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Archaeal *grpE*: Transcription in Two Different Morphologic Stages of *Methanosarcina mazei* and Comparison with *dnaK* and *dnaJ*

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Transcription of the heat shock gene *grpE* was studied in two different morphologic stages of the archaeon *Methanosarcina mazei* S-6 that differ in resistance to physical and chemical traumas: single cells and packets. While single cells are directly exposed to environmental changes, such as temperature elevations, cells in packets are surrounded by intercellular and peripheral material that keeps them together in a globular structure which can reach several millimeters in diameter. *grpE* transcript levels determined by Northern (RNA) blotting peaked after a 15-min heat shock in single cells. In contrast, the highest transcript levels in packets were observed after the longest heat shock tested, 60 min. The same response profiles were demonstrated by primer extension experiments and S1 nuclease analysis. A comparison of the *grpE* response to heat shock with those of *dnaK* and *dnaJ* showed that the *grpE* transcript level was the most increased, closely followed by that of the *dnaK* transcript, with that of the *dnaJ* gene being the least augmented. Transcription of *grpE* started at the same site under normal and heat shock temperatures, and the transcript was consistently ~700 bases long. Codon usage patterns revealed that the three archaeal genes use most codons and have the same codon preference for 61% of the amino acids.

A gene encoding a GrpE homolog has recently been cloned from the genome of the archaeon *Methanosarcina mazei* S-6 and sequenced (6). It is the first example of a *grpE* heat shock gene found within the domain Archaea. The Archaea are organisms phylogenetically distinct from Bacteria (eubacteria) and Eucarya (eucaryotes) (41). However, Archaea resemble eucaryotes in several molecular biological features more than they resemble bacteria and are closer to eucaryotes than bacteria (15, 28, 30, 31, 41). A case in point is gene regulation, since there are data indicating that Archaea use a transcription regulation machinery similar to that of eucaryotes (10, 24, 28, 33). Whether this is the case also for archaeal heat shock genes has not been established. To address this question, it is necessary to first determine the expression pattern of the known archaeal heat shock genes and then proceed to analyze the regulation mechanisms at the molecular level.

Among the Archaea, *M. mazei* is particularly interesting because it is one of a small group of organisms, the methanosarcinae (22, 23), that undergo a growth cycle with morphologic conversions (3, 25, 32, 42). The cycle includes a unicellular form or stage, named single cells, and two multicellular forms, called lamina and packets. The latter are globular structures with zonal heterogeneity and intercellular connective material that extends the outer surface of the packets and wraps the cells together. This connective material is composed chiefly of a heteropolysaccharide (14, 16) which confers to the packets resistance to traumas of various types: mechanical, physical (e.g., heat), and chemical (unpublished observations). This resistance is in contrast to the relative fragility of lamina (25) and especially to the fragility of single cells (9). Conversion of one form into another is induced by environmental changes, among which changes in catabolic substrate and divalent cations are the best studied (3, 5, 12, 22, 23, 25, 37, 42).

It may be postulated that packets are a form of resistance phenotype which are shielded from the environment by the intercellular and peripheral material and by the overall three-dimensional structure, with a central zone in which the cells are covered by layers of more-peripheral cells. In contrast, single cells are more directly exposed to the environment and thus are more dependent on a prompt protective reaction mounted by heat shock or stress genes, whose role in the cell's response to stressors and survival is well documented (8, 29, 36, 44). In this work, we have compared the expression of *grpE* with that of the adjacent heat shock genes *dnaK* and *dnaJ* (19, 20) in response to heat shocks of various durations in single cells and packets.

MATERIALS AND METHODS

Cultures and media. *M. mazei* S-6 was grown in 100-ml serum bottles containing 50 ml of S6-2 alpha medium at 37°C and 1-atm (1 atm = 101.29 kPa) N_2-CO_2(80:20) (3, 5, 12, 22, 23). For packets, the medium also contained 250 mM methanol, while 4.9 mM Mg^{2+}, 27 mM Ca^{2+}, and 40 mM trimethylamine were added for single cells (3, 25).

S6-2 alpha medium was composed of 0.4 g of KH_2PO_4 per liter, 0.1 g of $MgCl_2 \cdot 6H_2O$ per liter, 0.04 g of $CaCl_2 \cdot 2H_2O$ per liter, 1.0 g of NH_4Cl per liter, 0.25 g of L-cysteine HCl per liter, 0.25 g of $Na_2S \cdot 9H_2O$ per liter, 2.0 g of yeast extract per liter, 2.0 g of Trypticase per liter, 3.8 g of $NaHCO_3$ per liter, 10 ml of trace mineral solution, and 1.0 ml of 0.1% resazurin. The pH was 7.0. The trace mineral solution contained the following (in grams per liter): nitrilotriacetic acid, 1.5; $MgCl_2 \cdot 6H_2O$, 3.0; $MnCl_2 \cdot 4H_2O$, 0.5; NaCl, 1; $FeCl_2 \cdot 4H_2O$, 0.1; $CoCl_2 \cdot 6H_2O$, 0.1; $CaCl_2$, 0.1; $CuCl_2 \cdot 2H_2O$, 0.01; $ZnCl_2$, 0.1; $AlCl_3$, 0.01; H_3BO_3, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.01; and $NiCl_2 \cdot 6H_2O$, 0.02.

Cultures for heat shock experiments were placed in a 45°C water bath and incubated with gentle mixing every 5 min.

RNA isolation. Samples (25 ml) of single-cell or packet cultures were pelleted by centrifugation (600 × *g*) at 4°C for 10 min and resuspended in 1.0 ml of denaturing solution containing 4.0 M guanidine isothiocyanate, 0.75 M sodium citrate, 10% *N*-lauryl sarcosine, and 0.1% 2-mercaptoethanol (1, 34). In addition, for packets, four sonications of 10 s each were done to obtain proper cell

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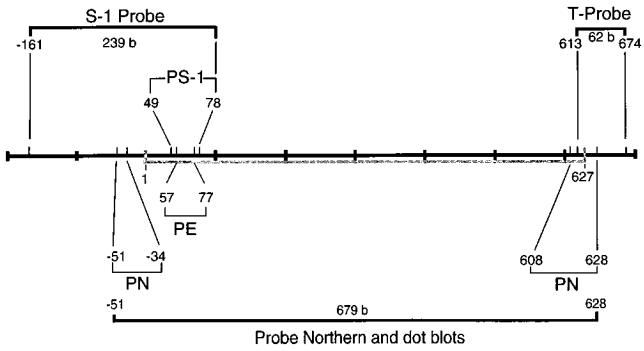

FIG. 1. Schematic representation of the *M. mazei* S-6 *grpE* gene and the probes used for this study. Shown from top to bottom are the probe used in the S1 nuclease analysis for mapping the 5′ (S-1 Probe) and the 3′ (T-Probe) ends of the transcript; the primer used to prepare the S-1 probe (PS-1); the stretch of genomic DNA (with 100-base intervals shown by short vertical lines) in which *grpE* resides, with the protein-coding region indicated (double line); the primer used in the primer extension experiments (PE); the primers used to generate by PCR the probe for the Northern and dot blotting tests (PN); and the latter probe (Probe Northern and dot blots). Negative digits denote bases upstream of the *grpE* translation start codon ATG (of which the A is base number 1).

disruption. RNA extraction was accomplished by using 1.0 ml of water-saturated phenol, 0.3 ml of 49:1 (vol/vol) chloroform-isoamyl alcohol solution, and 0.1 ml of 2 M sodium acetate (1, 34). After being mixed, the solution was kept on ice for 15 min and then centrifuged (670 × *g*) at 4°C. The RNA was precipitated from the aqueous phase by addition of an equal volume of 100% cold isopropanol and incubation at −20°C for 30 min. The RNA pellet obtained after 10 min of centrifugation (11,600 × *g*) at 4°C was dissolved in 0.3 ml of denaturing solution and precipitated with 0.3 ml of 100% cold isopropanol at −20°C for 30 min. The RNA was pelleted again by centrifugation for 15 min at 4°C and washed with 75% ethanol. The RNA was dried in a speed-vacuum apparatus (Speed Vac Concentrator; Savant Instruments Inc., Hicksville, N.Y.) and then dissolved in nuclease-free water to reach a concentration of 10 μg/μl. The quality and concentration of the RNA were determined by analysis in an ethidium bromide-stained 1.2% agarose gel and by spectrophotometric readings (ratio A_{260}/A_{280}). The RNA was stored at −70°C.

Northern (RNA) blotting. Ten micrograms of total RNA per sample was separated on a 1.2% agarose-formaldehyde denaturing gel (1, 34). The gel was stained with ethidium bromide and then photographed on a UV transilluminator (UVP, Inc., San Gabriel, Calif.). The gel was then treated with 0.05 N NaOH for 20 min, washed in H_2O, and transferred overnight with 20× standard saline citrate (SSC; 1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) to an N-Hybond nylon membrane (Amersham Life Sciences, Arlington Heights, Ill.) with an upward capillary system. The nylon membrane was dried for 1 h, cross-linked in a UV fluorescence analysis cabinet (Spectroline CX-20; Spectronics Corporation, Westbury, N.Y.) at a 254-nm wavelength for 2 min, soaked in 2× SSC for 5 min, and prehybridized for 1 h at 58°C with formamide hybridization solution containing 25 mM K_2HPO_4, 5× SSC, 5× Denhardt solution (25 mg of Ficoll 400, 25 mg of polyvinylpyrrolidone, and 25 mg of bovine serum albumin in 50 ml), 50 mg of salmon sperm per ml (sonicated 5 min), a 50% volume of formamide, and a 16.5% volume of diethylpyrocarbonate-treated water. Hybridization was performed with a PCR-generated probe prepared by using two synthetic oligonucleotides matching the *M. mazei grpE* sequence, one 18-mer (from bases −51 to −34) and one 21-mer (from bases 608 to 628), which yielded a 679-base-long probe (Fig. 1). The probe was ^{32}P labelled (10^6 cpm/ml) by random priming with a commercial kit (Promega Co., Madison, Wis.). After being incubated overnight at 58°C, the membranes were washed at 58°C in a 2× SSC–0.1% sodium dodecyl sulfate (SDS) solution for 15 min and twice for 15 min each in a 0.2× SSC–0.1% SDS solution. The membranes were wrapped in Saran Wrap and autoradiographed for 24 to 48 h at −70°C in a light-proof cassette equipped with an intensifying screen (autoradiography cassette; Rossi Walling, Albany, N.Y.).

Primer extension experiments. The 5′ end of the *grpE* transcript was mapped by using a primer extension kit (Promega) (1, 34). A 21-mer oligonucleotide that was complementary to nucleotides 57 through 77 of the *grpE* coding sequence (Fig. 1) was 5′ end labelled with [γ-^{32}P]ATP. A concentration of 0.1 pmol of this probe was hybridized to 10 μg of total RNA from *M. mazei* single cells or packets grown at 37°C or heat shocked at 45°C for various times. Annealing was carried out at 50°C for 20 min. The oligonucleotide was extended at 42°C for 90 min with reverse transcriptase. The primer-extended products were analyzed on a 6% polyacrylamide–7 M urea sequencing gel. The same unlabelled oligonucleotide served as a primer for a sequencing ladder that was run simultaneously. Sequencing was carried out by the dideoxynucleotide-chain termination method with α-^{35}S-dATP (1, 34).

Mapping of the transcription initiation site by S1 nuclease analysis. Mapping the 5′ end of the *grpE* transcript was carried out by S1 nuclease analysis (1, 34). Twenty picomoles of a 30-mer synthetic oligonucleotide encompassing bases 49 to 78 downstream of the ATG start codon (Fig. 1) was 5′ end labelled with T4 polynucleotide kinase (New England Biolabs, Beverly, Mass.). This DNA was then annealed at 40°C for 15 min to the original 11-kb double-stranded-DNA clone and extended with DNA polymerase (Klenow fragment; New England Biolabs) for 30 min at 37°C. This product was then digested with the enzyme *Sac*I (New England Biolabs), which cut 161 bases upstream of the *grpE* ATG start codon, yielding a fragment of 239 bases (Fig. 1). The probe was precipitated and rehydrated under alkaline conditions and run in a low-melting-point 1.0% agarose denaturing gel containing 50 mM NaOH and 0.1 mM EDTA (pH 8.0) for 2.5 h at 30 V. The band containing the probe was excised from the gel and purified by standard phenol extraction. Radiolabelled, single-stranded probe was hybridized to 25 μg of total RNA in 80% formamide–40 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) (pH 6.4)–0.4 M NaCl–1 mM EDTA (pH 8.0) at 30°C for 15 to 18 h. The remaining single-stranded fragments were digested with S1 nuclease (Promega) for 60 min at 30°C. After ethanol precipitation, samples were resuspended in 3.0 μl of 1× TE buffer (10 mM Tris-Cl, 1 mM EDTA; pH 7.4)–3.0 μl of loading dye containing 98% formamide, 10 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue. The entire 6.0-μl sample was loaded and electrophoresed in a 6% polyacrylamide–7 M urea sequencing gel for 1 h at 80 W. The gel was exposed to X-Omat AR film (Eastman Kodak Company, Rochester, N.Y.) for 16 h and developed.

Mapping of the 3′ end of the *grpE* transcript. The termination site was mapped by S1 nuclease analysis (1). A synthetic 62-mer oligonucleotide complementary to the 3′ end of the mRNA template was used (Fig. 1; T-probe). The oligonucleotide was purified and 3′ end labelled by using [α-^{32}P]dATP (Amersham) and terminal deoxynucleotide transferase (Promega). The probe was lengthened with one radioactive adenine to a final length of 63 bases after S1 nuclease digestion. Hybridization was done with 0.3 ng of labelled probe at 37°C for 18 h, using 25 ng of total RNA from cells grown at 37°C and from cells heat shocked at 45°C for 60 min. The preparation was treated with 300 U of S1 nuclease (Promega) at 37°C for 15 min. The protected fragments were resolved by electrophoresis on a 6% acrylamide–7 M urea gel.

Dot blotting and phosphor image analysis. Dot blotting of the RNA was performed according to standard procedures (1, 34). Briefly, 10 μg of total RNA per sample was denatured for 15 min at 65°C in formamide hybridization solution, diluted to a final concentration of 10× SSC solution, and then dot blotted by filtration through an N-Hybond nylon membrane (Amersham) using a manifold apparatus with a filtration template for dot blots (Minifold I; Schleicher & Schuell, Keene, N.H.). The nylon membrane was dried at 23°C and cross-linked via irradiation at a 254-nm wavelength for 2 min in a UV fluorescence analysis cabinet (Spectroline CX-20). Hybridization was done by adding random-primed ^{32}P-labelled PCR-generated probes (1.10×10^6 cpm/ml). For *grpE*, the probe prepared for the Northern blotting experiments was used (Fig. 1). For *dnaK* transcripts, a 1,448-bp PCR-generated probe was prepared by using two 18-mers, one from bases 12 to 29 and the other from bases 1443 to 1460 on the *dnaK* gene. For *dnaJ* transcripts, an 882-bp PCR-generated probe was prepared by using two 18-mers, bases 25 through 42 and 890 through 907 on the *dnaJ* gene. The hybridization procedure was done in the same manner as for Northern blotting. The hybridization was done successively with the radiolabelled *grpE*, *dnaK*, and *dnaJ* probes with intervening strippings. Stripping after each hybridization was done according to the manufacturer's instructions (Amersham). Briefly, the membranes were boiled in 0.1% SDS for 15 min and autoradiographed overnight to ensure that the probe had been completely removed. Computation of the intensity of the dot blots was done with a PhosphorImager apparatus (model 425; Molecular Dynamics, Sunnyvale, Calif.) (13).

Codon usage and amino acid sequence analyses. Comparative analyses of codon usage and amino acid sequences were done as previously described (6).

Nucleotide sequence accession number. The nucleotide sequences of the *grpE* gene and its flanking regions have been submitted to EMBL-GenBank under accession no. X74353.

RESULTS

***grpE* transcript in the two *M. mazei* S-6 morphologic stages, single cells and packets.** Nine independent experiments with RNA from single cells and a probe for *grpE* (Fig. 1) showed a positive hybridization band corresponding to a monocistronic transcript. The range of values was between 650 bases (once) and 750 bases (twice), with the most frequent result being 700 bases (four experiments, the results of one of which are shown in Fig. 2, top panel). Two independent experiments with packets RNA gave values of 800 and 700 bases (Fig. 2, bottom panel). This hybridization band was barely detectable in RNA from single cells and packets grown at the optimal growth

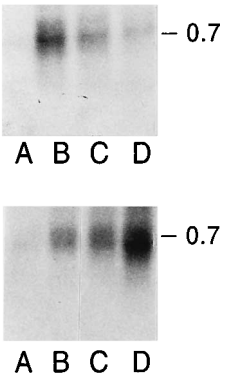

FIG. 2. Northern blotting analysis. Total RNA (10 μg per lane) from single cells (top panel) or packets (bottom panel) grown at 37°C (lane A) or heat shocked at 45°C for 15, 30, and 60 min (lanes B through D, respectively) was electrophoresed in a 1.2% agarose-formaldehyde gel. Hybridization was done with a radiolabelled PCR-generated probe of 679 bases (Fig. 1). The approximate transcript size (in kilobases) is indicated on the right.

temperature of 37°C and increased after heat shock at 45°C. The greatest increase in single cells was caused by a 15-min heat shock (compared with 30 and 60 min) (Fig. 2, top panel). In contrast, the transcript level in packets peaked after a 60-min heat shock (Fig. 2, bottom panel).

Transcription initiation site. To map the initiation site of the transcript identified by Northern blotting, primer extension experiments using a primer complementary to nucleotides 57 through 77 in the *grpE* coding region were performed (Fig. 1). The same site, a C located 44 bases upstream of the translation start codon, was found for single cells and packets (Fig. 3). The results also showed that there was a low level of transcription in non-heat-shocked single cells and that the level increased after heat shock. This increase was higher after a heat shock of 30 min than after a heat shock of 60 min. In packets, the transcript was clearly present only after a heat shock of 60 min. These temporal patterns paralleled those observed by Northern blotting.

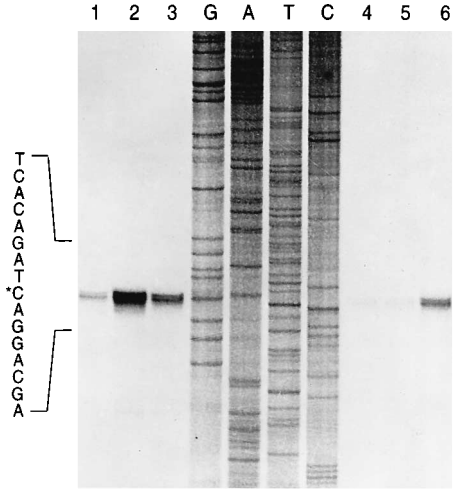

FIG. 3. Mapping of the transcription initiation site by primer extension. A radiolabelled oligonucleotide primer complementary to bases 57 through 77 within the *grpE* coding region (Fig. 1) was used with 10 μg of total RNA from single cells (lanes 1 to 3) or packets (lanes 4 to 6) per test. Single cells and packets had been grown at 37°C (lanes 1 and 4) or heat shocked at 45°C for 30 (lanes 2 and 5) or 60 (lanes 3 and 6) min. The primer-extended products were electrophoresed in a 6% acrylamide sequencing gel in parallel with the products of a sequencing reaction that was done with the same primer and the dideoxy-chain termination method (lanes G, A, T, and C). These lanes show the complementary (anti-sense) strand sequence. The coding (sense) strand sequence and the initiation site (asterisk) are shown on the left.

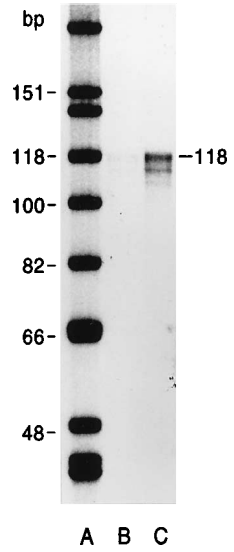

FIG. 4. Mapping of the transcription initiation site by S1 nuclease analysis. Lanes: A, ladder, with marker sizes shown on the left; B and C, protected products obtained with the probe shown in Fig. 1 and total RNA from single cells grown at 37°C or from cells heat shocked at 45°C for 30 min, respectively. The length of the major protected product is 118 bases, starting on the *grpE* coding region (78 bp downstream of the translation start codon's first base), which maps the initiation site at the A located 40 bp upstream of the translation start codon (Fig. 5). The other, minor band corresponds to a protected product that would map the site 4 bases downstream.

To corroborate the results of the primer extension experiments, S1 nuclease analysis was applied, using the probe shown in Fig. 1. By this method, the transcription initiation site was located to an A 4 bases downstream of the C identified as the initiation site by primer extension (Fig. 4 and 5).

Transcription termination site. S1 analysis showed one major and two lighter bands (Fig. 6) corresponding to termination sites located on bases 42, 36, and 21, counting from the third base (not included) of the *grpE* translation stop codon (Fig. 5).

***grpE* response to heat shock in comparison with the *dnaK* and *dnaJ* responses.** To compare the responses of the three genes to heat shocks of various durations, quantitative dot blotting was used along with hybridization measurements by

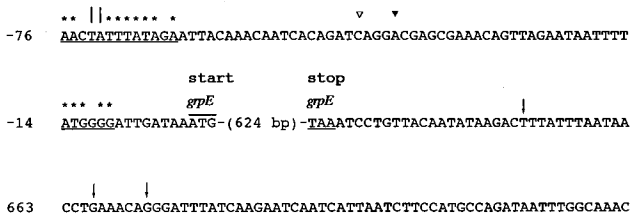

FIG. 5. Nucleotide sequence of the 5′- and 3′-flanking regions of *grpE*. The transcription initiation sites demonstrated by primer extension (base −44) and by S1 nuclease analysis (base −40) are indicated (open and closed triangle, respectively). Also shown are the putative promoter (bases −76 to −64) and ribosome-binding site (bases −14 to −9) (underlined), with bases that are identical to those in the respective consensus sequence for methanogens (asterisks) and positions within the promoter at which there is no base preference (4) (vertical lines) indicated, and the translation start (overlined) and stop (underlined) codons and the transcription termination sites determined by S1 nuclease analysis (arrows).

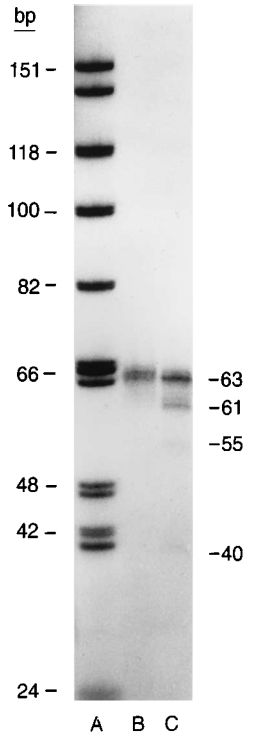

FIG. 6. S1 nuclease mapping of the 3′ end of the *grpE* transcript. Lane A, reference ladder, with the lengths of the standards indicated on the left; lane B, the probe used in this analysis; lane C, protected fragments and probe, with their lengths indicated on the right in base pairs (63 corresponds to the probe). The results shown are for total RNA from *M. mazei* S-6 single cells heat shocked at 45°C for 60 min.

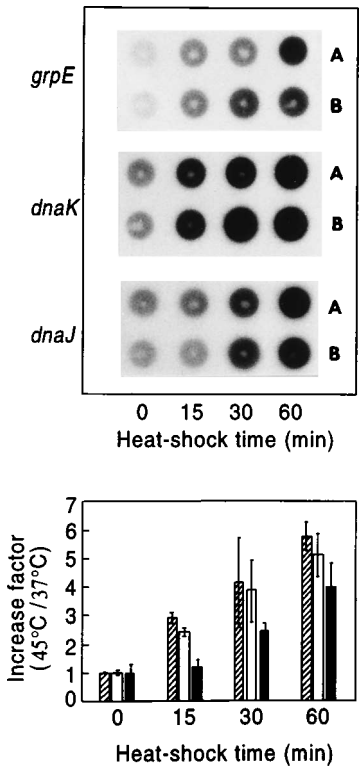

FIG. 7. Comparison of the responses of *grpE*, *dnaK*, and *dnaJ* to heat shock. Total RNA extracted from packets grown at 37°C or heat shocked at 45°C for the indicated times was dot blotted (10 μg per dot) and then hybridized with probes for *grpE*, *dnaK*, and *dnaJ*. The dot blot autoradiograms of two independent experiments, A and B, and the corresponding PhosphorImager readings are shown (top and bottom panels, respectively). Bars at 15, 30, and 60 min represent the factor of increase in the readings (arithmetic mean ± range; $n = 2$) compared with that at 0 min (no heat shock). Readings of dot blots with the *grpE*, *dnaK*, and *dnaJ* probes (hatched, open, and solid bars, respectively) are shown.

means of storage phosphor technology. RNA was extracted from non-heat-shocked packets grown at 37°C and also from packets which had been heat shocked at 45°C for 15, 30, and 60 min. The results of two independent experiments are displayed in Fig. 7. The transcript levels of the three genes augmented in parallel with the length of the heat shock. However, the increase of the *grpE* and *dnaK* transcript levels was faster than that of the *dnaJ* transcript; the increase in the *dnaJ* transcript level was clear only after a heat shock of 30 min. At this time, the factor of increase for the *dnaJ* transcript was approximately 2.5. In contrast, a similar factor of increase for the *dnaK* and *grpE* transcripts was already reached after a heat shock of 15 min.

Codon usage analysis. The codon usage pattern of the *M. mazei grpE* gene is displayed in Fig. 8, along with the patterns of the other two studied genes of the *dnaK* locus of this archaeon. The three genes show the same codon preference for 11 of 18 amino acids (61%); Trp and Met were excluded because the former is not present in these genes' products and Met has only one possible codon. The same preferences were observed when *grpE* and *dnaK* were compared, and the agreement increased to 72 and 78% for *dnaK-dnaJ* and *grpE-dnaJ*, respectively. The three genes use all codons with only five exceptions for *grpE* and *dnaJ* and six exceptions for *dnaK* (which is considerably longer than the other two genes); CUA (Leu) and UGG (Trp) are not used by any of these genes; and 8 of 16 cases (50%) of missing codons involve a codon with A in the third position, while 6 of 16 (38%) involve a codon with G in this position (the other two missing codons end in U or C).

DISCUSSION

The *grpE* transcript in *M. mazei* S-6 at the optimal growth temperature as well as after heat shock was found to be ~700 bases long, which corresponds to a monocistronic message. This was demonstrated by Northern blotting and by mapping the transcription initiation and termination sites.

Transcription, as demonstrated by primer extension, would start at a pyrimidine-pyrimidine (TC) dinucleotide. We examined 47 transcription initiation sites reported in the literature for 30 non-heat shock genes in 11 different methanogens and found a pyrimidine-pyrimidine dinucleotide 13 times (the most frequently found dinucleotide, occurring 17 times, was a pyrimidine-purine dinucleotide). A TC dinucleotide like that of the *M. mazei grpE* was found twice, the most frequent dinucleotides being TT (eight times), TG (seven times), and AT (six times). The information available for the *grpE* gene is limited to bacteria, and the dinucleotides found at the transcription initiation sites are CA (pyrimidine-purine) for *Bacillus subtilis* (40) and AA (purine-purine) for *Escherichia coli* (18).

The transcription initiation sites determined by primer extension for heat-shocked and non-heat-shocked cells are the same, which is reminiscent of what has been reported for *E. coli grpE* (18). The site determined by S1 nuclease analysis is close to that determined by primer extension, within the expected variations of the methods. The sizes of the *grpE* transcripts detected by Northern blotting and the transcription initiation sites are the same in single cells and packets. How-

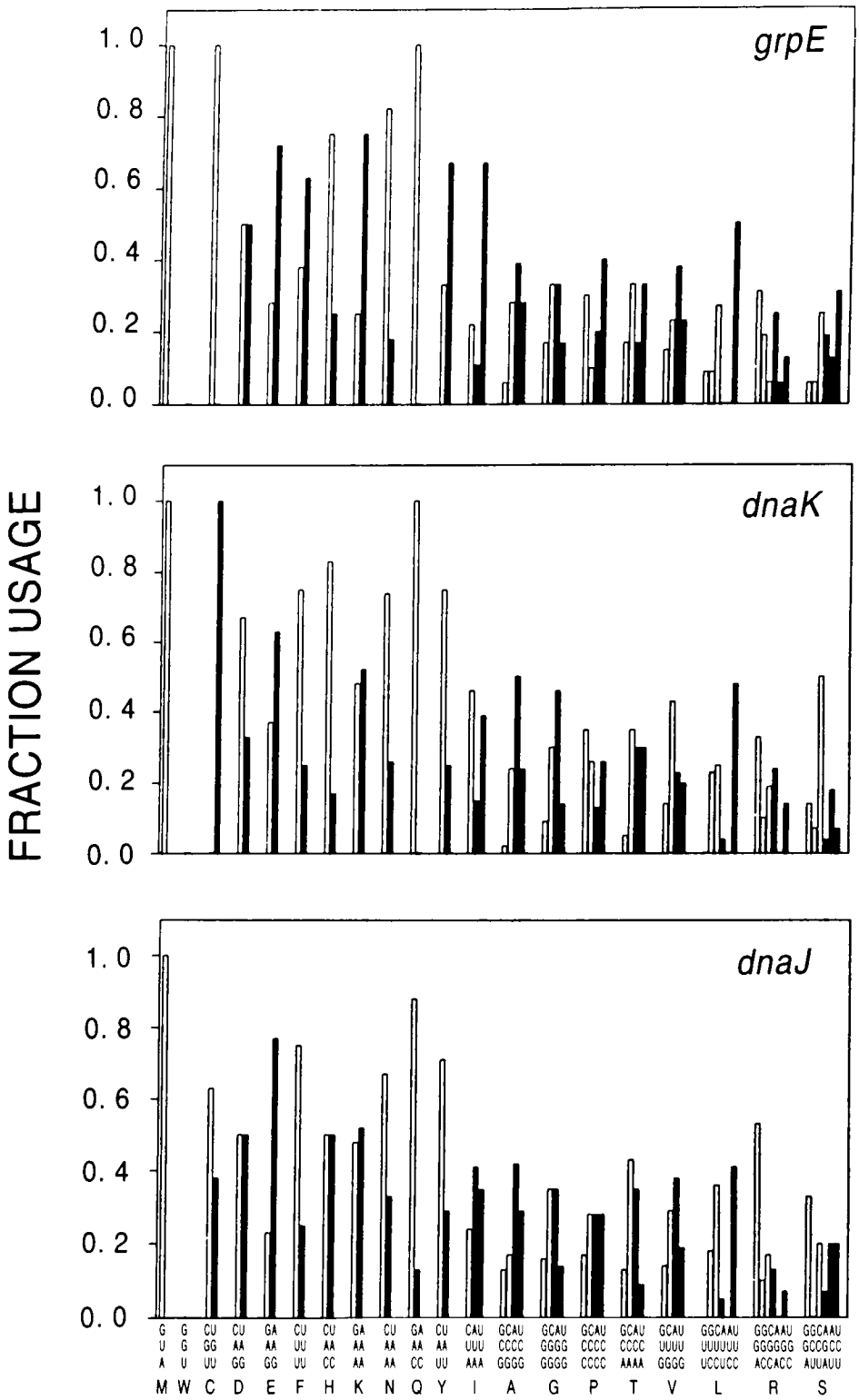

FIG. 8. Codon usage patterns for *M. mazei grpE*, *dnaK*, and *dnaJ*. The fractions of codons with G or C (open bars) and A or U (solid bars) in the third position are indicated. The codons possible for each amino acid are indicated along the horizontal axis. Single letters at the bottom indicate amino acids, which are in alphabetical order for the amino acids with two, four, or six possible codons (from left to right).

ever, in packets, a longer heat shock is necessary to induce levels of gene expression comparable to those observed in single cells. Furthermore, the *dnaK* transcript increase after heat shock in single cells was twice as great as that in packets (data not shown). The mechanism responsible for the differences between single cells and packets remains to be elucidated. Intrinsic properties, distinctive of the cells of the single-cell stage and the cells in the packets, might contribute to the differences observed between the two morphologies in the promptness and levels of expression of *grpE* and *dnaK*. Such distinctive mechanisms are suggested by the observation that *dnaJ* expression levels are similar in packets (Fig. 7) and single cells (unpublished data), while expression of *grpE* and *dnaK* is more pronounced in single cells. Also, the possibility that cells in the center of the packets are less accessible to heat ought to be explored. It can be postulated that single cells, without a protective envelope, have the *dnaK* and *grpE* genes ready to respond quickly at all times, on the alert, as it were. In contrast, packets would have these genes down-regulated to preclude rapid activation. In this way, cells in packets would avoid diversion of energy and nutrients by heat shock genes from the pools required for other functions, such as cell division and synthesis of the extracellular protective material.

The data suggest the organization depicted in Fig. 5 for the *grpE* gene of *M. mazei* S-6. A putative promoter is centered around a base that is 26 bp upstream of the transcription initiation site determined by primer extension, or 31 bp in front of the initiation site determined by S1 nuclease analysis. This promoter sequence resembles the consensus sequence generated for promoters in methanogens (4). No sequences resembling bacterial heat shock promoters (7) could be identified. A ribosome-binding sequence, also similar to the corresponding consensus obtained for methanogens (4), is located 8 bp upstream of the translation start codon. The coding region would encode a protein whose amino acid sequence has identities and conservative substitutions with regard to bacterial homologs that fall within the range observed among the latter (6) and between them and a recently described homolog from the mitochondria of *Saccharomyces cerevisiae* (2, 11, 17) (data not shown). The overall organization of the *grpE* chromosomal region in *M. mazei* is similar to those of *B. subtilis* (39, 40), *Clostridium acetobutylicum* (26), *Borrelia burgdorferi* (38), and *Staphylococcus aureus* (27). In these bacteria and in *M. mazei*, *grpE* is clustered with *dnaK* and *dnaJ*, located upstream of the former gene and preceded by another gene whose role is still incompletely understood. The archaeal *dnaK* locus, however, has distinctive characteristics: it is followed in the 3′ direction by a *trkA* homolog (21), and the distance between *grpE* and the nearest gene on either flanking region is longer than the bacterial equivalents. In the bacterial clusters, the length of the intergenic region between *grpE* and *dnaK* ranges between 21 and 68 bp, while in *M. mazei* it is 431 bp. Likewise, the separation between *M. mazei grpE* and its preceding gene (*orf147* [6a]) is 127 bp, whereas the equivalent regions in *B. subtilis*, *S. aureus*, *C. acetobutylicum*, and *Chlamydia trachomatis* (35) are 72, 31, 20, and 0 bp long, respectively.

Codon usage patterns revealed that the three archaeal heat shock genes use practically all codons with few exceptions, which is in contrast to the homolog genes from the other two phylogenetic domains, Bacteria and Eucarya. The three archaeal genes share the same codon preference for 61% of the amino acids. The agreement is even greater between *dnaK-dnaJ* (72%) and *grpE-dnaJ* (78%). These percentages are remarkable, as they contrast with those calculated for the agreements between the three *M. mazei* genes and pools of *E. coli* and *C. trachomatis* genes (35). The archaeal genes prefer the same codons as *E. coli* and *C. trachomatis* genes for 39 and 17% of the amino acids, respectively. The agreement between the two bacterial gene pools is 33%, that between the three *M. mazei* heat shock genes and the *trkA* gene is 39%, and that between the heat shock genes and an S-layer gene recently described for this archaeon (43) is 28%. The significance of the codon usage patterns and the high degree of agreement in codon preferences distinctive of the three archaeal heat shock genes in translation regulation, for example, remains to be determined.

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