

Specialized Transduction

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EARLY OBSERVATIONS

Genetic transduction is the virus-mediated transfer of nonviral genetic information to a recipient cell. The foreign genes are packaged within a shell of virus proteins and introduced into the recipient by infection. Transduction is traditionally subdivided into two subclasses, specialized and generalized, and the editors of this volume have seen fit to respect tradition. The term specialized transduction originally called attention to the propensity of coliphage λ , the first discovered specialized transducer, to mediate transfer of only a limited set of host genes (47, 48). Generalized transduction, discovered first in *Salmonella* phage P22 (79), a relative of λ , and subsequently in coliphage P1 (44), an unrelated temperate coliphage, has no such limitation (see chapter 130 in this volume). There is a second important distinction between specialized and generalized transduction. The limited set of host genes that λ naturally transduces can be stably incorporated into its own chromosome, where these genes are then replicated and packaged as efficiently as if they were native phage genes. By contrast, generalized transducing phages usually do not incorporate the genes they transduce into their own chromosomes but occasionally package a segment of host DNA in place of the phage chromosome. Because of this difference, use of the term “specialized transduction” has broadened to encompass any high-efficiency virus-mediated replication and packaging of a nonviral gene no matter how the nonviral gene is incorporated into a virus chromosome. In this broader sense, probably all viruses are capable of specialized transduction. However, in this chapter I concentrate on natural examples of specialized transduction.

Lambda is a temperate bacteriophage that lysogenizes *Escherichia coli* by inserting its chromosome into the host chromosome (see chapter 125). Lambda phage is a member of a large family of temperate phages that share a common chromosome organization and exchange genetic information in nature (4, 14, 32). Most of what we know of specialized transduction comes from the study of λ , although examples have been found in other members of the λ family and in unrelated temperate phages (see below). Transducing particles are found at a frequency of approximately $\leq 10^{-4}$ per induced cell in phage stocks prepared by induction of a λ lysogen and are typically detected by infection of a host carrying a mutation in a selectable gene. These particles are not normally detectable in phage stocks prepared by infection (47, 48) (but see below). Only host genes that are linked to the prophage attachment site (*attB*) are transduced. The first examples of genes transduced by λ are the *E. coli gal* and *bio* operons, which flank *attB* (47, 74). Transductants frequently retain the recipient copy of the transduced gene and become λ lysogens. In contrast, most transductants that arise by infection with generalized transducing phage lose the recipient allele and are usually not lysogenic. Lysogenic induction of these partially diploid λ -mediated transductants yields phage stocks, called high-frequency transducing lines, in which the number of transducing particles can exceed the number of induced cells. The transducing phages in such lines have undergone a chromosomal rearrangement in which a segment of phage DNA located in the central region of the phage chromosome has been replaced by a segment of host DNA located near the prophage attachment site (2, 7, 9). The amounts of phage DNA lost and host DNA acquired typically differ among different isolates, but once formed, the rearranged chromosomes are stable during further propagation (47, 48). The origin of such high-frequency transducing lines has been understood for some time and is described below. The virtues of such phage are also known: they facilitate the analysis of host gene structure and function by providing a concentrated and easily manipulable source of a specific DNA sequence. Indeed, the concentration of a specific host sequence in a transducing phage stock is hundreds of times higher than that in a bacterial culture. In addition, transducing phage have been extremely useful in elucidating the structure of the λ chromosome and the functions of particular

phage genes and sites (reviewed in reference 10). The use of specialized transducing phages has undergone explosive growth in the last 25 years, largely because of the development of simple, general methods that allow the incorporation of nearly any DNA sequence into a λ chromosome. I refer the reader to several reviews and manuals in which these methods are described (3, 5, 36, 49, 54, 57, 59).

MODEL

Our current understanding of λ -mediated specialized transduction begins with Campbell's model of λ lysogeny (8). According to this model, incorporation of host genes into a virus chromosome is the result of two successive recombinations. The first inserts a circular form of the virus chromosome into the host chromosome to form a prophage. The second excises a segment of the prophage together with an adjacent segment of host DNA from the host chromosome (abnormal excision; Fig. 1). Subsequent work has shown that prophage insertion requires a phage-encoded site-specific recombinase, integrase, that promotes recombination between the phage attachment site, *attP*, and *attB* (see chapter 125). Integrase also promotes excision by recombining the attachment sites that bracket the prophage (normal excision; Fig. 1). However, normal excision differs from abnormal excision in that the latter has little or no site specificity, is not known to require any phage or host proteins (71), and is estimated to occur at less than 10^{-6} of the frequency of normal excision. If the excised DNA molecule contains a site called *cos* (for cohesive end site) and is within the size range for λ packaging (about 36 to 51 kbp [63, 69]), a replica of the molecule can be packaged into a virus particle and transferred by infection to a new host (reviewed in reference 18). The *in vivo* substrate for packaging is an oligomer of several directly repeated phage chromosomes that can be produced by rolling-circle replication of the excised molecule (20). The DNA between adjacent *cos* sites is cleaved from the oligomer and packaged into virus particles in a reaction that requires a large set of proteins encoded by both λ and *E. coli* genes. Cleavage occurs by a pair of staggered single-strand cuts within each *cos* site, and the packaged chromosome is therefore a linear DNA molecule with short, complementary, single-strand ends (30). After injection of the packaged transducing chromosome into a recipient cell, the left and right ends anneal by complementary base pairing to reform the *cos* site, and the single-strand breaks are sealed by DNA ligase (21, 31). The circular chromosome can insert into the host chromosome to form a lysogen. In fact, high-frequency lines are most conveniently propagated as prophages when abnormal excision has deleted essential phage genes. If essential genes have been lost, lytic growth will require a "helper" phage to provide the missing functions. Helper phages can also supply missing *cis*-acting sites by recombining with the transducing phage. Such recombination can promote replication of a transducing phage chromosome that lacks a replication origin, or insertion of one that lacks an attachment site.

This model accounts for most of the early observations of specialized transduction. (i) The location of the host gene replacement (in the central region of the phage chromosome) is the result of the steps shown in Fig. 1: circularization of the infecting phage chromosome by end joining, insertion by recombination at *attP*, abnormal excision of the prophage, and *cos* cleavage during packaging. Genetic mapping, electron microscopy, and sequencing of transducing phage chromosomes show that one end of the host substitution is located within the attachment site, as expected from the model (17, 34, 43). (ii) The variable location in the phage chromosome of the other end of the host substitution and the variable amount of host DNA acquired reflect the low sequence specificity of abnormal excision. Recent evidence shows that the sites used in abnormal excision share very short regions of sequence similarity, and some resemble cleavage sites for the enzyme DNA gyrase (40). (iii) The absence of transducing activity for genes located far from *attB* is a result of the upper size limit for packaging and the rarity of chromosomal rearrangements that place distant host genes into a packageable size range. It is clearly not the result of some special character of the region around *attB*, since λ inserted at other host chromosome sites transduces markers linked to these sites (55). (iv) The partially diploid character of many transductants arises from the circularization of the transducing phage chromosomes by end joining and the insertion of the circles into the host chromosome to form lysogens containing a duplication of the transduced segment. Haploid transductants, which are less frequent, can arise by a multiple recombination in which the allele carried by the transducing

phage is substituted for the allele carried by the host. The model does not explain the absence of transducing particles in lysates produced by infection, unless it is assumed that insertion is rare during the lytic cycle of phage development. However, more recent results cast doubts on this assumption (see below).

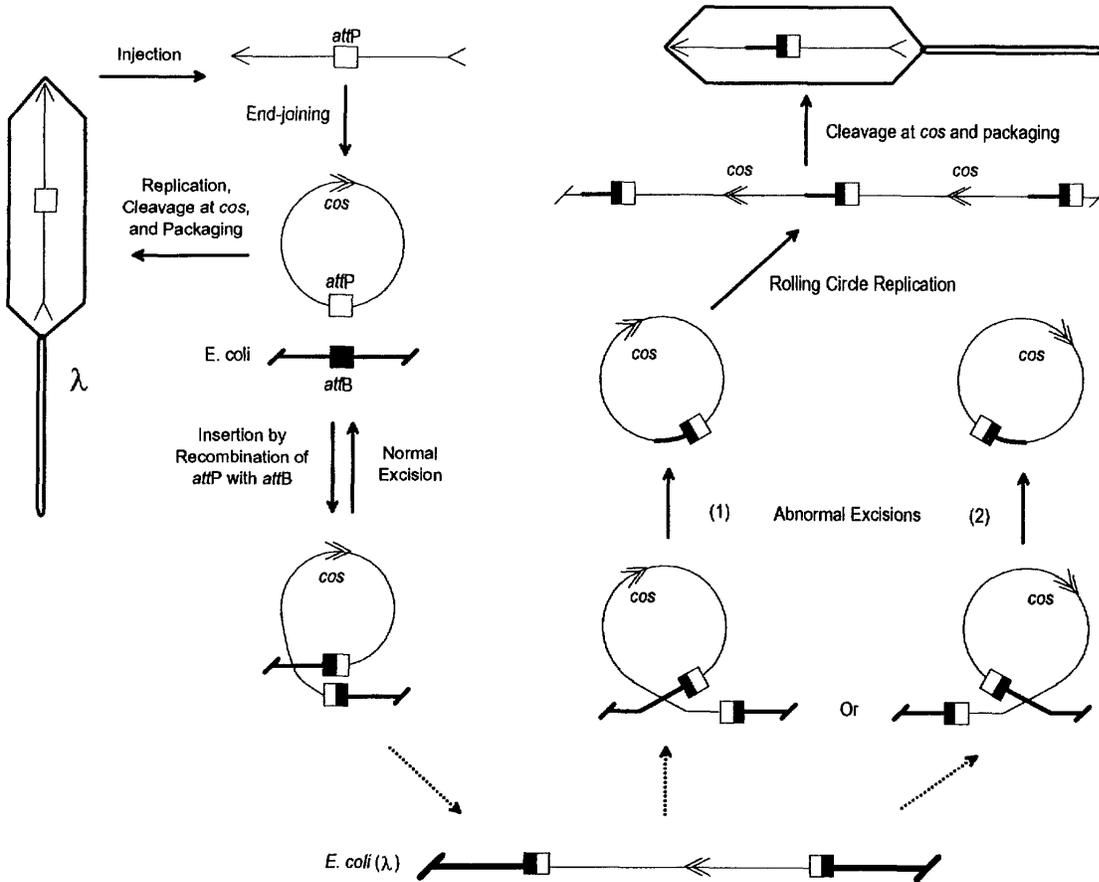


FIGURE 1 Model for prophage insertion and production of transducing phage lines. Thin lines, phage DNA; thick lines, segment of bacterial DNA; arrowheads, left and right cohesive ends of the λ chromosome; double arrowheads, cohered ends or *cos* sites; squares, attachment (*att*) sites; irregular hexagon, phage head; elongated oval, phage tail. On the left side, a phage particle injects its chromosome into the cell (not shown), and the chromosome circularizes by end joining. In the lytic pathway of phage development (leftward arrow), phage genes encoding replication, lysis, and virion proteins are expressed; the chromosome replicates; and the replicas are cleaved and packaged into progeny phage particles (see reference 52 for a discussion of the lysis-lysogeny decision). In the lyso-

genic pathway, phage gene expression is repressed, and the circular chromosome inserts into the bacterial chromosome by recombination between *attP* and *attB*. Normal prophage excision reverses the last step. Abnormal excisions that lead to transducing phage lines are shown on the right side (the inserted prophage is redrawn, as indicated by the dotted arrows). Abnormal excision 1 generates a circular chromosome carrying host DNA from the left of *attB*, rolling-circle replication leads to a concatemer of identical chromosomes joined head to tail, and *cos* cleavage and packaging produce a transducing phage particle. Abnormal excision 2 generates a circular chromosome carrying host DNA from the right of *attB*, and this chromosome is replicated and packaged as shown for excision 1.

Some of the transducing phage in λ stocks produced by lysogenic induction have a different structure and are formed in a way that does not require excision. The chromosomes of these phage lack one of the normal λ ends and are therefore unable to circularize by end joining (45, 64). Consequently, transduction does not occur by insertion of the entire phage chromosome into the recipient bacterial chromosome but instead by substitution of donor for recipient DNA through homologous recombination (72). Therefore, the transductants are not partial diploids, do not contain transducing phage chromosomes, and do not produce

high-frequency transducing phage lysates upon lysogenic induction. The chromosomes of these phage (called λ *doc*, for defective, one cohesive end) are produced by an aberration of the normal packaging mechanism in which one of the DNA ends arises by *cos* cleavage and the other arises by a less specific cleavage of host DNA (45, 64). Lambda *docL* chromosomes have the λ left end and host DNA from the right of *attB*, while λ *docR* chromosomes have the λ right end and host DNA from the left of *attB*. The production of a λ *docL* particle is diagrammed in Fig. 2. After induction of a lysogen and replication of the prophage together with adjoining bacterial DNA, the *cos* site of an unexcised prophage is cleaved, and prophage and host sequences to the right are packaged. The resulting particles are incomplete when released from the cell, because unpackaged and uncleaved DNA that protrudes from the head prevents the addition of essential virus proteins. When the excess DNA is digested with nuclease and the missing proteins are added, the incomplete particles become infectious and capable of transducing markers that are closely linked to and are to the right of *attB* (62, 64). Packaging of λ *docR* chromosomes is thought to begin at randomly located DNA nicks or ends to the left of the prophage, DNA to the right is packaged within a phage head, and the other chromosome end is formed by *cos* cleavage (62). As expected, the production of both λ *doc* particles is enhanced by mutations that block prophage excision (22, 23, 62). Lambda *doc* particles, unlike standard, two-cohesive-end transducing λ , are also found in stocks produced by infection, provided that the infecting phage is insertion proficient and the other conditions particular to λ *doc* formation are fulfilled (62). This result shows that infecting λ does insert into the host chromosome during the lytic cycle of phage development. The result also makes it more difficult to understand why two-cohesive-end transducing chromosomes are absent in stocks of λ produced by infection.

Although many different sequences can be joined during abnormal excision (37, 40), preferential joining has been found in a few cases. The best analyzed case occurs during the formation of the transducing phage λ *att*², in which bacterial sequences located to the left and right of an inserted prophage preferentially recombine to give a unique transducing phage chromosome that is larger than the prophage (19, 23, 56). This excision is promoted by the homologous recombination pathways of phage or host. Lambda *att*² particles were detected only after induction of prophages carrying deletion mutations and not after induction of wild-type prophages, because the undeleted DNA is too large to be packaged into an infectious virus after abnormal excision. A second, apparently similar case of preferential abnormal excision in coryneophage γ has been reported, but this case has not been analyzed as extensively (6).

SPECIALIZED TRANSDUCTION BY VIRUSES OTHER THAN λ

Campbell's model (8) suggests that any virus that regularly joins its DNA to host DNA and uses specific virus nucleotide sequences as chromosome packaging signals will be naturally capable of specialized transduction. Although this prediction has not been extensively tested, high-frequency transducing lines have been looked for and found in the lambdoid phages P22, ϕ 80, and $\phi\gamma$ (24, 25, 41, 61, 73); in coryneophage γ (6); and in *Bacillus* phages SP β and H2 (77, 78). In phage P22, in which the structure of transducing phage chromosomes has been extensively analyzed, the formation of high-frequency transducing lines can be accounted for by a modification of the Campbell model that takes account of the "headful" packaging mechanism of this phage (15, 33).

Induction of lysogens for phage P22 also leads to the production of transducing particles that resemble λ *docL* in some respects. The transduced genes are close to and preferentially to one side of P22 *attB*, the production of transducing particles is greatly enhanced by blocking normal prophage excision, and transductants that produce high-frequency transducing lines are infrequent (41, 42, 60, 76). However, these particles, unlike λ *docL* (Fig. 2), are infectious upon release from the cell. This is because P22, unlike λ , does not require a specific site to terminate packaging and to cleave DNA. Packaging normally initiates at a prophage site analogous to *cos*, and DNA segments equivalent to a headful are sequentially cleaved and packaged into phage particles.

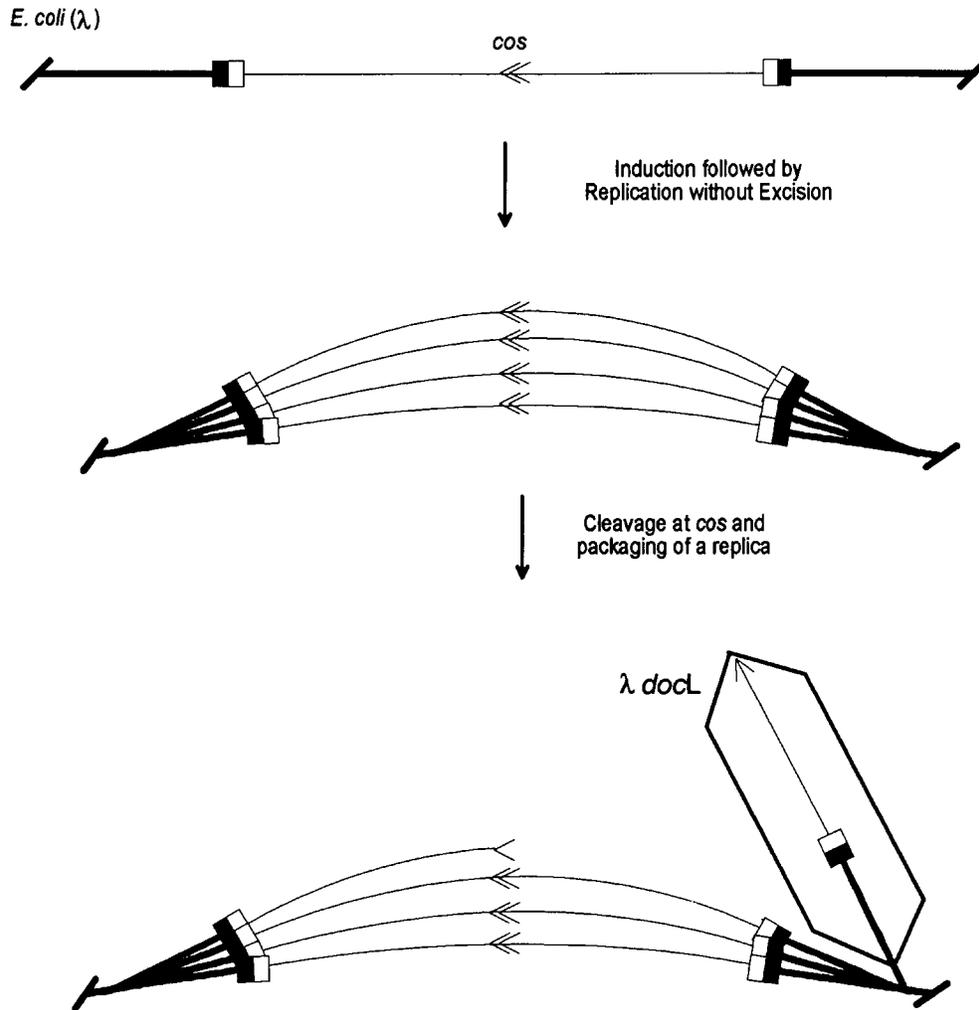


FIGURE 2 Model for the formation of λ *docL* transducing phage by the packaging of unexcised prophage and host DNA (62, 64). symbols are described in the legend to Fig. 1. After lysogenic induction, bidirectional DNA replication initiates within the inserted prophage shown at the top, and replication forks travel into flanking host DNA to produce the multiply forked structure diagrammed in the middle. *cos* cleavage and packaging initiating within one of the prophage replicas produce the incomplete transducing particle shown at the bottom. The direction of packaging shown (from *cos* rightwards) is preferred by λ . Lambda *docL* is not infectious when released from the cell because the protruding DNA blocks the addition of essential phage proteins, and the particles must be completed in vitro (see text). Unexcised P22 prophages are believed to form a similar type of transducing particle except that excess DNA is cleaved away from the head after packaging, and the particles are therefore infectious when released.

Specialized transducing derivatives of coliphage P1 have been isolated (39, 46; reviewed in reference 75), despite the fact that this phage does not have an efficient mechanism for joining its DNA to host sequences. It is believed that in some cases, the joint between phage and host DNA is formed by the action of a transposase, but in other cases, the mechanism of joining is unknown. P22 transducing phage that were formed by the action of a transposase have also been isolated and characterized (16, 67).

The best studied example of specialized transduction, apart from λ , is found in eukaryotic retroviruses (reviewed in reference 68). Recent evidence suggests that the chromosomes of transducing retrovirus particles arise by cotranscription of an inserted provirus and an adjoining host gene, splicing and packaging of the joint transcript, and illegitimate recombination of the transducing fragment with a nontransducing virus chromosome (66). In contrast to λ , many different host genes have been found in natural retrovirus isolates, presumably because the insertion specificity of retroviruses is lower than that of λ .

Many viruses package segments of the host chromosome that are not joined to viral DNA, and the resulting particles are responsible for generalized transduction by these viruses (35) (see chapter 130). The absence of these particles in stocks of λ was undoubtedly important in allowing recognition of specialized transduction as a new phenomenon, and it reflects the λ requirement for a specific DNA site for both initiation and termination of packaging. Generalized transducers, such as P22 and P1, prefer to initiate packaging at a specific site analogous to λ *cos*, but unlike λ , they do not have a sequence requirement for terminating packaging and cleaving away unpackaged DNA (reviewed in references 65 and 75). Thus, P22 and P1 package host chromosome segments into infectious particles, albeit with low efficiency. Lambda does not do this, in part because both the initiation and the termination steps of packaging are inefficient when *cos* is absent. Nevertheless, incomplete generalized transducing particles are present in λ lysates, and they are revealed when the lysate is treated with DNase in the presence of λ capsid proteins. Such treatment digests protruding DNA and allows completion of the particles by addition of essential proteins as described above for λ *docL*. The decrypted particles transduce many different host markers regardless of their linkage to *attB* (62, 64).

APPLICATIONS

Although specialized transducing phage are useful tools for studying host gene structure and function, the restricted set of genes that is naturally transduced by λ originally limited the range of applications. This limitation was largely eliminated by the development of highly successful genetic methods for positioning genes of interest near a λ prophage, and several of these methods and the many transducing phage lines they were used to isolate are cited in the earlier edition of this volume (see Table 1 of reference 70). These methods have been supplemented by newer techniques that combine the packaging power of phages λ and P22 with the low insertion specificity of phage Mu (26, 59). However, in many cases, the preferred method for isolating a desired transducing phage line is one of the efficient and user-friendly *in vitro* procedures for splicing DNA fragments into phage chromosomes and packaging the resulting products into virus particles (see reference 49 for a list of commonly used vectors). Such methods have allowed the isolation of specialized transducing derivatives of phages that do not insert their DNA into the host chromosome as part of their normal life cycle and of phages that transduce genes of organisms that are not their natural hosts.

One very useful application of this technology has been the construction of an ordered set of λ transducing phages that covers the entire *E. coli* chromosome (38) (see chapter 109). This set allows rapid mapping of clones of unknown *E. coli* genes to a precision of about 20 kb by hybridization (50). Although functional analysis of the genes carried by these phages can be difficult because of their insertion-defective and partially virulent character, a method that facilitates isolation of viable transductants has been developed (29). Another useful application that combines old and new transducing phage technology has greatly facilitated analysis of the control of bacterial gene expression. The expression of many genes depends on the relative concentration of *cis*- and *trans*-acting regulatory elements. Transfer of a gene encoding a regulatory element from a multicopy plasmid to a transducing prophage reduces the

concentration of the element and allows exploration of the relationship between relative concentration and gene expression. Such transfer has been simplified by the development of plasmids and phages in which the sequence of interest is flanked by regions of homology containing selectable markers. This arrangement allows rapid and selective transfer of sequences that lie between the regions of homology by genetic recombination (58).

EVOLUTION

Genetic transduction enlarges the gene pool available for recombinational exchange and increases the probability of such exchange for both virus and host. Transduction probably confers a selective advantage on both organisms, and if it does, both virus and host are likely to have evolved in ways that conserve and maximize this advantage. What might these advantages be in the case of specialized transduction? Lysogens of specialized transducing phages have two copies of a short segment of the bacterial chromosome, and this facilitates the generation of adaptive variants of one of the duplicated genes. However, it is not clear whether duplications arising by this mechanism would contribute significantly to the natural level of chromosomal duplications, which is already considerable (1). Possible advantages for the phage are easier to imagine. The *attP* sites of several temperate phages overlap perfect or nearly perfect duplications of segments of the host chromosome (12, 13). The host copy of the duplication contains the cognate *attB* site (28, 61a). Several of the duplicated segments are portions of tRNA genes, which serve as preferred insertion sites for many accessory genetic elements besides phages (27, 51, 53). However, protein-coding genes also contain *attB* sites, and segments of these can be duplicated in the cognate *attP* (12). For both the tRNA and the protein-coding genes, duplication prevents loss of gene function after prophage insertion within the gene and thus avoids impairment of the lysogen. It is probable that the bacterial DNA was originally translocated to the phage chromosome by abnormal prophage excision (Fig. 1). Campbell and his collaborators (12) have suggested that temperate phages evolve new insertion specificities in this way. First, abnormal excision places a new sequence adjacent to the original *attP*. Then, the phage integrase alters to recognize the new host sequence. The ability to change *att* sites could be advantageous for a phage parasitizing a bacterial population whose members already contain a prophage at the original site. Indeed, a common view of the origin of phages is that they arose by accretion and adaptation of functional modules originally of host origin (11, 14). If this is so, primordial λ was born transducing.

DEDICATION

This article is dedicated to the memory of Dr. Nat Sternberg, my coworker and friend. His presence and his contribution to this field will be sorely missed.

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