INTEGRATION OF THE λ GENOME IN THE ABSENCE OF SITE-SPECIFIC INTEGRATION FUNCTION: ISOLATION OF AN ABNORMALLY PERMUTED PROPHAGE λ

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Summary: The mechanism of integration of λbio11, which is deleted of all the known λ recombination genes, was studied using bio deleted hosts as recipients. The presence of recBC DNase and exoI in the recipient cells affected the fate of λbio11 DNA. In nine of ten immλ^+ transductants, insertion of the λbio11 genome took place somewhere between J and N and the remaining one had abnormally permuted prophage λ. In this lysogen (#42), the sequence of prophage genes was similar to that of vegetative phage λ. The properties of lysogen #42 were compared with those of other lysogens.

Introduction: The main features of site-specific integration and excision of λ phage have been elucidated from studies on phage mutants that cannot perform these processes (1). However, there is another type of virus-host interaction which remains to be clarified: namely, non-specific integration and excision of a viral genome (2). To understand the mechanisms of these processes, we studied the interaction of integration-defective (int) λ phage mutant with hosts and found a lysogen which has abnormally permuted prophage λ.

Abbreviations: bio, biotin; exoI, exonucleaseI; recBC DNase, product of the recB and recC genes; gam, phage gene whose product inhibits host recBC DNase; cI, gene that codes for the λ repressor; sus=sensitive to amber suppressors; del, deletion; T^r, ability to grow at 41°C. The other genetic symbols are those used by Taylor and Trotter (3) for E. coli and by Szybalski and Herskowitz (4) for λ.

Materials and Methods: The bio insertion-deletion phage, λcI857bio11, was used in this work as int⁻ phage(5). This phage carries a temperature-sensitive cI mutation and is deleted of λ recombination genes, int through gam. Inserted bio genes include at least intact bioB⁺ genes. The bioB⁺ gene product can convert dethiobiotin to biotin (6). To minimize the integration of the λbio genome through the homology of the bio region, we used bio deleted hosts as recipients (see Table 1 and ref. 7). Integration of λbio11 results in transduction and therefore a low frequency of integration can readily be detected by selecting transductants which can grow on minimal plates supplemented with dethiobiotin (6).

Results: First, the effect of the host Rec recombination system on integration of the λint phage genome was examined by measuring the frequency of transduction of the bioB⁺ gene carried by λbio11 into various bio deleted strains (Table 1). The results can be summarized as follows: 1) The most efficient recipient strain is JC7623, which lacks recBC DNase and the

Table 1 Transduction of the bioB⁺ gene by λbio11

Recipient strain	recA	recBC	sbcB	Frequency of bioB⁺ transductants	Fraction of immλ⁺ among bioB⁺
AB1157 rec⁺	+	+	+	3.2×10^{-5}	118/127
JC7623 rec⁺	+	-	-	5.2×10^{-4}	1/209
JC5495 rec⁻	+	-	+	$< 5 \times 10^{-7}$	—
JC5547 rec⁻	-	-	+	$< 5 \times 10^{-7}$	1/1
HfrH rec⁺	+	+	+	2.2×10^{-5}	not tested

For transduction experiments, bacteria were grown to about 2×10^9 cells per ml in broth, resuspended in 0.01 M $MgSO_4$, starved, and mixed with an equal volume of phage suspension in 0.01 M $MgSO_4$ to give an input multiplicity of about 8. The recipient strains were deleted of blu-attλ-bio regions. After adsorption (30 min at 30 °C) the mixture was diluted and plated on minimal agar containing dethiobiotin. The frequency of transduction is expressed as the ratio of bioB⁺ transductants to survivors. All bioB⁺ transductants are still bio⁻.

exoI (8), 2) In rec⁻ mutants (JC5495 or JC5547) the frequency of bioB⁺ transduction is at least 100-fold less than that in rec⁺ recipients, indicating that the host Rec system promotes transduction of the bioB⁺ gene carried by λbio11 into the bio deleted hosts, 3) In AB1157, where recBC DNase is functioning, 93 % of the bioB⁺ transductants were λcI857 lysogens, 4) In strain JC7623 (no recBC DNase, no exoI) the proportion of bioB⁺ transductants which are λ lysogens is drastically reduced to less than 0.5 % of the total bioB⁺ transductants. These results demonstrate that the presence of recBC DNase and exoI in the cells affects the fate of λbio11 DNA and changes the proportion of immλ⁺ lysogens among bioB⁺ transductants (9).

All the bioB⁺ transductants immune to the λ phages were able to produce phage at 41 °C, indicating that most of the prophage genome is present within the bioB⁺ transductants. Int-promoted insertion breaks of the phage chromosome at attP, P', thereby generating a characteristic prophage map which differs from the phage map by a cyclic permutation of the markers (1). Since λbio11 has no int gene at all, it seems likely that a prophage permutation in some bioB⁺ transductants must have been different from that of int promoted insertion. Ten independently isolated bioB⁺ transductants of the HfrH rec⁺ (gal-bio)del strain were purified on minimal plates supplemented with dethiobiotin and were incubated at 41 °C for 1 to 2 days. Prophage maps were constructed by testing these temperature-resistant strains for residual phage markers (see legend to Table 2). One of ten transductants (lysogen #42) gave unusual results on deletion mapping (Table 2), indicating that insertion of the λbio11 genome occurred between A and R (Fig. 1a). On the contrary, in nine of ten

Table 2 Prophage deletion mapping

Lysogen No.	T^r strain No.	*bioB* gene	N	*cI*	P	Q	R	A	F	J	*
42	42- 1	+	-	-	-	-	-	+	+	+	30
	16	+	-	-	-	-	+	+	+	+	8
	9	+	+	-	-	-	+	+	+	+	2
	48	+	-	-	-	+	+	+	+	+	1
	45	+	-	-	+	+	+	+	+	+	2
	42- 59	-	-	-	-	-	-	-	-	-	45
	122	-	-	-	-	-	+	-	-	-	1
	63	-	-	-	+	+	+	-	-	-	1
	67	-	-	-	+	+	+	+	-	-	1
	52	-	-	-	-	-	-	+	+	+	1
415	415- 1	+	-	-	-	-	-	-	-	-	1
	2	+	-	-	-	-	-	-	-	+	1
	4	+	+	-	-	-	-	-	-	+	2
	5	+	-	-	-	-	-	+	+	+	1
	6	+	-	-	-	-	+	+	+	+	1
59	59- 1	+	-	-	-	-	-	-	-	-	2
	3	+	-	-	-	-	-	-	-	+	9
	11	+	-	-	-	-	-	+	+	+	3

Survivors after treatment with *bioB*$^+$ lysogens at 41°C were isolated on minimal plates supplemented with dethiobiotin or on nutrient broth plates. Only one T^r survivor was taken from each culture; hence each isolate is independent. The presence of a given λ marker in a prophage deletion mutant was determined by spotting 10^6 to 10^7 particles of a λ*sus* mutant on a nutrient broth plate containing approximately 10^8 cells from an exponential culture of the bacterial deletion mutant. The presence of a given allele in a particular deletion mutant generally resulted in confluent lysis at the location of the phage spot (7). *, Number of independent isolates.

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bioB$^+$ transductants, the prophage map of the inserted λ*bio*ll genome was similar to that of normal λ lysogen, *i.e.* the insertion of the λ*bio*ll genome took place somewhere between J and N at least (Fig. 1b). The typical results obtained with two *bioB*$^+$ lysogens of this group (#415 and #59) are shown in Table 2.

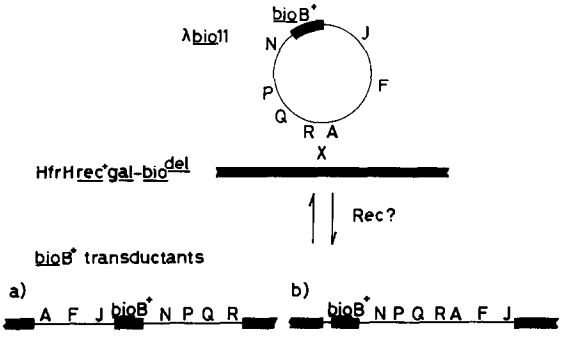

Fig. 1. Insertion and excision of the λbio11 chromosome. The dark thick lines represent a bacterial gene and a bacterial chromosome. The narrow continuous line represents a λ chromosome. Insertion is thought to occur by breakage and reciprocal exchange of the two participating chromosomes at a specific crossover locus, a) between A and R (lysogen #42), and b) between N and J (lysogen #415 or #59, see Table 2). Genes A, F, J, N, P, Q and R are phage markers (4) and genes bio and gal are bacterial markers (3).

The general properties of the abnormally permuted prophage lysogen (#42) were compared with those of other lysogens (Table 3). Strain #42 was sensitive to λcIcl7 and was therefore a single lysogen (7). The average number of viable phages liberated after heat induction of this lysogen was extremely small (about 10^{-6}/cell). Inefficient phage production is assumed to be a consequence of insertion by a cross between A and R. The frequency of spontaneous curing was about 1,000-fold higher in this lysogen (#42) than in normal λcI857 lysogen; normal λcI857 lysogens or λcI857 within trpC lysogens are efficiently cured by a brief heat pulse (7, 10), but this was not observed in #42 or in #415. Two phage functions, Int and Xis, are normally required for prophage excision (1). These functions are also required for prophage excision from various secondary sites (7, 10). To test this point, the abnormal lysogen was superinfected with λimm^{21}, λimm^{21}int,

Table 3 General properties of abnormally permuted λ lysogen

Lysogen	HfrH	#42	#415
Prophage gene order	N-R-A-J	A-J-N-R	N-R-A-J
Frequency of spontaneous curing	1.4×10^{-6}	1.5×10^{-3}	1.4×10^{-4}
Heat-pulse curing	3.7×10^{-1}	1.8×10^{-2}	8.9×10^{-4}
Burst-size (phage/cell)	$1.4 \times 10^{+2}$	1.1×10^{-6}	9.3×10^{-1}
Superinfection curing			
λ*imm*^{21}	5.4×10^{-1}	2.8×10^{-3}	1.8×10^{-3}
λ*imm*^{21}*int*29	2.1×10^{-2}	2.4×10^{-3}	2.1×10^{-4}
λ*imm*^{21}*xis*6	2.2×10^{-2}	3.2×10^{-3}	5.0×10^{-4}

The lysogens were HfrH (λ*c*I857 within the normal attachment site), #42=HfrH (*gal*-*bio*)*del* (λ*c*I857*bio*11 with abnormal permutation) and #415=HfrH (*gal*-*bio*)*del* (λ*c*I857*bio*11 with normal permutation). The frequencies of spontaneous λ phage curing, heat-pulse curing and superinfection curing and burst-size were measured as described previously (7).

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and λ*imm*^{21}*xis*. It was found that none of these λ*imm*^{21} phage superinfections had any effect on this abnormally permuted prophage curing.

Discussion: We studied specialized transduction with λ*bio*11 and observed two classes of transductants, *i.e.* *bio*B^{+}*imm*λ^{+} and *bio*B^{+} *imm*λ^{-} (Table 1). In the *rec*BC*sbc*B strain, *bio*B^{+} *imm*λ^{-} transductants constituted more than 99 % of the total transductants. This may be the result of increased availability of a particular type of λ*bio*11 DNA, such as DNA molecules with a linear structure, due to the absence of *rec*BC DNase and *exo*I (9). Circular λ*bio*11 DNAs are resistant to degradation by *rec*BC

DNase and exoI, and survive within the wild type cells. The integration of these molecules gives bioB$^+$immλ$^+$ transductants (Table 1).

An int$^-$ point mutant can be integrated into several abnormal chromosomal locations at an exceedingly low frequency and this integration is probably promoted by the host Rec system (2). The prophage gene order of these abnormally situated λ has not been determined. The present work shows that one bioB$^+$ transductant out of ten, obtained after infecting λbio11, had abnormally permuted prophage λ. In this lysogen (#42), the phage genome was inserted close to the junction of the two ends of the mature linear molecule (=cos) (11) and the sequence of prophage markers was similar to that of vegetative phage λ. Formation of this lysogen seems to depend on host Rec function (Table 1) and indicates the presence of homology between the phage A-R region and the E. coli chromosome. Sternberg & Weisberg studied the formation of generalized transducers of λ phage and their results suggest the presence of many cos-like sites on the E. coli chromosome (12). Study of this abnormally permuted prophage λ will be valuable for understanding the evolution of a specific λ integration-excision system. Moreover, the integration of λ genome not promoted by phage int may be a good model for studies on the mechanism of the primitive integration system of small oncogenic animal viruses.

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