The *Vibrio cholerae* mannitol transporter is regulated post-transcriptionally by the MtlS small RNA

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Supplemental Information

Materials and Methods

5' RACE. Wild type *V. cholerae* were grown in mannitol medium at 37 °C with aeration until mid-exponential phase. Total RNA was isolated from mid-exponential phase cultures using RNeasy Mini Kit according to the manufacturer's protocols, with a modification to include sRNAs (<200 nt) in the final preparation (Qiagen). DNA was removed from all samples using the DNAfree kit, according to the manufacturer's instructions (Applied Biosystems). 5' RACE assays were carried out using the RLM-RACE kit (Ambion) according to the manufacturer's instructions. Briefly, 2 μg total RNA was treated with calf intestinal phosphatase at 37 °C for 1 h. The reaction was terminated by phenol chloroform extraction followed by isopropanol ammonium acetate precipitation. Precipitated RNAs were then treated with tobacco alkaline pyrophosphatase at 37 °C for 1 h. 5' RACE adapter (5'-GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA-3') was added to the reaction and ligated at 37 °C for 1 h. The RNA was then reverse-transcribed with random decamers and M-MLV reverse transcriptase. The products of reverse transcriptase were amplified by the use of 1 μL aliquot of the RT reaction and 20 pmol *mtlA* RACE Outer (5'-CCATGAACATGGGAATGTCGGT-3') and the 5' RACE Outer (5'-GCTGATGGCGATGAATGAACACTG-3') primers, 250 μM dNTPs, 1 unit of Easy A polymerase (Stratagene), and 1 x Easy A buffer. Cycling conditions were 94 °C/3 min; 35 cycles of 94 °C/30 s, 58 °C/30 s, 72 °C/30 s; 72 °C/7 min. 2 μL of this product mixture was then used for a second amplification reaction with 20 pmol *mtlA* RACE Inner (5'-ATAGGCCCAACCAAAGAGGC-3') and 5' RACE Inner primer (5'-CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG-3'), 250 μM dNTPs, 1 unit Taq polymerase (NEB) and 1x Taq buffer. Cycling conditions were the same as above. The PCR products were cloned into pCR 2.1 TOPO vector (Invitrogen). After transformation, the plasmids from individual bacterial colonies were miniprepped (Qiagen) and sequenced with primer M13R (5'- CAG GAA ACA GCT ATG AC-3'). A fraction of sequences obtained (10/31) indicated a start site of transcription that correlates with the predicted start site 74 nt upstream of the MtlA start codon (Softberry BPROM; http://linux1.softberry.com). One 5' end mapped to 24 nt upstream of the MtlA start codon. The remaining 30 sequences obtained had 5' ends that mapped 60-100 nt downstream of the MtlA start codon and most likely represent degraded transcripts.

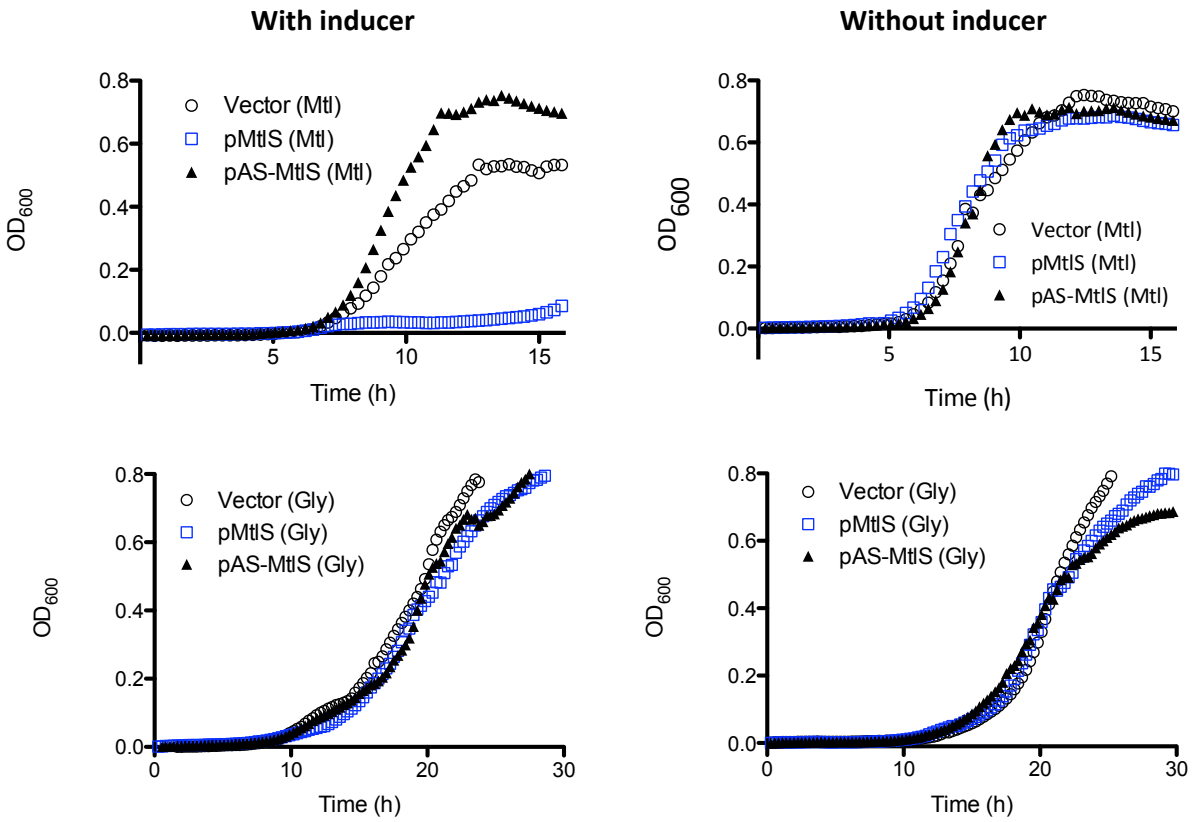

Figure S1. Overexpression of MtlS impairs growth in mannitol medium. Strains of *V. cholerae* Δ*tcpA mtlA*-FLAG containing an arabinose-inducible copy of MtlS, an RNA complementary to MtlS (AS-RNA) or an empty vector control were grown in LB media with (left) or without (right) arabinose (0.02%) to mid-exponential phase, then washed with M9 salts and diluted into fresh mannitol (Mtl) or glycerol (Gly) medium, with (left) or without (right) arabinose (0.02%), to OD_{600} = 0.01. Cultures were grown in a 96-well plate at 37 °C with constant aeration, and the OD_{600} were measured every 17 minutes. The experiment was performed in triplicate; the data of one representative trial is shown.

Figure S2. The *mtlA* mRNA and MtlS sRNA transcripts share 70 nucleotides of perfect complementarity. Red asterisk indicates the start of transcription for *mtlA* as determined by 5' RACE experiments. Blue asterisk indicates the start of transcription for MtlS and dashed blue line indicates the 3' end of MtlS; the ends of MtlS were previously identified during sRNA-Seq (1).

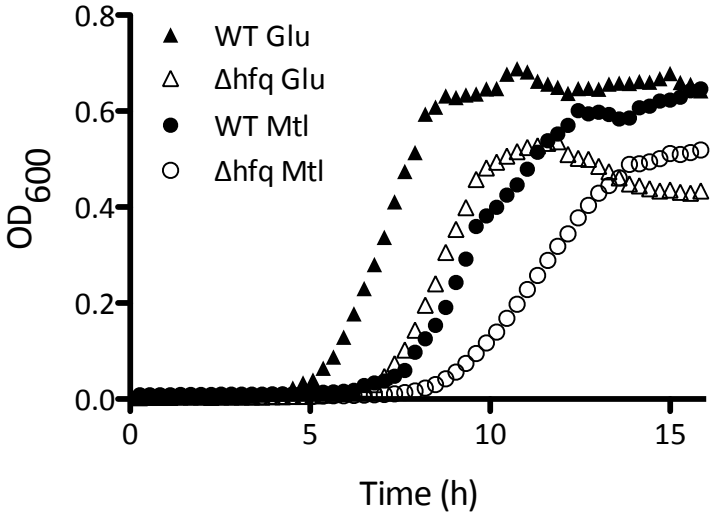

Figure S3. *V. cholerae* lacking *hfq* exhibit a slight delay in reaching exponential phase growth. *V. cholerae* Δ*tcpA mtlA*-FLAG strain and the *hfq* mutant derivative were grown overnight on LB agar, then washed with M9 salts and diluted into fresh glucose or mannitol medium to OD_{600} = 0.01. Cultures were grown in a 96-well plate at 37 °C with constant aeration, and the OD_{600} were measured every 17 minutes. The experiment was performed in triplicate; the data of one representative trial is shown.

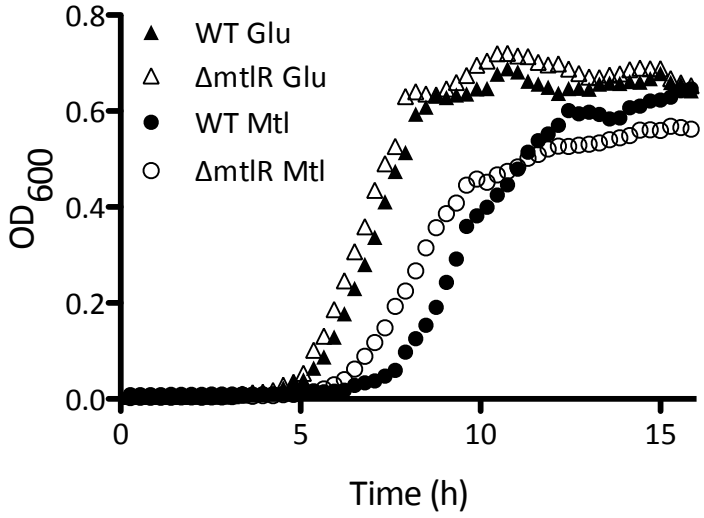

Figure S4. MtlR represses *V. cholerae* growth in mannitol medium. Strains of *V. cholerae* Δ*tcpA mtlA*-FLAG and an *mtlR* derivative were grown overnight on LB agar**,** then washed with M9 salts and diluted into fresh glucose or mannitol medium to OD_{600} = 0.01. Cultures were grown in a 96-well plate at 37 °C with constant aeration, and the OD_{600} was measured every 17 minutes. The experiment was performed in triplicate; the data of one representative trial is shown.

1. **Liu, J. M., J. Livny, M. S. Lawrence, M. D. Kimball, M. K. Waldor, and A. Camilli.** 2009. Experimental discovery of sRNAs in *Vibrio cholerae* by direct cloning, 5S/tRNA depletion and parallel sequencing. Nucleic Acids Research **37:**e46.