Structure and Replication of ϕX174

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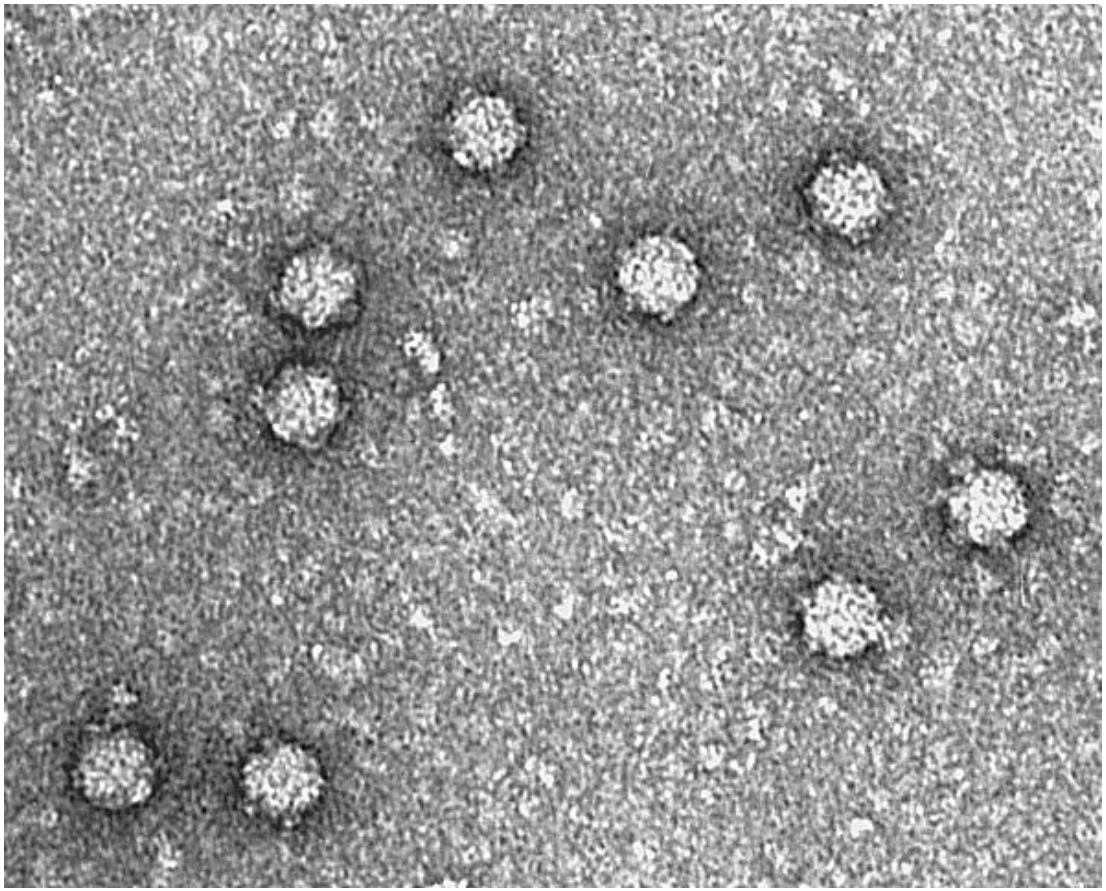
Outline

- Classification
- Morphology of ΦX174
- **Genetic Map of ϕX 174**
- **Overlapping Gene**
- **The Replication Cycle of ϕX174**

Classification

- Phi X 174 is a virus that infects the bacterium E. coli Hence phi X 174 is a bacteriophage.
- Group II (ssDNA)
- Family : Microviridae
- Genus : Microvirus
- Species : φX174

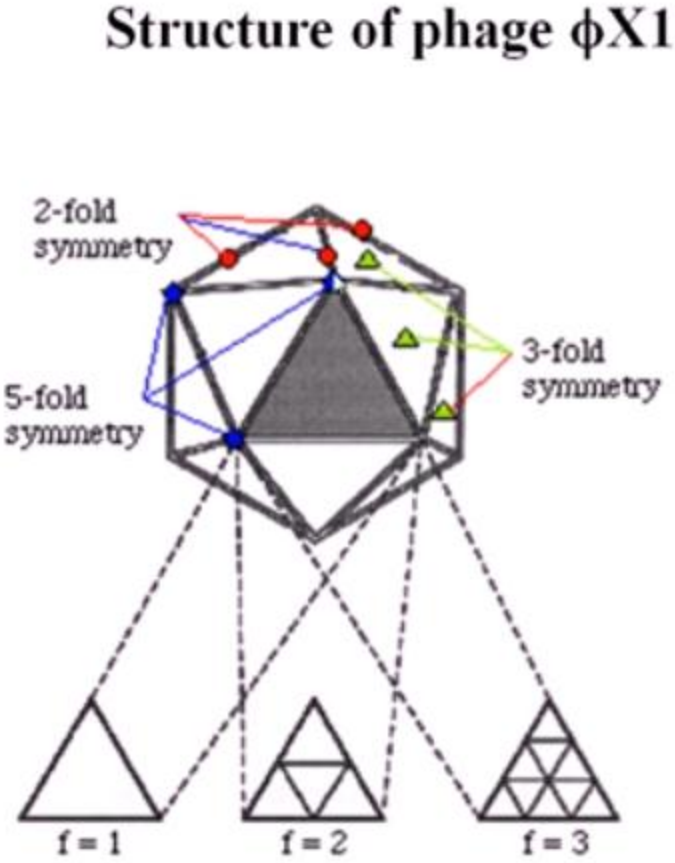
Electron Micrograph of ΦX174

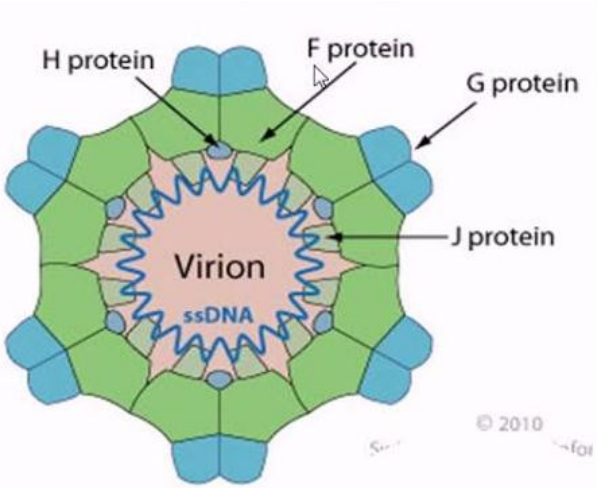


Morphology of ΦX174

- The capsid is icosahedral i.e. spherical.
- The genome consists of **circular ssDNA**.
- Genome size ranges from 4.6 to 6.1 kb.
- Diameter is 25 - 27 nm.
- Tailless icosahedral bacteriophage.
- Microviridae provided the **first evidence of overlapping genes.**
- Studies on replication of these phages led to the discovery of rolling circle replication

- **60 molecules** of major coat **protein F**(48.4KD) form the capsid (25-27nm in diameter).
- **5 molecules of G protein** (19.0KD) and 1 molecule of H protein (35.8KD) form spikes.
- **Protein J (4.0KD) binds to the phage genome for condensation of DNA during packeging**.





Frederick Sanger
(1918 - 2013)

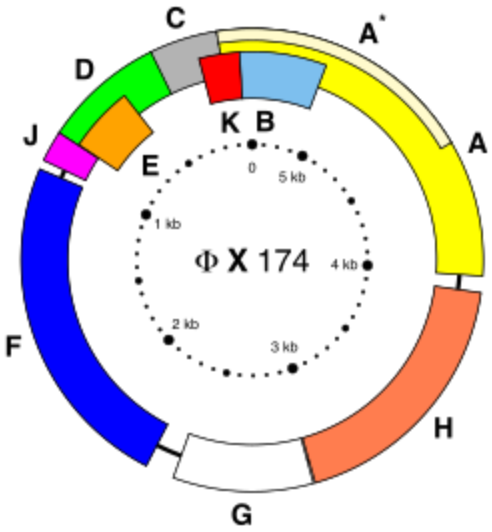
Fred Sanger sequenced the genome of ΦX174 bacteriophage in 1977.

Walter Fiers
(born 1931)

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

Genetic Map of ϕX 174

The genome is a circular ssDNA of 5386 nucleotides, coding for 11 proteins



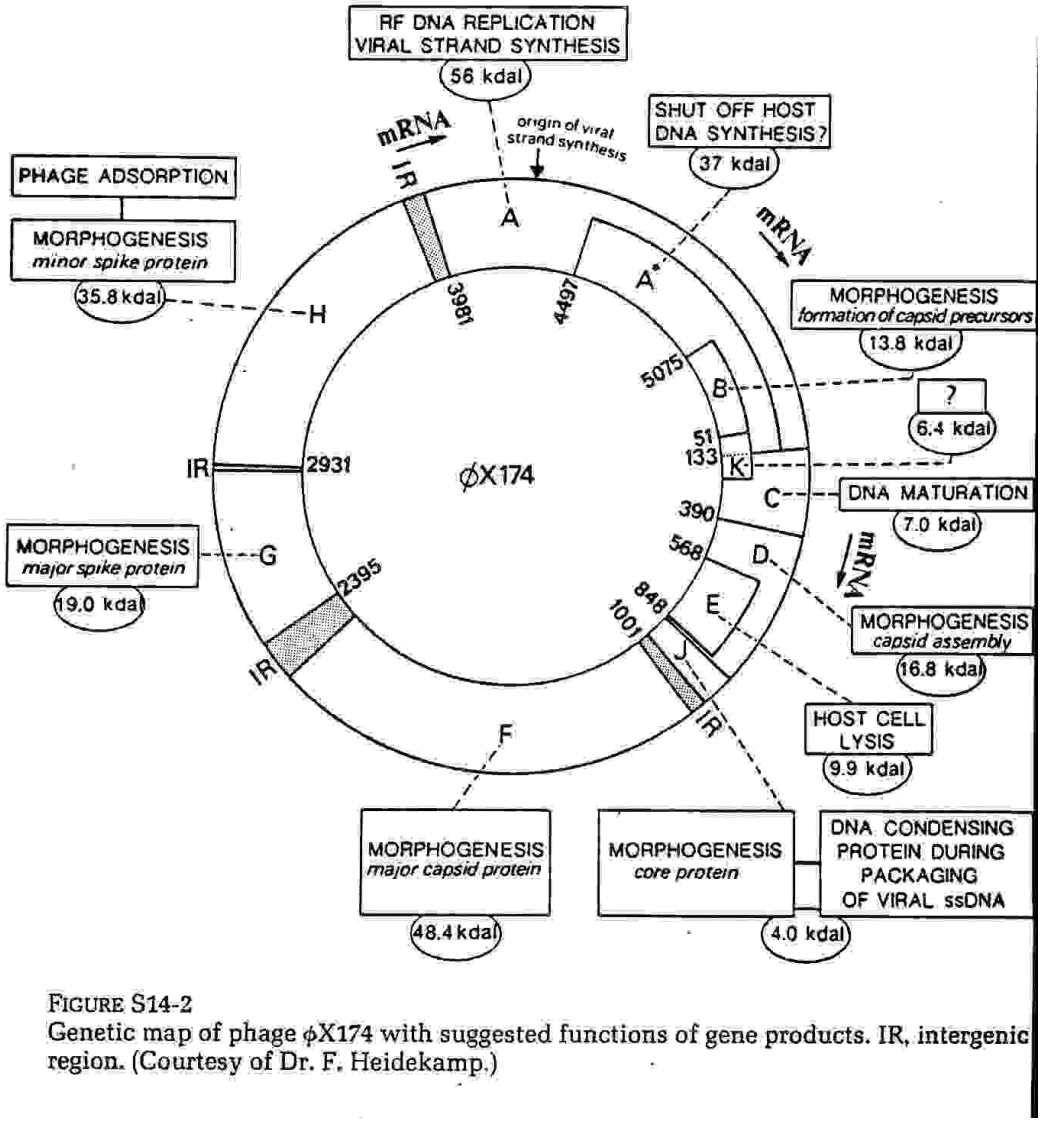


FIGURE S14-2
Genetic map of phage ϕX174 with suggested functions of gene products. IR, intergenic region. (Courtesy of Dr. F. Heidekamp.)

- 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 because of overlapping gene.

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.

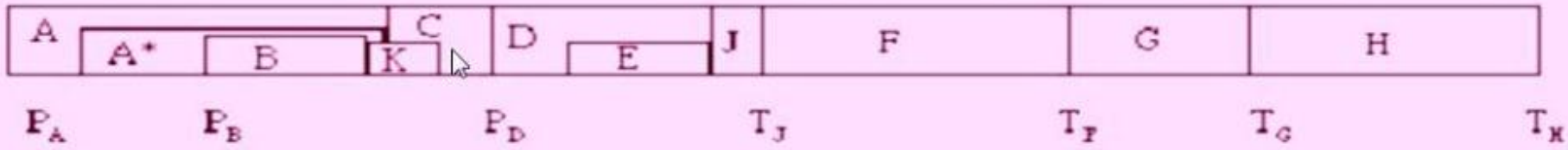
For example,

the sequence **…GAGCCGCAACTTC…** Can be read in three different reading 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…



- Gene A (viral genome replication) contains an internal translation initiation site to encode protein A* (shutting down host DNA synthesis).
- B (capsid morphogenesis) is encoded within A in different reading frame.
- K is at the end of gene A and extend into gene C (DNA maturation).
- E (host cell lysis) is totally within D.
- The termination codon of D overlaps initiation codon for J.

A Replication initiation,

A* Termination of host

DNA replication,

B Capsid morphogenesis,

C Phage maturation,

D Phage assembly,

E Host cell lysis

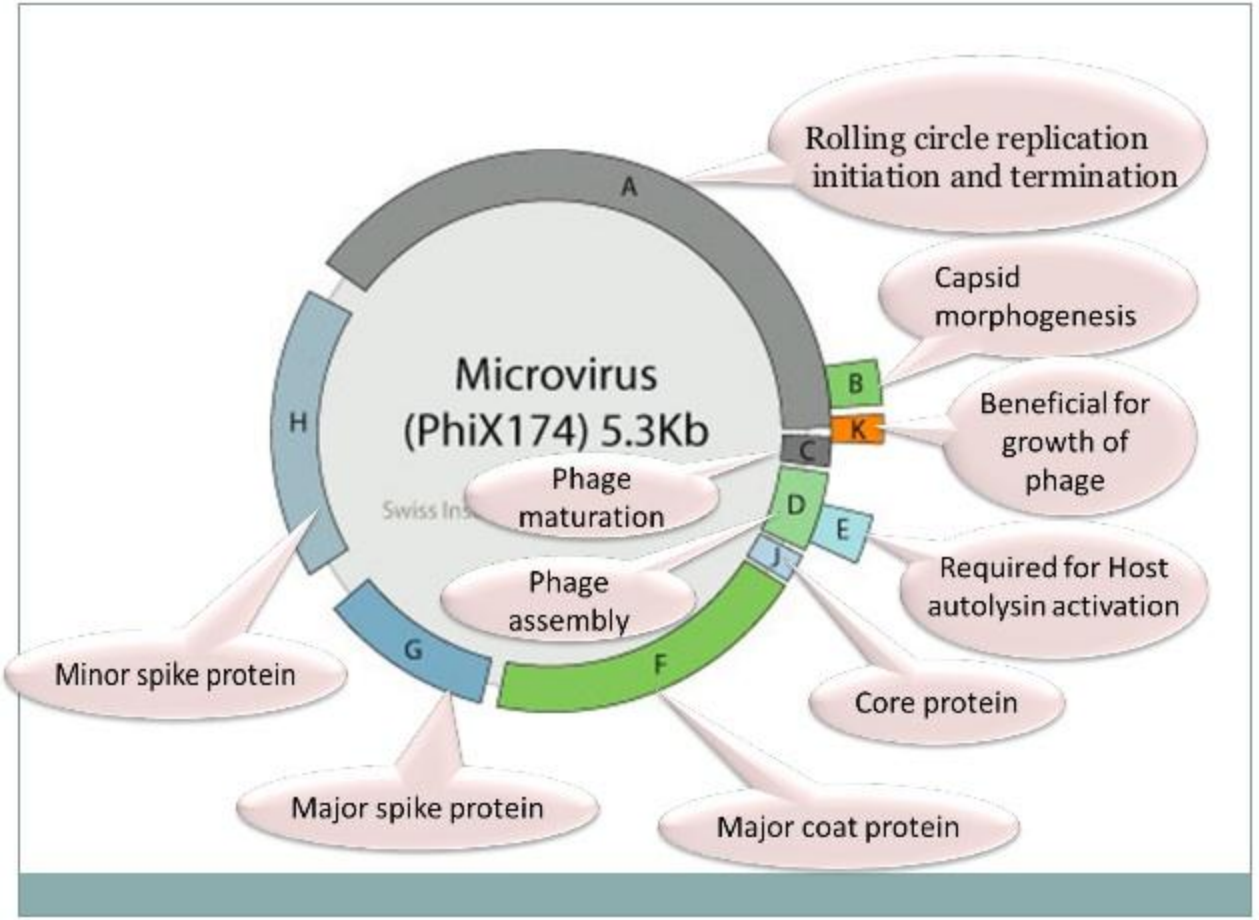
F Major coat protein,

G Major spike protein

H Minor spike protein

J Core protein

K Growth of phage



Applications

- It has been used as a model organism in many evolution experiments.
- ΦX174 is regularly used as a positive control in DNA sequencing due to its relatively small genome size in comparison to other organisms, its relatively balanced nucleotide content
- ΦX174 is also used to test the resistance of personal protective equipment to blood borne viruses
- ΦX174 has also been modified to enable peptide display (phage display) from the viral capsid G protein
- The ΦX174 genome was the first phage to be cloned in yeast, which provides a convenient dry dock for genome modifications

Subunit Composition of DNA Polymerase III Holoenzyme

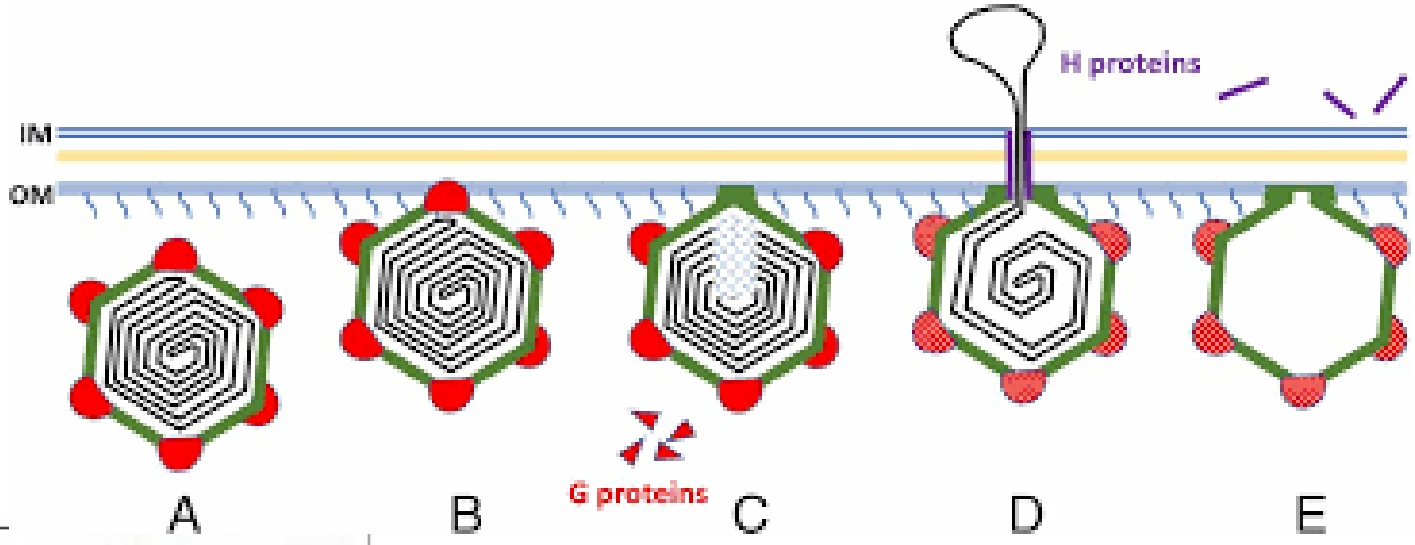
Table 1. Subunit composition of DNA Polymerase III holoenzyme

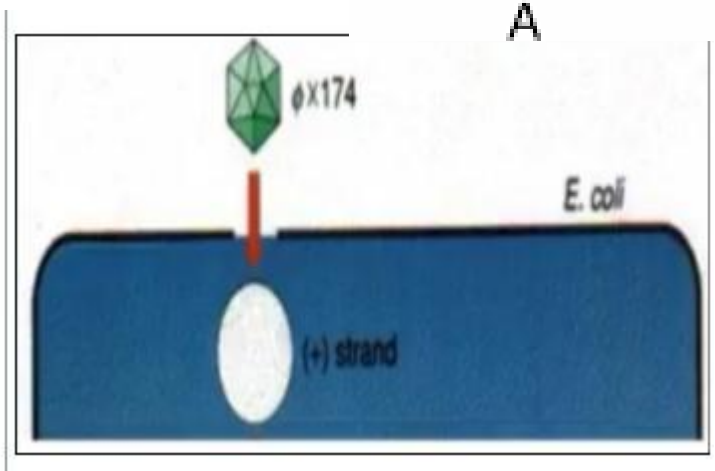
Subunit	Molecular Mass (kDa)	Function	Subassemblies
α	129.9	DNA polymerase	Core, Pol III′, Pol III*
ε	27.5	3′ to 5′ exonuclease	Core, Pol III′, Pol III*
θ	8.6	Stimulates ε exonuclease	Core, Pol III′, Pol III*
τ	71.1	Dimerizes core Binds γ complex	Pol III′, Pol III*
γ	47.5	Binds ATP	γ Complex (DNA-Dependent ATPase), Pol III*
δ	38.7	Binds to β	γ Complex (DNA-Dependent ATPase), Pol III*
δ′	36.9	Binds to γ and δ	γ Complex (DNA-Dependent ATPase), Pol III*
χ	16.6	Binds to SSB	γ Complex (DNA-Dependent ATPase), Pol III*
ψ	15.2	Binds to x and γ	γ Complex (DNA-Dependent ATPase), Pol III*
β	40.6	Sliding clamp	Pol III*

Adapted from Kelman and O'Donnell (1995).

ATTACHMENT OF PHAGE ΦX174

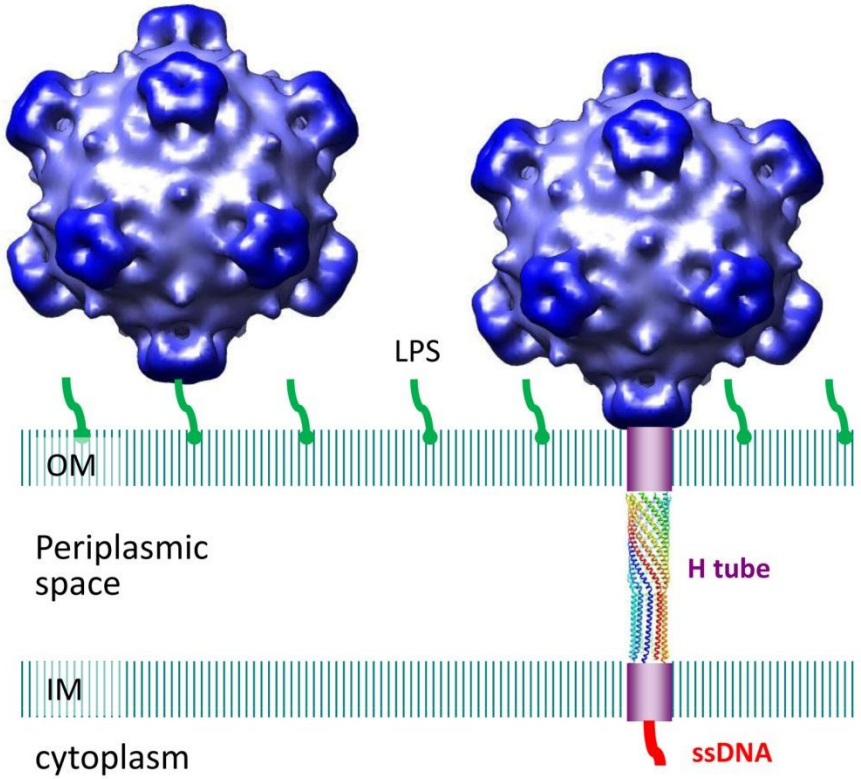
Phage φX174 recognizes the receptor **lipopolysaccharide** in the outer membrane of rough strains of ***Enterobacteriaceae*** , such as ***E.coli*** and ***Salmonella typhimurium*** , by the minor coat protein H.





ENTRY OF PHAGE ΦX174 GENOME

- 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.
- At least one H protein enters into the host cell with the viral DNA.



The Replication Cycle of ϕX174

Table 17-8
Replication cycle of ϕX174

Stage	Time, (minutes, at 33°)	Events
1 SS→RF	0–1	adsorption and penetration; viral SS→parental RF; transcription of RF
2 RF→RF	1–20	parental RF→~60 progeny RF
	25	RF multiplication stops; host DNA synthesis stops
3 RF→SS	20–30	~35 rolling circles→~500 viral SS→phage particles
	40	cell lysis

104. Kornberg A (1978) *CSHS* 43:1; Meyer RR, Shlomai J, Kobori J, Bates D, Rowen L, McMacken R, Ueda K, Kornberg A (1978) *CSHS* 43:289; Eisenberg S, Scott JF, Kornberg A (1978) *CSHS* 43:295.

Replication of ϕX174

- Replication of ϕX174 genome occurs in 3 stage

Stage 1

- Synthesis of (-) strand complementary to the (+) strand of to form the replicative form (RF) by host enzymes.

Stage 1

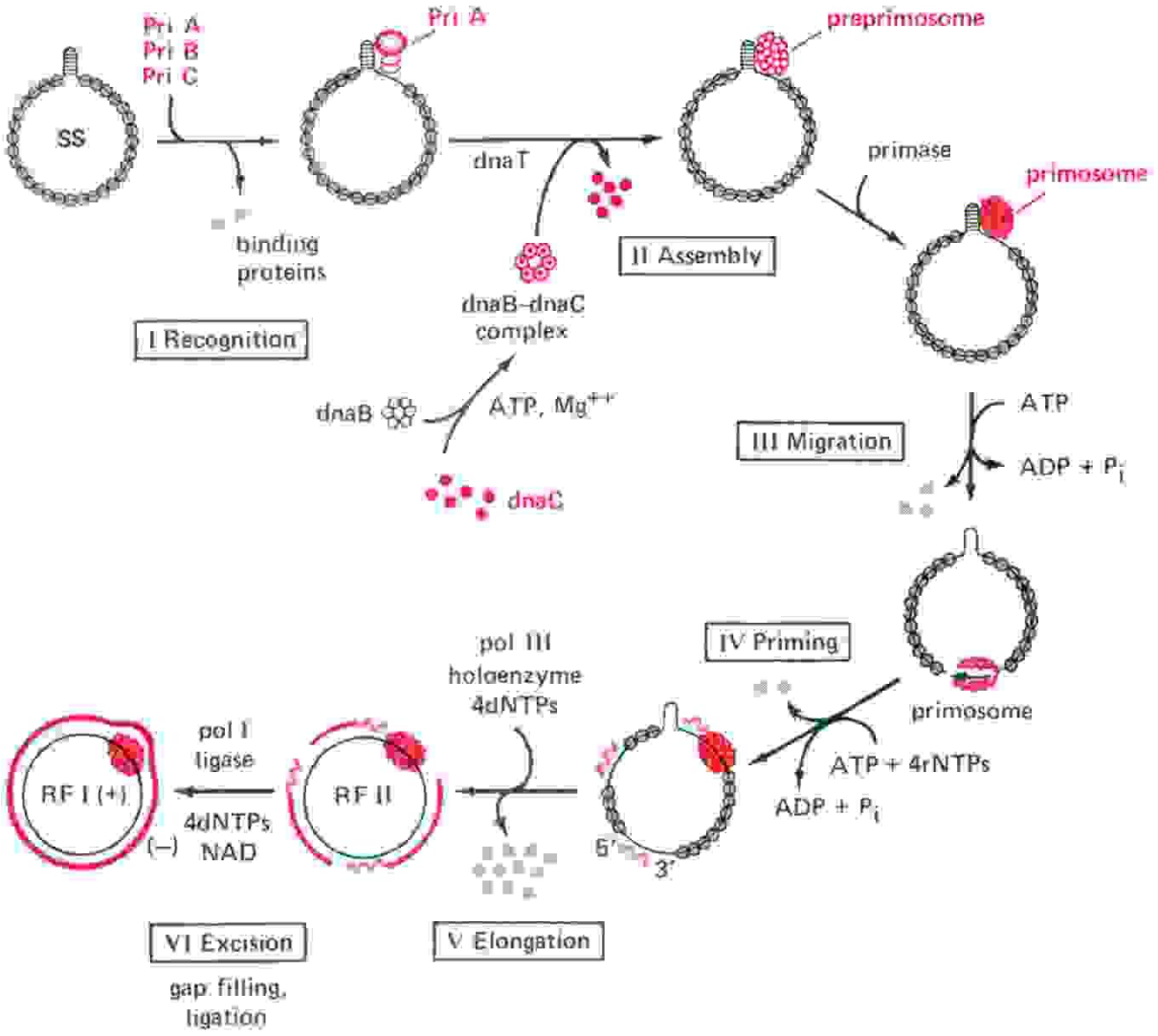


Figure 8-8
Scheme for assembly and migration of the primosome and the stepwise displacement of SSB in the ϕX SS → RF reaction.

Replication of ϕX174

Stage 2

- Replication of the RF involves rolling circle replication and requires phage encoded protein A to synthesize new plus strands. These then serve as a templates for minus strand synthesis to generate the new RFs.

The RF to RF Pathway

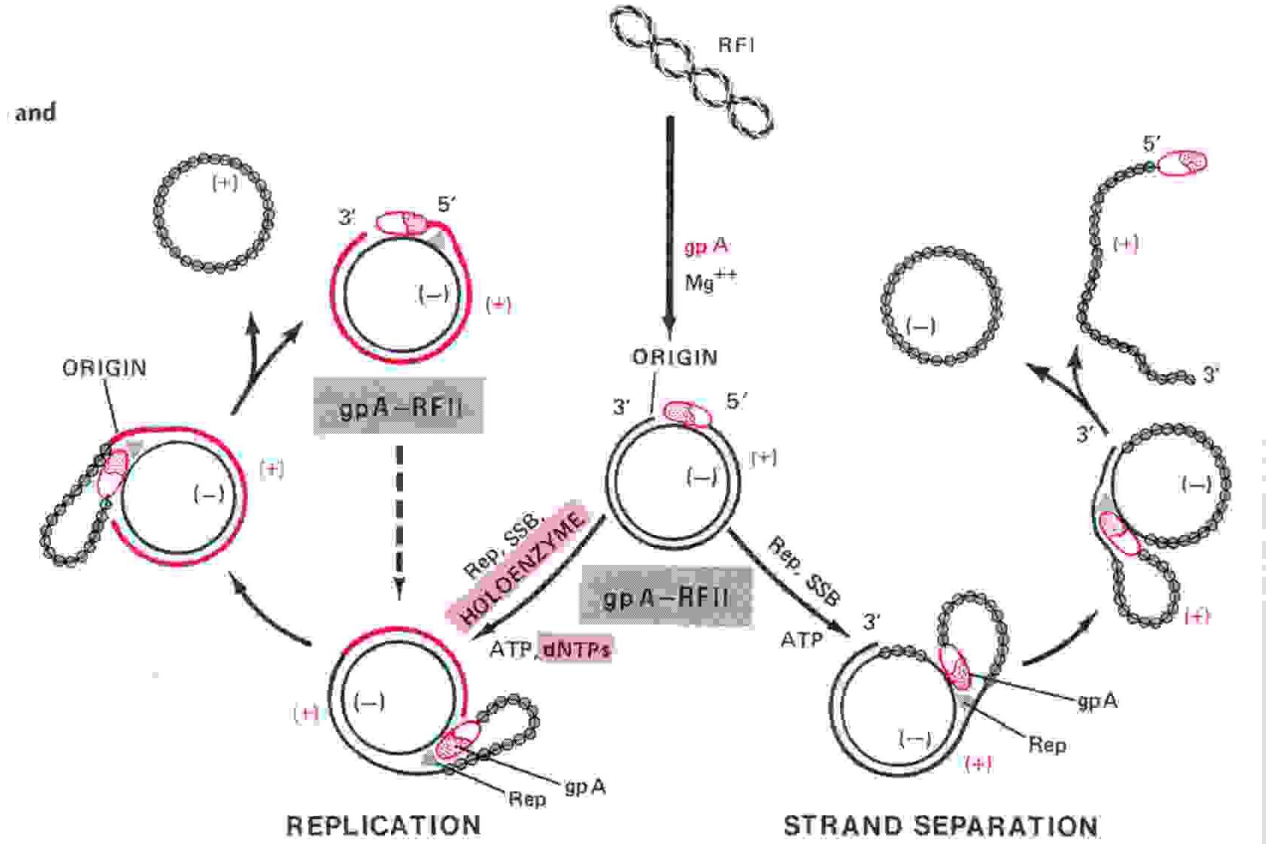


Figure 8-13
Scheme for gpA action, illustrating its multiple functions. The looped rolling-circle intermediate form is used in strand separation, uncoupled from replication, as well as in the synthesis of viral (+) strands. Rep = the Rep helicase.

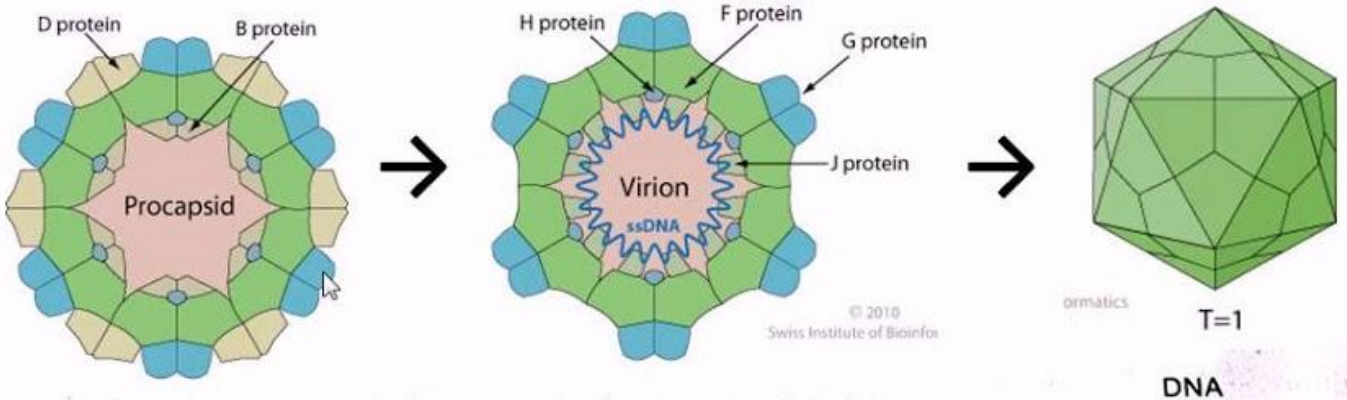
https://www.youtube.com/watch?v=ZDqsojQ8A5k

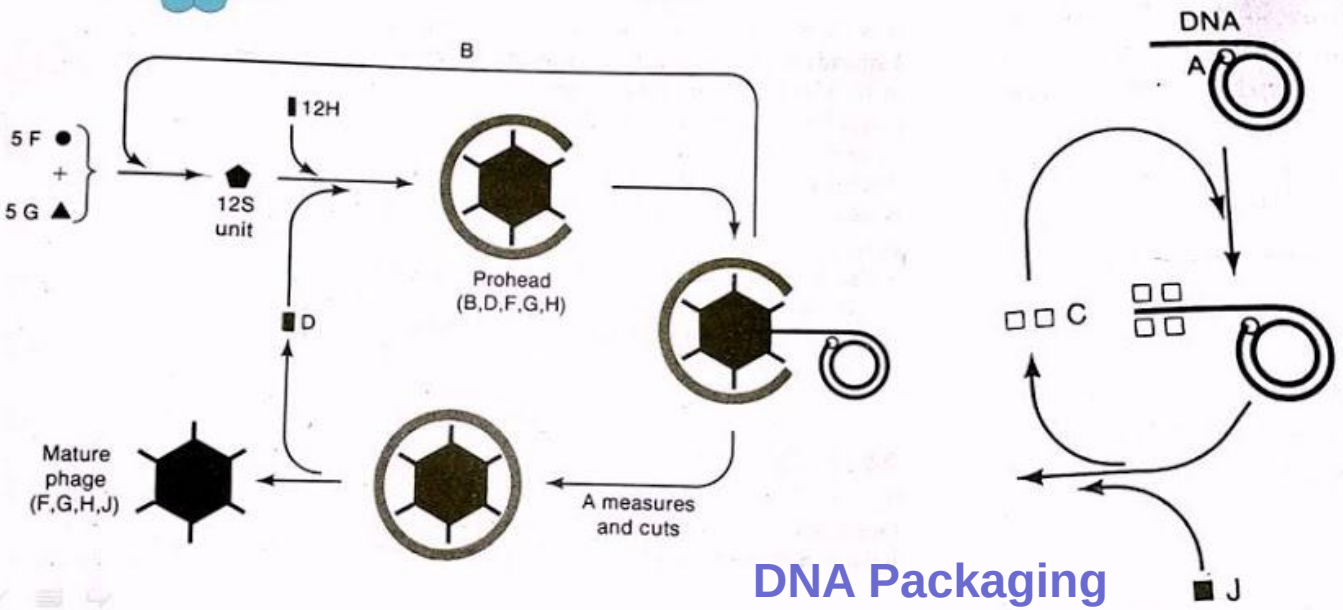
Replication of ϕX174

Stage 3

- Asymmetric replication of progeny ssDNA plus strand.
- RF synthesis continuous until sufficient structural proteins have been synthesized and assembled into empty precursor particle.

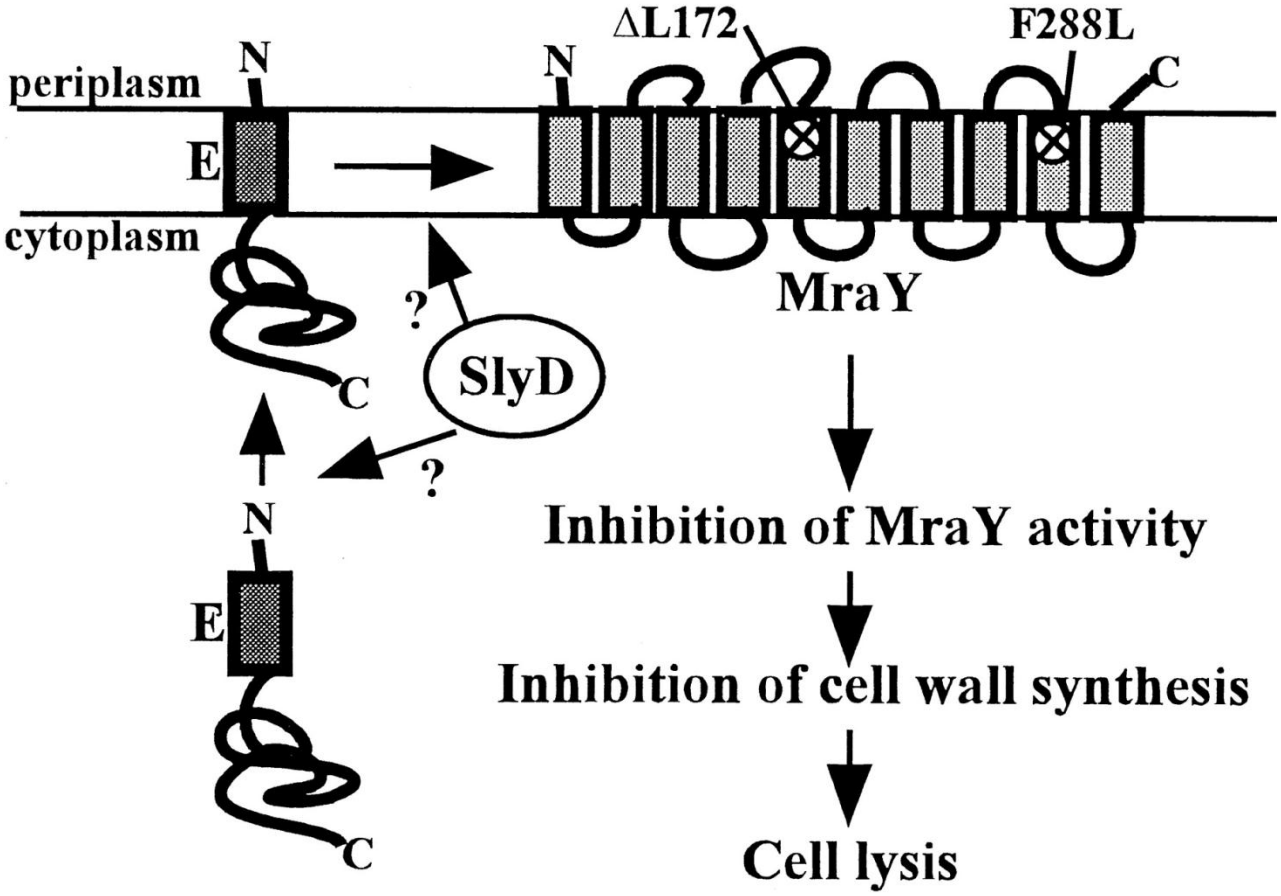
Assembly of mature virions



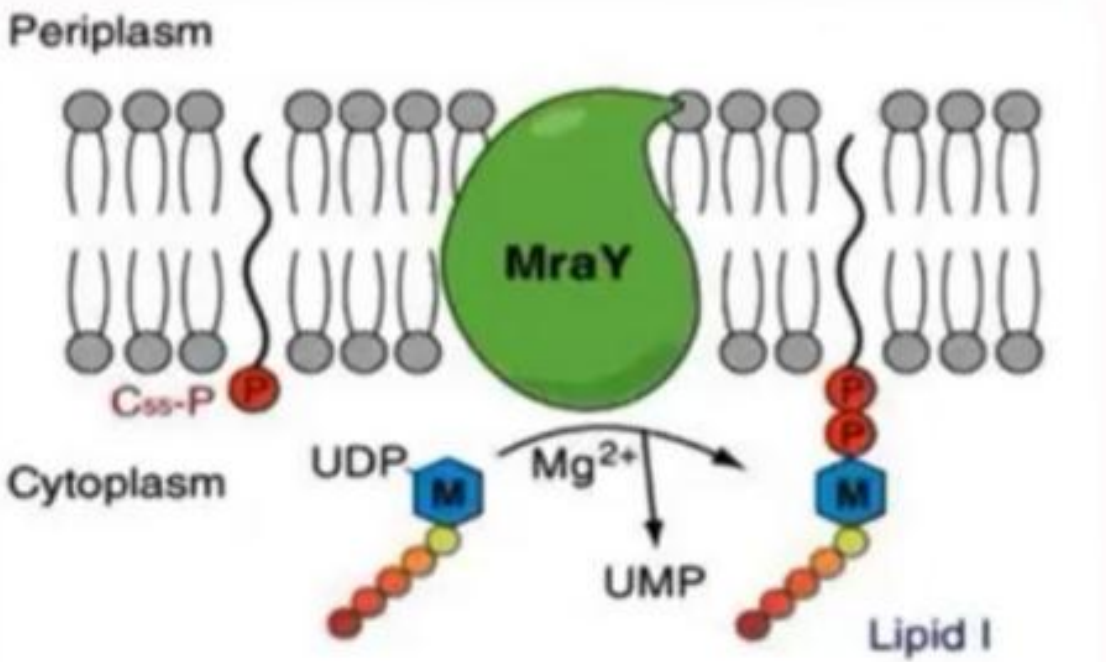


DNA Packaging

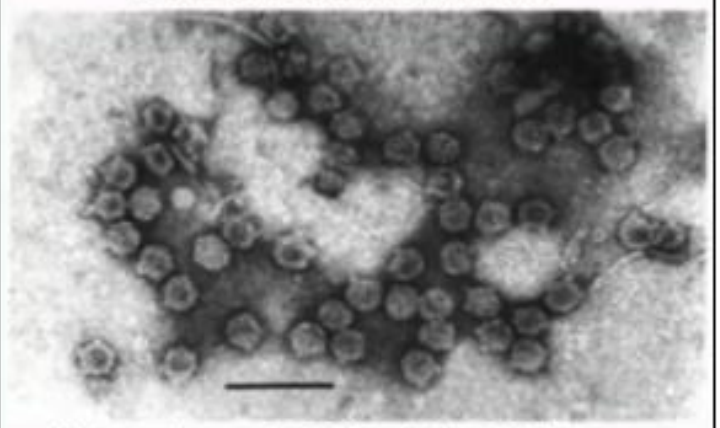
Lysis of Host Cell



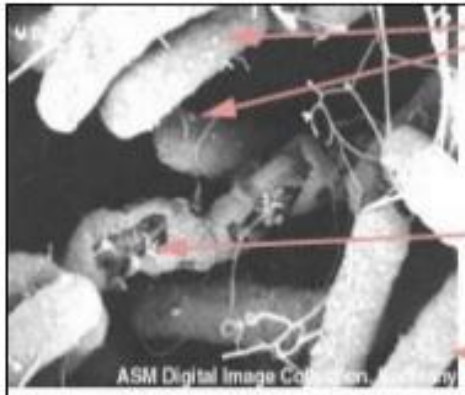
Mra Y (phospho-MurNAc-pentapeptide) translocase



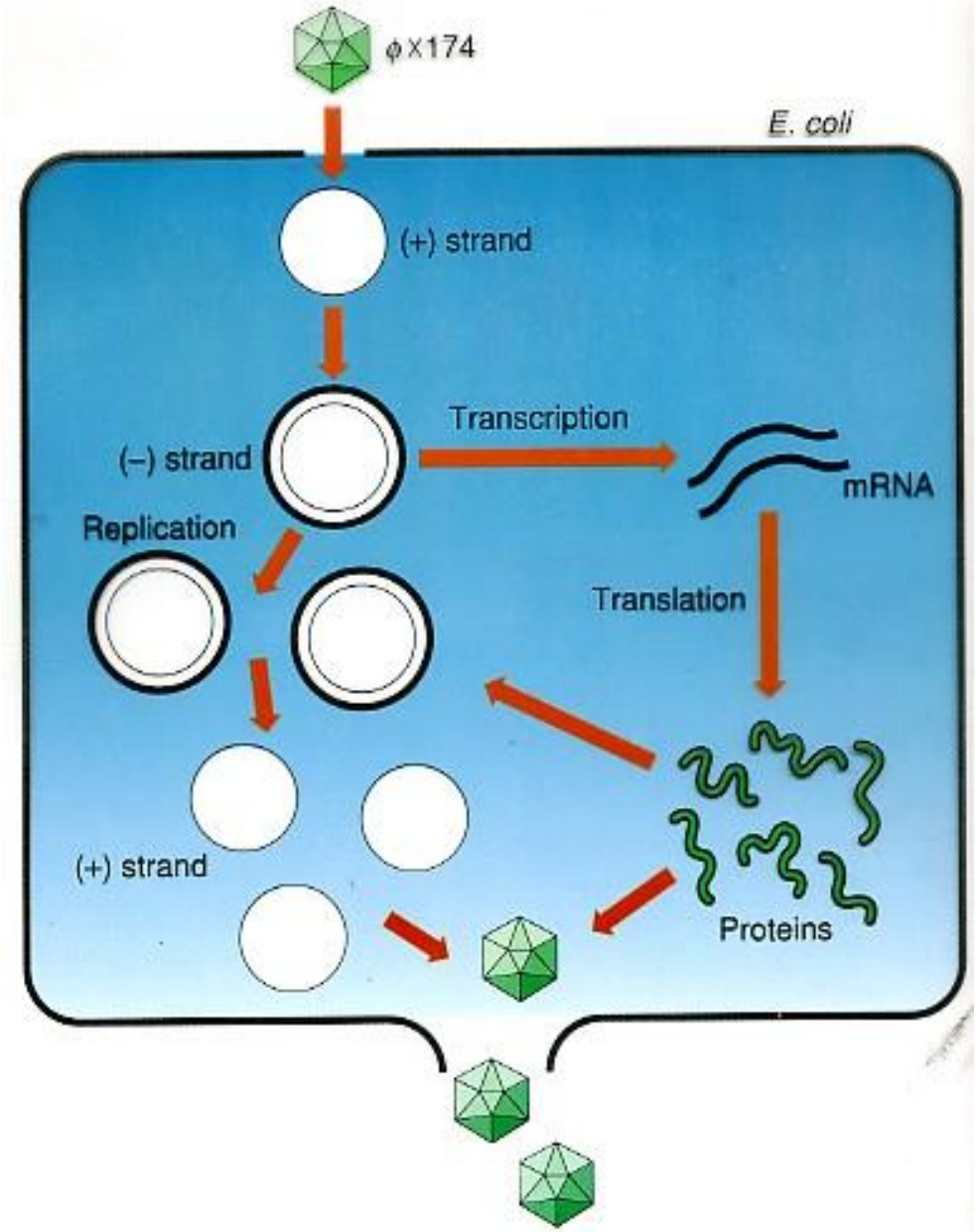
Release of mature virions



Electron micrograph of *Microviridae* ΦX174.
The bar represents 100 nm.



This scanning electron micrograph (SEM) shows *Escherichia coli* cells with disrupted cell envelopes, presumably due to phage release.


ϕX174
E. coli
(+) strand
Transcription
(–) strand
mRNA
Replication
Translation
(+) strand
Proteins

The Replication Proteins of E. coli used by ϕX174

TABLE 11-5
Replication proteins of *E. coli* used by phage ϕX174

Polypeptide	Mass kdal	Subunits	Function	Unamplified yield: molecules/cell	Unamplified yield: mg/kg[a]	Amplification[d]
SSB	74	4	single-strand binding	300	20	
protein i	80	4	prepriming	150	0.5	
protein n	25	1	prepriming			
protein n′	75	1	site recognition, ATPase	80	0.3	
protein n″	11	1	prepriming			
dnaC	29	1	prepriming			
dnaB	250–300	4–6	mobile promoter, ATPase	20	0.3	10–100
primase	60	1	primer formation	100	0.2	
holoenzyme[c]-α	140	1				
β	40	1				
γ[b]	52	1				10
δ	32	1	synthesis	20	0.5	
ε	25	1				
θ	10	2				
pol I	109	1		300	10	70
ligase	74	1	ligation	300	10	500
gyrase	400	4				
nalA(A)	210	2	supertwisting			
cou(B)	190	2				
rep	65	1	helicase	50	0.6	10
dUTPase	64	4	dUTPase	350	3	

[a]Mg protein/kg wet weight of cells.
[b]*dnaZ* (γ polypeptide)
[c]See Table 5-3 for more details.
[d]Normal protein level was increased this many times by introducing a plasmid or phage vector containing the encoding gene.