PRACTICAL-IV
(DBOTL22)
(MSC BOTONY)

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M.Sc. Botany (Final) Practical-II

CELL BIOLOGY, MOLECULAR BIOLOGY AND PLANT BIOTECHNOLOGY

1. The Study of Structure and functions of cell organelles with diagrammatic sketches

(a) Lysosomes

Every eukaryotic cell has a group of cytoplasmic organelles, the lysosomes (Fig. 1). Their main function is intracellular or extracellular digestion. Lysosomes show greater polymorphism. The primary lysosomes are dense particles of 0.4 μm, formed from small golgi vesicles and are not involved in the digestive processes. The formation of primary lysosome can be blocked by puromycin.

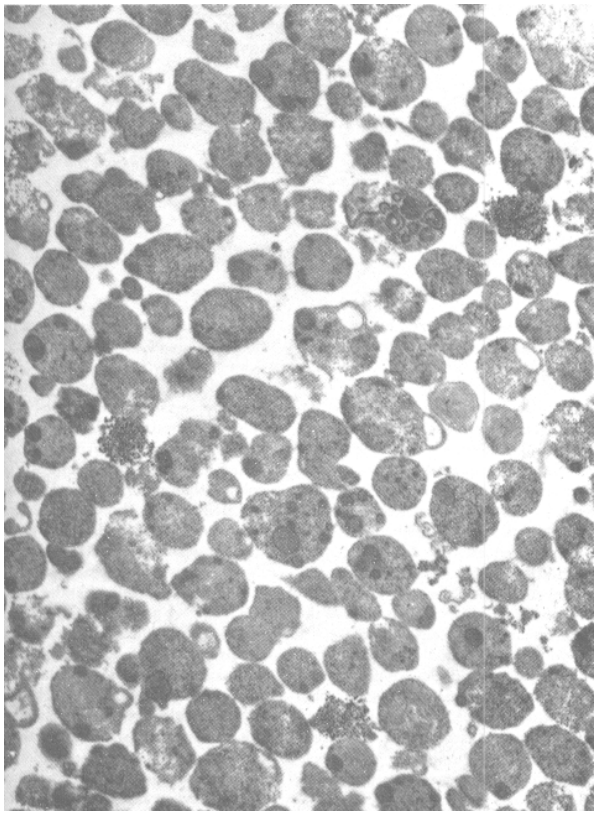


Fig. 1. Lysosomes isolated by differential centrifugation from rat liver, showing the dense particles and the variety of other dense material contained within the single membrane of the lysosome. x60,000 (Courtesy of C. De Duve).

Secondary lysosomes (digestive vacuoles) result from the association of primary lysosomes with vacuoles containing phagocytized material. The engulfed material is digested by the lysosomal enzymes. At present, 50 lysosomal hydrolases are known. The digested material is incorporated into the cell to be used again in metablic pathways. If digestion is incomplete residual bodies are formed. In amoeba and other protozoa, the residual bodies are eliminated by defecation.

Lysosomes regularly engulf bits of cytosol (mitochondrion or protein of ER) which is degraded by a mechanism called microautophagy. This is a mechanism by which the cell can achieve the degradation of its own constituents without irreparable damage. Autophagy is common during the period of rapid change (metamorphosis in insects and amphibians).

The lysosomal enzymes are secreted into ER lumen, they are glycosylated and are associated with golgi complex. Newly synthesized enzymes removed from golgi cisternae and delivered to GEBL (Golgi-Endoplasmic Lysosome) region and to lysosomes by a vesicular carrier. They contian high levels of degrading enzymes which catalyze the break down of proteins, nucleic acids, lipids and carbohydrates. In Eukaryotic cells, lysosomal enzymes breakdown old organelles, making room for new organelles, e.g. mitochondria are replaced in some tissues every 10 days.

In addition to breaking down organelles and other structures within cells, lysosomes eliminate other cells, the cell has engulfed in a process called phogocytosis (Fig. 2). When a white blood cell, phagocytizes a passing pathogen, lysosomes fuse with the resulting food vesicle releasing their enzymes into the vesicle and degrades the material within.

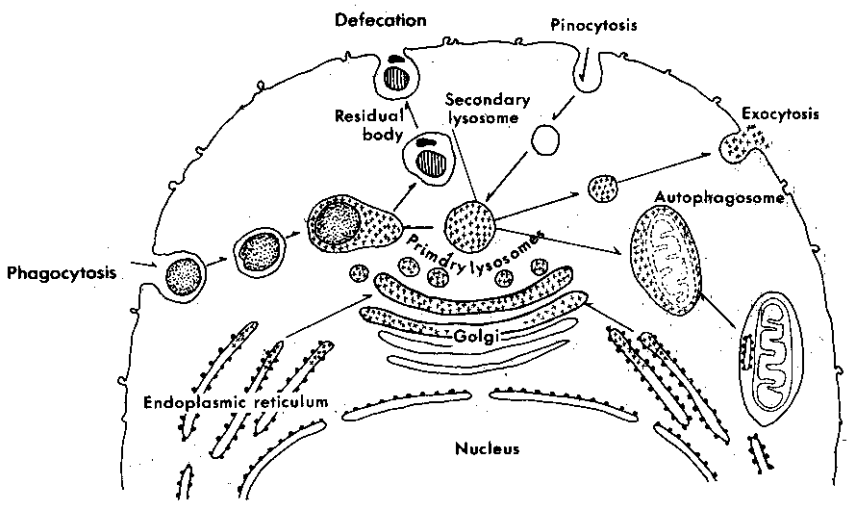


Fig. 2. Diagram representing the dynamic aspects of the lysosome system. Observe the relationships between the processes of phagocytosis, pinocytosis, exocytosis, and autophagy.

Residual bodies are formed if digestion is incomplete. In some cells like *Amoeba* and other protozoa, these residual bodies are eliminated by defecation.

Autophagic vacuoles are formed by the fusion of primary lysosomes and engulfing of degenerated intracellular organelles. Autophagic vacuoles cause the digestion of endogenous material. The products of digestion pass through the membrane of lysosome and are incorporated into the cell.

Functions: During starvation, lysosomes mobilise food from the storage organ. They take part in intracellular digestion. During metamorphosis, embryonic organs are digested (tail in tadpole of amphibians) by lysosomes. Genetic diseases result from the absence of even a single lysosomal hydrolase enzyme, e.g. bones are deformed due to accumulation of glycosaminoglycon in lysosomes. Arthritic joints may be caused by malfunctioning of lysosomes. They remove old organelles and control secretory activity of the endocrine glands. Lysosomes are important in medicine as they are involved in many diseases and syndromes. Acute release of lysosomal enzymes cause myocardial infarct. In pathological conditions, rheumatoid arthritis, silicosis and gout, release of lysosomal enzymes are found from macrophages. Lysosomes of leukocytes and macrophages are essential to the defense of the organism against bacteria and viruses. There are about 20 congential diseases called storage diseases due to accumulation of glycogen and glycolipids in lysosomes. Lysosomes in plant cells are involved in seed germination.

Mitochondria

They are present in the cytoplasm of eukaryotic cells. Mitochondria are called as chondriosomes and the power house of the cell as most of the energy is generated by oxidation of food materials. The number of mitochondria per cell varies from 50 to several thousands depending upon the type of organism. Plant cells have fewer number of mitochondria in comparison to animal cells.

They are 1 μm to 10 μm in length and 0.2 μm to 1.0 μm in width, made up of two unit membranes (Fig. 3). Outer membrane is smooth $60A^o$ to $75A^o$ thick. The inner membrane goes into folds called cristae. Cristae extend into mitochondrial matrix. It is $50\text{-}70A^o$ thickness. The space between the two membranes is intermembrane space. On the external surface of the outer membrane and on internal surface of inner membrane are found minute structure called microbodies. These microbodies on the outer surface are called subunits of Parson and inner surface are knob like structures called oxysomes. Each oxysome consists of head, stalk and base.

The cavity of mitochondria is occupied by dense fluid material called mitochondrial matrix. It is the site of Kreb's cycle. The matrix contains mitochondrial DNA, similar to bacterial DNA.

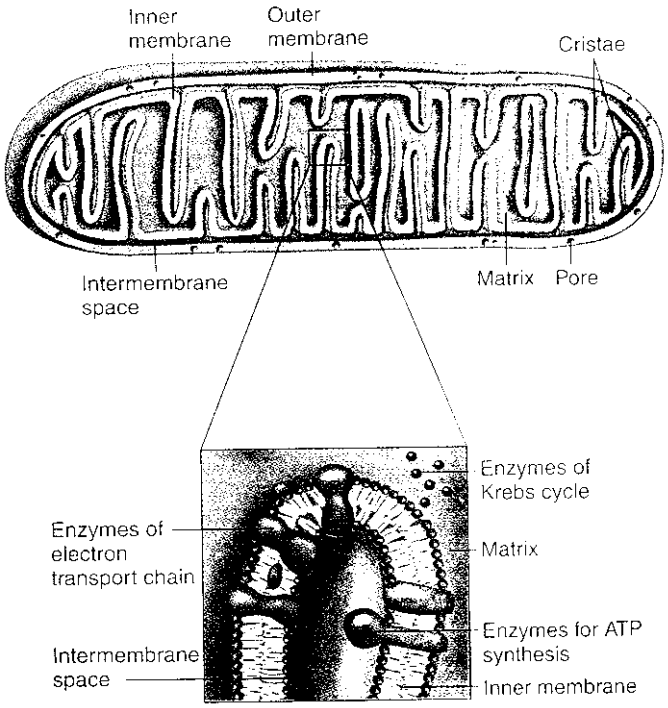


Fig. 3. Anatomy of a mitochondrion. The organization and structure of a single mitochondrion is shown. Core regions that are entirely enclosed within an inner membrane are known as the matrix. The matrix contains the mitochondrial DNA and enzymes of the Krebs cycle. Individual inner membrane foldings are called cristae. A single crista is magnified to show how enzymes of the electron transport chain carry out oxidative phosphorylation between the matrix and the intermembrane space.

Mitochondrial genome -- Mt DNA is mostly circular double stranded with a few exceptions. Numerous mitochondrial DNAs have been sequenced. It has 16s and 12s rRNA. Each haploid yeast cell has 20 mt DNA molecules. Huge variation exists in number of mitochondria and mt DNA molecules. The replication of mtDNA molecule can occur through out the cell cycle.

The size and gene content of mtDNA varies from organism to organism (Table 1and 2).

Table 1
Mitochondrial DNA sizes

Organism	Size (kb)
Plasmodium	6
Yeast	75
Drosophila	18
Pea	110
Human	16.5

Table 2

Comparison of Some Functions Encoded in mtDNA

Organism	Oxidative phosphorylation Genes	tRNAs	Genome Size (kb)
Yeast	7	25	75
Marchantia (liverwort)	14	29	186.0
Human	13	22	16.5

Like size and gene contents, the shape of mtDNAs varies mtDNAs of most species are circular, but mtDNAs of ciliated protozoans *Tetrahymena* and *Paramecium,* the alga *chlamydomonas* and the yeast are linear. The single cell eukaryotes contain a single mitochondrion.

A comparison of mitochondrial genome organization in humans, yeast and liverwort illustrates the details of mtDNA diversity (Fig. 4). 16.5 kb human mitochondrial genome carries 37 genes. In addition to 13 polypeptide encoding genes, there are 22 tRNA genes, the remaining 2 genes code for small rRNAs. There are no introns in human mtDNA.

Yeast Mt DNA is four times larger than human. Long introns A-T rich sequence spaces separate the genes in yeast mt DNA, 25% of yeast mitochondrial genome constitute introns. mtDNA of *Marchantia polymorpha* was the first plant mt DNA to be sequenced. It has many genes than non-plant mtDNAs. Most mitochondrial genome contains 16 genes for ribosomal proteins and 29 genes for unknown function.

Functions: Major part of ATP is synthesized in mitochondria. They provide raw materials for synthesis of chlorophyll, cytochrome, alkaloids and steroids. They are the sites of amino acid synthesis and fatty acid synthesis.

Although mitochondria in different eukaryotic organisms play similar role in the conversion of food to energy, evolution has produced mtDNAs with an astonishing diversity in the content and organization of their genes. Mitochondrial evolution has led to some remarkable variations on the basic mechanisms of gene expression.

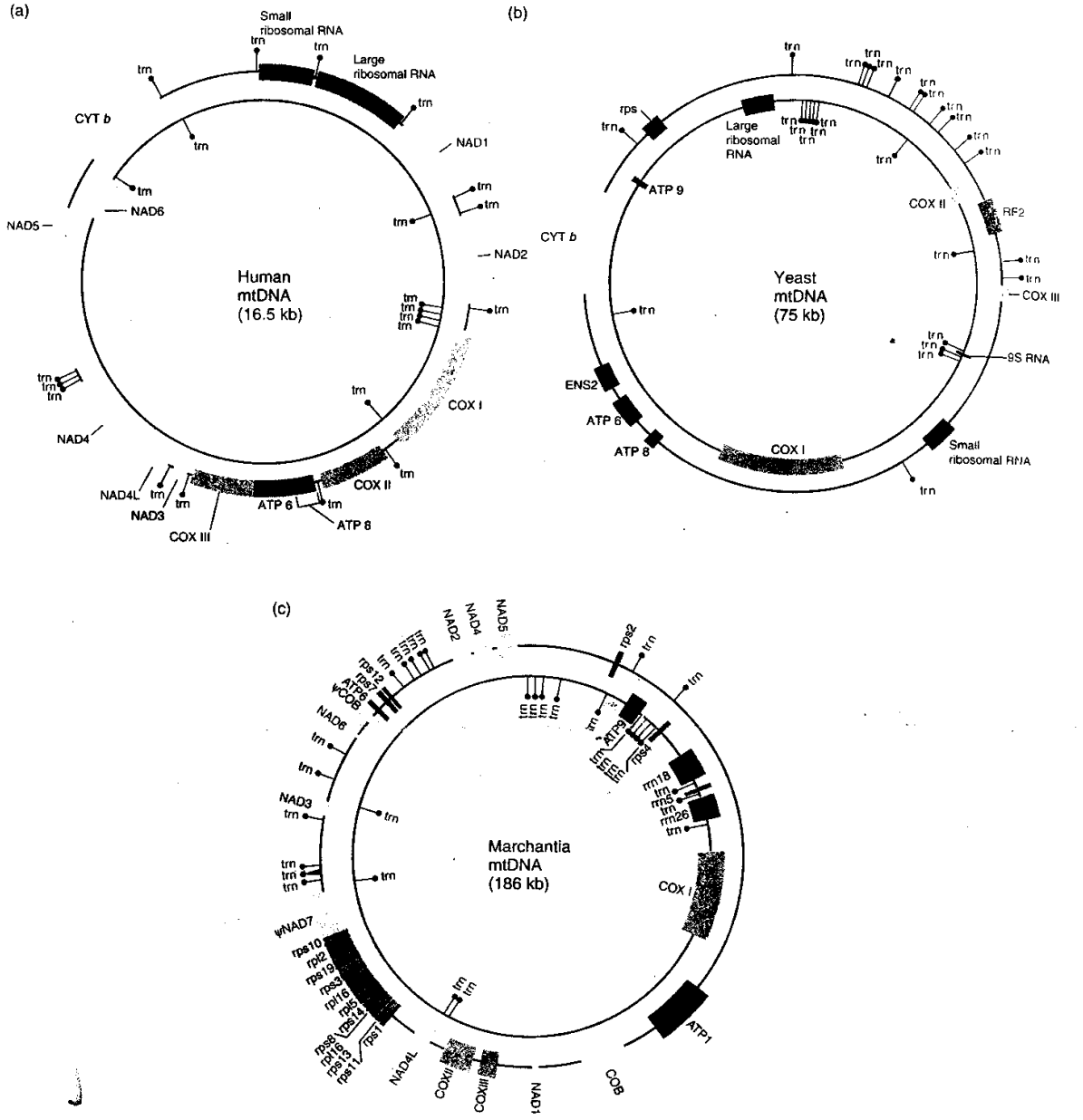


Fig. 4. Mitochondrial genomes of three species. Comparison of mitochondrial genomes of humans, baker's yeast, and liverwort. Human mtDNA is 16.5 kb long, while that of baker's yeast is 75 kb long, and liverwort is 121 kb long. Important differences illustrated in the diagrams are the presence of long intergenic sequences in yeast mtDNA and their absence from human mtDNA and differences in the numbers of genes in the mt genomes from these three organisms. The color scheme allows you to quickly see differences in numbers of genes for various functions in these three species. The following color scheme is used green, genes coding for cytochrome oxidase proteins, red, genes coding for ATPase subunit proteins; yellow, genes coding for NADH complex proteins; tan, genes coding for cytochrome complex proteins; purple, genes coding for ribosomal proteins of ribosomal RNAs, tRNA genes (tm) are indicated by black ball and stick. Genes shown on the outer and inner circles are transcribed in opposite directions.

Chloroplast

Chloroplasts are distributed through out the cytoplasm of green cells of plants. They are present in mesophyll and palisade cells of angiosperms, gymnosperms, pteridophytes and green cells of lower plants.

Chloroplasts may be spherical, oval or disc shaped (Fig. 1). They are 4-6 μm in diameter and 1 μm in thickness. Chloroplast is enclosed in two smooth membranes separated from one another by internal spaice or periplastidial space. The internal structure of a chloroplast can be differentiated into grana and stroma. The inner membrane surrounds a large central ground substance, colloidal in nature called stroma. The lamellar system with sac like green bodies are called grana.

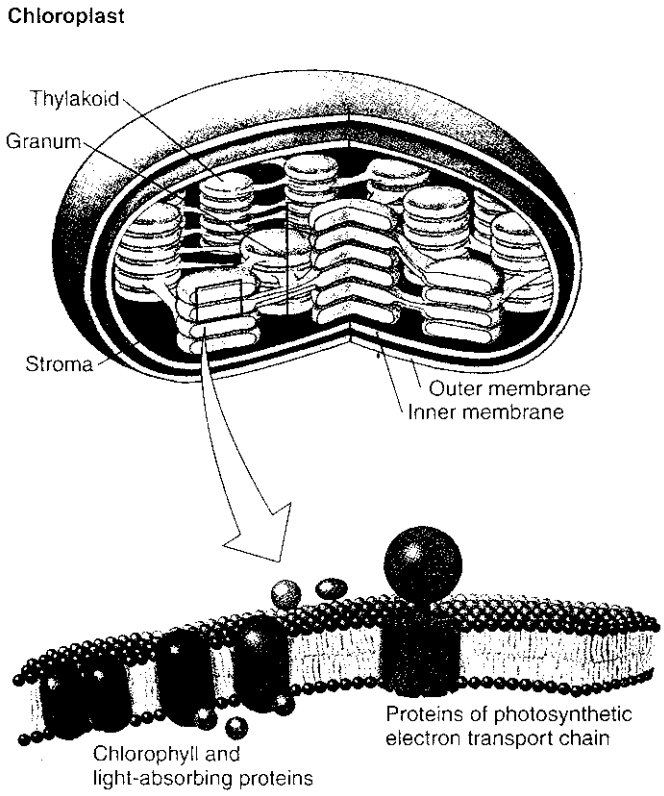


Fig. 5. Anatomy of a Chloroplast. Like a mitochondrion, a chloroplast also has an outer membrane and an inner membrane, but the inner membrane is not folded. The space found within the inner membrane-containing the chloroplast DNA and photosynthetic enzymes-is called a stroma. The stroma contains thylakoid membranes surrounding the thylakoid lumes. The magnified portion of a thylakoid membrane indicates the presence of photosynthetic enzymes.

The number of grana vary from 10-100 in each chloroplast. Granum consists of 10 or more thylakoids, like stack of coins arranged one above the other. The space enclosed by thylakoid is lumen. The thylakoid membranes connecting one granum with the other are called intergranal lamella.

In thylakoids, chlorophyll, carotonoid molecules are present. They are sites of light reaction of photosynthesis. Stroma is the site of dark reaction with necessary enzymes. The products of light reaction, ATP, NADPH, H^+ diffuse into stroma from grana. Ribosomes, DNA, RNA are also found. Due to the presence of these nuclear materials they are self replicating and are semiautonomous in nature.

The chloroplast DNA varies in size from 120-217 kb (Table 3). It is circular, like genes of yeast mtDNA and shows introns.

Table 3

Chloroplast DNA sizes

Organism	**Size (kb)**
Chlamydomonas reinhardtii	196
Marchantia (liverwort)	121
Nicotiana tabacum (tobacco)	156
Oryza sativa (rice)	135

The liverwort chloroplast genome is sequenced (Fig. 6). It contains 92 protein encoding genes and 36 genes for tRNAs and rRNAs. The similarity with bacterial genome is, cpDNA genes are organied in clusters that resemble bacterial operons. cpDNA encodes proteins that carry out photsynthetic electron transport and other aspects of photosynthesis, RNA polymerase and chloroplast gene expression. Inhibitors of bacterial translation, such as chloramphenicol and streptomycin inhibit translation in chloroplasts as they do in mitochondria.

Because of morphological and molecular likenesses, it is probable that mitochondria and chloroplasts started out as free-living bacteria that merged with the ancestors of modern eukaryotic cells to form cellular community. It is now accepted as **endosymbiont theory**.

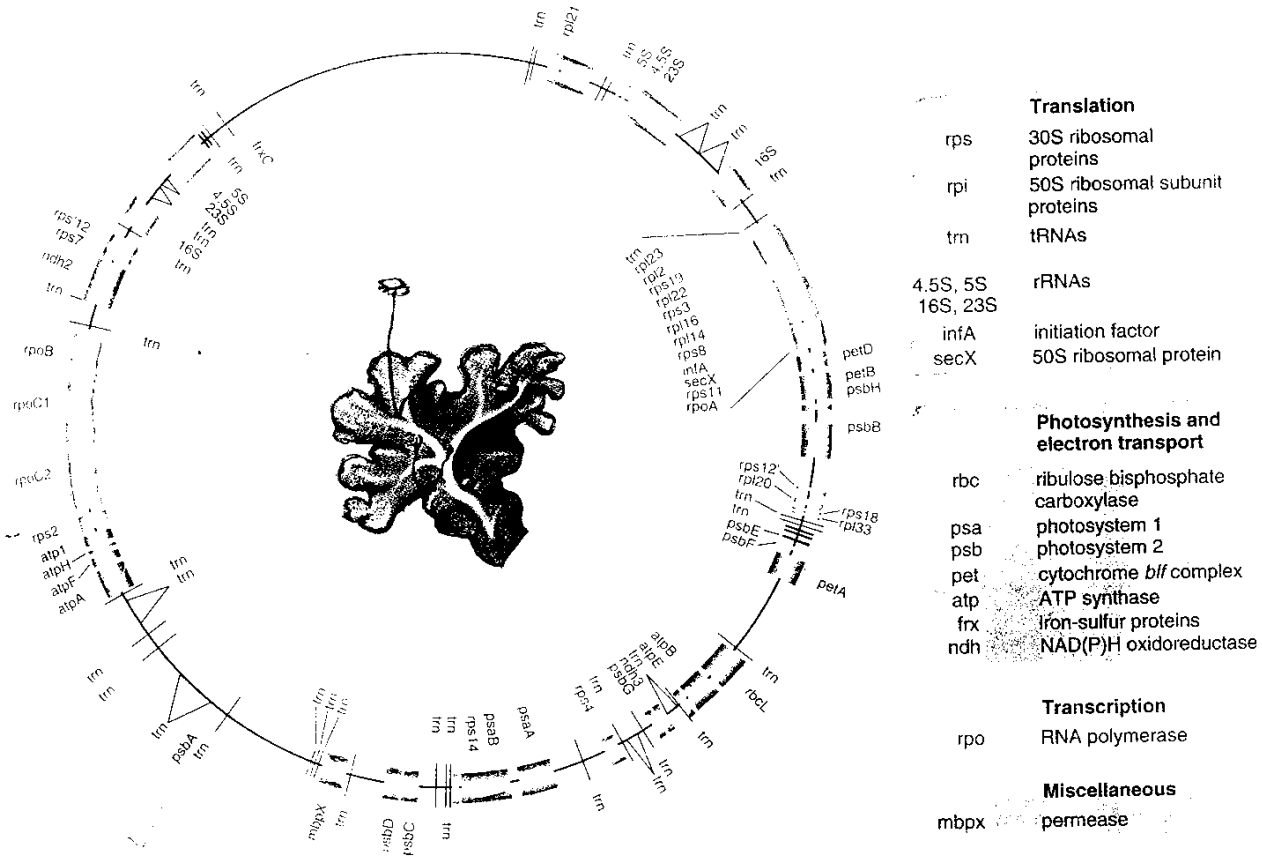


Fig. 6. Chloroplast genome of the liverwort, *M. polymorpha*. The relative locations and symbols of some of the 128 genes are indicated. Genes are color-coded according to general function.

Microtubules

Microtubules are universally present in the cytoplasm of eukaryotic cells and are characterized by their tubular appearance in different cell types. The first observation was made by Robertis and Franchi, 1953. The stability of different microtubules varies. Cytoplasmic and spindle microtubules are rather labile, whereas those of cilia and flagella are resistant to various treatments.

Cytoplasmic microtubules are uniform in size and are straight (Fig. 7). They are 25 nm in diameter and several micrometers in length. The wall of microtubule consists of linear filamentous structures about 5 nm in diameter. Although they show the same morphological characteristics, they differ in other properties, e.g. microtubes of cilia and flagella are much more resistant to various treatments, cytoplasmic microtubules disappear if stored at 0^{o}C after treatment with colchicine.

Fig. 7. Electron micrograph of the pancreas of a chick embryo showing cytoplasmic microtubules, and the replication of centrioles; *c*, the two centrioles; *dc*, daughter centrioles; *cl*, cilium; *m*. microtubules. X50,000 (Courtesy of J. Andre).

Microtubules are composed of protein subunits. Tubulin is the principal protein of cilia and flagella. Two different monomers A and A are present in flagella. The isolation of tubulin has permitted specific antibodies against this protein to be produced. These antibodies may be used as immunofluroscent probes for localizing microtubules in the cytoplasm of a wide variety of cultured cells (Fig. 8).

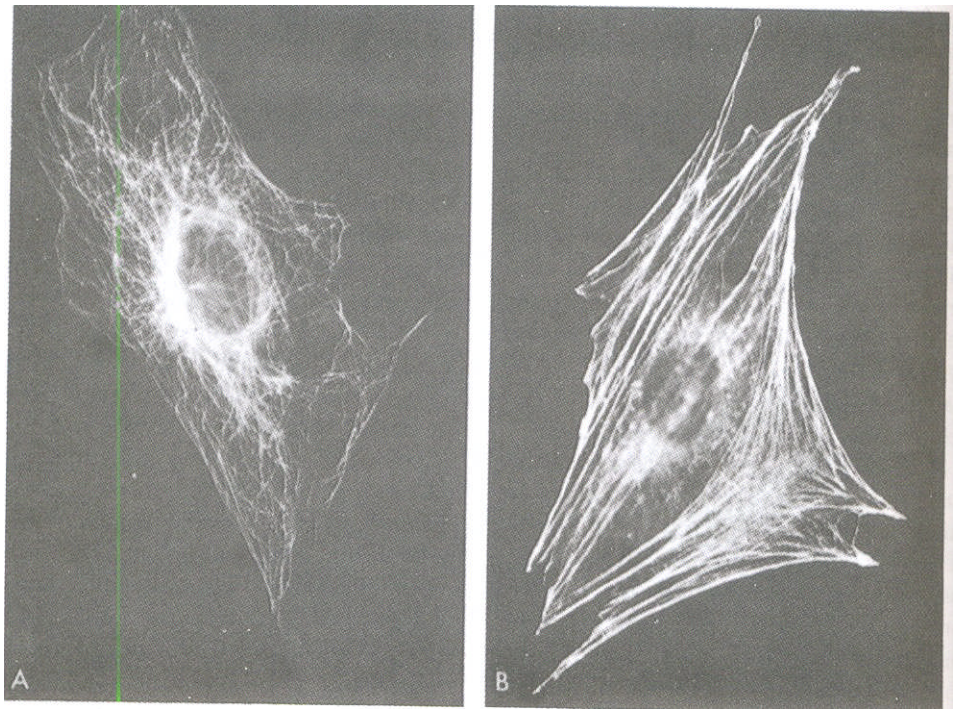


Fig. 8. Cultured 373 cells stained by immunofluorescence with antibodies against tubulin (A) and actin (B). x600. (Courtesy of M. Osborn and K. Weber)

FUNCTIONS

Mechanical Function: The shape of some cell processes has been correlated to the orientation and distribution of microtubules. They determine the shaping of the cell.

Morphogenesis: They shape the cell during cell differentiation.

Cellular polarity and motility: The determination of intrinsic polarity of certain cells is related to the microtubules. The directional gliding of the cell can be replaced by a random movement.

Circulation and Transport: They are involved in the transport of macromolecules in the cells interior.

Thus several functions related to the primitive forms of cell motility have been attributed to microtubules. They play mechanical function, shape of the cell, cell differentiation in nerve cells, spermiogenesis. The polarity and directional gliding of cultured cells depend on microtubules. They play role in the contraction of the spindle movement of chromosomes and centrioles, ciliary and flagellar motion etc.

2. MOLECULAR BIOLOGY ASSIGNMENTS

(a) DNA Structure, Replication and Repair

1. Which bases in nucleic acids are purines and which are pyrimidines?

Ans: Purines: adenine, guanine. Pyrimidines: cytosine, thymine, uracil.

2. In a nucleic acid which carbon atoms are connected by a phosphodiester group?

Ans: The 3' and 5' carbon atoms.

3. How many hydrogen bonds are there in an AT and a GC base pair?

Ans: AT, 2; GC, 3.

4. What chemical groups are at the end of a single polynucleotide strand?

Ans: A 5′-phosphate and a 3′-hydroxyl group.

5. In what sense are the two strands of DNA antiparallel?

Ans: The sugars have opposite orientation in the two strands; thus, at one end of a double-stranded DNA molecule one strand terminates with a 3′-OH group and the other strand with a 5′-P group.

6. Analysis of a DNA sample from a bacterium indicates that 18% of the bases are A What fraction is C?

Ans: If 18% is A, 18% is also T, for a total of 36% for A + T. Therefore, G + C will be 64%, and C will be half that, or 32%.

7. One of the complementary strands of two DNA molecules is given below. Which DNA molecule would have the lower temperature for strand separation? Why?
1. AGTTGCGACCATGATCTG 2. ATTGGCCCCGAATATCTG

Ans: Molecule 1, because of the long tract of GC pairs in molecule 2.

8. ^{15}N-labeled DNA from phage T4 is mixed with T4 DNA of normal density. The solution is then heat-denatured and renatured. The resulting DNA is analyzed by centrifugation in a CsCl density gradient. How many bands will be observed and what will their relative proportions be?

Ans: Pairing will occur randomly. Thus, there are three bands, in the ratios 1 $^{15}N^{15}N$: 2 $^{14}N^{15}N$: 1 $^{14}N^{14}N$.

9. The sequence 5′ – AGTCTGACT-3′ in DNA is equivalent to which sequence in RNA?

A. 5′-AGUCUGACU-3′
B. 5′-UGTCTGUTC-3′
C. 5′-UCAGUCUGA-3′
D. 5′-AGUCAGACU-3′

Ans:- A

10. Which of the following correctly describes A-DNA?

A. a right-handed antiparallel double helix with 10 bp/turn and base lying perpendicular to the helix aixs.
B. a right-handed antiparallel double helix with 12 bp/turn.
C. a right-handed antiparallel double helix with 11 bp/turn and bases tilted with respect to the helix axis.
D. a globular structure formed by short intramolecular helices formed in a single stranded nucleic acid.

Ans:- C

11. Denaturation of double stranded DNA involves…

A. breakage into short double-stranded fragments.
B. separation into single strands.
C. hydrolysis of the DNA backbone.
D. cleavage of the bases from the sugar-phosphate backbone.

Ans:- B

12. Which of the following is common to both *E. coli* and eukaryotic chromosomes?

A. the DNA is circular.
B. the DNA is packaged into nucleosomes.
C. the DNA is contained in the nucleus.
D. the DNA negatively supercoiled.

Ans:- D

13. In what region of the interphase chromosome does transcription take place?

A. the telomere
B. the centromere
C. euchromatin
D. heterochromatin

Ans:- C

14. What can be concluded about the nucleic acids in the table below?

Molecule %U	%A	%T	%G	%C	
1	28	28	22	22	0
2	31	0	31	17	21
3	15	15	35	35	0

Ans:- We must first look to see if U or T is present, for this determines whether the molecule is RNA or DNA, respectively. Molecule 2 is RNA. Now we look at base composition. In double stranded molecules, A bands with T (or U) and G bonds with C. This relationship holds for molecules 1 and 3, so they are double stranded, and molecule 2 is single stranded. Finally, the melting temperature increases with the amount of G-C, so the melting temperature of 3 is greater than that of 1.

15. Which of the following is an example of highly repetitive DNA.

A. Alu element
B. histone gene cluster
C. DNA minisatellites
D. dispersed repetitive DNA

Ans:- C

16. In which mode of replication does a parental circle generate a circle with a linear branch?

Ans:- Rolling circle replication.

17. Name three enzymatic activities of DNA polymerase I.

Ans:- Polymerasing activity, 5′-3′ exonuclease, 3′-5′ exonuclease.

18. In what direction does a DNA polymerase move along a template strand?

Ans:- From 3′ end to 5′ end

19. What are the precursors for DNA synthesis?

Ans:- Deoxynucleoside triphosphates

20. Which enzymatic activity of pol I and of Pol III is responsible for proof reading?

Ans:- 3′ $\rightarrow$ 5′ exonuclease activity

21. How do organisms solve the problem that all DNA polymerases move in the same direction along a template strand, yet double stranded DNA is antiparallel?

Ans:- One strand is copied from 3′ to 5′ end and other strand is copied in the direction opposite to that of the movement of the replication fork by synthesis in short pieces.

22. Which strand, leading or lagging, is usually separated from the replication fork by a gap?

Ans:- Lagging strand.

23. What is the fundamental difference between the initiation of replication and of rolling circle replication?

Ans:- Rolling circle replication is initiated by single strand break 8 replication does not need such a break.

24. If UV-irradiated phage are incubated in a buffer or exposed to light prior to plating, how will the efficiency of plaque b formation change?

Ans:- It will be unaffected, since phage particles do not contain any repair enzymes.

25. Which repair system cleaves thymine dimmer's?

Ans:- Photoreactivation.

26. Which enzymes are required for excision repair in *E. coli*?

Ans:- Uvr endonuclease, polymerase I and DNA ligase.

(b) Bacterial Genetics

1. How does an Hfr cell differ from an F^+ cell?

Ans: In an Hfr cell F is integrated into the chromosome.

2. Is there only one or several possible sites of integration of F in the chromosome?

Ans: Many. Each integration site produces a particular Hfr cell line.

3. Distinguish F^+ and Hfr transfer with respect to the amount of genetic material (DNA) transferred and the intactness of the transferred unit.

Ans: An Hfr cell can transfer more DNA, but usually only a fragment of the chromosome is transferred, An F^+ or F′ cell generally transfers the intact plasmid.

4. In a time-of-entry experiment, recombination frequency is determined for a particular gene at various times. How are these frequencies used to determine the time of entry of that gene?

Ans: The values are extrapolated back to the time axis, the time corresponding to a frequency of zero is the time of entry.

5. Genes *p*, *f*, and *q* have times of entry of 7, 11, and 19 minutes, respectively. What is the gene order and what are the map distances, in time units, with respect to the transfer origin?

Ans: The order is p f q and the map distances are origin-7-*p*-4-*f*-8-*q*

6. How are F′ plasmids produced?

Ans: Aberrant excision of F from an Hfr cell, such that adjacent bacterial genes are contained in the circular plasmid.

7. Could a single stranded DNA molecule with base sequence 5′-GATTGCCGGCAATC-3′ fold back on itself to form a hairpin.

Ans: Yes

8. It has been demonstrated experimentally that most highly repetitive DNA sequences in the chromosomes of eukaryotes are not transcribed. What does this indicate about the function of highly repetitive DNA?

Ans:- Highly repetitive DNA is junck DNA, not expressed.

9. How does a geneticist, doing interrupted matting experiments, know that the locus for the drug-sensitivity allele, used to eliminate the Hfr bacteria after conjugation, has crossed in the F-strain?

Ans:- When the drug sensitivity locus pass into the F-strain there will be general decline in the recovery of recombinants after selection because both Hfr and F-members of a conjugation event can be killed.

10. Three Hfr strains of E. coli (P4x, KL 98, and Ra-2) are mated individually with an auxotrophic F-strain, using interrupted mating techniques. Using these data construct a map of the *E. coli* chromosome, including distances in minutes.

Approximate Time of Entry

Donor Loci	Hfr P4X	Hfr KL98	Hfr Ra-2
Gal+	11	67	70
Thr+	94	50	87
Xyl+	73	29	8
Lac+	2	58	79
His+	38	94	43
Ilv+	77	33	4
Argg+	62	18	19

11. How many different petri plates and selective media are needed?

Ans:- Lac - Gal, 9 mts; gal - his, 27 mts; his - arg G, 24 mts; argG – xyl, 11 mts, xyl – ilu 4 mts; ilu – thr, 12 mts; thr – lac, 8 mts.

For every interruption, a complete medium plate and one each of the seven selected plates are needed.

12. A mating between his^+, leu^+, thr^+, pro^+, str sensitive cells (Hfr) and his-leu-thr-pro-, str resistance cells (F-) is allowed to continue for 25 minutes, the mating is stopped and the genotypes of the recombinants determined. The results appear at the top the next column. What is the first gene to enter and what is the probable gene order?

Ans: The leu-pro his. We see the cells that are thr^+ are most frequent. The chance of conjugation being interrupted increases with the length of time for mating. Therefore, genes far from origin of

transfer appear less frequently. Since we see no his^+ and we stopped the mating at 25 mts, his must be after 25 minutes on the map.

c. Gene Expression

1. If the following sequence of bases represents the start of a gene, what is the sequence of the transcribed RNA, what is its polarity, and what is the polarity of the DNA?

GCTACGGATTGCTG
CGATGCCTAACGAC

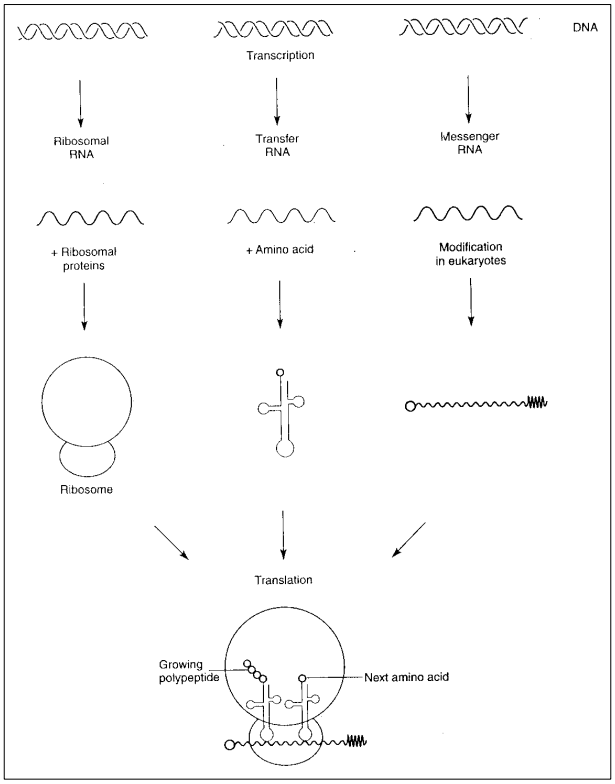
Ans:- Begin by writing the complementary strand to each DNA strand: CGAUGCCUAACGAC for the top and GCUACGGAUUGCUG for the bottom. Now look for the start codon, AUG. It is present only in the RNA made from the top strand, so the top strand must be transcribed. The polarity of the start codon is 5′-AUG-3′. Since transcription occurs 5′-3′, and since nucleic acids are antiparallel, the left end of the top strand is the 3′-end.

2. What are the transcription start and stop signals in eukaryotes and prokaryotes? How are they recognized? Can a transcriptional unit include more than one transnational unit (gene)?

Ans:- The transcription start signal is the promoter, recognized by the sigma factor of RNA polymerase transcription stop signals are terminators, some of which require the factors, olycistronic transcripts are common n prokaryotes.

3. Diagram the relationship of the three types of RNA at a complementarily?

Ans:-



Relationship among the three types of RNA—ribosomal, transfer, and messenger—during protein synthesis. All three types are found together at the ribosome during protein synthesis.

4. In what ways does the transcriptional process differ in eukaryotes and prokaryotes?

Ans:- Although, the overall process is similar in prokaryotes and eukaryotes, differences exist in number of different RNA polymerases and transcription factors in eukaryotes; exact DNA sequences recognized; polycistronic nature of prokaryotic DNA; and post-transcriptional process in eukaryotes.

5. What would be the effect on the final protein product if an intervening sequence were removed with an extra base? One base too few?

Ans:- Removing one base too many or few would result in a shift in the reading frame during translation. Thus radically alter the protein product.

6. What is heterogeneous nuclear in RNA? Small nuclear ribonucleoproteins?

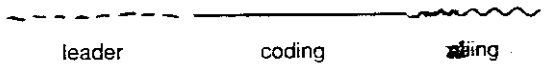
Ans:- Heterogenous nuclear mRNAs are eukaryotic transcripts before post-transcriptional modifications. Small nuclear ribonucleoproteins are the components of the eukaryotic mRNA splicing apparatus.

7. What is a stem-loop structure? An inverted repeat? A tandem repeat? Draw a section of DNA double helix with an inverted repeat of seven base pairs.

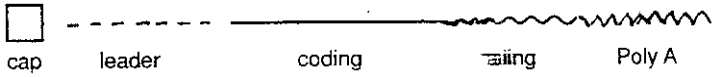
Ans:- When a single strand of DNA or RNA forms a short interval double helical part, an inverted report is read outward on both strands of helix from central point. A tandem repeat is a segment of nucleic acid repeated consecutively.

5′TCCGGTCCGGTCCGG3′
3′AGGCCAGGCCAGGCC5′

8. Draw a typical mature mRNA molecule of prokaryote and eukaryote. Label all regions.



Leader prokaryote coding trailing



Cap Eukaryote Poly A

Both molecules will have 5′ and 3′ ascending regions, the leader and trailing sequences, resperacy. Eukaryotic mRNA has a 5′ cap and a 3′ poly A tail.

9. For the RNA sequence below, determine the sequence of both strands of the DNA from which it was transcribed. Indicate the 5′ and 3′ ends of the DNA with an arrow, which strand was transcribed.

5′-CCAUCCAUGACACCCUUGCUAACGC-3′

d. Protein synthesis and Genetic code

1. Synthetic mRNA is made from a mixture of ribonucleotides supplied to a cell free system in the relative proportion of 3U : 2G : 1A.

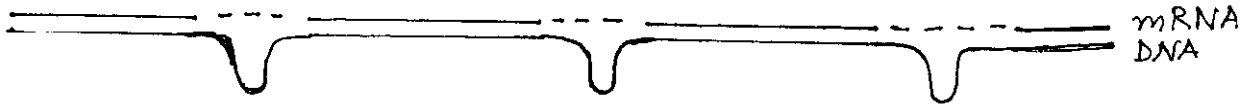
What fraction of the resulting triplets would be: a) UGA b) uuu

Since 6 is the total proportion

Ans: $UGA = \frac{3}{6} \times \frac{2}{6} \times \frac{1}{6} = \frac{6}{216}$

$UUU = \frac{3}{6} \times \frac{3}{6} \times \frac{3}{6} = \frac{27}{216}$

In the drawing of gene below, solid lines (_____) represent coding regions and dashed lines (- - - - - - -) represent introns. Draw what an RNA-DNA hybrid would look like if cytoplasmic mRNA is hybridized to a nuclear DNA.



3. What amino acids could replace methionine by a one base mutation?

Ans:- The code for methionine (internal as well as initiation) is AUG. If the A is replaced, we would get UUG (Leu), CUG (Leu), and GUG (Val); if the U is replaced we would get AAG (LYS)

< ACG (THr), and AGG (Arg); and if the Gis replaced, we would get AUA (Ile), AUU (Ile), and AUC (Ile). Hence, one base change in the codon for methionine could result in six different amino acids.

4. If an artificial mRNA contains two parts uracil to one of cytosine, name the amino acids.
Ans:

12 6 6 3
--- phenylalanine, ---- serine; ---- leucine; ---- proline
27 27 27 27

5. What amino acids could replace methionine by a one-base mutation?

Ans: The codon for methionine (internal as well as initiation) is AUG. If the A is replaced, we would get UUG (Leu), CUG (Leu), and GUG (Val); if the U is replaced we would get AAG (Lys), ACG (Thr) and AGG (Arg); and if the G is replaced, we would get AUA (Ile), AUU (Ile), and AUC (Ile). Hence, a one-base change in the codon for methionine could result in six different amino acids.

6. How do prokaryotic and eukaryotic ribosomes recognize the 5′ end of mRNAs? Could eukaryotic mRNAs be polycistronic?

Ans: In prokaryotes, the Shine-Dalgarno sequence is used for mRNA-ribosomal alignment. In eukaryotes, the scanning hypothesis describes the need for a 5′ cap and scanning behavior. Because a 5′ cap is needed for ribosomal alignment, only one polypeptide can be synthesized per mRNA (there is only one site of ribosomal attachment per eukaryotic mRNA).

7. How many single-base deletions are required to restore the reading frame of an mRNA? Give an example.

Ans: Three.

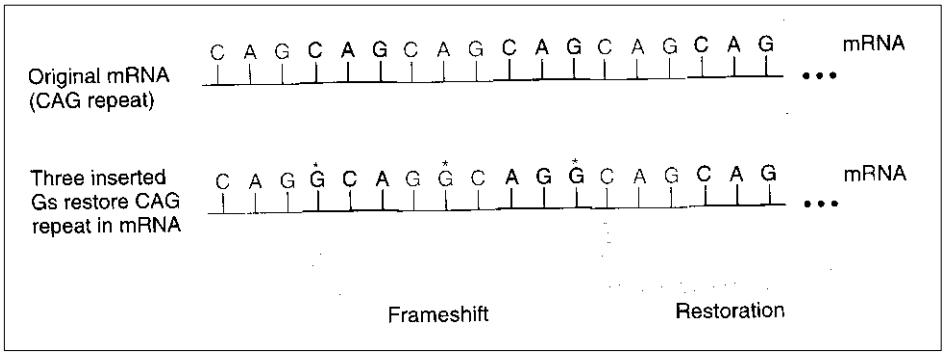


Fig. 1. The coding frame of CAG repeats is first shifted and then restored by three additions (insertions), *Asterisks* (*) indicate insertion.

8. A "nonsense mutation" is one in which a codon for an amino acid changes to one for chain termination. Give an example. What are its consequences?

Ans: 5′-UUA-3′ → 5′-UAA-3′. The consequence is that the growing polypeptide will be terminated at an improper point, probably producing a nonfunctioning enzyme or protein.

9. What are the consequences of having a prokaryotic initiation tRNA recognized by an internal methionine codon?

Ans: There would be blockage of further protein synthesis because of the N-terminal formyl group that prevents a peptide bond. The growing peptide would be stopped at that point.

10. What is the role of EF-Ts in elongation? EF-Tu? What are their eukaryotic equivalents?

Ans: EF-Tu brings a tRNA to the A site at the ribosome. EF-Ts is involved in recharging EF-Tu (see Fig. 10). The eukaryotic equivalent is eEF1.

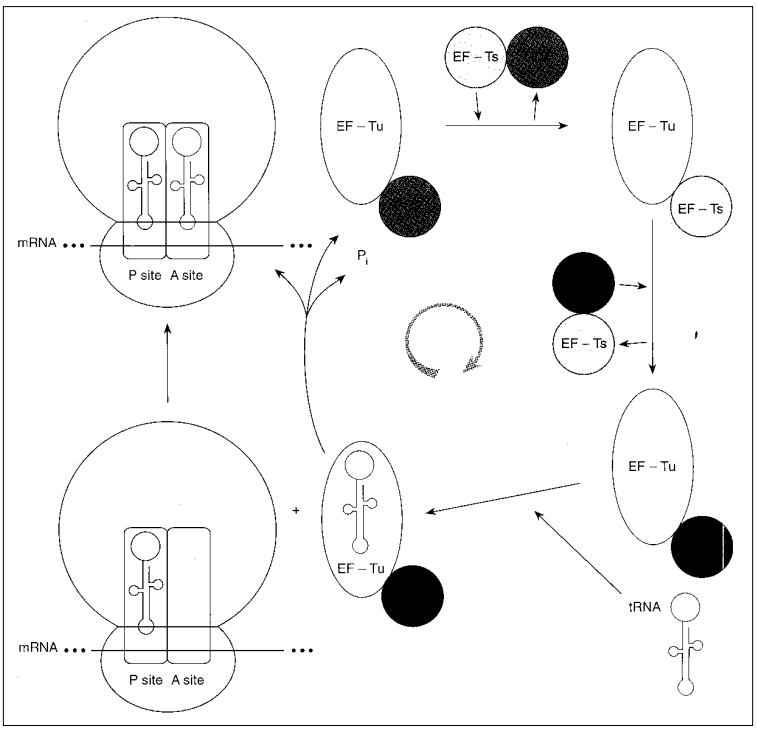


Fig. 2. The EF-Ts/EF-Tu cycle. EF-Ts and EF-Tu are required for the attachment of a tRNA to the A site of the ribosome. At **top center**, we have EF-Tu attached to a GDP. The GDP is then displaced by EF-Ts, which in turn is displaced by GTP. A tRNA attaches and is brought to the ribosome. If the codon-anticodon fit is correct, the tRNA attaches at the A site, with the help of the hydrolysis of GTP to GDP + P_i. The EF-Tu is now back where we started.

11. What are the roles of RF1 and rF2 in chain termination? What are their eukaryotic equivalents?

Ans: RF1 and RF2 recognize nonsense codons. Their eukaryotic equivalent is cRF.

12. What is a signal peptide? What role does it play in eukaryotes? What is its fate?

Ans: A signal peptide is a sequence of amino acids at the amino-terminal end of a protein that signals that the protein should enter a membrane (see Fig. 11.24). The situation in eukaryotes is more complex because there are so many different membrane-bound organelles, each having their own membrane-specific requirements. Signal peptides are usually cleaved off the protein after the protein enters or passes through the membrane.

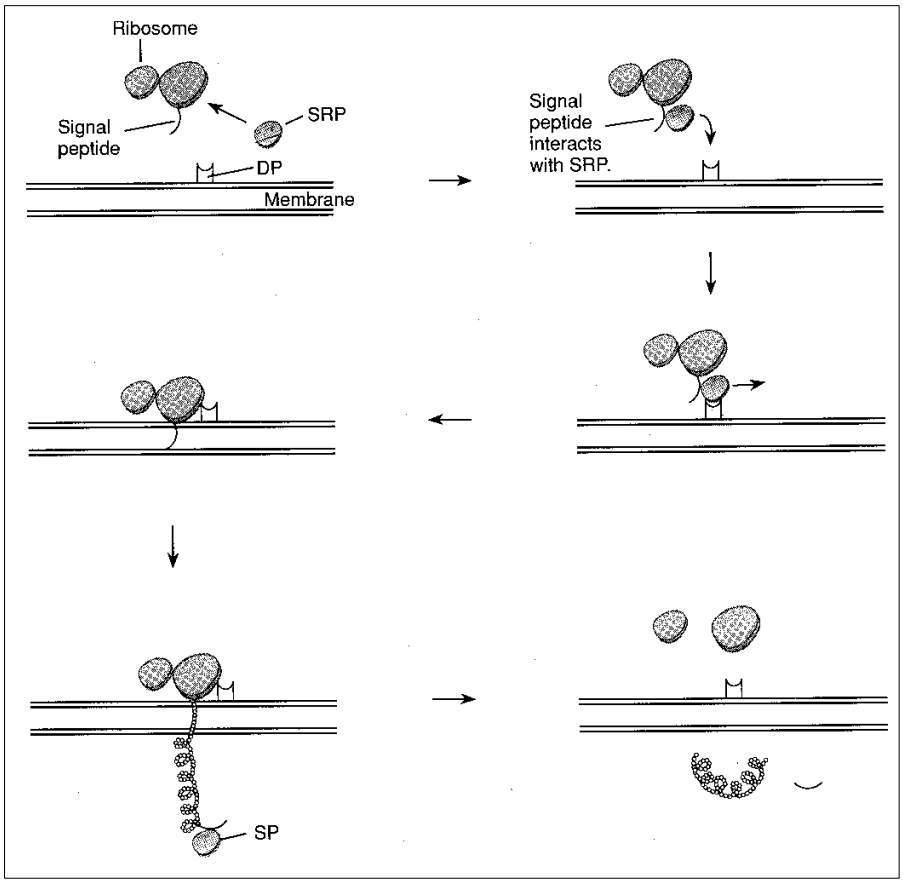


Fig. 3. The signal hypothesis. A signal peptide is recognized by a signal recognition particle (SRP) that draws the ribosome to a docking protein (DP) on the membrane. The peptide synthesized then passes directly into and through the membrane. A signal peptidase (SP) on the other side of the membrane

removes the signal peptide, which has completed its function. When translation is completed, the ribosome drops free.

13. A protein has leucine at a particular position. If the codon for leucine is CUC, how many different amino acids will appear as a result of single base substitutions?

Ans: 6. Begin with CUC, then list all possible codons in which the first C is changed; then list all possible codons in which only the U is changed. Finally, list all codons that result in a change of the second C.

List 1	**List 2**	**List 3**
AUC : *ile*	CAC : *his*	CUG
UUC : *phe*	CGC : *arg*	CUA all *leu*
GUC: *val*	CCC: *pro*	CUU

14. A transcribed DNA strand has the following sequence:

3′-TACTAACTTACGCTCGCCTCA-5′

a. What is the sequence of RNA made from this strand?
b. What is the sequence of amino acids made by the RNA?

Ans: a. 5′-AUG AUU GAA UGC GAG CGG AGU-3′
b. N-met-ile-glu-cys-glu-arg-ser

First determine the sequence of the RNA complementary to the given DNA strand. Don't forget about polarity; as the strand is written, the 5′ end of the RNA will be on the left. Blocking off successive groups of three bases allows the determination of the codons. Use the code to determine the amino acid sequence.

15. Which of the following list of feature correctly apply to the genetic code?

A. Triplet, degenerate, nearly universal, comma-less, non-overlapping.
B. Triplet, universal, comma-less, degenerate, non-overlapping.
C. Overlapping, triplet, comma-less, degenerate, nearly universal.
D. Overlapping, comma-less, non-degenerate, nearly universal.

Ans:- A

16. Which of the following statements about tRNAs is false?

A. most tRNAs are about residues long and have CCA as residues 74, 75 and 76.
B. Many tRNAs contain the modified nucleosides pseudouridine, dihydrouridine, ribothymidine and inosine.

C. tRNAs have a common L-shaped tertiary structure with three nucleotides at one end able to base pair with an anticodon on a messenger RNA molecule.
D. tRNAs have a common coverleaf secondary structure containing three single stranded loops called the D-, T-, and anticodon loop.

Ans:- C

17. Which three statement are true? The aminoacyl tRNA synthetase reaction …

A. joins AMP to the 3′-end of the tRNA.
B. is a two step reaction.
C. joins any amino acid to the 2′ - or 3′ hydroxyl of the ribose of residue A 76.
D. is highly specific because the synthetases use identify element in the tRNA as to distinguish.
E. join AMP to the amino acid to produce an intermediate.
F. releases Ppi in the second step.

Ans:- B, D and E.

18. Which one of the following statement correctly describes initiation of protein synthesis in *E. coli*.?

A. the initiator tRNA binds to the shine- Dalgarno sequence.
B. three initiation factors are involved and IF2 binds to GTP.
C. the intermediate containing IF1, IF2, IF3, initiator tRNA and mRNA is called the 30s initiation complex.
D. binding of the 50s sub-unit releases IF1, IF2, IF3, GMP and Ppi.
E. the initiation process is complete when and empty p site.

Ans:- B.

19. Coli release factor 1 (RF1) recognizes which codons?
A. UAA only
B. UAG only
C. UGA only
D. UGA and UAA.
E. UAG and UAA.
F. UAG and UGA.

Ans:- E.

20. Assume that an average polypeptide contains 400 amino acid residues. How many base pairs will be required to code for 50,000 polypeptides.

Ans: 60×10^6 (400 x 3 x 50000)

21. If a DNA-RNA hybrid were made, would the strands be parallel or antiparallel?

Ans: Antiparallel.

22. Define coding strand and antisense strand.

Ans: A coding strand is a segment of a DNA strand that is copied by RNA polymerase. An antisense strand is a DNA strand that is complementary to a coding strand.

23. Write down the two RNA sequences which could conceivably result from complete transcription of the following DNA duplex.

5′ A G C T G C A A T G 3′
3′ T C G A C G T T A C 5′

Indicate the 5′ and 3′ ends of each transcript.

Ans: PPP-5′-AGCUGCAAUG-3′ and PPP-5′-CAUUGCAGCU-3′

24. What parts of a mRNA molecule are not translated?

Ans: Leaders, spacers, and the unnamed region following the last stop codon of an mRNA are untranslated regions.

25. How many codons could be contained in a four-letter code?

Ans: $4^4 = 256$

26. What are the three stop codons?

Ans: UAA, UAG, UGA.

27. What is the principal start codon and to what amino acid does it correspond?

Ans: AUG; methionine.

28. Which of the following properties are essential for the function of a tRNA molecule: (1) recognition of a codon; (2) recognition of an anticodon; (3) ability to distinguish one amino acid from another; (4) recognition of DNA molecules?

Ans: 1.

29. Which chain-termination codon could be formed by a single base change from UCG, UUG, and UAU?

Ans: UAG.

30. Ribonuclease contains 124 amino acids. What is the least number of nucleotides you would expect to find in the gene encoding the protein? The first amino acid is not methionine.

Ans: (3 x 124) + 3 (termination) + 3 (initiation) = 378.

31. With what polarity is mRNA read?

Ans: The 5′ end is read first.

32. What is the direction of synthesis of a polypeptide chain?

Ans: The amino end is made first.

30. Which site, A or P, can never be occupied unless the other site is also occupied?

Ans: The A site.

33. A polysome has a 3′ and 5′ end. At which end will the polypeptide chain attached to the ribosome be longer?

Ans: The 5′ end, since ribosomes move along the mRNA from the 5′ end to the 3′ end.

(e) Gene Regulation

1. Would synthesis of an enzyme that is needed continually be regulated?

Ans:- Not always especially it needed in large quantities.

2. What is the biochemical action of an inducer?

Ans:- It binds to a repressor.

3. Which enzymes of the lac operon are regulated by the repressor.

Ans:- B Galaetosidase, (Laczz product) lactose permease lac Y produce and transacetylase C (lac A product)

4. What term describes a gene that is expressed continually, even though its transcription may be autoregulated?

Ans:- It is constitutive.

5. Is the lac repressor itself made constitutively or is it induced?

Ans:- Constitutive.

6. Is it necessary for a repressor gene to be adjacent to the operator?

Ans:- No, repressor is a diffusible protein.

7. Is it necessary for the operator to be very near the promoter?

Ans:- Yes, since binding of the repressor to the operator interferes with binding of RNA polymerase to the promoter.

8. When glucose is present, is the concentration of cyclic AMP high or low?

Ans:- Low.

9. Does the binding of cAMP-CRP to DNA affect the beinding of a repressor in any way?

Ans:- No

10. Which one of the following statement about catabolic-regulated operons is false?

A. cAMP receptor protein (CARP) and catabolite activator protein (CAP) are different names for the same protein.
B. When glucose is present in the cell cAMP levels fall.
C. CRP binds to cAMP and as a result activates transcription.
D. CRPbinds to DNA in the absence of cAMP.
E. CRP can bend DNA resulting in activation of transcription.

Ans: A

11. Which two of the following statement about transcription factors are true?

A. the helix turn helix domain is a transcriptional activation domain.

B. dimerization of transcription factors occurs through the basic domain.
C. leucine zippers bind to DNA.
D. it is often possible to get functional transcription factors when DNA binding domains and activation domain from separate transcription factors are fused together.
E. The same domain of a transcription factors can act both as a repressor and as an activation domain.

Ans:- D and E.

PLANT BIOTECHNOLOGY
3. STERILIZATION TECHNIQUES

Principle

The culture medium, especially when it contains sugar, will also support the growth of micro-organisms like bacteria, fungi etc. so if they come in contact with medium either in cellular form or in spore form, the microorganisms grow faster then the higher plant tissue due to their brief life cycle and will kill the tissue. The micro-organisms may come from glass vials, instruments, nutrient medium used for culture and even from plant material itself. Therefore the surface of plant tissue and all non-living articles including nutrient medium should be sterilized.

Procedure

(i) **Sterilization of Non-living Articles:-** The routine sterilization procedure of non-living articles such as nutrient medium, glass gods, distilled water, instruments (wrapped with brown paper) is by autoclaving under steam at a pressure of 15 1b/in^2 and a temperature of 120^{o}C for 15 minutes.

Thermolabile compounds are often added in the medium and such medium is sterilized either at room temperature or in cold by passing through bacterial filter.

An alternative method of sterilizing glass goods and instruments is by heating in an oven at 150^{o}C for 3-4 hrs.

(i) **Sterilization of Plant Material:-** Plant material which si to be cultured, should be surface sterilized to remove the surface borne micro-organisms. This procedure is done in front of a laminar air flow or inside the inoculation chamber before the plant material is inoculated on to the culture medium.

(1) Thoroughly washed plant material or explant in tap water is immersed in 5% u/v solution of liquid detergent such as Tween for 10-15 minutes. Then wash the material thoroughly in tap water and finally in distilled water. This step can be

done in the general laboratory. Subsequent steps are done infront of a laminar air flow or the presterilized inoculation chamber.

(2) Dip the explants in 70% ethylalcohol for 60 seconds.
(3) Immediately transfer the material into an autoclaved jaw bottle and pour 0.1% mercuric chloride ($HgCl_2$) 5-10% sodium hypochlorite (u/v) solution. Keep them for 10-15 minutes. During that period, the bottle is frequently swirled for shaking so that all surfaces of plant material come equally in contact with sterilant.

(4) After 10-15 minutes, decant the sterilant and wash the explants thoroughly with several canges of autoclaved distilled water to remove all traces of sterilant.

(5) Then the explants are ready for culture.

PREPARATION OF MS STOCK SOLUTION

Aim

To prepare stock solutions for media preparation.

Principle

As it is not possible to weigh and mix all the constituents just before the preparation of medium. It is time consuming and a tedious job. If 100 ml or 200 ml medium is to be prepared then it is very difficult to weigh some constituents that are used in very small quantity for one litre medium. So it is convenient to prepare the concentrated stock solutions of macro salts, micro-salts, vitamins, amino acids, hormones etc.

Procedure

Macro salts should be made at ten/twenty times of (x 10@ x 20) their final concentration in the medium while micro-nutrients can be prepared at thousand times (x1,000) of final strength. While making the stock solutions it is advisable to dissolve each constituent completely before adding another otherwise precipitation of salts may occur.

Stock solution of Macro-salts (x 20)

To make 1,000 ml of this stock solution, dissolve the salts one after another in 800 ml of double distilled water (DDH_2O) and then make up the volume. The solution is filtered and can be stored in refrigerator (10-16^{O}C) for a long period until the solution is totally used.

Stock solution of Micro-salts (x 1,000)

The minor elements are weighed and dissolved in 100ml double distilled water and prepare a stock solution of 1000 x concentration.

Stock solution of Iron-EDTA (x200)

Dissolve at first 745 mg of Na_2EDTA in 75 ml boiling distilled water then add gradually 557 mg of $FeSO_47H_2O$. Keep on a magnetic stirrer for at least 1hr in hot condition until the colour of the solution changes to golden yellow. Finally make the volume to 100 ml and store in refrigerator (5^oC). This solution specifically must be stored in an amber coloured bottle.

1. MS – Major :

Constituents	Amount mg/l	Amount (gm) to be taken for stock (x20)	Final volume of stock (ml)
NH_4NO_3	1650	33	1000
KNO_3	1900	38	
$CaCl_2$, $2H_2O$	440	8.8	
KH_2PO_4	170	3.4	
$MgSO_4$ $7H_2O$	370	7.4	

2. Stock solution of Micro salts (x1000)

Constituents	Amount mg/l	Amount (x100) to be taken for stock solution	Final volume of stock (ml)
H_3BO_3	6.2	620	100
$Na_2MoO_42H_2O$	0.25	25	
$CaCl_2$, $6H_2O$	0.025	2.5	
$CuSO_45H_2O$	0.025	2.5	
$ZnSO_472O$	8.6	860	
$MnSO_44H_2O$	22.3	2230	

3. Stock Solution of MS vitamins (x1000)

Constituents	Amount mg/l	Amount (x50) to be taken for stock solution	Final volume (ml)
Thiamine HCl	0.1	5	50
Nicotinic acid	0.5	25	
Pyridorine HCL	0.5	25	

4.

Hormone	Required amount for stock solution (mg)	Amount of solvent required to dissolve 1N NaoH	Amount of DD4	Final Cane mg/ml
Auxins	10	1ml	9ml	0.5
2,4-dichloropheroxy acetic acid (2,4-D)	10	1ml	9ml	0.5
Indole acetic acid (IAA)	10	1ml	9ml	0.5
α-Naphthalene acetic acid (NAA)	10	1ml	9ml	0.5
Cytokinins Kinetin (6-furfuryl amino purire)	10	1ml	9ml	0.5
BAP (6-Benzl-amino-purine)	10	1ml	9ml	0.5

Stock solution of MS vitamins : (x1,000)

A 1,000 x stock solution of vitamin were prepared by dissolving Thiamine HCL, Nicotinic acid & Pyridonine HCl in 50 ml of distilled water.

Stock solution of Glycine (x 1,000)

Dissolve 40mg glycine in 20ml of DDH_2O store at O^OC for 15 days.

Stock solution of kI (x 1,000)

Dissolve 83mg of kI in 100ml of DDH_2O. Store in refrigerator ($10 – 16^O$).

Stock solution of Meso-Inositol (x 500)

Dissolve 1gm meso-inositol in 20ml DDH_2O. Store at O^OC for 15 days.

Stock solution of Hormones

Auxins and cytokinins are not directly dissolved in water. So they are first made soluble in water miscible solvents and then water is added to get the final volume.

4. PREPARATION OF MS MEDIUM

Aim : To prepare 1000ml MS medium.

Principle

To vivo plant cells, tissues and organs get their appropriate nutrient and growth requirements from the intact plant body for their organized growth and development. Isolated cell, tissues and organs also need nutrients for their *in vitro* growth and development. So nutrients are supplied artificially according to the medium formulated. The main objective of medium preparation is to culture the cell, tissue and organ *in vitro*.

Procedure

Media should be prepared with care and the following procedure is recommended.

To make 1 litre of MS medium –

(i) Dissolve 30 gms cane sugar in 200ml DDH_2O.

(ii) Take DDH_2O in another flask and add in sequence the appropriate amount of stock solution as follows :

Stock solution of macro salts	50 ml
Stock solution of micro salts	1 ml
Stock solution of kI	1 ml
Stock solution of FE-EDTA	5 ml
Stock solution of MS vit	1 ml
Stock solution of Glycine	1 ml
Stock solution of meso-inositol	2 ml

Desired concentration of auxin and/or cytobinin are added from stock solution according to the formula.

$$\frac{\text{Desired concentration}}{\text{Stock concentration}} = \text{amount (ml) of stock solution to be taken for 1 litre medium}$$

If the quantity of the medium is less than 1 litre, then hormones are added using another formula.

$$\frac{\text{Required concentration} \times \text{volume of medium}}{\text{Stock concentration} \times 1{,}000} = \text{Amount (ml) of stock solution to be added}$$

(iii) Pour filtered sucrose solution and salt, vitamins, amino acid, hormone solution mixture into a one litre measuring cylinder. Make the final volume to one litre with DDH_2O. Shake well to mix up uniformly.

(iv) Adjust the pH of the liquid medium 5.6–5.8 with the aid of 0.1 (N) HCl as 0.1 (N) NaOH. This operation is done using the pH meter.

(v) Add 8% agar to the liquid medium to make solid medium. Heat to $60^{O}C$ to dissolve the agar completely.

(vi) Dispense the culture medium into culture tube (20 ml/tube) wide mouth conical flask (25-40 ml/flask). Insert non-absorbent cotton plug wrapped with gauge cloth. Cover the plug with the help of brown paper and rubber band.

(vii) Medium is finally sterilized by autoclaving.

SEED CULTURE

Aim: To culture given seed for the production of seedlings.

Principle: Surface sterilized seeds are used to develop healthy and disease free seedlings *in vitro*.

Requirements: Surface sterilized seeds, MS medium, forcep's, filter paper, petriplates, sterile DDW.

PROCEDURE

Surface sterilization of seeds

Seeds are washed by submerging in water with few drops of liquid detergent between for 5 minutes. The seeds are rinsed under running tap water to remove detergent. They are transferred into Laminar airflow hood where 70% Ethanol is added for 2 min. The seeds are thoroughly washed with sterile distilled water. Then add 0.1% $HgCl_2$ and treat the seeds for 10 min. Wash the seeds repeatedly with sterile DDW.

Inoculation

With the help of sterile forceps, seeds are inoculated on to the test tubes with autoclaved MS media. The culture is maintained in the culture room at $25 \pm 2^{O}C$.

Result

Seedlings with healthy cotyledenay leaves were observed with in 7 days.

5. CALLUS CULTURE FROM SEEDLING EXPLANTS

Aim: To induce callus formation in seedling explants

Principle

Callus tissue is good source of genetic or karyotypic variability, so it may be possible to regenerate a plant from genetically variable cells of the callus tissue.

Procedure:

(i) Aseptic preparation of plant material.

(ii) Selection of suitable nutrient medium supplemented with appropriate ratio of auxins and cytokinins (or) appropriate auxins.

(iii) Incubation of culture under controlled physical condition.

The *in vitro* grown seedling is taken as an plant material. It is cut aseptically with sterile blade under the laminar flow into small segments. The explants are finally transferred asceptically on a suitable nutrient medium solidified with agar. For healthy callus growth usually both an auxin and a cytokinin are required.

Hormonal concentration in the medium (Indole 3 – Acetic Acid).

Auxin – IAA – 1.5 mg/lit

Cytokinin – BAP – 2 mg/lit

Incubation

Culture tubes with small segments of leaves, internodes, nodes, cotyledons are incubated at 25 $\pm$ 2^{o}C for 15 days.

Result

Proliferating cell mass (or) callus is observed in the culture tubes.

6. CLONING VECTORS
(PBR 322, cosmid and yeast artificial chromosome)

One of the most important elements in r-DNA technology is the vector. There are different types of cloning vectors for use with different types of host cells. There are plasmid vectors, cosmids, phages, phagemids, yeast artificial chromosome, transposons etc. Any vector should have the following features.

1. It must contain a replicon that enables it to replicate in host cells.

2. It should have several marker genes, to differentiate the transformed cells from non-transformed cells.

3. It should have unique cleavage site for the insertion of foreign DNA into the marker gene.

4. For the expression of cloned DNA, the vector should contain suitable control elements such as promoters, terminators etc.

PBR 322

This is one of the artificial cloning vector, widely used cloning vector (Bolivar and Rodriguez, 1977a, b). It is a plasmid vector with 4.36 kb kb double stranded DNA (Fig. 12). It contains Col EI origin of replication. It contains two antibiotic resistance genes, one is ampicillin resistance (amp^R) gene coding for β-lactamase and the other is tetracycline-resistance gene (tet^R). The plasmid contains 20 unique recognition sites for restriction enzymes. Cloning of a DNA fragment into any of these sites results in the insertional inactivation of either one of the antibiotic resistance markers.

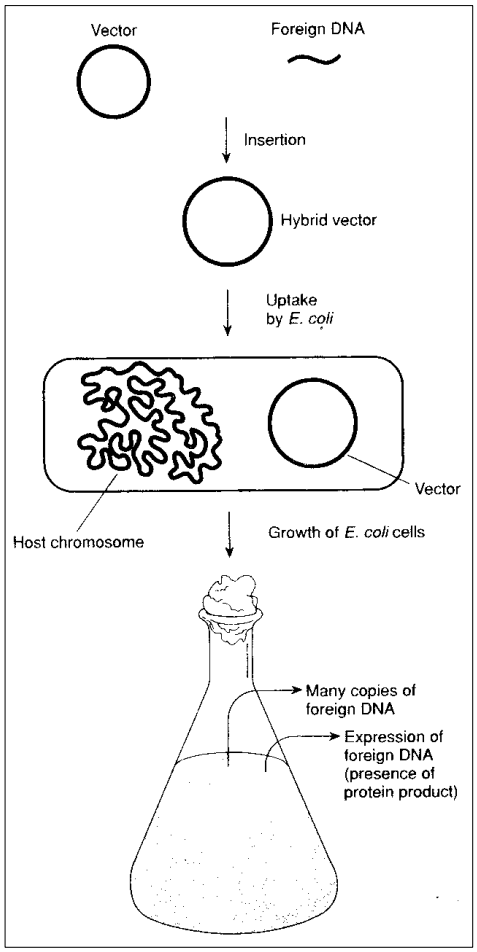


Fig. 12. Overview of recombinant DNA techniques. A hybrid vector is created, containing an insert of foreign DNA. The vector is then inserted into a host organism. Replication of the host results in many copies of the foreign DNA and, if the gene is expressed, quantities of the gene product. (All DNA shown is double-stranded).

Three types of cells are obtained:

1. Cells that have not been transformed and so contain no plasmid molecules and will be amp^S tet^S.

2. Cells that have been transformed with PBR 322 but without inserted DNA, they are amp^R, tet^R. These are transformed cells.

3. Cells that contain a recombinant DNA molecule i.e., DNA fragment has been inserted into the PBR 322 at tet^R gene cluster. These cells lose tetracycline resistance, since the fragment has inserted in the middle of tetracycline resistance gene cluster. There are recombinants amp^R tet^S. Colonies that do not grow on tetracycline agar are recombinants, whereas both transformants and recombinant will grow on ampicillin agar. By comparing the replica plates, recombinant can be picked up from agar ampicillin plates.

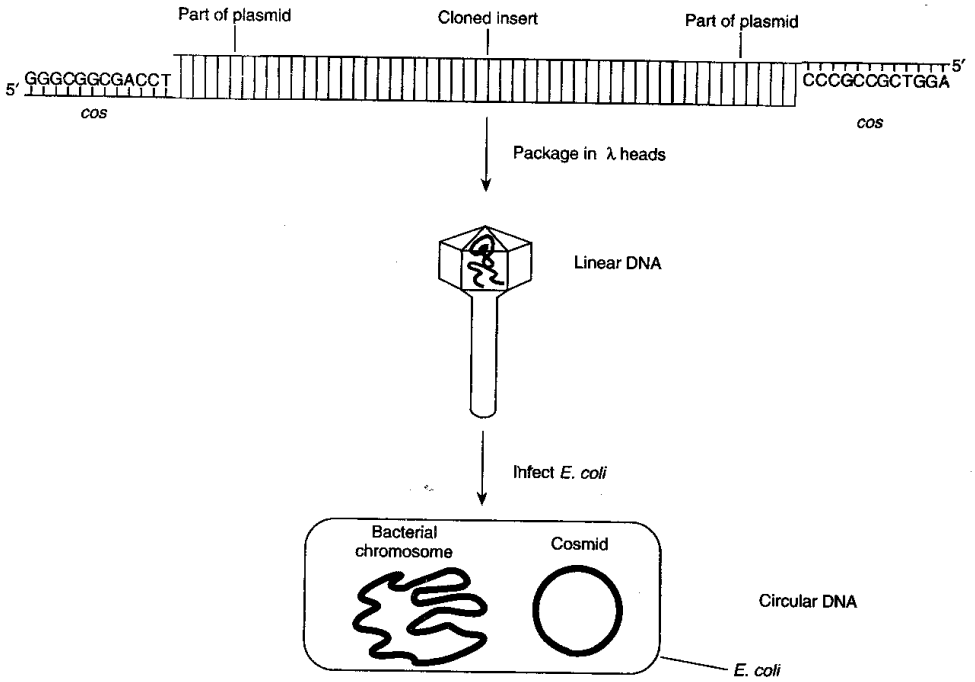


Fig. 2. A cosmid is a plasmid with *cos* sites that can be transferred into bacteria within phage lambda heads, a very efficient method of infection. The *cos* sites are single-stranded; they renamed to a *circle* when inside the host.

Cosmids: Cosmids are plasmid vectors (Fig. 2.) that contains a bacteriophage lamda *cos* site, that directs insertion of DNA into phage particles. The development of cosmid vector is based on the observation that-200 bp DNA sequence called *cos* is required for DNA packaging into phage particle. For successful packaging of DNA, there must be two COS sequences separated by 38-52

kb cosmid cloning vectors with DNA inserts of 30-45 kb can be packaged into λ phage particles. λ phage head can hold upto to 45 kb of DNA. Following absorption of these phage particles on *E. coli* host cells, the cosmid vector circularizes via the cohesive ends and replicates as a plasmid. Cosmid vectors possess an origin of replication, a selectable genetic marker and suitable cloning sites. The advantages of cosmids are that relatively large size of insert DNA (upto 45 kb) can be cloned. Therefore, cosmids are ideal vectors for genome mapping.

Yeast artificial chromosome: The development of yeast artificial chromosomes (YAC) by David Burke *et al*., (1987) extended the cloning range to several thousand bases (1000-2000 kb). A typical YAC consists of centrome elements (CEN4) for chromosome segregation during cell division, two telomere sequences (TEL) for chromosome stability, an origin of replication and one or more growth selectable markers (URA3) (Fig. 3). Normal eukaryotic chromosomes are linear. In yeast, the sequence elements required for circular plasmid vectors to be replicated and stage maintained in an extrachromosomal state have been identified as Autonomously replicating sequences (ARS). The most commonly used YAC for genome mapping of higher eukaryotes is PYAC4. The left and right arms have yeast chromosomal elements to enable replication in a yeast host, as well as markers TRP and 4RA, which permits selection for transformants. The two arms can be ligated to ECOR I ends of a large fragment of genome DNA insert to create a recombinant chromosome. This can be maintained as a linear chromosome in yeast, and is referred to as a yeast artificial chromosome. Yeast transformants containing recombinant YAC molecules can be identified by red/white color selection.

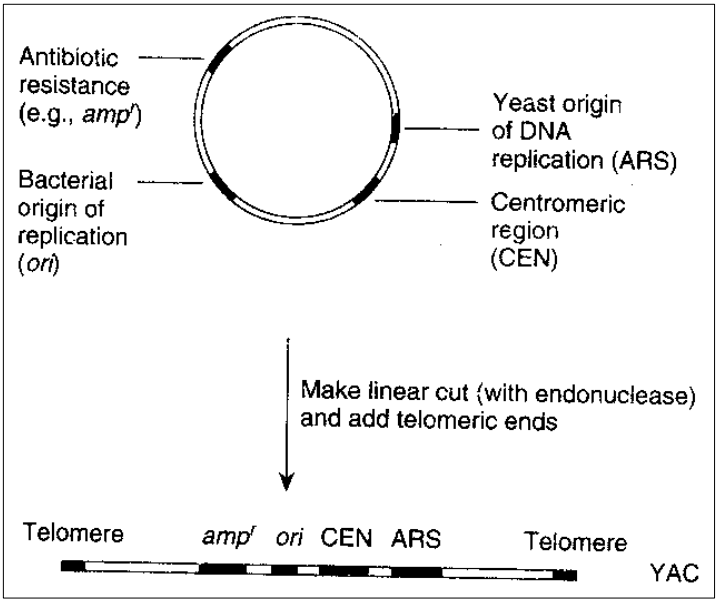


Fig. 3. *Escherichia coli* plasmid pBR322 modified for use in yeast. This plasmid survives and replicates in both yeast and *E. coli* because it contains the origin of replication for both, as well as a yeast centromeric region (CEN). When it is made linear and telomeres are added, the yeast artificial chromosome (YAC) becomes suitable for cloning large pieces of DNA.

Transformed yeast containing recombinant YAC molecules – Red colony.

Non-transformed yeast – white colony.

The advantage of this vector is, it can accommodate large inserts of DNA.

(7) The study of features of Ti plasmid – schematic representation

Ti Plasmid: Gene transfer methods are grouped as vector mediated or direct gene transfer. Vector mediated gene transfer is the transfer of genetic information between plants and transfer from other organisms to plants using vectors. Plant gene vectors being exploited for transfer of genes are plasmids of *Agrobacteria.*

Agrobacterium tumefaciens in gram negative soil bacterium, which transfers Ti plasmid into the host cells, where it becomes integrates into the plant DNA. The Ti plasmids are large, circular DNA molecules upto 200 kb in length. The T-DNA carries genes for opine synthesis (nos or ocs) and phytohormone biosynthesis and oncogenic (onc) region which cause crown gall tumour formation (Fig. 1).

This Ti plasmid have major regions for virulence, origin of replication, conjugation, oncogenicity and catabolism of opines (Fig. 2). Ti DNA is bordered by 25-bp highly conserved direct repeats of DNA. Any DNA sequence located between the borders is transferred to the plant. Oncogenes, necessary for tumor production, can be deleted, disarming the T-DNA. In this disarmed plasmids, foreign genes can be inserted (Fig. 3).

Virulence genes: They are necessary for T-DNA transfer. They are present in 8 operons vir A – vir H. They are silent, till they are induced by plant factors. Wounding is necessary for synthesis of these compounds e.g. acetosyringone and β-hydroxy acetosyringone. Dicot species produce these phenolic compounds, while monocots do not. In such cases, these compounds are added in the medium to induce vir genes. Besides the products of certain bacterial chromosome genes are also required, as they help in the attachment of **Agrobacteria** to host plant cells.

Agrobacterium plasmids are disarmed by deleting T-DNA encoded oncogenes and replacing them with foreign genes of interest. They are co-integrate vectors. The binary vector system consists of the autonomously replicating plasmids within *A. tumefacieus*, a shuttle vector that contains genes of interest between T-DNA border and helper Ti plasmid that provides vir gene products to facilitate transfer into plant cells. The helper Ti plasmids provide necessary vir gene products for transferring the T-DNA to the host plant cell. Most recently developed plant transformation vectors are binary vectors.

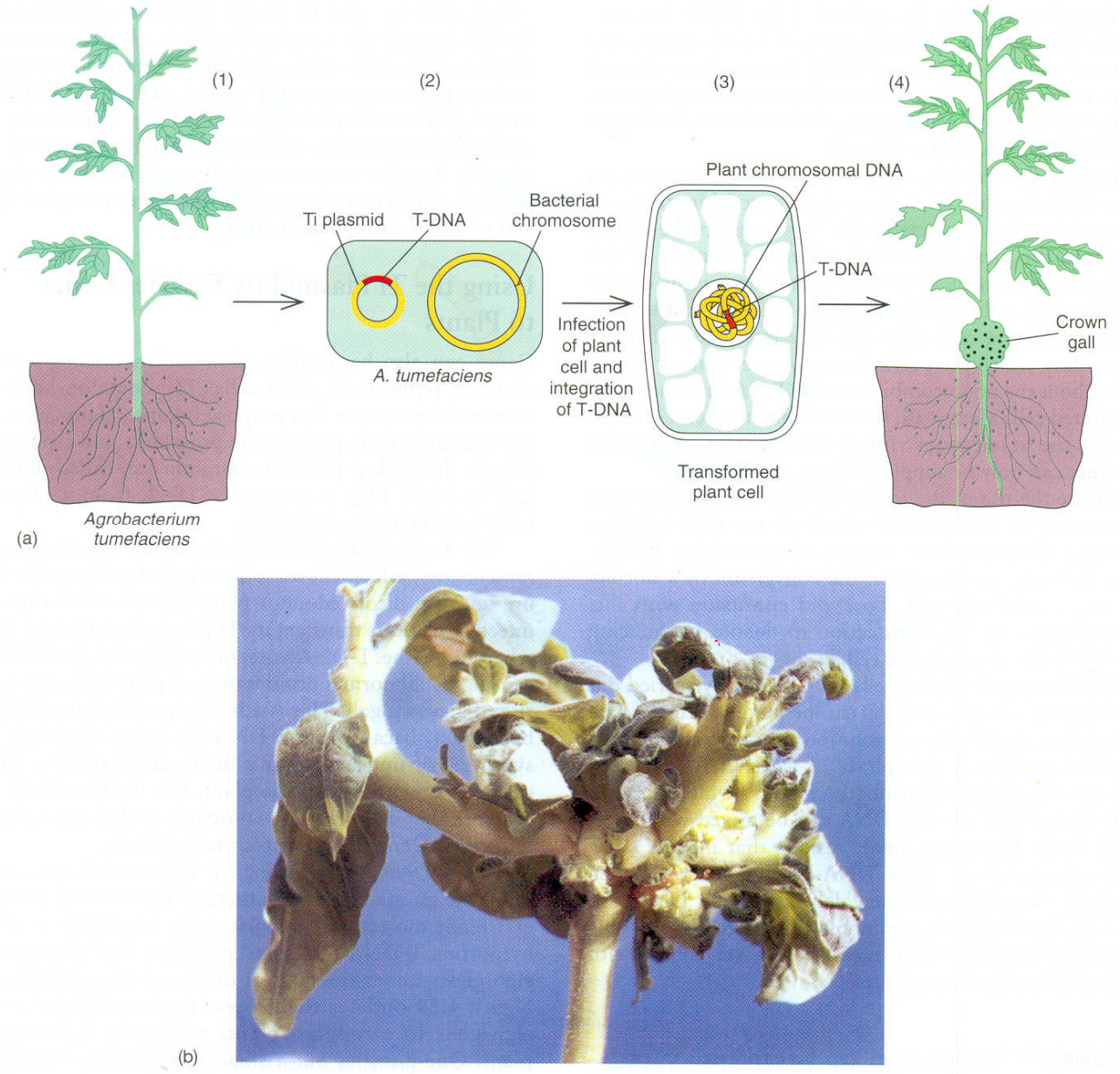


Fig. 15. Genetic organisation of Ti plasmid.

Ti plasmid has three important regions: (1) T-region with genes for hormone and opine biosynthesis and flanked by 24 bp direct repeat sequences is transferred to the plant cells; (2) Virulence region codes for proteins involved in T-DNA transfer; (3) Opine catabolism region codes for proteins involved in the uptake and metabolism of opines.

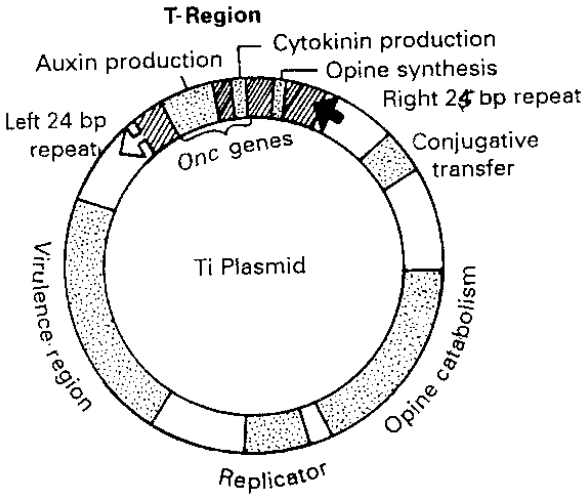


Fig. 2. Genetic organisation of Ti plasmid.

Ti plasmid has three important regions: (1) T-region with genes for hormone and opine biosynthesis and flanked by 24 bp direct repeat sequences is transferred to the plant cells; (2) Virulence region codes for proteins involved in T-DNA transfer; (3) Opine catabolism region codes for proteins involved in the uptake and metabolism of opines.

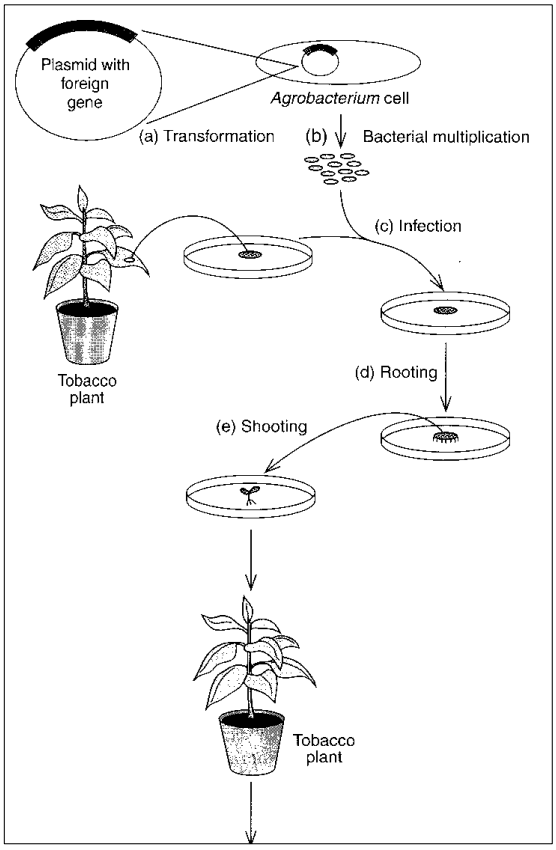


Fig. 3. **Using a T-DNA plasmid to introduce a gene into tobacco plants. (a)** A plasmid is constructed with a foreign gene (red) under the control of the mannopine synthetase promoter (blue). This plasmid is used to transform Agrobacterium cells, **(b)** The transformed bacterial cells divide repeatedly, **(c)** A disk of tobacco leaf tissue is removed and incubated in nutrient medium, along with the transformed *Agrobacterium* cells. These cells infect the tobacco tissue, transferring the plasmid bearing the cloned foreign gene, which integrates into the plant genome. **(d)** The disk of tobacco tissue sends out roots into the surrounding medium. **(e)** One of these roots is transplanted to another kind of medium, where it forms a shoot. This plantlet grows into a transgenic tobacco plant that can be tested for expression of the transplanted gene. (Source: (a) M Dell-Chilton, "A Vector for introducing New Genes into Plants:" Copyright (c) 1983 Scientific American, Inc. Reprinted by permission).

8. ANALYSIS OF PROTEINS BY SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Electrophoresis initially described by Arne Tiselius (1937) is the process by which charged particles move through a media in the presence of an electric field at a given pH. Many biological molecules possess ionisable groups at any given pH, they exist in solution as cations (+) or anions (-) under the influence of an electric field, these charged particles will migrate either to cathode or anode depending on their net charge. This phenomenon is known as electrophoretic mobility. It is technique that separates changed molecules (DNA, RNA and Protein) on the basis of migration in a appropriate matrix, subject to electron field.

Instrumentation: A wide range of Electrophoretic equipment is available. It consists of two items i.e., electrophoretic tank and a power supply. Vertical or horizontal electrophoretic tanks are commercially available. A well designed tank includes a cover to prevent evaporation of the buffer. A stabilized voltage supply is necessary (Fig. 1.).

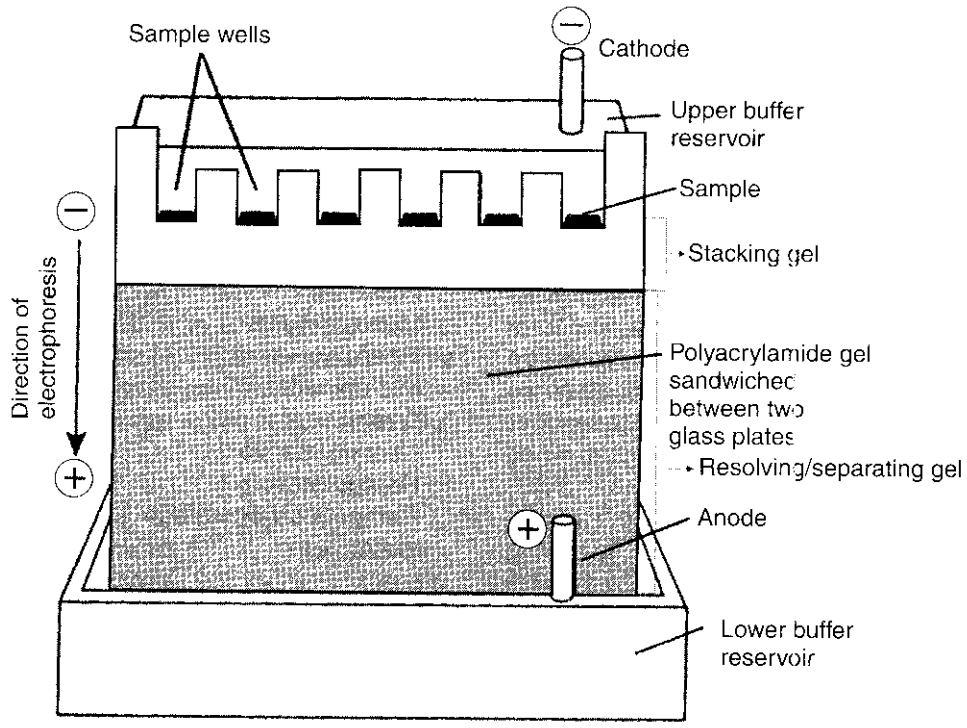


Fig. 1. Native polyacrylamide gel electrophoresis

Polyacrylamide gel: Polyacrylamide shows many advntages over starch gel because of its synthetic nature and pore size. The gel is formed by the polymerization of two nomomers, acrylamide and a cross linking agent N_2N_1 methylene-bis-acrylamide. Pore size in the gel can be

changed by changing the concentration of both polyacrylamide and bis-acrylamide. Polyacrylamide gel electrophoresis (PAGE) is performed either in cylindrical glass tubes or in flat beds.

Electrophoresis of Proteins: Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used method for analysis of protein mixtures qualitatively. Samples to be run on SDS-PAGE are boiled for 5 minutes in sample buffer containing P-mercaptoethanol and SDS. The mercaptoethanol reduces disulphide bonds thus denatures the protein. An ionisable tracking dye, bromophenol blue is mixed with the sample buffer. Once the sample is loaded, current is passed through the gel. This forces all the ionic species to migrate. The negatively changed complexes continue to move towards anode. After the dye has reached to the bottom of the gel the current is turned off and gel is removed from between the glass plates and shaken in an appropriate stain sol for a few hours and washed in destain solution. The destain solution removes unbond dye from the gel. Stained proteins are observed as clear blue bands on destaining, the RF value for each band was calculated using the following formula:

$$RF = \frac{\text{Distance of the band from origin}}{\text{Distance of the dye from origin}}$$

A typical gel lake 1 – 1½ hr to prepare and set, 8 hrs to run at 30 mA and 2-3 hrs staining time. Vertical slab gels are run since it allows upto 20 different samples to be loaded. The molecular man of a protein can be determined by comparing the mobility with those of standard proteins of known molecular mass.

Equipment and Materials

Equipment

1. Slab gel electrophoresis apparatus
2. Glass plates (14 x 16 x 0.3 cm)
3. Plexiglass spacers, 1 cm wide and 1 mm thick
4. Plexiglass comb with 10 or 12 teeth
5. Power supply
6. Clamps
7. Vacuum pump
8. Gadient mixer
9. Microsyringe
10. Staining boxes

SOLUTIONS

Solution A: 29.2% (w/v) acrylamide, (w/v), N_2N_1-methylene bisacrylamide, in distilled water, stored at 4^oC in dark bottles.

Solution B: 1.5 M Tris-HCl buffer, pH 8.8, containing 0.4% SDS (w/v) (Tris 18.15 g, 0.4 g SDS, distilled water to 100 ml, adjust pH to 8.8 with 6 N HCl).

Solution C: 10% (w/v) Ammonium persulphate, freshly prepared.

Solution D: 0.5 M Tris-HCl containing 0.4% SDS (w/v), pH 6.8 (6.05 g Tris, 0.4 g SDS, distilled water to 100 ml adjust pH to 6.8 with 6 N HCl).

TEMED

Electrophoresis Buffer: 0.025 M.Tris, 0.192 M glycine, 0.1% SDS, pH 8.3 (3.0 g Tris, 14.4 g glycine, 1.0 g SDS, distilled water to 1000 ml, pH 8.3).

Sample Solubilizing Buffer: 62 mM Tris-HCl, pH 6.8, 2.3% (w/v) SDS, 10% (v/v) glycerol and 5% b-mercaptoethanol. Heat sample for 3-5 min in boiling water.

Standard Molecular Weight Markers: Kits are commercially available.

Fixing Solution: 50% (v/v) methanol, 10% (v/v) acetic acid in distilled water.

Staining Solution: 0.2% (w/v) Coomassie Blue R-250 in the fixing solution. Filter the solution immediately before use. It can be reused 8 to 10 times.

Destsaining Solution: 5% (v/v) methanol, 7% (v/v) acetic acid in distilled water.

Experimental Procedure:

1. Clean the glass plates by soaking overnight in a detergent, wash thoroughly with water, and then with ethanol.

2. Grease three plexiglass spacers (both sides) and place them along two side edges and along the bottom edge of the dry plate.

3. Assemble the two glass plates and the three spacers tightly with 6 clamps.

4. Place the plates vertically on a flat surface.

5. Connect the gradient mixer to the two glass plates by inserting the teflon tube between the two glass plates at the top edge.

6. Prepare the polyacralamide gradient gels (seperating gel) by mixing the mixtures as follows:

Acrylamide (%)	**5**	**10**	**15**
Solution A (ml)	1.5	3	4.5
Solution B (ml)	3	3	3
Distilled water (ml)	4.5	25	25
Solution C (µl)	25	25	25
TEMED (µl)	5	5	5

Note: Solutions should be deaerated before adding TEMED.

7. Pour the high concentration mixture (9.0 ml) (e.g. 15%) into the mixing chamber of the gradient mixer (the chamber mearest the out-let) and the low concentration (e.g. 10%) mixture into the other chamber. Start the stirrer and open the valve between these two chambers.
8. Pump the gradient into the casting apparatus using a flow rate sufficient to complete delivery well within the polymerization time, but not too fast. Ten minutes should be enough to fill the system.

9. When all the gradient has been delivered, add about 3 ml of distilled water to overlay the polymerized mixture.

10. Without disturbing the casting apparatus, disconnect the teflon tube and gradient mixer and rinse them out immediately to remove any traces of unpolymerized gel mixture.

11. Allow the gradient gel slab to polymerize completely without disturbance for at least 1-2 hours.

12. Pour out liquid and unpolymerized gel or remove by using microsyringe with long needle.

13. About 30 min before starting electrophoresis, the stacking gel of 4.6% acrylamide is prepared by mixing:

Solution A ..	1.5 ml
Solution D ..	2.5 ml
Distilled Water	6.0 ml
Solution C ..	25 µl
TEMED ..	5 µl

Note: Deaerate the mixture before adding TEMED. Polymerization will occur within 30 min.

14. Pour stacking gel and insert comb.

15. Allow stacking gel to polymerize, suck off the unpolymerized gel mixture and remove bottom clamps and bottom spacer.

16. Remove comb carefully by sliding vertically upwards.

17. Suck out unpolymerized gel again.

18. Rinse with distilled water 2 times.

19. Place slab gel plates in the electrophoresis apparatus, connect leads and add electrophoresis buffer to lower chamber.

20. Prepare samples by diluting (1:2) in sample solubilizing buffer, and place in boiling water bath for 2 min. Typically load 20-50 μl sample, containing approx. 10,000 cpm of radioactivity or 10-30 μg protein.

21. Load 10ml of samples by using micropipettes.

22. Load 10 μl molecular weight standard (5 μg total protein)

23. Fill upper chamber with SDS electrophoresis buffer and add 2 drops of 0.1% Bromophenol blue.

24. Use a syringe with bent needle to remove bubbles from under gel between the glass plates.

25. Electrophoresis the gradient gel at constant 150 V until the tracking dye has reached 0.5 cm from the bottom of the gel (approx. 3-5 h total time).

26. After completion, open plates by running a large spatula along edges to free gel from spacers.

27. Fix, stain and destain gel as follows:

- Incubate slab gel in 3-5 volumes of staining solution in staining boxes for a few hours at room temperature.

- Remove stain by placing gel in first destain (fixing solution) for a few hours. The gel will shrink due to dehydration.

- Place gel in second destain (5% methanol – 7% acetic acid) overnight with several changes. This will revert the gel to previous size.

28. Dry on gel dryer for preservation or for autoradiography.

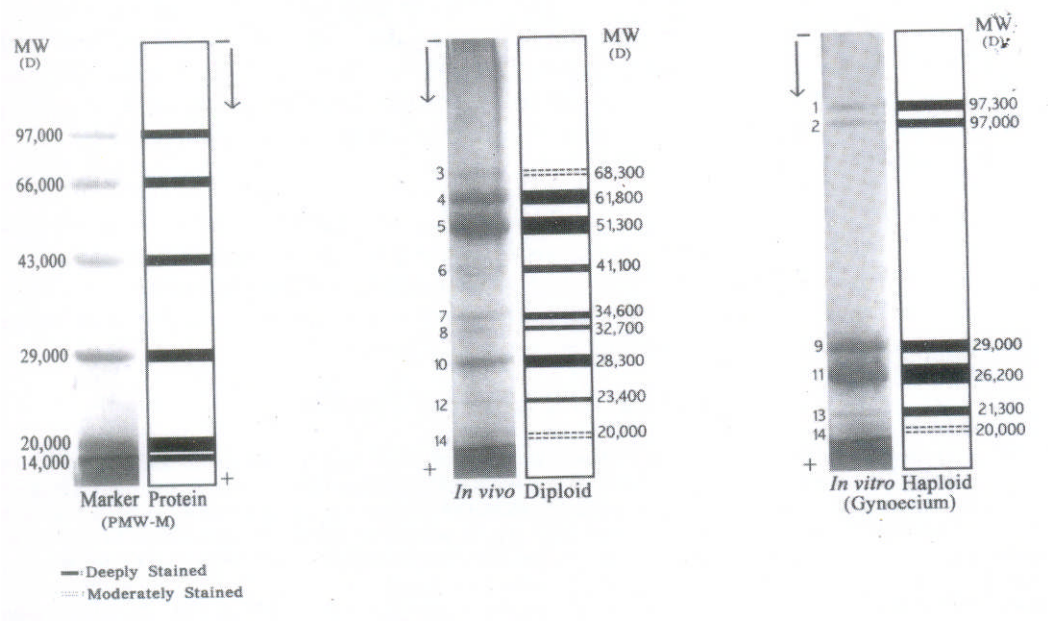


Fig. 2. Electrophoretogram of protein patterns of diploid and gynogenic haploid plants of mulberry var S_{54} analysed using standard protein marker PMW-M (Arrows indicate migration of bands towards anode). (Courtesy of Gayatri *et al*., 2002)

The analysis of the electrophoretic data revealed that the haploid plants are characterized by limited number of protein bands as compared to those of the diploid plant. Further, deeply stained and slow moving bands, 4 and 5 are specific to diploid plant and fast moving bands, 9 and 11 are specific to haploid plant. However, bands, 1, 2 and 13 are purely specific to haploid plant and are absent in the diploid plant.

9. SOUTHERN BLOTTING

When DNA segments are generated by endonuclease digestion, a desired gene must be located. We look for the gene either before or after it is cloned. In a DNA digest, there are many fragments. To proceed further, we have to transfer the electrophoresed fragments to another medium for probing. Nitrocellulore filters are excellent for hybridization, because DNA fragments bind to these members. The transfer procedure, devised by E.M. Southern is called **Southern Blotting**.

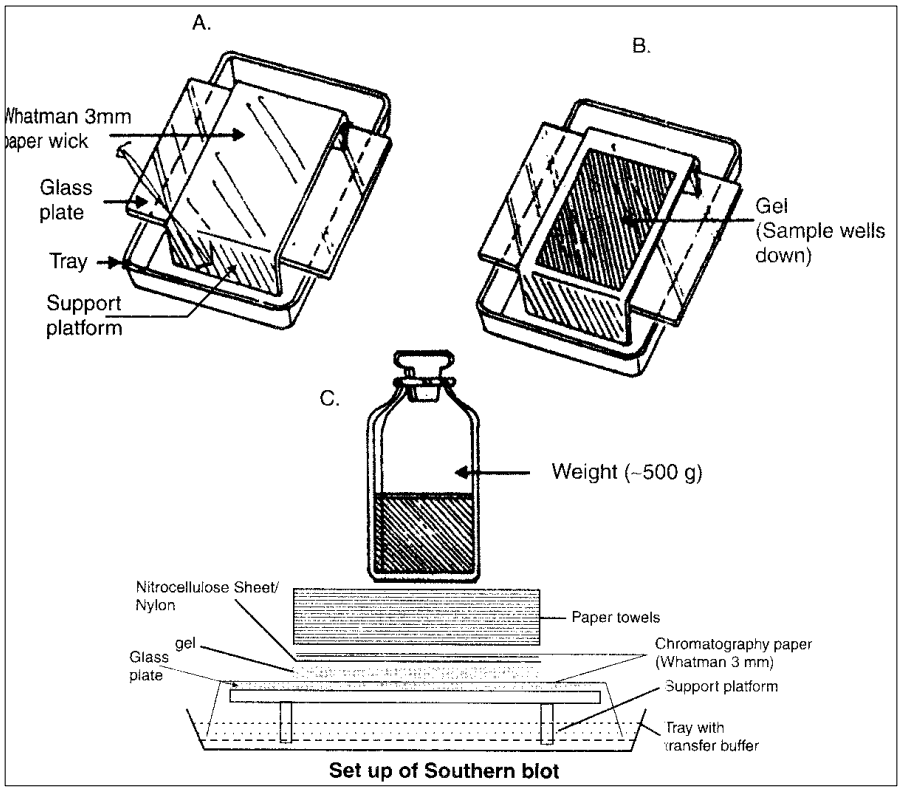


Fig. 20. Different stages in set up of a Southern blot. A-Whatman 3 MM paper wick draped over glass plate in a tray. B-Gel is placed over paper wick. C-Diagrammatic representation of different components in a Southern pyramid for blotting.

It is a method of transferring electrophoresed DNA fragments from the gel onto a nitrocellulose membrane, and subjecting it to hybridization by using a specific probe.

Steps involved in Southern Blotting

1. The given DNA sample is digested with restriction enzymes.

2. The resultant fragments are run on agarose or polyacrylamide gel. Agarose gel was used to separate DNA fragments of 20 kb, Polyacrylamide is preferred to separate smaller fragments, Pulse field gel electrophoresis was used to separate 1000-2000 kb.

3. The fragments are separated based on their size under electric field. Smaller fragments run faster than larger once.

4. The gel is stained with ethidium bromide so that the fragments flouresce in presence of uv light.

5. Marker DNA fragments are also run for comparison.

Blotting procedure

1. The separated DNA fragments are treated with alkali and the DNA is denatured.
2. The DNA fragments are now transferred to a nitrocellulose filter by placing the gel on top of a buffer then lying the nitrocellulose filter membrane on the top of gel

Order:

Weight
↑
Stack of dry filter papers
↑
Nitrocellulose membrane
↑
Gel

3. A stack of dry filter papers are placed on the membrane and a weight is placed over it.
4. The DNA becomes trapped to the nitrocellulose membrane as the buffer passed to dry filter papers through capillary action.

5. The nitrocellose membrane is baked in oven at 80^{o}C in vacuum to fix the DNA.
6. The Radio labbled probe is used to identity the specific sequence containing DNA.
7. Then the membrane is washed to remove the unhydridized probe.
8. Then x-rays are passed on to the membrane to identity where probe hybridized to single struded DNA of specific sequence, through autoradiography.

10. POLYMERASE CHAIN REACTION

The polymerase chain reaction (PCR) developed into a technique in 1985 by kary Mullis is a rapid procedure for *in vitro* enzymatic amplification of a specific segment of DNA (fig.1.). The number of applications of PCR include direct cloning from genomic DNA or cDNA, *in vitro* mutagenesis and engineering of DNA etc. The PCR technique basically involves three nucleic acid segments. The segment of double stranded DNA to be amplified and two single stranded oligonucleotide primers flanking this segment. Additionally there is a protein component (Taq DNA polymerase), appropriate deoxyribo-nucleoside triphosphates (dNTPs), a buffer and salts. Taq DNA polymerase is a thermostable enzyme isolated from Thermus aquaticus growing in hot springs. This enzyme does not lose its activity at high temperatures.

The primer is added in vast excess compared to the DNA to be amplified. They hybridize to opposite strands of DNA< so that synthesis by DNA polymerase extends areas the segment of DNA between them. Unlimited supply of amplified DNA is obtained by repeating the reaction which is made possible by regular denaturation of freshly synthesised double stranded DNA molecules by heating it to 90-98^{o}C. AT this high temperature, the two strands separate and the mixture with two primers recognising the two strands and bordering the sequence to be amplified is cooled to 40-60^{o}C. This allows the primer to bind to their complementary strands through renaturation.

Material required:

1. Sterile water
2. 100 mM MgCl2
3. 10x amplification buffer
4. 2 mM dNTP mix
5. 50 uM Oligunucleotide primers 1 and 2 (50 pmol/µl in sterile water: store at –20^{o}C)
6. 15 µg/ml template DNA
7. 2.5 µ/µl Taq DNA polymerase
8. Automated thermal cycler

1. Prepare a cocktail by mixing
   80 µl 10x amplificates buffer with MgCl2 to a final concentration of 2 mM.
   80 µl 2 mM dNTP mix
   8 µl 50 uM Oligonucleotide
   8 µl 50 mM Oligonucleotide 2
   544 µl 15 µg/ml template DNA.

2. Add 1 µl of 2.5 U/µl Taq DNA polymerase to each tube

3. Heat samples for 90 seconds at 94^{o}C (in either a water bath or a automated thermal cycler). This is the denaturation step.

4. Incubate the samples 2 mins. at 42^{o}C. This is the annealing step of the cycle.

5. Incubate the samples 3 min. at 72^{o}C. This is synthesis or extension step of the cycle.

6. Repeat steps 3 to 5 for another 29 cycles. The number of cycles necessary depends on both the efficiency of the reaction and the amount of template DNA in the reaction.

7. Electrophoresis 10 µl from each sample on a 1.4% agarose gel and stain with ethidium bromide.

Precautions: For applications that amplify rare templates, reagent purity is the most important parameter and avoiding contamination in every step is critical.

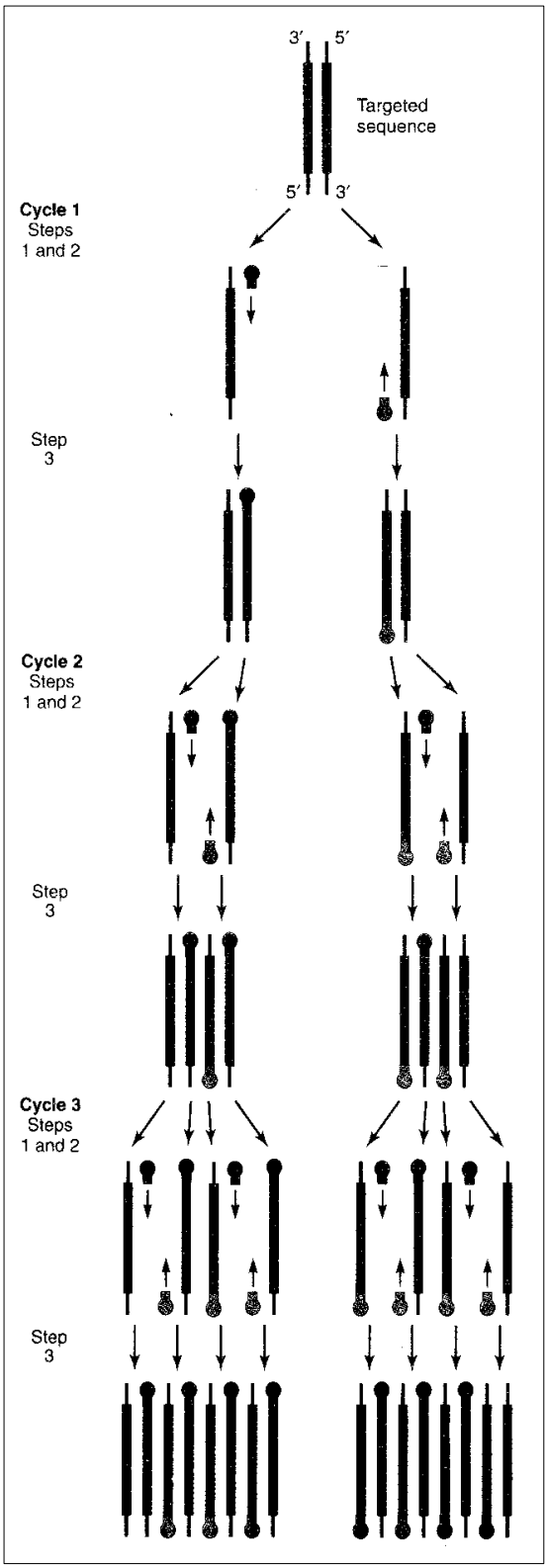


Fig. 1. Polymerase chain reaction. DNA is denatured, (step 1), primer oligonucleotides that are complementary to end sequences on the two strands anneal (step 2), and DNA replication takes place (step 3). Each step in the cycle is controlled by temperature changes. The targeted sequence is shown as *red* on one stand *blue* on the other. Primers are shown as either *green* or *yellow* lollipops. A green primer begins the copying of the red strand into a complementary blue strand; a yellow primer begins the copying of a blue strand into a complementary red stand. In three cycles, one double-stranded region of DNA becomes eight. The process requires the addition of primers, deoxynucleotide triphosphates, and DNA polymerase, as well as changing temperature cycles.

11. SYNTHESIS OF r-DNA MOLECULE

Restriction endonuclease enzymes cut DNA molecules at specific sites called palindromes. They are 4-8 base pair sequences with inverted repeats. Different enzymes recognize different but specific sequences. The first enzyme was isolated from *Haemophilus influenzae* in 1970. Now many types of restriction endonucleases have been isolated from a wide variety of bacteria (Fig.1)

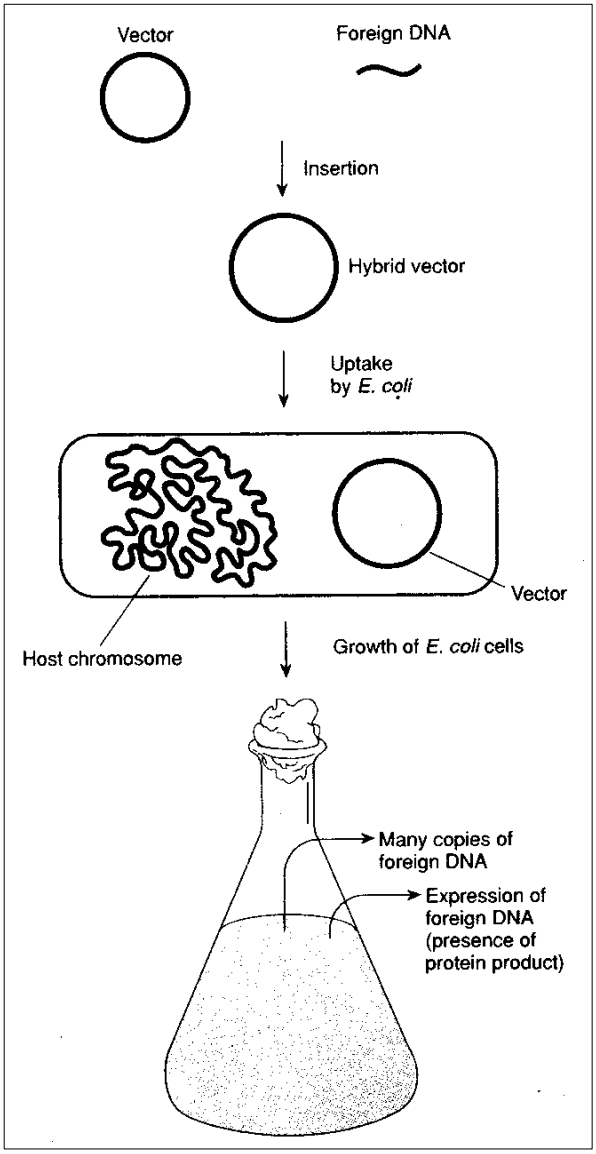


Fig. 1. Overview of recombinant DNA techniques. A hybrid vector is created, containing an insert of foreign DNA. The vector is then inserted into a host organism. Replication of the host results in many copies of the foreign DNA and, if the gene is expressed, quantities of the gene product. (All DNA shown is double-stranded).

The enzymes cut DNA resulting staggered cuts and blunt end cuts. (overhang) (fig 2).

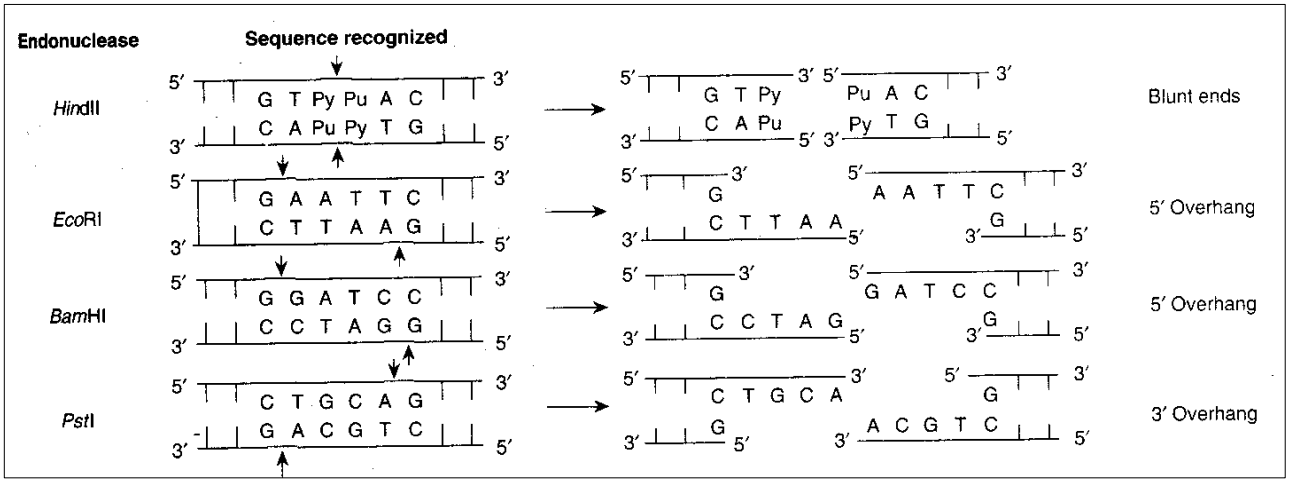


Fig. 2. Sequences cleaved by various type II restriction endonucleases. Py is any pyrimidine and Pu is any purine. *Arrows* denote places where endonucleases cleave the DNA. In 1971, K. Danna and D. Nathans showed that a restriction endonuclease would consistently cut DNA into pieces of the same size. The precision and repeatability of enzyme action made enzymes useful for further research. Not all restriction endonucleases make staggered cuts with 3' and 5' overhangs; some produce blunt ends.

The number of Type II enzyme cleavage sites in DNA molecule depends on the size of DNA, its base composition and GC content of the recognition sequences. Different DNA modifying enzymes are involved during the synthesis of r-DNA molecule.

- DNA ligase from *E. coli* and bacteriophage T_4 seals single stranded nicks.
- Bacteriophage T_4 polynucleotide kinase catalyzes the transfer of phosphate of ATP to a 5′ terminus of DNA or RNA.
- Alkaline phosphatase removes 5′ phosphate group from DNA, thus modifies the termini of DNA.
- DNA polymerase-I directs the synthesis of complementary nucleic acids using single stranded DNA as a template since *in vitro* enzymatic synthesis of DNA is important in genetic manipulation experiments.
- Terminal transferase purified from calf thymus can add oligodeoxynucleotide tails to the 3′ ends of DNA duplexes. Thus, it adds d(A) sequence to 3′ ends of one population and oligod (T) to 3′ ends of another population of DNA. The two types of DNA molecules can anneal to form a dimeric circle.

If suitable enzymes are not available for manipulation of the DNA, the cleavage sites can be added as linker or adaptor molecules (fig 3 & 4). The oligonucleotide dCCGAATTCGC will self associate to give a duplex structure containing ECORI recognition sequence.

Adaptor molecules are chemically synthesized DNA molecules within performed cohesive ends with a blunt ended foreign DNA, synthetic adaptor molecule is ligated. The adaptor is used in 5′ hydroxyl form to prevent self-polymerization. The foreign DNA plus ligated adaptor is phosphorylated at 5′ terminus and ligated to the vector, cut with a particular enzyme.

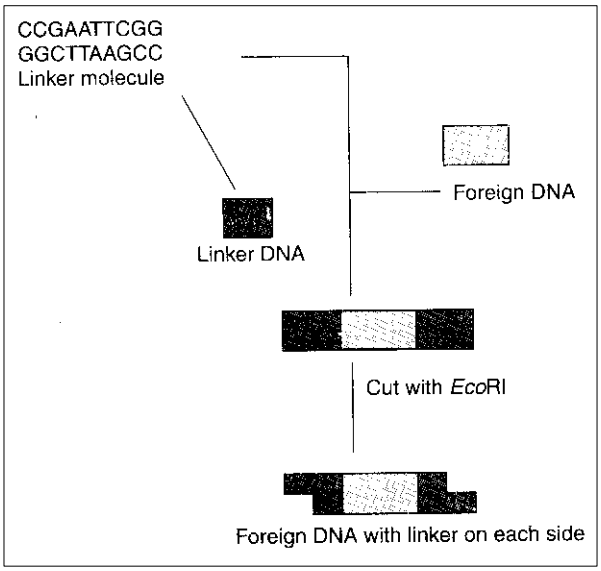


Fig. 3. The use of linker for construction of DNA fragments with cohesive termini.

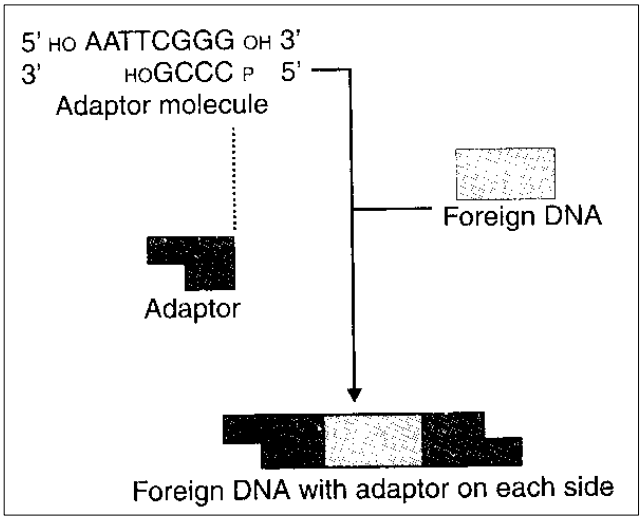


Fig. 4. The use of adaptors for construction of DNA fragments with cohesive termini.

12. GENE CLONING AND GENETIC ENGINEERING

Gene Cloning

Insertion of a fragment of DNA, carrying a gene, into a cloning vector and subsequent propagation of the recombinant DNA molecule in a host organism is called as gene cloning (Fig. 1).

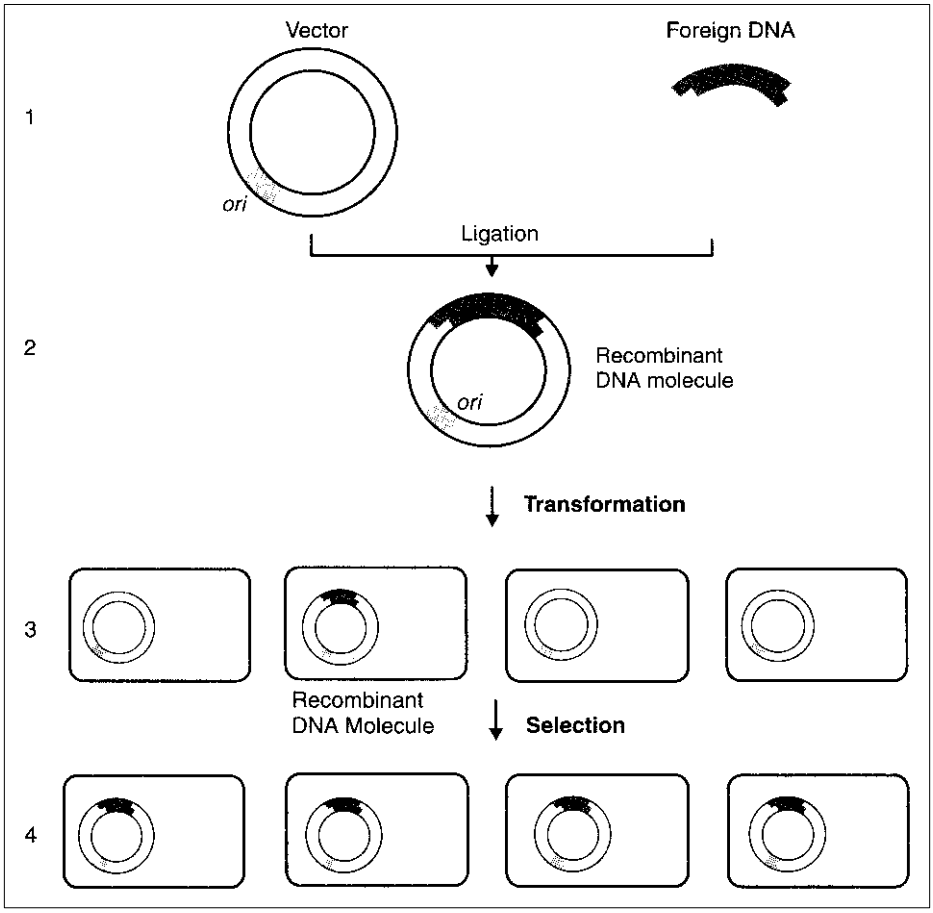


Fig. 1. Fundamental steps of a cloning experiment. The cloning experiment involves (1) the production and preparation of vector and foreign (passenger) DNA, (2) the ligation of vector and passenger DNA so as to construct a recombinant DNA molecule, (3) the transformation of suitable host cells, and (4) the selection of those cells which have acquired recombinant DNA molecules.

The basis steps in a gene cloning experiment are:

1. A fragment of DNA, containing the gene to be cloned, is inserted into a circular DNA molecule called a vector, to produce a chimaera or recombinant DNA molecule.
2. The vector acts as a vehicle that transports the gene into a host cell.

3. Within the host cell the vector multiples, producing numerous identical copies not only of itself but also of the gene that it carries.

4. When the host cell divides, copies of the recombinant DNA molecule are passed to the progeny and further vector replication takes place.

5. After a large number of cell divisions, a colony or clone of identical host cells is produced. Each cell in the clone contains one or more copies of the recombinant DNA molecule: the gene carried by the recombinant molecule is now said to be cloned.

CLONING STRATEGIES

Any DNA cloning procedure has four essential parts.

1. A method for generating DNA fragment.

This can be achieved by restriction endonuclease digestion or mechanical shearing or duplex cDNA synthesis or by direct chemical synthesis.

2. Reactions which join foreign DNA to the vector.

This can be done by homopolymer tailing or ligation of cohesive termini produced by restriction endonuclease or by blunt end ligation or by linker molecules.

3. A method of introducing the artificial recombinant into a host cell in which it can replicate:

   This is done either by transfection with recombinant phage DNA or transformation with recombinant plasmid DNA or by *in vitro* packaging into phage coat, transduction with recombinant phage or cosmid.

4. A method of selecting or screening for a clone of recipient cells that has acquired the recombinant.

Screening

Once a genomic library or cDNA library is available, screening of the libraries for isolation of specific gene sequence is done.

This can be done by

(a) Genetic methods
(b) Immuno chemical methods
(c) Nucleic acid hybridization methods
(d) Hybrid released translation (HRT) and hybrid arrested translation (HART).

Once you get the desired clone or gene, the gene is then characterized by DNA sequencing.

Genetic Engineering in Plants

To introduce a desired gene or set of genes by conventional methods requires a sexual cross between two lines and repeated back-crossing. This is restricted to plants that can hybridize only. Recombinant DNA techniques promise to overcome these limitations by enabling plant geneticists to identify and clone specific genes for desirable traits.

Genetic engineering aims of isolating DNA fragments and recombining them. The term recombinant DNA is used to refer to composite DNA molecules, that result from the physical combination of DNA segments derived from different sources (see synthesis of r-DNA). Gene cloning or genetic engineering is essentially the insertion of a piece of DNA into a cell, in such a way that inserted DNA is replicated. It can be performed by either direct gene transfers or vector mediated. Therefore genetic engineering is manipulation of genes in the laboratory to produce useful plants, animals or products. It is completely dependent on r-DNA technology as mentioned earlier. Thus genetic engineering is the application of recombinant DNA methods to confer new traits on organising by introducing new genes into their cells (See Synthesis of r-DNA molecule).

Through genetic engineering Insect, virus, disease, herbicide resistant plants, plants for quality like nutritional status, improved storage, plants for production of chemicals and pharmaceuticals can be produced. Genetic engineering of Bt gene is shown in the development of transgenic plants resistant to insects (Fig. 2)

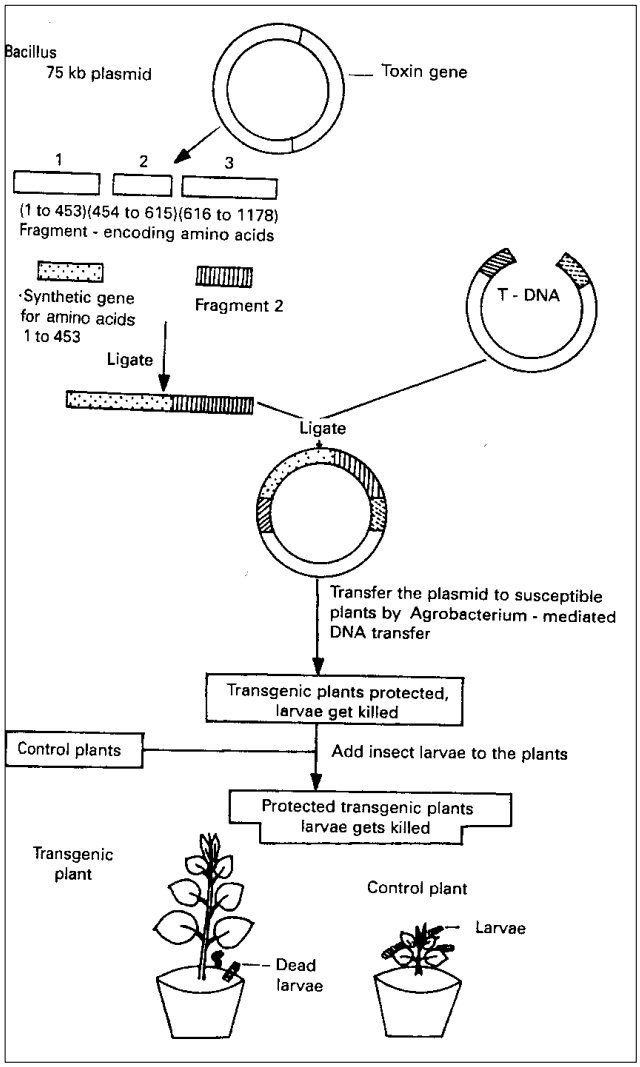


Fig.2. Expression of *Bacillus thuringiensis* toxin gene in transgenic plants to protect them against insect damage.

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