Cryptic Prophages

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DEFINITION AND EXAMPLES

The defining characteristic of temperate phages is their ability to enter a prophage state, where most of the phage genes are unexpressed and the prophage replicates as part of the host genome. Classically, the presence of the prophage was manifested by the occasional breakdown (either spontaneous or induced) of latency, so that every culture of a prophage-bearing (lysogenic) strain contains some free infectious phage liberated by lysis of a few cells in the culture.

Prophages occasionally mutate to lose some of the functions essential for lytic growth, in which case the strain no longer liberates infectious particles. Such strains have been called *defective lysogens*, and their prophages are termed *defective prophages* (13). Because a lysogenic strain can also lose its entire prophage, the concept of defective lysogeny became needed only because some defective lysogens retain prophage-determined traits, such as the superinfection immunity that results from expression of repressor genes. The term "cryptic prophage" was introduced to denote a particular type of defective prophage that does not confer immunity to superinfection but nonetheless harbors a partial prophage, demonstrable by recombination with a genetically marked superinfecting phage. This distinction between defective prophages and cryptic prophages has had limited utility, and here we will use the terms as synonyms.

Defective lysogens frequently arise spontaneously during laboratory propagation of lysogenic strains. Lambda prophages disabled by mutation or deletion have been deliberately sought for various purposes. It was early shown that various defective lysogens, apparently with point mutations in their prophages, were blocked at different stages of the phage life cycle (3, 22, 37, 43, 55). Complementation analysis of λ phage functions was performed by synthesizing double lysogens with one defective prophage on the chromosome and a second on an F′ element (27). These inherently awkward approaches were largely superseded with the advent of conditionally lethal mutations (11, 23). A conditionally lethal mutant prophage under nonpermissive conditions (e.g., an amber mutant in a nonsuppressing host) is effectively a defective prophage, although unconditionally defective prophages include some types, such as promoter mutations, that are not accessible as conditional lethals. Numerous X-ray-induced mutants of prophage λ were isolated in the unfulfilled hope that X-ray might preferentially induce deletions, allowing deletion mapping of the prophage (A. del Campillo-Campbell, quoted in reference 12). Successful deletion mapping was accomplished by isolation of nested sets of deletions selected for loss of function of specific genes in or near the prophage (1, 26, 30, 46, 52) and by the complementary approach of studying specialized transducing phages (10).

Specialized transducing phages, which arise by abnormal excision from the lysogenic chromosome of a partial prophage connected to flanking DNA, are frequently defective. Phages that have picked up antibiotic resistance transposons can also be defective, sometimes with deletions of large blocks of phage DNA at the site of transposon insertion. Phage P1, which ordinarily lysogenizes by plasmid formation, spawned such deletion derivatives bearing chloramphenicol resistance derived from Tn*9* (P1 *cry*) or tetracycline resistance from Tn*10* (P1 *tet*), which survive integrated into the chromosome (50, 51).

Besides these laboratory creations, natural *Escherichia coli* strains frequently contain λ-related sequences presumed to be cryptic prophages (2). The most intensely studied are those present in the K-12 strain, whose chromosome contains, beside λ itself, four elements (DLP12, Rac, Qin, and e14) that are related in sequence either to λ or (in the case of e14) to a λ relative, phage 21. All these elements are shorter than λ (the shortest, e14, being 14 kb compared to the 49 kb of λ). From comparison of the available sequence, plus recombinational studies, their genetic structures can be inferred. Assuming that each element entered the K-12 genome as an

active λ-related phage, each element must have lost substantial segments of the functional phage genome through deletion. Insertions of IS elements are also frequent. Additional deletions within some of these prophages have been observed during laboratory propagation of K-12 (53). Figure 1 shows genetic maps of some of these elements.

MODES OF DETECTION

Defective λ lysogens bearing single point mutations remain immune to superinfection by λ. They are distinguishable from λ-resistant host mutants by their sensitivity to virulent mutants of λ (λ *vir*) or heteroimmune lambdoid phages (such as λ imm^{434}). Many such isolates lyse on induction, in some cases liberating phage-related particles visible in the electron microscope.

Some natural strains likewise lyse on induction, liberating phagelike particles. This is true of *E. coli* 15, whose lysates contain two types of phagelike particles, each packed with a unique DNA species carried by the bacterium in plasmid form (29, 36). Neither type has been shown to form plaques. The phagelike particles and free tails can kill susceptible cells and thus qualify as colicins. This strain also harbors a large plasmid related in DNA sequence to phage P1 and displaceable by P1 prophage, but not packaged into virions.

Most natural prophages are not recognizable by such criteria. Three of the four cryptic prophages of K-12 (e14, Qin, and Rac) have repressor genes, presumably present in ancestral lambdoid phages but with a specificity that has not been encountered among the known lambdoid phages. Nor do they make the strain sensitive to DNA-damaging agents (like UV light) that induce derepression, at least not to the same extent that λ prophage does. However, derepression is induced in these elements, some of which were discovered through observation of its sequelae. In the case of e14, 14-kb circles of the excised element were found in induced cells, and later, chromosomes cured of e14 were detected (9, 31). With Rac, a phage-coded recombinase that can act on bacterial chromosomes is derepressed by conjugation (42). And mutations in the repressor gene (*dicA*) of Qin derepress another gene (*dicB*) whose product inhibits cell division (6).

When a cryptic prophage is bracketed by *attL* and *attR* sites and functional integrase and excisionase are available (either from the cryptic prophage itself or by complementation), the prophage boundaries are precisely defined. This is true of e14 (which excises) and of Rac (which can recombine with active λ, giving a recombinant phage, λ reverse, with the Rac *attP* site and flanking DNA derived from the two ends of the inserted prophage). Rac can be excised by superinfection with λ reverse (7). DLP12 has a full-length *int* gene and a truncated *xis.* Specific excision has not been shown, but a sequence from the *argU* tRNA gene at its left end is repeated on the right and could represent *attR.* No such information is available for Qin, which is delineated only approximately by knowledge of functions and sequences related to lambdoid phages on the one hand and standard bacterial genes on the other. As laboratory deletions frequently remove *att* sites, there is no expectation that all cryptic prophages should retain them.

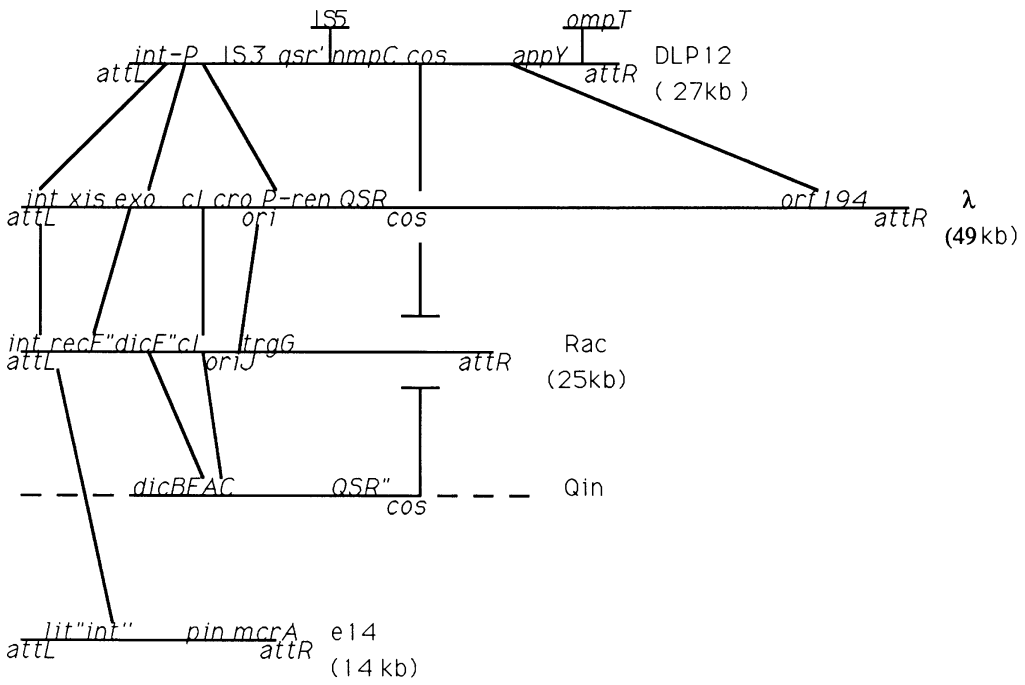

FIGURE 1 Lambdoid prophages of *E. coli* K-12, located at 12 min (DLP12), 17 min (λ), 25 min(e14), 30 min (rac), and 35 min (Qin). All but λ are defective. Homologous genes or segments or segments of the different phages are connected. (For *cI*Rac, homology and location are assumed on indirect evidence.) Genes are shown above the lines and recognition sites below the lines. Genes in quotation marks are identified only by sequence. Genes affecting hose phenotype are *nmpC* (new outer membrane protein), *trgG* (K^+ transport), *dicB* (division control), *lit* (phage T4 development), *pin* (DINA inversion), and *mcrA* (restriction). Major sources: DLP12 (42, 44); λ (20); Rac (18, 21; C. C. Chu, L. Satin, A. J. Clark, M. Faubladier, and J.-p. Bouché, personal communication); Qin (8, 24); e14 (35, 39, 47, 50).

Another way to detect cryptic prophages is through their interactions with superinfecting phages. Both DLP12 and Qin were found by the recombinational rescue of *QSR* function by λ phages mutant in one or more of these genes. The first such recombinant for DLP12 (called p4) was unwittingly selected because the recombinant fitted better with the λ packaging constraints when recombination was selected between other markers that changed the genome size. Later, selection for pseudorevertants of double amber mutants or deletions in gene *Q* gave the same type of recombinant, in which λ genes *Q, S,* and *R* are replaced by analogs from DLP12 or Qin (33, 38). In both DLP12 and Qin, the *QSR* analogs have no significant homology to λ, but there is homology in the flanking DNA. In the case of phage P2, infection of *E. coli* B produced recombinants (called P2HyDis) with a different repressor specificity from P2; as *E. coli* B does not spontaneously produce phage, the prophage bearing these determinants may be defective (19). *E. coli* B can be cured of the prophage by superinfection with P2. Furthermore, it has long been known that P2 does not readily insert at the chromosomal site of K-12 that is preferred in *E. coli* C. It turns out that a similar site in K-12 is already occupied by a defective remnant, 639 bp in length, of P2 DNA (5).

Another type of interaction takes place during prophage insertion. The cryptic prophages of K-12 can serve as secondary insertion sites for λ (through homologous recombination), and a Qin prophage in *E. coli* C has been detected by this property (40).

However, surveys at this level could mislead. Without information on the extent of the homology or the part of the genome involved, no certain conclusion can be drawn as to whether some of the hybridizations might register host sequences that have been incorporated by the phage rather than the other way around. As a specific possibility, many phages (including some λ derivatives) have picked up bacterial insertion sequences that are also found in the host chromosome; it would be unwarranted to conclude that the latter are of phage origin.

If the genomes of large double-stranded DNA phages originated as chimeras of various functional modules derived either from the host or from accessory DNA elements (14, 16), phage homology with genome DNA need not indicate the presence of prophage residua. Of the K-12 elements DLP12, Qin, Rac, and e14, the only one that might credibly have a nonphage origin is e14. The others all contain phage genes (frequently incomplete) in the same order found in active phages, and it is hard to imagine a reason for that arrangement other than derivation from an active prophage. With e14, the only known homology with another lambdoid phage is in its attachment sites and putative integrase gene.

Another possible manner of surveying natural strains is to focus on a specific chromosomal attachment site and to look for phage-related DNA in that position. This was effectively done for the Atlas prophages, which were discovered when a DNA segment near *trp* was examined for variation among a collection of natural *E. coli* strains (ECOR collection [45]). Atlas prophages are present in 23 of 72 strains examined (but not in K-12). At least two of these are active (able to form plaques), but most of the others are shorter and probably defective (A. B. Stolzfus, Ph.D. thesis, University of Iowa, Iowa City, 1991).

EFFECTS ON HOST

Cryptic prophages frequently have shown up as loci of mutations affecting host phenotype. This is true of the four cryptic prophages of *E. coli* K-12, although all except Rac were initially detected by other means. The mutations may either inactivate expressed prophage genes or activate unexpressed genes.

An unexpressed gene of DLP12 (*nmpC*), present also in some lambdoid phages, is homologous to (and probably derived from) the gene for the major host outer membrane protein, *ompC* (34). Host *ompC* mutants grow slowly, and some faster growing derivatives that come up have mutations in DLP12 that activate *nmpC,* whose product then can functionally replace OmpC. The *nmpC* gene is not essential for plaque formation by those phages that have it; its product may render the cell surface unable to adsorb phage through replacing OmpC, a common receptor for lambdoid phages.

Two other genes, *appY* and *ompT,* lie at the right end of the DLP12 prophage (41). Overexpression of *appY* cloned on a multicopy plasmid enhances expression of various *E. coli* genes including the alkaline phosphatase gene *appA* (4). The *ompT* gene is expressed from the DLP12 prophage and encodes a protease located in the outer membrane (32). It is not clear that either *appY* or *ompT* is useful to *E. coli.*

The Rac prophage came to light because of its gene for general recombination. In λ, the analogous genes (*exo,* β) promote RecA-independent general recombination and increase the rate of replication. As in λ, the Rac recombination gene, *recE,* is regulated by repression, which can be eliminated by zygotic induction during bacterial conjugation, by mutational inactivation of the repressor gene, or by long deletions that place *recE* under control of nearby bacterial promoters. Zygotic induction, causing a temporary restoration of recombination proficiency to a *recB recC rac*$^-$ recipient mated to an Hfr *rac*$^+$ donor, was the characteristic by which Rac was first identified (42). Mutations (*sbcA*) that permanently activate *recE* expression were selected on *recB recC* strains; *recB recC* mutants are hypersensitive to DNA-damaging agents such as mitomycin C, and the *recE* enzyme Exo VIII can substitute for the *recB recC* protein ExoV in increasing resistance.

Another gene in the Rac prophage (*trgG*) encodes a protein that cooperates with the TrgA protein of the host to form one of two systems catalyzing a high rate of K^+ transport (21). The system is not essential for *E. coli* growth but does have a higher affinity for K^+ than the other available system (TrgH), which could be advantageous under some circumstances. The conditions used to select for activation of *nmpC* and

recE suggest possible evolutionary pathways whereby a phage-borne gene might be coopted by the bacterial host. Obviously, the same selective conditions set up in the laboratory must sometimes arise in nature, and the result should be the same in the short run. There is no good evidence that cooption of prophage genes has ever been a step in an effective long-range evolutionary strategy.

The *dic* genes of the Qin prophage have been discussed in terms of cooption because they can affect cell growth, but the observed effects are negative (8). The *dicB* gene interferes with cell division and seems analogous and similarly located to the λ *kil* gene, whose expression is lethal to the cell. The *dicA* and *dicC* genes regulate *dicB* and have some homology to λ *cI* and λ *cro.*

The e14 element has no genes with defined functions analogous to those of other lambdoid phages. Even the integrase and excisionase functions have not been directly shown to reside in e14 (although e14 is at least necessary for their expression [9]), and the identified segment with *int* homology could be a pseudogene (S. J. Schneider, Ph.D. thesis, Stanford University, Stanford, Calif., 1992). Some genes that have been identified are *lit* (which regulates translation of T4 late genes), *mcrA* (a restriction gene), and an invertible segment P with its cognate site-specific recombinase encoded by *pin.* The P-*pin* system is homologous to counterparts in phages P1 and Mu-1, as well as in strains of *Salmonella* that can switch surface proteins (phase variation).

FIGURE 2 Structure of C32, the most common type of λ *cry* prophage. Homologous recombination has replaced prophage DNA from an IS*2* near *xis* through *cos* with bacterial DNA from the IS*2* at 9 min through the *cos* site of DLP12. From reference 49 with permission.

INTERACTIONS WITH ACTIVE PHAGES

Prophages can interact with superinfecting phages on both the physiological and the genetic level. Some of these affect heterologous phages (such as the exclusion of T4 *rII* mutants by the λ prophage *rex* gene), whereas others act on related phages (such as superinfection immunity or alterations of cell surface affecting phage attachment). The *nmpC* gene in DLP12 is not the ideal example here, because it is unexpressed unless activated by mutation. In lambdoid phage PA-2, *nmpC* is expressed from the prophage and interferes with attachment of phage of the carried type (48). The ε phages of *Salmonella* likewise alter the cell surface by introducing biosynthetic enzymes for surface polysaccharide phage receptors, so that ε15, for example, renders the cell resistant to superinfection by another ε15 (54). If the superinfecting phage gained access to the cell, it would be controlled by the prophage repressor, but in fact it does not get that far. Superinfecting λ mutants defective in certain genes can induce expression of their homologs in repressed or defective prophages at a level which is sometimes adequate to complement the defect (as in the case of gene *R*).

With respect to defective phages, the recombinational interactions have received most attention. The simplest is marker rescue. In general, rescue into an active phage requires homology on both sides of the rescued gene, not necessarily in the gene itself. The cryptic phages of *E. coli* K-12 (like the natural lambdoid phages) include segments homologous to various lambdoid phages. Thus rescue of the *q′* gene from DLP12 is accompanied by rescue of *s′* and *r′*, because there is no λ homology within the *qsr′* segment. Rescue of *cos* into λ is sometimes accompanied by rescue of *qsr′* and sometimes not, because *cos* lies within a λ-homologous segment. Whereas homologous exchange is the rule, there are clear examples where homologous exchange on one side of the rescued marker is accompanied by an apparent heterologous exchange on the other (discussed in reference 15). These are rare events, and little is known about mechanism.

The *recE* gene of Rac can be rescued into a λ deleted for the *rec* genes (28). Here the prophage includes no λ homology to the left of *recE*, but an excised prophage would have λ homology further to the left. The recombinant phage obtained has the structure expected if the prophage had excised and then recombined. Technically, all that can be said is that the event requires site-specific recombination at the *att* site; the actual intermediate might be a tandem double lysogen rather than an excised prophage.

A more complex example comes from homologous recombination between a λ prophage and the cryptic element DLP12. It was first noted that the survivors of a λ lysogen subjected to heavy doses of UV irradiation included a few individuals that had lost part, but not all, of the prophage. Such individuals (whose incomplete prophages were dubbed "cryptic") were much more frequently obtained from lysogens of a particular λ strain (called λ crypticogen) than from the reference λ used in most laboratories (25). Further study showed that λ crypticogen differs from reference λ by an IS*2* insertion upstream of the *xis* gene (56).

The basis of this phenomenology is now understood (49). The IS*2*, behaving as a portable stretch of homology, recombines with an IS*2* in the bacterial chromosome (most frequently the one located at about 9 min), and the λ prophage recombines near *cos* with DLP12 (Fig. 2). Substitution of the bacterial DNA between the IS*2* and DLP12*cos* for the λ DNA between IS*2* and *cos* produces a strain that still has the λ DNA from *cos* through *attR* and from *attL* through *xis* to the IS*2* (Fig. 1) and bears a duplication of the bacterial DNA from 9 to 12 min. This is the most common type of cryptic formed by this mechanism. In principle, homology could be provided by Rac or Qin, but use of those elements as recombination points has not been observed.

The orientation of IS*2*-*cos* in the λ prophage is the same as that in the 9- to 12-min segment, and one class of possible mechanisms would require that the orientation be the same. However, cryptics with the same duplication are readily generated when λ *crg* is inserted in the opposite orientation, as though the cryptics are formed through double crossover between two copies of the bacterial chromosome (17).

The duplication shown in Fig. 2 is the type most commonly found. Rarer types include crossovers at another IS*2* element (producing a longer duplication) and individuals where one crossover is in *cos* and the other appears to take place between heterologous DNA sequences. The rare cryptics coming from wild-type λ are in the latter class. The exact position of the crossover near *cos* varies. It can be either to the right or to the left of *cos* and occasionally takes place to the left of *qsr′*, giving strains that include λ genes *Q* through *att.*

CRYPTIC PROPHAGES AND MOLECULAR PHYLOGENY OF NATURAL STRAINS

Mobile elements such as transposons and prophages can be used as molecular markers to trace the history of the strains that harbor them. Their utility in this respect is restricted to a limited evolutionary timespan and is complicated (especially for phages with unique insertion sites) by reinfection and reinsertion into strains that have lost all or part of an element. Surveys of natural strains have some potential value in showing how frequent reinsertion may have been.

The junction between host DLP12 and *argU* has been found in many natural isolates of *E. coli* and also in bacteria as distant from it as *Salmonella typhimurium* and *Shigella boydii* (41). This could have come about if this part of the cryptic prophage has been preserved for some advantage it imparts to its bearer or if lysogenization at this site has been frequent.

A survey of Atlas prophages (defective and active) is in some ways more informative, because the presence of Atlas could be compared with molecular phylogenies constructed on other bases. The results show that Atlas prophages are distributed among lines not closely related to one another. Detailed analysis of the relationships between prophages and their hosts suggests multiple independent lysogenizations at this site (Stoltzfus, Ph.D. thesis).

SUMMARY AND CONCLUSIONS

Cryptic prophages have been aptly described as genetic debris that clutters the bacterial genome (53). From the cell's perspective, all prophages might be considered junk DNA; cryptic prophages appear to be junk from the phage's perspective as well. Although the available information is somewhat anecdotal, perhaps the most noteworthy feature of cryptic prophages is their prevalence. They are present in most bacterial strains examined and compose several percent of the *E. coli* K-12 genome. The clear implication is that in nature either the rate of lysogenization is high or the decay rate of cryptic prophages is slow. Available evidence (e.g., from Atlas prophages) indicates a high rate of lysogenization.

In a laboratory strain in pure culture, a cryptic prophage is simply an element doomed to extinction. However, in natural populations cryptic prophages are not genetically dead but can recombine with active phages and contribute to the phage gene pool. Indeed, it is plausible that the most common confrontation allowing generation of new recombinant types is infection by an active phage of a strain harboring a related cryptic prophage (11).

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