Lambda phage

MIC 2004

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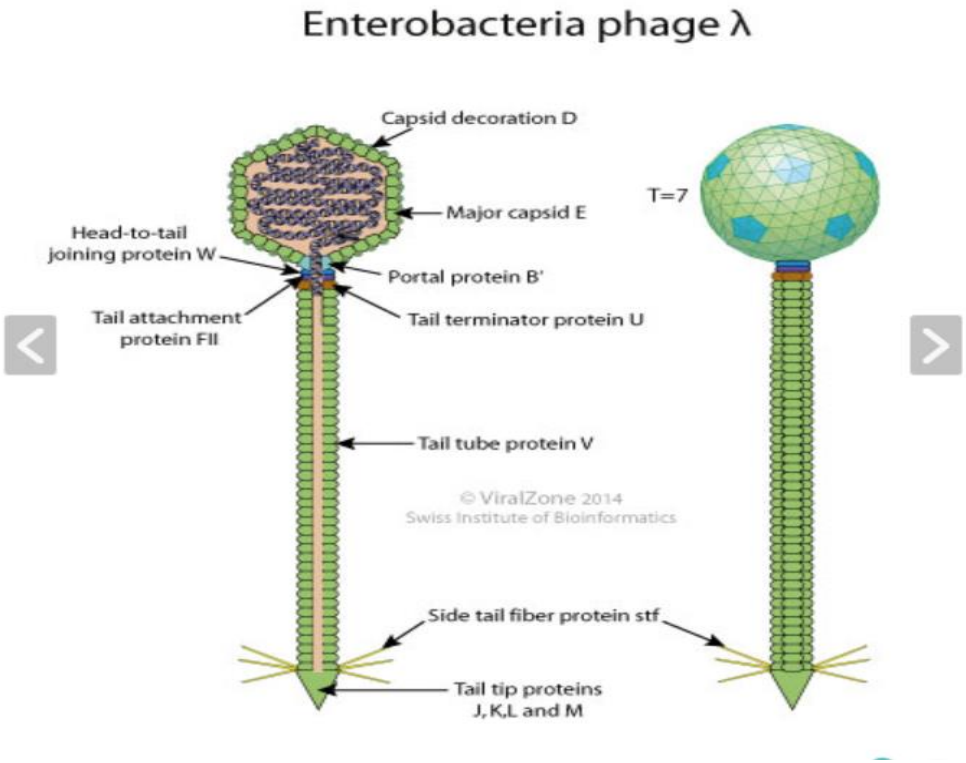
Bacteriophage lambda λ (Wikipedia.com)

- It was discovered by Esther Lederberg in 1950
- Temperate phage: lytic and lysogenic lifecycle
- Classification

Group I: ds DNA
Order: *Caudovirales*
Family: *Siphoviridae*
Genus: *Lambdavirus*
Species: ***Escherichia virus Lambda***

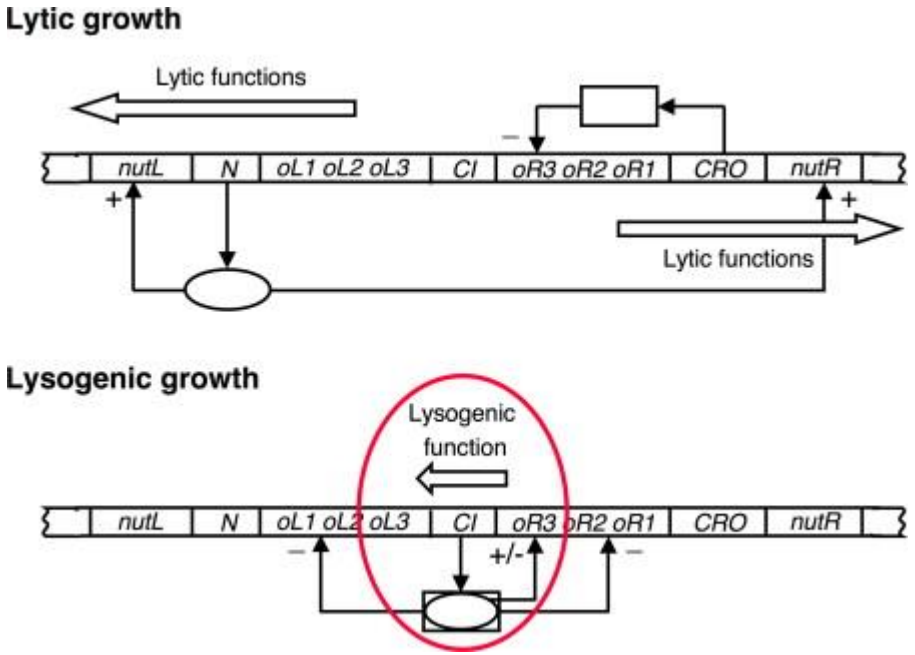
Structure (www.viralexpasy.com)

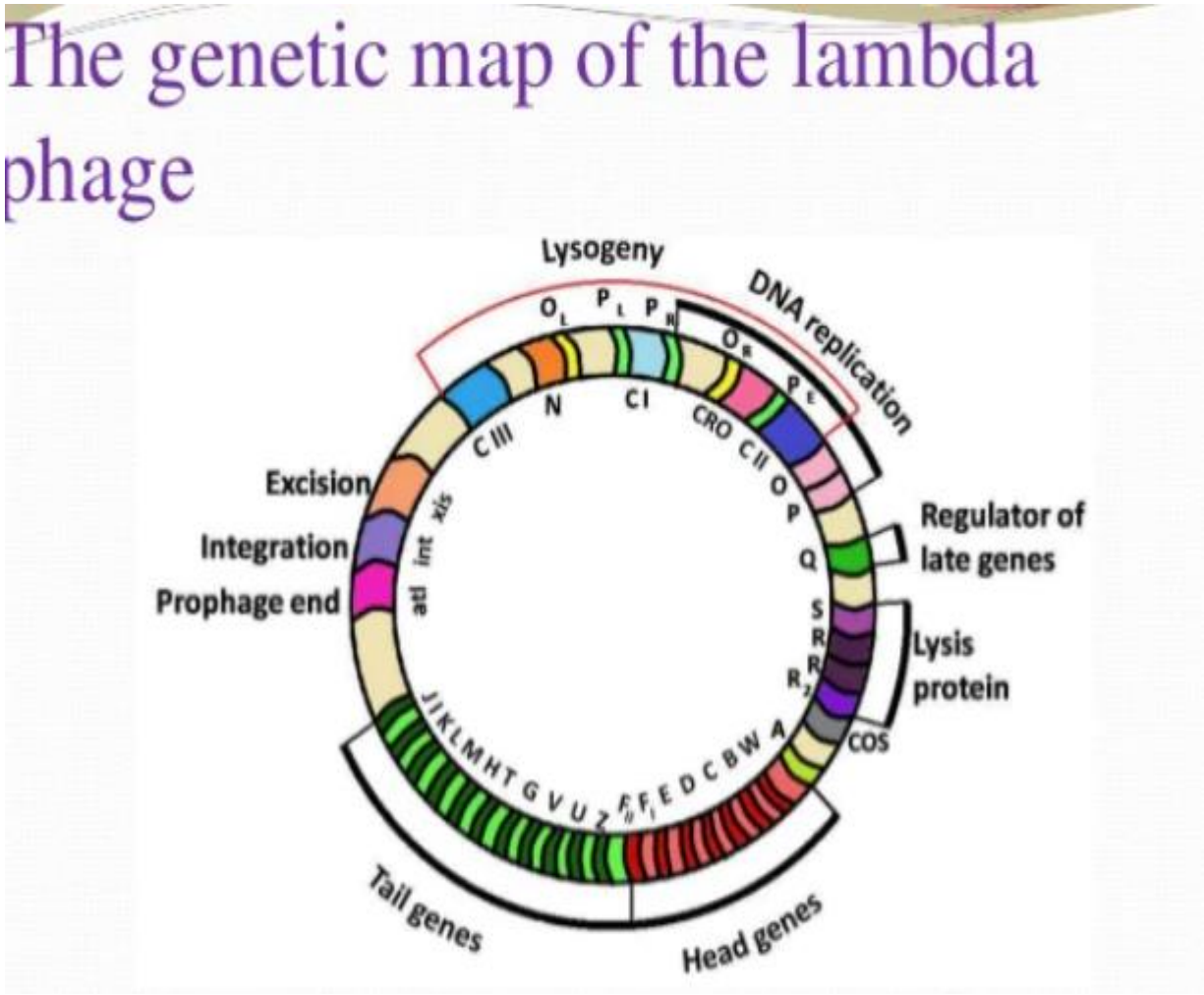
- The virus particle consists of a head and a non contractile tail that can have tail fibers (55nm head, 180 um tail, 25 nm tail fibre- no sheath).
- The whole particle consists of 12–14 different proteins with more than 1000 protein molecules total and one DNA molecule located in the phage head.
- Non-enveloped, head-tail structure. The head is about 60 nm in diameter and consists of 72 capsomers (T=7, laevo).
- **GENOME**
- Linear, dsDNA genome of about 48 kb, containing about 70 genes with terminal repeats 12 bp known as cos sites. The cos site circularizes the DNA in the host cytoplasm. In its circular form, the phage genome, therefore, is 48,502 base pairs in length



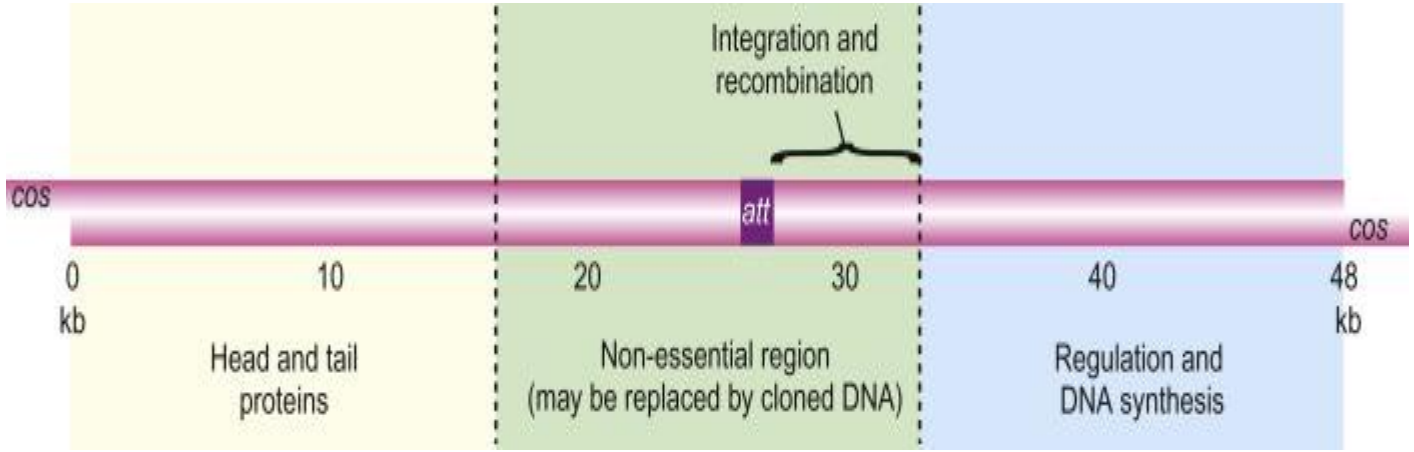
Three operon clusters

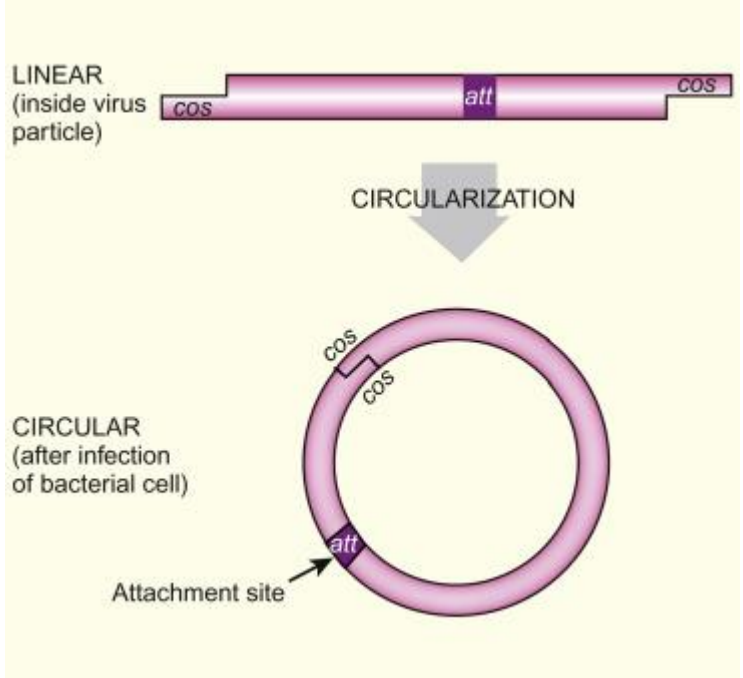
- Right operon: head, tail synthesis and DNA replication
- Left operon: Integration and recombination of lysogeny\
- Immunity operon: decision regarding lytic/lysogeny





Genome





David P. Clark, Nanette J. Pazdernik,Chapter 7 - Cloning Genes for Analysis, Editor(s): David P. Clark, Nanette J. Pazdernik,Molecular Biology (Second Edition),Academic Press,2013,Pages 194-226,

- Immediate Early genes: N and Cro
- Delayed early genes: located left to N: CIII
- Late genes
- 3 regulatory genes

❖N

❖Cro

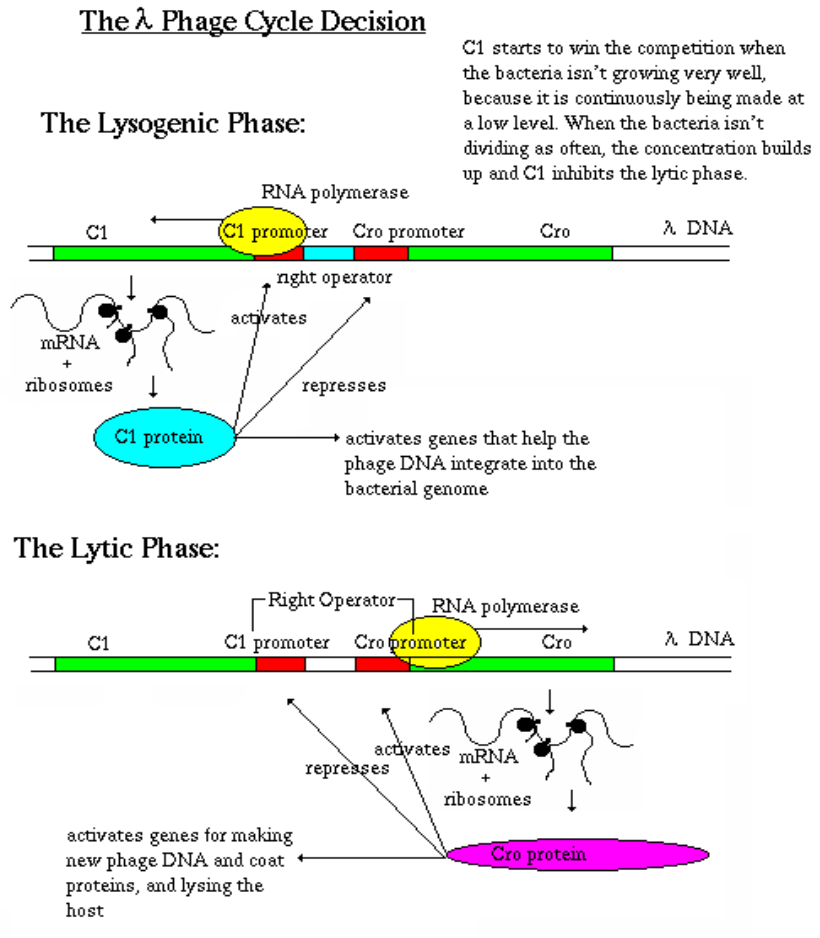
❖Q

- 3 promoters

❖PL, PR1 and PR2

lytic-lysogenic decision:

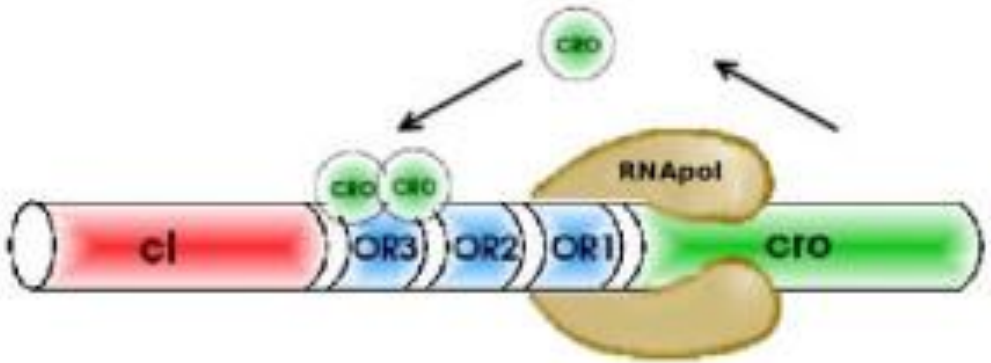
- At the most basic level, the decision depends on the amount of two phage encoded proteins called **Cl and Cro**, and their binding to their promoter control regions.
- When Cl is bound, the expression of the lytic gene is repressed and the phage follows the lysogenic pathway. For this reason Cl is also known as *Cl repressor or lambda repressor*.
- The expression and binding of Cro will lead to lytic development. Cro is made from PR and Cl made from either PRE or PRM. Both Cro and Cl binds to the same DNA sequences called promoters.
- Lambda contains two operators that bind Cro and Cl.
  - OR – overlaps PRM and PR promoters.
  - OL – overlaps with PL.
- OR is the major player in the lytic-lysogenic decision, while OL is not a part of the decision. OR is composed of 17 base pair sequences called OR1, OR2 and OR3. Cl repressor binds to OR1, ten times better than it binds to OR2 and OR3. At increasing concentrations of Cl, it will bind to OR2 and eventually OR3.
- When Cl repressor binds to OR, it stimulates the PRM Promoter and the production of Cl repressor and inhibits the PR promoter and the production of Cro, leading to lysogeny.
- Cro binds to OR3 first, then OR2 and finally at high concentration to OR1. When Cro is bound to OR, it inhibits the PRM Promoter and the production of Cl, leading to lytic growth.



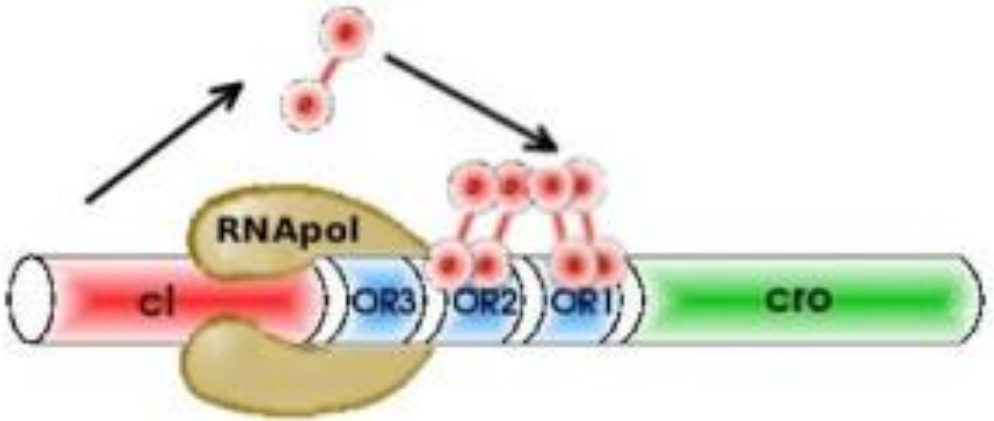
Lytic versus lysogeny

- In cells with sufficient nutrients, protease activity is high, which breaks down cII. This leads to the lytic lifestyle.
- In cells with limited nutrients, protease activity is low, making cII stable. This leads to the lysogenic lifestyle. cIII appears to stabilize cII, both directly and by acting as a competitive inhibitor to the relevant proteases.

Genetic switch



(a) The situation in a *lytic infection*. *CRO* protein occupies *OR3*, preventing RNA Polymerase from initiating transcription from the *cI* promoter. RNA Polymerase transcribes the *cro* gene, producing more *CRO* protein, which silences *CI* transcription.



(b) The situation in a *lysogenic cycle*. *CI* protein induces *cI* gene transcription and *cro* gene silencing. The *CI* repressor protein binds *OR2* and *OR1*, preventing RNA Polymerase from transcribing the *cro* gene, and promoting *cI* transcription. Unlike *CRO*, *CI* has an activation domain that promotes RNA Polymerase binding to its own promoter.

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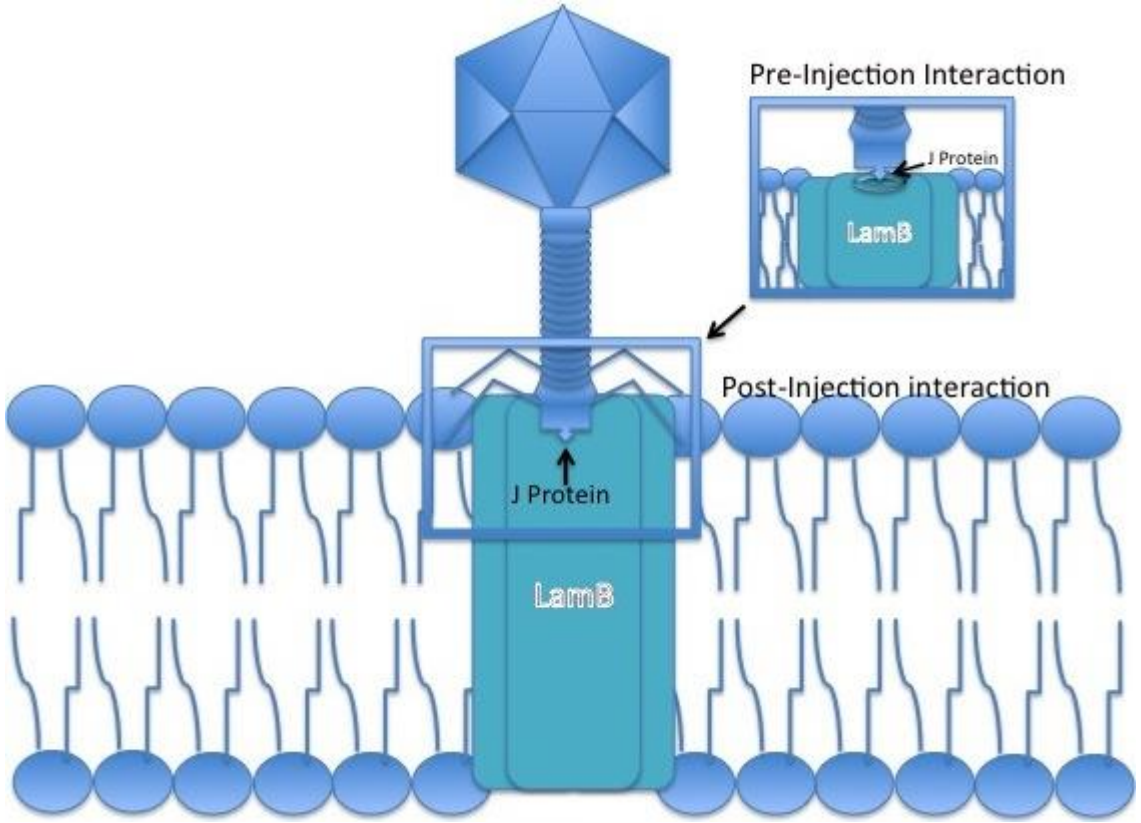
Entry

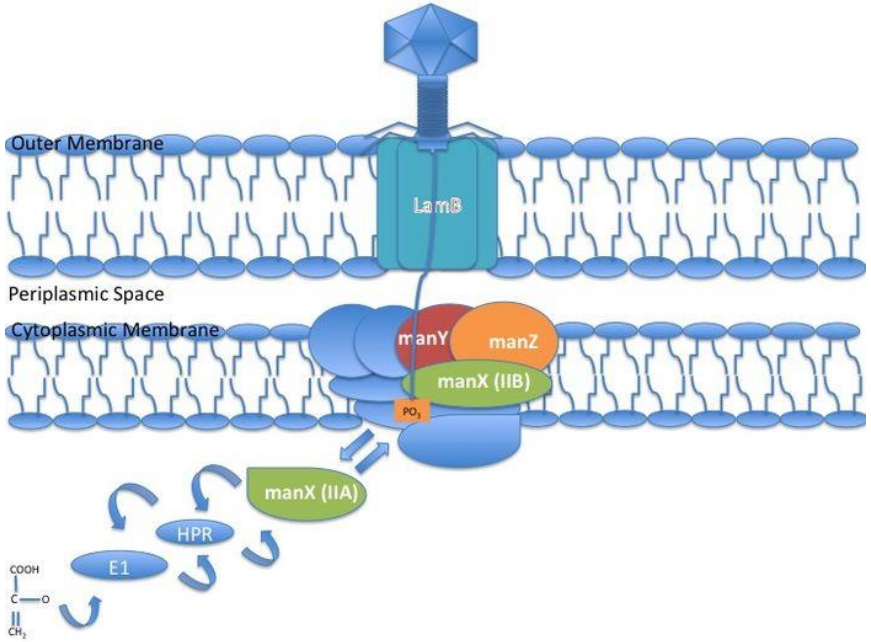
- Bacteriophage Lambda binds to an *E. coli* cell by means of its J protein in the tail tip. The J protein interacts with the maltose outer membrane porin (the product of the *lamB* gene) of *E. coli*, a porin molecule, which is part of the maltose operon.
- The linear phage genome is injected through the outer membrane.



https://www.wikiwand.com/en/Lambda_phage

Entry

- The DNA passes through the mannose permease complex in the inner membrane (encoded by the manXYZ genes) and immediately circularises using the *cos* sites, 12-base G-C-rich cohesive "sticky ends".
- The single-strand viral DNA ends are ligated by host **DNA ligase**.
- No host DNA degradation (T4/T7 phage feature)



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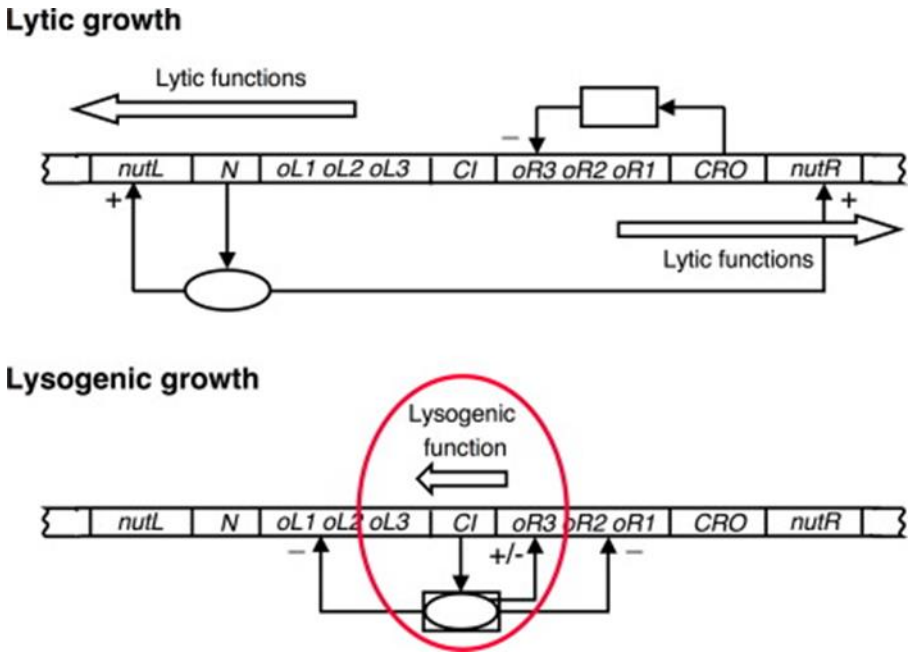
- Host DNA gyrase puts negative supercoils in the circular chromosome, causing A-T-rich regions to unwind and drive transcription.
- Transcription starts from the constitutive PL, PR and PR' promoters producing the 'immediate early' transcripts. At first, these express the N and cro genes, producing N, Cro and a short inactive protein.
- Cro binds to *OR3*, preventing access to the P_{RM} promoter, preventing expression of the *cI* gene. N binds to the two *Nut* (N utilisation) sites, one in the *N* gene in the P_L reading frame, and one in the *cro* gene in the P_R reading frame.



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- The N protein is an antiterminator, and functions by engaging the transcribing RNA polymerase at specific sites of the nascently transcribed mRNA. When RNA polymerase transcribes these regions, it recruits N and forms a complex with several host Nus proteins. This complex skips through most termination sequences. The extended transcripts (the 'late early' transcripts) include the N and cro genes along with cII and cIII genes, and xis, int, O, P and Q genes
- The cIII protein acts to protect the cII protein from proteolysis by FtsH (a membrane-bound essential *E. coli* protease) by acting as a competitive inhibitor. This inhibition can induce a bacteriostatic state, which favours lysogeny. cIII also directly stabilises the cII protein.
- On initial infection, the stability of cII determines the lifestyle of the phage; stable cII will lead to the lysogenic pathway, whereas if cII is degraded the phage will go into the lytic pathway. Low temperature, starvation of the cells and high multiplicity of infection (MOI) are known to favor lysogeny

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Lytic life cycle

- Rightward transcription expresses the *O*, *P* and *Q* genes. O and P are responsible for initiating replication, and Q is another antiterminator that allows the expression of head, tail, and lysis genes from $P_{R'}$.
- The 'late early' transcripts continue being written, including *xis*, *int*, *Q* and genes for replication of the lambda genome (*OP*). Cro dominates the repressor site, repressing synthesis from the P_{RM} promoter (which is a promoter of the lysogenic cycle).
- The O and P proteins initiate replication of the phage chromosome : genome replication
- Q, another antiterminator, binds to *Qut* sites. Q is similar to N in its effect: Q binds to RNA polymerase in Qut sites and the resulting complex can ignore terminators, however the mechanism is very different; the Q protein first associates with a DNA sequence rather than an mRNA sequence.The Qut site is very close to the PR' promoter, close enough that the σ factor has not been released from the RNA polymerase holoenzyme. Part of the Qut site resembles the -10 Pribnow box, causing the holoenzyme to pause.Q protein then binds and displaces part of the σ factor and transcription re-initiates.The head and tail genes are transcribed and the corresponding proteins self-assemble.
- Transcription from the $P_{R'}$ promoter can now extend to produce mRNA for the lysis and the head and tail proteins.
- Structural proteins and phage genomes self-assemble into new phage particles.
- Products of the genes *S*,*R*, *Rz* and *Rz1* cause cell lysis. S is a holin, a small membrane protein that, at a time determined by the sequence of the protein, suddenly makes holes in the membrane. R is an endolysin, an enzyme that escapes through the S holes and cleaves the cell wall. Rz and Rz1 are membrane proteins that form a complex that somehow destroys the outer membrane, after the endolysin has degraded the cell wall. For wild-type lambda, lysis occurs at about 50 minutes after the start of infection and releases around 100 virions.

https://www.wikiwand.com/en/Lambda_phage

Genome replication: lytic cycle

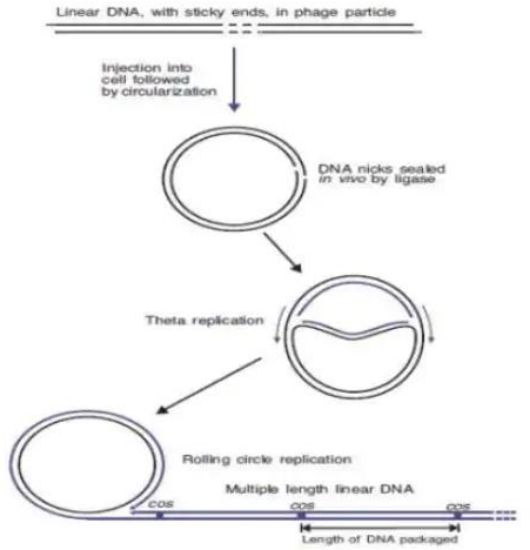
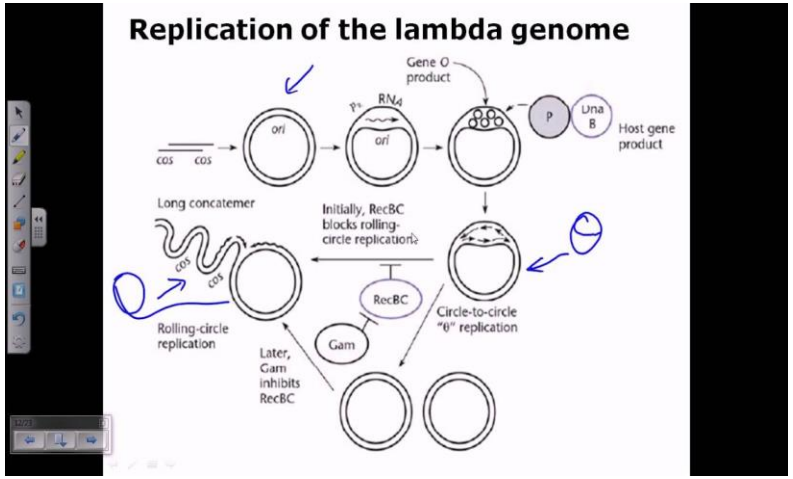


Figure 2.26 Replication of bacteriophage lambda DNA.

Pg 65

- **Lytic replication**
- For the first few replication cycles, the lambda genome undergoes θ replication (circle-to-circle).
- This is initiated at the ori site located in the O gene. O protein binds the ori site, and P protein binds the DnaB subunit of the host replication machinery as well as binding O. This effectively commandeers the host DNA polymerase.
- Soon, the phage switches to a rolling circle replication . The DNA is nicked and the 3' end serves as a primer to produce concatemer.
- These concatemers are cleaved at their cos sites as they are packaged. Packaging cannot occur from circular phage DNA, only from concatomeric DNA.



http://shomusbiology.weebly.com

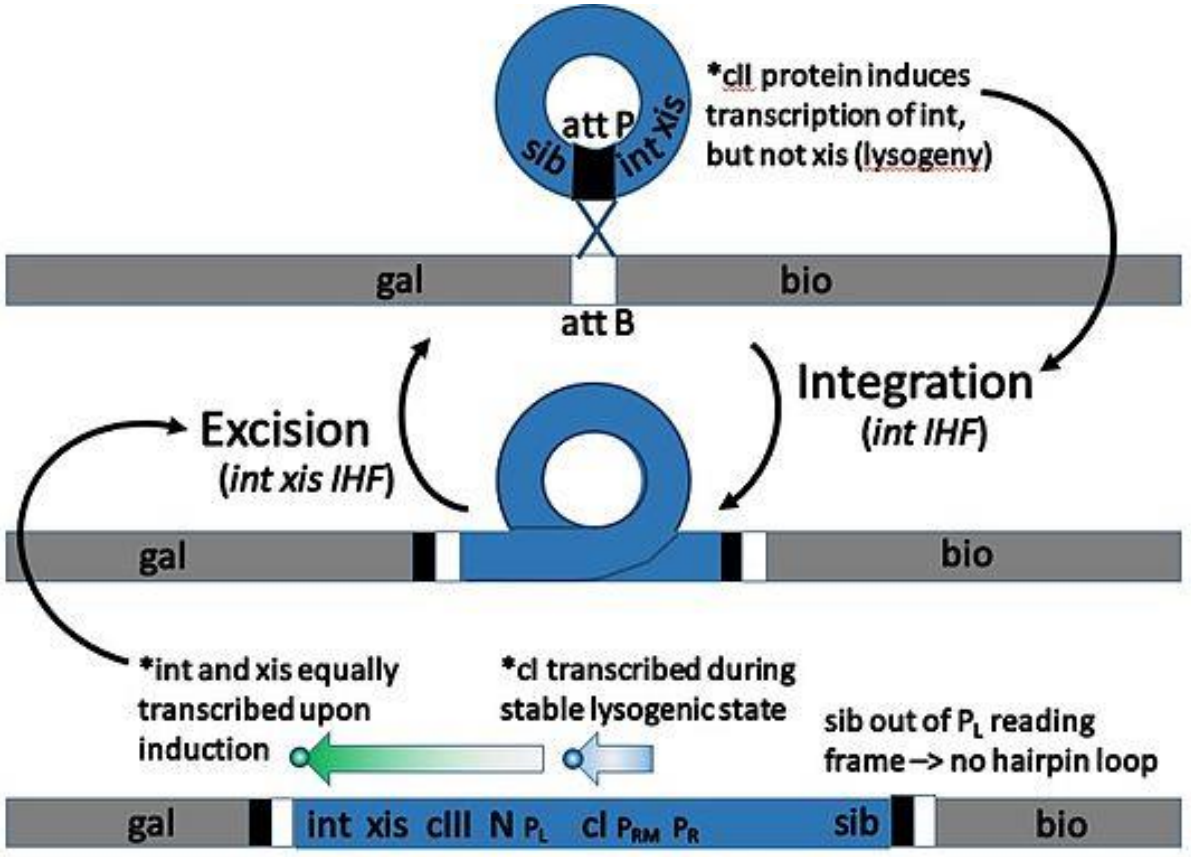
The lambda lysogenic pathway

- If active Cll prevails, it activates PRE and PI promoters leading to production of repressor CI and the integrase protein. Eventually Cl activates PRM ensuring that a continuous supply of CI is made.
- The expression of late genes is prevented by the action of the lambda repressor, Cl. Lambda repressor binding to the operator sequences OR and OL blocks transcription from PL and PR. Since PR is blocked, the lambda Q protein is not made and transcription of the late genes does not occur.

- Lambda recombines into the chromosomes using a specific site on the phage called attP and a specific site on bacterial chromosome called attB. When the lambda DNA is in the chromosome, it is bound by attL and attR, which are hybrid attP/attB sites.

- Once the lambda DNA is recombined into the chromosome, it is replicated and stably inherited by daughter cells as part of the bacterial chromosome. It stays in quiescent.

Excision

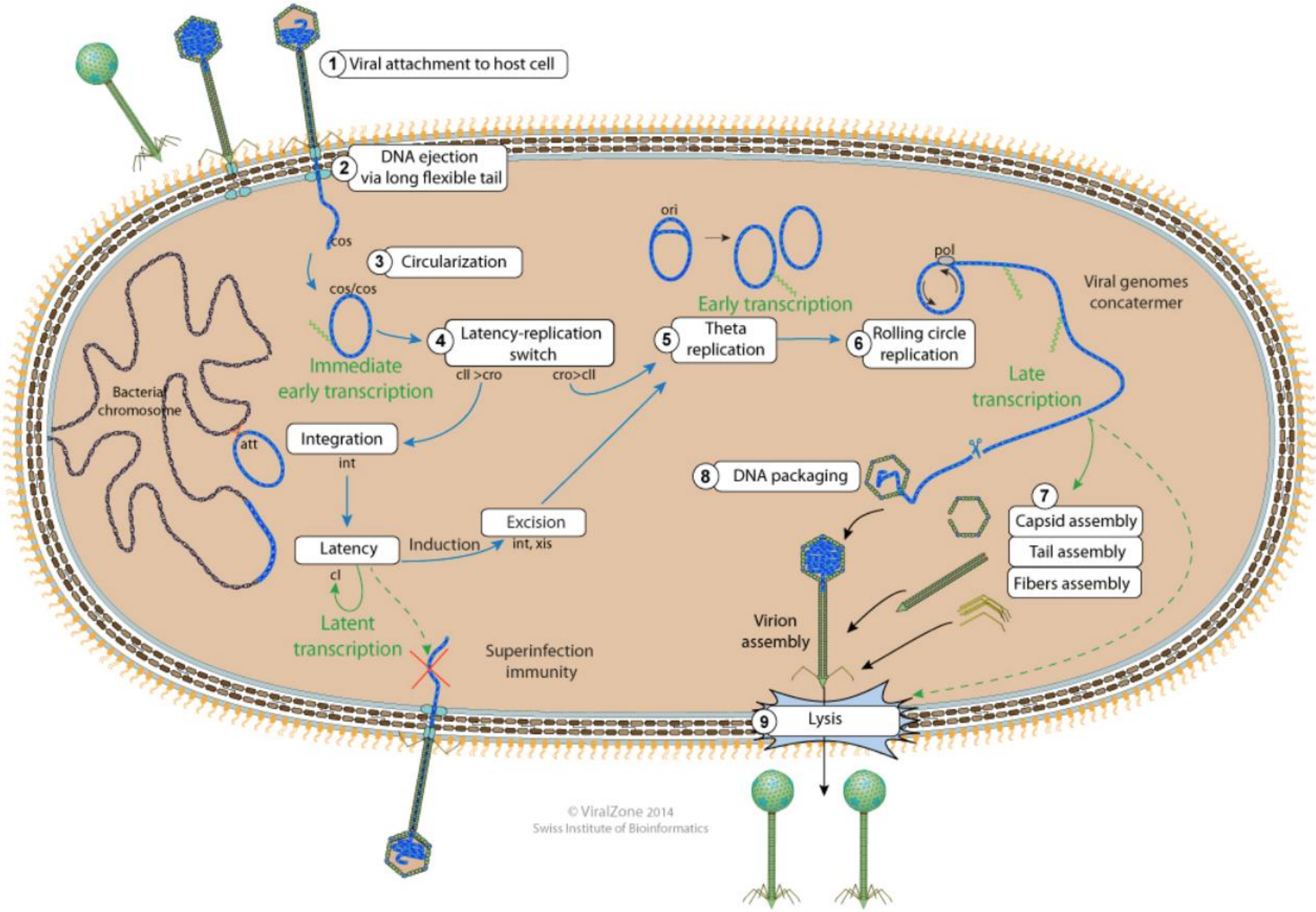


Induction: UV exposure: The host cell, containing a dormant phage genome, experiences DNA damage due to a high stress environment, and starts to undergo the SOS response.
RecA (a cellular protein) detects DNA damage and becomes activated. It is now RecA*, a highly specific co-protease.
Normally RecA* binds LexA (a transcription repressor), activating LexA auto-protease activity, which destroys LexA repressor, allowing production of DNA repair proteins. In lysogenic cells, this response is hijacked, and RecA* stimulates cI autocleavage. This is because cI mimics the structure of LexA at the autocleavage site.
Cleaved cI can no longer dimerise, and loses its affinity for DNA binding.
The PR and PL promoters are no longer repressed and switch on, and the cell returns to the lytic sequence of expression events

Superinfection

- If a cell is a lambda lysogen, another lambda phage that infects is not able undergo lytic development and produce phage. The incoming phage can inject its DNA, however the DNA is immediately shutdown and no transcription or translation of the lambda initiates. The Lysogens are immune to infection by another lambda phage particle, which is called superinfection. Superinfection is blocked because the lysogen is continuously producing Cl repressor.

- The lysogen actually produces more repressor than it needs to shut down one phage. This extra repressor binds to the superinfecting phage DNA at OL and OR and prevents transcription from PL and PR


1 Viral attachment to host cell
2 DNA ejection via long flexible tail
cos
3 Circularization
cos/cos
4 Latency-replication switch
cII >cro
cro>cII
Immediate early transcription
Bacterial chromosome
att
Integration
int
Latency
cI
Induction
Excision
int, xis
Latent transcription
Superinfection immunity
ori
Early transcription
5 Theta replication
6 Rolling circle replication
pol
Viral genomes concatermer
Late transcription
8 DNA packaging
7
Capsid assembly
Tail assembly
Fibers assembly
Virion assembly
9 Lysis
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Applications (Wikipedia)

- Model organisms for microbial genetics and molecular biology
- Cloning vector: insertional and replacement
- Specialized transduction
- Phage display technology
- Its site-specific recombinase (int) for the shuffling of cloned DNAs by the gateway method . Gateway Cloning Technique (Invitrogen) allows transfer of DNA fragments between different cloning vectors while maintaining the reading frame. Using Gateway, one can clone subclone DNA segments for functional analysis. The system requires the initial insertion of a DNA fragment into a plasmid with two flanking recombination sequences called “att L 1” and “att L 2”, to develop a “Gateway Entry clone” (special Invitrogen nomenclature).
- Red operon, including the proteins Red alpha (also called 'exo'), beta and gamma in the DNA engineering method called recombineering.
- **Recombineering** (**recombi**nation-mediated genetic engi**neering**) is a genetic and molecular biology technique based on homologous recombination systems

References

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