture/nutrient medium under controlled conditions of temperature and light.

Some common culture media: The main components of most plant tissue culture media are inorganic nutrients (macro and micro nutrient) organic supplements carbon source, growth regulators and a solidifying agent. White's medium (1953) is one of the earliest plant tissue culture medium. Various culture media recommended by different workers are mentioned in the following table showing amount of components in mg/1. Or ppm. (Table-7)

Components	White's medium (1953)	Nitsch's medium (1969)	Chu's medium (1978)	Gamborgetat medium (1983)
$Mg\ S0_4\ 7H_2O$	750	185	185	400
KH_2PO_4	–	68	400	250
$NaH_2PO_4H_2O$	19	x	x	x
KNO_3	80	950	2830	2100
NH_4NO_3	x	720	x	600
$CaCl_2$	x	x	166	450
$(NH_4)2\ SO_4$	x	x	463	x
H_3BO_3	1.5	x	1.6	3
$MnSO_4\ 4\ H_2O$	5	25	4.4	x
$ZnSO_4\ 7\ H_2O$	3	10	1.5	2
$Na_2MnO_4\ 2H_2O$	x	0.25	x	0.25
$CaSO_4\ 5H_2O$	.01	0.025	x	0.025
$CoCl.\ 6H_2O$	x	0.025	x	0.025
KI	0.75	x	0.8	0.8
$Fe\ SO_4$	x	27.8	27.8	x
$Na_2EDTA,\ 2H_2O$	x	37,3	37.3	x
Surose (g)	20	20	50	25
Thiamine HCI	0.01	0.5	1	10
Pyridoxine HCl	0.01	0.5	0.05	1
Nicotinic acid	0.05	5	0.05	1
pH	5.8	5.8	5.8	5

Table 7

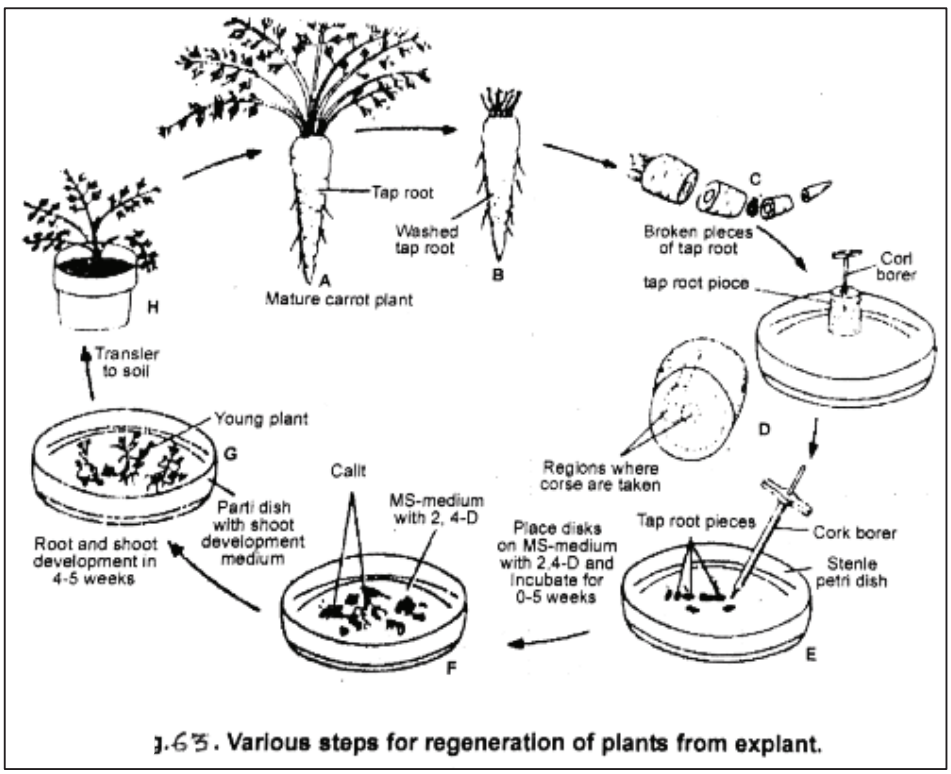
To prepare White's culture medium: This medium was formulated for root culture. These days commercially available media of reputed companies like Hi Media Laboratories Pvt. Ltd. 23 Vadhani Industrial Estate, Mumbai- 400 086. SIGMA Chemical Company, St. Louis, USA. containing desired macronutrients, micronutrients, vitamins, amino acids, growth regulators etc. are used. The dry powder of the medium is dissolved in distilled water. Agar, Sugar and other components are added in it. Distilled water is again added to prepare the final volume. Its pH is adjusted and autoclaved.

To demonstrate the regeneration of plant from explant.

Requirements: Explants (may be Anther, ovule, ovary, meristematic tissue etc.) flasks, liquid medium of White or Nitsch, sterile petri dishes, incubator, glass tubes, forceps, pot containing soil and water with nutrient solution, growth chamber etc.

Method: Take a culture tube with explant having 20 ml liquid medium/semi solid medium and incubate at 25-27°C in light for 3-4 weeks. It will grow into callus and/or differentiate into shoot may appear. These young plantlets are now transferred to pots containing soil, water and nutrient solution. The pots are kept in a growth chamber or a green house.

Observations: Young plants in the pot start growing into mature plants (Fig.91)



j.63. Various steps for regeneration of plants from explant.

Fig 91

Application of tissue culture technique:

Tissue culture techniques are effectively used in agriculture, industries animal husbandries, fisheries, dairy etc. It is of immense utility in the following:

1. Disease free plant production. Shoot tips and root tips of infected plants are made free from pathogens and then cultured in nutrient media to produce disease free plants.

2. Micropropagation: In this method small explants are vegetatively propagated into (as per table 7) nutrient medium to yield propagule or callus. The callus is transferred to rooting medium to develop plantlets, followed by field trials and finally desired plants are obtained.

3. With the help of another culture, haploid plants are raised useful in breeding experiments to stud! mutagenesis because the mutations are expressed immediately of genes are recessive.

4. Induction and selection of mutants are achieved through tissue culture technique.

5. Production of secondary metabolites like alkaloids, steroids, terpenoids, phenolics, essential oils etc are obtained in much more higher quantities of that obtained by whole plants. For this suspension culture technique is employed.

6. Protoplast culture: In this technique one can introduce foreign genes into the naked protoplast to get genetic variability of great agriculture V agronomic value in new plants.

ANTHER CULTURE

Anther culture is done to obtain homozygous plants. Homozygosity could also be achieved even in self-incompatible species. Development of haploids through anther or pollen culture can be used to transfer the genotypes of inbred lines into cytoplasm that caused male sterility. It also helps in the study of pairing relationships between chromosome.

Yamada et al (1963) were the first to raise haploid tissue from anther cultures of *Tradescantia reflexa*: Guha and Maheshwari (1964, 1966) were able to obtain haploid embryos in vitro from isolated anthers of Datura innoxia. Bourgin and Nitsch (1967) obtained the first haploid plants from isolated anthers of *Nicotiana*.

The selected buds are surface sterilized, and anthers are removed. Then anthers are quickly dip in absolute ethyl alcohol and its pollen are tested in 1% acetocarmine for correct stage. If the stage is desired, then the anthers of remaining stamens are detached and placed horizontally on the culture medium. In plants having minute flowers, the anthers are removed under a stereoscopic microscope aseptically. Only perianth is removed and rest of the buds with anthers are inoculated on simple media containing mineral salts, vitamins and sugars.

The anther cultures are maintained in alternating periods of light (12-18 hr) and dark (12-6 hr). The anther wall turns brown and after 3-8 weeks burst open due to the pressure exerted by growing pollen callus. Individual plants (3-5cm) or shoots are transferred to medium which supports a good root system. The rooted plants are ultimately transplanted to soil.

SELECTED QUESTIONS

1. Define biotechnology.
2. What is recombinant DNA technology.
3. What are the basic methodologies in genetic engineering.
4. What are plasmids? Differentiate them from cosmids.
5. Define transduction and transformation.
6. What are the vectors used for gene cloning in Plants.
7. What are the common media used for tissue culture.
8. What is anther culture? Explain their uses.
9. What are different types of waste treatments.
10. Name the biological organism involved in anaerobic digestion.

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Chapter 32
Nano Biotechnology

Thrust areas of nano biotechnology are (1) Nanotoxicology risk management & Nano biosynthesis (3) Nano biomolecular Interaction, Nano bioremediation (5) Nano emulsion and its application, (6) Nano sensor for food and environmental analytics (7) Nano aquaculture Centre for Bio separation Technology established in 2005 at the cost of 270 lakh by the department of science and technology. Nano metrology equipment includes (1) atomic force microscope (2) Scanning tunnelling microscope (3) Scanning probe microscope besides (4) High resolution optical microscope further enhanced with thin film metal evaporation unit, chemical vapour deposition unit, spin coater, Rota evaporator, high speed Centrifuge, milli pore distilled water plant, precision five digit Weighing balance, Microwave oven, chemical fume hood and work bench. Semiconductor parameter analyser and advance Image analysis software with nano laboratory setup.

Nanotechnology conceived in 1950s. It is engineering on a scale invisible to the naked eye. By manipulating individual molecule and atom nano engineers core cable to make materials with unique properties that promise enormous developments across every industry.

Swiss researchers Gerd Binnig (1980) and Heinrich Rohrer in 1981 saw developed scanning tunnelling microscope to see things at a single atom level. In nature almost everything happens

at a nanoscale. 1nm=1 millionth of mm. There are problems for Which nanotechnology might be the only solution. e.g. in keeping up with current trend for ever more powerful computers or delivering drugs to disease cells in human body. Gold nanoparticles uses ranging from also known as colloidal gold have many staining glass to treating arthritis and detecting cancer cells.

Bucky balls are made up of 60 carbon atoms with remarkable properties. They are hard to break and difficult to compress Carbon nanotube is one of the best and most useful products of the nanotechnology revolution. In 2005 bikes with frames made of carbon nanotube were very strong and rigid and make them extremely light. Each bike frame weighed less than 1 kg (2.2 lb).

So, tennis rackets and golf clubs' core to be made these tubes are about 100 times stronger than steel and are conductive or semiconductive Cheap solar panels and touch Screens are made. Tiny florescent crystals called quantum dots are use to track the movements of cells and molecules. Quantum dots and revolutionizing revolutionize the computer industry to increase data storage and speed up data retrieval. Nanoparticles are used in dust repellent paints, Sun screens and sports equipment Nano magnets have successfully been inserted into mouse blood cells.

To see detailed image of blood vessels with the use of magnetic resonance Imaging (MRI). Nano machines including motors made from DNA and nano scale computers form nano scale devices. self-propelled nano robots are made to perform Surgery.

Thus, nanotechnology is providing solutions to problems once thought impossible to solve. Ref-D. K. Science. ed. Adam Hart Davis, London, Delhi etc.

Nano liquid fertilizer: It is liquid nano DAP containing nitrogen of Phosphorus.

Nano urea Contains 500 ml of nano DAP equivalent to 50 kg bag of DPA (diammonium Phosphate/diphenylamine). It costs about Rs.6007-per 500 bottle of Nano DPA.

Nanoparticles Synthesis: Nanoparticles synthesis refers to methods for creating nanoparticles.

It can be derived from larger molecules or synthesized by growing particles from the time molecular distribution in Liquid or vapour phase Nanoparticle synthesis can be done by various methods like physical Chemical or biological approach Plant Nano particles Synthesis can be done with intracellular and extracellular mechanisms. Intracellular mechanism requires to Culture, monitor, track and harvest nanoparticles. It may result in adulteration with pathogens and other bio molecules.

Extracellular methods involve the addition of plant extracts of roots, leaves, stems etc to a boiling metal salt, various plant extracts were used for the synthesis of copper, gold, platinum nanoparticles. Plant nanoparticles are used in agriculture Cosmetics, biomedical and industrial sectors, silver nanoparticles produced from *Ocimum sanctum* leaf extract yielded spherical nanoparticles of 3-20 nm with a maximum absorption at 436nm Nanoparticles have extremely small size.

They can exist in single, fused, aggregated or agglomerated forms with spherical, tubular and irregular shapes Nano sensors are chemical or mechanical censors that can be used to detect the presence of chemical species, nano particles of monitor physical parameters. It is used as a sensing device to detect measure/sense the chemical and physical properties of matter like temperature, force, flow etc. at the nanoscale.

Senescence

Plants have a specific life span during which they develop, grow and finally die. Before death some deteriorative process occur in them which terminate their functional life. These processes are known as senescence. Salicylic acid, abscisic acid and Jasmonic acid including ethylene increases in volume. When a plant controls environmental stress senescence is Characterized by stable growth arrest and other Phenotypic alteration includes senescence. Thus, it is a process of

in plants. Ethylene regulates growth and senescence. It occurs in Leaves and flowers. Cytokinins are used to delay aging or senescence in plants Apoptosis is a form of programmed cell death while senescence is a process by which cells stop growth and cell division due to aging of the cells.

SECTION 4
Ecology. Environment

Chapter 33
Ecology

Ecology deals with the various principles between organism and their environments. Haeckel (1886) have used the term ecology. Charles Elton (1927) defined ecology as scientific natural history. Krebs (1972) defined ecology as "the scientific approach in the study of Living things and environmental interactions and evolution. The word Ecosystem has "eco" implies the environment and "s" as system-implies an interacting, interdependent complex Ecosystem may be categorise Luto natural ecosystem and artificial ecosystem. Natural ecosystem includes terrestrial and aquatic. Aquatic has two components: fresh water and marine ecosystem.

An ecosystem is a community of organisms and their physical environments interacting together. Environment involves both living organisms as well as non-living components. Living components are known as biotic factor. Non-living or abiotic factor of an ecosystem can be climatic or edaphic Energy flow travels through an ecosystem's food chain. Food chains initially provide through the input of the ecosystem itself e.g. the amount of Sunlight available for plant's life and the nutrient level of the soil. Without abiotic factors no ecosystem can provide for biotic factors.

Ecosystems are constantly changing. Human threats to biodiversity including deforestation. Pollution, transmission of disease across natural borders Introduction of non- indegenous species and reduced natural habitat through overpopulation. Natural threats include the migration of species into a particular region affects only one species.

Kinds of Ecosystem: -(1) Natural and (2) artificial ecosystem.

Natural ecosystem has been divided into Terrestrial and Aquatic

Aquatic may be fresh water or marine.

Artificial ecosystem is maintained artificially by addition of energy and planned manipulation, natural balance is disturbed regularly.

Structure and Function of an ecosystem: Structure means composition of biological community and Function means the rate of bio energy flow: It involves production of respiration rate of the community and in rate of the nutrients and materials cycle. In this way any ecosystem are Studied together. Structure of ecosystem has two major components 1) Biotic and 2) Non-biotic

Biotic components- It is tropic structure in which living organisms are differentiated based on their mutual relationship. From the nutritional view an ecosystem has two components viz- Autotrophic and heterotrophic. Natural resources mean the material which can be transformed in such a way that it becomes useful and valuable coming from nature and has some utility for man.

Land Utilization: About 162 million hectares land are used in agriculture which is about 51% of total land. A part of land like arid, rocky, sandy deserts, high mountainous uneven lands are not in use, much of the land is being used in towns and Cities as residential land. The land is also needed for transport, industries and sports etc. Therefore, we should make efforts for integrated planning to use land.

Chapter 34
Soil Degradation, Soli Profile

All the vital components required by plants are present. top layer of soil. It lies at a depth of 10-20 centimetres over the face & land. A healthy soil consists of bacteria, fungi, Algae, Worms protozoa, insects etc. There are many ways by which topsoil is Lost or degraded termed as soil erosion. massive irrigation is also harmful to fertility due to salination. Several methods have been devised by ecologists for management soil degradation.

These are biological methods and mechanical method. Biological method suggests normal farming used for soil protection. Growth & grasses are suitable in low rain fall area.

Mechanical method suggests to check the velocity of wind by tree plantation, growing vegetation alongside, construction of drains etc. and in addition-biological method suggests crop rotation, Dry forming.

Mechanical method includes afforestation, stream bank protection broad based ridge terrace etc

SOIL PROFILE

Soil profile is the sequence of the layers superimposed one above the other through the soil mantle. A soil horizone is a layer which is approximately parallel to the soil surface soil profile consists of five major horizons, viz (1) O-horizon (2) A-horizon (3) B-horizon (4) C horizon (5) R-horizon. It has been depicted in the following fig 92.

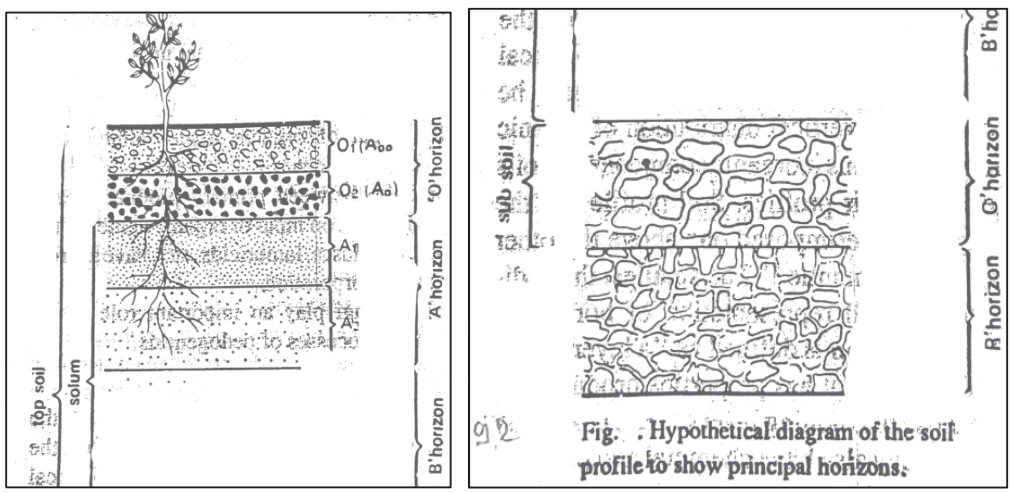


Fig 92

Soil Classification – Soils are classified as residual soil and transported soil.

Residual soils are those which occurs at the same place where their rock is present. Transported soils are (1) Colluvial (2) Alluvial (3) Glacial (4) Aeolian. Transporting agent of Colluvial soil is gravity, Alluvial soil is running water, glacial soil is large masses of snow (glaciers) and Aeolian soil is wind (Fig. 92).

Soil Erosion: Soil Erosion is a natural process. It takes about 500-1000 years for an inch of top layer to build up. There are many ways by which the fertile topsoil is lost and wasted. Chief accounts of soil erosion are water and wind.

Soil Conservation:

(1) Soil conservation is based on production of soil from impacts of raindrops.

(2) To prevent water from moving down the slope.

(3) To encourage more water to enter the soil.

(4) To increase the size of soil particle production in the wind velocity by growing vegetation which might catch and hold the moving particles of soil.

(5) Action in the wind velocity by growing vegetation which might catch and hold the moving particles of soil.

Methods of soil conservation:

Following methods are applied

(1) Biological method. It includes contour farming, crop rotation, dry farming layer farming etc

(2) Mechanical method: It includes contour tracing, bench tracing and afforestation. Contour farming creates ridges at the same level. The water is held in furrows and stored, which reduces erosion.

(3) Mulching: is effective in wind and water erosion. Mulch is protective layer formed by basal part of herbaceous plants, especially cereals attached to the soil. After harvest, 2 to 3 inches mulches, reduce soil moisture. Increase amount of soil moisture by addition of organic matter to soil.

Soil reclamation. It involves replacement of exchangeable sodium by calcium. Source of calcium may be gypsum, calcium chloride, and irrigation water containing calcium ions. Soil reclamation gives stability to agricultural granular sand. Soil reclamation is process of reclaiming soil quality, lost fertility, minerals, nutrients, moisture for use in agriculture.

ASP = Activated sludge Process

It is a multi-chamber reactor that are use highly concentrated microorganisms to degrade organics and remove nutrients from wastewater, producing quality effluent sludge by water treatment plant. The products are used in agriculture for reinstating eroded areas in forestry and urban landscaping applications.

ETP-Effluent Treatment Plant.

ETP is a process design to treating the industrial wastewater for its reuses or safe disposal to the environment. Atomic absorption spectroscopy (ASS) of sludge unveiled that it contains potential amount of Ca, Zn, Al, Fe, Cu, Co.

Chemical used in the ETP are alum, Ferric Chloride, Ferric-Sulphate and other Chemicals. Anaerobic bacteria are used in the plant.

Pyrolysis and Gasification

Pyrolysis is the decomposition of the biomass by heat in absence of oxygen. Pyrolysis generates gas, tar and char. While gasification converts the carbon containing materials into a gaseous output, Biomass gasification is a process of Converting solid biomass fuel into a gaseous combustible- gas through a sequence of

thermochemical reactions. Combustion of biomass refers to burning organic materials, at 500°C or above, like wood, dry leaves etc to generate heat and light. Heating takes place in absence oxygen.

Chapter 35
Ecological Factors

Majority of the ecologists prefer to classify four types of ecological factors, viz: a) climatic factor, (2) Topographic factor (3) Edaphic factor, and (4) Biotic factor.

Climatic factor includes light, temperature of air, humidity of air, wind and rainfall. Light directly or indirectly affects the plants life in chlorophyll production, on transpiration, stomatal movement and overall development of plant.

Rainfall: Water from soil comes from rainfall. Amount of rain fall determines the vegetation in any area. The region with scanty rainfall are seen with xerophytic vegetation and deserts.

Wind: Wind is air in motion. Wind is directly involved in transpiration, dispersal of pollen, seeds and fruits. Air moves from region of high pressure to low pressure and from poles towards equator. A wind of high velocity causes breaking, tearing of branches and leaves and sometimes uprooting.

Topographic Factor: Main topographic factor or phytogeographic factor includes mountain chains steepness of slope etc with change in altitude above sea level. There are changes in the temperature, wind Velocity, intensity of solar radiation etc- Mountains also affects the climate through, rain fall, wind velocity etc. Exposure of slope also affects the kinds of plants growing there.

Edaphic factor: Edaphic factors are concerned with Structure and composition of soil. Soil is made up of several component, Soil is not merely a group of mineral particles. It also has a biological system of living organisms. Soil water, soil organic matters and biological system having bacteria, fungi, algae, protozoa nematodes etc. According to DOKUCHAYEV (1889) boil is result of actions and reciprocal influences of parent rocks, climates topography, plants, animals and age of the land.

Chapter 36
Bioremediation and Bioethics

Bioremediation

Bioremediation is the use of living organisms, mainly microorganisms, to prevent or degrade environmental pollutants through waste treatment. Bioremediation is ideal alternative technology for removing pollutants and restoring contaminated site.

Three different bioremediations research are emerging worldwide. Europeans are expanding their traditional waste and water treatment system to cope with specific chemical pollutants. U.S.A. Focuses on site specific cleanup of soil and water contaminated with petroleum and genobiotics. Japanese considers global environmental problems.

Bioethics

Bioethics is the study of ethical, social and legal issues that arise in biomedicine and biomedical research. The term bioethics is made of Bios and ethos that is moral, nature, behaviour. This term was coined in 1927 by Fritz Jahr in an article regarding the use & animals and plants in Scientific research. Bioethics promotes a set of principles to guide the interaction between the human race and living things.

Biogas production: Biogas is renewable energy source. It can be made from different, mass like poultry, droppings crop waste and cattle dung by controlled anaerobic degradative Biogas can be processed to produce bio-methane which can be injected into natural gas pipeline. Biogas can be used for Power and heat generation use & biogas can reduce greenhouse gas emissions. It has a huge potential for renewal sources Biogas is produced under anaerobic digestion.

Chapter 37
Ecological Pyramids

Ecological Pyramids: Ecological pyramids involve producers like Herbivores, Carnivores may be demonstrated by means of ecological pyramids. Ecological pyramids are of three types.

(1) Pyramids of number (2) Pyramids of biomass and (3) Pyramids of energy.

Pyramid of numbers: It shows the relationship between herbivores and carnivores, at successive trophic level.

Pyramids of biomass: It shows the quantitative relationship of the standing crop. There is gradual decrease in biomass of Organism at successive level from the production to the top carnivores.

Pyramid of energy: the number and weight of the Organism at any level depends on the amount of fixed energy present. It's a picture of the rate of passage of food mass through the food chain. (Fig 93).

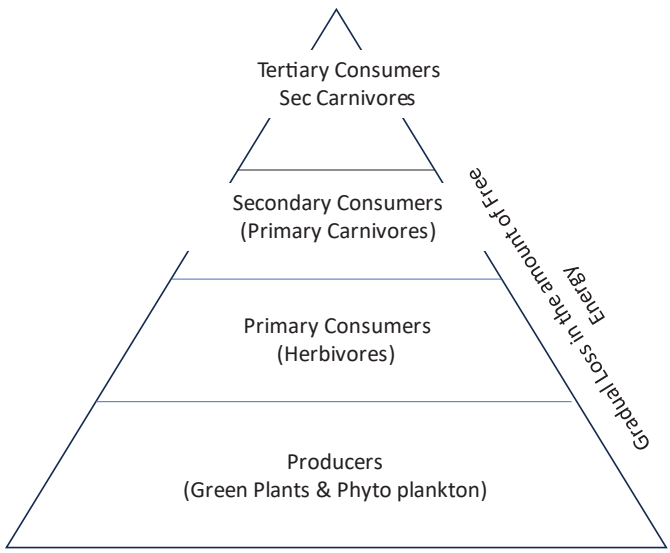


Fig 93. Pyramid of Energy

Food Chain: Food chain involves transfer of food energy from the producer via herbivores →carnivores →decomposer with repeated eating is known as food chain.

Producers utilize the radiant energy of sunlight, which is transformed into chemical energy that is ATP during photosynthesis.

These green plants occupy the first Tropic level in any food chain and are called primary producer. The energy stored in food matter by green plants is used by herbivores - a second Tropic level and called primary consumer (herbivores). Herbivores are eaten by carnivore- the secondary consumer level. These in turn may be eaten by other carnivores at tertiary consumer level. Some organisms are omnivorous, eating the producers and the carnivores both. This chain of eating and being eaters is called food chain.

Food Webs: Interlinking of various food chains is called Food Web. Food web relates the feeding relationship among species within a community revealing the dynamics & energy transfer in an ecosystem. Food web ensures that there is plentiful supply of food so that all plants, insect and animals have enough. A food web provides alternative pathways of food availability. There are two types of food web.

(1) A grazing food web based on photosynthetic plants and

(2) A detrital food web based on decomposers like bacteria and fungi.

Chapter 38
Ecological Adaptation

Ecological Adaptation: An ecological adaptation is any morpho-logical, Physiological or behavioural trait of an organism that allows it to survive or reproduce in a habitat. Adaptation is a biological mechanism by which an organism gradually gets more acclimated to its surroundings - Example of structural adaptation is the way some plants have adapted to live in dry and hot deserts. Some plant have adapted to its climate by storing water in their shoots and

Leaves. There are three types of adaptation: (1) structural, (2) Physiological and (3) behavioural

Ecological adaptation in hydrophytes: Features of hydrophytes

Morphological-Roots absent or poorly developed stem-long, slender spongy and flexible, Leaves - Long or ribbon shaped, linear or finely dissected as in *Ceratophyllum*. Flowers and seeds-less common where flower develops, seeds are rarely formed.

Anatomical features-cuticle absent or poorly developed.

Epidermis-Single Layered of thin-walled parenchymatous cells.

Cortex-has air cavities (aerenchyma) which increases buoyancy.

Vascular bundles-Poorly developed, Tracheid present.

Mechanical tissues are generally absent. Mesophyll is single layered in *Potamogetone*.

Warming (1909) recognised three major groups of Plants, viz:

(1) Hydrophytes, (2) terrestrial plants and (3) marshy plants i.e. halophytes.

Hydrophytes- Here abundant water is available to plants. They are divided into five categories, (1) Free floating hydrophyte e.g.,- Azolla, Eichhornia, Salvinia, Pistia etc

(2) Rooted hydrophytes with floating leaves, eg.- *Marsilea, Trapa, Nelumbo* etc.

(3) Rooted emergent hydrophytes, e.g., Ranunculus, Cyperus etc.

(4) Rooted submerged hydrophytes, e.g., *Hydrilla, Vallisneria*, *Potamogetone*.

(5) Submerged floating hydrophytes, eg.- *Ceratophyllum*, *Urticularia*, *Najas* etc.

Xerophytes: Daubenmire (1959) defined xerophytes as a plant which grow on substrata that usually become depleted of water to a depth of 2 centimetres during a normal season. Xerophytes are classified as (1) Ephemeral or drought escapers, succulent and (3) Non-Succulent perennials.

Ephemeral: In this group of plants avoid and not withstand dry Season and escapes dryness in internal and external environments

Succulents: These plants suffer from dryness in external environment only. Fleshy stems, leaves; roots serve as water storage organ, which stores water during rainy season, e.g., Aloe, Opuntia, Euphorbia etc.

Non-Succulent perennials -These plants suffer from dryness in their external and internal environments. It has extensive root system ability to reduce transpiration and reduction in size

of leaf blades, e.g., *Calotropis, Accacia, Casuarina, Nerium* etc.

General features of xerophytes: Root system extensive. may be 30 metres long, rolling and folding leaves, heavy cuticular epidermis, wax coating on leaves, sunken Stomata etc.

Chapter 39
Halophytes

Plants which grow on saline soils containing magnesium Chloride, magnesium sulphate and other salts, some of them produce negatively geotropic roots which come out of the mud surface for entry of oxygen gas. These roots are called Pneumatophores containing breathing pores for gaseous exchange. Some halophytes show Viviparous germination of seeds where seed germination starts before its shedding from parent plants. Halophytes occur mall tropical seas specially on muddy shores as in lagoons, inlets, estuaries etc These littoral swamp form a vegetation called mangrove vegetation. In subtropical or tropical areas Mangroves are very much prevalent or sea shores of Kerala. Mumbai and in Andaman Nicobar Island. Rhizophora, and

Sonneratia are common halophytes.

BSI (Botanical survey of India). It was established in 1890 with the objectives of exploring the plant resources and identifying Plants species with economic value. It is situated at Kolkata in

West Bengal.

Chapter 40
Succession

Environment is always changing over a period of timing due to variation in climatic and physiographic factors. This process continues and successive communities develop. This is ecological succession.

Clements (1916) defined succession as the natural process by which some locality becomes successively colonised by different communities of plants. Odum (1971) called it as ecosystem development rather than ecological succession. Process of succession would become clearer by studying various kinds of series in different habitat condition- on the basis of areas colonised by plants, Plant Succession can be of two categories:

(1) Hydrosere and

(2) Xerosere.

Hydrosere: Its stages are well studied in ponds or lakes. Stages of hydrosere in ponds are as follows:

Phytoplankton Stage: Some green algae, diatoms, blue green algae and bacteria are the first organism to colonised in the pond.

Rooted Submerged Stage: Due to death and decomposition of Phytoplanktons and mixing with the silt results in the soft mud at the bottom of the ponds. This facilitates the growth of hydrilla. *Potomogetone, Vallisneria, utricularia* etc.

Rooted Floating stage: Rooted hrdrophytes like *Nelumbium*, *Nymphaea, Limnanthemum* etc with their large floating leaves develop.

Reed swamp stage: Plants like *Typha, Scirpus, Saggitaria* etc. grow. They have well developed rhizomes. Water level is very much reduced.

Sledge Meadows stage: Plants of graminae and cyperaceae like *Cyperus Juncus, corex* etc., colonize the area.

Wood Land Stage: Disappearance of marshy vegetation leads to driver soil. Terrestrial plants like *Populus, Almus, Salix* like shrubs appear in this area (vanishing pond).

Forest Stage: Now climax communities appears like *Quercus, Acer* like many trees. (Fig 94).

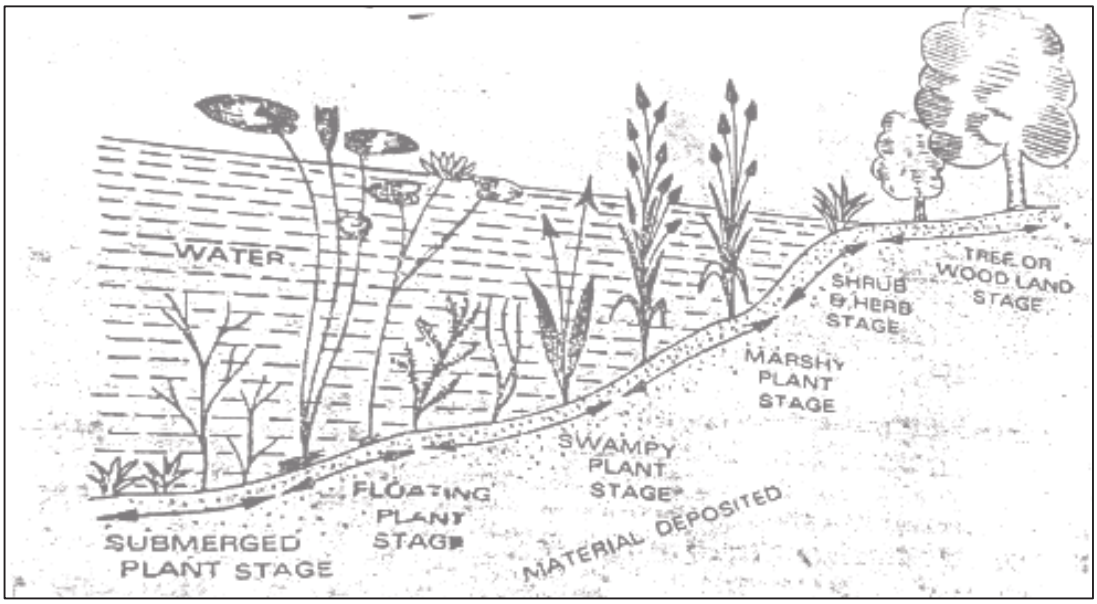


Fig. 94. Plant succession in a pond.

Xerosere: It originates on bare rock surface. The various stages and their plant species appearing on rock surface are as follows:

(1) Crustose Lichen Stage: Lichens like *Rhizocarpon, Leconora* appear first which produces acids due to which weathering of rock starts.

(2) Foliose Lichen Stage: *Parmella, Dermatocarpon*, lichen appear,

(3) Moss Stage: Some xerophytic mosses like *Torula, Polytrichum* appear in this stage. Thickness of soil layer on rock area seen.

(4) Herb Stage: *Poa Aristida, Festuca* like herbaceous plants appear at this stage.

(5) Shrub stage: In this stage shrubs like *Phyto corpus*, *Rhus* appear.

(6) Forest stage: Finally xerophytic trees invade this area and forest community dominates.

Chapter 41
GANGA Action Plan

Ganga action plan started in 1986 with the objective of pollution abatement from river Ganga. It is centrally sponsored Scheme. The main action was to lower the pollution, improve the quality of water in Ganga River by diversion and treatment of domestic sewage and toxic wastes from identified point source of Pollution. It started on 14th January 1986 by Shri Rajiv Gandhi, India's then Prime Minister. Lack & funds made it difficult to set up adequate sewage treatment infrastructure of the required capacity. Lack of data on the water use and wastewater generation ensured that the plans failed.

Yamuna Action Plan

It is bilateral plan between the government of India and Japan. It was introduced in 1993. It is one of the largest river restoration projects in India. Purpose of the plan is the abatement of severe pollution of the river Yamuna by raising sewage treatment. Capacity caused by rapid population growth, industrialisation, urbanisation in the towns of river basin which includes Delhi -the capital of India.

Ministry of environment and forest has initiated Yamuna Action and Ganga Action plan.

Chapter 42
Epiphytes, Environmental Pollution

Epiphytes are non-parasitic plants grow on other plants such as trees, mosses, ferns, bromeliads like lichen and *Peperomia*. Epiphytes are also known as air plants, because they as red in the soil. Epiphytes derive nutrients from rainwater, air and from other sources. The majority of epiphytic plants are flowering plants (angiosperms), they include orchids, tillandsias and members of Bromeliaceae family. Mosses, ferns and Liverworts are also epiphytes and are found on tropical and temperate regions, Leaves and seeds are eaten by insects, tree frogs and birds. Squirrels and monkeys may eat some species of epiphytes.

Rafflesia is an obligate epiphyte. It has a single large flower and lacks chlorophyll. Lipstick plants is herbaceous and perennial native to Thailand and Malaysian peninsula, Sumatra and Bornio.

Epiphytes grow on another plant, but not parasitic. They use their roots to anchor themselves rather than for nutrient uptake.

Epiphytes may be holoepiphytes or hemiepiphytes. Holoepiphyte completes their life cycle with coming in contact with soil. Hemiepiphyte spends half of its life without soil and half of its life with soil.

Environmental Pollution: Pollution is necessary evil of all development. It is man-made problem. Pollution is undesirable change in the physical, chemical or biological features of air, water and soil that may affect the life of any living organism. Following pollutants pollute our air, water and land.

(1) Deposited matter like smoke, dust, soot, etc.

(2) Gases e.g.-NO, NO_2, SO_2, CO, Halogens, etc.

(3) Acid droplets of nitric acid and sulphuric acid etc.

(4) Metals - It includes mercury, leads, Iron, Zinc etc.

(5) Agrochemicals - Pesticides, insecticides, herbicides etc.

(6) solid waste produced in households, kitchen etc.

(7) Radioactive waste and

(8) Noise

METHODS OF WASTE DISPOSAL

In the past the waste from affluent societies was just dumped, burned or placed in landfill sites whereas less affluent societies recycled out of economic necessity. Incineration and landfill are still much used but now there is increasing pressure on everyone to recycle as much waste as possible. Even better is to reuse materials such as glass, metal and plastic wherever possible.

Method	Advantages	Disadvantages
Landfill	Efficient method to deal with large volumes. Filled land can be used for building or other community purposes.	Local residents may object to new sites. Once filled needs time to settle and may require maintenance as methane released.
Open dumping	Convenient and inexpensive.	Causes air and ground water pollution. Health hazard- encourages rodents and insects. Unsightly.
Ocean. dumping	Source of nutrients. Convenient and inexpensive.	Danger to marine animals. Pollutes the sea.
Incineration	Reduces volume. Requires minimal space. Produces stable, odourless residue. Can be used a source of energy.	Expensive to build and operate. Can cause pollutants e.g. dioxins, if inefficiently burned. Requires energy.
Recycling	Provides a sustainable environment.	Expensive. Difficulty in separating different materials – not possible in all cases.

TYPES OF NUCLEAR WASTE

There are various types of nuclear waste. Some are radioisotopes from researchdaboratones of hospitals Some are spent fuel rods from nuclear powerstations and some are materials that have come into contact with radioactive material and become contammated themselves. Essentially nuclear waste can be divided into high level waste and low level waste. Low level waste includes items such as rubber gloves paper towels and protective clothing that have been used in areas where radioactive materials are fiandled

The level of activity is low and the halftives of the radioactive isotopes are generally short. High level waste has high activity and generally the isotopes

have long half-lives so the waste will remain active for a tong period. Most high level waste comes from spont fuel rods or the reprocessing of spent nuclear fuel

Recycling

Metals: Mainly aluminium and steel. The metals are sorted then melted and either re-used directly or added to the purification stage of metals formed from their ores. This is particularly important for metals such as aluminium which require large amounts of energy to produce direct from ore.

Paper: Sorted into grades. Washed to remove inl's etc., made into a slurry to form new types of paper such as newspaper and toilet rolls. However energy is required to transport the paper and it may be more efficient to compost.

Glass: Sorted by colour, washed, crushed then melted and moulded into new products. Glass is not degraded during the recycling process so can be recycled many times.

Plastics: After sorting plastics are degraded to monomers by pyrolysis, hydrogenation, gasification and thermal cracking then repolymerized. Recycling causes less pollutants and uses less energy that producing new plastics from crude oil but sorting the plastics can be problematic.

STORAGE AND DISPOSAL OF NUCLEAR WASTE

Low level waste Different methods are used to dispose of low level waste. Although many governments have now banned the practice some is simply discharged straight into the sea where it becomes diluted. Since the decay produces heat it is better to store it in vast tanks of cooled water called 'ponds' where it can lose much of its activity. Before it is then discharged into the sea it is filtered through an ion exchange resin which removes strontium and caesium, the two elements responsible for much of the radioactivity. Other methods of disposal include keeping the waste in steel containers inside concrete-lined vaults.

High level waste During reprocessing of spent fuel about 96% of the uranium is recovered for re-use. About 1% is plutonium which is a valuable fuel. The remaining 3% is high level liquid waste.

One method used to treat this is to vitrify it. The liquid waste is dried in a furnace and then fed into a melting pot together with glass making material. The molten material is then poured into stainless steel tubes where it solidifies. Air flows round the containers to keep them cool. Because of the high activity and long half-lives some of the waste will remain active for hundreds if not thousands of years. The problem is how to store it safely for this length of time. Currently the best solution seems to be burying it in deep remote places that are geologically stable such as disused mines or in granite rock. The concern is that the radioactive material may eventually leach into the water table andthen into drinking water.

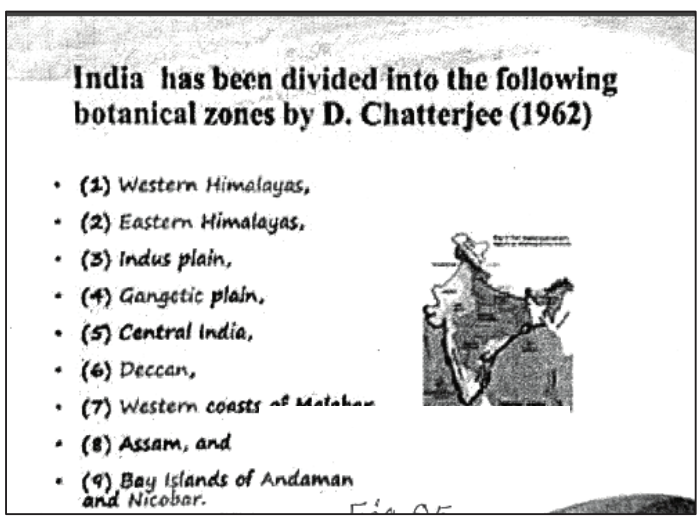
Chapter 43
Phytogeography & Forest

Phytogeography is the study of the distribution of plants in terms of their origin, dispersal and evolution. It shows records of specimen According to Wulff (1943) Phytogeography is the study of distribution of plants in their habitats and elucidating of origin and history of development of the flora. Croizal has mentioned in the year 1952 that Phytogeography is the study of migration and evolution of plants in time and space. It can be said that phytogeography is concerned with the geographic distribution of plant species and their influence on the earth's surface. The purpose of the Phytogeography is to learn how Plants migrate but ultimately to ascertain how they evolve while migrating Alexander von Humboldt referred as the father of Phytogeography.

Phytogeographic Region of India.

Following are the Phytogeographic regions of India

1. Western Himalayas
2. Eastern Himalayas,
3. Indus plain.
4. Gangetic Plain
5. central India
6. Deccan
7. Western Ghats of Malabar.
8. Assam and
9. Bay Island of Andaman and Nicobar (Fig. 95).



(Fig. 95).

Plant Phenology: It is the study of the timing of plant lifecycle events, such as, flowering, fruiting and leaf bud burst. It helps to understand how Plants respond to environmental changes or climate change. It is studied through field observations and satellite remote sensing. Field observations involve recording the timing of events like Leaf unfolding, bud burst and leaf fall. Satellite remote sensing can measure the spectral features of vegetation across the globe Plant phenology is sensitive to environmental factors like temperature, water availability and day length.

Changes in the plant phenology can have cascading effects on other organisms including animals that depend on plants. These changes can lead to agricultural losses, evolutionary shifts and negative impacts on demography.

Robert Marsham is the founding father of Phenology in the U.K.

Stages of phenology: Germination, bud bloom, flowering etc are called phenological stages. Phenological events can provide valuable data for planning, organizing and timely execution of certain standard and special agricultural activity that require advanced information on the dates of specific stages of crop development.

Forest

Forests are essential for ecological balance. It is renewable natural resources. It attracts rainfall. The main product of forest are wood, paper, pulp, timber, board, packing materials for fruits, matches, sports goods etc. Minor forest products are dyes, gums, resins, tannins, lac, fibres, *kathha* etc. Forests provide food in the form of roots, Leaves, tubers, fruits etc to tribal people. It provides protection to wildlife. It maintains the gene reserve of important plants. Forests help mitigate the effects of climate change by absorbing Carbon dioxide and releasing oxygen Forests help in maintaining temperature and oxygen Level of the atmosphere and play a significant role in balancing the oxygen level in the atmosphere. Forest is the house & many living organisms Forests can be classified as tropical, evergreen, deciduous and dry forests based on the climate condition and types of trees present. Forest also comprise non-living components sum as lakes, ponds, soil, rocks etc. A Forest is defined as an area forming on ecosystem Forests help in preventing global warming. The increase amount & carbon dioxide (greenhouse gas) in the atmosphere results in the greenhouse effect and thus causes global warming.

Chapter 44
Energy, E.I.S, G.I.S., Ecological Footprints

Energy is important input for development. According to law of conservation of energy, energy can be converted in form but not created or destroyed. Energy is ability to do Work, Sources of energy are renewable and non-renewable. Renewable energy and its sources are solar energy, wind energy, water energy, petro plants, animal dung etc. They are produced in nature and can be harvested through proper planning and management.

Non-renewable energy and its sources are available in limited amount. It includes mineral oil, coal, natural gas and nuclear power. Coal, natural gas and mineral oils are organic and are also known as fossil fuels-Conventional Source of energy.

Coal- out 6000 billion tons of coal lies under the earth, about 200 million tons of coal had been used. Coal major fields in India are Bihar, Jharkhand, Madhya Pradesh and other states of India.

Oil and Natural gas: There are many regions in the wood rich in mineral oil. They are USA, Mexico, USSR, Iraq, Saudi Arabia, Kuwait, Iran etc. In India Assam was only state where mineral oils were drilled. Natural gas reserves have been found, Tripura, Rajasthan, Orissa, Maharashtra, Tamil Nadu and Andhra Pradesh.

Thermal Power: Thermal power stations are located in India are in many states.

Hydro Power: The generation of hydroelectric power in India stated from first five year Plan. Several river valley projects were launched and dams were constructed from which electricity are generated.

Nuclear Power: A small amount of radio active material can produce enormous amount of energy. For example, one ton of Uranium (Uranium 35) would produce as much energy as three million tons of coal. Nuclear reactors are required for atomic energy.

In India there are six nuclear power plants located in Maharashtra, Tamil Nadu, Rajasthan, Gujrat, Karnataka and Uttar Pradesh.

Non-conventional source of energy:

Solar energy: Photovoltaic conversion system converts solar directly into electricity through silicon solar cell. It may be installed in forest, deserts, rooftops, street-lamp post etc.

Wind energy: Wind energy may be converted into electrical and mechanical energies. Today wind energy, is utilized in pumping water in rural areas and others remote areas.

Tidal energy from Ocean: Tidal power depends on harnessing of rise and fall of sea level tidal action. France Constructed tidal electric Plant in 1966. India could intensify work on ocean thermal energy plant.

Energy from urban waste: Solid waste can be converted into energy source. Sewage in cities are used for generating gas and electricity. Bagasse Rich husks and other farm wastes are also being used to produce electricity. In several areas Gobar Gas plants are installed to produce energy specially for household uses.

EIS Environmental Impact Assessment It is a tool used to assess the significant effect of a project on the environment. It makes sure that effects on environment due to project are to avoid, reduce or offset those effects. An EIS is a document required by the 1969 National Environmental Policy act for certain action under United States.

Following are the steps in the EIA process:

Screening, scoping. Impact Assessment, Mitigation, Public participation, Reporting, Decision making, monitoring and compliance. The goal of EIA is to identify, predict and assess the potential effect / impacts of the proposed project on the environment including its natural resources, ecosystems and human health as well as social, cultural and economic factors. The scope of EIS includes a survey and review of prior studies done by others to describe the physical, Chemical and biological conditions of the study area.

GIS Geographic Information Service.

GIS are computer system that capture, store, analyse and display data that is linked to a specific location on earth. It can help users make better decisions by providing foundation for mapping and analysis. It can show many different kind of data on one map such as streets, buildings and vegetations. It is used to create, manage, analyse and map all types of data. GIS facilitates basic cartographic tasks such as creating legends, north arrows and distance scales. It performs the difficult jobs of accurately projecting a map to the shape of the earth's surface. There are several methods used to enter data into a GIS where it is stored in a digital format. Benefits of GIS are as such:

Effective planning, Efficient design, Betts information management, enhanced mapping capabilities, improved Communication, manage natural resources, Streamline service and logistics (Logistics), scope of GIS is unlimited.

Civil Engineering, Public Works Administration, Property mapping are main domains of GIS. Government uses GIS data and GIS based Solutions for urban planning, zoning and land use projects, natural disaster and health event response, roadway system and building design, utility, distribution, energy production and waste and resource management.

GPS (Global Positioning system) are used to find the exact location of things. GIS (Geographic Information system) are used to record Information on to maps.

Ecological Footprint: It tracks the use of productive Surface areas like Cropland, Grazing land fishing ground, built up Land, forest area and carbon demand on land. It measures the amount of biologically productive land or water that enables the population to sustain itself. India represents 6% of the world's ecological foot paint. The ecological footprint is a method that determines that how humans are on natural resources. Ecological footprint in a measure of how much a person Population on product consumes resources from the environment and how much waste it produces. Following are the factors that can impact an ecological footprint: Infrastructure, food and wates consumption energy. Finally Ecological footprint is a measure that indicates how much resources form the environment are required to support a specific way of life or business.

Carbon Footprint

Carbon footprint is the total amount of greenhouse gases that are generated by our action. The average carbon foot point for a person in USA is 16 tons- one of the highest in the world. According to WHO a carbon footprint

is a measure of the impact & your activities have on the amount of CO_2 produced through the burning of fossil fuels and expressed as aa weight of CO2 emission produced into us.

Carbon footprint can be reduced by following measures: Buy energy efficient appliances, switch to renewable energy resources, replace old refrigerators, use less water, reduce the dependence on air conditioners and buy food locally.

Chapter 45
Biodiversity

Walter G. Rosen used the term Biodiversity or biological diversity in 1986. It is variability among living organisms from all sources. It includes diversity within species, between species and of ecosystem.

Following is the species diversity:

1. Point diversity: It is diversity of micro habitat.
2. Alpha diversity: St is local diversity and includes varieties of animals occurring in a particular habitat. E.g. a plant may be considered as unit of alpha diversity and a Leaf as an area of point diversity.
3. Gamma diversity: It is diversity of larger unit like an island or land scape. A group of plants occurring together as an area of gamma diversity
4. Epsilon diversity: Itis regional diversity and includes total diversity of a group of areas of gamma diversity. It includes a forest within which the plants are located as an area of Epsilon diversity.

Biodiversity may be discussed under following heads:

1. Genetic diversity: It deals with genetic variation within species of a single population.
2. Species diversity: It deals with the full range of species on earth from microorganisms to multicellular organisms of Plants, animals and fungi.

Community diversity: It includes variations in the biological communities in which communities exists and interaction among these.

Importance of Biodiversity: Bio-diversities are required to determine the origin of life

Aldo Leopod (1949) had stated that a thing is right when it tends to preserve the integrity, stability and beauty of the biotic community. Present human activities are destroying the earth's biodiversity and hence existing political, economic, technological and ideological structures must be changed.

Endemic and Endanger species: These organisms are one that lives in a particular region and no where else on the earth. Endanger species or organisms are close to extinction to the fact that its members are declining.

Banyan tree is endemic in India.

NBPGR (National Bureau of Plant genetic resources). It is a unit under ICAR- Department of Agricultural Research and Education (DARE), Ministry of Agriculture, Govt. of India. Its headquarter is in India. It acts at the national Level for management and acquisition of PGR for agriculture and to carry out related research for sustainable growth of agriculture.

FAO (Food and Agricultural organisation): It plays a crucial role in restoring rural lively hood building resilience and participatory approaches to the Policy making. Its goal is to achieve food security for all.

Environmental auditing.

It is a systematic documented periodic and objective process in assessing an organisation's activities and services related to. In nutshell, environmental audit provides an assessment of the environmental performance of a business or organisation of environment.

Main objective of environmental audit is to help the safeguard and minimise risk to human health. An ISO 14001 audit is an essential part of supplementing the 15014001 environmental management system (EMS). It is systematic independent objective and documented process for gathering facts to identify areas for improvement and ensure you have best practice processes in place. It recommends you to carryout regular internal and external audits.

Circular Economy.

Circular economy is oriented to nature as its role model. It aims to keep raw materials in a close loop. Thus, resources are maximally used. The need for new ones is reduced waste is avoided and the life cycle 8 products, is increased. The Waste & today becomes the raw materials of tomorrow. Circular economy focuses on reducing waste and pollution with repair, reuse and reduction, restoring natural system

Characteristics of Smart City.

A smart city is a complex interconnected system that applies modern technologies to manage wide variety of city services like public and private transport system, energy and water resources, civil protection plan, communicating incidents etc more efficiently.

Environmental Ethics

Environmental ethics is a branch of applied philosophy that studies the conceptual foundation of environmental values and more concrete issues surrounding social attitudes actions and policies to protect and sustain biodiversity and ecological system Environmental ethics explores the moral relationship, humans have with earth, plants and animals. Justice, sufficiency and solidarity are classic ethical principles.

Wet land

A wetland is an area of land that is covered by water or saturated with water. Wetland's water also come from a nearby river or lake. Seawater can also create wetlands especially in coastal areas. Wetland provides food and clean water and protect us from flooding. Largest wetland in India is Sundarbans in the delta of Ganga River and Brahmaputra on the way of Bengal. Renuka wetland in Himachal Pradesh is the smallest wetland in India.

Ramsar site

Ramsar site is a wetland site designated to be of international importance. These wetlands are protected under strict guidelines of the Ramsar convention on wetlands India has 54 sites of international importance. Ramsar name is after the city of Ramsar in Iran, where the convention was signed on 2nd February 1971 in Ramsar by UNESCO which came into effect for 21st December 1975.First Indian Wetland under Ramsar convention was chilka lake in 1981. Ramsar's head quarter is in Switzerland.

Chapter 46
Artificial Intelligence (AI)

John Mc Carthy in 1950 coined the term Artificial Intelligence and known as father of A1. Artificial neural network are computing system that constitute animal brains. It is based on the collection of connected units or nodes called artificial neurons in biological brain. Artificial neural network is an attempt to stimulate the network of neurons that make up a human brain so that the computer will be able to learn things and make a decision in a human like manner. A neural network is a method in artificial intelligence that teaches computers to process data in a way that is inspired by the human brain. It is a type of machine learning process. It attempts to solve complicated problems like summarizing documents or recognising faces with greater accuracy. Artificial Intelligence in Plant science

Artificial intelligence has emerged as a fundamental component of global agricultural research. It is capable of learning intricate structure and patterns in Large data sets. It is used to accelerate the process of breeding new plant varieties. Top uses of Artificial Intelligence in agriculture are:

1. Drones or Agricultural Robots are used in agriculture for a variety of purposes like crop monitoring, weather detection soil analysis, pesticide spraying, crop mapping etc.

2. Farming and harvesting.

3. Automatic weeding,

4 crop and Soil management,

5 Plant sorting,

6-Harvesting etc.

AI is also used to detect, identify, count or measure key biological features of plants

Internet of Things (IOT)

IOT refers to devices embedded with sensors so they can measure and transmit data via a network. Things could be a tractor shed, weather Station, Sheep or even a person. It is an interconnected network of computing and digital devices. These objects are embedded with other devices and system These objects include - Smart thermostats, smart watches, voice assistants, automated vacuum cleaners, security systems etc. IOT can be used to monitor farm animals and crops and to apply Pesticides to coops

Information and communication Technology (ICT) in Botany

This technology supports farmers by facilitating access to markets through real time data on market price, weather forecast information on pests, seed varieties and planting techniques. Agriculture is developing and applying innovative ways to use ICT in the rural domain with a primary focus on agriculture. ICT culms to bridge the gap between parents, educators and students by encouraging Sustainable co-operative and

transparent communication methods. It can change traditional classroom into small classroom and improve teaching and learning.

There are many ICT application for crops and horticultures. Electronic fencing for banana plot, wild animal voice sound weather forecast alert for grapes and mangoes, sorting of fruits sequencial timer fumigation, communication with overseas market for grapes, health care for plants and soils, post harvest measurement and storage etc

Digital Technology

Claude Shannon is credited for having laid down the foundation of digitalization that converted technology from analogue format to digital format. Digital India programme was launched on July 1, 2015 by Honourable Prime Minister - Sri Narendra Modi. It is a flagship programme of the government of India with a vision to transform India in a digitally empowered society and knowledge economy. The main benefits of digital technology are

(1) Improved customer experience

(2) increased productivity

(3) Improved business models

(4) Improved agility and innovation

(5) Better Collaboration and

(6) Increased transparency.

The use of information and communication technologies play a Crucial role in education, providing new and innovative beams learning processes more of support to teachers, students and broadly.

Advantage of technology in education can be categorised: Makes Students more engaged and helps them:

1. Retain information.
2. Accommodates multiple learning styles.
3. Encourages collaboration.
4. Provides instant feedback for teachers.
5. Prepares students for future.

Social media and mobile devices may lead to eye strain and difficulty in focusing on important tasks, serious health conditions like depression on developing children and teenagers.

Chapter 47
Plant Parasites

Angiosperm or flowering plants are generally autotrophe but some are heterotrophs also. Plants which depends upon other plants for their food requirements are heterotrophs and the mode of nutrition is heterotrophic Such parasitic plants have been categorised as such if stem parasite: It may be :

1. Semi-parasite such as *Loranthus* or total (holo) parasite like *Cuscuta, Viscum* etc
2. Root parasite which depends upon roots of other plants for their requirement. They are also of two types, viz, Plant soot parasite for example striga and total or holo soot parasite like Orobanche.

Following are the description of some parasitic seed plants.

Loranthus: It is also known as Dendrophthoe and it is semi stem parasite on mango trees. Its systematic positions are as follows:

Order- Santanales

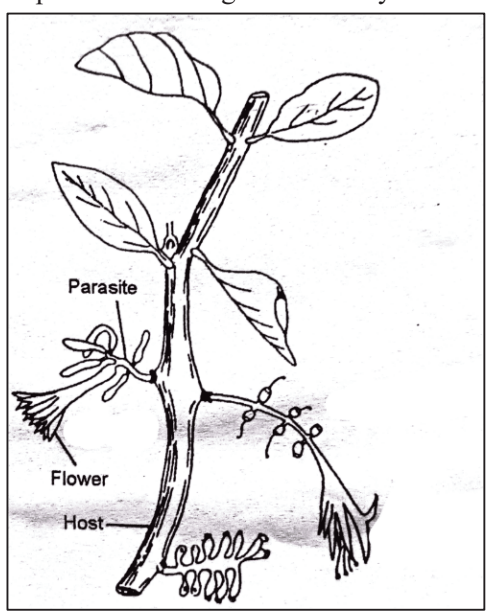
Family - Loranthaceae

Genus - *Loranthus*

Species - L. falcata or L. longiflorus

It posses Chlorophyll and synthesizes its own food by obtaining minerals and water from host plant. The plant is shrub, thick stem and arise in cluster. Flowers are born in cluster; fruits are fleshy containing single seed. Seeds are often eaten by animals or buds and are dispersed to different branches of the tree and to other trees also where they germinate give rise haustoria and established there (Fig.96).

Eradication: 30-40% diesel oil in Soap water are spread to eradicate the parasite from mango trees, Injection of 2-4-D and copper sulphate into affected branches are effective.



Parasite Flower Host
Fig. 96 Showing Loranthus parasitizing on host plant.

OROBANCHE

This plant is holo root parasite affecting tomato tobacco, brinjal, cabbage, turnip etc. It belongs to

Order : Orchidales

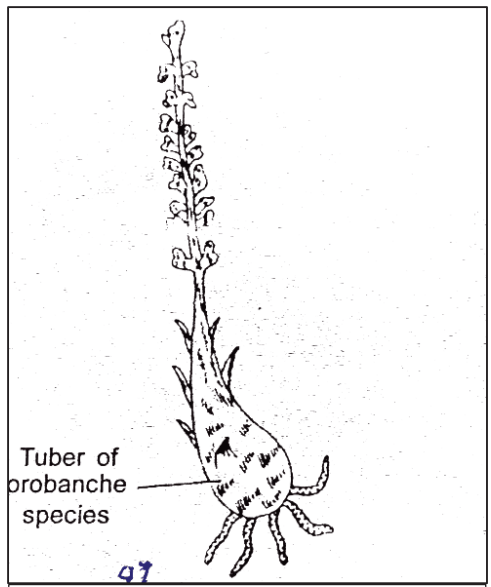
Family : Orobanchaceae

Genus : *Orobanche*

Species: *O. indica, O. cernua*

It has fleshy and stout stem about 10-15 inches in length, covered with brown leaves. Its parasitic roots (haustoria), penetrates the roots of the host and absorbs the nutrients from the host plant (Fig.97).

Control. Uprooting of parasitic plants before flowering is useful. Spraying of copper sulphate solution (25%) on the soil yields good result for the elimination of parasite.



Tuber of *Orobanche*
Fig. 97 Showing *Orobanche* species

STRIGA

It is semi root parasite on sugarcane, maize etc. Its systematic positions are as such:

Order : Personales

Family : Scrophulariaceae

Genus : *Striga*

Species : *S. densiflora*

It is obligate parasite. Plants are 2"-9" long and develop in cluster. It is found in rabi and kharif seasons.

Striga seeds germinate below the soil surface and produce underground stems and roots. Roots of parasite produce haustoria and penetrate the host root and absorbs water and nutrients from the host root. Later it lives as semi-parasite.

Seeds are dispersed through rain, irrigation water and wind.

Eradication. 2-3% copper sulphate solution are soaked into the soil to kill the parasite. Spraying of *Fernoxone* (80% Host sodium salt of 2-4-D) is very effective to kill the parasite. 1% plant tetrachloride acetic acid and other chemicals are effective to eradicate the parasite (Fig. 98 and 99)

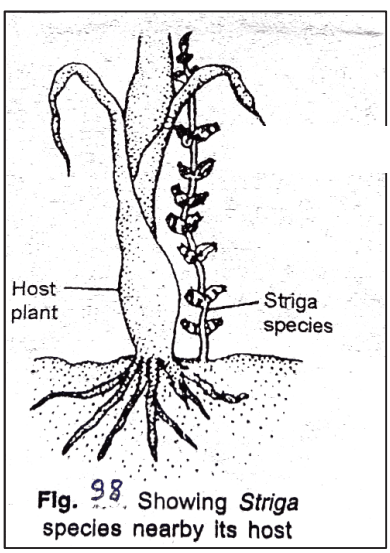


Fig. 98 Showing *Striga* species nearby its host

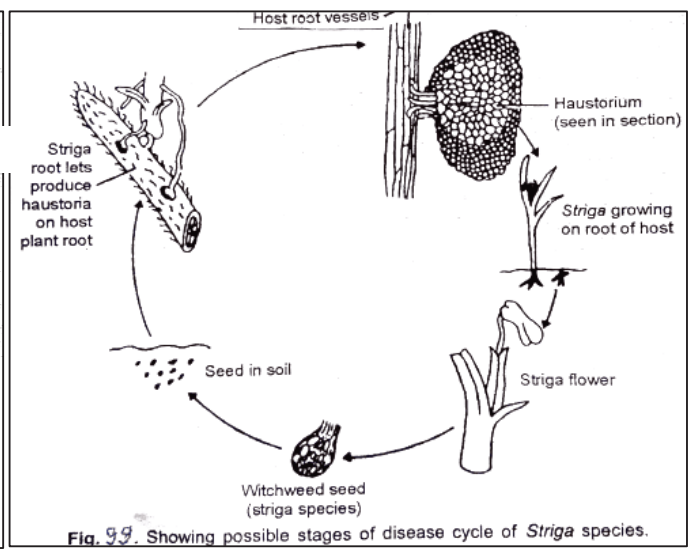


Fig. 99. Showing possible stages of disease cycle of *Striga* species.

CUSCUTA

Systematic position of *Cuscuta* (Amarbel) is like this:

Order : Solanales

Family : Convolvulaceae

Genus *: Cuscuta*

Important species are *C. compestris, C. reflexa, C. indicona* etc. The plant is thread like twining parasite found on fruits and ornamental plants like citrus, potato, carrot, radish, babool etc. It is non-chlorophyllous, rootless, leafless, yellowish thread like stem twining around the host. It is holo stem parasite plant. When stem of Cuscuta comes in contact with host, minute haustoria-root like structures - penetrates into the host cortex to absorb food.

Flowers of the parasitic plant occurs in clusters. Fruit is a capsule bearing seeds which germinates like seeds of other plants.

Control. Cuscuta (Dodder) and affected host plants should be destroyed properly. Some suitable weedicides like 2% nitrophenol should be sprayed. (Fig.100, 101).

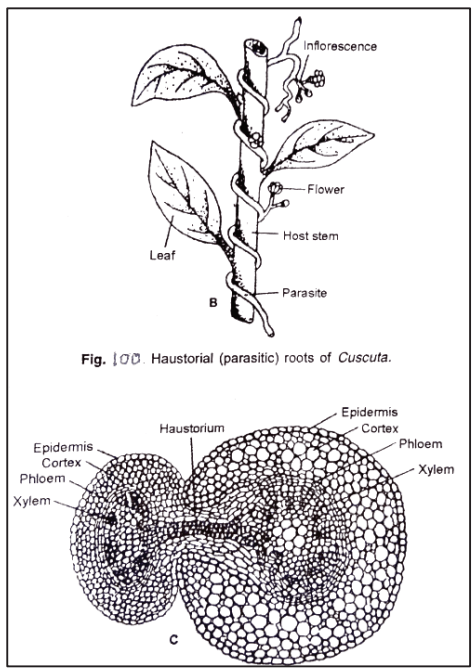


Fig. 100. Haustorial (parasitic) roots of *Cuscuta*.

Fig 101 T. S of host and parasite showing haustoria

Chapter 48
Phylogenetic Analysis

It is to show the evolutionary history between set of species or taxa during specific time. It is the study of evolutionary development of a species or a group of organism or a particular group of any organism.

It makes it hard to believe, some species are related until you take a look at a phylogenetic tree. For example, the echidna shares a common ancestor with the platypus, despite looking more like a hedgehog. The echidnas evolved from an aquatic ancestor and the platypus lost the venomous trait.

El Nino: (It means little boy in spanish)

South American fisherman first noticed periods of unusually warm water on the pacific Ocean in 1960s. El nino typically beaks around December. It can affect our weather significantly- During El Nino, fishes are affected.

The warm water also brings tropical species like yellow tail and albacore tuna in the areas that are normally too cold. El nino is also called La nina. It pushes more warm water towards Asia, bringing cold, nutrient rich water to surface.

It Attract more cold-water species like Salmon and squid. Low pressure zone contributes to increase the rainfall.

Stomules: Small portion of chloroplast send tube like projections called stromules. Its role is in immunity against bacterial and viral infection. KIS 1 gene is involved in chloroplast stromule biogenesis.

Air pressure: Evangelista Torricelli discovered air pressure in 17th century and also discovered mercury barometer. Air pressure/ barometric pressure is 760 mm of mercury or 29.9219 inches of mercury;

Gene TBXT is involved in tail length in certain animals. Human lost their tail about 25 million years ago. Now this TBXT is missing in human being due to genetic code “snippet" known as Aluy which was randomly inserted into early human and non-tailed apes. When Aluy pairs with TBXT, produces two types of RNA critical to cellular structure that produces tailless in people and apes. Aluy is also called Jumping gene as they can move around and insert themselves repeatedly and randomly in human code

Data generation: It is the process of creating data using computer software and algorithms to address research question or analyse various operating conditions. Data generating process refers to the procedures to creating data based on specific - algorithm, which allows for the simulation and investigation of various scenarios to understand the underlying patterns and models or probabilities associated within data.

Data retrieved is a process of accessing and collecting information from a storage location such as a database or network. Dala retrieval means obtaining data from a data base management System (DBMS), for example an object oriented database. In this case it is considered that data is represented in a standard way and there is no ambiguity in data-

Mass spectrometry: It is an -analytical tool useful for measuring the mass to charge ratio. of one or more molecules present in the sample. These measurements can often be used to calculate the exact molecular weight of the sample components as well.

Formula for mass spectrometry: It is : $m/e = H^2r^2/2v$ i,e., 'm' is the mass of the ion, e is the charge of the ion, H is the magnetic field strength & 'r' is the radius of the semicircle and 'V' is the accelerating potential. Main advantage of using mass spectroscopy is in research.

BLAST (Basic Local Alignment Search Tool). It is an algorithm and program for comparing primary biological sequence information such as amino acid sequence of protein, or nucleotide of DNA and or RNA sequence

FASTA (Fast Adaptive Shrinkage Threshold Algorithm). It is basically coded text language of nucleotides and amino acid sequences.

Gene Bank: Gene bank format allows for the storage of information in addition to DNA/ Protein sequence. It holds the much information than the FASTA format.

Chapter 49
Bioinformatics

Bioinformatics: Review

It has now become possible to obtain the images of molecule structure with the helps of atomic force microscope just like as x-ray to image bones and organs inside the human body. It is now easy to design a biomolecule and try its feasibility. This has been possible with the application of knowledge of computer to biology. This is computational biology or Bioinformatics.

Bioinformatics may be defined as a branch of Biology concerned with the development and application of computers to gather, store, analyse and visualise biological information. It is recent and most advanced branch of biology in the field of computer. In other words, we can say that bioinformatics is the application of computer technology to the management of biological information.

Bioinformatics involve use of applied mathematics, statistics, computer science, chemistry,

biochemistry etc. to solve biological problems usually on the molecular level.

The most pressing tasks in bioinformatics are the analysis of sequence, information as follows:

1. RNA sequences of genes of various organisms.
2. Methods to predict the structure and function of newly discovered proteins and structural RNA sequences.
3. Development of protein models.
4. Aligning similar proteins and generating phylogenetic trees to analyse phylogenetic relationships.

Tools of bioinformatics: The main tools of bioinformatics is computer. Thus, the knowledge

of computer is very important for the study of bioinformatics.

Organisation of a computer: Computer is an electric machine which can take in and analyse

information only in terms of number. It performs all these operations accurately and speedily.

The computer system has two parts (i) Hardware and (2) Software.

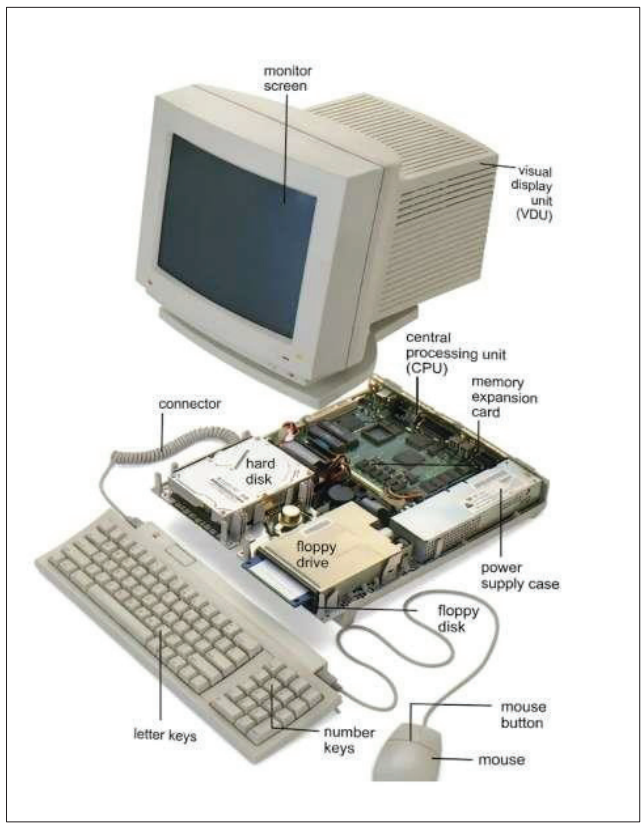
The hardware consists of physical circuits, central processing unit (CPU), input and output devices, keyboard, monitor, Printer, hard disc, main memory etc.

The software consists of different programs to perform the desired activities.

The software can be divided into two main groups as given below.

(a) System software and

(b) application software.



System software: It can be furthers divided into operating system and language translators.

An operating system is a program used for proper functioning of a computer and hence overall manager of the computer. It manages the resources like memory, CPU time devices etc. It accepts the commands from the users and responds them. The example of operating systems are UNIX, LINUX, WINDOWS, DOS etc.

The language translators are used to convert program written in high level language to machine language-understood by computer. It consists of assemblers, compilers and interpreters

Application software: It consists of programs written for doing application-oriented tasks.

They are divided into

(1) text processors/word processors
(2) desk top publishing (DTP).
(3) Data processing software (DPSW),
(4) Computer added design (CAD) software
(5) Educational software
(6) Entertainment software, (7) Expert systems etc.

Data base and its types

The collection of integrated data is called database. It contains information related to a particular

application. In bioinformatics it is most important part of the process. There is a huge data created by scientist world over. This data is made available to anyone interested in it. Currently the data bases are Gene bank, EMBL, DDBJ for data about genome, PIR, SWISS-PROT for data about protein molecules. Typical data base has three sections, viz., Header, Feature and Sequence.

The data base is accessed by typing a specific keyword through the search engine like Google. Specific part of the sequence can also be search. Structural data base stores three dimensional structure of a molecule.

Motif data base is a pattern of a group of sequence. Protein motif data base is the example.

Genome data base stores the information about genome of a particular organism or the species.

Human genome data base is of great importance in research on genetic engineering gene therapy etc.

Proteome data base includes 2D gel images of proteins which indicates protein content of an individual cell.

RNA expression data base: In this data base the changes is array of a specific RNA are measured and stored.

Application of Bioinformatics and its significance.

Bioinformatics help in the following areas:

1. **Phylogeny**: Data base for the extinct or remote organism and their details can be prepared to know the evolutionary sequence of the organism and their phylogeny can be traced.
2. **Functional genomics**: Genomics are the study of genes and their activities in an organism. Functional genomics find out the nucleotides, their sequences and their relationship which are helpful in gene sequencing, gene therapy, cloning etc.
3. **Protein structure**: The varied arrangements of amino acids of a particular protein can be studied by using various tools of bioinformatics. Modelling and restructuring of protein can be done with the techniques of bioinformatics.
4. **DNA micro arrays**: Several DNA models are studied and then arranged in the form of arrays. These arrays are then probed by labelling the DNA model. Gene expression and genome studies are made with this technique.
5. **Chemo informatics**: There are immense data base of drugs and chemicals. Discovery of new drugs, their various chemical combinations specific effects etc. can be studied through this technique.
6. **Pharmacogenomics**: It is combination of pharmacology and genomics. Genes and their relationship with affectivity of a drug is covered under this area.
7. **Medical informatics:** It is also known as clinical informatics. It involves application of computer knowledge in the field of medicine and hence better mode of treatment comes under this area.
8. **Human genome project:** Most important application of bioinformatics is in human genome project. The aim is to developing disease-free human beings, developing gene therapy for curing the congenital and genetic disorders and so on.
9. **Simulation of cell** is possible with this technique. Intracellular molecular activities can be visualised by bioinformatics technique. It is also helpful in biochemical assays of the cells and their activities.

Useful Websites of Bioinformatics.

S No	Information	Websites
1	National centre for biotechnology (NCBI)	http:\\www.ncbi.nlm.nih.gov
2	DNA methylation data, patterns & profiles	http:\\www.methdb.de\
3	European molecular biology laboratory (EMBL)	http: \\ www.ebi.ac.uk\
4	Expasy at Swiss Institute of Bioinformatics.	http:\\ www.expasy.ch\
5	All known nucleotides and protein sequences	http:\\ www.ddbj.nig.ac.jp\
6	Mouse genetics and genomics	http:\\ www.informatic.jzx.orx\
7	Structure data determined by x-ray crystallography and NMR	http:\\ www.rcsb.org.pdb

Artificial neural Networks: It is a method in Artificial intelligence that teaches computers to process data inspired by the human brain. It's a type of machine learning machine used to interconnect neurons in layered structure that resembles the human brain. In plant science it is used to analyse complex data sets and extracts patterns to support agricultural production, weed control, seed traits and quality of harvested seeds.

Chapter 50
Hybridoma and Monoclonal Antibodies

Adalimumab is a human antibody that targets TNF-a and is used to treat autoimmune diseases like rheumatoid arthritis and Psoriasis. Hybridoma is a hybrid cell that is created by fusing. antibody producing lymphocyte with a tumour cell. It is used to continuous culture of a specific monoclonal antibody.

Monoclonal antibodies are proteins made in the lab that can bind to specific target in the body, like an antigen on cancer cells. They are used to treat and diagnose a variety of diseases including cancer, infectious diseases and immunology.

Monoclonal antibodies are used to treat cancer by killing cancer cells or stopping them to grow. It is also used to treat pneumonia, hepatitis C, HIV etc. Monoclonal antibodies are also used in imaging vehicles to help identify targets. The first monoclonal antibody is CD_3 (Ortho clone OKT_3) consists of murine monoclonal antibody against T-cells.

Immune System protects our body from various infections. Improper functioning of immune system can cause discomfort, diseases or even death. The immune disorders are of three types, viz;

(1) Acquired immunodeficiency disease (AIDS)

(2) Allergic of hypersensitivity and

(3) Chronic hepatitis.

Differences and Significance

Antigens	Antibodies (Immunoglobulins)
1. It is foreign agent that stimulates antibody formation. 2. It is a protein or polysaccharide molecules 3. Produce disorders or allergic reactions	1. Antibody is produced by an animal in response to presence of antigen. 2. Each antibody is a protein molecule 3. Antibody occurs on surface of a Plasma cell and also in body fluid. Protects body form disorders.

Appendix

LONG AND SHORT QUESTIONS

1. Explain in detail, root pressure theory.
2. Give an account of cohesion theory of Dixon.
3. Define transpiration. Explain the mechanism of transpiration.
4. Describe mechanism of closing and opening of stomata.
5. Comment on: "Transpiration is necessary evil".
6. Write notes on:
   a) Factors affecting transpiration.
   b) Capillarity theory of water translocation.
   c) Types of transpiration.
   d) Apoplast and symplast pathway.
   e) Structure of root hair.

MULTIPLE CHOICE QUESTIONS

1. Translocation of water through xylem in the tall trees, is due to

a) Capillarity
b) Root pressure
c) Metabolic activity of xylem cells
d) Transpiration pull and cohesion of water molecules

2. Most widely accepted theory of ascent of sap is

a) Root pressure theory
b) Pulsative activity of cortex
c) Transpiration pull
d) Capillarity

3. The metal ion influxed into guard cells is

a) Iron
b) Potassium
c) Magnesium
d). Sodium

4. Increase in CO, concentration around the leaf causes

a) Closing of stomata completely
b) No effect on stomata
c) Opening of stomata completely
d) Opening of stomata partially

5. Stomata open during day time because of

a) Conversion sugar into starch
b) Accumulation of CO2 in the guard cells.
c) Movement of K+ ions from guard cells to the surrounding.

d) Photosynthesis and production of sugar.

6. Root pressure is maximum when

a) Transpiration is low and absorption is high
b) Transpiration is high and absorption is low
c) Transpiration and absorption are low
d) Transpiration and absorption are high

7. Exudation of xylem sap on cutting the stem is due to

a) Transpiration
b) Guttation
c) root pressure
d) None of the above

8. Root hair absorbs water from the soil due to

a) Turgor pressure
b) Osmosis
c) Ion-exchange
d) None of the above

9. Transpiration pull is maximum when

a) Stomata are open, atmosphere is dry
b). Stomata are open, atmosphere is humid
c) Stomata are open, soil is dry
d) Stomata are closed atmosphere is humid

10. Who made the statement that "transpiration is necessary evil"

a) J. C. Bose
b) Curtis
c) Levitt

11. Root hair is found above the

a) The root cap
b) Zone of maturation
c) Zone of elongation
d) Zone of adventitious roots

12. During the absorption of water by the roots, the osmotic potential of the sap is:

a) Lower than pure water and soil solution
b) Higher than pure water and soil solution
c) Lower than pure water but higher than soil solution
d) Lower than soil solution but higher than pure water

ANSWERS

1. d	2. c	3. b	4. a	5. d	6. a
7. c	8. B	9. A	10. B	11. C	12. B

Exercise
(Related to Respiration)

LONG AND SHORT QUESTIONS

1. What is respiration? Explain the types of respiration.
2. Describe in brief, the ultra structure of mitochondrion. Add a note on its role in respiration.
3. Describe EMP pathway. State its significance.
4. Describe TCA cycle and give its significance.
5. Describe in brief the electron transfer system (ETS).
6. What is fermentation? Write a note on alcoholic fermentation.
7. Explain the mechanism of anaerobic respiration.
8. Describe terminal oxidation. Add a note on its significance.
9. Differentiate between:
   a) Aerobic and anaerobic respiration.
   b) Photosynthesis and respiration.
   c) Glycolysis and Krebs cycle.
   d) Fermentation and respiration.
10. Give schematic representation of:
    a) Glycolysis.
    b) Krebs cycle.
11. Write notes on:
    a) Alcoholic fermentation.
    b) Lactic acid fermentation.
    c) Significance of respiration.
    d) Oxidative phosphorylation.
    e) Terminal oxidation.

MULTIPLE CHOICE QUESTIONS

1. Cytochromes are

a) O_2 acceptors b) H_2 acceptors

c) Electron acceptors d) None of the above

2. Krebs-Cycle occurs within

a) Cytoplasm b) Mitochondria

c) Chloroplast d) Ribosomes

3. Glycolysis occurs in

a) Cytoplasm b) Mitochondria

c) Golgi complex d) Ribosomes

4. If a starved plant is provided with hexoses, the rate of respiration will

a) Increase b) Decrease

c) No change d) Increase or decrease

5. When yeast carry on anaerobic respiration, they produce

a) O_2 b) H_2O

c) CO_2 d) N_2

6. In cellular respiration, O_2 is used as the final receptor of

a) Carbon b) Hydrogen

c) Nitrogen d) Iron

7. During aerobic respiration the oxidation of glucose results in the formation of

a) H_2 & CO_2 b) O_2 & CO_2

c) CO_2 & H_2O d) H_2 & O_2

8. More energy is released by the process of aerobic respiration than by fermentation because fermentation

a) Occurs in microorganisms which have very few enzymes.

b) Requires oxygen both as a hydrogen donor and acceptor

c) Because incomplete oxidation of organic food

d) None of these

9. To start cellular respiration the living cells, require supply of

a) CO_2 only b) O_2

c) Glucose only d) Glucose & ATP

10. The final electron acceptor in respiration is

a) H_2 b) O_2

c) Cytochrome d) Dehydrogenases

11. Which among the following is most appropriate reason for storing green coloured apples at low temperature.

a) The rate of respiration is reduced b) The rate of photosynthesis is reduced

c) The rate of both the processes is reduced. d) The rate of both is zero

12. Plants require the mineral components for ATP synthesis are

a) Iron & Phosphorus b) Nitrogen & Phosphorus

c) Copper & Calcium d) Calcium & Potassium

13. Respiration occurs in

a) All living cells both in light & dark b) All living cells only in light

c) All living cells only in dark d) Only in non-green cells in light & dark

14. The incomplete oxidation of sugars in anaerobic respiration results in the formation of

a) Fructose and water b) H_2O and CO2

c) Alcohol and CO2 d) Glucose and CO_2

15. Enzymes taking part in EMP pathway are present in

a) Mitochondria b) Cytoplasm

c) In both a &b d) Vacuole

16. Link between glycolysis and Krebs Cycle is

a) Citric acid b) Acetyl Co Enzyme A

c) Succinic acid d) Fumaric acid

17. In glycolysis along with 2 Mols. Of ATP, 2 Mols. of the following are formed

a) $FADH_2$ b) $NADH_2$

c) $NADPH_2$ d) $FMNH_2$

18. Which of the following involves loss of two protons and two electrons?

a) Carboxylation b) Deamination

c) Dehydrogenation d) None of these

19. The importance of Krebs Cycle is in the production of

a) Acetyl Co Enzyme A b) Water

c) ATP d) H_2

20. Oxidative phosphorylation occurs in

a) Stroma of chloroplast

b) Grana of chloroplast

c) Outer membrane of Mitochondria

d) Inner membrane of Mitochondria

ANSWERS

1 c	**6 b**	**11 a**	**16 b**
2 b	**7 c**	**12 a**	**17 c**
3 a	**8 c**	**13 a**	**18 c**
4 a	**9 d**	**14 c**	**19 d**
5 c	**10 b**	**15 b**	**20 d**

Exercise
(Photosynthesis)

LONG AND SHORT QUESTIONS

1. What is photosynthesis? Give its significance.
2. Define ATP. Give its structure and function.
3. Describe the ultra-structure of chloroplast.
4. What is phosphorylation? Explain non-cyclic photophosphorylation. Add a note on its significance.
5. Distinguish between cyclic and non-cyclic photophosphorylation.
6. Describe in brief, the importance of non-cyclic photophosphorylation.

OR

Explain in which way non-cyclic photophosphorylation is efficient than cyclic.

7. Explain Hill reaction.
8. Explain the source of O_2 evolved in photosynthesis.
9. Describe various types of pigments involved in photosynthesis.
10. Explain the role of pigments in photosynthesis.
11. Describe the nature and role of light in photosynthesis.
12. What are C_4 plants? Describe the process of CO_2 fixation in case of C_4 plants.
13. Write notes on:
    a) Kranz anatomy.
    b) Efficiency of C_4 plants.
    c) Calvin cycle.

MULTIPLE CHOICE QUESTIONS

1. During dark phase of photosynthesis

a) O_2 is released b) PGAL is synthesized
c) Hydrogen is released d) ATP is produced

2. The first step in the process of photosynthesis is

a) Absorption of light but PS-I only
b) Absorption of light by PS-II only
c) Absorption of light by both PS-I & PS-II
d) Production of ATP & NADPH.

3. The immediate electron donor to PS-I is

a) Plastoquinone
b) Plastocyanin
c) Ferredoxin
d) Quinone

4. In the process of photosynthesis, chlorophyll serves as

a) End product
b) Raw material
c) Energy converter
d) Hydrogen donor

5. During light reaction of photosynthesis

a) Water molecules split
b) CO_2 reacts with H_2
c) PGAL is synthesized
d) O_2 is combined with CO_2

6. The C_4 plants are different from C_3 plants with reference to the

a) Types of end product
b) Substance accepts CO, in dark reaction
c) No. of ATP consumed in dark reaction
d) Types of pigments

7. Which of the following is not a limiting factor for photosynthesis?

a) O_2
b) CO_2
c) Chlorophyll
d) Light

8. Leaves appear green because they

a) Absorb green light
b) Reflect green light
c) Both absorb and reflect green light
d) None of these

9. Our present-day view regarding photosynthesis is that it

a) Converts light energy into chemical energy

b) Creates useful energy

c) Fixes CO_2 into carbohydrates with the help of assimilation power (ATP & $NADPH_2$)

d) Reverse the action of respiration

10. The specific function of light energy is to

a) Activate Chlorophyll

b) Split water

c) Reduce carbon dioxide

d) Synthesis glucose

11. In case of C_4 pathway in the first step

a) CO_2 combines with PGA

b) CO_2 combines with PEP

c) CO_2 combines with RuDP

d) CO_2 combines with RMP

12. During photosynthesis when PGA is changed into PGAL, it is

a) Oxidation

b) Reduction

c) Hydrolysis

d) Electrolysis

13. ATP formation during photosynthesis is

a) Phosphorylation

b) Photophosphorylation

c) Oxidative phosphorylation

d) None of these

14. Besides water and light, which is more essential as a raw material for food formation?

a) CO_2

b) O_2

c) NAD

d) Minerals

15. Which of the following is the least effective in photosynthesis?

a) Sunlight

b) Blue light

c) Red light

d) Green light

16. Tropical plant like sugarcane shows high efficiency CO_2 fixation because of

a) Calvin cycle

b) EMP pathway

c) HSK pathway

d) TCA cycle

17. O_2 evolved in photosynthesis is

a) Equal to CO_2 consumed
b) Less than the CO_2 consumed
c) More than the CO_2 consumed
d) None of these

18. Photophosphorylation is a process in which

a) Chemical energy is used to produce ATP
b) Light energy is converted into chemical energy
c) CO_2 is reduced
d) NADP is formed

19. The primary acceptor of CO_2 is

a) PGA
b) Glucose
c) RuDP
d) RuMP

20. In photosynthesis the first event is

a) Photolysis of water
b) Photoexcitation of chlorophyll
c) Reduction of CO_2
d) Production of $NADPH_2$

ANSWERS

1 b	2 c	3 b	4 c
5 a	6 b	7 a	8 b
9 c	10 a	11 b	12 b
13 b	14 a	15 d	16 c
17 a	18 b	19 c	20 b

MULTIPLE CHOICE QUESTION

1. The term gene was coined by

a) Johanssen
b) Watson
c) Mendel
d) Sutton and Boveri

2. Chromosome theory was proposed by

a) Watson and Crick
b) Sutton and Boveri
c) Avery and MacLeod
d) Beadle and Tatum

3. Nucleoside is nucleotide without

a) N_2 base b) Sugar

c) Phosphate d) Hydrogen bond.

4. Successive nucleotides of the same strand of DNA are joined by

a) H_2 bonds b) N_2 bases

c) Sugars d) Phosphodiester bonds

5. In one turn of DNA nucleotide pairs are

a) 10 b) 20

c) 34 d) 40

6. Diameter of DNA is

a) 10 A° b) 20 A°

c) 30 A° d) 40 A°

7. The first animal cloning done was of

a) Sheep b) Horse

c) Frog d) Dog

8. Agrobacterium is used for

a) Gene cloning b) Biopiracy

c) Gene library d) Producing transgenic plants.

9. Combination of plasmid DNA and desired gene is called

a) r-DNA b) c-DNA

c) RNA d) T-DNA

10. Enzyme joining two DNA strands from different origin is

a) Polymerase b) Ligase

c) Restriction d) Restriction exonuclease

11. Formation of an entire organism from a single cell is called

a) Callus culture b) Organogenesis

c) Totipotency d) Clone.

12. For root formation growth promoter used is

a) 2,4-D b) Cytokinin

c) gibberellin d) Auxin

13. Anther culture means production of plants from

a) Pollen grains b) Anthers

c) Tapetum d) Filament

14. A patent granted for Basmati rice to U.S.A. is

a) Biopatent b) Both biopatent and biopiracy.

c) Biopiracy d) Bioethics

15. Producing organism exactly like original organism is

a) Gene cloning b) Tissue Culture

c) Cloning d) Genomics.

16. To produce insect resistant cotton plant the gene used was from

a) Anabaena b) Rhizobium

c) Agrobacterium tumefaciens d) Bacillus thuriengensis

ANSWERS

1 a	2 b	3 c	4 d
5 a	6 b	7 c	8 d
9 a	10 b	11 c	12 d
13 a	14 b	15 c	16 d

Exercise
(Related to molecular Biology)

LONG AND SHORT QUESTIONS

1. Describe the structure of DNA.
2. Describe the process of replication of DNA.
3. Explain the structure of RNA and mention its types.
4. Differentiate between DNA and RNA.
5. Define genetic engineering. Explain the method in brief.
6. Explain the process of cloning.
7. Give an account of the process to produce transgenic plants.
8. Give applications of transgenic plants.
9. How is the gene library constructed?
10. Give an account of gene bank.
11. Explain the steps involved in DNA recombinant technology.
12. Describe the steps involved in making DNA finger print.
13. What is biopiracy? Enumerate various examples of biopiracy.
14. Explain the following terms:
    a) Bioethics and biowar
    b) Organogenesis
    c) Callus.
    d) Totipotency
    e) Explant.
15. Write notes on
    a) Cistron, recon, and muton.
    b) mRNA
    c) tRNA
    d) Restriction endonuclease
    e) Applications of genetic engineering.
    f) Ligase

g) Applications of DNA finger printing.

h) Types of tissue culture

i) Sterilization technique in tissue culture.

j) Disease free plant production.

k) Anther culture.

l) Protoplast culture.

m) Somatic hybridization.

Exercise
(Related to Bioinformatics)

LONG AND SHORT ANSWER QUESTIONS

1. Explain the collection of data base.
2. Describe any two applications of Bioinformatic
3. What is internet? What are its uses?
4. Describe the various components of a computer
5.Explain the different types of databases.
6. Why is database important in bioinformatics?
7. Define the term bioinformatics.
8. What is an operating system? What are its uses?
9. State any two applications of Computational Biology.
10. What is the importance of memory in a computer?
11. Define and explain in short, the term 'Algorithm'.

MULTIPLE CHOICE QUESTIONS

1. The main device in a computer is

a) CPU b) Peripherals c) Memory d) All of these

2. Accessing the data from world over is possible due to:

a) www b) Internet c) CPU d) All of these

3. The environment for running of computer program is made available by.

a) Internet b) Operating system c) Memory d) CPU

4. Collection of the essential data in bioinformatics is known as:

a) Files b) Database c) Directory d) Error

5. Bioinformatics is also known as:

a) Programming b) Computational Biology

c) Software development d) None of these

6. The most important component in bioinformatics is:

a) Operating system b) Database

c) Both of these d) None of these

7.Which is not done in Bioinformatics?

a) Simulation b) Genome assembly

c) Protein designing d) All of these

8. Human genome project was done with

a) Computer animation b) Computational Biology

c) Both of these d) None of these

9. Bioinformatics can be useful in:

a) Designing a biomolecule b) Understanding a gene sequence

c) Decoding the genome d) All of these

10. Speed of a computer depends upon

a) CPU b) Memory c) RAM d) Floppy drive.

ANSWERS

1 A	2 B	3 B	4 B	5 B
6 B	7 D	8 D	9 D	10 C

DISCUSSION QUESTIONS (Related to ECLOGY)

A. <u>Fill in the blanks:</u>

1. _____ is the only planet that has shown signs of life.
2. Our planet that was born of _____
3. The Greeks called the Earth as_____ or the Goddess Earth.
4. Earth can be divided in to _____

spheres

5. The _____is the solid, rocky crust covering entire planet.
6. _____ per cent of the Earth's water is in the oceans.
7. Most of the planet's life is found from_____
8. Abiotic elements are the _____
9. _____ are the living elements in the environment.
10. _____ are also called as autotrophs.

11. The complex process converts solar energy into chemical energy is known as _____

mts. below the ground.

elements in the environment.

12. _____ is given out as a by-product of photosynthesis.

13. _____ are organisms that live on the refuse and dead organic matter.

Answers

(1) Earth, (2) fire. (3) Gaia. (4) four. (5) lithosphere. (6) Ninety- seven. (7) three metres. (8) non-living. (9) Biotic elements. (10) Producers. (11) photosynthesis (12) Oxygen. (13) Detritivores

B. Match the following:

A	B
1. Hydrosphere.	a) Plants
2. Biosphere.	b) Life
3. Autotrophs.	c) Water
4. Carnivores.	d) Flesh-Eaters
5. Herbivores.	e) Plant-Eaters

Answers (1) c, (2) b, (3) a, (4) d, (5) e

C. Choose the correct alternative:

1. _____ these are also called the primary consumers.

(a) **Herbivores**, (b) Carnivores, (c) Detrivores (d) Predators.

2. The biosphere is composed of _____

(a) Gases, (b) Water, (c) Soil, (d) **Living organisms**.

D. Short notes:

(a) Producers. (b) Consumers (c) Food chain.

(d) Food Web. (e) Energy transfer in ecosystem.

E. Short Questions:

1. Write a note on the biotic components of environment.

2.Describe the process of photosynthesis with help of a suitable diagram.

3. What are the different types of consumers?

4. How is energy received, transformed and transferred in an ecosystem?

F. Long Questions:

1. Write a note on the four spheres of environment.

2. What are the various components of environment?

Chromosomal Aberration in Human being (1956)

According to Joe Hin Tjio and Albert Levan, male has 44 +XY = 46 Chromosomes and female has 44+XX = 46 chromosomes. If 44+ X =45 it is female called Turner's syndrome -characterised by short stature, webbed neck, lack of secondary sex characters.

If 44+Y=45- It is male called Klinefelter's syndrome with tendency towards femaleness, enlarged breast, small testis. No hair develops.

Male with 44+ XXY= 47 or 44 + XXXXY are rare. An individual with extra x and y chromosome

{44 +XXYY) display hermaphroditism has both testicular and ovarian tissues.

Showing intersexual development of genitalia males with 44 + XXYY are extremely tall. One among 20,000 (Twenty thousand) males was found to have XX chromosomes in place of XY chromosomes.

Similarly, one among Twenty thousand (20,000) female have XY chromosome in place of XX chromosomes. It may be due to transfer of a segment of Y chromosome having testis determining factor (TDF) to X Chromosome or due to mutation in TDF which represses testis development

'It has been reported in the "Cell" journal in 2025 that Y chromosome has SRY gene responsible for testis development - may vanish after 11 million years. X Chromosome has 900 genes and Y chromosome has 55 genes. SRY twins SOX9 gene to make male traits to develop. Mutation in SRY or SOX9 gene can interfere with their ability to activate the testis and express the ovarian pathway, can lead to sex reversal.