JOURNAL OF BACTERIOLOGY, July 1999, p. 4062–4070 Vol. 181, No. 13
0021-9193/99/$04.00+0

Site-Specific Recombination of Temperate *Myxococcus xanthus* Phage Mx8: Regulation of Integrase Activity by Reversible, Covalent Modification†

VINCENT MAGRINI, MICHAEL L. STORMS, AND PHILIP YOUDERIAN*

Department of Microbiology, Molecular Biology, and Biochemistry, University of Idaho, Moscow, Idaho 83844-3052

Received 22 April 1998/Accepted 10 December 1998

Temperate *Myxococcus xanthus* phage Mx8 integrates into the *attB* locus of the *M. xanthus* genome. The phage attachment site, *attP*, is required in *cis* for integration and lies within the *int* (integrase) coding sequence. Site-specific integration of Mx8 alters the 3′ end of *int* to generate the modified *intX* gene, which encodes a less active form of integrase with a different C terminus. The phage-encoded (Int) form of integrase promotes *attP* × *attB* recombination more efficiently than *attR* × *attB*, *attL* × *attB*, or *attB* × *attB* recombination. The *attP* and *attB* sites share a common core. Sequences flanking both sides of the *attP* core within the *int* gene are necessary for *attP* function. This information shows that the directionality of the integration reaction depends on arm sequences flanking both sides of the *attP* core. Expression of the *uoi* gene immediately upstream of *int* inhibits integrative (*attP* × *attB*) recombination, supporting the idea that *uoi* encodes the Mx8 excisionase. Integrase catalyzes a reaction that alters the primary sequence of its gene; the change in the primary amino acid sequence of Mx8 integrase resulting from the reaction that it catalyzes is a novel mechanism by which the reversible, covalent modification of an enzyme is used to regulate its specific activity. The lower specific activity of the prophage-encoded IntX integrase acts to limit excisive site-specific recombination in lysogens carrying a single Mx8 prophage, which are less immune to superinfection than lysogens carrying multiple, tandem prophages. Thus, this mechanism serves to regulate Mx8 site-specific recombination and superinfection immunity coordinately and thereby to preserve the integrity of the lysogenic state.

Like other temperate bacteriophages, phage Mx8 of *Myxococcus xanthus* integrates into a preferred chromosomal locus, *attB*, to generate a linear prophage. Maintenance of the Mx8 prophage depends not only on the stability of its integrated state but also on the repression of phage lytic genes. Repression of the phage lytic genes confers a selective advantage upon the lysogenic host by conferring immunity to superinfection.

Surprisingly, lysogens with single Mx8 prophages maintain a lower level of immunity than lysogens with tandem prophages. A wild-type stock of Mx8 plates with efficiencies of 10^{-5} on lysogens with a single integrated copy of the Mx8 genome and $<10^{-8}$ on lysogens with two or more tandem copies of the Mx8 genome. Thus, the relative level of superinfection immunity conferred upon a lysogen is dependent upon the number of integrated Mx8 prophages and presumably the dosage of the *imm* gene, which encodes the primary Mx8 repressor (29).

In the accompanying paper (19), we show that, unlike the case for most other temperate phages, the integration of Mx8 is unusual for two reasons. First, the Mx8 bacterial attachment locus, *attB*, contains two nearby attachment sites, *attB1* and *attB2*. Plasmids with the Mx8 *int-attP* genes prefer to integrate into the *attB1* site much more than into the *attB2* site, and plasmid integration often is accompanied by a deletion between the *attB1* and *attB2* sites. Second, the Mx8 phage attachment site, *attP*, lies within the *int* coding sequence (34). Thus, integration of the Mx8 prophage results in a change in the primary structure of the *int* gene and generates a recombinant *intX* gene predicted to encode a product with a new C terminus.

To explain these unusual features of Mx8 site-specific recombination, we have explored the ability of integrase to mediate site-specific recombination between different pairs of attachment sites. We find that Mx8 Int can promote a variety of recombination events between different *att* sites with different efficiencies. We also show that the substitution of a new 3′ end of *int* upon the integration of Mx8 does not result in the inactivation of integrase but rather in a reduction in its specific activity. This novel genetic switch allows *M. xanthus* lysogens carrying single or multiple Mx8 prophages to coordinate the rate of prophage excision with the level of superinfection immunity.

MATERIALS AND METHODS

Bacterial strains. The multiple-mutant strain *M. xanthus* DZ1 (5) is the preferred host for the growth of phage Mx8 and was used to assay plasmid integration. *Escherichia coli* JM107 (38) was used for the construction of plasmids and the preparation of plasmid DNA. Plasmids were constructed by standard cloning procedures (30) and introduced into *E. coli* (33) or *M. xanthus* (13) hosts by electroporation. Derivatives of *M. xanthus* DZ1 with integrated plasmids were grown in CTPM medium (36) with kanamycin (40 μg/ml) and/or the combination of spectinomycin (800 μg/ml) and streptomycin sulfate (1 mg/ml). Derivatives of *E. coli* with plasmids were grown in Luria-Bertani medium supplemented with ampicillin (100 μg/ml), kanamycin (40 μg/ml), or spectinomycin and streptomycin (50 μg/ml each). Antibiotics were from Sigma Chemical Co. Oligonucleotides used for plasmid construction and mutagenesis were made by Biosource Inc. Methods for the PCR amplification of *M. xanthus* genomic DNA with the *attP*, *attB*, *attL*, and *attR* primer pairs are described in the accompanying paper (19). Methods for the growth and assay of phage Mx8 have been described previously (20, 29).

Simple derivatives of plasmid pAY721. Plasmids with portions of the Mx8 genome were derived from DNA isolated directly from the wild-type strain of Mx8 (21), from Km^r *int-attP* plasmid pAY721 (29), which is a 2.2-kilobase-pair

* Corresponding author. Mailing address: Department of Microbiology, Molecular Biology, and Biochemistry, University of Idaho, Moscow, ID 83844-3052. Phone: (208) 885-0571. Fax: (208) 885-6518. E-mail: pay@uidaho.edu.

† This work is dedicated to the memory of Hatch Echols, teacher and friend.

TABLE 1. Plasmids

Plasmid	bp[a]	Vector[b]	Relevant genotype	Source or reference
pBGS18		Km^r		31
pAY1099		Sp^r Sm^r		18
pLITMUS28		Ap^r		New England Biolabs
pLITMUS29		Ap^r		New England Biolabs
pAY721	4585–6809	pBGS18/Km^r	*uoi*$^+$ *int*$^+$ *attP*$^+$	This study
pAY952	4585–6809	pGB2/Sp^r Sm^r	*uoi*$^+$ *int*$^+$ *attP*$^+$	This study
pAY980	4585–6809	pBGS18/Km^r	*uoi*$^+$ Δ*int-5403/5851 attP*$^+$	19
pAY994	5851–6809	pBGS18/Km^r	*attP*$^+$	This study
pAY999	4585–6469	pBGS18/Km^r	*uoi*$^+$ *intX*$^+$ *attR*$^+$	This study
pAY1311	5851–6469	pBGS18/Km^r	*attR*$^+$	This study
pAY1299	5851–6809	pLITMUS28/Ap^r	*attP5*	This study
pAY1300	4585–6809	pBGS18/Km^r	*attP5*	This study
pAY971	5073–6809	pAY703/Km^r	*int*$^+$*-5085*	This study
pAY972	5208–6809	pAY703/Km^r	*int*$^+$*-5208*	This study
pAY973	4979–6809	pAY703/Km^r	*uoi*$^+$ *int*$^+$	This study
pAY1301	5073–6809	pAY1099/Sp^r Sm^r	*int*$^+$*-5085*	This study
pAY1302	5208–6809	pAY1099/Sp^r Sm^r	*int*$^+$*-5208*	This study
pAY1303	4979–6809	pAY1099/Sp^r Sm^r	*uoi*$^+$ *int*$^+$	This study
pAY1304	5073–6809	pAY1099/Sp^r Sm^r	*int*$^+$*-5085 attP5*	This study
pAY1305	5208–6809	pAY1099/Sp^r Sm^r	*int*$^+$*-5208 attP5*	This study
pAY1306	4979–6809	pAY1099/Sp^r Sm^r	*uoi*$^+$ *int attP5*	This study
pAY1307	5851–6469	pLITMUS28/Ap^r	*attR5*	This study
pAY1308	5073–6469	pAY1099/Sp^r Sm^r	*intX*$^+$*-5085 attR5*	This study
pAY1309	5208–6469	pAY1099/Sp^r Sm^r	*intX*$^+$*-5208 attR5*	This study
pAY1310	4979–6469	pAY1099/Sp^r Sm^r	*uoi*$^+$ *intX*$^+$ *attR5*	This study
pAY1048	5851–6469	pBGS18/Km^r	Δ*attP-1048*	This study
pAY1044	6398–6469	pBGS18/Km^r	Δ*attP-1044*	This study
pAY1046	6398–6809	pBGS18/Km^r	Δ*attP-1046*	This study
pAY1042	6447–6809	pBGS18/Km^r	Δ*attP-1042*	This study
pAY1041	6447–6469	pBGS18/Km^r	Δ*attP-1041*	This study
pAY1039		pBGS18/Km^r	*attB1*$^+$ *attB2*$^+$	This study
pAY1037		pLITMUS28/Ap^r	*attL1*$^+$	This study
pAY1038		pBGS18/Km^r	*attL1*$^+$	This study

[a] Coordinates of Mx8 DNA are those of GenBank accession no. U64984.
[b] Antibiotic resistance determinants retained from the vector are indicated.

(kb) subclone of Mx8 DNA in plasmid pBGS18 (31), or from amplified products of *M. xanthus* DZ1 chromosomal DNA and are listed in Table 1. Plasmid pAY952 (18) is an Sp^r Sm^r, integration-proficient derivative of plasmid pGB2 (6) with the same insert of Mx8 DNA as that in pAY721. Plasmid pAY980 is a derivative of pAY721 with a substitution of the 86-bp *Stu*I-*Pst*I fragment of pLITMUS28 for the *Stu*I-*Pst*I fragment internal to *int* (bp 5403 to 5851) (19). Plasmid pAY994, a Δ*int attP*$^+$ derivative of pAY721, contains the *Pst*I-*Hin*dIII fragment representing the 959 bp at the 3′ end of *int* (bp 5851 to 6809). pAY994 was made by subcloning this fragment from pAY721 into pBGS18. The *attP* core sequence (bp 6447 to 6469) is located approximately in the middle of this fragment. pAY999 is the *attR*$^+$ derivative of plasmid pAY721 with the *intX* gene in place of *int* (in which the *int* coding sequences represented by the P′ arm of *attP*, POP′, were replaced with those represented by the B′ arm of *attB*, BOB′). To construct pAY999, genomic DNA isolated from a DZ1 lysogen carrying the Mx8 prophage was amplified with 5′ primers having the sequences GGGAAG CTTGAATTCATAAAAGCCCGCCTCACCGAA and TCAGCGCTTCAGGT CCGGGACTGGGAC; the product containing *attR* was cleaved with *Nco*I and *Hin*dIII and ligated in place of the *Nco*I-*Hin*dIII fragment of pAY721 to replace the 3′ end of *int*. Plasmid pAY1131 was made by subcloning the *Pst*I-*Hin*dIII fragment with *attR* from pAY999 into pBGS18. Plasmid pAY1300 is an otherwise isogenic derivative of pAY721 with the *attR5* mutation. Primer pairs with the sequences CGGCTCTAGACCGGTACTCCTCGCCACCCCTGCCCCAG CAAGTT and GCGGTGCGCATCGGGGAGGCGT and the sequences CCG GTCTAGACGCGGTACTCCTGGGCTCCTTCTA and CCCAAGCTTCCTA GGTAGCGGAAGGGCTCTC were used to amplify plasmid pAY721, yielding products of 646 and 355 bp, respectively. The 646-bp product was cleaved with *Pst*I and *Xba*I, and the 355-bp product was cleaved with *Xba*I and *Hin*dIII. The cleaved products were ligated to the *Pst*I-*Hin*dIII backbone of pLITMUS28 to make plasmid pAY1299, and the smaller *Pst*I-*Hin*dIII fragment of pAY1299 was ligated to the larger *Pst*I-*Hin*dIII fragment of pAY721 to make pAY1300.

Plasmids that express *int* or *intX* (*attP5*) from the *mgl* promoter. Plasmids pAY971, pAY972, and pAY973 express *int* from the constitutive *mglBA* promoter and are described in the accompanying paper (19). Sp^r Sm^r derivatives of these plasmids with the *attP5* mutation were constructed in two steps. First, plasmids pAY971, pAY972, and pAY973 were cleaved with *Eco*RI and *Xba*I to yield DNA fragments of 2.9, 2.8, and 3.4 kb; these were ligated to the *Eco*RI-*Xba*I backbone of Sp^r Sm^r plasmid vector pAY1099 (18) to make pAY1301, pAY1302, and pAY1303, respectively. Second, primers with the sequences GCG GTGCGCATCGGGGAGGCGT and CCCGAATTCTCAGGTAGCGGAAG GGCTCT were used to amplify plasmid template pAY1300 (*attP5*). After treatment with *Pst*I and *Eco*RI, the 1-kb fragment with the *attP5* mutation was ligated to the larger *Pst*I-*Eco*RI fragments of plasmids pAY1301, pAY1302, and pAY1303 to replace the *attP*$^+$ allele and to yield pAY1304, pAY1305, and pAY1306, respectively.

An otherwise isogenic set of plasmids that express *intX* from the *mgl* promoter and carry the *attP5* mutation was constructed in two additional steps. First, primer pairs with the sequences CGGCTCTAGACCGGTACTCCTCGCCACC CCTGCCCCAGCAAGTT and GCGGTGCGCATCGGGGAGGCGT and the sequences CCGGTCTAGAGCCGGTGGCCTCCGGCGTGACAGGCCGGC GTTCTAA and GGGAAGCTTGAATTCATAAAAGCCCGCCTCACCGAA were used to amplify plasmid templates pAY721 and pAY999, respectively. The 646-bp product of the pAY721 amplification was treated with *Pst*I and *Xba*I, and the 366-bp product of the pAY999 amplification was treated with *Xba*I and *Hin*dIII. The pair of cleaved fragments was ligated to the *Pst*I-*Hin*dIII backbone of pLITMUS28 to generate the construction intermediate, pAY1307, with the *attP5* mutation in the *attR* core. Second, the smaller *Pst*I-*Eco*RI fragment of pAY1307 was ligated to the larger *Pst*I-*Eco*RI fragments of plasmids pAY1301, pAY1302, and pAY1303 to make plasmids pAY1308, pAY1309, and pAY1310, respectively.

Plasmids with deletions extending into *attP*. To localize the elements required for *attP* function in reactions with *attB*, we constructed derivatives of plasmid pAY994 with different portions of the 3′ end of *int*, including the *attP* core. Primers with the sequences AGCGGATAACAATTTCACACAGGA and CCC CCAAGCTTACGGGTTCAAGTCCCGTA were used to amplify template plasmid pAY994. After cleavage of the PCR product with *Pst*I and *Hin*dIII, the resulting 637-bp fragment was ligated to the *Pst*I and *Hin*dIII sites of pBGS18 to make pAY1048 (bp 5851 to 6469), with the left arm of *attP* and the *attP* core. Primers with the sequences AAAAAACTGCAGAAAAGAAAAACCCCAGC AAGTCCGAGAACTTGCTGGGGCAGGGGTGGCGAGGAGTACGGGA CTTGAA and CCCCCAAGCTTACGGGTTCAAGTCCCGTA were used in a

similar way to make pAY1044 (bp 6398 to 6469), with the *attP* core and the Mx8 terminator for *trnD*. Primers with the sequences AAAAAACTGCAGAAAAG AAAAACCCCAGCAAGTCCGAGAACTTGCTGGGGCAGGGGTGGCGA GGAGTACGGGACTTGAA and CCCAAGCTTAGGTAGCGGAAGGGCT CTC were used to make pAY1046 (bp 6398 to 6809), with the Mx8 terminator, the *attP* core, and the right arm of *attP*. pAY1042 (bp 6447 to 6809) and pAY1041 (bp 6447 to 6469) are smaller versions of pAY1046 and pAY1044 without the terminator and were made in a similar way with primer pairs having the sequences AAAAAACTGCAGAGGAGTACGGGACTTGAA and CCCA AGCTTAGGTAGCGGAAGGGCTCTC and the sequences AAAAAACTGC AGAGGAGTACGGGACTTGAA and CCCCCAAGCTTACGGGTTCAAGT CCCGTA, respectively.

Plasmids carrying the Mx8 prophage *attL* (*attL1*) junction site. Chromosomal DNA isolated from the Km^r electroporant DZ1(1308) was amplified by PCR with *attL* primers having the sequences GGGGGAATTCGTCGACTGCGCAG GTCCGCGGAGGA and CCCAAGCTTCCTAGGTAGCGGAAGGGCTC TC. After treatment with *Hin*dIII and *Eco*RI, the 526-bp PCR product was ligated to pLITMUS29 to make plasmid pAY1037. The smaller *Acc*65I-*Xho*I fragment of pAY1037 was ligated to the larger *Acc*65I-*Sal*I fragment of pBGS18 to make the Km^r *attL* plasmid pAY1038. Plasmid pAY1039 was made by subcloning the *Eco*RI-*Hin*dIII fragment made by amplification of *M. xanthus* DZ1 DNA with *attB* primers (19) into plasmid pBGS18.

Construction and phenotypic analysis of lysogens of DZ1 carrying single and multiple, tandem Mx8 prophages. To construct lysogens of DZ1, wild-type Mx8 (10^7 phage/ml) was spotted on a lawn of host DZ1, and plates were incubated at 32°C for 96 h. Single colonies were purified from the zone of phage clearing. Lysogens of Mx8 carry either a single Mx8 prophage or multiple, tandem prophages (24). A lysogen with a single Mx8 prophage ("low yielders") was distinguished from a lysogen with multiple, tandem Mx8 prophages ("high-yielders") by amplification of chromosomal DNAs isolated from candidate lysogens with the *attR*, *attL*, and *attP* primer pairs. Whereas DNA isolated from the multiple, tandem-prophage lysogen yielded products in all three amplification reactions, DNA isolated from the single-prophage lysogen did not yield an abundant product in the *attP* reaction.

To measure phage released spontaneously from single- and tandem-prophage lysogens, cultures inoculated with single colonies of a lysogen were grown to an exponential density of 4×10^8/ml in CTPM medium at 32°C, cells were pelleted by low-speed centrifugation, and culture supernatants were treated with chloroform. Numbers of plaques formed by serial dilutions of the supernatants on host DZ1 were scored after incubation at 32°C for 48 h. Reported titers are the averages for three independent determinations.

To show that phage released spontaneously from lysogens have an intact *int* gene, high-titer phage stocks were prepared from single plaques. DNA prepared from these stocks was cleaved separately with restriction endonucleases *Eco*RI (which cleaves at sites flanking *int*) and *Mlu*I (which cleaves within *int*), as well as several additional endonucleases. In all (20 of 20) cases, the restriction patterns of DNA from phage released by spontaneous induction were identical to those of wild-type Mx8 DNA.

Nucleotide sequences of the *int* and *uoi* genes and the *attB* locus and their accession numbers. The sequence of a region of the Mx8 genome including *uoi* and *int* has been assigned GenBank accession no. U64984 (29). The sequence of the *attB* locus, determined by Tojo et al. (34), has been assigned GenBank accession no. D26557.

RESULTS

Site-specific integration results in a change in the C terminus of integrase that decreases its activity in promoting integration (*attP* × *attB* recombination). When a plasmid with the functional *int-attP* genes integrates into the *attB* locus, the *int* gene acquires a new 3′ end because *attP* lies within the *int* coding sequence (see Fig. 1 in accompanying paper [19]). We designate this altered *int* gene, with a new coding sequence for the C terminus of its product, *intX*. To test whether the *intX* gene is functional, we amplified its 3′ coding region from the DNA of strain DZ1(pAY721) by using primers specific for the amplification of *attR*. This 3′ end was subcloned in place of the phage-encoded 3′ end of *int* on plasmid pAY721 to make the otherwise isogenic plasmid pAY999.

When electroporated into DZ1, the *intX-attR*$^+$ plasmid pAY999 gives rise to Km^r recombinants at a very low but detectable efficiency. Unlike *int*, the *intX* gene has an internal *attR* site, not an internal *attP* site. Thus, when we measure the ability of pAY999 to integrate, we are assaying the ability of *intX* to promote *attR* × *attB* recombination. Consequently, the results show only that the pAY999-encoded IntX integrase can catalyze *attR* × *attB* recombination inefficiently at best.

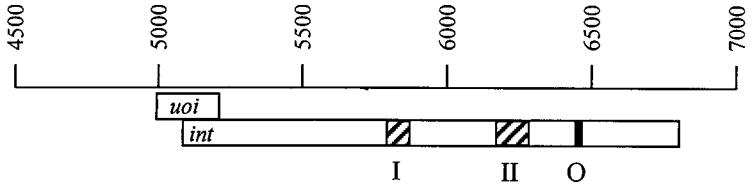
To determine whether *intX* can promote *attP* × *attB* recombination, we examined whether the IntX integrase made from one plasmid (pAY999) can catalyze the integration of a second plasmid with an inactive *int* gene but an active *attP* site. As shown in Fig. 1, the electroporation of the host *M. xanthus* DZ1 with plasmid pAY721, carrying the functional *int-attP* genes, results in Km^r recombinants at a high efficiency. In contrast, the otherwise isogenic deletion derivative pAY980, missing a central region of *int*, does not result in Km^r recombinants. However, when Km^r plasmid pAY980 and Sp^r Sm^r *int-attP*$^+$ plasmid pAY952 are coelectroporated into DZ1, Km^r Sp^s Sm^s recombinants arise at a high frequency. This result shows that pAY980 retains an active *attP* site that can be complemented in *trans* for integration by integrase made from plasmid pAY952 (19). Similarly, plasmid pAY994, which retains 959 bp at the 3′ end of *int*, including the *attP* core, is also complemented efficiently by pAY952 upon coelectroporation, indicating that it also retains a functional *attP* site (data not shown).

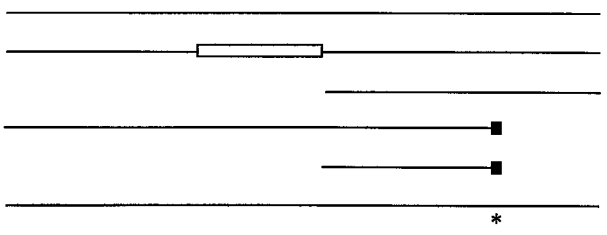
When Km^r plasmid pAY999 is coelectroporated with Km^r *attP*$^+$ plasmids pAY980 and pAY994 (Table 2), Km^r electroporants are obtained at low but measurable efficiencies, showing that IntX can promote *attP* × *attB* recombination. In addition, eight of eight Km^r electroporants resulting from the coelectroporation of host DZ1 with pAY999 (*intX*$^+$ *attR*$^+$) and pAY980 (*int*Δ*5403/5851 attP*$^+$) carry a single copy of pAY980, and not pAY999, integrated into *attB* (Fig. 2). Clearly, however, IntX promotes *attP* × *attB* recombination at a much lower efficiency than does Int.

Separating the *int* and *attP* functions: construction of *attP5*, a mutation that inactivates *attP* but not *int*. Because the Mx8 *attP* site lies within *int* and the *attR* site lies within *intX*, it is difficult to measure the relative activities of the Int and IntX integrases in vivo, because plasmids that produce these proteins are also potential substrates for the reactions that these integrases catalyze. To separate the *trans*-acting *int* function from the *cis*-acting *attP* function, we constructed a mutation within the *int* gene that inactivates *attP* but has no effect on *int*.

As shown in Fig. 2, the Mx8 *attP* site shares a 17-bp core sequence with the two bacterial attachment sites, *attB1* and *attB2*, within which site-specific recombination occurs (19). The core is comprised of an asymmetric 7-bp sequence flanked by two 5-bp dyad symmetry sequences. Using PCR, we introduced into the common core five different single-base-pair substitutions each of which changes a base pair in the third positions of the *int* codons. Because these changes replace wild-type *int* codons with synonymous codons, they should not change the amino acid sequence of integrase. As expected, an integrated plasmid that expresses a mutant *int* gene with these changes produces active integrase that functions in *trans* (see below).

Because the *attP5* mutation alters the common core sequence, however, it should inactivate the *attP* site. The integrases in the λ Int family, to which Mx8 Int belongs, catalyze four-strand exchange events between sites with a similar structure, a central asymmetric sequence flanked by short inverted repeats. For three integrases in this family, phage λ Int (7), the yeast 2μm FLP recombinase (1), and phage HK022 Int (14), cleavage of these sites is known to occur on opposite single strands of DNA at the junctions between the central asymmetric sequence and its flanking repeats. Efficient completion of the four-strand exchange reaction catalyzed by these recombinases will occur inefficiently if the two central asymmetric sequences of a pair of sites are not identical, because homology





Plasmid	bp	EOE (10^3 μg $^{-1}$)
721	4585-6809	26
980	4585-6809 Δ *int*	<0.005
994	5851-6809	<0.005
999	4585-6469	0.002
1311	5851-6469	<0.002
1300	4585-6809 *attP-5*	<0.01

FIG. 1. Plasmids with the *intX* and *attP5* mutations cannot integrate efficiently. Coordinates of Mx8 DNA inserts (base pairs), based on GenBank accession no. U64984 (29), are shown above the open boxes representing the *uoi* and *int* coding sequences (top) which, like the coliphage λ *xis* and *int* genes (10), overlap. The hatched regions in the *int* gene correspond to conserved domains I and II of integrases (2, 17); conserved domain II includes the active-site tyrosine residue (26). The filled region within *int*, O, is the *attP* common core. The extents of Mx8 DNA inserts present in each plasmid are shown as horizontal thin lines (bottom left). Mutant plasmids are derived from pAY721, which contains a 2.2-kb region of the Mx8 genome with the functional *int-attP* genes. pAY980 is a derivative of pAY721 with a deletion, Δ*int-5403/5851* (open box), that removes bp 5403 to 5851 internal to *int*, including a portion of a portion of domain I (19). pAY999 contains the *attR* site and encodes the recombinant IntX integrase, with an altered C terminus (small black box). pAY994 and pAY1131 contain smaller regions of *int* and *intX* with minimal, functional *attP* and *attR* sites, respectively. pAY1300 is an otherwise isogenic version of pAY721 in which the *attP* site has been inactivated by the *attP5* mutation (asterisk) (see Fig. 2). These plasmids were used in experiments to measure the relative abilities of Int and IntX to mediate a subset of site-specific recombination reactions involving different combinations of *att* sites (see Fig. 3). The efficiencies of electroporation (EOE) of the plasmids are the numbers of Km^r recombinants arising per microgram of DNA electroporated into host DZ1 and are the averages of at least three independent determinations (see Materials and Methods). *attP-5* is *attP5*.

in this region is required for the proper resolution of the Holliday junction formed during the recombination event (3, 23). This information predicts that, because the *attP5* mutation alters the sequence of the *attP* core in the predicted region of branch migration between recombining *attP5* and *attB* sites, this mutation should abolish *attP* × *attB* (integrative) recombination. Consistent with this prediction, pAY1300, an otherwise isogenic derivative of pAY721 with the *attP5* mutation, cannot integrate into the *M. xanthus* genome (Fig. 1).

Expression of *int* or *intX* from the *mgl* locus promotes integrative (*attP* × *attB*) recombination in *trans*. To show that the *attP5* mutation does not inactivate *int*, we constructed a plasmid that expresses the mutant *int* (*attP5*) gene from the constitutive *mgl* promoter, integrated this plasmid at the *mgl* locus by homologous recombination, and assayed the ability of Int made from this plasmid to promote the integration of *int-attP*$^+$ plasmid pAY994 into the *attB* locus.

Plasmid pAY703 has the *mglBA* genes and can integrate into the *mgl* locus of DZ1 by homologous recombination to give rise to Km^r recombinants after electroporation of this host (20). We have shown that when the phage Mx8 *mox* gene (20), the *M. xanthus sglK* gene (36), or the *E. coli glk* gene (unpublished results) is subcloned into pAY703 and the subclones are integrated by homologous recombination into the *M. xanthus mgl* locus, the genes are expressed constitutively as part of the *mglBA* operon. Also, in the accompanying paper (19), we show that when we add functional *int-attP* genes to pAY703, we give this plasmid the option of integrating either at the *mgl* locus by homologous recombination or at the *attB* locus by site-specific recombination. Site-specific recombination prevails over homologous recombination, and the majority of Km^r electroporants carrying a plasmid with both the *mgl* locus and functional

TABLE 2. The IntX integrase can promote integrative (*attP* × *attB*) recombination[a]

Plasmid(s) [relevant genotype(s)]	EOE (10^3 μg^{-1})
pAY980 (Δ*int-5403/5851 attP*$^+$)	<0.005
pAY994 (*attP*$^+$)	<0.005
pAY999 (*intX*$^+$ *attR*$^+$)	0.002
pAY999 (*intX*$^+$ *attR*$^+$) + pAY980 (Δ*int-5403/5851 attP*$^+$)	0.07
pAY999 (*intX*$^+$ *attR*$^+$) + pAY994 (*attP*$^+$)	0.17

[a] The efficiency of electroporation (EOE) of each plasmid alone or in coelectroporations was measured as the numbers of Km^r recombinants of host DZ1 as described elsewhere (19).

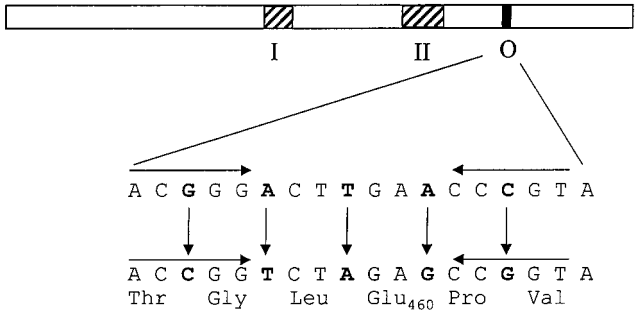


FIG. 2. *attP5* mutation. Within the *int* coding sequence, the *attP* common core (O) is comprised of an asymmetric 7-bp sequence flanked by two 5-bp dyad symmetry sequences (horizontal arrows). To inactivate *attP* and create *attP5*, we introduced the five single-base-pair substitutions shown in bold; these mutations change five codons within *int* to synonymous codons.

int-attP genes carry the plasmid integrated at the *attB* locus, not at the *mgl* locus (19).

As described in Materials and Methods, we constructed plasmids pAY1305 and pAY1306, which carry the *int* (*attP5*) gene immediately downstream of the *mglA* gene (Fig. 3). These plasmids should express *int* from the constitutive *mgl* promoter and couple the termination of *mglA* translation with the initiation of *int* or *uoi* translation, beginning at either the second start codon of *int* (GTG-5208 [GTG at bp 5208]; pAY1305) or the start codon of the upstream *uoi* gene (GTG-4991; pAY1306). When these plasmids are electroporated into host DZ1, Sp^r Sm^r recombinants are found to carry these plasmids integrated at the *mgl* locus, not at the *attB* locus. Thus, when chromosomal DNA is isolated from these recombinants and amplified, reactions with the *attB* primer pair yield a 591-bp product, indicating that these recombinants retain an intact *attB* locus (19). This information shows that *int*$^+$-*attP5* plasmids pAY1304 and pAY1306, unlike their *int-attP*$^+$ counterparts without the *attP5* mutation, pAY971 and pAY973 (19), are defective in site-specific integration.

To show that these plasmids express active integrase in *trans*, we measured the efficiency with which Sp^r Sm^r derivatives of strain DZ1 with these plasmids integrated at the *mgl* locus could complement Km^r Δ*int-attP*$^+$ plasmid pAY994 for integration. As shown in Fig. 3, electroporation of pAY994 into strain DZ1::pAY1304, which expresses the *int*$^+$ (*attP5*) gene from the *mgl* promoter, results in about 8×10^5 Km^r electroporants μg^{-1}. This efficiency is significantly (>20-fold) higher than that for the integration of control *int*$^+$-*attP*$^+$ plasmid pAY721, which expresses *int* in *cis* from the *int* promoter (Fig. 1). This efficiency is also much higher than that for a deletion derivative of pAY721, pAY759, which should be missing the *uoi* promoter (19). Similar results are observed when pAY994 is electroporated into host DZ1::pAY1304, which expresses the version of the *int* (*attP5*) gene that initiates translation at the first *int* start codon (data not shown). The simplest interpretation of these results is that Int activity is expressed at higher levels from the constitutive *mgl* promoter than from its own promoter.

The data in Fig. 3 also reveal that strain DZ1::pAY1306, in which both *uoi* and *int* are expressed as part of the *mgl* operon, promotes the integration of plasmid pAY994 much less (<50-fold) efficiently than does strain DZ1::pAY1305. This result suggests that the expression of *uoi* inhibits the ability of Int to mediate the *attP* × *attB* integration reaction in *trans*, supporting the ideas that the *uoi* gene encodes Mx8 excisionase and that excisionase inhibits integrative recombination.

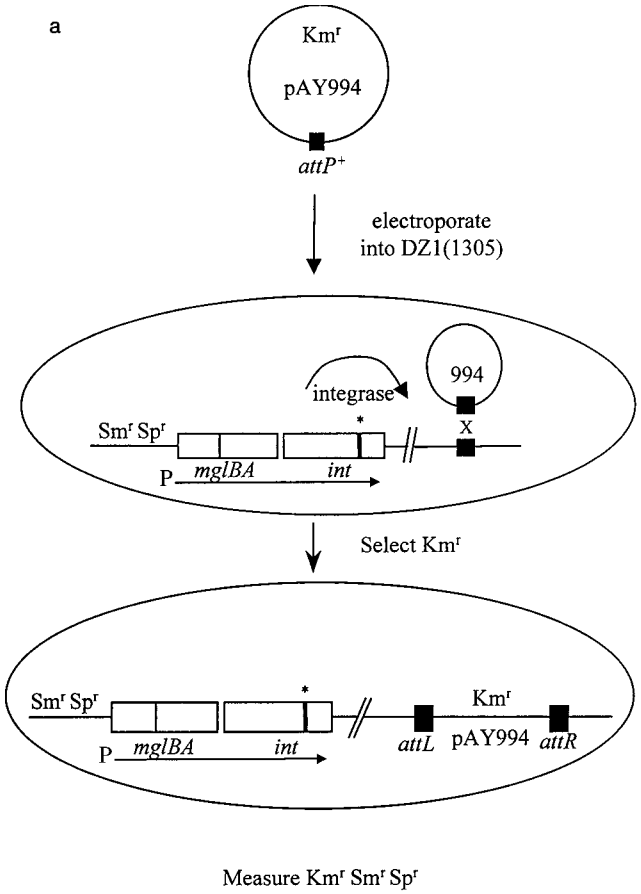
We also constructed plasmids pAY1309 and pAY1310, which express *intX* (*attP5*) from the *mgl* promoter, without and with *uoi*, respectively. Because the *attP5* mutation affects the common core, it changes the *attR* site internal to *intX* in the same way that it changes the *attP* site internal to *int*. This mutation also should prevent the *attR5* site from participating in productive site-specific recombination reactions with substrates that have the wild-type common core. When these plasmids are electroporated into host DZ1, again, Sp^r Sm^r recombinants are found to carry these plasmids integrated at the *mgl* locus. When pAY994 is electroporated into hosts with these plasmids, Km^r recombinants arise at very low but detectable frequencies (Fig. 3). Analysis of these Km^r recombinants by PCR shows that they carry pAY994 integrated at *attB*, because these recombinants have acquired an *attL* site (data not shown). These results confirm independently that the product of the prophage *intX* gene can promote *attP* × *attB* recombination but at an efficiency much lower than can the product of the phage *int* gene. They also show that the presence of the *uoi* gene has little effect on the ability of IntX to mediate *attP* × *attB* recombination, in contrast to its pronounced inhibition of the ability of Int to mediate the same reaction. Again, strain DZ1::pAY1308, which expresses *intX* from its first start codon, yielded results similar to those obtained with strain DZ1::pAY1309 (data not shown).

Int promotes *attP* × *attB* recombination more efficiently than *attR* × *attB*, *attL* × *attB*, or *attB* × *attB* recombination. Prophage integration depends on a recombination reaction that is catalyzed by integrase and that proceeds in the highly favored, forward direction, *attP* + *attB* → *attR* + *attL*. For phages λ, P22, φ80 (17), and HK022 (37), this directionality of the recombination reaction depends on the difference between the structures of the *attP* and *attB* sites. Whereas the function of the *attB* site (BOB′) depends almost solely on the common core, O, the function of the *attP* site (POP′) depends on the common core, O, and both flanking P and P′ arm sequences. This is because the P and P′ arms have stronger binding sites for integrase and weaker binding sites for the integrative host factor required for the efficient catalysis of the integration reaction, whereas the B and B′ arms lack such sites (8, 11, 27, 28). Thus, the directionality of the integration reaction depends on this fundamental, functional asymmetry between the *attP* and *attB* sites, which facilitates the assembly of the catalytically active, asymmetric intasome complex (4).

Consequently, for phage λ, Int promotes site-specific recombination between the *attP* and *attB* (POP′ × BOB′) sites much more efficiently than between the *attR* (POB′), *attL* (BOP′), or *attB* (BOB′) site and an *attB* (BOB′) partner site, because only the *attP* × *attB* combination of substrates facilitates the proper assembly of the intasome. As shown in Fig. 3, when Mx8 integrase is expressed constitutively from the *mgl* promoter in strain DZ1::pAY1305, it can stimulate the integration of plasmids pAY1311 (*attR*), pAY1038 (*attL*), and pAY1039 (*attB*), but at lower efficiencies than that of plasmid pAY994 (*attP*). These results suggest that, as is the case for many other temperate phage *attP* sites, both the P and the P′ arms of the Mx8 *attP* site contribute significantly to *attP* function in the integrative recombination reaction.

Two secondary conclusions may be drawn from the additional data presented in Fig. 3. First, the expression of *uoi* together with *int* in host strain DZ1::pAY1306 inhibits not only *attP* × *attB* recombination but also the other three site-specific recombination reactions involving *attR*, *attL*, or *attB* and an *attB* partner. Second, IntX made in DZ1::pAY1309 or the combination of IntX and Uoi made in DZ1::pAY1310 promotes these other recombination reactions less efficiently than Int.

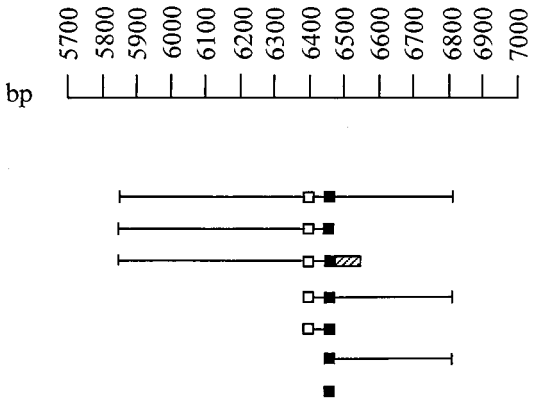
Both arm sequences flanking the *attP* core contribute significantly to *attP* function. To confirm that both the P and the P′ arms of the *attP* site are important for *attP* function, we constructed a variety of deletion derivatives of Km^r *attP*$^+$ plasmid pAY994, electroporated these derivatives into host strain DZ1::pAY1305, which produces integrase constitutively from the *mgl* promoter, and measured the frequencies of Km^r electroporants. As shown in Fig. 4, plasmid pAY1048, missing the right (P′) arm of the *attP* site, integrates into this host to give rise to Km^r recombinants with a >10-fold lower efficiency than does plasmid pAY994. This result is consistent with the finding that *attR* plasmid pAY1311, with the prophage *attR* (POB′) site, also gives rise to fewer recombinants than does plasmid pAY994. Plasmid pAY1042, missing the left (P) arm of the *attP* site, also is impaired in its ability to be complemented in *trans* for integration. Plasmid pAY1041, with deletions of both *attP* arms, can be complemented for integration only very



b

	Frequency of Km^r electroporants (10^3 μg^{-1})			
	pAY994 *attP*	pAY1311 *attR*	pAY1038 *attL*	pAY1039 *attB*
none	<0.08	<0.06	0.01	0.04
1305 (P — *mglB* *mglA* *int* 5208)	820	44	1.2	0.5
1306 (P — *mglB* *mglA* *uoi/int*)	14	1.1	0.3	0.3
1309 (P — *mglB* *mglA* *intX* 5208)	0.1[a]	0.7	0.2	0.7
1310 (P — *mglB* *mglA* *uoi/intX*)	0.2[a]	0.3	0.3	0.3

FIG. 3. Expression of the mutant *int* (*attP5*) gene promotes site-specific recombination in *trans*. (a) When Sp^r Sm^r plasmid pAY1305 is integrated into the *mgl* locus of host DZ1 by homologous recombination, it expresses the *int* (*attP5*) gene from the constitutive *mgl* promoter (P). To show that the product of this mutant *int* gene functions in *trans*, strain DZ1::pAY1305 was electroporated with Km^r plasmid pAY994, and Km^r electroporants were selected. These recombinants arise upon site-specific recombination between the *attP* site of pAY994 and the *attB* locus to yield strains in which the linear, integrated plasmid pAY994 sequences are flanked by *attL* and *attR* sites. (b) DZ1 and Sp^r Sm^r derivatives of DZ1 carrying plasmids pAY1305, pAY1306, pAY1309, and pAY1310 were electroporated with plasmids pAY994 (*attP*), pAY1311 (*attR*), pAY1038 (*attL*), and pAY1039 (*attB*). The structures of the first four plasmids, which express *int* (*attP5*) or *intX* (*attR5*) from the *mgl* promoter, without and with *uoi*, are shown on the left; the *attP5* (*attR5*) mutation is indicated by asterisks. On the right are given the efficiencies of electroporation, expressed as the numbers of Km^r electroporants arising per microgram of plasmid DNA and representing the average determinations from at least three independent experiments.



Plasmid	*att* Site	EOI
994	POP'	32
1048	POV'	1.2
1311	POB'	1.7
1046	TOP'	0.5
1044	TOV'	0.006
1042	VOP'	0.4
1041	VOV'	0.02

FIG. 4. Both arms of the *attP* site contribute to *attP* function in the *attP* × *attB* integrative recombination reaction. Plasmids carrying various portions of *attP* or *attR* were electroporated into host strain DZ1::pAY1305, and the efficiencies with which they gave rise to Km^r recombinants were determined. The coordinates of Mx8 DNA inserts in Km^r plasmids with portions of *attP* (or *attR*) are shown on the top left, numbered as in GenBank accession no. U64984 (29). The extents of DNA inserts in the plasmids are represented as horizontal lines. All of the plasmids include the common core (filled boxes), and all but two of the plasmids carry the t_{Mx8} terminator (open boxes). Attachment sites in these plasmids carry various combinations of the left (P) and right (P′) arms of *attP*, a deletion of the left arm of *attP* that retains the t_{Mx8} terminator (T), the right (B′) arm of *attB1* (hatched box), and/or vector sequences (V or V′) flanking the common core. Efficiencies of integration (EOI) are the average frequencies of Km^r Sp^r Sm^r recombinants recovered per microgram of plasmid DNA divided by the average frequencies of Km^r recombinants obtained from parallel electroporations of *int-attP*$^+$ plasmid pAY721 and represent the average determinations from at least three independent experiments.

poorly, at a very low efficiency comparable to that observed for plasmid pAY1039, carrying the *attB* (BOB′) sequence (Fig. 4).

In the accompanying paper (19), we show that when a plasmid with the Mx8 *attP* site integrates into the *attB* locus, the natural terminators for the *trnD* genes are replaced by a phage-encoded terminator embedded within the *int* coding sequence. To explore whether this Mx8-encoded terminator is important for *attP* function, we constructed two additional deletion derivatives of plasmid pAY994, pAY1046 and pAY1044, retaining this terminator but missing left-arm sequences upstream of the terminator. The data in Fig. 4 show that this terminator is not sufficient for P-arm function and may contribute little to *attP* function, because pAY1046 integrates at the same lower efficiency in this assay as does otherwise isogenic plasmid pAY1042, which lacks the terminator. Similarly, plasmid pAY1044 is as defective for integration as plasmid pAY1041. We obtained similar results from experiments in which we measured the efficiencies of formation of Sp^s Sm^s Km^r recombinants after coelectroporation of each of these plasmids with Sp^r Sm^r plasmid pAY952 (data not shown).

DISCUSSION

Unlike the situation for most other integrative elements, Mx8 site-specific recombination involves an *attP* site located within the *int* gene. In this study, we have shown that the *attP* site is required in *cis* for integration. Also, two lines of evidence show that sequences flanking both sides of the *attP* core are required for efficient Mx8 integration, as for the integration of other temperate phages. When an *int* gene with an inactive, internal *attP* site is expressed from the constitutive *mgl* promoter, it promotes *attP* × *attB* recombination between a second plasmid and the chromosome more efficiently than *attR* × *attB*, *attL* × *attB*, or *attB* × *attB* recombination (Fig. 3). When *int* is expressed from the *mgl* locus, it promotes recombination between a plasmid with *attP* and the *attB* locus more efficiently than between plasmids with deletions of one or both arms flanking the *attP* core and the *attB* locus (Fig. 4).

The *attB* locus on the *M. xanthus* genome is also unusual, because it has two *attB* sites. Integrated plasmids with the Mx8 *attP-int* genes are found at the *attB1* site much more frequently than at the *attB2* site, and integration often is accompanied by a deletion between the *attB1* and *attB2* sites. The result that Mx8 integrase can stimulate *attR* × *attB* recombination at a reasonably high efficiency (Fig. 3) may account in part for the spectrum of events that we observe when plasmids with the Mx8 *int-attP* genes integrate into the bipartite *attB* locus. For example, after integration into the *attB2* site, a subsequent *attR* × *attB1* recombination event would generate an integrated plasmid accompanied by a deletion between the two sites. However, this factor cannot be the only one that accounts for this bias, because simple integration events into the *attB1* site are more frequent than the sum of integration events into the *attB2* site and integration events accompanied by deletion. Therefore, we must conclude that differences in the sequences flanking the *attB1* and *attB2* cores also contribute to this site preference and that the *attB* arms may influence the assembly of the phage Mx8 intasome more than they influence such assembly in other phage site-specific recombination systems. Consistent with this idea, we found that *int*, when expressed in *trans* from the *mgl* locus, can mediate *attB* × *attB* recombination events at a considerable efficiency (Fig. 3).

It comes as no surprise that the expression of the *uoi* gene together with *int* inhibits *attP* × *attB* recombination. The *uoi* gene is predicted to encode an excisionase that resembles phage P22 excisionase in sequence (17) and likely changes the directionality of site-specific recombination.

Upon the integration of Mx8, the *attP* × *attB* integration reaction per se changes the structure of the *int* gene and thereby reduces the specific activity of the enzyme that catalyzes this reaction. Thus, Mx8 integrase is among the class of enzymes with specific activities regulated by reversible covalent modification. Most often the reversible covalent modification of an enzyme involves the modification of a single or only a few amino acid side chains, usually by a different enzyme. For example, the regulation of the specific activity of *E. coli* isocitrate dehydrogenase involves the phosphorylation and dephosphorylation of a serine residue by the AceK kinase-phosphatase (15, 16). The reversible modification of Mx8 integrase is unusual in two respects. This modification involves not only the activity of integrase, the specific activity of which it itself mod-

ifies, but also a dramatic change in the primary amino acid sequence of the integrase protein.

Several other phage integrases result in the reversible covalent modification of the coding sequence of an enzyme. The lambdoid coliphages e14 (9) and 21 (35) integrate into an *attB* site located within the 3′ end of the *E. coli* K-12 *icd* gene, which encodes isocitrate dehydrogenase. Integration of these phages results in the replacement of an alternative 165-bp 3′ end of *icd*. However, unlike the change in Mx8 *int* caused by integration, the change in *icd* is conservative and is predicted to have little effect on the specific activity of isocitrate dehydrogenase. Thus, the new 165-bp 3′ end is predicted to encode a new enzyme C terminus differing in amino acid sequence by only two conservative changes (9).

The purpose of this novel mechanism of reversible covalent modification is even more intriguing and provides us with a new example of how temperate phages have coadapted with their hosts in elegant ways to maintain the integrity of the lysogenic state. The purpose of this modification is to coordinate the different levels of superinfection immunity exhibited by Mx8 lysogens with the different levels of (potentially virulent) phage that are released spontaneously from lysogens as a result of excisive recombination.

M. xanthus lysogens are of two classes, low yielders and high yielders (24), which carry single and multiple, tandem prophages, respectively. Lysogens with single Mx8 prophages maintain a lower level of immunity than do those with tandem prophages. A wild-type stock of Mx8 plates with efficiencies of 10^{-5} on low yielders, with a single integrated copy of the Mx8 genome, and 10^{-9} on high yielders, with two or more tandem copies of the Mx8 genome. Thus, the relative level of superinfection immunity conferred upon a lysogen is dependent upon the number of integrated Mx8 prophages and presumably the dosage of the *imm* gene, which encodes the primary Mx8 repressor (29).

Low-yielder lysogens of DZ1 with a single Mx8 prophage express the *intX* gene. When such lysogens are grown to an exponential density of 4×10^8 cells/ml and treated with chloroform, a titer of 10^2 phage/ml is found in the cell-free supernatant. In contrast, high-yielder lysogens of DZ1 with multiple, tandem Mx8 prophages release 10^6 phage/ml (24 and data not shown). These high yielders express both the less active, IntX form of integrase and the more active, Int form of integrase because, in addition to *intX*, they can carry an intact *int* gene at the (*attP*) junction of the two tandem prophage genomes. These results again suggest that the IntX integrase has a lower specific activity than the Int integrase, in this case for promoting excisional (*attL* × *attR*) recombination of the Mx8 prophage.

In lysogens with either single or multiple prophage genomes, the titer of spontaneous phage released by each type of lysogen is lower than the titer of wild-type phage required to form plaques on each type of lysogen. The rare derivatives of wild-type Mx8 that form plaques on a lysogen are virulent mutant phages, which can lyse an exponential culture of a lysogen in short order. The simple correlation between the rate of spontaneous phage release and the level of superinfection immunity of single- and multiple-prophage lysogens explains why the *attP* site is located within the Mx8 *int* gene. Lysogens of *M. xanthus* with Mx8 prophages can coordinate the rate of excisional prophage recombination with the relative level of immunity conferred by the Mx8 prophages, which is dependent upon the copy number of integrated prophage genomes. Such a mechanism limits the rate at which virulent Mx8 mutants, which threaten the life of a lysogen, are released from the lysogen. It is likely that this amazing mechanism of phage-host adaptation is not a unique feature of Mx8 and *M. xanthus*. Because *Sulfolobus shibatae* virus SSV1 is the only other integrative element known to have an *attP* site within its *int* gene (22, 25), it is inviting to speculate that SSV1 also enjoys the same adaptation. Other myxophages may also share this mechanism. Many wild-type strains of *M. xanthus* carry long arrays of tandem repeats of an 80-kb sequence called Mxα, which gives rise to particles capable of mediating generalized transduction (32). These strains include *M. xanthus* FB, which appears to carry five tandem copies of the Mxα genome. Our wild-type strain DK1622, which was derived from FB by the use of UV mutagenesis and generalized transduction with Mx8 (12), carries only a single copy of the Mxα element (32). Recently, we have found that lysates of a strain derived from FB include phages that form plaques on host DZ1 (which carries a single Mxα prophage), suggesting that phage Mxα may share this mechanism (unpublished results). If this is the case, then the Mxα *int* gene should also promote the site-specific recombination of a plasmid and carry an *attP* site within its coding sequence, predictions that we are now testing.

ACKNOWLEDGMENT

This work was supported by a grant from the National Institute of General Medical Sciences (GM53392) to P.Y.

REFERENCES

1. **Andrews, B. J., G. A. Proteau, L. G. Beatty, and P. D. Sadowski.** 1985. The FLP recombinase of the 2 micron circle DNA of yeast: interaction with its target sequences. Cell **40:**795–803.
2. **Argos, P., A. Landy, K. Abremski, J. B. Egan, E. Haggard-Ljungquist, R. H. Hoess, M. L. Kahn, B. Kalionis, S. V. Narayana, and L. S. Pierson.** 1986. The integrase family of site-specific recombinases: regional similarities and global diversity. EMBO J. **5:**433–440.
3. **Azaro, M. A., and A. Landy.** 1997. The isomeric preference of Holliday junctions influences resolution bias by lambda integrase. EMBO J. **16:**3744–3755.
4. **Better, M., C. Lu, R. C. Williams, and H. Echols.** 1982. Site-specific DNA condensation and pairing mediated by the int protein of bacteriophage lambda. Proc. Natl. Acad. Sci. USA **79:**5837–5841.
5. **Campos, J. M., J. Geisselsoder, and D. R. Zusman.** 1978. Isolation of bacteriophage MX4, a generalized transducing phage for *Myxococcus xanthus*. J. Mol. Biol. **119:**167–178.
6. **Churchward, G., D. Belin, and Y. Nagamine.** 1984. A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. Gene **31:**165–171.
7. **Craig, N. L., and H. A. Nash.** 1983. The mechanism of phage lambda site-specific recombination: site-specific breakage of DNA by Int topoisomerase. Cell **35:**795–803.
8. **Craig, N. L., and H. A. Nash.** 1984. *E. coli* integration host factor binds to specific sites in DNA. Cell **39:**707–716.
9. **Hill, C. W., J. A. Gray, and H. Brody.** 1989. Use of the isocitrate dehydrogenase structural gene for attachment of e14 in *Escherichia coli* K-12. J. Bacteriol. **171:**4083–4084.
10. **Hoess, R. H., C. Foeller, K. Bidwell, and A. Landy.** 1980. Site-specific recombination functions of bacteriophage lambda: DNA sequence of regulatory regions and overlapping structural genes for Int and Xis. Proc. Natl. Acad. Sci. USA **77:**2482–2486.
11. **Hsu, P. L., W. Ross, and A. Landy.** 1980. The lambda phage *att* site: functional limits and interaction with Int protein. Nature **285:**85–91.
12. **Kaiser, D.** 1979. Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76:**5952–5956.
13. **Kashefi, K., and P. L. Hartzell.** 1995. Genetic suppression and phenotypic masking of a *Myxococcus xanthus frzF−* defect. Mol. Microbiol. **15:**483–494.
14. **Kolot, M., and E. Yagil.** 1994. Position and direction of strand exchange in bacteriophage HK022 integration. Mol. Gen. Genet. **245:**623–627.
15. **LaPorte, D. C., and T. Chung.** 1985. A single gene codes for the kinase and phosphatase which regulate isocitrate dehydrogenase. J. Biol. Chem. **260:**15291–15297.
16. **LaPorte, D. C., and D. E. J. Koshland.** 1983. Phosphorylation of isocitrate dehydrogenase as a demonstration of enhanced sensitivity in covalent regulation. Nature **305:**286–290.
17. **Leong, J. M., S. E. Nunes-Duby, A. B. Oser, C. F. Lesser, P. Youderian, M. M. Susskind, and A. Landy.** 1986. Structural and regulatory divergence among site-specific recombination genes of lambdoid phage. J. Mol. Biol. **189:**603–616.
18. **Magrini, V., C. Creighton, D. White, P. L. Hartzell, and P. Youderian.** 1998.

The *aadA* gene of plasmid R100 confers resistance to spectinomycin and streptomycin in *Myxococcus xanthus*. J. Bacteriol. **180:**6757–6760.
19. **Magrini, V., C. Creighton, and P. Youderian.** 1999. Site-specific recombination of temperate *Myxococcus xanthus* phage Mx8: genetic elements required for integration. J. Bacteriol. **181:**4050–4061.
20. **Magrini, V., D. Salmi, D. Thomas, S. K. Herbert, P. L. Hartzell, and P. Youderian.** 1997. Temperate *Myxococcus xanthus* phage Mx8 encodes a DNA adenine methylase, Mox. J. Bacteriol. **179:**4254–4263.
21. **Martin, S., E. Sodergren, T. Masuda, and A. D. Kaiser.** 1978. Systematic isolation of transducing phages for *Myxococcus xanthus*. Virology **88:**44–53.
22. **Muskhelishvili, G., P. Palm, and W. Zillig.** 1993. SSV1-encoded site-specific recombination system in *Sulfolobus shibatae*. Mol. Gen. Genet. **237:**334–342.
23. **Nunes-Duby, S. E., M. A. Azaro, and A. Landy.** 1995. Swapping DNA strands and sensing homology without branch migration in lambda site-specific recombination. Curr. Biol. **5:**139–148.
24. **Orndorff, P., E. Stellwag, T. Starich, M. Dworkin, and J. Zissler.** 1983. Genetic and physical characterization of lysogeny by bacteriophage Mx8 in *Myxococcus xanthus*. J. Bacteriol. **154:**772–779.
25. **Palm, P., C. Schleper, B. Grampp, S. Yeats, P. McWilliam, W. D. Reiter, and W. Zillig.** 1991. Complete nucleotide sequence of the virus SSV1 of the archaebacterium *Sulfolobus shibatae*. Virology **185:**242–250.
26. **Pargellis, C. A., S. E. Nunes-Duby, L. M. de Vargas, and A. Landy.** 1988. Suicide recombination substrates yield covalent lambda integrase-DNA complexes and lead to identification of the active site tyrosine. J. Biol. Chem. **263:**7678–7685.
27. **Ross, W., and A. Landy.** 1982. Bacteriophage lambda int protein recognizes two classes of sequence in the phage *att* site: characterization of arm-type sites. Proc. Natl. Acad. Sci. USA **79:**7724–7728.
28. **Ross, W., and A. Landy.** 1983. Patterns of lambda Int recognition in the regions of strand exchange. Cell **33:**261–272.
29. **Salmi, D., V. Magrini, P. L. Hartzell, and P. Youderian.** 1998. Genetic determinants of immunity and integration of temperate *Myxococcus xanthus* phage Mx8. J. Bacteriol. **180:**614–621.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Spratt, B. G., P. J. Hedge, H. S. Te, A. Edelman, and J. K. Broome-Smith.** 1986. Kanamycin-resistant vectors that are analogues of plasmids pUC8, pUC9, pEMBL8 and pEMBL9. Gene **41:**337–342.
32. **Starich, T., and J. Zissler.** 1989. Movement of multiple DNA units between *Myxococcus xanthus* cells. J. Bacteriol. **171:**2323–2336.
33. **Taketo, A.** 1988. DNA transfection of *Escherichia coli* by electroporation. Biochim. Biophys. Acta **949:**318–324.
34. **Tojo, N., K. Sanmiya, H. Sugawara, S. Inouye, and T. Komano.** 1996. Integration of bacteriophage Mx8 into the *Myxococcus xanthus* chromosome causes a structural alteration at the C-terminal region of the IntP protein. J. Bacteriol. **178:**4004–4011.
35. **Wang, H., C. H. Yang, G. Lee, F. Chang, H. Wilson, A. del Campillo-Campbell, and A. Campbell.** 1997. Integration specificities of two lambdoid phages (21 and e14) that insert at the same *attB* site. J. Bacteriol. **179:**5705–5711.
36. **Weimer, R. M., C. Creighton, A. Stassinopoulos, P. Youderian, and P. L. Hartzell.** 1998. A chaperone in the HSP70 family controls production of extracellular fibrils in *Myxococcus xanthus*. J. Bacteriol. **180:**5357–5368.
37. **Yagil, E., S. Dolev, J. Oberto, N. Kislev, N. Ramaiah, and R. A. Weisberg.** 1989. Determinants of site-specific recombination in the lambdoid coliphage HK022. An evolutionary change in specificity. J Mol. Biol. **207:**695–717.
38. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.