Cloning and characterization of the \textit{groE} heat-shock operon of the marine bacterium \textit{Vibrio harveyi}

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The DNA region of the \textit{Vibrio harveyi} chromosome containing the heat-shock genes \textit{groES} and \textit{groEL} was cloned, and the genes were sequenced. These genes are arranged in the chromosome in the order \textit{groES–groEL}. Northern hybridization experiments with RNA from \textit{V. harveyi} and a DNA probe carrying both \textit{groES} and \textit{groEL} genes showed a single, heat-inducible transcript of approximately 2200 nt, indicating that these genes form an operon. Primer extension analysis revealed a strong, heat-inducible transcription start site 59 nt upstream of \textit{groES}, preceded by a sequence typical for the \textit{Escherichia coli} heat-shock promoters recognized by the \(\sigma^{32}\) factor, and a weak transcription start site 25 nt upstream the \textit{groES} gene, preceded by a sequence typical for \(\sigma^{70}\) promoters. Transcription from the latter promoter occurred only at low temperatures. The \textit{V. harveyi groE} operon cloned in a plasmid in \textit{E. coli} cells was transcribed in a \(\sigma^{32}\)-dependent manner; the transcript size and the \(\sigma^{32}\)-dependent transcription start site were as in \textit{V. harveyi} cells. Comparison of \textit{V. harveyi groE} transcription regulation with the other well-characterized \textit{groE} operons of the \(\gamma\) subdivision of proteobacteria (those of \textit{E. coli} and \textit{Pseudomonas aeruginosa}) indicates a high conservation of the transcriptional regulatory elements among these bacteria, with two promoters, \(\sigma^{32}\) and \(\sigma^{70}\), involved in the regulation. The ability of the cloned \textit{groESL} genes to complement \textit{E. coli groE} mutants was tested: \textit{V. harveyi groES} restored a thermoresistant phenotype to \textit{groES} bacteria and enabled \(\lambda\) phage to grow in the mutant cells. \textit{V. harveyi groEL} did not abolish thermosensitivity of \textit{groEL} bacteria but it complemented the \textit{groEL} mutant with respect to growth of \(\lambda\) phage. The results suggest that the GroEL chaperone may be more species-specific than the GroES co-chaperone.

INTRODUCTION

All organisms analysed so far respond to a sudden increase in temperature by transiently enhanced synthesis of heat-shock proteins (Hsps) (Neidhardt \textit{et al.}, 1984). Most prominent among the Hsps are molecular chaperones and ATP-dependent proteases, which help to restore homeostasis by either refolding or degradation of the non-native proteins (Bukau & Horwich, 1998). The primary structure of most Hsps is highly conserved during evolution, suggesting that they serve similar and very important functions in all organisms, from bacteria to man (Lindquist & Craig, 1988).

The chaperonin GroEL (Cpn60) and co-chaperonin GroES (Cpn10) constitute the GroE chaperone machine, which takes part in the process of folding and assembly of proteins (reviewed by Hartl & Hayer-Hartl, 2002; Houry, 2001) and is found in bacteria, mitochondria and chloroplasts (Ellis & van der Vies, 1991). The GroE chaperone system assists the folding of polypeptides in compact conformations, recognizing intermediates exposing hydrophobic surfaces (Hartl & Hayer-Hartl, 2002). Although purified GroEL is able to bind to a wide range of proteins, \textit{in vivo} there is evidence that it has high affinity for a defined set of substrates. While \textit{in vitro} GroE binds to about 50 % of soluble \textit{Escherichia coli} proteins in their unfolded or partially folded state (Viitanen \textit{et al.}, 1992), only about 300 \textit{E. coli} proteins require the chaperonin for folding \textit{in vivo}, and only about 50 protein substrates have been identified (Houry \textit{et al.}, 1999). So far, consensus sequences characteristic for GroEL substrates have not been identified (Houry, 2001) and the problem of GroE substrate-specificity is unsolved. Similarly, little is known about the species-specificity of the GroE system.

Because of their chaperonin reactions, their importance for growth at all temperatures (Fayet \textit{et al.}, 1989), and the importance of GroEL as a major antigen of pathogenic bacteria, the genes encoding these proteins have been cloned.
from a wide variety of bacteria. Cloning and sequencing of these genes served in studies oriented at characterization of protein structure and function (Zeilstra-Ryalls et al., 1991) as well as in comparative studies on the regulation of heat-shock-gene expression (Segal & Ron, 1996b).

The GroE proteins and regulation of their genes have been studied most intensively in E. coli. The groES and groEL genes form an operon essential for E. coli viability at all temperatures (Fayet et al., 1989). The groE operons of E. coli and other bacteria tested are arranged in the order promoter–groES–groEL (Segal & Ron, 1996b), with several bacteria having an additional, monocistronic groEL operon [e.g. Synechocystis sp. (Lehel et al., 1993), Synechococcus vulgaris (Furuki et al., 1996), Rhizobium meliloti (Rusanganwa & Gupta, 1993), Anabaena sp. (Rajaram et al., 2001)]. To our knowledge, there is only one case (Mycobacterium bovis) of groES without groEL (Segal & Ron, 1996b). In some species, several groE operons have been found [e.g. Rhodobacter sphaeroides (Lee et al., 1997), Bradyrhizobium japonicum (Babst et al., 1996)]. In E. coli, the groE operon belongs to the main heat-shock regulon, regulated by the σE factor (Yura et al., 1993). Under heat-shock conditions, the groES genes are efficiently transcribed from a heat-shock promoter located upstream of groES by the RNA polymerase cooperating with the σE factor (Cowing et al., 1985). The groE operon has a second promoter, located immediately downstream from the heat-shock one, which can be utilized under normal growth conditions by RNA polymerase cooperating with the vegetative sigma factor, σ20 (Zhou et al., 1988).

The strategies of regulation of the groE operons in bacteria are diverse and, in contrast to the E. coli system, poorly understood (Segal & Ron, 1996b; Schumann, 1996). Generally, regulation of groE transcription occurs by two different mechanisms: (i) alternative sigma factors, like σ20 in E. coli, or/and (ii) transcriptional repressors. The latter mechanism, first described in Bacillus subtilis, is facilitated by the HrcA repressor, which binds to an inverted repeat named CIRCE (Controlling Inverted Repeat of Chaperone Expression) (Mogk et al., 1997; Schumann, 1996). This element has been identified in the promoter regions of many groE genes of Gram-positive as well as Gram-negative bacteria (Segal & Ron, 1996b; Schumann, 1996).

V. harveyi is a free-living Gram-negative γ purple bacterium found in diverse marine environments, at various geographical locations, at depths between the surface and about 1000 m (Baumann & Schubert, 1984; Ruby et al., 1980). Its use as a bioindicator of environmental pollution is under current investigation, especially for monitoring the presence of mutagenic substances. V. harveyi cells are highly sensitive to various mutagens and hypersensitive strains have been constructed, for use in a modified Ames test (Czyk et al., 2000). However, non-mutagenic toxic substances would not be detected in this type of test. Heat-shock proteins have been proposed as general markers of cellular stress and their use for environmental monitoring is often suggested (Ait-Aissa et al., 2000; Ryan & Hightower, 1996; Sanders & Martin, 1993). One of the approaches is to construct indicator organisms which carry reporter genes (e.g. chloramphenicol acetyltransferase or β-galactosidase) under the control of heat-shock promoters responding to various stresses (Ait-Aissa et al., 2000). V. harveyi seems to be a good candidate for such an indicator organism, because of its wide distribution in marine environments and the availability of genetic data for this species, mainly due to investigations focused on luminescence (Freeman & Bassler, 1999) and the heat-shock response. Its main heat-shock proteins (DnaK, DnaJ, GroEL, GroES and IbpA/B) have been identified (Klein et al., 1995, 2001) and it has been shown that transcription of the heat-shock dnaKJ genes is regulated by a σ32 homologue, in a manner similar to that observed in E. coli (Klein et al., 1998).

We have undertaken to clone the V. harveyi groE genes and characterize them for the following reasons: (i) to understand how the groE genes of the γ purple bacteria other than the model E. coli are regulated; (ii) to select a heat-shock gene for constructing a promoter fusion for biomonitoring purposes; (iii) to better understand species-specificity of chaperonins. In this work we report the whole sequence of the V. harveyi groE operon and show that it is transcribed as a single bicistronic transcript starting from two promoters, σ32 and σ70. We also analyse the complementation of mutations in the groESL genes of E. coli by the homologous V. harveyi genes.

METHODS

Bacteria, bacteriophages, plasmids and media. Bacteria, phages and plasmids used in this study are described in Table 1. All E. coli strains were grown on L agar plates or in Luria broth (LB) (Sambrook et al., 1989). The V. harveyi strain was grown in BOSS medium (1% peptone, 0.3% beef extract, 0.1%, v/v, glycerol, 3% NaCl, pH 7.3). Bacteria used for RNA preparation were grown in P medium (0.1 M Tris/HCl pH 7.4, 0.02 M NH₄Cl, 2 mM MgSO₄, 5 mM KCl, 1 mM CaCl₂, 3×10⁻⁴ M FeCl₃, 0.3% glucose, 0.3% Casamino acids, 2 μg thiamin ml⁻¹); for V. harveyi cells, the medium was supplemented with 0.5 M NaCl. When appropriate, the following antibiotics were added: ampicillin (Amp) (200 μg ml⁻¹), kanamycin (Kan) (30 μg ml⁻¹), tetracycline (Tet) (10 μg ml⁻¹) and spectinomycin (Spc) (30 μg ml⁻¹). Stocks of recombinant β-phages were prepared as described by Sambrook et al. (1989).

DNA manipulations. Recombinant DNA techniques were performed by standard protocols (Sambrook et al., 1989). DNA fragments, when necessary, were purified using the QiAquick Gel Extraction Kit (Qiagen), according to the manufacturer’s protocol. DNA was sequenced using Sanger’s method (Sanger et al., 1977) and the Pharmacia Biotech T7 Sequencing Kit, as described by the manufacturer, or automatically by the Laboratory of Sequencing of DNA and Biophysics, Warsaw, Poland. Sequencing was carried out using double-stranded plasmid or β DNA and the oligonucleotide primers (17-mers) synthesized by Interactiva. The sequences were analysed with the PC Gene and BLAST programs on the NCBI server.

Southern hybridization. DNA was transferred from agarose to positively charged nylon membrane (Boehringer Mannheim) using 10× SSC solution (1× SSC is 150 mM NaCl plus 15 mM sodium
were resolved on agarose gels, to control the integrity of the RNA. Described previously (Lipinski et al., 1998), the membrane (Boehringer Mannheim) using 20% formamide and high-stringency conditions. The probes were drawn at the indicated time points and immediately lysed by mixing 8 mM EDTA, pH 7 with 50% formamide and high-stringency conditions. The probes were prepared as follows: (i) the XbaI–PstI fragment of the plasmid pDK1, which contains the 1500 bp ClaI fragment carrying the 3' end of the V. harveyi groEL gene; (ii) the 3500 bp EcoRI–HindIII fragment from pDK5 cloned in EcoRI–HindIII sites; (iii) the 2260 bp Smal fragment of the plasmid pDK5, which carries groES operon of V. harveyi, was purified from agarose gels, and following the procedure recommended by the manufacturer of the enzyme. The oligonucleotide (·122) 5’-GCCTTTACCACAGTCT-3’ was used for primer extension experiments and for obtaining the accompanying sequencing ladder. The number in parentheses indicates the position of the 5' end relative to the A (on the opposite DNA strand) of the groE start codon. The primer was purchased from Interactiva. Kpnl–MluI fragment of the plasmid pDK5, which carries groES and most of the groEL gene of V. harveyi, was purified from agarose and labelled with digoxigenin-11-dUTP, using the random-priming method and the Boehringer Mannheim DNA Labelling and Detection Kit. This was used as a probe in hybridization experiments carried out as described by the manufacturer of the kit. Each lane in Northern hybridization contained 30 µg RNA extracted from V. harveyi cells or 5 µg RNA extracted from E. coli cells.

**Primer extension experiments.** Primer extension experiments were carried out according to the procedure described before (Klein et al., 1998). 32P-end-labelled oligonucleotide was prepared using T4 polynuclease kinase (Fermentas) and [γ-32P]ATP, 4500 Ci mmol⁻¹ (166–5 TBq mmol⁻¹), purchased from ICN, and following the procedure recommended by the manufacturer of the enzyme.

The oligonucleotide (+122) 5’-GCCTTTACCACACAGTCT-3’ was used for primer extension experiments and for obtaining the accompanying sequencing ladder. The number in parentheses indicates the position of the 5' end relative to the A (on the opposite DNA strand) of the groE start codon. The primer was purchased from Interactiva.

**Complementation tests.** E. coli strains with groE mutations (E. coli CG2244 groES619 and CG2241 groEL44) were transformed with high- or low-copy-number plasmids carrying groE genes of V. harveyi.
(a) E. coli B178  V. harveyi  KY1620 B178

![DNA gel image with bands labeled T1, T1', and T2.]

(c) V. harveyi

![DNA gel image with bands labeled T1, T1', and T2.]

(d) Pσ^{32} -35 -10

725 CAGGCTCTACCCCTGCGATCCCTAGCTTTG CACCCCTATA

784 CGCGGGCAATATGAAATTTCAGAGAGAGACGGCAGATGAACATTCG

Pσ^{70} -35 T1, T1'
Plasmids were constructed by standard procedures using plasmid pDK5 (described in Results) as a source of the *V. harveyi* groE genes (Table 1). Growth of λlb2 phage was assayed by plating. Efficiency of plating (EOP) was the ratio of plaque-forming units on a tested strain to plaque-forming units on a permissive strain (*E. coli* NM538). Transformants were tested for ability to grow at 42°C (*groEL* strains) or 45°C (*groES* strains): 10 μl drops of diluted cultures were spotted on L agar. Plasmid pOF39 carrying *E. coli* groE genes was used as a positive control.

**RESULTS**

**Cloning and sequencing of the *V. harveyi* groE genes**

In order to clone the *groEL* and *groES* genes of *V. harveyi*, we previously constructed a *V. harveyi* genomic library in the λEMBL1 vector and selected phage clones which were able to complement mutations in both *groE* genes of *E. coli* for phage λ growth (Kuchanny et al., 1998). In one of these clones (λ*groES*vibrio) we identified and subcloned a 1500 bp ClaI DNA fragment carrying the 3' end of the *groEL* gene of *V. harveyi* (Kuchanny et al., 1998). In this work, employing the 1500 bp ClaI fragment as a probe in Southern hybridization experiments with λ *groES*vibrio DNA, we found, cloned in vector pUC19 and sequenced a 3500 bp SalI fragment containing the whole *V. harveyi* groEL gene. We then decided to clone the groE genes from the chromosomal DNA and not from the phage, since we had found that the phage λ *groES*vibrio1 did not contain the transcriptional regulatory elements of the groE genes (results not shown).

In the next step, we performed Southern hybridization with *V. harveyi* chromosomal DNA using the 3500 bp SalI fragment as a probe and identified a 4800 bp SacI fragment which we subsequently cloned in the pGEM-3Zf vector (results not shown). The resulting hybrid plasmid, named pDK5, was used in further work. The major part of the insert was sequenced and the sequence (GenBank accession number AY246431) revealed that it carries both groE genes, which are arranged in the order groES–groEL, and upstream of the groES gene there is an N-terminal end of an ORF (orf > 540).

Based on the sequencing data, the *groES* gene has a length of 306 nt and encodes a protein consisting of 102 amino acid residues, with a predicted molecular mass of 10 875 Da, and 81% and 88% similarity to *E. coli* and *V. cholerae* GroES proteins, respectively. The *V. harveyi* groEL gene has a coding sequence of 1644 nt. The predicted GroEL protein contains 548 amino acids, its calculated molecular mass is 57 549 Da and it has 85% and 87% similarity to the GroEL protein of *E. coli* and *V. cholerae*, respectively. The calculated isoelectric points of the *V. harveyi* GroES and GroEL proteins are respectively 4.9 and 4.47. Upstream of the groES gene there are two potential promoters: a distal one, similar to the *E. coli* heat-shock promoters recognized by σ32 factor, and a proximal one, similar to the *E. coli* vegetative promoters recognized by σ70 (Fig. 1d). Six nucleotides before the groES translation initiation codon there is a potential ribosome-binding site (AGGAG) (Fig. 1d). At 131 nt downstream from the groEL translation stop codon we found an inverted repeat which may represent a rho-independent transcription termination signal. There are no putative transcription termination sites downstream of the groES gene and no promoter-like sequences preceding the groEL translation initiation codon but there is a potential Shine-Dalgarno sequence (AGGA) starting 8 nt before the groEL translation initiation codon. At the C-terminus of the predicted GroEL protein, there is a GMR motif (Gly-Gly-Met repeats), present in almost all known GroEL homologues and in many Hsp70 proteins (McLennan et al., 1993).

The orf > 540, located upstream of the groES position and oriented in the opposite direction to the groE genes, encodes the N-terminal end of a polypeptide of at least 180 amino acids which shows 88% similarity to the conserved hypothetical protein from *Vibrio cholerae*, encoded by gene VC2671 located downstream from the groE operon at chromosome I, section 240 (Heidelberg et al., 2000).

**Transcriptional analysis of the groE gene region**

The *in vivo* transcription of the groE genes was investigated by Northern analysis. Total RNA was prepared from heat-shocked and non-heat-shocked *V. harveyi* cells, and also from *E. coli* cells in which *V. harveyi* groE genes were expressed from the pDK5 plasmid. The heat-shock temperature used for *V. harveyi* and *E. coli* cells was 42 and 39°C, respectively, the difference being due to the fact that *V. harveyi* does not grow well above 39°C (Klein et al., 1995).
The RNA was hybridized with a digoxigenin-labelled probe which carries both groE genes of \textit{V. harveyi}. The Northern analysis of the \textit{V. harveyi} RNA revealed a single heat-shock-induced transcript of approximately 2.2 kb (Fig. 2). A heat-shock-induced transcript of the same size was found in \textit{E. coli} cells carrying \textit{V. harveyi} groE genes cloned in pDK5, but not in cells carrying the pGEM-3Zf vector (Fig. 2). A faint band present in \textit{E. coli} B178(pDK5) cells, migrating above the groE mRNA, probably resulted from a non-specific binding of the probe to 23S rRNA. These results indicate that the groE genes form an operon, like the groE genes of \textit{E. coli}, and that this operon is regulated by heat shock similarly in \textit{V. harveyi} and \textit{E. coli} cells.

To find if the groE operon of \textit{V. harveyi} has, similarly to groE of \textit{E. coli}, an additional promoter recognized by $\sigma^{70}$, we performed primer extension experiments at a lower temperature (23°C). Indeed, we found a weak transcript (T2) starting at A$_{806}$, 25 nt upstream from the groE translational start (Fig. 1c, d). This transcript could be detected in cells grown at 23°C, was barely visible at 30°C but was absent in the cells heat-shocked at 39°C for 10 min (Fig. 1c). The T2 transcript is preceded by a sequence similar to the consensus sequence of $\sigma^{70}$-dependent promoters (Fig. 1d) (Hawley & McClure, 1983). Our results indicate that the groE operon of \textit{V. harveyi} has, besides the heat-shock promoter, a $\sigma^{70}$-dependent vegetative promoter.

### Complementation of \textit{E. coli} groES and groEL mutations with the respective genes of \textit{V. harveyi}

Mutations in the groES and groEL genes of \textit{E. coli} give two major phenotypic effects: (i) inability to maintain growth of \lambda phage and (ii) thermosensitivity (Ts phenotype) of the mutant bacteria (Georgopoulos et al., 1990).

To find out whether the \textit{V. harveyi} GroEL and GroES proteins could function in \textit{E. coli}, we tested the ability of the \textit{V. harveyi} groES and groEL genes to complement mutations in the respective genes of \textit{E. coli}. We found that the cloned \textit{V. harveyi} groES gene enabled \textit{E. coli} groES mutants to form normal, large colonies at 45°C, while mutant cells transformed with vector plasmid and non-transformed mutants formed small colonies (thermosensitivity of the \textit{E. coli} B178 groES619 mutant manifests as growth impairment rather than total lack of growth) (Fig. 3, Table 2). The cloned \textit{V. harveyi} groES gene enabled \lambda phage to grow in the mutant cells (Table 2). The \textit{V. harveyi} groEL gene did not complement the Ts phenotype of the \textit{E. coli} groEL bacteria but it complemented the groEL mutant with respect to growth of \lambda phage (Table 2). The lack of complementation was not due to inefficient expression of GroEL, since phage \lambda grew well (Table 2) and overproduction of GroEL was visible on Coomassie-stained SDS-PAGE gels (not shown). These
results indicate that GroEL protein is more species-specific than GroES co-chaperone.

The complementation described depended on the copy number of plasmids carrying the Vibrio genes: (i) the Ts phenotype of the E. coli groES bacteria could be complemented by the low-copy-number plasmids but not by the high-copy-number ones; (ii) groEL mutation (with respect to λ phage growth) was complemented much better (approx. 70-fold) by the high-copy-number plasmids (Table 2).

**DISCUSSION**

The groES and groEL genes of V. harveyi were cloned and sequenced and showed high levels of homology to the corresponding E. coli genes. The genes are arranged in the order groE–groEL, as in E. coli and many other species of eubacteria (Segal & Ron, 1996b), including *V. cholerae* (Mizunoe *et al.*, 1999).

Since by Northern blotting we were able to detect only one 2.2 kb transcript using the probe containing both genes (Fig. 2), and primer extension experiments showed transcriptional starts before the groES gene (Fig. 1), we conclude that the genes form an operon. The size of the transcript is exactly as predicted taking into account the transcription start point T1 and an inverted repeat found 131 nt downstream from the groEL translation stop codon, which may represent a rho-independent transcription termination signal. Such organization seems typical for γ-purple proteobacteria, and in that subdivision of bacteria has been demonstrated so far for *E. coli* (Zeilstra-Ryalls *et al.*, 1991), *Pseudomonas aeruginosa* (Fujita *et al.*, 1998), *Pasteurella multocida* (Love *et al.*, 1995) and *V. harveyi* (this work).

Table 2. Complementation of *E. coli* groE mutations by the cloned groE genes of *V. harveyi*

<table>
<thead>
<tr>
<th>Plasmid [vector]</th>
<th>Plasmid genotype</th>
<th>Genotype of E. coli cells</th>
<th>Growth of λclb2 (EOP)</th>
<th>Complementation of Ts phenotype</th>
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<tbody>
<tr>
<td>pDK3 [pUC19]</td>
<td>groES&lt;sup&gt;+&lt;/sup&gt; groEL&lt;sup&gt;+&lt;/sup&gt; (V. harveyi)</td>
<td>groES</td>
<td>&lt;10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>pDK7 [pGEM]</td>
<td>groES&lt;sup&gt;+&lt;/sup&gt; groEL&lt;sup&gt;+&lt;/sup&gt; (V. harveyi)</td>
<td>groES</td>
<td>0.87</td>
<td>–</td>
</tr>
<tr>
<td>pDK8 [pGB2]</td>
<td>groES&lt;sup&gt;+&lt;/sup&gt; groEL&lt;sup&gt;+&lt;/sup&gt; (V. harveyi)</td>
<td>groEL</td>
<td>0.74</td>
<td>+</td>
</tr>
<tr>
<td>pDK9 [pGEM]</td>
<td>groES&lt;sup&gt;+&lt;/sup&gt; (V. harveyi)</td>
<td>groES</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>pDK10 [pGB2]</td>
<td>groES&lt;sup&gt;+&lt;/sup&gt; (V. harveyi)</td>
<td>groEL</td>
<td>0.014</td>
<td>–</td>
</tr>
<tr>
<td>pOF39 [pBR322]</td>
<td>groES&lt;sup&gt;+&lt;/sup&gt; groEL&lt;sup&gt;+&lt;/sup&gt; (E. coli)</td>
<td>groES</td>
<td>&lt;10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>groEL</td>
<td>0.87</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.61</td>
<td>+</td>
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</table>
transcript size of *P. aeruginosa* groE genes is also 2.2 kb (Fujita et al., 1998).

We have shown, by Northern analysis (Fig. 2) and primer extension (Fig. 1), that the *V. harveyi* groE genes are transcriptionally activated by heat shock. Primer extension experiments identified potential heat-shock-induced transcripts (T1 and T12) starting at 59 and 57 nt upstream of the translational start of groES (Fig. 1). They are preceded by a typical heat-shock promoter sequence, designated Pσ70 (Fig. 1d). Since we have previously identified a σ32 homologue in *V. harveyi* (Klein et al., 1995) and σ32 has been shown to be required for the transcription of *V. harveyi* groE in *E. coli* cells (this work), we conclude that Pσ32 functions as a σ32-dependent promoter. High induction of the transcript under stress conditions suggests that the σ32 promoter of the groE gene may be a good candidate for use in a promoter fusion with a reporter gene; further experiments will show whether *V. harveyi* carrying such a construct could be used for environmental monitoring.

Presence of a weak transcript (T2) starting 25 nt upstream from the groES translational start, preceded by the sequence characteristic for σ70-dependent promoters, indicates that the *V. harveyi* groE operon has an additional, σ70-dependent promoter. This promoter functions only at low temperature and is switched off during heat shock, which may be caused by occlusion of the promoter with polymerase cooperating with σ32, since the −35 region of Pσ70 overlaps with the start of the heat-shock transcript T1 (Fig. 1d).

Cowing et al. (1985) identified a σ32-dependent heat-shock transcript of the *E. coli* groE operon starting at −72 nt upstream from the groES translational start and Zhou et al. (1988) showed the presence of the σ70-dependent transcript initiating about 25–30 bases downstream of the σ32 transcript. The level of the σ70 transcript was significant at low temperature (17°C) only. In this case also, as in the *V. harveyi* groE operon, the σ32 transcript starts in the −35 region of the σ70 promoter. In the groE operon of *P. aeruginosa*, another member of the γ subdivision, overlapping consensus sequences for σ32 and σ70-dependent promoters were found upstream of the groES gene (Fujita et al., 1998). A comparison of the proteobacterial groE operons characterized so far allows us to conclude that in the γ subdivision of proteobacteria, transcriptional regulation is based on the presence of two, σ32 and σ70-dependent, promoters, while in other subdivisions the strategies are more diverse: either one of these two promoters is found [Cowdria ruminantium – σ70 (Lally et al., 1995), B. japonicum – σ32 (Babst et al., 1996)], or there is a combination of two promoters plus a CIRCE regulatory element [R. sphaeroides (Lee et al., 1997)], or one of the promoters is accompanied by CIRCE [Agrobacterium tumefaciens (Segal & Ron, 1996a)].

The *V. harveyi* groE genes were functionally analysed by testing their ability to complement mutations in homologous genes of *E. coli*. We found that both *V. harveyi* groE genes were able to support the growth of λ phage in the *E. coli* groE mutants (Table 2). This rescue of the *E. coli* groE mutants occurred efficiently when either one or both *V. harveyi* groE genes were present, which suggests that the *V. harveyi* GroE proteins are able to cooperate with the co-chaperones from *E. coli*. Complementation of the *E. coli* groE mutations with respect to λ phage growth was observed previously for the groE genes of *Chromatium vinosum* (Ferreryra et al., 1993), *Actinobacillus pleuropneumoniae* (Vezina et al., 1997) and *Bacillus stearothermophilus* (Schon & Schumann, 1993).

The groES gene of *V. harveyi*, expressed from the low-copy-number plasmids (pDK8/10), but not from the high-copy-number plasmids (pDK7/9), complemented the Ts phenotype of the *E. coli* groES mutants (Table 2, Fig. 3). This shows that *V. harveyi* GroES is fully functional in *E. coli* cells and also indicates that high amounts of GroES are not tolerated at the higher temperature. In contrast to groES, the *V. harveyi* groEL gene did not complement the Ts phenotype of the *E. coli* groEL mutant (Table 2), which suggests that the GroEL chaperone is more species-specific than the GroES co-chaperone. What could be the reason for this difference in behavior between the groES and groEL genes, and why would this difference be important for Ts complementation but not for phage growth? A simple explanation could be that the GroE chaperonin system participates in multiple cellular processes, and even a minor impairment of each of them would have a cumulative final effect of serious consequences for the cell as a whole, under stress conditions (heat shock), while in phage infection GroE is involved only in one process (capsid formation: Georgopoulos et al., 1990). It is also possible that some specific chaperone substrate(s) in *E. coli* cells is not recognized by *V. harveyi* GroEL. It should be noted that GroEL, not GroES, is responsible for substrate recognition (Hartl & Hayer-Hartl, 2002). Surprisingly, Mizunoe et al. (1999) found that the *V. cholerae* groEL gene complemented the Ts phenotype of an *E. coli* groEL mutant. A positive effect of the cloned groE genes on the Ts phenotype of *E. coli* groEL bacteria was also observed for *C. vinosum* (Ferreryra et al., 1993) and *B. stearothermophilus* (Schon & Schumann, 1993). These results show that the problem of species-specificity of chaperone proteins is complex and cannot be simply explained in terms of protein homology: for instance, homology between GroEL proteins of *E. coli* and *V. harveyi* is 83 % and there is no complementation, while GroEL of *B. stearothermophilus* can complement, though identity is only 71 %.

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