NAME OF THE COURSE WORK
VIROLOGY

UNIT-II
BACTERIAL VIRUSES

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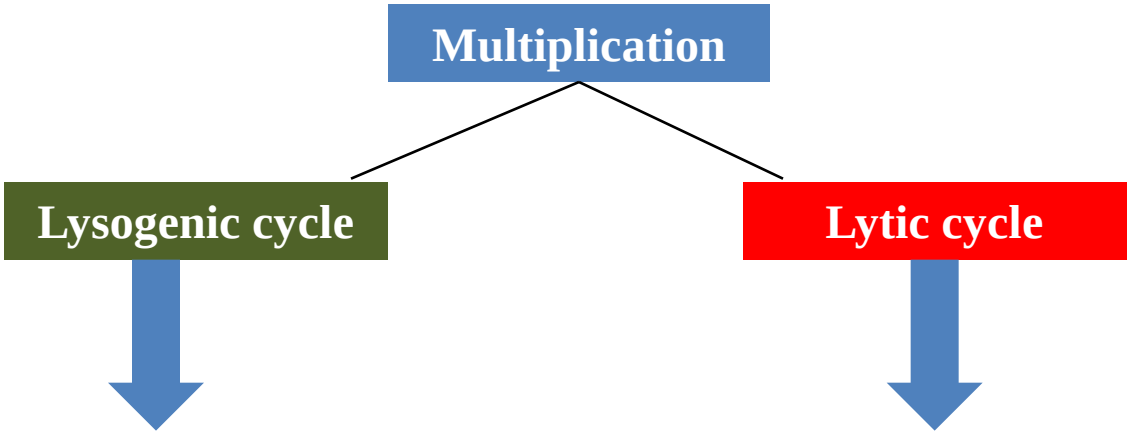
- Viruses which infects bacteria
- Use bacterial replication system for its multiplication
- Multiplied by either lysogenic or lytic cycle



- Viral DNA merges with Cell DNA and does not destroy the cell.
- There are no symptoms of viral infection.
- Temperate viral replication takes place.
- Progeny viruses released by budding from host cell

- Viral DNA destroys Cell DNA, takes over cell functions and destroys the cell.
- The Virus replicates and produces progeny phages.
- There are symptoms of viral infection.
- Virulent viral infection takes place.

PHI × 174 - BACTERIAL VIRUS

- It is a ssDNA Bacteriophage. It belongs to Type D Group (contains a head which is made up of capsomeres but lacks tail).
- Classification
  - Group : Group II (ssDNA)
  - Family : *Microviridae*
  - Genus : Microvirus
  - Species: phi X 174 phage
  -
  - Discovered by R.L.Sinsheimer at California Institute of Technology.
  - It is one of the ssDNA phages of *E. coli* which has been most extensively studied.

- The phage particles are naked and icosahedral having a diameter (without spike) of 24-29nm. The Weight of a virus particles is 6.2 x 10 million Daltons.

- The capsid is made up of capsomeres, each consisting of five structural units.

- Morphologically, the capsomeres are probably angular ,hollow and pentagonal from the center of which projects a single spike situated at one apex of the icosahedran hence, there are 12 spikes in one phage particle.

- Individual spike is constituted by H protein (encoded by one gene) and G protein(encoded by five genes)

- These interact with F gene protein. The H gene protein assists the adsorption of phage to bacterial cell, in addition to functioning as pilot protein and helping the injection of DNA into the bacterial cell

- Capsid encloses a single and circular(+) ss DNA (molecular weight 1.7x 10 million daltons)
- Consisting of eleven genes arranged in an order A,B,C,D,E,J,F,G,H, A* and K. Gene A and K overlap to each other.
- The gene B lies with in gene A, and gene E is present T with in gene D. The gene C overlaps with genes A and D. There fore , from two genes four proteins are encoded instead of two.

Gene A	: Replication of phage by introducing single stranded break in the DNA
Genes B,C,D	: Synthesis and packaging of ssDNA of progenies.
Gene E	: Bacterial cell lysis.
Gene J	: Internal protein.
Gene F	: Major capsid protein.
Gene G	: Synthesis of spike protein.
Gene H	: Synthesis of spike protein, adsorption, injection of phage DNA.
Gene A	: overlaps.
Gene K	: overlaps.

ADSORPTION

- The cell wall surface of *E. coli* contains specific receptor sites. For example , the receptor site of phi x 174 present on outer membrane of cell wall of *Salmonella typhimurium* is a lipopolysaccharide.
- The phage gets absorbed on bacterial cell surface through any one of 12 icosahedral verticles.
- The spikes differ each other in specificity for attachment.
- For the attachment to host cell wall the phage needs Ca^{++} or Mg^{++} ions. Therefore, to find out the specific receptor site, the adsorption may be reversible.

REPLICATION AND PHAGE PRODUCTION

- Synthesis
- After the introduction of phage DNA into the bacterial cell, there starts synthesis of viral DNA and protein.
- Complex in Phi x 174.
- The phage genome is in circular form immediately after infection which is accomplished in three different stages:

  (a) Synthesis of a replicative form (RF) of viral DNA,

  (b) Replication of parental RF to progeny RF DNA and

  (c) Conversion of RF molecules into rolling circle molecule.

(a)Formation of replicative form of DNA

- DNA replication of phi x 174. Soon after penetration, the ssDNA of phage synthesizes a synthesized RF DNA and results in formation of the double stranded DNA .
- The newly synthesized RF strand is known as (-) strand. Synthesis of RF of DNA is completed in certain stages.
- Soon after entry of ss DNA , the DNA unwinding proteins extend ss DNA leaving the hair pin duplex which is a promoter region.
- This step requires for the presence of ssDNA , dna B protein, dna C protein, unwinding protein and protein factors (the X, Y and Z and ATP). These proteins from an intermediate substrate for the synthesis of dna G protein (MW 60,000 daltons).

Injection

- There starts eclipse period. Viral DNA is injected into the bacterial cell. Phage gene H encodes proteins which act as pilot protein, conveys the DNA into the bacterial cell.

- DNA but not The unwinding proteins of which about 800 molecules are present, binds to ss dsDNA.
- This protein establishes the ssDNA in a state which can act as a template for synthesis of its complementary strand .
- DNA polymerase is used since this process occurs before transcription of mRNA.
- It allows the initiation of DNA synthesis at the point of origin of replication present in ds DNA. The presence of RNA primer is ssDNA and is essential for the synthesis of DNA.

- However, after pre-priming with dnaB and dnaC proteins, the dnaG protein catalyses the RNA priming in phi x 174.
- The RNA polymerase is not required for the synthesis of RNA primer. On RNA primer a complementary(-) strand is extended by using the (+) ssDNA as the template.
- Chain elongation of primed DNA takes place by DNA polymerase III holo enzyme (i.e. DNA polymerase III +DNA co polymerase) in okazaki fragments .
- This structure is known as RF II which is converted to RF when gap is sealed
- DNA polymerase through 5'--->3' exo nuclease activity removes the RNA primer from (-) DNA strand.
- This gap is filled up by the activity of DNA polymerase - I.
- The two ends of DNA molecule are ligated by the enzyme DNA ligase which results in a circular DNA molecule.
- Thereafter, the cellular polymerase forms a pool of progeny RF molecules (RF→RF). The ssDNA are also synthesized.

(b) Replication of RF (RF→RF)

- The RF molecules produced in this way replicate to form a pool of progeny RF molecules.
- For the replication of parental RF molecule, the A gene protein is needed that makes a nick on the viral strand of RF molecule.
- Possibly the protein acts as hairpin in (-) DNA super helix existing at palindromic sequences. For the replication of RF molecule the host cell machinery is required that includes proteins encoded by rep gene and dnaB, dnaC, dnaE, dna G, dna H and dna Z genes.
- Probably the origin of replication is present in gene A ,Two models, *reciprocatory strands* model and *rolling circle model*, have been suggested for the replication of RF of DNA duplx.

RECIPROCATORY STRAND MODEL

- After the formation of RNA primers synthesis of a new strand begins at the origin of replication present on gene A.
- In phi x 174 the RNA priming is catalyzed by dna G protein when a short sequence of DNA is formed .
- Positive super helical twists accumulate which impose the strain .
- The viral strand is synthesized around the genome continuously in unidirectional and in clock wise directions.
- The growing point moves for its origin to terminus around the circular genome.
- Moreover, the complementary strand primed by RNA is synthesized in discontinuous manner

- Synthesis of complementary strand is lagged behind the viral strand, there occurs the repeated exchange reactions between the nascent strand and bacterial strand.
- The two parental strands are completely un wound.
- A strand exchange reaction releases two separate, circular but incomplete duplexes.
- One duplex has several gaps that separate nascent complementary strand of DNA segments (many C strand gaps).

ROLLING CIRCLE MODEL

- Dressler and Wolfson (1970) suggested that the rolling circle mechanism results in replication of RF-→RF as well as RF--->ss.
- Replication of RF ---> RF takes place by semi- conservative method. A nick is made on outer strand, and the 5’ end tail serves as template for synthesis of a small DNA segment .
- The growing point moves from its joint by the enzyme DNA ligase to produce a dsDNA molecule.
- In turn each strand of dsDNA molecules acts as a template for the synthesis of complementary DNA molecule.

(c) SYNTHESIS OF SSDNA (RF ---> SS)

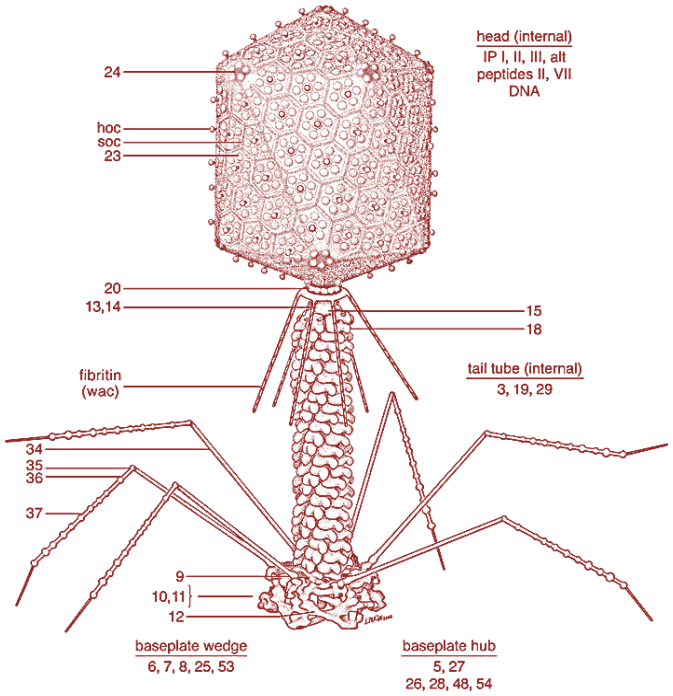
- For the first time Gilbert and Dressler (1968) suggested the rolling circle mechanism for ssDNA synthesis of phi x 174.
- They demonstrated that during the first round of replication RF molecules are produced.
- The rolling circle mechanism operates during the first stage of replication and produces ss DNA molecules.
- However no complementary strand is synthesized on the tail during RF→ss stage of synthesis.

- To begin replication a virus coded enzyme makes a specific single stranded nick in the plus strand of the RF .This generates the 3’-OH end and 5’ phosphate ends on the strand.
- The de oxynucleotides (d ATP, d GTP, d CTP ,d TTP) are added to the 3’-OH end . The open single strand rolls off the circle as a free tail with the progress of synthesis.
- By using the minus strand as template and de oxynucleotide precursor a new plus strand is synthesized.
- With the rolling of the strand , structural proteins of phage bind to elongating tail.
- Nuclease acts with in the hair pin loops present on DNA and release the plus strand which becomes circularized by binding the cut ends and forming the complete hair pin loop.
- The enzyme ligase fills up the gap.

(d) ASSEMBLY AND RELEASE

- The phage proteins are synthesized in bacterial cell. Soon after synthesis ,
- Late in the life cycle of the single-stranded DNA phage phi X, the synthesis of positive strand DNA is coupled to the maturation of progeny virions.
- DNA synthesis and packaging take place in a replication-assembly complex, which we have purified to homogeneity and characterized.

T4 Phage



Structure of the T4 virion

- Structure of the T4 virion based on negative stain and cryo-electron microscopy, and crystallographic data. The locations of the protein components are indicated by gene number.
- The portal vertex composed of gp20 is attached to the upper ring of the neck structure, inside the head itself.
- The internal tail tube is inside the sheath and itself contains a structural component in its central channel.
- The baseplate contains short tail fibers made of gp12; these are shown in a stored or folded conformation.

T4 gene maps

- The outer circle shows the approximate positions of characterized genes on the 169,903 bp DNA, drawn as a circle.
- The next three genome segments show three maps derived from determining distances of chromosomal ends from rI, rII and rIII respectively .
- Note that the relative length of the small rI molecules was 0.77, not 0.68 of the normal T4 chromosome.
- The next (full) circle indicates the positions of genes derived from genetic crosses .
- The innermost circle indicates the positions of heteroduplex loops after annealing of heat-denatured T2, T4 and T6 DNA

Transcription

- The upper panel shows the transcripts initiated from early, middle and late promoters by sequentially modified host RNA polymerase.
- Hairpins in several early and middle transcripts inhibit translation of the late genes present on these mRNAs.
- The lower panel depicts the pathways of DNA replication and recombination detailed in this review.
- Hatched lines represent strands of homologous regions of DNA and the arrows point to possible positions of endonuclease cuts.
- Replication can be only initiated from cuts marked by filled arrows Cuts indicated by open arrows cannot be used to initiate replication forks.

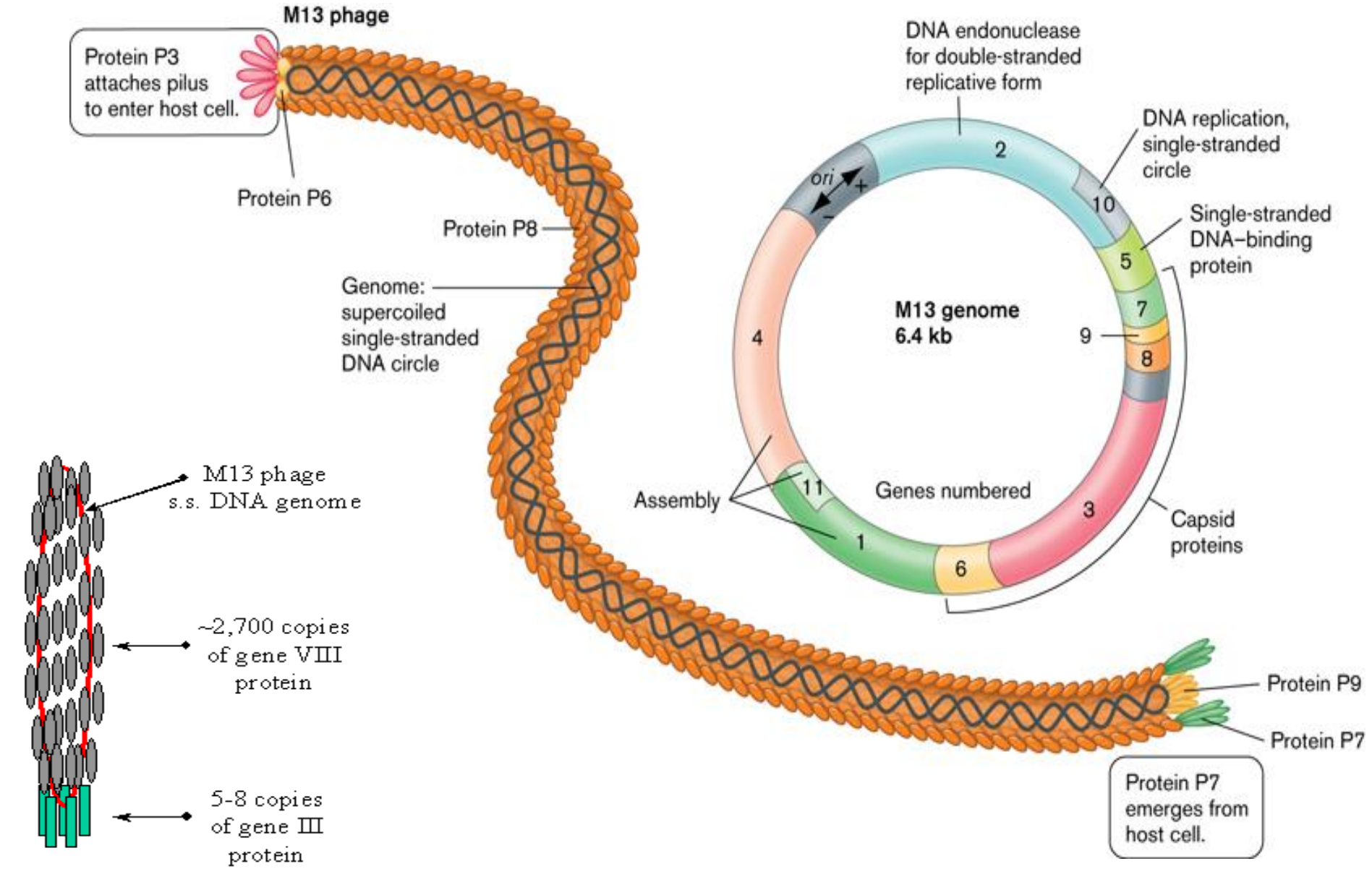
- The T4 phage initiates infection of an *E. coli* bacterium by recognizing the lipopolysaccharide cell surface receptors with the distal ends of its LTFs.
- The recognition signal is then transmitted through the LTFs to the baseplate attachment protein, gp9, and then to the baseplate itself.
- Subsequently, the STFs unravel from underneath the baseplate and bind irreversibly to the lipopolysaccharide cell surface receptors, thus securely anchoring the baseplate to the cell membrane.
- The baseplate changes its conformation from hexagonal to star-shaped, causing contraction of the tail sheath.
- The contracted tail sheath drives the head closer to the cell surface and, therefore, exerts a force onto the tail tube directed toward the cell membrane. This force is transmitted through the gp27 cylinder and the N-terminal domain of gp5 to the β-helix needle, causing the latter to puncture the outer membrane of the cell .

- As the tail sheath contraction progresses, the β-helix needle spans the entire 40 Å width of the outer membrane, thereby enlarging the pore in the membrane.

- Subsequently, when the β-helix needle comes into contact with the periplasmic peptidoglycan layer, it dissociates from the tip of the tube, thus activating the lysozyme domain of gp5. The latter digests the cell wall, allowing penetration of the tail tube to the inner membrane .

- The gp27 trimer, forming the tip of the tail tube, probably interacts with a specific receptor molecule on the cytoplasmic membrane to initiate release of DNA from the phage head through the tail tube into the host cell.

M13 Phage


M13 phage
Protein P3 attaches pilus to enter host cell.
Protein P6
Protein P8
Genome: supercoiled single-stranded DNA circle
DNA endonuclease for double-stranded replicative form
DNA replication, single-stranded circle
Single-stranded DNA–binding protein
M13 genome 6.4 kb
ori
Assembly
Genes numbered
Capsid proteins
Protein P9
Protein P7
Protein P7 emerges from host cell.
M13 phage s.s. DNA genome
~2,700 copies of gene VIII protein
5-8 copies of gene III protein

- Gene II encodes pII, which binds in the IG region (located between genes IV and II/X; not shown) of dsDNA and makes a nick in the strand, initiating replication by host proteins.
- pX is required later in infection for the switch to ssDNA accumulation.
- Gene V encodes the ssDNA binding protein pV.
- Genes VII and IX encode two small proteins located at the tip of the virus that is first to emerge from the cell during assembly.
- Gene VIII encodes the major coat protein, and genes III and VI encode pIII and pVI, which are located at the end of the virion and mediate termination of assembly, release of the virion, and infection.
- Gene I encodes two required cytoplasmic membrane proteins, pI and pXI, and gene IV encodes pIV, a multimeric outer membrane channel through which the phage exits the bacterium.
- Note that the genome is in fact circular, but is shown in a linear presentation here for clarity.

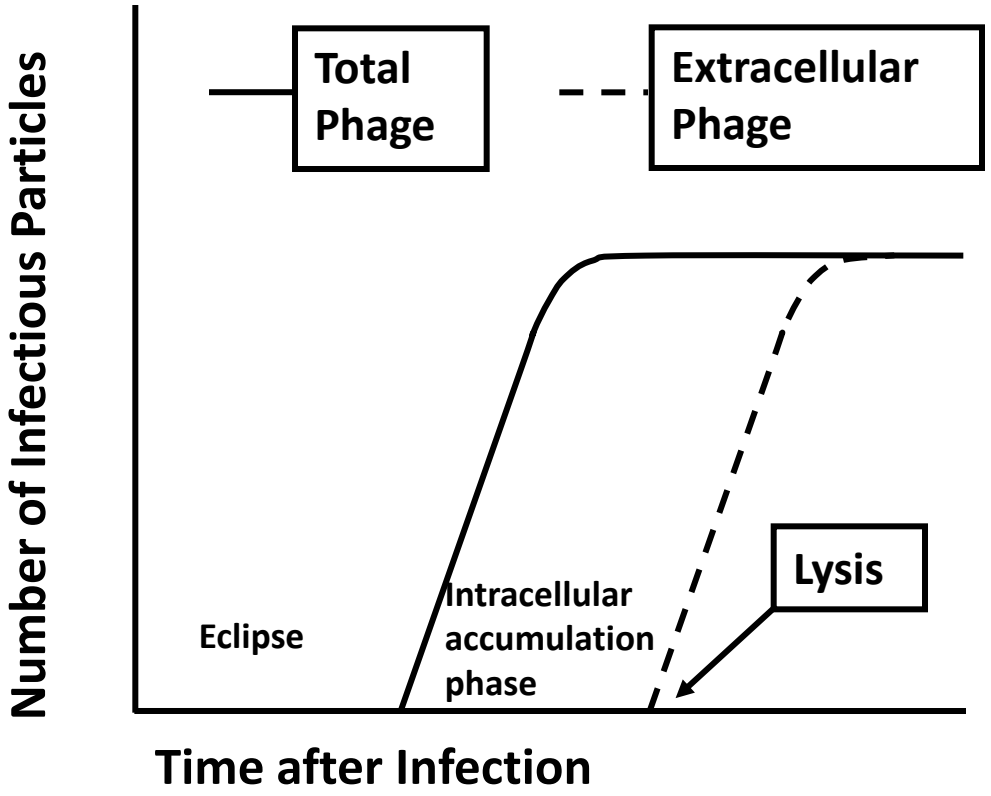
Life Cycle of M13 Phage

- Phages exhibit two different types of life cycle.
- In the virulent or lytic cycle, intracellular multiplication of the phage culminates in the lysis of the host bacterium and release of progeny virions.
- In the temperate or lysogenic cycle the phage DNA becomes integrated with the bacterial genome replicating synchronously with it causing no harm to the host cell.

- Sequential binding of pIII to the tip of the F-pilus and then the host Tol protein complex results in depolymerization of the phage coat proteins, their deposition in the cytoplasmic membrane (where they are available for reutilization), and entry of the ssDNA into the cytoplasm.
- The ssDNA is converted by host enzymes to a double-stranded RF, the template for phage gene expression.
- Progeny ssDNA, coated by pV dimers (except for the packaging sequence hairpin (PS) that protrudes from one end), is the precursor of the virion.
- A multimeric complex that spans both membranes composed of pI, pXI, pIV, and the cytoplasmic host protein thioredoxin-mediates conversion of the pV-ssDNA complex to virions and secretion of virions from the cell.
- This process involves removal of pV dimers and their replacement by the five coat proteins that transiently reside in the cytoplasmic membrane.

Lytic M13 Phage Multiplication Cycle

- Eclipse
  - Early genes
  - Phage DNA synthesis
  - Late genes
- Intracellular accumulation
- Lysis and Release

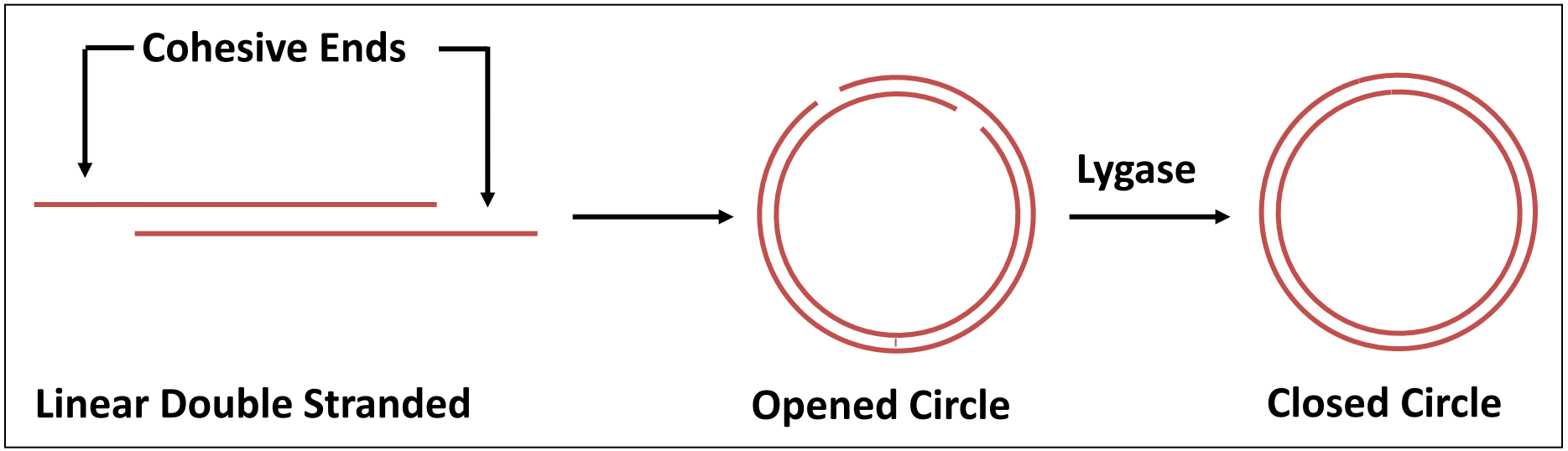


Lysogenic or Temperate Phage

- Lysogenic or temperate phages are those that can either multiply via the lytic cycle or enter a quiescent state in the cell.
- In this quiescent state most of the phage genes are not transcribed; the phage genome exists in a repressed state. The phage DNA in this repressed state is called a prophage because it is not a phage but it has the potential to produce phage.
- In most cases the phage DNA actually integrates into the host chromosome and is replicated along with the host chromosome and passed on to the daughter cells. The cell harboring a prophage is termed a lysogenic bacterium.

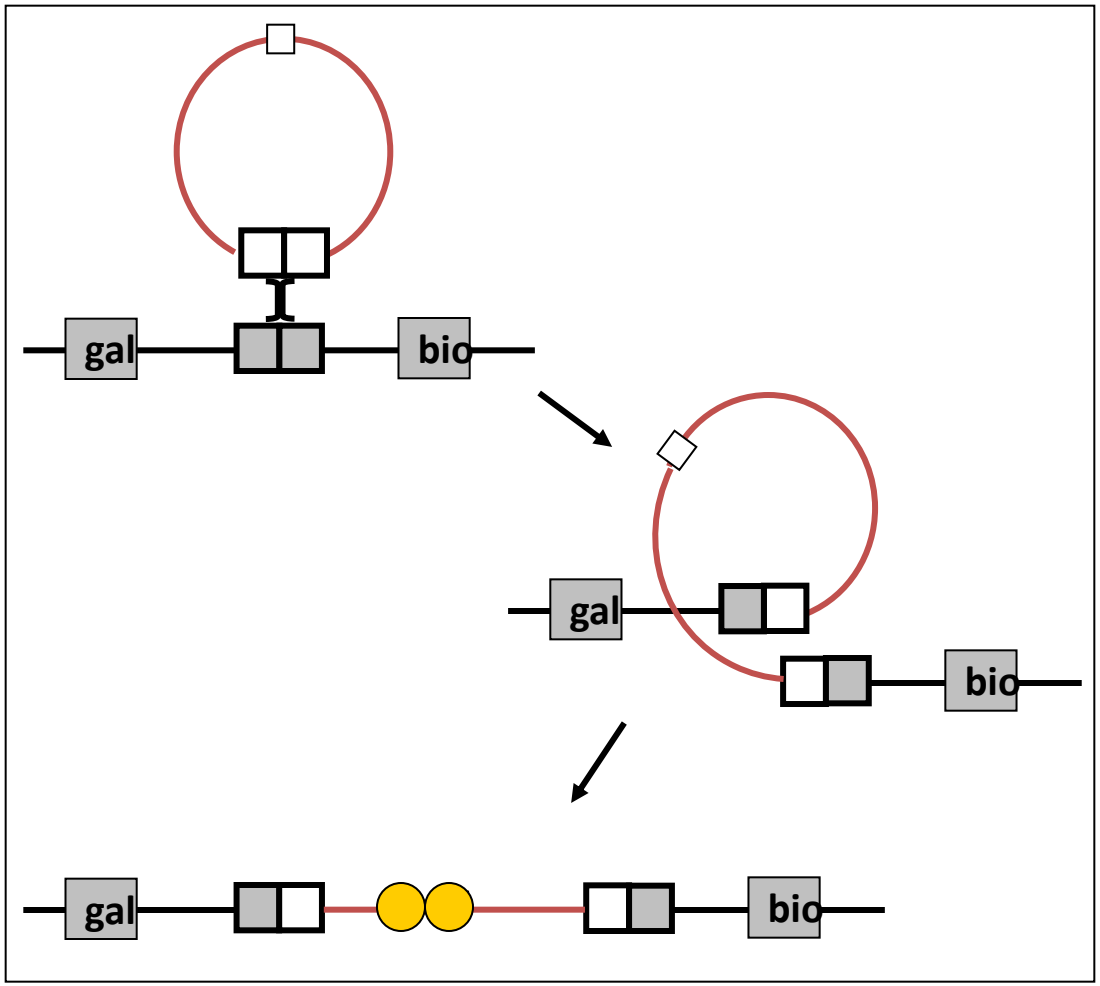
Events Leading to Lysogeny

- Circularization of the phage chromosome
  - Cohesive ends



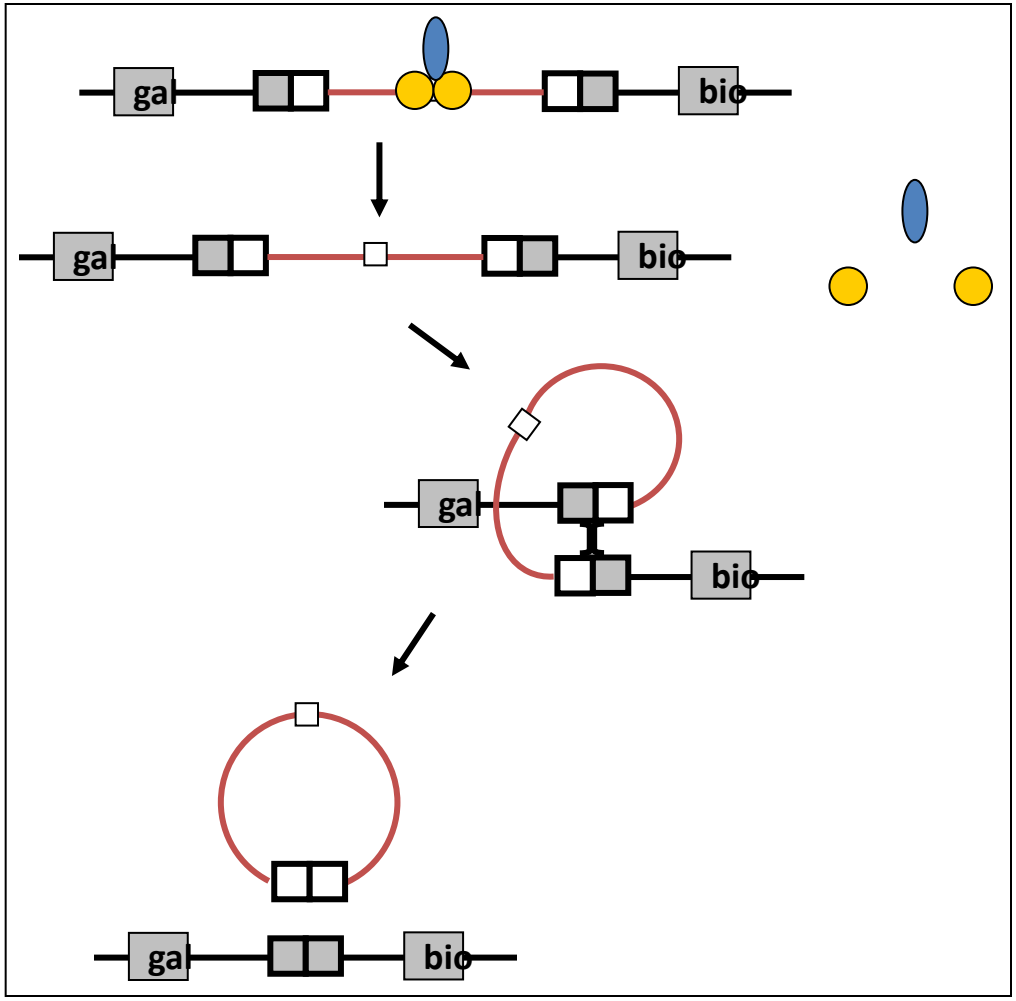
- Site-specific recombination
  - Phage coded enzyme

- Repression of the phage genome
  - Repressor protein
  - Specific
  - Immunity to superinfection



Termination of Lysogeny

- Induction
  - Adverse conditions
- Role of proteases
  - recA protein
  - Destruction of repressor



Application of M13 phage

- Used in treatment of bacterial infections
- Used for the identification of pathogenic bacteria (phage typing)
- Used in molecular biology

Lambda Phage

- Lambda phage is a virus particle consisting of a head, containing double-stranded linear DNA as its genetic material, and a tail that can have tail fibers.
- Bacteriophage lambda (λ) was discovered by Joshua and Esther Lederberg.

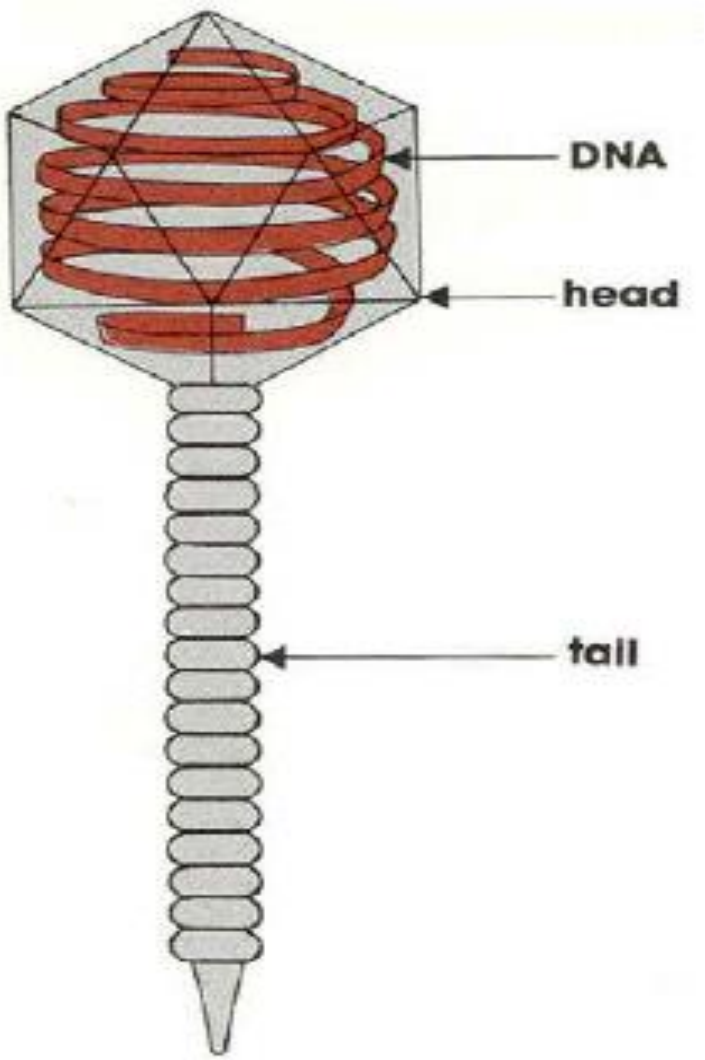
Order : *Caudovirales*

Family *: Siphoviridae*

Genus : λ-like viruses

Species : Enterobacteria phage λ

Morphology



- Head: icosahedral symmetry
- Tail: Helical Symmetry
- one tail fiber
- Capsid not enveloped
- Linear dsDNA (phage)
- Infected *E.coli* cell

- The l genome is circular and 48502 base pairs long.

- Genes and open reading frames are shown as coloured boxes, promoters as arrowheads, transcripts as coloured lines above or below the genes, and terminators as circles.

- For clarity, only a few genes have been named. Groups of genes are colour-coded; lysogenic genes are red, early lytic genes expressed from P_R are green, early lytic genes expressed from P_L are blue, and late lytic genes expressed from P_R' are purple.

- Regions of the late lytic transcript encoding proteins involved in cell lysis and in phage head and tail formation are indicated.

- The genes required for integration and excision (*int* and *xis*) are shown in orange.

- Transcripts made from cII-activated promoters (P_{RE}, P_{I} and P_{aQ}) are in yellow.

- The cohesive ends (*cos*) and phage attachment site (*attP*) are represented by a black circle and black rectangle, respectively.

Head & Tail genes

- The head and tail genes code for the structural proteins of the bacteriophage capsid as well as the terminase enzyme required to process rolling circle multimers into unit genome-length pieces during packaging.

Nu1	DNA packaging
A	DNA packaging
W	head-tail joining
B	capsid component
C	capsid component
Nu3	capsid assembly
D	head-DNA stabilization
E	capsid component
Fi	DNA packaging
Fii	head-tail joining
Z	tail component
U	tail component
V	tail component
G	tail component
T	tail component
H	tail component
M	tail component
L	tail component
K	tail component
I	tail component
J	tail component
lom	tail:host specificity
orf-401	outer host membrane

N	early gene regulator
rexb	exclusion
rexa	exclusion
cI	repressor
cro	antirepressor
cII	regulator
ren	exclusion
Nin 146	pept unknown
Nin 290	pept unknown
Nin 57	pept unknown
Nin 60	pept unknown
Nin 56	pept unknown
Nin 204	pept unknown
Nin 68	pept unknown
Nin221	pept unknown
Q	late gene regulator

Regulation genes

- The regulation region includes the immunity region as well as the genes that are responsible for controlling the switch between lysogenic and lytic growth.

Replication genes

- The replication region includes two replication protein genes **O** and **P** and the origin of replication.

O	**DNA replication**
P	**DNA replication**

Recombination genes

orf28	
ral	restriction alleviation
ea10	ssb
cIII	regulation
kil	host-killing
gam	recombination
bet	recombination
exo	exonuclease
orf60a	
orf63	
orf61	
ea22	
ea8.5	
xis	excision
int	integration protein
ea31	
ea47	

Lysis genes

orf-64	
S	cell lysis
R	cell lysis
Rz	cell lysis

Life cycle of lambda phage

- Lytic cycle
- Lysogenic cycle

- Lysis: Infection by phage produces many progeny and breaks open (lyses) the host bacterium.

- Lysogeny: After infection, the phage DNA integrates into the host genome and resides there passively.

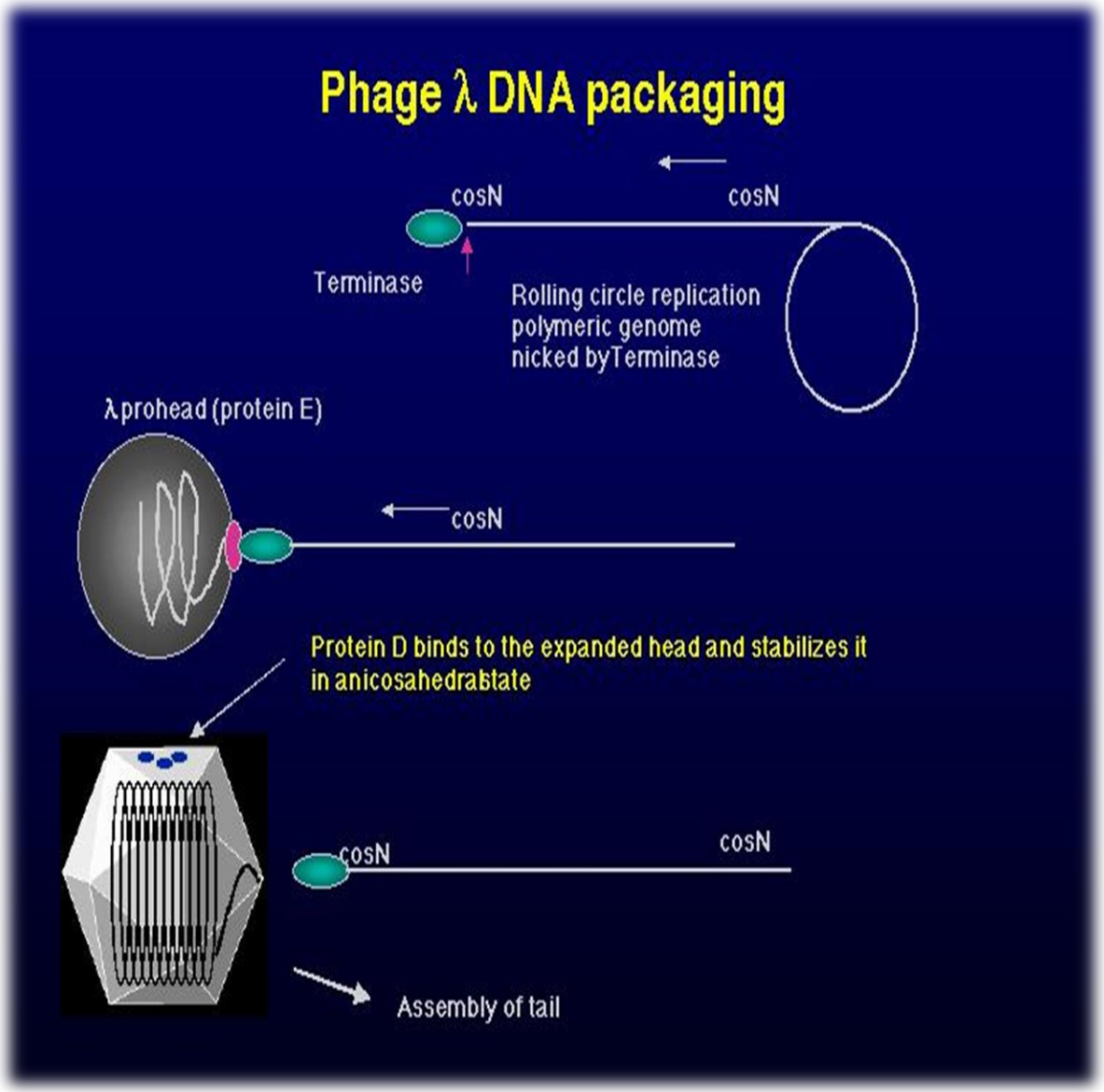
- Bacteriophage lambda can do either.

  - No progeny
  - No lysis of the host
  - Can subsequently lyse (lysogeny)

Lytic cycle

1. Adsorption specific with surface of the cell (outer memb.)
2. Injection
3. Circulation cos site ,nuclease host
4. Replication : bidirectional
5. Protein coding : endonuclease plus st.
6. Syn new minus strand and new plus st.
7. Lytic : rolling circle : long DNA for multiple phage genome
8. Code structeral protein DNA phage
9. Packaging
10. Phage code endolysin destroy peptidoglycan

    Cell lysis



Lytic cycle

- ❑ Nick
- ❑ Rolling circle
- ❑ Polymeric genome
- ❑ Endonuclease
- ❑ Cohesive end

Transcription

- In bacteriophage infection cycle, gene expression can be classified into 3 distinct phases.

- The 3 phases in bacteriophage lambda are:

  - Very early expression
  - Early expression
  - Either late lytic or late lysogenic expression

- The **first** phase involves synthesis of proteins that will take over the host cell.
- These proteins often include a phage-specific RNA polymerase.
- The **second** phase involves replication of the bacteriophage.
- The **third** phase is the assembly and packaging of mature bacteriophage capsids.

Very Early Expression

- Transcription from P_L proceeds in a leftwards direction until it is terminated at t_L1. This t ranscript allows expression of the N protein, which is an anti-terminator.

- Transcription from P_R proceeds in a rightwards direction until it is terminated at t_R1. This transcript allows expression of the Cro protein.

- Transcription from P'_R proceeds in a rightwards direction but it is soon terminated at t'_R before it transcribes through any coding sequences.

Early Expression

In addition to Cro, the early transcript of P_R codes for:

- The CII protein - a transcriptional activator required for lysogenic growth.
- The O & P proteins - required for replication of the bacteriophage.
- The Q protein - another antiterminator protein; Q is required for late gene expression .
- Transcription continues as far as the terminator, t_R3.

- Transcription continues as far as the terminator, t_R3.

In addition to N, the early transcript of P_L codes for:

•The CIII protein -- required to protect the activator protein, CII

•The Xis protein -- normally required for excision of a prophage

•The Int protein -- normally required for integration of a prophage.

Late Expression

- During lysogenic growth, late gene expression depends on the action of the CII and CIII proteins. CII is an unstable and protease-sensitive protein. CIII helps to protect CII from degradation.

Activation of transcription from P_{RE}

CI is expressed directly. Once the protein starts to accumulate in the cell, it will activate its own transcription as a result of binding to O_R to activate P_{RM}

Replication of Bacteriophage Lambda

- Bacteriophage lambda contains a linear dsDNA genome.

- The ends of the genomic DNA are single-stranded and are **cohesive**, i.e. they are complementary to one another. The two cohesive ends known as ***cos*** sites.

Early replication

- Bacteriophage lambda initially replicates by means of **theta** form intermediates.
- The origin of replication (***ori***) is located within the **O** gene, whose product is required for replication.
- The gene **P** product is also required for replication.
- The gene **P** product has a function analogous to that of **DnaA**as a function analogous to that of **DnaC.**
- It helps **DnaB** to bind to the "melted" DNA.
- Thereafter, the other components of a bacterial replisome can bind and replication ensues.
- This mode of replication continues for 5 - 15 minutes after replication.

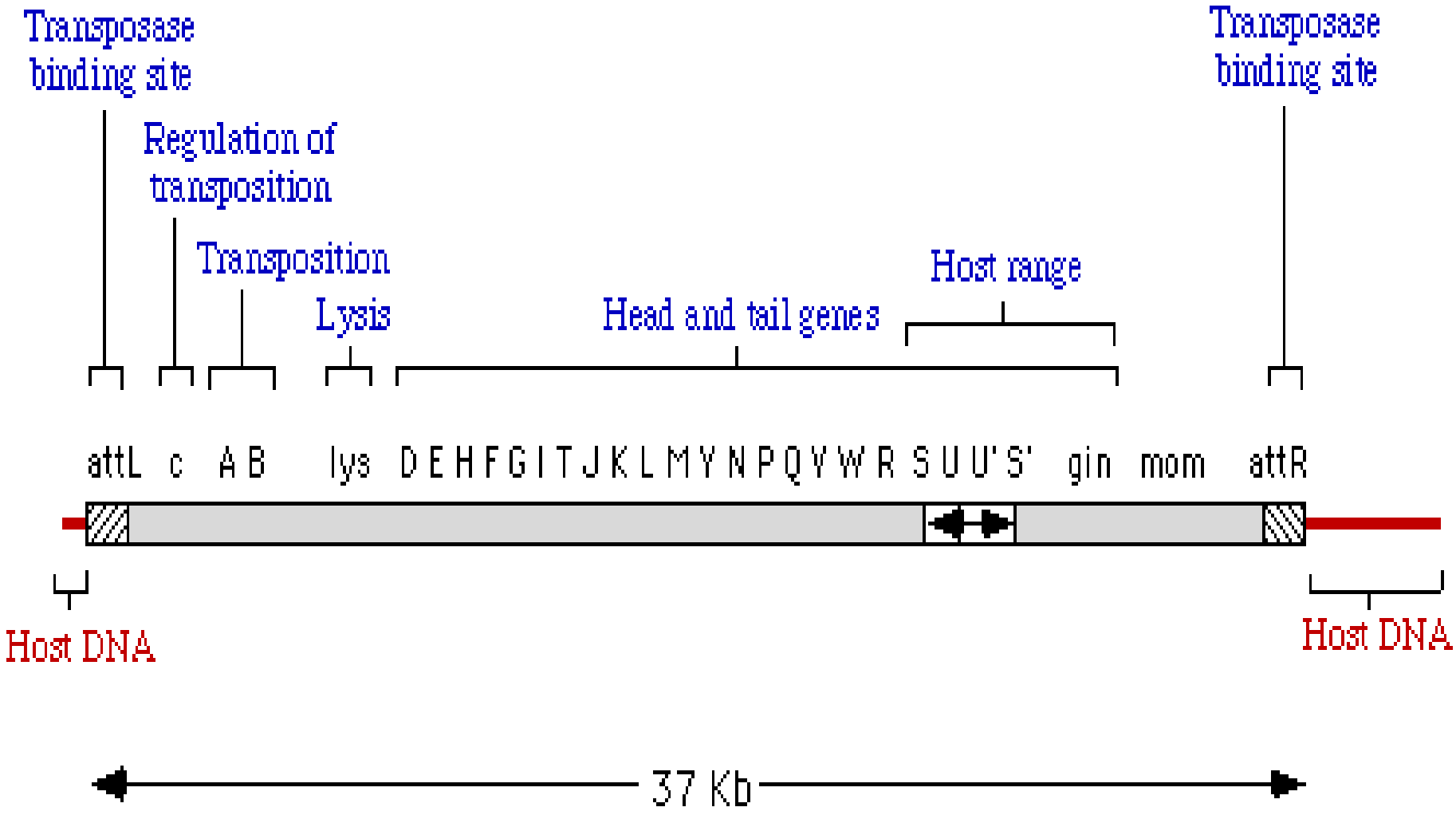
Late replication

- After 15 minutes, bacteriophage lambda switches to replication by a rolling circle mechanism.
- The action of **Terminase** which consists of two protein subunits coded by the lambda ***A*** and ***Nu1*** genes.
- Gene ***A*** codes for a 74 kDa protein; ***Nu1*** codes for a 21 kDa protein.
- **Terminase** recognizes the ***cos*** sites (in its double-stranded form) and cleaves them to generate new cohesive ends.
- After the first ***cos*** site has been recognized, the second one must be located within 75% to 105% of the unit length of the phage chromosome.
- Bacteriophage lambda derived cloning vectors can only be used to clone DNA fragments that are less than 15 kb in size.
- The nutritional status of cells is certainly important and there are proteases other than *ftsH* which can breakdown cII and are regulated by the cell cycle. Although a model organism for the study of bacterial infection, λ still holds its mysteries.

Mu Phage

Group : Group I (dsDNA)
Order : Caudovirales
Family : Myoviridae
Genus : Mu-like viruses
Species : Mu Phage

Genetic map of bacteriophage Mu



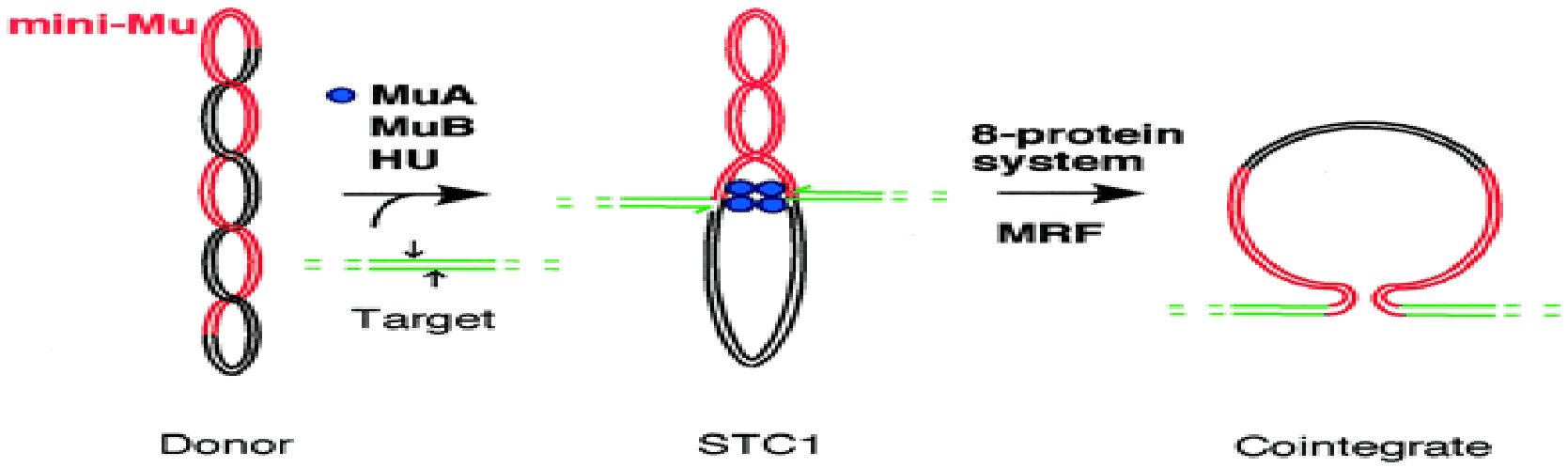
The life cycle of phage Mu is shown in the cartoon below.

(A)When Mu infects a sensitive host, the linear DNA enters the cell and the Mu DNA (i.e. not including the variable sequences of DNA acquired from the previous host) is inserted into the recipient genome via a non-replicative, "cut and paste" mechanism.

(B) Lysogens of wild-type Mu are quite stable and are not induced by UV or other DNA damaging agents. However, derivatives of Mu with a temperature sensitive repressor -- Mu c(Ts) -- can be induced by shifting the lysogen to 42˚ C.

(C) When the repressor is inactivated, the A and B proteins are expressed and Mu transposes by a replicative mechanism to 50 - 100 new sites on the chromosome. Meanwhile, late phage gene products are made (including phage heads, tails, lysis proteins, etc).

The phage DNA is packaged by a headful mechanism, beginning by cutting the dsDNA in host sequences located about 100 bp from the left end of Mu. The length of Mu DNA is about 37 Kb and about 39 Kb are packaged into each head, so about approximately 2 Kb of host DNA is included on the right end of the packaged DNA. After assembly of the phage, the host is lysed, releasing 50-100 phage particles.



Replication of Mu by transposition

- In the first stage, the phage-encoded transposition proteins aided by the histone-like protein HU promote transfer of 3′-OH ends of miniMu (red) to each strand of target DNA (green).

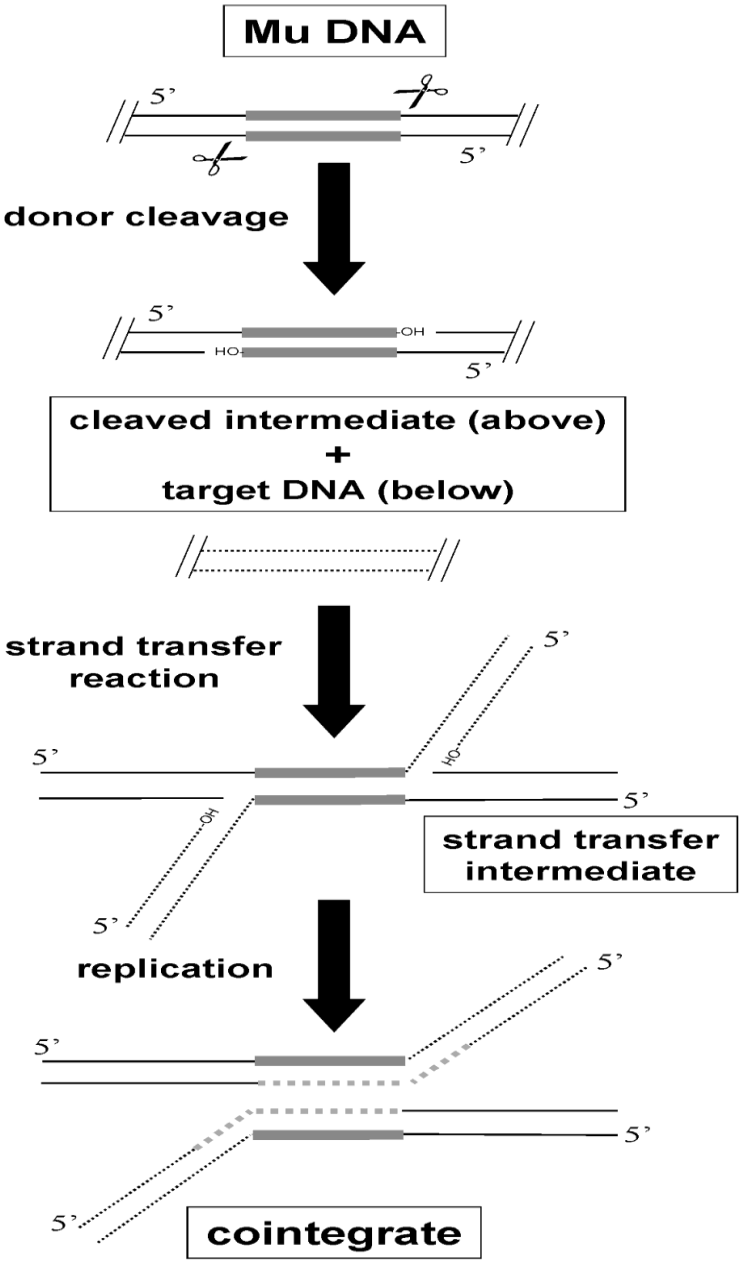
- Two sites, 5 bp apart on target DNA, that will be subjected to a nucleophilic attack by each Mu end are indicated by arrows.

- Strand exchange produces a fork at each Mu end, the target providing 3′-OH ends (indicated by half arrows) that can potentially serve a primers for leading strand synthesis.

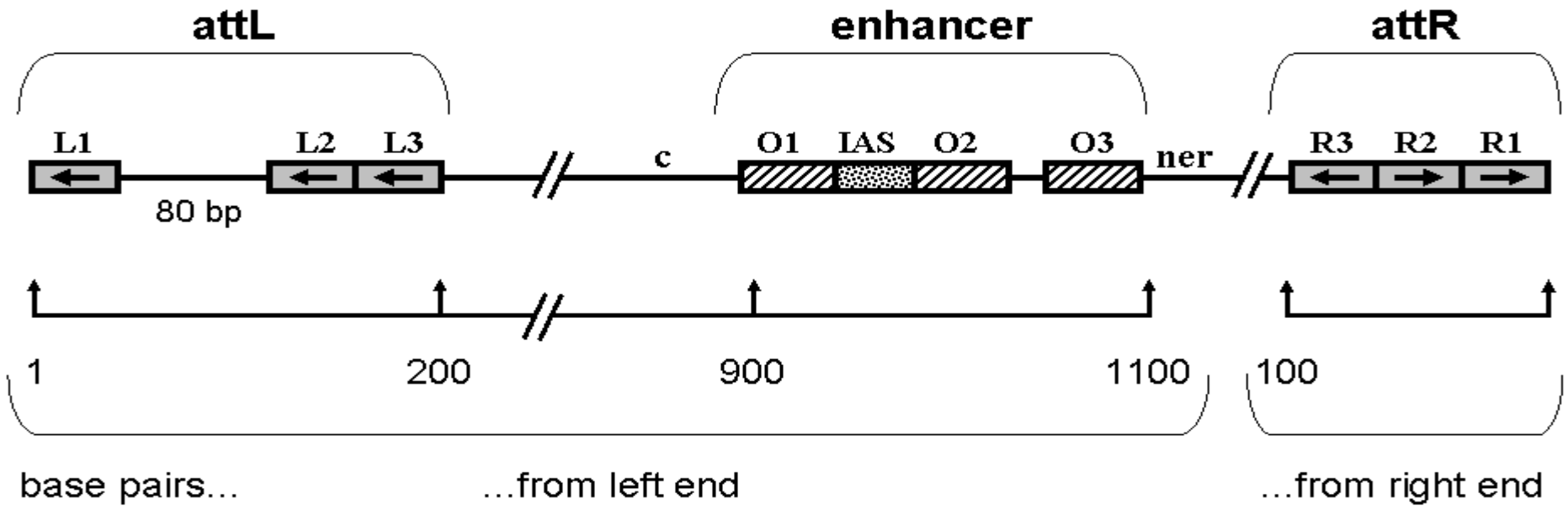
- MuA transposase, which has been assembled into an oligomeric transpososome, remains tightly bound to both Mu ends in the strand exchange product (strand transfer complex, STC1).

- Host factors then initiate Mu DNA synthesis from one end to duplicate Mu and form the final cointegrate product. The DNA synthesis phase was initially reconstituted in an eight-protein system supplemented with partially purified host factors (MRF)

The Shapiro model for transposition



The transposase targets



- The att sites, located at phages extremities, and the enhancer sequence are recognized and bound by the phage transposase.
- Each att site is formed by three binding sites for Mu A (see section, 'Replicative transposition').
- These structures are also recognized by phage repressor which shows overlapping binding specificity with the transposase.

Mu Phage applications

- Practical significance of constructing plasmid-less recombinant bacterial strains for use in applied microbiology and biotechnology are increasing due to the potential for genetic instability to reduce the number of active recombinant alleles in plasmids (Friehs 2004) and restrictions on the application of plasmids in large-scale industries.
- In vivo chromosomal editing methods, which are primarily based on homologous and/or site-specific recombination of DNA as well as on transposition mechanisms, have been used to engineer plasmid-less bacteria.
- The increase in genomic copy number of native or previously modified target genes is an important tool for chromosomal editing and the construction of stably maintained bacterial genomes.

P1 Phage

Group : Group I (ds DNA)

Order : Caudovirales

Family : Myoviridae

Genus : P1 – like viruses

Species : P1 Phage

- Temperate phage, such as P1, have the ability to exist within the bacterial cell they infect in two different ways. In lysogeny, P1 can exist within a bacterial cell as a circular DNA in that it exists by replicating as if it were a plasmid and does not cause cell death.
- Alternatively, in its lytic phase, P1 can promote cell lysis during growth resulting in host cell death. During lysogeny new phage particles are not produced.
- In contrast, during lytic growth many new phage particles are assembled and released from the cell.
- By alternating between these two modes of infection, P1 can survive during extreme nutritional conditions that may be imposed upon the bacterial host in which it exists.

- A unique feature of phage P1 is that during lysogeny its genome is not incorporated into the bacterial chromosome as is commonly observed during lysogeny of other bacteriophage. Instead, P1 exists independently within the bacterial cell, much like a plasmid would.
- P1 replicates as a 90 kilobase (kb) plasmid in the lysogenic state and is partitioned equally into two new daughter cells during normal cell division.
- P1 encodes a site-specific recombinase, Cre, that is widely used to promote cell-specific or time-specific DNA recombination via flanking loxP sites

Genetic and physical organization of the P1 genome

- Boxes with internal triangles show positions and orientations of genes, color-coded by function: yellow, plasmid maintenance; red, repression of early functions; pink, immunity control, not *c*1 itself; magenta, source of tRNAs; brown, DNA methylation; deep blue, transcriptional activation of late genes; grey, defective IS*1*; green, all other.
- Black boxes are intergenic regions of defined function: recombination sites, iterons to which RepA binds, plasmid centromere, and origin of DNA packaging, the direction of packaging being indicated by an arrowhead at the *pac* site. Bidirectional replication determined at the phage (lytic) origin, *oriL*, and at the plasmid origin, *oriR*, are indicated by black arrowheads above the genome map. C1 operator sites are marked with red flags pointing to the left or right.

- Thin lines with terminal deep blue half arrowheads indicate the start sites and directions of the transcripts from particular late promoters. GATC sequences that overlap transcriptional promoters and clustered 5-GATC sequences (two or more sites with pairwise separation of not more than 50 bp), substrates for Dmt or Dam methylation, are marked above the gene map by brown lollipops that are filled in the case of sites shown to alter function upon methylation.
- Hooks indicate Rho-independent transcriptional terminators. They face the starts of transcripts that they terminate. The map refers to the genome of P1 *c*1-100 *mod749*::IS*5* without its nonintegral part, IS*5*

Applications of phage P1

- P1 possesses a site-specific recombination system.
- P1 has served as a model organism for different aspects of phage and biology such as DNA restriction modification, site-specific recombination, plasmid replication, partition, incompatibility and addiction