Cra and CRP Have Opposing Roles in the Regulation of the fruB in Vibrio cholerae

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Figure S1. Experimental fruB TSS differs from TSS from published RNA-Seq. Sequence of the antisense strand of the fruB-cra intergenic region, written 5’ to 3’. Coding regions of fruB and cra are written in blue and green bolded text respectively. 5’ RACE was used to determine the transcription start site of fruB (fruB TSS-2; written in blue and underlined) to be 133 nt upstream of the start codon (which is at position +242, relative to TSS-1). fruB_5’RACE_GSP1 and fruB_5’RACE_GSP2 reverse primers were used to reverse-transcribe transcripts in 5’ RACE experiments. RNA used for 5’ RACE was extracted from V. cholerae cultured in 1X M9 + 0.4% wt/vol fructose. This TSS differs from that identified by Papenfort et al., 2015 (fruB TSS-1; written in blue and underlined), which lies 241 nt upstream of the fruB start codon (1). The cra TSS (which lies on the sense strand) is written in green and underlined. Putative Cra and CRP binding sites are written in purple and red bolded text respectively. Putative fruB -10 and -35 hexamers are underlined. IGR4 (107 nt), a putative product of transcriptional processing, is highlighted in teal. Coordinates relative to the fruB TSS-1 +1 site are included on the left side of the sequence.
Figure S2. Consensus between fruB 5’ RACE samples extracted from fructose cultures and sequence upstream of fruB’s start codon. The TSS is expected to lie directly downstream of the poly-dG tail synthesized during 5’ RACE, which is highlighted by the green box. fruB’s start codon is highlighted by the yellow box. RNA for 5’ RACE was extracted from V. cholerae cultured in 1X M9 + 0.4% wt/vol fructose, and fruB_5’RACE_GSP1 and fruB_5’RACE_GSP2 were used as primers in reverse transcription reactions. Amplified fragments were sequenced and aligned using CLC Sequence Viewer 7. 24 total sequences were analyzed across two separate experiments, and five sequences suggest that the TSS lies approximately 133 nt upstream of fruB’s start codon.
**Figure S3.** Consensus between *fruB* 5’ RACE samples extracted from glucose cultures and sequence upstream of *fruB*’s start codon. The TSS is expected to lie directly downstream of the poly-dG tail synthesized during 5’ RACE, which is highlighted by the green box. *fruB*’s start codon is highlighted by the yellow box. RNA for 5’ RACE was extracted from *V. cholerae* cultured in 1X M9 + 0.4% wt/vol glucose, and fruB_5’RACE_GSP1 and fruB_5’RACE_GSP2 were used as primers in reverse transcription reactions. Amplified fragments were sequenced and aligned using CLC Sequence Viewer 7. 12 sequences were analyzed, and five sequences suggest that the TSS lies approximately 133 nt upstream of *fruB*’s start codon.
**Figure S4. FPr levels are highest in fructose media.** Western blots of FPr-FLAG (JL436) in 1X M9 supplemented with fructose and (A) glucose or (B) mannitol. Cultures were grown overnight in 1X M9 with the indicated mixtures of fructose and glucose or mannitol, totaling 0.4% wt/vol. The following day, cultures were back-diluted into fresh 1X M9 with the same mixtures of carbon sources as before. Back-dilutions were grown to mid log phase before protein extraction. 5 μL of the 100 μL protein extraction was included in loaded samples. Rabbit α-FLAG antibodies were used in Western blot analysis. Protein levels were normalized to REVERT Total Protein Stain.
Figure S5. Consensus sequence between *cra* 5' RACE samples and sequence upstream of *cra’s* start codon. The TSS is expected to lie directly downstream of the poly-dG tail synthesized during 5' RACE, which is highlighted by the green box. *cra’s* start codon is highlighted by the yellow box. RNA for 5' RACE was extracted from *V. cholerae* cultured in 1X M9 + 0.4% wt/vol fructose, and *cra_5’RACE_GSP1* and *cra_5’RACE_GSP2* were used as primers in reverse transcription reactions. Seven sequences were analyzed, and four sequences suggest that the TSS lies approximately 57 bp upstream of *cra’s* start codon.
Figure S6. Schematic of $P_{cra}$ transcriptional reporter design. The TSS of $cra$ is indicated by the sideways arrow. This TSS was first determined by Papenfort et al., 2015 using RNA-Seq, and we observed the same TSS in this work using 5’ RACE (RNA for 5’ RACE was extracted from cultures grown in fructose media). The region of the $cra$ promoter included in $P_{cra}$ is indicated by the single-headed arrow. Exact coordinates for this region are listed to the right of the arrow, with numbering based on the $cra$ TSS as +1. The red bar depicts a putative CRP binding site, which lies 14 nts upstream of $cra$’s start codon.
Figure S7. Cra levels remain unchanged when IGR4 is overexpressed. Western blot of Cra-HA pJML05 (JL530) and Cra-HA pJML05::IGR4 (JL531) in 1X M9 plus 0.4% wt/vol fructose or mannose. Protein was extracted using BPER. Rabbit α-HA antibodies were used in Western blot analysis. Protein levels were normalized to REVERT Total Protein Stain. The blots shown represent one of two experiments that are both included in the bar graph.
Figure S8. MtlA expression in the absence of Cra. Western blot of MtlA-FLAG (JL2) and Δcra MtlA-FLAG (JL538) in 1X M9 plus 0.4% fructose, glucose, or mannitol. Rabbit α-FLAG antibodies were used in Western blot analysis. Protein levels were normalized to REVERT Total Protein Stain. The blots shown represent one of two experiments that are both included in the bar graph.
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Table S1 continued

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**Plasmids**

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<td>pJL1::lacZ(Ec)</td>
<td>pJL1 derivative with RBS and coding region of $E.\ coli\ lacZ$ inserted into the VC2338 fragment of pJL1 in an antisense orientation; Ap$^R$</td>
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<td>pJML05::IGR4</td>
<td>pJML05 derivative in which the IGR4 +1 site directly proceeds the PLlacO-1 promoter</td>
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aSm$^R$, streptomycin resistance; Ap$^R$, ampicillin resistance. Coordinates of DNA fragments included in lacZ fusions are listed in parentheses following the first mention of the fusion. These coordinates are relative to the +1 site of the indicated gene as identified in Papenfort et al., 2015.
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Cloning of *V. cholerae* $P_{fruB}$-lacZ($Ec$)

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Cloning of *V. cholerae* $P_{fruB_{min}}$-lacZ($Ec$)

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Cloning of *V. cholerae* $P_{fruB_{null}}$-lacZ($Ec$)

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<td>LIU127 (R0)</td>
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<td>LIU640 (fwd vector)</td>
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<td>AAC AGC TAT GAC C</td>
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Table S2 continued

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cra 5’ RACE

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fruB 5’ RACE

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qRT-PCR

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\(^a\)Twd, forward; rev, reverse; gBlock, dsDNA fragment; GSP, gene-specific primer.

\(^b\)Underlined regions indicate homology tails for fragment ligation using DNA fragment assembly.
References


