

Project 1: Identification of the ligand binding domain(s) of the methyl accepting chemotaxis proteins in *Vibrio cholerae* (Vc)

Flagellar motility in Vc is controlled by the chemotaxis system, allowing the microbe to move in response to environmental cues. Chemotaxis is regulated by chemoreceptors that sense environmental cues, and transduce this ligand-binding information to regulate a signal transduction cascade that affects flagellar rotation. The core signal transduction proteins consist of CheW, the receptor-kinase coupling protein, CheA, the kinase and CheY, the response regulator that interacts with the flagellar motor.

This proposal seeks to understand the ligand(s) bound by methyl-accepting chemotaxis proteins in Vc. The list of these proteins and their VC gene numbers are shown below. Detailed information on each gene including DNA and amino acid sequences can be obtained from: http://www.tigr.org/tigr-scripts/CMR2/name_search_test.spl.

<i>Vibrio cholerae</i> El Tor N16961		
Locus	Gene Symbol	Common Name
VC0098		methyl-accepting chemotaxis protein
VC0216		methyl-accepting chemotaxis protein
VC0282		methyl-accepting chemotaxis protein
VC0449		methyl-accepting chemotaxis protein
VC0512		methyl-accepting chemotaxis protein
VC0514		methyl-accepting chemotaxis protein
VC1248		methyl-accepting chemotaxis protein
VC1289		methyl-accepting chemotaxis protein
VC1298		methyl-accepting chemotaxis protein
VC1313		methyl-accepting chemotaxis protein
VC1394		methyl-accepting chemotaxis protein
VC1403		methyl-accepting chemotaxis protein
VC1405		methyl-accepting chemotaxis protein
VC1406		methyl-accepting chemotaxis protein
VC1413		methyl-accepting chemotaxis protein
VC1535		methyl-accepting chemotaxis protein
VC1643		methyl-accepting chemotaxis protein
VC1859		methyl-accepting chemotaxis protein
VC1868		methyl-accepting chemotaxis protein
VC1898		methyl-accepting chemotaxis protein

VC1967		methyl-accepting chemotaxis protein
VC2161		methyl-accepting chemotaxis protein
VC2439		methyl-accepting chemotaxis protein
VCA0008		methyl-accepting chemotaxis protein
VCA0031		methyl-accepting chemotaxis protein
VCA0068		methyl-accepting chemotaxis protein
VCA0176		methyl-accepting chemotaxis protein
VCA0268		methyl-accepting chemotaxis protein
VCA0658		methyl-accepting chemotaxis protein
VCA0663		methyl-accepting chemotaxis protein
VCA0773		methyl-accepting chemotaxis protein
VCA0864		methyl-accepting chemotaxis protein
VCA0906		methyl-accepting chemotaxis protein
VCA0923		methyl-accepting chemotaxis protein
VCA0974		methyl-accepting chemotaxis protein
VCA0979		methyl-accepting chemotaxis protein
VCA0988		methyl-accepting chemotaxis protein
VCA1031		methyl-accepting chemotaxis protein, authentic frameshift
VCA1034		methyl-accepting chemotaxis protein
VCA1056		methyl-accepting chemotaxis protein
VCA1069		methyl-accepting chemotaxis protein
VCA1088		methyl-accepting chemotaxis protein
VCA1092		methyl-accepting chemotaxis protein

Project 2. Identification of cyclic nucleotide (cAMP, cGMP, c-di-GMP) binding proteins binding proteins in *V. cholerae*.

Sequence analysis of many bacterial genomes showed that proteins with GGDEF and/or EAL domains are highly prevalent. Recent studies showed that the GGDEF and EAL domains control the cellular levels of cyclic-di-GMP through the opposing activities of di-guanylate cyclases (DGCs) and phosphodiesterases (PDEs), respectively. It has been shown recently that a cyclic guanosine signaling compound called cyclic di-guanosine-monophosphate (c-di-GMP) is as an essential modulator of cell surface structures and in turn biofilm formation in medically important microorganisms. Other cyclic nucleotides cAMP and cGMP are required for many cellular functions as well.

The overall objective is to determine the proteins that are capable of binding to these cyclic nucleotides.

Below is some information on cNMP binding proteins and related references.



Proteins that bind cyclic nucleotides (cAMP or cGMP) share a structural domain of about 120 residues [1, 2, 3]. The best studied of these proteins is the prokaryotic catabolite gene activator (also known as the cAMP receptor protein) (gene *crp*) where such a domain is known to be composed of three alpha-helices and a distinctive eight-stranded, antiparallel beta-barrel structure. There are six invariant amino acids in this domain, three of which are glycine residues that are thought to be essential for maintenance of the structural integrity of the beta-barrel. cAMP- and cGMP-dependent protein kinases (cAPK and cGPK) contain two tandem copies of the cyclic nucleotide-binding domain. The cAPK's are composed of two different subunits, a catalytic chain and a regulatory chain, which contains both copies of the domain. The cGPK's are single chain enzymes that include the two copies of the domain in their N-terminal section. Vertebrate cyclic nucleotide-gated ion-channels also contain this domain. Two such cations channels have been fully characterized, one is found in rod cells where it plays a role in visual signal transduction.

1. Körner H. , Sofia H.J. , Zumft W.G.
Phylogeny of the bacterial superfamily of Crp-Fnr transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs.
FEMS Microbiol. Rev. 27: 559- 592 (2003) [[PubMed: 14638413](#)]
2. Busby S. , Ebright R.H.
Transcription activation by catabolite activator protein (CAP).
J. Mol. Biol. 293: 199- 213 (1999) [[PubMed: 10550204](#)]
3. Kaupp U.B.
The cyclic nucleotide-gated channels of vertebrate photoreceptors and olfactory epithelium.
Trends Neurosci. 14: 150- 157 (1991) [[PubMed: 1710853](#)]

This web site <http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gvc> can be used to retrieve gene sequences.

Project 3. Characterization of the protein encoded by VCA0849.

VCA0849 is predicted to encode for a protein of 3263 amino acid in length. The goal of the project is to characterize this protein. Below the coordinates for the VCA0849 on the genome are given. The sequence could also be obtained from : <http://us.expasy.org/cgi-bin/niceprot.pl?Q9KL97>.

TIGR Locus Name:	VCA0849
Primary Locus Name:	None
SWISS-PROT/TrEMBL AC:	<u>Q9KL97</u>
Putative identification:	hypothetical protein
Coordinates:	800820 to 791029
DNA Molecule Name:	Chromosome 2 V.cholerae EI Tor N16961
Gene length:	9792
Protein length:	3263
Molecular Weight:	341491.81
pI:	3.7102
Percent GC:	50.14%
Kingdom:	Bacteria
Family:	Proteobacteria