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MINIREVIEW

Chromosomal Insertion Sites for Phages and Plasmids

ALLAN M. CAMPBELL

Department of Biological Sciences, Stanford University, Stanford, California 94305

INTRODUCTION

Bacteriophages insert their DNA into host chromosomes either through transposition (as in phage Mu) or through site-specific recombination (as in phage λ). Whereas Mu can insert almost anywhere along the chromosome, λ has a single highly preferred chromosomal site. Certain plasmids also insert into chromosomes by site-specific recombination. The site-specific recombinases used generally belong to the integrase family, whose members show some sequence homology and conservation of reaction mechanism, indicating descent from a common ancestor (2, 8, 12, 19).

This minireview examines some chromosomal sites with known nucleotide sequences. The purpose is not to prepare an exhaustive catalog but to look for common trends.

The focus is on chromosomal (*att*B) sites, not on the phage (*att*P) sites. Nevertheless, interpretation requires comparisons between the two, as well as some discussion of reaction mechanisms. The prototype is coliphage λ (47).

λ insertion requires a minimal *att*B segment, 21 bp in length, and a 234-bp *att*P sequence. For optimal activity, the *att*P partner must be supercoiled. The λ *att*B sequence

CTGCTT**ttTtatAct**AACTTG
GACGAA**aaAataTga**TTGAAC

is identical to *att*P in the underlined 15 bp (identity segment). The integration reaction requires a phage-coded protein, integrase (Int), and a bacterial protein, integration host factor. The excision reaction (in which the prophage comes out of the chromosome) requires in addition the phage-coded excisionase (Xis) and host protein Fis (4). In the reaction, several integrase molecules bind to *att*P, and the complex then binds to *att*B. The C-terminal domains of integrase molecules recognize certain base pairs in *att*B and *att*P (shown in capital letters) that approximate a consensus sequence (Table 1) and cause strand exchange between the two upper strands, branch migration 7 bp to the right, and strand exchange between the two lower strands, in that order. The nucleotides shown in boldface make up the overlap segment in which branch migration takes place. The sequences recognized by Int are called core sites. *att*B is denoted BOB′ and the *att*P core is COC′, where B, B′, C, and C′ are core sites and O is the intervening overlap segment DNA.

Integrase binding to B and B′ is too weak to observe easily on *att*B DNA. The N-terminal domain of Int binds strongly to arm sites in *att*P which are located some distance from the reaction site, at positions −140 and −110 to the left and +60, +70, and +80 to the right. Integration host factor, Xis, and Fis are DNA-bending proteins that facilitate positioning of Int molecules so that the C terminus is near a core site once its N terminus binds to an arm site. Base changes in specific arm and core sites can weaken the interaction and prevent the reaction (24). Mutations within the overlap segment of nucleotides not needed for core binding interfere with branch migration if introduced into one of the two partners but allow normal reactivity if included in both partners (47).

The four core sites, B, B′, C, and C′, are equivalent and interchangeable by most tests, although some quantitative effects of substitutions (dependent on the recombining partner) have been seen (18, 35). Both the difference between insertion and excision and the direction of branch migration are attributable to the disposition of arm sites on *att*P, not to sequence differences among the core sites (16).

SECONDARY SITES

Whereas λ has one highly preferred *att*B site in *Escherichia coli* K-12 (its host of origin), it can occasionally insert at other sites. Insertion at secondary sites is observable in strains from which the preferred site is deleted (41). Secondary sites resemble the preferred site in the overlap segment, the core sites, or both (47). In one cycle of insertion-excision, the overlap segment of *att*P can be replaced with that from a secondary site. Compared with wild-type λ, such excisants ("site affinity" or *saf* mutants) insert better at the secondary site (although still more poorly than λ at *att*B) and more poorly at the preferred site, because branch migration is impeded by mismatches (34). Secondary-site insertions and *saf* mutants corroborate molecular studies in defining the position and extent of the overlap segment.

*att*B SEQUENCES AND CROSSOVER POINTS

Examination of the chromosomal sequences flanking λ *att*B indicate that it lies in intergenic DNA (26, 37). Many other elements insert into actual or potential protein-coding genes or tRNA genes. In these cases, the phage *att*P DNA duplicates the sequence information in the 3′ end of the host gene, either precisely (tRNA genes) or approximately (protein-coding genes), so that, after insertion, the cell continues to make a functional gene product in which the information 3′ of the insertion point comes from the element and the host-derived 3′ end becomes an inactive pseudogene distal to the inserted prophage.

In λ, the 15-bp identity segment lies within the 21-bp *att*B sequence, so that the core sites extend beyond the identity segment on both sides. B, B′, C, and C′ each have slightly different sequences, from which a consensus has been inferred (Table 1). Other phages, especially those inserting into genes, can have much longer segments of identity or strong similarity. In these cases, there is room for the entire core within the identity segment. In fact, in those few cases for which information is available, the core extends beyond the 5′ end of the identity segment, so that B′ and C′ are identical but B and C differ from each other. Since the

TABLE 1. Sequences of *att*B sites and *att*P cores

Element (reference)	Suggested consensus[a]	*att*B[b]	*att*P[b]
λ (26)	CAACTTNNT(3)ANNAAGTTG	CtgCTTttTtatActAAcTTG	CAgCTTttTtatActAAGTTG
HK022 (49)	TNANCCTTT(7)AAAGGNTNA	gtgcaCTTTaggtgaaAAAGGtTgA	TcAtCCTTTaggtgaatAAGttgtA
21 (40)	ATATGNNGC(1)GCNNCATAT	AgATGatGCtGCgcCATAT	ATATactGCtGCgcCATAT
e14 (10)	Same as for 21	AgATGatGCtGCgcCATAT	AacTaatGCtGCgcCAcAT
P22 (30, 34a)	CTTCGCATT(3)AATGCGAAG	CagCGCATTcgtAATGcGAAG	CTTataATTcgtAATGCGAAG
Atlas[c] (42)	AANNTACCA(3)TGGTANNTT	AAcaTgtCAgtgTGGTAcaTg	AAacTACCAgtgTGGTAcaTg
ϕ80 (30)	AACANTTTN(1)NAAANTGTT	AACAcTTTcttAAAtgGTT	AAcAcTTTcttAAAtTGTc
P2 (5)	AAATNNNCC(7)GGNNNATTT	AAATaagCCcgtgtaaGGgagATTa	AAATaagCCcgtgtaaGGgagATTT
HP1 (19)	AAGGGATTT(1)AAATCCCTT	AAGGGATTTaAAATCCCTc	AtGGtATTTaAAATCCCTc
pSE101 (11)	ACCGNCCGC(7)GCGGNCGGT	ACCGcCCGCcttgtaaGCGGaCGGT	ACCGgagatcttgtaaGCGGaCGGT
pMEA100[c] (32)	ACCNTCCGC(7)GCGGANGGT	AgCgTCCGCctgaaaaGCGGAaGGT	ACCcaagaCctgaaaaGCGGAaGGT

[a] N, unspecified. The number of unspecified central bases is given in parentheses.

[b] Bases agreeing with the consensus are capitalized. Lowercase letters are used for bases that either disagree with the consensus or lie at unspecified positions. Identity segments are underlined.

[c] There is no direct evidence localizing the crossover points for Atlas or pMEA100.

transition from identity to heterology is generally abrupt, this creates a problem if B and C are to approximate the same consensus. Either the consensus bases must be preferentially conserved or B must agree with the consensus at some positions and C must agree in others. For this reason, it seemed worthwhile to go through the exercise of identifying candidate sequences even for some elements for which direct evidence is lacking.

Table 1 identifies such candidate sequences. Except for the Atlas phages and pMEA100, these are all elements for which the crossover point has been located to within a few nucleotides. The best 9-bp symmetries that maximized the fit to both *att*P and *att*B core sites were selected, allowing separation by 1 to 7 bp and requiring that the center of symmetry lie within the identity segment.

If these criteria seem biased toward forcing a λ-type model onto other systems, they are indeed. An unbiased assessment of each system for consensus bases or conspicuous repeats has some merit, but it risks focusing on coincidences irrelevant to the process of interest. Not only is the λ model well demonstrated, but the criteria are mechanism based. The symmetry of the λ *att*B core is not highly conspicuous. Many other DNA sequences, such as terminators or tRNA genes, have larger and more perfect reverse repeats. The importance of the symmetrical distribution of λ *att*B core sites about the center of the overlap segment is that it allows each integrase molecule to relate to its cutting site in the same manner. With other systems, significance has sometimes been attached to presumed crossover sites flanked by reverse repeats that were not centered in this manner, their possible importance resting on unstated assumptions as to mechanism. The choice of a 9-bp segment is arbitrary, based only on the fact that λ core recognition sites span that length. There seems to be no strong reason to assume that different integrases should recognize the same positions within *att*B; hence the separation between the core sites is allowed to vary between 1 and 7 bp.

Among the sequences shown in Table 1, the best candidate core sites always turned out to be separated by an odd number of nucleotides and the center of symmetry lay at least 4 bp within the identity segment, as expected for a 7-bp overlap segment. Some members of the integrase family (not those known to be used in phage insertion) have overlap segments of 6 bp (Cre of phage P1) (21) or 8 bp (FLP of yeast 2 μm plasmid) (1). In those systems, only core recognition is required; recombination takes place between *att*B sites without need of the arm sites present in *att*P. Even λ can tolerate a 6- or 8-bp overlap, although the efficiency is markedly decreased (17, 47). Nevertheless, it is worth noting how many facts about the elements in Table 1 fit the hypothesis that they all use a 7-bp overlap.

The first seven phages in Table 1 are considered lambdoid phages because (except for the defective element e14) they share a common genetic map with λ and (except for Atlas, for which the point is untested) produce viable hybrids with it. Phage HK022 integrase resembles λ integrase in the N terminus and probably recognizes the same arm sites. Different recognition of core sequences is inferred from the fact that HK022 integrase shows only weak activity on most combinations of λ sites. Systematic study of chimeric core sites localized the major determinants of specificity to several bases in the B′ sequence (34). The consensus sequence shown resembles that of λ in that the sequences CTT . . .AAG are the same distance from the center of symmetry. The implied overlap segment does not resemble that of λ.

Phage 21 and e14 insert within the isocitrate dehydrogenase (*icd*) gene of *E. coli*, 165 bp from the 3′ end. Both elements include an alternative 3′ end extending rightward from the crossover point. The sequence identity at the crossover point is 16 bp for phage 21 and 11 bp for e14, with 10 bp in common. The similarity extends beyond the 3′ end of the *icd* gene, ending about 20 bp downstream from its transcriptional terminator, for a total of 225 bp (10, 40). There seems to be some difference in core recognition between phage 21 and e14, because 21 will not react with an *att*B site whose B end is replaced by the C site of e14 (15), but the e14 sequence does not fit any other consensus sequence better than that shown for 21. Some substitutions of consensus bases in phage 21 *att*B destroy its ability to act as a substrate (13).

Phage P22 inserts into a threonine tRNA gene. The 46-bp identity segment extends from the anticodon loop through the 3′ end. The consensus sequence indicated in Table 1 utilizes the symmetrical anticodon stem sequences as part of the core sites. The B and C sites both approximate the consensus, but in different nucleotide positions.

The Atlas prophages were discovered in sequencing the *trp-tonB* segment of numerous *E. coli* isolates (33, 43). They are inserted within an open reading frame whose product is nonessential for host viability. As with phage 21, the lysogen includes an imprecise duplication of the 3′ end of the open reading frame; for Atlas, this is 121 bp long. Sequence

comparison of *att*B from nonlysogens with the phage-host junctions in lysogens indicates that insertion took place within a 28-bp identity segment at the 5′ end of the 121-bp duplication. As with phage 21, the best consensus sequence (shown in Table 1) extends into heterologous DNA 5′ from the crossover point.

Lambdoid phage φ80 inserts into DNA of unknown function with a 17-bp identity (29, 30). The defective lambdoid prophage DLP12 is inserted within an arginine tRNA gene (31).

P2 is a coliphage unrelated to λ. In *E. coli* C, P2 inserts preferentially within a 27-bp identity segment (*loc*I) and less frequently at secondary sites that are similar but not identical to *loc*I (5, 50). In *E. coli* K-12, *loc*I is occupied by a defective prophage, and infecting P2 inserts only at secondary sites. Secondary-site insertion in both C and K-12 place the crossover point at or near the right end of the central 7-bp segment shown in Table 1. Furthermore, in a *saf* mutant derived from one secondary site (*loc*II), the 7-bp segment has the *loc*II sequence CGTGGAA rather than CGTGTAA, indicating that at least the substituted base lies within the overlap segment, and in one of three *loc*II lysogens examined, the sequence CGTGTAA appears at both prophage ends, as though mismatch correction had occurred within the overlap segments.

On the simplest assumptions, a secondary-site lysogen is equally likely to be crossed over at either end of the overlap segment, whereas in P2 the observed exchanges were all at the right end of the segment suggested in Table 1. A tentative interpretation is that when branch migration is stalled by mismatching, resolution can occur near the end where branch migration initiates (6).

The next three elements in Table 1 (*Haemophilus* phage HP1 and actinoplasmids pSE101 and pMEA100) insert within tRNA genes. Insertion has been studied more thoroughly for HP1 than for any other phage except λ and Mu. The phage inserts into the anticodon loop of a leucine tRNA gene which is the second of three cotranscribed tRNA genes and duplicates the 3′ DNA of the operon, for a total of 182 bp. The first 62 bases are identical to host DNA and include the 3′ end of the leucine tRNA gene; this is followed by an intergenic segment with six mismatches to host DNA and 93 identical base pairs that include a lysine tRNA gene (46).

Molecular studies identified the overlap segment as TTT AAAA. From deletion analysis, the sequence AGGGATTT AAAATCCC is sufficient to give normal activity in vitro, whereas further truncation reduced or abolished it (19). The consensus sequence in Table 1 extends 1 to 2 bp beyond this minimal sequence. The overlap segment is identical to the anticodon loop, so that the stem-determining DNA sequences are part of the B and B′ core sites.

In *Saccharopolyspora erythraea*, plasmid pSE101 inserts into a 46-bp identity segment extending 3′ from the anticodon loop of a threonine tRNA gene (11). In *Streptomyces lividans*, pSE101 inserts at various secondary sites that resemble the anticodon loop segment of *S. erythraea*. Sequencing of junction fragments showed that crossing over had occurred at or near the left end of the 7-bp central segment of the *att*B sequence proposed in Table 1. As with phage P2, the proposed *att*B sequence implies that the lysogens recovered from insertion at mismatched sites result from abnormal resolution.

In several other actinoplasmids, insertion occurs in tRNA genes with identity segments extending from the anticodon loop through the 3′ end of the gene. These include pMEA100 (32) and several other plasmids whose sequences are not shown in Table 1: SLP1 (28, 36), pSE211 (12), pIJ408 (42), and pSAM2 (7, 25). In none of these cases has the crossover point been localized precisely. However, use of insertion mutation (SLP1) or comparison of species with some downstream heterology (pSAM2) restricts the crossover site to the 5′ portion of the identity segment.

Reiter et al. (39) point out that the identity segments of many elements inserted in tRNA genes, including that of their archaebacterial virus-like element SSVI, extend from the anticodon loop through the 3′ end. In all of these cases a candidate core site centered on the anticodon loop can be constructed. In satellite phage P4 (38) and retronphage φR73 (44) the identity segment is shorter, extending from the TψC loop through the 3′ end. In both cases the center of the loop is 5′ of the identity segment, precluding the use of its stems as part of a candidate core site.

DISTRIBUTION AND ORIENTATION OF *att*B SITES FOR LAMBDOID PROPHAGES

Jacob and Wollman (22) reported that among 13 independently isolated coliphages whose *att*B sites had been mapped, seven were UV inducible with sites located in one segment of the bacterial chromosome whereas six others were noninducible and located elsewhere. Subsequent work showed that their inducible phages were lambdoid phages and located other lambdoid prophages in the same segment. The lambdoid phage *att*B sites that appear on the K-12 map (3), including the loci of the defective lambdoid prophages resident in K-12, all lie between 6 and 44 min. The clustering is significant at the 99% confidence level (equation 3 of reference 23). Sampling bias is an unlikely explanation, considering the diversity of sources (14).

At nine of these sites, the prophage orientation can be inferred from genetic or molecular data. Remarkably, eight of the nine sites are oriented in the same direction, and all nine have the same orientation with respect to the usual direction of replication fork movement (14).

EVOLUTIONARY IMPLICATIONS

The limited available data allow four tentative generalizations. (i) When an *att*B site lies within a host gene, the 3′ end of the gene is duplicated in the phages. (ii) The crossover site is at the 5′ end of the identity segment, so that the core sites recognized by integrase extend into heterologous 5′ DNA. (iii) The core sites of the various elements are highly diverse. (iv) The *att*B sites for lambdoid phages are clustered on the *E. coli* genome and oriented nonrandomly.

As with all genetic structures, the properties of *att* sites reflect their evolutionary origins and the operation of natural selection. Thus, the explanations for each of the above generalizations may be sought either in historical origin or in current function.

3′ duplications. The need for a duplication is obvious if the target gene is unique and essential. Proof that a tRNA target gene is essential has been provided for SLP1 (45). A functional explanation may be sought for why the duplicated end is 3′ rather than 5′. This does avoid the necessity of duplicating the 5′ regulatory signals; however, 3′ DNA may contain downstream genes (as in HP1) or signals for processing or termination (as in phage 21 and perhaps SLP1). One advantage of a 3′ duplication is that a 5′ duplication would create an expressed pseudogene, which could be deleterious.

A long duplication can create a problem for the element if

it extends beyond the normal location of the arm sites in *att*P. Either the element must use sequences within the duplication as arm sites (which, for a precise duplication, destroys the ability to distinguish insertion from excision and regulate the two processes differentially in the manner that λ does) or the arm sites must lie beyond the duplication, extending the dimensions of *att*P beyond those of λ. The latter strategy has been suggested for phage 21 on the basis of nucleotide sequence comparisons (40) and has been demonstrated by functional assay for HP1 (20).

Crossover at the 5′ end. When phages carry extended sequences homologous to the 3′ ends of genes, the phage-borne sequences must have been derived in some manner from the host. Two simple scenarios can be invoked: (i) abnormal excision from an ancestral phage inserted 3′ to the present target gene, with subsequent evolution of integrase to recognize a sequence within the bacterial portion (14), or (ii) insertion at the current site, followed by abnormal excision. The latter possibility requires that the host of origin bore a preexisting duplication of the target gene, the abnormal excision being selected because it allowed lysogenization of other host strains in which the gene was unique; it has the advantage that it explains the location of the crossover point at the 5′ end of the duplication without additional steps. It also fits the notion that the frequent use of tRNA genes as insertion sites relates to the suitability of anticodon stem sequences as elements of core sites (19).

One consequence of the location of the crossover point is that the B and C sequences differ from each other. It is intriguing to speculate whether some use is made of this difference.

Variation of core sequences. The diversity of core sites (Table 1) is noteworthy considering that they all are recognized by integrase proteins with related sequences and presumed common ancestry. Only with λ and HK022 do we see specificities that are related but slightly different. This probably reflects the ease with which DNA-binding proteins, such as λ repressor (48), can change specificity.

Location of *att*B sites. A possible historical explanation for the clustering of *att*B sites would be that the *E. coli* chromosome has a chimeric origin, with one segment derived from an ancestral host of lambdoid phages (14).

Orientation of lambdoid phages. Historical explanations for the nonrandomness of prophage orientation can be contrived, but a functional explanation relating either to the insertion process or to the survival or stability of lysogens to replication fork movement or to direction of transcription may prove more fruitful (14). Some *att*B sites (such as that of λ) are probably not transcribed. However, there is a global correlation between directions of transcription and replication (9), and in the four cases in which lambdoid phages insert within known genes (DLP12, P22, 21, and Atlas), the prophage orientation with respect to *att*B transcription is the same.

Primordial *att*B sites. The wide distribution of elements inserting into tRNA genes prompted the suggestion that these were the sites used by ancestral phages (39). It is amusing to note that the 7-bp overlap seen in λ equals the length of the anticodon loop and that in phage HP1 the two coincide (19).

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