Mercury Operon Regulation by the *merR* Gene of the Organomercurial Resistance System of Plasmid pDU1358

GIUSEPPINA NUCIFORA,* LIEN CHU, SIMON SILVER, AND TAPAN K. MISRA

Department of Microbiology and Immunology, University of Illinois College of Medicine, P.O. Box 6998, Chicago, Illinois 60680

The structural basis for induction of the mercury resistance operon with inorganic mercury and with the organomercurial compound phenylmercuric acetate was addressed by DNA sequencing analysis and by *lac* fusion transcription experiments regulated by *merR* in *trans* from broad-spectrum-resistance plasmid pDU1358 (Hg^{2+} and phenylmercury responding). The *lac* fusion results were compared with those from a narrow-spectrum-resistance (Hg^{2+} responding but not phenylmercuric responding) operon and the pDU1358 *merR* deleted at the 3′ end. The nucleotide sequence of the beginning region of the broad-spectrum *mer* operon of plasmid pDU1358 was determined, including that of the *merR* gene, the operator-promoter region, the *merT* and *merP* genes, and the first 60% of the *merA* gene. Comparison of this sequence with DNA sequences of narrow-spectrum *mer* operons from transposon Tn*501* and plasmid R100 showed that a major difference occurred in the 3′ 29 base pairs of the *merR* gene, resulting in unrelated C-terminal 10 amino acids. A hybrid *mer* operon consisting of the *merR* gene from pDU1358, a hybrid *merA* gene (determining mercuric reductase enzyme), and lacking the *merB* gene (determining phenylmercury lyase activity) was inducible by both phenylmercury and inorganic Hg^{2+}. This shows that organomercurial lyase is not needed for induction by organomercurial compounds. A mutant form of pDU1358 *merR* missing the C-terminal 17 amino acids responded to inorganic Hg^{2+} but not to phenylmercury. Thus, the C-terminal region of the MerR protein of the pDU1358 *mer* operon is involved in the recognition of phenylmercury.

Bacterial cells can express mechanisms to counteract the toxic effects of mercury ions and organomercurial compounds. These resistance mechanisms are often encoded on plasmids or transposons and are highly specific for the Hg^{2+} ion or mercury covalently bonded to an organic component, as for example in phenylmercuric acetate (PMA) (4, 25, 26).

Three mercury resistance operons from gram-negative bacteria have been cloned and sequenced: the narrow-spectrum (Hg^{2+} resistance but not PMA resistance) resistance determinants from plasmid R100 (originally from a *Shigella* sp.) and from transposon Tn*501* (originally from a *Pseudomonas* sp.) (2, 3, 17, 18) and the broad-spectrum (resistance to both Hg^{2+} and PMA) resistance determinant from pDU1358 (originally from a *Serratia* sp. [7; this work]). The operons contain several homologous genes: *merT* and *merP*, which code for proteins that transport the toxic ion into the cell, and *merA*, coding for mercuric reductase, which reduces Hg^{2+} ions to volatile and less toxic metallic Hg^0. Plasmid pDU1358 confers resistance to organomercurial compounds such as PMA and has an additional gene, *merB*, which codes for the enzyme organomercurial lyase. The lyase cleaves the covalent C-Hg bond of PMA to give less toxic benzene and Hg^{2+}, which is reduced by the reductase.

The expression of these genes is under the control of a *trans*-acting activator-repressor protein, encoded by the *merR* gene, which is transcribed divergently from the structural genes. The MerR proteins from different plasmids can cross-complement a *merR* mutant operon on plasmid R100 (4, 6). Expression of the structural genes can be induced by subtoxic levels of Hg^{2+} ions in narrow-spectrum *mer* operons or by either Hg^{2+} ions or organomercurial compounds

such as phenylmercury, methylmercury, and ethylmercury in broad-spectrum *mer* operons.

The regulatory protein MerR of the narrow-spectrum *mer* operons from Tn*501* and R100 binds to the operator-promoter region of the operon (9, 22, 23), possibly acting as a repressor by precluding access to RNA polymerase and repressing transcription. However, when Hg^{2+} ions are present, a complex is formed at the operator-promoter region of the DNA between MerR, RNA polymerase, and Hg^{2+}. The complex activates transcription of the downstream structural genes (6, 9, 22). The *mer* operator of Tn*501* has recently been analyzed with deletion mutants carrying mutations that result in constitutive synthesis of *mer* gene products (11). Recently published observations on the chromosomally encoded *mer* resistance operon of *Bacillus* sp. strain RC607 indicate that expression of the structural genes is regulated differently (8, 27), although the regulatory protein is significantly homolgous to that of the gram-negative systems.

No specific information is available on the induction of the broad-spectrum *mer* operon with organomercurial compounds, and it is not clear whether activation depends on direct interaction between the regulatory protein (MerR) and organomercurial inducers, such as PMA. A low constitutive level of lyase could produce Hg^{2+}, which actually acts as inducer.

In this report, we present the DNA sequence of the region of the broad-spectrum organomercury resistance determinant of plasmid pDU1358, starting with the *merR* gene and continuing beyond the operator-promoter region through the *merT* and *merP* genes for 1,029 base pairs (bp) into the *merA* gene. (The sequence has been submitted to the GenBank data base under accession number M24940.) Together with the previously published sequence of the distal region of this *mer* operon (7), this completes the *mer* operon sequence of

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* Corresponding author.

pDU1358 except for approximately 300 bp within *merA*. We also report complementation tests and in vitro recombination experiments which demonstrate that the ability of the plasmid pDU1358 MerR protein to respond to PMA resides in its carboxyl end, distal to the site for the Hg^{2+} response.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Escherichia coli* strains MC1061 [F^- *araD139* Δ(*ara leu*)7696 Δ*lacY74 galU galK hsdR rpsL* (14)], DU5111 [Δ(*lac-proXIII*) *thi rpoB* Mu) harboring plasmid pDU1161 (encoding resistance to ampicillin, chloramphenicol, and kanamycin and supersensitivity to Hg^{2+} [21]), and WB373 (phage M13-sensitive host [1]) were used.

Recombinants of plasmid pBR322 were selected on LB agar plates (13) for resistance to tetracycline (12 μg/ml), and derivatives of phage M13 were selected by screening single plaques on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) plates and agarose gel electrophoresis of DNA from infected-cell lysates (16). The cultures were grown either in LB broth (strains MC1061 and DU5111) or in 2XNY broth (strain WB373). Growth was monitored by measuring the culture turbidity with a Klett-Summerson colorimeter.

The inducibility of the mercury operon was determined by addition of Hg^{2+} or PMA to exponentially growing cultures 60 min before harvesting the cells and assaying the β-galactosidase levels (15).

Plasmids. Plasmid pDU1003 containing the narrow-spectrum mercury resistance operon from plasmid R100 (21), plasmid pHG103 (7) containing the broad-spectrum mercury resistance operon from pDU1358, and plasmid pDU1161 (21), a derivative of R100, were used.

The construction of the plasmid containing the pDU1358-R100 hybrid mercury operon is outlined in Fig. 3. Plasmid pGN101 contained the proximal region of the *mer* operon of pDU1358 ligated at the conserved *Eco*RI site in the *merA* gene to the distal region of the *mer* operon of pDU1003. The correct recombinant was selected by agarose gel analysis after digestion with *Hind*III (which cuts twice in the pDU1358 *mer*, giving a 310-bp fragment, but has no recognition sequence in the R100 *mer* operon).

One of the M13 deletions generated for sequencing of pDU1358 *mer* by Bal31 exonuclease treatment (mGN1-267) was used to construct a recombinant operon containing a *merR* gene deleted at the 3' end (see Fig. 3). Derivative mGN1-267 carries a *merR* gene lacking 54 nucleotides at the 3' end and was digested with *Pvu*I and *Hpa*I and ligated to plasmid pGN101, also digested with *Pvu*I and *Hpa*I. The desired recombinant was selected by size and by the loss of the recognition sequence for *Sal*I, lost in the deleted nucleotides.

DNA sequencing. A modification (16) of the dideoxy chain termination method (24) was used to sequence both strands of the 2.9-kilobase (kb) region of the mercury resistance operon from plasmid pHG103 (7). The 2.9-kb *Bgl*II-*Eco*RI fragment from plasmid pHG103 was cloned into M13 vector mTM010 (16), and overlapping ordered deletions were generated by using exonuclease Bal31.

RESULTS

DNA sequence. Figure 1 shows the 2,924 bp of the broad-spectrum mercury resistance operon of plasmid pDU1358 from approximately 160 bp 3' to the terminal *Bgl*II site in pHG103 (7) to the *Eco*RI site in *merA*. The first 676 bp

shown are apparently not part of the *mer* system, and the function of this segment of DNA has not been studied. The pDU1358 *mer* operon starts with the termination codon of *merR* and continues to the *Eco*RI site that is conserved in the *merA* sequences of R100, Tn*501*, and pDU1358. Following a gap of approximately 300 bp that have not been sequenced, the pDU1358 *merA* gene continues in the sequence of Griffin et al. (7). The major reading frames are indicated with the direction of transcription and the predicted amino acid sequences of the translated gene products. The operator-promoter region and the coding regions of *merR*, *merT*, *merP*, and the proximal part of *merA* were identified by comparison with the sequences of the *mer* operons of R100 and Tn*501* (5, 12, 17, 18).

The sequence in Fig. 1 from the end of the *merR* gene (position bp 677) through the end of the sequence shown (*Eco*RI site in *merA*) was compared with that of the previously sequenced *mer* operons from Tn*501* and R100 (2, 17, 18). The comparisons of the sequences were made with the nucleotide alignment program of Wilbur and Lipman (28) with parameters *K*-tuple = 4, window = 20, and gap penalty = 10. All three sequences are very closely homologous. The pDU1358 sequence showed 1,967 of 2,244 identical base pairs when matched with Tn*501* (87.7% identify) and somewhat fewer (1,790 of 2,247) identical base pairs when matched with the R100 sequence (79.7% identity). For comparison, the Tn*501* and R100 sequences are 81.5% identical (1,829 of 2,244 bp) over this region. Thus, the pDU1358 *mer* sequence shown is more similar to that of Tn*501* than to that of R100. The only region where the pDU1358 DNA sequence is more dissimilar from that of Tn*501* than are the Tn*501* and R100 sequences is the 3'-most 29 bp of the *merR* gene, where there are only 6 differences between Tn*501* and R100, 20 differences between pDU1358 and R100, and 21 differences between pDU1358 and Tn*501*. This is the region where the amino acid sequence of the organomercurial compound-responding *merR* product of pDU1358 differs from that of the organomercurial compound-nonresponding *merR* product of R100 (see below).

The pDU1358 *mer* operon (like that of Tn*501*) lacks a *merC* gene. The termination codon of *merP* of pDU1358 is followed after 55 bp by the ribosome-binding site and ATG initiation codon of *merA*. The R100 sequence has an additional 438 bp representing the *merC* gene (17, 25) that is missing in Tn*501* and pDU1358.

Comparison of the predicted amino acid sequences of MerR from pDU1358 with those from R100 and Tn*501* (Fig. 2) shows strong homology, with the exception of the carboxyl-terminal nine amino acids, suggesting that this region is not essential for induction by inorganic mercury. A cysteine at position 115 is missing in pDU1358 MerR. The three cysteines at positions 82, 117, and 126 are conserved in the three MerR sequences. These three cysteines might be involved in Hg^{2+} binding.

Inducibility of the *mer* operon: *trans* activation of the mercury operon with *merR* from R100 and pDU1358. The ability of the pDU1358 *merR* product to respond to Hg^{2+} and PMA as inducers was studied in complementation tests between pDU1161, a derivative of plasmid R100 with an inactivated *merR* gene, and with a transcriptional *merA-lacZ* fusion (7, 21).

In addition, in order to test the activation of the pDU1358 broad-spectrum *merR* in the absence of the *merB* lyase gene, a hybrid plasmid, pGN101, was constructed and used to complement pDU1161. Plasmid pGN101 (Fig. 3) contains the pDU1358 broad-spectrum *merR* gene, the transport

TTAATCTGCTCAACAAGATAGTGATAATCC TGTTGTAATTTAGCAATAACTGGCTAGGTA AAGAGGCAAACTATTATCCTCAAGAATGGT ACTCAGTCGGCTAATAACGGCAGCTCCTCG 120

GGGAACGCTAATGCCAAATTCCAGCAGAAA AGCATGCATTTGATTGGTTGTTTTCACCTT ATCCTGAACCAGGGATTCACGGACACGATG CAGAGCCCGCATTGCCTGCTGAGATTCCGT 240

SalI
TCTGGCCTGCACAAAACGCATAGACGGACG CGATGCAGCTTCACAAATAGCTTCGGCGTC GACAAATTCGTTTTTATTGCTTTTAACGAA CGGGCGGACAAATTGTGGTGATAATCAGCTT 360

TGGGAATGCCCCAACTCTTCCAACTTGCGT GCCATAAAGTGAGAACCGCCACAGGCTTCC ATTGCGATGGTTGTAGCCGGGCCATGTCGCC AAAAATTCGATCAACTTTGCCCGGGTAAAT 480

TTTTTACGGTAAACAGCCTTCCCGCGACGA TCCTGCCAATGAATATCGAAAGAGTTTTTA CCCAGATCGATACCAATGAGCGCCAATGTT TTTCCATGATAGTTCTCCGAATGAAAGCCT 600

end merR * V A D A G R P E K E G Q L S
GTCCTCAGCATAGTACCGGGAAGGACGGAG TGACCATCTCATTAATAAAGCACGCTAAG GCGTAACTACCCTCGGCTACACGCGTCGG CACCACGCGGCTCTTTCTCCCCTTGCAGCG 720

HindIII
A I L P C S V N G Q R A H C A F V L E S L V T E M R A L D T M K E R V D Q L K H
AAGCCAATCAGCGCGCAAGAAACGTTCCCTT GCCCGCCCATGGCCAGCGGCGAACACAAGTTCGG ATACCACGCGTTTCCATGCGCGCGCCAGGTCGG TCATTTTTTCGCGCCACGCCCTGAAGCTTGT 840

E A L S S A E E C H T G D D L R L L E A I E D L S F G L R Q A S K V F R V R T V
GCCTCGGCCAGCCTGCTGGCCTTCCTGCCAGT GGGTGCCGGTCATCCAGCCTCAGCAGCTCTG CGATCTCGTCGAGGCTGAATCCGAGCCGGCT GGCCTGATTTCACGAAGCGCACCCGCGTCA 960

D A E G Y R R I S G Y P K D P E P L L G K R Q Y F R I T E V N V G A A K A F V G
CATCCCGCCTCGCCATAGCGCGGCGATGCTCC CATAGGGCTTGTGCCGGCTCGCGGCAACAAGC CCTTGCGCTGATAGAACCGGATTGTTTCCA CGTTGACCCCGGCCGGCGCTTGGCGAAAACGC 1080

I T L N E L N K E M start merR   HindIII   start merT M S E P Q N
CAATAGTCAGATTCTCCCAAATTTTTTTCCA TATCGGCTTGACTCGGTACATTGGTACGGAA GTAAGCTTAAGCTATCCAATCCAGATTTGA AAGGACAAGCGGATGTCTGAACCTCAAAAC 1200
              -35   -10 -10   -35

G R G A L F T G G L A A I L A S A C C L G P L V L I A L G F S G A W I G N L T V
GCCGCGCGGCGGCTCTTCACTGGCGGGCTA CCCGCGCATCCTCGCCTCGGCCTTGCTGCCTG GGGCGCCTCGGTTCTGATCGCCCTCGGGTTC AGCGGCGCTTGGATCGGCCAACTTGACCGTG 1320

L E P Y R P I F I G A A L V A L F F A W R R I Y R P A Q A C K P G D V C A I P Q
TTGGAACCTTATCGCCCGATCTTCATCGGC GCGGCGGTTGGTGGCGCTGTTTTTCGCCTGG CGGCGCATCTACCGACCGGCGCAAGCCTGC AAACCAGGGGATGTGTGTGCGGATTCCCCAA 1440

V R A T Y K L I F W V V A A L V L V A L G F P Y V M P F F Y * <-merT merP-> M K K L
GTGCGCGCCTACTTACAAGCTCATTTTCTGG GTGGTGGCCGCGCTGGTTCTGGTCGCGCTC GGATTTCCCTACGTCATGCCATTTTTCTAT TAATCACAGGAGTTCATTATGAAAAAACTG 1560

F A S L A I A A V V A P V W A A T Q T V T L S V P G M T C S A C P I T V K K A I
TTTGCCTCTCTCGCCATCGCTGCGGTTGTT GCCCCCGTGGTGTGGGCGGCCACCCAGACCGTC ACGCTGTCGGTACCGGGCATGACCTGCTCC GCTTGTCCGATCACCGTTAAGAAGGCGATT 1680

HpaI
S K V E G V S K V N V T F E T R E A V V T F D D A K T S V Q K L T K A T E D A G
TCCAAGGTCGAAGGCGTCAGCAAAGTTAAC GTGACCTTCGAGACACGCGAAGCGGTTGTC ACCTTCGATGATGCCAAGACCAGCGTCCAG AAGCTGACCAAGGCCACGGAAGACGCGGGC 1800

Y P S S V K K * end merT   start merA M T H L K I T G
TATCCGTCCAGCGTCAAGAAGTCAGCCACT GAAAACGCCAGCGCCAGCACATCTGACGCCC TTGTCTGCTACCACAAACGAAAAAGGATCT GTCCGATGACCCATCTAAAAATCACCGGCA 1920

M T C D S C A A H V K E A L E K V P G V Q S A I V S Y A K G A A Q L A L D P G T
TGACCTGCGACTCGTGCGCCGCGCACGTCA AGGAAGCGCTGGAAAAGGTACCCGGCGTCC AATCTGCCATAGTGTCCTATGCCAAGGGG CGGCCCAGCTCGCCCTTGATCCAGGCACAG 2040

A P D A L T A A V A G L G Y K A M L A D A P P T D N R T G L F D K V R G W M G A
CGGCCGGACGCACTGACCGCGGCGGTGGCTG GCCTGGGCTACAAAGCGATGCTCGCCGGATG CCCCGCGGACCGACAACCGCACTGGGCTGT TCGACAAGGTGCGCGGCTGGATGGGTGCCG 2160

A D K G S G A E R P L Q V A V I G S G G A A M A A A L K A V E Q G A Q V T L I E
CCGACAAGGGCACCGCGGCCGAGCGCGGGT TCCAAGTCGCCGGTGATCGGCAGCGGCGGGAG CCCGGCATGGCCGGCAGCACTGAAGCCGGTCG AGCAAGGCGCGCAGGTCACGCTGATTGAGC 2280

R G T I G G T C V N V G C V P S K I M I R A A H I A H L R R E S P F D G G M P P
GCCGGCACCATCGGCGGCACCTGCGTCAACG TCGGTTGTGTGCCGGTCCAGCATCATGATCC GCCGCGCGCACATCGCCCATCTGCGCCGGG AAAGCCCATTCGACGGCGGCATGCCACCCA 2400

T P P T I L R E R L L A Q Q Q A R V E E L R H A K Y E G I L D G N S A I T V R H
CACCGCCGACGATCTTGCGCGAGCGGCTGC TGGCCCAGCAGCAGGCCGTGTGTCGAAGAAC TCCGTCATGCCAAGTACGAAGGCATCCTGG ACGGCAATTCAGCCATCACCGTTCGTCACG 2520

G E A R F K D D R D L S V S L N E G G E R V V M F D R C L V A T G A S P A M P P
GTGAAGCCCGTTTCAAGGACGACCGAGACC TTACCGTTAGTTTGAACGAGGGTGGTGAGC CGGTCGTCATGTTCGACCGCTGCCTGGTCG CCACGGGTGCCAGTCCGGCCATGCCCCCA 2640

I P G L K E S P Y W T S T E A L V S D T I P E R L A V I G S S V V A L E L A Q A
TTCCCGGCCTGAAAGAGTCACCCTACTGGA CTTCGACCGAGGCCTTGGTCAGCGACACCA TTCCCGAACGCCTAGCCGTGATCGGCTCGT CGGTGGTCGCGCTGGAACTGGCGCAAGCCT 2760

F A R L G S Q V T I L A R N T L F F R D D P S I G E A V T A A F R A E G I K V L
TCGCCCGGCTCGGCAGCCAGGTCACGATCC TAGCTCGCAACACCCTGTTCTTCCCCGGACG ACCCGTCCATCGGCGAGGCGGTCACAGCCG CTTTCCGTGCCGAAGGGATCAAGGTACTGG 2880

EcoRI
E H T Q A S Q V A H V N G E F
AACACACGCAAGCCAGCCAGGTCGCGCATG TGAACGGCGAATTC 2924

FIG. 1. DNA sequence of the 2,924 bp of the plasmid pDU1358 broad-spectrum *mer* operon. Only one strand is shown. Putative transcription initiation signals, some restriction nuclease sites (used in these studies), and predicted amino acid sequences are shown. Starting codons are boxed, and stop codons are indicated by asterisks. Following a 300-bp gap after bp 2924, the *merA* sequence continues in Griffin et al. (7).

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┌──────────────────────────────────────────────────────────────────────┐
│ pDU1358  MEKNLENLTIGVFAKAAGVNVETIRFYQRKGLLPEPDKPYGSIRRYGEAD        50  │
│                                                                        │
│ Tn501    **N*******************************L****************           │
│                                                                        │
│ R100     **N*******************************R****************           │
│                                                                        │
│                                                                        │
│ pDU1358  VIRVRFVKSAQRLGFSLDEIAELLRLDDGTHCEEASSLAEHKLQDVREKM       100  │
│                                                                        │
│ Tn501    ****************************E***************K******           │
│                                                                        │
│ R100     *V**K***************************************K******           │
│                                                                        │
│                                                                        │
│ pDU1358  TDLARMETVLSELVFACHARQGNVSCPLIASLQGEKEPRGADAV          144     │
│                                                                        │
│ Tn501    A*******A*****C****R************GASLAGSAMP                    │
│                                                                        │
│ R100     A*************C****K*************AGLARSAMP                     │
└──────────────────────────────────────────────────────────────────────┘
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FIG. 2. Alignment of the predicted amino acid sequences of the MerR regulatory protein from pDU1358 with those of MerR from R100 and Tn501. Asterisks indicate amino acids identical to those of pDU1358.

genes, and a hybrid pDU1358-R100 functional *merA* gene (but no *merB* lyase). Cells harboring this hybrid operon were resistant to inorganic mercury but not to PMA (Fig. 4). The ability of a *trans*-acting *merR* from pHG103 or pGN101 (along with control pDU1003) to activate the *mer* operon in pDU1161 was tested by monitoring the transcriptional activation of the *merA-lacZ* fusion in pDU1161 with increasing concentrations of Hg^{2+} or PMA as an inducer. The results show similar induction of the *merA-lacZ* fusion in all three complementation systems, with a dependence on the Hg^{2+} concentration from 0.2 to 2 μM (Fig. 5A).

Similar experiments were done with PMA as the inducer (Fig. 5B). As expected, the narrow-spectrum *merR* gene from pDU1003 was not induced by the organomercurial compound PMA in the range of concentrations tested (0.2 to 2 μM). In contrast, the pDU1358 broad-spectrum *merR* gene activated expression of β-galactosidase in pDU1161 both with the wild-type operon (pHG103), in which *merB* was also present, and, to a lesser extent, with the hybrid operon
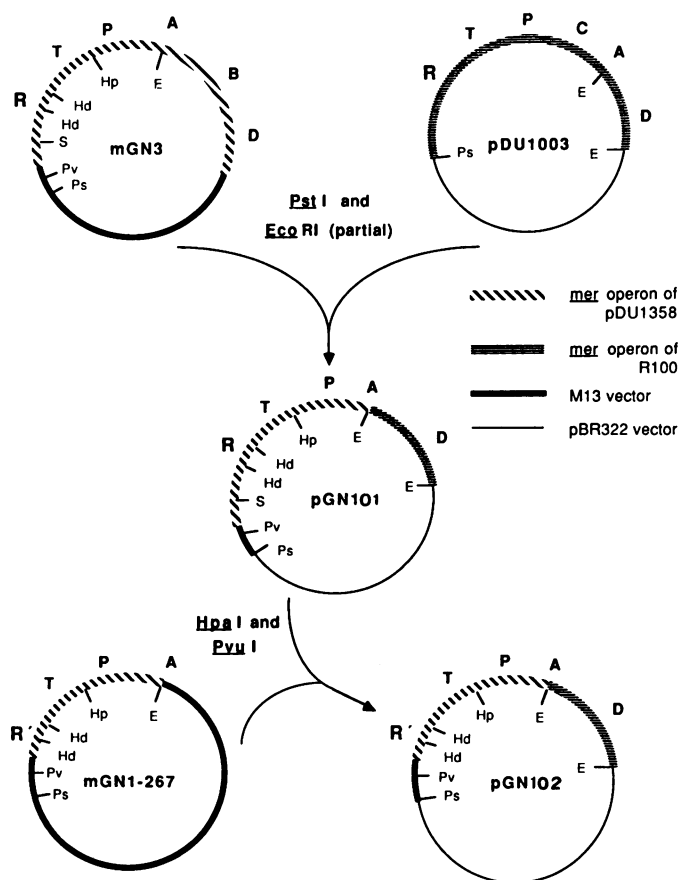

FIG. 3. Construction of plasmids pGN101 and pGN102. For the construction of these plasmids, M13 derivatives were used as donors of the *mer* operon containing the broad-spectrum *merR*. Phage mGN3 replicative form (RF) DNA containing the intact *mer* operon of plasmid pDU1358 was digested with *Pst*I and *Eco*RI and ligated to plasmid pDU1003, digested with *Pst*I and partially digested with *Eco*RI. The two restriction enzymes cut in both molecules outside the *mer* operon in the vector (*Pst*I) and at corresponding sites in *merA* (*Eco*RI). The correct recombinant, containing the *Pst*I-*Eco*RI fragment of the broad-spectrum *mer* operon replacing the comparable fragment of the R100 *mer* operon, was selected by screening the size of the recombinants and by digestion of the recombinants with *Hind*III, which cuts twice in the pDU1358 *merR*, giving a 310-bp fragment; *Hind*III has no recognition sequence in the R100 *mer* operon. For construction of plasmid pGN102, M13 derivative mGN1-267 (created by Bal31 digestion for sequencing) was used. In this construct, the pDU1358 *merR* gene lacks 54 bp at the 3' end. mGN1-267 was digested with *Pvu*I and *Hpa*I and ligated to pGN101 digested with the same two restriction enzymes. The correct recombinant was selected by size and by the inability to be digested by *Sal*I, which has a recognition sequence 383 bp 3' of *merR* of pDU1358 (see Fig. 1) that is lost in the *merR*-deleted recombinant. *mer* genes are shown as R, T, P, A, B, D, and R' (3'-deleted version of *merR*). Restriction enzyme sites: E, *Eco*RI; Hd, *Hind*III; Hp, *Hpa*I; Ps, *Pst*I; Pv, *Pvu*I; S, *Sal*I. (Not drawn to scale.)
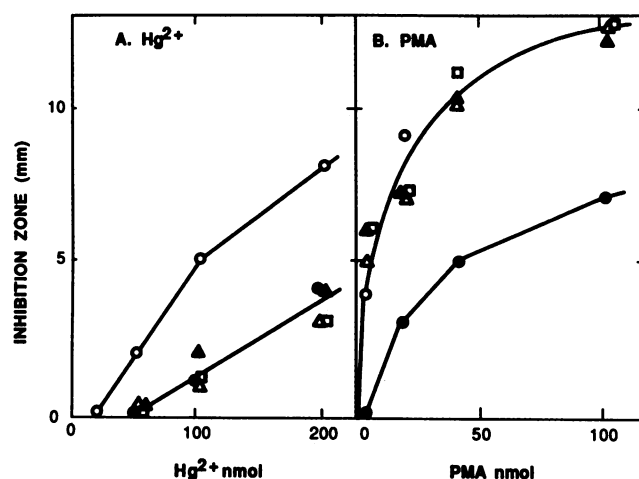
FIG. 4. Hg^{2+} and PMA resistance measured on petri dishes. A 0.2-ml amount of late-log-phase LB cultures of *E. coli* DU5111 with plasmid pDU1161 (○) and *E. coli* MC1061 with plasmid pHG103 (●), pDU1003 (△), pGN101 (▲), or pGN102 (□) was spread on LB agar petri dishes, and disks containing Hg^{2+} or PMA were placed on the agar surface. Inhibition zone diameters (minus 6.5 mm for disk diameter) were measured after incubation at 37°C for 16 h.

FIG. 6. Inducibility of the *mer* operon with the deleted *merR* mutant. The plasmids supplying *merR* function in *trans* were pGN101 (●) and pGN102 (○) with Hg^{2+} as the inducer or pGN101 (▲) and pGN102 (△) with PMA as the inducer. □, Control plasmid pDU1161 alone with Hg^{2+} as the inducer.

(pGN101), in which *merB* was absent. These results suggest interaction between PMA and *merR* in activation of the broad-spectrum *mer* operon.

Recognition of different inducers by the pDU1358 broad-spectrum MerR protein. Comparison of the predicted amino acid sequence of the MerR protein from pDU1358 with those of the MerR proteins from R100 and Tn501 (Fig. 2) showed a lack of homology at the carboxyl terminus. In contrast, the rest of the sequence had an overall conservation of more than 90%.

We investigated the possibility that this C-terminal region plays a role in induction with PMA by replacing the wild-type pDU1358 *merR* in plasmid pGN101 with a deletion mutant lacking 54 bp at the 3' end in plasmid pGN102 (Fig. 3). In plasmid pGN102, the C-terminal 17 amino acids (starting with Leu-Ile-Ala) (Fig. 2) are missing and are replaced with 7 amino acids (Ile-Ser-Arg-Ser-Leu-Trp-Asn). These seven amino acids are encoded by nucleotides from the M13 phage in which the Bal31 deletion was generated. Despite the deletion of the 17 carboxyl-end residues, the MerR protein was capable of regulating expression of the
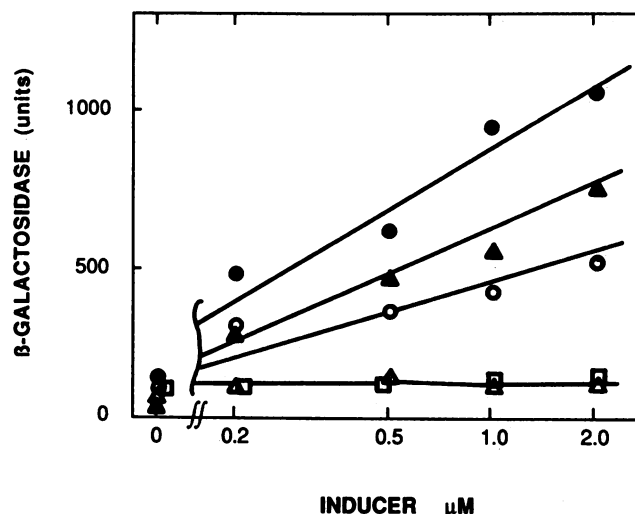
R100 operon in pDU1161. Plasmid pGN102 expressed the same level of resistance to mercury as did plasmid pGN101 (Fig. 4A). However, the level of induction was affected by the altered carboxyl end of the protein. With Hg^{2+} as the inducer, the production of β-galactosidase was reduced in the complementation experiment with plasmid pGN102, and the activity of PMA as an inducer was completely lost (Fig. 6).

DISCUSSION

A major question concerning gene regulation today is the topology of regulatory proteins and how the domains involved in inducer recognition and binding and DNA target recognition are arranged within the three-dimensional structure of the protein. For the *merR* gene product of pDU1358, five domains must be considered. (i) A helix-turn-helix motif is presumed to be involved in binding to the operator DNA. Its sequence has been deduced from homologies with other regulatory proteins (5, 23). Since MerR proteins from other *mer* operons can recognize the R100 *mer* operator sequence,
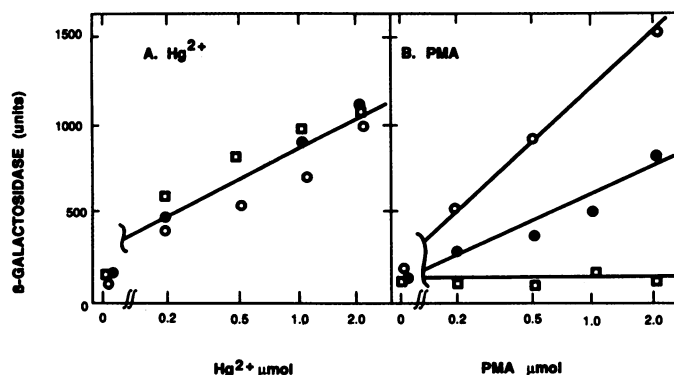

FIG. 5. Inducibility of the *mer* operon measured as expression of *lacZ* with different *merR* genes. The inducer was (A) Hg^{2+} or (B) PMA. The plasmids supplying *merR* function in *trans* were pDU1003 (□), pHG103 (○), and pGN101 (●).

A. Match of ends of merR with K=3;window=20;gap penalty=7. 68 matches

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pDU1358  759  ATGCGCGGCAAGGGAACGTTTCTTGCCCGCTGATTGCTTCGCTGCAAGGGGAGAAAGAGC  700
              :::::::      ::::::::::::: ::::::::::::: :: :: :: :: :: :     :
Tn501    196  ATGCGCGAAGGGGGAACGTTTCCTGCCCGCTGATCGCGTCACTACAGGGTGGAGCAAGCT  137

pDU1358  699  CGCGTGGTGCCGACGCGGTGTAGCCGAGGGTAGTTACGCCTTAGCGTGCTTTATTTAATG  640
              : ::::      : :: ::::::::::::::::::::: :
Tn501    136  TGGCAGGT--------------------TCGGCTATGCCTAGCGTGCTTTATTTTCCG  97
```

B. Match of ends of merR with K=3;window=20;gap penalty=15. 57 matches

```
pDU1358  759  ATGCGCGGCAAGGGAACGTTTCTTGCCCGCTGATTGCTTCGCTGCAAGGGGAGAAAGAGC  700
              :::::::      ::::::::::::: ::::::::::::: :: :: :: :: :: :     :
Tn501    196  ATGCGCGAAGGGGGAACGTTTCCTGCCCGCTGATCGCGTCACTACAGGGTGGAGCAAGCT  137

pDU1358  699  CGCGTGGTGCCGACGCGGTGTAGCCGAGGGTAGTTACGCCTTAGCGTGCTTTATTTAATG  640
              : ::: :: :    : :::  :: :: :
Tn501    136  TGGCAGGTTCGGCTATGCCTTAGCGTGCTTTATTTTCCG  97
```
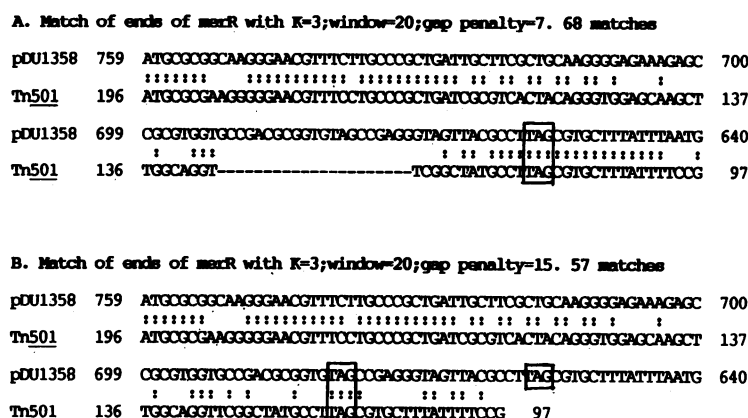
FIG. 7. NUCALN (28) alignments of the 3'-terminal ends of the *merR* genes of pDU1358 and Tn*501*. The coding strand, complementary to the strand presented in Fig. 1, is shown. The nucleotide sequence numbering is the same as in Fig. 1. (A) Alignment with a low gap penalty of 7, showing the proposed 21-bp gap. (B) Alignment with a higher gap penalty of 15, showing no gap. The aligned TAG stop codons are boxed along with the additional in-frame TAG in the pDU1358 sequence.

the DNA recognition amino acids are presumably conserved among many MerR proteins. (ii) Since MerR protein exists as a homodimer (22, 23), there must be residues at the interface between the subunits determining subunit-subunit interactions. There are no data concerning where these determinants lie. (iii) Inorganic Hg^{2+} acts as an inducer of R100, Tn*501*, and pDU1358 *mer* transcription, so the amino acid residues interacting with Hg^{2+} ions are probably conserved. (iv) This report shows that the response to the phenylmercury ions requires the carboxyl-terminal end of MerR, which can be removed without abolishing the response to Hg^{2+} (Fig. 6). The Cys-126 residue shown by our laboratory (K. P. Yoon, unpublished data) to be essential for response to Hg^{2+} is only 2 amino acids from the beginning of the deletion (of 17 amino acids) that removed the phenylmercury response. (v) A fifth function comes from the findings of O'Halloran and Walsh (22, 23) that the same DNA region is protected from DNase I attack by MerR protein in the presence or absence of Hg^{2+}. In the absence of MerR function, the *mer* operon transcription is about 1% of its maximum induced rate (100% in the presence of Hg^{2+} and MerR). In the presence of MerR, the transcription is repressed to less than 0.1% (21). Activation by Hg^{2+} may not involve a shift in position of MerR protein on the DNA. The addition of Hg^{2+} activates *mer* transcription by allowing a specific interaction between MerR and another component, probably RNA polymerase (22).

The broad-spectrum mercury resistance operon of plasmid pDU1358 differs from those of R100 and Tn*501* with the additional inducibility by PMA (7). Griffin et al. (7) reported Southern blot DNA-DNA hybridization experiments with pDU1358 DNA in which a probe containing the *merR* gene of R100 was used. In these experiments, hybridization was not detected. However, when these experiments were repeated with the same probe, hybridization was seen when either *Hind*III or *Eco*RI was used to cut the pDU1358 plasmid DNA (data not shown). The small 310-bp *Hind*III fragment within *merR* of pDU1358 (Fig. 1) was apparently lost in the experiments of Griffin et al. (7).

When the *merR* genes of Tn*501* and pDU1358 were aligned by the Wilbur and Lipman (28) method with parameters that allowed gaps, a 21-nucleotide gap appeared followed by a perfect match of 19 bp, including the 3'-most 7 bp of *merR* (ends with TAG), followed by 12 bp, including 7 T residues

(Fig. 7A), potentially a rho-independent transcription termination site. When the Wilbur and Lipman (28) gap penalty parameter was increased to suppress gaps, a different TAG chain terminator codon aligned with the Tn*501* sequence (Fig. 7B), and this was preceded by 29 unrelated nucleotides.

Possibly the 29 unrelated bp of Tn*501* were replaced in a single substitution event with the 50 bp of pDU1358 from bp 659 (in the *merR* stop codon) to 708 (Fig. 2). One can speculate that a phenylmercury-binding "cassette" might have been inserted toward the 3' end of the *merR* gene, in a region which is not needed for the Hg^{2+} response. The DNA sequence alignment in Fig. 7 suggests this possibility.

The *merR* deletion mutant lacking the C-terminal 17 amino acids of MerR was fully resistant to inorganic Hg^{2+} but sensitive to phenylmercury (Fig. 4), whereas the results with the *lac* fusion in Fig. 6 show lesser response to Hg^{2+} as an inducer with the deletion mutant *merR* than with wild-type pDU1358 *merR*. It is possible that the deletion of the C-terminal 17 amino acids reduced the ability of MerR to respond to Hg^{2+} quantitatively (Fig. 6), but that *merR* function is not rate limiting for mercury ion resistance. Barrineau et al. (2) also found that a *merR* deletion mutant (in this case missing the C-terminal 15 amino acids of R100 MerR) conferred full Hg^{2+} resistance. It seems that a balance between adequate mercury transport activity and intracellular mercuric reductase determines the mercury resistance level (10, 19, 21).

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