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MINIREVIEW

Untwist and Shout: a Heavy Metal-Responsive Transcriptional Regulator

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BACTERIAL MERCURY RESISTANCE

How do living systems deal with chemicals inherently toxic to their fundamental biochemical processes? Among chemicals deleterious to life, metals have an ancient history of use. Metal-containing compounds were among the earliest specifics for human maladies, especially contagious ones, and their effectiveness is still exploited in common antiseptics and disinfectants. The microbial targets of these agents have acquired resistances to them even as they acquired resistances to widely used antibiotics. Genes conferring genetically and biochemically distinct resistances to arsenic, antimony, bismuth, borate, cadmium, cobalt, copper, lead, mercury, nickel, silver, and zinc have been described in many genera of the eubacteria. Frequently these loci are encoded by plasmids and/or transposons, and there is ample evidence of their extensive dissemination in both pristine and chemically impacted environments (37).

Presently, one of the best understood metal resistance loci is that conferring resistance to mercury compounds (*mer*) (37, 41). This system is of special interest because the resistance mechanism results in the transformation of mercury compounds from the toxic form (either ionic or organic) to the less toxic elemental form, Hg(0) (23). Exposure to mercury increases the incidence of mercury resistance in both soil and water eubacterial populations (4), as well as in the primate oral and intestinal flora (40). Current molecular studies emphasize the loci carried by two transposons of gram-negative bacteria, Tn*21* and Tn*501*, and by an IncC plasmid, pDU1358, and a chromosomal locus occurring in a marine *Bacillus* species. The Tn*21* *mer* operon lies just upstream of the integron locus within the large, multiple resistance aggregate of the IncFII plasmids prevalent in the genus *Escherichia* (39). Tn*501* was originally isolated from a *Pseudomonas* plasmid (6). Mercury resistance loci resembling Tn*501* *mer* and others less closely related to either Tn*21* or Tn*501* occur in both fermentative and nonfermentative gram-negative bacteria (11). The *Bacillus* chromosomal *mer* locus is related to one found on plasmids in the staphylococci (19), and these gram-positive *mer* loci are clearly, though distantly, related to those of gram-negative bacteria (42).

The mercury resistance (*mer*) operon of gram-negative bacteria consists of the gene coding for the regulatory protein, *merR*, which controls its own transcription and that of the divergently transcribed structural genes (6, 17, 23, 41) (Fig. 1). The latter include genes encoding a Hg(II) transport system (*merTP*) which brings Hg(II) through the cytoplasmic membrane. Hg(II) is then accessible to the Hg(II) reductase (*merA*), a cytosolic, NADPH-dependent, flavin adenine dinucleotide-containing disulfide oxidoreductase which reduces Hg(II) to volatile Hg(0), which diffuses away through the cell surface. The most promoter-distal gene in the operon of gram-negative bacteria, *merD*, encodes a small protein with some homology to *merR*; recent evidence suggests that it may also play a role in structural gene expression (24). The Tn*21* operon (but not that of Tn*501*) contains an additional gene, *merC*, encoding an inner membrane protein. The cloned *merC* of the *Thiobacillus mer* operon confers Hg(II) uptake ability on *Escherichia coli* (18), but deletion of *merC* from Tn*21* has no phenotypic effect (13a).

Many mercury resistance loci (though not Tn*21* or Tn*501*) carry an additional gene, *merB*, encoding the enzyme organomercurial lyase which cleaves the C-Hg bond yielding Hg(II), the substrate for mercuric reductase (41). Mercury resistance loci which contain the *merB* gene (called broad-spectrum loci) are inducible by both inorganic and organic Hg compounds (13, 26), whereas narrow-spectrum loci, such as Tn*21* and Tn*501*, which lack *merB* are inducible only by inorganic mercury. When *merB* is found, it typically lies between *merA* and *merD* (Fig. 1) (13); nothing is known about the transport of organomercurial compounds. Thus, the *mer* operon is modular, with the basic elements consisting (in gram-negative bacteria) of MerA, the mercuric ion reductase, and the two transport proteins, MerP and MerT (or MerC), and/or the organomercurial lyase, MerB (23). The *mer* loci of gram-positive bacteria encode a cytosolic Hg(II) reductase and a Hg(II) transport system (3, 19, 42). Interestingly, in gram-positive bacterial systems, *merR* is not divergently transcribed but is the first element in a transcript which includes the structural genes (15, 38).

REGULATION OF *mer* GENE EXPRESSION

The regulatory protein, MerR. The synthesis of this multifaceted detoxification pathway depends upon the properties of the metal-responsive transcriptional regulator, MerR. In the operons of gram-negative bacteria, this 144-amino-acid polypeptide regulates operon expression in the following three ways. (i) In the absence of Hg(II), MerR represses initiation of the *merTPCAD* transcript. (ii) In the presence of Hg(II), MerR activates initiation of the *merTPCAD* transcript. (iii) MerR represses its own transcription regardless of whether Hg(II) is present (Fig. 1).

The behavior of MerR mutants reveal physically distinct domains for its binding to DNA and for interacting with the inducer Hg(II) (Fig. 2). A predicted helix-turn-helix domain (residues 9 to 30) is associated with DNA binding. The

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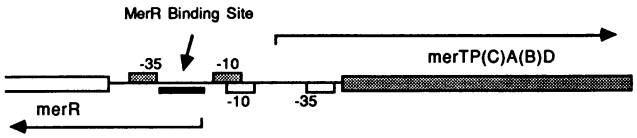

FIG. 1. General structure of the *mer* operon (not to scale). See text for gene designations.

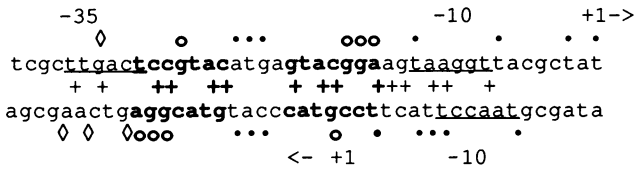

FIG. 3. Tn*21*/Tn*501* *mer* operator-promoter region. Boldface type indicates palindrome. Underlined positions on the top strand are RNA polymerase recognition hexamers for the *merTP(C)AD* promoter; underlined positions on the bottom strand are the −10 hexamer for the divergent *merR* promoter. In vivo start positions (+1) of the *merTP(C)AD* and *merR* transcripts of Tn*21* are indicated by arrows. Symbols: +, identified loss- or gain-of-function mutation; ◇, RNA polymerase contacts; o, MerR contacts; Hg(II)-induced sensitivity to $KMnO_4$ oxidation or copper phenanthroline cleavage (•). See specific references in the text.

dominant impairment of activation and repression functions (33) in the Glu-22→Lys (Glu22Lys) mutant of $MerR_{Tn21}$, as well as its loss of DNA binding ability (34), is consistent with the second helix of this helix-turn-helix motif recognizing the dyad symmetry region in the *merOP* DNA (see below). Purified MerR forms a dimer which is stable against reducing agents and binds only one Hg(II) per dimer (28, 35, 36, 43). Since there are three conserved cysteine residues per monomer (indicated with # in Fig. 2), none of which are involved in internal or intersubunit disulfide bonds in the native protein (36), there are six potential ligands for Hg(II). Biochemical (14, 28), genetic (14, 33), and physical (43, 44) studies indicate that Hg(II) binds to MerR in an unusual tricoordinate geometry which, at least in *Bacillus* MerR, involves Cys-79 from one monomer and Cys-114 and Cys-123 from the other monomer (14) (the corresponding MerR positions in gram-negative bacteria are Cys-82, Cys-117, and Cys-126 (Fig. 3)]. Thus, the purified protein and its inducer ligand can form an asymmetric complex. While in vivo genetic evidence does support nonequivalent roles for Cys-82 and for Cys-117 and Cys-126 (33), as yet there is no other evidence as to whether such an asymmetric MerR-Hg complex actually activates transcription in vitro or in vivo or even whether the one-Hg(II)-per-MerR-dimer stoichiometry is maintained when the protein binds to DNA. In vitro, MerR does activate transcription in response to Cd(II) and to Zn(II), but only at concentrations several orders of magnitude higher than for Hg(II) (32).

Residues involved in activation per se are distributed throughout MerR. The region from residues 50 through 90 contains several pairs of acidic residues, and although none of the reported activation mutations occurs in these acidic residues, many such mutants lie at other positions within this region (Fig. 2) (33). Mutations effecting loss of activation (Ala60Val and Ala60Thr) and others resulting in constitutivity (Ala89Val) occur in this central domain (33). Of the nonconserved regions among the various MerR proteins, the most C terminal is particularly noteworthy. Although a missense mutation resulting in constitutivity lies as far out as residue Ser-131 (Fig. 2), earlier studies indicated that the 14 C-terminal residues of MerR Tn*21* (5) could be deleted and replaced with 19 nondescript amino acids without loss of activator function. The MerR proteins of gram-positive bacteria end just 9 to 12 residues beyond their equivalent to Cys-126. Thus, there appears to be very little constraint on variability in this C-terminal region, making it difficult to assign specific roles to this region. The overall picture of MerR is consonant with that of other procaryotic repressors in having separate domains for interaction with DNA and with the inducer ligand. However, the activation function of this protein does not appear to be closely associated with the DNA binding domain but extends from the middle of the protein to near (but not including) the C terminus.

Thirty-two of the 130 common residues shared by MerR proteins of gram-positive and gram-negative bacteria are exactly conserved (Fig. 2). The three Hg(II)-ligand cysteines are conserved in number and in relative spacing. In addition to these residues, there are two prominent clusters of conserved residues from Val-19 to Leu-32 (the putative recognition helix of the helix-turn-helix domain) and from Arg-53 to Ala-89 (a region implicated in interaction with RNA polymerase). MerR is related to a chromosomally encoded sensor of oxidative damage in *E. coli* (1) and, thus, is the second gene in the operon to have a chromosomal "cousin", the other being *merA*, which is a homolog of glutathione reductase (41).

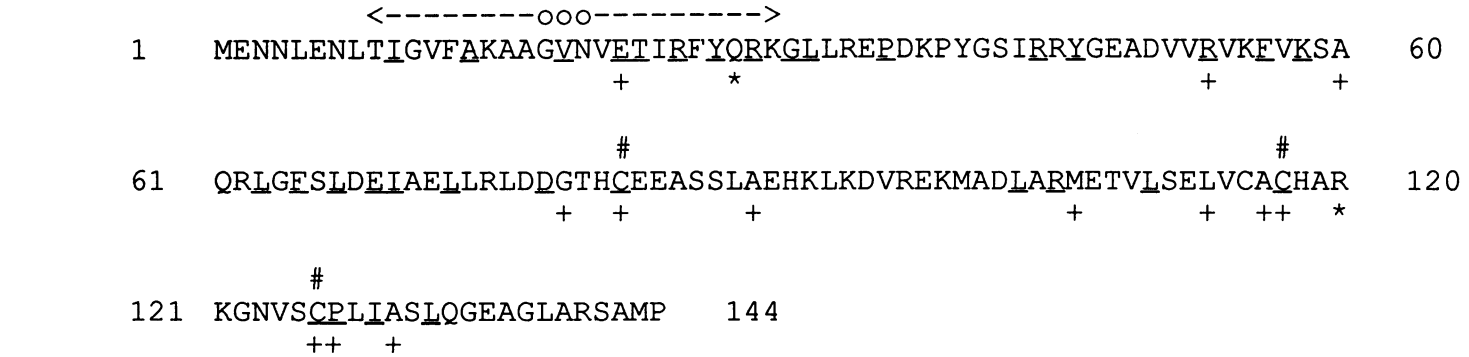

FIG. 2. Sequence and functional domains of Tn*21* MerR. The 32 underlined residues are conserved in all known MerR genes in both gram-positive and gram-negative bacteria. Symbols: #, cysteines involved in Hg(II) binding; ←o→, helix-turn-helix motif; +, observed loss- or gain-of-function missense mutation; *, nonsense mutation.

The *mer* operator-promoter. MerR binds (29, 36) to a region of dyad symmetry which lies within the spacer region, between the −10 and −35 sigma-70 RNA polymerase recognition hexamers of the structural gene promoter, $P_{TP(C)AD}$ (Fig. 3). In this position in the loci of gram-negative bacteria, MerR also occludes the +1 position of its own (divergent) transcript. Thus, with respect to the *merTP(C)AD* promoter, the MerR binding site lies well within the range of consensus positions for repressors and well outside of that range for activators (8). With respect to its own promoter, the MerR binding site, lying within the beginning of the transcript, is also within the common range for repressor binding sites.

The arrangement of RNA polymerase recognition hexamers and their spacing in the *mer* operon is unusual. The −10 hexamers of the divergent promoters overlap, but the −35 hexamers do not (Fig. 3). There is but one set of candidate hexamers for the $P_{TP(C)AD}$ promoter and apart from the nonoptimal (19-bp) spacing, they are in good agreement with consensus sigma-70 recognition sites. Single-base deletions in the spacer lead to up-promoter mutations (21). For the *merR* promoter (P_R), however, there are two putative candidate sets of recognition hexamers, both with reasonably good correspondence to the sigma-70 consensus and both with nonoptimal interhexamer spacing of either 15 or 19 bp. Genetic evidence (30) currently indicates that it is the more-start-site-proximal (9, 16) hexamer set (with 15-bp spacing) that functions as P_R in vivo (Fig. 3). Despite this less-than-optimal spacing, in the absence of *merR*, P_R is a more active promoter than $P_{TP(C)AD}$ in vivo (25) and in vitro (28). One of the most intriguing observations of the *mer* system is that induction with Hg(II) has no effect on expression of *merR* (25, 28, 30). When MerR is present, neither in vitro runoff transcripts nor *merR'-phoA* or *merR'-lacZ* transcriptional fusions detect any change in the transcription from P_R when Hg is added. The mechanism whereby the tightly overlapped P_R promoter maintains its apparent independence of changes affecting initiation at $P_{TP(C)AD}$ remains to be determined.

Genetic analyses (30, 31) indicate that the leftward offset position of the MerR binding site within the spacer region is optimum for activation; moving the operator to the right within the spacer region reduces activation efficiency. This suggests MerR may contact RNA polymerase, albeit from a different "face" than the majority of described activators (which bind on the 5′ side of the −35 hexamer) (8). The fact that mutants whose dyad is offset toward the right (but whose spacer length is not changed) are impaired in both activation and repression suggests that contact between MerR and RNA polymerase is important for both processes, although the distance between dyad center and the −10 hexamer of $P_{TP(C)AD}$ may also be important in activation.

The conservation of the MerOP site is very strong, with the most prominent elements retained being the central bases "GTACnnnnGTAC" of the interrupted palindrome. Similarly, genetic analyses indicate that these inner bases in each dyad arm are more important in repression of both promoters and in activation of $P_{TP(C)AD}$ (30, 31).

Interactions of MerR with MerOP. In vitro-purified MerR binds its cognate linear DNA (MerOP) with a dissociation constant of 10^{-10}. The addition of Hg(II) increases the dissociation rate from 3- to 100-fold (15, 28) (note that there is some controversy on this point [36]). Dimethylsulfate footprinting identified nine guanine residues (28) within the dyadic region which are protected by purified MerR on relaxed DNA in vitro; only four residues (G's at −18, −19, −30, and −31) are protected by MerR alone on supercoiled DNA in vivo (Fig. 3) (16). Bases G-18 and G-19 are just slightly deprotected upon Hg(II) addition (16, 28), suggesting that once MerR is bound to DNA, Hg(II) does not decrease its occupancy, although it may cause a shift in the conformation of the MerR-MerOP complex.

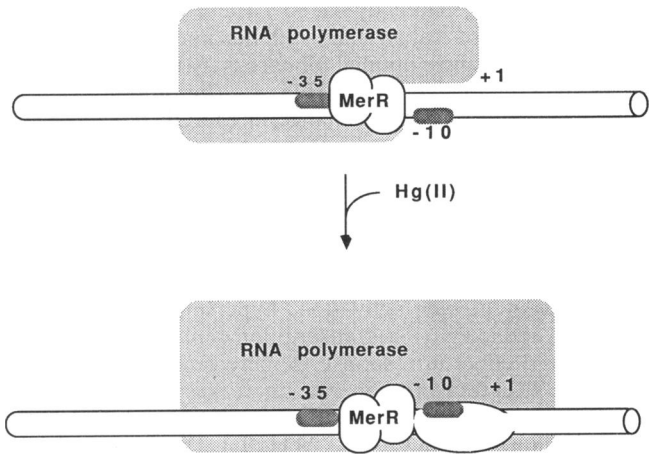

FIG. 4. Model of the MerR-Hg(II)-mediated activation of the *mer* structural gene promoter, $P_{TP(C)AD}$. See details in text.

One of the most surprising observations arising from the DNA footprinting is that when MerR is bound to the wild-type Tn*21* MerOP, sigma-70 RNA polymerase is also bound, clearly occupying the −35 region, regardless of whether Hg(II) has been added (9, 16) (Fig. 4). Thus, although MerR binding in the −10 and −35 spacer would be expected to prevent access of RNA polymerase to $P_{TP(C)AD}$ the protein can paradoxically sequester sigma-70 RNA polymerase at the inactive promoter. It is likely that MerR also actively does something either to RNA polymerase or to the DNA to prevent transcription until Hg(II) is added, since repressed expression is 10-fold less than derepressed (i.e., *merR*Δ) expression. Active interference with open-complex formation could involve direct interaction with RNA polymerase, or it could arise from MerR constraining the DNA locally so as to minimize transient spontaneous underwinding.

DNA footprinting has revealed another surprising facet of the MerR activation mechanism. As expected, induction with Hg(II) in vivo (or in vitro in the presence of RNA polymerase, MerR, and nucleoside triphosphates) increases the reactivity to potassium permanganate of several T residues in the −12 to +2 region of $P_{TP(C)AD}$ consistent with the formation of an open promoter complex (9, 16, 28). However, in addition to these bases, in vivo Hg(II) induction results in increased $KMnO_4$ oxidation of several bases lying in the dyad region, well upstream of the region typically melted when an open complex is formed in other promoters (16, 22). Sensitivity of bases in the center of the dyad to the chemical nucleases 5-phenyl orthophenanthroline copper and methylpropidium EDTA-Fe is also markedly enhanced upon Hg(II) treatment of the MerR-MerOP DNA complex in vitro (9). A long-held hypothesis for the mechanism of MerR activation of $P_{TP(C)AD}$ is that the protein fosters distortion of the DNA to compensate for the unusually long (19 bp) spacer region and thereby allow RNA polymerase to form an open complex (6, 28). Ansari et al. (2) have recently demonstrated that purified MerR alone induces a slight bend in the DNA upon binding and that when Hg(II) is added, MerR under-

winds the *merOP* DNA by ca. 33°. These combined actions could suffice to realign the −10 hexamer with the −35 hexamer at the angle optimal for access by RNA polymerase in forming the open complex (Fig. 4). The precise extent of this novel conformational change in MerOP remains to be determined. Also as yet unresolved is the question of whether the DNA distortion alone is sufficient for activation or does MerR also "tweak" RNA polymerase itself in some fashion during the activation process.

In some manner (yet to be determined), these activities of MerR and RNA polymerase give rise to the third unusual phenomenon of *mer* activation, hypersensitivity. Both in vitro (32) and in vivo (8a) transcription of *mer* mRNA exhibit a threshold effect in response to increasing Hg(II) concentrations. The transcription rate increases ninefold over a four- to fivefold increase in Hg(II), with the midpoint of the sigmoidal response at ca. 10^{-8} M Hg(II). This hypersensitive response is occasionally seen in enzyme catalysis and can be indicative of cooperativity in the reaction mechanism. Such a response has not been reported in transcriptional activation; its exact basis in the mechanism of *mer* regulation is under investigation.

Transcriptional elongation in *mer*. While the most obvious control of *mer* expression takes place at the initiation of transcription, the *mer* operon of gram-negative bacteria is also subject to modulation of transcriptional elongation. The first hint that early genes were more highly expressed than those more distal came from a comparison of the relative amounts of the gene products. The estimates from minicell protein labelling indicated that the relative amounts of MerT, MerP, MerC, and MerA are 4:50:1:1 (10). MerD is undetectable in minicells, but MerD-LacZ protein fusion measurements indicate that it is produced at 5% the level of MerA (20). Since there are no obvious translational impediments in the sequences preceding the less abundant proteins and since MerP (unpublished observations), MerA (41), and MerD (24) are sufficiently stable to have been purified, mRNA synthesis or turnover seemed likely factors affecting the apparent natural polarity of gene expression in the operon.

Indeed, the 5′ end of the *merTPCAD* transcript is about 40-fold more abundant in induced cells than in uninduced cells; in contrast, the 3′ end of the transcript is only about 2-fold more abundant in induced cells than in uninduced cells (10). The mRNA half-lives are quite similar for all genes (1.0 to 2.5 min), suggesting that no region is preferentially degraded. However, for the first 4 to 5 min after induction, the synthesis rate of the more-promoter-proximal 1,700 nucleotides of mRNA (including *merT*, *merP*, and *merC*) is fivefold greater than that of the subsequent *merA-merD* mRNA (ca. 2,100 nucleotides). This is surprising since at its normal rate of transcription (12) RNA polymerase should transit the entire 3,800-bp Tn*21* *mer* operon within 80 to 90 s. The low initial transcription rate of *merAD* gradually rises about twofold 5 to 10 min after induction but never reaches a level equivalent to that of the early genes before repression is restored (ca. 12 to 15 min) as a result of consumption of the inducer Hg(II) by newly synthesized Hg(II) reductase. We have not observed a unique termination endpoint in the *merC-merA* region. However, two regions with significant conservation of functionally important elements of the MerR binding site occur in the N terminus of *merA*. This premature termination is counterintuitive and results in the greater synthesis of the proteins for Hg(II) uptake than for Hg(II) reduction. It may be that the Hg(II) transport process works less efficiently than mercuric reductase and, thus, is required in greater abundance.

The mysterious MerD. The role of the most promoter distal of the structural genes, *merD*, has been a puzzle since it was first noted in the DNA sequence (7). The similarity of its N terminus to that of MerR suggested that MerD might be a coregulator (7). Like MerR, MerD is made in very small amounts (20). Unlike *merR*, deletion of *merD* has little or no effect on the expression of mercury resistance. However, *mer-lac* fusions in *trans* to a multicopy operon expressed less LacZ than if the multicopy *mer* operon had a deletion in *merD* (27). These observations suggest that an excess of MerD may antagonize the activator function of MerR. Consistent with this hypothesis is the recent finding that purified MerD protein binds to the MerOP DNA, albeit with a considerably lower affinity than does MerR (24). There is as yet no information on the Hg(II) binding ability of MerD, although the protein does have three cysteine residues. The availability of the purified protein should rapidly dispel much of the mystery which has surrounded *merD* and reveal yet another layer of regulation ("retro"?) in this surprisingly tightly controlled system.

Summary. In closing, it is important to distinguish what *mer* regulation is not. Thus, while it would seem quite appropriate for a heavy metal-responsive regulator, MerR is not a "finger" or a "fist," nor is it apparently a "zipper" or given to "looping." Further, its relationship with MerD cannot presently be understood, in the simple sense, to be similar to those of the so-called two-component regulators. Although *mer* is a relatively new model system used to study transcriptional regulation, work from several laboratories building on and extending the insights and techniques devised for the more classical regulated systems now reveal that the *mer* system has some remarkable properties. Currently, MerR is the only known prokaryotic activator which stably sequesters sigma-70 RNA polymerase at an inactive promoter. The "in-the-spacer" binding position of MerR and its ligand-induced helix underwinding and hypersensitive induction kinetics are also novel among described prokaryotic activators. In general, the behavior of the gene products of the *mer* operon challenges the conventional wisdom about what happens to proteins whose sulfhydryl groups react with Hg(II); similarly, *mer* regulation requires some expansion of our models of how transcriptional activation works. The question of whether other systems (including the versions of the *mer* operon found in gram-positive bacteria and the related oxidative stress regulator of gram-negative bacteria) will prove to have similar properties is a subject of active enquiry.

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