Properties of Lambda Transducing Bacteriophages Carrying R100 Plasmid DNA: Mercury Resistance Genes

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Three λ mer (resistance to Hg^{2+} and mercurials) transducing phages were prepared from three independent cointegrate isolates of bacteriophage λ and plasmid R100. DNA heteroduplex and restriction nuclease analyses of the λ mer DNA showed that all three phages had resulted from λ insertion at kilobase coordinate 8.6 of plasmid R100, followed by loss of different lengths of λ DNA and replacement with different lengths of R100 DNA. Two of the λ mer phages were defective, containing deletions from λatt through the λN gene and into the λrex gene; the third, VA λ 14, was an N^+ Spi $^-$ plaque-forming phage. With VA λ 14, N -dependent transcription of R100 *mer* from the λp_L promoter suggested that transcription of *mer* proceeded in the direction from *IS1b* toward the sulfonamide resistance determinant (i.e., from a plasmid promoter in restriction nuclease fragment *EcoRI*-H toward fragment *EcoRI*-I). Phage-directed protein synthesis in a UV-irradiated *lind* $^-$ lysogen showed the Hg^{2+} -inducible synthesis of three major polypeptides of molecular weights 68,000, 11,500, and 8,500 and three minor ones of molecular weights 54,000, 33,000, and 13,500. The largest of the major polypeptides is identified as the subunit of the mercuric reductase enzyme. The functions of the smaller polypeptides are not known. Hg^{2+} reductase enzyme assays confirmed the regulation of *mer* synthesis during phage infection.

Plasmid-determined mercuric resistance in bacteria is due to the enzymatic transformation of Hg^{2+} to Hg^0 , which is volatilized from the growth media (22, 24). In *Escherichia coli*, mercuric resistance is always plasmid determined and never chromosomal; it is also always accompanied by resistance to the organomercurials merbromin and fluorescein mercuric acetate (22, 29). These latter resistances do not result from enzymatic degradation and volatilization, but are thought to be due to alterations in cellular permeability to the organomercurials (29). In all tested cases, resistance to Hg^{2+} and the organomercurials is inducible and appears only after exposure to Hg^{2+} or organomercurials (23, 24). Cloning of restriction nuclease fragments of plasmid NR1 (identical to R100) and restriction nuclease analysis of Tn3 mutants of plasmid R100 have shown that the determinants for the mercuric-mercurial resistance system are found on the *EcoRI* restriction nuclease fragments H and I (12, 16; T. Foster, H. Nakahara, A. A. Weiss, and S. Silver, in preparation).

During studies of R100:: λ cointegrates, we found two cointegrate molecules in which λ had

inserted between the genes governing sulfonamide resistance (*sul*) and mercuric resistance (*mer*) in the resistance determinant of R100 (8). Preliminary studies indicated that the *mer* genes could be readily isolated as part of specialized λ transducing phages from these cointegrate molecules (7, 8). We recently found that a third R100:: λ cointegrate molecule also had λ inserted between *sul* and *mer*, and we report here the characterization of the λ mer phages isolated from all three cointegrate molecules. Such a characterization provides information about the number and size of the proteins whose synthesis is induced by mercuric ion as well as DNA structural information about the site of λ insertion and the R100 DNA carried by the λ mer phages. A preliminary report on these phages has been presented (7).

MATERIALS AND METHODS

Strains. Most of the bacteria, plasmids, and phages used in this work are listed in Table 1; the rest have been described elsewhere (8). Isolation of the original R100::ED λ 4 cointegrates and the λ mer phages ED λ 26 and ED λ 27 was described earlier (8).

TABLE 1. *Strains used*

Strain	Description	Source or Reference
Bacteria		
Ymel(P2)	<i>mel-1 supE57 supF58</i> (P2)	B. Bachmann
ED7133	ED2149(pEDR120)	This work
NO1348	<i>uvrA galK</i>	R. Jaskunas
NO1626	λ ind ⁻ lysogen of NO1348	R. Jaskunas
VA7486	ED2149(pEDR120)	This work
ED2149	Δ (<i>nia gal att\lambda bio</i>) <i>tsx</i>	8
ED3814	<i>supF Lac\Delta x74 tsx Str</i> ^r	8
ED395	<i>Lac\Delta U124</i> mutant of W3110	8
N3098	<i>lig-7(Ts) supF</i>	4
J53	<i>proA metF</i> (λ)	23
VA7582	W3110 (ED\lambda26) (ED\lambda7)	This work
VA7596	ED395 (ED\lambda27) (ED\lambda7)	This work
VA7598	ED395 (VA\lambda14) (ED\lambda4)	This work
JC3272	<i>his trp lys str gal lac \lambda'</i> (λ def)	1
Plasmids		
R100	Tet ^r Cml ^r Str ^r Sul ^r Mer ^r	8
pEDR120	R100::ED\lambda4	This work
pVAR30	Tet ^r Cml ^r Str ^r Sul ^r Mer ^r mutant of pEDR120	This work
pVAR31	Tet ^r Cml ^r Str ^r Sul ^r Mer ^r mutant of pEDR120	This work
pVAR33	Tet ^r Cml ^r Str ^r Sul ^r Mer ^r mutant of pEDR120	This work
pEDR29	R100::ED\lambda4	8
pEDR30	R100::ED\lambda4	8
Phages		
ED\lambda4	λ b519b515cI857 <i>Sam7</i>	8
ED\lambda7	λ cI857 <i>Sam7</i>	N. Murray
ED\lambda26	λ mer from pEDR29	8
ED\lambda27	λ mer from pEDR30	8
NMA500	λ cI857b519 <i>nin5</i>	N. Murray
VA\lambda14	λ mer from pEDR120	This work
λ b2c ⁻		J. Gross
λ b221cI857 <i>int6</i>		A. Campbell
λ h80c ⁻		J. Gross
λ papa		R. Jaskunas
λ vir		M. Monk

Since the λ mer strains were ED\lambda4 derivatives, the phage stocks were titered on the Su⁺ strain ED3814 as described (8).

Media. Most media and all chemicals have been defined (8). L-[³⁵S]methionine was obtained from New England Nuclear Corp. Resistance to mercuric ion was tested in L agar or minimal media containing 70 μ M HgCl₂. Results of mercuric resistance tests on plates are dependent upon inoculum size. With this concentration of mercuric chloride, we spread no more than 2×10^7 cells per plate during transductions with λ mer phages. Resistance patterns of transductant clones were also tested by disk inhibition zones (28, 29).

Construction of strain VA7486. Equal volumes of exponentially growing cultures of JC3272 (which is λ immune) and ED7133 were mixed at 33°C for 90 min, and portions of the mixture were streaked onto medium selective for Tet^r [Str^r] transconjugants. A plasmid pEDR120-containing derivative of JC3272 was purified on this medium and then used as donor

to transfer pEDR120 into a new ED2149 recipient. ED2149 was made temporarily immune to λ by infecting it during exponential growth at a multiplicity of 5 with λ b221cI857*int6* and then using it 90 min later as the recipient. The new ED2149(pEDR120) derivative (designated VA7486) was selected and purified as a Tet^r [His⁺ Trp⁺ Lys⁺] transconjugant.

Lysogenization of ED2149(R100) with VA\lambda14. Overnight cultures of ED2149(R100) in L-broth containing maltose were prepared for transduction as described (8). Purified VA\lambda14 was mixed with ED2149(R100) at a multiplicity of 1 and incubated for 30 min at 33°C. λ b2c⁻ and λ h80c⁻ were then added at multiplicities of 2, and the mixture was diluted and spread on mercury medium. After overnight incubation at 33°C, 20 colonies were purified on the same medium, and their immunity to λ was tested.

Preparation of purified phages. All three λ mer phages contained λ cI857 and *Sam7* genes. Large preparations, therefore, were made from strains VA7582, VA7596, and VA7598 as concentrated lysates by the heat pulse and chloroform lysis methods described by Miller (17). The lysates were further concentrated by CsCl step gradient centrifugations and purified by sequential CsCl density gradient centrifugations. Pilot runs determined that ED\lambda4 for VA\lambda14 and ED\lambda7 for the other two phages allowed good separation of the λ mer and helper phage bands at equilibrium in CsCl centrifugations.

Protein synthesis and gel electrophoresis. Irradiation of bacteria, λ infection, and incorporation of isotope were accomplished by following the protocol of Jaskunas et al. (11). For each 10^9 cells, 10 to 30 μ Ci of L-[³⁵S]methionine (specific activity ~600 Ci/mmol) was added 15 min after the preincubation period. After a 10-min labeling period, isotope incorporation was diminished by the addition of unlabeled L-methionine to a final concentration of 0.5 mg/ml. Labeled cells were washed three times with 0.01 M tris(hydroxymethyl)aminomethane (pH 6.8) containing unlabeled L-methionine at 20 μ g/ml, and then suspended in 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 6.8) containing 2% sodium dodecyl sulfate, 2 mM ethylenediaminetetraacetic acid, 1% mercaptoethanol, and 10% glycerol. Samples were boiled for 2 min immediately before use. Sodium dodecyl sulfate-polyacrylamide gels were prepared and used according to Thomas and Kornberg (27). The homogeneous 18% polyacrylamide gels were fixed overnight in methanol-acetic acid-water (5:1:5), placed on Schleicher and Schuell 470-7 paper, dried for 2 h on a Hoeffer Slab Gel drier, soaked in water for 45 min, and redried. This latter soaking was necessary to remove acetic acid, which otherwise caused the gel to stick to the film used in radioautography. Radioautography was done with Kodak RP Royal X-Omat X-ray film. Molecular weights were determined by comparison of mobilities of unknown proteins with those of the following standard proteins: bovine serum albumin, 68,000; citrate synthase, 50,000; malate dehydrogenase, 37,500; myokinase, 21,000; myoglobin, 16,000; and cytochrome c, 12,000.

To test the induction effect of Hg²⁺, HgCl₂ was added to the prewarmed minimal maltose solution used to dilute the preincubated phage-cell mixtures.

With such a toxic inducer, a careful balance is required, since mercury toxicity reduces overall [^{35}S]methionine incorporation and can prevent the protein synthesis required for induction. Preliminary experiments showed that total [^{35}S]methionine incorporation in the presence of ED λ 4 was nearly normal with either 0.5 μM or 1.0 μM HgCl_2 , but was reduced three- to fourfold by 2 μM HgCl_2 . Therefore, we adopted 0.8 μM as the standard HgCl_2 concentration for induction experiments.

Electron microscopic heteroduplex analysis. Heteroduplex molecules were prepared from purified phage stocks, spread, and mounted on collodion grids using the formamide technique of Davis et al. (6). Grids were examined and molecules were photographed in a Phillips 301 electron microscope; molecules on the enlarged negative films were traced and measured with a Keuffel and Esser map measure. The size of wild-type λ was taken as 49 kilobase pairs (kb; 1 kb = 1,000 base pairs of DNA) in length (P. Phillipsen and R. Davis, manuscript in preparation). Single-stranded lengths were calculated by using the 2.9-kb *nin5* loop (3) of the reference phage NMA500 (c1857b519*nin5*) as the standard. Our measured value of *nin5* was 2.9 ± 0.04 kb when compared to a ϕX174 single-stranded DNA standard of 5.375 kb (21). The distance of 5.1 kb from the *nin5* loop to the right end of λ (3) was used as the double-stranded DNA standard. Only measurements falling within ± 1 standard deviation were included in the calculations. In determining coordinates from heteroduplexed molecules, preference was given to measurements of double-stranded regions. This method gives the most reliable results (19).

EcoRI digestion of λ mer DNA. This was carried out as described by Willetts and McIntire (J. Mol. Biol., in press). The notation for the λ EcoRI fragments is that of Murray and Murray (18), except that the fused fragment generated by the b519 deletion is denoted AE. The notation for the R100 EcoRI fragments is that of Tanaka et al. (26).

Mercuric reductase assays. Mercuric reductase measurements were carried out as previously described (5, 22, 29). Cells, either uninfected or previously infected with λ mer phage, were centrifuged, resuspended, and added to assay buffer containing, usually, 10 μM $^{203}\text{Hg}^{2+}$. Mercuric reduction ($\text{Hg}^{2+} \rightarrow \text{Hg}^0$) was followed as loss of radioactivity from the assay buffer by counting samples periodically by liquid scintillation spectroscopy (5, 22, 29).

RESULTS

Sources of λ mer phages. The three λ mer phages were isolated from three independent cointegrates of R100 and phage ED λ 4: ED λ 26 from plasmid pEDR29, ED λ 27 from plasmid pEDR30, and VA λ 14 from plasmid pEDR120. Dempsey and Willetts (8) reported the isolation of cointegrates pEDR29 and pEDR30 and showed that the ED λ 4 insertion site was between the *mer* and *sul* genes of R100 in both cointegrates (see R100 map, Fig. 1). Plasmid pEDR120 was isolated at the same time and by the same methods.

The molecular weight of pEDR120 was determined to be 88×10^6 by comparing the position of the peak of ^3H -labeled DNA in sucrose density gradients of cleared lysates of ED2149 (pEDR120) with that of ^{35}S -labeled R17 phage in the manner described previously (8). This value is that expected for a cointegrate molecule containing a single ED λ 4 and a single R100 molecule.

To locate the ED λ 4 integration site, deletion mutants of pEDR120 were isolated by heat treatment and analyzed as described earlier for pEDR29 and pEDR30 (8). Representative deletion types recovered are plasmids pVAR30, pVAR31, and pVAR33, whose phenotypes are listed in Table 1. Since we obtained *Mer*⁻, *Sul*⁻, and *Mer*⁻ *Sul*⁻ deletions independently, this analysis located the ED λ 4 insertion site in pEDR120 between *mer* and *sul* (Fig. 1) and suggested that pEDR120 might also serve as a source of λ mer transducing phages. Before heat induction of strains containing pEDR120 to isolate λ mer phages, the plasmid was transferred via strain JC3272 back into strain ED2149, using the temporary immunity procedure described in Materials and Methods. This was done to avoid any possibility of a second ED λ 4 insertion in the ED2149 chromosome. The new strain with pEDR120 in ED2149 was VA7486. A low-frequency transducing lysate was then prepared from this strain. The λ mer phage (VA λ 14) was isolated as a double lysogen with ED λ 4 in ED395 by transduction of ED395 with this lysate as described previously (8).

Characterization of the λ mer phages. Buoyant densities in CsCl were determined for each of the three phages by using as density references λ papa (1.499) and λ b2c⁻ (1.482). The relative DNA lengths were calculated from the values obtained using the equation of Bellett et al. (2; Table 2). During analysis of the fractions from analytical CsCl gradients for plaque formation and *mer* transducing ability we observed that VA λ 14 fractions showed a peak of extremely tiny plaques in the same fractions as the peak of *mer* transducing particles.

Strain ED2149(R100) was lysogenized with VA λ 14 to confirm its plaque-forming ability. In this bacterial strain the chromosomal *att* λ site is deleted so that lysogenization usually occurs via a *recA*-mediated recombination between the DNA of the transducing phage and the homologous region of plasmid DNA. Several lysogens were found among 20 strains examined that were sensitive to λ vir and immune to λ b2c⁻. These strains yielded λ mer transducing phages when heat induced without helper phages, and the phage lysates were able to make plaques, showing that VA λ 14 is N⁺. However, the plaques

FIG. 1. Map of R factor R100, redrawn from Dempsey and Willetts (8), and including the *EcoRI* fragments determined by Tanaka et al. (26). The new *IS1b* coordinates (15) are shown, as are the approximate locations of the fertility inhibition gene (*finO*), the replication genes (*repA* and *repB*), the transfer genes (*tra*), and the genes determining resistances to mercuric ion (*mer*), sulfonamides (*sul*), streptomycin (*str*), fusidic acid (*fus*), chloramphenicol (*cml*), and tetracycline (*tet*). The revised location of *finO* in fragment B (at coordinate 81.3) will be discussed elsewhere (W. B. Dempsey and S. A. McIntire, manuscript in preparation). The arrow at coordinate 8.6 in fragment K indicates the *EDλ4* insertion site in *pEDR29*, *pEDR30*, and *pEDR120*, as determined in this report.

TABLE 2. Buoyant densities and calculated lengths of *λmer* phages

Phage	Density	Calculated length	
		% of wild type	kb
EDλ26	1.486	89.1	43.7
EDλ27	1.489	91.6	44.9
VAλ14	1.497	98.3	48.2

formed were very tiny, a characteristic of *Spi*⁻ (i.e., *red*⁻ *gam*⁻) phages (13, 14, 30). Since *red*⁻ phages fail to form plaques on *E. coli lig* mutants (9) and *red*⁻ *gam*⁻ phages do form plaques on *E. coli* P2 lysogens (13), we tested the VAλ14 lysate on the *lig*(Ts) strain N3098 and on *Ymel*(P2). It failed to form plaques on N3098 and did form plaques on *Ymel* (P2), confirming that VAλ14 is *red*⁻ *gam*⁻.

Electron microscopic analysis of heteroduplex molecules confirmed the results of genetic analysis. Heteroduplexes of each *λmer* phage DNA with NMλ500 DNA showed a deletion of λ DNA

from *attPOP'* rightward. Figure 2 is a schematic diagram of the heteroduplexes observed, and Table 3 presents the measurements for each *λmer* phage. Conversion of these measurements into kilobase coordinates (by subtracting the right end, C + 2.9 + 5.1, from total λ length) showed that EDλ26 and EDλ27 carried deletions from *attλ* (28.1 kb) through 37.7 and 37.5 kb, respectively, thus removing the λ*N* gene at 35.8 kb and λ*p_L* at 36 kb (see ref. 25). The deletion in VAλ14 extended to 34.2 kb, consistent with its being *N*⁺ *red*⁻ *gam*⁻.

We know from earlier work that the orientation of EDλ4 in pEDR30 is *sul*-*λint*-*λJ*-*mer* (8). This also is consistent with the observation that EDλ27 carried the *mer* genes in place of the *λint-red* region. Since the electron microscopic analysis of all three *λmer* phages showed that they were similar, the orientations of EDλ4 in pEDR29, pEDR30, and pEDR120 must have been identical.

Analysis of the DNA fragment sizes produced

FIG. 3. A schematic representation of the fragments generated by *Eco*RI from ED λ 4 and the three λ mer phages. The lines represent DNA lengths; the arrows are *Eco*RI cut sites; and the numbers below the lines are the fragment sizes in kb. The heavy line represents DNA derived from plasmid R100; the hatched area represents IS1b. The letters below each line are the fragment names assigned by other workers to ED λ 4 and R100 *Eco*RI-generated fragments (18, 26); letters in parentheses indicate that only a part of the fragment is present. Lambda genes are indicated above the line, as are the R100 insertion site coordinate and the IS1b coordinates.

FIG. 4. Inducible synthesis of Hg²⁺ reductase activity. Strain J53(λ) was grown to 4×10^8 cells per ml and infected at a multiplicity of 5 phage per bacterium with ED λ 27. After 15 min, 2.2 μ M Hg²⁺ was added to a portion of the infected cells. Samples were removed, centrifuged, and assayed for the rate of loss of 10 μ M ²⁰³Hg²⁺ as described in the text.

Functioning of λmer phages. The best-defined function of the *mer* region of plasmid R100 is the mercury volatilization activity that results from the enzymatic reduction of Hg^{2+} to Hg^0 by the soluble mercuric reductase enzyme (22, 24). This enzyme is synthesized inducibly in plasmid-containing cells (23, 24). Figure 4 shows the time course for inducible synthesis of the reductase activity following infection of *E. coli* with phage ED λ 27. No reductase activity was found with uninfected *E. coli* cells (Table 4). Very low-level reductase synthesis was found in the absence of Hg^{2+} with the $\lambda N^- \lambda p_L^-$ phages ED λ 26 and ED λ 27 (Fig. 4, Table 4). Induction with 2 μM Hg^{2+} caused synthesis of reductase activity (Fig. 4, Table 4). Not surprisingly, with the λN^+ phage, VA λ 14, somewhat different results were found since infection of a lambda-sensitive strain gave reductase activity without induction by Hg^{2+} (Table 4), consistent with the reductase gene being transcribed from the λp_L promoter. The relative (i.e., uninduced/induced) level of reductase synthesis upon infection of a nonlysogenic host differed somewhat from experiment to experiment and seemed to depend upon the growth medium used (76 and 86% in broth versus 36 and 54% in minimal media in one experiment). As expected for genes being transcribed from λp_L , infection of a λ^+ or a λind^- lysogen with VA λ 14 showed less than 10% of the reductase activity seen in the nonlysogen (data not shown). These results are consistent with the polypeptide synthesis results discussed below.

Stable lysogens of bacterial strains NO1348 or J53 with phage ED λ 26, ED λ 27, and VA λ 14 were selected on agar plates containing Hg^{2+} . These lysogens were also resistant to merbromin and fluorescein-mercuric acetate. We have not seen segregation of these resistances from one another under any condition to date. All lysogens showed only inducible synthesis of the mercuric

reductase enzyme.

λmer -directed protein synthesis. The three λmer phages were prepared from strains VA7582, VA7596, and VA7598 and obtained free of helper phages by repetitive banding in CsCl. The phages were then used to direct protein synthesis in a UV-irradiated λ -sensitive host, NO1348, or a λind^- lysogen, NO1626, in the presence and absence of mercuric ion. A λind^- lysogen contains an active λ repressor protein which is not destroyed by UV irradiation, and thus the repressor prevents expression from the leftward and rightward promoters of both the resident and incoming λ molecules. Protein synthesis was measured by the incorporation of [^{35}S]methionine, and estimates of polypeptide sizes were made from the mobilities of the bands obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and radioautography. Control experiments included analysis of incorporation in the absence of phage and incorporation in the presence of ED λ 4.

The radioautogram in Fig. 5 shows the results obtained during infection of strain NO1626 in the absence (left side) and in the presence (right side) of induction by Hg^{2+} . The dark λ band seen with ED λ 4 and all λmer phages is the lambda repressor protein from the *cl* gene (molecular weight = 26,000). The dark λ band present with ED λ 4 and VA λ 14 but absent from ED λ 26 and ED λ 27 infected cells is the λrex protein (molecular weight = 30,000); this observation is consistent with the amount of DNA deleted from each phage. In the presence of 0.8 μM $HgCl_2$ (Fig. 5, right side) three new labeled polypeptides of molecular weight 68,000, 11,500, and 8,500 appeared and are therefore induced by Hg^{2+} . These molecular weights are the mean values from seven experiments using the standards listed in Materials and Methods. Earlier calculated values (7) were based on an erroneous molecular weight for the glutamic dehydrogenase standard used. These three polypeptides may be products of a *mer* "operon." Known functions of the mercurial resistance genes include mercuric reductase enzyme and resistance to nondegraded organomercurials, so three new polypeptides were not unexpected. The 68,000-molecular weight polypeptide corresponds to the subunit size of the mercuric reductase that has been purified from another *E. coli* plasmid (22). Additional faintly labeled polypeptides with molecular weights of 54,000, 33,000, and 13,500 were seen sometimes after Hg^{2+} induction of λmer -infected cells. Whether these are additional but unstable *mer* polypeptides or have precursor relationships to the three strongly labeled polypeptides is not known. There was evidence for "processing" of the mercuric reductase with de-

TABLE 4. Mercuric reductase synthesis after λmer infection^a

Phage	Induced with 2 μM Hg^{2+}	Rate of Hg volatilization (nmol/min per 10^8 cells)
No phage	—	<0.005
ED λ 26	—	0.01
	+	0.18
ED λ 27	—	0.02
	+	0.16
VA λ 14	—	0.18
	+	0.20

^a Strain NO1348 was centrifuged, resuspended, and infected with phage as indicated. Fifteen minutes after phage addition at a multiplicity of 8.6, 2 μM Hg^{2+} was added for induction, and after a subsequent 35-min incubation at 37°C, the cells were centrifuged, resuspended, and assayed for the rate of loss of 7 μM ^{203}Hg .

FIG. 5. Radioautograph of ^{35}S -labeled proteins made after infecting strain NO1626 (λ ind⁻) with λ mer phages. The left side of the photograph (A to E) shows the proteins synthesized in the absence of Hg^{2+} induction; the right side (F to J) shows the proteins synthesized in the presence of $0.8 \mu M HgCl_2$. A and F, No phage added; B and G, ED λ 4; C and H, VA λ 14; D and I, ED λ 27; and E and J, ED λ 26.

creased molecular weight during purification (22).

We also noted that the 68,000-, 11,500-, and 8,500-molecular weight polypeptides were clearly but faintly present in the VA λ 14 track in the absence of Hg^{2+} (Fig. 5C), yet absent in the ED λ 26 and ED λ 27 tracks (Fig. 5E and D, re-

spectively). The presence of these polypeptides with VA λ 14 suggests that the high multiplicity used in the infection (11) overrides the λ immunity, allowing *mer* gene(s) expression from λ p_L. In ED λ 26 and ED λ 27, λ p_L is deleted, and presumably the *mer* promoter per se does not allow visible protein synthesis in the absence of

mercuric ions.

During infection of the lambda-sensitive strain NO1348, [³⁵S]methionine incorporation into the 68,000-, 11,500-, and 8,500-dalton polypeptides was also seen with phage VAλ14 without Hg²⁺ induction (7), but only after induction with phages EDλ26 and EDλ27 (data not shown). These findings and the enzyme assay results presented above confirm that EDλ26 and EDλ27 carry the *mer* promoter, and that the mercuric resistance genes of VAλ14 can be expressed from the λ_{PL} promoter in the absence of λ repression.

DISCUSSION

Agarose gel electrophoresis of restriction endonuclease *Eco*RI-treated λ*mer* DNA showed that the fusion fragment R100-K-λ-D was of similar size in all three λ*mer* phages. The size of this fragment (1.7 kb) allows us to calculate that the site of EDλ4 insertion in each of the parental R100:λ cointegrate molecules was at kilobase coordinate 8.6 in the R100 *Eco*RI fragment K. Inasmuch as the EDλ4 insertion site and orientation were indistinguishable in the three independently isolated cointegrates, it seems likely that there is a hot spot for *int*-promoted λ integration at this point. The physical analysis also confirmed that each λ*mer* phage carried the portion of R100 DNA previously shown to code for mercurial resistance, namely, *Eco*RI fragments H and I (12, 16).

We found that during λ*mer*-directed protein synthesis in a λ*ind*⁻ lysogenic strain, mercuric ions induced synthesis of three distinct polypeptides. The molecular weights of these polypeptides were 68,000, 11,500, and 8,500. The largest polypeptide was assigned to the reductase protein since purified reductase subunits have been shown to have this weight (22). From studies in progress, we know that another component of mercuric resistance is a mercury transport system that brings Hg²⁺ to the intracellular reductase enzyme. This transport function is coded for by part of the *Eco*RI fragment H (Foster et al., manuscript in preparation). One or both of the nonreductase polypeptides may be involved in this function. A possible function for one of the faintly labeled polypeptides (molecular weights, 54,000, 33,000, and 13,500) may be as a component of the *mer* "inducer" protein that has been hypothesized from genetic studies (Foster et al., manuscript in preparation). Since the prosthetic group of mercuric reductase is flavine adenine dinucleotide (22), one of the proteins may be a specific flavoprotein synthetase for joining the flavin component to the reductase. This type of activity would likely be

present only in small amounts.

Mercuric ion can induce the mercuric resistance genes to function with all three phages in either host; phages EDλ26 and EDλ27 do not visibly express the mercuric resistance genes in the absence of mercuric ion. These data indicate that the entire mercuric resistance and control system is carried by EDλ26 and EDλ27; since the length of R100 DNA carried by VAλ14 is greater than or equal to that carried by the other two phages (Fig. 3), VAλ14 must also carry this entire system.

The reason then that the mercury genes are expressed by VAλ14 at detectable levels even during infection in the absence of Hg²⁺ must reside in the extra λ genes it carries, namely *N* and *p_L*. It is likely, then, that the *mer* sense strand is attached to the left strand of λ, such that the *mer* genes can be transcribed from λ_{PL} in the direction *IS1b* toward *sul*. This is in agreement with the direction of transcription proposed from genetic studies by Foster et al. (manuscript in preparation). As noted above, in the VAλ14 infection of a λ-lysogenic host, the high multiplicity of infection accounts for the expression of the mercury genes from λ_{PL}, i.e., there are more λ molecules present than can be immediately turned off by the resident represor.

In EDλ26 and EDλ27, the R100 DNA is fused to the *rex* gene (see Fig. 3). Since *rex* and *cI* are transcribed efficiently from *prm* in infections of a λ*ind*⁻ lysogenic strain (the data in Fig. 5 show that these two phages synthesize *cI* protein), one might expect transcription originating at *prm* to go through *cI* into the fused *rex*-R100 DNA and into the *mer* genes. However, when EDλ26 and EDλ27 were used to infect a λ*ind*⁻ lysogen, no uninduced *mer* gene expression was detected. We assume that this lack of expression in EDλ26 and EDλ27 was due to the known polar effect of *IS1* (10, 20), which is thought to be the result of rho-dependent transcription termination outside of *IS1* itself. *N* gene product antagonizes rho-dependent termination, and, since VAλ14 is *N*⁺ *p_L*⁺, the polar effect of *IS1* was not expressed in that strain. In light of this it seems possible that an *N*-sensitive transcription termination signal exists between *IS1b* and the *mer* genes.

It is hoped that the existence of these λ*mer* specialized transducing phages will facilitate subsequent genetic and molecular analysis of the structure and function of the mercurial resistance determinants, which appear an ideal case of a polygenic, tightly regulated plasmid resistance system. Furthermore, the DNA from the phages provides starting material for in vitro analysis of the functioning of the *mer* regulatory system.

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ADDENDUM IN PROOF

We have examined heteroduplexes of λ mer DNA with λ gal DNA carrying an IS1 insertion within galT. As expected, the molecules contained a duplex region of IS1 length; however, we could detect no R100-1 DNA beyond IS1b and conclude that our λ mer phages carry identical lengths of R100-1 DNA from coordinate 8.6 through most or all of IS1b. We thank N. Grindley for the lysogen of λ gal::IS1.

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