A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-13; Unit I)

Introduction of Recombinant DNA Technology

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Definitions

- Genetic Engineering means the introduction of manipulated genetic material into a cell in such a way as to replicate and be passed on to progeny cells
- Biotechnology means application of scientific and engineering principles to biological processes to provide goods and services
- Biotechnology is the use of living organisms in industry or industrial-type processes

Definitions continued….

- Biotechnology consists of “the controlled use of biological agents, such as, micro-organisms or cellular components, for beneficial use.” —U.S. National Science Foundation
- “The application of biological organisms, system or processes” constitutes Biotechnology. –British biotechnology
- Biotechnology may be defined as “ the use of living organisms in systems or processes for the manufacture of useful products; it may involve algae, bacteria, fungi, yeast, cells of higher plants and animals or subsystem of any of these or isolated components from living matter.”-Gibbs and Greenhalgh, 1983

- The basic problem before advent of modern gene manipulation was:
- 1. Detection of uptake of exogenous DNA
- 2. Maintenance of exogenous DNA in transformed cell
- Composite molecules in which foreign DNA has been inserted into a vector molecule are sometimes called **DNA chimeras** because of their analogy with the chimera of mythology- a creature with the head of a lion, body of a goat ant the tail of the serpent.

Cloning of a DNA fragment requires following steps:

- Vector preparation
- Insert fragment preparation
- Ligation
- Transformation
- Screening of recombinant vector

Genetic Engineering

- GE was born in 1973 (stanley Cohen of Stanford University of Medicine, Stanford, California; Herbert Boyer of the University of California School of Medicine, Sanfrancisco
- H. Boyer constructed first recombinant DNA molecule using RE and Ligase (Cloned antibiotic resistant gene from salmonella).

Recombinant DNA

- Spliced DNA formed from two or more different sources that have been cleaved by RE and joined by ligases.
- Genetically engineered DNA made by recombining fragments of DNA from different organisms. The joining together of genetic material from two different organisms.
- Genes: Initially hereditary unit
- Physical properties was not known
- 1940s, gene was shown that is made up of DNA
- To study in detail (like structure, behavior and activity of gene) manipulation is required
- New branch in 1970 rDNA technology was born
- Definition: rDNA is any artificially created DNA molecule which bring together DNA sequences that are not usually found together in nature.
- rDNA technology refers to creation of chimeric DNA and subsequently introduction into living cells.

Definition of recombinant DNA technology

- In UK… the formation of heritable material by the insertion of nucleic acid molecules, produced by whatever means outside the cell, into virus, bacterial plasmid or vector system so as to allow their incorporation into the host organism in which they don't naturally occur but in which they are capable of continued propagation.

- The propagation of recombinant DNA inside a particular host cell so that many copies of the same sequence are produced is known as **cloning**.
- *E.coli* is the primary cloning host because:
- Technically culture is easier than other organism
- Gene manipulation in this bacterium is easier.
- Cloning techniques extended to a range of M.O., such as Bacillus subtilis, Psuedomonas spp., yeast, filamentous fungus and then higher eukaryotes.
- Mid 1980s all cloning was cell based (DNA molecule required host organism for amplification)
- In 1983 PCR was invented (*In vitro* amplification of DNA sequences using pure enzymes).

Application

- In medicine, development of new drugs, vaccine etc.
- Example is HGH (Human Growth hormone) used to treat growth defects.
- HGH purified from pituitary glands removed from cadavers.
- However, many pituitary glands are required to produce enough HGH to treat just one child.
- Some children treated with pituitary derived HGH leads to Creutzfeld-Jacob syndrome originating from cadavers.
- By recombinant DNA technology HGH gene cloned into *E.coli*, expressed and purified in more pure form that does not develop CJ syndrome in children.

Host Controlled Restriction and Modification

- Till 1970's GE was not possible
- No method was available to cut DNA into discrete fragment
- HCR & M leads to the discovery of RE
- Favorite organism of Molecular Biologists *E.coli* K12
- First studied in this regard but turned out to be unfortunate choice due to its endonuclease complex in behavior
- Break through came to the study of *H. influenzae*- endonuclease behaves more simply.

3re terms

- EOP (Efficiency of Plating)
- Colony formation
- Plaque formation

- Host controlled restriction and modification are most readily observed when bacteriophage are transformed from one bacterial host strain to another
- *E.coli* C infected by λ phage, EOP= 10^{-4}. This phenomenon is called Host restriction. Again λC infected on *E.coli* C, EOP=1. This phenomenon is called Host control modification

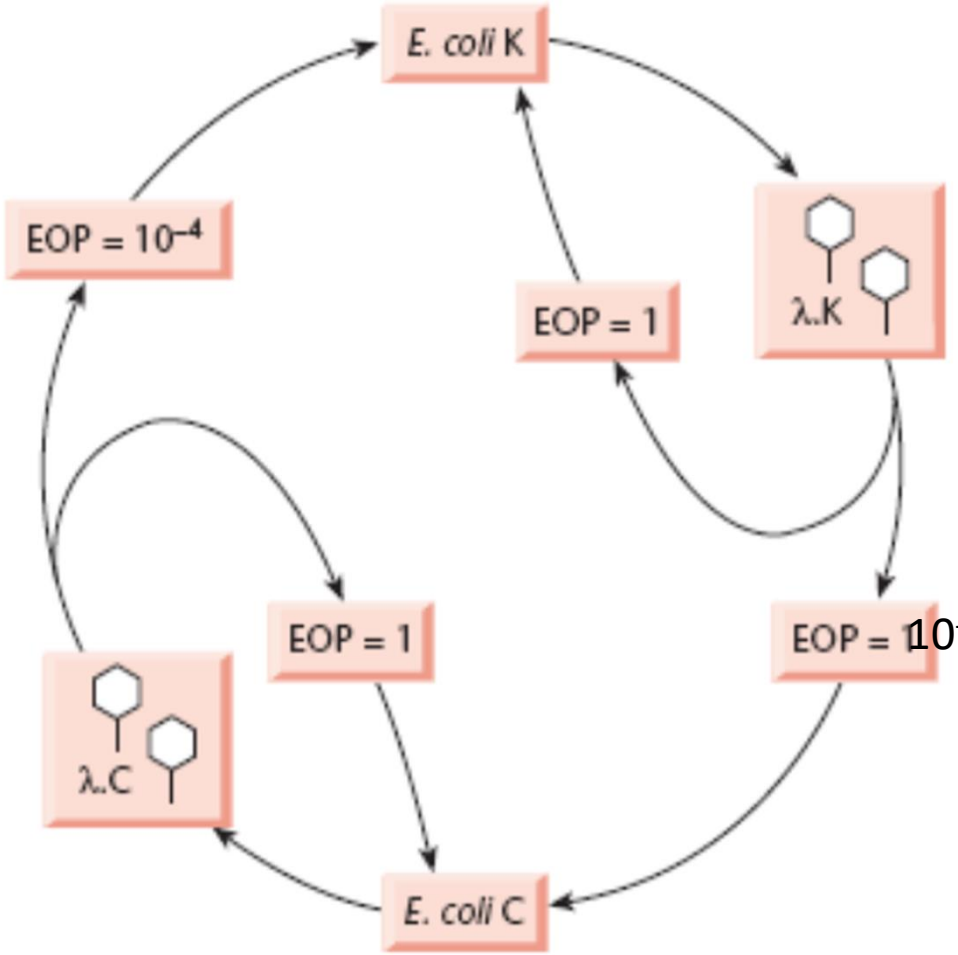

Fig. 3.1 Host-controlled restriction and modification of phage λ in *E. coli* strain K, analysed by efficiency of plating (EOP). Phage propagated by growth on strains K or C (i.e. λ.K or λ.C) have EOPs on the two strains, as indicated by arrows.

Mechanism

- The restricted phages absorb to restricted host and inject their DNA normally.
- When the phage are labeled with P^{32}, it is apparent that their DNA is degraded soon after injection and the endonuclease that is primarily responsible for this degradation is called a **restriction endonuclease** or **Restriction Enzyme** (Lederberg and Meselson 1964).
- The restrictive host must of course protects its DNA from potentially lethal effects of the RE and so its DNA must be modified.
- Modification involves the certain bases (adenine and Cytosine)
- Adenine to 6-methyl amino purine
- Cytosine to 5-methyl cytosine
- These modified bases are generally present in the recognition sequence of RE
- Enzyme responsible for methylation is called methylase.
- Host DNA is methylated in order to protect its DNA from RE action

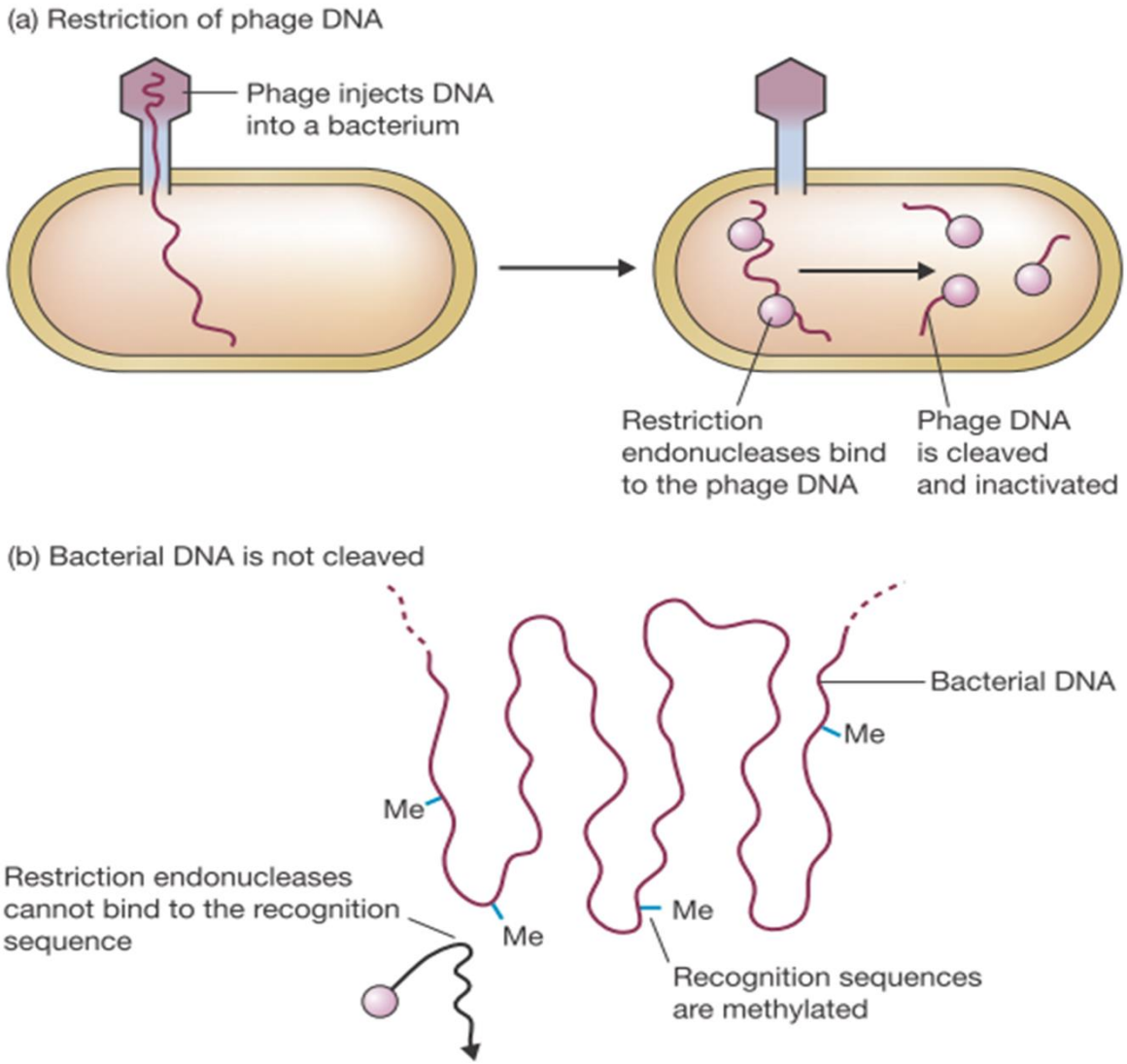

Figure 4.8

The function of a restriction endonuclease in a bacterial cell: (a) phage DNA is cleaved, but (b) bacterial DNA is not.

Acknowledgement and Suggested Readings:

1. Kuby Immunology; Sixth Edition; Kindt, Goldsby and Osborne; W. H. Freeman and Company
2. Fundamental Immunology; 5th edition; William E., Md. Paul (Editor) ; Lippincott Williams & Wilkins Publishers
3. Roitt's Essential Immunology; Tenth Edition; Roitt and Delves; Blackwell Science
4. Cellular and Molecular Immunology; 6th Edition; Abbas, Lichtman and Pillai; Saunders Elsevier

Thanks