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Gene structure and transcriptional regulation of *dnaK* and *dnaJ* genes from a psychrophilic bacterium, *Colwellia maris*

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Abstract The *dnaK* and *dnaJ* genes, encoding heat shock proteins, were cloned from a psychrophilic bacterium, *Colwellia maris*. Significant homology was evident comparing DnaK and DnaJ of the psychrophilile with the counterparts of mesophilic and thermophilic bacteria. In the DnaJ protein, three conserved regions of the Hsp40 family were observed. A putative promoter similar to the σ^{32} consensus sequence was found upstream of the *dnaK* gene. The G + C content in the 5′-untranslated region of the *dnaK* gene was much lower than that in the corresponding region of mesophilic bacteria. Northern-blot analysis and primer-extension analysis showed that both genes were transcribed separately as monocistronic mRNAs. Following several temperature upshifts from 10 to 26°C, maximum induction of the *dnaK* and *dnaJ* mRNAs was detected at 20°C, suggesting that this temperature induces the heat shock response in this bacterium. In addition, the level of the induction of the *dnaJ* gene was much lower than that of the *dnaK* gene. These findings together revealed several specific features of the heat shock response at a relatively low temperature in psychrophiles.

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Introduction

A variety of environmental stresses induce the synthesis of a set of highly conserved proteins called heat shock proteins (HSPs) (Parsell and Lindquist 1993; Yura and Nakahigashi 1999). These proteins play important roles in assisting the folding, assembly, and degradation of proteins, not only under stress conditions, but also under normal growth conditions (Hartl 1996). The DnaK/J system is one of the best-characterized chaperone machineries. This system functions in preventing the aggregation of proteins, assisting the folding of newly synthesized proteins, and facilitating the proteolytic degradation of unstable proteins (Motohashi et al. 1999; Hartl and Hayer-Hartl 2002; Tatsuta et al. 2000). Although various types of environmental stress promote the expression of HSP genes, high temperature is undoubtedly a major factor in the induction of these genes. The temperature range for the induction of HSP genes varies depending on the organism and is related to the optimum growth temperature of the organism.

Colwellia maris strain ABE-1 (JCM 10085) is a psychrophilic bacterium that grows at 10–15°C, and its lethal temperature is about 25°C (Yumoto et al. 1998). Relatively low temperatures, such as 20°C, cause high-temperature stress to *C. maris*, whereas these temperatures can, in general, induce tolerance to low temperature in most mesophilic organisms (Aguilar et al. 1999; Chamot et al. 1999; Sakamoto et al. 1997; Yamanaka 1999). Thus, it is interesting to study the mechanisms of the enhancement of thermal tolerance of psychrophiles at low temperature. Although many reports have described the HSPs of mesophilic and thermophilic bacteria (Segal and Ron 1996), little is known about the mechanisms of the response of psychrophilic bacteria to high-temperature stress. Recently,

we cloned the *groESL* genes from *C. maris* (Yamauchi et al. 2003). The GroES and GroEL proteins of *C. maris* were found to be evolutionarily conserved with those of mesophilic and thermophilic bacteria. Although the temperatures for the induction of the *groESL* operon of *C. maris* were much lower than those of mesophilic bacteria, a putative σ^{32} promoter was located in the upstream region of the *groES* gene, as is often the case in mesophilic bacteria. The *groESL* gene was also cloned from another psychrophilic bacterium, *Pseudoalteromonas haloplanktis* TAC 125 (Tosco et al. 2003).

In the present study, to clarify the mechanisms of gene regulation of HSPs that are expressed at relatively low temperatures, we cloned the genes for the DnaK and DnaJ homologues from *C. maris* and investigated the expression of these genes under high-temperature stress.

Materials and methods

Bacteria and culture conditions

The pychrophilic bacterium *Colwellia maris* strain ABE-1 (JCM 10085) was grown at 10°C for 3 days in nutrient medium containing 1% Bacto Peptone, 1% meat extract, and 0.5 M NaCl (Ochiai et al. 1979). *Escherichia coli* JM109 was grown at 37°C in LB medium supplemented with 50 μg ml^{-1} ampicillin when required.

DNA preparation

The genomic DNA of *C. maris* was isolated and purified according to the method described by Williams (1988). Cells from 80-ml cultures were harvested by centrifugation at 1,800 *g* at 4°C for 15 min. They were suspended in 5 ml of buffer containing 50 mM Tris-HCl pH 8.5, 50 mM NaCl, and 5 mM NaEDTA and were then treated with 7 mg ml^{-1} lysozyme at 37°C for 45 min. The cells were disrupted by incubation with 0.8% N-lauroylsarcosine at 37°C for 20 min. The lysate was extracted twice with phenol:chloroform:isoamylalcohol (25:24:1) and then DNA was precipitated with 99.5% ethanol. The DNA pellet was dissolved in TE buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA).

Amplification of the *dnaK* gene fragment by PCR

Degenerate primers were designed to encode amino acid sequences conserved among 15 bacterial DnaK homologues. The sense primer, 5′-ATHATHGGNATHGA-3′ (FW), corresponding to amino acid residues 4 (I) to 8 (D) of *E. coli* DnaK, and the anti-sense primer, 5′-GCNGGNACNGTDAT-3′ (RV), corresponding to amino acid residues 148 (I) to 155 (N) of *E. coli* DnaK, were used for PCR. Amplification was carried out in a 50-μl reaction mixture containing 1×PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, each FW and RV primer at 250 μM, and 1.25 U *Taq* DNA polymerase (Gibco BRL, Gaithersburg, Md., USA) in a DNA thermal cycler 480 (Applied Biosystems, Foster City, Calif., USA) for 28 cycles. Cycling conditions were as follows: initial three cycles of denaturation at 95°C for 1 min, annealing at 40°C for 1 min, and extension at 72°C for 1 min, followed by 25 cycles of incubation at 95°C for 1 min, 45°C for 1 min, and 72°C for 1 min. The amplified DNA fragment was cloned in a T-vector (Novagen, Darmstadt, Germany) and sequenced using an automatic DNA sequence analyzer (ABI 310, Applied Biosystems).

Cloning of the full-length *dnaK* and *dnaJ* genes

For Southern-blot analysis, the genomic DNA was digested with restriction enzymes (New England Biolabs, Beverly, Mass., USA) and separated by electrophoresis in a 1% agarose gel and then transferred to a nylon membrane (Hybond-N^+; Amersham, Buckinghamshire, UK). The DIG-labeled probes were hybridized with the digested fragments at 48°C overnight. The following three DNA fragments were used as probes: the 422-bp PCR-amplified fragment corresponding to the first half of *dnaK* [Fig. 1, probe (pb) 1], the 253-bp *Hin*cII-*Hin*dIII fragment for the latter half of *dnaK* (Fig. 1, pb 2), and the 357-bp *Eco*RI-*Hin*cII fragment corresponding to the *dnaJ* (Fig. 1, pb 3). The membranes were washed, and the hybridized signals were detected with a DIG-detection kit (Boehringer Mannheim, Mannheim, Germany) as described in the supplier's instructions. The DNA fragments whose sizes were similar to those of the hybridized fragments were ligated to the plasmid vector pBluescript (pBS) II KS (+) (Stratagene, La Jolla, Calif., USA), and the resultant constructs were introduced into *E. coli* JM109. The plasmids carrying the *dnaK* or *dnaJ* genes were identified by hybridization with the DNA probes and sequenced as described above.

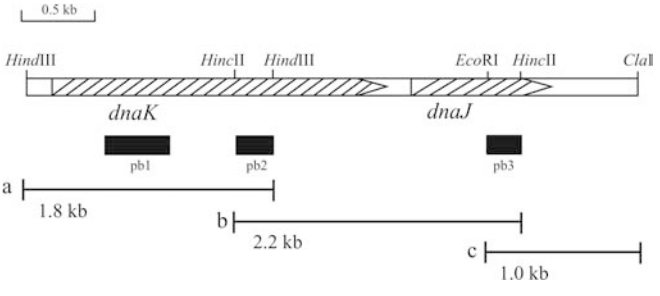

Fig. 1 Restriction map of the genomic region containing the *dnaK* and *dnaJ* genes in *Colwellia maris*. The DNA fragments used for Southern- and Northern-blot analyses are shown as *black boxes*: probe 1 (*pb 1*) Southern- and Northern-blot analyses, *pb 2* Southern blot analysis, *pb 3* Southern- and Northern-blot analyses. Three DNA fragments used for DNA sequencing are indicated by *bars a, b, and c*. The locations of the *dnaK* and *dnaJ* genes are shown as *hatched boxes*

RNA preparation and Northern-blot analysis

Total RNA was purified from cells that had been incubated at the indicated temperatures for 60 min or at 20°C for the indicated time. After incubation, 10 ml culture was mixed with an equal volume of ethanol containing 10% (w/v) phenol. Cells were collected by centrifugation and resuspended in 600 µl TE-SDS buffer (50 mM Tris-HCl pH 8.0, 5 mM EDTA pH 8.0, and 0.5% SDS). After addition of acid phenol, the suspension of cells was incubated at 65°C for 10 min and extracted with phenol:chloroform:isoamylalcohol (25:24:1) three times. DNA was removed by digestion with DNaseI (Nippon Gene, Tokyo, Japan), and total RNA was precipitated with 99.5% ethanol. To remove impurities, one-fourth of the sample volume of 10 N LiCl was added to each 1 µg μl^{-1} RNA sample, and the mixture was incubated at 4°C overnight. RNA pellets were dissolved in H_2O and stored at −80°C.

Total RNA was separated by electrophoresis in a 1% agarose gel containing 0.6 M formaldehyde and then transferred onto a nylon membrane. DIG-labeled DNA probes including a part of the *dnaK* (Fig. 1, pb 1) or *dnaJ* (Fig. 1, pb 3) gene were used for hybridization. The membranes were washed, and the hybridized signals were detected with a DIG-detection kit (Boeringer Mannheim), and signals from hybridized mRNA were determined with a chemiluminescence analyzer (Las-1000UVmini; Fuji Film, Tokyo, Japan)

Primer extension

Two 5′-DIG-labeled oligonucleotides, 5′-CAGCAACA-CATGAGTTAGTTG-3′ and 5′-TTCATAATAATCA-CGTTTTGACAT-3′, which were complementary to nucleotides +32 to +52 of the *dnaK* initiation codon and +1 to +24 of the *dnaJ* initiation codon, were used for the primer extension analysis. Total RNA was isolated from *C. maris* cells grown at 10 or 20°C for 60 min as described above. Twenty micrograms total RNA for *dnaK* or 40 µg for *dnaJ* was mixed with 4 µl of 5× first-strand buffer, 1 µl of 0.1 M DTT, 2 pmol of oligonucleotide, 10 mM dNTP, and 200 U of Superscript III RNaseH$^-$ reverse transcriptase (Invitrogen, Carlsbad, Calif., USA) in a final volume of 20 µl. The extension reaction was carried out at 55°C for 1 h, followed by incubation at 70°C for 15 min to inactivate the reverse transcriptase. The reaction mixtures were denatured at 95°C for 3 min and loaded onto a 6% sequencing gel. The length of the extension products was determined by comparing the mobility of the products with that of the product of a sequencing reaction generated with the same primers.

Nucleotide sequence accession number

The nucleotide sequence reported here has been deposited in the DDBJ, GenBank, and EMBL databases (accession no. AB084455).

Results and discussion

Nucleotide and amino acid sequences of *dnaK* and *dnaJ* genes

Figure 1 shows the DNA fragments obtained during the cloning of *Colwellia maris dnaK* and *dnaJ* genes. Using the three DNA probes specific to the first half of *dnaK*, to the latter half of *dnaK*, and to *dnaJ*, we identified three DNA fragments containing the *dnaK* and/or *dnaJ* genes (Fig. 1).

The *dnaK* gene of *C. maris* consists of 1,917 bp and encodes a polypeptide consisting of 638 amino acid residues with a deduced molecular mass of 68,734 Da. The *dnaJ* gene consists of 1,140 bp and encodes a polypeptide consisting of 379 amino acid residues with a deduced molecular mass of 40,994 Da. Putative ribosome-binding sites (AGGAG and GAAGT) were found 13 and 15 bp upstream of the initiation codons of the *dnaK* and *dnaJ* genes, respectively. Two inverted repeats were found 11 and 91 bp downstream of the termination codon of the *dnaK* gene. Inverted repeats were also found 7 and 64 bp downstream of the termination codon of the *dnaJ* gene. These inverted repeats might act

Table 1 Comparison of the amino acid sequences of DnaK and DnaJ from *Colwellia maris* with those of homologues in other bacterial species

Organism	DnaK (%)	DnaJ (%)	Accession no.[a]
Psychrophilic bacterium			
C. psychroerythraea[b]	92.8	97.4	
Mesophilic bacteria			
Escherichia coli	79.6	69.0	NP_414555, P08622
Salmonella typhimurium	79.8	69.8	Q56073, Q60004
Vibrio cholerae	80.3	73.0	O34241, NP_230503
Bacillus subtilis	54.3	50.7	S09500, B41874
Thermophilic bacterium			
B. stearothermophilus	55.1	42.4	Q45551, JC4739

[a]Sequences were obtained through the GenBank database using the accession numbers listed
[b]Sequences were obtained through the TIGR microbial database

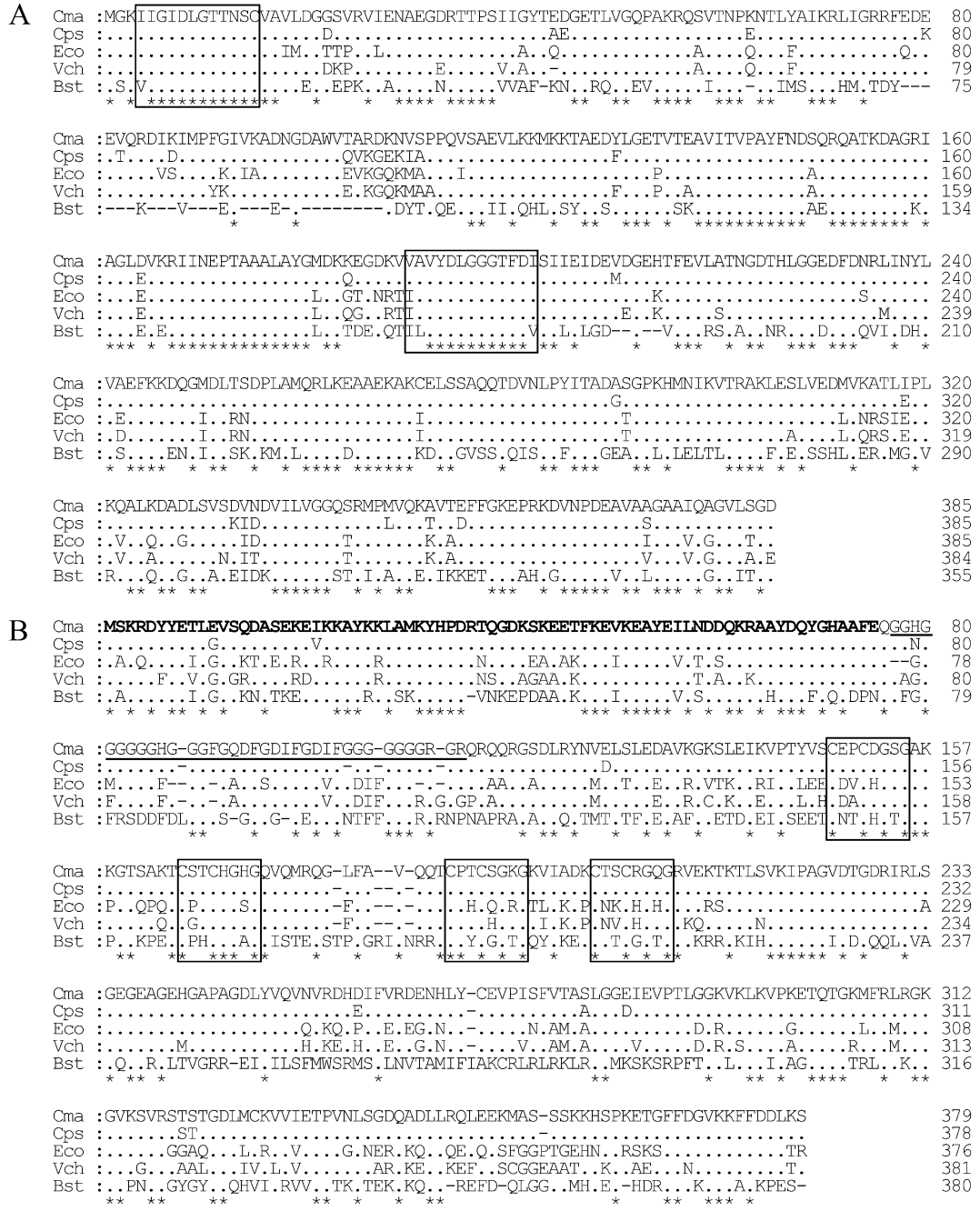

Fig. 2a, b Comparison of the ATPase domain (1–385) of DnaK (**a**) and DnaJ proteins (**b**) from *C. maris* with those from other bacterial species. The deduced amino acid sequences from *C. maris* (*Cma*) are aligned with those from *C. psychroerythraea* (*Cps*), *Escherichia coli* (*Eco*), *Vibrio cholerae* (*Vch*), and *Bacillus stearothermophilus* (*Bst*). Amino acid residues identical to those of proteins of *C. maris* are indicated by *dots*. *Asterisks* show amino acid residues identical in all four bacteria. **a** Two possible ATP-binding domains are *boxed*. **b** Three conserved domains of the Hsp40 family are indicated as follows: the amino acid region of the J domain is in *boldface*, the G/F-rich regions are *underlined*, and the C-rich regions are *boxed*

as the terminators of the *dnaK* and *dnaJ* genes. The *dnaK* gene was located 244 bp upstream of the *dnaJ* gene. This order of *dnaK-dnaJ* is found in several proteobacteria, for example in *Escherichia coli*. In several Gram-positive bacteria, the *grpE* gene forms an operon together with downstream genes such as *dnaK* and *dnaJ* (Iizumi and Nakamura 1997; Wetzstein et al. 1992). Although we sequenced over 367 nucleotides of the region upstream of the translational start codon of the *dnaK* gene, no open reading frame homologous to the *grpE* gene was found. It appears that the *dnaK* gene does not form a *grpE-dnaK-dnaJ* operon in *C. maris*.

The DnaK protein of *C. maris* exhibited a high degree of overall similarity with the corresponding proteins of other bacteria (Table 1). In particular, with the DnaK homologue of the psychrophilic bacterium *C. psychroerythraea*, about 90% homology was

observed. The N-terminal ATPase domain, including two ATP-binding domains, is highly conserved in DnaK of *C. maris* (Fig. 2a).

The DnaJ protein also exhibited a high degree of overall similarity with the corresponding proteins of other bacteria, in particular, about 90% homology with that of the psychrophilic bacterium *C. psychroerythraea* (Table 1). The DnaJ protein of *C. maris* contained all of the three conserved domains of the Hsp40 family, namely, the N-terminal J domain (amino acids 2–75), the G/F-rich region (amino acids 76–112), and the C-rich domain (four repeats of the sequence CXXCXGXG) (Fig. 2b). The J domain, especially H33-P34-D35, plays an essential role in the interaction with its counterpart, DnaK/Hsp70 (Pellecchia et al. 1996). The G/F-rich region is involved in an important role in modulating substrate-binding activity of DnaK (Wall et al. 1995). The C-rich region binds two zinc atoms and appears to participate in binding unfolded polypeptide (Szabo et al. 1996). Thus, DnaK might interact with DnaJ and serve as a molecular chaperone in *C. maris*, as does DnaK in other bacterial species. The high degree of identity in the amino acid sequences of the DnaK and DnaJ proteins between *C. maris* and mesophilic and thermophilic bacteria suggests that DnaK and DnaJ might be evolutionarily conserved among thermophiles, mesophiles, and psychrophiles.

Expression of the *dnaK* and *dnaJ* genes

We analyzed the mRNA level to examine the expression of the *dnaK* and *dnaJ* genes of *C. maris* in response to high-temperature stress (Fig. 3). Cells that had been grown at 10°C were incubated at various temperatures for 60 min. A predominant 2.1-kb transcript and a weak 1.4-kb transcript of the *dnaK* gene were detected (Fig. 3a). The 2.1-kb transcript should correspond to a full-length transcript of the *dnaK* gene, and the 1.4-kb transcript might be a degradation product of the 2.1-kb transcript or a premature termination product. Although a certain level of *dnaK* transcripts was found in cells grown at 10°C, maximum levels of the transcripts were observed when the cells were incubated at 18–20°C (Fig. 3a). The level of the *dnaK* transcripts reached a maximum (about fivefold) increase after 60 min at 20°C (Fig. 3b and Table 2).

A *dnaJ*-specific probe was hybridized to a 1.3-kb transcript (Fig. 3a). Judging from the size, this transcript could be reasonably assigned as the full-length *dnaJ* transcript. No detectable bands were found around 3.4 kb, a size that would correspond to that of the bicistronic transcript of the *dnaK* and *dnaJ* genes. Thus, the size of the transcriptional products did not accord with those of the *dnaK* and *dnaJ* operon found in the most mesophilic and thermophilic bacteria (Segal and Ron 1996). The level of the *dnaJ* transcript increased at 18–20°C and reached a maximum, with about a 1.5-fold increase, after 30–60 min at 20°C. Thus, the level of induction of *dnaJ* at 20°C was much lower than that of *dnaK* (Fig. 3b; Table 2). The ratio of the level of the *dnaK* transcript to that of the *dnaJ* transcript was about 8 at 10°C, while the ratio was about 30 in cells incubated at 20°C for 60 min (Table 2). Thus the degree of regulation differs markedly between *dnaK* and *dnaJ*. The results of transcript size and expression level analyses indicate that the *dnaK* and *dnaJ* genes are regulated separately as monocistronic messengers in *C. maris*.

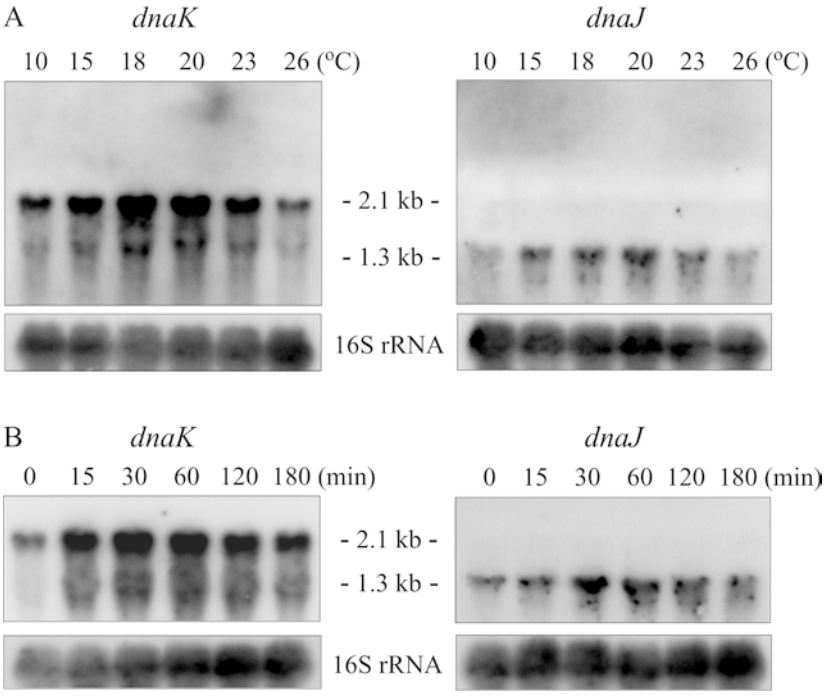

Fig. 3a, b Northern-blot analysis of the *dnaK* and *dnaJ* genes. **a** Total RNA was extracted from cells that had been exposed to various temperatures for 60 min. **b** Total RNA was extracted from cells that had been exposed to 20°C for various periods of time. The amounts of RNA applied per lane were 10 μg for *dnaK* and 20 μg for *dnaJ*. Membranes were hybridized with DIG-labeled probes including a part of the *dnaK* (Fig. 1, pb 1) or *dnaJ* (Fig. 1, pb 3) gene. The transcript sizes are marked *between the panels*. Hybridization with 16S rRNA was used as a loading control (*lower panels*)

Table 2 Quantitation of the mRNAs of *dnaK* and *dnaJ* in *C. maris*

Gene	Relative amount of mRNA[a]	
	10°C	20°C (60 min)
dnaK	4.7 ± 0.7	24.7 ± 3.0
dnaJ	0.6 ± 0.1	0.9 ± 0.2

[a]The values are the ratio of the level of the *dnaK* or *dnaJ* mRNA to that for 16S rRNA background

Identification of the promoter regions of the *dnaK* and *dnaJ* genes

To identify the promoter regions of the *dnaK* and *dnaJ* genes, we determined the initiation sites for their transcription by primer extension (Fig. 4). Two transcriptional initiation sites for the *dnaK* gene were found. They were located 83 and 92 nucleotides upstream of the translational initiation site of *dnaK*. The levels of both extension products were increased after a temperature upshift from 10 to 20°C. The sequences of the −35 and −10 regions of the putative promoter for *dnaK* were TTTATCTATTGAA and CCCCATTTC, respectively, which are similar to the consensus sequence of the σ^{32}-dependent promoter in *E. coli* (Table 3; Hawley and McClure 1983). A similar promoter sequence is also found in the upstream region of the *groES* gene in *C. maris* (Yamauchi et al. 2003). These results indicate that the *dnaK* gene might be mainly regulated by the σ^{32} system in *C. maris*, like *dnaK* genes in mesophilic bacteria. In *E. coli* and *Vibrio cholerae*, the expression of the *groESL* and *dnaK* genes is regulated by σ^{32}, whose synthesis, activity, and stability are, in turn, regulated by high temperature (Yura and Nakahigashi 1999). The mechanism of the regulation of these heat shock genes by σ^{32} in *C. maris* remains to be elucidated.

In contrast, one transcriptional initiation site was found for the *dnaJ* gene, being located 40 nucleotides upstream of the translational initiation site of *dnaJ*. The sequences of the −35 and −10 regions of the putative promoter for the *dnaJ* gene were similar to the consensus sequences of the σ^{70}-dependent promoter in *E. coli*, which is a housekeeping promoter (Table 3). In addition, no clear σ^{32} consensus promoter sequence was found in the upstream region of the transcriptional initiation site of the *dnaJ* gene. Thus, the regulatory mechanism of the *dnaJ* gene differs from that of the *dnaK* gene in *C. maris*, and the difference of induction between the *dnaK* and *dnaJ* genes might be due to the different types of promoter.

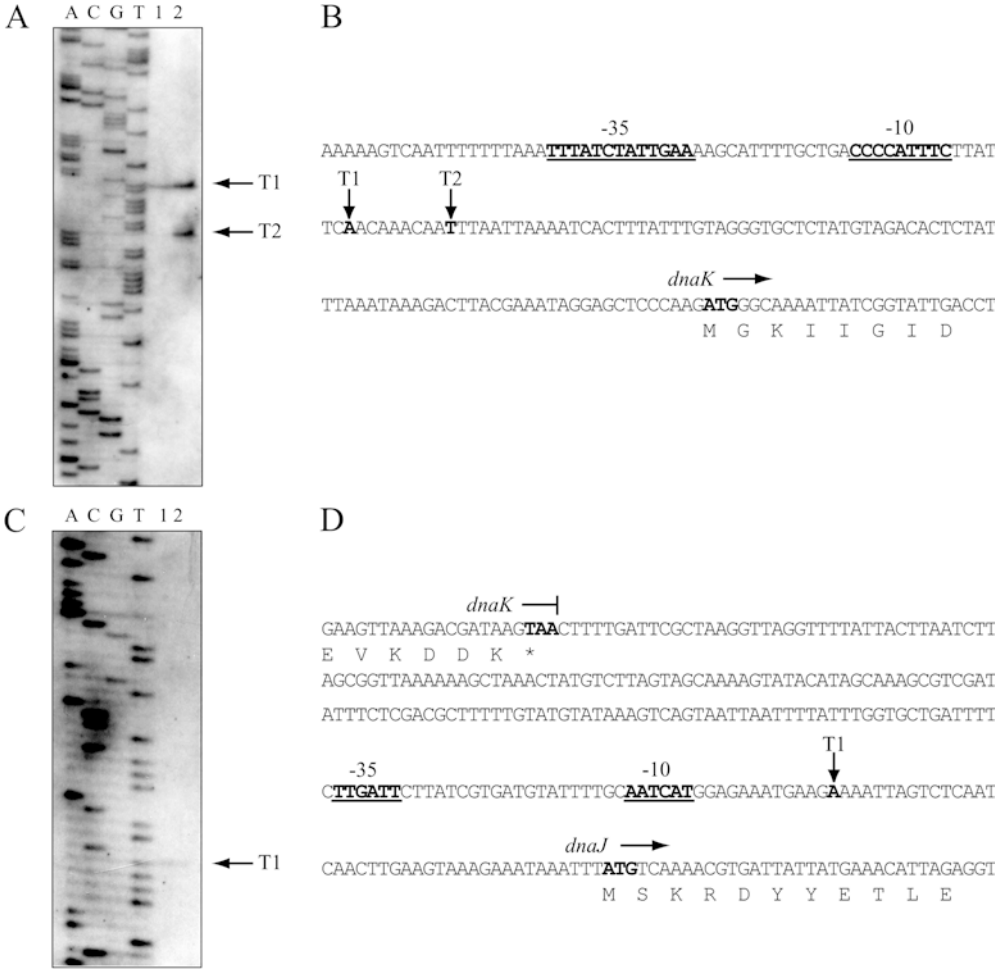

Fig. 4a–d Determination of the transcriptional initiation sites of the *dnaK* and *dnaJ* genes by primer extension. Total RNAs from *C. maris* incubated at 10°C (*lane 1*) and 20°C (*lane 2*) for 60 min were subjected to primer extension analysis using oligonucleotides specific to *dnaK* (**a**) and the *dnaJ* (**c**). The extension products were marked T1 or T2. Nucleotide sequence of the promoter regions of *C. maris dnaK* (**b**) and *dnaJ* (**d**). The *vertical arrow* indicates the transcriptional start site. Potential −10 and −35 sequences of the promoters are *underlined*. The translational initiation codons of *dnaK* and *dnaJ* are indicated in *boldface*

Table 3 Comparison of the putative promoters of *dnaK* and *dnaJ* genes from *C. maris* and *E. coli*

Genes	−35 Region	Spacing (nucleotides)	−10 Region
C. maris dnaK	TTTATCTATTGAA	---14---	CCCCATTTC
C. maris groESL	TAGCACTTGTGAA	---12---	CCCCACTTC
E. coli dnaK	TCTCCCCCTTGAT	---14---	CCCCATTTA
E. coli groESL	TTTCCCCCTTGAA	---13---	CCCCATTTC
σ^{32} Consensus	TNNCNCCCTTGAA	--13–15--	CCCCATNTA
C. maris dnaJ	TTGATT	---20---	AATCAT
σ^{70} Consensus (*E. coli*)	TTGACA	--16–19--	TATAAT

Table 4 The G + C content of *dnaK* and *dnaJ* genes and the 5′-untranslated region of the *dnaK* gene from *E. coli*, *S. typhimurium*, and *C. maris*

	dnaK (%)	*dnaJ* (%)	5′-Untranslated region of *dnaK* (%)
C. maris	40.9	41.6	29.6
E. coli	51.1	55.2	43.8
S. typhimurium	52.6	54.0	43.3

G + C content of *dnaK* and *dnaJ* genes

The G + C contents of the coding regions of *dnaK* and *dnaJ* of *C. maris* were 40.9 and 41.6 mol%, respectively. These values were about 10 mol% lower than those of mesophilic bacteria such as *E. coli* (51.1 and 55.2 mol%) and *Salmonella typhimurium* (52.6 and 54.0 mol%) (Table 4). Similarly, the G + C content of the coding regions of *dnaK* and *dnaJ* in another psychrophilic bacterium, *C. psychroerythraea*, was about 10 mol% lower than those of mesophilic bacteria (39.5 and 42.2 mol%). The low G + C content was especially marked in the promoter region. The G + C content in the 5′-untranslated region of the *dnaK* gene was 29.6 mol%, whereas that of the corresponding region in *E. coli* was 43.8 mol% (Table 4). In addition, the G + C content in the upstream region of the *dnaJ* gene was low (25.0 mol%). Low G + C content in the promoter region is also found in the *dnaK* and *dnaJ* genes of *C. psychroerythraea* and in the *groESL* genes of *C. maris* and *P. haloplanktis* TAC 125 (Tosco et al. 2003; Yamauchi et al. 2003). Recently, several genes have been identified in *C. maris*, for example, the isocitrate lyase (ICL) gene and isocitrate dehydrogenase isozyme (IDH) gene (Watanabe et al. 2002; Ishii et al. 1993). The G + C contents of the untranslated regions of the *icl* and *idh* genes of *C. maris* were found to be almost the same as those of their coding regions (data not shown). Thus, the particularly low G + C content in the untranslated region may be specific to HSP genes in *C. maris*. The low G + C content results in a decrease in the melting point of the region (Wada and Suyama 1986). Since a low melting point in the promoter region facilitates the unfolding of the DNA double strands, it might allow the expression of these heat shock genes to be induced at relatively low temperatures. It will be of interest to examine the correlation between low G + C content and expression of heat shock genes at low temperature by further comparison of nucleotide sequence as well as gene manipulation.

In conclusion, the psychrophilic bacterium *C. maris* expresses DnaK and DnaJ proteins homologous to those of other bacteria. The *dnaK* gene is highly expressed upon incubation of the cells at 18–20°C, reflecting the heat shock response in this bacterium. On the other hand, the *dnaJ* transcript was only slightly increased by the same treatment. Discrepancies of the expression level and the transcript size indicate that the *dnaK* and *dnaJ* genes are expressed separately and do not form an operon, in contrast to other bacteria. Identification of the transcription initiation sites revealed that the *dnaK* gene possesses a σ^{32}-dependent promoter, whereas the *dnaJ* gene has a σ^{70}-dependent promoter. Specific low G + C content in the 5′-untranslated regions of both genes was noted.

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