Lysogeny

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Secondary article

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Introduction (History, Terminology, Types, Pseudolysogeny)

Lysogeny is the harbouring of a quiescent bacteriophage genome within the genome of a growing bacterial host. Most of the phage genome (prophage) is transcriptionally inactive (or it would kill the cell). A bacterial host harbouring a prophage is a lysogen. Phages that can establish lysogeny are temperate, as distinguished from virulent phages that cannot. (A recent practice by some authors of using 'lysogenic' and 'lytic' for 'temperate' and 'virulent' should be discouraged.) The act of establishing lysogeny is lysogenization.

Naturally lysogenic bacteria were reported within a few years of the discovery of phages. They were recognized by the presence of small amounts of phage in culture fluids and by the fact that this ability to produce phages is stable through single colony isolation. The presence of phage was detected by plaque formation on a phage-sensitive (non-lysogenic) indicator strain. It was soon discovered that some infected cells of the sensitive host did not lyse but instead survived as lysogens.

Whereas some early investigators cited lysogeny as evidence for an endogenous origin of phages (Bordet, 1925), others discounted the phenomenon as an artefact of uncontrolled reinfection (d'Herelle, 1922). Convincing proof that prophages are perpetuated endogenously was provided by Lwoff and Gutmann (1950), who isolated the products of a single cell division into separate droplets of medium and showed that the potentiality to produce phages was perpetuated throughout the period of study (up to 19 cell doublings).

The prophage may either be inserted into the host chromosome or persist extrachromosomally as a plasmid. Insertion requires the agency of phage-coded proteins, either site-specific recombinases (as in lambda) or transposases (as in Mu). With certain filamentous phages, the cell survives infection, but progeny phages are extruded continually through the surface of the growing cell, a condition sometimes called pseudolysogeny.

Pseudolysogeny differs from true lysogeny in that (a) the phage genome is not quiescent but transcriptionally active, and (b) phages are extruded from growing cells rather than being released from rare cells that lyse. It also differs from a carrier state, where phages are perpetuated by reinfection of some cells of a partially resistant host (although the term pseudolysogeny has been applied by some authors to the carrier state as well).

The prophage in a lysogenic strain may undergo mutation or deletion so that phage production becomes impossible. Such incomplete prophages (called defective) arise in the laboratory and are frequently encountered in natural bacteria.

The Temperate Cycle

Alternative states

A temperate phage has two alternative lifestyles – lytic and lysogenic. Each infected cell decides which course to follow. The system is delicately poised, so that, over a wide range of ambient conditions, a significant fraction of the cells goes in either direction. Once a cell commits to becoming lysogenic, most genes of the lytic pathway are turned off. Established lysogens have a low constant probability of switching back to the lytic pathway and producing phages. The mechanisms of gene regulation are diverse, but can be illustrated by coliphage lambda.

Gene expression in established lysogens

The state of a lysogen, stably harbouring a prophage, is described first, and mechanisms for establishing that state are then discussed. In a lambda prophage, only one short transcript is made at high rate, and only one of its gene products (the repressor, encoded by gene *cI*) is needed to maintain the lysogenic state. Repressor controls two operators (*o*L and *o*R), each of which contains three repressor-binding sites. Repressor occupancy of these sites prevents transcription from the adjacent promoters (*p*L and *p*R) that control early gene expression. Late gene expression is controlled indirectly, because products of the early transcripts turn on late gene transcription. The *cI* gene itself is transcribed from a promoter (*p*M) adjacent to *o*R. Repressor bound to a subsite of *p*R activates *cI*

transcription, creating a self-sustaining feedback loop. The positions of some relevant genes and sites are shown in **Figure 1**.

Because a lysogen is full of repressor molecules, it is immune to external infection by phages of the carried type. Among lambda-related phages, many different immunity specificities are observed. Phage 434, for example, makes a repressor that binds its own operators but not those of lambda; nor does lambda repressor recognize the 434 operators.

The pathway to commitment

How does a lambda-infected cell decide to become lysogenic rather than to lyse? The critical events at the individual cell level are still not fully understood, but the major players have been identified. These include, in addition to repressor, the products of phage genes *N*, *cII*, *cIII* and *cro*, their deoxyribonucleic acid (DNA) recognition sites, and interacting host proteins (in particular, proteases). Upon infection, the first genes expressed are *N* and *cro*. gp*N* is an antiterminator that extends transcription beyond *t*L1 (the first site for transcriptional termination) on the left and *t*R1 on the right (by interacting with messenger ribonucleic acid (mRNA) at the *mut*L and *mut*R sites, respectively). Cro is a secondary repressor that binds *o*L and *o*R, and (like the repressor encoded by *cI*) blocks transcription. Unlike repressor, it does not stimulate *cI* transcription.

Antitermination by gp*N* allows transcription of *cII* and *cIII*. cII is the primary determinant that switches the cell to the lysogenic mode. At high concentration, cII turns on leftward transcription from *p*L, which extends through *cI* to allow repressor synthesis (as well as neutralizing rightward transcripts with antisense RNA). cII is highly susceptible to digestion by cellular proteases; cIII partially stabilizes it. The instability and high concentration dependence mean that cII can commit a cell to lysogeny only when its rate of synthesis is high. The stable Cro protein, which accumulates with time, gradually shuts down rightward transcription to the point that such a rate cannot be attained, thus committing the cell to lyse. So there is a race between cII and Cro, with some uncertainty as to what tips the balance in an individual cell. The complex regulation lets the cell temporize between lytic and lysogenic pathways before commitment to either.

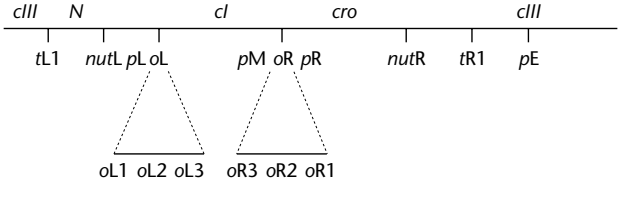

Figure 1 Map of the early regulatory region of bacteriophage lambda, showing the location of regulatory genes (above the line) and regulatory sites (below the line).

Table 1 gives a closer look at *o*R. Repressor and Cro bind to all three sites, but with different affinities. Repressor binds strongly and cooperatively to *o*R2 and *o*R1, whereas Cro binds most strongly to *o*R3. Thus at moderate concentrations repressor stimulates its own synthesis, but at high repressor concentrations repressor synthesis shuts off. An established lysogen alternates between these two states, maintaining repression but avoiding repressor concentrations so high that it would be hard to destroy when lysis is induced. Likewise, in a cell committed to the lytic pathway, not all *o*R2 and *o*R1 sites are filled with Cro at all times.

cII induces transcription from other promoters besides *p*E; in particular, from a promoter (*p*I) for the integrase gene, whose product inserts prophage DNA into the host chromosome. Use of the same effector for turning on both integrase and repressor coordinates the process of lysogenization, ensuring prophage insertion in just those cells where lytic development is repressed.

Other phages

Phages other than lambda exhibit significant variations on this regulatory scheme, always with the net result that some infected cells lyse to produce phages while others survive as lysogens. For example, among phages related to lambda, P22 has, in addition to the elements seen in lambda, an antirepressor gene, whose product can neutralize its own repressor and those of related phages, as well as a repressor of the antirepressor; and the gp*N* analogue (*nun*) of phage HK022 is a terminator rather than an antiterminator. An unrelated phage, P4, uses antisense RNA rather than a DNA-binding protein as repressor.

Lambda-type Lysogeny

Phage lambda

Types of lysogeny can be classified according to the relationship of the prophage to the host chromosome, and the mechanism whereby that relationship is attained. The lambda prophage is spliced into the chromosome and passively replicated therein. Integration results from site-specific recombination between circularized phage DNA and the circular host chromosome. Recombination occurs with high preference between specific 'attachment' sites on the phage (*attP*) and the chromosome (*attB*).

Circularization of the linear phage DNA occurs immediately after injection, whether the lysogenic or lytic pathway is ultimately taken. Circularization takes place through annealing of complementary single-stranded ends (which were generated when the phage DNA was pack-

Table 1 Control of transcription initiation by binding of regulatory molecules of *o*R

Rate of leftward transcription from *p*M (cI) (*cI*←)	Repressor (R) or Cro (C) bound at			Rate of rightward transcription from *p*R (cro) (→*cro*)
	*o*R3	*o*R2	*o*R1	
Low	–	–	–	High
High	–	R	R	Off
Off	R	R	R	Off
Off	C	–	–	High
Off	C	C	C	Off

*o*R3, *o*R2 and *o*R1 are binding sites for repressors and Cro within *o*R.
Adapted from Ptashne (1992).

aged) and subsequent ligation. Site-specific recombination is mediated by the phage-coded integrase protein. Integrase recognizes a specific sequence, about 20 base pairs (bp) surrounding the crossover point in the bacterial (*attB*) and phage (*attP* core) partners. These 20 bp are very similar in sequence in the two partners. The N-termini of many phage integrases also recognize other sequences in the phage partner (arm sites) which are iterated several times on either side of the core and whose locations span a total of more than 200 bp.

The reaction proceeds by cutting of the upper strands of the reacting partners and ligation crosswise to give a Holliday structure. The lower strands are then cut and religated crosswise at a position 7 bp to the right. In the product of the cutting reaction, protein is bonded covalently to the DNA through a tyrosyl OH, thus preserving the phosphodiester bond energy for later ligation. For optimal reaction, the two partners must be identical in sequence in the 7 bp between the two cutting sites. At one time this requirement was assumed to indicate branch migration through the 7 bp, but more recent results favour a strand-swapping mechanism in which free DNA ends 3 bp in length switch partners before ligation (Nunes-Duby *et al*., 1997). Cutting and ligation on the left thus places the Holliday branchpoint in the middle of the 7-bp region, from which the same steps (cutting, ligation, swapping) follow on the right. The left–right orientation of the reaction depends on the disposition of the arm sites, not on the core sequence.

Figure 2 shows the overall process. The locations of the crossover points on phage and host chromosomes are determined through sequence recognition by the phage integrase. The orientation of the prophage is fixed by DNA–DNA recognition in the 7 bp between the two cutting sites.

Our understanding of why the reaction proceeds in a forward direction remains incomplete. The reaction will not readily reverse (which *in vivo* would excise the prophage from the chromosome), although *in vitro* the DNA substrate and products should be isoenergetic,

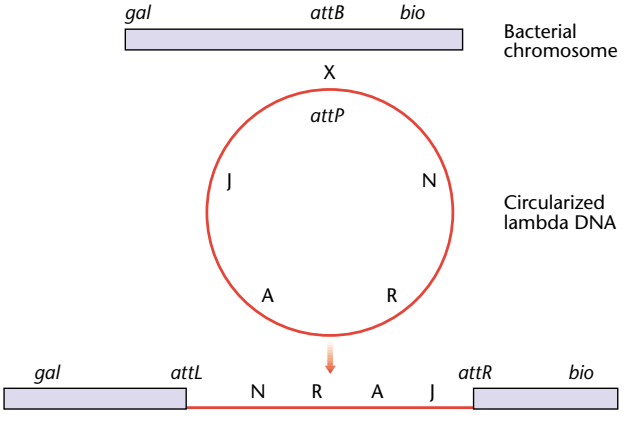

Figure 2 Overall view of lambda integration into a linear segment of the circular bacterial chromosome, based on the Campbell model (Campbell, 1962).

except for supercoiling. The excision reaction requires, in addition to integrase, a second phage-coded protein, excisionase. In whatever way excisionase may work, excision is not a true reversal of integration (which would entail cutting first on the right, then on the left), but rather a separate process that again proceeds from left to right.

Other phages

Many phages apart from lambda and its relatives (e.g. coliphages P2 and P4 and *Haemophilus* phage HP1) as well as some plasmids integrate by site-specific recombination. Usually a member of the integrase family is used, although integration by enzymes from the other family of site-specific recombinases (the DNA invertase–resolvase family) is known (Thorpe and Smith, 1998). Members of the integrase family are widely distributed among diverse phages infecting bacteria and archaea (Esposito and Scocca, 1997).

Mu-type lysogeny

In Mu-type lysogeny, as with lambda, the prophage is inserted into the chromosome, but the mechanism is by transposition rather than site-specific recombination. The packaging mechanism of coliphage Mu generates virion DNA in which host DNA flanks phage DNA at both ends. To insert, phage DNA transposes to a chromosomal site, where it is flanked by different host DNA. The transposase recognizes the ends of Mu but is not specific for the chromosomal target site. Thus, each Mu lysogen has its private chromosomal site, which may lie either within a gene or between genes. Intragenic insertion disrupts the gene (a mutation, from the host's perspective), hence the name Mu for 'mutator phage'. The transposition mechanism entails replication of the target site, so that inserted Mu is flanked by oligonucleotide repeats in direct orientation.

Transposition is also used to replicate Mu DNA during its lytic cycle. Whereas transposition during lysogenization is conservative ('cut and paste'), the same transposase also catalyses replicative transposition. Transposase synthesis is repressed in lysogens, although transposition to new chromosomal sites occasionally happens.

Mu-type lysogeny is used by Mu and the related phage D108 and also by another group of phages infecting *Pseudomonas* (Akhverdian *et al*., 1998).

P1-type Lysogeny

The P1 prophage is not integrated into the host chromosome but is instead maintained as an autonomous plasmid. Both its replication and its segregation at cell division are stringently controlled, so that each daughter cell generally receives a single copy of the P1 plasmid. Its relation to the cell is thus similar to that of the bacterial chromosome, and P1 has been studied as a model for control of replication and DNA partitioning.

P1 virion DNA is circularly permuted and terminally redundant. Soon after infection, the DNA ends undergo homologous recombination, producing a circular molecule which replicates either extensively (lytic growth) or under stringent control. As in lambda, the critical determinant of lysogeny is the establishment of repression, but regulation is more complex. P1 repressor binds to at least seven separate sites on P1 DNA, compared with two in lambda. And even when most of its genes are repressed, the P1 plasmid still expresses the functions needed for origin-specific initiation and for partitioning, as well as a negative regulator of a system that has the same net effect as the antirepressor of phage P22 (Yarmolinsky and Sternberg, 1988).

Filamentous Phages: *Xanthomonas* and *Vibrio* Phages, Cholera Toxin

Filamentous phages (such as coliphage M13) contain a covalently closed single + -strand of DNA. The virions attach to the tips of pili encoded by conjugative plasmids (such as F), and phage DNA enters the cell. The single-stranded circle is next replicated by host enzymes to become double stranded and then undergoes rolling-circle replication using a phage-coded initiation protein. Replication produces monomer circles of + -strand DNA; these complex with a phage-coded DNA-binding protein and are extruded through the intact cell envelope of growing cells. As they traverse the inner membrane, the DNA-binding protein is exchanged for the phage coat protein.

In addition to this pseudolysogenic state, some filamentous phages can establish true lysogeny, in which the prophage is integrated into the chromosome (by site-specific recombination with the double-stranded replicative form) and viral functions are repressed. This is true of several phage isolates that infect the plant pathogen *Xanthomonas* (Dai *et al*., 1987) and also the *Vibrio cholerae* phage CTXϕ (Waldor *et al*., 1997; Lynn and Waldor, 1998). The circuitry connecting repressor with integration is unknown. The *Vibrio* phage repressor is similar in sequence to other phage repressors, but the integrase is unlike any known recombinase. This phage carries the gene for cholera toxin, which is among those expressed during autonomous replication but not in the lysogenic state. In lysogens, it can be turned on by a host regulatory protein (ToxR) in response to certain growth conditions.

Induction (Techniques, Agents, Mechanisms)

Definition

One of the seminal early findings was that, for many phages, certain treatments induce the majority of lysogenic cells to switch into the lytic pathway. Technically, induction is the elicitation of switching rather than switching itself, but common usage has blurred this distinction, leading to oxymorons such as 'spontaneous induction'.

Mechanism

Most inducing treatments work by damaging DNA. Simple examples are irradiation with ultraviolet light, exposure to radiomimetic agents such as mitomycin C, and thymine starvation. In *Escherichia coli* all these agents induce expression of host genes for DNA repair functions by stimulating the autoproteolytic activity of the LexA

protein, which ordinarily represses all these genes, directly or indirectly. This stimulation is effected through the coprotease activity of the RecA protein, which is activated by DNA breakdown products. The repressors of many temperate phages mimic LexA in this respect. For example, lambda repressor, like LexA, spontaneously cleaves itself into two inactive fragments under conditions of high pH; at neutral pH, the same cleavage requires activated RecA. Mutations at the cleavage site prevent both *in vitro* digestion and *in vivo* induction of the lytic cycle.

Once sufficient repressor has been destroyed, transcription from the early phage promoters is initiated. An induced lambda lysogen resembles a recently infected nonlysogen, with two major differences. (1) Depending on conditions, a proportion of recently infected cells choose to become lysogens. Derepressed lysogens seldom revert to lysogeny, first because RecA remains activated and able to destroy any newly made repressor and second because lysogenization is rare in singly infected cells. (2) Immediately after derepression, the prophage remains in the chromosome. In lambda-type lysogeny, lytic development requires excision, so that autonomous replication can generate the proper substrate for packaging into virions. As discussed above, lambda excision requires expression of phage integrase and excisionase. During lysogenization, cII activates the *p*I promoter, whose transcript expresses integrase alone. However, following induction, transcription from the *p*L promoter gives coordinate expression of integrase and excisionase. Excision is unnecessary with Mu-type lysogeny.

Experimental use

Induction provides a potential means to synchronize phage development in all cells of a culture. Induction by DNA damage is not ideal for this purpose because the time and extent of repressor destruction vary among cells and because phage development may be affected in other ways. It has proven more convenient to isolate mutants that make a thermolabile repressor, and then to induce derepression by raising the temperature. For this reason, a particular mutant of lambda (*cI ts*857) has virtually replaced the wild type in many laboratories.

Natural function

Inducibility by DNA damage may have adaptive value: when the host is threatened, the lysogen responds by producing virions that can infect new hosts. Inducibility is accordingly widespread among phages that affect different hosts, including the cholera phage CTXϕ, but in any case phage is produced spontaneously at a low rate. A small constant proportion of the cells in a lysogenic culture (typically 10^{-2} to 10^{-4}) switch to the lytic pathway in each generation. Little is known about why a particular cell switches. Spontaneous lambda phage production is *recA*-dependent, suggesting a common mechanism for spontaneous and induced switching.

Evolutionary Aspects

Selection to survive

Mathematical population biologists have addressed the question of long-term survival of temperate and virulent phages. On the simplest assumptions, a virulent phage should establish a steady state with its host, rather than causing its extinction; however, there are problems of stability and of long-term survival in the presence of competing hosts. The temperate lifestyle does not solve these problems completely. Temperate phage survival is promoted when the phage carries some genes of selective value to the lysogenic host. Examples of phage-carried genes whose primary effect is on the host phenotype (such as the cholera toxin gene) are numerous. That such genes remain phage-borne rather than being incorporated into the host genome suggests that they are of only intermittent value to the host.

Components of natural phage populations

In many phage groups (e.g. lambda and its relatives), genome comparisons strongly indicate extensive genetic recombination in nature. Lysogeny provides additional venues for recombination unavailable to virulent phages. An infecting phage can recombine with a related prophage, and the prophage may be defective. In a purely asexual population, defective prophages would be an evolutionary dead-end, but natural recombination allows them to function as active components of the gene pool. In nature, confrontations between an infecting phage and a prophage (active or defective) are probably much more common than simultaneous infection by two related phages.

Tracing phylogenies

Like any other part of the genome, an inserted prophage constitutes a scorable genetic marker that identifies a bacterial lineage. Insertion at the same site by closely related phages seemed to have recurred frequently in different lineages identifiable by host gene polymorphisms. Thus their value as markers is restricted to a limited time scale.

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