2011

Searching for crp: Investigating Candidate Genes for the cAMP Receptor Protein in Bdellovibrio bacteriovorus

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Searching for *crp*: Investigating Candidate Genes for the cAMP Receptor Protein in *Bdellovibrio bacteriovorus*.

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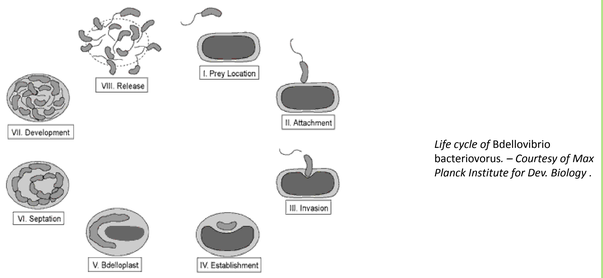
Abstract

Bdellovibrio bacteriovorus is a δ-proteobacter with an obligatory predatory lifestyle, consuming a wide variety of Gram-negative bacteria. Its unusual life cycle and genome make it a particularly interesting organism to study. In this experiment, three candidate *crp* homologs – ***Bd0446***, ***Bd2590***, and ***Bd2602*** – were evaluated by complementation studies in *Escherichia coli*, and characterized by bioinformatic analysis. Based on the complementation studies, none of these genes function as a *crp* homolog in *E. coli*. However, comparative bioinformatic analysis strongly suggests that either ***Bd2590*** or ***Bd2602*** may indeed code for the cyclic AMP receptor protein in *Bdellovibrio,* and merit further investigation. Additionally, a fourth homologue, ***Bd2591***, was identified, and remains to be explored.

Life Cycle

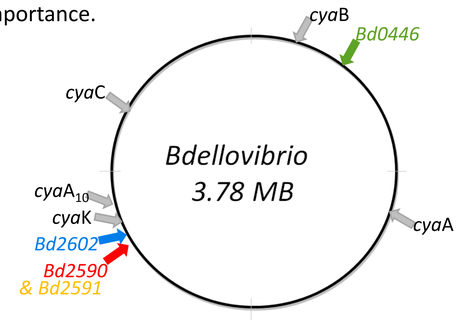


Life cycle of Bdellovibrio bacteriovorus. – *Courtesy of Max Planck Institute for Dev. Biology .*

Bdellovibrio bacteriovorus is a δ-proteobacter that lives as an obligate predator. Wild-type *Bdellovibrio* preys upon Gram-negative bacteria, such as *Escherichia coli*. *Bdellovibrio's* unique life cycle is characterized by two stages: attack phase (AP) and growth phase (BD). While in AP, *Bdellovibrio* is free-swimming, motile, and hunts for bacterial prey. During BD, *Bdellovibrio* inhabits the periplasm of the invaded prey cell, and digests its host from within using an array of hydrolytic enzymes. The original invading cell then grows and septates via filamentation. Finally, the progeny lyse the host cell and emerge as new AP bdellovibrios[1,2].

The cAMP-CRP System in *Bdellovibrio*

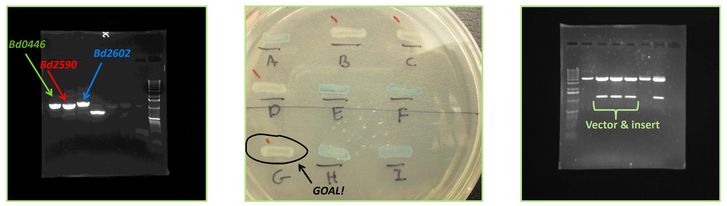
Bdellovibrio displays stage-specific differential regulation of two of its *cya* genes, *cya*A and *cya*K[3]. These *cya* genes code for the adenylate cyclase protein, which synthesizes cyclic AMP (cAMP). Together, cAMP and its receptor protein, CRP, serve as part of a global gene regulatory system. Until recently, there were no identified *crp* genes; however, three possible *crp* homologs were identified earlier this year. Clearly, investigating and characterizing these genes is of immediate importance.



A Question of Complementation

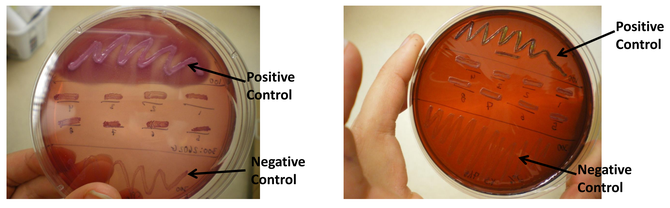
Three candidates – ***Bd0446***, ***Bd2590***, and ***Bd2602*** – were identified as possible *crp* genes. Given that *Bdellovibrio's cya* genes can complement a Δ*cya* mutation in *Escherichia coli*, it was hoped that a viable *crp* from *Bdellovibrio* would restore that function in Δ*crp E. coli.* In this fashion, a Δ*crp* strain of *E. coli* could be used to explore whether or not any of these candidates complement such a mutation; if so, that would be strong evidence confirming a *crp* homolog.

PART I: Vector Construction



Each gene was amplified by PCR, cloned into Invitrogen's TOPO-4 plasmid vector (which carries a gene for Kanamycin resistance), and transformed into One-Shot TOP10 *E. coli* (Invitrogen), screening for Kan^R colonies. Resulting transformants were then patched onto selective media to confirm insertion of the target gene into the vector. Colonies that grew white on LB + xgal plates were expected to carry plasmids with the desired gene. Further verification was achieved by plasmid restriction enzyme digests by EcoRI-HF (New England BioLabs), which were subsequently run on an agarose gel. Plasmids with the target gene had been cut twice, resulting in two pieces; plasmids without had been cut into only once.

PART II: Testing target genes in PD300



Each plasmid was extracted from TOP10 and electroporated into PD300, a Δ*crp* strain of *E. coli*[4], screening for Kan^R colonies. Resulting transformants were then patched onto selective media to test the genes' ability to complement the deletion of PD300's *crp* gene. Pink colonies on MacConkey + 2% maltose, and green on EMB + 2% maltose, would have "fixed" the mutation in PD300. The color change from white to pink is due to a change in pH, caused by maltose fermentation.

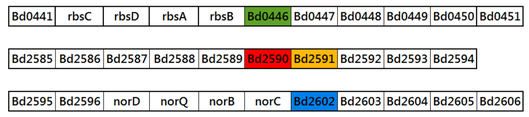
Conclusions and Future Work

It was determined from complementation studies that none of the three candidate genes complement the Δ*crp* mutation in *E. coli* strain PD300. Thus, it can be concluded that if any of these genes are indeed a *crp* homolog, it is dissimilar enough to *E. coli's crp* as to not function within a host *E. coli*. For future work, it will be vital to create mutant strains of *Bdellovibrio* lacking in these candidate genes. It will be particularly relevant to do so for ***Bd2590*** and ***Bd2602***, who, based on bioinformatic analysis, appear to be the most likely *crp* homologs.

Additionally, the identification of ***Bd2591*** as another potential *crp* is very exciting, and it will be worthwhile to test its complementationability in PD300. Now that there are four recognized potential *crp* homologs, possibilites abound.

Bioinformatic Analysis

PART I: "Genography"

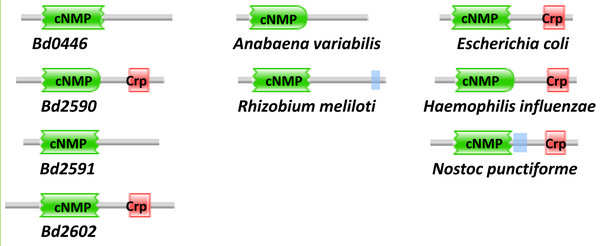


Using the Genome Reviews online database[5], a map was constructed of genes nearby ***Bd0446***, ***Bd2590***, and ***Bd2602***. One neighboring gene, ***Bd2591***, was annotated as another potential *crp* homolog.

PART II: BLAST Analysis

Using the National Center for Biotechnology Information's BLAST program[6], **basepair analysis** of ***Bd0446***, ***Bd2590***, ***Bd2602*** and ***Bd2591*** was performed. No significant similarity was shown; neither between the four genes, nor between any of them and known *crp* homologs in other bacteria.

PART III: Domain Analysis



Using the Wellcome Trust Sanger Institute's Pfam program[7], domain analysis was performed on the predicted protein products for ***Bd0446***, ***Bd2590***, ***Bd2602*** and ***Bd2591***, and then compared to that of known Crps in similar organisms. A pattern emerged: most known Crps analysed were between 200 and 270 amino acids long. All had a cNMP-binding domain that began 20-40 residues from the start of the chain, and extended for 89-91 residues. For many, an additional Crp domain followed shortly after, and extended for 29-31 residues. Both ***Bd2590*** and ***Bd2602*** follow this pattern, while ***Bd0446*** and ***Bd2591*** appear to have only the cNMP-binding domain.

Literature

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7. Wellcome Trust Sanger Institute <http://pfam.sanger.ac.uk/>. Accessed 15 Aug 2011.

I would like to express my gratitude and appreciation to my mentor, Dr. Mark Martin, for his patience and guidance; to the Univeristy of Puget Sound for funding my research, and to the Murdock Charitable Trust for financially supporting Dr. Martin's and our work with Bdellovibrio bacteriovorus. Finally, I would like to express my deep disappointment in my lab lackey, Scott Anderson, who failed to entertain me. I intend to return him for a full refund.