A *Vibrio harveyi* insertional mutant in the *cgtA* (*obg*, *yhbZ*) gene, whose homologues are present in diverse organisms ranging from bacteria to humans and are essential genes in many bacterial species

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The *cgtA* gene product is a member of the subfamily of small GTP-binding proteins that have been identified in diverse organisms ranging from bacteria to humans. In bacteria that sporulate or display another special developmental programme, this gene (referred to as *cgtA*, *obg* or *yhbZ*) appears to be involved in the regulation of these processes. However, this gene has also been found to be essential in all bacterial species investigated to date, although its role in bacteria that do not sporulate and do not undergo a specific development remains unknown. Here the authors characterize a *Vibrio harveyi* mutant bearing a transposon insertion into the *cgtA* gene. This mutant reveals a multiple phenotype: it grows more slowly than the wild-type strain in a rich medium; its growth is completely inhibited in minimal media; its survival in 3% NaCl is dramatically reduced; it is very sensitive to UV irradiation; it is more susceptible to mutation upon treatment with different mutagens; its luminescence is decreased; its quorum-sensing regulation is less effective than in the wild-type strain; and the elongated shape of the mutant cells may suggest problems with the regulation of cell division and/or DNA replication. These defects in diverse cellular processes found in the insertional *cgtA* mutant of *V. harveyi* indicate that in a bacterium that does not sporulate and does not display other special development programmes, the CgtA protein is involved in the regulation of many crucial biochemical reactions, possibly at the stage of signal transduction.

Keywords: *cgtA* gene, GTP-binding protein, bioluminescence, signal transduction

INTRODUCTION

GTP-binding proteins play crucial roles in the regulation of fundamental processes in cells of all living organisms. In fact, small monomeric GTP-binding proteins have been found in every organism examined thus far. In eukaryotic cells, these proteins are involved in a number of essential processes, such as signal transduction, protein synthesis and translocation or cell cycle regulation (for a review see, for example, Sprang, 1997). However, perhaps surprisingly, relatively little infor-

mation is currently available about the roles of GTP-binding proteins in prokaryotes.

The best-studied prokaryotic GTP-binding protein is Era (for 'Escherichia coli Ras-like protein'). This protein is essential for bacterial growth, and mutants in the *era* gene have pleiotropic phenotypes, including alterations in the regulation of carbon metabolism, the stringent response, and cell division (Lerner & Inouye, 1991; Britton *et al.*, 1997, 1998). Recent studies have demonstrated that Era bears an RNA-binding motif (Chen *et al.*, 1999) and binds to the 30S ribosomal subunit (Sayed *et al.*, 1999).

Apart from Era, a subfamily of small GTP-binding proteins was discovered recently in bacteria. Interestingly, members of this subfamily have homo-

Abbreviation: DAPI, 4',6-diamidino-2-phenylindole.

The GenBank accession number for the sequence reported in this paper is AF247677.

logues in diverse organisms ranging from bacteria to humans (Okamoto & Ochi, 1998). This subfamily is Obg/Gtp1, and examples of bacterial members of it are Obg from *Bacillus subtilis*, *Streptomyces griseus* and *S. coelicolor*, CgtA from *Caulobacter crescentus*, and YhbZ from *Escherichia coli* and *Haemophilus influenzae*. Among this group, the *B. subtilis* obg gene product is the best-studied protein. Genetic studies led to proposals that Obg may regulate initiation of sporulation (Trach & Hoch, 1989; Vidwans *et al.*, 1995), may be involved in the control of DNA replication (Kok *et al.*, 1994), and is necessary for stress-dependent activation of transcription factor σ^B (Scott & Haldenwang, 1999). It was proposed that Obg can function by sensing the intracellular GTP level (Kok *et al.*, 1994) and may be required to stimulate the activity of the phosphorelay system (Vidwans *et al.*, 1995). Therefore, it is likely that Obg may be involved in signal transduction.

Functions of *obg* and *cgtA* genes have been investigated to date in bacteria that display a development programme, i.e. in *B. subtilis*, *S. griseus*, *S. coelicolor* and *C. crescentus*. However, members of the Obg/Gtp1 subfamily, with a high homology to Obg and CgtA, were found to be essential also in bacteria that do not sporulate and do not differentiate. For example, the *yhbZ* gene of *E. coli* was demonstrated to be essential (Arigoni *et al.*, 1998), whereas its role in this bacterium is unknown. Investigation of the role of an essential gene is complicated by the fact that it is not possible to obtain viable null mutants. In the case of *B. subtilis*, apart from a temperature-sensitive *obg* mutant (Kok *et al.*, 1994), a strain in which the only functional *obg* gene copy is under control of an IPTG-inducible promoter has been constructed (Vidwans *et al.*, 1995). This strain is viable only in the presence of the inducer in the medium. Without IPTG-induced transcription of *obg*, the levels of the Obg protein were depleted during the cell growth; however, cells appeared to grow normally for several generations before there was any significant change relative to the same strain grown in the presence of IPTG (Vidwans *et al.*, 1995). This allowed investigation of the influence of *obg* gene function on sporulation, but such a phenotype would cause difficulties in studies on many other processes potentially affected by this gene function. Moreover, using an IPTG-inducible transcription of a given gene, it is difficult to obtain a level of the gene product comparable to that found in the wild-type strain.

Vibrio harveyi is a Gram-negative, free-living, luminescent marine bacterium. Here we describe a *V. harveyi* mutant which bears a transposon insertion in the *cgtA* (*obg*, *yhbZ*) gene. To our knowledge, this is the first reported viable bacterial mutant in which a gene belonging to the Obg/Gtp1 subfamily has been disrupted by insertion.

METHODS

Bacterial strains and plasmids. *V. harveyi* wild-type strain BB7 (Belas *et al.*, 1982) and its *cgtA::Tn5TpMCS* derivative,

BB7X (Czyż *et al.*, 2000a), were used. Plasmid pSupTn5pMCS (MacKenzie *et al.*, 1995) bears a *Tn5*-derived transposon carrying a trimethoprim-resistance gene. Plasmid pUC19 has been described previously (Yanisch-Perron *et al.*, 1985). All genetic engineering procedures used for plasmid construction described below were performed according to Sambrook *et al.* (1989). For construction of plasmid pAC1, bearing a chromosomal DNA fragment flanking the site of transposon insertion, a procedure described by MacKenzie *et al.* (1995) was employed. Briefly, chromosomal DNA isolated from *V. harveyi* BB7X was digested with *EcoRI* and mixed with *EcoRI*-digested pUC19. Following ligation, the transformation of *E. coli* MC1061 (Meissner *et al.*, 1987) was performed using selection for resistance to both ampicillin and trimethoprim.

Culture media. Luria-Bertani (LB) medium (used for *E. coli* cultivation) and BOSS medium (a rich medium used for *V. harveyi* cultivation) have already been described by Sambrook *et al.* (1989) and Klein *et al.* (1998), respectively. Minimal medium 3 (Węgrzyn & Taylor, 1992) was used, but the concentration of NaCl was 3%, and the following carbon sources were employed: 1% glucose plus 1% Casamino acids; 1% glucose; 1% (v/v) glycerol; 1% sodium succinate; or 1% sodium acetate. If not indicated, *V. harveyi* strains were cultivated at 30 °C.

DNA sequencing. A fragment of plasmid pAC1 (bearing the *V. harveyi* BB7X DNA region flanking the site of transposon insertion) was sequenced automatically using a Perkin-Elmer ABI 310 sequencer. The ABI Prism BigDye Terminator Cycle Sequencing Reaction Kit with Ampli Taq DNA Polymerase FS was employed. The primers used for sequencing reactions were as follows: primer TN, 5'-TTC AGG ACG CTA CTT GTG TA-3'; and primer pUCR, 5'-AGC GAA TAA CAA TTT CAC ACA GG-3'.

Electron microscopy. *V. harveyi* cells were examined by electron microscopy using two techniques: negative staining with phosphotungstic acid (PTA), and ultrathin sectioning. For the first technique, cells were prepared by negative staining with 0.2–1% PTA (neutralized with KOH to pH 7.5) on carbon-coated copper grids according to Quintarelli *et al.* (1971). Briefly, the grid was placed on the top of a drop of bacterial culture and left for 30 s, and then transferred to the top of a drop of the PTA solution for 30 s. Excess stain was removed with filter paper and the grid was dried at room temperature. For the second technique, the basic methods used to fix and embed cells for thin sectioning were according to Spurr (1969). Sections were stained with saturated uranyl acetate dissolved in 50% ethanol, and then with lead citrate for 2 min at room temperature. The grid was dried at room temperature. In both techniques, the grids were examined and photographed using a Philips CM 100 electron microscope operating at 60 kV.

Light microscopy. *V. harveyi* cells were examined using differential interference contrast under a Nikon Eclipse E800 microscope. Staining with 4',6-diamidino-2-phenylindole (DAPI) was carried out essentially as described previously by Hause *et al.* (1993). Briefly, culture samples were mixed with DAPI solution (25 µg ml⁻¹) and left in the dark for 20 min. For staining with ethidium bromide, culture samples were mixed with the dye solution (100 µg ml⁻¹) and left in the dark for 20 min. In both staining procedures, before examination under a microscope, the stained cells were placed on a 0.5% agar layer on a microscope slide and covered with a coverslip.

Southern blotting. Southern-blotting analysis was performed according to Sambrook *et al.* (1989) using the Random Primer Fluorescein Labelling Kit with Antifluorescein-AP (NEN Life

Fig. 1. Homology of the N-terminal (99 amino acids) part of the *V. harveyi* CgtA protein (the sequence was deduced on the basis of the partial *cgtA* gene sequence) with corresponding regions of certain proteins belonging to the Obg/Gtp1 subfamily from diverse organisms. The wu-blastp software was used for comparison of protein sequences taken from the SWALL database. Identity (white letters on black background) or similarity (black letters on grey background) of particular amino acid residues in the given proteins from different organisms to the N-terminal part of the *V. harveyi* CgtA protein (first line) is shown (black letters on white background indicate no identity and similarity to the *V. harveyi* CgtA sequence). The first and the last residues of the compared regions of proteins are indicated at the beginning and at the end of the respective sequence. The abbreviations of the names of species are as follows (the symbols in parentheses represent SWALL database accession numbers): V.h., *Vibrio harveyi*; A.a., *Aquifex aeolicus* (O67849); B.s., *Bacillus subtilis* (P20964); B.b., *Borrelia burgdorferi* (O51722); C.e., *Caenorhabditis elegans* (O45691); C.j., *Campylobacter jejuni* (CAB72580); C.c., *Caulobacter crescentus* (O30861); C.p., *Chlamydia pneumoniae* (Q9Z808); C.t., *Chlamydia trachomatis* (O84423); D.r., *Deinococcus radiodurans* (AAF09676); E.c., *Escherichia coli* (P42641); H.i., *Haemophilus influenzae* (P44915); H.p., *Helicobacter pylori* (Q9ZMD3); H.s., *Homo sapiens* (BAA91783); L.i., *Lawsonia intracellularis* (O87884); M.t., *Mycobacterium tuberculosis* (P17909); M.g., *Mycoplasma genitalium* (P47624); M.p., *Mycoplasma pneumoniae* (P75215); P.sp., *Pseudomonas* sp. BG33R (AAD47628); R.p., *Rickettsia prowazekii* (Q9ZCB6); S.ce., *Saccharomyces cerevisiae* (P38860); S.p., *Schizosaccharomyces pombe* (CAB55178); S.co., *Streptomyces coelicolor* (P95722); S.gr., *Streptomyces griseus* (P95758); S.sp., *Synechocystis* sp. PCC6803 (P72931); T.m., *Thermotoga maritima* (Q9WXV3); T.p., *Treponema pallidum* (O83724); U.u., *Ureoplasma urealyticum* (AAF30873).

Science Products) for probe labelling and detection. The templates for preparation of probes were the *EcoRI*–*PvuII* fragment of plasmid pSupTn5pMCS (encompassing a trimethoprim-resistance gene from the transposon) and the *EcoRV*–*BglII* fragment of plasmid pAC1 (encompassing a part of the *V. harveyi* *cgtA* gene).

Measurement of bioluminescence. Bacteria were grown to high cell density in BOSS medium. Then the cultures were diluted 10000-fold in the fresh medium and cultivation was continued. Samples were withdrawn at intervals; each sample was centrifuged (2000 g, 5 min) and the pellet was resuspended in an equal volume of 3% NaCl. The number of bacteria was determined by plating and luminescence was monitored in a scintillation counter using chemiluminescence mode as described previously (Bassler *et al.*, 1994). The relative light units were calculated as counts min⁻¹ ml⁻¹ per cell.

Survival of cells under starvation conditions. Bacterial cultures growing in BOSS medium were centrifuged; pellets were washed with 3% NaCl and resuspended in an equal volume of this solution. The suspensions were incubated at 30 °C; samples were withdrawn at indicated times and the number of living cells was determined by plating on BOSS plates.

UV sensitivity assay. Bacteria were cultivated in BOSS medium, centrifuged and resuspended in 3% NaCl. Then 1 × 10⁸ cells were irradiated with different UV doses followed

by incubation in BOSS medium in the dark for 2 h and titration on BOSS plates (the plates were incubated overnight in the dark).

Measurement of RNA synthesis. The experiments were performed as described previously by Węgrzyn *et al.* (1991). Briefly, bacteria were grown in BOSS medium to mid-exponential phase; [³H]uridine was added to 2 µCi ml⁻¹ (74 kBq ml⁻¹) and incubation was continued. At indicated times samples (50 µl each) were withdrawn to paper filters and transferred immediately to ice-cold 10% trichloroacetic acid (TCA). After 5 min incubation in an ice bath, the filters were transferred to ice-cold 5% TCA for 5 min, and then washed twice in 96% ethanol. After drying at room temperature, the radioactivity of the samples was measured in a scintillation counter. To induce the stringent response, serine hydroxamate was added to the culture up to 1 mg ml⁻¹.

RESULTS

Determination and analysis of a partial nucleotide sequence of the *V. harveyi* *cgtA* gene and a deduced amino acid sequence of its product

We have reported previously the isolation of a number of random insertional *V. harveyi* mutants obtained after transposon mutagenesis (Czyż *et al.*, 2000a, b). Here we

Fig. 2. Morphology of cells of *V. harveyi* wild-type strain (a, c, e, g, i) and the *cgfA::Tn5TpMSC* mutant (b, d, f, h, j). The cells were photographed under an electron microscope (a, b, c, d) or under a light microscope using differential interference contrast (e, f, g, h, i, j). The specific treatments of the cells were as follows: staining with PTA (a, b), ultrathin sectioning (c, d), staining with DAPI (g, h), and staining with ethidium bromide (i, j). The bar in (a) represents 2 μ m for the electron micrographs (a–d); the bar in (e) represents 7.5 μ m for the other photographs (e–j).

aimed to characterize one of these mutants, strain BB7X. To determine which gene had been disrupted by a transposon insertion in this strain, *V. harveyi* BB7X total DNA was digested with *EcoRI* and the fragment bearing a part of the transposon and flanking DNA sequence was subcloned into pUC19, using selection for ampicillin (an ampicillin-resistance gene is present in the vector) and trimethoprim (a trimethoprim-resistance gene is present in the fragment of the transposon). Then, the DNA region adjacent to the transposon was sequenced using primers complementary to the end of the transposon and to the pUC19 vector. Thus, both DNA strands of this region have been sequenced. The sequence obtained (GenBank accession number AF247677) revealed that the transposon had been inserted into a gene that has a high homology to the *yhbZ* gene of *E. coli* and *H. influenzae* (data not shown). The insertion occurred after 99th codon of the gene (data not shown).

Analysis of the putative amino acid sequence of the N-terminal 99 amino acids of the investigated gene product indicated a high percentage of identity/similarity to small GTP-binding proteins from diverse organisms ranging from bacteria to humans (Fig. 1). This analysis indicated that the gene product belongs to the Obg/Gtp1 subfamily. The best-investigated bacterial members of this subfamily of proteins are Obg (for 'Spo0B-associated GTP-binding protein') from *B. subtilis*, *S. griseus* and *S. coelicolor* and CgtA (for 'Caulobacter GTP-binding protein') from *C. crescentus*, although our knowledge of the roles of these proteins and the corresponding genes is very limited. To avoid further confusion in the nomenclature of homologous proteins, we propose to keep the name CgtA for the *V. harveyi* member of the Obg/Gtp1 subfamily (however, the name CgtA would be for 'common GTP-binding protein' rather than for 'Caulobacter GTP-binding protein').

It was intriguing that *cgtA* (*yhbZ*, *obg*)-null mutants of other bacteria investigated to date were not viable (Arigoni *et al.*, 1998), whereas we have isolated a viable *cgtA* insertional mutant of *V. harveyi*. Therefore, we aimed to characterize this mutant in more detail, as such studies could provide important information about the role and function of the *cgtA* gene product and homologous proteins. To verify whether the transposon is located in the single locus in *V. harveyi* strain BB7X, and thus whether all observed phenotypes result solely from disruption of the *cgtA* gene, we performed Southern-blotting analysis. Total DNA isolated from strain BB7X was digested with *EcoRI*, subjected to agarose gel electrophoresis, transferred to a nylon membrane and hybridized to a fluorescein-labelled probe specific to a fragment of the transposon. If the transposon was located in the single locus we should observe one specific band, whereas location of the transposon in two or more regions of the chromosome should result in appearance of multiple bands after the Southern blotting. Indeed, we observed a single band (data not shown), which indicates that the transposon is located in a unique site in the chromosome of strain

BB7X, and the subcloning and sequence analysis described above determined that this site is the *cgtA* gene.

One could argue that *V. harveyi* might contain a homologue of the *cgtA* gene whose expression could partially suppress effects of *cgtA* dysfunction, making the insertional mutant described in this report viable, in contrast to other bacteria devoid of such a homologue. To test such a possibility, we performed Southern-blotting analysis using *EcoRI*-digested chromosomal DNA of the wild-type *V. harveyi* (strain BB7). Following agarose gel electrophoresis, transfer of DNA to nylon membrane, and hybridization with a fluorescein-labelled probe prepared using the evolutionarily conserved 5' region of the *cgtA* gene of *V. harveyi* as a template (the *EcoRV*-*BglII* fragment of plasmid pAC1), we observed a single band (data not shown). This makes the hypothesis of the presence of a homologue of the *cgtA* gene in *V. harveyi* chromosome less likely.

Morphology of colonies and cells of the *cgtA* mutant

The *cgtA*::Tn5TpMCS mutant formed considerably smaller colonies on plates with nutrient agar medium relative to the otherwise isogenic wild-type strain (data not shown). Moreover, the colonies of the mutant were less luminescent than those of the wild-type *V. harveyi* (data not shown).

The morphology of mutant cells also differed from that of the wild-type bacteria. This was revealed using electron microscopy and light microscopy with interference contrast and after staining with ethidium bromide and DAPI (Fig. 2). Generally, the *cgtA* mutant cells were significantly longer than normal *V. harveyi* cells, i.e. they had a tendency to form filaments. Such a morphology of cells may suggest problems with cell division and/or regulation of DNA replication. The long *V. harveyi* *cgtA*::Tn5TpMCS cells stained quite uniformly with the DNA-binding dyes, suggesting that there are problems with regulation of DNA replication rather than with segregation of genomes to daughter cells. Frequent formation of chains of cells (Fig. 2j) supports the suggestion of problems with cell division in the mutant.

Quorum sensing by the *cgtA* mutant

Luminescence in *V. harveyi* is regulated by the quorum-sensing mechanism (for reviews see Bassler & Silverman, 1995; Swift *et al.*, 1998). Due to this regulation, the luminescence is induced only when bacteria are at high cell density because expression of the appropriate genes is dependent on the presence in the medium of an autoinducer. This autoinducer is produced by *V. harveyi* cells and its synthesis is regulated according to a positive feedback mechanism. Therefore, only cells growing at high cell density can produce a sufficient amount of the inducer.

Wild-type *V. harveyi* and the *cgtA* mutant were grown to high cell density (to allow efficient luminescence), and then the cultures were diluted 10000-fold in fresh

Fig. 3. Luminescence of *V. harveyi* wild-type cells (circles) and the *cgtA::Tn5TpMSC* mutant (squares) at different cell densities. Cultures growing exponentially in BOSS medium were diluted 10000-fold at time 0, and luminescence of samples withdrawn from the cultures at the indicated cell densities was measured. The relative light units were calculated as counts $\text{min}^{-1} \text{ml}^{-1}$ per cell. The values presented are means from three experiments. In all cases, the standard deviation was below 10% of the mean.

medium. Cultivation was continued, and luminescence and cell density were measured at intervals. In the culture of the wild-type cells, the luminescence decreased after dilution and then became more effective due to the increase in the cell density of the culture (Fig. 3). The same phenomenon was observed in the culture of the *cgtA* mutant; however a significantly higher cell density had to be achieved to stimulate luminescence (Fig. 3). These results indicate that the quorum-sensing regulation may be less efficient in the *cgtA* mutant in comparison to the wild-type strain.

Sensitivity of the *cgtA* mutant to mutagenic agents

V. harveyi strains, including BB7 and BB7X, have previously been proposed as bioindicators of mutagenic pollution of the environment (Czyż *et al.*, 2000a). Higher sensitivity of strain BB7X (characterized in this report as the *cgtA::Tn5TpMSC* mutant) to UV irradiation in a preliminary plate test has been reported (Czyż *et al.*, 2000a). Here the survival of bacteria after irradiation with various UV doses was measured in more detail. We found that the *cgtA* mutant was significantly more sensitive to UV irradiation than the wild-type (Fig. 4). Higher mutability of strain BB7X in response to various mutagens, relative to the isogenic wild-type strain BB7, was reported previously (Czyż *et al.*, 2000a). We conclude that DNA repair is impaired in the *cgtA* mutant.

Inhibition of growth of the *cgtA* mutant in minimal media

As mentioned above, the *cgtA* mutant formed smaller colonies on nutrient agar plates than the wild-type strain. We also observed slower growth of the *cgtA* mutant in the rich liquid medium relative to the wild-type strain (data not shown). Moreover, we found that

Fig. 4. Sensitivity to UV irradiation of *V. harveyi* wild-type cells (circles) and the *cgtA::Tn5TpMSC* mutant (squares). Bacteria from exponentially growing cultures were UV irradiated (with the indicated doses) and the number of survivors was determined by plating.

Fig. 5. Survival of *V. harveyi* wild-type cells (circles) and the *cgtA::Tn5TpMSC* mutant (squares) in 3% NaCl. Bacterial cultures growing in BOSS medium were centrifuged; the pellets were washed with 3% NaCl and resuspended in equal volumes of this solution. The bacterial suspensions were incubated at 30 °C and samples for titration (on BOSS plates) were withdrawn at the indicated times.

in contrast to wild-type bacteria the *cgtA* mutant was not able to grow in minimal media with various carbon sources (see Methods) (data not shown).

Survival of the *cgtA* mutant during starvation

Because of the inhibition of growth of the *cgtA* mutant in minimal media, we measured its viability under starvation conditions. Bacteria were incubated in 3% NaCl (this salt concentration is optimal for *V. harveyi*) for several days and the number of survivors was measured by plating of the cell suspensions. Viability of the *cgtA* mutant cells was dramatically decreased under these conditions relative to the wild-type (Fig. 5).

The insertion in the *cgtA* gene does not influence the stringent response or the heat-shock response

Since the *cgtA* mutant revealed defects in its growth in minimal media, and its viability in 3% NaCl was significantly impaired, we asked whether the stringent response, a bacterial response to amino acid and carbon source starvation, is affected by *cgtA* dysfunction. The first effect of the stringent response is production of a specific nucleotide, guanosine tetraphosphate (ppGpp), which by interacting with RNA polymerase inhibits synthesis of stable RNAs (for a review see Cashel *et al.*, 1996). We measured RNA synthesis in cells growing in normal conditions and after induction of the stringent response, achieved by addition of serine hydroxamate to the medium. However, we did not observe any significant differences between the *cgtA* mutant and the wild-type strain. In both strains addition of serine hydroxamate resulted in rapid inhibition of RNA synthesis (data not shown).

We also investigated the response of the *cgtA* mutant to another stress agent, increase in temperature. It was demonstrated previously that *V. harveyi* shows a typical heat-shock response, although at temperatures somewhat lower than *E. coli* (Klein *et al.*, 1995). However, we failed to detect any difference in the heat-shock response between the *cgtA* mutant and the wild-type strain (data not shown).

DISCUSSION

In this study we have characterized a *V. harveyi* strain in which the *cgtA* gene, an *obg* homologue, has been disrupted by transposon insertion. To our knowledge, this strain is the first example of an insertional mutant in genes encoding bacterial members of the Obg/Gtp1 proteins. Although it seems likely that the transposon insertion inactivated the *cgtA* gene, we cannot be sure that strain BB7X is a true null mutant. The insertion occurred after the 99th codon of the *cgtA* gene; thus it is possible that synthesis of the N-terminal part of CgtA is sufficient to retain some functions of this protein. Another possibility could be that the phenotypes of the mutant do not result directly from the *cgtA* gene dysfunction but are polar effects of the transposon insertion. This might occur if any important genes were located downstream of *cgtA* in the same operon. However, analysis of the structure of the *E. coli* operon containing the *yhbZ* gene (a *cgtA* homologue) revealed that it consists of four genes: *rplU*, *rpmA*, *yhbE* and *yhbZ*, where the *cgtA* homologue is the most distal gene. Considering the high homology between *E. coli yhbZ* and *V. harveyi cgtA* genes, and the close relation of these two bacterial species, it is likely that the organization of a homologous operon in *V. harveyi* resembles that of *E. coli*. If this is true, no polar effects of the *cgtA*::Tn5TpMCS mutation should occur.

The *V. harveyi cgtA* mutant revealed multiple defects in several cellular processes. Formation of cell filaments (Fig. 2) may suggest problems with regulation of cell division and/or DNA replication at least under certain

conditions. In fact, it was proposed previously that the Obg protein of *B. subtilis* may be involved in the regulation of DNA replication (Kok *et al.*, 1994). The same *B. subtilis* protein was recently demonstrated to be involved in activation of the σ^B factor (Scott & Haldenwang, 1999). This factor is the general stress-response σ subunit of RNA polymerase that is activated when intracellular ATP levels fall or the bacterium experiences environmental stress. Activity of σ^B is regulated by a set of kinases and phosphatases, called the Rsb proteins, which catalyse the release of σ^B from an anti- σ factor. It was suggested that Obg may interact with one or more σ^B regulators (Scott & Haldenwang, 1999). The *cgtA* mutant of *V. harveyi* is not able to grow in minimal media and its viability in 3% NaCl is dramatically decreased (Fig. 5), suggesting that induction of expression of certain genes that are normally active under conditions of starvation and energy deprivation may be impaired. Therefore, one may speculate that activation of an appropriate σ factor (e.g. a homologue of *E. coli* σ^S) could be dependent on the *cgtA* gene function, similarly to the *obg*-dependent activation of *B. subtilis* σ^B . However, it is worth noting that in the case of the *B. subtilis obg* gene dysfunction the σ^B factor was no longer activated in response to environmental stress, but it retained the ability to be activated by the ATP-responsive pathway (Scott & Haldenwang, 1999). Thus, we should also consider that the mechanism of *cgtA*-dependent *V. harveyi* growth in minimal media and survival under starvation conditions might be significantly different from that of *B. subtilis*.

Colonies of the *cgtA* mutant of *V. harveyi* were less luminescent than those of the wild-type. However, calculation of the relative luminescence per single bacterium in a liquid culture of high cell density revealed significantly smaller differences (Fig. 3). This could suggest defects in the quorum-sensing regulation rather than in the luminescence reaction itself. Indeed, the *cgtA* mutant required significantly higher cell density for efficient quorum-sensing-dependent induction of luminescence relative to the wild-type strain (Fig. 3). The quorum-sensing regulation in *V. harveyi* requires activation of kinases and phosphatases (Freeman & Bassler, 1999a, b; Freeman *et al.*, 2000). Therefore, it seems likely that the *cgtA* gene product may be involved in the process of signal transduction during the quorum-sensing regulation.

Currently it is difficult to explain the mechanism of decreased efficiency of DNA repair in the *cgtA* mutant (Fig. 4, and Czyż *et al.*, 2000a). One possibility is that decreased efficiency of light emission in this mutant results in less efficient photoreactivation, since it was proposed recently that bioluminescence may stimulate photolyase activity (Czyż *et al.*, 2000b). On the other hand, CgtA might be involved in the regulation of expression of genes encoding DNA repair proteins.

In conclusion, the *cgtA*::Tn5TpMCS mutant of *V. harveyi* reveals multiple defects in important cellular processes, although it is still viable in rich media, in contrast to null mutants in the homologous genes of

other bacteria investigated to date, which are not viable. Most, if not all, of the processes affected by the *cgtA* dysfunction in *V. harveyi* and reported in this article involve regulation of gene expression in response to various environmental signals. The *V. harveyi cgtA* gene product is a potential GTP-binding protein as it has a high homology to such proteins identified in many other organisms (Fig. 1). Therefore, it seems likely that *V. harveyi* CgtA protein is involved in signal-transduction processes. According to this speculation, bacterial responses to environmental stresses that apparently do not require protein phosphorylation/dephosphorylation-mediated signal transduction, e.g. the stringent response and the heat-shock response, appear to be normal in the *cgtA* mutant of *V. harveyi*.

ACKNOWLEDGEMENTS

We thank Jerzy Bohdanowicz and Beata Lewandowska for their help during light microscopy studies. We are grateful to Joanna Potrykus and Katarzyna Potrykus for critical reading of the manuscript. This work was supported by University of Gdańsk (grant BW/1190-5-0120-0 to A.C.). G.W. acknowledges also a financial support from the Foundation for Polish Science (subsydium 14/2000).

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Received 15 May 2000; revised 11 September 2000; accepted
19 September 2000.