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Isolation and Characterization of Specialized Lambda Transducing Bacteriophage Carrying the *metBJF* Methionine Gene Cluster

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Secondary attachment site lysogens of $\Delta att^{\lambda}\Delta$*ppc-argECBH* strains of *Escherichia coli* with λ*c*I857 integrated into the *bfe* gene (88 min) were isolated. Of 20 such lysogens examined, 2 produce lysates with transducing phage containing the *metBJF* gene cluster (87 min). Reintroduction of the *ppc-argECBH* chromosome segment (which lies between the *bfe* and *met* genes) into these strains virtually abolishes the production of *met* transducing phage. All of the phage examined have lost essential genes from the left arm of the λ chromosome. Approximately 85% of the phage appear to have the same genetic composition, containing the *metBJF* gene cluster, but not the closely linked gene *cytR*, and having lost phage genes *G* and *J*. Analytical CsCl density gradient centrifugation of five representatives of this major class of phage shows four of them to have identical densities (lighter than λ), while the fifth cannot be resolved from λ. The four apparently identical phage were isolated from three separate lysates, which suggests the existence of preferred sites for illegitimate recombination on the bacterial and phage chromosomes. Three specialized transducing phage that carry *cytR* in addition to *metB*, *metJ*, and *metF* have also been studied. Each of these viruses has a different amount of phage deoxyribonucleic acid. Two of them have less deoxyribonucleic acid than λ, whereas the third has about the same amount. The *metB*, *metF*, and *cytR* genes of the transducing phage have been shown to function in vivo. The phage-borne *metB* and *metF* genes are subject to *metJ*-mediated repression.

The methionine biosynthetic pathway of *Enterobacteriaceae* is rather complex, with both divergent and convergent branches (8). The structural genes for the enzymes of the pathway are dispersed on the *Escherichia coli* chromosome, and mutations at a single locus (*metJ*) cause constitutive synthesis of all of them (13, 18). Although the *metJ* gene has no known function other than that of regulation of the methionine biosynthetic enzymes, there is no direct evidence that the product of *metJ* is a repressor. The availability of purified *met* deoxyribonucleic acid (DNA) would assist the investigation of this regulatory system by allowing the measurement of messenger ribonucleic acid levels, specific DNA binding properties of putative repressors, and perhaps in vitro regulation of transcription and translation. We thus set about to isolate lambda *met* transducing phage to supplement the few φ80d*met* phage (17, 24). By using the technique of Shimada et al. (25, 26), we isolated lysogens with lambda inserted in *bfe* in a strain with the *ppc-argECBH* region deleted to shorten the distance between the prophage and the *metBJF* gene cluster. This paper describes a new type of *bfe* insertion lysogen and the properties of several λd*met* transducing phage isolated from such lysogens.

MATERIALS AND METHODS

Materials, media, and strains. *O*-succinyl homoserine was synthesized by the method of Flavin and Slaughter (9). 5-Methyltetrahydrofolate ($^{14}CH_3$) from the Amersham/Searle Corp. was purified before use by a procedure similar to that of Coward et al. (5). All other chemicals were from standard commercial sources.

LB broth medium, methionine-free medium, and standard minimal medium were prepared as described by Greene et al. (12). Tryptone broth medium was prepared as described by Gottesman and Yarmolinsky (11), using deionized water that contained 0.01% antifoam (FG-10, Dow Corning).

Strains employed for the construction of secondary attachment site lysogens were derived from KS302 [HfrH no suppressor, Δ(*gal-bio*) *thi*] (26), and

strains employed for the analysis of λd*met* transducing phage were derived from JJ100, which is AB301 (*metB1 relA1*) cured of λ. Derivative strains are described as used in the text. λ*c*I857 was used.

Enzyme assays. Semiquantitative methionine adenosyltransferase (EC 2.5.1.6) assays were performed by a modification of the procedure of McKensie and Gholson (19). Reaction mixtures (0.1 ml) with [^{14}C]adenosine 5'-triphosphate were incubated with toluenized cells and, after 20 min at 37°C, 50-μl volumes were applied to Whatman P81 phosphocellulose disks. The disks were washed three times with 0.1 M lithium formate, pH 3.0, and counted. Cystathionine γ-synthase (EC 4.2.99.9) was assayed and protein was determined as previously described (12, 13). N^5,N^{10}-methyltetrahydrofolate reductase (EC 1.1.1.68) was assayed by measuring the oxidation of N^5-methyltetrahydrofolate to N^5,N^{10}-methylenetetrahydrofolate by menadione, using a modification of the procedure of Donaldson and Keresztesy (7) with measurement of formaldehyde exchangeable radioactivity by the procedure of Taylor and Weissbach (28).

Isolation of lysogens and preparation of specialized transducing phage. Secondary attachment site lysogens with prophage λ integrated within the *bfe* gene were isolated by a modification of the procedures described by Kirschbaum and Konrad (16) and Shimada et al. (25).

Low-frequency transducing (LFT) lysates of secondary attachment site lysogens were prepared by heat induction of cultures for 15 min at 42°C followed by incubation at 39°C with vigorous shaking for 2 to 3 h. At the end of the induction period, the cell debris was removed by centrifugation and the supernatant was stored over chloroform. High-frequency transducing (HFT) lysates of defective λd*met* transducing phage and helper phage were made by the procedure of Zubay (29) except for use of the heat shock procedure described above for LFT lysates and tryptone broth medium. Bacteriophage from high-titer HFT lysates were purified by CsCl density gradient centrifugation by the procedure of Miller (21) with minor modifications. The procedure of Bellett et al. (2) was used for the precise determination of phage density.

Genetic manipulations. Recipient cells for transductions with λd*met* were grown to midlog phase in LBλ medium (LB medium supplemented with 10 mM $MgSO_4$, 5 mM $CaCl_2$, and 0.25% maltose), washed twice with 0.9% saline, and starved by incubation in 0.1 M $MgSO_4$ for 45 min at 32°C. Mixtures of transducing phage and recipient bacteria were diluted, spread on selection plates, and incubated at 32°C for 48 h. Since LFT lysates of the secondary site lysogens have very low concentrations of phage, after phage absorption it was necessary to concentrate the recipient cells before spreading on selection plates.

The presence of specific λ genes in the defective transducing phage was tested by marker rescue with a series of nine amber mutants of λ*c*I857 (having mutations in genes *A, C, E, F, G, J, N, P*, or *Q*). Lysogens under examination were infected with each of the λ*c*I857 amber strains, and the infected cells were heated at 42°C for 10 min and spotted on LBλ plates with a soft agar layer containing HfrH (no suppressor). The plates were incubated at 37°C overnight, and complementation was scored by the appearance of hundreds of plaques or confluent lysis in the area of the applied spot.

To isolate *metJ* derivatives of the transducing phage, single λd*met* lysogens of *metJ* cells were constructed. About 5 × 10^8 of the *metJ*$^+$/*metJ* heterodiploid cells were spread on minimal plates containing 0.04 M DL-methionine (27) that had been seeded with about 2 × 10^9 to 4 × 10^9 λ*b2c*. After incubation at 32°C for 35 h, 10 to 20 colonies appeared on each plate. Colonies were picked, purified, and tested for immunity and for MetJ phenotype by semiquantitative assay of methionine adenosyltransferase activity of cells grown on complete medium. The presence of *metJ* transducing phage was verified by the production of HFT lysates with the expected transducing activity. It is not clear whether these *metJ* phage arose by spontaneous mutation or by incorporation of the bacterial *metJ* allele into the prophage chromosome.

All other genetic manipulations were done as described by Miller (21) with minor modifications.

RESULTS

Isolation and characterization of specialized *met* transducing phage. To shorten the distance between *bfe* and *metBJF*, the *ppc-argECBH* deletion (20) of strain 514-2R$^+$1 was introduced by P1 transduction into two *metB* derivatives of KS302. The resultant Δ*ppc-argECBH*Δ*att*^{λ} strains were infected with λ*c*I857, grown on LB medium, infected with bacteriophage BF23, and spread on plates seeded with λ*b2c*. Resistant colonies were obtained at a frequency of 3 × 10^{-8} from cells subjected to the BF23, λ*b2c* selection procedure. In approximately 25% of these strains, the BF23 resistance appeared to result from the insertion of λ*c*I857 into *bfe* as judged by the return of BF23 sensitivity after curing by a heat pulse (3, 16). Lysates prepared by heat induction of 20 of these strains were tested for their ability to transduce *metB* and *metF* cells to prototrophy. Only two of the strains (JJ3004 and JJ4021) gave lysates with significant levels of *met* transducing phage. Lysates of JJ3004 and JJ4021 contain very few plaque-forming units (PFU) (2 × 10^3 to 3 × 10^3/ml), but do contain an unusually high proportion of transducing phage carrying the *metB* and *metF* genes of the host bacterium (approximately two transducing phage per 100 PFU). Reintroduction of the *ppc-argECBH* chromosome segment (strains JJ8004 and JJ8021) essentially abolishes the production of *met* transducing phage, although lysates of these cells contain reasonable numbers of phage that carry the *ppc-argECBH* gene cluster (approximately two transducing phage per 1,000 PFU). The curing rates before and after a

heat pulse and the burst sizes of JJ3004 and JJ4021 are significantly lower than those of representatives (JJ3013 and JJ4014) of the 18 lysogens that failed to yield *met* transducing phage, suggesting different secondary attachment sites in these two types of lysogens.

Approximately 440 lysogenic *met* transductants originating from five independently prepared LFT lysates of either JJ3004 or JJ4021 have been analyzed. More than 99% of these transductants contain specialized transducing phage that are capable of transducing both *metB* and *metF E. coli* strains to the prototrophic state. Most of these *met* transductants are incapable of producing PFU or transducing particles upon heat induction. However, superinfection of the lysogens with a helper phage at the time of induction results in the production of both PFU and *met* transducing particles. These results indicate that the specialized *met* transducing phage are defective and that most of the lysogens do not carry a helper phage.

One hundred singly lysogenic *met* transductants isolated from five independently prepared LFT lysates of JJ3004 and JJ4021 were tested for phage genes by marker rescue with nine amber mutants of λ. All of the transducing phage had lost phage genes mapping to the left of the attachment site on the vegetative phage chromosome. About 85% of the phage gave identical patterns of marker rescue, complementing mutations in genes *A, C, E, F, N, P,* and *Q* while failing to complement mutations in genes *G* or *J*. The remaining 15% gave a variety of marker rescue patterns. According to the Campbell model for prophage integration and excision (4), the λ prophage inserted within the *bfe* gene of strains JJ3004 and JJ4021 must have the orientation diagramed in Fig. 1.

Production of HFT lysates and properties of the λ*dmet* phage. Several λ*dmet* phage were selected for a more detailed study. To produce high-titer lysates of these viruses, the lysis-defective mutation *amS*7 (10) was introduced by crosses with λ*c*I857*amS*7. The HFT lysates prepared from these transductants were purified on block CsCl gradients and banded on equilibrium density CsCl gradients. Two phage bands were clearly visible in the equilibrium CsCl density gradients of HFT lysates of λ*dmet*102, λ*dmet*117, λ*dmet*122, λ*dmet*126, λ*dmet*128, and λ*dmet*132, but only single bands were seen in the gradient tubes of λ*dmet*127 and λ*dmet*136. Fractionation of those equilibrium gradients with two bands showed the transducing activity to be associated with the upper, lower-density bands and the plaque-forming activity with the lower, heavier-density bands.

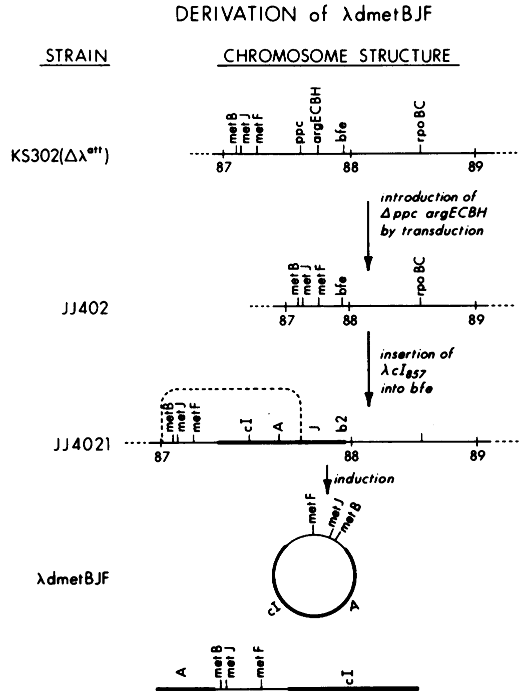

FIG. 1. *Diagram of the events leading to the formation of λdmet transducing phage. The deletion Δ(ppc-argECBH) of strain 514 2R+1 (20) was introduced into strain KS302 by P1 transduction. The resulting transductant, JJ402, was lysogenized by λcI857, and the phage BF23-resistant lysogen, JJ4021, was isolated as described in the text. During the induction of JJ4021, specialized λdmet transducing phage were formed through aberrant excision of the prophage (dotted line). The genetic structure of a representative λdmet phage of this type is shown at the bottom of the figure.*

All of the specialized transducing phage listed in Table 1 are able to transduce *metB* and *metF* auxotrophs to prototrophy, indicating that they carry functional *metB* and *metF* genes. When these phage are used to transduce *metJ* recipients, the resultant lysogens regain the ability to repress the synthesis of methionine adenosyltransferase, which shows the presence of a functional allele of *metJ* on the phage chromosome. The *glpK* locus is about 0.5 min counterclockwise from *metB* on the *E. coli* chromosome (1, 6). None of the lysogens of the *met* transducing phage in a glycerol kinaseless host can utilize glycerol as a carbon source. Thus, it appears that none of the segment of bacterial DNA is large enough to include a functional *glpK* locus.

Approximately halfway between *glpK* and *metB* on the map of Bachmann et al. (1) lies the *cytR* gene, which codes for the repressor protein controlling the synthesis of two cytidine-cata-

TABLE 1. *Properties of phage particles*

Phage	Bacterial genes	Phage genes[a]	Density (g/cm)[b]	Calculated genome size (kilobases)
λd*met*102	*metBJF*	*N, P, Q, A-F*	1.4839	43.8
λd*met*117	*metBJF*	*N, P, Q, A-F*	1.4839	43.8
λd*met*122	*metBJF*	*N, P, Q, A-F*	1.4838	43.8
λd*met*126	*metBJF*	*N, P, Q, A-F*	1.4840	43.8
λd*met*127	*metBJF*	*N, P, Q, A-F*	1.4915 (approximate)	46.8 (approximate)
λd*met*128	*cytR metBJF*	*N, P, Q*	1.4754	40.5
λd*met*132	*cytR metBJF*	*N, P, Q, A*	1.4880	45.4
λd*met*136	*cytR metBJF*	*N, P, Q, A-C*	1.4903 (approximate)	46.3 (approximate)

[a] Marker rescue experiments were performed as described in the text with phage containing amber mutations in gene *N, P, Q, A, C, E, F, G,* or *J*.

[b] Density and genome sizes of the phage particles relative to λ*c*I857*am*S7 were measured by the method of Bellett et al. (2), using λ*c*I857*am*S7 and λ*b2c* as reference markers. λ*c*I857*am*S7 was taken to have a density of 1.4915 g/cm^3 (2) and a genome size of 46.8 kilobases (20).

bolizing enzymes, cytidine deaminase and uridine phosphorylase (1, 22). A spontaneous *cytR* mutant of the *metB* strain JJ100 was isolated by procedures described by Munch-Petersen et al. (22). The *metB cytR* isolate, JJ90, was transduced to *metB*$^+$ by using HFT lysates, and single lysogens of each of the defective transducing phage were isolated. Cultures were grown, and cytidine deaminase assays were performed on cell extracts. Only extracts of JJ90 lysogens containing λd*met*128, λd*met*132, or λd*met*136 have cytidine deaminase activities similar to that of the *cytR*$^+$ strain JJ100, suggesting that these phage carry *cytR*$^+$ alleles. This conclusion is supported by the observation that heat pulse-cured derivatives of these strains have the CytR phenotype of their parent strain JJ90.

Five of the specialized transducing phage (λd*met*102, λd*met*117, λd*met*122, λd*met*126, and λd*met*127) are of the predominant class, carrying bacterial *metB*, *metJ*, and *metF* genes and phage genes *N, P, Q, A, C, E,* and *F*, but not *G* or *J*. Four of these viruses have the same density and may be identical. If these phage are identical, the same excision event must have occurred three times since they were isolated from three different lysates. The fifth phage (λd*met*127), with the same apparent gene content, has a larger amount of DNA. The remaining viruses all have bacterial genes *metB*, *metJ*, *metF*, and *cytR*, but each has a different content of phage genes. Thus, there is no obvious preferred recombination site on the phage chromosome in those viruses with enough bacterial DNA to include *cytR*.

For a quantitative study of the expression of the *met* genes of the specialized transducing phage, a series of heterodiploid lysogens with various combinations of *metB*, *metF*, and *metJ* alleles was constructed. Table 2 shows the cystathionine γ-synthetase or methylenetetrahydrofolate reductase activities of some λd*met*102 lysogens and of nonlysogenic control strains. Similar results have also been obtained with derivatives of λd*met*117. Although the differences in cystathionine γ-synthetase activities are more striking than those of methylenetetrahydrofolate reductase, both enzymes show the same qualitative results. When both the phage and the bacteria contain a *metJ* allele, the phage-borne *metB*$^+$ or *metF*$^+$ genes are expressed at about half the level of the corresponding genes in the chromosome of a *metJ* nonlysogen. The reduced expression of the *met* genes in the prophage may be caused by the presence of two copies of a partially functional *metJ* allele in the lysogenic strains, but regardless of the reason for the difference, it is clear that the phage-borne *metB* and *metF* genes can direct the synthesis of substantial amounts of enzymes. If the lysogens contain a functional *metJ* gene either in the bacterial or prophage chromosomes, expression of the *metB* and *metF* genes of the specialized transducing phage is repressed. In addition to confirming the existence of functional *metB*, *metF*, and *metJ* genes on the λd*met* phage, these results suggest that the control sites of the *metB* and *metF* genes are intact.

DISCUSSION

Only a few of the *bfe* insertion lysogens (2 out of 20 isolates tested) can produce specialized *met* transducing phage. All of the specialized *met* transducing phage that were tested are missing genes from the left arm of the λ chromosome. From this observation and the Campbell model for prophage excision (4), the orientation of the prophage inserted in *bfe* must be as diagramed in Fig. 1. Kirschbaum and Konrad (16) and Ikeuchi et al. (14) used λ insertion into *bfe* to isolate specialized transducing phage

TABLE 2. *Enzyme activities of λdmet lysogens*[a]

Strain	Relevant genotype		Enzyme activity[b] (nmol/min × mg of protein)	
	Cell	Phage	Cystathionine synthetase	Methylenetetrahydrofolate reductase
JJ116	*metB*$^+$ *metJ*$^+$		2.2	
JJ118	*metB*$^+$ *metJ*		53.3	
JJ100	*metB metJ*$^+$		0.0	
JJ131	*metB metJ*		0.0	
JJ255	*metB metJ*	*metB*$^+$ *metJ*	24.5	
JJ201	*metB metJ*	*metB*$^+$ *metJ*$^+$	1.6	
JJ261	*metB metJ*$^+$	*metB*$^+$ *metJ*	1.9	
JJ116	*metF*$^+$ *metJ*$^+$			0.03
JJ118	*metF*$^+$ *metJ*			0.33
JJ122	*metF metJ*$^+$			0.03
JJ126A	*metF metJ*			0.02
JJ293	*metF metJ*	*metF*$^+$ *metJ*		0.18
JJ275	*metF metJ*	*metF*$^+$ *metJ*$^+$		0.04
JJ285	*metF metJ*$^+$	*metF*$^+$ *metJ*		0.04

[a] The *metJ* allele was isolated as a spontaneous mutant of JJ100 to yield JJ131. All other strains were constructed by the transduction of JJ100 or its derivatives. Phage are derivatives of λ*dmet*102. Cultures were grown at 32°C.

[b] Enzyme activities were measured at 37°C.

containing the *rpoB* gene (β subunit of ribonucleic acid polymerase), which lies on the other side of *bfe* from the *met* genes. The λ*drif* phage isolated by both laboratories are also missing phage genes from the left arm of the λ chromosome (14, 15), which implies an opposite orientation of the inserted phage from that shown in Fig. 1. The 18 insertion lysogens that do not generate detectable levels of *met* transducing particles may be similar to the *bfe* insertion lysogens isolated in these laboratories. Mazaitis et al. (20) described four λ*dargECBH* phage isolated from lysates of mixed secondary attachment site lysogens. Two of these *arg* transducing phage contain the *bfe* gene and appear to arise from a secondary integration site that lies clockwise from *bfe* on the *E. coli* chromosome. The other two *arg* phage apparently both arose from another secondary attachment site that is either in *bfe* or between *argH* and *bfe*. This site may be the same as that which gave rise to the *met* phage described here. Thus, there are at least three secondary prophage attachment sites in the 88-min region of the *E. coli* chromosome.

Lysates of the two *bfe* insertion lysogens used in this work (JJ3004 and JJ4021) contain few PFU with an unusually high proportion of *met* transducing phage (about two per 100 PFU). Within the resolution of the complementation tests done, about 85% of the λ*dmet* phage have the same gene content, and four out of the five phage of this type that were studied in more detail have the same DNA content. If these four phage are identical, the same excision event must have occurred at least three times since they were isolated from three independent lysates. These results suggest the existence of a hot spot for illegitimate recombination on the bacterial chromosome near *metB*. Although this phenomenon is of interest, it has rendered the isolation of different types of transducing phage more difficult. The remaining 15% of the λ*dmet* phage consist of a number of different types, but excision events within the *met* gene cluster are rare. Only 2 of the more than 400 λ*dmet* phage examined have contained *metF* but not *metB*.

Two laboratories have previously isolated specialized transducing phage containing *met* genes from the 87-min region of the *E. coli* chromosome (12, 24). The specialized *met* transducing phage isolated here are in a pure λ background, whereas the previously isolated *met* phage are either in λh80 (24) or in φ80 (17). The λ*dmet* phage also have some differences in gene content from the other *met* transducing phage and, if the structure diagramed in Fig. 1 is correct, the orientation of the *met* genes is the opposite of that in φ80 *dmet* and λh80 *dmet* (23). Because of these differences, the new viruses should be useful for various purposes, such as a probe for the hybridization of ribonucleic acid from in vitro transcription of φ80 *dmet* or vice versa.

The *metB*, *metF*, and *metJ* genes of the

λd*met*102 and λd*met*117 transducing phage are expressed in vivo, and the *metB* and *metF* genes are subject to *metJ*-mediated repression. DNA from these phage has been shown to direct the in vitro synthesis of the *metB* gene product (J. R. Johnson, J. H. Krueger, and R. C. Greene, Fed. Proc. 35:1567, 1976). Thus, these phage may provide useful probes for the study of expression and regulation of *met* genes.

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