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Structure and Expression of the *dnaKJ* Operon of *Buchnera*, an Intracellular Symbiotic Bacteria of Aphid[1]

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Buchnera sp., an intracellular symbiont of the pea aphid (Acyrthosiphon pisum Harris), is a close phylogenetical relative of Escherichia coli, and synthesizes a large amount of symbionin, a GroEL homolog. The other heat shock protein homologs, which are not expressed as much as symbionin, have not been studied yet. In this study, we cloned the dnaK and dnaJ genes of Buchnera, and revealed that its DnaK and DnaJ are structurally very similar to those of E. coli. Amino acid residues and motifs proposed so far to be essential for the function of the E. coli DnaK and DnaJ were completely conserved in the Buchnera counterparts. However, Buchnera dnaKJ operon could not fully complement mutations of either dnaK or dnaJ of E. coli. This is probably because of a difference in net charge of DnaK and DnaJ between Buchnera and E. coli, and a unique structure of Buchnera DnaJ that prevents heterologous components from operating in concert. Buchnera dnaK and dnaJ formed an operon whose transcription is governed by a promoter structurally homologous to heat shock promoters of E. coli, although the cellular amount of dnaKJ mRNA was not affected by heat shock. Two inverted repeats flanking both sides of E. coli dnaJ were also found in the gene of Buchnera at the corresponding positions, suggesting that expression ratio of DnaK to DnaJ is regulated in a similar manner in the two organisms.

Key words: aphid, *Buchnera*, DnaJ, DnaK, symbiosis.

Buchnera species are a group of bacteria living in specialized cells of aphids (Homoptera, Insecta) (*1*). Phylogenetically, they are close relatives of Enterobacteriaceae and are classified into the γ-subdivision of Proteobacteria (*2*). The intracellular symbiosis of *Buchnera* with aphid was suggested to date back to 180-250 million years ago (*3*). During this period, *Buchnera* species have lost the ability to live outside the host's cells, and have become adapted to the intracellular environment provided by the host. As a result, the protein pattern expressed by the bacterium is quite different from that of *Escherichia coli*, a close relative of *Buchnera* (*2*). *Buchnera* species selectively synthesize a large amount of the protein named symbionin (*4*). Molecular cloning of the gene encoding symbionin (*symL*) revealed that this protein is a homolog of an *E. coli* heat shock (HS) protein GroEL, which functions as molecular chaperone (*5*). It has been suggested that symbionin functions as not only a molecular chaperone, but also a component of a signal transducing pathway unique to this symbiont (*6*).

In *E. coli*, the structures of the promoters for several HS genes are highly conserved (*7*). RNA polymerase containing the HS-specific sigma factor σ^{32} recognizes those HS promoters and starts transcription (*7, 8*). Although the cellular level of σ^{32} under normal conditions is kept low by the regulatory mechanisms operating both in synthesis, especially in translation, and in degradation, the level is dramatically elevated under stress such as HS, leading to induction of many HS proteins (*8-10*). It was suggested that many Gram-negative bacteria share with *E. coli* this mechanism of regulation of expression of the HS genes (*11*). Recently, we found that *Buchnera* has a gene encoding σ^{32}, and that transcription of *symL* is governed by a promoter that is homologous to the HS promoters of *E. coli* (*12*). In view of the fact that symbionin is the only HS protein that is synthesized constitutively in a large amount in *Buchnera* (*4*), it is possible that the other HS genes of this bacterium are controlled differently from *symL*, whose expression perhaps depends on σ^{32} (*12*).

DnaK is a major HS protein of bacteria. It has the weak ATPase activity, and binds to substrate proteins to alter or maintain their conformation (*13*). This chaperone activity of DnaK is modulated by interaction with other HS proteins, DnaJ and GrpE (*14*). In *E. coli*, the *dnaK* and *dnaJ* genes form an operon, whose transcription is governed by two strong HS promoters and a weak σ^{70}-cognate promoter (*7*). The DnaK/DnaJ/GrpE complex, through binding to σ^{32}, interferes with its binding to core RNA polymerase, and makes it sensitive to degradation (*15*). As expected from this negative feedback system, *E. coli* strains with

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Abbreviations: aa, amino acid; HS, heat shock; IR, inverted repeat; nt, nucleotide; *ts*, temperature sensitive.

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mutation in either *dnaK* or *dnaJ* overproduce the HS proteins (*10*). The fact that *Buchnera* vigorously synthesizes symbionin constitutively may suggest that the symbiotic bacterium has lost at least a part of the negative feedback mechanism operating in *E. coli*. To test this hypothesis, we examined the structure and expression of the *dnaK* and *dnaJ* genes of *Buchnera*.

MATERIALS AND METHODS

Bacteria—Buchnera cells were collected from the pea aphid, *Acyrthosiphon pisum* (Harris), by the method described previously (*6*). *E. coli* strains NRK156, KY1455, and KY1456, derivatives of strain MC4100 (*16*), were constructed by introducing *dnaK756*, *dnaK204*, and *dnaJ*::Tn*10-42*, respectively (*17*). All the *E. coli* strains used in this study were cultured in LB medium.

Western Blotting—Total proteins of bacteria were separated by 10% SDS-PAGE, and then transferred to PVDF membrane. The membrane was soaked in buffer containing either rabbit anti-*E. coli* DnaK antibody (StressGen Biotechnologies) or rabbit anti-*E. coli* GroEL antibody (StressGen Biotechnologies), and reaction on the membrane was detected using the ABC reagent (Vector), as described elsewhere (*18*).

Molecular Cloning—According to the nucleotide (nt) sequence of *E. coli dnaK*, we synthesized two synthetic primers, DNAK1 and DNAK2, which encode amino acid residues highly conserved among many bacterial DnaKs (from M1 to I7, and from A144 to Q150 of *E. coli* DnaK) (*19*). Using these primers, a DNA fragment encoding the N-terminal part of DnaK was amplified by PCR from the *Buchnera* genomic DNA. Subsequently, DNA segments flanking this region were amplified from the genomic DNA using the cassette PCR technique (*20*). PCR-amplified fragments were subcloned into pBluescriptII SK− (Stratagene) to generate pBUK1, pBUK2, pBUK3, and pBUJ1 (Fig. 1), and then sequenced using a Hitachi SQ-5500 autosequencer.

Northern Blotting—Buchnera cells were isolated from the host and incubated at 40°C for 0, 5, 10, or 20 min, and the total cellular RNA was extracted from the cells. Each sample containing 2 μg of RNA was subjected to agarose gel electrophoresis, and transferred from the gel to nylon membrane. The insert of pBUK1 (Fig. 1) was labeled with ^{32}P and then hybridized with the *dnaKJ* mRNA on the membrane. Signals were detected by autoradiography.

Primer Extension Analysis—The 5′-end of DNAK3 primer (Fig. 2) was labeled with ^{32}P. Using this labeled primer, cDNA complementary to the *dnaKJ* mRNA was synthesized with AMV reverse transcriptase from the *Buchnera* total RNA prepared just after the isolation of symbionts from the host. The size of cDNA was estimated by comparing the position of its signal with a sequence ladder for the *dnaKJ* operon developed alongside the lane for the cDNA on PAGE.

Complementation Test—A PCR primer DNAK5 (5′-AT-CGAATTCTAAATAGGAGAAACTTTAATGGGTA-3′) which hybridized to the region around the translational start codon of *Buchnera dnaK* was synthesized. Using this, a DNA fragment encoding the entire DnaK and the N-terminal part of DnaJ was amplified by PCR from the *Buchnera* genomic DNA attached to a PCR cassette at a *Hin*dIII site (underlined in Fig. 2). The PCR fragment was digested with *Eco*RI and *Hin*dIII, and recombined with pUC18 together with the *Hin*dIII-*Sal*I fragment of the pBUJ1 insert (Fig. 1) to generate the plasmid pBUKJ1 which contained the entire coding region of both *dnaK* and *dnaJ* of *Buchnera*. The plasmid pKV8000 (constructed by Chieko Wada, Institute for Virus Research, Kyoto University) is a derivative of B10-a carrying the entire *E. coli dnaKJ* operon. Each *E. coli* strain was transformed with either pBUKJ1, pKV8000, or pUC18, spread on an LB agar plate containing ampicillin (50 μg/ml) and incubated at 30°C for 16 h. A colony formed on each plate was picked up, spread on a fresh LB agar plate containing ampicillin (50 μg/ml), tetracycline (25 μg/ml), and 0.2 mM isopropylthiogalactoside and incubated for 16 h at an indicated temperature to examine its colony formation ability.

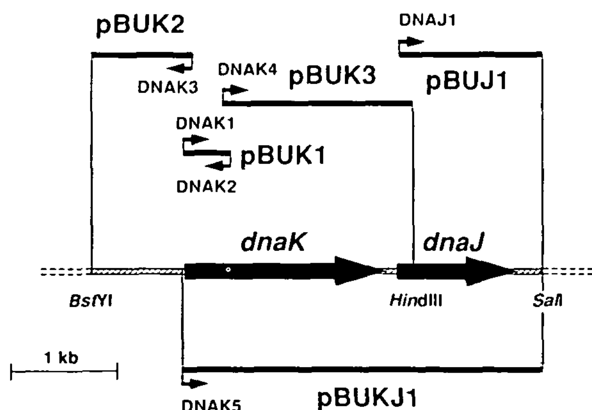

Fig. 1. **Experimental strategy in molecular cloning of the *Buchnera dnaKJ* operon.** Coding regions of the *dnaK* and *dnaJ* genes of *Buchnera* are indicated by thick arrows directed from 5′ to 3′. Solid lines above these arrows indicate individual PCR fragments subcloned into the respective plasmids. An insert of pBUKJ1, used in the complementation test, is shown below the thick arrows. Small arrows represent oligonucleotides synthesized as PCR primers.

RESULTS

Structure of DnaK and DnaJ of Buchnera—We found that *Buchnera* isolated from the insect host contains a protein which can specifically cross-react with antibody raised against *E. coli* DnaK (Fig. 3). This suggested that DnaK of *Buchnera* is structurally similar to that of *E. coli*. According to the protein database, the amino acid sequence of the N-terminal half of DnaK is highly conservative among different organisms. Consequently, as the first step to determine the nucleotide sequence of *Buchnera dnaK* gene, we synthesized a pair of PCR primers whose sequences coded for parts of the N-terminal half of *E. coli* DnaK (Figs. 1 and 2). By PCR using these primers, we successfully obtained a DNA fragment corresponding to a part of *Buchnera dnaK*. As shown in Fig. 1, flanking sequences of this DNA segment were subsequently amplified by cassette PCR (*20*) using additional synthetic primers. In the course of this study, we found that the gene encoding DnaJ of this bacterium is present in a region downstream of *dnaK*, just as is the case with *E. coli*. Finally, we determined the entire sequences of these genes (Fig. 2).

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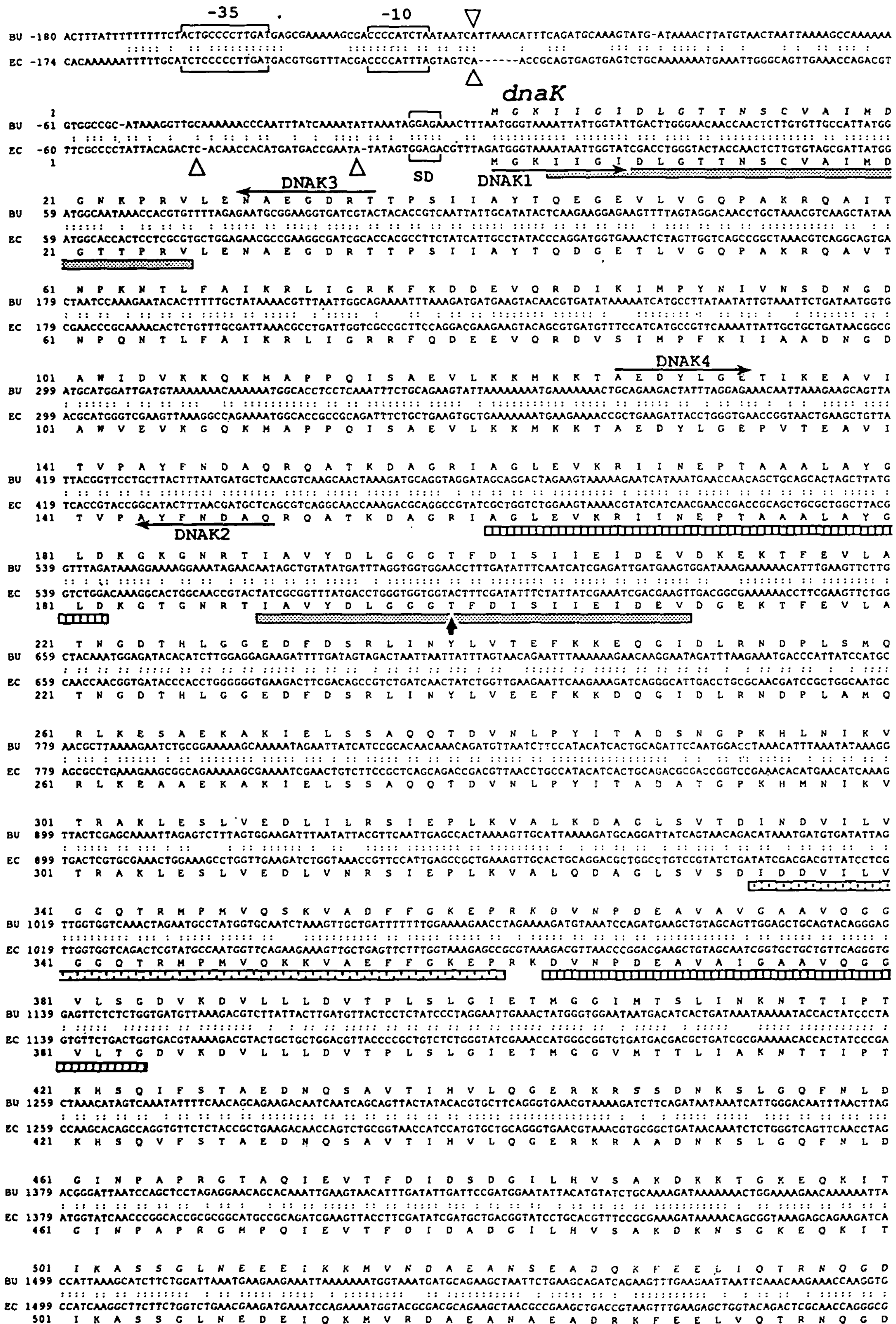

Fig. 2 (continued on next page)

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It turned out that the primary structure of *Buchnera* DnaK is very similar to that of *E. coli* DnaK (Fig. 2 and Table I). The residues which are essential to the function of DnaK of *E. coli*, such as D8, E171, D194, and D201 for Mg^{2+} binding (*21-23*), T199 which is essential for ATP hydrolysis and is autophosphorylated *in vitro* (*24*), and

Fig. 2

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Fig. 2. **Alignment of the *dnaKJ* operons.** Alignment of amino acid sequences was achieved mainly using the GENETYX-MAC program with final minor manual adjustment. Regions of *E. coli* DnaK corresponding to the phosphate binding motifs, connection motif, and adenosine binding motif are indicated by shaded boxes, a hatched box, and a dotted box, respectively. The autophosphorylation site of *E. coli* DnaK (T199; Ref. *22*) was marked by a vertical arrow. Regions of *E. coli* DnaJ corresponding to the J-domain, G/F motif, and four repeats of the CXXCXGXG motif were indicated by an open box, a closed box, and a pair of large vertical brackets, respectively. Nucleotide sequences encoding the proteins are written between the alignment of amino acid sequences, with a colon when a residue was shared by the two sequences. Transcription start sites are marked by open triangles, and the conserved "HS promoter 1" sequence is enclosed by vertical brackets. The two IRs mentioned in "DISCUSSION" are indicated by pairs of horizontal arrows. The sites hybridized to the synthetic PCR primers are shown by thin arrows, and the *Hin*dIII site used in cassette PCR is underlined. BU, *Buchnera*; EC, *E. coli*.

TABLE I. **Structural similarity of DnaK and DnaJ of *Buchnera* to those of *E. coli*.**[a]

	DnaK		DnaJ	
	Buchnera	*E. coli*	*Buchnera*	*E. coli*
Total aa number[b]	637	638	377	376
Molecular weight[b]	70,041	69,114	42,348	41,100
Predicted pI[b]	5.22	4.68	9.33	7.72
Similarity of aa sequence[c]				
Total aa number	636		375	
Identical	530 (83.3%)		275 (73.3%)	
Homologous[d]	622 (97.8%)		358 (95.5%)	
Identity of the nt sequence of encoding gene[e]				
Total nt number	1,908		1,125	
Identical	1,331 (69.8%)		729 (64.9%)	
1st position[f]	526 (82.7%)		282 (75.2%)	
2nd position[f]	589 (92.6%)		323 (86.1%)	
3rd position[f]	216 (34.0%)		124 (33.1%)	

[a]Each value listed in this table was calculated using the computer program GENETYX-MAC (Version 8.0). [b]Including the first methionine residue. [c]Only the pairs of aa residue aligned in Fig. 2 were analyzed; those pairing with a gap were omitted. [d]"Identical" + "Similar." [e]Only the residues encoding the aa residue used in the above analysis were compared. [f]Each "Identical" nt was classified by the position in the codon.

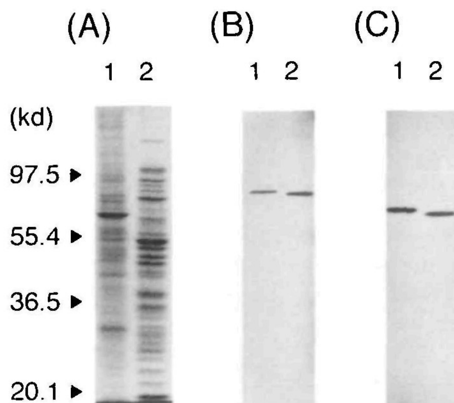

Fig. 3. **Expression of HS protein homologs in *Buchnera*.** (A) SDS-PAGE of total protein. Two micrograms of total protein of *Buchnera* (lane 1) and *E. coli* (lane 2) was subjected to 10% SDS-PAGE and visualized by Coomassie Blue staining. (B, C) Immunoblotting. Either 1 μg (B) or 250 ng (C) of total protein of *Buchnera* (lane 1) and *E. coli* (lane 2) was subjected to 10% SDS-PAGE, and DnaK (B) and GroEL (symbionin) (C) were detected by immunoblotting with antibodies against those proteins of *E. coli*.

A174, which is required for synergistic activation of its ATPase activity by DnaJ and GrpE (*23*), were completely conserved. The residues forming characteristic motifs in the N-terminal half of *E. coli* DnaK (*25*) were also highly conserved in that of *Buchnera*. Although the predicted pI of *Buchnera* DnaK was a little more basic than that of the *E. coli* DnaK (Table I), the high similarity in primary structure between the two suggested that *Buchnera* DnaK might functionally substitute for that of *E. coli*.

Not only *Buchnera* DnaK, but also its DnaJ was very similar to that of *E. coli* (Fig. 2 and Table I). However, as found in DnaK, the predicted pI of *Buchnera* DnaJ was also more basic than that of *E. coli* DnaJ (Table I). It is known that there are some conserved motifs in DnaJ and its homologs. As shown in Fig. 2, the amino acid residues in the N-terminal "J-domain" which is necessary for DnaJ's binding to DnaK (*26*) were especially highly conserved in *Buchnera* DnaJ. In addition, four repeats of CXXCXGXG sequence, which are proposed to be involved in the association of DnaJ with several substrate proteins (*27*), were also conserved in *Buchnera* DnaJ. On the other hand, a cluster

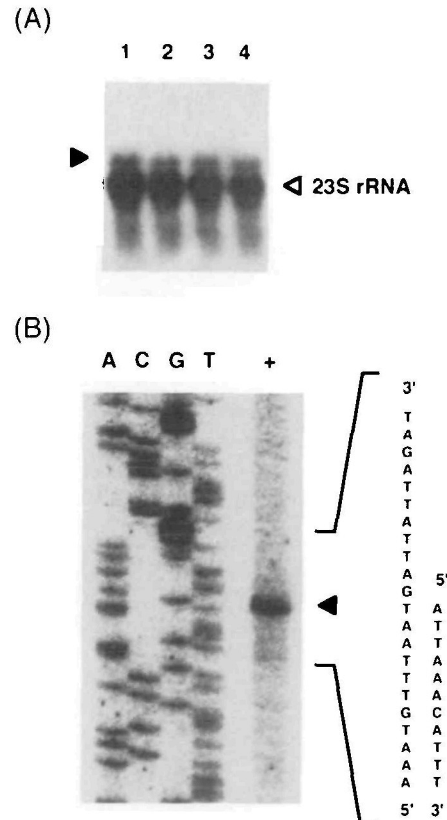

Fig. 4. **Transcription of the *Buchnera dnaKJ* operon.** (A) Northern blotting. *Buchnera* cells were incubated at 40°C for 0-20 min after isolation from the host insects, then the total RNA was subjected to electrophoresis, and hybridized with a probe specific to the *dnaK* sequence. The signal of *dnaKJ* mRNA is indicated by a closed triangle. The position corresponding to the 23S rRNA is indicated by an open triangle. Lanes 1, 2, 3, and 4: cells incubated for 0, 5, 10, and 20 min. (B) Primer extension analysis. The cDNA complementary to the *dnaKJ* mRNA (lane +) was separated alongside the sequence ladder (lanes A, C, G, and T) on polyacrylamide gel. The nucleotide sequence around the transcription start site is shown on the right.

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of glycine residues (from G77 to G87 of *E. coli* DnaJ) in the G/F motif (*28*), which had been found commonly in many bacterial DnaJ proteins (*29*), was missing from *Buchnera* DnaJ, although a region corresponding to the DIF repeats following the glycine cluster of *E. coli* DnaJ, which are essential to the function of DnaJ (*28*), was conserved almost completely.

Structural Characteristics in Buchnera dnaKJ Operon—As shown in Fig. 2, *dnaK* and *dnaJ* were tandemly arranged in the *Buchnera* genome with a short intergenic sequence between them, which suggested that these two genes may form an operon, as in *E. coli*. Northern blotting with a hybridization probe corresponding to a segment of *dnaK* indicated that the size of mRNA encoding DnaK of this bacterium is about 3.2 kb (Fig. 4A). An RNA of the same size was also detected in an experiment using a segment of *dnaJ* as a hybridization probe (data not shown). The size of these mRNAs agreed well with the summation of the size of *dnaK* and *dnaJ*, suggesting that these two genes form a transcriptional operon, and that transcription starts just upstream of *dnaK* and ends just downstream of *dnaJ*. A very strong signal at the position corresponding to the 23S rRNA (Fig. 4), which was also detected with a *dnaJ* specific probe (data not shown), is probably due to degradation of the transcript described above.

Primer extension analysis suggested that there is only one promoter operating for transcription of the *dnaKJ* operon of *Buchnera* (Fig. 4B). The 5′ end of the *dnaKJ* mRNA was the adenine residue residing at 121 bp upstream of the translation start codon of *dnaK* (Figs. 2 and 4B). The nucleotide sequence around the promoter of *Buchnera dnaKJ* operon coincided with that of the HS promoter 1 of *E. coli* (*7*). On the contrary, the regions corresponding to the HS promoter 2 and the σ^{70}-cognate promoter (*7*) were not identified in the *Buchnera* sequence. Although the promoter of *Buchnera dnaKJ* operon was structurally similar to the HS promoters of *E. coli*, its transcription was totally unaffected by incubation at 40°C (Fig. 4A).

Incomplete Ability of Buchnera dnaK and dnaJ to Complement E. coli Mutants—The similarity in primary structure suggested that *dnaK* and *dnaJ* of *Buchnera* might functionally complement the respective mutants of *E. coli*. We constructed a plasmid pBUKJ1 which contained the entire sequence of the *Buchnera dnaKJ* operon (Fig. 1), and introduced it into *ts* mutant strains of *E. coli* to examine whether expression of the *Buchnera dnaKJ* operon could support growth of the mutants at restrictive temperatures. As expected, the growth-limiting temperature of a *dnaK* mutant strain KY1455 (*dnaK204*) was elevated significantly when the cell was transformed with the pBUKJ1 plasmid. However, the transformant could not survive at temperatures over 40°C, while the mutant transformed with pKV8000, containing the entire *E. coli dnaKJ* operon, could grow even at 43°C (Table II). On the other hand, a complementation test using the *E. coli* mutant strain NRK156 (*dnaK756*) or KY1456 (*dnaJ*::Tn*10-42*) revealed that *Buchnera dnaKJ* operon could not complement these mutations at all (Table II). The *E. coli* strain MC4100 transformed with pBUKJ1 grew normally at any temperature tested (Table II).

TABLE II. **Complementation of *E. coli* mutants.**

Host	Plasmid	Colony formation[a]			
		30°C	37°C	40°C	43°C
MC4100 (wt)					
	pKV8000	++	++	++	++
	pBUKJ1	++	++	++	++
	pUC18	++	++	++	++
NRK156 (*dnaK756*)					
	pKV8000	++	++	++	++
	pBUKJ1	++	++	++	−
	pUC18	++	++	++	−
KY1455 (*dnaK204*)					
	pKV8000	++	++	+	+
	pBUKJ1	++	+	−	−
	pUC18	++	−	−	−
KY1456 (*dnaJ*::Tn*10-42*)					
	pKV8000	++	+	+	−
	pBUKJ1	++	−	−	−
	pUC18	++	−	−	−

[a]Colony formation ability of each *E. coli* strain transformed with the indicated plasmid on an LB plate was tested at the respective temperature. ++, vigorous; +, faint; −, no colonies.

DISCUSSION

In this study, we performed the cloning and characterization of *dnaK* and *dnaJ* of *Buchnera*. As shown in Table I, these genes encoded proteins whose primary structures were very similar to those of the corresponding *E. coli* proteins, as found for other genes of *Buchnera* so far cloned (*5, 30*). All the amino acid residues and motifs proposed to be necessary for the function of DnaK and DnaJ of *E. coli* were also found in these *Buchnera* proteins (Fig. 2). However, our observations suggested that neither *dnaK* nor *dnaJ* of this symbiont could fully complement defective mutation in the respective genes of *E. coli* (Table II). Immunoblotting showed that the products of these *Buchnera* genes were expressed in the *E. coli* transformants (data not shown), suggesting that the inability of these *Buchnera* genes to complement the respective *E. coli* mutants fully is probably due to the intrinsic nature of the product proteins.

The growth-limiting temperature of NRK156, a *dnaK ts* strain of *E. coli*, did not change even if the strain carried the pBUKJ1 plasmid. Although the other *dnaK* mutant strain KY1455 could grow at slightly higher temperatures than otherwise if transformed with pBUKJ1, the transformed cells could not survive at 40°C, while the transformant with pKV8000, containing *E. coli* wt *dnaK*J operon, could grow at 43°C (Table II). *Buchnera* DnaK is structurally similar to that of *E. coli*, but the predicted pI of the former was significantly more basic due to minor substitutions of amino acid residues (Table I). The basic charge of *Buchnera* DnaK possibly reduces the electrostatic affinity of this protein for DnaJ/GrpE of *E. coli*, and thus inhibits the association among the proteins which is essential for full activity as a molecular chaperone at higher temperatures (*14*). Likewise, the *Buchnera dnaKJ* operon did not change the growth-limiting temperature of an *E. coli dnaJ* mutant KY1456 at all, although almost all the elements that had been proposed to be essential for the function of *E. coli* DnaJ were found in *Buchnera* DnaJ (Fig. 2). It was reported that the G/F motif, a 35-aa region following the J-domain of *E. coli* DnaJ, is necessary for stabilization of the binding between DnaJ and its substrate (*28*). Although the cluster of glycine residues itself was not responsible for this

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function (*28*), the existence of this motif in many DnaJ species may suggest its involvement in some function of the protein, probably by endowing it with flexibility (*31*). In *Buchnera* DnaJ, most of the glycine residues in this region have been replaced by other residues with larger side chains. A kind of rigid structure involving these residues may affect the higher-order structure of DnaJ by interfering with intramolecular interaction between functional domains, resulting in a decrease in its affinity for DnaK. In addition, the predicted difference in pI of *Buchnera* DnaJ from that of the *E. coli* counterpart was more striking than that between the two DnaKs (Table I). More basic pI and/or the difference in higher-order structure may inhibit *Buchnera* DnaJ's binding to *E. coli* DnaK. In view of the fact that the *Buchnera*-aphid symbiosis tends to be disrupted at higher temperatures (*32*), it is possible that at such temperatures as are employed for the complementation tests, the *Buchnera* DnaK/DnaJ complex is not functional even in the symbiont cell.

Two pairs of inverted repeats (IR), each of which probably forms a stable hairpin-loop, are present in the *dnaKJ* operon of *E. coli* (*33*). One is present at the intergenic region between *dnaK* and *dnaJ*, and probably regulates the molar ratio of DnaK to DnaJ, and the other, proposed to be a transcriptional terminator, is present just downstream of *dnaJ*. These two IRs were also present at the corresponding regions of the *Buchnera dnaKJ* operon (Fig. 2). Although the nucleotide sequence of the second IR just downstream of *dnaJ* is not precisely conserved, probably this element also functions as a transcriptional terminator in *Buchnera*, because the size of the *dnaKJ* mRNA detected indicated that transcription is terminated around the IR (Fig. 4A). It should be emphasized that the sequence of the first IR at the *dnaK-dnaJ* intergenic region is highly conserved between *Buchnera* and *E. coli* (Fig. 2), because non-coding regions of their *dnaKJ* operons in general are largely divergent structurally from each other. The expression ratio of *dnaK* to *dnaJ* in *Buchnera* may be regulated by a similar mechanism to that in *E. coli* by a hairpin-loop formed by this IR.

Primer extension analysis suggested that there is only one promoter operating for the *dnaKJ* operon in *Buchnera* (Fig. 4B). The unique promoter of the *Buchnera dnaKJ* operon structurally resembled the HS promoter 1 of *E. coli* (*7*). On the other hand, we could not identify a region corresponding to the HS promoter 2, or the σ^{70}-cognate promoter, of *E. coli* in the *Buchnera* sequence because the nucleotide sequence of the 5′ flanking region of *dnaK*, except for the region mentioned above, was highly divergent between the two organisms (Fig. 2). Recently, we demonstrated that transcription of *symSL* is governed by a promoter whose structure agrees well with the consensus of the HS promoters of *E. coli* (*12*). Taken together with this, our findings in this study suggested that transcription of the HS genes of *Buchnera*, in general, is governed by a common factor, probably σ^{32}, just as in *E. coli*. However, the cellular amounts of *dnaKJ* mRNA (Fig. 4A) and *symSL* mRNA (*12*) were not affected at all by incubation at higher temperature. In addition, the cellular level of σ^{32} is very small in *Buchnera* (*12*), although the bacterium produces a large amount of symbionin constitutively (*4*). Moreover, immunoblotting suggested that the amount of DnaK protein is not affected by HS (data not shown), indicating that expression of *Buchnera dnaK* is probably independent of translational regulation. All these data suggested that in *Buchnera*, HS genes are constitutively expressed to a certain extent under the influence of the small amount of σ^{32} that is continuously produced. It may be the case that *Buchnera* produces large amounts of HS proteins constantly in this manner to survive incessant attack by the host's defense system. Alternatively, large amounts of HS proteins may be required to endure deleterious mutations in a number of genes that have been accumulated during the history of intracellular symbiosis with the host (*34*).

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