Purification and Functional Characterization of MerD
A COREGULATOR OF THE MERCURY RESISTANCE OPERON IN GRAM-NEGATIVE BACTERIA

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Mercury resistance operons (mer) from transposons Tn21, Tn501, and plasmid pDU1358 are highly homologous and inducible with Hg²⁺. The regulatory gene merR is transcribed from one promoter, which is divergently oriented from the promoter for the other mer genes. MerR, the product of the regulatory gene, negatively regulates its own expression as well as the expression of the other genes. MerR activates transcription of the operon in the presence of inducing concentrations of Hg²⁺. The most promoter distal gene, merD, which is cotranscribed with the structural genes, down regulates the mer operon. A frame-shift mutation in merD, created by deletion of 3 bp and an inserted 16 bp sequence upstream of the major inverted repeats present at the 3' end of the merD sequence, resulted in increased synthesis of the structural gene transcript and higher level of resistance to Hg²⁺ by a factor of about 2. MerD protein was overproduced using a T7 expression system. The overproduced protein was present in the pellet fraction, when cell lysates were centrifuged at a low speed. Approximately 80% pure MerD protein was recovered from the pellet fraction by extracting with a buffer solution containing 5 M urea. The purified protein migrated as a 13,500 molecular weight protein on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the N-terminal amino acid sequence corresponded to that deduced from the DNA sequence of merD. MerD bound specifically with the mer promoter sequence. DNase I footprinting experiments identified a common mer operator sequence for MerR and MerD.

The genes encoding resistances to mercurial compounds are often found on plasmids and transposons in bacteria. Two classes of mercury resistance determinants from Gram-negative bacteria have been characterized: narrow-spectrum resistance, conferring resistance to inorganic mercury salts; and broad-spectrum resistance to inorganic mercury salts as well as some organomercurial compounds, as for example phenylmercuric acetate. The regulation of expression of three highly homologous mercury resistance determinants derived from transposons Tn21 and Tn501 and from plasmid pDU1358 have been studied (for recent reviews see Refs. 1–4). These operons consist of a regulatory gene, merR which is separated from the other genes by an operator-promoter region. These genes are expressed from two divergently oriented promoters. Both Tn501 and pDU1358 mer operons contain two genes, merT and merP, encoding Hg²⁺ transport proteins. The Tn21 mer operon contains an additional gene, merC, encoding a separate Hg²⁺ transport protein (5). The transport genes are followed by the merA gene, encoding mercuric ion reductase, and an additional gene, merD. The mer operon of pDU1358 contains the gene, merB (encoding organomercurial lyase) which is located between merA and merD. Organomercurial lyase cleaves the carbon-mercury bond in such compounds as CH₂Hg⁺ and CH₃Hg⁺; Hg²⁺ is released and subsequently reduced to relatively volatile Hg₀ by the mercuric ion reductase. Hg²⁺ is then diffused out of the cells.

The expression of the mercury resistance operons from the three systems described above is regulated by the transacting activator-repressor protein MerR. MerR binds to the operator-promoter site, repressing transcription from the promoter, PmerT, as well as from its own promoter, PmerR (6, 7). To induce expression from PmerT, MerR requires the presence of activating Hg²⁺ (8, 9). In addition, the broad-spectrum mercury resistance system of plasmid pDU1358 is activated by organomercurials such as phenylmercuric acetate (10, 11).

In vitro transcription assays from the Tn501 operator-promoter site have shown that purified MerR and Hg²⁺ are necessary and sufficient for initiation of transcription by the RNA polymerase from PmerT (12).

merD is the most promoter distal of the structural genes in the three mer systems, and its deletion or interruption by transposon insertion reduces the level of resistance to Hg²⁺ by approximately 20% (9, 13, 14). The role of merD in the expression of the operon is not defined. In fact, only recently the product of the merD gene has been visualized (14). Two notable features of this gene are; (a) a series of inverted and directly repeated sequences that, although not identical, are found in all the three versions of merD, and (b) the predicted homology of MerD with MerR in the putative DNA binding region of MerR (13, 14). These features tend to suggest that merD might have a regulatory role. Recently, in studies of the induction of the pDU1358 mercury operon (14), we have shown indirectly that the presence of merD represses in trans the induction of the operon itself.

Here, we report that interruption of the merD reading frame upstream from the major inverted repeats at the 3' end of the merD sequence results in the elevation of net synthesis of the structural gene transcript with consequent increase of resistance to Hg²⁺ by a factor of approximately 2. We have partially purified MerD protein and have demonstrated that MerD and MerR specifically bind to a common operator DNA site in the mer operon.
ATGACGGCCTAAGGCGGTTCGCATTGCCGCTATGCGGGCAGCGCCTG

---1---

CGAGCTTTGTGGAACGCTAAGCTTCTATGCCCTCGGCTGCG

---2---

GCCACGGGTTGACGCTGCTCAGGGCGGCGGCGGATGC

---3---

TGCTTCGTGCCGGCGGCGCCGCTCAGCGGCACTGGCCGCG

---4---

GGCTGGCGGGGTGGGACGCTGGCCTGGGACGCTGGCCTGG

---5---

GATAGAAGCTCCTGCGAAGTGGAAGAGATCGGCCTGGCGGC

---6---

GCCGGGACACTCCGGCGCCGATCTCTGCGCGCTCGCGCGCGCG

---7---

CGCGCGGCAAGGGAGGCGGAGATGGAGTGCAGCGCGCGCGCG

---8---

CGCGCGGCAAGGGAGGCGGAGATGGAGTGCAGCGCGCGCGCG

---9---

CGCGCGGCAAGGGAGGCGGAGATGGAGTGCAGCGCGCGCGCG

FIG. 1. Sequence of mer D reading frame showing insertion of an oligonucleotide. Two copies of an 8-bp AsnI linker were added to the blunted BglII site. Blunting of the 3′-protruding BglII ends resulted in the deletion of 3 bp in the reading frame (shown in lower case letters). The site of insertion did not affect the repeated sequences (numbered 1 to 4). The added base pairs as well as the putative start and stop codons are underlined. The sequence was confirmed by nucleotide sequencing. The only coding strand of the gene is shown.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—Escherichia coli strains MC1061 [ harboring merB (araBAD (araBAD-lexA) 7670 laciX71 galU galK rpsL thi (15), DU5111 [Δ(lac-pro-XIII)] thi rpsB Mu] harboring plasmid pDU161 (Ap&, chloramphenicol& kanamycin, Hg²⁺ hyper sensitive (9) and BL2 (DE3)) harboring plasmid gal (λc0657 indu Sam7 mini lacUV5-T7 gene 1)] harboring plasmid pLysE (lysosome-producing) (16) were used. Cells were grown at 37°C with shaking, as necessary. Derivatives of plasmid pBHR32 were selected on LB agar plates (10) for resistance to tetracycline (12 μg/ml) or ampicillin (100 μg/ml).

pG1200 (Hg²⁺, phenylmercuric acetate&, tetracycline, Ap+) containing the entire broad-spectrum mer operon of pDU3388 (10, 14) was used to construct mutant derivatives with variations in merD gene. pG1200, a derivative of pG1200 with a 3′-deletion and a 16-bp insertion in the merD gene was obtained by cutting pG1200 DNA with BglII, polishing the ends using T4 DNA polymerase, and dNTPs (removing 3 bp) and inserting two copies of the Asnl linker 5′-GGACGTTCC-3′ (Fig. 1). The sequence shown in Fig. 1 was confirmed by nucleotide sequencing. The frame-shift mutation changes the amino acid sequence starting from the 57th residue of MerD, and the deduced amino acid sequence terminates following the 84th residue (TGA stop codon). There are two PvuII sites on the pG1200 DNA, one located 106 bp upstream of the start of merD gene and the other one in the ba gene. pDH1, a merD deletion derivative of pG1200, was obtained after digesting pG1200 DNA with PvuII and self-ligation of the larger DNA fragment containing the mer operon.

Resistance to Mercuroic Ions—The resistance to mercuroic ions of cells harboring wild type and variant merD genes was determined in LB medium by following the turbidity of the bacterial culture after addition of Hg²⁺ (5–65 μM).

β-Galactosidase Assays—The production of β-galactosidase in complementation systems containing plasmid pDU161 (9) was measured as described by Miller (17).

Purification of MerD—A DNA fragment containing the merD gene and the ribosome binding sequence was fused with the 5′-flank gene at the BamHI site of pS7-3a for overproduction of MerD protein under the control of T7 RNA polymerase using E. coli (BL2 (DE3)) harboring pLysE as host (16). Cells containing the cloned merD gene were grown in 2XN medium (NZ amine, 25 μl/g; yeast extract; 10 g/liter; and NaCl, 2.5 g/liter) supplemented with 50 μl/ml carbenicillin. A 5-ml overnight culture was inoculated into 1 liter of 2XNY. The cells were grown for 2.5 h (50-60 Klett units), induced for the production of T7 RNA polymerase with 1 μM IPTG for 1.5 h and then harvested by centrifugation in a Sorvall centrifuge (G3 rotor; at 7,000 rpm for 10 min, 4°C). All subsequent manipulations were done at 4°C unless stated otherwise. The cell pellet was washed twice with buffer A (20 μM Tris-HCl, pH 7.5, 10 μM MgCl₂, 1 mM EDTA, 1 mM 2-mercaptoethanol, and 5% glycerol). The cells were resuspended in 20 ml of buffer A and lysed by passing through a French pressure cell at 14,000–16,000 p.s.i., and then centrifuged in a Sorvall centrifuge (SS-34 rotor) at 18,000 rpm for 15 min. The pellet was resuspended in 10 ml of 0.1 M Tris-HCl, pH 8.5, containing 5 μl/ml ethylenediaminetetraacetic acid (EDTA) and 10 μl/ml ethylenediaminetetraacetic acid (EDTA) to solubilize MerD protein. MerD was separated from the insoluble fraction by centrifugation at 18,000 rpm for 1 hour. The supernatant was removed from the supematant fluid by dialysis against 200 volumes of buffer A with three changes. The MerD protein preparation was stored frozen in small aliquots at −70°C, thawed, and used once for in vitro studies. We were unable to estimate what fraction of the total protein was renatured and properly folded for biological activity after dialysis of urea at the final step of purification. It is noteworthy that MerD could not be solubilized from the pellet fraction in buffer systems containing salts (NaCl or KCl) as high as 2 M.

Protein samples were subjected to SDS-PAGE using 15% polyacrylamide (18) and polypeptides were identified by staining with Coomassie Blue R-250.

DNA Binding Assay—Protein-DNA binding was studied by the gel mobility shift assay (19, 20). A 273-bp DNA fragment containing the promoter region (including 45 bp of the merR gene and 157 bp of the merT gene, additional sequences from the M13 vector) was obtained by digestion of the replicative form (RF) DNA of M13 derivative with StyI and PstI. The fragment was used as an unlabeled mer operator-promoter DNA or labeled at the 5′ end with [γ-32P]dCTP (3000 CI/mmole; Amersham). A 475-bp fragment was derived from the digestion of M13 (mT1001) (21) RF DNA with AvaI, and labeled as above. A 200-bp fragment containing the tet promoter was obtained after digestion of pBR322 DNA with HindIII and NsiI. All DNA fragments were separated on polyacrylamide gels, and electrophoresed. Binding reactions were carried out at 30°C in a solution containing 10 μM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM EDTA, 50 μg/ml bovine serum albumin, 0.05% Nonidet P-40, 20 μg/ml sonicated salmon sperm DNA, 5% glycerol, 4.5 μl of the total labeled DNA fragments, and varying amounts of MerD. Reaction ingredients were mixed at 0°C, and then the mixture was incubated at 25°C for 15 min. Samples were loaded immediately onto a 5.5% polyacrylamide gel and subjected to electrophoresis at 4°C. TBE (44.5 mM Tris, 44.5 mM borate acid, and 1 mM EDTA) was used as gel and electrode buffer. The gel was prerun before loading samples. After electrophoresis, the gels were dried and exposed to XAR-5 film (Kodak) for autoradiography.

DNAase I Protection Footprinting—A modification of the procedure described by Galas and Schmitz (22) was used. Single-end-labeled operator-promoter DNA was incubated with or without MerD in a total volume of 20 μl of solution, as above. Partial digestion of the bound and unbound DNA was carried out in a total volume of a 50 μl of solution containing 20 μM Tris-HCl (pH 8.0), 3 mM MgCl₂, 5 mM CaCl₂, 80 mM NaCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.5 μg/ml of bovine serum albumin, and 0.1 μg/ml DNase I. The reaction mixtures were incubated for 1 min at 25°C. The DNA was terminated by adding 10 μl of 50% TMA, 10 μl EDTA followed by phenol-chloroform extraction and ethanol precipitation. The DNA samples were denatured with 90% formamide at 90°C for 5 min and then analyzed in sequencing gels.

RESULTS

Mercury Resistance of Cells Containing mer Operon with Mutations in merD—Cells containing the narrow-spectrum mer operon (R100- and Ts301-derived) with interruption of merD by transposon insertion or deletion in merD are slightly less resistant to Hg²⁺ compared to cells containing the intact mer operon (9, 13). On the other hand, when the effect of the merD gene was measured in trans as induction of the reporter gene product, β-galactosidase (expressed from the PmerT promoter), produced from a merA-lacZ fusion (pDU116) with a Ts5 insertion in merD), a 2-4-fold increase in β-galactosidase activity was observed. The results indicated that the merD gene is involved in the expression of the mer operon, and the expression of the mer operon is regulated by a transcriptional activator, MerR, which is regulated by the level of mercury.
ase activity was noticed in the absence of merD as compared to that in its presence (14). To investigate further, whether the MerD protein has any role in conferring resistance to mercury salts, we used cells with two variants of merD for growth in the presence of varying concentrations of Hg\(^{2+}\). As shown in Fig. 2, cells containing the intact mer operon (plasmid pGN120) and those containing pDH1 (merD deletion mutant) were resistant to approximately 35 and 30 \(\mu\)M Hg\(^{2+}\), respectively. Interestingly, cells harboring plasmid pGN124 (frame-shift mutation in merD by insertion of a small DNA oligomer) were resistant to approximately 60 \(\mu\)M Hg\(^{2+}\). The resistance pattern was reproducible from several experiments and was correlated with the rate of reduction of \(^{209}\)Hg\(^{2+}\) to \(^{209}\)Hg\(^{0}\) (data not shown). Note that Hg\(^0\) quickly diffuses out of the cell and volatilizes out of the aqueous medium (2).

Effect of MerD on in Vivo Transcription from the PmerT—β-Galactosidase fusion with merA (expressed from the merT promoter) in plasmid pDU1161 was used to assay quantitatively the effects of merD on transcription. The plasmid pDU1161 was derived from R100 by MucdAMP lac insertion in merA and then, by inactivating the merR gene by Tn5 insertion (9). The merR defect in pDU1161 was complemented by wild type merR from the plasmids supplied in trans. Since pDU1161 lacks a functional merD, the effect of different merD mutations could be assayed in trans. Fig. 3 shows the results of the induction experiments using Hg\(^{2+}\) as the inducer. Cells harboring pGN124 (frame-shift mutation in merD) consistently produced a 2-fold higher level of β-galactosidase as compared to cells harboring plasmid pGN120 (intact mer operon). On the other hand, cells containing plasmid pDH1 (merD deleted) produced the highest level of β-galactosidase. These results suggest that MerD represses transcription from the PmerT.

Purification of MerD—MerD protein was overproduced using a T7 expression system, where T7 RNA polymerase is inducible from a lacUV5 promoter. The basal level of T7 RNA polymerase synthesized in uninduced cells is inactivated by titration with lysozyme produced by the same cells (16). We were unable to overproduce MerD from the lacUV5 promoter or the tac promoter in Escherichia coli). Overproduced MerD protein was primarily present in the pellet when cell lysate was fractionated by centrifugation and purified as described under “Experimental Procedures.” Protein of approximately 80% purity was obtained (Fig. 4, lane 4). The relative molecular weight of the purified protein was about 13,500, which coincided with the value predicted from the nucleotide sequence of merD (10). Following SDS-PAGE, the purified protein was electroblotted, and the N-terminal amino acid sequence was determined (Applied Biosystems 477A protein

![Fig. 3. Inducibility of mer operon measured as expression of lacZ with different variants of merD in trans. Exponentially growing cells (50–60 Klett units) were induced with different concentrations of HgCl\(_2\) for 1 h prior to the measurement of β-galactosidase activity. Cells harboring different plasmids are shown.](http://www.jbc.org/)

![Fig. 4. SDS-PAGE of hyperexpressed and purified MerD.](http://www.jbc.org/)
sequencer). The sequence was determined to be MNA-YTYSRLAL, confirming the N-terminal sequence of MerD deduced from the nucleotide sequence (10).

**Binding of MerD Protein to the mer Promoter Region—In vivo transcription results suggest that MerD negatively regulates expression from the PmerT. In gel mobility shift assays, a 273-bp radiolabeled DNA fragment containing the mer promoter region and a 475-bp radiolabeled DNA fragment derived from the M13 replicative form DNA (as a nonspecific control) were used (see "Experimental Procedures"). The molar ratio of the mer promoter DNA: the nonspecific M13 DNA was approximately 14:1. When the DNA fragments were incubated with 0.05 or 0.1 μg of total MerD protein, the migration of the 273-bp DNA was specifically retarded (Fig. 5, lanes 2 and 3). However, the mobilities of the DNA fragments were retarded using higher concentrations of MerD (0.5 or 1 μg of MerD, lanes 4 and 5). In order to confirm the specificity of binding of MerD with the mer promoter region, we challenged radiolabeled prebound DNA with excess mer promoter DNA (unlabeled) or with a 200-bp tet promoter DNA (unlabeled) (lanes 6 and 7). Prebound MerD exchanged with unlabeled mer promoter DNA, but not with the unlabeled tet promoter DNA, demonstrating that MerD binds specifically the mer promoter region.

**Mapping of the mer Promoter-MerD Binding Sequence—**

Binding of MerR (Tn501-encoded) with the mer operator sequence has been mapped (23). In order to determine whether MerR and MerD bind the same sequence, we incubated the 273-bp radiolabeled DNA with MerD or MerR (R100-encoded) and performed DNase I footprinting analysis (Fig. 6). MerR- and MerD-protected sequences span from +22 to −8, and +28 to −10, respectively, relative to the start of the merR mRNA, confirming that MerD and MerR bind to sequences overlapping the PmerT and ε transcriptional region of the merR, which encompasses a 7-bp inverted repeat. It should be noted that the R100 MerR protects the same sequence from DNase I digestion as reported for Tn501 MerR (23). Hg2+ did not have any effect on DNase I protection by MerR or MerD (data not shown).

**DISCUSSION**

The role of merD in the regulation of the mer operon has been proposed, but direct experimental data demonstrating any specific interaction of MerD with the mer DNA was lacking (13, 14, 25). In this paper, we report partial purification (>80%) of MerD protein after overexpressing it from a T7 expression system. We have shown that the purified protein binds to the mer operator site, previously identified as the site for specific binding of the trans-acting activator/repressor protein, MerR. N-terminal amino acid sequence of 11 residues determined from the purified protein matched with the sequence predicted from the DNA sequence.

MerR is a homodimer of 144 amino acid polypeptides, which activates the transcription of the mer operon after binding with Hg2+, apparently by bending the promoter (PmerT) DNA structure (12, 26). The −10 and −35 consensus sequences of PmerT are separated by 18 bp, which is 2 bp longer than the 17-bp optimum distance for efficient transcription by the E. coli σ70 RNA polymerase (27, 28). 3 conserved cysteine residues (C82, C117, and C126) are most critical for binding of Hg2+ with MerR. The MerR-Hg2+ complex bound to the operator distorts the DNA structure at the promoter region,

**Fig. 6.** Top, DNase I protection footprinting of protein-DNA complexes. 10 ng of 32P-labeled DNA was used in lanes 1–3. Lanes 1: no protein added; 2, 0.6 μg of MerR; 3, 0.1 μg of purified MerR (from R100, approximately 99% pure). Lanes 2, 7: chemical "G" reaction according to Maxam and Gilbert (24), using the same mer operator-promoter fragment. Nucleotide sequences are shown on the right; MerR-protected sequence is delineated with an overline (A) and an underline (G). Bottom, nucleotide sequence of the mer operator-promoter region of the plasmid pDU138. merR mRNA and mer mRNA start sites are indicated with arrows pointing the direction of transcription (by analogy with Tn501 mer operon, Ref. 7). Consensus −10 and −35 sequences for the mer promoter are marked on the top strand. Consensus −10 sequence of the merR promoter is shown on the bottom strand. Palindromic sequences are underlined on the top strand.

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**Fig. 5.** Binding of MerD to mer promoter. 32P-Labeled DNA fragments containing about 4 ng of mer operator-promoter and 0.5 ng of a nonspecific M13 vector DNA (see "Experimental Procedures") were used in all lanes. Lanes: 1, 2, 3, 0.0, 0.05, 0.1, 0.5, and 1.0 μg of MerD protein, respectively; 6, same as in lane 5, except challenged with 200 ng of the unlabeled mer operator-promoter DNA (50-fold excess of 32P-labeled fragment) following incubation of MerD with the 32P-labeled DNA; 7, same as in lane 6, except challenged with 200 ng of an unlabeled tet promoter fragment. V, M13 vector DNA fragment; F, mer operator-promoter fragment; C, MerD-mer operator-promoter DNA complex.
bringing the −10 and −35 sequences closer for efficient transcription from the PmerT by the RNA polymerase (12, 29, 30). MerD is a 120 (Tn21) or 121 (pDU1358, Tn501) amino acid polypeptide. The highest amino acid sequence similarity between MerR and MerD is found in the N-terminal region containing the helix-turn-helix motif, presumed to be involved in binding with the operator DNA (14, 23). The conserved sequence in MerD at the N terminus suggests that it can bind with an operator DNA site. DNase I footprinting data suggests that MerR and MerD bind to the same operator site. Apparently, when MerD concentration reaches a critical level in the cell, it binds with the operator DNA, resulting in partial deactivation of transcription from PmerT, eventually lowering the synthesis of structural gene products and MerD protein itself. Note that merD and mer structural genes are cotranscribed. It may be hypothesized that MerD is involved in fine tuning of the expression of the mer operon as a coregulator.

Earlier attempts to delineate the role of merD on Hg⁺ resistance or on the expression of the mer operon were based on studies with deletions in merD or interruption by low insertion sequences of transposons [9, 13, 14]. Mutation in merD by insertion of a small DNA cligeron (net increase of 13 nucleotides within the coding sequence, and upstream of the major inverted repeat sequences) resulted in about a 2-fold increase in Hg⁺ resistance (Fig. 2). This increased resistance to Hg⁺ is correlated with the stability of mRNA (data not shown). It is known that secondary structure at the 3’ end of the mRNA protects it from degradation by exonucleases resulting in net increase in translation products (31–33).

When the level of β-galactosidase was measured to determine Hg⁺-induced activation of the mer operon in trans complementation experiments (expressed from PmerT of pDU1161, Fig. 3), three factors were likely involved in regulating the synthesis of β-galactosidase, e.g. MerR, MerD, and the concentration of the inducer Hg⁺. Since MerR was expressed from the same constitutive promoter, its concentration was perhaps the same in cells with all the variants of mer operon supplied in trans. β-Galactosidase activity in cells containing the intact mer operon (pGN120) was always lower as compared to that in cells harboring plasmids with mutations in merD (pGN124 or pDH1). This difference in β-galactosidase level can be attributed to the negative control of transcription by MerD. However, the difference in β-galactosidase activities induced by the pDH1- and pGN124-encoded merR gene product in trans may be difficult to reconcile. Of the three possible factors regulating the synthesis of β-galactosidase, as mentioned above, MerD (full length) is absent in both cases. A truncated 84-amino acid MerD protein (with 56 residues from the N terminus of MerD plus 28 unrelated residues resulting from the insertion of the linker) is presumably synthesized in cells containing pGN124. Note that the entire merD sequence is deleted in pDH1. The truncated MerD protein may bind to the operator DNA and function as a negative regulator, albeit less efficiently as compared to the full length MerD. Further experiments with mutations close to the 5’ end of the merD gene need to be done to investigate the above possibility. An alternative possibility is that differential levels of the inducer available inside the cells containing pDH1 and pGN124 result in differential activation of the merA-lacZ transcription (Fig. 3). The inducer (Hg⁺) concentration is most likely higher in cells harboring pDH1 as compared to that in cells containing pGN124 due to slower rate of Hg⁺ reduction in the former case (see “Results”).

We conclude that merD encodes a protein that binds within the mer operator and therefore may compete for binding with MerR. Our data also suggest that the inverted repeat structures at the 3’ end of merD contribute to the stability of the mer transcript.

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