Mutation in tcpR gene (Vc0832) of *Vibrio cholerae* O1 causes loss of tolerance to high osmolarity and affects colonization and virulence in infant mice

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*Vibrio cholerae*, the agent of cholera, multiplies and colonizes human intestinal tract where it survives high osmolarity due to bile and other sodium salts. In this work, by TnphoA mutagenesis, a mutant of *V. cholerae* O1 which could not grow and form colonies on LB agar containing 400 mM NaCl has been characterized. The mutant, designated CD83, adhered normally to freshly isolated rabbit intestinal discs, colonized poorly the gut of infant mice and was avirulent in the same model, whereas the parent strain CD81 would colonize the gut and cause death of mice. Attenuation of virulence of CD83 was not attributable to its inability to produce cholera toxin, as no difference was found in the cholera toxin produced *in vitro* by the mutant and parent strains. Molecular cloning and sequencing of the mutated gene revealed that insertion of transposon occurred in tcpR gene (Vc0832) of *V. cholerae*. Complementation of the mutant with wild-type tcpR gene resulted in restoration of the ability to survive at high salt concentration (400 mM NaCl), and to colonize the gut and restore virulence. The results suggest that tcpR plays a role in survival of *V. cholerae* in the small intestine of host as the osmolarity in the intestinal lumen is thought to be equivalent to 300 mM NaCl or higher.

**INTRODUCTION**

The diarrhoeal disease cholera in humans is caused by *Vibrio cholerae* strains O1 and O139. Vibrios live in the aquatic environment as well as transiently in the lumen of the intestine of humans. It is becoming increasingly clear that vibrios respond to changes in their environment by modulating gene expression of regulatory genes and virulence factors (Reidl & Klose, 2002). Extensive molecular and genetic analysis has led in recent years to the elucidation of events in the pathogenesis of cholera, important virulence factors and virulence regulatory systems of *V. cholerae*. Cholera toxin (CT) and toxin-coregulated pili (TCP) are considered to be the most important virulence factors. While CT is responsible for massive diarrhoea, TCP helps the vibrios to colonize the intestine (Taylor et al., 1987). The coordinate expression of CT and TCP is controlled by a unique regulatory system consisting of transcriptional activators ToxR, ToxT and TcpP (DiRita et al., 1991; Krukonis et al., 2000). The AraC-like transcriptional activator ToxT directly activates both the ctx and tcp gene clusters, as well as additional genes like aldA and tagAB (DiRita et al., 1991; Higgins et al., 1992). *V. cholerae* strains lacking ToxT make no CT or TCP and are avirulent (Champion et al., 1997). Transcription of the toxT gene is controlled by the two transmembrane transcriptional activators ToxR and TcpP (Higgins & DiRita, 1994; Hase & Mekalanos, 1998). The genes involved in TCP assembly (tcpABCDEFGHIJKLMNOPQRSTUVWXYZ) and those associated with ToxR regulatory cascade such as acfABCD, aldA, tagAB, toxT and tcpP are located within the *Vibrio* pathogenicity island (VPI) on the *V. cholerae* chromosome I (Kovach et al., 1996; Karaolis et al., 1998; Heidelberg et al., 2000).

Extensive investigations are required to understand the survival and adaptation systems employed by vibrios in the two contrasting environments. *V. cholerae* is a halotolerant micro-organism whose growth is stimulated by sodium, and it survives under a wide range of conditions of salinity and pH. *V. cholerae* strains are mostly isolated from environmental sites with NaCl concentrations between 0.2 and 2.0 % (Colwell & Huq, 1994) and are known to survive *in vitro* in 0.25–3.0 % salt, the optimal salinity being 2 % (Miller et al., 1984). Bile is a major constituent of human small intestine and because of bile and other sodium salts, osmolarity in the intestinal lumen is thought to be equivalent to 300 mM NaCl or higher (Gupta & Chowdhury, 1997). All organisms survive variation in salinity by employing homeostatic mechanisms, however, such mechanisms have not been.
investigated in *V. cholerae*, despite their potential importance for survival in the environment and during infection process (Williams et al., 1998).

Recently, three genes *nhaA*, *nhaB* and *nhaD* have been identified in *V. cholerae*, which encode *NhaA*, *NhaB* and *NhaD* sodium proton antiporters respectively (Vimont & Berche, 2000; Dzioba et al., 2002). These genes are presumably involved in the survival and persistence of free-living bacteria in their natural habitats. The genes responsible for the survival of vibrios and their colonization in intestine in response to high salt concentration have not been elucidated. In this investigation, we are reporting that mutation in tcpR gene (Vc0832) of *V. cholerae*, located within the VPI, resulted in sensitivity of vibrios to grow at high salt concentrations, reduced colonization and loss of virulence in an infant mice model of cholera.

**METHODS**

**Bacteria, plasmids and media.** A spontaneous streptomycin-resistant derivative, CD81 of *V. cholerae* 0395, was used as parent strain (Singh et al., 1994). CD83 was isolated by transposon mutagenesis of CD81 and characterized as salt sensitive. *Escherichia coli* strain DH5α was used for standard DNA manipulations. All bacterial strains and plasmids used in this investigation are listed in Table 1. Bacteria were stored at −70 °C in LB medium containing 50 % glycerol and grown in Luria–Bertani (LB) or brain-heart infusion (BHI) or Terrific broth (TB) (Singh et al., 1994). Plates were made with 1·5 % bacto agar. Syncase sucrose minimal medium supplemented with 0·2 % Casamino acids was used for preparation of cholera toxin (Finkelstein & LoSpalluto, 1969). Filter-sterilized solutions of 50 μg ampicillin (Ap) ml⁻¹, 12·5 μg tetracycline (Tc) ml⁻¹, 50 μg kanamycin (Km) ml⁻¹, 30 μg gentamicin (Gm) ml⁻¹, 300 μg streptomycin (Sm) ml⁻¹, and 40 μg 5-bromo-4-chloro-3-indolyl phosphate (XP) ml⁻¹ were added in the medium when required. All bacterial dilutions were made in PBS.

**TnphoA mutagenesis and selection of mutants.** Random insertions of TnphoA into CD81 chromosome were accomplished by plasmid pRT291, a derivative of pRK290, that carries a copy of TnphoA, complemented with wild-type tcpR, Km'. The plasmid was transferred by conjugation and transposition was allowed to occur at 37 °C for 18 h. Chromosomal inserts of TnphoA were obtained by conjugal transfer of incompatible plasmid pPH1JI and simultaneous selection for kanamycin and gentamicin resistance, which allowed selection of pPH1JI, retention of TnphoA and elimination of pRT291. As TnphoA was transposed from pRT291 to the chromosome, all colonies were streptomycin-, kanamycin- and gentamicin-resistant and susceptible to tetracycline. CD81 isolates carrying TnphoA inserts were screened by alkaline phosphatase activity on LB agar containing glucose (0·2 %) and XP, at 37 °C. Transposon insertions that resulted in the production of fusion proteins directed by signal sequences were detected as blue colonies. TnphoA mutants along with CD81 were grown on LB agar plates containing different NaCl concentration (100–400 mM). Plates were incubated at 37 °C overnight. One of the mutants, designated CD83, could not grow on higher concentrations of NaCl and was selected.

**Survival of bacteria on different concentrations of NaCl.** CD81, CD83 and CD84 were grown in LB broth containing 100 mM NaCl at 37 °C for 18 h, then washed and suspended in sterile water. A 10⁻⁸–10⁻⁶ dilution of each bacterium was prepared and 0·1 ml of each dilution was plated on LB agar plates containing 100–400 mM NaCl and incubated for 18 h at 37 °C. The dilutions giving 100–500 colonies on the plates were counted and c.f.u. ml⁻¹ were determined.

**Adherence, colonization and virulence.** Adherence of parent (CD81) and mutant (CD83) strains to freshly isolated rabbit intestinal discs was measured as described previously (Jacob et al., 1993). The adherence index is the number of adherent vibrios expressed as a percentage of the total number of vibrios to which the disc of intact rabbit intestinal mucosa was exposed. Intestinal colonization of parent and mutant strains was measured in 5-day-old mice by oral infection with a single strain (Jacob et al., 1993). Infant mice were fed with 10⁸–10⁶ vibrios in 0·1 ml PBS. Ten infant mice were taken in each group and three mice were killed at each time-point for viable counts of vibrios in the intestine. The intestine was dissected out and homogenized in PBS. After suitable dilution, samples were plated on LB agar plates containing streptomycin. The virulence of parent and mutant strains was measured by their ability to cause mortality of 5-day-old mice. Bacterial dilutions containing 10⁶, 10⁵ and 10⁴ vibrios were inoculated orally to a group of four mice each and mortality was observed up to 72 h.

**Preparation and assay of cholera toxin.** Toxin was obtained by growing CD81, CD83 and CD84 in syncase sucrose minimal medium supplemented with Casamino acids at 30 °C for 18 h in a shaker. Bacteria-free culture filtrate was obtained by membrane filtration (Millipore; 0·22 μm pore size). The presence of toxin was assayed in

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**Table 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Relevant characteristics</th>
<th>Reference/source</th>
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<tr>
<td><strong>V. cholerae</strong></td>
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<tr>
<td>CD81</td>
<td>Wild-type, Sm', derivative of 0395, O1 classical</td>
<td>Singh et al. (1994)</td>
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<tr>
<td>CD83</td>
<td>Derivative of CD81, salt-sensitive, tcpR::TnphoA, Km', Sm'</td>
<td>This study</td>
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<tr>
<td>CD84</td>
<td>Derivative of CD83, complemented with wild-type tcpR, Km'</td>
<td>This study</td>
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<td><strong>E. coli</strong></td>
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<tr>
<td>DH5α</td>
<td>Host strain used for cloning</td>
<td>Gibco-BRL</td>
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<td><strong>Plasmid</strong></td>
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<tr>
<td>pUC18</td>
<td>Cloning vector, Ap'</td>
<td>Bangalore Genei</td>
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<tr>
<td>pRT291</td>
<td>Derivative of plasmid pRK290, carrying TnphoA, Km', Tc'</td>
<td>Taylor et al. (1989)</td>
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<tr>
<td>pPH1JI</td>
<td>Incompatible plasmid to pRT291, Gm'</td>
<td>Beringer et al. (1978)</td>
</tr>
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<td>pSM1</td>
<td>N- and C-terminal ends of mutated gene of CD83 cloned in pUC18, Ap', Km'</td>
<td>This study</td>
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<tr>
<td>pSM2</td>
<td>pUC18 containing a 456 bp BamHI PCR fragment (tcpR) in-frame with lacZ</td>
<td>This study</td>
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two ways. (1) The presence of biologically active toxin was measured in vivo in adult albino guinea pigs. Culture filtrate was diluted in BHI and

0·1 ml was injected intradermally. Induration was recorded after 18 h. BHI alone caused no induration (Craig, 1965; Singh et al., 1994). (2) Toxin was estimated by ELISA essentially as described previously (Harlow & Lane, 1988; Singh et al., 1994). Culture filtrate was serially diluted and coated in immunoplate wells (NUNC) and titrated using rabbit anti-cholera toxin IgG and horseradish-peroxidase-conjugated goat anti-rabbit IgG (Sigma) and O-phenylenediamine dihydrochloride as substrate.

DNA manipulation, analysis and sequencing. Plasmid and chromosomal DNA preparation, DNA ligation, bacterial transformation, agarose gel electrophoresis and Southern blotting were carried out by the standard techniques described by Sambrook et al. (1989). Restriction enzymes, biochemicals, T4 DNA ligase, etc., were purchased from Promega, New England Biolabs and Sigma. The nucleotide sequence was determined by dye-oxide chain termination method with the ABI PRISM Dye Terminator Cycle Sequencing kit (Perkin-Elmer) and the ABI PRISM 377 automatic sequencer (Applied Biosystems). Internal TnphoA primers K36 (5'-ATCGCTAAGAGAATCACGCAG-3') and G2 (5'-CCCGAAGAAACACAGATTACG-3') were used to sequence fragments cloned in pUC18 vector. K36 located 155 nucleotides away from the +1 position and moving outward, was used to read the N-terminal junction of the transposon and the disrupted gene whereas G2, located 153 bases from the C-terminal end of the transposon, was used to read the C-terminal junction point of the gene and transposon.

Cloning of the transposon insertion site. Since TnphoA has no internal EcoRV site, this restriction enzyme was used to clone both the N- and C-terminal of transposon disrupted gene. Chromosomal DNA from the mutant was digested with EcoRV enzyme and ligated with Smal-digested pUC18; the ligation mixture was transformed into E. coli DH5α, and clones were selected on LB agar plates containing ampicillin and kanamycin. One of the clones, designated pSM1, was used for further studies.

Expression and complementation. Amplification of tcpR gene from CD81 was done using the following primers: VcTcp1 (5’-GGATCCGGATCCATGACTAGATGTTACATG-3’) and VcTcp2 (5’-GGATCCGGATCCATGACTAGATGTTACATG-3’) (BamHI sites are in bold). The design of the primers was such that the amplified tcpR gene (from +1) without its own ribosome-binding site when cloned in right orientation would exist in-frame with lacZ. In a final volume of 50 μl, 10 ng chromosomal DNA, 200 μM dNTPs, 200 ng each primer, and 1·5 U Taq polymerase (Promega) in 1× reaction buffer were mixed. The PCR mixture was subjected to a denaturation step (5 min at 94°C) followed by 30 cycles of amplification (1 min denaturation at 96°C, 1 min annealing at 52°C, and 90 s elongation at 72°C) and a termination step (5 min at 72°C). The PCR product was purified using Qiagen PCR purification kit and cloned in the pUC18 vector in-frame with lacZ so as to use the ribosome-binding site of the vector. This design allowed expression of TcpR as a fusion protein with LacZ. Clones in the correct orientation were selected by digestion with Ndel enzyme and expressed in E. coli DH5α. Culture was induced with 1 mM IPTG at 30°C. Expression of protein was visualized by SDS-PAGE (16%), stained with 0·25 % Coomassie brilliant blue R-250 and size of expressed protein was determined by comparative mobility of the protein with commercial molecular mass markers in the same gel (Singh et al., 1994). One of the clones, designated pSM2, showing expression of the fusion protein, was electroporated in CD83 for complementation (Schodel et al., 1991). Briefly, exponential-phase cells were spun down, washed in 2 mM CaCl2 and finally suspended in 1 mM CaCl2. Plasmid (10–100 ng) was added to the cell suspension and electroporated once at 2·5 kV, 25 μF and 200 Ω in a gene pulser (Bio-Rad). Immediately, the cells were gently resuspended in LB and allowed to grow for 1 h at 37°C without shaking before plating on LB agar plates containing 100 μg ampicillin ml⁻¹. The ability of the cloned gene to complement the mutation was tested by growing the complemented strain (CD84) in the presence of 400 mM NaCl along with parent and mutant strains. The adherence to rabbit intestinal disc, colonization and virulence of CD84 was measured as described above.

RESULTS AND DISCUSSION

Identification and characterization of salt-sensitive mutant

After TnphoA mutagenesis, the culture was plated on LB agar plates containing kanamycin and gentamicin and single isolated colonies were streaked on LB agar plates containing 400 mM NaCl. One of the mutants, CD83, was sensitive to 400 mM NaCl and did not form colonies (Fig. 1). When streaked in presence of XP, CD83 gave a blue phenotype, suggesting an in-frame insertion of TnphoA in a gene encoding secretory or membrane protein. Adherence, colonization and virulence of CD84 were measured. It adhered to rabbit intestinal mucosa normally as the parent strain (data not shown) but did not multiply and colonize as efficiently as CD81 (Fig. 2). CD81 multiplied in the gut and increased in number whereas the cell number of the mutant was lower. The difference in colonizing ability was probably not due to difference in growth rates of the mutant and its isogenic parent strains as no difference in growth was found between the two strains grown in broth containing different concentrations of NaCl (Fig. 3). This difference from the results obtained on plates and shown in Fig. 1 was consistently observed. Apparently, CD83 loses plating efficiency on agar at 400 mM NaCl but retains the capacity to grow in broth containing the same salt concentration. As none of the mice fed orally with the mutant died but those inoculated with the parent strain died of cholera, the virulence of CD83 was significantly attenuated (Table 2). The attenuation of virulence of CD83 in the suckling mouse model does not appear

![Fig. 1. Viable counts of parent CD81 (squares), mutant CD83 (circles) and complemented CD84 (triangles) strains on different concentrations of NaCl in LB agar medium.](http://jmm.sgmjournals.org)
to be due to the inability of the mutant to produce cholera toxin. When production of cholera toxin produced by the mutant CD83 was measured in vitro, induration was 18 mm and the inverse ELISA titre was 1280 for all three strains (CD81, CD83 and CD84), suggesting that the loss of virulence of CD83 was not due to a defect in toxin biosynthesis.

Identification of the gene responsible for high salt tolerance

Since TnphoA contains no EcoRV site, cloning of the EcoRV-digested genomic DNA of CD83 in SmaI-digested pUC18 vector yielded recombinants containing whole TnphoA along with N- and C-terminal ends of the mutated gene. Authenticity of the clone, pSM1, was confirmed by RFLP analysis and hybridization with V. cholerae genomic DNA and transposon-specific 2·6 kb BglII probe (Singh et al., 1994) (data not shown). Subsequently, pSM1 was sequenced with internal TnphoA primers K36 and G2. K36 and G2 read 155 and 153 bases of the transposon and entered N-terminal and C-terminal ends of the mutated gene, respectively (Fig. 4). The sequence of N- and C-terminal ends of the gene was merged. It revealed 100 % homology (http://www.tigr.org) with tcpR gene (Vc0832) of TCP-pathogenicity island located on the large chromosome (Heidelberg et al., 2000). The nucleotide sequence was translated and analysed by DNASTAR and compared with proteins in databases. ORF tcpR has 456 bases and encodes a protein of 17·7 kDa with 151 amino acids. The transposon was inserted at 216 bp downstream of the initiation codon of tcpR. TcpR appears to be a transmembrane protein and is composed of hydrophilic residues with an $\alpha$-helical region at the N-terminal end. The C-terminal end is hydrophobic. The probable location of TcpR is predicted to be the inner membrane. TcpR showed no significant homology to any known or hypothetical protein in public domain. However, some weak homology was found to membrane-associated sensory histidine kinase of Clostridium acetobutylicum in the N-terminal region between 16–64 amino acid residues.

PCR cloning of tcpR and expression in E. coli

Vc0832 (tcpR) was PCR-amplified with primers VcTcp1 and VcTcp2 tagged with BamHI site and checked by agarose gel electrophoresis. Amplified product of 456 bases was purified, cloned in BamHI-restricted pUC18 vector and transformed in DH5az. Recombinants were selected on LB agar plates.
containing ampicillin. Digestion of recombinant plasmid, pSM2, with BamHI released an insert of 456 bases. A single site of NdeI is present in the tcpR gene 11 bases away from +1 and therefore this enzyme was used to check the orientation of the insert with respect to the lacZ promoter. Digestion of pSM2 with NdeI yielded a fragment of 679 bases that is comprised of 445 bp of the tcpR and 234 bp of the plasmid vector. It suggested that the insert was cloned in the correct orientation vis-à-vis lacZ, and hence in-frame with lacZ in the vector.

To see the expression of TcpR, an exponential phase culture of DH5α(pSM2) was induced with 1 mM IPTG at 30 °C and run on SDS-PAGE (16%) along with uninduced culture and DH5α(pUC18). As shown in Fig. 5, a protein of about 19.8 kDa is observed in the induced culture which was absent in uninduced culture. The induced protein is a fusion protein of TcpR with LacZ (456 bp of tcpR and 55 bp of lacZ).

**Complementation of tcpR::TnphoA mutant by a cloned tcpR gene**

Plasmid pSM2, carrying tcpR, was electroporated into CD83 and ampicillin-resistant transformants were selected which were also kanamycin-resistant. A transformant, CD84, was checked for growth in the presence of a high salt concentration along with parent and mutant strains. As shown in Fig. 1, the ability to grow on 400 mM NaCl was restored in CD84 along with its virulence and ability to colonize the intestine (Table 2, Fig. 2). CD84 was resistant to kanamycin and found to contain TnphoA when probed with Tn-specific probe in Southern hybridization as described before (Singh et al., 1994). Hence the complemented strain seems to acquire a copy of the wild-type tcpR gene along with the resident disrupted copy of the gene. No plasmid could be detected from CD84, suggesting that the complementation was due to homologous recombination between the chromosomal tcpR::TnphoA and pSM2 resulting in the replacement of mutant allele with the wild-type allele. The restoration of the wild-type phenotype in the complemented strain CD84 demonstrates that the observed phenotype of CD83 was due to the lack of TcpR. Whole-cell proteins of parent, mutant and complemented strains were analysed on SDS-PAGE (16%). As opposed to fusion protein of 19.8 kDa (Fig. 5), TcpR protein of 17.7 kDa encoded by wild-type tcpR gene was observed in the parent and complemented strains but absent in the mutant (Fig. 6).

For successful infection of host, V. cholerae must survive in and colonize the intestine. Because of bile and other sodium salts, the osmolarity in the intestinal lumen remains high (Gupta & Chowdhury, 1997) and vibrios must have some mechanism to overcome this environment for survival. In this investigation, it has been shown that the tcpR gene may have a role in survival of V. cholerae at high osmolarity. The poor colonization and attenuation of virulence of CD83 appears to be due to the inability of the mutant to survive in the intestinal environment and to associate into micro-

![Fig. 5. SDS-PAGE profile of total proteins of E. coli (pSM2) showing induction of 19.8 kDa fusion protein (arrow). Lanes: A, E. coli DH5α(pUC18); B and C, E. coli (pSM2) induced; D, E. coli (pSM2) uninduced.](http://jmm.sgmjournals.org)

![Fig. 6. SDS-PAGE profile of total proteins of V. cholerae CD81 (C), CD84 (B) and CD83 (A); arrow shows the presence of 17.7 kDa protein in CD81 and complemented strain CD84 but absent in CD83 mutated in the tcpR gene.](http://jmm.sgmjournals.org)
colonies on the villous surface (Kirn et al., 2000). The difference in colonization of intestine by CD81 and CD83 was not due to difference in growth rates because the two strains grew in vitro at approximately the same rate.

The observed phenotype of the mutant CD83 is apparently due to insertional mutagenesis of tcpR gene by TnphoA and inactivation of the gene function. It may be argued that insertion of TnphoA in tcpR gene may have resulted in polar effects causing attenuation of toxT downstream of tcpR. It is well known that the expression of TCP and cholera toxin is influenced by environmental conditions and is coordinately regulated by transcriptional regulators such as ToxR, ToxT and TcpP (DiRita et al., 1991; Krukonis et al., 2000; Reidl & Klose, 2002) and attenuation of toxT suppresses expression of cholera toxin (Champion et al., 1997). However, this does not seem to be the reason. It has been reported in this investigation that biosynthesis of cholera toxin was not affected in the mutant, CD83. Hence the observed effect seems possibly due to disruption in the tcpR gene. Complementation of CD83 with wild-type tcpR gene restored parent-strain-like characteristics.

TcpR is one of the open reading frames within the tcpR operon constituting the pilus-colonizing factor TCP in V. cholerae and it will be very interesting and important to elucidate the specific role of membrane proteins encoded by genes within this operon. TcpR, a membrane protein, shows no significant homology to any protein in databases and hence it is very difficult to provide clues as to its physiological role. However, on the basis of some homology to C. acetobutylicum sensory histidine kinase, TcpR may be predicted to function as sensory protein. As the vibrios move in the gut, passing through various microenvironments, TcpR might be involved in sensing high osmolarity in the intestine and may stimulate the expression of toxR regulon for biosynthesis of OmpU and OmpT porins. It is well documented that the levels of OmpU and OmpT are affected by osmolarity and bile (Miller & Mekalanos, 1988; Provenzano et al., 2000) and that the cells expressing OmpU are more resistant to bile than cells expressing OmpT (Provenzano & Klose, 2000). It will be interesting to examine the expression of porins in tcpR mutant CD83.

The ability of vibrios to use sodium in bioenergetic processes appears to play a key role in both the environmental and the pathogenic phases (Hase & Mekalanos, 1999). It has been argued that one of the functions of the cholera toxin is to generate a high Na⁺ environment in the lumen of the intestine (Bakeeva et al., 1986). Furthermore, the induction of sodium bioenergetics could play a role in the persistence of V. cholerae in the environment, as it might increase the resistance of the bacterium to various environmental factors (Brown & Sireenko, 1997). Recently, the role of Na⁺/H⁺ antiporters in the ion homeostasis of all living cells has been supported. In V. cholerae, three antiporters NhaA, NhaB (Vimont and Berche, 2000) and NhaD (Dzioba et al., 2002) have been identified. These antiporters exchanged Na⁺ ions for protons across the membrane in an electrogenic process that results in the expulsion of sodium driven by the electrochemical proton potential and presumably involved in the survival and persistence of free-living bacteria in their natural habitats. The mechanism by which TcpR may regulate Na⁺ homeostasis needs to be investigated.

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