Final Technical Report

DE-FG03-96ER20226, Genetics in Methylotrophic Bacteria
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1. Specific Aims

The specific aims of this project are to determine the functions of regulatory genes of the Mox system in *M. extorquens* AM1, and to study the regulatory regions of Mox genes.

2. Progress

The methanol oxidation system in *Methylobacterium extorquens* AM1 includes 5 regulatory genes, *mxaB*, *mxbDM*, and *mxcQE*. We have mapped transcripts and transcriptional start sites and have investigated sigma factors using the genome sequence.

a) Transcripts. We have mapped the transcripts for the Mox gene clusters, *mxcQET* and *pqqFG*, by RT-PCR and as expected, the genes in these clusters are cotranscribed.

b) Transcriptional start sites. We have mapped transcriptional start sites for the following Mox promoters : *mxaW*, *mxcQE*, *mxbDM*, and *pqqFG*. A comparison of the –10 and –35 regions for these promoters shows strong conservation for the –35 region, which strongly resembles a standard *E. coli* σ^{70} –35 sequence. However, the –10 region is not conserved between these promoters and it does not resemble the *E. coli* σ^{70} –10 sequence. These –10 regions are significantly higher in GC ratio, but otherwise the exact sequence is not conserved. Each of these promoters contains a GAAAA sequence within 50 bp upstream, and we are currently carrying out site-directed mutagenesis to analyze the role of this sequence in expression of these promoters.

c) Sigma factors. We have isolated mutants in each of the two genes predicted to encode heat shock sigma factors (*rpoH1* and *rpoH2*). These mutants grow normally on C1 compounds and express C1 promotors normally, but are defective in survival after heat shock response. It appears that both of these are involved in heat shock survival. We have isolated a mutant in another possible regulatory gene, with similarity to CbbR, a regulator of the Calvin-Benson-Bassham cycle in other bacteria. We have shown that this protein is involved in regulation of serine cycle genes, but not in regulation of Mox genes.

d) Mxc region

RT-PCR was used to establish that three genes in this region are cotranscribed, *mxcQ* (a putative sensor kinase)*, mxcE,* (a putative response regulator)*,* and *tenA* (a putative regulatory protein). Mutation in *tenA* showed that although growth is normal in this mutant, the cell cannot induce Mox operons in response to the presence of C_1 compounds. Therefore, *tenA* appears to mediate the induction response.

e) Upstream elements

Analysis of the regions upstream of the transcriptional start sites revealed –10 and –35 regions roughly similar to the *E. coli* sigma-70 consensus sequence. Upstream of this region no conserved repeats or inverted repeats were found. However, an A-rich hexanucleotide was always present near the –35 region. This sequence was analyzed by mutation and in each case,

deletion of the sequence or conversion of the As to Gs caused a dramatic decrease in the promoter activity. Therefore, it appears this sequence element is essential for Mox promoter activity.

f) Downstream elements

The growing literature describing important elements in mRNA sequences upstream of the translational start site prompted us to investigate the long leader sequence of the *mxaF* operon (180 bp). No significant inverted repeats were found in this region, although a series of predicted weak hairpin structures were present. Sequential deletion analysis for each of these predicted weak hairpins identified a region of 20-35 bp upstream of the translational start site that was essential for promoter activity. Mutation of the hairpin structure within this region did not alter promoter activity, suggesting that the sequence was not involved in secondary structure effects on expression. A search of the genome sequence for possible small RNA-encoding regions did not identify any matches to this region, suggesting that an RNA-duplex mechanism was not involved. It is possible that this region is necessary for mRNA stability, a hypothesis that will be tested in the future.

g) New regulatory genes

The hierarchy data we had previously suggested that at least one unknown negative regulator of the Mox system must be present. We have carried out a transposon mutant search for new regulatory genes of the Mox system, and a candidate for a negative regulator was identified. This genes contained an open reading frame annotated as a potential regulatory gene, and was shown to be involved in negative regulation of the Mox system using reporter gene fusions to Mox promoters.

We have also used DNA-binding columns (coupled to the *mxaF* promoter target sequence) and gel shift assays in attempts to identify proteins that bind to this region. However, so far the only protein that binds is RNA polymerase.

3. Publications

Zhang, M., and M.E. Lidstrom. Promoters and transcripts for genes involved in methanol oxidation in *Methylobacterium extorquens* AM1. Microbiology. 2003 149:1033-40.

Zhang M, FitzGerald KA, Lidstrom ME. 2005. Identification of an upstream regulatory sequence that mediates the transcription of *mox* genes in *Methylobacterium extorquens* AM1. Microbiology 151(Pt 11):3723-8.