Bacteriophage Lambda and its Relatives

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Bacteriophage lambda is a virus that infects the bacterial species *Escherichia coli*. It is a temperate virus, in that it can either make virion progeny in its host, or establish a state in which its chromosome is integrated into the host chromosome and its own replicative genes are turned off.

Secondary article

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Introduction

Bacteriophage lambda (bacteriophage λ) has occupied a central position in molecular genetic research since the 1950s, and has played an important role in understanding many of the central premises of molecular biology. It is a temperate phage that was discovered by Esther Lederberg (1951) when it was accidentally lost from its host (*Escherichia coli* strain K12) during mutagenesis with ultraviolet light. We now know λ as the prototypical member of a family of related phages that share similar lifestyles, called the 'lambdoid phages'. The study of these phages was critical in the initial steps toward attaining our current understanding of the mechanisms and control of gene expression and was also seminal in the early development of genetic engineering technology in the 1970s.

The first DNA sequence to be determined directly was the λ cohesive end sequence, and the λ genome was the first double-stranded (ds)DNA genome to be completely sequenced. In addition, research with these phages was crucial in laying the foundations for our current knowledge in many other diverse arenas. We name only the following few as examples: the specificity and mechanism of action of transcriptional repressors and activators, the mechanisms of homologous and site-specific recombination and DNA replication, autoregulation of gene expression, messenger RNA structure and function, RNA polymerase function and its control by antiterminator proteins, the use of conditional lethal mutations, virus–host interaction, and molecular chaperone action (see Hendrix *et al*., 1983, for more detailed discussions of these early experiments with phage λ).

The lambdoid phages are temperate viruses, which means that they have two alternate lifestyles. During lytic growth a cascade of phage genes is expressed, culminating about 50 min after infection with lysis of the infected cell and release of 100–150 progeny virions per cell. However, upon infection of its *E. coli* host, λ has the option of establishing a lysogenic state in which the phage chromosome is physically integrated into the bacterial host's chromosome.

Although λ itself has only been found once in nature, lambdoid (λ-like) phages appear to be common both as virions and lysogens. All characterized members of the lambdoid phage family infect only the *Enterobacteriaceae*, and each phage type is specific to a particular bacterial species or subtype. Many lambdoid phages have been isolated, and at least some aspect of the molecular genetics of over 20 members have been studied in the laboratory. In addition, fragments of lambdoid phage genomes are ubiquitous in the chromosomes of their host bacterium. For example, *E. coli* K12 contains three lambdoid 'defective prophages' in addition to the lambda prophage. Studies of other natural isolates have shown that 30 of 78 independent *E. coli* isolates carry at least a fragment of a lambdoid phage genome at the phage 21 integration site (see below). It is not known whether these phage genome fragments are simply prophages in a state of evolutionary decay or whether they may serve some function for the host.

The Virion

The phage λ virion is made up of an icosahedral 'head' that is ~63 nm in diameter and a noncontractile 'tail' ~150 nm long (this places it in the *Siphoviridae* taxonomic group). A tapered 'tail tip' structure is at the distal end of the tail, and four nonessential ~85-nm-long 'side-tail fibres' extend from the junction of the tail shaft and tip structures. The virion has a mass of about 67 Md, a buoyant density of 1.508 g mL^{-1} in CsCl density gradients and a sedimentation coefficient of about 360 S.

Like all viruses, λ exists outside cells as an inert virion, shown in **Figure 1**. The dsDNA chromosome is packed tightly into the head portion and the tail serves to bind to the cellular receptor and act as a conduit for the DNA to enter the target cell. The exact physical arrangement of the DNA within the head is unknown, but it is packed so that

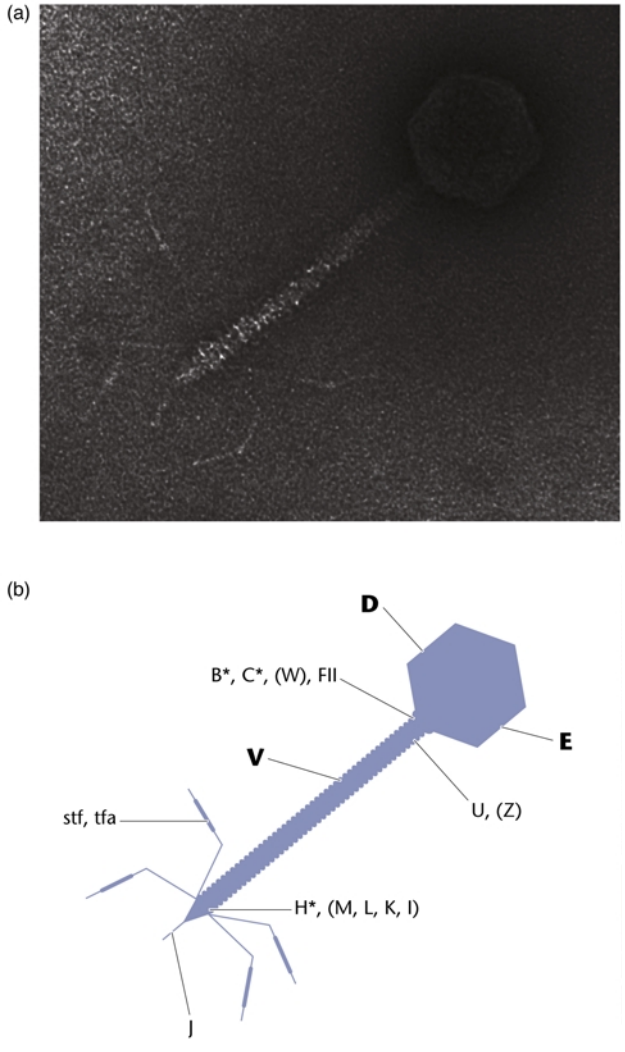

Figure 1 The bacteriophage λ virion. (a) Electron micrograph of a phage λ virion negatively stained with uranyl acetate. The head is about 63 nm in diameter. (b) Diagram of the λ virion with the locations of the structural components indicated by gene name. Larger gene names indicate more molecules of the encoded protein are present; parentheses indicate gene products which are likely (but not yet proven) to be virion components; asterisks indicate covalently modified proteins.

average centre-to-centre distance of adjacent DNA double helices is about 2.7 nm. The right end of the DNA protrudes part way down the tail. Twenty-three head and tail genes make the protein products that are involved in building progeny virions in infected cells. All of these, except *stf* and *tfa*, are absolutely required for virion assembly; the *stf* and *tfa* encoded fibres allow the virion to adsorb to hosts more rapidly. Curiously, the λ derivative used in all laboratories has a frameshift mutation in the *stf* gene that is not present in the original *E. coli* K12 λ prophage; the resulting loss of the side-fibres gives rise to larger plaques on agar plates, perhaps because the virion diffuses through agar faster without them.

The main building block of the head is the product of gene *E*, which is arranged with icosahedral symmetry and about 420 E protein molecules are present in each virion. An equal number of gene *D* protein molecules decorate the outside of the head shell and give it additional physical strength. V protein is the building block of the tail shaft, and J protein at the tail tip is thought to recognize the main cellular receptor. Not all proteins required for the assembly of the virion are in the final structure, for example the Nu1 and A proteins are required for DNA packaging and the G and G-T proteins are required for tail shaft assembly, but none of these is found in the virion.

To date, many lambdoid phages appear to have virions that are very similar to that of λ; however, phages P22 and HK97 represent lambdoid phages which have morphogenetic genes and virions or virion parts that are not obviously homologous to those of λ (see discussion of evolution below.)

The function of the virion is to act as a vehicle to carry the genome safely from one host to the next. Accordingly, the virion is quite resistant to most nucleases and proteases which might otherwise inactivate it before it could deliver its genome to the next host. Lambda virions are also resistant to inactivation by choloroform, an agent that rapidly inactivates virions with membrane envelopes. Some of the known instabilities of lambda virions relate to properties it must have to successfully deliver the genome to the cell it infects. Thus lambda is inactivated slowly in the presence of debris from lysed cells, and this is due at least in part to abortive interactions between virions and receptors to which it would normally attach in order to inject its DNA into the cells. The fact that the DNA is tightly packed in the head – which may facilitate delivery of the DNA – means that virions are susceptible to disruption by agents which destabilize the DNA. Thus virions are stabilized by Mg^{2+} ions or the polyamine putrescine, and if these are removed the heads typically rupture and release their DNA, presumably as a result of charge–charge repulsion in the DNA.

The Lambdoid Phage Chromosome

The λ chromosome is a single dsDNA molecule 48 503 bp long and contains about 63 recognizable open reading frames. Of these, all are presumed to produce proteins, and the predicted proteins have been observed experimentally for at least 45 genes. The roles of most of these genes are known, and 28 are essential for lytic growth and are tightly turned off in a lysogen.

Genes are closely packed on the lambdoid phage genomes, with only small or no intergenic spaces in many locations. **Figure 2** shows that genes are clustered according

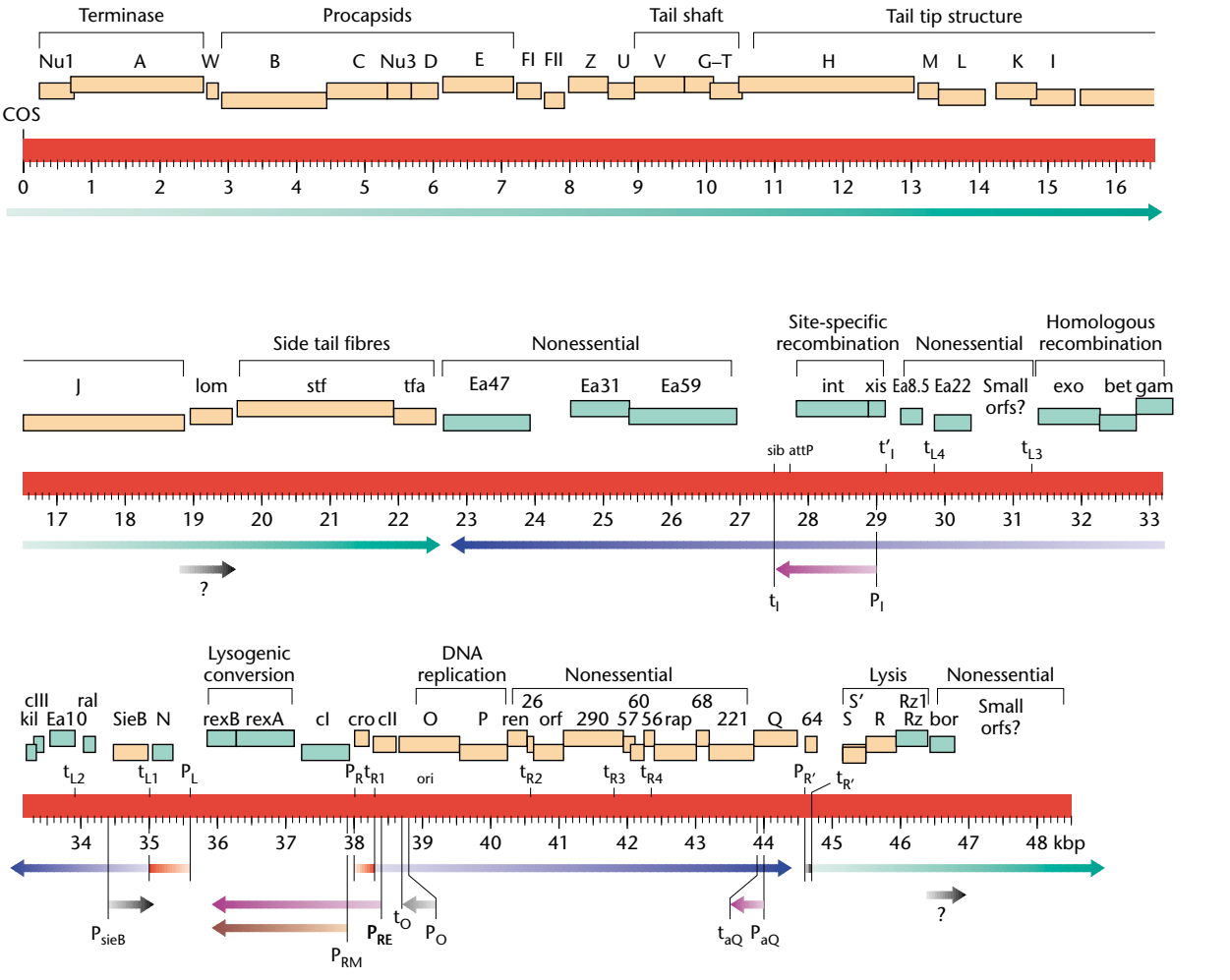

Figure 2 Map of the bacteriophage λ chromosome. The linear virion chromosome is shown with a scale in kilobase pairs (kbp) below. Rectangles above indicate known genes; with yellow ones transcribed rightward and green ones leftward. Important sites (e.g. P, promoters; t, terminators) on the DNA are indicated below the genes, and arrows below the kbp scale indicate the transcripts made from the λ chromosome: brown, made only in a lysogen; black, made in lysogen and pre-early conditions; orange, pre-early; blue, early; green, late; purple, made in response to high CII levels; grey, made in response to DNA replication.

to function on the λ chromosome. All of the virion assembly genes are clustered, as are genes involved in replication, recombination and cell lysis. Lambda's DNA exists in several forms, a linear molecule with complementary 12 nucleotide 5′ overhangs at the two ends (called 'cohesive ends') in the virion, circular and concatemeric forms during replication and lytic gene expression, and a linear prophage form that is a circular permutation of the virion form (see below). Other members of the lambdoid family have different virion DNA termini such as 3′-terminal overhangs (HK97) or blunt ends on a terminally redundant and partially circularly permuted genome (P22).

The Lytic Growth Cycle

Injection

Lambda virions adsorb, probably via the J protein (host range mutations lie in gene *J*), to the LamB protein of *E. coli*. The LamB protein is an outer surface protein that normally functions in maltose transport. The side-tail fibres interact with the host OmpC protein to facilitate rapid LamB contact. Little is known about the actual transport of the DNA from the virion into the bacterial cytoplasm, but it has been observed that transport occurs right end first and is complete in < 5 min at 37°C, the H protein is concomitantly released from the virion, and mutations that affect this process map in phage gene *H* and

host gene *ptsM* (another component of a sugar transport apparatus). The emptied virion 'ghost' remains bound to its receptor until cell lysis. Different lambdoid phages often use different host surface receptors, and even less is known about injection by short-tailed members of the lambdoid phages such as P22, whose tail fibres have endorhamnosidase activity that degrades its receptor, the O-antigen polysaccharide on the outside of its *Salmonella* host.

Lytic gene expression

All characterized lambdoid phages carry a linear chromosome in their virion that rapidly circularizes upon injection (annealing and ligation of the cohesive ends in λ or homologous recombination between terminal redundancies in P22). These circles contain exactly one copy of the genome and are the substrate for gene expression and DNA replication. After circularization, lytic growth gene expression occurs as a cascade with three phases: 'pre-early' transcription in which the host RNA polymerase initiates on phage promoters P_L, P_R and $P_{R'}$, 'early' transcription in which transcription from the first two promoters is extended past default terminators by the action of the pre-early N protein, and 'late' transcription in which the protein product of the early *Q* gene extends the $P_{R'}$ transcript (see **Figure 2**). The only pre-early protein products are encoded by the *N* and *cro* genes. The N protein (in association with several host proteins and a specific site on the nascent mRNA) binds to RNA polymerase, causing it to fail to terminate at downstream terminators (Greenblatt *et al*., 1993), and the cro protein is a repressor that blocks transcription of the *cI* gene and so pushes development toward lytic gene expression (Ptashne, 1987). N protein action results in the expression of genes whose products participate in recombination, replication and turning on the late genes.

DNA replication

The λ *O* gene product (and parallel proteins of other lambdoid phages) binds to the origin of replication (a reiterated sequence inside the *O* gene), from which it recruits the host replication apparatus. In λ the O protein binds the phage-encoded P protein which in turn binds the host DnaB initiation helicase; in P22 its P analogue is itself a homologue of the host DnaB protein. This initiation complex serves to cause bidirectional replication forks made up of the host's DNA replication apparatus to traverse around the circle, resulting in circle duplication. Circle-to circle replication proceeds for about 15 min, until there are about 50 circles in the infected cell, at which point replication switches to 'rolling circle' mode in which unidirectional replication forks fail to terminate and several hundred genome copies are made that are present as the concatemeric products of this type of replication. It is these concatemers that are the substrate for assembly of progeny virions.

Particle assembly and DNA packaging

Like other large dsDNA viruses, lambdoid phages assemble a protein procapsid first, insert the DNA into that preformed shell and then add tails to make the completed virions. As mentioned above, different lambdoid phages actually have one of three known sets of distinct types of head genes, but they all assemble procapsids that contain a grommet-like 'portal' at only one of the 12 icosahedral vertices. In λ the B protein makes up the portal structure. DNA insertion into the procapsid is a complex process and the details vary among the different phages, but in all cases a phage-encoded enzyme called 'terminase' (contains A and Nu1 proteins in λ) recognizes a specific site on the concatemeric DNA (called *cos* in λ). Terminase cleaves the DNA at that site, one of the DNA ends thus produced is inserted through the portal structure and is translocated into the interior of the procapsid in a process that requires adenosine triphosphate (ATP) cleavage. Terminase stays bound to the translocating complex and it and the portal appear to be the components of this DNA translocase. During or before DNA entry the major head subunit undergoes a conformational change that expands and stabilizes the shell, and protein cleavages (λ – Nu3, B and C proteins; HK97 – major capsid protein) and covalent crosslinks (λ – C to E protein; HK97 – between major capsid proteins) often occur also.

When the head is full of DNA a second DNA cleavage is made to release the intravirion 'mature' chromosome from the concatemer. A poorly understood mechanism only allows this second cleavage when the head has more than a critical amount of DNA within its shell. After DNA is packaged, D protein decorates the outside of the head and W and FII make the head competent to bind to assembled tails. Meanwhile, tails are assembled independently with initiation of assembly at the head distal end and assembly proceeding to the proximal end. Several interesting features of tail assembly are the determination of the length of the shaft by the length of the 'tape measure' gene *H* protein and the transient participation of a protein produced from the *G-T* gene by programmed translational frameshifting. Heads and tails join to one another spontaneously to form mature virions.

Cell lysis

Lysis of infected cells is largely mediated by an enzyme that cleaves the polysaccharide portion of the peptidoglycan cell wall (the transglucosylase product of the *R* gene in λ; a true lysozyme in some others like P22). A second accessory enzyme that cleaves the peptide portion of the cell wall has

been proposed to be the product of two genes (*Rz* and *Rz1* in λ), that are, curiously, encoded by different reading frames of the same region of DNA. The cell wall-degrading enzymes are released into the periplasmic space through pores created in the cytoplasmic membrane by the phage-encoded 'holin' (S protein in λ). The time of lysis is precisely controlled by the ratio of initiations at two alternate in-frame translation start sites for gene *S*, since one start makes the active holin subunit and the other makes an inhibitor of the active holin.

Lysogeny

There are three important components of the λ lysogenic lifestyle: the chromosome must physically integrate into the host chromosome, a gene expression pattern must be established that culminates in turning off expression of all the lytic genes, and finally a stable gene expression pattern must be maintained that keeps the lytic gene expression cascade from turning on.

Prophage repression

In the lysogenic state the lambdoid phages express only a very small number of genes. The only one that is critical to maintaining this state is the *cI* gene encoding the prophage repressor. This repressor blocks P_L- and P_R-initiated transcription by the host RNA polymerase by binding to operator O_L and O_R which overlap these promoters and so blocks the whole cascade of lytic gene expression described above. Indeed the repressor made by a resident prophage will bind to the operators of a chromosome injected by another λ virion, thereby blocking initiation of its lytic gene expression. Thus, a prophage confers 'immunity' to 'superinfecting' λ phages.

However, not all lambdoid prophage repressors have the same operator specificity, so not all lambdoid phages are blocked in this way by a λ prophage. To date about a dozen different operator specificities have been demonstrated; phages with different specificities are said to be 'heteroimmune'. The lambdoid prophage repressors occupied a central role in the initial development of molecular biology as we know it today. The phenomenon of heteroimmunity allowed models for specificity of repressor function to be developed long before actual demonstration of their action in the test tube. Later, the λ prophage repressor was one of the first to be physically isolated and characterized, and the repressor of its close relative phage 434 was the first transcription factor whose atomic structure when bound to DNA was determined (Anderson *et al*., 1987).

Expression of the *cI* gene in the prophage state was one of the first characterized examples of an autoregulated gene. The operators recognized by the CI protein each have three separate binding sites. The differential affinities of these sites allow for the specific activation of *cI* gene transcription from P_M when two CI protein dimers are bound but repression when a third dimer is bound. Thus, neither too little or too much CI protein is made in the lysogen (Ptashne, 1987).

In addition to the prophage repressor, the lambdoid phages produce a few other proteins in the lysogenic state that alter the host in some way. These are called 'lysogenic conversion' genes. In λ the known ones are *rexA*, *rexB*, *sieB*, *lom* and *bor*. The *rex* and *sieB* genes protect the lysogen from infection by some other bacteriophages. The *lom* and *bor* gene products both appear to increase the pathogenicity of the host *E. coli*, probably at least in part by providing outer surface proteins that change the ways the bacteria interact with mammalian hosts. Other related phages make enzymes that alter the outer surface polysaccharides of their hosts. Thus, lambdoid phages are thought to give some benefit to their bacterial hosts when in the quiescent prophage state.

The lytic growth/lysogeny decision

All injected λ chromosomes initiate the lytic gene expression cascade described above; however in some infected cells after a few minutes it is shut down and a lysogenic state is established. This 'decision' between growing lytically and becoming a lysogen was one of the initial motivations for the study of phage λ (e.g. Kaiser, 1957).

The Cro and CII proteins are major competing players, in that the cro repressor, a product of a pre-early 'lytic' gene, shuts off the *cI* maintenance promoter P_M, and the CII protein activates a stronger upstream CI-producing promoter P_E (see **Figure 2**). The phage-encoded CIII protein in turn inhibits host-mediated rapid proteolysis of CII protein. If P_E expression becomes high enough to produce a critical level of CI protein, CI protein can turn off the pre-early promoters and block further lytic development. When this happens, programmed rapid proteolytic decay (by host proteases) of the products of the 'lytic' genes *N*, *O* and *xis* occurs, and a CII protein stimulated antisense RNA mediates a downregulation of gene *Q*. There are additional inputs to this genetic switch. Glucose levels control cyclic AMP levels, which in turn affect the synthesis of a host protease that speeds CII protein decay, the host RNAaseIII affects *cIII* expression, and phage DNA replication appears to stimulate the synthesis of an antisense RNA that speeds decay of the *cII* messenger RNA. All these inputs, summarized in **Figure 3**, allow the phage to assess at least some aspects of the physiological state of the infected cell, and to ensure that this molecular switch is 'all or nothing' (Ptashne, 1987).

In addition, the CII protein activates a promoter that results in high-level expression of the *int* gene, which encodes the only phage protein (integrase) that is required for physical integration of the phage chromosome into the

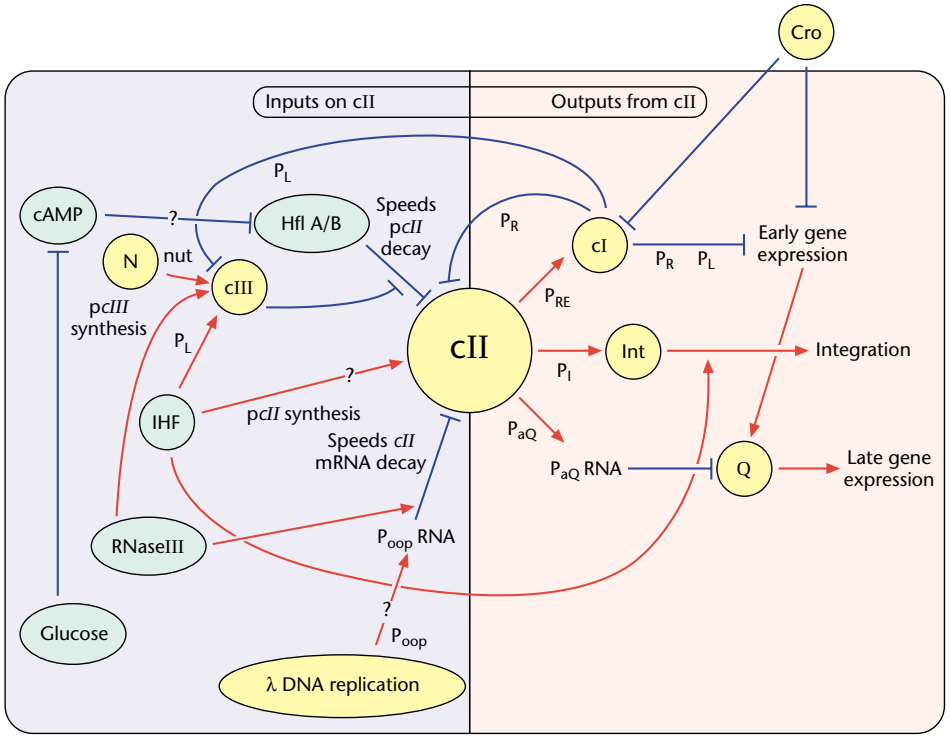

Figure 3 Lambda's lysis–lysogeny decision. The *cII* gene protein plays a central role in the lysis–lysogeny decision. Yellow circles represent phage-encoded functions and green circles represent host functions. Red arrows indicate positive effects and blue lines indicate negative effects. Question marks indicate areas of mechanistic uncertainties.

host chromosome, thus ensuring that a lysogenic state is not established without an integrated prophage. Integration occurs through a single recombination event between a circularized λ DNA molecule and the bacterial chromosome at a single, precise location on the phage chromosome (*att*P) and on the bacterial chromosome (*att*B) (originally proposed in Campbell, 1962). Different lambdoid phage integrases catalyse integration at different sites in the bacterial chromosome, and they often carry a duplication of the host integration target region so that integration does not harm the host by disrupting a critical gene (e.g. P22 integrates into a *Salmonella* threonine tRNA gene, and it carries a copy of the 3′ portion of this tRNA gene adjacent to *att*P so that an intact tRNA gene is reconstituted upon integration). The integration mechanism is well studied and is the prototypic example of site-specific recombination in molecular biology (Nash, 1996).

Induction of lytic growth in a lysogen

The lysogenic state is not a dead end for the virus. Environmental signals can cause the prophage to be 'induced' or to initiate the lytic gene expression cascade with the consequent death of the host cell and the production of progeny virions. In the case of may prophages, including λ, ultraviolet light causes induction (there may in some cases be other inducing signals, but they are not well understood). The pathway of induction is multistep: the DNA damage caused by ultraviolet light stimulates the host SOS response which results in the synthesis of high levels of the host's RecA protein which then binds to the cI repressor causing the CI protein to inactivate itself autoproteolytically. At least one other lambdoid phage, P22, has a potential alternate induction mechanism in the form of a phage 'antirepressor' gene, which when expressed makes a protein that inactivates the prophage repressor; however, the signal that might turn on the antirepressor gene is not known.

With no active repressor present, the prophage pre-early promoters are recognized by the host RNA polymerase and the lytic gene cascade begins. The only difference from an infection is that the prophage DNA has to be excised from the bacterial chromosome and circularized. This is ensured by a mechanism that causes the *int* and *xis* portion of P_L-initiated mRNA to be specifically stabilized when the transcript comes from integrated rather than circularized DNA. Thus, integrase and the Xis protein, which are both required to reverse the integration reaction, are made at high levels until excision takes place.

Bacteriophage Evolution and the Lambdoid Phages

The lambdoid phages were first defined as a group of phages which could successfully recombine with lambda to give viable hybrids. Subsequent work showed that these phages have their essential genetic functions arrayed along their genomes in the same order, making it easy to understand how a simple recombination between two of them could result in a hybrid phage with a complete set of essential genes. However the two genes that carry out the same function at the same genome positions of two lambdoid phages are often not identical. This was seen initially in differences, for example, of prophage immunity, correlating with differences of repressor genes and the respective operators, and differences of host range, correlating with different receptor specificities in the tail fibres (see Casjens *et al*., 1992, for more details).

Most of what we know about the evolution of lambda and its relatives comes from the comparison of whole phage genomes. This was first done in the late 1960s using the method of DNA heteroduplex mapping with the electron microscope, and it has been done more recently by direct comparisons of genomic sequences. The main conclusion from both approaches is the same – that each of these phages is a genetic mosaic with respect to the other members of the family. Thus, in a comparison between two lambdoid genomes there will typically be regions that match each other at a very high level, bounded by sharp transitions to regions that match at a very different level and sometimes that have no recognizable similarity. These transitions are taken to be relics of illegitimate (nonhomologous) recombination events in the ancestry of one or both of the two phages being compared. The locations of the sequence transitions are not random but occur at boundaries between functional units in the sequence, at gene boundaries in many cases, but also at boundaries of portions of genes encoding functional domains of proteins, and in the case of the head and tail genes only at the boundaries between groups of genes encoding interacting structural proteins.

In the case of most functional positions on the genome, the different genes that occupy that position in different phages can be seen, or imagined, to share a common ancestry. However, perhaps surprisingly, at some positions there are genes carrying out analogous functions but which clearly do not share common ancestry. The lysis genes and the recombination genes of λ and P22 provide good examples of this. Thus the lysis proteins of these phages carry out the same function of cleaving a bond in the peptidoglycan of the cell wall, but they do so by a different enzymatic mechanism and presumably have distinct evolutionary origins. Similarly, the recombination genes carry out the same function for the two phages, but differences in properties of the proteins and organization of the genes suggest distinct evolutionary origins.

The head genes of the lambdoid phages provide an interesting 'intermediate' example. HK97, λ and P22 exemplify three distinct variations of the phage head gene cluster; these genes dictate variations in head assembly mechanism and structure. While these three types may represent independent evolutionary origins, there are at the same time similarities of the gene function and organization which suggest that they may represent independent lines of descent from an ancient ancestor. Interestingly, the λ head gene organization is found in the nonlambdoid *E. coli* phage Mu, and the HK97 head gene organization is found in the *Streptomyces* phage ϕC31.

In the picture of lambdoid phage evolution that emerges, there are two sources of variety in the population: mutation and recombination. These are formally analogous to the 'antigenic drift' and 'antigenic shift' of the influenza A viruses. Mutation leads to the accumulation of sequence differences in a (presumably) time-dependent fashion, and the differences seen in actual comparisons of genes vary from one or a few nucleotide differences to differences that are so great that common ancestry can no longer be deduced from the sequences alone. Recombination of two types can be inferred. The illegitimate recombination described above probably occurs very rarely and even more rarely results in a viable recombinant phage, but when it is successful, it produces a large, saltatory change in the combination of different alleles present in the resulting phage. Homologous recombination, when it happens between two identical sequences, produces identical progeny phages. However, once a combination of mutation and illegitimate recombination has introduced variety into the lambdoid population (as has perhaps been the case now for a couple of billion years), homologous recombination between sequences that two phages share will almost always generate a novel version of a lambdoid phage by reassortment of the flanking sequences. Since homologous recombination between phages of the lambdoid family is a very frequent event, the rate at which new lambdoid phage designs are being generated and submitted to the scrutiny of natural selection is large indeed.

As more genome sequences become available, the picture of the genetic structure of the lambdoid population – and the evolutionary inferences that can be drawn from it – becomes increasingly detailed. At the same time, similar information emerging for other families of phages with different gene organizations argues that those phage families evolve in much the same way as outlined here for the lambdoid family. The question then arises how the different groups of phages are related to each other, if at all. Information on this point is still fragmentary, but it nevertheless seems clear that most and probably all of the dsDNA phages are related through a common ancestral pool of genes, and furthermore, that the exchange of genetic material that occurs so vigorously within the

lambdoid family and within other phage families, also extends to exchange between different phage families (e.g. side-tail fibre genes *tfa* and *stf* of λ that are highly similar to those of T4), with the result that all the dsDNA phages are linked both by common ancestry and by ongoing horizontal genetic exchange.

Propagation

Although lambda does not grow as prolifically as do some other phages, it is relatively easy to grow and purify large enough amounts of virions to carry out physical studies or to purify large amounts of DNA, as well as to grow the much lesser amounts required for genetic studies. Lambda's long use as a model experimental system and more recently as a cloning vector means that numerous detailed recitations of the appropriate methods are available (see, for example, Hendrix *et al*., 1983). Other methods can be found in various manuals of cloning and general molecular biological procedures.

Application as a DNA Cloning Vector

The DNA cloning concept had been developed (but had no name) by the lambdoid phage research community in the 1960s, with successful attempts to 'trick' *E. coli* into putting one or a few *E. coli* genes onto the phage λ chromosome. This could be done because when prophages are induced, the excision process is not perfectly accurate and at a low frequency the host genes adjacent to *att*B (biotin biosynthesis and galactose utilization genes on either side of the λ integration site and the tryptophan biosynthesis genes near the φ80 attachment site) would be joined to the phage chromosome in the resulting virions. These virions could deliver these specific host genes to a new bacterial host in a process called 'specialized transduction', and these phages could also be propagated from a single individual (cloned) and be used to study these host genes and their regulation.

In 1968, Peter Lobban, a graduate student in Dale Kaiser's laboratory in the Biochemistry Department at Stanford University, proposed in his PhD preliminary examination that, with the then recently characterized enzymes terminal transferase, DNA polymerase and DNA ligase, foreign DNA could be joined to a phage chromosome in the test tube, and he proceeded to 'clone' proline biosynthesis genes into a P22 phage 'vector' (the words 'DNA cloning' and 'vector' would only be invented later) (Lobban and Kaiser, 1973). Even in its earliest formative stages, many others quickly realized the enormous potential of this methodology, and, with the substantial background of knowledge of phage λ molecular genetics, the subsequent advent of restriction enzymes allowed various λ and 'cosmid' cloning vectors to be devised. Soon *in vitro* packaging made the process more efficient, and today various commercial enterprises vie to offer the most useful and efficient λ cloning systems. These systems stand as obvious monuments to the ultimate practical value of basic research, done simply out of curiosity about how nature works at a molecular level.

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