Structure &Multiplication of TMV and T_4 Bacteriophage

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PART -I

Tobacco Mosaic Virus (TMV): Structure and Replication

Introduction

- TMV is a plant virus which infects a wide range of plants, especially tobacco and other members of the family Solanaceae.
- The infection causes characteristic patterns, such as "mosaic"-like mottling(spots) and discoloration on the leaves (hence the name).
- TMV was the first virus that was crystalized in1935 by W.M.Stanley in the U.S.A.

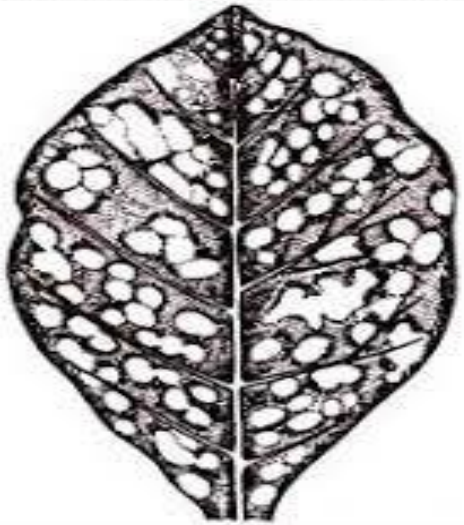

Fig. 1. Tabacco leaf showing the moasic symptoms.

STRUCTURE OF TMV

- TMV is a simple rod-shaped helical virus, consisting of centrally located single- stranded RNA (5.6%) enveloped by a protein coat (94.4%). The rod is considered to be 3,000 Å in length and about 180 Å in diameter.
- The protein coat is called ‘capsid’. R. Franklin estimated 2,130 sub-units, namely, capsomeres in a complete helical rod and 49 capsomeres on every three turns of the helix; thus there would be about 130 turns per rod of TMV.
- The diameter of RNA helix is about 80 Å and the RNA molecule lies about 50 Å inward from the outer-most surface of the rod.
- The central core of the rod is about 40 Å in diameter. Each capsomere is a grape like structure containing about 158 amino acids and having a molecular weight of 17,000 dalton as determined by Knight.

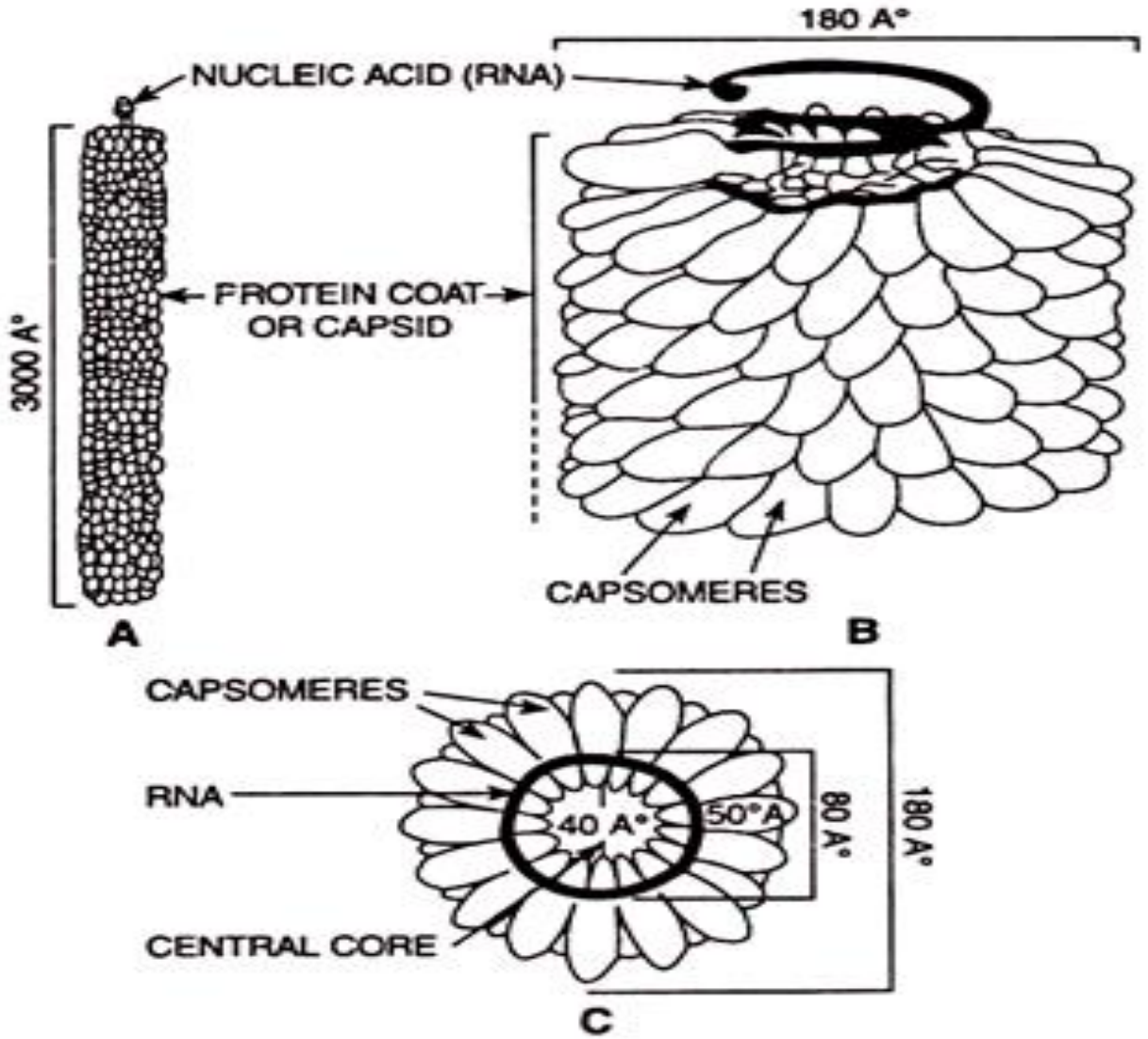

FIG. 13.20. Tobacco mosaic virus (TMV). **A.** surface view; **B.** an enlarged portion showing RNA-capsomere arrangement; **C.** view in section.

The Reproductive cycle of TMV consists of Following steps as-

- 1. Entry into host cell
- 2. Uncoating
- 3. Intracellular Development
- 4. Assembly (Maturation)
- 5. Release

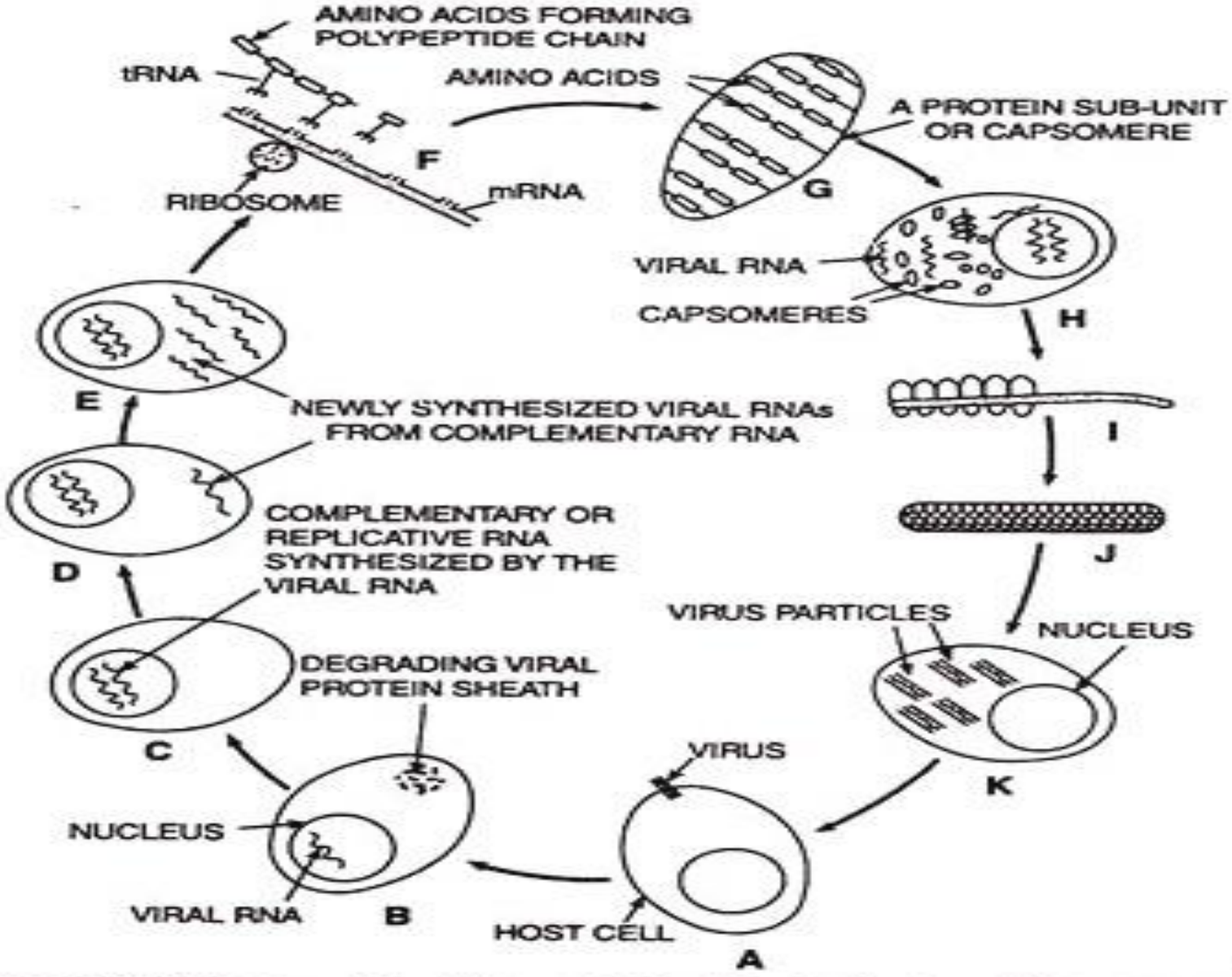

FIG. 13.21. Replication of TMV (diagrammatic). **A.** Virus particle entering inside the cell of the host plant; **B. & C.** Viral RNA enters inside the nucleus and synthesizes its complementary copy; **D. & E.** Complementary RNA synthesizes new viral RNA that comes in the cytoplasm; **F.** Polypeptide chain synthesis; **G., H. & I.** Arrangement of capsomeres around viral-RNA; **J.** Complete virus particle; **K.** Host cell containing many virus particles.

Life-Cycle (Replication) of Tobacco Mosaic Virus (TMV):

1.Entry into host cell :TMV can not directly enter the host cell. It requires damage to plant cells. It enters through breaches (Gap) in the cell wall.

2.Uncoating: It is a process in which capsid is removed and nucleic acid is released into the cell cytoplasm. Inside the host cell, the protein coat dissociates and viral nucleic acid becomes free in the cell cytoplasm.

3.Biosynthesis :The viral-RNA first induces the formation of specific enzymes called RNA polymerases the single-stranded viral-RNA synthesizes an additional RNA strand called replicative RNA.

- This RNA strand is complementary to the viral genome and serves as ‘template’ for producing new RNA single strands which is the copies of the parental viral-RNA.

REPLICATION STEPS

- The new viral-RNAs are released from the nucleus into die cytoplasm and serve as messenger-RNAs (mRNAs). Each mRNA, in cooperation with ribosomes and t-RNA of the host cell directs the synthesis of protein subunits.

4. Assembly: After the desired protein sub-units (capsomeres) have been produced, the new viral nucleic acid is considered to organize the protein subunit around it resulting in the formation of complete virus particle, the virion.

5. Release: No 'lysis' of the host cell, as seen in case of virulent bacteriophages, takes place. The host ells remain alive and viruses move from one cell to the other causing systemic infection. When transmitted by some means the viruses infect other healthy plants.

Symptoms associated with TMV infections

- **Stunting**.
- **Mosaic pattern** of light and dark green (or yellow and green) on the leaves.
- Malformation of leaves or growing points.
- Yellow streaking of leaves (especially monocots)
- Yellow spotting on leaves.
- Distinct **yellowing** only of veins.

Significance of TMV

- TMV can be a major problem because, unlike most other viruses, it does not die when the host plant dies and can withstand high temperatures.
- It can also survive in crop debris on the soil surface and infect a new crop planted on contaminated land.
- Ultimately, effective TMV management should be done by using virus-free seedlings or plants and implementing strict hygiene procedures.

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PART -II

Structure & Multiplication of T_4 Bacteriophage

Introduction

- Bacteriophages are viruses that parasitize bacteria. Bacteriophages were jointly discovered by Frederick Twort (1915) in England and by Felix d'Herelle (1917) at the Pasteur Institute in France.
- Felix d'Herelle coined the term “Bacteriophage”. Bacteriophage means to eat bacteria, and are called so because virulent bacteriophage can cause the compete lysis of a susceptible bacterial culture.
- They are commonly referred as “phage”. Phages are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery.
- They occur widely in nature and can readily be isolated from feces and sewage. There are at least 12 distinct groups of bacteriophages, which are very diverse structurally and genetically.

Examples of phages

- T-even phages such as T_2, T_4 and T_6 that infect E.coli .
- Temperate phages such as lambda and mu.
- Spherical phages with single stranded DNA such as PhiX174.
- Filamentous phages with single stranded DNA such as M13.

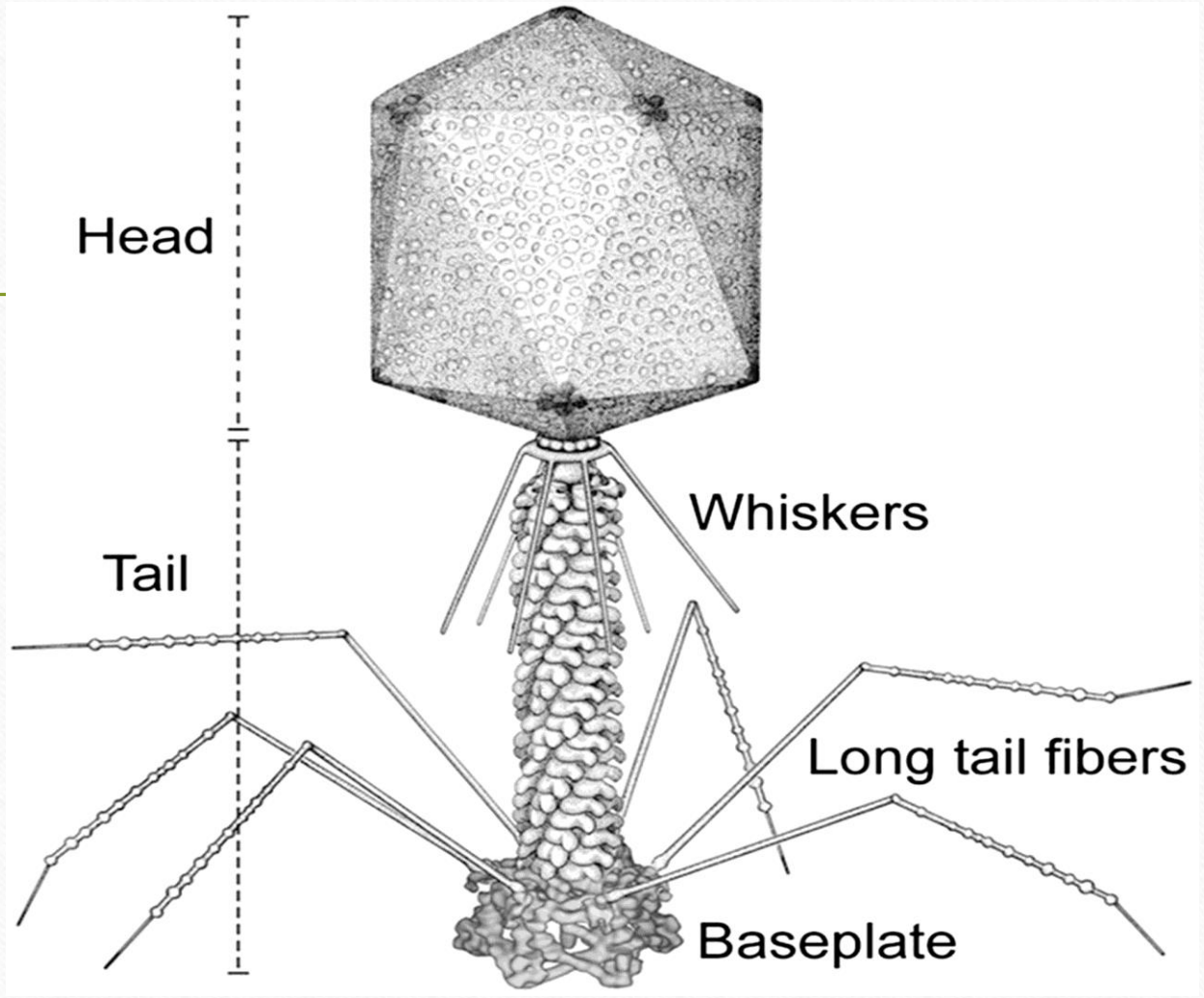
Important Characteristics of Some Bacteriophages:

Phage	Nucleic acid type	Head	Tail
T_1, T_3, T_5, T_7	Double stranded DNA	Hexagonal	short, non-contractile
T-even	Double stranded DNA	Polygonal	contractile tail
$\phi \times 174$	Single stranded DNA	Hexagonal	tail absent
f_2, R_{17}, f_r	Single stranded RNA	Hexagonal	tail absent

Characteristics of T_4 Bacteriophage

- Bacteriophage T_4 (phage T_4) is a virulent phage; it uses the metabolic machinery of the host cell to produce progeny viruses and kills the host in the process.
- Bacteriophage T_4 is a large & dsDNA virus.
- T_4 bacteriophage infect the colon bacillus, *Escherichia coli* bacteria.
- It does not have a probacteriophage form,
- The T_4 chromosome is approximately 168,800 base pairs long and contains about 150 characterized gene.

Structure of T_4 bacteriophage



Structure of T_4 Bacteriophage

- With the help of electron microscope, the morphology of the bacteriophage has been studied.
- The T even phages show complex symmetry. These viruses are generally tadpole shaped i.e., a ‘head’ followed by a ‘tail’.
- The head is hexagonal and like a prism in outline. This shape is also known as elongated icosahedron. It is 950 A° in length and 650 A° in width.

- The head has a 2-layered protein wall that encloses the double stranded DNA. The wall is 35 A° thick and is composed of about 2000 similar capsomeres. DNA is tightly packed in the head and is about 50 µ long.
- The tail has a complex structure and proteinaceous in nature. It is made up of a cubical, hollow, cylindrical core.
- This core is 800 A° long, 70 A°in diameter and has 25 A° wide central canal. This core is surrounded by a contractile sheath. The sheath is 165 A° in diameter.

The Replication cycle of virulent phage is divided into five sequential phases :

- 1. Adsorption
- 2. Penetration
- 3. Synthesis of phage components
- 4. Maturation and assembly
- 5. Release of progeny viruses

1.Adsorption

- The phage particles come into contact by random collision and a phage attaches to a specific receptor site on the host cell membrane by means of tail fibres.
- Adsorption occurs within minutes of contact.

2. Penetration

- After adsorption of phage to bacteria, the tail sheath of phage contracts and the base plate and tail fibres are held firmly against the bacterial cell.
- As a result the hollow core is pushed downwards through the already weakened part of cell-wall caused by a phage muramidase present on the base plate.
- The viral nucleic acid passes down the hollow tube similar to injection through a syringe. The tube does not penetrate the cell wall and the empty head (capsid) and tail remain outside as shell or ghost.

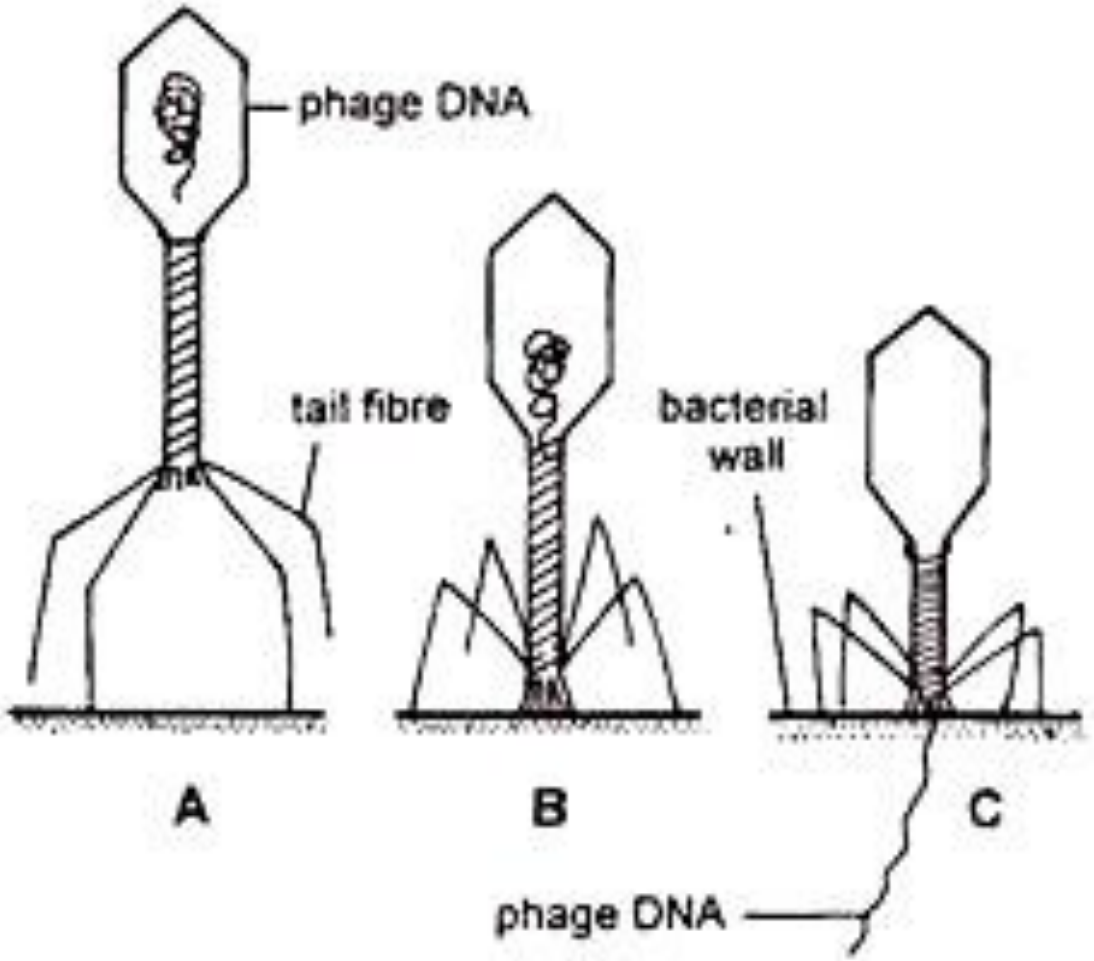


Fig. 2. (A-C). Process of bacteriophage infection : A. Infection in which the phage attaches to the host baterium, (B-C). The nucleic acid core is emptied into the bacterial cell.

3. Synthesis of phage components:

- After the release of nucleic acid into the bacterial cell, the viral genome directs the biosynthetic machinery of host cell to shut down the normal cellular metabolism and to produce components of new virus particles.
- This is effected by synthesis of specific enzymes (called early proteins) necessary for synthesis of phage components.
- Subsequently, late protein, subunits of phage head and tail appear. Some of the components appear in the nucleus and others in the cytoplasm of host cell.

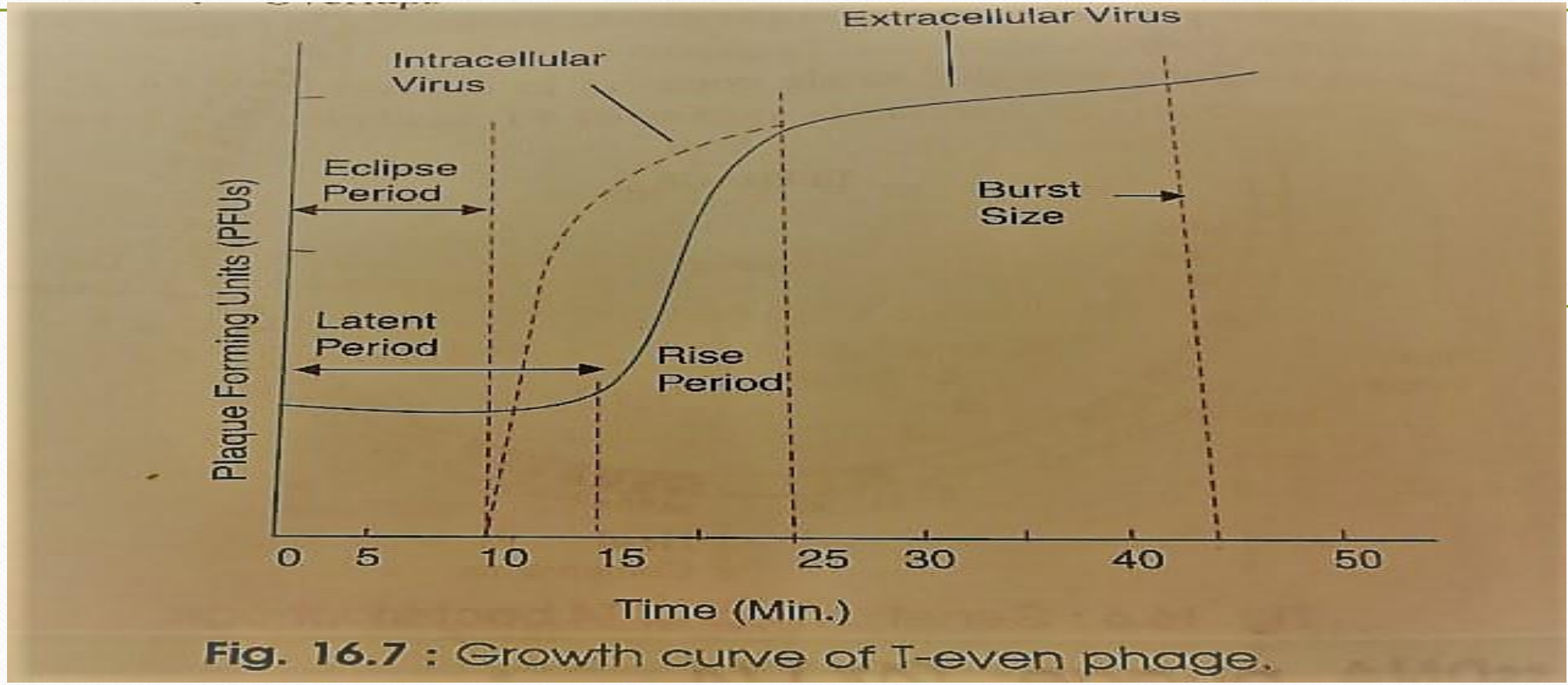


Fig. 16.7 : Growth curve of T-even phage.

During the first 10 minutes after infection of the phage DNA, no phage could be recovered from the infected bacterium. This time interval is called as eclipse period. During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell.

4. Maturation and Assembly:

- During maturation there is spontaneous assembly of phage DNA head protein and tail protein of phage.
- Each component of phage nucleic acid acquires a protein coat and finally the tail structures are added forming a virion (infective virus particle).

5. Release

- The progeny phages are rapidly released by the lysis of the infected bacterium. Phage enzyme (probably muramidase) weakens the cell wall during replication of phage.
- As a result the infected bacterium assumes a spherical shape. Muramidase concentration rises in the late stage of growth cycle, which acts on the already damaged cell-wall causing lysis of cell with release of progeny phage.

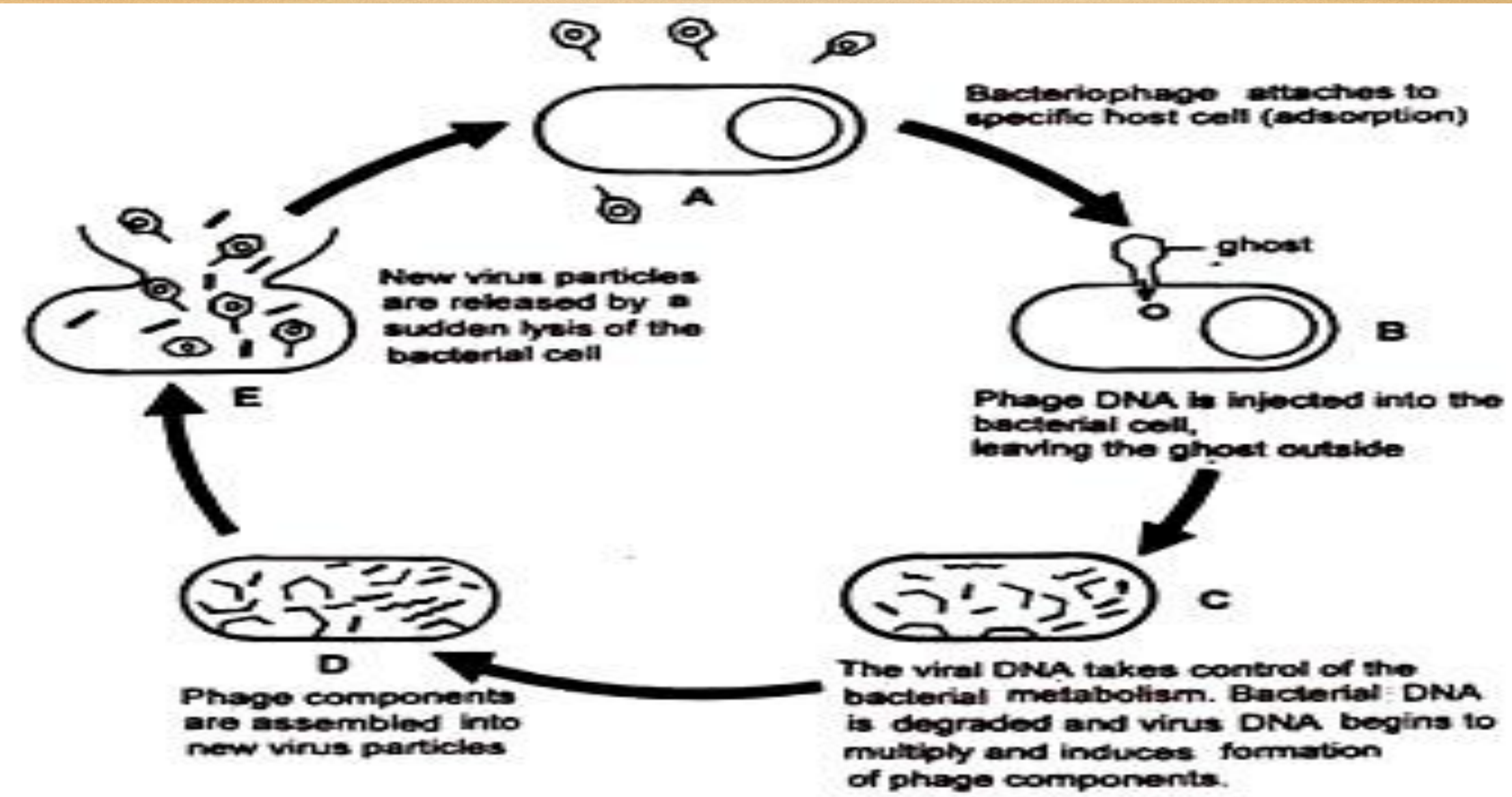


Fig. 3. (A-E). The lytic cycle of bacteriophage.

Biological Importance of Bacteriophages:

- Bacteriophages have been used in prophylaxis and medical treatment against several pathogenic bacterial diseases e.g., cholera, plague, dysentery, enteric fever etc.
- They are also used in the diagnosis of certain infections like plague, cholera etc.
- Bacteriophages feed on pathogenic bacteria present in polluted water. So, they can also be used as scavengers.
- In many cases bacteriophages determine the micro-flora of the soil. Thus, they play an important role in agriculture.
- In space microbiology, lysogenic cultures are used as radiation detectors and are used in USSR spaceship Vostok 2.
- Bacteriophages are very harmful during the process of manufacturing of antibiotic and milk products because they kill beneficial bacteria by their lysogenic activity.

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