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DNA Sequence Analysis of the *dnaK* Gene of *Escherichia coli* B and of Two *dnaK* Genes Carrying the Temperature-Sensitive Mutations *dnaK7*(Ts) and *dnaK756*(Ts)

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The DNA sequence of the *dnaK* gene of *Escherichia coli* was analyzed. The nucleotide sequence of the wild-type *dnaK* gene of *E. coli* B differed from that of *E. coli* K-12 in 15 bp, none of which altered the amino acid sequence. Two temperature-sensitive *dnaK* mutations were examined by cloning and sequence analyses. Results showed that one *dnaK* mutation, *dnaK7*(Ts), was a one-base substitution of T for C at nucleotide position 448 in the open reading frame yielding an amber nonsense codon. The other mutation, *dnaK756*(Ts), consisted of base substitutions (A for G) at three nucleotide positions, 95, 1364, and 1403, in the open reading frame resulting in an aspartic acid codon in place of a glycine codon.

To determine the mechanism of regulation of macromolecular synthesis in *Escherichia coli* cells, Itikawa and Ryu (16) isolated a temperature-sensitive *dnaK* mutant carrying a mutation [*dnaK7*(Ts)] by selection for thymineless death at 43°C. Like *groES* mutant cells (34), the mutant showed severely inhibited syntheses of both DNA and RNA as well as an inability to propagate phage lambda at the permissive temperature (16). Another temperature-sensitive *dnaK* mutant carrying a mutation [*dnaK756*(Ts)] was originally selected for the inability to propagate phage λ (12).

The *dnaK* gene product (DnaK) is a major heat shock protein and is perhaps the most highly conserved protein in nature (2, 22). DnaK has weak DNA-independent ATPase activity and autophosphorylating activity (39). These mutants also show a loss of phosphorylation of glutaminyl-tRNA synthetase and threonyl-tRNA synthetase at the nonpermissive temperature (17, 36).

Bardwell and Craig (2) determined the nucleotide sequence of the open reading frame (ORF) of the *dnaK* gene in *E. coli* K-12 and found remarkable conservation between the *dnaK* gene and a major heat shock-induced gene, the Hsp70 gene of *Drosophila* and other species. Cowing et al. (7) studied the promoter regions of the *E. coli* operon *dnaK* and two others for heat shock proteins and identified three promoters within 179 bp upstream of the *dnaK* gene together with consensus sequences. Sunshine et al. (29) isolated a temperature-sensitive *dnaJ* mutant carrying a mutation [*dnaJ259*(Ts)]. The *dnaJ259*(Ts) mutation also affected the syntheses of both RNA (35) and DNA at 43.5°C and propagation of phage λ at the permissive temperature (29). The *dnaJ* gene has been shown to be located at 0.3 min on the *E. coli* chromosome map together with the *dnaK* gene (1), between the *thr* and *leu* genes, in the order *thr dnaK dnaJ leu* (38). Georgopoulos (11) isolated a phage λ *imm*^{21} carrying the *dnaK* gene of ligation of chromosomal DNA fragments and λ *imm*^{21} no. 540 (21) after their treatment with the restriction enzyme *Hin*dIII. The restriction map of the *dnaK* gene obtained from the DNA sequence data of Bardwell and Craig (2) and Cowing et al. (7) revealed that the *dnaK-dnaJ* operon is included in a 7.3 kb *Bam*HI fragment of *E. coli* DNA (20).

Ezaki et al. (8) cloned and sequenced the *dnaK* gene of *seg* mutants and found that it contained one base substitution. Cegielska and Georgopoulos (6) examined the functional domains of the *dnaK* protein by using altered *dnaK* genes carrying deletions or insertions. In this article, we report the nucleotide sequences of the *dnaK* gene of *E. coli* B and of two altered *dnaK* genes carrying the temperature-sensitive mutations *dnaK7*(Ts) and *dnaK756*(Ts).

MATERIALS AND METHODS

Bacterial strains, plasmids, and phage. The bacterial strains, plasmids, and phage used and their sources are listed in Table 1.

Media. LB medium (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, 1 g of glucose, all per liter, pH 7.0) was used for bacterial growth. For preparation of λ phage DNA, recipient cells were grown in TB medium (pH 7.4), which contained, per liter, 10 g of tryptone, 5 g of NaCl, 10 mM $MgSO_4$, and 2 g of maltose. λ Agar medium contained, per liter, 10 g of tryptone, 2.5 g of NaCl, and 12 g (bottom layer) or 5 g (upper layer) of agar and was adjusted to pH 7.0. 2× YT medium contained, per liter, 16 g of tryptone, 10 g of yeast extract, and 5 g of NaCl and was adjusted to pH 7.6 with 1 M NaOH. Tryptone, yeast extract, and agar were obtained from Difco Laboratories.

Cloning of wild-type and mutant *dnaK* genes. It was necessary to determine the nucleotide sequence of the wild-type *dnaK* gene of *E. coli* B because the *dnaK7*(Ts) mutant was originally isolated from the *E. coli* B strain H/r30RT (16). Libraries of chromosomal DNA extracted from the wild-type strains H/r30RT (*E. coli* B) and WK41 (*E. coli* K-12) were constructed with the λ phage derivative EMBL 3 as a cloning vector (9). The chromosomal DNA was partially digested with *Sau*3AI and ligated with *Bam*HI-digested EMBL3 DNA. The ligated DNA sample was packaged into λ phage particles by using Gigapack II Gold Packaging Extract (Stratagene, San Diego, Calif.). The phage were used to infect cells of the mutant strains WK45 [*dnaK7*(Ts)] and WJ45 [*dnaJ259*(Ts)] in

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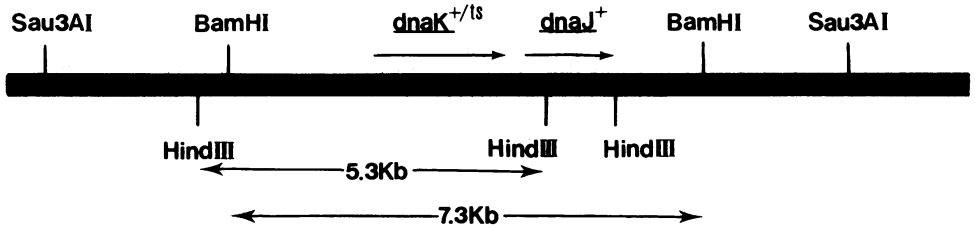

FIG. 1. Physical arrangement of the *dnaK-dnaJ* operon located in the 0.3-min region of the *E. coli* chromosome. The solid bar represents the *Sau*3AI fragment of about 13 kb encompassing the *dnaK-dnaJ* operon. The positions of the restriction sites on this fragment are shown.

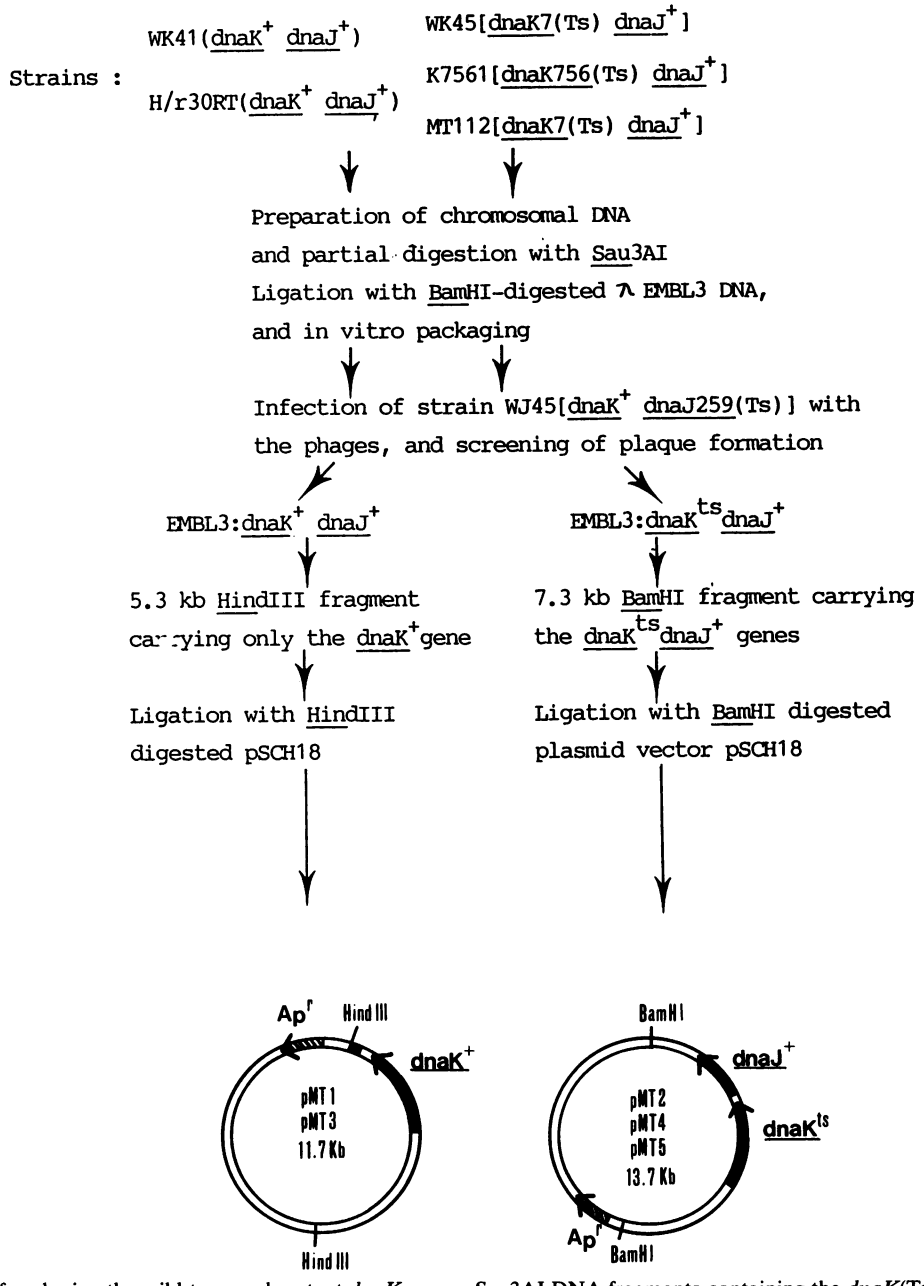

FIG. 2. Strategy for cloning the wild-type and mutant *dnaK* genes. *Sau*3AI DNA fragments containing the *dnaK*(Ts)-*dnaJ*$^+$ operon were ligated with *Bam*HI-digested λ EMBL3. Subclonings of the *dnaK*$^+$ gene and *dnaK*(Ts)-*dnaJ*$^+$ segments were performed with the cloning vector pSCH18.

TABLE 1. Strains, plasmids, and phage

Strain, plasmid, or phage	Genetic characteristics	Reference or source
E. coli K-12		
WK41	Wild type	14
WK45	WK41 *dnaK7*(Ts)	14
WJ45	WK41 *dnaJ259*(Ts) *met*	14
K7561	*thr thi thy lacY tonA supE dnaK745*(Ts)	25
JM83	*ara* Δ(*lac-proAB*) *rpsL thi* φ80*dlacZ* ΔM15	31
JM109	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* λ⁻ (*lac-proAB*) [F′ *traD36 proAB lacI*^{q} *lacZ* ΔM15]	37
E. coli B		
H/r30RT	*thy argF*	16
MT112	H/r30RT *dnaK7*(Ts)	16
MT1121	MT112 *sup*(Am)	This study[a]
Plasmids		
pSCH18	Vector for cloning, Ap^r	18
pUC119	Vector for cloning, Ap^r	32
Phage (λ EMBL3)	Vector for genome DNA library	9

[a] Strain MT1121 was isolated from spontaneous temperature-resistant revertants of strain MT112 as a strain carrying both *dnaK7*(Ts) and an amber nonsense suppressor, *sup*(Am), as described in the text.

TABLE 2. Complementation test

Plasmid (containing cloned *E. coli* gene or vector)	Recipient[a]	
	WK45 [*dnaK7*(Ts)]	WJ45 [*dnaJ259*(Ts)]
pMT1 (*dnaK*$^+$ of WK41)	+	−
pMT2 [*dnaK7*(Ts)-*dnaJ*$^+$ of WK45]	−	+
pMT3 (*dnaK*$^+$ of H/r30RT)	+	−
pMT4 [*dnaK756*(Ts)-*dnaJ*$^+$ of K7561]	−	+
pMT5 [*dnaK7*(Ts)-*dnaJ*$^+$ of MT112]	−	+
pSCH18 (vector)	−	−

[a] Temperature-sensitive mutant strains WK45 [*dnaK7*(Ts)] and WJ45 [*dnaJ259*(Ts)] were used as recipients for the transformation test with recombinant plasmid DNA derived from the manipulated pSCH18. Transformants were selected at 43°C on Luria-Bertani agar medium containing 50 μg of ampicillin per ml (Banyu-Seiyaku). Symbols: +, growth of transformants at 43°C by complementation with the wild-type *dnaK* or *dnaJ* gene; −, no growth of transformants.

which λ phage carrying a fragment of *dnaK*$^+$-*dnaJ*$^+$ operon are able to propagate and to form plaques.

For cloning the mutant *dnaK* genes carrying the *dnaK7* (Ts) and *dnaK756*(Ts) mutations, libraries of chromosomal DNA fragments from the mutant strains WK45 [*E. coli* K-12 *dnaK7*(Ts)], MT112 [*E. coli* B *dnaK7*(Ts)], and K7561 [*E. coli* K-12 *dnaK756*(Ts)] were constructed by manipulation of cellular DNA as described for cloning of the wild-type *dnaK* gene. Recombinant phage carrying the *dnaK*(Ts)-*dnaJ*$^+$ fragment of the operon were selected by infection of strain WJ45 [*dnaK*$^+$ *dnaJ259*(Ts)], in which λ phage carrying the *dnaK*(Ts)-*dnaJ*$^+$ fragment could multiply.

Subcloning of the wild-type *dnaK* and mutant *dnaK* genes. For subcloning the wild-type *dnaK* gene, a 5.3-kb *Hin*dIII-*Hin*dIII restriction fragment of the recombinant λ EMBL 3 containing the *dnaK*$^+$ gene (Fig. 1) was ligated with a plasmid vector pSCH18 DNA that had been digested with *Hin*dIII and treated with APase. The DNA mixture was introduced into JM83 cells by transformation. White colonies were picked up. Recombinant plasmid DNA was extracted and examined for the presence of the 5.3-kb segment of the inserted DNA. The recombinant plasmids (pMT1 and pMT3) carrying the *dnaK*$^+$ gene were also identified by transfer to WK45 cells, which produced colonies at 43°C after transformation (Fig. 2, left side). Subcloning of the mutant *dnaK* genes was performed by the same method as described above except that a 7.3-kb *Bam*HI-*Bam*HI restriction fragment carrying *dnaK*(Ts)-*dnaJ*$^+$ genes (Fig. 1) was ligated with pSCH18 DNA treated with *Bam*HI and that strain WJ45 cells were used as recipients for transformation to identify the recombinant plasmids. As shown in Fig. 2 (right side), three additional plasmids carrying the *dnaK*(Ts)-*dnaJ*$^+$ operons pMT2, pMT4, and pMT5 were obtained. Complementation tests of the five derivatives of pSCH18 verified that these plasmids carried the *dnaK*$^+$ gene or *dnaK*(Ts)-*dnaJ*$^+$ operon (Table 2).

DNA sequencing. Cloned DNA segments were sequenced by the dideoxy method (26, 27) with the phagemid vector pUC119 and *E. coli* JM109 as host cells. A 2.3-kb *Nru*I-*Hin*dIII restriction fragment containing the *dnaK* gene was extracted from the subcloned plasmids (pMT1, pMT2, pMT3, pMT4, and pMT5) and digested with four restriction enzymes to obtain segments of appropriate length for DNA sequencing. DNA fragments were isolated by electroelution. The restriction enzymes used and the sizes of the six fragments prepared are shown in Fig. 3. The protruding

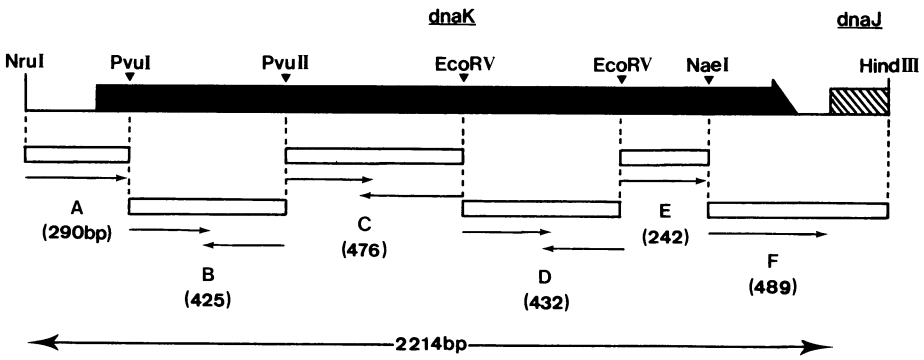

FIG. 3. DNA sequencing strategy. Shown is a restriction map of the *Nru*I-*Hin*dIII fragment encompassing the *dnaK* gene and part of the *dnaJ* gene. Directions and extents of sequences analyzed are shown by arrows. Thick solid arrow indicates the ORF of the *dnaK* gene. Numbers of base pairs of segments are shown in parentheses. Fragments carrying the *dnaK*$^+$ gene were obtained from plasmids pMT1 and pMT3, and those of the *dnaK*(Ts) gene were obtained from plasmids pMT2, pMT4, and pMT5, as shown in Fig. 2.

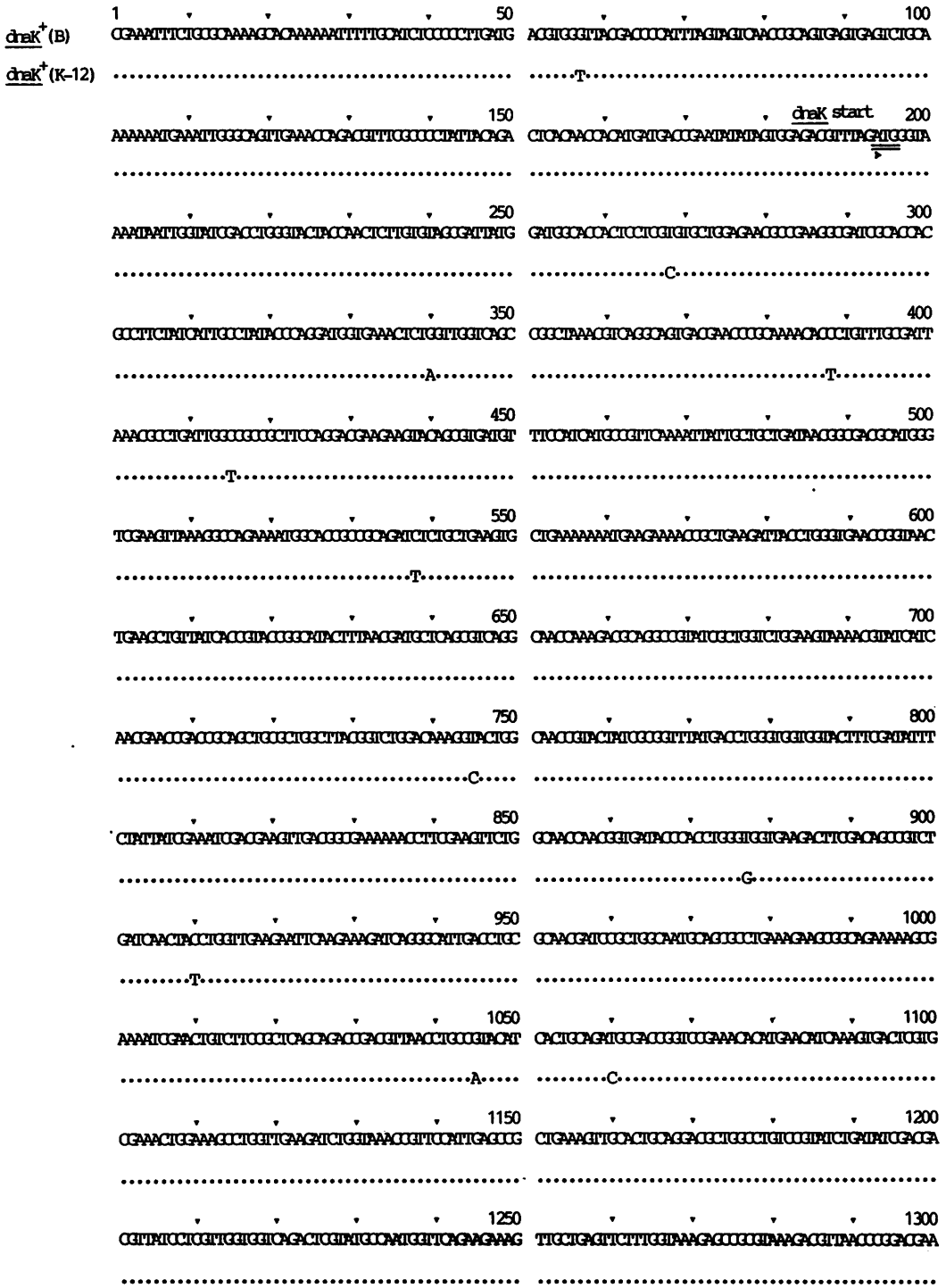

FIG. 4. DNA nucleotide sequence of the *dnaK* gene and its flanking regions. The *dnaK* genes of *E. coli* B and *E. coli* K-12 are aligned. Homologies are indicated by dots on the sequence of *E. coli* K-12. The ORF of the *dnaK* gene is from nucleotide positions 193 to 2107 (total, 1,914 bp). The ORF of the *dnaJ* gene is from nucleotide position 2199 (3, 24).

single-stranded regions of the DNA fragment were blunt ended with the Klenow fragment of *E. coli* DNA polymerase I, and the small DNA fragment was ligated to the *Sma*I site on the pUC119 phagemid vector. The direction of insertion of the DNA fragment was determined by digestion of the recombinant pUC119 DNA with an appropriate restriction enzyme. The recombinant pUC119 plasmid was introduced into strain JM109 grown in 2× YT medium, and transformants were selected on Luria broth plates containing 50 μg of ampicillin per ml. Single-stranded DNA containing a part of the *dnaK* gene was obtained by infection of the transformed cells with the helper phage M13K07.

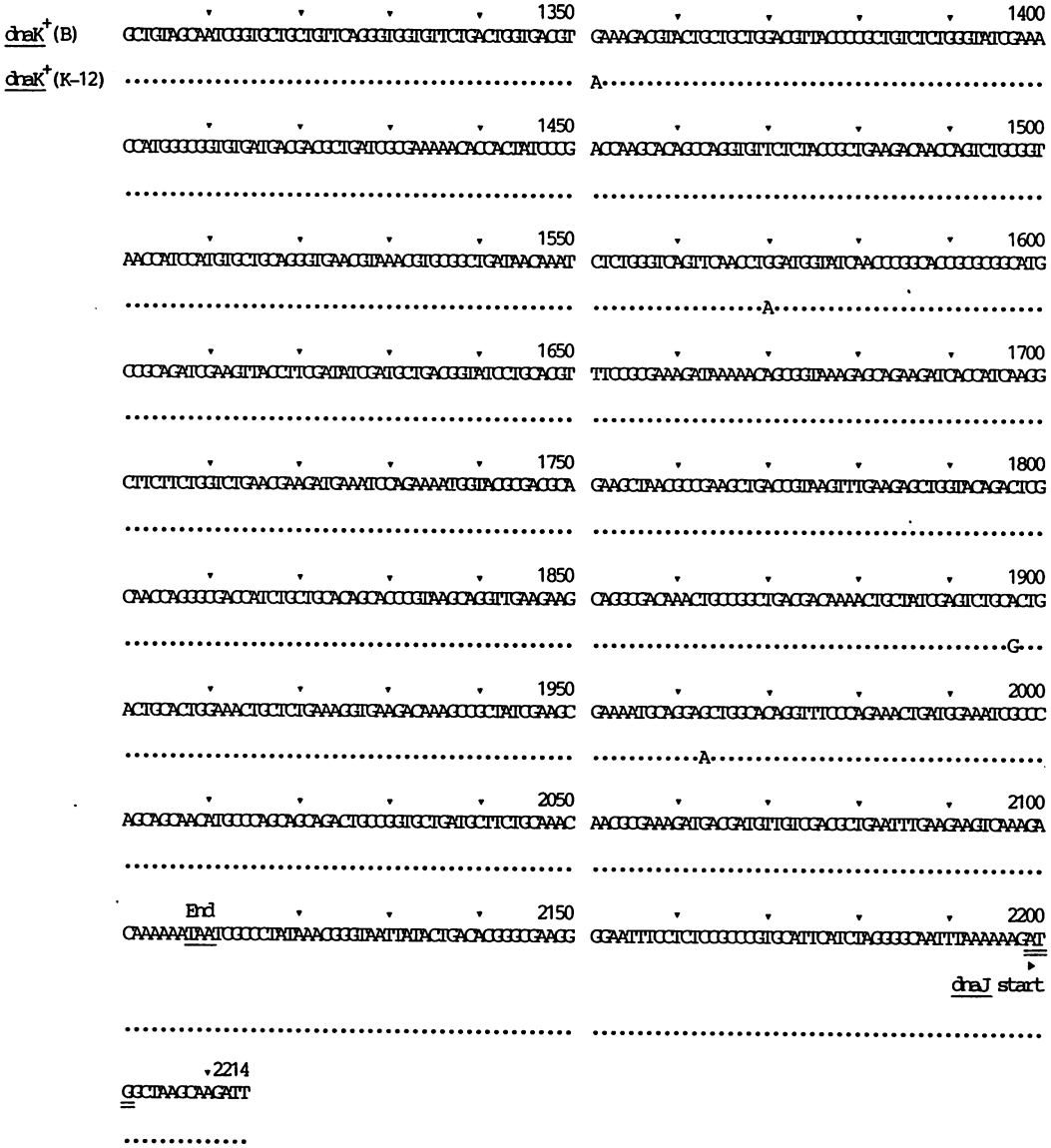

FIG. 4—*Continued.*

DNA-directed translation analysis. For determination of the in vitro transcription-translation products of the subcloned *dnaK* genes, DNA samples of pSCH18 vector, pMT1 (*dnaK*$^+$), and pMT2 [*dnaK7*(Ts)-*dnaJ*$^+$] were prepared and used with a prokaryotic-DNA-directed translation kit (Amersham). The proteins synthesized were labeled with [^{35}S]methionine and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Assay of temperature-resistant revertants by amber nonsense suppression. Temperature-sensitive clones were isolated from spontaneous temperature-resistant revertants of strain MT112 by amber nonsense suppression, and their *dnaK* gene products were examined by SDS-PAGE.

Nucleotide sequence accession number. The nucleotide sequence data reported in this article will appear in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under accession number DO1141.

RESULTS

DNA sequencing. The nucleotide sequence of 2,214 bp containing the wild-type *dnaK* gene of *E. coli* B is shown in Fig. 4, in comparison with that of *E. coli* K-12. The nucleotide sequence data revealed that 15 bp (at nucleotide positions 57, 268, 340, 388, 415, 538, 745, 877, 910, 1045, 1060, 1351, 1570, 1897, and 1963) in the *dnaK* gene of *E. coli* B differed from those of the *E. coli* K-12 gene. None of the 14 changes in the ORF region resulted in alterations in the amino acid sequence.

The nucleotide sequence data for the temperature-sensitive *dnaK* mutant gene, *dnaK7*(Ts), were obtained by using plasmids pMT2 and pMT5. The results showed that this mutation resulted from a one-base substitution of T for C at nucleotide position 448 in the ORF yielding the amber nonsense codon TAG for CAG (Gln) at the 150th codon (Fig. 5). Nucleotide sequencing of another *dnaK*(Ts) mutant gene carrying a *dnaK756*(Ts) mutation showed that this gene differed from the

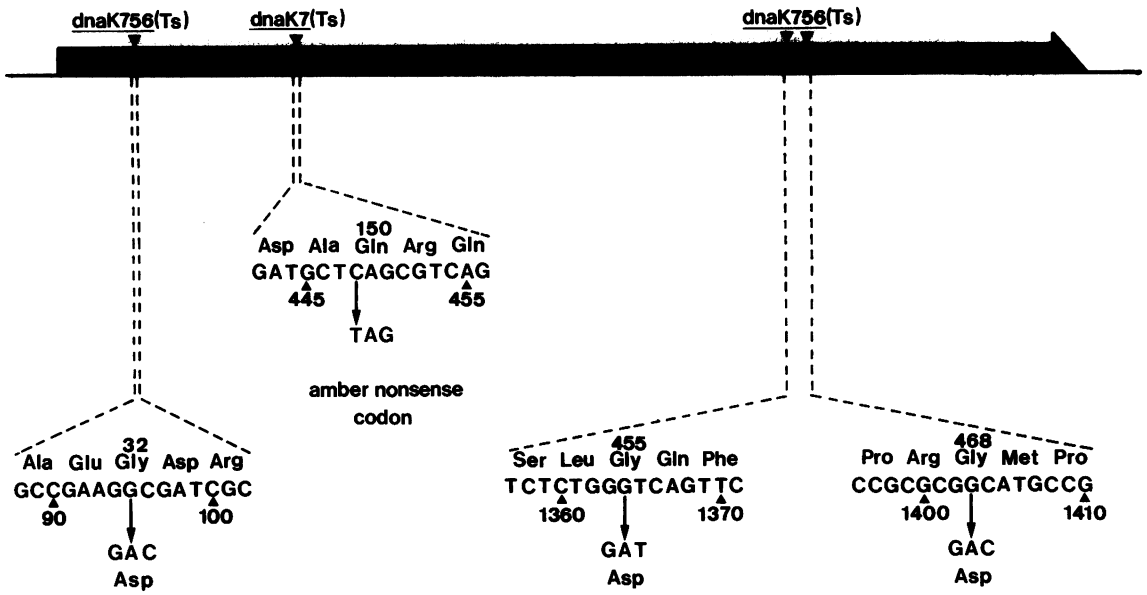

FIG. 5. Site of the *dnaK7*(Ts) mutation in the coding frame of the *dnaK* gene. Thick solid arrow represents the 1,914 bases in the intact ORF of the *dnaK* gene. Top numbers indicate positions in the amino acid sequence. Bottom numbers indicate positions in the nucleotide sequence. Sites of the three missense mutations in the coding frame of the *dnaK756*(Ts) mutant gene are also shown.

wild-type *dnaK* gene in harboring base substitutions of A for C at nucleotide positions 95, 1364, and 1403 in the ORF that resulted in missense mutations at the three sites. These missense mutations caused substitution of an aspartic acid codon for a glycine codon, as shown in Fig. 5.

Identification of the gene product of the *dnaK* mutant gene carrying a *dnaK7*(Ts) mutation. The product of the *dnaK* gene carrying the mutation *dnaK7*(Ts) was examined by DNA-directed translation analysis. DNA samples were prepared by purification of the plasmid DNAs of pSCH18 (vector), pMT1 (*dnaK*$^+$), and pMT2 [*dnaK7*(Ts) *dnaJ*$^+$] and used with a prokaryotic-DNA-directed translation kit. The proteins synthesized and labeled with [^{35}S]methionine in this system were subjected to SDS-PAGE. As shown in Fig. 6,

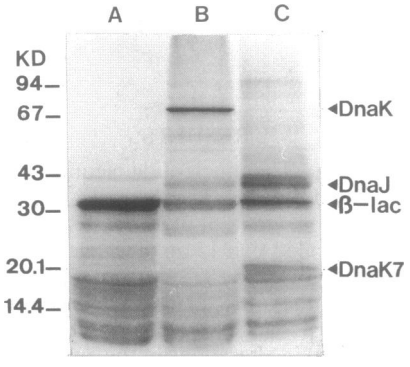

FIG. 6. Gene products encoded by plasmid DNAs carrying the *dnaK*$^+$ and *dnaK7*(Ts) genes. For DNA-directed translation analysis, DNA samples were prepared and purified after multiplication of the plasmids pSCH18 (vector), pMT1 (*dnaK*$^+$), and pMT2 [*dnaK7* (Ts) *dnaJ*$^+$] and used with a prokaryotic-DNA-directed translation kit. Proteins synthesized and labeled with [^{35}S]methionine were analyzed in an SDS–7.5 to 20% polyacrylamide gradient gel. (A) pSCH18 DNA (vector); (B) pMT1 (*dnaK*$^+$); (C) pMT2 [*dnaK7*(Ts) *dnaJ*$^+$]. β-lac, β-lactamase.

fluorograms of the gels indicated that DnaK protein of normal size (69 kDa) was synthesized in the reaction mixture containing pMT1 DNA as a template (Fig. 6B). When pMT2 DNA was used as a template, a premature DnaK protein with a molecular mass of about 20 kDa and a normal DnaJ protein (40 kDa) were detected (Fig. 6C). The results showed that transcription and translation of the *dnaK* gene occurred in this system and that the *dnaK7*(Ts) mutation is a nonsense mutation resulting in premature termination of the peptide.

Assay of the amber nonsense mutation of *dnaK7*(Ts). Fifty spontaneous temperature-resistant revertants of the strain MT112 [*thy argF dnaK7*(Ts)] were isolated at 43°C, and each was infected with the T4 amber phage, which forms plaques only in cells with an amber suppressor mutation. Seven of the 50 revertants permitted multiplication of T4 amber (14%). At the same time, the arginine-requiring phenotype of the amber nonsense mutation in the *argF* gene of strain MT112 was also suppressed in all seven revertants. To determine the effect of amber suppression on synthesis of the DnaK protein, we labeled cellular proteins with [^{35}S]methionine at 30, 40, and 43°C and then extracted the proteins and examined them by SDS-PAGE. As shown in Fig. 7, cells of the parental strain H/r30RT synthesized GroEL and DnaK proteins well at 40 and 43°C (Fig. 7B and C). The mutant strain MT112 synthesized GroEL but not DnaK protein (Fig. 7E and F). The other temperature-resistant revertant carrying the amber nonsense suppressor mutation [*sup*(Am)], MT1121, synthesized small amounts of the DnaK protein of 69 kDa at 30 and 43°C and also synthesized the GroEL and Lon proteins (Fig. 7G and I).

DISCUSSION

In this study, we cloned the wild-type *dnaK* gene of *E. coli* B and that of *E. coli* K-12 and then determined their nucleotide sequences in order to compare the two sequences. The sequence of the *dnaK* gene of *E. coli* K-12 has already been reported (2, 7). We found that the sequences in

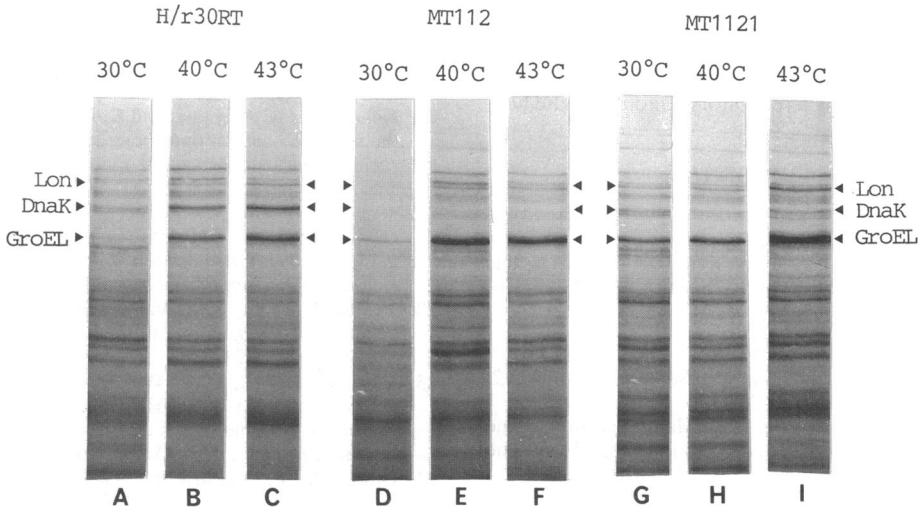

FIG. 7. Effects of the amber nonsense suppressor on synthesis of 69-kDa DnaK protein in the *dnaK7*(Ts) mutant cells. From among the spontaneous temperature-resistant revertants, strain MT1121 carrying both *dnaK7*(Ts) and an amber nonsense suppressor were isolated and analyzed. Cells were grown in M9CA medium at 30°C, and in the mid-log phase they were shifted to 40 or 43°C. After incubation for 5 min at these temperatures, the cells were labeled with [^{35}S]methionine for 6 min. The proteins were extracted, separated by SDS-PAGE, and autoradiographed. The positions of Lon, DnaK, and GroEL were identified by use of molecular mass standards in a separate gel. Strain H/r30RT cells were labeled at 30°C (A), 40°C (B), and 43°C (C); strain MT112 cells were labeled at 30°C (D), 40°C (E), and 43°C (F); strain MT1121 cells were labeled at 30°C (G), 40°C (H), and 43°C (I).

E. coli B and *E. coli* K-12 differed in 15 bp that did not alter the amino acid sequence. Thus, the DnaK protein is conserved and retains its primary structure in two subspecies of *E. coli* (Fig. 4). Cowing et al. (7) have identified three promoters (P1, P2, and P3) within 179 bp in the upstream region of the *dnaK* gene of *E. coli* K-12. Our sequence data showed that only 1 bp was different, at nucleotide position 57 (between the −35 region and the −10 region of P1) in *E. coli* B. This difference did not seem to affect the promoter activity since DnaK protein synthesis occurred well after heat shock, as seen in *E. coli* cells (as shown in Fig. 7; see also reference 15).

Our sequence data indicated that the *dnaK7*(Ts) mutation was an amber nonsense mutation resulting from a one-base substitution of T for C at nucleotide position 448 in the ORF causing premature termination and yielding a peptide of 149 amino acids instead of the normal DnaK protein of 683 amino acids (Fig. 5). The results were confirmed in two ways in which the product of premature termination was demonstrated by analysis with DNA-directed transcription and translation systems, as shown in Fig. 6. In this assay, a premature DnaK protein with a molecular mass of about 20 kDa was detected together with normal-size DnaJ protein due to expression of the plasmid DNA of pMT2 carrying the *dnaK7*(Ts)-*dnaJ*$^+$ segment (Fig. 6C). The nature of the *dnaK7*(Ts) mutation was also studied with strain MT112 by isolating revertants carrying the amber nonsense suppressor. Seven of 50 spontaneous temperature-resistant revertants were able to propagate the T4 amber phage and also showed suppression of an arginine-requiring phenotype due to the amber nonsense mutation as denoted by Ichikawa and Kondo (13). The slower growth rate of the *dnaK7*(Ts) mutant compared with that of wild-type cells at the permissive temperature (16) is probably related to this amber mutation and the low efficiency of suppression.

Deletion mutants of the *dnaK* gene have been isolated, and their cellular growth at 30°C has been examined (4, 5, 19). Results showed that during several generations of growth at 30°C, suppressor mutations accumulated in the *dnaK* deletion mutant strains. In the *dnaK7*(Ts) mutant cells, various suppressor mutations including the amber suppressor presumably act to support cellular growth, because mutant cells of strain MT112 could grow at 43°C because of the amber nonsense suppressor, as described here, and WK45 cells could grow at 40°C because of a mutation in the *sukA* or *sukB* gene located at 76 min on the *E. coli* chromosome (15). Cell growth of the mutant carrying the *dnaK7*(Ts) mutation at 30°C might be supported by some suppressors with low efficiencies.

Ezaki et al. (8) showed that the *seg-1* and *seg-2* mutations are located in the *dnaK* gene and that the active *dnaK* gene product is essential for replication of the mini-F plasmid. The *seg-1* mutation is homologous to one of the three missense mutations in the *dnaK756*(Ts) mutant gene.

Cegielska and Georgopoulos (6) studied the functional domains of DnaK protein by mutational analysis. First they investigated the potential domains for enzymatic activity by using a set of truncated DnaK proteins. From comparative analyses of the wild-type DnaK and mutant DnaK756 proteins, they suggested that the DnaK polypeptide is organized into at least two distinct functional domains: the amino-terminal portion is required for ATPase activity, while the carboxyl-terminal portion is responsible for autophosphorylating activity. When the mutant phenotypes of both *dnaK* mutants were compared by Tilly et al., one marked difference was pointed out (30). They have reported that the DnaK protein modulates the heat shock response negatively, because a *dnaK756*(Ts) mutant was unable to turn off induced synthesis of heat shock protein at 43°C. With the *dnaK7*(Ts) mutant cells, no such prolonged synthesis of heat shock protein was observed (15).

The cellular function of the DnaK protein is thought to be that of a molecular chaperone because the DnaK protein is highly homologous with the Bip protein (immunoglobulin heavy-chain binding protein) as described by Nicholson et al. (23). The DnaK protein is known to be required for renaturation of denatured λ repressor (10) and heat-inactivated RNA polymerase in an ATP hydrolysis-independent manner (28).

The DnaK protein seems to play a role as a molecular chaperone to sustain cellular functions through association with components of synthetic machines to form their functional structures in addition to having a regulatory function in the heat shock response.

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REFERENCES

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47:**180–230.
2. **Bardwell, J. C. A., and E. A. Craig.** 1984. Major heat shock gene of *Drosophila* and the *Escherichia coli* heat inducible *dnaK* gene are homologous. Proc. Natl. Acad. Sci. USA **81:**848–852.
3. **Bardwell, J. C. A., K. Tilly, E. Craig, J. King, M. Zylicz, and C. Georgopoulos.** 1986. The nucleotide sequence of the *Escherichia coli* K-12 *dnaJ*$^+$ gene. A gene that encodes a heat shock protein. J. Biol. Chem. **261:**1782–1785.
4. **Bukau, B., and G. C. Walker.** 1989. Cellular defects caused by deletion of the *Escherichia coli dnaK* gene indicate roles for heat shock protein in normal metabolism. J. Bacteriol. **171:**2337–2346.
5. **Bukau, B., and G. C. Walker.** 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.
6. **Cegielska, A., and C. Georgopoulos.** 1989. Functional domains of the *Escherichia coli dnaK* heat shock protein as revealed by mutational analysis. J. Biol. Chem. **264:**21122–21130.
7. **Cowing, D. W., J. C. A. Bardwell, E. A. Craig, C. Woolford, R. W. Hendrix, and C. A. Gross.** 1985. Consensus sequence for *Escherichia coli* heat shock gene promoters. Proc. Natl. Acad. Sci. USA **82:**2679–2683.
8. **Ezaki, B., T. Ogura, H. Mori, H. Niki, and S. Hiraga.** 1989. Involvement of DnaK protein in mini-F plasmid replication: temperature-sensitive *seg* mutations are located in the *dnaK* gene. Mol. Gen. Genet. **218:**183–189.
9. **Frischauf, A.-M., H. Lehrach, A. Poustka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170:**827–842.
10. **Gaitanaris, G. A., A. G. Papavassilrou, P. Rubock, S. Silverstein, and M. E. Gottesman.** 1990. Renaturation of denatured λ repressor requires heat shock protein. Cell **61:**1013–1020.
11. **Georgopoulos, C. P.** 1977. A new bacterial gene (*groPC*) which affects DNA replication. Mol. Gen. Genet. **151:**35–39.
12. **Georgopoulos, C. P., and I. Herskowitz.** 1971. *Escherichia coli* mutants blocked in lambda DNA synthesis, p. 553–564. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Ichikawa, H., and S. Kondo.** 1969. Comparative studies of radiation and chemical mutagenesis I. Jpn. J. Genet. **44**(Suppl. 2)**:**57–59.
14. **Itikawa, H., H. Fujita, and M. Wada.** 1986. High temperature induction of the stringent response in the *dnaK*(Ts) and *dnaJ*(Ts) mutants of *Escherichia coli*. J. Biochem. **99:**1719–1724.
15. **Itikawa, H., Y. Mishina, M. Wada, and H. Fujita.** 1992. Genetic mapping and biochemical characterization of suppressor mutations *sukA* and *sukB* for a *dnaK7*(Ts) mutation of *Escherichia coli* K-12. Jpn. J. Genet. **67:**17–27.
16. **Itikawa, H., and J.-I. Ryu.** 1979. Isolation and characterization of a temperature-sensitive *dnaK* mutant of *Escherichia coli* B. J. Bacteriol. **138:**339–344.
17. **Itikawa, H., M. Wada, K. Sekine, and H. Fujita.** 1989. Phosphorylation of glutaminyl-tRNA synthetase and threonyl-tRNA synthetase by the gene products of *dnaK* and *dnaJ* in *Escherichia coli* K-12 cells. Biochimie **71:**1079–1087.
18. **Iwasaki, H., T. Shiba, A. Nakata, and H. Shinagawa.** 1989. Involvement in DNA repair of the *ruvA* gene of *Escherichia coli*. Mol. Gen. Genet. **219:**328–331.
19. **Kang, P. J., and E. A. Craig.** 1990. Identification and characterization of a new *Escherichia coli* gene that is a doseage-dependent suppressor of a *dnaK* deletion mutation. J. Bacteriol. **172:**2055–2064.
20. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
21. **Murray, K., and N. E. Murray.** 1975. Phage lambda receptor chromosomes for DNA fragments made with restriction endonuclease III of *Haemophilus influenzae* and restriction endonuclease I of *Escherichia coli*. J. Mol. Biol. **98:**551–564.
22. **Neidhardt, F. C., R. A. VanBogelen, and V. Vaughn.** 1984. The genetics and regulation of heat-shock proteins. Annu. Rev. Genet. **18:**295–329.
23. **Nicholson, R. C., D. B. Williams, and L. A. Moran.** 1990. An essential member of the HSP70 gene family of *Saccharomyces cerevisiae* is homologous to immunoglobulin heavy chain binding protein. Proc. Natl. Acad. Sci. USA **87:**1159–1163.
24. **Ohki, M., F. Tamura, S. Nishimura, and H. Uchida.** 1986. Nucleotide sequence of the *Escherichia coli dnaJ* gene and purification of the gene product. J. Biol. Chem. **261:**1778–1781.
25. **Saito, H., and H. Uchida.** 1977. Initiation of the DNA replication of bacteriophage lambda in *Escherichia coli* K-12. J. Mol. Biol. **113:**1–25.
26. **Sanger, F., A. R. Coulson, B. G. Barrell, A. J. Smith, and B. A. Roe.** 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143:**161–178.
27. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
28. **Skowyra, D., C. Georgopoulos, and M. Zylicz.** 1990. The *E. coli dnaK* gene product, the hsp70 homolog, can reactivate heat-inactivated RNA polymerase in an ATP hydrolysis-dependent manner. Cell **62:**939–944.
29. **Sunshine, M., M. Feiss, J. Stuart, and J. Yochem.** 1977. A new host gene (*groPC*) necessary for lambda DNA replication. Mol. Gen. Genet. **151:**27–34.
30. **Tilly, K., N. McKittrick, M. Zylicz, and C. Georgopoulos.** 1983. The *dnaK* protein modulates the heat-shock response of *Escherichia coli*. Cell **34:**641–646.
31. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
32. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. Methods Enzymol. **153:**3–11.
33. **Wada, M., H. Fujita, and H. Itikawa.** 1987. Genetic suppression of a temperature-sensitive *groES* mutation by an altered subunit of RNA polymerase of *Escherichia coli* K-12. J. Bacteriol. **169:**1102–1106.
34. **Wada, M., and H. Itikawa.** 1984. Participation of *Escherichia coli* K-12 *groE* gene products in the synthesis of cellular DNA and RNA. J. Bacteriol. **157:**694–696.
35. **Wada, M., Y. Kadokami, and H. Itikawa.** 1982. Thermosensitive synthesis of DNA and RNA in *dnaJ* mutants of *Escherichia coli* K-12. Jpn. J. Genet. **57:**407–413.
36. **Wada, M., K. Sekine, and H. Itikawa.** 1986. Participation of the *dnaK* and *dnaJ* gene products in phosphorylation of glutaminyl-tRNA synthetase and threonyl-tRNA synthetase of *Escherichia coli* K-12. J. Bacteriol. **168:**213–220.
37. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
38. **Yochem, J., H. Uchida, M. Sunshine, H. Saito, C. P. Georgopoulos, and M. Feiss.** 1978. Genetic analysis of two genes, *dnaJ* and *dnaK*, necessary for *Escherichia coli* and bacteriophage lambda DNA replication. Mol. Gen. Genet. **164:**9–14.
39. **Zylicz, M., J. H. LeBowitz, R. McMacken, and C. P. Georgopoulos.** 1983. The *dnaK* protein of *Escherichia coli* possesses an ATPase and autophosphorylating activity and is essential in an *in vitro* DNA replication system. Proc. Natl. Acad. Sci. USA **80:**6431–6435.