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Secondary Lambda Attachment Site in the Threonine Operon Attenuator of *Escherichia coli*

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We have determined the nucleotide sequence of a secondary λ attachment (*att*) site which overlaps the *Escherichia coli* threonine (*thr*) operon attenuator. The secondary *att* site shows uninterrupted homology (8 out of 15) with the 15 base-pair "common core" sequence found in λ and at the primary bacterial attachment site. These 8 base pairs also overlap the *thr* operon attenuator. Comparison of the secondary *att* site with the flanking prophage sites shows that the crossover site for λ integration lies within the −7 to 0 region of the core. Sequences on both sides of the core show no obvious homology with analogous sequences of the λ or primary bacterial *att* sites. The core sequence of the left prophage *att* site is completely homologous to the wild-type core and also shows the same 8-base pair overlap with the *thr* operon attenuator. The position of the *thr* operon attenuator, relative to the left prophage *att* site, indicates that ribonucleic acid transcripts, initiated at a λ promoter, are terminated efficiently at the *thr* attenuator. It is also possible that this prophage *att* site is able to undergo *int* dependent site-specific recombination with another nearby secondary *att* site. Evidence is also presented which suggests that a base or sequence to the left of position −6 in the core is necessary for excisive recombination.

The integration and excision reactions of bacteriophage λ represent one of the most thoroughly characterized examples of site-specific recombination. Both genetic (16, 37) and biochemical (20, 23, 24, 26, 31, 32) analyses have yielded information concerning this specialized recombination process. The current model for λ integration proposes that a single reciprocal genetic exchange occurs involving unique sites (*att* sites) in both bacteriophage and bacterial DNA (4). These sites are designated POP′ (*att*P) and BOB′ (*att*B), respectively. The resulting recombinant prophage attachment sites, which flank the λ insertion, are designated BOP′ (*att*L) and POB′ (*att*R). During the excision process, the reverse reaction proceeds, utilizing the two prophage sites as substrates. The net result of excision is the reformation of *att*BOB′ and *att*POP′ and the construction of autonomous λ and bacterial chromosomes. The integration process requires the presence of a phage gene product, *int* (12, 14, 40) in addition to at least two host specific proteins (30, 39). Excision is catalyzed by the *int* protein and host factors, but also requires *xis* (15, 22), another phage specific protein.

The four attachment sites demonstrate distinctive behavior when compared in recombination studies (13, 31). Each site appears to be functionally unique in respect to exchange frequencies with other attachment sites, yet analyses of mutant attachment sites have long suggested that homologous stretches of DNA are part of these loci (37, 38). Direct DNA sequencing analysis of the four sites resolved this apparent contradiction. Landy and Ross (26) demonstrated that each attachment site contains a 15-base pair (bp) "common core" sequence and that the four flanking sequences are nonhomologous. It is these differences in the structures of P, P′, B, and B′ which appear to account for the uniqueness of each site. Since the flanking sequences are different and the core sequences are identical, the integration reaction between POP′ and BOB′ must take place within or immediately adjacent to the core sequence (26).

When the *gal-att*BOB′-*bio* region of the *Escherichia coli* chromosome is deleted, the integration of λ is reduced significantly (2, 34). As demonstrated by Shimada, Weisberg, and Gottesman (34), the residual integration is the result of recombination at specific secondary *att* sites (designated ΔOΔ′). Several of these sites lie in structural genes and are utilized at different frequencies. Integration at secondary *att* sites utilizes the phage *att*POP′ site and requires *int* protein. Similarly, excision from these sites requires *int* and *xis* proteins and usually restores

the function of the interrupted gene (34-36). All secondary *att* sites studied to date show partial homology to the wild-type core, whereas the Δ and Δ′ sequences appear to show little or no homology with P, P′, B, and B′ (3, 5, 6, 19). Thus, it is thought that the core sequences determine the efficiencies of the integration and excision reactions with secondary *att* sites. Since secondary *att* sites represent variants of BOB′, comparison of primary and secondary *att* sequences should provide information on the functional roles played by the core and flanking sequences in site-specific recombination.

During the course of our studies on the genetic regulation of the threonine (*thr*) operon, we isolated a secondary-site prophage which mapped within or near the *thr* operon controlling elements (10, 11). Starting with this lysogen, it was possible to isolate plaque-forming transducing phages which carried ΔOP′ and the *thr* structural genes (λ p*thr* as shown in Fig. 1) or POΔ′ and bacterial DNA to the right of the secondary *att* site (λ *spi*, in Fig. 1). By *int-xis*-mediated site-specific recombination, it was also possible to construct recombinant λ p*thr spi* transducing phages which carried the intact ΔOΔ′ site (9). We have utilized these transducing phages to determine the DNA sequences of ΔOΔ′, the flanking prophage ΔOP′ and POΔ′ sites, and another possible secondary *att* site which lies near the original ΔOΔ′ site.

MATERIALS AND METHODS

Phage and bacterial strains. The isolation and characterization of λ p*thr* and λ *spi*, which carry ΔOP′ and POΔ′ respectively, has been described previously (9). λ p*thr spi*, containing ΔOΔ′, was constructed by *int-xis*-mediated site-specific recombination between λ p*thr* and λ *spi* (9). pJG39 is a pVH51 (17) derivative carrying the ΔOΔ′ region of λ p*thr spi* (J. F. Gardner, S. L. Lynn, and W. S. Reznikoff, manuscript in preparation) and was used as a source of DNA for sequence analysis. del-104 was isolated as a Thr^+, lysogenic revertant of L79 which does not undergo *int*-dependent heat pulse curing ($<10^{-5}$ as opposed to $\sim 10^{-3}$ for L79 [10, 34]). λ p*thr* del-104, which carries the ΔOP′ sequence of this lysogen, was isolated as described previously (9). All bacteriophage were grown on strain MO.

Restriction enzymes and gel electrophoresis. *Rsa*I and *Taq*I were isolated by published procedures (28, 33). *Bam*HI was a gift from R. Jorgensen, and *Hha*I was purchased from New England Bio-Labs. Conditions for analytical and preparative agarose and acrylamide gel electrophoresis were as described previously (9).

DNA Sequencing. Labeling of 5′ ends was carried out as described by Maxam and Gilbert (29) except that [γ-^{32}P]ATP was synthesized by the method of Johnson and Walseth (21). Alkaline phosphatase and T4 polynucleotide kinase were gifts from R. Gumport and O. Uhlenbeck.

DNA fragments labeled at a single 5′ end were generated by digestion of labeled fragments with a second restriction enzyme followed by purification on 5% (wt/vol) polyacrylamide gels (29). DNA sequences were then determined by the chemical modification and cleavage method of Maxam and Gilbert (29).

RESULTS

Restriction mapping of *att* sites. The isolation and characterization of λ transducing phages carrying the *thr* operon secondary *att* sites have been described previously (9). Restriction fragments carrying the secondary *att* sites were identified by comparing restriction digests of λ, λ p*thr*, λ p*thr spi*, and λ *spi*. When the four samples of DNA are digested by a single restriction endonuclease or a combination of restriction endonucleases and subjected to acrylamide or agarose electrophoresis, each digest yields a unique fragment, which carries the *att* site of that phage. Restriction endonuclease cleavage sites in the regions near the four *att* sites are shown in Fig. 2. Cleavage sites in the P and P′ arms have been mapped by Landy and Ross (26), and sites in the Δ and Δ′ arms were mapped previously (9) or determined from DNA sequencing of ΔOΔ′ (8).

ΔOΔ′ has been mapped within a 160-bp *Hha*I-*Taq*I fragment from λ p*thr spi* (9). This fragment also carries the *thr* operon attenuator, and the entire region has been sequenced (8). However, the exact position of ΔOΔ′ within this fragment could not be determined unambiguously, since the sequences of ΔOP′ and POΔ′ were not known. Although it was not possible to establish a unique site for ΔOΔ′, the *thr* attenuator (from positions 0 to −33; see Figure 4) (8) contains the 8-bp sequence 5′-GCTTTTTT-3′ which shows perfect homology with positions −7 to 0 of the wild-type core sequence. (In the commonly used numbering system (26), the sequences are numbered with 0 as the central position of the 15-bp core with positive numbers extending rightward and negative numbers extending leftward). Thus, it was suspected that this sequence represented the core of ΔOΔ′.

POΔ′ of λ *spi* has been mapped within a 740-bp *Hind*III fragment (9) and ΔOP′ from λ p*thr* has been mapped within a unique 2,000-bp *Rsa*I fragment by comparison of *Rsa*I digests of λ, λ p*thr*, λ p*thr spi*, and λ *spi* (data not shown). Direct DNA sequencing of ΔOΔ′ from λ p*thr spi* (8) has shown that a *Rsa*I site maps approximately 10 bp to the right of the suspected core in the Δ′ arm of λ p*thr spi*. This site also appears in the Δ′ arm of the λ *spi Hind*III fragment and was utilized for sequence determination of POΔ′. Previous DNA sequencing of ΔOΔ′ also showed that a *Rsa*I site lies in the amino terminus of

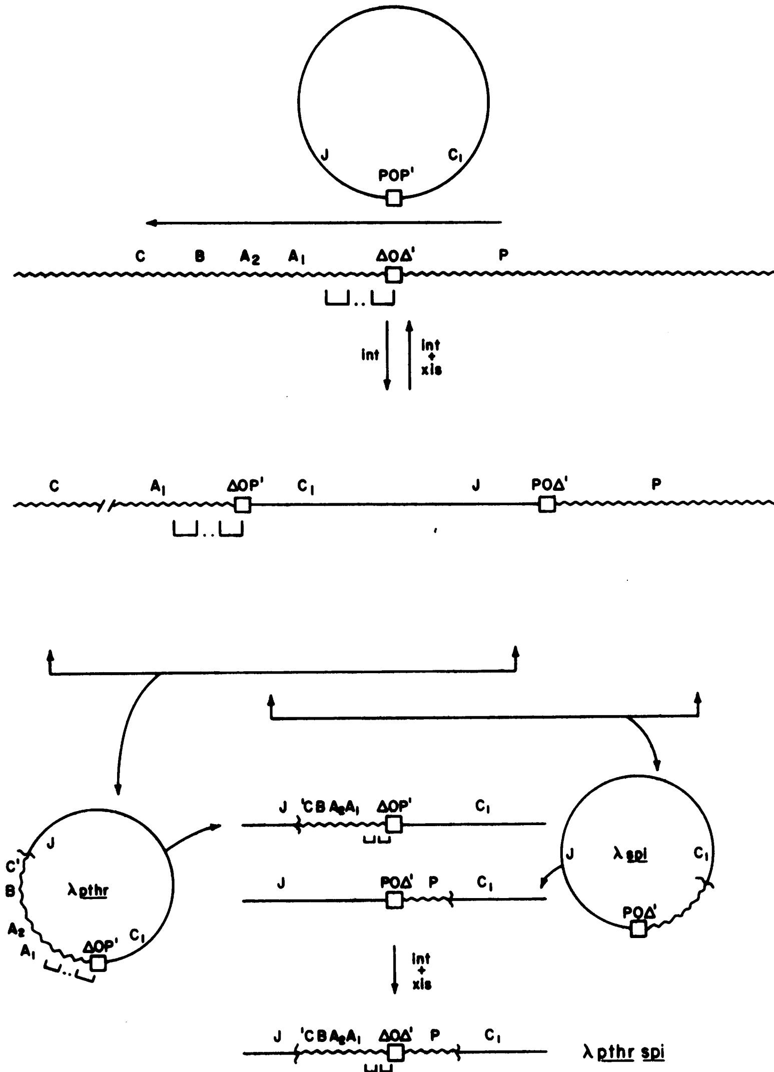

FIG. 1. *Origin of transducing phages carrying ΔOP', POΔ', and ΔOΔ'. The thr operon, composed of the promoter (P), and the structural genes (A1, A2, B, and C) and the secondary att site (ΔOΔ') are indicated. The arrow indicates the direction of transcription. The location of ΔOΔ' is indicated between thrP and the structural genes (9) and overlaps the rotational symmetry (⌊——⌋..⌊——⌋) of the thr attenuator (see text). Int-dependent integration of λ into ΔOΔ' generates the prophage secondary att sites ΔOP' and POΔ' and renders the cell phenotypically Thr^-. The sequence of the thr attenuator at the ΔOP' bacterial-prophage DNA junction is unchanged and overlaps the core of ΔOP' by 8 bp (see text). The transducing phages carrying the prophage att sites arise by incorrect excisions between phage and bacterial DNA (↑__↑) to generate λ pthr (ΔOP') and spi (POΔ') transducing phages. λ pthr spi, carrying the intact ΔOΔ' site, was generated by int-xis-mediated site-specific recombination between λ pthr and λ spi. The construction of these phages has been described previously (9).*

the *thrA1* structural gene (8) approximately 75 bp from the suspected core region of ΔOΔ'. This site also appears in the Δ' arm of λ p*thr* and defines one end of the *Rsa*I-2,000 bp fragment. In addition, the *Rsa*I-2,000 bp fragment also contains the *Bam*HI site mapped by Landy and Ross (26) at position +230 in the P' arm of λ. Both the *Rsa*I and *Bam*HI sites were used for determining the ΔOP' sequence.

DNA sequence analyses. The restriction endonuclease sites used for DNA sequence analyses and the extent of the sequences obtained

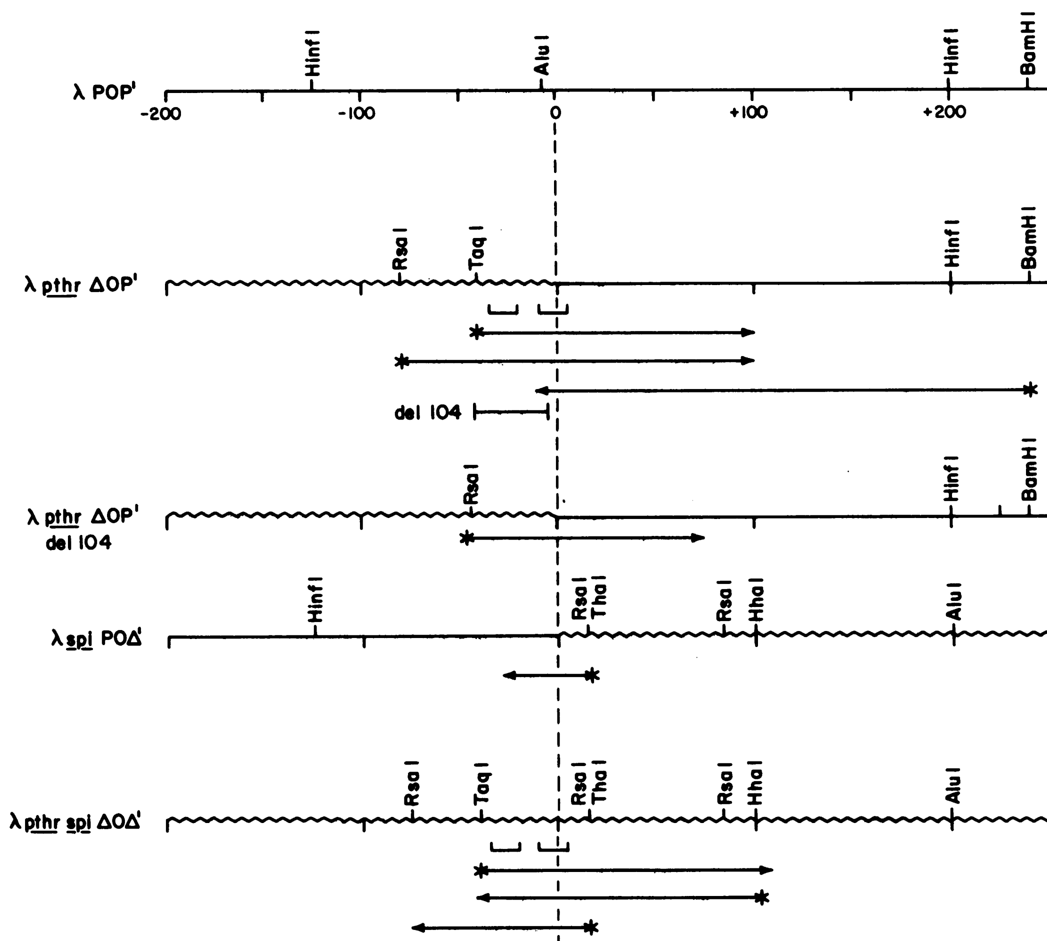

FIG. 2. *Restriction map and DNA sequencing of secondary att sites. Map distances are given in bp from the center of the core (0) with positive numbers extending to the right and negative numbers to the left. The restriction sites in the P and P′ arms have been mapped by Landy and Ross (26), and the sites in the Δ and Δ′ arms were mapped previously (9) or obtained from DNA sequencing of ΔOΔ′ (8).* ⌴··⌴, *Rotational symmetry of the thr attenuator. The deletion in del-104* (⊢—⊣), *which deletes most of the thr attenuator, is missing the TaqI site at position −36 in the Δ arm of ΔOP′. Regions sequenced are indicated below each map. Symbols:* *, *5′-^{32}P-labeled end;* →, *direction and extent of sequence;* ——, *phage DNA;* ∿, *bacterial DNA.*

for ΔOP′, POΔ′, and ΔOΔ′ are shown in Fig. 2. Figure 3 shows the sequences of the secondary phage *att* sites, BOB′, and POP′. The junctions of bacterial and phage DNA, which define ΔOP′ and POΔ′, were determined by comparison of the P′ or P sequences of these phages with those of λ. The sequences obtained for the P arm of λ *spi* and the P′ arm of λ p*thr* agree completely with those found by Landy and Ross (26) from positions −50 to +200.

Bidwell and Landy (3) and Christie and Platt (5) have shown that the ΔOP′ and POΔ′ sequences generated by λ integration at the secondary *galT* and *trpC* sites show sequence homology with the wild-type core region found in λ and at the primary bacterial attachment site. The sequence at the junction of phage and bacterial DNA in λ p*thr* (ΔOP′; Fig. 3) is identical to the wild-type core sequence while the sequence at the phage bacterial junction in λ *spi* (POΔ′) shows an uninterrupted stretch of 8 bp which are homologous to the equivalent bases in the wild-type (and ΔOP′) core from positions −7 to 0.

A total of 200 bp of sequence in the region of the *thr* secondary *att* site has been obtained. Figure 4 shows the sequence from positions −50 to +70. The core region of the secondary attachment site was determined by matching the Δ and Δ′ sequences of ΔOΔ′ with those of ΔOP′ and

	-20 … -10 … 0 … +10 … +20
λ POP′	CGTTTCTCGTTCAGCTTTTTTATACTAAGTTGGCATTATAA
λ pthr ΔOP′	CTGTCAGGTGCGGGCTTTTTTATACTAAGTTGGCATTATAA
λ spi POΔ′	CGTTTCTCGTTCAGCTTTTTTCTGTGTTTCCTGTACGCGTC
λ pthr spi ΔOΔ′	CTGTCAGGTGCGGGCTTTTTTCTGTGTTTCCTGTACGCGTC
del 104 ΔOΔ′	TGTTACCTCGTTACCTTTGGTCGAAAAAAAAAGCCCGCACT
gal-bio BOB′	TCCGTTGAAGCCTGCTTTTTTATACTAACTTGAGCGAAACG

FIG. 3. *DNA sequences within and adjacent to the core regions of POP′, BOB′, and the thr secondary att sites. The 15-bp core sequences are indicated in boldface type. *, Bases not homologous to the wild-type core. The core sequence of λ pthr (ΔOP′) is completely wild type, and the λspi (POΔ′) and λpthr spi (ΔOΔ′) cores are wild type from positions −7 to 0. The region of overlap between the cores of ΔOP′ and ΔOΔ′ and the thr attenuator are from positions −7 to 0. The proposed secondary att site used in the generation of del-104 is indicated.*

-50 -40 -30 -20 -10 0 +10 +20 +30 +40 +50 +60 +70

CGTTACCTTTGGTCGAAAAAAAAAGCCCGCACTGTCAGGTGCGGGCTTTTTTCTGTGTTTCCTGTACGCGTCAGCCCGCACCGTTACCTGTGGTAATGGTGATGGTGGTGGTAATGGTGGTG

attenuator

FIG. 4. *DNA sequence of the thr secondary att site. Position 0 is the center of the 15-bp core (indicated in boldface type). Inverted repeats (→····←) and direct repeats (⊢—⊣··⊢—⊣) are indicated below the sequence. The criteria for determining these structural features were a minimum of 6 bp with no mismatches or a single-base mismatch with 3 or more bp on each side. The inverted repeat of the thr attenuator is within positions −33 to 0 and is also found intact in the equivalent region of ΔOP′ of λ pthr. The thr promoter is to the right of the sequence (at approximately position +130; Wynsma and Gardner, unpublished data) and the inverted repeats from positions −14 to +68 code for part of the leader RNA which is implicated in regulation of transcription termination at the thr attenuator (8).*

POΔ′ of λ p*thr* and λ *spi*, respectively. The core sequence of ΔOΔ′ is identical to that found in POΔ′ of λ *spi* and thus shows the same uninterrupted 8-bp homology with the wild-type core sequence. Excision of λ from this secondary *att* site would also generate the same ΔOΔ′ sequence if the crossover event occurred in the region of 8-base homology from −7 to 0 in the ΔOP and POΔ′ sites. Similarly, this suggests that the integration of λ into the *thr* secondary site occurred by a crossover in the region of −7 to 0 (Fig. 3) to generate the respective ΔOP′ and POΔ′ sequences.

The mutation carried on λ p*thr* del-104 was isolated as a spontaneous, lysogenic Thr+ revertant of L79, the original *thr* secondary-site lysogen. This mutation is a deletion which originates in the core of ΔOP′ and deletes 39 nucleotides, terminating 21 bases before the initiation codon of the *thrA1* gene (Fig. 5). The core sequence of this mutant shows a G → C change at the −7 position. Since the sequences at the deletion termini show homology (CCTTT at −46 to −42 and GCTTT at positions −7 to −3 of the core; Fig. 5), the exact bases involved in the deletion event are not known. Interestingly, the deletion affects the excision properties of the prophage since the frequency of Thr^+ survivors after *int*-dependent heat pulse curing is more than 100-fold less than the frequency of Thr^+

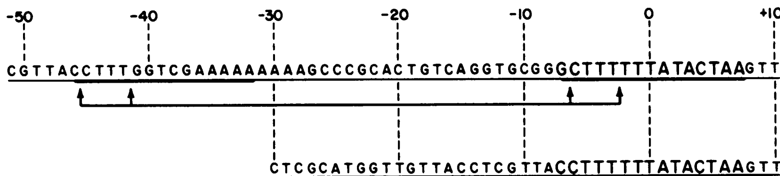

FIG. 5. *Generation of del-104. The wild-type core of ΔOP′ (underlined and boldface type) and the core of the proposed secondary att site (underlined and centered at position −40) are shown on the top line. The new ΔOP′ core sequence, formed by the del-104 deletion, is shown on the bottom (underlined and boldface type). The net change in the core sequence of del-104 is a G → C change at position −7. The homologous regions of ΔOΔ′ and ΔOP′ involved in the site-specific recombination event are indicated by the vertical arrows. The thr attenuator, which includes positions −33 to 0, is almost completely deleted in del-104.*

survivors from L79, the parent lysogen.

DISCUSSION

The integration of bacteriophage λ at secondary attachment sites is mediated by the same enzyme system involved in the primary site recombination. However, the frequency of recombination at these sites varies over a wide range (34), reflecting differences in the DNA sequences of these sites. DNA sequence analyses of secondary attachment sites in λ (19), *galT* (3), *trpC* (5), and near *rrnB* (6) and analyses in this report show varying degrees of homology with the wild-type core sequence, whereas the flanking Δ and Δ′ arms display minimal homology with each other or the B, B′, P, and P′ arms. Since secondary attachment sites show few sequence similarities, except for the partial core homologies, it has been proposed that the Δ and Δ′ arms are nonessential for *int*-dependent recombination and that the core regions themselves comprise the recognition elements of these sites (3). Recent evidence (31) has also suggested that sequences immediately adjacent to the core are also involved. Therefore, the reduced efficiency of recombination at secondary *att* sites is thought to be a consequence of the sequence differences relative to the wild-type core region. Some biochemical support for this model has been provided by recent DNA binding studies and in vitro recombination studies (20, 32).

Since secondary *att* sites represent natural variants of the wild-type *att* site, comparisons of the sequences of several secondary *att* sites may yield information on the structural features which are necessary for site-specific recombination. As pointed out by Bidwell and Landy (3), all secondary *att* sites sequenced to date (λ *b* deletions, *galT*, *trpC*, and *rrnB*) show uninterrupted homologies with the wild-type core varying from 3 to 11 nucleotides. The sites always contain at least three contiguous T residues, and a T usually appears at positions −2 and −4. In general, the most conserved region of these cores lies in the region of −7-to-0. The *thr* secondary *att* site follows this pattern since the conserved region from −7 to 0 (GCTTTTTT) is wild type in sequence. This sequence also appears in the Δ arm of ΔOΔ′, in an inverted order (from −26 to −33), as part of the *thr* attenuator (Fig. 4), and it is possible that this sequence may also serve as a secondary *att* site. In addition, there are other regions of extensive rotational symmetry in the vicinity of the core ΔOΔ′. Interestingly, the wild-type chromosomal *att* site as well as the *galT* and *trpC* secondary *att* sites also displays regions of rotational symmetry near their core regions. DNA sequencing of other secondary *att* sites should demonstrate whether regions of rotational symmetry are important structural features of secondary *att* sites.

Comparison of ΔOΔ′ with ΔOP′ and POΔ′ revealed that all three sites contain uninterrupted homology with the wild-type core sequence in the −7 to 0 region. In addition, the core sequence of ΔOP′ is wild type, and the core sequence of POΔ′ is identical to that of ΔOΔ′. This allows the placement of the crossover during λ integration within the region of −7 to 0, but the exact position of the DNA exchange site cannot be determined since both core sequences contain the uninterrupted homology from −7 to 0. An examination of the eight secondary *att* sites sequenced to date shows that it is not possible to identify a unique crossover site which is common to all *att* sites. This is compatible with models which describe integration as occurring by staggered or flush cuts in the DNA (3). Again, DNA sequencing of other secondary *att* sites should provide more information on the features of *att* sites which are important for recombination and whether the apparent conservation of the −7 to 0 region is a common feature of these *att* sites.

It is interesting to note that the −7 to 0 region of ΔOΔ′, which shows the homology with the wild-type core, is also part of the *thr* operon attenuator (Fig. 4). Furthermore, integration of λ into ΔOΔ′ does not alter the attenuator se-

quence at the junction of bacterial and phage DNA at ΔOP′. However, *thr* regulatory DNA, including the promoter and the coding sequence of the leader peptide, is separated from the attenuator by the λ insertion so that these sequences lie to the right of the POΔ′ junction of the prophage (Fig. 1). This explains several observations made previously on the properties of this lysogen (L79) and the isolation of *thr* regulatory mutations. First, the prophage in L79 was mapped outside of the *thr* operon but exerted a severe polar effect on the expression of the *thr* operon structural genes (9, 10). This indicated that the prophage site was within *thrP* or between *thrP* and the first structural gene. Second, it was possible to isolate pseudorevertants which were still lysogenic. Characterization of these mutations showed that they mapped between the prophage and the first structural gene. Most of these mutant lysogens displayed normal *int*-dependent curing frequencies (9). However, cured strains showed derepressed, constitutive synthesis of the *thr* operon enzymes in contrast to cured derivatives of L79, which always displayed regulated expression of the *thr* operon structural genes. This indicated that the mutations affected a regulatory function(s) of the *thr* operon. Direct DNA sequencing of these mutations isolated from recombinant λp*thr spi* (8) or from λ *pthr* (K. Chapman, T. Wynsma, and J. Gardner, manuscript in preparation) phages has shown that most are base substitutions or small deletions or insertions in the *thr* attenuator.

These observations can be explained as follows. The orientation of the prophage in L79 is such that transcription initiated at the *int* promoter (P_I; 1, 18), approximately 1,350 bp to the right of ΔOP′, proceeds toward ΔOP′ and the *thr* structural genes. Since the structure of the *thr* attenuator at ΔOP′ is intact, but other *thr* regulatory signals are absent, transcription is terminated efficiently at the *thr* attenuator and the lysogen is phenotypically Thr$^-$. When the lysogen is cured, excision of the prophage restores the wild-type sequence at ΔOΔ′ and results in normal regulation and expression of the *thr* operon. In the pseudorevertants, transcripts initiated at P_I are not terminated efficiently, and read-through transcription into the *thr* structural genes is sufficient to restore the Thr$^+$ phenotype. The mutations in most of the pseudorevertants disrupt the rotational symmetry of the attenuator and therefore destabilize the secondary RNA structure thought to be essential for signaling termination (8, 27). When such a lysogen is cured, excision of the prophage regenerates a ΔOΔ′ sequence which retains the attenuator mutation. Since the attenuator is defective, these derivatives show derepressed, constitutive synthesis of the *thr* operon structural genes.

This model predicts that it should be possible to isolate mutations which affect both the *thr* attenuator and the core sequence of ΔOP′, since both sites share an 8-bp common sequence. Strain del-104 was isolated as a Thr$^+$ lysogenic revertant which does not undergo *int*-dependent prophage curing. DNA sequencing of this mutant revealed that it deletes 39 bp, including the *thr* attenuator. The deletion originates at the core of ΔOP′ and terminates 21 bp preceding the *thrA1* gene (Fig. 5). Since both the core (G → C change at −7) and the flanking Δ sequence have been changed by the deletion, it is not possible to determine what region of the sequence is important for excision. However, this does indicate that a base or sequence to the left of −6 is important for excisive recombination.

Since one endpoint of the del-104 deletion originates in the core of ΔOP′, this deletion may be analogous to the λ *b* deletions isolated previously (7, 11, 25). Formation of these deletions is *int* dependent, and the endpoints originate in the core of POP′ and delete λ DNA either to the right or to the left. In addition, all four of the deletions sites in the P or P′ arms of λ DNA show homology, ranging from 3 to 11 bp, with the wild-type core. These sites in λ DNA are considered secondary *att* sites, which fortuitously reside in the λ chromosome, and it has been proposed that these deletions arose by intramolecular site-specific recombination between POP′ and the various sequences in the λ chromosome (25). Therefore, the deletion in del-104 could have been formed by an intramolecular or intermolecular event involving ΔOP′ and the putative secondary *att* site.

Interestingly, the sequence CCTTTGGTCG-AAAAA appears in the Δ arm of the original lysogen at the leftward terminus site of the deletion. This sequence contains a 4-bp uninterrupted homology (CTTT) and shares a total 8-bp homology with the wild-type and ΔOP′ core sequences. If the del-104 deletion was generated by site-specific recombination, the DNA exchange occurred within the −3 to −6 region of the ΔOP′ core, but it is not possible to specify a unique site because of the homologies between the *att* sites. In addition, if the above sequence is considered as a secondary *att* site, there are three contiguous T residues in the conserved region of the sequence (Fig. 3). Thus, the bacterial sequence involved in generation of the del-104 deletion shows properties common to other secondary *att* sites. We are currently isolating other mutants which are phenotypically similar to del-104. One predicted class of mutations are those which are base substitutions in the −7 to

0 region of ΔOP′, which overlaps the *thr* attenuator. Such mutations will change the sequence of the conserved region of the core, and it should be possible to study the effects of these mutations on the frequency of site-specific recombination.

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