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Genetic Organization and Regulation of Antimicrobial Efflux Systems Possessed by *Neisseria gonorrhoeae* and *Neisseria meningitidis*

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Abstract

Efflux pumps can make a significant contribution to the capacity of bacteria to resist the action of antibiotics. Certain efflux pumps also recognize antimicrobial agents that are present in their respective hosts and their ability to export toxic agents could enhance bacterial survival during infection prior to appearance of cellular or humoral host defensive systems. This review is concerned with the principal efflux pumps possessed by two closely related strict human pathogens, *Neisseria gonorrhoeae* and *Neisseria meningitidis.* Specific emphasis is placed on the organization of the structural genes encoding the *mtr* and *far* efflux pumps, the substrates (often host-derived) recognized by these pumps, and the *cis*- and *trans*-acting transcriptional factors that regulate efflux pump gene expression in gonococci and meningococci. The overriding theme of this review is that the efflux pumps possessed by these pathogens likely contribute to their pathogenic mechanisms by providing a means to escape a number of antimicrobial compounds that bathe mucosal surfaces.

Introduction

N. gonorrhoeae and *N. meningitidis* are the two human pathogenic species of the genus *Neisseriae.* As strict human pathogens that are incapable of persisting for prolonged periods outside of the human host, except under laboratory conditions, they have likely adapted strategies to survive host antimicrobial systems that exist on the mucosal surfaces that they infect or colonize. For *N. gonorrhoeae* (the gonococcus), biblical passages in the Old Testament suggest that it has caused the sexually transmitted disease gonorrhea for thousands of years (Sparling *et al.*, 1990). In contrast, the earliest description of disease (cerebrospinal meningitis and/or meningococcemia) likely caused by *N. meningitidis* (the meningococcus) can be traced to the writings of Vieusseaux in 1805 (Vieusseaux, 1805). Despite significant differences in the frequency of gonococcal versus meningococcal disease in the United States (>250,000 vs. ca. 3,000 cases, respectively) and the severity of their clinical presentations, these pathogens have significant biochemical, genetic and structural similarities. Included in these similarities is their possession of homologous genes that encode multi-drug efflux pump systems.

The overriding interest in bacterial efflux pumps has been their contribution to the development of microbial resistance to clinically useful antibiotics (Levy, 1992). To be sure, this is an exceptionally important concern given the global public health problem of antibiotic resistance expressed by pathogenic bacteria. However, when viewed in the context of microbial evolution throughout the millennia, it is likely that efflux pumps developed as a microbial defense mechanism against antimicrobials present in the environment and that this ability contributes to the persistence of bacteria in hostile environments. For strict human pathogens such as gonococci and meningococci, these environmental antimicrobial compounds are likely present or become available at the different mucosal surfaces that these pathogens infect (gonococci and meningococci) or transiently colonize without causing disease (meningococci). The main tenet of our work dealing with efflux pumps possessed by gonococci and meningococci is that their capacity to remove host-derived antimicrobials (Figure 1) promotes bacterial survival and persistence at mucosal surfaces. Given this ability, it is our belief that antimicrobial efflux systems should be viewed in the larger context of bacterial pathogenesis in that they serve as virulence factors. Accordingly, this review will concentrate on the genetic organization of the principal efflux pumps possessed by gonococci and meningococci, the antimicrobials recognized by these pumps and the bacterial transcriptional control systems that modulate their gene expression.

Results from several laboratories during the past ten years (as outlined in the present series of reviews) has clearly established that a single bacterial species can encode a multitude of structurally similar or dissimilar efflux pumps. More recent advances through analyses of genome sequence data bases has revealed other potential efflux systems. Without question, it is likely that only the “tip of the iceberg” has thus far been revealed and future efforts will establish the function and expression of many new

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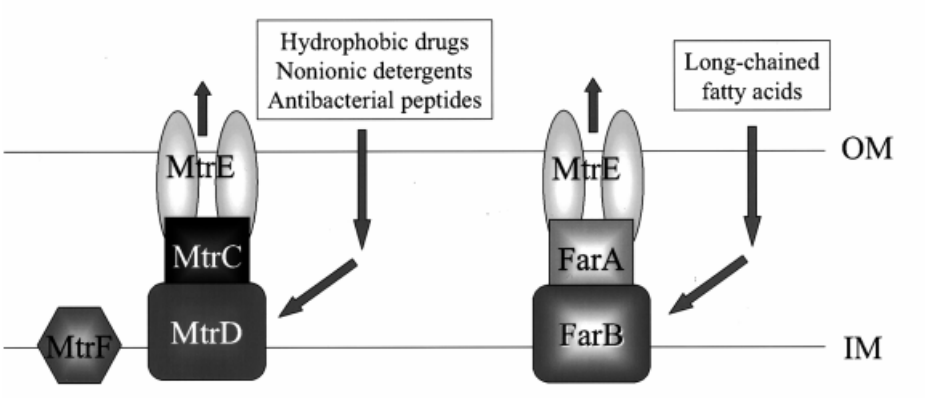

Figure 1. Based on the model developed for other bacterial efflux pumps, we propose that the MtrC-MtrD-MtrE and FarA-Far-B-MtrE proteins form efflux pumps that can capture certain antimicrobial agents (summarized in the boxed areas) in either the periplasm or cytoplasmic membrane. We also suggest that MtrF is a cytoplasmic membrane protein that is important for export of high levels, by the MtrC-MtrD-MtrE pump, of certain antimicrobial agents by resistant strains. Its possible interaction with the MtrC-MtrD-MtrE pump is now under investigation.

efflux pumps. With respect to gonococci and meningococci, experimental evidence (Hagman *et al.*, 1995; Lucas *et al.*, 1995; Lee and Shafer, 1999; Rouquette *et al.*, manuscript in preparation) exists for two functional efflux pumps (*mtr* and *far*), but others been predicted from the annotated meningococcal genome sequences (Parkhill *et al.*, 2000; Tettelin *et al.*, 2000).

Gene Organization and Proteins of Gonococcal Efflux Pumps

The *mtr* (*m*ultiple *t*ransferable *r*esistance) system was originally described in 1973 (Maness and Sparling, 1973). They studied a laboratory-derived mutant that expressed increased resistance to multiple hydrophobic drugs, dyes and detergents and found that the resistance phenotype was transferable to a wild-type strain in single-step transformation experiments. The *mtr* system likely played an important role in the gradual and step-wise, chromosomally-mediated resistance of gonococci to penicillin that was first noticed in the 1960s by physicians whose patients failed penicillin therapy. Elegant work by Sparling's group in the mid-1970s revealed that the combined action of three mutations (*penA, penB* and *mtr*) was required for such resistance (Sparling *et al.*, 1975). Similarly, decreased susceptibility of gonococci to other antibiotics used previously (e.g., tetracycline) or presently in certain developing countries (e.g., single-dose azithromycin therapy in Uruguay) can develop because of the *mtr* system (Zarantonelli *et al.*, 1999). Thus, as stressed by others (Levy, 1992; Nikaido, 1996), the capacity of efflux pumps to act synergistically with other resistance mechanisms is a significant clinical problem for effective management of infections.

Initially, the phenotype endowed by *mtr* was suggested to be the result of a mutation that decreased the permeability of the gonococcal cell envelope (Guymon *et al.*, 1975), but later studies by us and our collaborators (Hagman *et al.*, 1995, 1997; Lucas *et al.*, 1995; Delahay *et al.*, 1997; Veal *et al.*, 1998; Shafer *et al.*, 1998) showed that it defined a chromosomal locus that encodes an efflux pump (see Figure 2) that uses the proton-motive force to export structurally diverse antimicrobial compounds. These hydrophobic agents (HAs) include antibiotics (e.g, azithromycin, erythromycin and rifampin), nonionic detergents (including the popularly used spermicide, nonoxynol-9), certain antibacterial peptides (Protegrin-1 and LL-37), bile salts, and gonadal steroidal hormones.

Since many of the substrates recognized by the *mtr* system exist on mucosal surfaces, we proposed that it is

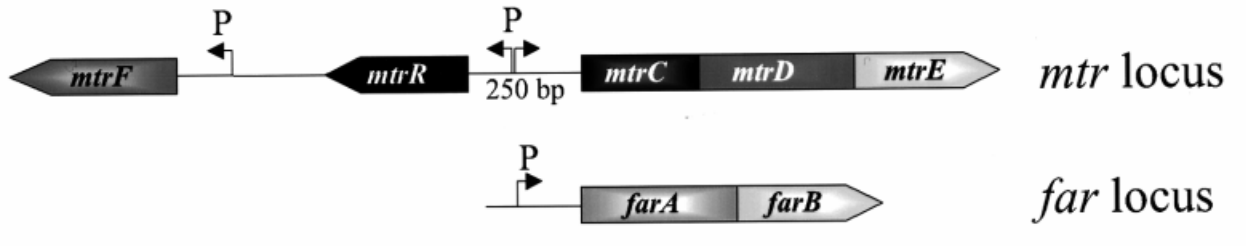

Figure 2. Shown is the genetic organization of the *mtr* and *far* loci possessed by gonococci; the *mtrC-mtrD-mtrE* genes represent a single transcriptional unit. Upstream but transcriptionally divergent is the *mtrR* gene, which encodes a DNA-binding protein that negatively regulates transcription of the *mtrC-mtrD-mtrE* operon through binding to its promoter (P); other P elements are shown upstream of *mtrF* and *farA-farB*. The promoters for *mtrR* and *mtrC-mtrD-mtrE* overlap. A *cis*-acting 13 bp inverted repeat (IR) element is located within the *mtrR* promoter. Gonococci expressing high levels of resistance to HAs typically have a single bp (T:A) deletion in this IR element. Although not shown in this figure, all meningococcal strains examined to date have a 155-159 insertion sequence (Correia element) within the 250 bp intervening region, downstream of the *mtrC-mtrD-mtrE* promoter. Some strains also have a tandemly-linked copy of IS*1301*. The Correia element appears to decrease transcription of this efflux pump operon in meningococci.

important in the pathogenesis of gonococcal disease in that it would promote bacterial survival at sites that can be infected by gonococci (Shafer *et al.*, 1995). In this respect, 46% of all clinical isolates obtained from males with acute gonococcal urethritis examined in one study (Zarantonelli *et al.*, 1999) expressed HA-resistance (HA^R) due to the *mtr* system. HA^R mediated by the *mtr* system is a frequent property of isolates obtained from rectal infections (Morse *et al.*, 1982), presumably because such strains are exposed to high levels of antibacterial fatty acids and bile salts in the rectum. Our recent collaboration with King Holmes' group in Seattle revealed that one particular gonococcal strain was frequently isolated from the gay male population in that urban area. We found (Xia *et al.*, 2000) that these isolates always contained a *cis*-acting mutation in a promoter element (see below) that we previously (Hagman and Shafer, 1995) showed to enhance expression of the *mtr* efflux pump. This strain was probably maintained in the Seattle community since the mutation provides increased resistance to fecal-derived HAs. While the *mtr* efflux system is of likely importance in survival of gonococci in the rectum, where high levels of HAs are present and feces may trap gonococci, making treatment more difficult, examination of other strains isolated from both males and females with urogenital infections revealed that they also can express *mtr*-determined HA^R. Interestingly, a subset of gonococcal strains (termed envelope mutants) expressing hypersusceptibility to HAs was identified in the 1970s (Sarubbi *et al.*, 1975; Eisenstein and Sparling, 1978). These isolates often caused asymptomatic infections. These highly HA-susceptible strains could, however, donate HA^R to wild-type strains in transformation experiments. Our (Veal *et al.*, 1998) molecular analyses of these strains revealed that they can have loss-of-function mutations in genes that encode structural proteins of the *mtr* pump (see below). We also confirmed an earlier hypothesis (Eisenstein and Sparling, 1978) that these strains often contained a phenotypically-suppressed mutation that would abrogate the activity of a transcriptional repressor (MtrR) of the efflux pump genes; the natures of these repressor-inactivating mutations are described below.

Our studies and those of our collaborators have defined the genetic organization of the *mtr* locus in the antibiotic-sensitive *N. gonorrhoeae* strain FA19. The *mtrRCDE* gene complex and the components of the efflux pump (MtrC-MtrD-MtrE) are shown in Figures 2 and 1, respectively. The efflux pump formed by these proteins places it in the Resistance/Nodulation/Division (RND) category (Saier *et al.*, 1994); similar pumps are possessed by *E. coli* (e.g., AcrA-AcrB-TolC [Ma *et al.*, 1995]) and *P. aeruginosa* (e.g, MexA-MexB-OprM [Poole *et al.*, 1993]). Efflux pumps of the RND-class use the proton motive force of the cytoplasmic membrane for their energy-dependent export of toxic agents (Nikaido, 1994). MtrC, MtrD and MtrE are each essential for efflux activity, since loss of any one protein due to mutation results in hypersusceptibility of gonococci to HAs. Based on the model developed by Nikaido (Nikaido, 1994; 1996) for RND efflux pumps, we envision (Figure 1) that the MtrD protein serves as the cytoplasmic membrane transporter (Hagman *et al.*, 1997). It likely makes physical contact with the MtrC periplasmic protein, which has significant amino acid similarity with a class of proteins termed membrane fusion proteins (Saier *et al.*, 1994). MtrC is in turn linked to the MtrE outer membrane protein, which serves as the channel for export of agents to the extracellular environment (Delahay *et al.*, 1997). Recently, we (Veal and Shafer, manuscript in preparation) obtained evidence that a fourth Mtr protein (MtrF) exists. Our findings suggest, but have not proven, that MtrF is a cytoplasmic membrane protein (Figure 1) that is needed for export of high levels of HAs by resistant strains. It is important to note that MtrF displays significant similarity to hypothetical proteins produced by other bacteria and is, interestingly, similar (38% a.a. identity over 522 amino acids) to the AbgT protein (Hussein *et al.*, 1998) possessed by *E. coli*; AbgT is a cytoplasmic membrane protein involved in the import of p-aminobenzoyl-glutamate across the cytoplasmic membrane. Whether MtrF also exhibits transport activity (import or export of HAs across the cytoplasmic membrane) is now under investigation.

We suspected that gonococci would possess efflux pumps other than that encoded by the *mtrCDE* operon. This hypothesis was based on an earlier study (McFarland *et al.*, 1983) that certain clinical isolates expressed resistance to fatty acids but not other HAs such as erythromycin, Triton X-100 and crystal violet. These strains were stated to have an *mtr*-independent mechanism for resistance to long-chained fatty acids. As the gonococcal genome sequencing project progressed to nearly 95% completion, it became possible to search online (www.genome.ou.edu) for ORFs that would encode proteins similar to other efflux proteins. This effort revealed an ORF that would encode an EmrB-like protein (Lomovskaya and Lewis, 1992). We subsequently cloned and sequenced a locus from strain FA19 that contained two tandemly linked ORFs (Figure 2) that were predicted to encode proteins similar to the EmrA and EmrB efflux proteins of *E. coli* (Lomovskaya and Lewis, 1992). These proteins, along with the TolC (Fralick, 1996) outer membrane protein, mediate energy-dependent export of uncoupling agents from *E. coli;* a homologous efflux system (VceA-VceB) exists in *Vibrio cholerae* (Colmer *et al.*, 1998). Insertional inactivation of the *emrB*-like sequence did not increase gonococcal susceptibility to uncoupling agents, hydrophobic or hydrophilic antibiotics, but it did increase susceptibility to the long-chained fatty acids (oleic, linoleic and palmitic acid) studied by Morse's group (McFarland *et al.*, 1983). We named these ORFs *farA* and *farB* to signify their involvement in *f*atty *a*cid *r*esistance. We also determined that the gonococcal FarA-FarB pump uses the MtrE protein as its outer membrane protein channel (Figure 1), which is similar to the use of TolC by multiple efflux pumps possessed by *E. coli* (Fralick, 1996). These results offer an explanation as to how some gonococci could develop resistance to long-chained fatty acids, which are frequently found at the rectal mucosal surface, without developing constitutive resistance to other HAs (McFarland *et al.*, 1983).

Transcriptional Control of Efflux Pump Gene Expression

Increasing evidence from a variety of studies using different bacteria indicates that genes encoding efflux pump systems

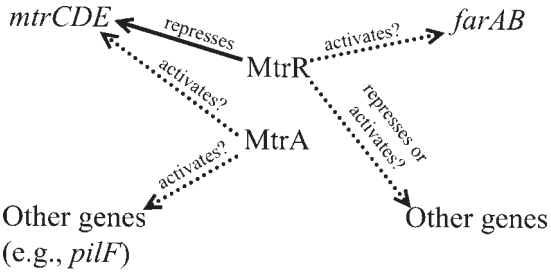

Figure 3. The MtrR and MtrA proteins can regulate expression of the efflux pump operons possessed by gonococci as well as other genes. Direct evidence for MtrR-repression of *mtrCDE* has been obtained (see solid line with arrow) but it may also activate (directly or indirectly) *farAB* or other genes as well as repressing other genes (dotted line with arrow). The MtrA transcriptional activator protein has a direct or indirect role in activating *mtrCDE* as well as other genes, notably *pilF* (dotted line with arrow). Expression of *pilF* is essential for induction of gonococci to high level resistance to HAs (Rouquette *et al.*, manuscript in preparation).

are under transcriptional control (Nikaido, 1996). It has been argued that constitutive over-expression of efflux pump genes is energy-draining, which would be disadvantageous under normal growth conditions. Under conditions where antimicrobial agents are not present, there would be no need to over-express efflux pump genes. In contrast, it would be beneficial for bacterial survival to respond to the presence of antimicrobial agents by over-expressing efflux pump-encoding genes. This model suggests that bacteria can utilize both negative and positive transcriptional regulators to modulate expression of efflux pump genes. Our biochemical and genetic studies strongly indicate that MtrR directly represses *mtrCDE* gene expression in gonococci (Hagman *et al.*, 1995; Lucas *et al.*, 1997). In contrast to this repressor activity, we (Rouquette *et al.*, 1999) have identified a second gene that encodes a protein (MtrA) required for induction of *mtrCDE* gene expression. Efflux pump gene expression can also be regulated by *cis*-acting factors that flank promoter elements used for efflux pump gene transcription. Below, the *trans*- and *cis*-acting factors that are known or presumed to be involved in efflux gene expression in gonococci and meningococci are described.

The *mtrR* gene, positioned upstream and transcriptionally-divergent from the *mtrCDE* operon (Figure 2), was first identified by Pan and Spratt (1994). They showed that it encodes a 210 amino acid protein that bears significant similarity to numerous transcriptional repressors, notably the tetracycline repressor family (Brow *et al.*, 1985), the AcrR and AcrS proteins that regulate expression of the *acrAB* and *acrEF* genes of *E. coli* (Ma *et al.*, 1994; 1996), and the LuxR transcriptional activator of *Vibrio harveyi* (Schwartzman *et al.*, 1992). Loss of the MtrR protein, through deletion or insertional-inactivation of *mtrR*, resulted in increased resistance of gonococci to certain HAs (Pan and Spratt, 1994; Hagman *et al.*, 1995) and enhanced expression of *mtrCDE* (Hagman and Shafer, 1995). These results argue that MtrR is a transcriptional repressor that negatively regulates expression of the *mtrCDE* gene complex (Figure 3). The MtrR protein possessed by wild-type strain FA19 (sensitive to HAs) contains a helix-turn-helix (HTH) motif in the *N*-terminal region (Pan and Spratt, 1994), suggesting that this region is important in DNA-binding activity. Using an MtrR-maltose-binding fusion protein, we found that MtrR binds a target DNA sequence that encompasses the promoter used for *mtrCDE* gene expression (Lucas *et al.*, 1997). Two important domains of MtrR appear to be needed for its function. First, the HTH domain (amino acid positions 32-53) is clearly important since radical amino acid substitutions within this region (e.g.,Gly-45 to Asp-45) result in increased HA^R (Hagman *et al.*, 1995) when introduced into strain FA19. This radical amino acid change in the HTH abrogates MtrR binding to its target site (Lucas *et al.*, 1997), the promoter that drives transcription of *mtrCDE*. A second group of missense mutations (such as His-105 to Tyr-105) alter the center domain of MtrR, and although their impact on MtrR function is not yet known, it is likely that this substitution modifies its capacity to form multimers needed for repressor activity.

The *E. coli emrAB* operon is negatively controlled by the EmrR repressor (Lomovskaya *et al.*, 1992), which is encoded by an upstream gene. Since efflux pump operons are often subject to transcriptional control systems (Nikaido, 1996), we were curious as to if and how the *farAB* gene complex in gonococci is regulated. We did not detect an *emrR* equivalent in the FA1090 genome sequence data base. Our initial studies dealing with the regulation of the *farAB*-encoded efflux pump have given us reason to suspect that the MtrR protein might regulate genes other than *mtrCDE* and that it may not always be a transcriptional repressor (Figure 3). Since we could not detect an *emrR*-like sequence in the FA1090 genome sequence, we asked whether MtrR might serve to negatively regulate expression of *farAB*. Accordingly, we examined expression of *farAB* in MtrR-positive strain FA19 and its isogenic MtrR-negative strain KH15. We fully expected that like *mtrCDE* expression, the expression of *farAB* would be elevated in strain KH15, but it was actually lower than that of FA19 (Lee and Shafer, 1999). Moreover, strain KH15 expressed a lower level of resistance to long-chained fatty acids than strain FA19, which is the exact opposite property for other HAs that are exported by the *mtrCDE*-encoded efflux pump. This suggests that somehow MtrR is involved in expression of *farAB*, either directly by serving as an activator of transcription or indirectly by repressing some other modulator of *farAB* gene expression. It is very likely that MtrR has the capacity to directly or indirectly control expression of genes other than *mtrCDE* and *farAB,* since its loss by gene deletion results in decreased production of 11 proteins and increased production of 3 proteins (Shafer *et al.*, unpublished observations) that have electrophoretic characteristics different from the known efflux pump proteins. The repertoire and identification of MtrR-regulated genes is now under investigation. It will be of interest to see how many of these genes encode proteins that are known or presumed to be involved in virulence. Given the recent finding (Tan *et al.*, 1999) that an MtrR homolog is required for full virulence of *Pseudomonas aeruginosa*, this possibility has merit.

While our understanding of how efflux pump operons can be negatively regulated by repressor proteins similar to MtrR has increased significantly, much less is known at the molecular level about how the operons can be transcriptionally activated. The question is: how do bacteria

that produce a repressor of efflux pump gene expression respond to toxic agents in their environment? Ma *et al.* (1996) posed this question in their experiments with the *acrAB* efflux pump system possessed by *E. coli*. They showed that growth in a sublethal concentration of antimicrobials stimulated expression of this efflux pump, even in the face of an active AcrR repressor. They provided genetic evidence that global transcriptional regulators (e.g., SoxS, MarA and Rob) stimulated expression of *acrAB*. We suspected that such a system might exist in gonococci. We analyzed the gonococcal genome database for ORFs that would encode transcriptional activators and found (Rouquette, *et al.*, 1999) one that would encode a protein, which we termed MtrA (Figure 3), similar to the AraC/XylS family (Gallegos *et al*., 1993). Insertional-inactivation of the wild-type *mtrA* gene in strain FA19 resulted in an uninducible phenotype. Additionally, a subset of gonococcal isolates that could not be induced were all found to contain an 11 bp deletion at the 5'-end of *mtrA*, which would result in a truncated MtrA protein. Analysis of the protein content of whole cell lysates from a fully inducible strain by two-dimensional isoelectric focusing/SDS-PAGE showed the induction and repression of several gonococcal proteins during growth in sub-lethal concentrations of HAs (Rouquette and Shafer, unpublished observations). Three of the inducible proteins were present only in an MtrA-positive background; these proteins were 25, 26 and 58 kDa. While the identity of the MtrA-dependent, inducible 25 and 26 kDa proteins have yet to be determined, the 58 kDa protein appears to be PilF. Evidence that PilF is needed for inducible HA^R in gonococci was obtained when a *pilF* insertional mutant was found to be uninducible. PilF has been localized to the cytoplasm and is an accessory protein in the pilus biogenesis system (Freitag *et al*., 1995). It has been implicated in the secretion of pilin monomers across the inner membrane. Taken together, our data suggest that it could also participate in the MtrA-dependent, inducible antimicrobial resistance in gonococci. This induction process also seems to require energy provided by the TonB-ExbB-ExbD protein complex, since deletion of the *tonB-exbB-exbD* locus in strain FA19 significantly reduced induction, but had no impact on basal levels of HA^R (Rouquette *et al*., manuscript in preparation).

Although the MtrR protein is likely important in determining levels of HA^R through regulating expression of the *mtrCDE* genes, a novel bypass system allows gonococci to express high levels of HA^R even when a wild-type *mtrR* gene is present. We (Hagman and Shafer, 1995) identified a 13 bp inverted repeat DNA sequence that apparently serves as a *cis*-acting transcriptional control element. This inverted repeat was identified in the divergent promoter regions of the *mtrR* and *mtrCDE* genes in HA-sensitive strain FA19 (see Figure 2 and legend). We determined that a single T:A bp deletion in this repeat sequence, in the presence or absence of a wild-type *mtrR* gene, resulted in high levels of HA^R in gonococci. This deletion was identified in all clinical isolates displaying high levels of HA^R (Shafer *et al*., 1995). We found that this single bp deletion, which would reduce the spacing between the -10 and -35 domains of the *mtrR* promoter from 17 to 16 nucleotides, repressed transcription of the *mtrR* gene but enhanced *mtrC* expression. More recently, we identified a clinical isolate from Uruguay that expresses decreased susceptibility to azithromycin, erythromycin and Triton X-100 contains a dinucleotide (TT) insertion within the 13 bp inverted repeated within the *mtrR* promoter (Zarantonelli and Shafer, unpublished observations). This insertion likely has a *cis*-acting impact on *mtrR* gene expression. Potential *cis*-acting sequences have also been identified upstream of the *farAB* efflux pump operon (Lee and Shafer, unpublished observations). These include repeat elements and a potential binding site for integration host factor. Their capacity to regulate *farAB* gene expression is now under investigation.

The importance of *cis*-acting regulatory elements in modulating efflux pump gene expression is further highlighted by our recent findings with meningococci. Interest in the efflux pumps possessed by the meningococcus is based on the capacity of the gonococcal *mtrCDE*-encoded efflux pump to recognize rifampin, an antibiotic used prophylactically to treat individuals exposed to an index case of meningococcal meningitis. We hypothesized that over-expression of a meningococcal *mtrCDE* efflux pump due to *cis* or *trans*-acting factors present in gonococci could have serious consequences in treating exposed individuals. Our molecular studies (Rouquette and Shafer, manuscript in preparation) on several different capsular serogroups of meningococci revealed the presence of an intact *mtrCDE* gene complex that would encode a functional efflux pump. These strains and one studied by Abadi *et al.* (Abadi *et al*., 1996) also contained a variety of nonsense or missense mutations in their respective *mtrR* gene that would in the gonococcus abrogate MtrR function leading to enhanced antibiotic resistance. However, these strains were sensitive to HAs. Moreover, despite having a full-length and expressed *mtrA* gene they could not be induced to higher levels of resistance to HAs. Thus, meningococci seem to have a naturally down-regulated *mtrCDE* efflux pump operon. Analysis of the DNA sequence that intervenes the *mtrR* and *mtrCDE* genes revealed that all meningococci contain a 155-159 bp insertion that is identical to the previously (Correia *et al.*, 1988) described Correia Element (CE); a minority of strains also contained a tandemly linked copy of IS*1301*. While this element exists in multiple copies in both the gonococcal and meningococcal genomes (Correia *et al*., 1988; Parkhill *et al*., 2000), we have never detected it in the *mtr* promoter region in the over thirty gonococcal isolates studied to date. Transcriptional analyses revealed that meningococci use a promoter element similarly positioned to that in gonococci but the presence of the downstream CE reduces transcription. Thus, acquisition of the CE by meningococci seems to have (thankfully) abrogated any potential effect of loss of MtrR. It is possible that during the course of its evolution, meningococci acquired an over-expressed *mtrCDE* gene complex from gonococci by transformation. While this de-repressed *mtrCDE* operon would have provided increased resistance to HAs, it may have also slowed the growth rate, which was detrimental for passage from a colonized host to a susceptible host. By placement of the CE downstream of the *mtrCDE* promoter, the benefits of having a down-regulated efflux pump operon (export of low level levels of HAs and faster growth rate) was achieved.

While much has been learned during the past decade regarding efflux pumps possessed by gonococci and meningococci, a large number of questions remain. These include: Are there additional pumps possessed by these pathogens and if so, are they expressed and what are their substrates? How are environmental signals that induce efflux pump gene expression received and what are the events that result in enhanced efflux pump production? What are the roles of accessory proteins in efflux pump action? Are there additional transcriptional factors that modulate pump gene expression? Answers to these and other questions should provide important insights regarding how gonococci and meningococci survive the various antimicrobials in their human host.

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