BHARATHIDASAN UNIVERSITY

Tiruchirappalli-620024

Tamil Nadu, India,

Programme: M.Sc., Biomedical Science

Course Title : Medical Virology

Course Code : BM59C19MV

Unit-II

ΦX174 Virus

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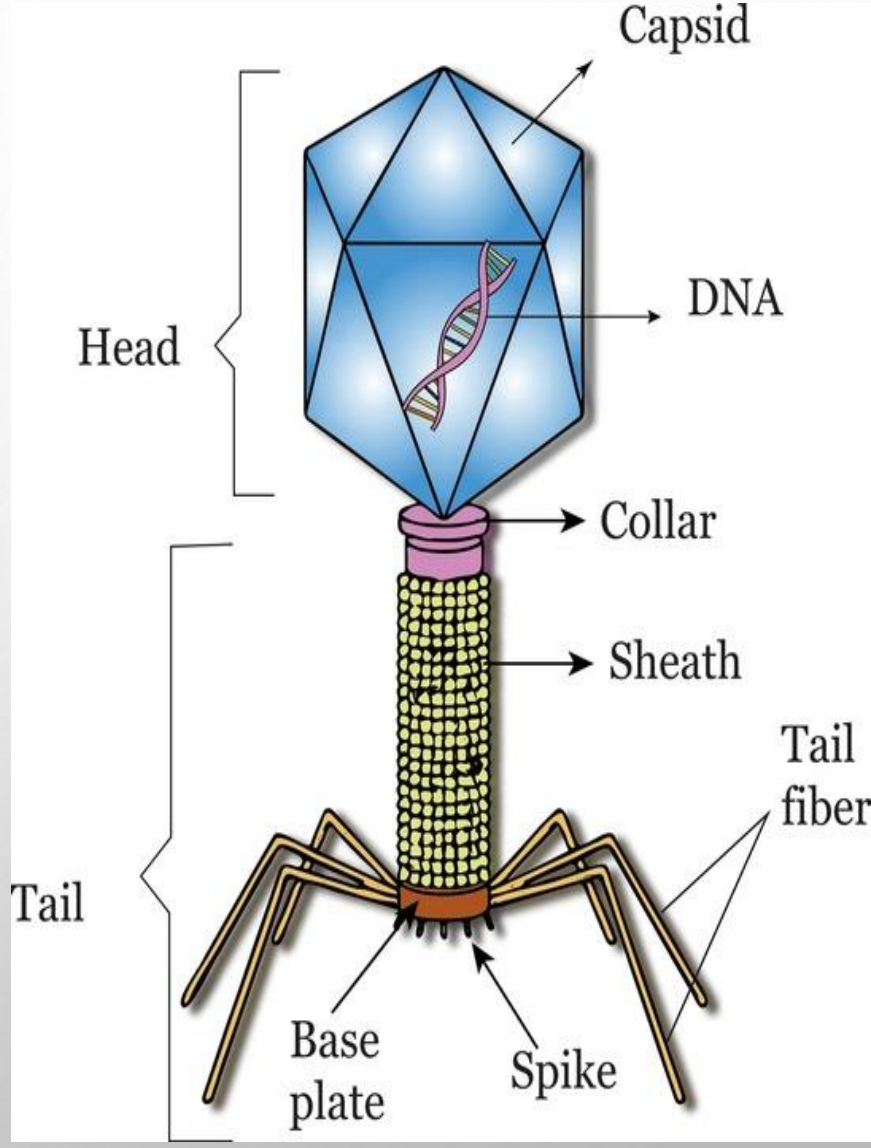
Bacterial virus:

ΦX174

BACTERIOPHAGE

- Type of virus that infects bacteria (phage-to eat).
- Replicate only in bacterial cells.
- Ubiquitous.
- With the help of electron microscopy, scientists studied the detailed visualisation of hundreds of phage types , some of which appears to have head,legs and tails.
- Phages are non-motile and depend upon Brownian motion to reach their targets.



DISCOVERY OF BACTERIOPHAGE

Fredrick Twort **1915**

Discovered an agent that kill bacteria. Research has been cut short-world war 2.

Félix d'Hérelle **1917**

An invisible, antagonist microbe of the dysentery& named the term.

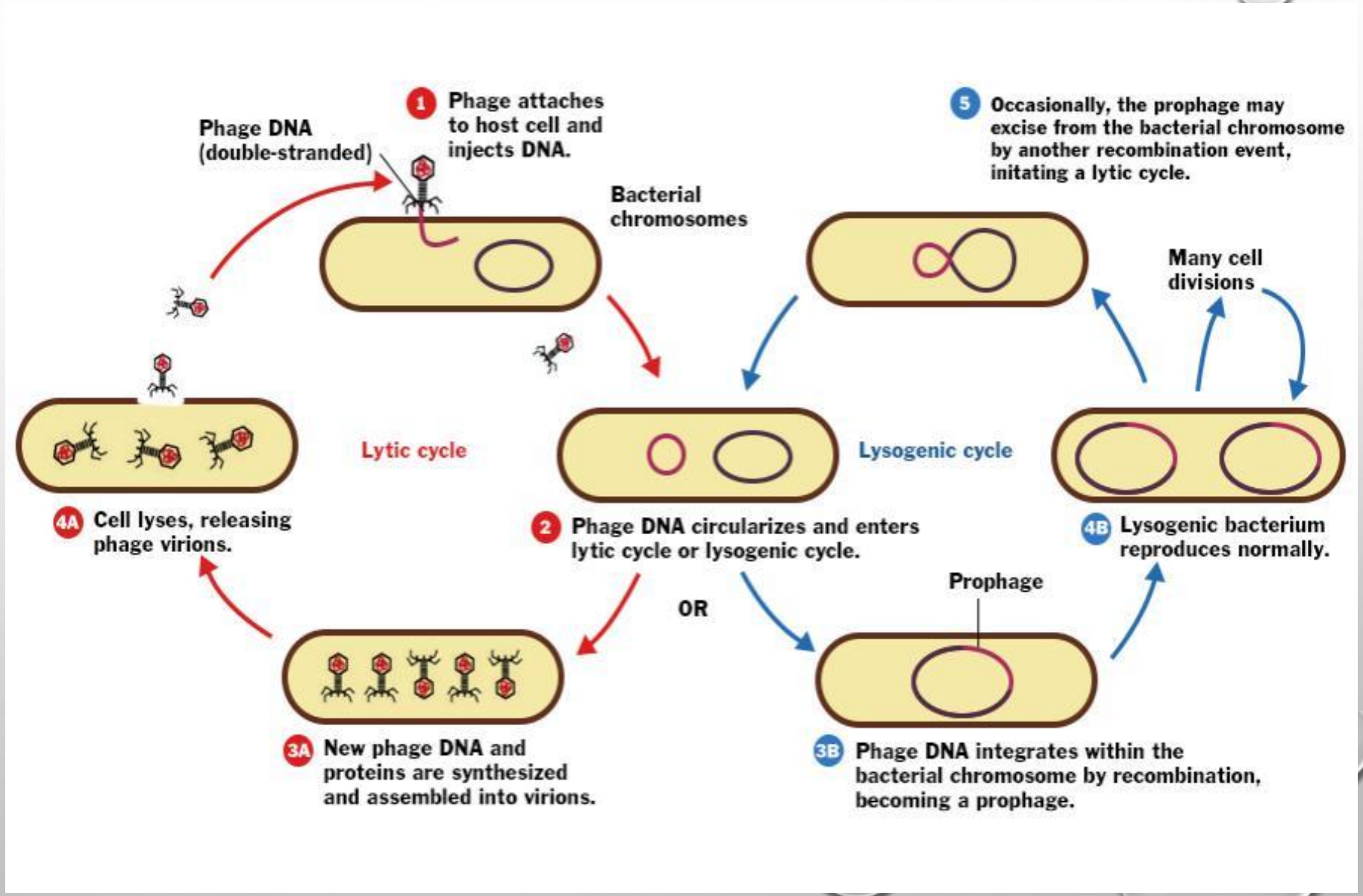
Ernest Hanbury Hankin **1896**

Report on Ganges and yamuna river- seemed to have some sort of antibacterial property against Cholera.

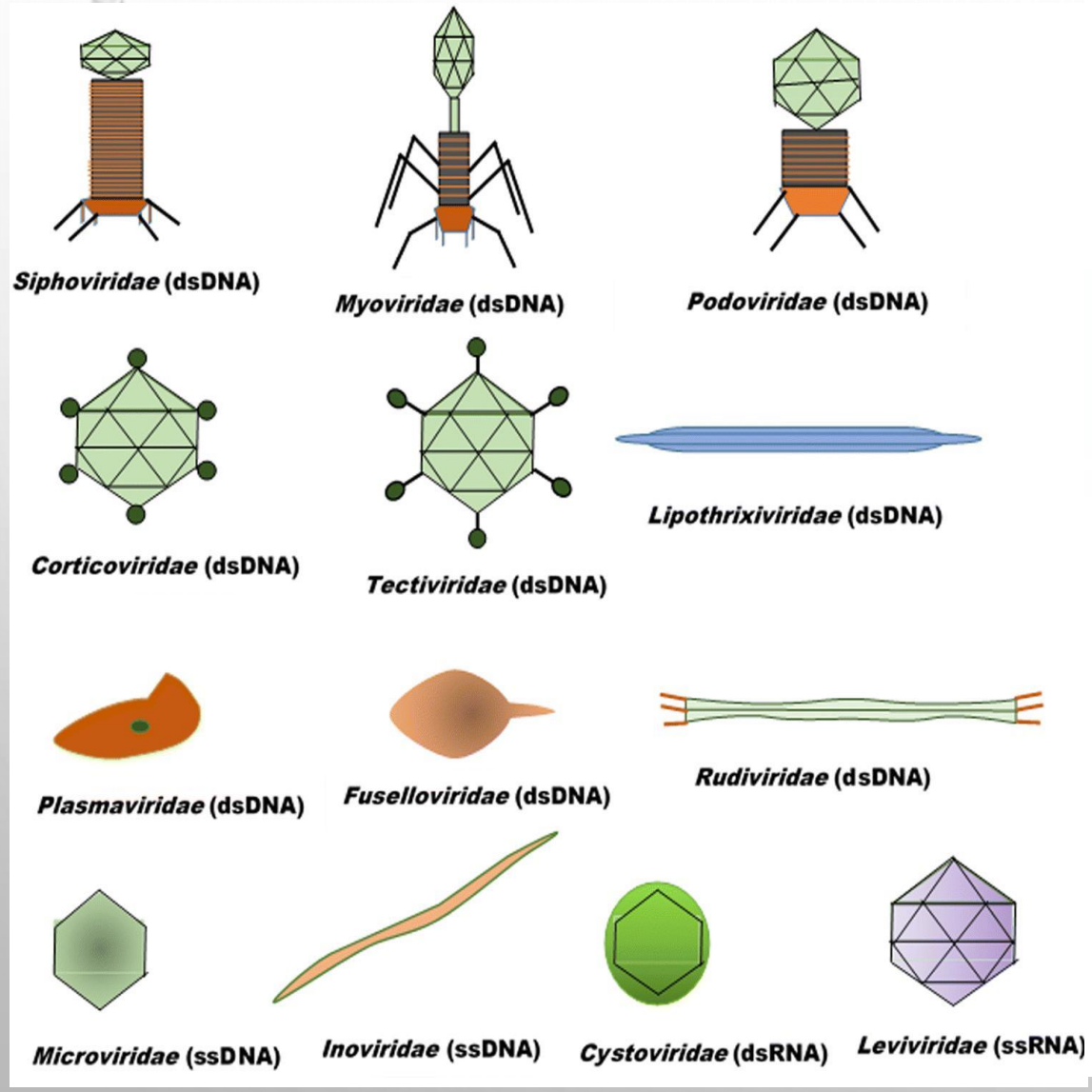
TYPES OF BACTERIOPHAGE

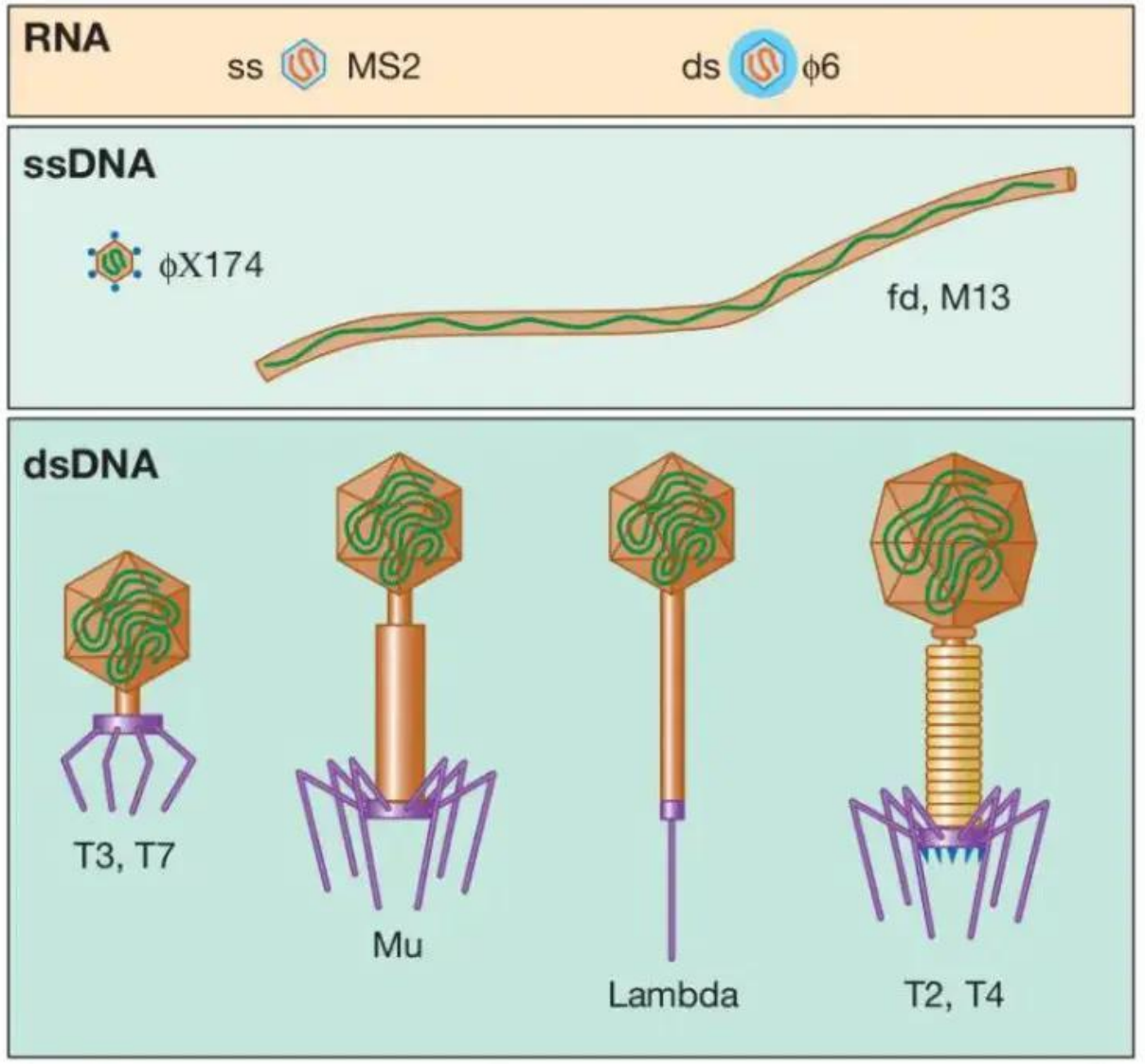
1.Virulent phages- **lytic cycle**

2.Temperate phages- **lysogenic cycle**



STRUCTURE OF BACTERIOPHAGE



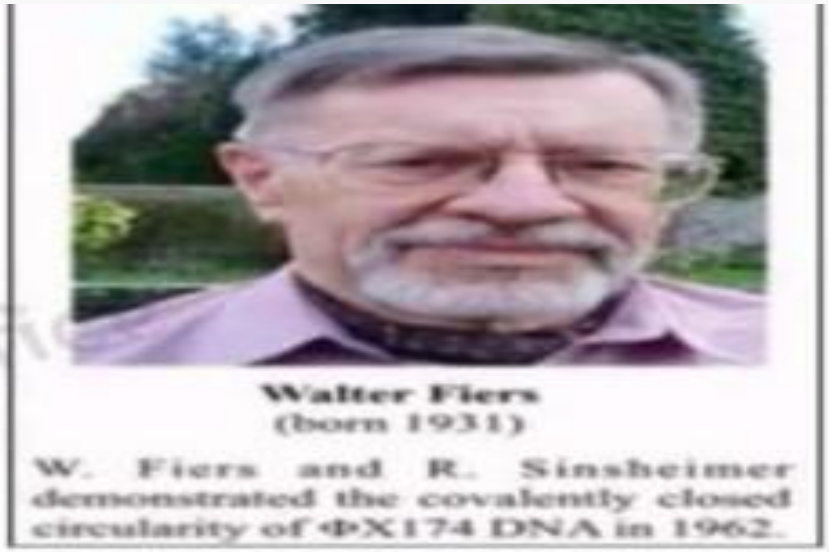


PHAGE THERAPY

ΦX174

Frederick Sanger
(1918 - 2013)

Fred Sanger sequenced the genome of ΦX174 bacteriophage in 1977.



Walter Fiers
(born 1931)

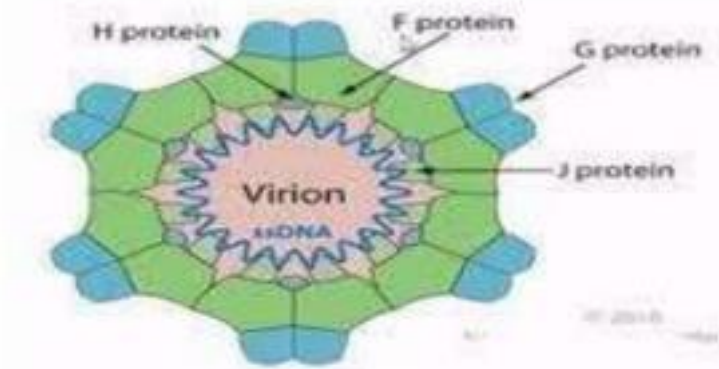
W. Fiers and R. Sinsheimer demonstrated the covalently closed circularity of ΦX174 DNA in 1962.

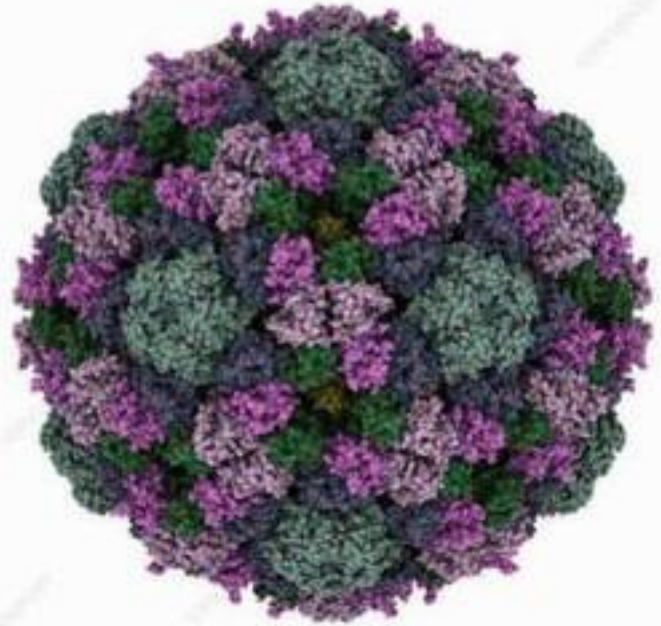
- It is a bacteriophage infects the bacterium *E.coli*
- In **1962**, **Walter fiers** and **Robert Sinsheimer** – demonstrated the physical, covalently closed circularity of ΦX174 DNA.
- The first DNA-based genome to be sequenced.This work was completed by **Frederick sanger** in **1977**.
- Studies of phage replication led to discovery of Rolling circle replication.
- Family: Microviridae provided the first evidence of overlapping genes.

Taxonomical arrangement

Morphology:

- The genome consists of circular ssDNA.
- Genome size ranges from 4.6 to 6.1 kb.
- The capsid is icosahedral (i.e.spherical).
- Diameter is 25-27nm
- Tailess icosahedral bacteriophage





Genome organization

- It contains 5386 nucleotides.
- These nucleotide encodes 11 proteins.
- Proteins A, A*, B, C, D, E, F, G, H, J, K are encoded.
- The 11 proteins encoded by phi X 174 DNA range in the size from the A protein which contains 513 amino acids, to the J protein, which contain only 38.
- The 11 protein together contain a total of 1986 amino acids.
- It is encoded with 10 genes but generates 11 proteins.
- This is beacause of overlapping gene.
- The gene is organized in such a way if one gene ends in a particular position, the succeeding gene starts with few nucleotide overlapping the terminal region of the first gene.
- This is called overlapping genes, where reading of genes are overlapped in their sequence.

- For example, the sequence

  ...GAGCCGCAACTTC...

Can be read in three different rading frames-

...GAG CCG CAA CTT C ... which encodes ....

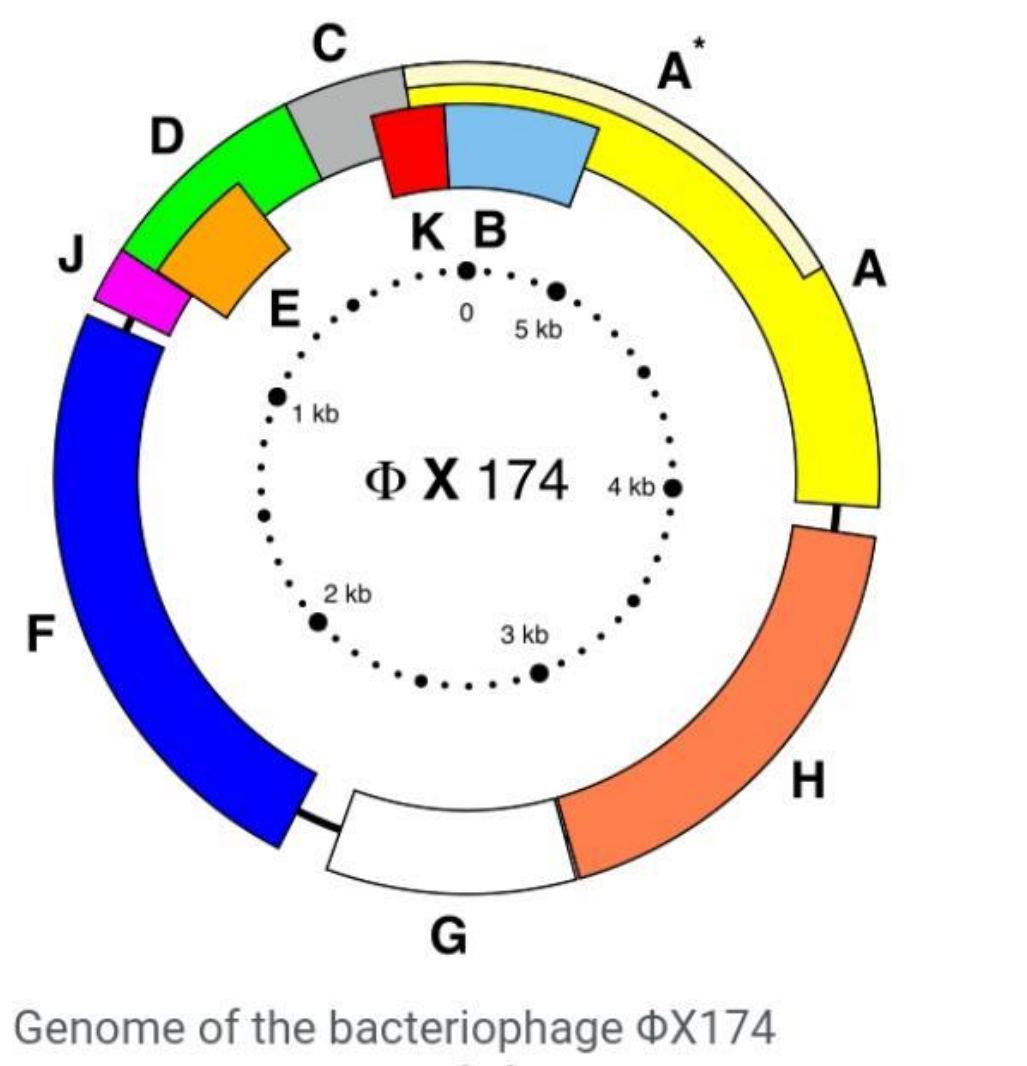
...Glu-Pro- Gln-Leu...

...G AGC CGC AAC TTC... which encodes....

...Ser-Arg-Asn-Phe...

...GA GCC GCA ACT TC...which encodes ....

...Ala-Ala-Thr...



Genome of the bacteriophage ΦX174

A- Replication initiation

A*- Termination of host DNA replication

B- Capsid morphogenesis

C- Phage maturation

D- Phage assembly

E- Host cell lysis

F- Major coat protein

G- Major spike protein

H- Minor spike protein

J- Core protein

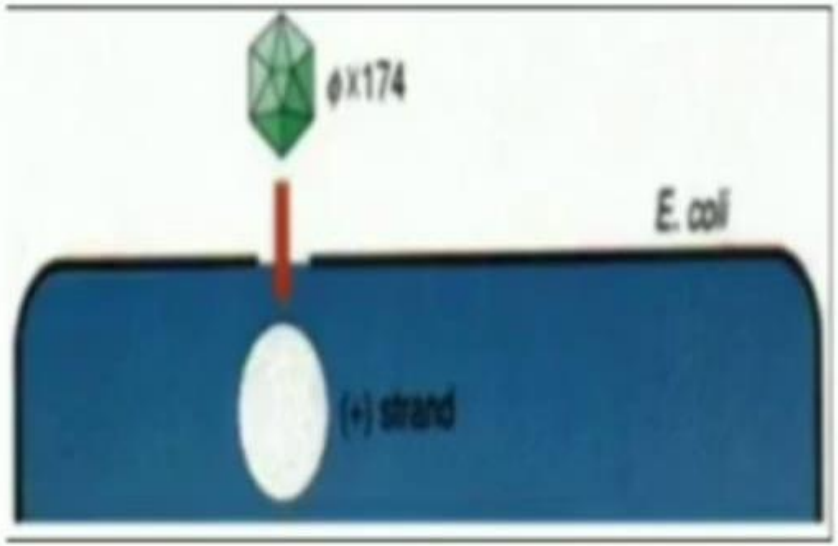
K- Growth of phage

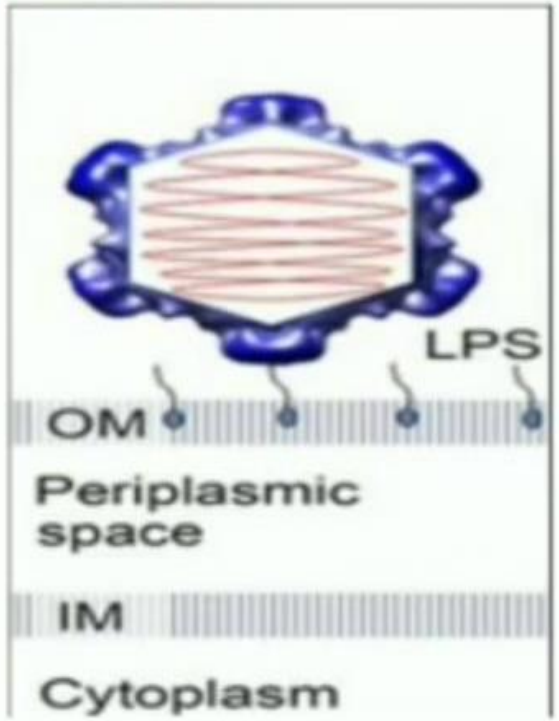
LIFE CYCLE

1.Attachment of bacteriophage
2. Entry of bacteriophage into cytoplasm
3. Replication of bacteriophage
4.Assembly of bacteriophage
5. Lysis of host cells

1.Attachment of bacteriophage:

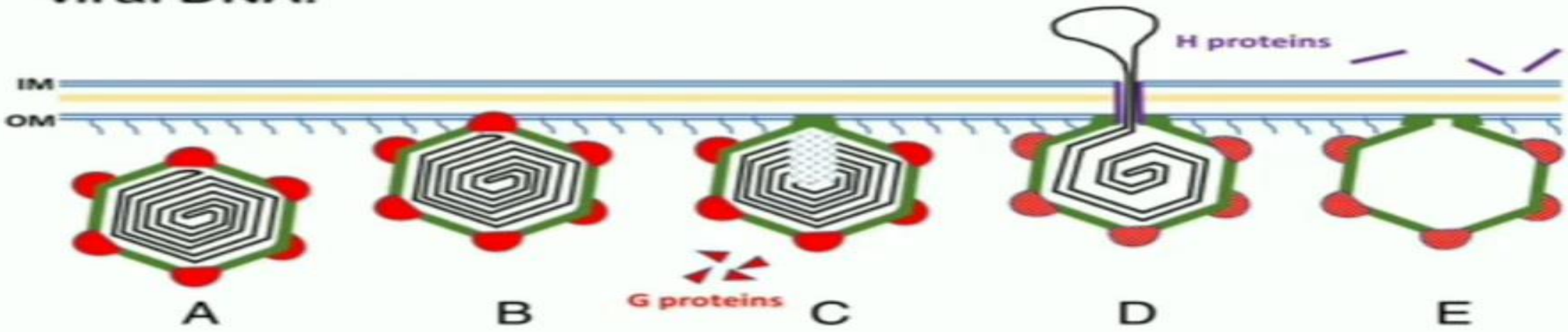
Phage φX174 recognizes the receptor lipopolysaccharide in the outer membrane of rough strains of *Enterobacteriaceae* , such as *E.coli* and *Salmonella typhimurium* , by the minor coat protein H.





2.Entry of Phage phiX174 into cytoplasm:

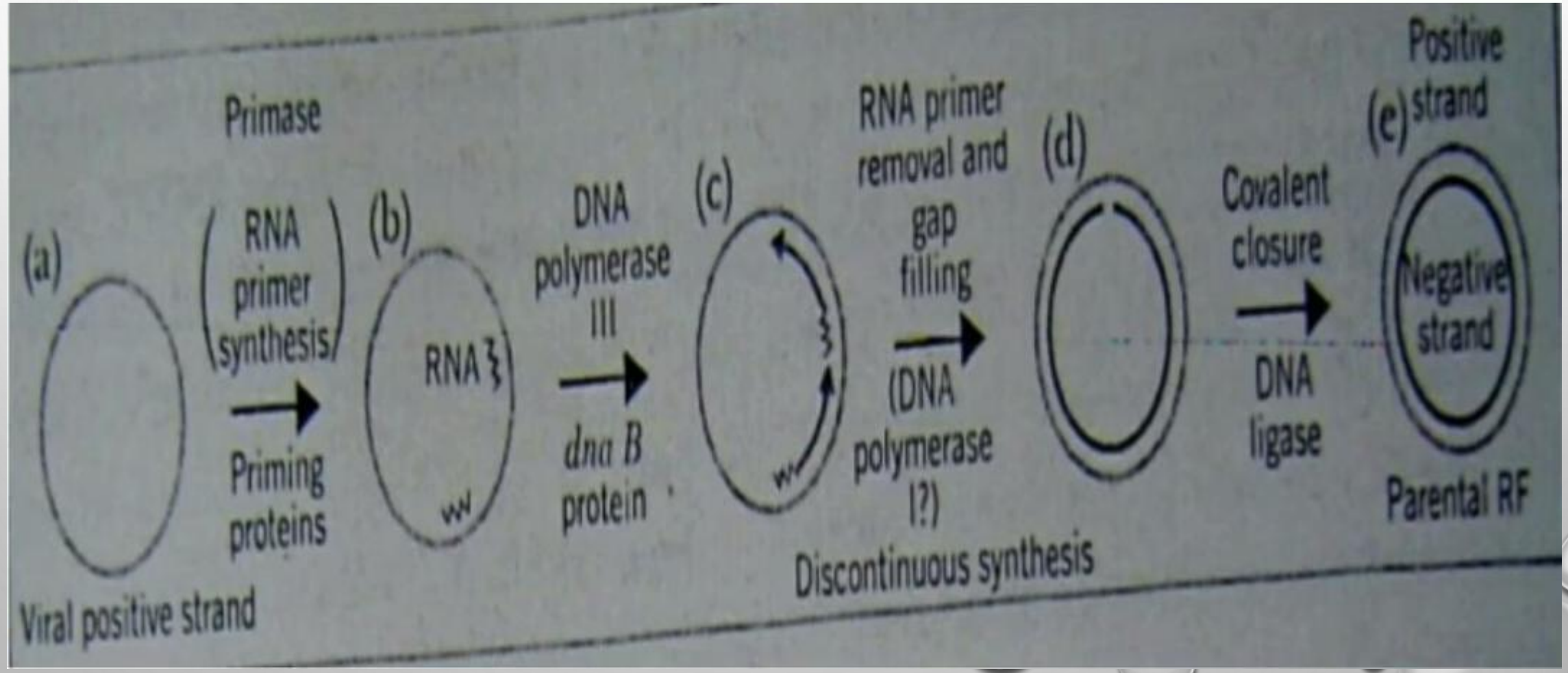
- The terminal spike protein, gp H spans the capsid.The outer part recognizes the LPS receptor. The inner part of the H protein is responsible for the injection of genome into the host cell.
- Atleast one H protein enters into the host cell with the viral DNA.



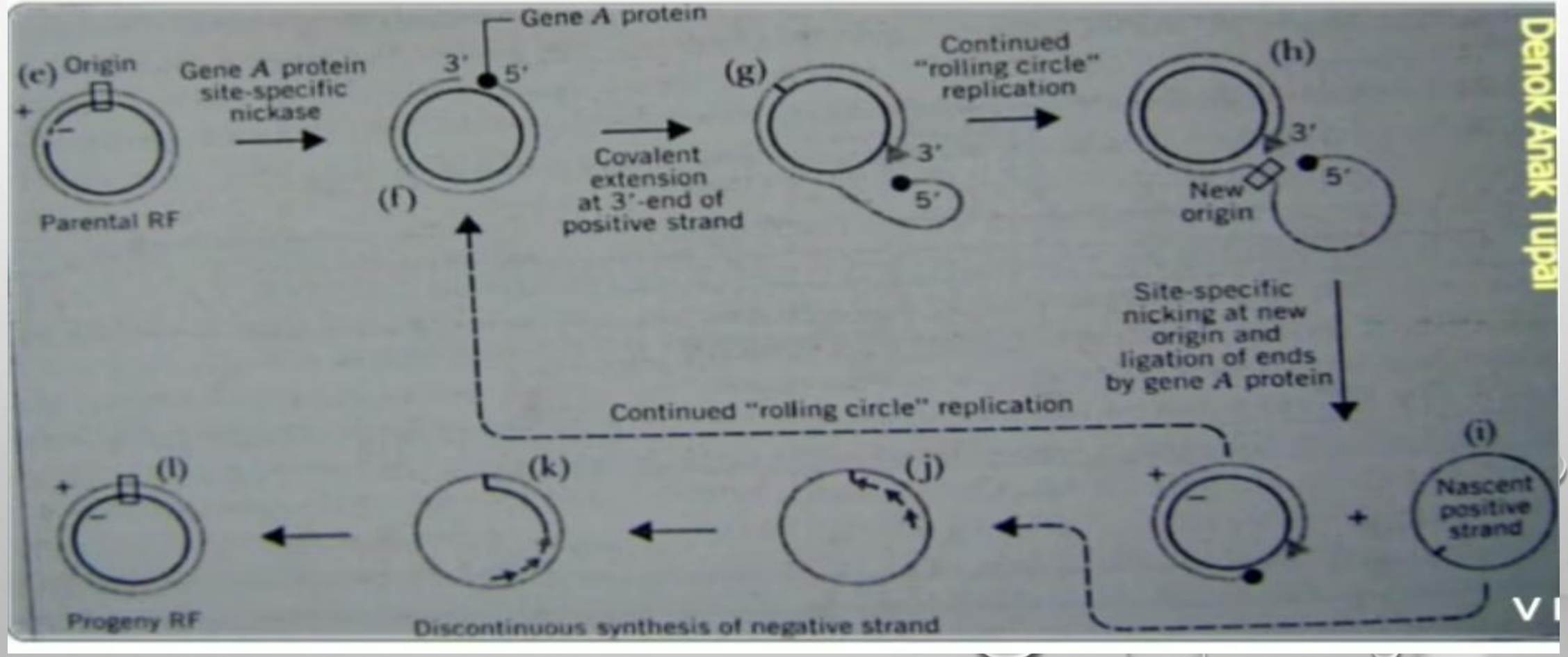
3.Replication of phiX174 genome:

Stages	Time (min)	Events
1. SS ⟶ RF	0-1	SS ⟶ parental RF
2. RF ⟶ RF	1-20	Parental RF ⟶ approx 60 progeny RF
3. RF ⟶ SS	20-30	RF ⟶ SS(+) DNA

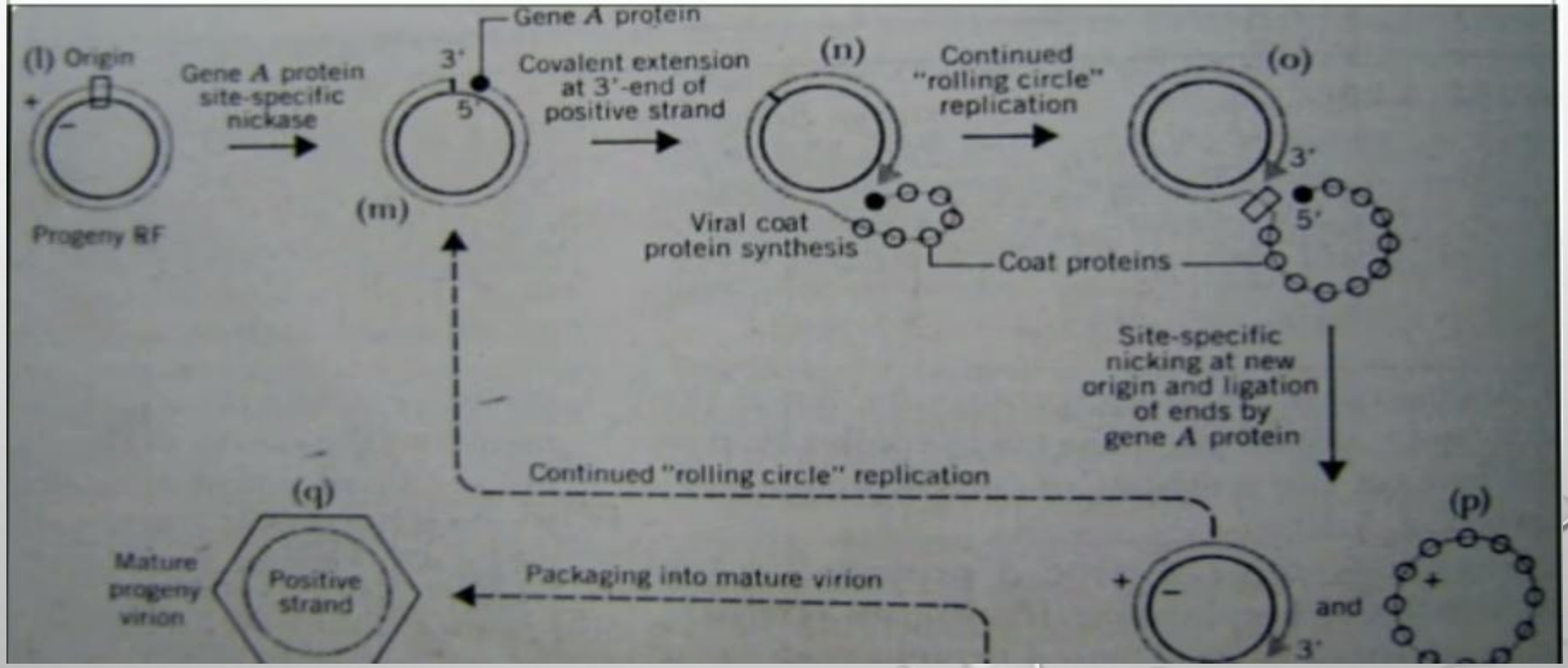
Stage 1: Synthesis of (-) strand complementary to the (+) strand to form the replicative form (RF) by host enzyme.



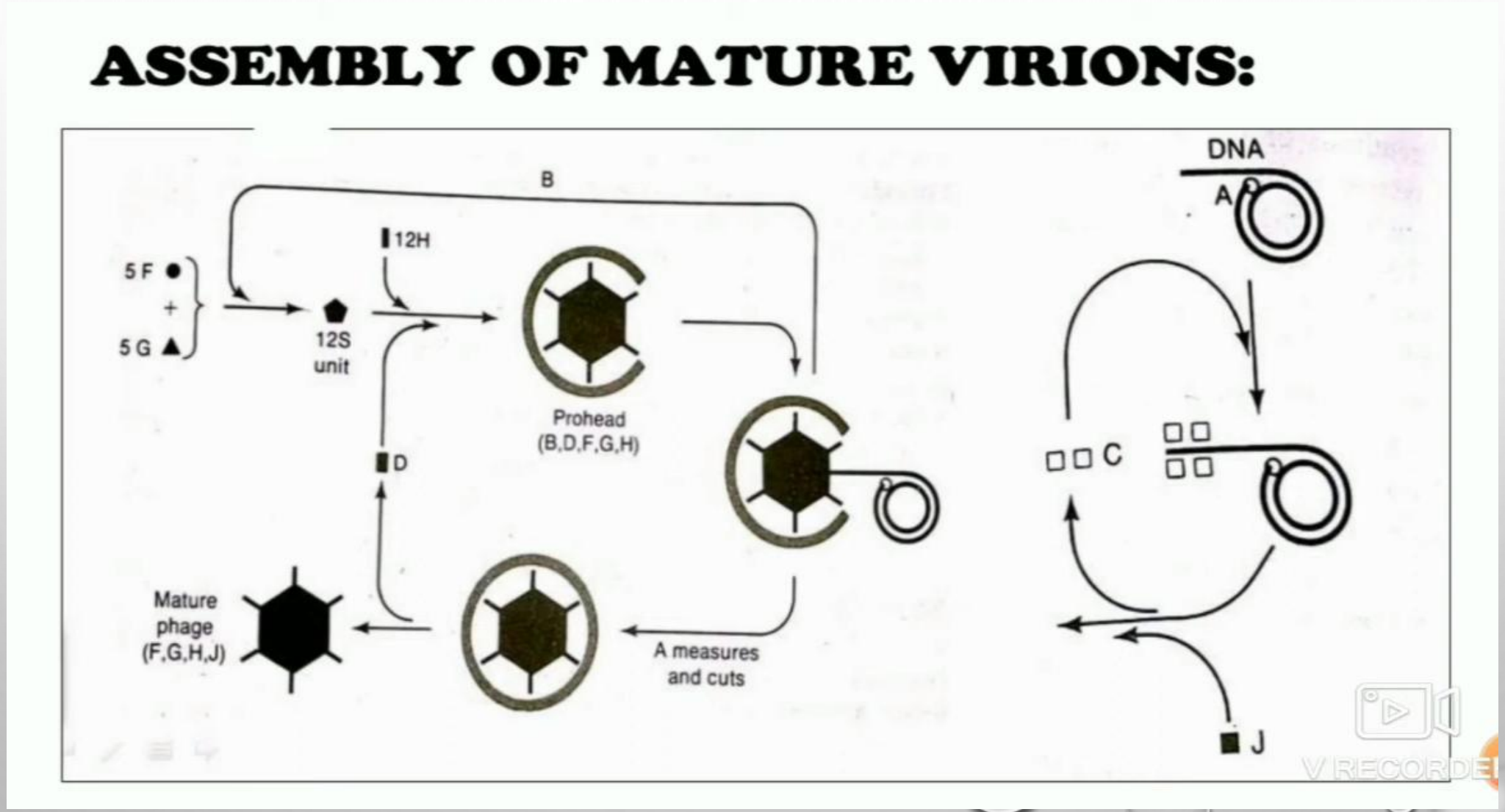
Second stage:
Replication of the RF involves rolling circle replication and requires phage encoded protein A .



Stage 3:
Formation of (+) strand DNA

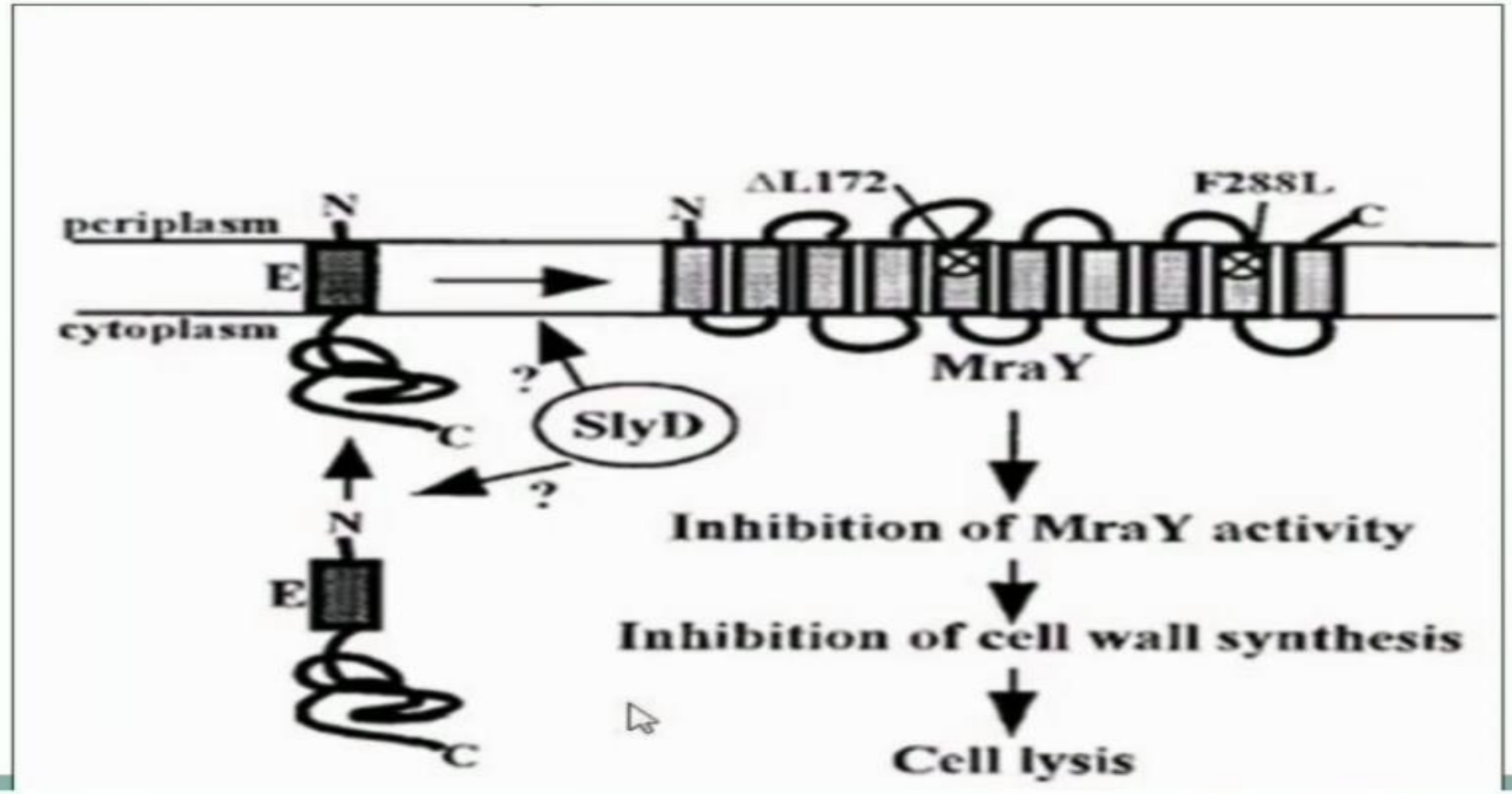


4.Assembly of bacteriophage:



5.Lysis of host cells:

RELEASE OF MATURE VIRIONS (LYSIS OF HOST CELL):



APPLICATIONS

- ***Genetic Research:*** first DNA-based genome to be sequenced, which was a significant milestone in molecular biology. Its genome has been used as a model for studying DNA replication, transcription, and the genetic code.
- ***Molecular Cloning:*** used as a vector in cloning experiments. Its small, circular genome and well-understood genetics make it a useful tool for introducing and manipulating genes in bacterial cells.
- ***Phage Display:*** employed in phage display technology, which allows researchers to study protein interactions and functions.
- ***Synthetic Biology:*** serves as a model for designing and constructing new biological systems and circuits.
- ***Viral Evolution Studies:*** Its evolution and interactions with bacterial hosts are studied to understand viral evolution and host-pathogen dynamics.

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• Kramer, N. E. (1990). "Bacteriophage Life Cycles: An Overview." In Advances in Microbial Physiology, Vol. 31, pp. 1-52. Academic


THANK YOU !