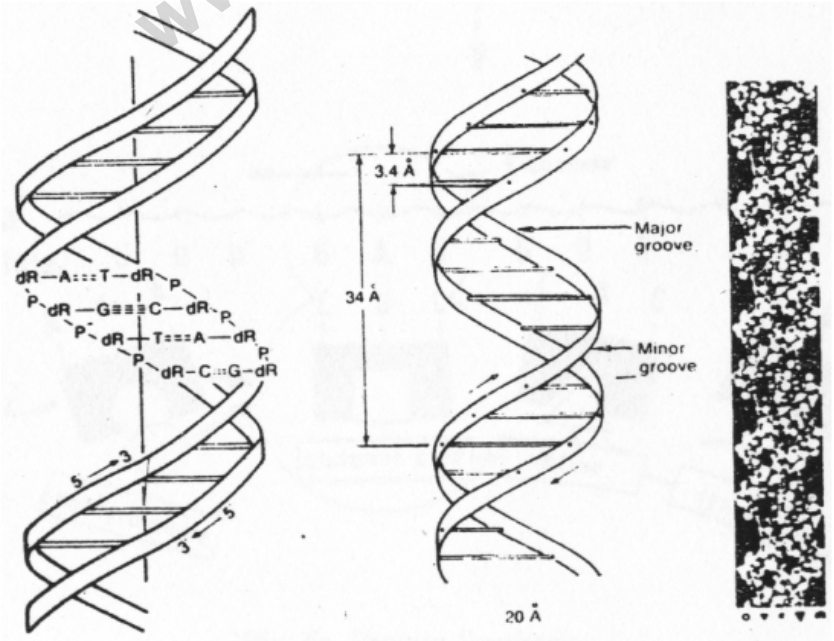
Chapter 6. MOLECULAR BASIS OF INHERITANCE

DNA largest macromolecule made of helically twisted, two, antiparallel polydeoxyribonucleotide chains held together by hydrogen bonds.

- X-ray diffraction pattern of DNA by Rosalind Franklin showed DNA a helix.
- Components of DNA are (i) deoxyribose sugar, (ii) a phosphate, and (iii) nitrogen containing organic bases.
- DNA contains four different bases called adenine (A), guanine (G) cytosine (C), and thymine (T).
- These are grouped into two classes on the basis of their chemical structure: (i) Purines (with a double ring structure) and (ii) Pyrimidines (with a single ring structure)
- 1953.James Watson and Francis Crick proposed three dimensional structure of DNA and won the Nobel prize.
- DNA double helix with sugar phosphate back bone on outside and paired bases inside.
- Planes of the bases perpendicular to helix axis.
- Each turn has ten base pairs.(34 A^0)
- Diameter of helix 20 A^0.
- Two strands of DNA antiparallel.
- DNA found both in nucleus and cytoplasm.
- Extranuclear DNA found in mitochondria and chloroplasts.
- Two chains complementary
- Two chains held together by hydrogen bond.
- Adenine-Thymine pair has two hydrogen bonds.
- Guanine-Cytosine pair has three hydrogen bonds.
- Upon heating at temperature above 80-90 degree two strands uncoil and separate (Denaturation)
- On cooling two strands join together (renaturation /annealing)
- DNA is mostly right handed and B form.
- Bacterial nucleoid consists of a single circular DNA molecule .



PACKAGING OF DNA HELIX

DNA of eukaryotes is wrapped around positively charged histone proteins to form nucleosome.

Nucleosome contains 200 base pairs of DNA helix.

Histone octamer =2(H2a+H2b+H3+H4)

Linker DNA bears H1 protein

Chromatin fibres formed by repeated units of nucleosomes.

Non histone proteins required for packaging.

Regions of chromatin, loosely packed and stains lightly called euchromatin.

Regions of chromatin, densely packed and stains darkly is called heterochromatin.

DNA AS THE GENETIC MATERIAL

Transformation experiment or Griffith effect.

- Griffith performed his experiments on Mice using *Diplococcus pneumoniae*.
- Two strains of bacteria are S-type and R-type cells.
- Experiments

 Living S-strain Injected into mice ⟶Mice killed

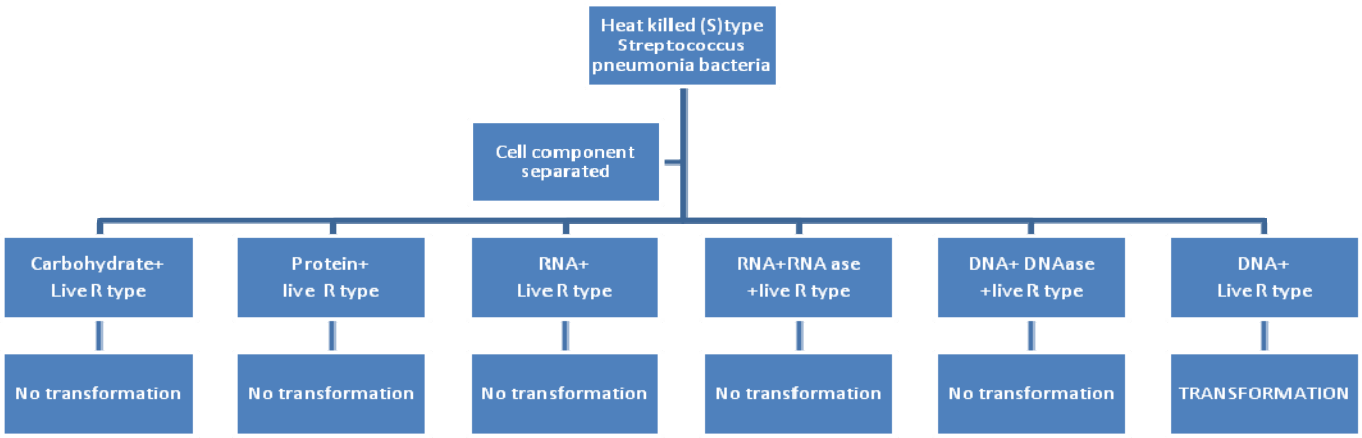
 Living R-strain Injected into mice ⟶ Mice lived

 Heat Killed S-strain Injected into mice ⟶Mice lived

 Living R-strain + Heat Killed S-strain Injected into mice⟶Mice killed

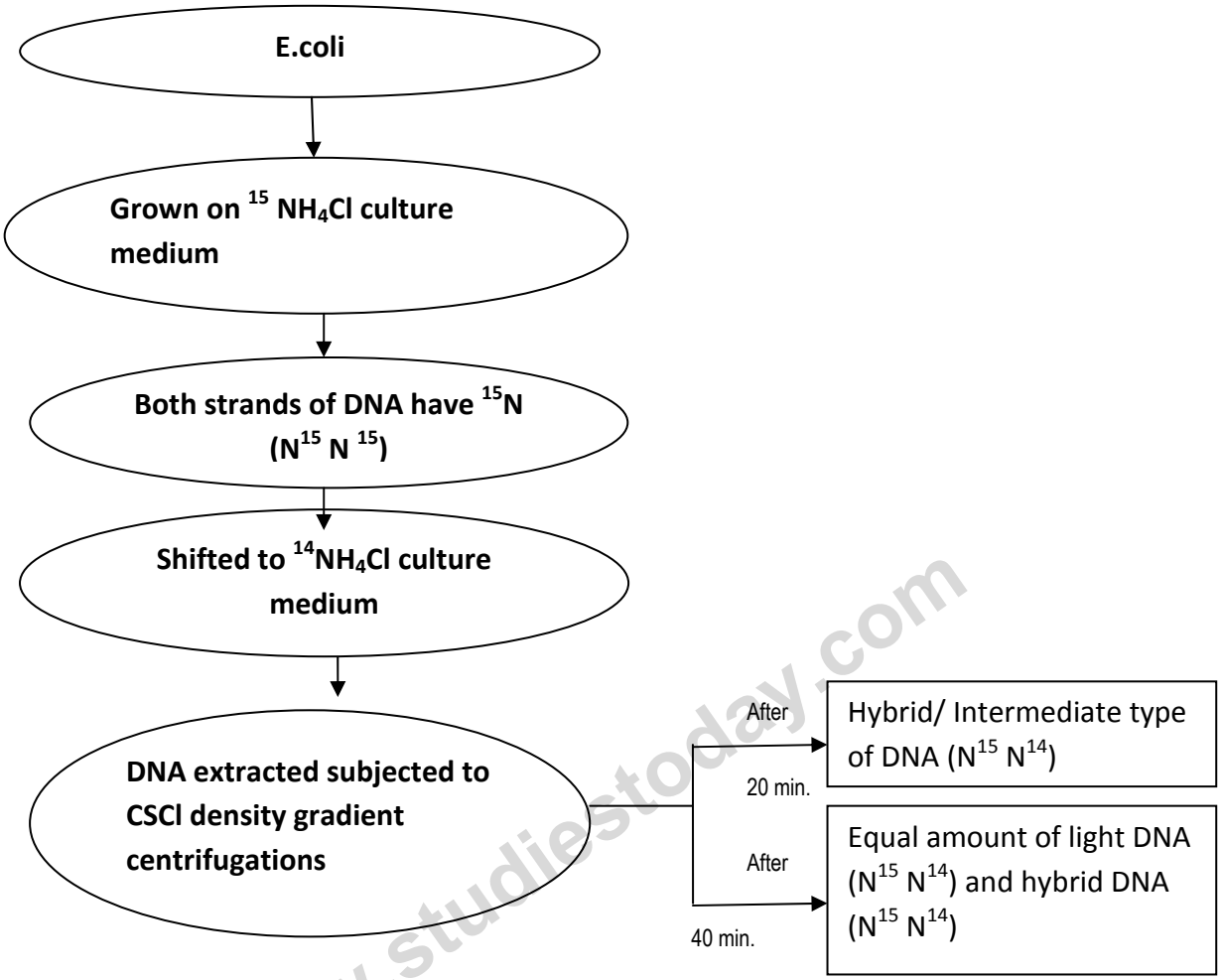
Griffith concluded that R type bacteria is transformed into virulent form.
Transformation - change in the genetic constitution of an organism by picking up genes present in the remains of its relatives.

BIOCHEMICAL CHARACTERISATION OF TRANSFORMING PRINCIPLE
Proved by Oswarld Avery, Colin Macleod, Maclyn Mc Carty



From this we conclude that DNA is the genetic material.

Semi conservative nature of DNA Mathew Messelson and Franklin start.



8. 3 Replication of DNA In Eukaryotes:

Definition: "Process by which DNA produces daughter DNA molecules which are exact copies of the original DNA." In eukaryotes, DNA is double stranded. The two strands are complementary to each other because of their base sequences.

Semi-conservative method of DNA replication Important points:

(i) Most common method of DNA replication.
(ii) Takes place in the nucleus where the DNA is present in the chromosomes.
(iii) Replication takes place in the S-phase (synthesis phase) of the interphase nucleus.
(iv) Deoxyribose nucleotides needed for formation of new DNA strands are present in nucleoplasm. At the time of replication, the two strands of DNA first separate. Each strand then acts as a **template** for the formation of a new strand. A new strand is constructed on each old strand, and two exactly identical double stranded DNA molecules are formed. In each new DNA molecule, **one strand is old** (original) while the **other is newly formed**. Hence, Watson and Crick described this method as **semi-conservative replication**. (A) An overall process of DNA replication showing replication fork and formation of new strands template and lagging template.

The various steps involved in this process are summarized as follows:
i. Mechanism of replication starts at a specific point of the DNA molecule, called **origin**.
ii. At origin, DNA strand breaks because of an **incision** (nick). This is made by an enzyme called **incision enzyme** (endonuclease).
iii. The hydrogen bonds joining the two strands are broken by the enzyme.
iv. The two strands start **unwinding**. This takes place with the help of a **DNA unwinding enzyme Helicases.** Two polynucleotide strands are thus separated.
v. The point where the two strands separate appears like a fork or a **Y-shape**. This is described as a **replicating fork**.
vi. A new strand is constructed on each old strand. This takes place with the help of a small **RNA primer** molecule which is complimentary to the DNA at that point.
vii. Each old DNA strand acts as a **template** (site) for the construction of new strand. The RNA primer attaches itself to the old strand and attracts the enzymes (**DNA polymerase III**) which add new nucleotides through ***base complementation***. The deoxyribose nucleotides are present in the surrounding nucleoplasm. New DNA strand is thus constructed opposite to each old strand.
viii. Formation of new complementary strand always begins at the 3' end of the template strand (original strand) and progresses towards the 5' end (ie in 3' - 5' direction). Since the new strand is ***antiparalle***l to the template strand, it is obvious that the new strand itself is always developed in the, 5'-3' direction. For this reason when the two original strands separate (then with respect to the origin of separation), one acts as 3'-5' template while the other acts as 5'- 3' template.
ix. Of the two, the replication of 3'-5' template begins first. Hence the new strand formed on it is called the **leading strand**. The other template (5'-3') must begin replication at the fork and progress back toward the previously transcribed fragment. The new strand formed on it is called the **lagging strand**.
x. Replication of the lagging strand takes place in small fragments called **Okazaki fragments**. These are then connected together by the enzyme **ligase.**
xi. Replication may take place in only one direction on the DNA helix (unidirectional) or in two directions (bidirectional).
xii. At the end of the process, two double stranded DNA molecules are formed from the original DNA molecule.

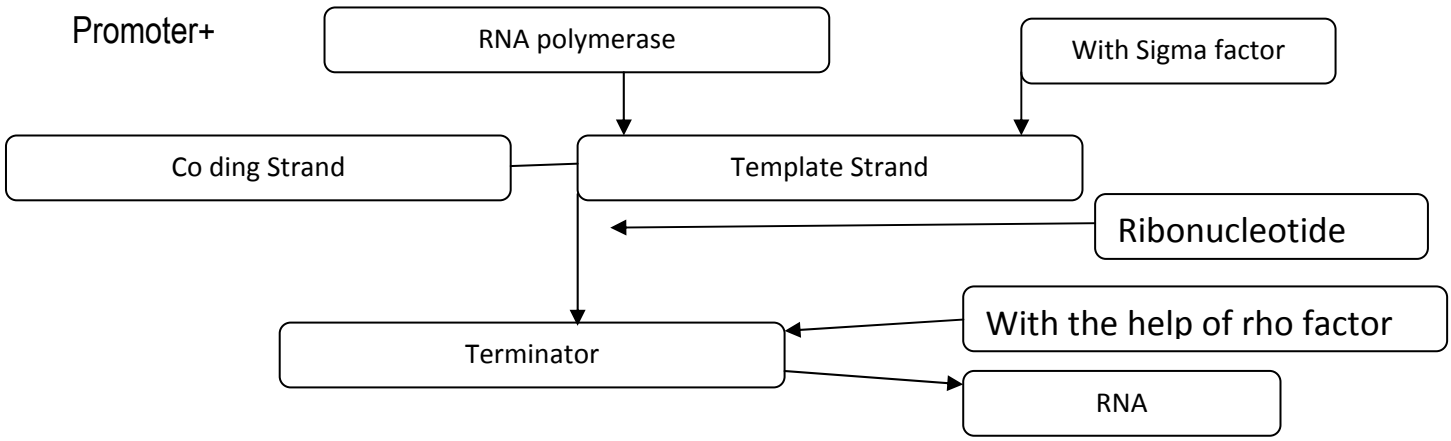
Three major types of RNA:

1. Messenger RNA or mRNA- has the information to make a protein. It is very unstable and comprises ~5% of total RNA polymer. Its length is highly variable, of the range 7503000 nucleotides.

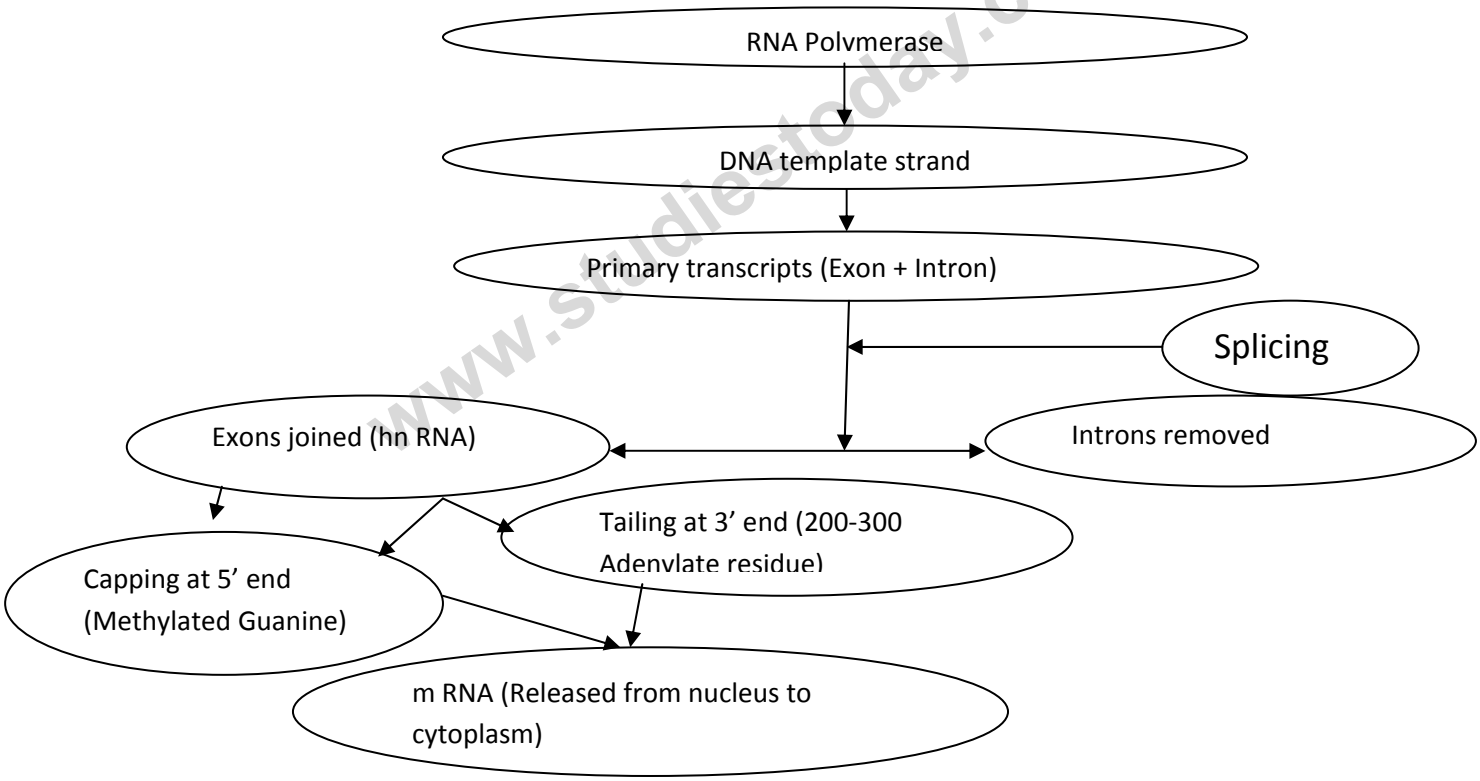
2. Transfer RNA or tRNA- small molecule, about 90 nucleotides long. It is highly folded into an elaborate 3-d structure and comprises about 15% of total RNA.

3. Ribosomal RNA or rRNA- 80% of the total RNA, is associated with subcellular structures called ribosomes in which the polymer length varies from 120-3000 nucleotides and is folded into an elaborate structure which give ribosomes their shape.

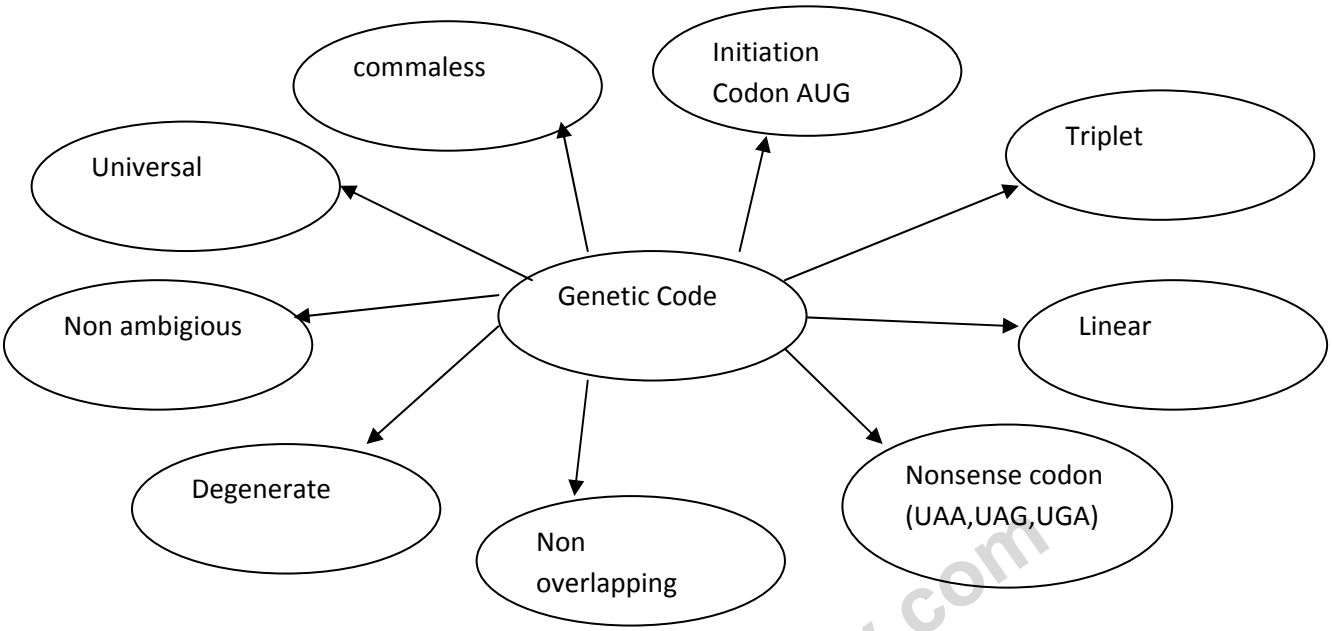
Transcription in Prokaryotes



Transcription in Eukaryotes



GENETIC CODE



Non ambiguous—Particular codon will always code for same amino acid.
Degenerate—Number of codons can code for one amino acid.
Universal—Specific codon codes for same amino acid in all organisms.

Translation:-

❖ Process of joining of amino acids by peptide bond to form a polypeptide.

1. Activation of amino acids
   - AA+ATP+E $\xrightarrow{Mg^{+2}}$ AA-AMP-E+ PPi
   - AA-AMP-E+tRNA → AA-tRNA+AMP+E

2. Initiation
   - Small subunit (40s) of ribosome binds with mRNA.
   - Charged t RNA specific for initiation codon reaches P site
   - Larger subunit (60s) of ribosome now combines with 40s-m RNA—t RNA ^{met} complex in the presence of Mg^{+2}

3. Elongation
   - Second t-RNA charged with amino acid occupies A site of ribosome.
   - Peptide bond formation between methionine and second amino acids with the help of enzyme peptide transferase.
   - Ribosomes moves over m RNA in 5'→3'

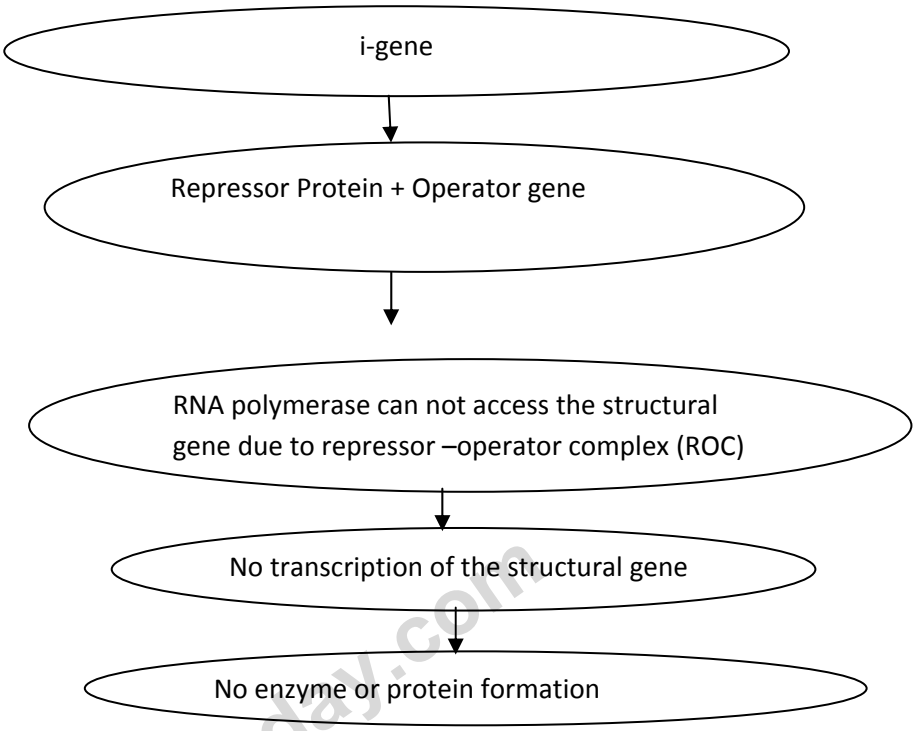
4. Terminator
   - Translation stops when non sense codons (Stop codons) reached.
   - No t RNA for stop codons (UAA,UAG,UGA)
   - Synthesized polypeptide is released with the help of release factor.

* AA—Amino acid
*ATP—Adenosine Triphosphate
*E—Pyrophosphate
AA—AMP-E-Amino acid adenylate enzyme complex
AA—t RNA—Amino acyl-t RNA complex

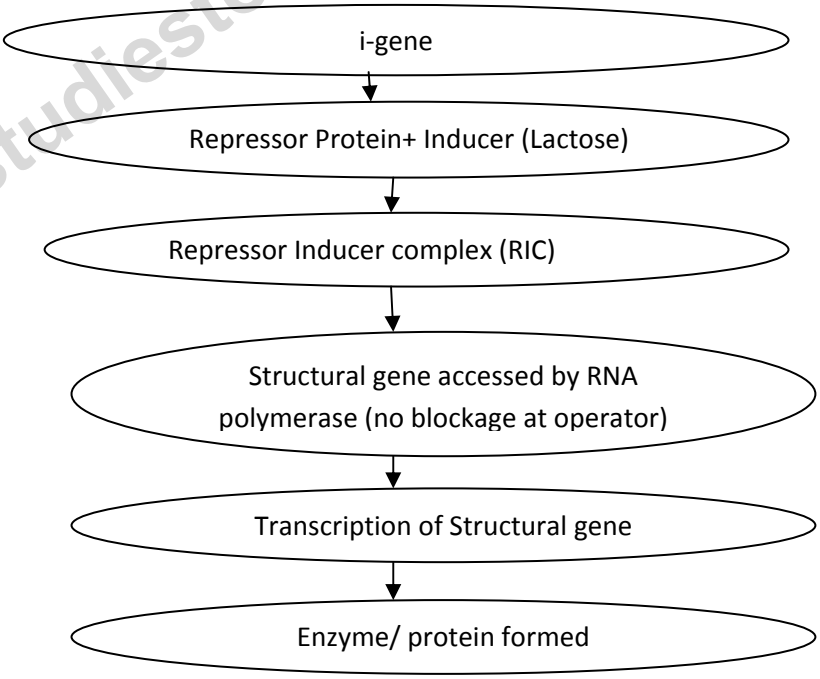
LAC OPERON

*Discovered by Jacob and Manod.
*Experimented on E.coli.
Refer to figure number 6.14 of page 117 of text Book

SWITCH OFF CONDITION



SWITCH ON CONDITION



Methodologies of Human Genome Project

Sequence annotation (Sequence the whole set of genome)

-Isolation of total DNA from the cell

-Fragmentation by restriction endonuclease

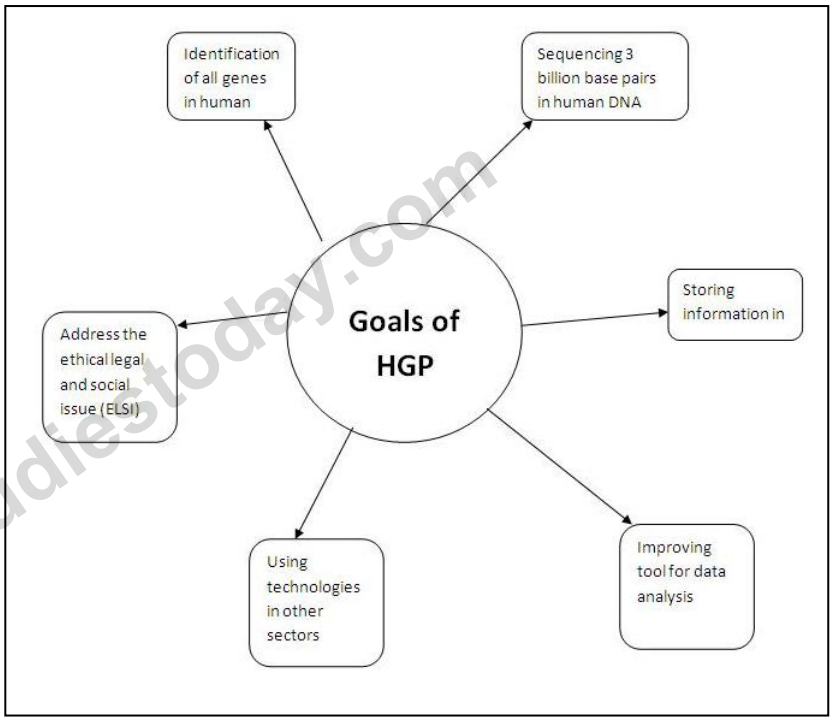
Fragments cloned in suitable host BAC/YAC

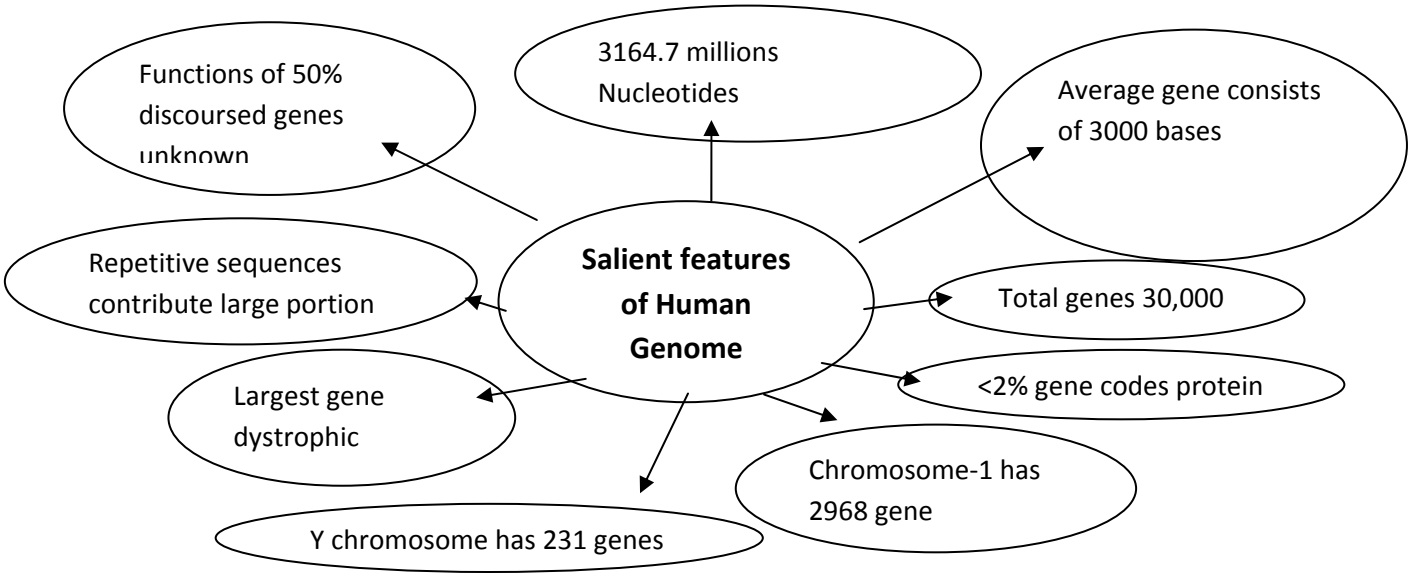
Fragments sequenced using automated DNA sequences.

Sequences arranged on the basis of overlapping regions.

Alignment of the sequences by specialized computer based programmes

Expressed sequence Tags (EST) (Identifying all the genes Expressed as RNA)





Application of Human genome project

-: Identification of defective genes.
-: Opportunity to offer early treatment.
-: Identification of genes that confer susceptibility to certain disease.
-: Prediction of protein that the genes produce.
-: Drug designing to enhance or inhibit the activities of the proteins.

TECHNIQUE FOR DNA FINGER PRINTING

- Technique developed by Dr.Alec Jeffreys.
- Process is also known as DNA typing/DNA profiling.

DNA extraction from the cells in high speed refrigerated centrifuge
↓
Amplification of DNA content by PCR (Polymerase chain reactions)
↓
DNA fragmentation by Restriction endonuclease
↓
Gel electrophoresis
↓
Double stranded DNA split into single stranded
↓
Southern blotting (Transferring separated DNA to nylon or nitrocellulose sheet)
↓
Nylon sheet immerse in a bath having probes/marker* (Hybridisation)
↓
Nylon membrane pressed on X-ray film (Autoradiography)
↓
Dark band develops at probe site

*Probes/ Markers are radioactive synthetic DNA complementary to VNTR

QUESTIONS

ONE MARK QUESTION

1.Name the genetic material in TMV.

2.Write the scientific name of the plant on which Taylor et al performed their experiment.

3.What would be the proportion of light and hybrid density DNA molecules after 80 minutes of a single cell of E. coli growth?

4.When does DNA replicate in the cell cycle ?

5.Name the amino acids having only one codon.

TWO MARK QUESTION

1.What is meant by semiconservative nature of DNA replication?

2. What are the functions of DNA polymerase?

3. What is frame shift mutation ? Name the type of mutation that does not affect protein synthesis .

4.What are the untranslated regions (UTRs) ?

5.Briefly describe polymorphism.

6. What do you mean by phosphodiester bond?

Ans: The bond which is formed between the 3'-OH of one deoxyribonucleotide and 5'-phosphate residue of an adjacent deoxyribonucleotide.

7. What type of transcription is found in retrovirus? Name the enzyme.

Ans: in retrovirus the genetic information flows from RNA to DNA and is called reverse transcription while the enzyme involved is called reverse transcriptase.

8. What would happen if histones were to be mutated and made rich in amino acids aspartic acid and glutamic acid in place of basic amino acids such as lysine and arginine?

Ans: If histone proteins were rich in acidic amino acids instead of basic amino acids then they would not have any role in DNA packaging in eukaryotes as DNA is also negatively charged molecule. The packaging of DNA around the nucleosome would not happen. Consequently, the chromatin fibre would not be formed.

9. Explain what happens in frameshift mutation. Name one disease caused by the disorder.

Ans: Mutation in which addition/insertion or deletion of one or two bases changes the reading frame from the site of mutation is called frameshift mutation. It may result in polypeptide with different sequences of amino acids. Disease caused by frameshift mutation - sickle-cell anemia.

10. Comment on the utility of variability in number of tandem repeats during DNA fingerprinting.

Ans: Tandemness in repeats provides many copies of the sequence for fingerprinting and variability in nitrogen base sequence in them. Being individual-specific, this proves to be useful in the process of DNA fingerprinting.

11. Why is lactose considered an inducer in lac operon?

Ans: Lactose binds to repressor molecule and prevents it from binding with the operator, as a result RNA polymerase binds to promoter-operator region to transcribe the structural genes. Thus the lac operon is switched on.

12. If a double-stranded DNA has 20 % of cytosine, calculate the % of adenine in the DNA.

Ans: cytosine = 20%, therefore guanine = 20%

According to Chargaff's rule,

A+T = 100 – (G+C)

A+T = 100 – 40. Since both adenine and thymine are in equal amount.

Therefore, Thymine= Adenine = 60%/2 = 30%

13. What is cistron?

Ans: Region of the DNA template (gene) coding for a single protein is called cistron.

THREE MARK QUESTIONS

1.Describe the discontinuous synthesis of DNA.

2. How is Lac operon "switched on" in an *E.coli* cell ?

3.Name the three RNA Polymerases found in eukaryotes and mention their functions.

4.Explain the two major approaches involved in the sequencing of genomes.

FIVE MARKS QUESTIONS

1.Describe the salient features of the double helical model of DNA.

2. Bring out the salient features of genetic code .

3.Describe in detail the steps in the technique of DNA finger printing.

4.Describe the process of replication of DNA.

5. What is satellite DNA ? Name their types. Mention their basis for classification of satellite DNA.

6. What are the differences between DNA and RNA?

Ans:

DNA	RNA
1. Polymer of deoxyribonucleotides consisting of two antiparallel strands. 2. Purine nucleotides are- adenine and guanine. Pyrimidine nucleotides are cytosine and thymine. 3. Main function is to carry all the hereditary characteristics. 4. Mainly present in nuclear material of chromatin fibre, mitochondria and chloroplast.	1. Polymer of ribonucleotides consisting of only a single strand. 2. Purine nucleotides are- adenine and guanine. Pyrimidine nucleotides are cytosine and uracil. 3. Main function is to perform protein synthesis. 4. Mainly present in cytoplasm, nucleolus and chromosome.

7. What are B-DNA, A-DNA and Z-DNA?

Ans:

B-DNA	A-DNA	Z-DNA
1. Most predominant form of DNA, the conformation described by Watson and Crick, present under physiological conditions in the body, right handed double helix. 2. Base pair per turn 10. 3. Diameter 2 nm.	1. In lower concentration of salts or in a partially dehydrated state, this form is present, found in some Gram positive bacteria, right handed double helix. 2. Base pair per turn 11. 3. Diameter 2.6 nm.	1. It has been discovered in synthetically made oligodeoxynucleotides, left handed double helix. 2. Base pair per turn 12. 3. Diameter 1.8 nm.

8. What do you mean by grooves of DNA?

Ans: DNA backbone is somewhat tilted from its vertical axis, it has two uneven grooves or furrowings i.e., one major groove (about 12 A˚) and one minor groove (about 6 A˚). They are the protein binding sites of DNA.

9. Recall the experiment done by Frederick Griffith. If the RNA, instead of DNA was the genetic material, would the heat killed strain of strain of streptococcus have transformed the r-strain into virulent strain? Explain your answer.

Ans: RNA is more labile and prone to degradation (owing to the presence of 2'-OH group in its ribose). Hence heat-killed S-strain may not have retained its ability to transform the R-strain.

10. What do you mean by selfish DNA?

Ans: DNA whose role appears to be to mediate its own replicationand survival within the genome, e.g. some satellite DNA, and transposable elements.

11. What are the differences between euchromatin and heterochromatin?

Ans:

euchromatin	heterochromatin
1. During interphase certain areas in chromatin are loosely coiled and stain less intensely. 2. These contain the genes or the coding DNA.	1. During interphase certain areas in chromatin remain tightly coiled or condensed and hence stain darkly. 2. These contain non-coding DNA like the repetitive DNA.