

T4 BACTERIOPHAGE AS A MODEL ORGANISM

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ABSTRACT

In order to study many of the essential processes of life, T4 bacteriophage has become a "model organism" due to many reasons. It's easy to maintain and breed T4 bacteriophage in a laboratory. It is presently the most vastly used organism in molecular genetics due to its rapid adsorption, penetration, DNA replication, formation of new particles, enzyme synthesis and completed genomic sequence. T4 bacteriophage play key role in molecular biology field in order to identify the chemical nature of the gene, discovering genes code for proteins, deciphering how the genetic code is read and elucidating the mechanism of DNA replication.

The reason of attraction towards T4 bacteriophage as therapeutic agent is that they are lethal and highly specific for targeted bacteria while on the other hand safe for humans. T4 bacteriophages are considered as natural antimicrobial agents to fight against *Escherichia coli* infections in animals and humans. Moreover research work on *Escherichia coli* and its T4 bacteriophages played an important part in the revolution of molecular biology. Endolysin or lysins of T4 bacteriophage are used as antimicrobial agents and T4 bacteriophage is used as a particle to deliver vaccine. T4 bacteriophage also play major role in detecting the pathogen.

KEYWORDS: T4 Bacteriophage, Escherichia Coli, Phage Therapy, Model, Organism

INTRODUCTION

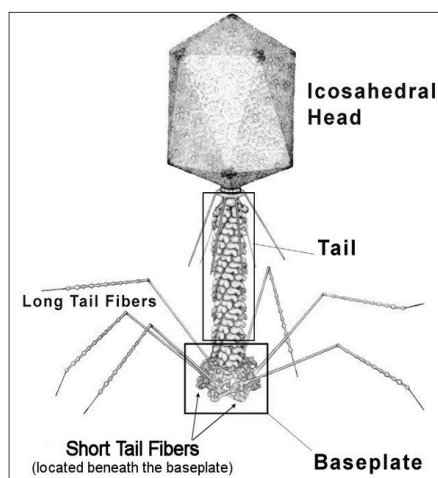


Figure 1: Schematic of T4 Bacteriophage

The *Escherichia coli* are infected by a bacteriophage named T4 bacteriophage. Length of Its DNA genome is about 169 kbp which is double stranded. DNA genome is held in an icosahedral head which is also called capsid. As compared to other bacteriophages T4 is a relatively big bacteriophage, which is about 200 nm long and 90 nm wide (most of the bacteriophages range in length from 25 to 200 nm). Its tail fibers permit attachment to host cell while the tail of T4 bacteriophage is hollow so that it can transmit its nucleic acid to the host cell thus infecting it during attachment as shown in Figure 1 T4 bacteriophage does not undergo lysogenic lifecycle but has the ability to undergo a lytic lifecycle (Miller *et al.*, 2003).

Infection and Lifecycle of T4 Bacteriophage

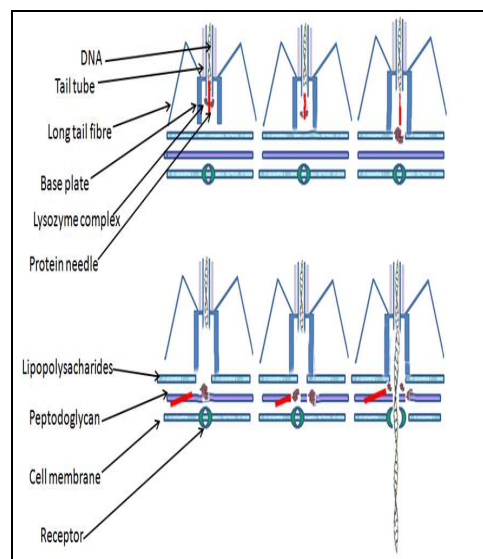


Figure 2: Injection Process of T4 Bacteriophage DNA into a Bacterial Cell

Long tail fibers (LTF) of T4 bacteriophage recognize surface receptors of *E. coli* thereby initiating the infective process. LTFs send a recognition signal to the base plate. The short tail fibers (STF) are then unraveled thus binding irreversibly to the *E. coli* cell surface. The conformation of base plate changes which results in contraction of tail sheath causing GP5 present at the end of the tail tube to puncture the outer cellular membrane. The periplasmic peptidoglycan layer is degraded by the activated lysozyme domain of GP5. When remaining part of the membrane is also degraded then DNA from the phage's head enters the *E. coli* by traveling through the tail tube as shown in Figure 2 (Tarahovsky *et al.*, 1994).

T4 bacteriophage takes about 30 minutes to complete lytic lifecycle i.e from entering a bacterium to its destruction (at 37 °C) and composed of:

- Adsorption and penetration (starting immediately)
- Arrest of host gene expression (starting immediately)
- Enzyme synthesis (starting after 5 minutes)
- DNA replication (starting after 10 minutes)
- Formation of new virus particles (starting after 12 minutes)

The host cell bursts open and releases the newly built viruses after the completion of lytic lifecycle thus destroying the host cell. The burst size of T4 bacteriophage is about 100-150 viral particles per infected host. T4 bacteriophage infects a host cell by their information afterwards blowing up the host cell thus propagating their progeny and increasing themselves (Tarahovsky *et al.*, 1994).

T4 Bacteriophage Unique Feature

There are some unique features in T4 bacteriophage which are as follows: (Oda *et al.*, 2004; Miller *et al.*, 2003; Madigan and Martinko, 2006; Tarahovsky *et al.*, 1994).

- Eukaryote-like introns
- High speed DNA copying mechanism, with only 1 error in 300 copies
- Special DNA repair mechanisms
- It infects *E. coli* O157:H7
- Genome terminally redundant

First of all genome is replicated and form several units and then there is end-to-end recombination of these genomic units which results in the formation of a concatemer. The concatemer is cut into same length at unspecific points during packaging. Thus several genomes are formed which correspond to Circular permutations of the original genome (Madigan and Martinko, 2006).

“Lysis from within” and “Lysis from without”

T4 bacteriophage signifies two totally different types of lysis, which are "Lysis from without" and "Lysis from within". Lysis from without occurs almost instantaneously by T4 bacteriophage adsorption at a threshold which is almost equal to the adsorption capability of that bacterium. None of the T4 bacteriophage is liberated in this case, on the contrary, the adsorbed T4 bacteriophages are lost. While Lysis from within is the usual lysis that is observed when the latent periods of lytic T4 bacteriophage end. The cell wall of the bacterium is attacked by T4 bacteriophage in such a way which permits swelling of the cell and its deformation into a spherical body. Lysis from within is initiated by adsorption of single or few T4 bacteriophage particles. In favorable conditions there is multiplication of one T4 bacteriophage particle within the bacterium up to a threshold value during the latent period. Threshold value is equal to the adsorption capacity and when this threshold value reaches only then there is abrupt destruction of protoplasmic membrane which results in liberation of the T4 bacteriophage particle and permits a rapid release of the cell contents with no deformation of the cell wall (Delbruck, 1940).

T4 Bacteriophage as Model Organism

E. coli is infected by a virus i.e bacteriophage T4 which has played key roles in few of the most important advances in the field of molecular biology including the recognition of the chemical nature of genes, explaining the mechanism of DNA replication, discovering genes code for proteins and even deciphering how the genetic code is read (Sriskantharajah, 2011).

- **T4 Bacteriophage as Model System**

In the development of modern genetics and molecular biology, T4 bacteriophages have been considered important model systems since the 1940s. Many investigators have taken benefit of T4 phage's practical degree of complexity and its capability to gain complete genetic and physiological information with quite easy experiments. T4 bacteriophage was considered useful in the formulations of many basic biological concepts. These comprise of clear-cut recognition of nucleic acids as the genetic material; defining gene by fine-structure through recombinational, mutational and functional analyses; demonstrating the triplet nature genetic code; mRNA discovery; the significance of recombination in DNA replication; mechanism of light-independent and light-dependent DNA repair; modification and restriction of DNA; translational bypassing; prokaryote's self splicing introns and many others (Karam *et al.*, 1994; Mathewe *et al.*, 1983). The benefit in view of T4 bacteriophage as a model system is T4 phage's complete inhibition of host gene expression thus allowing investigators to distinguish between phage and host macromolecular syntheses (Alberts and Miake-Lye, 1992; Alberts, 1987).

- **T4 Bacteriophage Therapy**

The reason of attraction towards T4 bacteriophage as therapeutic agent is that they are lethal and highly specific for targeted bacteria while on the other hand safe for humans. Moreover, T4 bacteriophages can develop rapidly to battle the antibiotic-resistant pathogenic bacteria emergence (Barrow and Soothill, 1997; Thacker, 2003). The Eli Lilly Company (Indianapolis, Ind.) in 1940s formed T4 bacteriophage products against *Escherichia coli* for human use (Sulakvelidze *et al.*, 2001). In a most recent paper from Brussow and colleagues (Chibani-Chennoufi *et al.*, 2004) systematical experiments were conducted with *E. coli* phages. T4 bacteriophages were tested against both non-pathogenic and pathogenic (EPEC and EHEC) strains of *E. coli* (Ochman and Selander, 1984). T4 coli phages which showed the complementary and widest infection range among the strains were chosen for the experiments.

- **T4 Bacteriophage Lysins as Antimicrobials**

Many of recent studies have revealed that rather than intact phage the T4 bacteriophage endolysins has been potentially used in therapeutics (Oliveira *et al.*, 2012). T4 bacteriophage endolysins are enzymes which hydrolyze the four major bonds in peptidoglycan component of cell wall thus spoiling the cell wall's integrity. Most of the phage lysins studied till date is in fact modular in structure that composed of two clearly divided functional domains which are N-terminal catalytic domain and C-terminal cell-wall binding domain. The C-terminal directs the enzyme to its target while the catalytic domain can consist of one or more than one of the following types of peptidoglycan hydrolases: muramidases (lysozyme), endopeptidases, glucosamidases and N-acetylmuramoyl- L-alanine amidases. The majority of the lysins are amidases (Brid *et al.*, 2010; Oliveira *et al.*, 2012).

- **T4 Bacteriophage Display**

Display technology of T4 bacteriophage is a powerful molecular tool that had a key impact on the discovery of drugs, immunology, pharmacology and plant science. Display technology is a technique by which foreign proteins, peptides or fragments of antibody are present at the T4 bacteriophage particles surface. As a result of transcriptional fusion, the heterologous protein or peptide is cloned with one of the coat protein genes into phagemid genome or a T4 bacteriophage. Thus T4 bacteriophages become vehicles for expression that along with carrying the nucleotide

sequence within them encode the expressed proteins thereby permitting the gene sequence to be retrieved and also have the ability to replicate (Malys *et al.*, 2002).

- **Function of T4 Bacteriophage in Vaccines**

An exciting and novel use of T4 bacteriophages is to deliver vaccines either as delivery vehicles for DNA vaccines or as a form in which immunogenic peptides are attached to the modified coat proteins of T4 bacteriophage. Display of T4 bacteriophage is helpful for the recognition of immunogenic mimotopes or epitopes on displayed peptides in turn which can become the base of peptide vaccines (Tao *et al.*, 2013).

- **T4 Bacteriophage for Detection of Pathogens**

E.coli can be detected by T4 bacteriophage. Small outer capsid (SOC) protein of T4 bacteriophage was used in order to present green fluorescent protein (GFP) which is an easily detectable protein marker present on the capsid of phage. The T4 e (-) phage was used to detect *E.coli*, which does not produce the lysozyme responsible for the lysis of host cell. The intensity of green fluorescence was increased by the propagation of T4 e (-)/GFP in the host cells thus it makes the differentiation of *E. coli* cells from other cells very effective and simple. This method allows the rapid and conclusive quantitation of *E. coli* cells in an hour (Tanji *et al.*, 2004).

CONCLUSIONS

Studies on T4 bacteriophage growth and use played a very important role in the history of molecular biology. The information which was accumulated during the 1940s, with the model species T4 bacteriophage, laid the basics for the evolving field. T4 bacteriophage research continues to break new ground in our understanding of the basic molecular mechanisms. T4 bacteriophage is one of the basic models of molecular biology for several decades and this virus is now actively used as object of structural biology, phage therapy, vaccine and phage display.

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