Some Mercurial Resistance Plasmids from Different Incompatibility Groups Specify *merR* Regulatory Functions That Both Repress and Induce the *mer* Operon of Plasmid R100

TIMOTHY J. FOSTER* AND FIONA GINNITY

Microbiology Department, Moyne Institute, Trinity College, Dublin 2, Ireland

Transcription of the *mer* genes of plasmid R100 is regulated by the product of the *merR* gene. The *merR* gene negatively regulates its own expression and also controls the transcription of the *merTCA* operon both negatively (in the absence of inducer) and positively (in the presence of inducer). We used transcriptional *mer-lac* fusions of R100-1 in complementation tests to measure the ability of the *merR* products of different mercury-resistant transposons and plasmids to functionally interact with R100-1. Plasmids from incompatibility groups C, B, S, L, and P, as well as the *Pseudomonas* transposons Tn*501* and Tn*3401*, regulated the expression of the R100 *mer* genes in a similar fashion to the R100-1 *merR* product itself, suggesting that these elements are closely related. Only plasmid R391 (IncJ) did not complement.

Resistance to mercurial compounds is a common plasmid-encoded property in bacteria (7, 20, 21, 24, 29). In gram-negative organisms this phenotype has been associated with plasmids from many different incompatibility groups and with several transposons (6, 17, 20, 22, 29). Two types of resistance determinant which enzymatically modify and volatilize mercurial compounds have been described. Broad-spectrum determinants confer resistance to mercuric ions and organomercurials, whereas narrow-spectrum determinants only confer resistance to Hg^{2+} (7, 20, 24, 29). Both types specify resistance to some organomercurials by an uncharacterized mechanism which does not involve volatilization (20, 29).

A common feature of both broad- and narrow-spectrum resistance determinants is an inducible flavoprotein called mercuric reductase which converts toxic Hg^{2+} to the relatively nontoxic and volatile Hg^0 (1, 9, 18, 19, 25). Broad-spectrum determinants specify a second enzyme, organomercurial lyase, which converts, for example, phenylmercury acetate to benzene and Hg^{2+} (19, 26, 27). The Hg^{2+} is subsequently reduced to Hg^0 by the accompanying reductase.

Genetic analysis of the narrow-spectrum determinant of plasmid R100 revealed the following operon structure (8, 14, 16). A regulatory gene *merR* specifies a protein which controls transcription of the *merTCA* operon both positively and negatively. Transcription of *merTCA* is repressed in the absence of inducer and is stimulated after incubation with Hg^{2+}. In addition, *merR* appears to regulate its own transcription negatively. Recent DNA sequence analysis of the Tn*501* and R100 (Tn*21*) *mer* regions revealed that the two determinants have a similar genetic organization and considerable DNA sequence homology (13a). The putative *merR* polypeptides were highly conserved, showing about 10% amino acid sequence divergence, whereas the region between *merR* and *merTCA* where transcriptional regulatory sites are located also was highly conserved (13a). In addition, Tn*501* and R100 specify immunologically related but

nevertheless distinguishable mercuric reductase enzymes (S. Silver and T. Kinscherf, personal communication). In contrast, genetic analysis of broad-spectrum determinants is lacking. However, several observations suggest that the basic genetic organization might be quite similar to that of R100 or Tn*501*, with additional Hg^{2+}-inducible genes specifying the organomercurial lyase subunits. (i) Plasmid R828, which specifies broad-spectrum Hg^r, complemented a *merR* mutant of R100, stimulating induction of the *mer* operon (8). (ii) The pattern of Hg^{2+}-inducible polypeptides encoded by R831 was identical to that of R100 with two additional polypeptides of 22 and 24 kilodaltons which probably corresponded to lyase subunits (12). (iii) The reductases of several broad-spectrum determinants are immunologically related to the R100 enzyme (Silver and Kinscherf, personal communication).

Transcriptional *lac* fusions have been formed by inserting Mu d(Ap *lac*) into the *merR* and *merA* genes of R100-1 (16). These fusions helped elucidate the mechanisms of regulation of *mer* gene expression (16). This paper describes experiments with the *mer-lac* fusions to investigate whether the *merR* product of Hg^r determinants from diverse sources could interact with R100.

MATERIALS AND METHODS

Bacterial strains and plasmids. The plasmids used are listed in Table 1. All experiments were performed in DU5111 [Δ(*lac-pro*)XIII *thi rpoB* Mu$^+$] (16).

Bacteriological media, growth conditions, and chemicals. Bacteriological media, growth conditions, and chemicals were as described previously (16).

Construction of the double-plasmid-carrying strains. The double-plasmid-carrying derivatives of DU5111 (Table 2) were constructed as follows. First, the Hg^r plasmids were transferred into DU5111 by conjugation or transformation. Matings were performed by mixing equal volumes of exponential broth cultures of a rifampin (Rif)-sensitive donor strain carrying the conjugative Hg^r plasmid and DU5111 Rif ^{r}, incubating the cultures for 2 h at 37°C, and then plating them on suitable selective plates. These contained rifampin

---

* Corresponding author.

TABLE 1. Plasmids

Plasmid	Phenotype[a]	*mer* genotype	Source and reference
pDU1124	Cm^r Sm^r Su^r Ap^r Hg^{ss} IncFII	R100-1 *merA*::Mu d(Ap *lac*)	16
pDU1161	Cm^r Sm^r Su^r Ap^r Ka^r Hg^s IncFII	R100-1 *merR25*::Tn5 *merA*::Mu d(Ap *lac*)	16
pDU1135	Cm^r Sm^r Su^r Ap^r Tc^r Hg^s IncFII	R100-1 *merR*::Mu d(Ap *lac*)	16
pDU1053	Tc^r Ka^r Hg^s	pDU1003 *merR25*::Tn5	16
pDU1059	Tc^r Ka^r Hg^{ss}	pDU1003 *merA31*::Tn5	16
pSM1	Cm^r Ka^r Tc^r Ap^r Tp^r Su^r Hg^r Pm^r IncC	*mer*$^+$	4
R55-1	Cm^r Su^r Hg^r IncC	*mer*$^+$	E. Lederberg (30)
R391	Ka^r Hg^r IncJ	*mer*$^+$	E. Lederberg (3, 20)
R724	Cm^r Tc^r Sm^r Su^r Hg^r IncB	*mer*$^+$	N. Datta (5)
R828	Cm^r Tc^r Sm^r Ap^r Ka^r Hg^r Pm^r IncS	*mer*$^+$	N. Datta (11, 20)
R830a	Ka^r Sm^r Hg^r Pm^r IncL	*mer*$^+$	N. Datta (11, 20)
R478	Ka^r Tc^r Cm^r Hg^r IncS	*mer*$^+$	N. Datta (11)
R702	Ka^r Tc^r Sm^r Hg^r IncP	*mer*$^+$	N. Datta (10)
R388::Tn*3401*	Tp^r Su^r Hg^r Pm^r IncW	*mer*$^+$	A. Radford (17)
pUB307::Tn*501*	Ka^r Tc^r Hg^r IncP	*mer*$^+$	P. Bennett
pRR130	Ap^r Hg^r '	*mer*$^+$	R. Rownd (13)

[a] The symbols for the drug resistance phenotypes are: Cm, chloramphenicol; Sm, streptomycin; Su, sulfathiazole; Ap, ampicillin; Ka, kanamycin; Tc, tetracycline; Tp, trimethoprim; Hg, mercuric chloride; Pm, phenylmercury acetate. r, Resistant; s, sensitive; ss, hypersensitive.

(40 μg/ml) and an antibiotic selective for the plasmid (Table 1). The nonconjugative plasmids pDU1053 and pDU1059 were introduced into DU5111 by transformation selecting for Tc^r.

Next, the R100-1 *merR*::Tn5 *merA*::Mu d*lac* plasmid pDU1161 or the R100-1 *merR*::Mu d*lac* element pDU1135 was transferred into DU5111 (R$^+$) by conjugation selecting for Rif^r and for a resistance marker that was unique to the incoming plasmid. The presence of the Hg^r plasmid resident in DU5111 was monitored by scoring for resistance to HgCl₂ and phenylmercury acetate (in the case of broad-spectrum strains) as well as for a marker unique to the resident plasmid where this was available.

β-Galactosidase assays. The expression of β-galactosidase by cultures that were either uninduced or induced by incubation for 30 min in 0.5 or 1.0 μM HgCl₂ was measured as described previously (16).

TABLE 2. β-Galactosidase activities of strains carrying R100-1 *mer-lac* fusions and other mercury-resistant plasmids

Other plasmid	β-Galactosidase activity (U)[a] of strains carrying a mercury-resistant plasmid and an R100-1 *mer-lac* fusion:			
	pDU1135 *merR*::Mu d(Ap *lac*)		pDU1161 *merR*::Tn5 *merA*::Mu d(Ap *lac*)	
	Uninduced	Induced	Uninduced	Induced
	232	223	32	28
pDU1053 *merR*$^-$	252	222	ND	ND
pDU1059 *merR*$^+$	33	28	ND	ND
pRR130	ND	ND	9	201
R702	33	28	8	298
R830a	65	67	13	434
pSM1	29	31	9	248
R828	ND	ND	9	126
pUB307::Tn*501*	61	70	13	307
R388::Tn*3401*	ND	ND	6	172
R478	28	20	7	254
R724	32	38	10	218
R55-1	25	20	10	287
R391	163	172	44	36

[a] The values are the means of at least two independent experiments. ND, Not done.

RESULTS

Regulation of the R100 *mer* genes by different Hg^r determinants. A transcriptional (operon) fusion was constructed by insertion of phage Mu d(Ap *lac*) into the *merA* gene of plasmid R100-1, whereby transcription and translation of *lacZ* and the formation of β-galactosidase was controlled by *merR* (16). The subsequent inactivation by the insertion of Tn5 into the *merR* gene of R100-1 *merA*::Mu d(Ap *lac*) led to micro-constitutive (unregulated) expression of β-galactosidase (16). In the presence of a compatible plasmid carrying the cloned R100-1 *merR*$^+$ gene on pDU1003 or pRR130 the expression of β-galactosidase was regulated in the same way as the original R100-1 *merR*$^+$ fusion (16).

We performed experiments to determine whether the *merR* products of Hg^r determinants from plasmids from different incompatibility groups (R55-1, R391, R724, R828, R830a, R478, R702, and pSM1) as well as transposons Tn*501* and Tn*3401* could control the R100-1 *mer* operon (Table 2). β-Galactosidase activity expressed by strains carrying pDU1161 (*merR*::Tn5 *merA-lac*) and a compatible Hg^r plasmid was measured in uninduced cultures and after induction. In all cases except R391 the micro-constitutive expression of β-galactosidase characteristic of pDU1161 alone was repressed in the uninduced cultures and was elevated after induction to the same extent as that in the control complementation experiments with pRR130.

In the case of pDU1161-R391, β-galactosidase was expressed at the same micro-constitutive level as that of the pDU1161 control (Table 2). Care was taken to ensure that all the cells in the pDU1161-R391 population retained both plasmids and were Hg^r. R391 is known to specify Hg^r by inducible volatilization activity (20). In addition, the *Escherichia coli* (R391) strain used here was checked for this property (S. Silver, personal communication).

Similar experiments were performed with R100-1 *merR*::Mu d(Ap *lac*). With this fusion, β-galactosidase was expressed constitutively, but in the presence of the cloned R100-1 *merR*$^+$ gene (on pDU1059) this activity was reduced 5- to 10-fold, suggesting that *merR* normally regulates its own expression autogenously. This repression of β-ga-

lactosidase could not be elevated by induction (16; Table 2). Each of the plasmids which were shown above to regulate the *mer* operon also repressed expression of the R100-1 *merR* gene. R391 had no effect. Thus the heterologous Hg^r determinants behaved similarly and consistently in both tests.

DISCUSSION

The *Pseudomonas* transposons Tn*501* and Tn*3401* as well as plasmids from incompatibility groups C, B, S, L, and P specifying broad- or narrow-spectrum mercurial resistance produce *merR* products which can act in *trans* to regulate the R100-1 *mer* genes in a manner indistinguishable from the R100-1 *merR* product itself. These plasmids provide inducer-repressor functions that can presumably bind to regulatory sites in the R100-1 *mer* region to control transcription of the *merTCA* genes and also a repressor function which can complement the autogenous regulation of R100-1 *merR*. This suggests that the regulatory elements of these determinants are closely related. Only plasmid R391 (IncJ) failed to complement.

These results confirm and extend previous observations that heterologous Hg^r determinants located on different plasmids can regulate the expression of the R100-1 *mer* genes. Thus Hg^s mutants of plasmids R828 and R702 have previously been shown to induce the R100-1 *mer* operon as measured by volatilization activity (8, 23). This paper also shows that these elements repress micro-constitutive expression of the operon. Thus the report that R702 failed to repress micro-constitutive reductase activity (23) is not consistent with these findings. This work also provides the first evidence that Tn*501* and R100-1 *mer* determinants can interact functionally. These two elements were compared at the DNA sequence level and shown to be closely related (13a).

Interesting questions have been raised about the evolution of mercury resistance. In contrast to tetracycline resistance determinants found in enteric bacteria which fall into at least four DNA homology groups with less than 60% sequence homology (2, 7, 15, 28) and where repressors fail to interact with heterologous operator sites (P. M. Bennett, personal communication), the majority of Hg^r determinants, including those originating in pseudomonads, are sufficiently related for their *merR* products to regulate the R100 *mer* operon. The regulatory regions of Tn*501* (originally from *Pseudomonas aeruginosa*) and R100 (from *Shigella flexneri*) *mer* determinants have considerable homology at the DNA sequence level (13a). The putative regulatory sites for controlling transcription of *merTCA* occur in a region of almost complete sequence identity, whereas the putative *merR* polypeptides show only about 10% amino acid sequence divergence (13a). On the basis of the data presented here we can predict that the regulatory regions of the plasmids which functionally interact with R100 will be similarly related. If this is true then severe constraints must have been imposed upon the divergent evolution of *mer* genes. Alternatively, their horizontal spread on plasmids and transposons may have occurred in the relatively recent past.

ACKNOWLEDGMENTS

ADDENDUM IN PROOF

A recent paper by Klock et al. (J. Bacteriol. **161**:326–332, 1985) has shown heterologous repressor-operon interactions among the four classes of tetracycline resistance determinants.

LITERATURE CITED

1. **Brown, N. L., S. J. Ford, R. D. Pridmore, and D. C. Fritzinger.** 1983. Nucleotide sequence of a gene from *Pseudomonas* transposon Tn*501* encoding mercuric reductase. Biochemistry **22**: 4089–4095.
2. **Chopra, I.** 1984. Antibiotic resistance resulting from decreased drug accumulation. Br. Med. Bull. **40**:11–17.
3. **Coetzee, J. N., N. Datta, and R. W. Hedges.** 1972. R factors from *Proteus rettgeri*. J. Gen. Microbiol. **72**:543–552.
4. **Coleman, D., R. D. Falkiner, M. E. Carr, G. Dowd, G. Dougan, and C. T. Keane.** 1984. Simultaneous outbreaks of infection due to *Serratia marcescens* in a general hospital. J. Hosp. Infect. **5**:270–282.
5. **Datta, N., and J. Olarte.** 1974. R factors in strains of *Salmonella typhi* and *Shigella dysenteriae* 1 isolated during epidemics in Mexico: classification by compatibility. Antimicrob. Agents Chemother. **5**:310–317.
6. **De La Cruz, F., and J. Grinsted.** 1982. Genetic and molecular organization of Tn*21*, a multiple resistance transposon from R100.1. J. Bacteriol. **151**:222–228.
7. **Foster, T. J.** 1983. Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol. Rev. **47**:361–409.
8. **Foster, T. J., H. Nakahara, A. A. Weiss, and S. Silver.** 1979. Transposon A-generated mutations in the mercuric resistance genes of plasmid R100-1. J. Bacteriol. **140**:167–181.
9. **Fox, B., and C. T. Walsh.** 1982. Mercuric reductase—purification and characterization of a transposon-encoded flavoprotein containing an oxidation-reduction-active disulphide. J. Biol. Chem. **257**:2498–2503.
10. **Hedges, R. W.** 1975. R factors from *Proteus mirabilis* and *P. vulgaris*. J. Gen. Microbiol. **87**:301–311.
11. **Hedges, R. W., V. Rodriguez-Lemoine, and N. Datta.** 1975. R-factors from *Serratia marcescens*. J. Gen. Microbiol. **86**: 88–92.
12. **Jackson, W. J., and A. O. Summers.** 1982. Polypeptides encoded by the *mer* operon. J. Bacteriol. **149**:479–487.
13. **Miki, T., A. M. Easton, and R. H. Rownd.** 1978. Mapping of the resistance genes of the R plasmid NR1. Mol. Gen. Genet. **158**:217–224.
13a. **Misra, T. K., W. M. Barnes, L. Haberstroh, and S. Silver.** 1984. Mercuric resistance operons of plasmid R100 and transposon Tn*501*: the beginning of the operon including the regulatory region and the last two structural genes. Proc. Natl. Acad. Sci. U.S.A. **81**:5975–5979.
14. **Nakahara, H., S. Silver, T. Miki, and R. H. Rownd.** 1979. Hypersensitivity to Hg^{2+} and hyperbinding activity associated with cloned fragments of the mercurial resistance operon of plasmid NR1. J. Bacteriol. **140**:161–166.
15. **Nguyen, T. T., K. Postle, and K. P. Bertrand.** 1983. Sequence homology between the tetracycline-resistance determinants of Tn*10* and pBR322. Gene **25**:83–92.
16. **Ni' Bhriain, N. N., S. Silver, and T. J. Foster.** 1983. Tn*5* insertion mutations in the mercuric ion resistance genes derived from plasmid R100. J. Bacteriol. **155**:690–703.
17. **Radford, A. J., J. Oliver, W. J. Kelly, and D. C. Reanney.** 1981. Translocatable resistance to mercuric and phenylmercuric ions in soil bacteria. J. Bacteriol. **147**:1110–1112.
18. **Rinderle, S. J., J. E. Booth, and J. W. Williams.** 1983. Mercuric reductase from R-plasmid NR1: characterization and mechanistic study. Biochemistry **22**:869–876.
19. **Schottel, J. L.** 1978. The mercuric and organomercurial detoxifying enzymes from a plasmid-bearing strain of *Escherichia coli*. J. Biol. Chem. **253**:4341–4349.
20. **Schottel, J. L., A. Mandal, S. Silver, and R. W. Hedges.** 1974. Volatilization of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. Nature (London) **251**:335–337.
21. **Silver, S.** 1981. Mechanisms of plasmid-determined heavy metal

resistances, p. 179–189. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), Molecular biology, pathogenicity and ecology of bacterial plasmids. Plenum Publishing Corp., New York.

22. **Stanisich, V. A., P. M. Bennett, and M. H. Richmond.** 1977. Characterization of a translocation unit encoding resistance to mercuric ions that occurs on a nonconjugative plasmid in *Pseudomonas aeruginosa.* J. Bacteriol. **129:**1227–1233.

23. **Summers, A. O., and L. Kight-Oliff.** 1980. Tn*1* generated mutants in the mercuric ion reductase of the IncP plasmid, R702. Mol. Gen. Genet. **180:**91–97.

24. **Summers, A. O., and S. Silver.** 1978. Microbial transformation of metals. Annu. Rev. Microbiol. **32:**637–672.

25. **Summers, A. O., and L. I. Sugarman.** 1974. Cell-free mercury(II)-reducing activity in a plasmid-bearing strain of *Escherichia coli.* J. Bacteriol. **119:**242–249.

26. **Tezuka, T., and K. Tonomura.** 1978. Purification and properties of a second enzyme catalyzing the splitting of carbon-mercury

linkages from mercury-resistant *Pseudomonas* K-62. J. Bacteriol. **135:**138–143.

27. **Tonomura, K., and F. Kanzaki.** 1969. The reductive decomposition of organic mercurials by cell-free extract of mercury-resistant pseudomonad. Biochim. Biophys. Acta **184:**227–229.

28. **Waters, S. H., P. Rogowsky, J. Grinsted, J. Altenbuchner, and R. Schmitt.** 1983. The tetracycline resistance determinants of RP1 and Tn*1721*: nucleotide sequence analysis. Nucleic Acids Res. **11:**6089–6105.

29. **Weiss, A. A., J. L. Schottel, D. L. Clark, R. G. Beller, and S. Silver.** 1978. Mercury and organomercurial resistance with enteric, staphylococcal, and pseudomonad plasmids, p. 121–124. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.

30. **Witchitz, J. L., and G. R. Gerbaud.** 1972. Classification des plasmides conferant la résistance à la gentamicine. Ann. Inst. Pasteur (Paris) **123:**333–339.