Unique organization and regulation of the mrx fimbrial operon in Xenorhabdus nematophila

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Xenorhabdus nematophila, a Gram-negative bacterium belonging to the Proteus clade of the family Enterobacteriaceae, forms a mutualistic association with the soil nematode Steinernema carpocapsae. The nematode invades insects and releases Xenorhabdus into the haemolymph, where it participates in insect killing. To begin to understand the role of fimbriae in the unique life cycle of Xenorhabdus, the organization and expression of the mrx fimbrial operon was analysed. The mrx operon contained only five structural genes (mrxC/CDGH), making it one of the smallest chaperone-usher fimbrial operons studied to date. Unlike the mrr operon of Proteus mirabilis, a site-specific recombinase was not linked to the mrx operon. The intergenic region between the major fimbrial gene (mrxA) and the usher gene (mrxC) lacked a mrrB-like gene, but contained three tandem inverted repeat sequences located downstream of mrxA. A 940 nt mrxA-containing mRNA was the major transcript produced in cells growing on agar, while an mrx polycistronic mRNA was produced at low levels. A canonical ρ70 promoter, identified upstream of mrxA, was not subject to promoter inversion. Fimbriae were not produced in an lrp-mutant strain, suggesting that the leucine-responsive regulatory protein, Lrp, plays a role in the regulation of the mrx operon. These findings show that the genetic organization and regulation of the mrx operon is in several respects distinct from other chaperone-usher fimbrial operons.

INTRODUCTION

The symbiotic-pathogenic bacterium Xenorhabdus nematophila forms a species-specific symbiosis with the soil nematode Steinernema carpocapsae (Forst et al., 1997; Forst & Clarke, 2002). Xenorhabdus, a member of the family Enterobacteriaceae, is carried in a specialized intestinal sac in the free-living infective juvenile form of the nematode. This symbiotic interaction protects X. nematophila from the competitive environment of the soil and carries the bacterium to the nutrient-rich haemolymph of a susceptible insect, where it produces a variety of toxins that are involved in killing the host. Xenorhabdus proliferates in the haemolymph, and produces a wide range of exoenzymes that convert the insect cadaver into a nutrient source for the nematode during development and reproduction. The nematode reproduces until the nutrient supply becomes limiting, at which time it is recolonized by Xenorhabdus. The bacteria also produce a wide range of antibiotic compounds that suppress contamination of the insect cadaver by soil microbes and reduce predation by invertebrate organisms (Webster et al., 2002; Zhou et al., 2002). During its life cycle, therefore, X. nematophila both survives in the haemoecoic insect environment and colonizes a specific location of the nematode intestine. Although the mechanism by which X. nematophila establishes the symbiotic association with its nematode partner is not well understood, several genetic loci affecting this interaction have been identified (Forst & Boylan, 2002; Heungens et al., 2002; Vivas & Goodrich-Blair, 2001).

Hair-like appendages called fimbriae participate in the specific attachment of pathogenic bacteria to host cells (Low et al., 1996). This facilitated attachment is a prerequisite for bacterial invasion and colonization, important early steps in pathogenesis (Stentebjerg-Olesen et al., 1999). The well-characterized Pap fimbriae of Escherichia coli (Tullus et al., 1992) and type 1 fimbriae of E. coli (Connell et al., 1996), Salmonella (Tinker et al., 2001) and Proteus (Zhao et al., 1997) are important factors in the colonization of the human intestinal and urogenital tracts. Fimbriae also play a role in the symbiotic interaction between Vibrio fischeri and the Hawaiian squid, Euprymna scolopes (Stabb & Ruby, 2003).

Pap and type 1 fimbriae are assembled by a chaperone-usher pathway (Soto & Hultgren, 1999). The operons encoding these fimbriae generally consist of between eight and eleven genes, which include regulatory, assembly and structural components. While the assembly and structural proteins
of the various fimbriae share similarities, the regulation of the operons can vary considerably (Blomfield, 2001). The pap operon is regulated by a complex mechanism involving local regulatory proteins (PapB, PapI), the leucine-responsive regulatory protein (Lrp), cyclic AMP receptor protein (CRP), and the differential site-specific methylation of two GATC sites situated upstream of the promoter (Hernday et al., 2002). The fim operon of E. coli and the mrf operon of Proteus mirabilis are regulated by a recombinase-mediated inversion of a promoter-bearing invertible element. The recombinase genes are linked upstream of the major fimbrial subunit gene. Lrp stimulates recombination of the fim invertible element, possibly by altering the local spatial organization of the DNA in this region (Blomfield, 2001). Both Pap and type 1 fimbriae are produced under prolonged static growth conditions, but generally are not produced on solid agar media (Old & Duguid, 1970; Gally et al., 1993).

X. nematophila produces fimbriae on nutrient agar, but not when grown under aerated conditions in LB broth (Binnington & Brooks, 1993; Moureaux et al., 1995). The X. nematophila fimbriae are 7 nm in diameter and up to 3 μm in length, consisting primarily of a 16 kDa major subunit protein (Moureaux et al., 1995). Purified fimbriae agglutinate erythrocytes of sheep and rabbit in a mannose-resistant pattern. Immunogold labelling indicates that fimbriae are present on X. nematophila inhabiting the nematode gut sac (Binnington & Brooks, 1993). Although there has been speculation that the fimbriae in X. nematophila are involved in the species-specific symbiotic association with Steinernema carpocapsae (Binnington & Brooks, 1993; Forst & Nealon, 1996; Moureaux et al., 1995), the role they play in the complex life cycle of the bacterium has not been established. To begin to understand the function of fimbriae in Xenorhabdus, we have characterized the mannose-resistant fimbrial operon (mrx) of a X. nematophila strain.

METHODS

Bacterial strains, media and growth conditions. Xenorhabdus nematophila AN6 (ATCC 19061), HGB321 (ATCC 19061 lrp::kan; kindly provided by H. Goodrich-Blair), and Escherichia coli XL-1 Blue MRF’ (Stratagene) were used in this study. The flhDC strain, ABB1 (flhDC::kan), was constructed by B. Boylan, following the method of Forst & Boylan (2002). The bacteria were grown in LB broth, and on LB agar and nutrient agar, which were prepared from standard recipes. Antibiotics were added to the media as indicated below. X. nematophila AN6 was cultured at 30°C, and E. coli was grown at 37°C.

Purification of DNA and RNA. Genomic DNA was extracted using the Edge BioSystems purification kit. Total RNA was extracted with the RNasy mini kit (Qiagen) or Trirreagent (Sigma). RNA samples used for RT-PCR were predigested with the RNase-free DNase reagent (Qiagen).

Sequence analysis of the mrx operon. To sequence the mrx operon, three separate partial libraries were constructed in pBluescript SK(+). mrxA and mrxD PCR probes based on partial genomic sequence information of Xenorhabdus strain 85816 (kindly provided by B. Goldman, Monsanto) were used to screen a X. nematophila AN6 library. The mrxA-containing clone, pBS4, carried a 9.3 kb EcoRI-HindIII fragment, which contained pnp, mrxA, and 212 bp downstream of the stop codon. The mrxD-containing clone, pBS21, carried a 5 kb HindIII insert, containing mrxC, mrxD, mrxG and 687 bp of the 5′ region of mrxH. To isolate the clone pBSH1, a probe derived from the mrxH sequence of AN6 was used, which carried a 4.5 kb EcoRII insert containing mrxG, mrxH, mrxJ and the downstream gene encoding the large subunit of carbamyl phosphate synthase. To complete the sequence between mrxA and mrxC, a 1245 bp PCR product was amplified from the beginning of mrxA to an internal sequence in mrxC. This PCR fragment contained a 439 bp overlap with mrxA and a 241 bp overlap of mrxC. The overlapping sequences of the PCR fragment were identical to that derived above and provided the sequence between the 3′ end of pBS4 and the 5′ end of pBS21. The organization of this region of the mrx operon was confirmed by overlapping PCR analysis. Automated nucleotide sequence analysis was performed at the Core DNA Facility at the University of Wisconsin-Milwaukee. Sequences were assembled with MacVector 5.0. Amino acid identity searches were performed using the NCBI BLAST website, and identity values were calculated using pairwise sequence alignment (http://genome.cs.mtu.edu/align/align.html). To search for putative Lrp-binding sites upstream of mrxA, the MEME (Bailey & Elkan, 1994) probability matrices algorithm was used (http://meme.sdsc.edu/meme/website/intro.html). This analysis was performed using individual Lrp-binding sequences of the papBA promoter.

Construction of mrxA-null strain (AHA1). An internal mrxA fragment was PCR amplified with the primer pair 5′-CGCAAGCAA-CTGCCAGCAC-3′ (forward) and 5′-CAGGTAGTTCAAGATGATG-ATGGC-3′ (reverse). A PolII site (underlined) was engineered into the forward primer. A natural EcoRV site exists within mrxA. The PCR product was ligated into pSTBlue-1 (Novagen), and a Pdr-EcoRV fragment (418 bp) from the resultant plasmid was ligated into the conjugal suicide vector pKnock-Cm (Alexeyev, 1999). This was followed by electroporation into E. coli S17-1 ppir and conjugal transfer into X. nematophila AN6. The desired mutant (AHA1) was selected on LB agar containing 50 μg ampicillin ml⁻¹ and 25 μg chloramphenicol ml⁻¹, and confirmed by Southern analysis.

PCR and RT-PCR reactions. PCR reactions (50 μl) contained 40 ng genomic DNA, 20 pmol primers, 2 mM dNTPs, 2-5 mM MgCl₂ and 2-5 units Tag DNA polymerase (Promega). Standard reactions were performed for 30 cycles, RT-PCR reactions (50 μl), using the AccessQuick RT-PCR system (Promega), contained 100 ng DNA-free RNA, 20 pmol primers and 1 unit reverse transcriptase. The reverse transcription reaction was conducted at 52°C for 45 min. To obtain semi-quantitative results, the PCR reaction was carried out for 25 cycles.

Primer extension analysis. Three primers were used in primer extension reactions to determine the start of transcription of mrxA and examine the potential existence of a promoter within the intergenic region between mrxA and mrxC. Primer 1 (5′-GCTTTGTC-AGGTGTTTA-TTCACCACC-3′) is internal to the start codon of mrxA. Primer 2 (5′-CGGGGATAGAGG-AAATGTGC-3′) is located in the middle of the intergenic region. Primer 3 (5′-GCCGATAATGCACACGAC-3′) is internal to the start codon of mrxC. These primers were end-labelled with [γ³²P]ATP and T4 polynucleotide kinase. The Primer Extension System-AMV Reverse Transcriptase (Promega) was used for the primer extension reaction. The primer was annealed to total RNA by incubating at 52°C for 20 min and cooling at room temperature for 10 min. The primer extension reaction was conducted at 42°C for 30 min. The extension product was separated on an 8% polyacrylamide denaturing gel and sized using a radiolabelled δX174 DNA marker ladder.

H. He, H. A. Snyder and S. Forst
**Northern blot analysis.** Total cellular RNA was denatured with formamide and formaldehyde, and resolved by gel electrophoresis on 1-25 % Seakem Gold agarose gel (BioWhittaker Molecular Application). The level of RNA in each lane was examined by staining the gel with SYBR Green II RNA gel stain (BioWhittaker Molecular Application). RNA was transferred to a nitrocellulose membrane, which was cut into three sections and hybridized with probes spanning \textit{mrxA}, the intergenic region between \textit{mrxA} and \textit{mrxC}, or \textit{mrxC}.

**Extraction of surface molecules.** For preparation of fimbriae, overnight cultures (100 µl) were spread on nutrient agar plates and incubated at 30 °C for 48 h. Cells were harvested by repeated resuspension in 0-5 ml PBS (1 ml total). The cell suspension was vortexed vigorously for 30 s and centrifuged at 15,000 g for 10 min at 24 °C. The supernatants containing released fimbriae were spun for 14 min at 353,000 g at 4 °C. The resulting fimbrial pellet was resuspended in 20 µl SDS loading buffer, incubated at 4 °C for over 1 h and loaded onto a 15 % SDS-PAGE gel. For N-terminal sequence analysis of MrxA, the protein was transferred onto Immobilon-P membranes (Millipore). Amino acid sequence analysis was carried out at the Protein and Nucleic Acid Facility of the Medical College of Wisconsin. To examine production of fimbriae and flagella under broth conditions, cultures were grown in LB broth. Cells were pelleted and processed as described above.

**Examination of fimbrial production by electron microscopy.** A 10 µl sample of bacterial suspension (see above) was placed on a 400-mesh copper-coated grid, and cells were allowed to settle for 30 s. After removing the liquid, one drop 0-8 % phosphotungstic acid (pH 6-5) was placed on the grid, which was incubated for 30 s, and then processed for electron microscopy.

**RESULTS**

**Sequence analysis of the \textit{mrx} operon**

The 11.4 kb region encompassing the \textit{mrx} operon and flanking genes is shown in Fig. 1. The number of amino acid residues in each protein is indicated under the respective gene. The \textit{mrx} operon consists of five structural genes (\textit{mrxACDGH}) and one putative regulatory gene, \textit{mrxJ}. BLAST analysis revealed that the \textit{mrx} genes were most closely similar to the \textit{mrf} and \textit{mrf} orthologous genes of \textit{Proteus mirabilis} (Zhao et al., 1997) and \textit{Photorhabdus temperata} (Meslet-Cladiere et al., 2004), respectively (Fig. 1). The \textit{mrx} operon consists of \textit{mrxA} (major subunit), \textit{mrxC} ( usher protein), \textit{mrxD} (chaperone), \textit{mrxG} (minor subunit) and \textit{mrxH} (adhesin). The ORF downstream of \textit{mrxH} encodes a protein that shares 42 % identity with MrpJ of \textit{P. mirabilis}, which functions as a repressor for flagellar synthesis (Li et al., 2001). This ORF was therefore designated \textit{mrxJ}. The gene located upstream of \textit{mrxA} encodes a protein that shares 88 % identity with purine nucleoside phosphorylase (Pnp) of \textit{E. coli}. The gene located downstream of the \textit{mrx} operon encodes a protein that shares 92 % identity with the large subunit of carbamoyl-phosphate synthase (CpsL) of \textit{Yersinia pestis}.

The \textit{mrx} operon was distinct from the \textit{mrp} and \textit{mrf} operons in several respects. Firstly, the \textit{mrx} operon did not contain

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**Fig. 1.** Comparison of the \textit{mrx} operon of \textit{Xenorhabdus nematophila} with \textit{mrf} of \textit{Proteus mirabilis} and \textit{mrf} of \textit{Photorhabdus temperata}. Major subunit genes are shown in black; genes involved in fimbrial assembly in grey; \textit{mrxJ} in light grey. Two arrows flanking the promoter indicate inverted repeat sequences. The number of amino acid residues for each Mrx protein is shown below the respective gene. Values above \textit{mrf} and \textit{mrf} operons show percentage amino acid identity with \textit{mrx}. Arrows above genes represent direction of transcription. CpsL, carbamoyl-phosphate synthase large chain; HP, hypothetical protein; p, promoter; Pnp, purine nucleoside phosphorylase; TR, transcriptional regulator.
Fig. 2. Production of MrxA in X. nematophila AN6 and mutant strains. AN6 (lane 1) and the mrxA mutant strain (lane 2) were grown on nutrient agar for 48 h. Cells grown in aerated LB broth for 15 h are shown in lane 3 (AN6), lane 4 (mrxA strain) and lane 5 (flhC strain). Surface structures were prepared as described in Methods. The identification of the OpnP (348 amino acid residues) and FlIC bands (313 amino acid residues) was determined previously (Forst & Boylan, 2002).

Production of Mrx fimbriae on agar medium

To ensure that the fimbriae produced on NB agar were encoded by the mrx operon, the N-terminal amino acid sequence of the major fimbrial subunit was determined. Fimbriae derived from the parental strain grown on nutrient agar produced a 16 kDa major subunit protein (Fig. 2, lane 1). The first 24 N-terminal residues of the 16 kDa protein were 100% identical to the N-terminal sequence of mature MrxA (Fig. 3, bold letters) and shared 76% and 73% identity with the N-terminal sequence of the major fimbrial subunits MrfA and MrpA of Proteus temperata and Proteus mirabilis, respectively. Met-1 to Ala-23 constitutes the signal peptide of MrxA (Fig. 3, underlined), which is cleaved between Ala-23 and Ala-24. Based on the amino acid sequence homology with other major subunit genes, the N-terminal region of MrxA is predicted to be involved in subunit–subunit interactions, while the C-terminal sequence (Gly-166 to Leu-179) is predicted to form a β-zipper motif recognized by PapD-like chaperones (Girardeau et al., 2000). Further proof that the mrx operon encodes the fimbriae produced on agar surfaces is obtained from a recently constructed strain in which mrxA was disrupted (He, 2002). The mrxA strain lacked fimbriae, as assessed by electron microscopy (Fig. 4), and did not

Fig. 3. X. nematophila mrxA gene sequence with flanking regions. The −35 and −10 regions of the ρ70 promoter are located upstream of mrxA. The leader peptide of the MrxA protein is underlined. Bold letters represent the N-terminal sequence determined by amino acid sequence analysis. Putative Lrp-binding sites located upstream of mrxA are shown by divergent arrows. Downstream inverted repeats are labelled with convergent arrows. rbs, Putative ribosome-binding site.

Fig. 4. Electron micrographs of X. nematophila AN6 and the mrxA-null strain, AHA1, showing lack of fimbriae in AHA1. Cells were grown on nutrient agar and processed as described in Methods.
produce the 16 kDa protein (Fig. 2, lane 2). These findings confirmed that fimbriae produced on NB agar were encoded by the mrx operon. We have also shown that MrxA was not produced in X. nematophila grown to stationary phase under aerated conditions in LB broth (Fig. 2, lane 3). Flagella were expressed in liquid medium, as indicated by the production of the major flagella subunit, FlIC (Fig. 2, lanes 3, 4), which was absent in the flhDC strain that does not produce flagella (Fig. 2, lane 5).

Identification of the mrxA promoter

A potential σ70 promoter region, containing −35 (TTGACT) and −10 (AATGAT) consensus sequences separated by 17 nt, was identified approximately 150 nt upstream of the AUG start site of the mrxA gene (Fig. 3). To determine whether this promoter regulated mrxA, primer extension analysis was performed using total RNA derived from cells grown on NB agar for 30 h (Fig. 5, lane 1). A single extension product of approximately 345 nt was obtained which coincides with the size (350 nt) predicted for a transcript initiated at the consensus σ70 promoter. To assess whether a second promoter existed in the mrxA–C intergenic region, primer extension analysis was performed using primers directed to the middle of the intergenic region (primer 2) and to the 5′ end of mrxC (primer 3). Neither of these primers generated a detectable extension product (Fig. 5, lanes 2 and 3).

Regulation of the mrxA promoter

The mrpA and mrfA promoters are located on invertible elements flanked by inverted repeat sequences. Unlike mrpA and mrfA, the region upstream of mrxA did not possess inverted repeat sequences. Since a MrpI-like recombinase had been identified elsewhere in the genome of X. nematophila (He, 2002), we assessed whether the mrxA promoter was subject to inversion, using a PCR-based assay (Fig. 6). If the promoter is oriented in the ‘ON’ direction, primer pair 1–3 (lanes 2 and 6) and primer pair 2–4 (lanes 3 and 7) would generate PCR products of 578 and 741 bp, respectively. In contrast, PCR products would not be produced either with primer pair 1–2 (lanes 4 and 8) or with primer pair 3–4 (lanes 5 and 9). Alternatively, if the promoter were in the ‘OFF’ orientation, primer pairs 1–2 and 3–4 would produce PCR fragments. Fig. 6 shows that the mrxA promoter was oriented in the ‘ON’ configuration, both in cells grown on agar and in cells grown in broth, indicating that it was not subject to promoter inversion.

The leucine-responsive regulatory protein (Lrp) is involved in the regulation of both papBA and fimA (Blomfield, 2001; Hernday et al., 2002). To assess whether Lrp was involved in mrxA regulation, fimbrial production was analysed in an lrp mutant strain (HGB321). The lrp strain lacked fimbriae, as determined by both electron microscopy and

**Fig. 5.** Promoter identification by primer extension. Total RNA was obtained from X. nematophila AN6 grown on nutrient agar for 30 h. Each reaction used 16 μg RNA. Primers used in the extension reaction were as follows: lane 1, primer located in mrxA; lane 2, primer located in the mrxA–C intergenic region; lane 3, primer located in mrxC.

**Fig. 6.** PCR-based assay for assessing promoter inversion. Locations of the four PCR primers used are indicated by arrows. Lanes 1 and 10, 1 kb DNA ladder; lanes 2–5, PCR products using DNA from cells grown on nutrient agar; lanes 6–9, PCR products using DNA from cells grown in LB broth. Primer pairs used in the experiment were as follows: lanes 2 and 6, primer pair 1–3; lanes 3 and 7, primer pair 2–4; lanes 4 and 8, primer pair 1–2; lanes 5 and 9, primer pair 3–4. p, mrxA promoter.
SDS-PAGE (data not shown). To further assess the role of Lrp in the regulation of mrxA, the upstream region was examined for putative Lrp-binding sites. While Lrp-binding sequences are not highly conserved, a consensus sequence, GWWNTTTWW (where W = A or T, N = any nucleotide) has been determined for the six Lrp-binding sites of papBA (Hernday et al., 2002). Using the papBA Lrp-binding sequences to search the upstream region of mrxA, eight putative binding sites were identified (Fig. 3). These putative Lrp-binding sites were arranged as divergent pairs, suggesting that dimeric Lrp binds to these sequences. Taken together, these results suggest that Lrp functions as a positive regulator of the mrx operon. Finally, the mrx upstream region lacked GATC methylation sequences and canonical CRP-binding sites.

**Processing of mrx transcripts**

To analyse further the regulation of the mrx operon, Northern blot analysis was carried out with total cellular RNA prepared from cells grown on nutrient agar. Probes directed to mrxA, the mrxA–C intergenic region and to mrxC were used in the analysis (Fig. 7). A major RNA transcript of approximately 940 nt was detected with the mrxA probe (Fig. 7, lane a). The size of this transcript was consistent with the production of an mRNA initiating at the σ70 promoter upstream of mrxA and terminating in the mrxA–C intergenic region. In contrast, RNA transcripts were not detected with either the mrxA–C (lane b) or the mrxC (lane c) probes. These results indicated that mrxA RNA was expressed at high levels in cells growing on agar, while polycistronic mRNAs were not present at levels that could be detected by Northern blot analysis.

To determine whether low levels of a polycistronic mrx mRNA were produced in cells grown on nutrient agar, an RT-PCR approach was taken. Polycistronic mRNA initiated at the mrxA promoter would contain mrxA, the mrxA–C intergenic region and downstream genes. RT-PCR primer pairs were designed to amplify overlapping fragments along the putative polycistronic mRNA as shown in Fig. 8. The primer pairs spanned the mrxA gene (fragment 1, Fig. 8), extended from the middle of mrxA to 3′ of the inverted repeats in the intergenic region (fragment 2, Fig. 8), encompassed most of the intergenic region (fragment 3, Fig. 8) or extended through the intergenic region to the sequence encoding the N-terminus of mrxC (fragment 4, Fig. 8). RT-PCR fragment 1 (Fig. 8, lane 1) and fragment 2 (lane 2) were produced at high levels. RT-PCR fragments 3 (lane 3) and 4 (lane 4) were also produced, but at levels that were significantly lower than those obtained with the upstream primers. Taken together, these findings show that an mrx polycistronic mRNA through mrxC is produced at low levels relative to the mrxA transcript.

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**Fig. 7.** Northern blot analysis. Total RNA was obtained from X. nematophila grown on nutrient agar; 30 μg RNA was used in each lane. Lane a, mrxA probe; lane b, mrxA–C probe; lane c, mrxC probe. A major RNA transcript of approximately 940 nt was detected with the mrxA probe. The locations of the probes are shown above the diagram of the mrxA–mrxC region.

**Fig. 8.** Analysis of mrx polycistronic mRNA by RT-PCR. The location of the four overlapping RT-PCR products is shown above the diagram of the mrxA–mrxC region. Lanes 1–4 show the production of the four different RT-PCR products. –RT, negative control (no reverse transcriptase).
**DISCUSSION**

In this study, we show that the *mrx* operon belongs to the chaperone-usher class of fimbrial operons. A typical organization of fimbrial operons includes a regulatory gene(s) linked to the 5′ end of the major subunit, a centrally located chaperone-usher region, and minor subunit and adhesin genes located at the 3′ end of the operon (Blomfield, 2001). The *mrx* operon is distinct from other chaperone-usher operons, since it lacks a linked regulatory gene, and does not possess *mrpB*, *mrpE* and *mrpF*-like minor subunit genes. Separation of regulatory and minor subunit genes from the major subunit-usher-chaperone operon also occurs in the *cfa* and *coo* fimbrial operons of *E. coli* (Mol & Oudega, 1996), and in this regard the *mrx* operon may resemble *cfa* and *coo*. Alternatively, the assembly of Mrx fimbriae may not require additional minor subunit proteins, and may therefore represent the minimal requirement for type 1 fimbrial production. It is of interest to note that Mrx fimbriae reach 3 μm in length, while Mrp and Pap fimbriae are shorter (0.5–1 μm). MrpB has been shown to function as a terminator for the assembly of the Mrp fimbriae (Li & Mobley, 1998). The increased length of the Mrx fimbriae may be due to the absence of a MrpB-like subunit in the *mrx* operon.

Results from Northern blot analysis and RT-PCR indicated that *mrxA* mRNA was highly abundant in cells grown on agar and that a polycistronic *mrx* mRNA was present at significantly lower levels. Tandem inverted repeats were identified downstream of *mrxA*. Transcription initiation at the *mrxA* promoter and partial transcription termination in the inverted repeat region could result in high levels of *mrxA* mRNA production relative to the downstream genes. Besides functioning in transcription termination, the inverted repeats may form stem–loop structures at the 3′ end of the *mrxA* RNA, thereby protecting against mRNA degradation by exonucleases. In the *pap* and *sfa* fimbrial operons of *E. coli*, mRNA transcripts for the major subunit genes are abundant, while the transcripts of the downstream genes are detected at lower levels. The high ratio of major to minor subunit transcripts is achieved by the combination of early transcription termination, post-transcriptional RNA processing and differential stabilization of cleaved RNA (Bäga et al., 1987, 1988; Hacker & Morschhäuser, 1994). The high abundance of *mrxA* transcripts and low level of polycistronic *mrx* mRNA may be achieved by similar mechanisms.

We have shown that the *mrx* operon is not regulated by promoter inversion or by regulatory genes linked to the structural operon. The finding that fimbriae are not produced in an *lpr* mutant strain, and that putative Lrp-binding sites are present upstream of *mrxA*, suggests that Lrp may function as a positive regulator of the *mrx* operon. Whether *mrx* transcription is directly controlled by Lrp and/or other regulatory proteins remains to be determined. Unlike the *pap*, *fim* and *mrp* operons, *mrx* is highly expressed in cells grown on solid agar surfaces. In *P. mirabilis*, the switching of the *mrpA* promoter to the ‘OFF’ position accounts for the lack of fimbrial production in cells grown on agar (Zhao et al., 1997). In *Salmonella typhimurium*, the expression of *fimW*, located at the 3′ end of the *fim* operon, increases in cells grown on agar, and a *fimW* mutant produced higher levels of fimbriae than the wild-type (Tinker et al., 2001). These results indicate that FimW functions as a negative regulator of the *fim* operon in cells grown on agar. *Sal. typhimurium* and *E. coli* can produce fimbrial structures called curli fibres when grown on agar surfaces at lower temperature (Römling et al., 1998). The mechanism by which curli is regulated is complex (Prigent-Combaret et al., 2001), and the environmental signals that stimulate production on agar surfaces are not well understood. The Cpx two-component signal transduction system has recently been shown to play a key role in surface sensing and adhesion (Otto & Silhavy, 2002). It is conceivable that Mrx fimbrial production involves a surface-sensing regulatory mechanism that is activated by growth on solid surfaces.

*Xenorhabdus* cells produce fimbriae when they inhabit the nematode intestinal vesicle (Binnington & Brooks, 1993), but are motile within the insect cadaver (Forst & Nealson, 1996). Motility can be initiated in *vitro* during exponential phase growth (Forst & Boylan, 2002; Kim et al., 2003). These findings suggest that the differential regulation of cell surface adhesion and swimming motility plays an integral role in the life cycle of *Xenorhabdus*. MrxJ may be involved in the coordinate regulation of fimbrial and flagellar synthesis, since it shares significant sequence identity with MrpJ, which functions to repress flagellar synthesis in *P. mirabilis* when Mrp fimbriae are produced (Li et al., 2001). We envision that fimbriae are produced late in the infectious cycle and are present on the bacterial surface when the dauer juvenile stage of the nematode is being colonized by *Xenorhabdus*. Future studies on the Mrx fimbriae will be directed at addressing the following questions: what is the mechanism by which the *mrx* operon is regulated, and what role do Mrx fimbriae play in the colonization of the nematode partner? These studies should provide novel ideas on the function of fimbriae and their regulation in pathogenic and symbiotic bacteria.

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