Extremophiles (2005) 9:145–150
DOI 10.1007/s00792-004-0429-9

ORIGINAL PAPER

**Kazuaki Yoshimune · Andrey Galkin
Ljudmila Kulakova · Tohru Yoshimura
Nobuyoshi Esaki**

Cold-active DnaK of an Antarctic psychrotroph *Shewanella* sp. Ac10 supporting the growth of *dnaK*-null mutant of *Escherichia coli* at cold temperatures

Received: 21 July 2004 / Accepted: 12 November 2004 / Published online: 15 December 2004
© Springer-Verlag 2004

Abstract *Shewanella* sp. Ac10 is a psychrotrophic bacterium isolated from the Antarctica that actively grows at such low temperatures as 0°C. Immunoblot analyses showed that a heat-shock protein DnaK is inducibly formed by the bacterium at 24°C, which is much lower than the temperatures causing heat shock in mesophiles such as *Escherichia coli*. We found that the *Shewanella* DnaK (SheDnaK) shows much higher ATPase activity at low temperatures than the DnaK of *E. coli* (EcoDnaK): a characteristic of a cold-active enzyme. The recombinant SheDnaK gene supported neither the growth of a *dnaK*-null mutant of *E. coli* at 43°C nor λ phage propagation at an even lower temperature, 30°C. However, the recombinant SheDnaK gene enabled the *E. coli* mutant to grow at 15°C. This is the first report of a DnaK supporting the growth of a *dnaK*-null mutant at low temperatures.

Keywords Antarctic bacteria · Psychrotroph · Heat-shock response · DnaK · *Shewanella* sp. Ac10

Communicated by K. Horikoshi

K. Yoshimune · A. Galkin · L. Kulakova · T. Yoshimura
N. Esaki (✉)
Institute for Chemical Research, Kyoto University,
Uji, Kyoto 611-0011, Japan
E-mail: esaki@scl.kyoto-u.ac.jp
Tel.: +81-774-383240
Fax: +81-774-383248

Present address: K. Yoshimune
Department of Applied Chemistry, Faculty of Engineering,
Oita University, Oita 870-1192, Japan

Present address: T. Yoshimura
Department of Applied Molecular Biosciences,
Graduate School of Bioagricultural Sciences,
Nagoya University, Nagoya, Aichi 464-8601, Japan

Introduction

Most organisms prevail over heat and other stresses by inducing heat-shock proteins (HSP) to prevent aggregation and misfolding of intracellular proteins under stress conditions. For example, the 70-kDa heat-shock proteins (HSP70) including bacterial DnaK not only directly catalyze refolding of misfolded proteins, but also negatively modulate heat-shock regulators, such as the RpoH of Gram-negative proteobacteria, and the heat-shock factors of eukaryotes. EcoDnaK cooperates with its cochaperones DnaJ and GrpE to maintain the homeostasis of heat-shock gene expression and keeps intracellular HSP concentrations at appropriate levels through the cooperation (Arsene et al. 2000; VanBogelen et al. 1987).

DnaK is an ATP-binding protein with ATPase activity that assists protein folding through interaction with ATP. Its affinities toward peptides and proteins are affected by ATP-binding and its hydrolysis. Thus, the ATPase activity of DnaK is crucial for its chaperone action.

Cold-adapted microorganisms including psychrophiles and psychrotrophs (Morita 1975) are capable of growing at temperatures as low as 0°C and have been isolated from various cold environments such as Antarctic seawater. Since they usually live in cold environments, they probably cause heat shock at temperatures much lower than those for mesophilic microorganisms. The DnaKs of cold-adapted bacteria are probably characterized by their cold activities, i.e., high ATPase activity at low temperatures.

We show here that an Antarctic psychrotroph, *Shewanella* sp. Ac10, indeed causes heat shock at a temperature as low as 24°C to accumulate intracellular DnaK, which we have shown to be a typical cold-active enzyme. We have found that the cold-active DnaK enables recombinant *Escherichia coli* cells to grow at 15°C on its heterologous expression.

Materials and methods

Basic methods and materials

The standard protocols of Sambrook et al. (1989) were used for DNA manipulations. PCR was done with *Ex taq* DNA polymerase from Takara Shuzo, Kyoto, Japan. DNA sequencing was done by the dideoxy chain-termination method with an Applied Biosystems Model 370A DNA sequencer. The *E. coli* MC4100 *dnaK*-null mutant Δ*dnaK52* (Paek and Walker 1987) was kindly provided by Dr. C. Wada of the Institute for Virus Research, Kyoto University. Plasmid pDNAJ, an expression vector for *E. coli* DnaJ (EcoDnaJ), was constructed by the introduction of the *Sal*I-*Pst*I insertion in pDNAJ-A (Ohki et al. 1986) into the corresponding sites of pMPM-T3 (Mayer 1995). EcoDnaK was purified according to our previous method (Yoshimune et al. 1998). Other materials purchased were oligonucleotides from Biologica, Nagoya, Japan; Ligation High (DNA ligation kit) from Toyobo, Osaka, Japan; plasmid pKK223-3 from Amersham Pharmacia Biotech, Buckinghamshire, England; a DNA sequencing kit from Perkin-Elmer Cetus, Perkin-Elmer, Wellesley, MA, USA; a gelatin-agarose gel from Sigma, St. Louis, MO, USA.

Sequence determination and cloning of SheDnaK gene

Shewanella sp. Ac10 was cultured in a medium (pH 7.2) containing 1.5% polypeptone, 0.1% yeast extract, 0.1% glycerol, 0.3% potassium phosphate, 0.01% $MgSO_4 \cdot 7H_2O$, and 3% sodium chloride with reciprocal shaking at 4°C. Its genomic DNA was used as a template for the PCR amplification of a fragment of the SheDnaK gene, by means of synthetic primers designed on the basis of the amino acid sequences of highly conserved regions among various DnaKs: 5′-GGTATCGATCTAGGTACAACTAACTCTTGTGT-3′ and 5′-TTCGATACCTAGTGATAAAGGCGTTACGTC-3′. The amplified 1-kbp fragment was further extended by gene walking with a TaKaRa PCR in vitro Cloning Kit (Takara Shuzo) and sequenced using the dideoxy chain termination method with an Applied Biosystems Model 370A DNA sequencer (Perkin-Elmer).

The SheDnaK gene was amplified by PCR with the primers 5′-GAG CCCGGGATGGGCAAAATTATTGGTATCGATTTAGG-3′ corresponding to the N-terminus (*Sma*I site, underlined), and 5′-TG CTGCAGTTAACTTACTTCGAACTCAGCATCAAC-3′ corresponding to the C-terminus (*Pst*I site, underlined). The PCR product was digested with *Sma*I and *Pst*I and ligated into the corresponding sites of pKK223-3. The SheDnaK gene in the resulting plasmid pACDK was confirmed to have the correct sequence.

Overproduction and purification of SheDnaK

The EcoDnaK-deficient mutant Δ*dnaK52* was used as a host to avoid contamination with EcoDnaK. The Δ*dnaK52*-pACDK cells were cultured at 37°C in a Luria–Bertani's (LB) medium supplemented with ampicillin (50 μg/ml) and 1 mM isopropyl 1-thio-β-D-galactoside (IPTG) for 12 h, and then sonicated in 20 mM Tris–HCl (pH 7.5) containing 20 mM NaCl, 0.1 mM EDTA, and 15 mM 2-mercaptoethanol. The cell extract containing 70 mg protein was applied to a gelatin-agarose column. After washing with 20 mM Tris–HCl (pH 7.5) containing 0.1 mM EDTA, 15 mM 2-mercaptoethanol, and 0.5 M NaCl, SheDnaK was eluted from the column with the same buffer supplemented with 3 mM ATP-Mg^{2+}. The homogeneity of the final SheDnaK preparation (2 mg) was confirmed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Immunoblot analysis of SheDnaK expression in *Shewanella* sp. Ac10 cells

A rabbit was injected with 0.5 mg of the purified SheDnaK with Freund's adjuvant, followed by an injection of the same amount of SheDnaK with incomplete Freund's adjuvant as a booster. *Shewanella* sp. Ac10 was grown at 4°C in the medium described above and then cultured at different temperatures for an additional 1 h. The cells were then harvested by centrifugation, suspended in 20 mM Tris–HCl (pH 7.5) containing 20 mM NaCl, 0.1 mM EDTA, and 15 mM 2-mercaptoethanol, and disrupted by sonication. The cell extracts were suspended in a cracking buffer (10 mM EDTA, 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, and 50 mM Tris–HCl pH 6.8) and then boiled for 5 min. The same amount of protein (10 μg) was subjected to SDS-PAGE. The proteins in the gel were transferred onto a PVDF membrane with a trans-blot SD Cell according to the manufacturer's instructions. The anti-SheDnaK antibodies prepared were diluted 5,000 times and used as a primary antibody for immunoblot analysis with a horseradish peroxidase-conjugated anti-rabbit IgG as a secondary antibody and an ECL Western blotting detection reagents (Amersham Biosciences Corp., Piscataway, NJ, USA).

ATPase assay

We determined the ATPase activities of SheDnaK by measuring the ADP formed from ATP in a mixture (50 μl) containing 0.1 M Hepes-NaOH (pH 7.5), 150 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 5 mM ATP, and SheDnaK. The reaction was carried out at various temperatures and stopped by addition of 5 μl of 20% perchloric acid. ADP was determined by reversed-phase HPLC with a COSMOSIL 5C18-MS column

(4.6×150 mm; Nacalai Tesque, Kyoto, Japan) in the same manner as described previously (Yoshimune et al. 2002).

Effect of expression of SheDnaK in *E. coli* Δ*dnaK52*

Escherichia coli Δ*dnaK52* transformed with pACDK alone or pACDK plus pDNAJ was cultured in an LB medium containing 0.1 mM IPTG at 15, 30 or 43°C. Antibiotics were also supplemented to the medium: 50 μg/ml ampicillin alone for pACDK or together with 20 μg/ml tetracycline for pACDK plus pDNAJ. The transformants were further infected with λ phage: 100 μl of the culture was mixed with 10 μl (10^3 PFU) of a λ phage suspension followed by incubation at 30°C for 15 min. The titers of the λ phages were assayed on LB agar plates supplemented with IPTG and appropriate antibiotics at concentrations described above after incubation at 30°C for about 12 h.

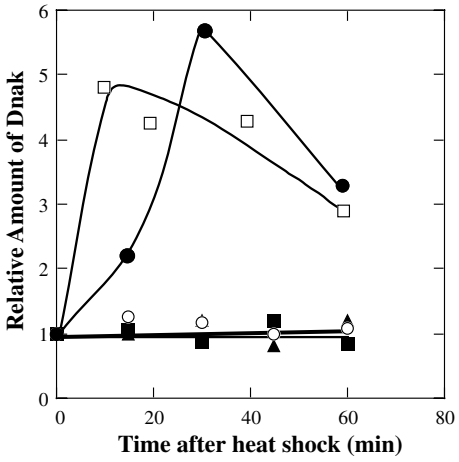

Fig. 1 Immunoblot analysis of DnaK produced by *Shewanella* sp. Ac10. *Shewanella* sp. Ac10 was grown at 4°C and subjected to heat shock at 10 (■), 15 (▲), 20 (○), 24 (●), or 28°C (□) for the indicated periods. The relative amounts of DnaK were quantified using the NIH image program and expressed with the ratio of the values for the heat-shocked cells to those for the untreated cells

Results and discussion

Heat-shock response and induction of DnaK synthesis in *Shewanella* sp. Ac10

Shewanella sp. Ac10 grows well at 4°C, its apparent optimum growth temperature being around 20°C, but not at temperatures over 30°C, as described in a previous report (Kulakova et al. 1999). We examined whether this psychrotroph shows a heat-shock response. The temperature causing the heat shock, if any, is most probably much lower than those observed with mesophiles such as *E. coli*. DnaK is not only a heat-shock protein, but also plays a key role in heat-shock response. Therefore, we examined DnaK as a test protein for the heat-shock response.

We determined the chromosomal DNA sequence for the SheDnaK gene, cloned it, purified SheDnaK, and prepared antibodies against it as described in Materials and methods. *Shewanella* sp. Ac10 was cultured at 4°C until the growth reached a mid-logarithmic phase. The temperature was then elevated or kept at 4°C, and the cultures were continued for various periods of time up to 1 h. The cell extracts were subjected to immunoblot analysis by application of the same amount of protein (10 μg) as described in Materials and methods. The amount of SheDnaK was invariable at temperatures between 4 and 20°C, but it increased about six times in 30 min at 24°C, which is close to the upper limit for the growth temperature of *Shewanella* sp. Ac10 (Fig. 1). This psychrotrophic bacterium faces thermal stress under these conditions, and heat-shock proteins are induced in the same manner as *E. coli*. However, the threshold temperature inducing heat shock is about 18°C lower than that for *E. coli*: 42°C.

The ability to cause heat-shock response is not necessarily common to all organisms. Some cold-adapted organisms are exceptional. For example, *Euplotes focardii*, an endemic psychrophilic ciliate in Antarctic coastal seawater, showed no obvious heat-shock response in terms of Hsp70 synthesis (La Terza et al. 2001). Similarly, no heat-shock response was found for the Antarctic psychrophilic yeast *Mrakia frigida* (Michelle and Kenneth 1998). Thus, *Shewanella* sp. Ac10 is distinct from these organisms. This probably arises from the difference in their habitats, whether or not they are permanently cold.

Sequences of SheDnaK

We determined the nucleotide sequence of the SheDnaK gene and found sequences characteristic of a heat-shock promoter, recognized by the σ^{32} factor at 87 (TAAAATGCTTGAA) and 59 (CCCCATATC) nucleotides upstream of the initiation codon. Their consensus sequences are TNtCNCcCTTGAA at the −35 region and CCCCATtTa at the −10 region (Cowing et al. 1985). SheDnaK is encoded by 1,926 nucleotides corresponding to 641 amino acids and has a predicted molecular mass of 69,024 Da (Fig. 2). Three putative domains can be assigned by comparison with those of EcoDnaK: the N-terminal ATPase domain in the region from 1 to 385; the central substrate-binding domain from 386 to 540; and the C-terminal variable domain from 543 to 637. SheDnaK exhibits 90% sequence identity with DnaK of a mesophilic counterpart in the same genera *S. oneidensis* (Heidelberg et al. 2002). The C-terminal domains are the least conserved portions of the two *Shewanella* DnaKs. It is therefore possible that the structure of the

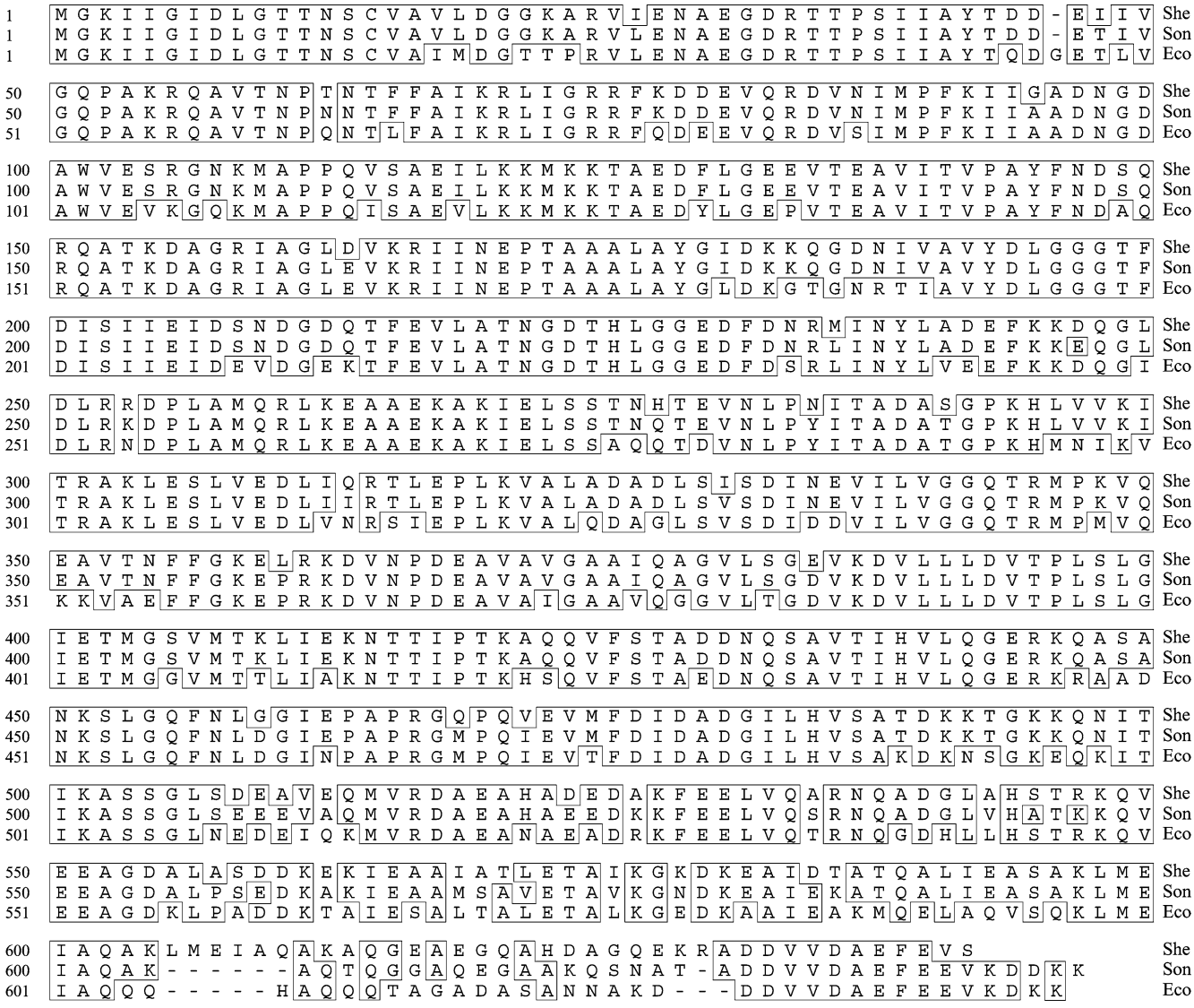

Fig. 2 Alignment of the amino acid sequences of DnaKs of *Shewanella* sp. Ac10 (She), *S. oneidensis* (Son), and *E. coli* (Eco). The conserved amino acid residues are *boxed*. The residue numbers for each DnaK are shown

C-terminal domain is responsible for the cold adaptation of SheDnaK.

Temperature dependence of ATPase activity of SheDnaK

It has been clearly shown that the ATPase activity of DnaK is essential for its function (MaCarty and Walker 1994). DnaK undergoes a conformational transition from a low-affinity state to a high-affinity one concomitantly with its ATPase action. Substrate polypeptides are weakly and tightly bound, respectively (Farr et al. 1998; Slepenkov and Witt 1998). We compared the ATPase activity of SheDnaK with that of EcoDnaK at various temperatures. EcoDnaK was almost inactive at temperatures as low as 15°C. However, SheDnaK was fairly active at this temperature with a relative activity of about 20% of the maximum value at 35°C, which is much lower than the optimum temperature for EcoDnaK, in agreement with the difference in upper-limit growth temperature for the two bacteria. The ATPase activity of EcoDnaK was highly temperature-dependent over the range relevant to viable temperatures for *E. coli* (Fig. 3). McCarty and Walker (1991) suggested that the sharp dependence of its ATPase activity on temperature is of physiological significance to be utilized for temperature sensing.

Complementation of the *dnaK*-null mutant of *E. coli* with the SheDnaK gene

The *dnaK*-null mutant of *E. coli* lacks the ability to grow at both ends of growth temperatures permissible for the wild-type strain: 15 and 43°C (Bukau and Walker 1990; Paek and Walker 1987). Moreover, the mutant cannot propagate λ phages even at the moderate temperature of

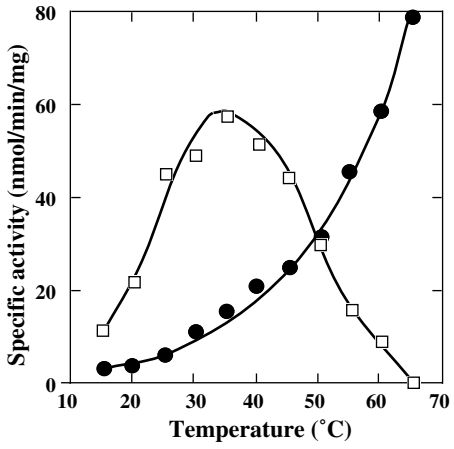

Fig. 3 Effect of temperatures on the ATPase activities of SheDnaK (□) and EcoDnaK (●). The DnaKs (20 μM) were pre-incubated with a mixture containing 0.1 M Hepes-NaOH (pH 7.5), 150 mM KCl, 5 mM $MgCl_2$, and 1 mM DTT at various temperatures for 5 s. The reaction was started with the addition of 5 μl of 50 mM ATP (the final concentration of ATP was 5 mM). The reaction mixture was incubated for 5 min, and the ADP formed was measured

30°C (Alfano and McMacken 1989; Bukau and Walker 1990; Zylicz et al. 1989; Paciorek et al. 1997). DnaK acts cooperatively with DnaJ and GrpE to rescue various unstable proteins from aggregation or misfolding at temperatures as high as 43°C.

We examined whether the recombinant SheDnaK gene rescues the *dnaK*-null mutant of *E. coli* (Δ*dnaK52*) to grow and propagate λ phages. However, the amount of EcoDnaJ in Δ*dnaK52* cells is reduced due to a polar effect of the chloramphenicol-resistant marker, downstream of the EcoDnaJ gene (Mogk et al. 1999; Szabo et al. 1994). Therefore, we introduced this gene with a plasmid pDNAJ together into Δ*dnaK52*. As shown in Table 1, SheDnaK supported the growth of Δ*dnaK52* at 15°C but not at 43°C. On the other hand, the recombinant SheDnaK failed to help the mutant to propagate λ phages at 30°C. Our results clearly show that SheDnaK is fully active in terms of its ATPase activity, not only at 30°C but also at 43°C as shown in Fig. 3.

Gene encoding molecular chaperones, which are transcribed with RpoH, a heat-shock transcription factor represented by σ^{32} of *E. coli*, are toxic, especially at low temperatures. DnaK and its co-chaperones are required to capture RpoH in order to stimulate digestion with a protease such as FtsH of *E. coli*. GrpE is not essential to arrest RpoH, if sufficient DnaK and DnaJ occur and do so together (Tomoyasu et al. 1998). Thus, SheDnaK supported the growth of Δ*dnaK52* at 15°C, only under co-expression with EcoDnaJ. It is interesting to note that *E. coli* is also enabled to grow at low temperatures by expression of the chaperonin from a psychrophile *Oleispira antarctica* (Ferrer et al. 2003) or trigger factor of *E. coli* (Kandror and Goldberg 1997). Therefore, our report presents another example of a molecular chaperone to support the growth of *E. coli* at low temperatures.

Table 1 Growth rates at 15 and 43°C of *E. coli* W3110 strain and Δ*dnaK52* strain harboring the plasmid carrying the gene of SheDnaK, EcoDnaK, or EcoDnaJ

	W3110	Δ*dnaK52* with the gene(s)				
		None	SheDnaK + EcoDnaJ	SheDnaK	EcoDnaK	EcoDnaJ
15°C	0.21	0	0.2	0.14	0.19	0
43°C	0.67	0	0	0	0.37	0

The growth was monitored with the turbidity at 600 nm, and the rates were calculated with the square-root model of Ratkowsky et al. (1983) and expressed as h^{-1}

Acknowledgements This work was supported in part by the Grant-in-Aid for Scientific Research 09460049 (to N.E.), and Grant-in-Aid for Scientific Research on Priority Areas (B) 13125203 (to N.E.) from the Ministry of Education, Culture, Sports, Science, and Technology, and by the Pioneering Research Project (to T.Y.) in Biotechnology of the Ministry of Agriculture, Forestry, and Fisheries.

References

Alfano C, McMacken R (1989) Heat shock protein-mediated disassembly of nucleoprotein structures is required for the initiation of bacteriophage lambda DNA replication. J Biol Chem 264:10709–10718

Arsene F, Tomoyasu T, Bukau B (2000) The heat shock response of *Escherichia coli*. Int J Food Microbiol 55:3–9

Bukau B, Walker GC (1990) Mutations altering heat shock specific subunit of RNA polymerase suppress major cellular defects of *E. coli* mutants lacking the DnaK chaperone. EMBO J 9:4027–4036

Cowing DW, Bradwell JC, Craig EA, Woolford C, Hendrix RW, Gross CA (1985) Consensus sequence for *Escherichia coli* heat shock gene promoters. Proc Natl Acad Sci USA 82:2679–2683

Farr CD, Slepenkov SV, Witt SN (1998) Visualization of a slow, ATP-induced structural transition in the bacterial molecular chaperone DnaK. J Biol Chem 273:9744–9748

Ferrer M, Chernikova TN, Yakimov MM, Golyshin PN, Timmis KN (2003) Chaperonins govern growth of *Escherichia coli* at low temperatures. Nat Biotechnol 21:1266–1267

Heidelberg JF, Paulsen IT, Nelson KE, Gaidos EJ, Nelson WC, Read TD, Eisen JA, Seshadri R, Ward N, Methe B, Clayton RA, Meyer T, Tsapin A, Scott J, Beanan M, Brinkac L, Daugherty S, DeBoy RT, Dodson RJ, Durkin AS, Haft DH, Kolonay JF, Madupu R, Peterson JD, Umayam LA, White O, Wolf AM, Vamathevan J, Weidman J, Impraim M, Lee K, Berry K, Lee C, Mueller J, Khouri H, Gill J, Utterback TR, McDonald LA, Feldblyum TV, Smith HO, Venter JC, Nealson KH, Fraser CM (2002) Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. Nat Biotechnol 20:1118–1123

Kandror O, Goldberg AL (1997) Trigger factor is induced upon cold shock and enhances viability of *Escherichia coli* at low temperatures. Proc Natl Acad Sci USA 94:4978–4981

Kulakova L, Galkin A, Kurihara T, Yoshimura T, Esaki N (1999) Cold-active serine alkaline protease from the psychrotrophic bacterium *Shewanella* strain Ac10: gene cloning and enzyme purification and characterization. Appl Environ Microbiol 65:611–617

La Terza A, Papa G, Miceli C, Luporini P (2001) Divergence between two Antarctic species of the ciliate Euplotes, *E. focardii* and *E. nobilii*, in the expression of heat-shock protein 70 genes. Mol Ecol 10:1061–1067

Mayer MP (1995) A new set of useful cloning and expression vectors derived from pBlueScript. Gene 163:41–46

McCarty JS, Walker GC (1991) DnaK as a thermometer: threonine-199 is site of autophosphorylation and is critical for ATPase activity. Proc Natl Acad Sci USA 88:9513–9517

McCarty JS, Walker GC (1994) DnaK mutants defective in ATPase activity are defective in negative regulation of the heat shock response: expression of mutant DnaK proteins results in filamentation. J Bacteriol 176:764–780

Michelle LD, Kenneth W (1998) Heat shock response in psychrophilic and psychrotrophic yeast from Antarctica. Extremophiles 2:41–49

Mogk A, Bukau B, Lutz R, Schumann W (1999) Construction and analysis of hybrid *Escherichia coli–Bacillus subtilis dnaK* genes. J Bacteriol 181:1971–1974

Morita RY (1975) Psychrophilic bacteria. Microbiol Rev 39:144–167

Ohki M, Tamura F, Nishimura S, Uchida H (1986) Nucleotide sequence of the *Escherichia coli dnaJ* gene and purification of the gene product. J Biol Chem 261:1778–1781

Paciorek J, Kardys K, Lobacz B, Wolska KI (1997) *Escherichia coli* defects caused by null mutations in *dnaK* and *dnaJ* genes. Acta Microbiol Pol 46:7–17

Paek KH, Walker GC (1987) *Escherichia coli dnaK* null mutants are inviable at high temperature. J Bacteriol 169:283–290

Ratkowsky DA, Lowry RK, McMeekin TA, Stokes AN, Chandler RE (1983) Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J Bacteriol 154:1222–1226

Sambrook J, Fritsch EF, Maniatis T (eds) (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 1.1–1.110

Slepenkov SV, Witt SN (1998) Peptide-induced conformational changes in the molecular chaperone DnaK. Biochemistry 37:16749–16756

Szabo A, Langer T, Schroder H, Flanagan J, Bukau B, Hartl FU (1994) The ATP hydrolysis-dependent reaction cycle of the *Escherichia coli* Hsp70 system DnaK, DnaJ, and GrpE. Proc Natl Acad Sci USA 91:10345–10349

Tomoyasu T, Ogura T, Tatsuta T, Bukau B (1998) Levels of DnaK and DnaJ provide tight control of heat shock gene expression and protein repair in *Escherichia coli*. Mol Microbiol 30:567–581

VanBogelen RA, Acton MA, Neidhardt FC (1987) Induction of the heat shock regulon does not produce thermotolerance in *Escherichia coli*. Genes Dev 1:525–531

Yoshimune K, Yoshimura T, Esaki N (1998) Hsc62, a new DnaK homologue of *Escherichia coli*. Biochem Biophys Res Commun 250:115–118

Yoshimune K, Yoshimura T, Nakayama T, Nishino T, Esaki N (2002) Hsc62, Hsc56, and GrpE, the third Hsp70 chaperone system of *Escherichia coli*. Biochem Biophys Res Commun 293:1389–1395

Zylicz M, Ang D, Liberek K, Georgopoulos C (1989) Initiation of lambda DNA replication with purified host- and bacteriophage-encoded proteins: the role of the *dnaK*, *dnaJ* and *grpE* heat shock proteins. EMBO J 8:1601–1608