Phi X174

MIC 204

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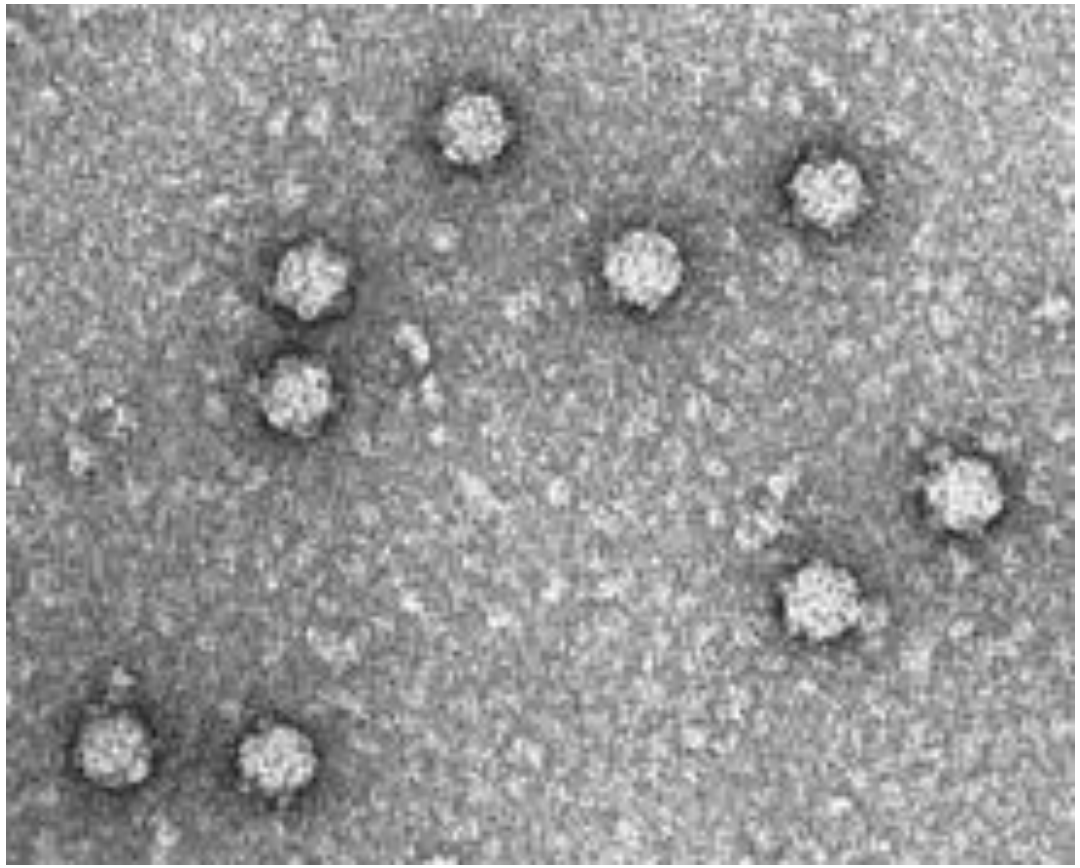
Classification

- Group II: ds DNA
- Family: Microviridae
- Genus: Microvirus
- Species: ϕ X174

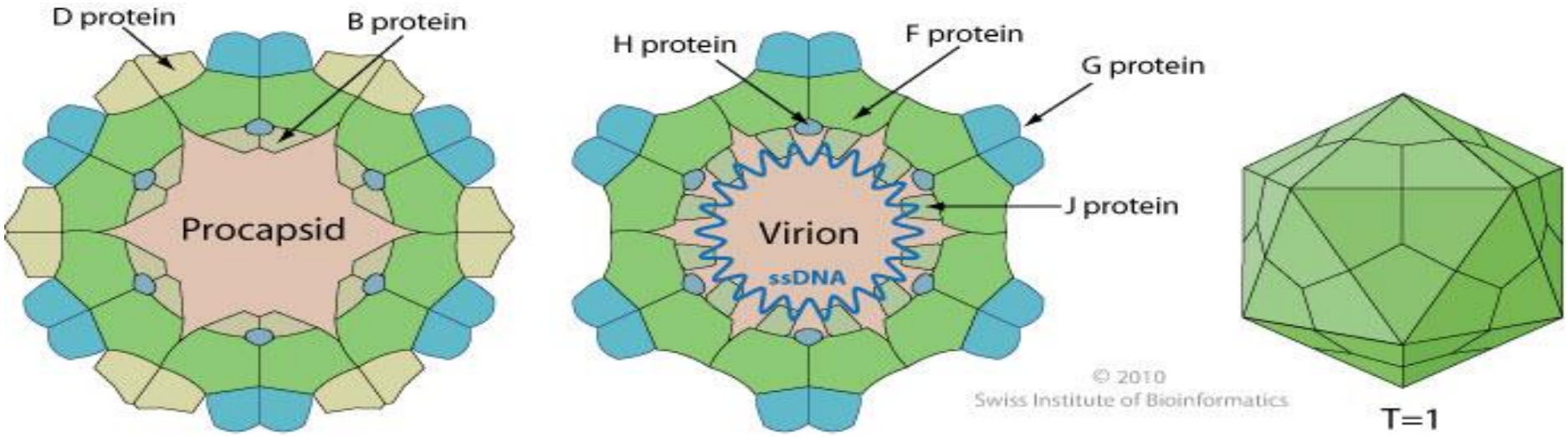


https://en.wikipedia.org/wiki/Phi_X_174

IMPORTANT POINTS

- First DNA-based genome to be sequenced. This work was completed by F
- Nobel prize winner Arthur Kornberg used ΦX174 as a model to first prove that DNA synthesized in a test tube by purified enzymes could produce all the features of a natural virus, ushering in the age of synthetic biology.red Sanger and his team in 1977
- In 2003, Craig Venter's group that the genome of ΦX174 was the first to be completely assembled in vitro from synthesized oligonucleotides- Synthetic Biology
- Because of the balance base pattern of the genome, it is used as the control DNA for Illumina DNA sequencers.
- In 2020, the transcriptome of ΦX174 was generated

Structure



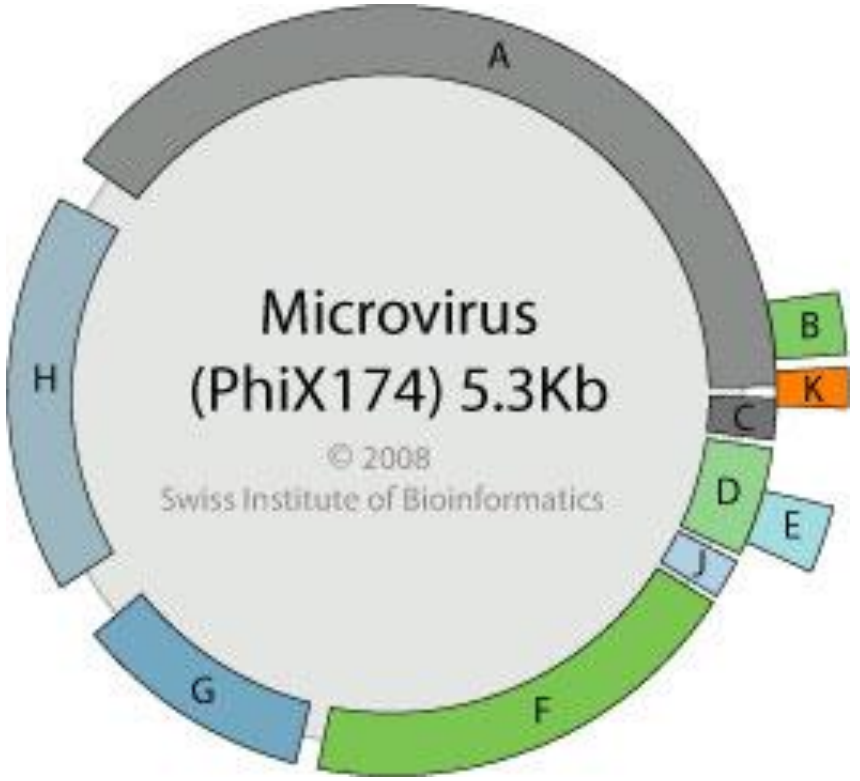
Non-enveloped, round, T=1 icosahedral symmetry, about 30 nm in diameter. The capsid consists of 12 pentagonal trumpet-shaped pentomers. The virion is composed of 60 copies each of the F, G, and J proteins, and 12 copies of the H protein. There are 12 spikes which are each composed of 5 G and one H proteins. The major capsid protein (F) has 426 amino acids, the major spike protein (G) has 175 amino acids, the small DNA-binding protein (J) has 25–40 amino acids, and the DNA pilot protein (H) has 328 amino acids. 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 packaging. Viralexpasy.com

Protein	Copies	Function[14]
A	—	Nicks RF DNA to initiate rolling circle replication; ligates ends of linear phage DNA to form single-stranded circular DNA
A*	—	Inhibits host cell DNA replication; blocks superinfecting phage; not essential
B	60 in procapsid	Internal scaffolding protein involved in procapsid assembly
C	—	DNA packaging
D	240 in procapsid	External scaffolding protein involved in procapsid assembly
E	—	Host cell lysis
F	60 in virion	Major capsid protein
G	60 in virion	Major spike protein
H	12 in virion	DNA pilot protein (or minor spike protein)
J	60 in virion	Binds to new single-stranded phage DNA; accompanies phage DNA into procapsid
K	—	Optimizes burst size; not essential

https://en.wikipedia.org/wiki/Phi_X_174

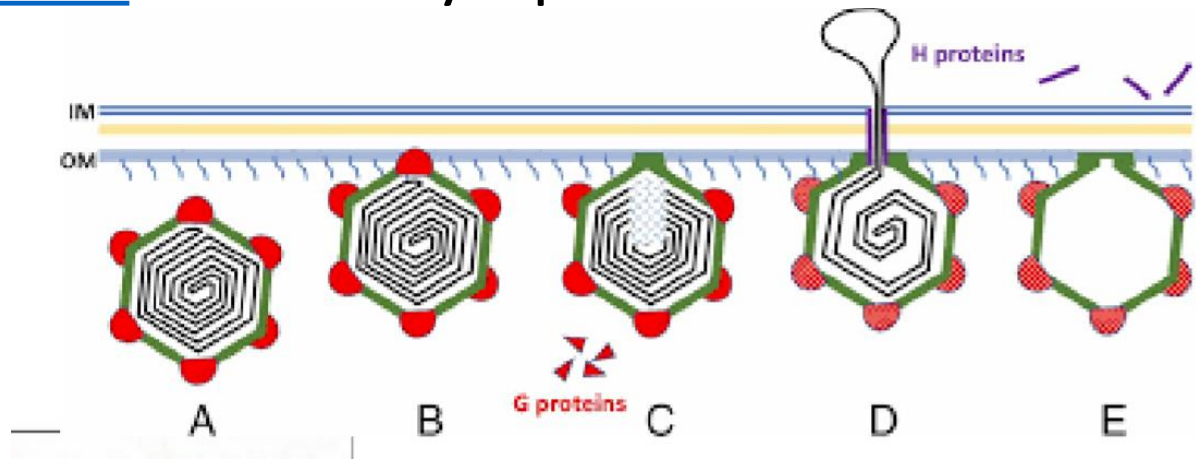
Genome

- Circular, ssDNA(+) genome of 4.4 to 6.1kb.
- Codes for 11 proteins
- Overlapping genes
- replication occurs via dsDNA intermediate and rolling circle.
- Viralexpasy.com



REPLICATION

- **CYTOPLASMIC**
- The viral particle attaches to host cell by binding host lipopolysaccharides. Infection begins when G protein binds to lipopolysaccharides on the bacterial host cell surface. H protein (or the DNA Pilot Protein) pilots the viral genome through the bacterial membrane of E.coli bacteria
- The proteins of the capsid perform Injection of the viral DNA through bacterial membranes into cell cytoplasm.



REPLICATION

- Host polymerase convert the (+)ssDNA viral genome into a covalently closed dsDNA called replicative form DNA I (**RF-I**).
- **Early** viral genes are transcribed by host RNA polymerase, producing viral replication proteins.
- Viral protein A cleaves RF-I(+) DNA strand at the origin of replication and covalently attaches itself to the DNA.

Replication Cycle

- (+)strand replication occurs by rolling circle, which is converted to dsDNA by host polymerase, generating **RF-II** molecules (amplification of RF-I).
- **Late** viral genes are transcribed by host RNA polymerase.
- Procapsid assembly in the cytoplasm.
- Viral protein C binds to replication complex, inducing synthesis and packaging of neo-synthesized (+)ssDNA genomes (**RF-III**) into procapsids.
- Procapsids maturation occurs in host cytoplasm
- Mature virions are released from the cell by lysis. Cell lysis is mediated by the phiX174-encoded protein E, which inhibits the peptidoglycan synthesis leading to an eventual bursting of the infected cell.

DNA Replication

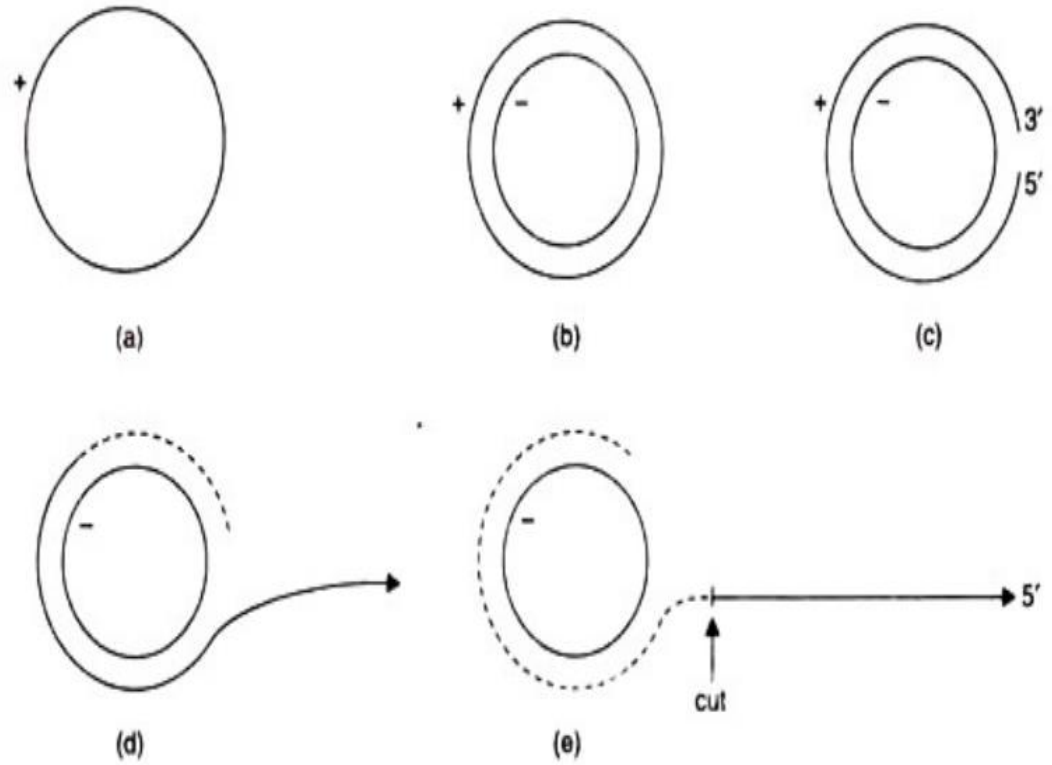
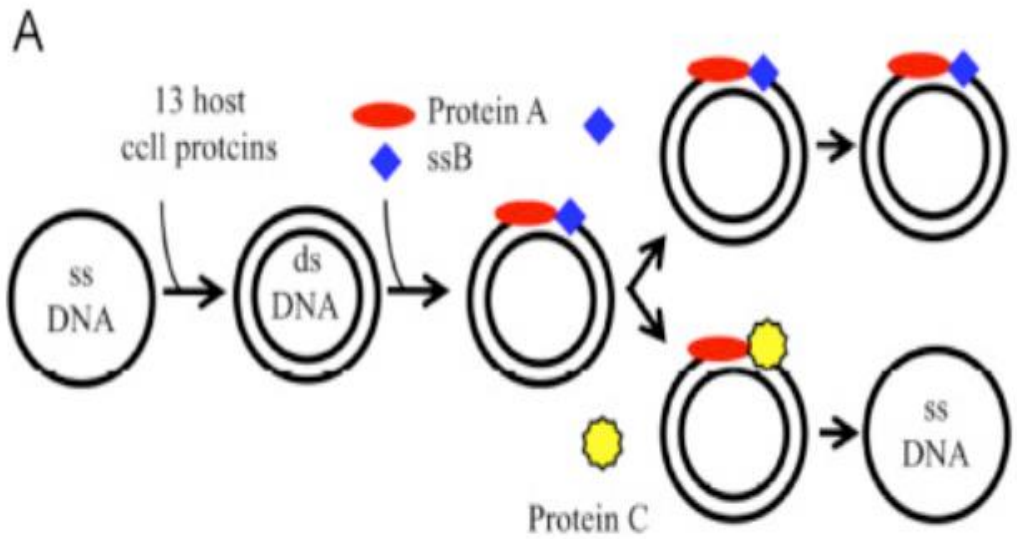


Fig. 14.12 The rolling circle method of DNA replication in ϕX174.



https://www.biologydiscussion.com/dna/dna-replication/rolling-circle-method-of-dna-replication-genetics/67533

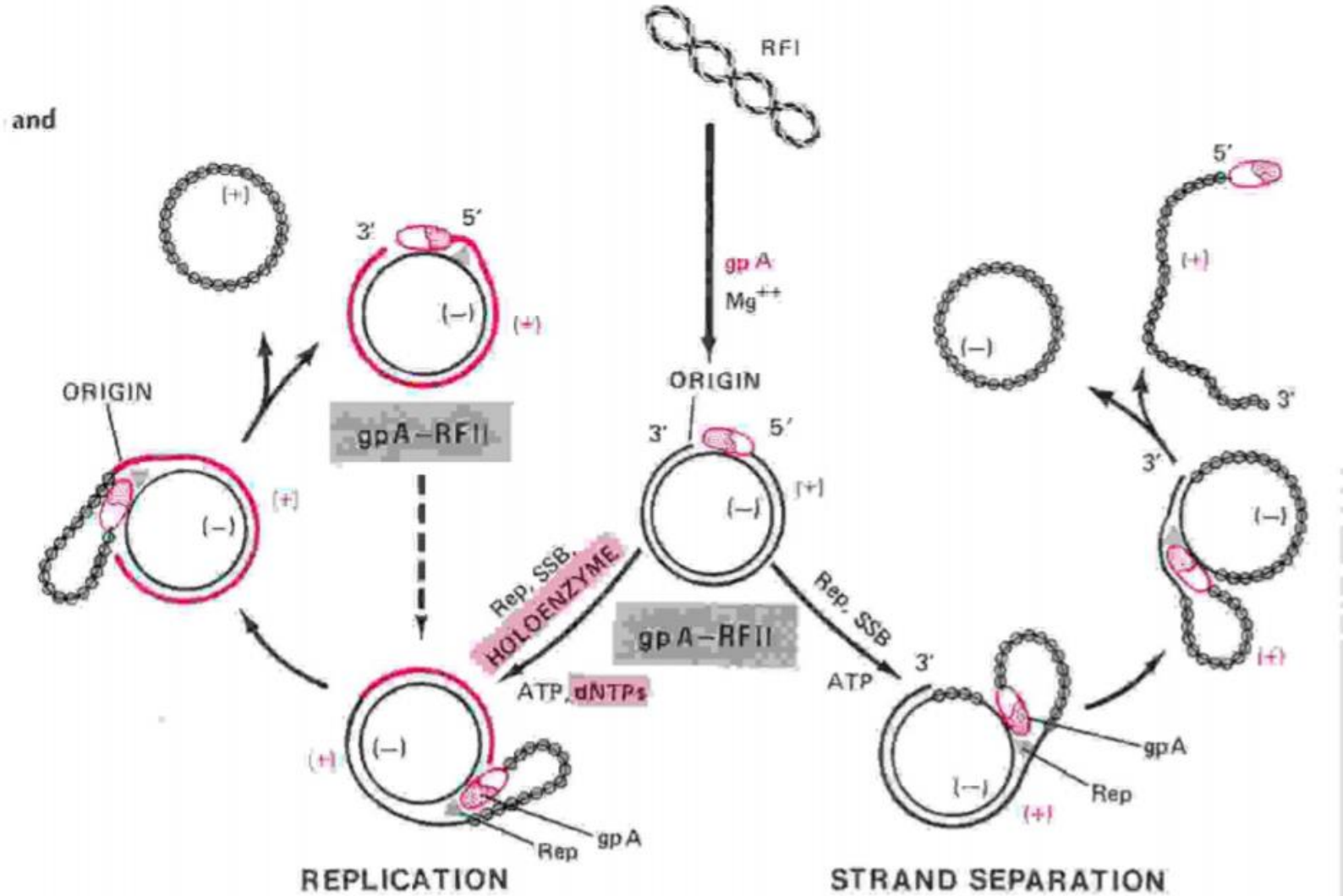
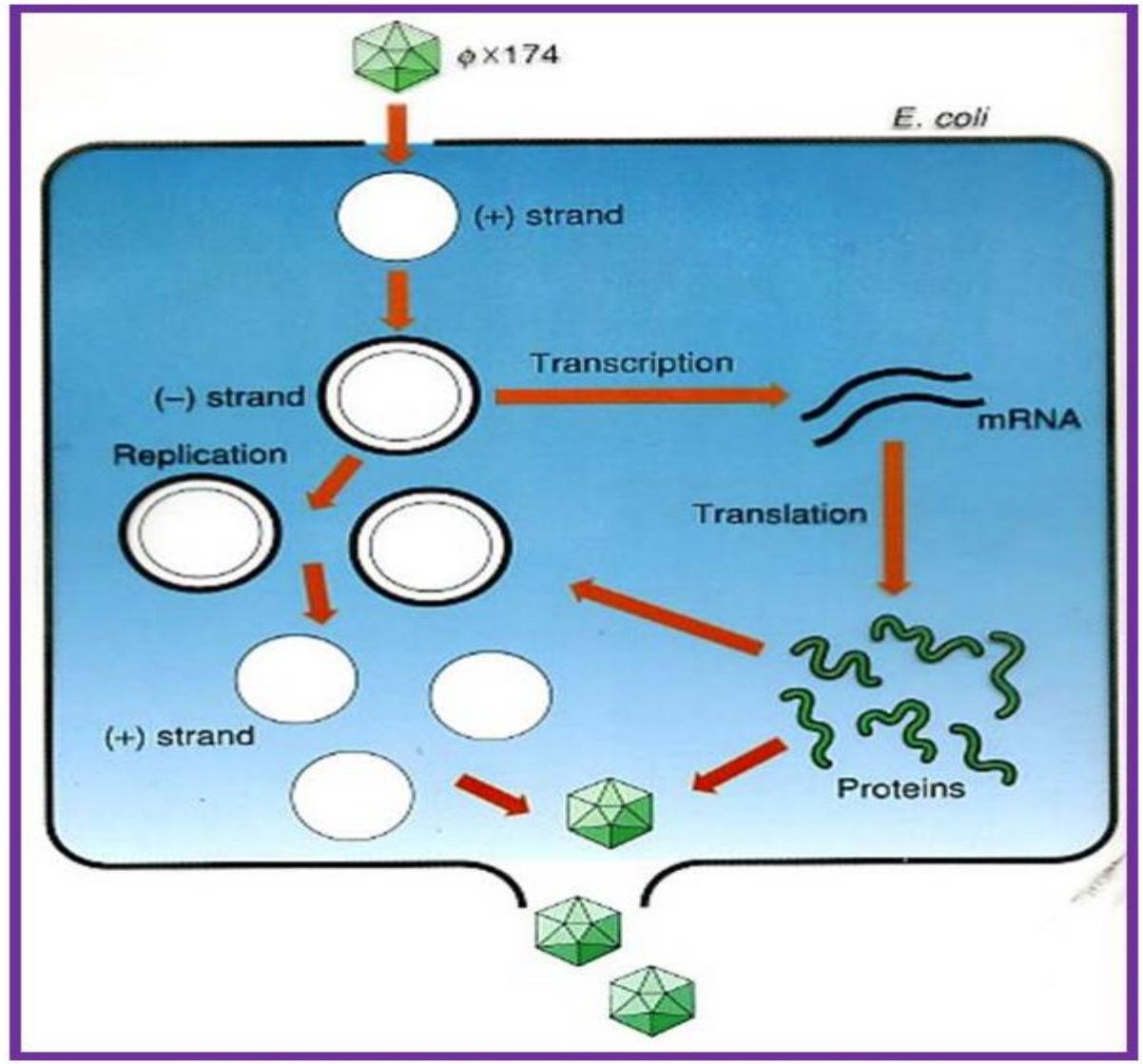


Figure 8-13
Scheme for gpA action, illustrating its multiple functions. The looped rolling-circle intermediate form is used in strand separation, uncoupled from replication, as well as in the synthesis of viral (+) strands. Rep = the Rep helicase.

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



Life cycle of the phage; http://www.dls.ym.edu.tw/

Published: 24 February 1977

Nucleotide sequence of bacteriophage φX174 DNA

F. Sanger, G. M. Air, B. G. Barrell, N. L. Brown, A. R. Coulson, J. C. Fiddes, C. A. Hutchison III, P. M. Slocombe & M. Smith

Nature **265**, 687–695 (1977) | Cite this article

Abstract

A DNA sequence for the genome of bacteriophage *φ*X174 of approximately 5,375 nucleotides has been determined using the rapid and simple 'plus and minus' method. The sequence identifies many of the features responsible for the production of the proteins of the nine known genes of the organism, including initiation and termination sites for the proteins and RNAs. Two pairs of genes are coded by the same region of DNA using different reading frames.

> Proc Natl Acad Sci U S A. 2003 Dec 23;100(26):15440-5. doi: 10.1073/pnas.2237126100. Epub 2003 Dec 2.

Generating a synthetic genome by whole genome assembly: phiX174 bacteriophage from synthetic oligonucleotides

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Affiliations + expand

PMID: 14657399 PMCID: PMC307586 DOI: 10.1073/pnas.2237126100

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Abstract

We have improved upon the methodology and dramatically shortened the time required for accurate assembly of 5- to 6-kb segments of DNA from synthetic oligonucleotides. As a test of this methodology, we have established conditions for the rapid (14-day) assembly of the complete infectious genome of bacteriophage X174 (5386 bp) from a single pool of chemically synthesized oligonucleotides. The procedure involves three key steps: (i). gel purification of pooled oligonucleotides to reduce contamination with molecules of incorrect chain length, (ii). ligation of the oligonucleotides under stringent annealing conditions (55 degrees C) to select against annealing of molecules with incorrect sequences, and (iii). assembly of ligation products into full-length genomes by polymerase cycling assembly, a nonexponential reaction in which each terminal oligonucleotide can be extended only once to produce a full-length molecule. We observed a discrete band of full-length assemblies upon gel analysis of the polymerase cycling assembly product, without any PCR amplification. PCR amplification was then used to obtain larger amounts of pure full-length genomes for circularization and infectivity measurements. The synthetic DNA had a lower infectivity than natural DNA, indicating approximately one lethal error per 500 bp. However, fully infectious X174 virions were recovered after electroporation into Escherichia coli. Sequence analysis of several infectious isolates verified the accuracy of these synthetic genomes. One such isolate had exactly the intended sequence. We propose to assemble larger genomes by joining separately assembled 5- to 6-kb segments; approximately 60 such segments would be required for a minimal cellular genome.

figures

References

- Doore, Fane. The microviridae: Diversity, assembly, and experimental evolution. Virology, Volume 491, April 2016, Pages 45-55
- https://www.biologydiscussion.com/dna/dna-replication/rolling-circle-method-of-dna-replication-genetics/67533
- https://en.wikipedia.org/wiki/Phi_X_174
- Viralexpasy.com