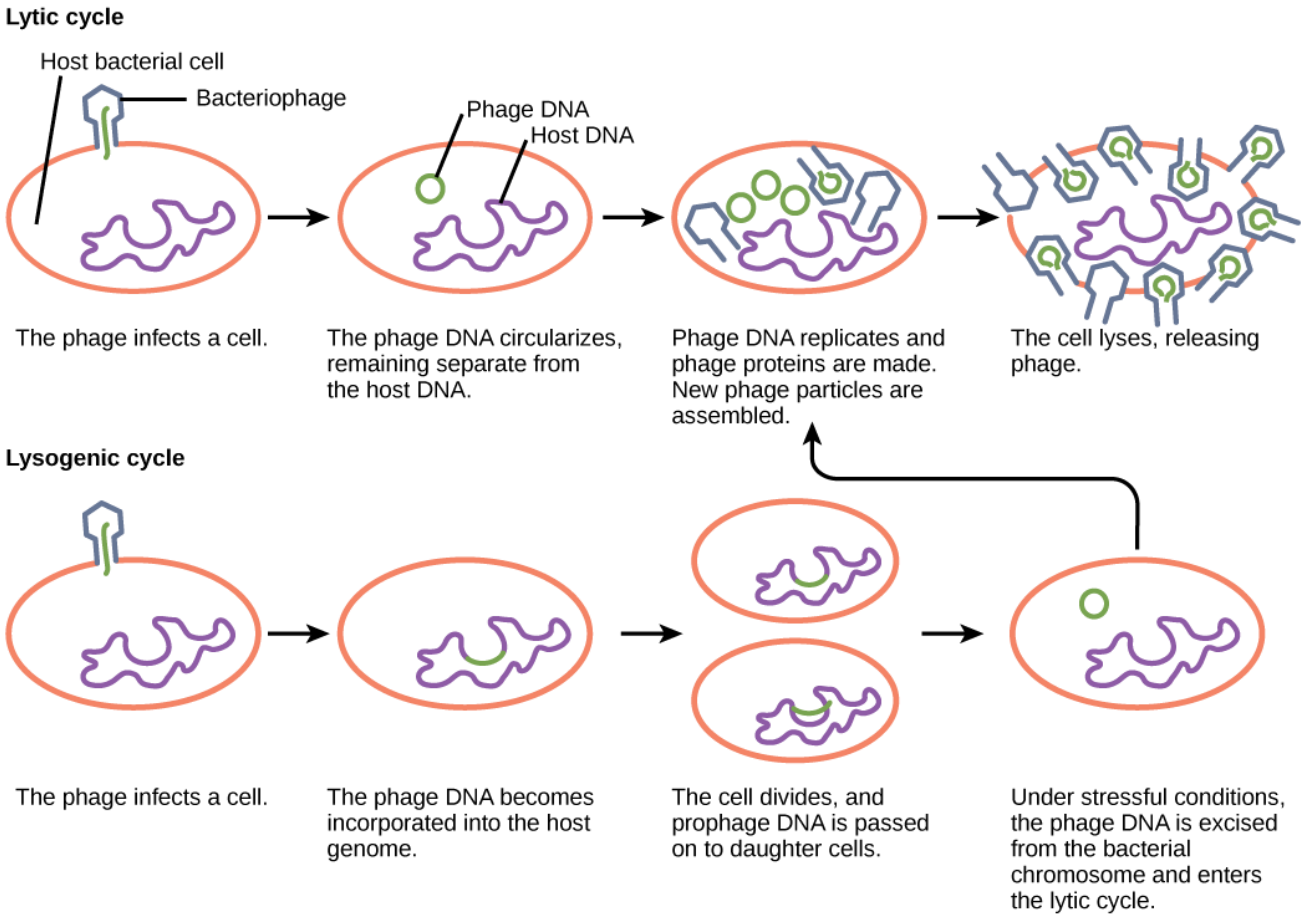
Bacteriophage- Introduction

Dr Shilpa Deshpande Kaistha
Department of Biotechnology
School of Life Sciences & Biotechnology
CSJM University Kanpur

Terminology

- **Virion: singular**
- **Virus: Plural**
- **pfu: plaque forming unit**
- **Titre: amount of virus in suspension**
- **Multiplicity of infection (MOI): the ratio of PFU/ml to CFU/ml**
- **Efficiency of Plating (EOP): the ratio of the plaque titer to the number of phage particles**
- **Virulent Phage: Phage showing only lytic life cycle**
- **Temperate Phage: Phage with lytic and lysogenic life cycle**
- **Prophage: state of phage co-existing with host**
- **Lysogenic bacteria: term of bacteria carrying prophage**
- **Phage conversion: phenotype change in lysogenic bacteria**

Life Cycle- Bacteriophage



Lytic Life Cycle

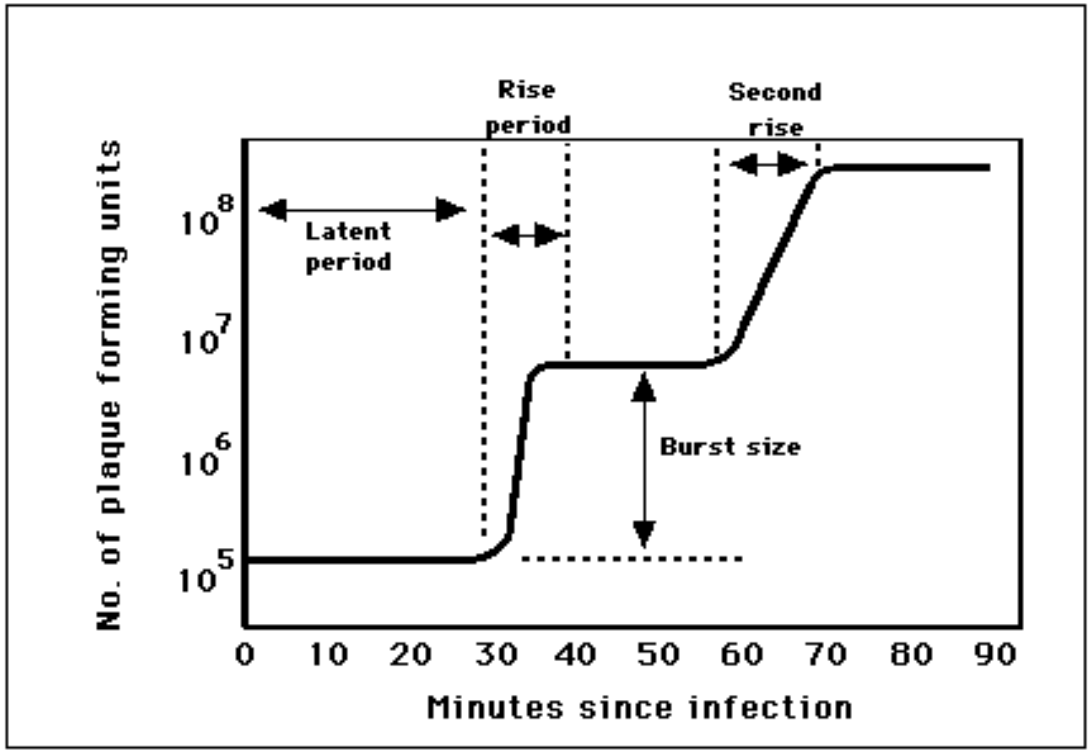
- During the **lytic cycle** of virulent phage, the bacteriophage takes over the cell, reproduces new phages, and destroys the cell. T-even phage is a good example of a well-characterized class of virulent phages. There are five stages in the bacteriophage lytic cycle (see Figure 1). **Attachment** is the first stage in the infection process in which the phage interacts with specific bacterial surface receptors (e.g., lipopolysaccharides and OmpC protein on host surfaces). Most phages have a narrow host range and may infect one species of bacteria or one strain within a species. This unique recognition can be exploited for targeted treatment of bacterial infection by phage therapy or for phage typing to identify unique bacterial subspecies or strains. The second stage of infection is entry or **penetration**. This occurs through contraction of the tail sheath, which acts like a hypodermic needle to inject the viral genome through the cell wall and membrane. The phage head and remaining components remain outside the bacteria.

https://courses.lumenlearning.com/suny-microbiology/chapter/the-viral-life-cycle/

Lysogenic cycle

- In a **lysogenic cycle**, the phage genome also enters the cell through attachment and penetration.
- Example of a phage with this type of life cycle is the lambda phage.
- During the lysogenic cycle, instead of killing the host, the phage genome integrates into the bacterial chromosome and becomes part of the host. The integrated phage genome is called a **prophage**. In other lysogeny, phage can remain as extrachromosomal element as in P phage
- A bacterial host with a prophage is called a **lysogen**.
- The process in which a bacterium is infected by a temperate phage is called **lysogeny**. It is typical of temperate phages to be latent or inactive within the cell. As the bacterium replicates its chromosome, it also replicates the phage's DNA and passes it on to new daughter cells during reproduction. The presence of the phage may alter the phenotype of the bacterium, since it can bring in extra genes (e.g., toxin genes that can increase bacterial virulence).
- This change in the host phenotype is called **lysogenic conversion** or **phage conversion**.

One step growth



Ellis, E. L. and M. Delbrück (1939). The Growth of Bacteriophage. J. Gen. Physiol. 22:365-384.

- One-step growth curve, developed by Max Delbruck and Emory Ellis (1939) using Escherichia coli-T4 bacteriophage system
- A one-step growth curve is a fundamental method for describing a new bacteriophage.
- It's used to determine the life cycle of a virus on a specific host.
- Only single or one cycle of virus growth is observed hence it is called as One-step multiplication curve

Procedure

- To limit the phage–host interaction in the experiment to a single infection cycle, phages and hosts have to be mixed in the right ratio. Prior to experiment, it is therefore necessary to determine the titer of the phage stock and to know the relation between cell density and optical density (i.e., obtain corresponding numbers of cells mL–1 and OD) of the host.
- Infection should be done at low MOI (multiplicity of infection = ratio of phage to host) e.g., between 0.1 and 0.01.
- At higher MOI, the probability of cells infected by more than one phage would increase and the total estimate of infected cells becomes less than the phage input.
- Plaque forming assay at different time points post phage infection- Double Layer Agar Overlay Assay

One Step Growth Curve

- Log no. of plaque forming unit/ml plotted against time, a curve is obtained, termed as one step growth curve
- It has three distinct phases
  - 1. Latent / Eclipse period
  - 2. Burst or Rise period
  - 3. Plateau period

Phases of One Step Growth Curve

- The latent period is described as the time period prior to the release of infection particles or appearance of extra cellular phages.
- In the latent period, attachment, entry, replication, transcription, translation and assembly of progeny phages occur.
- During this period there is no release of new phage particle therefore plaque count remains constant
- Latent period can be divided into two phases
  - Eclipse: This period immediately follows the penetration of viral particles into the host cell. Eclipse is characterized by the incapacity to detect free virions since viruses are actively transcribing and replicating inside the host. The eclipse usually lasts from minutes (bacteriophages) to hours or days (animal or plant viruses).
  - Intracellular accumulation: During the eclipse period all structural proteins and viral genomes have been produced and massively accumulated in the cytoplasm of the host cell. Both components self assembly to form new viral particles that accumulate intra cytoplasmatically

- **Rise Period**
  - In this period lysis occurs and librated a crop of new virus particle hence extracellular phages appear
  - During this phase, phage particle increase in their concentration rapidly.
  - T4 rise period = 10 min

Plateau Period

- This period represents the end of all infected host cell lysis.
- The newly liberated phage particles fail to meet uninfected host cells due to high dilution.
- Therefore during this phase, the plaque count remains constant.
- **Burst Size:** Average yield of infectious virus per cell is called burst size.
- Burst Size = pfu/ml at plateau divided by pfu/ml at latent period

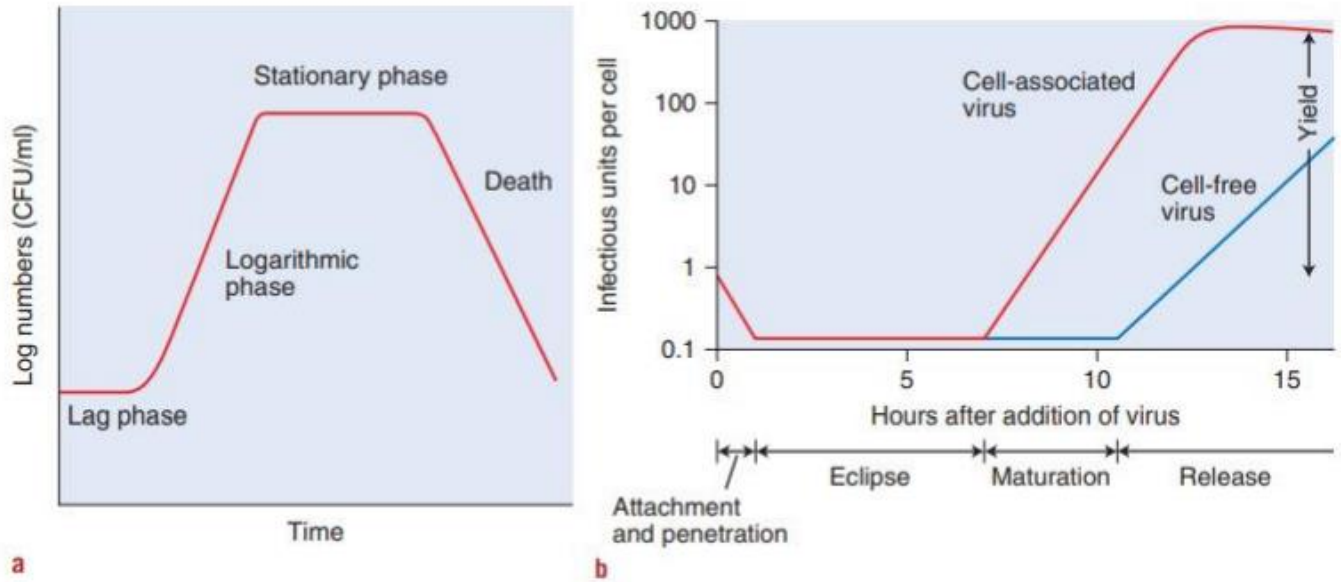


FIGURE 4-2 Typical bacterial growth versus a one-step growth curve of a naked virus. **(a)** Bacterial growth generally proceeds in a series of phases: lag, log (exponential growth in which the rate of multiplication is most rapid and constant), stationary, and death. Viruses require host cells for growth and reproduction. CFU/ml = colony forming units per milliliter. Modified from an illustration by H. Douglas Goff, Ph.D., University of Guelph. **(b)** Viruses are assembled from preformed "parts" when enough of the preformed parts have been made. Adapted from White, D. E., and Fenner, F. J. *Medical Virology,* Fourth Edition. Academic Press, 1994.