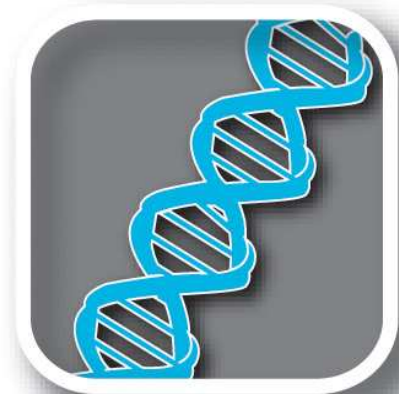


Bacterial Genetics

Dr. BIMAL CHAUHAN

Assistant Professor



Understanding Genetics

We resemble and differ because of Genetic configurations

Parents - Son - Daughter, how they resemble each other.

They breed true from Generation to Generation
But vary in small proportions in progeny.

Bacteria too obey the laws of Genetics

Beginning of Bacterial Genetics

- The principles of Genetics were applied to bacteria and viruses
- Advances in Genetic process also of lead to fundamental advances in Biology and Biochemistry.

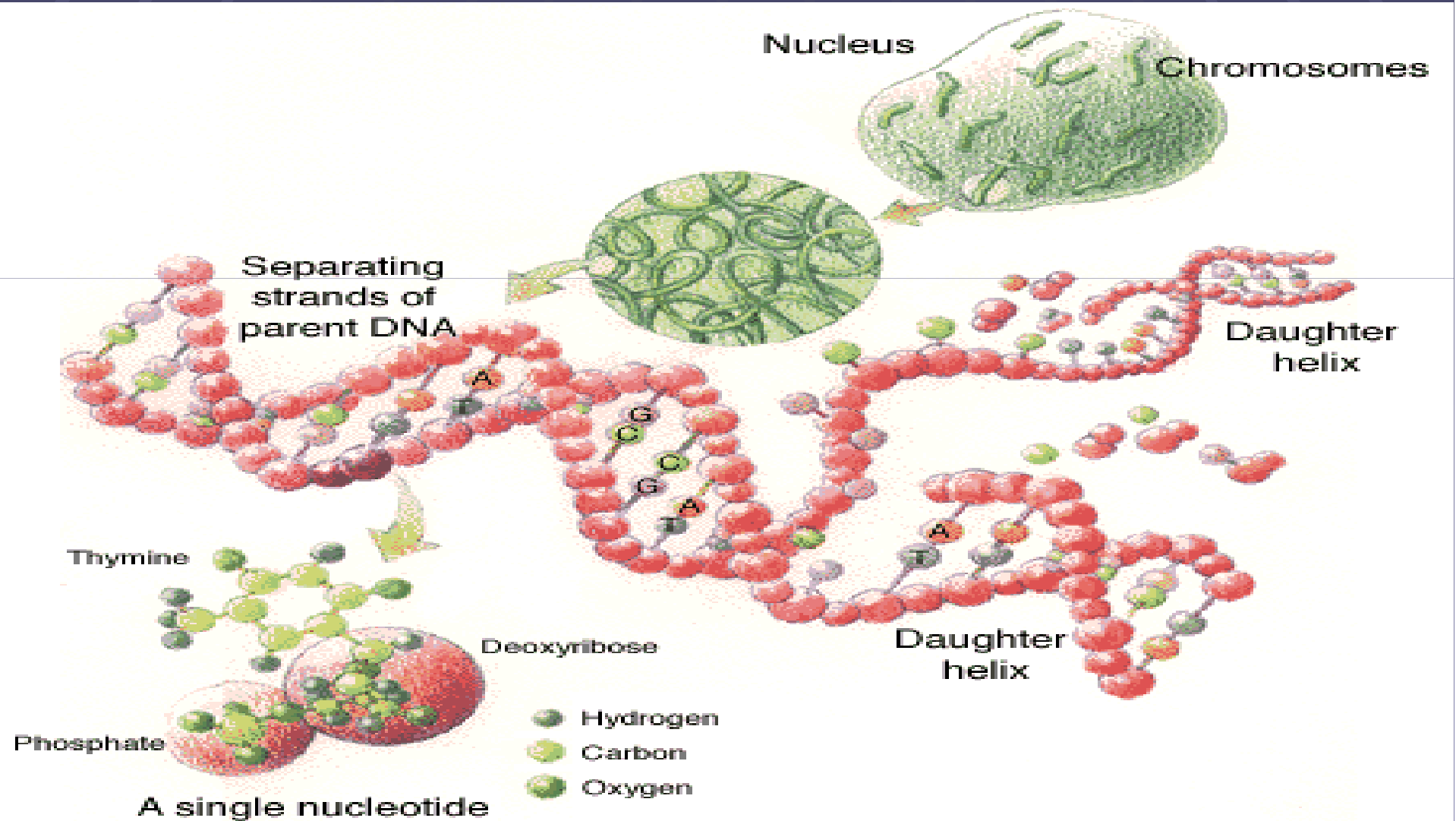
A Birth of New Branch of Science

Molecular Biology

DNA

A Complex Structure

Makes Life



Structure and Function of Genetic Material

➤ DNA & RNA

➤ DNA=deoxyribonucleic acid

➤ RNA=ribonucleic acid

➤ Basic building blocks:

➤ Nucleotides

➤ Phosphate group

➤ Pentose sugar

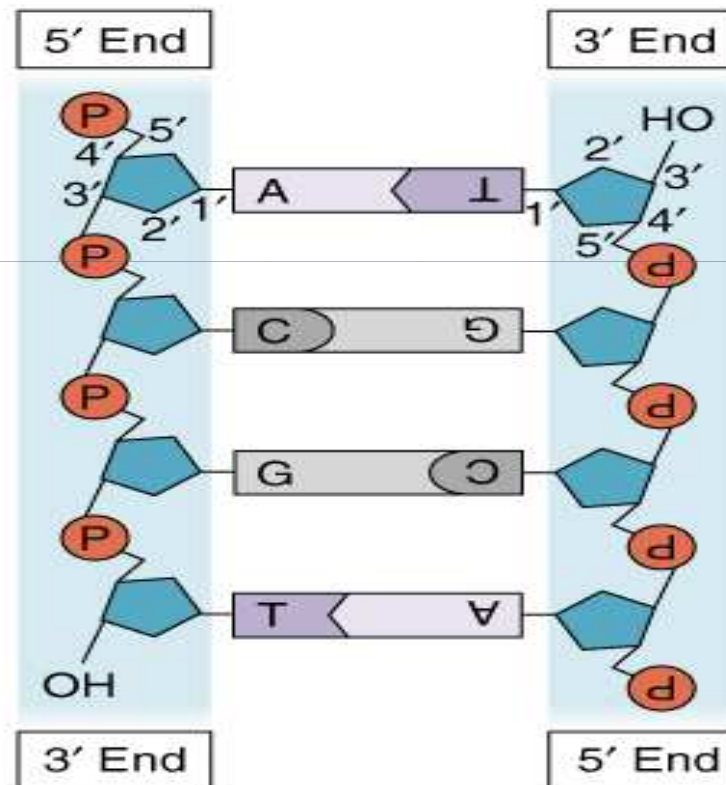
➤ Nitrogenous base

Structure of DNA

- Double stranded (double helix)
- Chains of nucleotides
- 5' to 3' (strands are anti-parallel)
- Complimentary base pairing
 - A-T
 - G-C

DNA Structure

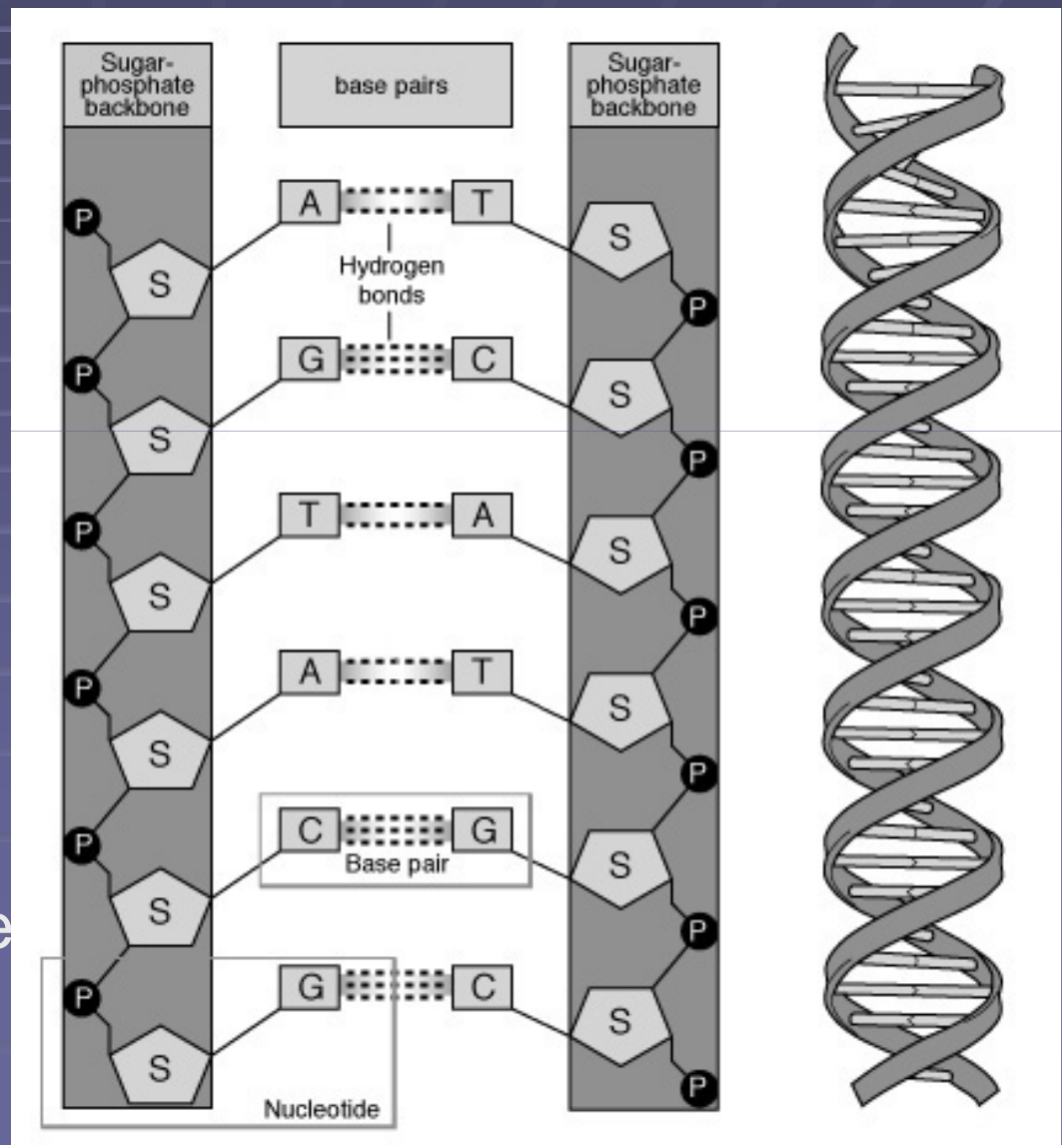
Phosphate-P
Sugar-blue
Bases-ATGC



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Structure of DNA

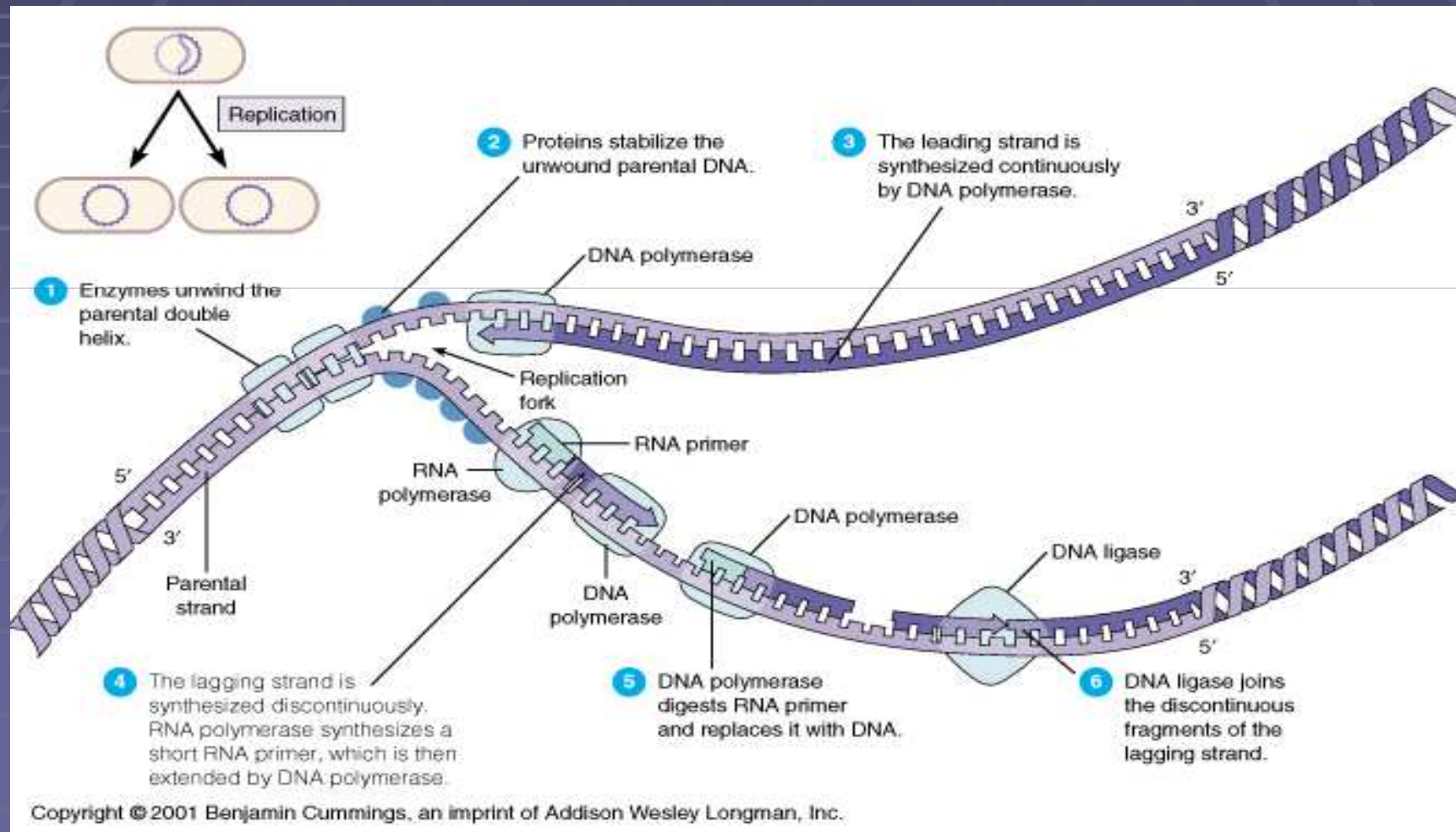
- Attached to each Deoxyribose and phosphate residues arranged alternatively
- Attached to each Deoxyribose are of four nitrogen bases
- Purines - Adenine, Guanine
- Pyrimidines
Thymidine and Cytosine



DNA Replication

- Bacteria have closed, circular DNA
- Genome: genetic material in an organism
- *E. coli*
 - 4 million base pairs
 - 1 mm long (over 1000 times larger than actual bacterial cell)
 - DNA takes up around 10% of cell volume

Replication Fork



Protein Synthesis



Central Dogma
of Molecular Genetics

Transcription

- One strand of DNA used as a template to make a complimentary strand of mRNA
- Promoter/RNA polymerase/termination site/5' to 3'
- Ways in which RNA & DNA differ:
 - RNA is ss
 - RNA sugar is ribose
 - Base pairing-A-U

How RNA differs from DNA

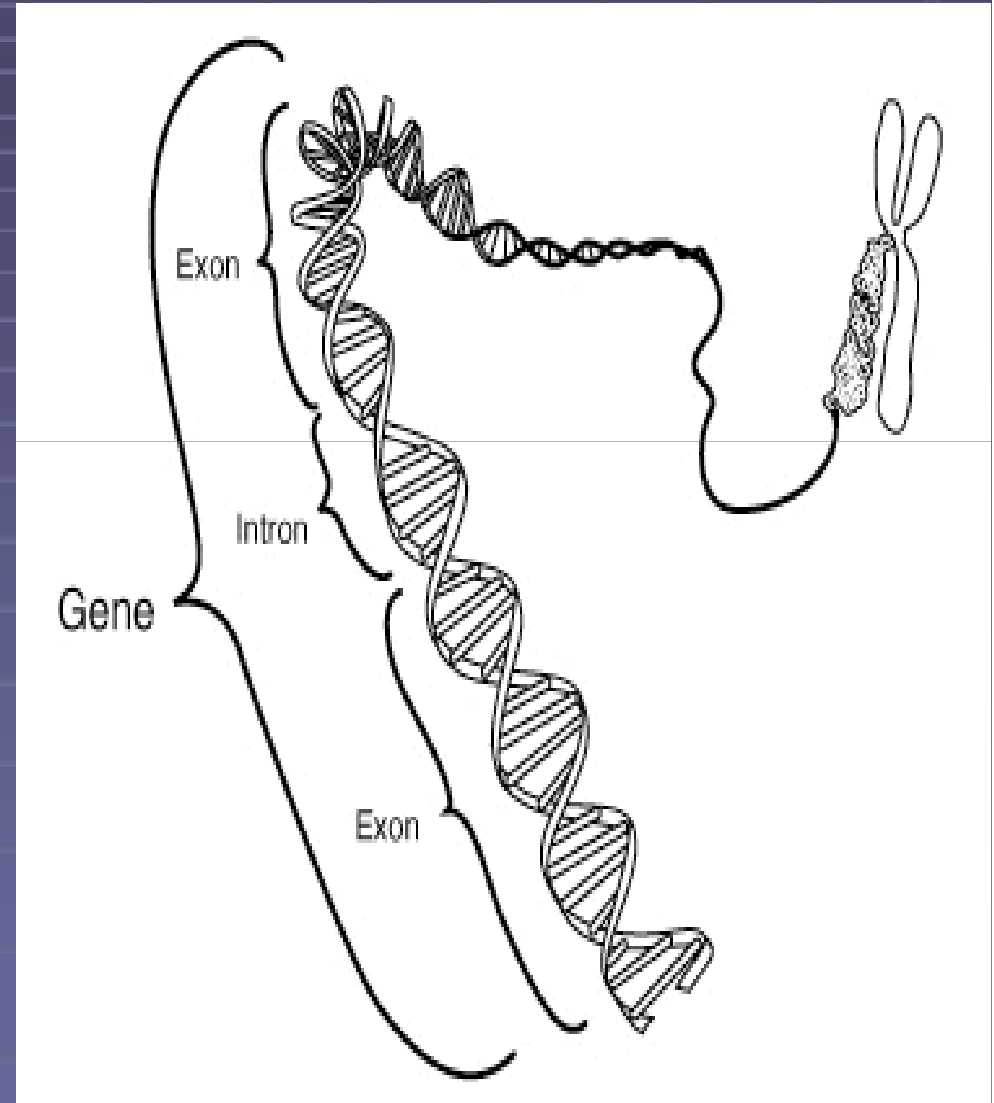
- RNA contains - Sugar Ribose instead of Deoxyribose
- Uracil is present instead of Thymine
- Types of RNA
 - Messenger RNA mRNA
 - Ribosomal RNA rRNA
 - Transfer RNA tRNA

What is a Code in Genetics

- **Code** is a unit consists of sequence of three Bases
- **Code** is triplet **A-T- C**
- A code can make single Amino acid
- More than one code present for making similar sequence of Amino acid
- AGA make Arginine
- AGC, CGU, CGG, also code for similar Amino acid
- Some Codons UAA don't code for any Amino acid called as Nonsense codon

What is a Gene

- Gene is a sequence of DNA carrying codons specifying for particular polypeptide.
- DNA contains many Genes(A combinations of hundreds and thousands of Nucleotides)



Bacterial Chromosome

- Contains a Double stranded molecules of DNA arranged in circular form.
- Length 1,000 microns.
- Bacterial DNA contains about 4,000kilobases
- 1 kb = 1000 base pairs (A-T) (G-C)
- Humans have about 3 million kb pairs.

How bacterial Genome differs from Higher forms of Life

- Several stretches of DNA don't appear to function as codons, occurs between the coding sequences of Gene. called as INTRONS.
- Coded are called as EXONS
- In transcription introns are excised when form RNA before translated by ribosomal proteins.

Extra chromosomal Genetic Elements

Bacteria possess Extra chromosomal genetic elements

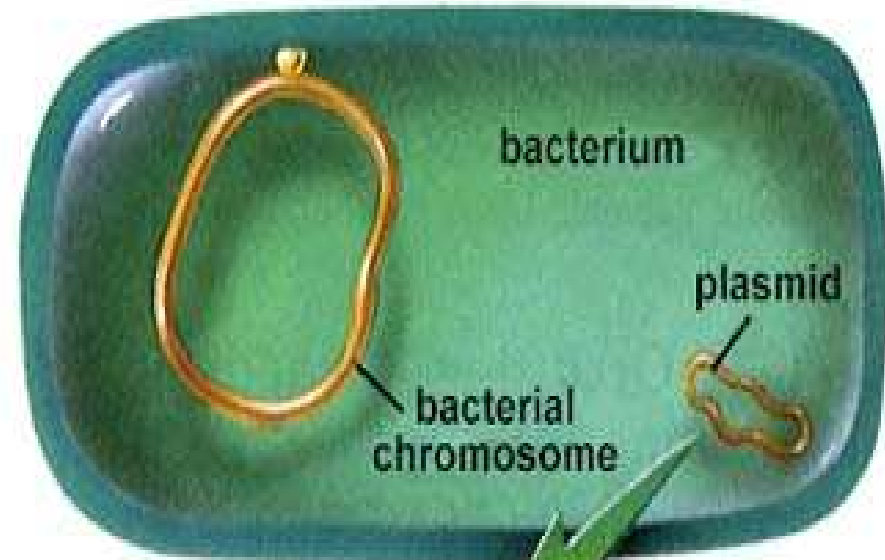
Not Essential for survival of Bacteria

But makes the Bacteria Resistant to antibiotics, and makes them survive

Able to produce toxins

Plasmids

- Plasmids are circular DNA molecules present in the cytoplasm of the Bacteria
- Capable of Autonomous replication
- Can transfer genes from one cell to other
- Act as vectors in Genetic engineering.
- Can also present in Yeasts



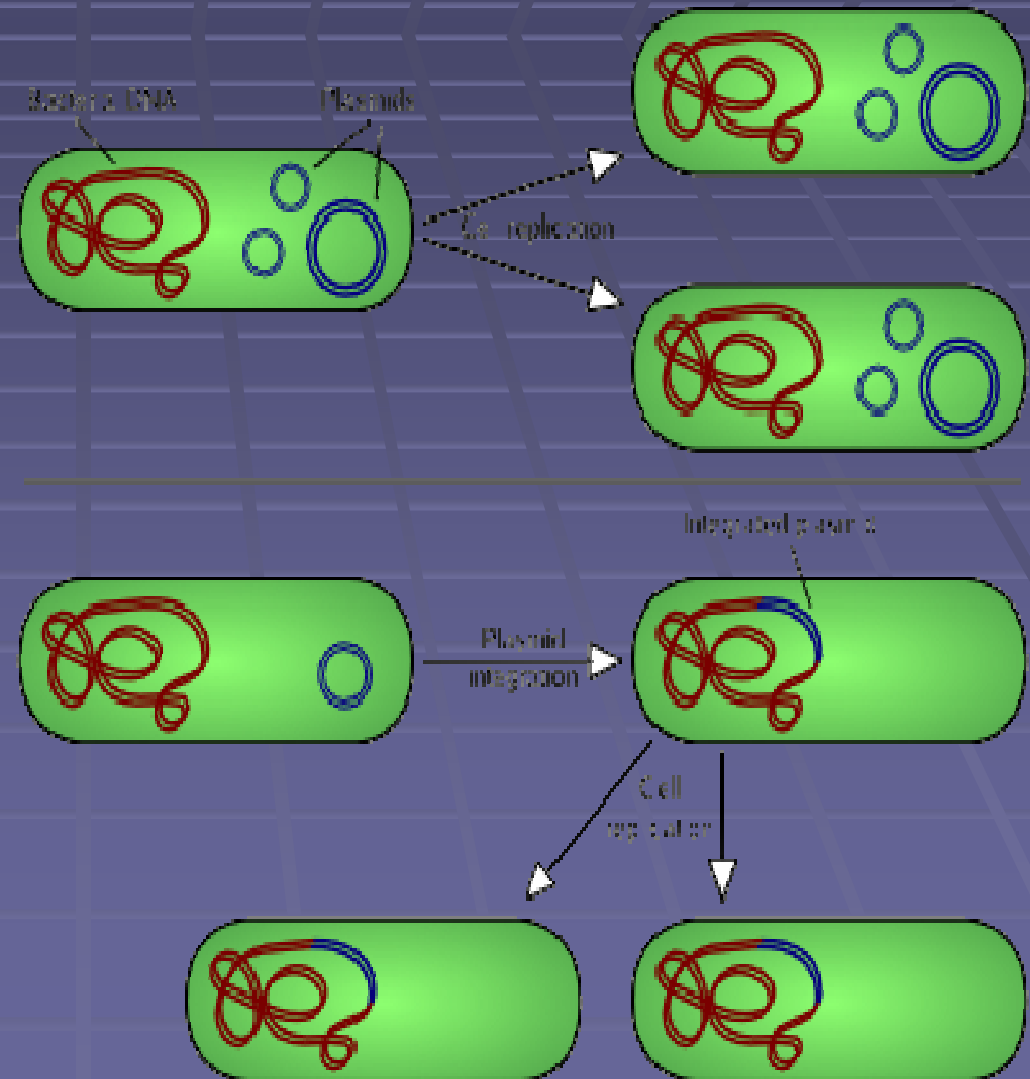
1 μ m

Plasmids

- Plasmid seem to be ubiquitous in bacteria, May encode genetic information for properties
 - 1 Resitance to Antibiotics
 - 2 Bacteriocins production
 - 3 Enterotoxin production
 - 4 Enhanced pathogen city
 - 5 Reduced Sensitivity to mutagens
 - 6 Degrade complex organic molecules

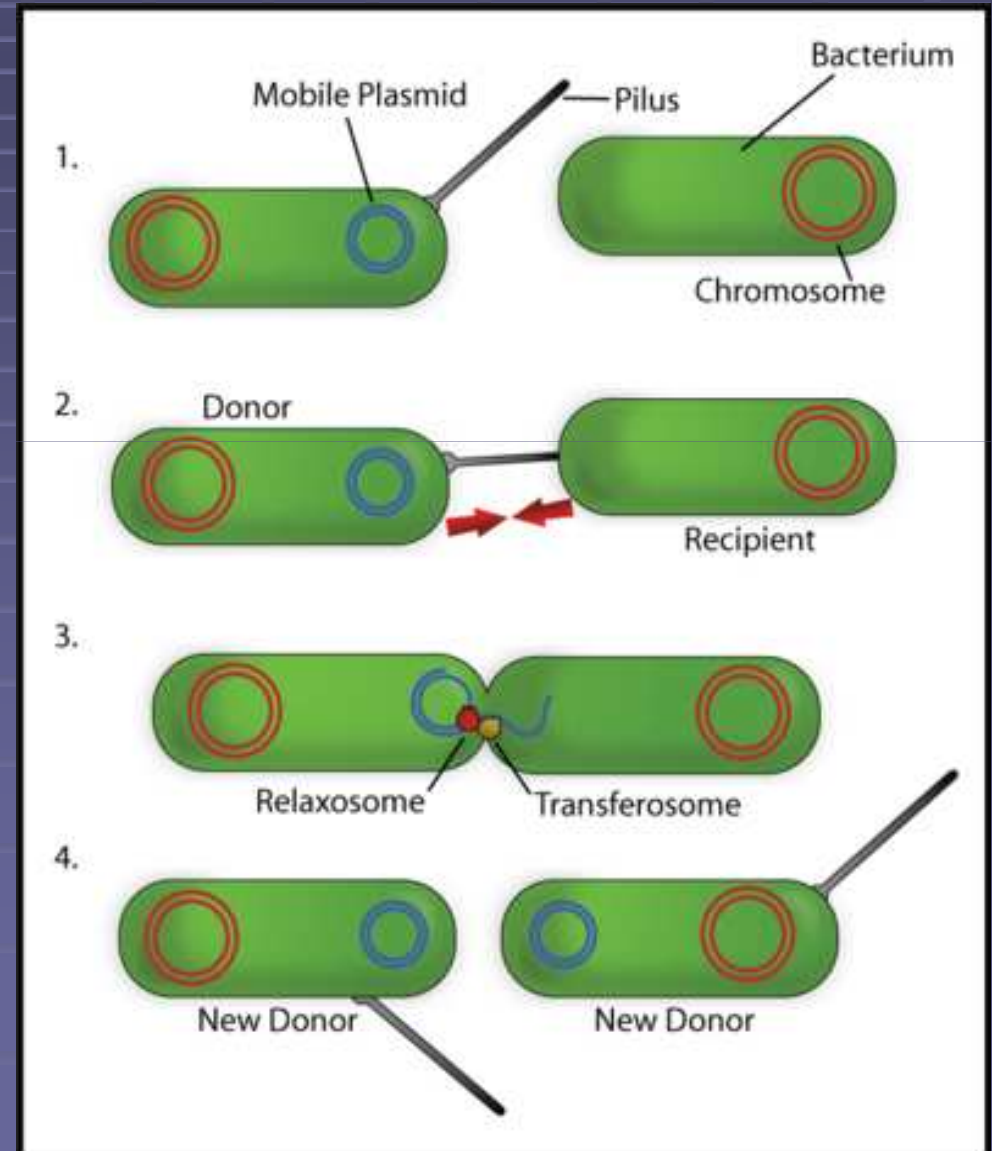
Plasmids

- Can be integrated with Chromosomal DNA
- Episomes - Integrated form of plasmid with DNA



Potentials of Plasmids

- Plasmids can be self transmissible and Non transmissible
- Transfers the Sex and Drug resistance with the help of restriction end nucleases



Genotypic and Phenotypic variation

- Genome – Sum total of Gene that make up the genetic apparatus of cell established as Genotype.
- Hereditary constitution of cell this transmitted to its progeny
- Phenotype – is the physical expression in a environment. Change according to environment.

What is Phenotypic expression

- Exhibit – different phenotypes
- Appearance differs in different situations.
- Eg Typhoid bacilli flagellated normally
- But grown in Phenol agar don't grow flagella So flagella are lost physical variation
- Lactose fermentation in E.coli dependent on Beta Galactosidase
 - When lactose present - test is positive
 - When lactose is absent - test turns negative

Principles of Genotypic variations

- Mutations
- Genotypic by transfer of genes

Transformation

Transduction

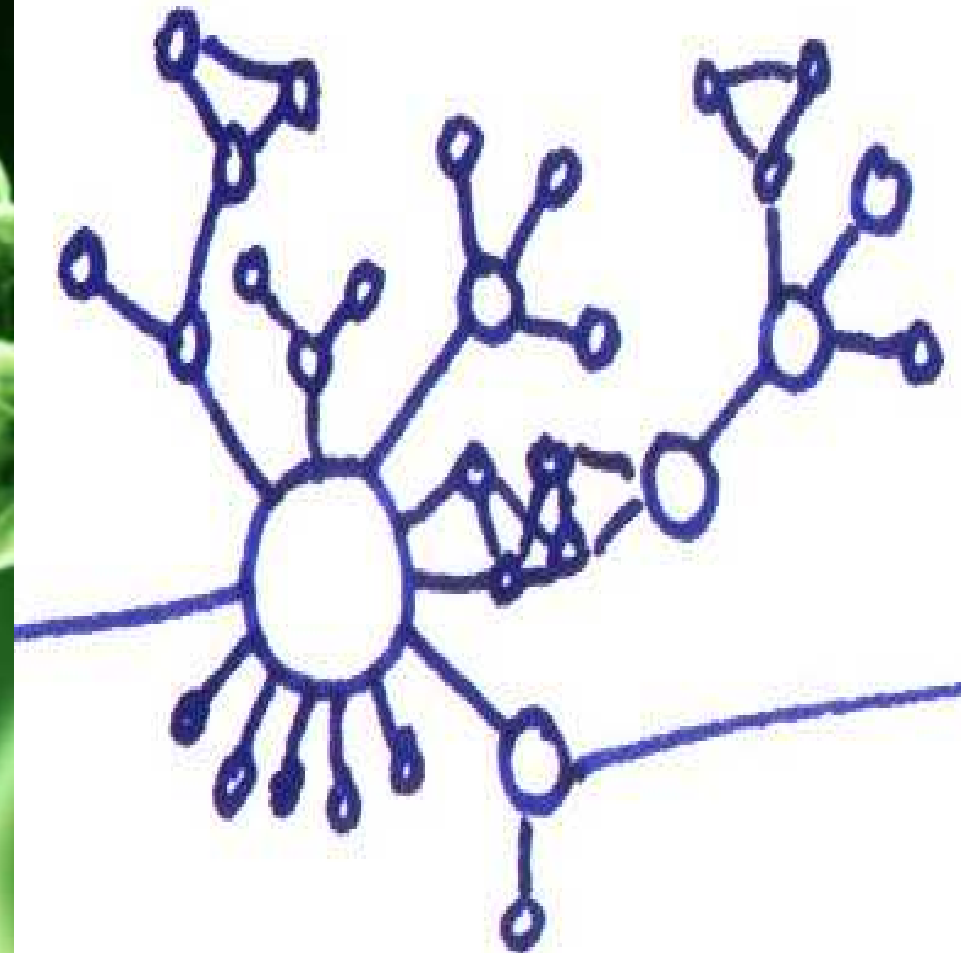
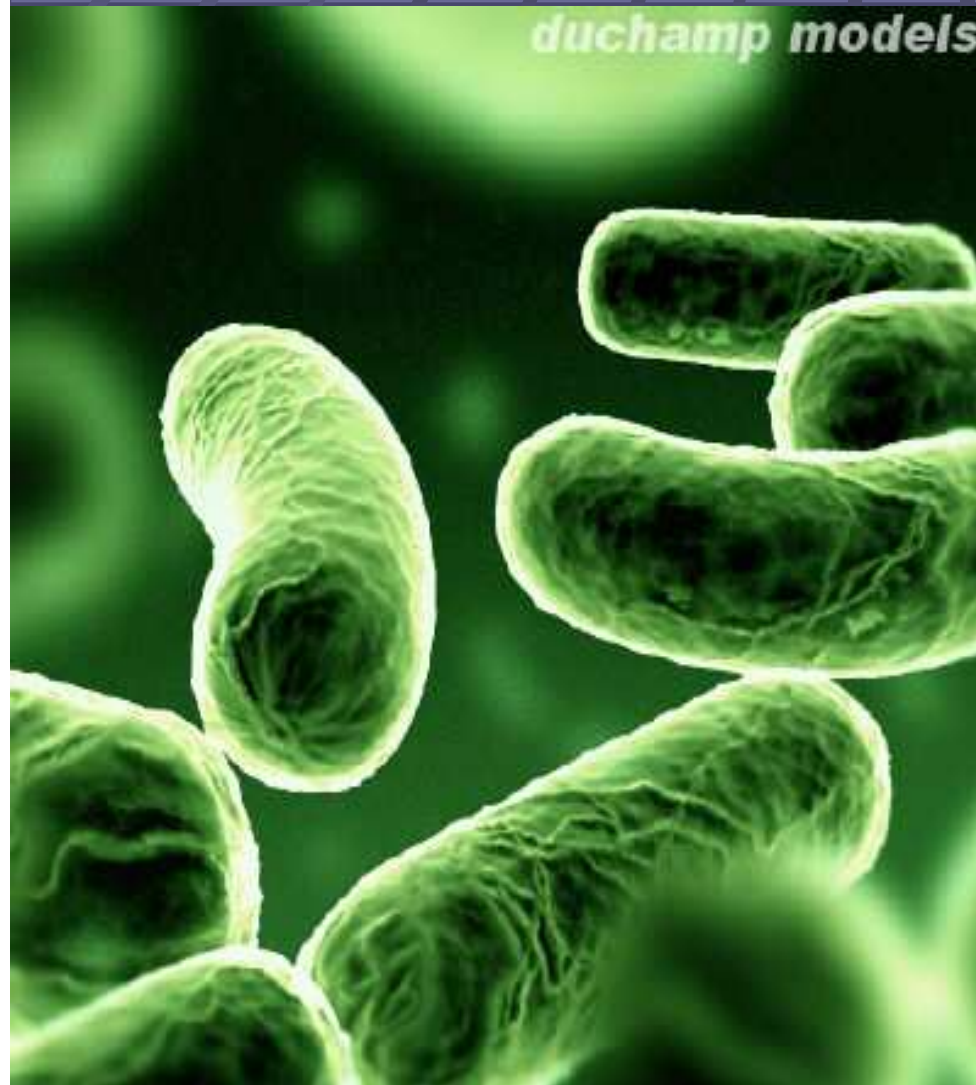
(Lysogenic conversion)

Conjugation

Mutations

- Mutation is a Random, Undirected, Heritable variation
- Caused by alteration in the Nucleotide sequence at some point of DNA which can occur due to
 - Addition
 - Deletion
 - Substitutionof one or more bases

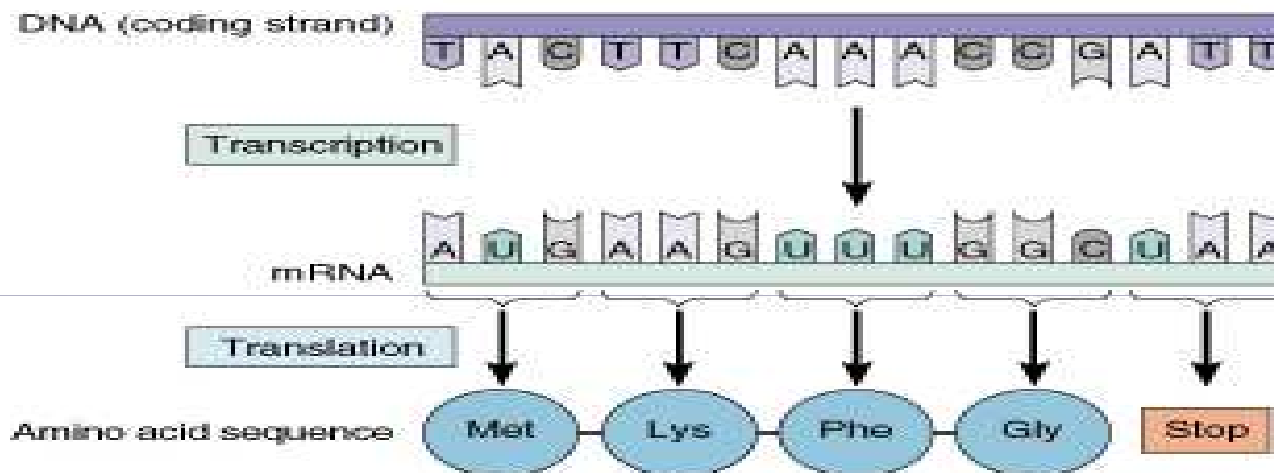
Mutations can occur in any sequence, inevitable, useful for Survival



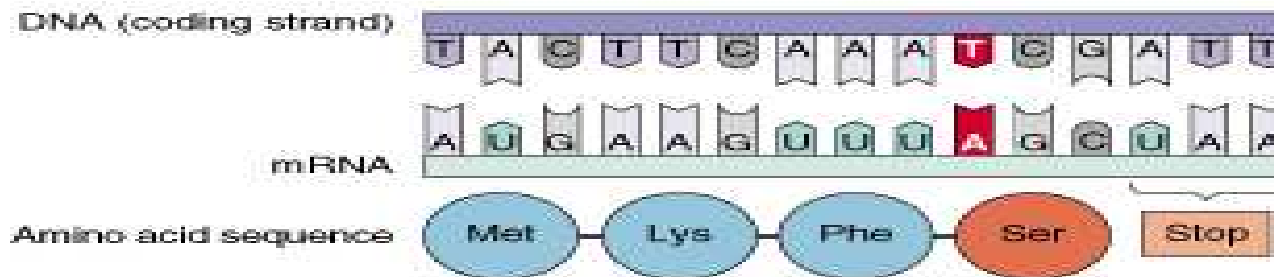
Multiple Mutations

- Causes extensive chromosomal rearrangement
- **Missense mutation** - Triplet code is acted so as to specify an Aminoacid different from that normally located at particular position in the protein
- **Nonsense mutation** - Deletion of nucleotide within a gene may cause premature polypeptide chain termination by nonsense codon
- Tran version is Substitution of purine for pyrimidine or vice versa in the base pairing

Normal DNA/Missense Mutation

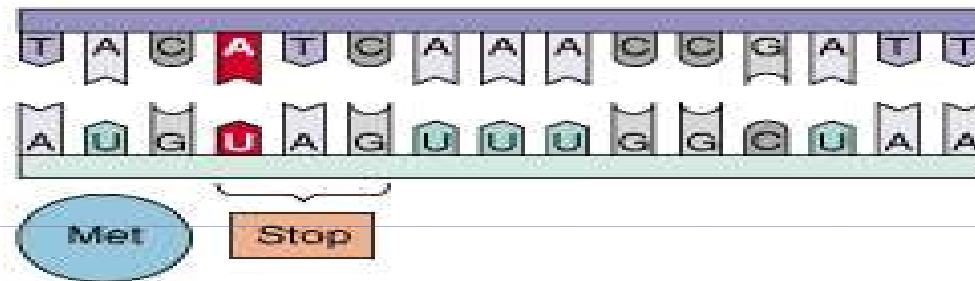


(a) Normal DNA molecule

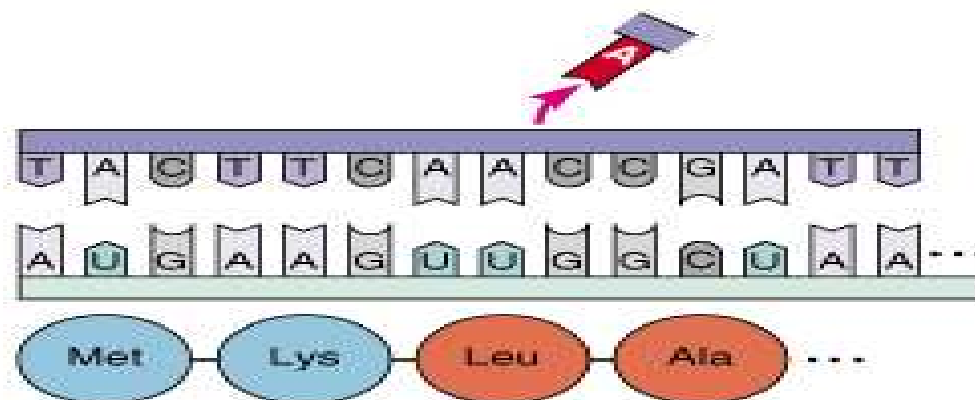


(b) Missense mutation

Nonsense Mutation/Frameshift Mutation



(c) Nonsense mutation



(d) Frameshift mutation

Mutagenic Agents

- U V rays
- Alkylating agents
- Arcidine Dyes

GENE TRANSFER Occurs by Complex Mechanisms

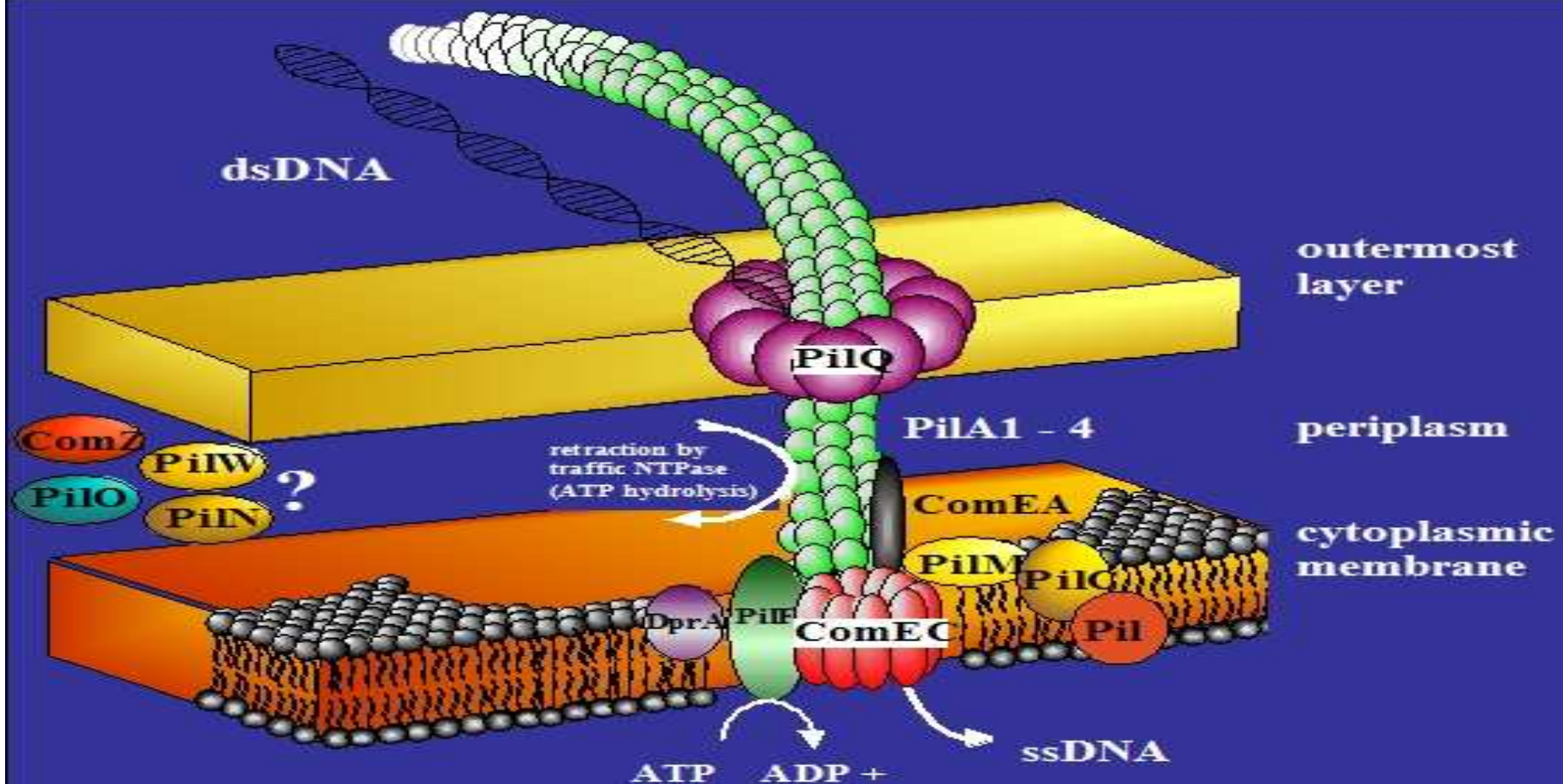
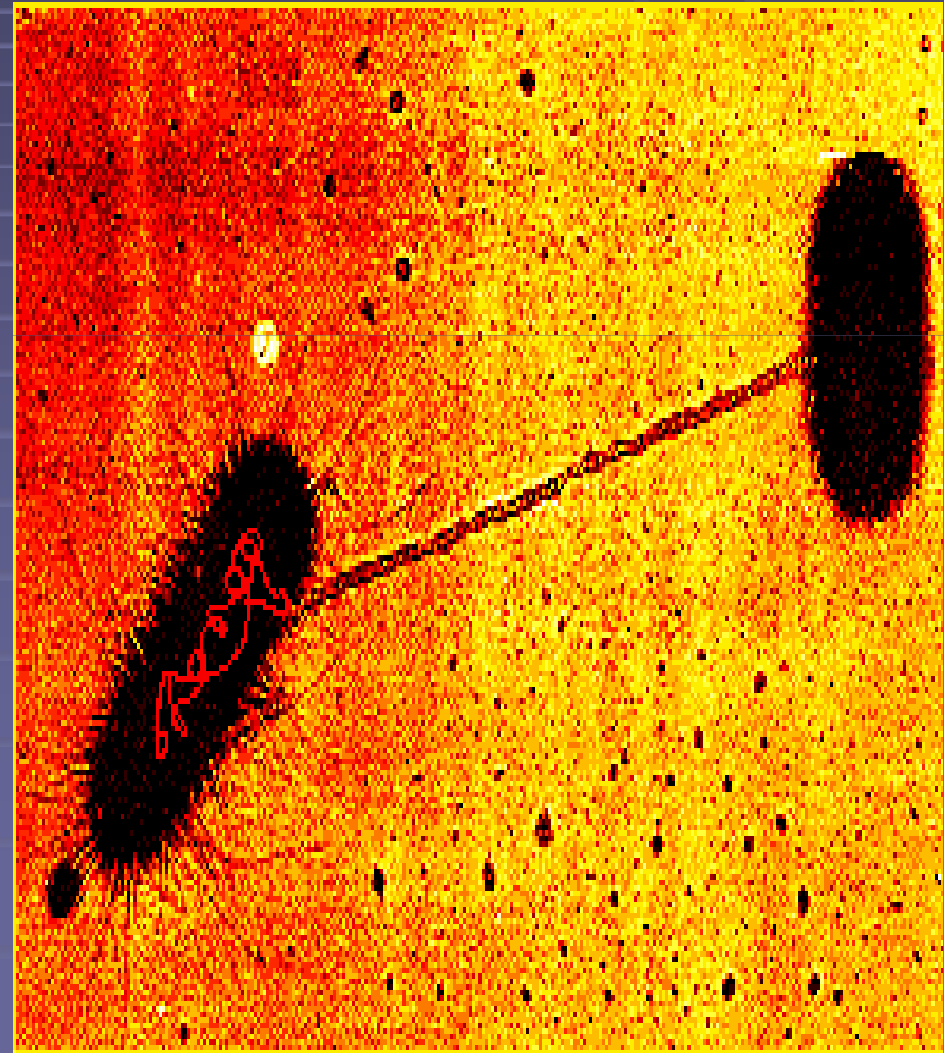


Fig. 2 Model of the transformation process in *Thermus thermophilus*

Transformation of Genetic material (Gene Transfer)

- Different Mechanisms
 - Transformation
 - Transduction
 - Conjugation

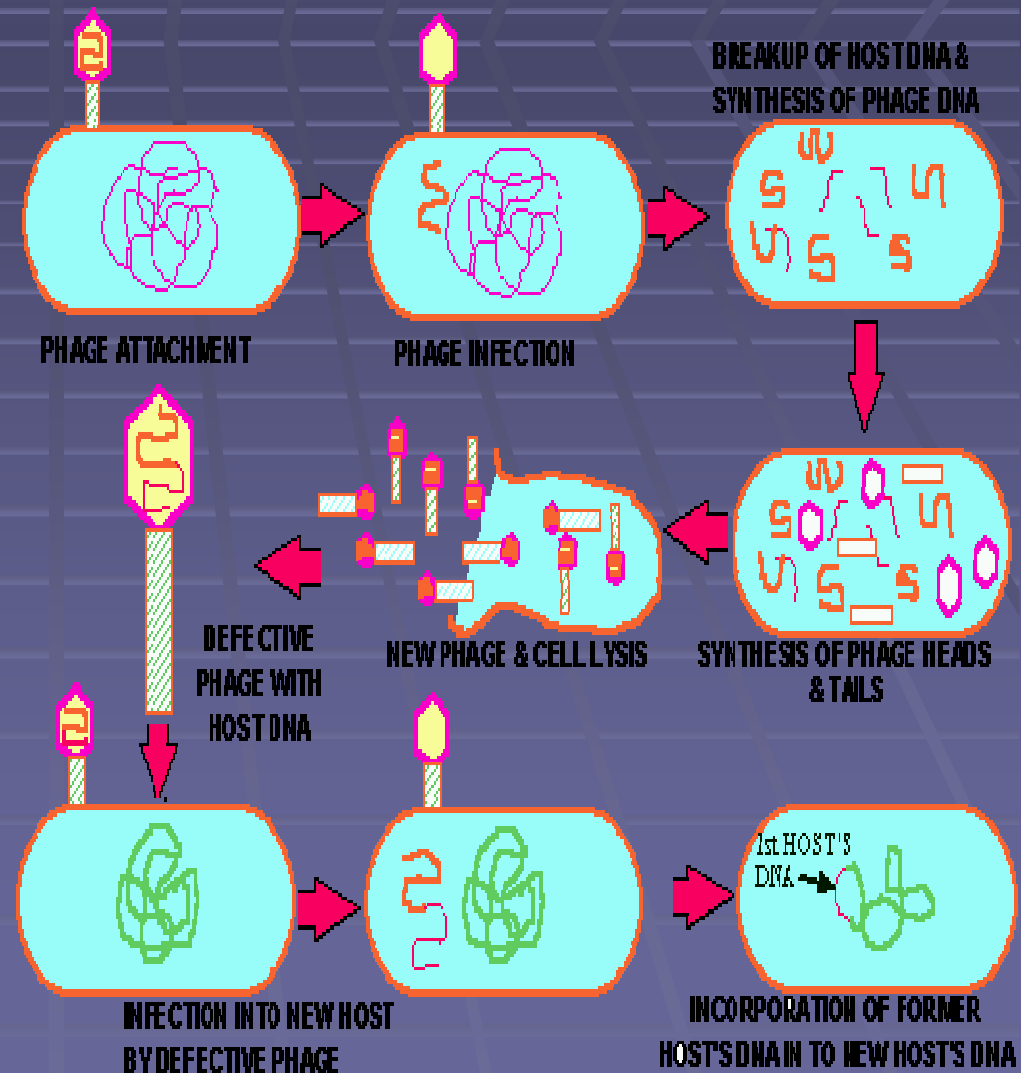


What is Transformation

- Transformation is defined as transfer of Genetic information through the agency of free DNA
 - Griffith experiment
 - Mice injected with Live non capsulated (R) Pneumococci
 - with heat killed capsulated (S) Pneumococci
 - Lead to death of Mice with isolation of Live capsulated Pneumococci
- It means that some factor from Dead pneumococci transferred to live non pathogenic Pneumococci

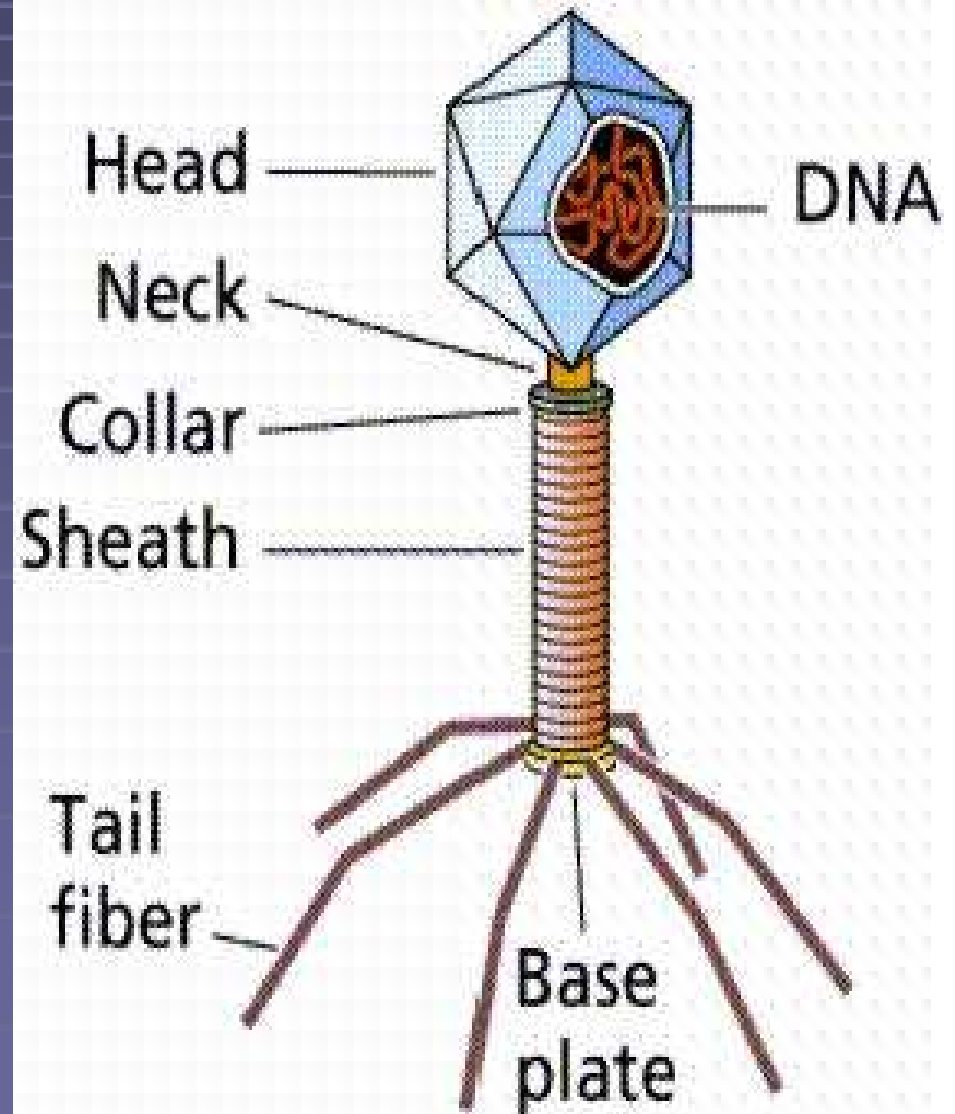
Transduction

- Transduction is defined as transfer of portion of DNA from one bacteria to another by Bacteriophages, is known as Transduction



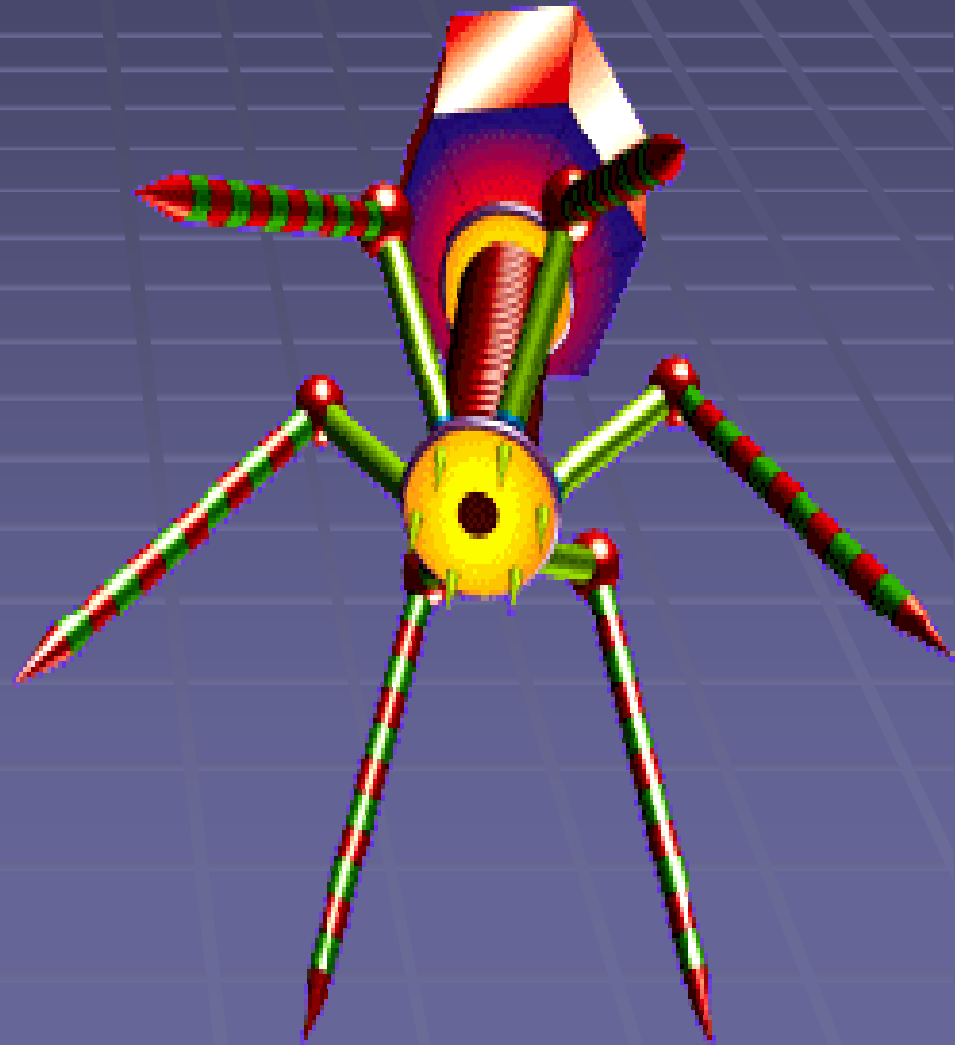
Bacteriophages

- Are viruses that parasitize bacteria and consists of Nucleic acid core and a protein coat
- A phage particle may have at its core besides its own nucleic acid and a segment of the Host DNA

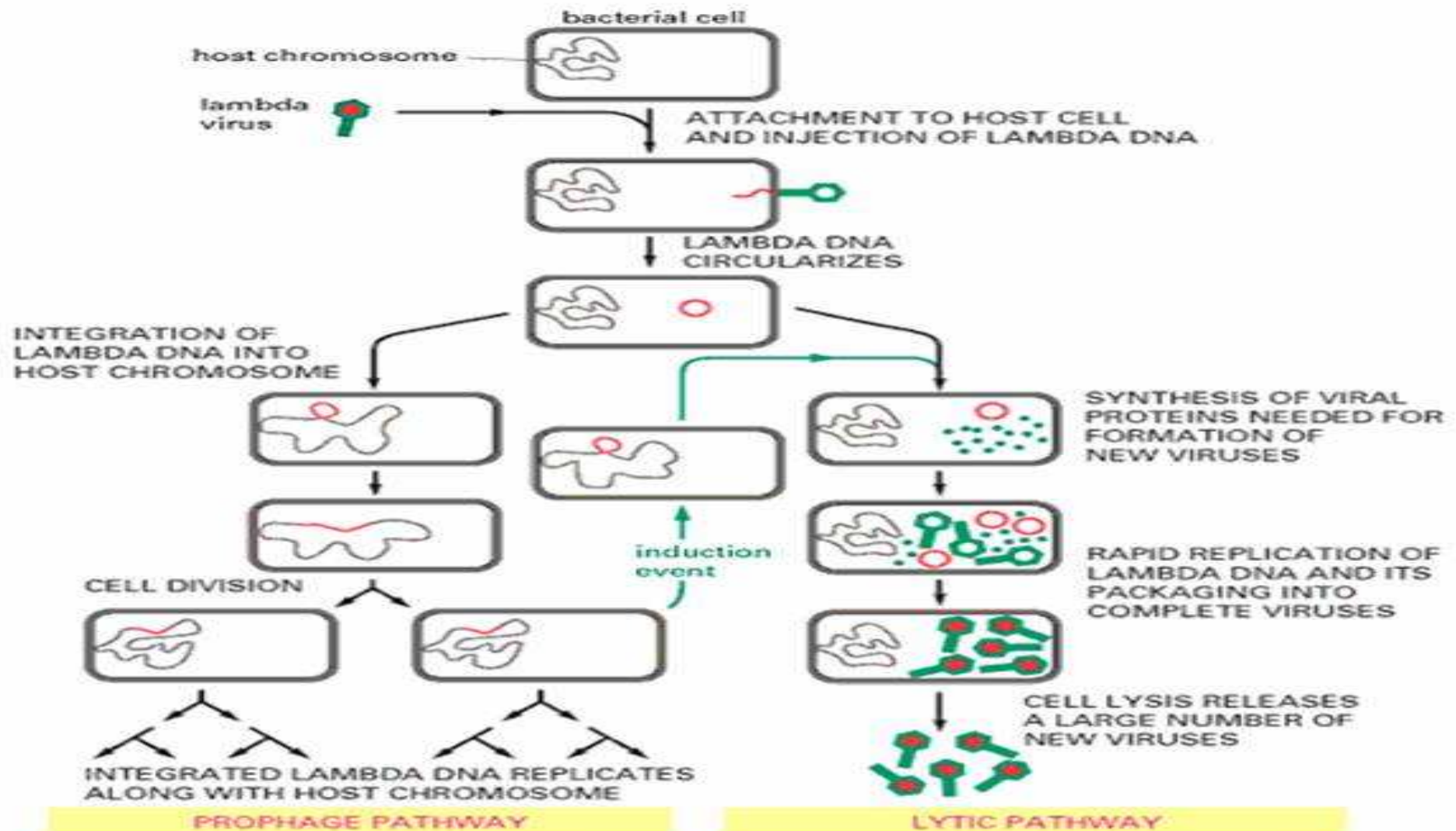


DNA transfer through Bacteriophages

- When the Phage particle infects another bacteria DNA transfer is effected and the recipient cell acquires new characters coded by donor DNA



Types of DNA transfer through Bacteriophages



Transduction

- Generalized involve any segment of DNA
- Restricted when specific Bacteriophages traduces only a particular genetic trait.
- Transduction effects Plasmids ,and Episomes
- Plasmid transfer induces Penicillin resistance in Staphylococcus
- Helps Genetic mapping, also in eukaryotic cell
- Helps Genetic Engineering

Transduction Types

- Two types of Transduction
 - 1 Lytic and 2 Lysogenic
 - 1 Virulent or Lytic cycle after large number of progeny are built up inside the host bacterium ruptures and phages are released

Transduction

Lysogeny

- Bacteria called as lysogenic bacterium, in lysogenic bacteria prophages have an additional segment of bacterial chromosome
- Codes for new characteristics
- Influences susceptibility for Bacteriophages
- Gains new antigen characters

Lysogenicity creates new characters

- Eg - Lysogenic conversion in Diphtheria bacilli which acquires toxigenicity by lysogenization with phage beta
- Elimination of phage for toxigenic strain renders nontoxigenic

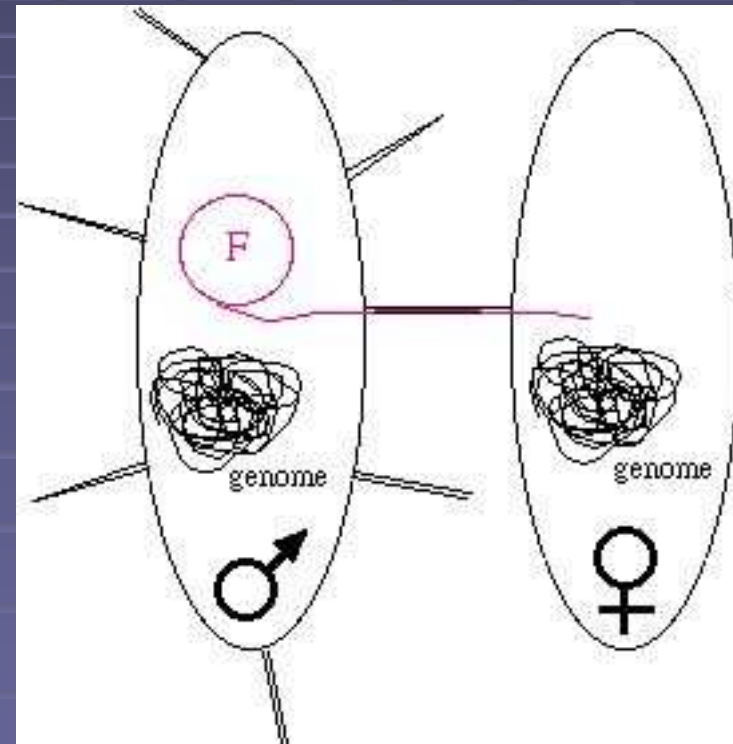
Conjugation

Laderberg - Tatum

- A process by which a Donor cell or male cell makes contact with another cell, the recipient or Female cell.
- **DNA is directly transferable**
- Plasmid Carry genetic information necessary for conjugation to occur.
- Only cell that contain such plasmids can act as donor. the cell lacking a corresponding plasmid act as recipient.
- Requires direct contact between donor and recipient

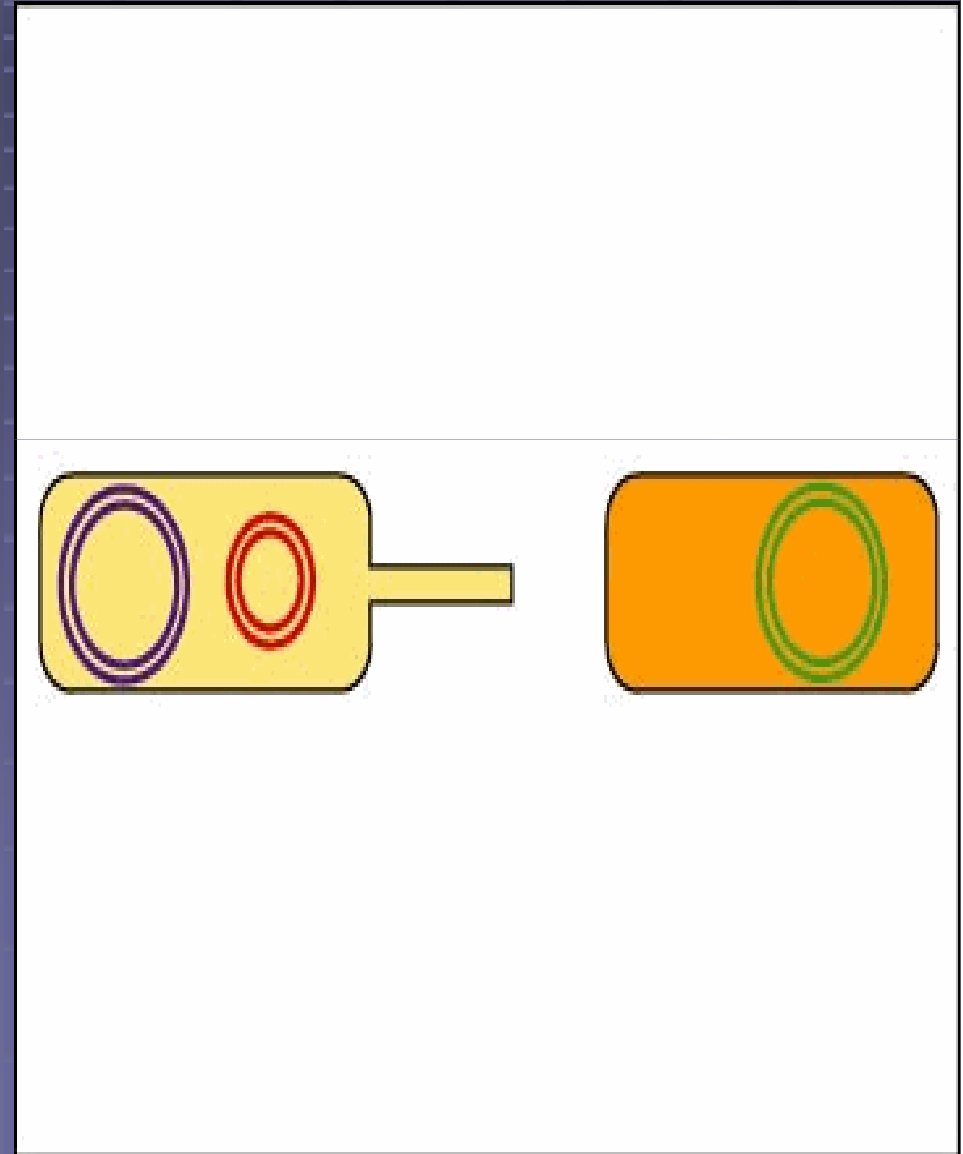
Conjugation - Transferring genes with plasmids

- Plasmids mediating conjugation carry genes coding for properties, of 1-2 microns long protein appendage termed Pilus on the Donor cell



Pilus helps Conjugation

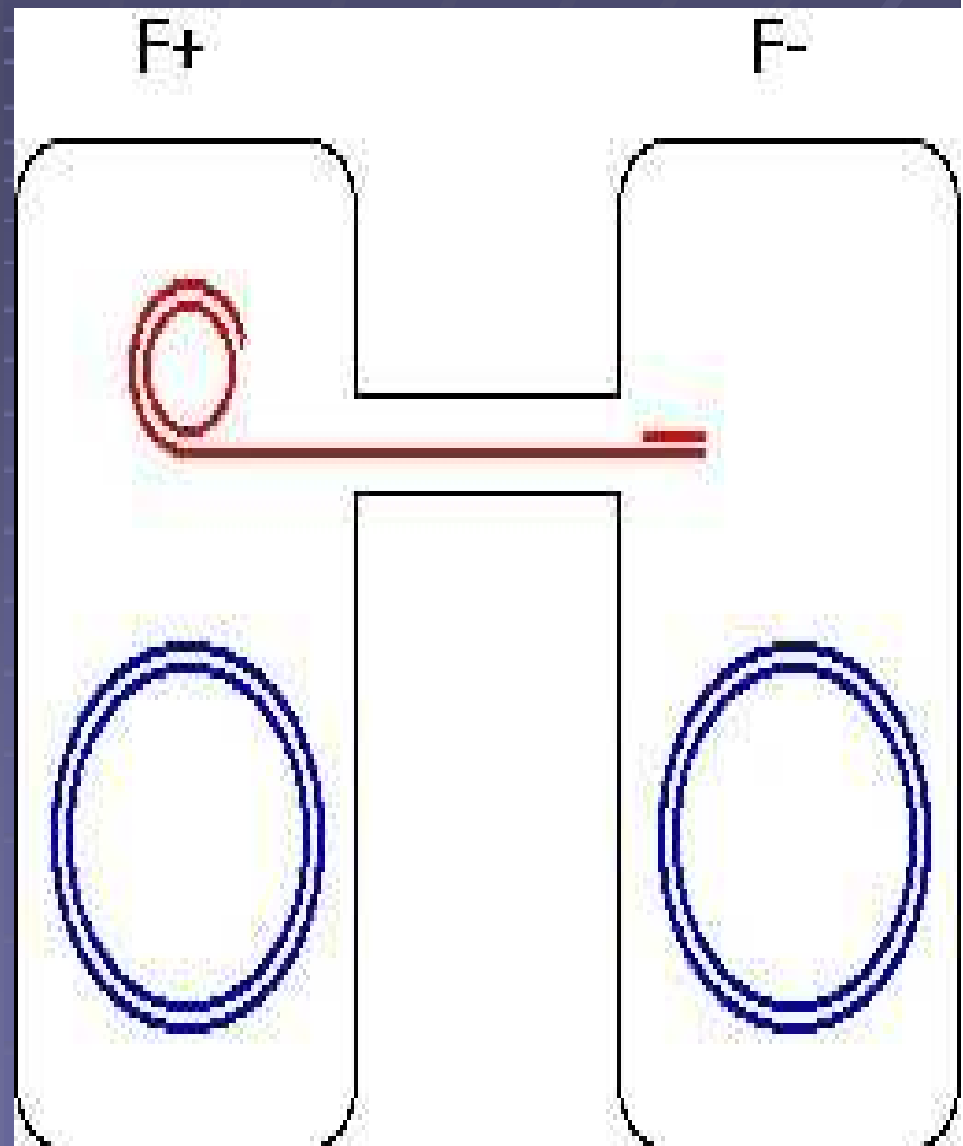
- Different types of Pilus are specified by different types of plasmids and can help in aid of plasmid classification.
- Only one strand of circular DNA of the plasmid nicked upon at a specific site and passed into a recipient.
- Spread to all other cells.



F factor

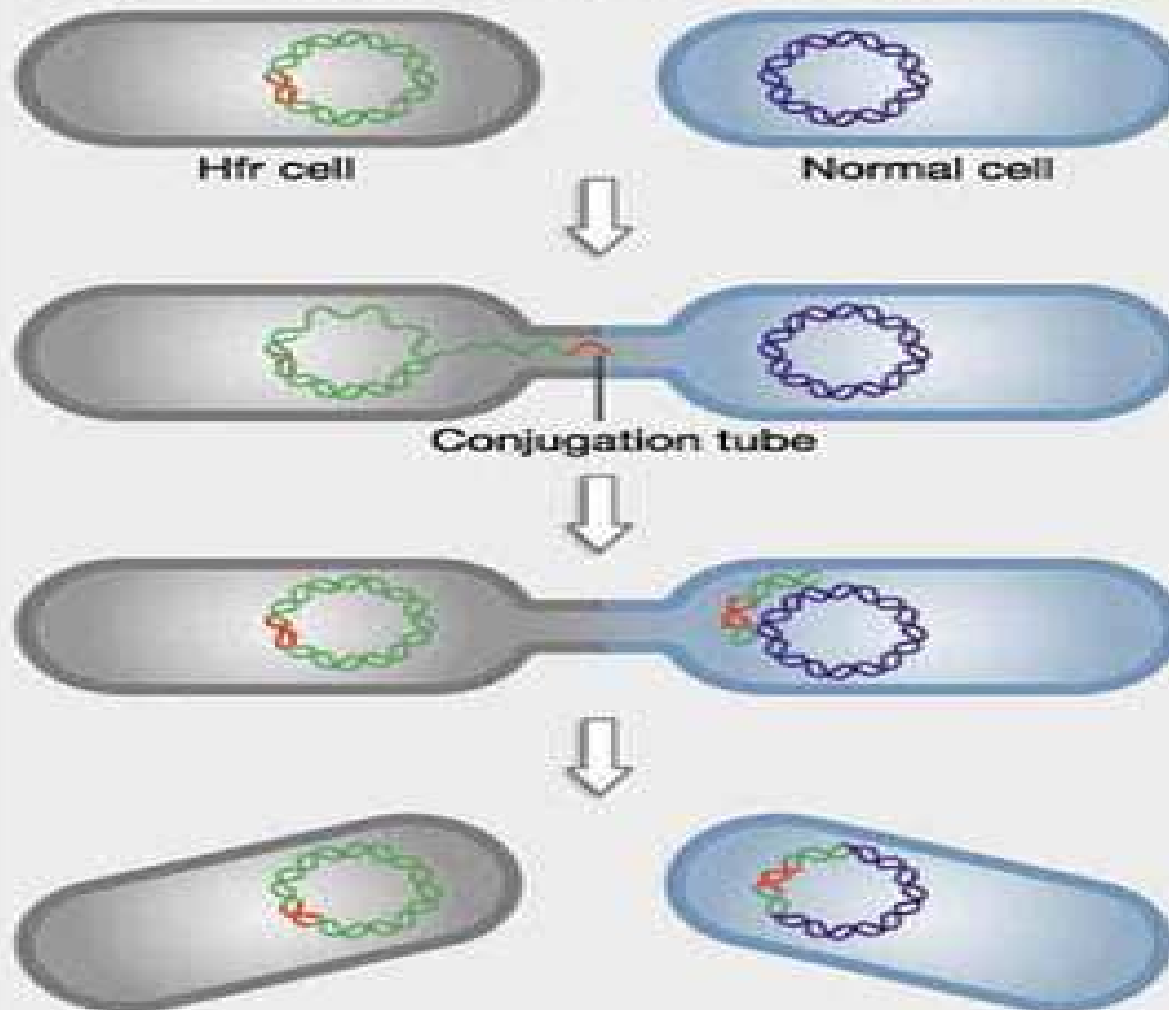
- Transfer factor that contains the genetic information necessary for synthesis of Sex Pilus and for self transfer without any other identifiable genetic materials such as drug resistance

T.V.Rao MD



Conjugation

BACTERIAL CONJUGATION AND RECOMBINATION



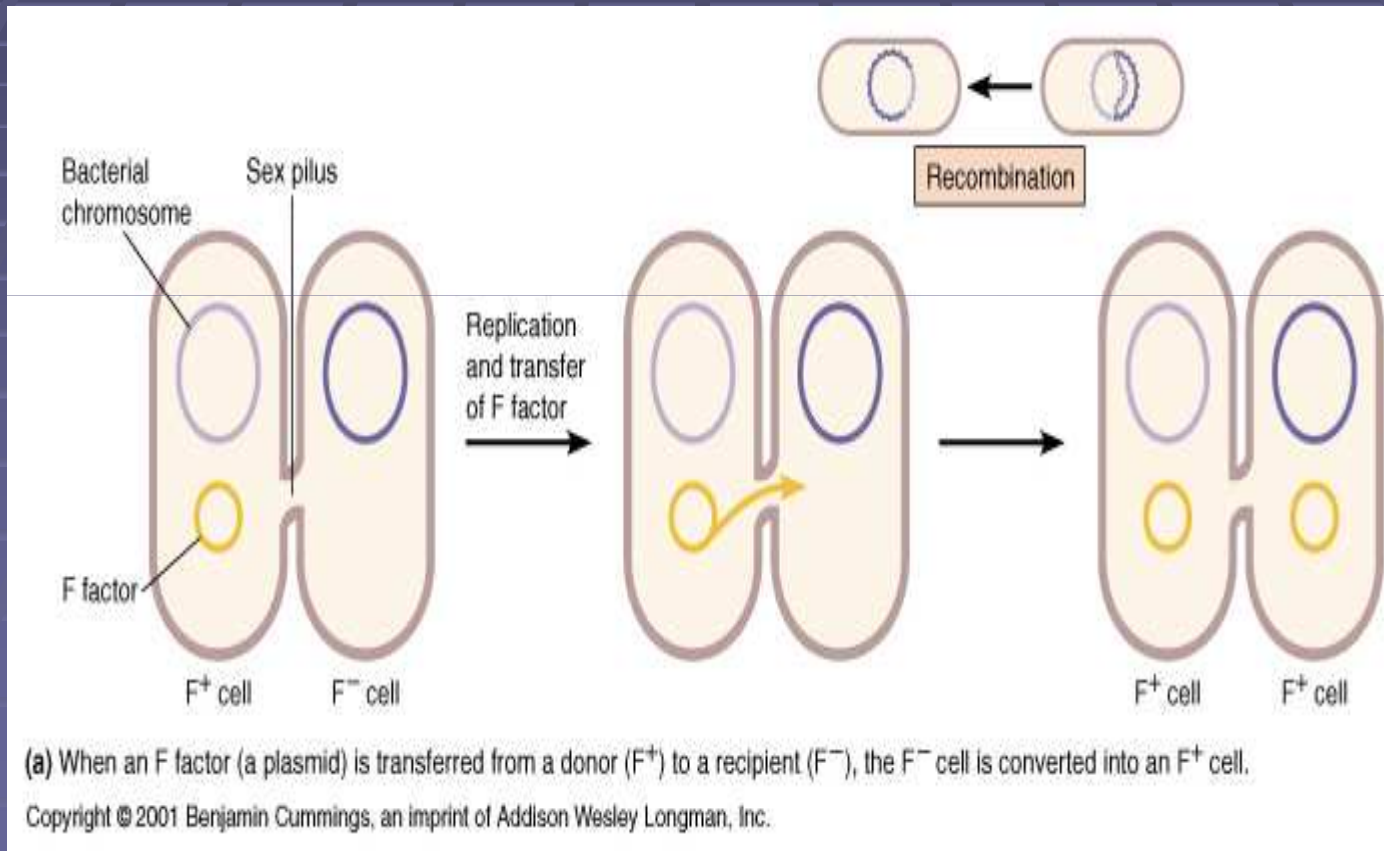
1. Hfr cells contain genes that allow them to transfer some or all of their chromosome to another cell.

2. Conjugation tube connects Hfr cell to normal cell. Copy of Hfr chromosome begins to move to recipient cell.

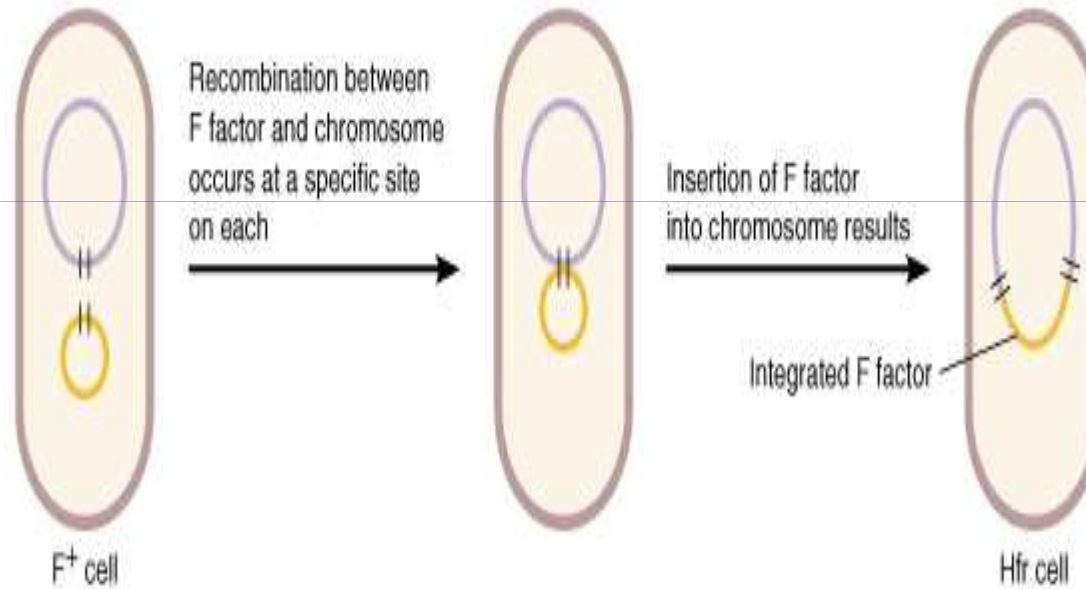
3. Homologous sections of chromosome synapse.

4. Cells separate. Section of Hfr chromosome integrates into recipient chromosome by crossing over.

Conjugation in *E. coli*



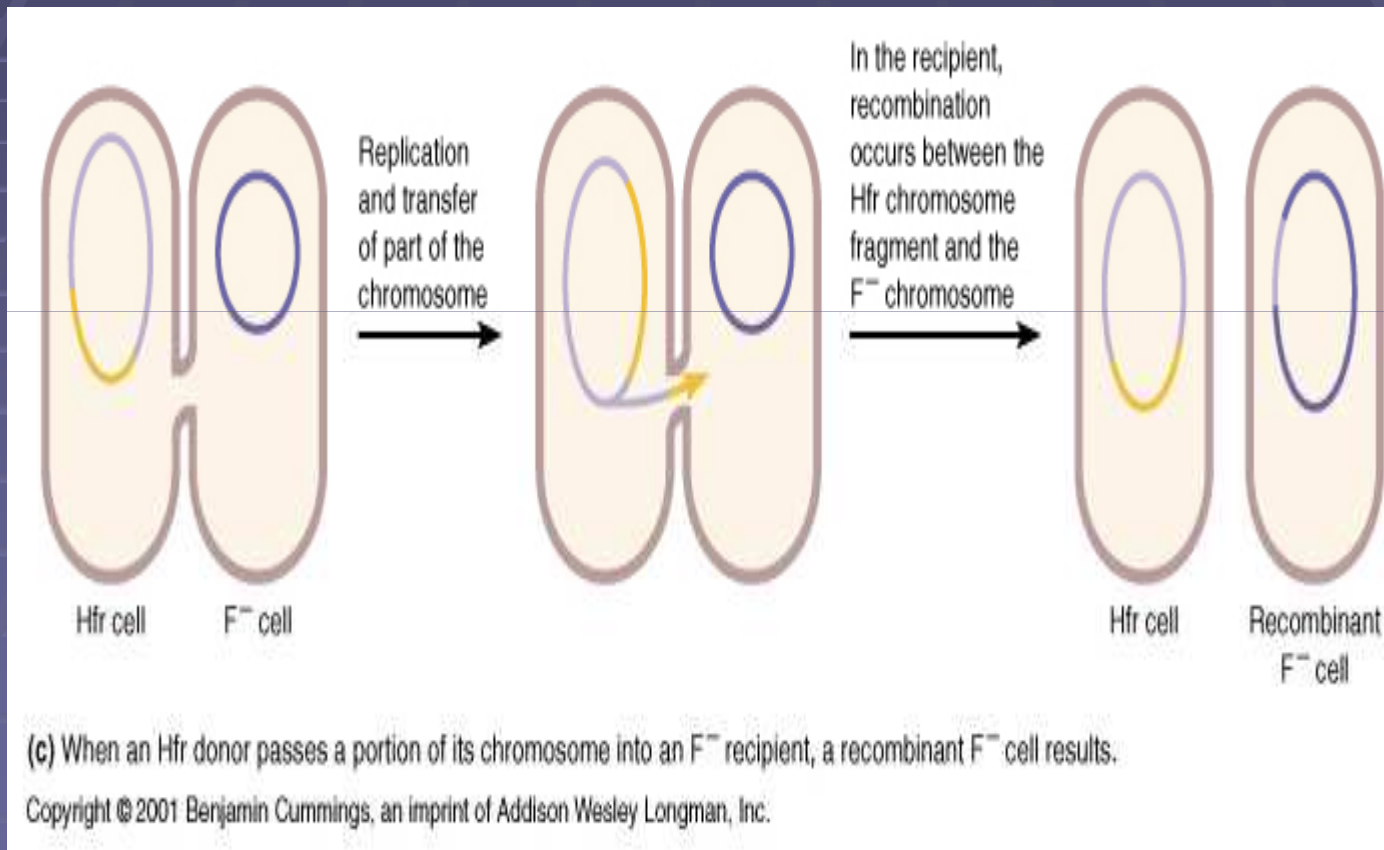
Conjugation continued...



(b) When an F factor becomes integrated into the chromosome of an F⁺ cell, it makes the cell a high frequency of recombination (Hfr) cell.

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Conjugation continued...



Resistance Transfer Factor RTF

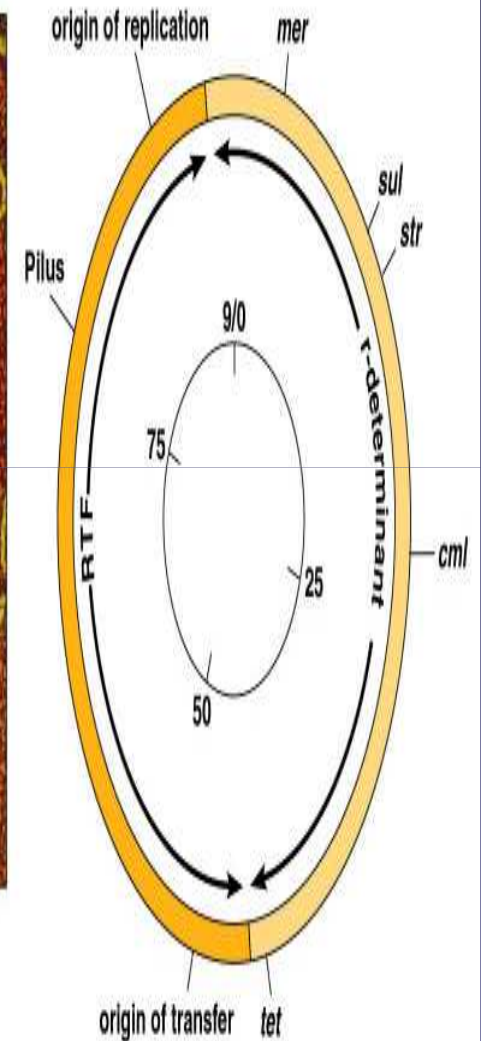
- Plasmids – helps to spread multiple drug resistance
- Discovered in 1959 Japan
- Infections caused due to Shigella spread resistance to following Antibiotics
 - Sulphonamides
 - Streptomycin
 - Choramphenicol,
 - Tetracycline

RTF

- *Shigella* + *E.coli* excreted in the stool resistant to several drugs in vivo and vitro
- Plasmid mediated – transmitted by Conjugation
- Episomes spread the resistance



(a)



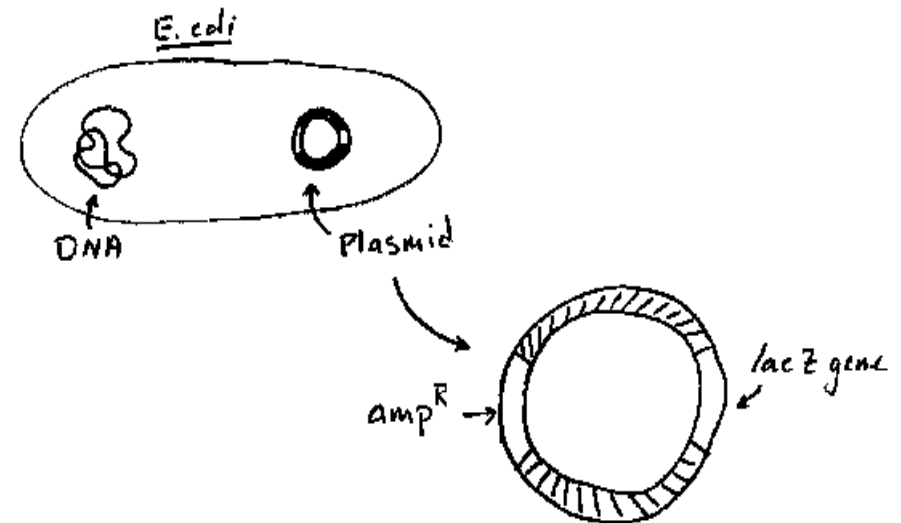
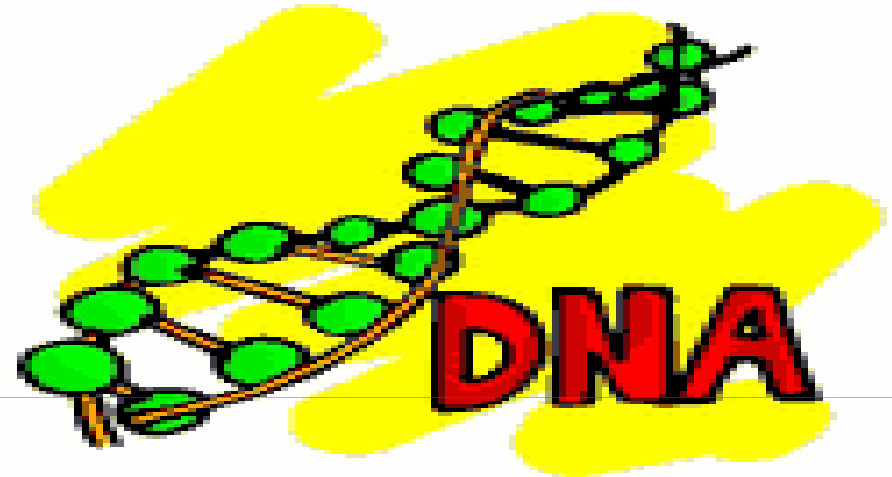
(b)

Composition of RTF

- Plasmid consists of two components
- A transfer factor RT, helps conjugational transfer and resistant determinants (r) to each of the several drugs
- RTF + r determinants are known as R factor

R factor

- R factor can contain several determinants as many as 8 or > 8 drugs
- Guide the cell for production of Enterotoxins too
- But R factors can be inhibited by
 - Bile salts
 - R factors can be transferred to animals



Genesis of R factors

- In discriminate use of Antibiotics in veterinary Medicine has increased the spread of R factors to Human
- Addition of Antibiotics to Animal feeds to be prohibited.

Colicinogenic (Col) Factor

- Coli form Bacteria produce Colicins
- Colicins are lethal to other Enterobacteriaceae
- Pyocins produce by Pseudomonas
- Diphtherocins produced by C.diphtheria
- Plasmid transmits col factor leads to self transfer of chromosomal segments

Genetic Mechanisms in Bacteria helps to spread the Infectious diseases



Other Mechanisms

- Use of Penicillin created resistant Staphylococcus by transduction
- R factors created resistance to several drugs, caused increased virulence
- Spread to several humans and animals

***Best option- To restrict use of
Antibiotics***

Transposable Genetic Elements

Structurally / Genetically – Discrete sequence of DNA – Move around in a cut and paste manner between Chromosomal and Extrachromosomal DNA molecules within cells.

Called as Transposons _ Jumping Genes

Genetic transfer due to Transposition

Small Transposons 1 – 2 Kb

Not self replicating and depend on Plasmid or Chromosome for replication.

A chunk of DNA is added by Transposons.

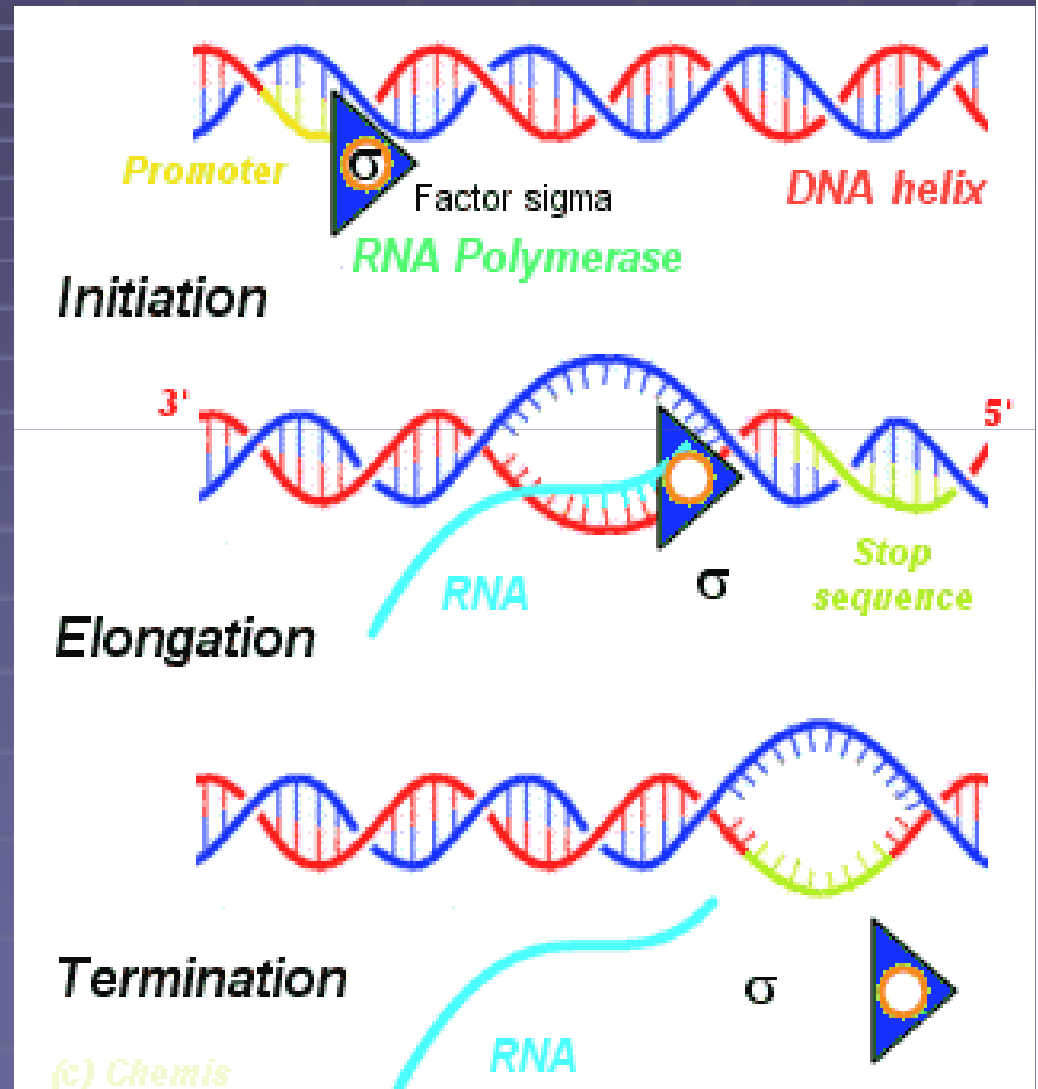
Transposons and R factor

- R forms may have evolved as a collection of Transposons
- Each carrying Genes that confers resistance to one or several Antibiotics
- Seen in Plasmids,
Microorganisms
Animals

Laboratory Manipulations are called as Genetic Engineering

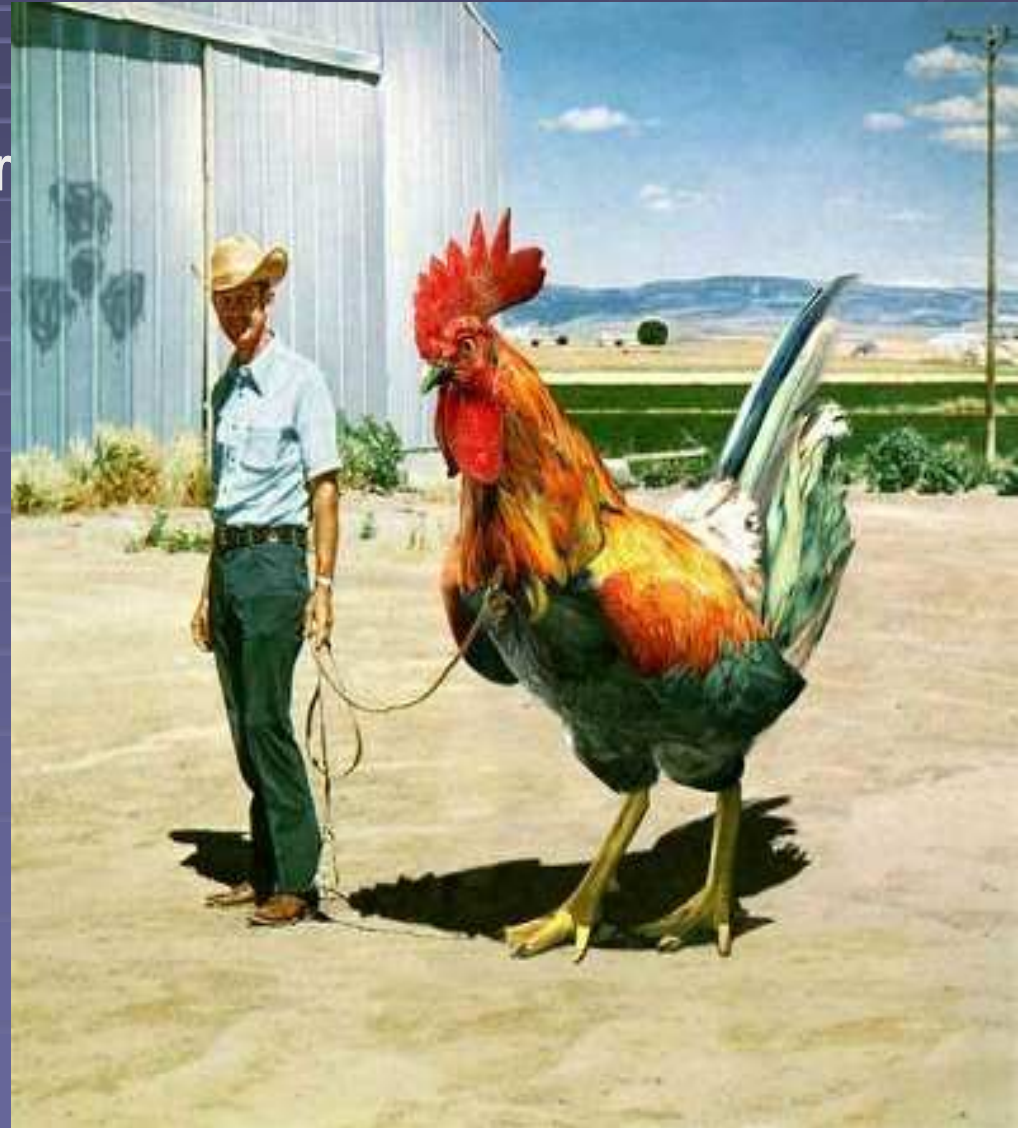
Molecular Genetics

- Analysis and manipulation of DNA using Biochemical and Microbiological techniques



Genetic Engineering

- Understanding Molecular genetics in Biochemistry fuels genetic Engineering
- Recombinant DNA (rDNA) techniques changed the ideals of Medicine
- Genetic Engineering await many surprises?

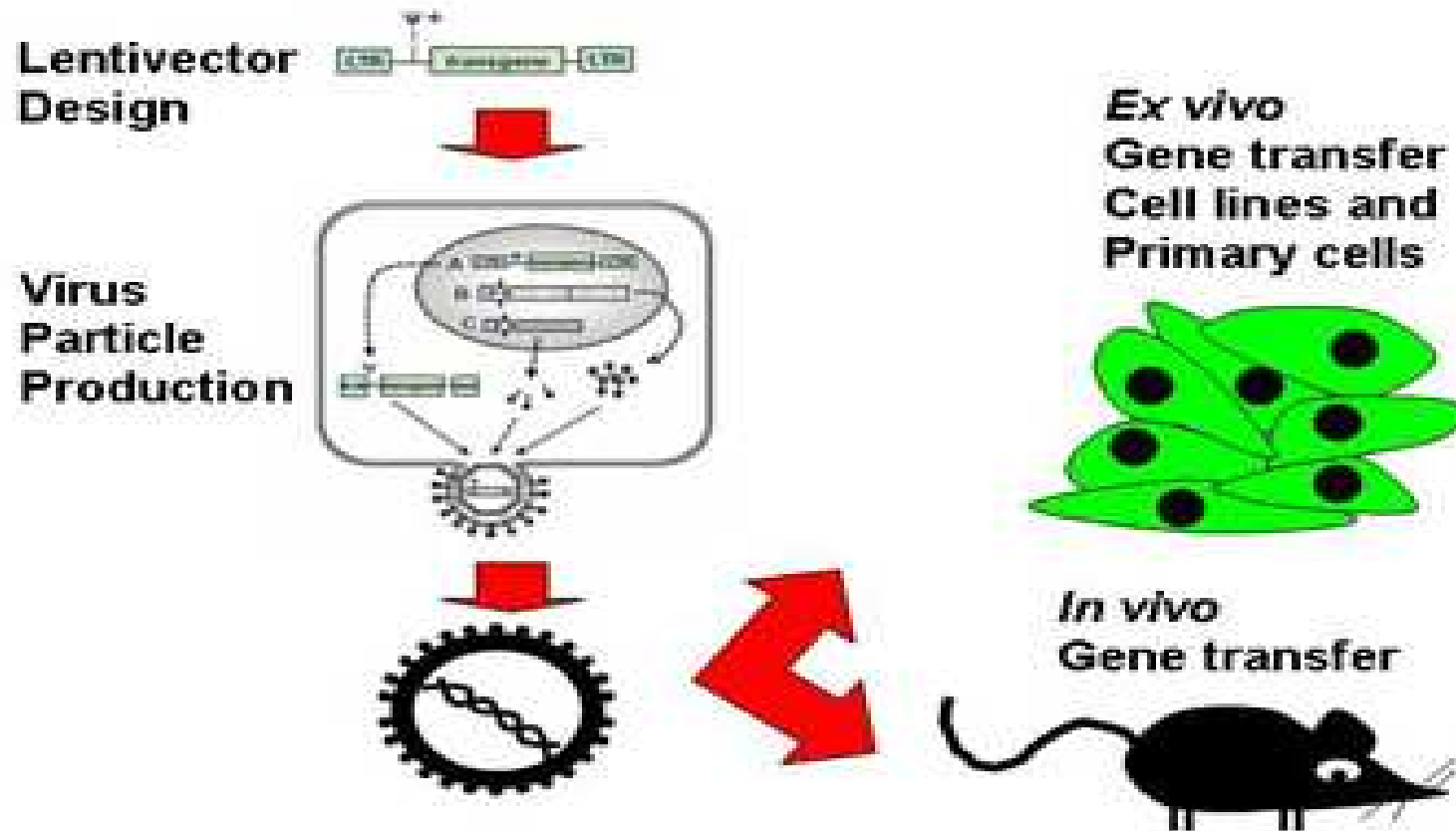


Genetic Engineering

- Isolation of Genes coding for any desired protein from Microorganism or from cell of higher life forms including human beings and their introduction into a suitable microorganism in which genes would function directing the production of specific proteins

***Genetic Engineering
changing the Diagnostic
and Therapeutic
Protocols in
MEDICINE***

Research on Gene transfer shapes the future of Science



Genitically Engineered Products

- Can prepare desired protein in pure form in economic way

Somatostatin

- Commercial preparations of
Cloned Human
Insulin

Interferons

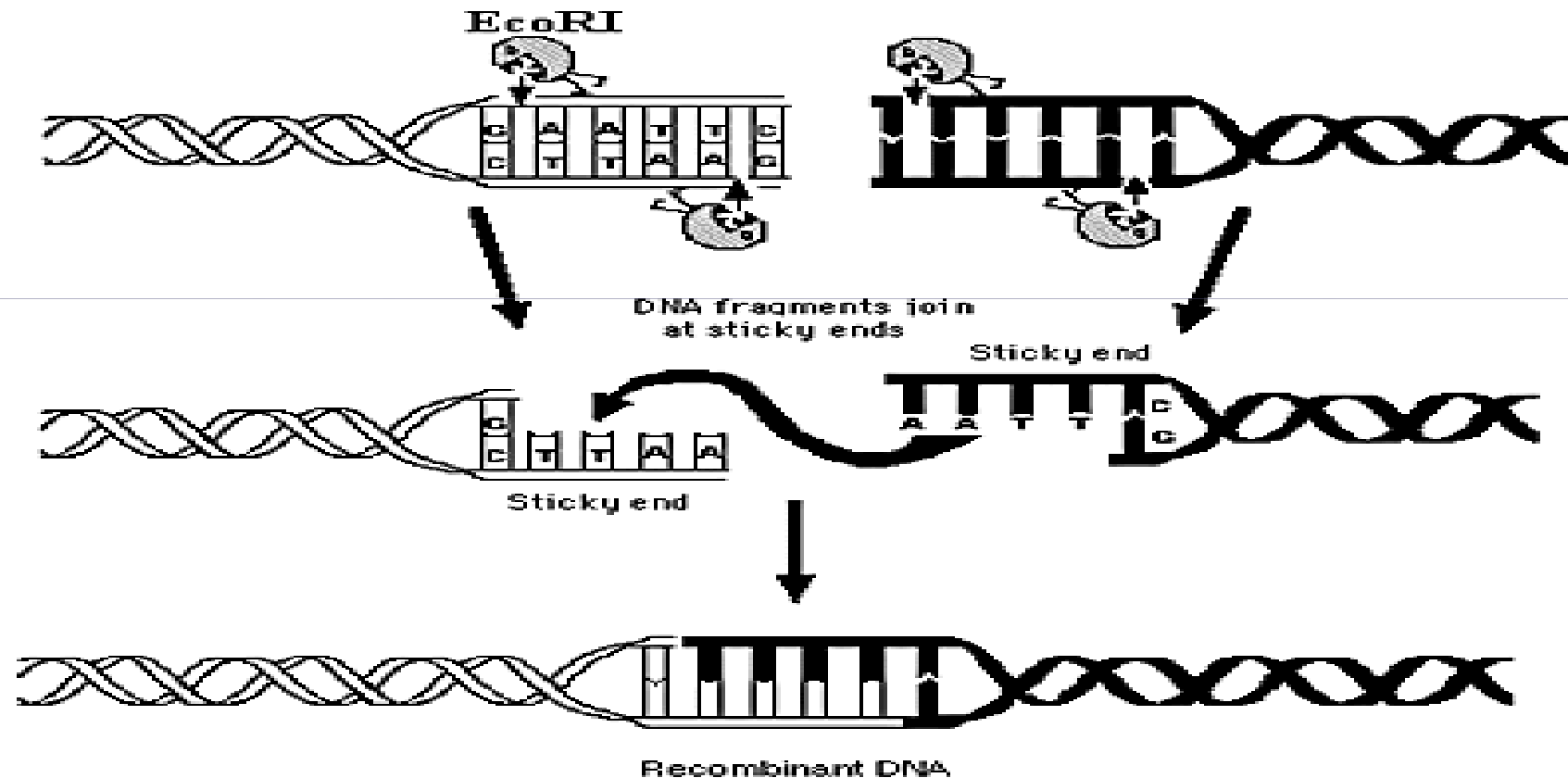
Hepatitis B vaccine



Restriction Endonucleases

- A restriction enzyme (or restriction endonuclease) is an enzyme that cuts double-stranded DNA. The enzyme makes two incisions, one through each of the sugar-phosphate backbones (i.e., each strand) of the double helix without damaging the nitrogenous bases. They work with cutting up foreign DNA, a process called

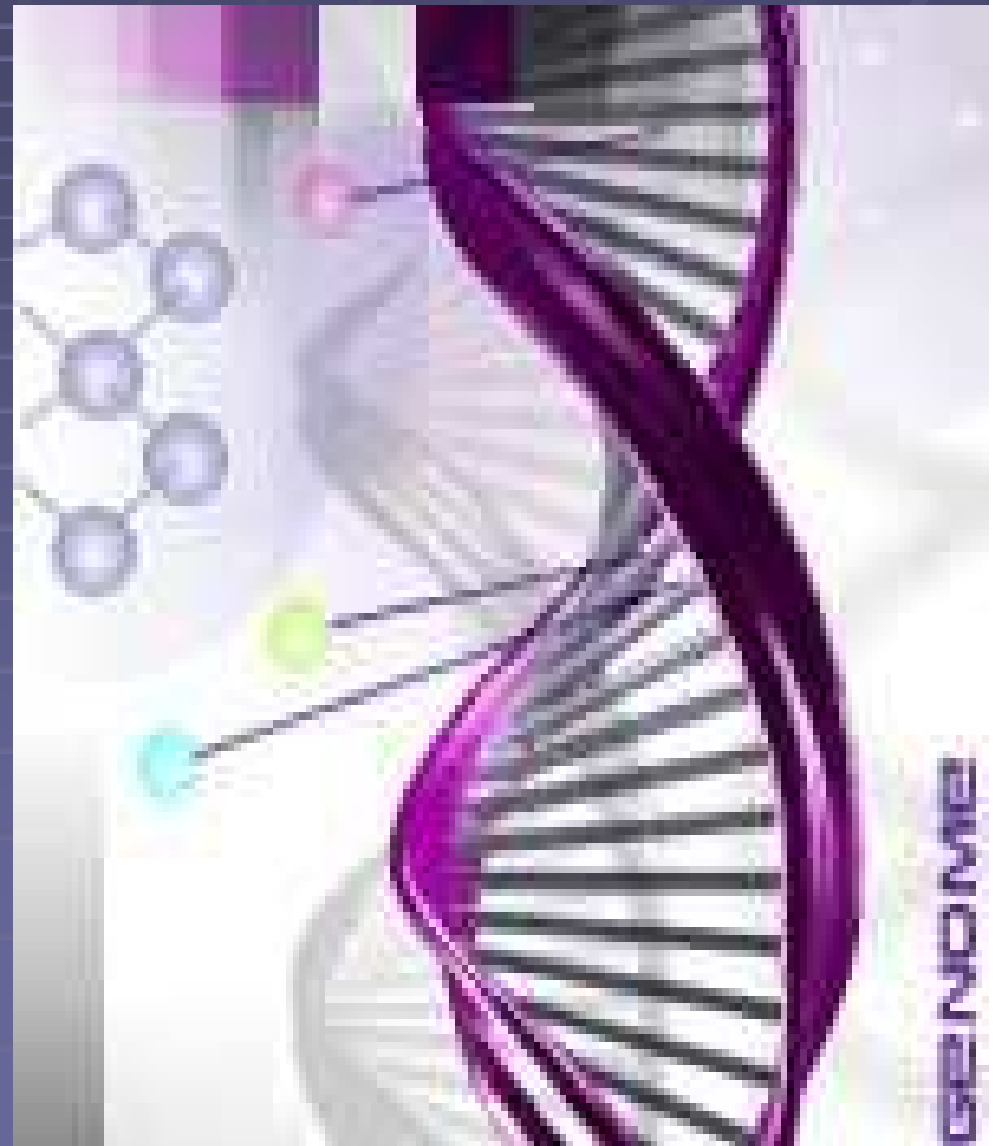
Restriction Endonucleases Made the advances in Genetic Engineering



**Restriction Enzyme
Action of EcoRI**

DNA Probes

- There are Radioactive Biotinylated otherwise labelled copies united single stranded DNA
Contains 20 -25 nucleotides
Helps detection of Homology DNA by Hybridisation.
Helps Diagnosis of Infectious Diseases
Minute quantities of DNA can be detected.



Blotting Techniques

- Drug fragments obtained by restriction enzyme digestion on separation Gel can be transferred to Nitrocellulose or nylon membranes
- Several methods
 - 1 Southern blotting
 - 2 Northern Blotting
 - 3 Western blotting

Western Blotting

- In Western Blot Protein (Antigen) mixture is separated by SDS (Sodium dodecyl sulfate – polyacrylamide gel electrophoresis) Blotted on to Nitro cellulose strips and identified by radio labeled or enzyme labelled antibodies as probes

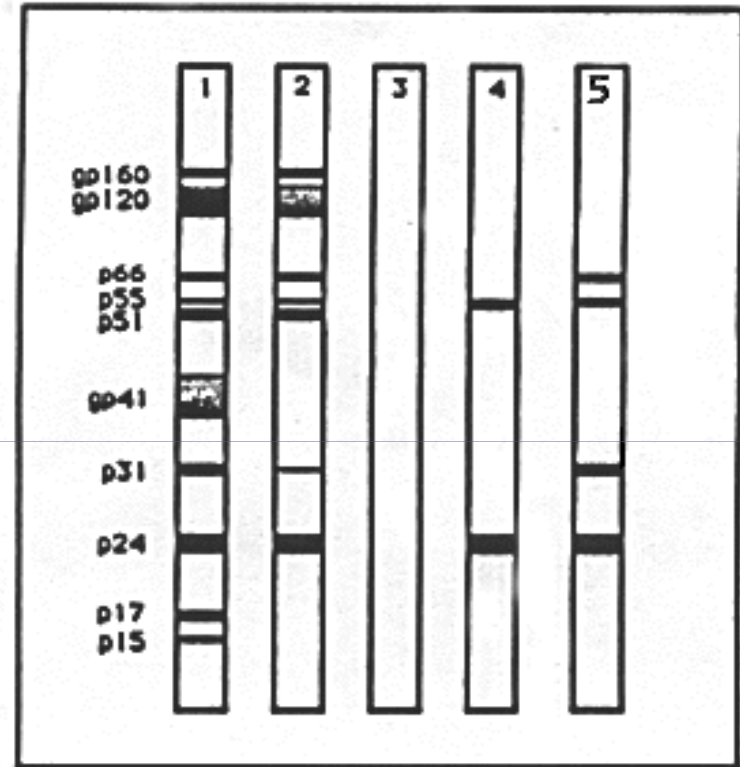


Figure:

Examples of reactions by an HIV-1 Western blot:

1. Positive control (strong)
2. Positive control (weak)
3. Negative control
4. Indeterminate profile
5. Indeterminate profile (highly suggestive)

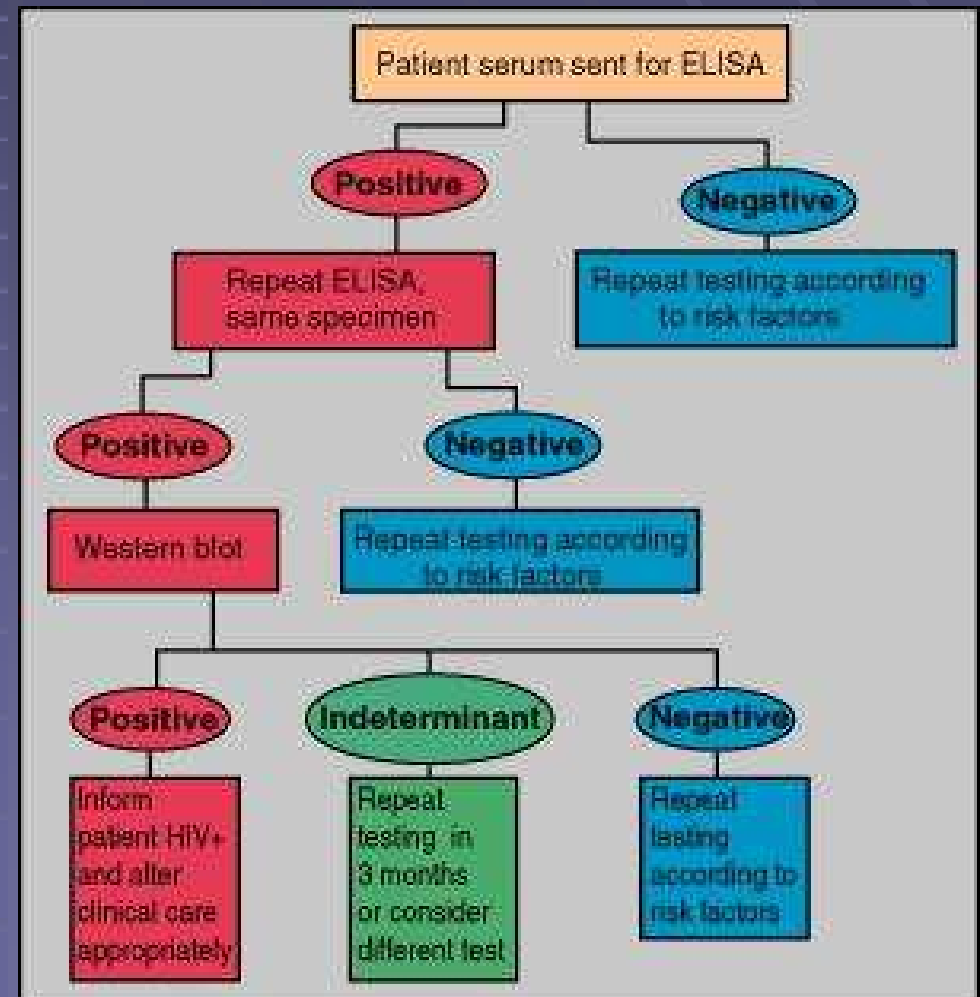
Western Blot to confirm HIV Infections made land mark Diagnostic tool

- Western Blot testing is confirmatory test for diagnosis of HIV/AIDS
- Identifies antibodies directed against different antigens in pathogen

Surface,

Core

RT antigen



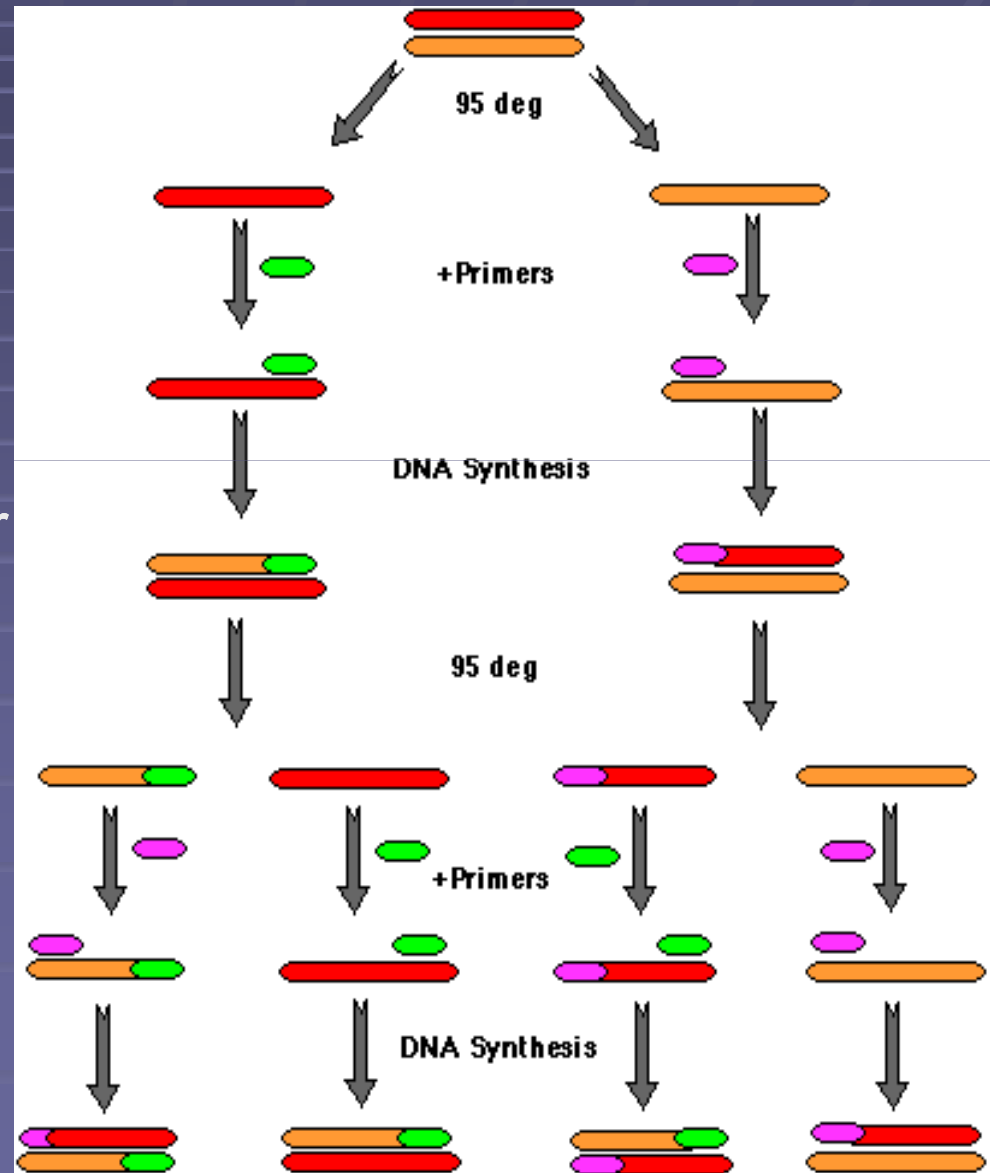
Polymerase chain reaction

Kary B Mullis 1983

- Rapid
- Automatic amplification of specific DNA sequences
- Nobel prize winning Technology 1993

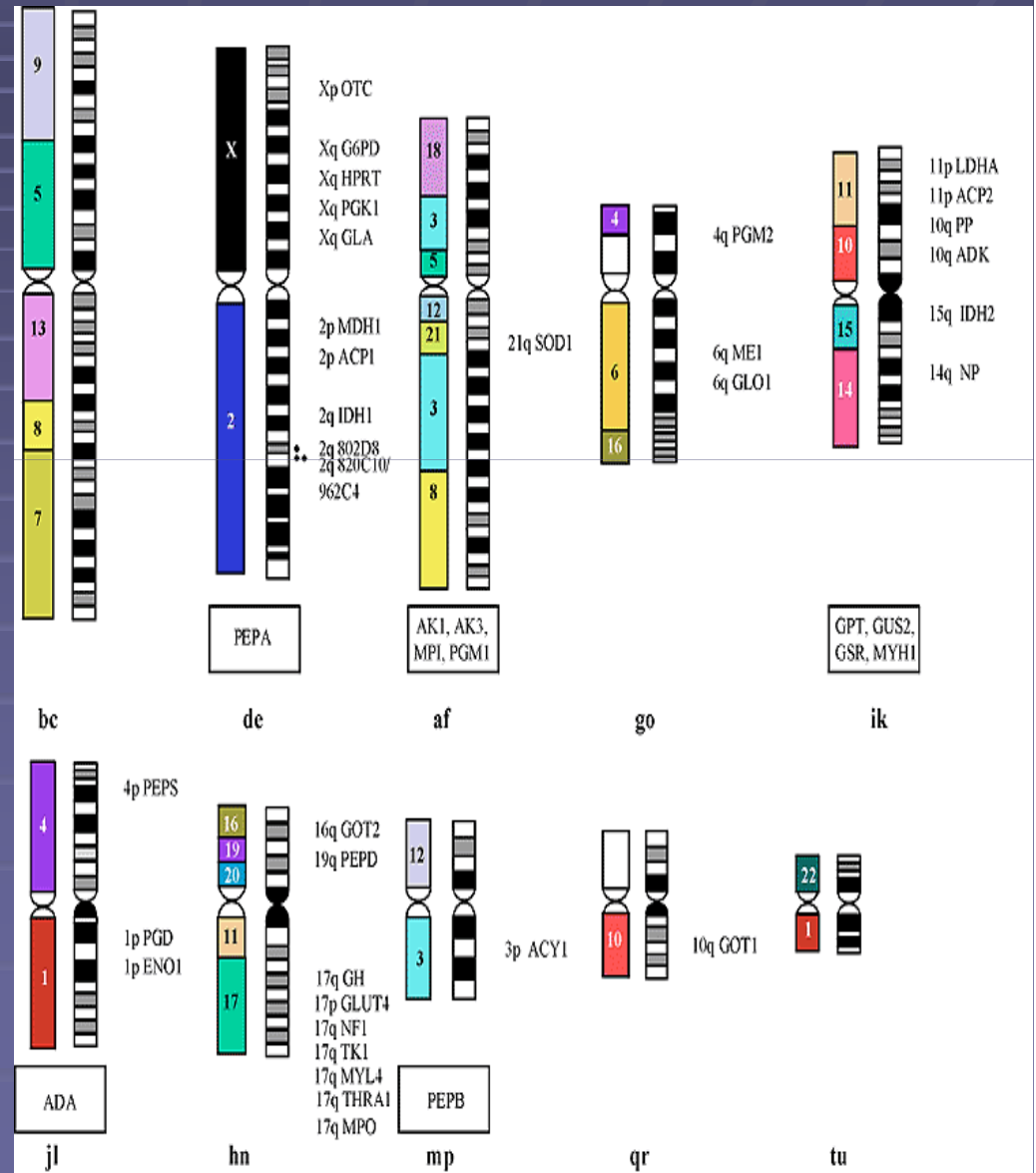
PCR -Sequences

- PCR consists of several cycles of sequential DNA replication where the products of first cycle becomes the template for the Next
- It makes available abundant quantities of specific DNA sequences starting

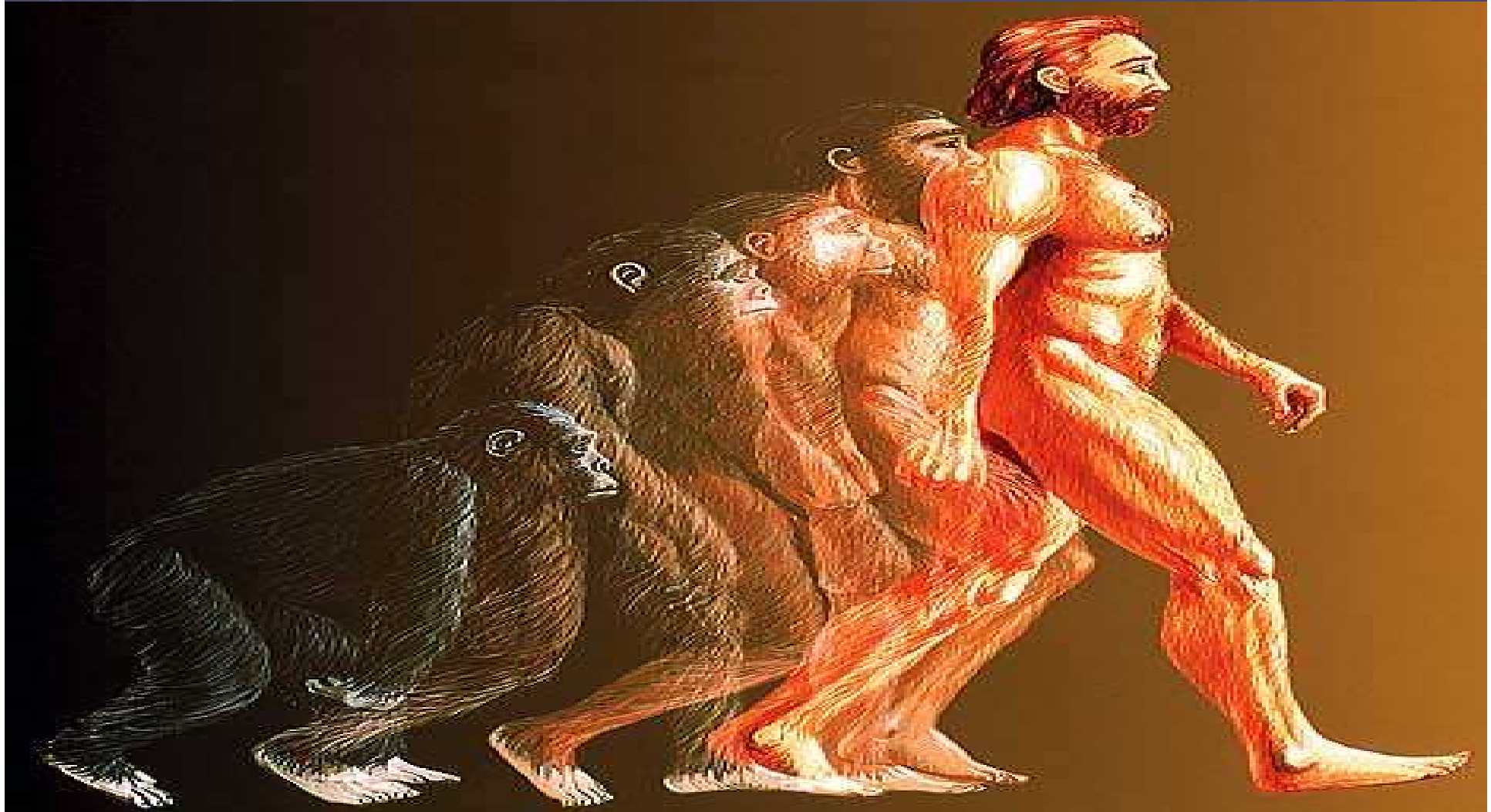


Genetic Mapping

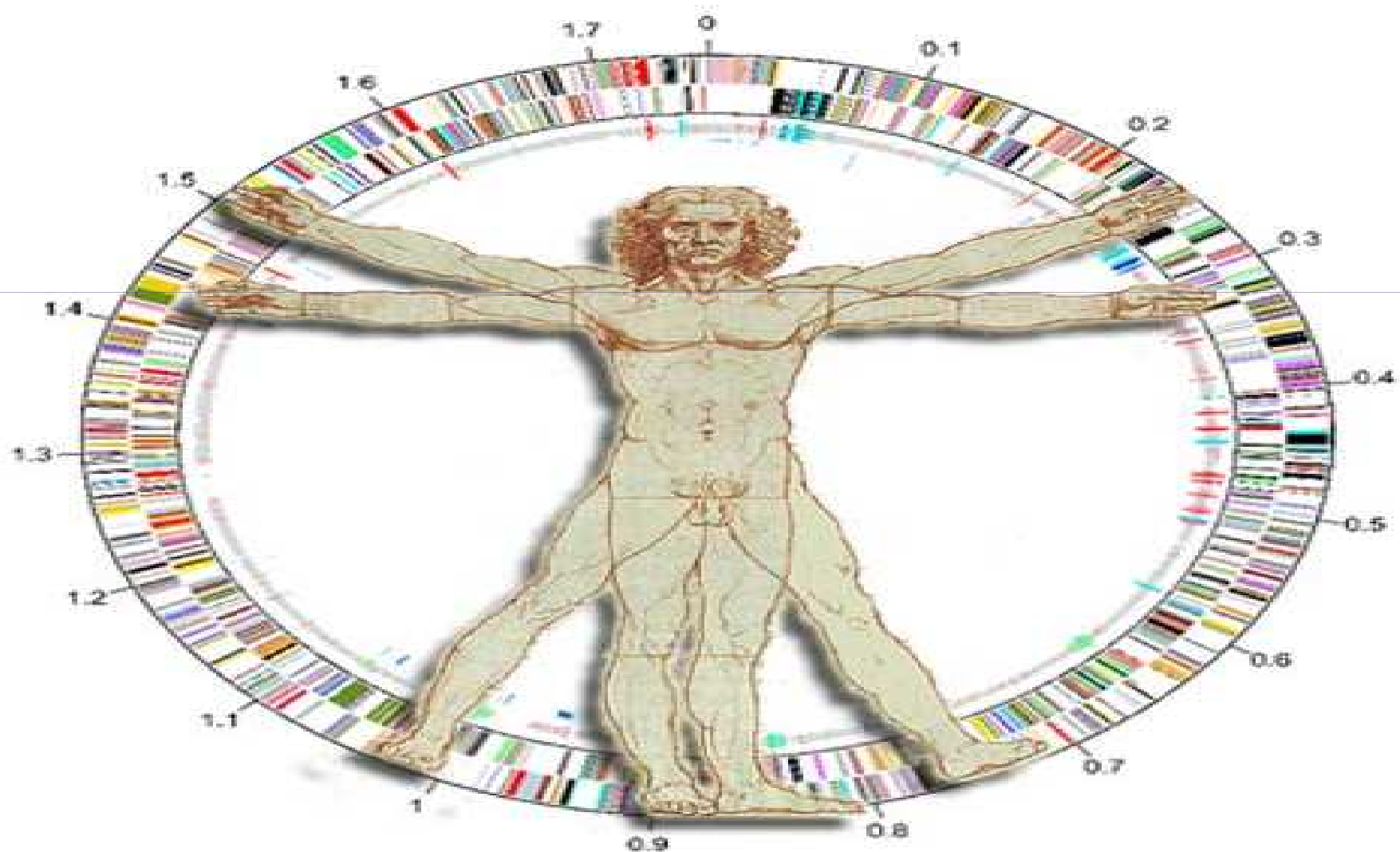
- Genetic sequences for Bacteriophages and virus
- Genetic mapping is done most of the Human Genes



*Genes Evolved and made us
Men
What NEXT ?*



Understanding of human Genome is Changing the Future of Medicine



Thanks

