Indian J.Sci.Res. 8(1) : 231-237, 2017 Review Article ISSN : 0976-2876 (Print)
ISSN : 2250-0138 (Online)

BACTERIOPHAGE λ: BIOLOGY AND APPLICATIONS

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ABSTRACT

Bacteriophage λ as a temperate phage was originally isolated from strain K-12 of *Escherichia coli* and are widely used for genetic recombination studies. The λ phage is made up of protein and nucleic acid as DNA. The head and tail are joined by the connector and the tail lacks tail sheath and tail fibre. Presence of maltose is necessary in growth medium for infection process because of the production of maltoporine, which acts as receptor site for bactiophage λ. The λ pahge genome consists of three distinct regions, the right operon, the left operon and immunity operon. These operons contain different types of genes that are responsible for DNA replication, head and tail synthesis, their growth and lysis. Apart from these, the operon also regulates the lytic or lysogenic condition of phage by producing repressor protein. With these properties bacteriophage λ completed its life cycle in lysogenic and lytic phase, which is sometimes useful in genetic recombination of bacteria by the process called transduction. A biotechnological application is also achieved by preparation of genomic and c-DNA libraries of λ phage as vector of useful novel genes of other organisms.

KEYWORDS: Bacteriophage λ, Operon, Lytic Cycle, Lysogenic Cycle, Genomic and C-DNA library

Bacteriophage λ is a temperate phage. The temperate phage λ was originally isolated by Lederberg and Lederberg (1953) from strain K-12 of *Escherichia coli*, a strain widely used for genetic recombination studies (Appleyard, 1954; Hendrix, 2002; Hendrix, et al., 2000). Phage λ was studies with the electron microscope by Kellenberger, et al., (1961). λ phage particle appear to be morphologically very similar to T5 but serologically λ is not related to any the T- phages (Kovall and Matthers, 1997). The λ phage is a double stranded DNA virus with a genome size of about 50 kb. In λ phage particle the DNA is arranged as a linear duplex with complimentary single stranded ends 12 nucleotides in length. After interring an *E. coli* host the λ DNA circularizes via base pairing of cohesive ends and is transcribed as a circular molecule. In the early phage of infection one of two alternative replication pathways is chosen. In one pathway lytic growth occurs, the circular DNA is replicated many fold and a number of λ phage gene product of translated. Using the nearly made proteins, progeny λ phage are for made and the host cell lysis, releasing many new infectious phage particles. In a second pathway lysogenic growth occurs. The phage DNA becomes integrated and transmitted to progeny bacteria like another *E. coli* chromosomal sequences. The usefulness of λ phage as a cloning vector. A number of λ vector in the unessential region is bordered by restriction enzyme site convenient for cloning foreign DNA, have also been constructed by standard mutant selection techniques. These restriction sites have also been removed by mutation from the essential λ phage arms which encoded head and tail assembly proteins and DNA replication proteins.

STRUCTURE OF BACTERIOPHAGE λ

The λ phage particles are half protein and half DNA. The symmetry of head is icosahedral and about 55 nm in diameter. The capsid consists between 300 to 600 subunits. The subunits appear to be arranged in clusters of 5 and 6 subunits, the pentons and hexons, respectively. The genome consists of linear duplex DNA molecule about 17 μm in length. It consists of about 46,500 base pairs and has a molecular weight about 32 x 10^6 daltons. The ends linear duplex has single stranded regions which are complementary to each other. Each single stranded region consists of 12 nucleotides. The tail is rigid and 135 nm long in length. The tail consists 35 discs or annuli; the tail lacks tail sheath and tail fibre. The head and tail joined to each other by the help of head-tail connector (Kovall and Matthers, 1997).

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Infection Process

In order to absorb bacteriophage λ in *E. coli* cell it is necessary that the cells should be present in maltose. Presence of maltose in growth medium leads to production of maltoporine as a maltoporine is responsible for transport of maltose. Maltoporine acts as receptor site for bacteriophage λ. After infection the linear λ DNA duplex which contain two complementary single stranded end both ends have 12 nucleotide are spontaneously circularizes and formed a nicked rings. Whose nicks have been covalently closed by the host enzyme polynucleotide ligase.

Genetic Map of Phage λ

The genome consists of three distinct regions, the right operon, the left operon and immunity operon (Hendrix, 2003). The right operon controls the vegetative functions, DNA replication, head synthesis, tail synthesis and lysis (Szybalski and Szybalski, 1979). The left operon is involved in integration with DNA determines whether the phage enters the lytic or the lysogenic cycle.

Head Genes

Genes nu1A, W, B, C nu3, D, E and located at the left end of the phage map are required for DNA maturation and phage head proteins.

Tail Genes

Z, U, V, Y, T, H, M, L, K, I and J, which code for the phage tail proteins are clustered just to the right of the head genes.

Recombination Genes

Site specific recombination at the attp site coded by W genes, int and xis (Scaife, et al., 1985). General recombination at normal frequency is controlled by three genes. These are exo (red) which codes for exonuclease, bet (red β) which determines the protein beta and gama (γ) whose protein inhibits host exonuclease V and allows phage growth in rec A hosts (Little, 1967).

Positive Regulation Genes

N and Q are positive regulation genes whose products increase the rate of transcription of other genes. The product of the N genes stimulates of transcription of genes CII, O, P, Q gam exo, bet, xis and int. The product of the Q gene increases transcription of the head, tail and lysis genes. Plague formation requires both N and Q genes. In the absence of these genes the number of phage particles produced is small, but not zero.

Negative Regulation Genes

The repressor required for maintenance of the prophage in the lysogenic condition is determined by gene CI. The products of the CII and CIII genes have an accessory role to CI in lysogenization. The CI product acts on two sites on the DNA. The Cro (f, red) gene produced reduces the expression of genes CI, N, exo and xis.

DNA Synthesis Genes

O and P are required for phage DNA replication. These genes rarely transcribe in the absence of N function. Mutants of genes N are therefore also deficient in DNA synthesis.

Lysis Genes

Lysis of the bacterial cell envelope, which marks the end of the lytic cycle, is controlled by two genes, S and R.

Immunity Region

Crosses of the λ phage with other lamboid phages, 434, 21 and 82 yield recombinants which posses and immunity region in the middle of the lambda genetic map. The recombinant λ imm 434 has the phage 434 sequence replacing the genes rex-CI Cro. The recombinant λ imm 21 has a longer substitution in which the λ genes N-rex-CI Cro-CII are replaced by DNA from phage 21. These recombinants have proved very useful in analysing the control of λ lysogeny and lytic development, because the substituted DNA confers selective immunity.

The left arms of λ and 434 between genes A to J are identical, as are the left arms of and 21 between genes Z and J. The two sequences are partially homologous form genes A and F.

Life Cycle of Bacteriophage λ

The phage has two types of life cycles, the lytic cycle and the lysogenic cycle (Jocob and Wollman; 1961). The phage DNA on entering the host cell may sometimes immediately multiply and enter the lytic cycle. During this cycle the phage genes are expressed and the phage DNA replicated, leading to the production of many phage particles. This part of the life cycle is virulent. The virus

exists in the viron form with a DNA core in a protein coat (head) and a tail. In the lysogenic cycle the viral chromosome becomes integrated into the host chromosome and called a prophage. When the viral DNA becomes a part of the host DNA it behaves like a gene of the genetic map of the host. It replicates along with the host chromosome and is inherited in the sameway as bacterial genes. In the host chromosome and called a prophage. When the viral DNA becomes a part of the host DNA it behaves like a gene of the genetic map of the host. It replicates along with the host chromosome and is inherited in the same way as bacterial genes. In the prophage condition the virus exists in harmony with the host cell and is non-infectious. Since the λ phage normally exists in this condition, it is called a temperate phage. Bacteria containing prophages are called lysogenic bacteria. Viruses whose chromosomes become prophages are called lysogenic viruses (Bertani, 1958).

Lytic Cycle

When phage DNA is injected into the bacterial cell it can enter either the lytic cycle or the lysogenic cycle. During the lytic cycle the linear DNA molecule under goes circularization and has converted into the circular form. The cyclic DNA molecule transcribes phage m-RNA which is then translated in an orderly sequence. The circular DNA molecule undergoes replication, which is of two types, early replication and late replication.

Early replication is bidirectional, while late replication is by the rolling circle mechanism. The multiple unit length DNA duplexes generated by late replication are cut up into unit length pieces. During the next phase, maturation, the assembly of phage particles takes place, each head containing one unit length DNA molecule. In the final phase of the lytic cycle, lysis of the bacterial cell takes place, releasing the phage particles.

Circularization of The Chromosome

The λ phage infects a linear DNA molecule into the bacterial cell. The two complementary single stranded ends of the linear DNA close simultaneously to form hydrogen bonded circle, which is then sealed by a DNA ligase. Most linear DNA molecules converted into covalently closed circle within 5 minutes after infection. The covalently closed circle is also known as RFI, supercoils and species I. An intermediate in the formation of the closed circle is the nicked circle form (RFII, species II). This consists of a circle with one open and one closed strand.

Transcription

Lytic development has two phages, the early period and the late period. The early period is the time before replication of phage DNA. The late period is the time from replication to maturation. There are three classes of genes, immediate early genes, delayed early genes and late genes. The immediate early genes, N and Cro, are transcribed during the early period. The delayed early genes lie to the left of N and the right of Cro. The CIII, gam, bet, exo, del, xis and int genes are on the left of the immunity operon (Murphey, 2007). The late genes are transcribed during the late period. The three classes of genes transcribe three corresponding classes of mRNA, immediate early, delayed early and late messengers. Early messengers are transcribed from both left and right strands, while late messengers are transcribed only from the right strand. Transcription of right strand proceeds from left to right, while transcription of the left strand proceeds in the opposite direction.

Transcription of the delayed early genes requires the gene N product (gpN). This protein acts as an antiterminator and neutralizes the terminators tL, tR1 and tR2. It thus controls the expression of most viral functions. This appears to be brought about by the interaction of gpN with RNA polymerase at the promoters, forming a rho-resistant complex. Prevention of termination permits transcription in both left ward transcription extends into the b2 region and right ward transcription to gene Q. The Q gene product (gpQ) is a late antiterminator which neutralizes the third right terminator (tR3). This permits transcription to proceeds through the vegetative component genes to gene J and into the b2 region.

Replication

The λ Phage has two modes of replication during autonomous replication during the lytic and as a prophage incorporated into the host DNA during the lysogenic cycle. λ DNA replication makes use of only exogenous precursors

and bacterial DNA is not broken down. Autonomous replication occurs into two stages, early replication producing cyclic DNA molecules and late replication producing long multiple length genomes. During early replication the cycle DNA molecule associates with the host cell membrane and replicates symmetrically, generating cyclic copies. Replication has a unique origin site (ori) located within gene o, at 81.7% of the λ genome length (Hendrix, 2003). The initiation of replication the requires the functions of viral genes o and p and host functions. The products of these genes may be involved in nicking the closed circle at the origin. Replication is divergent and bidirectional, proceeding in opposite directions from the point of origin. A replication intermediate of λ DNA engaged in early replication resembles the Greek letter theta (θ) and is called the θ structure. The structure has two branch points which represent the replication forks. The replication fork is the point at which the non replicated parental duplex joins the two daughter chromosomes. The distance between the two branch points is always the same on each branch. This suggests that each branch represents a newly replicated daughter strands. If the two branches are called a and a' and the third segments b' then a+b will always equal unit length, i.e. the characteristic length of the λ DNA molecule (17μm). The branches a and a' represent the replicated segments, while b is the segment which has yet to undergo replicated theta (θ) structures have also been reported in *E. coli* replication. This suggests that the λ phage and *E. coli* have similar replicating mechanisms. Replication terminates where the two forks meet. In late replication the progeny DNA molecules leave the cell membrane and switch over from the bidirectional to the rolling circle mode of replication. Initiation occurs at variable points on the cyclic DNA molecule. Multiple unit length DNA duplex called concatermers are generated. Recombination can also take place, producing oligomers of λ DNA. Concatemeric DNA is cut up into length pieces, resulting in the formation of mature molecules with cohesive ends. The cuts are made by phage gene A protein. This binds to the concatemers at sites corresponding to the cohesive ends and produces staggered single stranded cuts 12 nucleotides apart. The sticky ends are thus regenerated. The unit-length duplex produced contains exactly one phage genome. Control of transformation from early to late replication depends on phage genes under the control of the N gene. DNA molecules with defective N replicate continuously in cyclic form, without undergoing rolling circle replication. The function of the viral gene gam is required to inhibit the rec BC endonuclease V of the host, with would otherwise break down the concatemers (Murphey, 2007).

Maturation

During the maturation of the phage particles, the head and tail are assembled independently, genes nu1, A, W, B, C, nu3, D, E and F code for DNA maturation and phage head proteins. Genes Z, U, V, G, T, H, M, L, K, I and J code for phage tail proteins. The first stage in head assembly is the formation of prohead I, which consists of a protein core surrounded by a shell. The next stage is prohead II which is an empty particle. DNA is now packaged into the particle. The λ genome is cut from the concatemer during the insertion of the DNA into the protein capsid. The DNA is cut at the cos site to generate right and left cohesive ends, of which the former is inserted into the head shell. Insertion continues till the next cos site. Partially full particles with and an expanded head are called grizzled particles because their centres appears grizzled due the presence of DNA. When the particles become packed with the full complement of DNA, they are known as black particles. The head is now stabilized for the attachment of the tail. The mature particle is formed by the attachment of the head and tail.

Lysis

Two genes S and R are involved in the dissolution of the host cell wall. The gene S product stops metabolism of the bacterial cell, while the gene R product lysis the cell wall. About 100 viral particles are produced within an hour by an infected bacterial cell. Lysis of the cell wall releases the progeny phage particles and the host cell is destroyed.

Lysogenic Cycle

The two characteristic features of lysogenic bacteria are immunity and induction. The prophage, which

is incorporated into the bacterial genome, may be inserted just like any bacterial gene, may be carried indefinitely in the inert condition. The presence of the prophage confers immunity on the bacterial cell against infection. Lysogenic cells are not infected by other λ phage particles and therefore do the undergo lytic bacterial infection. The prophage can, however be induced under certain conditions to enter the lytic cycle. Induction may occur spontaneously or may be stimulated by UV irradiation or mitomycin c. The lysogenic state is brought about in two stages, establishment and maintenance. Establishment of lysogeny requires the integration and repressor of the phage genome into the bacterial chromosome and the beginning of repressor synthesis to prevent expression of genes bringing about lysis. Establishment requires the participation of three control genes, CI, CII and CIII. Maintenance of lysogeny only requires the repressor synthesis continue. This is brought about by the transcription and translation of a single gene CI.

Repressor

The prophage genome has only one function, synthesis of a repressor protein (MW of diam. 30,000). This protein is synthesized by the CI gene. Enough repressor is synthesized to prevent the infection of super infected phage for lytic development or for integration. The repressor protein binds to two operators, which control transcription of adjacent genes. The repressor thus prevents the transcription of all the λ prophage genes, except its own. Thus no mRNAs are produced for the vegetative and recombination regions. A specific promoter site, pre is activated by the combined products of the CII and CIII genes. This permits transcription of the CI gene and the synthesis of the repressor. The repressor blocks the left and right promoters (PL and PR). Blocking the left promoter gene in turn prevents the transcription of gene N, whose product has activator functions. Blocking of the right promoter gene prevents the transcription of the O, P and Q genes. These are the early transcriptional genes, without whose products the late genes can not be transcribed.

Integration

The process by which the λ phage DNA is integrated into the bacterial host cell DNA is called site specific recombination (Signer and Weil; 1968). The association between phage and host chromosomes is explained by the Campbell model. The site of attachment on the phage genome is called attP and the site on the bacterial chromosome where λ DNA integrates is known as att λ or att B. Each site consists of two half sites. Thus attB is represented BB' and attP as PP'. The dot between the two letters represents the point of crossover. The λ phage attachment site attP lies between the int and the J genes. The bacterial attachment sites attB is bordered on one side by the galactose loci, gal E, T, K and on the other site by a cluster of biotin genes (bio). Integration and excision of the λ chromosome appear to require staggered nicks produced by alter endonuclease (Gingery and Echols, 1968). During integration, the endonuclease appears to recognize and nicks symmetrical sequences in attP and attB. Each sequence is probably located in a duplex region less than 20 base pairs long. Which is common to both loci integration is completed with the ligation of the two pairs of staggered nicks. The phage DNA is joined to the bacterial DNA by covalent bonds. The phage DNA is now called the prophage. Integration results in the separation of bacterial markers by the prophage. Another result of integration is the formation of two recombinant attachment sites BP' and PB'. The reciprocal crossover between phage and host DNA is brought about by the product of the phage gene int. The int gene products bring about site specific recombinant between PP' and BB' resulting in integration.

Replication

Prophage replication takes place under the control of the host by the normal bacterial DNA replication mechanism. The replication of the prophage along with the bacterial DNA indirectly contributes to viral growth. Each bacterial cell carrying a prophage is a potential producer of phage particles (Taylor and Wegrzyn, 1995).

Excision

The prophage can enter the vegetative cycle only when it is released from the bacterial chromosome. This is brought about by the products of the int and the xis genes. Recombination between BP' and PB', under the influence of the int and xis gene products results in excision of the

prophage. Excision is thus the reversal of integration (Gingery and Echols, 1968). The circulation phage chromosome is liberated into the cytoplasm of the bacterial cell. The vegetative functions of the phage can be established and the phage can enter the lytic cycle.

Transduction

During excision the bacterial bio or gal gene is included in the progeny phage genome. The frequency of such occurrences is about one in a million. Since the phage capsid can accommodate only a limited amount of DNA, a part of the phage DNA is left out. This produces a chromosome which is deficient in some phage genes. The phage enhance inactive. Such a phage may inject its DNA into a bacterial cell and the DNA may be inserted into the bacterial genome (Gingery and Echols, 1968). The host now has an additional gene for the first bacterial cell. The process is known as specialized transduction.

Applications of Bacteriophage λ

1. λ phage plays an important role in the transmission of genetic information between bacteria by the process of specialized transduction (Sullia and Shantharam; 1998).
2. Many λ phage vectors used restriction endonuclease sites other than EcoRI to provide the essential left and right arm fragments, thus allowing for ligation to a variety of insert fragments.
3. λ phage is a very useful for study of site-specific recombination (int) for the shuffling of cloned DNAs by the gateway method (Murphey, 1998).
4. λ phage used as a molecular cloning vectors
   (i) λ phage is a very useful vector for genetic engineering experiments.
   (ii) λ phage has been used as important tools for molecular cloning in biotechnology.
5. λ phage used as libraries (i) Genomic libraries (ii) c-DNA libraries (Turner, et al., 1988).

Genomic Libraries

Genomic libraries are usually constructed using λ bacteriophage vectors and in vitro packaging. The vectors except large inserts, these by minimizing the total number of recombinant required to constitute the library. For example If a λ library with an average insert they encoded portions of opine. The c-DNA clones were used as probes to screen Drosophila, bovine and human genomic libraries. The bovine c-DNA was excised from the λ vector DNA by restriction endonuclease cleavage and recloned into a vector like PGEM.

C-DNA Libraries

λ phage vector is generally used to prepare c-DNA libraries. c-DNA libraries can be used to clone mRNA sequences even if the protein, amino acid sequence and nucleotide cloning sequence are entirely unknown and even if no homologous probe is available.

CONCLUSION

In conclusion, the temperate bacteriophage λ, a pre-existing cellular structure or metabolic processes, has 'learned' partial independence from its prokaryotic host, *E. coli.* It has been used heavily as a model organism, and has been a rich source for useful tools in molecular biology. Uses include its application as a vector for the cloning of recombinant DNA, the use of its site specific recombination. As an experimental system, λ phage has highly developed with excellent genetics and strong biochemistry. Each of the proteins involved in λ assembly has been cloned and purified which sets the stage for detailed characterization and development of a virus at the molecular level. λ phage is also used in the diagnostic laboratory for the identification of pathogenic bacteria (phage typing). Although phage typing is not used in the routine clinical laboratory, it is used in reference laboratories for epidemiological purposes. Recently, new interest has developed in the possible use of λ phage for treatment of bacterial infections and in prophylaxis. Whether λ phage will be used in clinical medicine remains to be determined.

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