

Chapter 17 Lecture Notes: The Virus: Bacteriophages

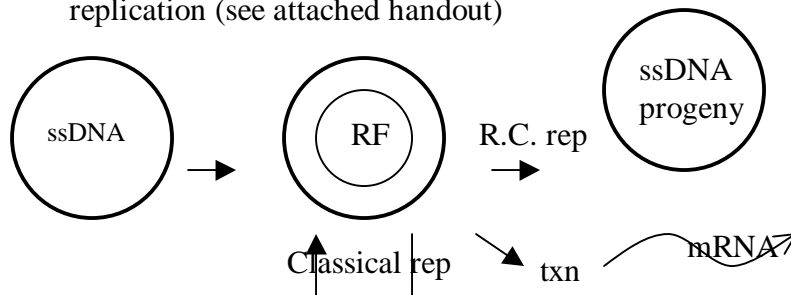
- I. General info: Bacteriophages (or phages) are viruses that infect bacteria.
- II. Virulent bacteriophages (lyse host cells as a result of expression of the phage genome)
- A. Lytic cycle = life cycle of viruses that result in the lysis of the host bacterium
- B. Experimentally followed via one-step growth curve in which infection of bacteria with phage is synchronized and then # of viable phage are quantitated at various time points. Fig 17-2

1. Latent phase = shortest period required for phage production.
 - a) eclipse period = no phage even inside cell
 - b) post-eclipse period = phage only inside cell
2. Rise period (burst) = phage are released
3. Plateau = no more phage released

C. What does a virulent phage need to do to reproduce? (Fig 17-5 and attached)

1. Adsorption = Attachment to host cell via specific receptors
 - a) Phage T4 uses LPS core polysaccharide
 - b) Phage lambda uses LamB (protein for maltose uptake)
 - c) Phage MS2 uses F-pili (conjugation pili)
 - d) Others use other proteins, teichoic acids, flagella
2. Penetration = injection of phage DNA into cell (eclipse period of latent period)
3. Synthesis of early mRNA (eclipse period of latent period)
 - a) encodes proteins for phage replication
 - b) encodes proteins for phage RNA polymerase
 - c) encodes proteins for turn off of host cell functions
4. Synthesis of phage nucleic acid (eclipse period of latent period)
 - a) ds DNA phage
 - (1) classical bidirectional followed by concatamerization of the end which are later cleaved into individual molecules when the DNA is packaged in the capsid (ex. T7)
 - (2) rolling circle replication (see attached handout)
 - b) ss DNA (F X174)

replicates through a ds replicative form (RF) using rolling circle replication (see attached handout)



c) ss RNA (+) (MS2)

After injection of + ssRNA → replicase protein (RNA dependent RNA polymerase) is translated → replicase protein synthesizes (-) strand RNA from (+) strand RNA → new (-) strand RNA serves as template for replicase to make lots of (+) strand RNA for viral progeny and for translation to make viral proteins

Figure 17-13

d) ss RNA (-) and ds RNA

(1) replicase is injected with RNA into cell because the genome that is injected can not be translated into protein (must first be copied to + strand RNA

5. Synthesis of proteins required for assembly of the viral capsid (eclipse period of latent period)

- a) structural proteins
- b) scaffolding proteins

6. Assembly of phage (post-eclipse period) → Phage nucleocapsids are assembled

7. Release of phage (rise/burst period)

- a) Usually from cell via cell lysis via viral specific lysozyme or endolysin
- b) Rarely secreted from cell without lysis

D. T4 life cycle as an example

1. General features of T4

- a) The virion is structurally complex, consisting of an icosohedral head attached to a tail and tail fibers.
- b) The nucleic acid is circularly permuted (see below) dsDNA of 165 kilobasepairs.
- c) The DNA contains a modified base called 5-hydroxymethylcytosine (HMC) that is glycosylated (Fig. 17-7)

2. Life cycle:

a) Adsorption of T4

- (1) Attachment to host cell LPS core polysaccharide via tail fibers.
- (2) contact of tail baseplate with cell wall

b) Penetration of T4 (Fig. 17-3)

- (1) lysosome-like enzyme creates "hole" in peptidoglycan
- (2) contraction of tail sheath
- (3) central tube is pushed through hole
- (4) injection of phage DNA through central tube and into cell

c) Synthesis of T4 early mRNA by host RNAP and middle mRNA by phage modified RNAP

- (1) encodes proteins for DNA synthesis
 - (a) for degradation of host DNA to nucleotides
 - (b) for formation of glycosylated HMC
 - (c) for DNA polymerization
- (2) encodes proteins (MotA and AsiA) that modify host RNA polymerase so that it recognizes phage promoters of middle genes
- (3) encodes proteins that modify host RNA polymerase so that it can transcribe late genes (for phage capsid)
 - (a) modification is such that only replicating DNA is transcribed late in the growth phase due to these proteins
 - (b) thus there is a link between replicating DNA and capsid assembly
- (4) encodes proteins for turn off of host cell functions

3. Synthesis of T4 DNA

a) Classical bidirectional: 5' ABCDEF//UVWXYZAB3'
3' abcdef//uvwxyzab5'

b) Digestion of the ends with a phage exonuclease to leave complementary 5' overhangs:

5' ABCDEF//UVWXYZ 3' 5' ABCDEF//UVWXYZ 3' 5' ABCDEF//UVWXYZ 3'
3' cdef//uvwxyzab5' 3' cdef//uvwxyzab5' 3' cdef//uvwxyzab5'

c) Concatamerization of the replicated molecules:

5' ABCDEF//UVWXYZABCDEF//UVWXYZABCDEF//UVWXYZ
3' abcdef//uvwxyzabcdef//uvwxyzabcdef//uvwxyz

d) Concatamers are later cleaved into individual molecules that are 2% larger than the actual genome (A-Z) so that each genome is terminally redundant (within a molecule the ends are the same). Note that each separate molecule has different ends. However, if you circularize the molecules they are genetically identical (circular permutation)

5' ABCDEF//UVWXYZAB3' 5' CDEF//UVWXYZABCD3' 5' EF//UVWXYZ
3' abcdef//uvwxyzab5' 3' cdef//uvwxyzabcd5' 3' ef//uvwxyz

4. Synthesis of T4 late proteins

- phage structural proteins
- proteins that help in assembly but are not part of the final capsid
- proteins involved in cell lysis and phage release

5. Assembly of T4

- Fig. 17-11
- Extreme condensation of DNA to fit into phage head (5000X)
- The DNA concatamer (102%) is put into the head (ATP-dependent) and then cleaved

6. Release of T4

- T4 protein disrupts plasma membrane
- Lysozyme attacks the cell wall peptidoglycan

III. Temperate bacteriophage: Lysogeny

A. Definitions

1. lysogeny → relationship between a phage and its host in which the phage genome remains in the host cell after infection and is maintained with the host genome so that a clone of infected cell develop
2. lysogen → bacterium that carries a lysogenic phage
3. temperate phage → phage that is capable of either setting up a lysogenic or lytic relationship with the host
4. prophage → term used to describe a temperate phage that is being maintained with the host genome
5. induction → process by which phage reproduction is initiated in a lysogen
6. lysogenic conversion → alteration of the phenotype of a lysogen by the prophage
 - a) phage β carries diphtheria toxin gene in the bacterium *Corynebacterium diphtheriae*
 - b) phage carries cholera toxin in *Vibrio cholerae*
 - c) phage carries shiga toxin in *Shigella dysenteriae*
 - d) phage e alters LPS of *Salmonella*
7. immunity → a lysogen is unable to be infected by another phage of the same type
8. cryptic virus → virus in bacterial genome that has lost the ability to leave the host

B. General life cycle of temperate phage (see attached):

1. Infection of host cell
2. Choice between lytic and lysogenic life
 - a) Lysogenic
 - (1) Infection of a population of cells that have limited nutrients
 - (2) Infection of a population at a high multiplicity of infection (MOI)
 - b) Lytic
 - (1) Infection of rapidly, growing unstressed cells
 - (2) see below too for induction of lytic growth
3. If lysogenic is chosen: Synthesis of:
 - a) repressor protein → to repress lytic phage genes
 - b) and in some cases integrase protein → for insertion of phage DNA into the host chromosome (however, note that some prophage are maintained extrachromosomally as plasmids)
 - c) Replication of phage DNA along with chromosomal DNA
4. What happens if the host cell dies or appears to be dying? Phage responds to environmental signals that indicate cell death is possible and inactivation of the repressor protein occurs to allow lytic growth again (better get out before the host is dead)

C. Most bacteria isolated from nature are lysogens for phage.

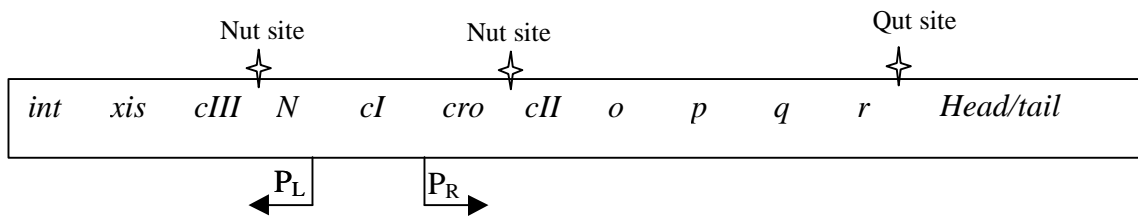
D. Phage lambda as a model

1. General features (Fig. 17-14)

- a) complex morphology: icosohedral head with tail
- b) ds DNA – linear with 12 bp single stranded cohesive ends that are complementary and join to form a circle in the host (Fig. 17-15)

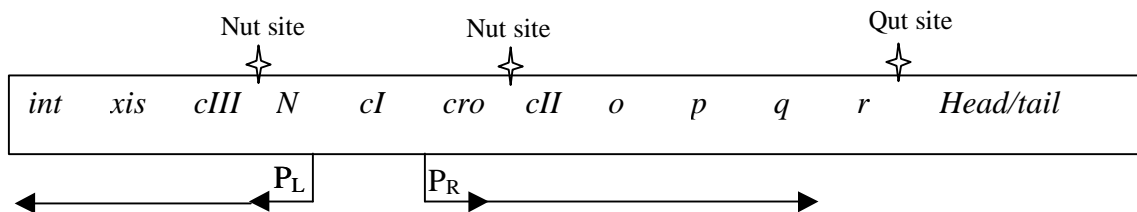
2. Life cycle:

- a) Attachment of lambda to LamB protein that usually binds maltose
- b) Injection of lambda of DNA
- c) Circularization of the DNA via cohesive ends and host DNA ligase
- d) Very early after infection
 - (1) host RNA polymerase transcribes N antiterminator gene from the P_L promoter and *cro* gene (encodes a repressor protein) from the P_R promoter



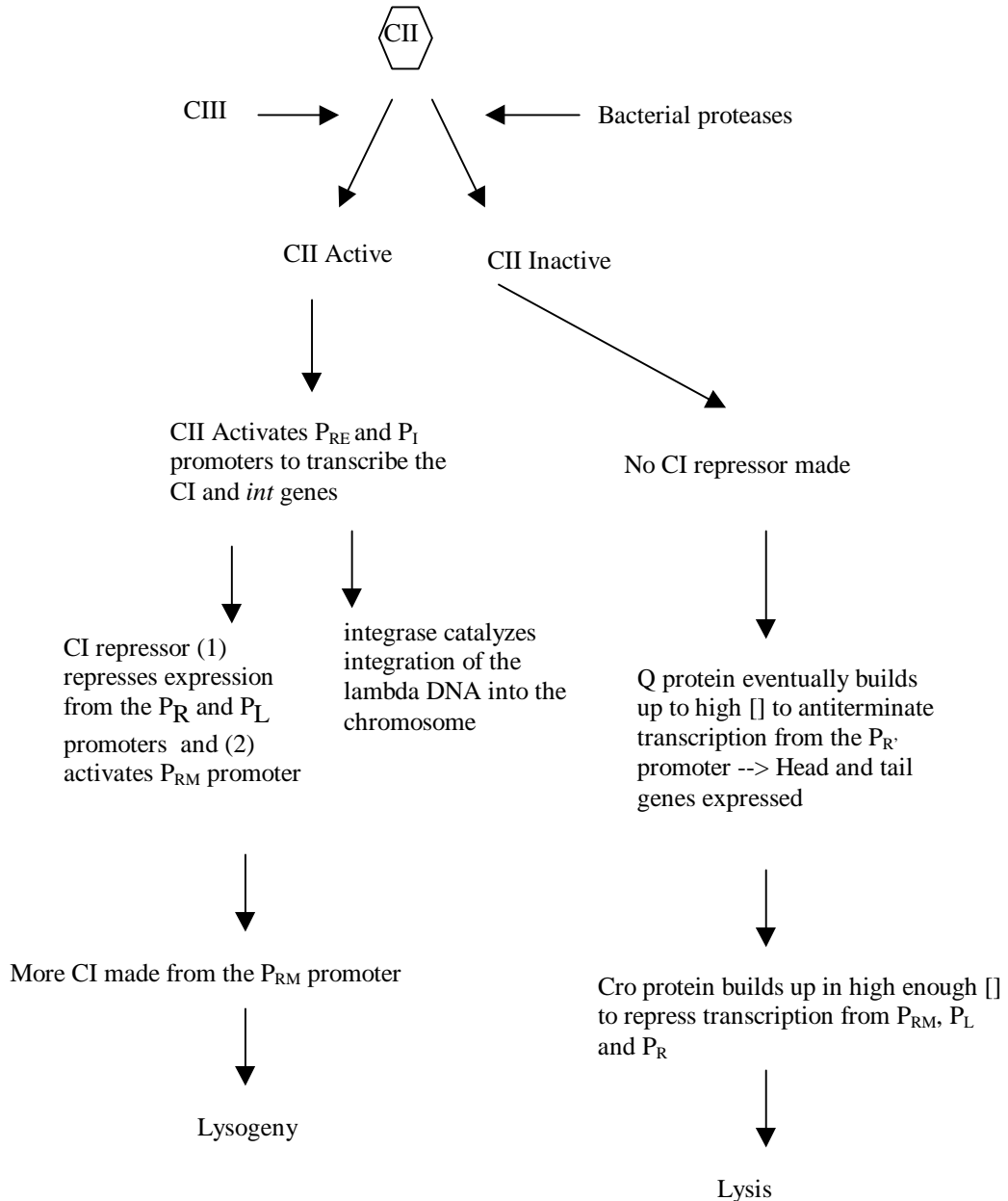
e) Early

- (1) N prevent the termination of transcription at the Nut sites so that the *CIII*, *xis*, *int* genes are transcribed from the P_L promoter
- (2) N prevent the termination of transcription at the Nut sites so that the *CII* (encodes a transcriptional activator protein that activates expression of the repressor protein), *O*, *P*, (initiation of DNA replication) and *Q* (antiterminator) genes are transcribed from the P_R promoter



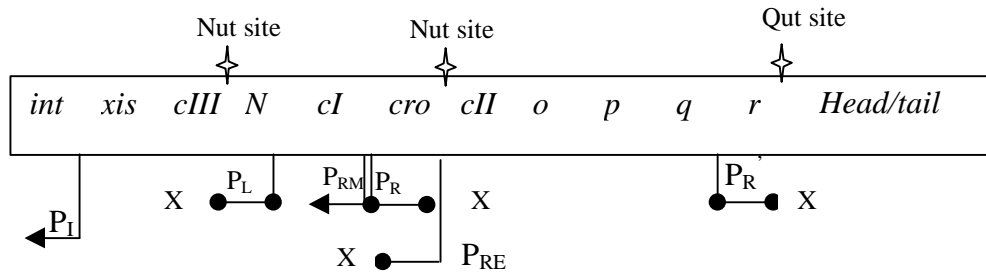
f) Choice between lysogeny and lysis lifestyles: ACTIVITY OF THE CII PROTEIN

CII is a highly unstable protein that is degraded by host proteases which are in turn regulated by growth medium conditions. The activity/inactivity of CII determines which pathway will be followed because CII activates the expression of the CI (repressor) protein, which then represses lytic growth.

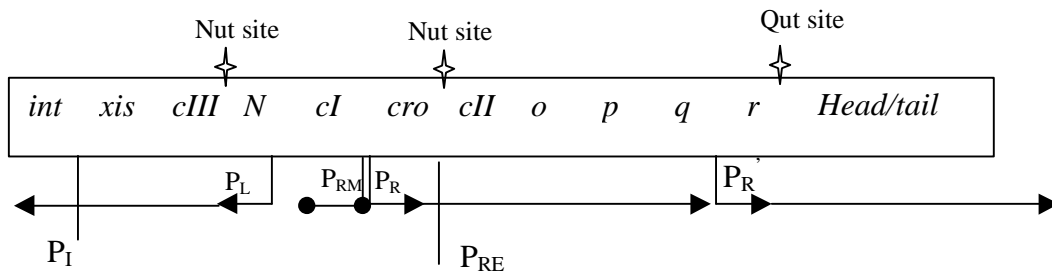


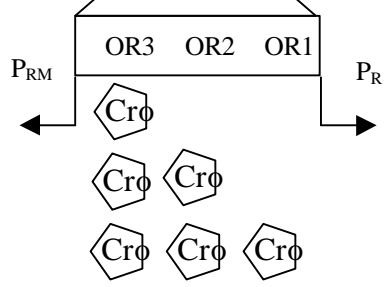
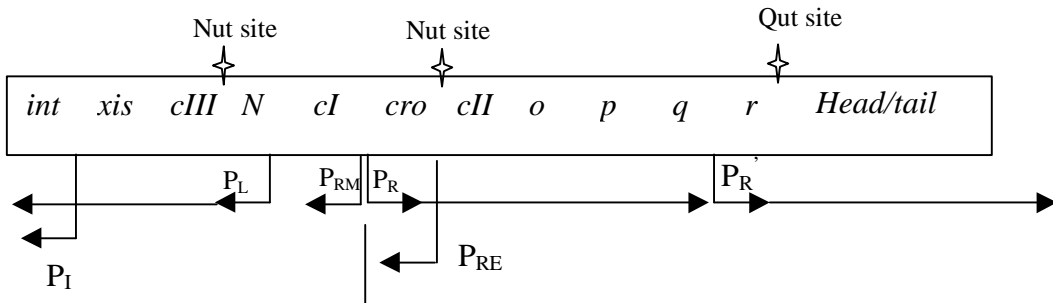
g) There is a “race” between Cro and CI binding to the region that is comprised of P_{RM} and P_R .

If $CI > Cro$: At low concentration of CI, CI will bind to P_R and P_L repress transcription of all phage genes (except *CI* and *int*). At moderate concentrations of CI, CI activates the transcription of P_{RM}

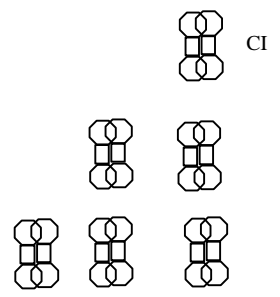


If $Cro > CI$: At low to moderate levels of Cro, Cro prevents the transcription of P_{RM} . At moderate to high levels of Cro, Cro also turns off P_R and P_L . However, by this point the genes are not needed.



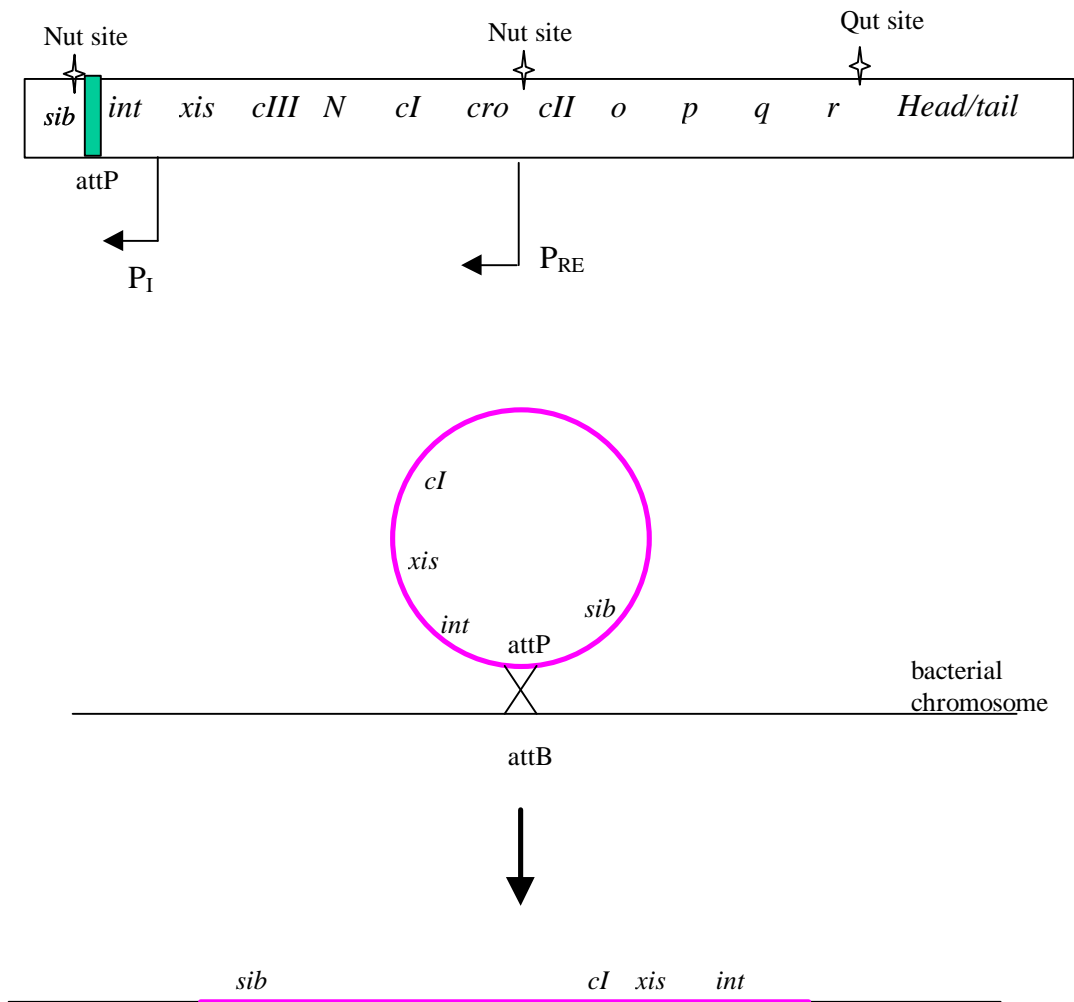


P_{RM}	OR3	OR2	OR1	P_R
off				on
off	X			on
off	X	X		off
off	X	X	X	off

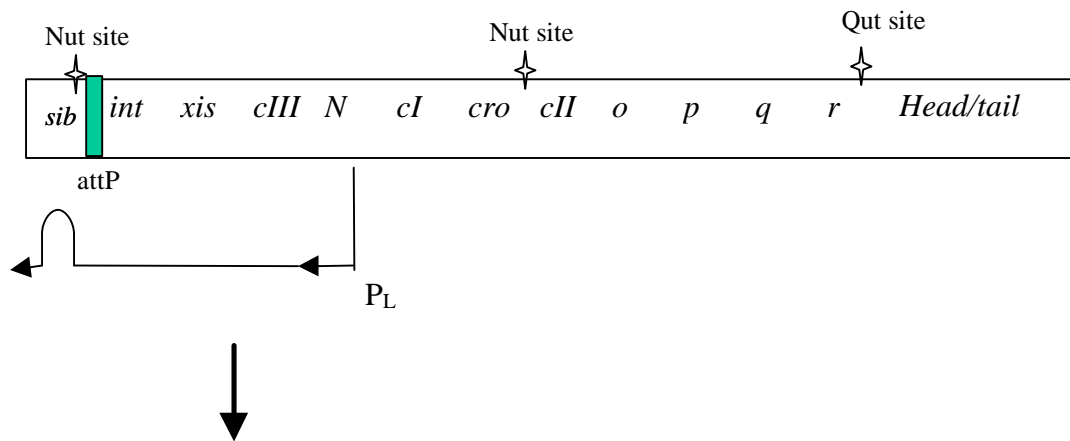


P_{RM}	OR3	OR2	OR1	P_R
off				on
off			X	off
on		X	X	off
off	X	X	X	off

h) Integration



i) Preventing integration during lytic growth



Hairpin structure causes RNaseIII to degrade mRNA from 3' end; thus no *int* mRNA left

j) Induction of prophage lambda

The presence of environmental stresses (including UV light, chemical mutagens) activated the RecA protein which binds to the CI protein and mediates self cleavage of CI → Xis protein produced which excises the prophage from the genome

