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An analogue of the DnaJ molecular chaperone in *Escherichia coli*

(heat shock protein/DNA-binding protein)

CHIHARU UEGUCHI, MINORU KAKEDA, HISAMI YAMADA, AND TAKESHI MIZUNO

Laboratory of Molecular Microbiology, School of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464, Japan

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ABSTRACT **_Escherichia coli_ DnaJ functions as a typical molecular chaperone in coordination with other heat shock proteins such as DnaK and GrpE in a variety of cellular processes. In this study, it was found that _E. coli_ possesses an analogue of DnaJ, as judged from not only its primary structure but also its possible function. This protein, named CbpA (for curved DNA-binding protein), was first identified as a DNA-binding protein that preferentially recognizes a curved DNA sequence. Cloning and nucleotide sequencing of the gene encoding CbpA revealed that the predicted product is very similar to DnaJ in amino acid sequence: overall identity is 39%. The _cbpA_ gene functions as a multicopy suppressor for _dnaJ_ mutations. The mutational lesions characteristic of a _dnaJ_ null mutant—namely, temperature sensitivity for growth and defects in λ phage and mini-F DNA replication—were all restored upon introduction of the _cbpA_ gene on a multicopy plasmid. An insertional mutant of _cbpA_ was also isolated, which showed no noticeable phenotype, particularly with regard to temperature sensitivity for growth. However, when this _cbpA::kan_ allele was combined with the _dnaJ_ null allele, the resultant strain was unable to grow at 37°C, at which strains carrying each mutation alone could grow normally. These genetic results are interpreted as meaning that the function(s) of CbpA in _E. coli_ is closely related to that of DnaJ.**

The heat shock response occurs when cells growing at a low temperature are shifted to a higher temperature and results in the induction of a subset of proteins called heat shock proteins. This response to temperature is nearly universal among prokaryotes and eukaryotes. This heat shock family of proteins has recently become central to the study of correct folding of nascent polypeptides, assembly of protein complexes, and uptake of proteins into organelles (1). Therefore, some heat shock proteins are widely recognized as "molecular chaperones" that play crucial roles even under physiological conditions (2, 3).

Although the heat shock response has been extensively studied in both prokaryotic and eukaryotic organisms, *Escherichia coli* is one of the best examples characterized in terms of the underlying molecular mechanism. Among *E. coli* heat shock proteins, DnaJ was first identified as a host factor required for DNA replication of λ phage many years ago (4–7). Subsequently DnaJ has been shown to be involved in a variety of cellular processes, including DNA replication [e.g., *E. coli* chromosome (6), P1 plasmid (8) and fertility factor F (9)], protein folding (10), and protein translocation (11). In these processes, DnaJ functions as a typical molecular chaperone in coordination with other heat shock proteins such as DnaK and GrpE (1). As in the case of DnaK (or Hsp70), DnaJ homologues have also been discovered in a number of organisms, including higher eukaryotes (for review, see ref. 12). Here, we provide evidence that *E. coli* itself possesses an analogue of DnaJ, which displays an extensive degree of amino acid sequence homology.

There have been numerous reports of DNA curvature playing potential roles in certain processes, such as DNA replication, recombination, and transcription (13). As an approach to explore such functions of curved DNA, we previously searched for *E. coli* proteins that preferentially recognize curved DNA sequences. As a result, the *E. coli* nucleoid protein H-NS was identified as a putative curved DNA-binding protein (14). During the course of these studies, we purified another DNA-binding protein (named CbpA). Determination of its N-terminal amino acid sequence suggested that CbpA was an analogue of DnaJ. Thus, cloning and nucleotide sequencing of the gene encoding this protein were done. A series of genetic studies were conducted, including the isolation of a null mutant of the *cbpA* gene, which indicated that CbpA is an analogue of DnaJ, as judged from not only its structure but also its possible function.

MATERIALS AND METHODS

Materials. DNA-manipulating enzymes were mainly from Takara Shuzo (Kyoto) or Toyobo (Osaka). [α-^{32}P]dCTP (110 TBq/mmol) and [γ-^{32}P]ATP (220 TBq/mmol) were from Amersham. Degenerate oligonucleotides (5′-dATGGAYRT-NAAYGARTARTARG) were synthesized with an automated Applied Biosystems DNA synthesizer (Center for Gene Research, Nagoya University).

Bacterial Strains and Plasmids. A set of derivatives of *E. coli* strain MC4100 [F$^-$, *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*] were used (9, 15–18). Plasmids pCU48 and pCU49 were constructed as follows: an *E. coli* chromosomal *Eco*RI fragment (3.2 kb) encompassing the *cbpA* gene was isolated from λ phage 5A12 and then cloned into a unique *Xba* I site of plasmid pKY184 (19) in both orientations (plasmids pCU48 and pCU49, respectively). Plasmid pMK13 was constructed as follows: an *Nde* I–*Mlu* I fragment encompassing the *cbpA* gene and ORF-2 (open reading frame) was isolated from pCU48 (see Fig. 2). This fragment was inserted into a unique *Sma* I site of an overexpression vector, pUSI2 (20). This vector contains the *tac* promoter in front of the *Sma* I site. Similarly, plasmids pCU55 and pCU56 were constructed as follows: an *Nde* I–*Eco*RI fragment carrying only the *cbpA* gene was prepared from pCU49-6 (a deletion derivative of pCU48, the *Eco*RI site being derived from the vector) and then inserted into a unique *Sma* I site of pUSI2. In the resultant plasmids, the *cbpA* gene was inserted in both orientations relative to the *tac* promoter (see Fig. 2).

Purification of CbpA. An *E. coli* strain CU211 [Δ*hns::neo* Δ(*pro-lac*) *thi ara*] (21) was grown in Luria broth and harvested at the late-logarithmic phase. The cells (115 g) were suspended in 300 ml of a 10 mM Tris acetate, pH 7.8/5.6 mM magnesium acetate/24 mM potassium acetate/150 mM su-

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Abbreviations: IPTG, isopropyl β-D-galactopyranoside; ORF, open reading frame.

crose/0.6 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride buffer. The suspension was passed through a French pressure cell twice at 10,000 psi (1 psi = 6.9 kPa). The lysate was centrifuged at 120,000 × *g* for 3 hr. The resultant cleared lysate was precipitated by ammonium sulfate (40–60% saturation). The precipitate was suspended in 50 ml of a 10 mM potassium phosphate, pH 6.9/2 mM 2-mercaptoethanol buffer and then dialyzed against the same buffer at 4°C. The sample (2.9 g of protein) was applied to a P11-phosphocellulose (Whatman) column (2 cm × 42 cm) previously equilibrated with the same buffer, and then proteins were eluted with a 700-ml linear potassium phosphate gradient (10–700 mM). Fractions containing CbpA, eluted around 400 mM potassium phosphate, were pooled and dialyzed against a 10 mM potassium phosphate, pH 7.5/2 mM 2-mercaptoethanol/1 mM EDTA buffer. The sample (19 mg of protein) was applied to a heparin-Sepharose CL-68 (Pharmacia) column (1 cm × 13 cm) previously equilibrated with the same buffer, and then proteins were eluted with a 260-ml linear NaCl gradient (0–800 mM). The fractions containing CbpA, eluted around 500 mM NaCl, were finally applied to a fast protein liquid chromatography column system (Pharmacia LKB) equipped with a Mono Q column after being dialyzed against a 10 mM potassium phosphate, pH 7.5/2 mM 2-mercaptoethanol/1 mM EDTA buffer. The chromatography was done with a linear NaCl gradient (0–800 mM). The fractions containing the highly purified CbpA protein, which was eluted around 200 mM NaCl, were combined and used.

RESULTS

Isolation of a DNA-Binding Protein from *E. coli*. We previously searched for *E. coli* proteins that could preferentially bind to a synthetic curved DNA sequence (named S10-15) by means of a DNA-binding gel-shift assay in the presence of an excess amount of a noncurved competitor sequence (named S15-12) (14). S10-15 (200 bp) contains $(dA)_5$ stretches, which were designed so as to be phased at 10-bp intervals in the DNA helix, whereas S15-12 (233 bp) contains $(dA)_5$ stretches at 15-bp intervals. This competitive gel-shift assay allowed us to identify proteins that preferentially recognize a DNA curvature, as has been described (14). During such studies, we isolated another curved DNA-binding protein candidate, as described below (Fig. 1*A*).

A cell extract of *E. coli* strain CU211 was fractionated by successive column chromatographies, with concomitant monitoring by means of a gel-shift assay with ^{32}P-labeled S10-15 sequence in the presence of excess amounts of competitors S15-12 (50 times by weight) and poly(dA·dT) (200 times). These chromatographies included ones on P11-phosphocellulose, heparin-Sepharose, and Mono Q columns (Fig. 1*A*). As a result, a protein with an apparent molecular mass of 34 kDa, which appeared to bind S10-15 sequence with strong affinity, was purified to near homogeneity on SDS/PAGE (Fig. 1*A*, lane 5). The preferential association of this protein with S10-15 sequence was further examined by means of a series of gel-shift assays, in which the purified protein was incubated with ^{32}P-labeled S10-15 and increased amounts of various competitors, including poly(dA·dT), poly(dI·dC), poly(dG·dC), and yeast tRNA (Fig. 1*B*). Large excesses of these competitors did not significantly abolish the binding to S10-15, although poly(dA·dT) affected it partially when added in excess (Fig. 1*B*). From these results, we assumed that this protein associates with the curved DNA sequence (S10-15) with strong affinity. Hence, this protein is named CbpA (*c*urved DNA-*b*inding *p*rotein). In this context, the amount of CbpA in cells (i.e., molecules per cells) was difficult to estimate, although this protein appears to be relatively minor in *E. coli* cells.

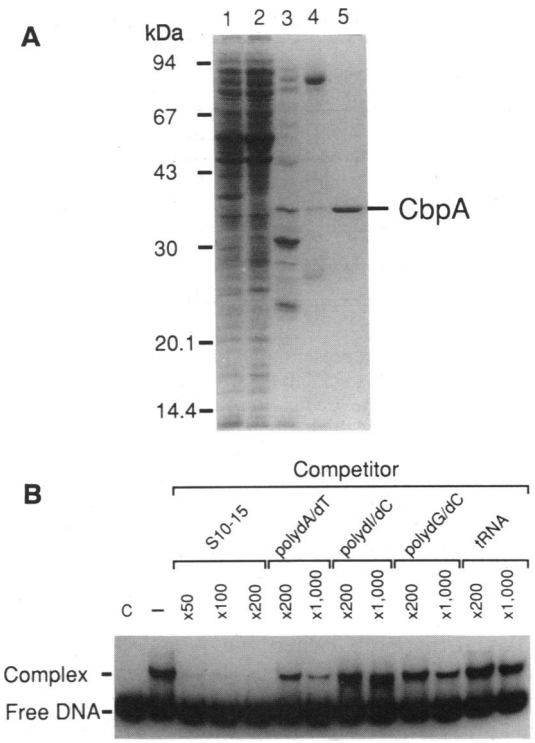

FIG. 1. Purification of CbpA and characterization of its DNA-binding property. (*A*) Proteins obtained at each purification step were analyzed by SDS/PAGE (15% acrylamide), followed by staining with Coomassie brilliant blue. Lanes: 1, cleared lysate after French pressure cell (36 μg of protein); 2, ammonium sulfate precipitation (40–60% saturation; 36 μg); 3, P11-phosphocellulose chromatography (17 μg); 4, heparin-Sepharose chromatography (5 μg); 5, Mono Q chromatography (1.2 μg). (*B*) DNA-binding property of CbpA was examined by a gel-shift assay. In each lane, radioactively labeled S10-15 sequence (4 ng) was incubated with purified CbpA (0.5 μg), except in lane C (control), in which labeled DNA alone was applied. Incubation was done without (lane denoted by –) or with excess amounts of competitor DNAs or RNA, as indicated. In all lanes both nonlabeled S15-12 sequence (50-fold) and poly(dA·dT) (50-fold) were added as basal competitors in addition to those indicated. After incubation, samples were immediately loaded on 6% nondenaturing polyacrylamide gel and then autoradiographed.

Cloning of *cbpA*. The N-terminal amino acid sequence of CbpA was directly determined as MELKDYYAIMGVKX-TDDLK (X denotes an undetermined residue). A degenerated oligonucleotide mixture (22-mer) was prepared as a probe, corresponding to the sequence MELKDYY. We then screened a selected set of a λ phage library, which was constructed for the whole *E. coli* genomic DNA (22). Two λ phages (named λ2F1 and λ5A12) hybridized with the probe. The overlapping DNA on these phages cover a 10-kb chromosomal region located at ≈22 min on the *E. coli* genetic map. The results of successive Southern hybridization analyses showed that the probe specifically hybridized with a 3.2-kb *Eco*RI fragment (data not shown, see Fig. 2). This *Eco*RI fragment was cloned in plasmid pKY184 and then sequenced (the nucleotide sequence data appears in the GenBank/EMBL/DDBJ Nucleotide Sequence Data Libraries under accession number D16500). As schematically shown in Fig. 2, determination of the complete nucleotide sequence (3211 bp) revealed the presence of several possible ORFs. Among these, an ORF of 891 bp (located between the *Nde* I and *Mlu* I sites) could encode a protein consisting of 297 amino acids, in which the predicted N-terminal sequence was exactly the same as that determined for CbpA (Fig. 3). The molecular mass (33,407 Da) calculated for this ORF agreed well with that estimated for CbpA on SDS/PAGE (see

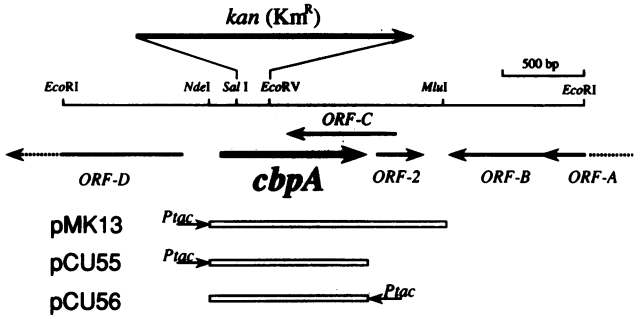

FIG. 2. Schematic representation of *E. coli* chromosomal region surrounding *cbpA*. This schematic structure was constructed from DNA sequencing of the *Eco*RI–*Eco*RI region. A set of predicted ORFs are indicated by thick (for *cbpA*) or thin (for other ORFs) arrows that also indicate the 5′–3′ direction. Each DNA segment indicated by an open rectangle was placed under control of *tac* promoter (Ptac), and plasmids carrying these constructs were designated pMK13, pCU55, and pCU56, respectively. Structure of an insertional mutant of *cbpA* is also schematically shown above the restriction map, in which the kanamycin-resistance gene (*kan*) was inserted in this chromosomal region as indicated.

Fig. 1). Furthermore, when the *Nde* I–*Mlu* I sequence encompassing the putative *cbpA* gene was placed under control of the *tac* promoter in its proper orientation in plasmid pMK13 (see Fig. 2), the cells harboring this plasmid overproduced a protein, the molecular weight of which is exactly the same as that of CbpA (data not shown). From these results, we assumed that this ORF encodes CbpA; hence, the corresponding sequence was designated as *cbpA*.

The *cbpA* gene is followed by a small ORF of 303 bp, named ORF-2. Beside ORF-2, several other ORFs could be predicted in the determined nucleotide sequence surrounding the *cbpA* gene (two of them are incomplete, Fig. 2). Whether or not these four ORFs (designated A–D) as well as ORF-2 represent a functional gene is not presently known.

CbpA Is an Analogue of *E. coli* DnaJ. A computer-aided search of the SwissProt data base revealed a high homology between the predicted amino acid sequence of CbpA and that of the *E. coli* DnaJ protein (23). Fig. 3 shows that optimal alignment of CbpA with DnaJ indicated 39% amino acid identity plus 17% conserved substitutions. Similar degrees of identity were also seen with the *Bacillus subtilis* and *Mycobacterium tuberculosis* DnaJ proteins (37% and 31% identity, respectively) (24, 26). Beside these bacterial DnaJ homologues, several yeast and human proteins that exhibit sequence similarity to DnaJ have been found (for review, see ref. 12 and the references therein). CbpA also exhibited significant sequence similarity to these putative eukaryotic DnaJ homologues, particularly in its N-terminal portion stretching ≈70 amino acids in length (for example, see YDJ1 in Fig. 3). Bacterial and yeast DnaJ homologues commonly contain a cysteine-rich region stretching for ≈70 amino acids positioned ≈140 amino acids from the N terminus. CbpA completely lacks this cysteine-rich region (Fig. 3). These analyses of the predicted amino acid sequence for CbpA indicated that it exhibits a significant degree of sequence homology with authentic DnaJ. Thus, CbpA can be regarded as an analogue of DnaJ, as far as the primary amino acid sequence is concerned.

The *cbpA* Gene Functions as a Multicopy Suppressor for *dnaJ* Mutations. An insertional mutant (*dnaJ*::Tn*10–42*) exhibits a temperature-sensitive phenotype for cell growth above 42°C and is resistant to λ phage at all temperatures (27). In addition, a mini-F replicon (derived from fertility factor F) is also unable to replicate in the *dnaJ* null mutant (9). The high degree of similarity between the CbpA and DnaJ amino acid sequences led us to examine whether or not overexpression of CbpA could suppress these phenotypes characteristic of the *dnaJ* mutant. To determine this unambiguously, we constructed a set of high-copy plasmids that carry a minimal region encompassing the *cbpA*-coding sequence alone (plasmids pCU55 and pCU56 in Fig. 2). Plasmid pCU55 contains the *cbpA* gene under the control of the *tac* promoter in its proper orientation, whereas the other appropriate control plasmid (pCU56) carries the same region in the opposite orientation relative to the *tac* promoter.

The *dnaJ* null mutant was transformed with either pCU55, pCU56, or a vector (pUSI2) at a permissive temperature (30°C). These transformants grown in Luria broth were serially diluted and then spotted onto Luria-agar plates containing 1 mM isopropyl β-D-galactopyranoside (IPTG) for growth at both the permissive and nonpermissive temperatures (30°C and 42°C, respectively). The results indicated that the temperature sensitivity for growth of the *dnaJ* mutant was suppressed only when pCU55 was introduced (data not shown)—i.e., when CbpA was supposed to be overexpressed. The temperature-sensitive phenotype, caused by another *dnaJ* lesion (*dnaJ259*) (4), was also suppressed by pCU55 under the same conditions as those tested (data not

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CbpA(EC): MELK-DYYAIMGVKPTDDLKTIKTAYRRLARKYHPDVS-KEPDAEARFKEVAEAWEVLSDEQRRAEYDQMWQHRNDPQFNRQFHHGDGQSFNAEDFDD   96
DnaJ(EC): MAKQ-DYYEILGVSKTAEEREIRKAYKRLAMKYHPDRNQGDKEAEAKFKEIKEAYEVLTDSQKRAAYDQ-YGHAAFEQGGMGGGGFGGGADFSDIFGD   96
DnaJ(BS): MSKR-DYYEVLGVSKSASKDEIKKAYRKLSKKYHPDIN-KEAGSDEKFKEVKEAYETLSDDQKRAHYDQ-FGHTDPNQ-GFGGGGFGGGDFGGFGFDD   94
YDJ1(SC): MVKETKFYDILGVPVTATDVEIKKAYRKCALKYHPDKN-PSEEAAEKFKEASAAYEILSDPEKRDIYDQ-FGEDGLSG-AGGAGGFPGGGFGFG--DD   93

CbpA(EC): IFSSIFGQHARQSRQRPATRGHDIEIEVAVFLEETLTEHKRTI------------------------------------------------------   139
DnaJ(EC): VFGDIFGGGRG--RQR-AARGADLRYNMELTLEEAVRGVTKEIRIPTLEECDVCHGSGAKPGTQPQTCPTCHGSGQVQMRQGF----FAVQQTCPHCQ   187
DnaJ(BS): IFSSIFGGGTRRRDPKLRARGADLQYTMTLSFEDAAFGKETTIEIPREETCETCKGSGAKPGTNPETCSHCGGSGQLNVEQNTPFGKVVNRRVCHHCE   192
YDJ1(SC): IFSQFFGAGGAQ-RPRGPQRGKDIKHEISASLEELYKGRTAKLALNKQILCKECEGRGGKKGAVKK-CTSCNGQGIKFVTRQMGPMIQRFQTECDVCH   189

CbpA(EC): ------SYNLPVYNAFGMIEQEIPKTLNVKIPAGVGNGQRIRLKGQGTPGENGGPNGDLWLVIHIAPHPLFDIVGQDLEIVVPVSPWEAALGAKVTVP   231
DnaJ(EC): GRGT--LIKDPCNKCHGHGRVERSKTLSVKIPAGVDTGDRIRLAGEGEAGEHGAPAGDLYVQVQVKQHPIFEREGNNLYCEVPINFAMAALGGEIEVP   283
DnaJ(BS): GTGK--IIKNKCADCGGKGKIKKRKKINVTIPAGVDDGQQLRLSGQGEPGINGGL-PDLFVVFHVRAHEFFERDGDDIYCEMPLTFAQAALGDEVEVP   287
YDJ1(SC): GTGDIIDPKDRCKSCNGKKVENERKILEVHVEPGMKDGQRIVFKGEADQAPDVIP-GDVVFIVSERPHKSFKRDGDDLVYEAEIDLLTAIAGGEFALE   286

CbpA(EC): TLKESILLTI---PPGSQAGQRLR-VKGKG--LVSKKQTGDLYAVLKIVMPPKPDENTAALWQQLADAPVVF                           297
DnaJ(EC): TLDGRVKLKV---PGETQTGKLFR-MRGKGVKSVRGGAQGDLLCRVVVETPVGLNERQKQLLQELQESFGGPTGEHNSPRSKSFFDGVKKFFDDLTR   376
DnaJ(BS): TLHGKVKI-----PAGTQTGTKFR-LRGKGVQNVRGYGQGDQHIVVRVVTPTNLTDKQKDIIREFAEVSGNLPDEQEM----SFFDKVKRAFKGD   372
YDJ1(SC): HVSGDW-LKVGIVPGEVIAPGMRKVIEGKGMPIPKYGGYGNLIIKFTIKFPENHFTSEENLKKLEEILPPRIVPAIPKKATVDECVLADFDPAKYNRT   383

YDJ1(SC): RASRGGANYDSDEEEQGGEGVQCASQ                                                                          409
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FIG. 3. Alignment of deduced amino acid sequence of CbpA with those of DnaJ homologues. The following sequences were aligned: CbpA [*E. coli* (EC), this study]; DnaJ [*E. coli* (EC), ref. 23]; DnaJ [*B. subtilis* (BS), ref. 24], and YDJ1 [*Saccharomyces cerevisiae* (SC), ref. 25]. Gaps (indicated by dashes) were appropriately introduced by computer for optimal alignment. Amino acid residues, conserved in three out of four sequences, are shaded.

shown). It is worth mentioning, however, that pCU55 failed to restore the temperature-sensitive phenotype seen at 42°C for a set of mutants carrying lesions in other heat shock genes. Such temperature-sensitive alleles so far examined are *dnaK204*, *dnaK756*, *grpE280*, *groEL44*, *groES619*, and *rpoH15* (data not shown).

We next examined whether or not CbpA overproduction could also permit λ phage propagation in the *dnaJ*-carrying mutant. Plaque-forming efficiency of the λ*vir* phage was examined at 37°C for the *dnaJ* mutant, as well as the wild type (strain MC4100) carrying appropriate plasmids (pCU55 or pUSI2) (Table 1). In agreement with previous reports (4–7), the plaque-forming efficiency of λ phage in the *dnaJ* mutant was very poor—i.e., the value was $\approx 10^{-5}$-fold relative to that in the wild type. However, when pCU55 was introduced into the *dnaJ* mutant, λ phage could propagate in the transformant as efficiently as in the wild type. Similarly, we examined whether or not overproduction of CbpA could permit mini-F replication in the *dnaJ* mutant. An attempt was made to transfer plasmid pMF21 harboring a mini-F replicon (30) into the *dnaJ* mutant as well as the wild type carrying appropriate plasmids (pCU55 or pUSI2). The transformant was selected at 30°C on Luria-agar plates containing kanamycin as a selective antibiotic for pMF21. To ensure the competency of each host used, a plasmid pSC101 derivative, pCU8 (29), was also analyzed similarly as a reference. The transformation efficiency of pMF21 on these hosts was expressed relative to that of pCU8 (Table 1). The results indicated that when pCU55 was introduced, pMF21 could replicate in the *dnaJ* mutant as efficiently as in the wild type.

Combining these results, we concluded that the *cbpA* gene can function as a multicopy suppressor for the *dnaJ* mutant, at least with regard to the temperature sensitivity for growth and the defects in λ phage and mini-F replication. In this respect, certain *dnaK* and *grpE* mutants are known to also be unable to support λ phage and mini-F replication (5–7, 9, 31). However, the *cbpA* gene did not function as a multicopy suppressor for these particular mutants (Table 1). These results can be explained by assuming that the possible function(s) of CbpA are closely related to that exerted by DnaJ.

Table 1. Multicopy suppression by *cbpA* examined for mutations of a set of heat shock genes

E. coli strain*	Plasmid	Relative plaque-forming efficiency of phage λ*vir*†	Relative transformation efficiency of plasmid pMF21‡
MC4100 (wild type)	pUSI2	1.0	2.0
	pCU55	0.96	1.3
KY1456 (*dnaJ*$^-$)	pUSI2	8.8×10^{-6}	$<1.2 \times 10^{-3}$
	pCU55	1.16	0.97
NRK156 (*dnaK*$^-$)	pUSI2	1.5×10^{-7}	$<1.1 \times 10^{-4}$
	pCU55	$<1.0 \times 10^{-9}$	$<6.4 \times 10^{-5}$
KY1454 (*grpE*$^-$)	pUSI2	1.0×10^{-5}	$<1.8 \times 10^{-3}$
	pCU55	$<1.0 \times 10^{-9}$	$<2.2 \times 10^{-3}$

*Host strains were transformed with indicated plasmids and then used as described.

†Bacterial hosts were infected with λ*vir* phage and then spread with soft agar on Luria-agar plates containing ampicillin at 50 μg/ml and 1 mM IPTG. Plates were incubated at 37°C (or 30°C for strain KY1454). The number of plaques in each bacterial host was counted and expressed relative to that of MC4100 carrying pUSI2.

‡Transformation with plasmid pMF21 was done by the RbCl method (28) and then incubated at 30°C on Luria-agar plates containing ampicillin at 50 μg/ml, kanamycin at 25 μg/ml, and 1 mM IPTG. Transformation with pSC101 derivative (pCU8, ref. 29) was also done under the same conditions (chloramphenicol at 25 μg/ml was used for pCU8). Number of colonies transformed by pMF21 was counted for each host and expressed relative to that of colonies transformed by pCU8.

An Insertional Mutation of *cbpA* Exhibits a Synthetic Phenotype in Combination with the *dnaJ* Mutation. To further investigate the function of CbpA, we constructed an insertional mutation of *cbpA*. A portion of the *cbpA*-coding sequence was replaced by the kanamycin-resistance gene (*kan*) on the *E. coli* chromosome (Fig. 2). This mutant strain was constructed by a standard method via homologous recombination in a *recD* background (32). The *cbpA*::*kan* allele in the *recD*-carrying strain was successively transferred into strain MC4100 and other appropriate strains by P1 transduction. It should be noted that this insertional mutation does not disrupt any other putative ORFs, including ORF-C (see Fig. 2). In any case, the resultant *cbpA*::*kan*-carrying strain was not noticeably changed in phenotype, particularly with regard to temperature sensitivity for growth, λ phage, and mini-F replication (data not shown). We then attempted to construct a strain that harbored both the *cbpA* and *dnaJ*-null alleles. Such a strain could be established when bacteria were grown at 30°C. However, the resultant *cbpA*::*kan*/*dnaJ*::Tn*10–42*-carrying mutant was unable to grow at 37°C, at which temperature both strains carrying each single mutation could grow normally (data not shown). The same genetic analyses were also conducted for the *dnaK756*, *grpE280*, *groEL44*, *groES619*, and *rpoH15* alleles. None of these mutations, except for *rpoH15*, exhibited such a temperature hypersensitivity for growth, even when combined with the *cbpA* null mutation. The *rpoH* gene encodes a heat shock-specific σ factor (33), and the strain carrying the *rpoH15* allele can grow only below 30°C. Although the *cbpA*::*kan*/*rpoH15*-carrying double mutant was stably established, it grew at a very poor rate at 30°C, thereby forming very tiny colonies on Luria-agar plates, even after prolonged incubation, as compared with those of the parental strains. Although the results of these genetic experiments could be explained by a possible polar effect of the *cbpA*::*kan* allele on ORF-2 expression (see Fig. 2), these results are again compatible with the hypothesis that the function(s) of CbpA is closely related to DnaJ function.

DISCUSSION

Cloning and nucleotide sequencing of *cbpA* revealed that *E. coli* possesses an analogue of the DnaJ heat shock protein. Several lines of evidence support this conclusion, as described below. Although DnaJ may not be absolutely essential for bacterial growth, a *dnaJ* null mutant (*dnaJ*::Tn*10–42*) does not grow as well as the wild type at temperatures >42°C (27). However, when *cbpA* was introduced with a high-copy plasmid into the *dnaJ* mutant, this particular mutational lesion of *dnaJ* was phenotypically suppressed. In this context, it should also be noted that the *cbpA*::*kan*/*dnaJ*::Tn*10–42*-carrying mutant exhibited an intriguing phenotype. When both *cbpA* and *dnaJ* mutations were combined, the resultant double mutant was unable to grow at 37°C, at which temperature the strains harboring each mutation singly could grow normally. Thus, the *cbpA* null allele appears to somehow render the *dnaJ* mutant more sensitive to temperature for growth. We will tentatively refer to this particular phenomenon as "synthetic temperature hypersensitivity." These two lines of genetic observations with regard to *dnaJ* and *cbpA*—namely, "multicopy suppression" and synthetic temperature hypersensitivity—are interpreted *a priori* as meaning that both products, DnaJ and CbpA, play a similar and/or overlapping function for *E. coli* survival. This hypothesis was further supported by the fact that these phenomena are specific for *dnaJ*. In other words, the temperature sensitivity for growth, caused by mutations of other heat shock genes (*dnaK*, *grpE*, *groEL*, *groES*, and *rpoH*), was not suppressed by CbpA

overexpression. Further, mutational lesions in these heat shock genes did not lead to such a synthetic temperature hypersensitivity, even when combined with the *cbpA* null mutation. In the latter case, however, an interesting exception was seen for the *rpoH15* allele (a missense mutation of the 32-kDa σ factor σ^{32}). The *cbpA::kan/rpoH15* mutant grew at very poor rate at 30°C, as compared with the individual parental strains. In the *rpoH15* background, the amount of DnaJ in cells is probably small because σ^{32} is known to be required for expression of *dnaJ* (34). Under these particular conditions, the compensatory functioning of CbpA may be crucially required for *E. coli* survival; therefore, the simultaneous loss of CbpA in the *rpoH* mutant may be harmful for *E. coli* growth. In any event, these lines of genetic observations are also compatible with the hypothesis that the cellular function(s) of CbpA is closely related to that of DnaJ.

The *dnaJ* null mutant is unable to support λ phage and mini-F DNA replication. The defects in the λ phage and mini-F DNA replication in the *dnaJ* mutant were suppressed by the overexpression of CbpA. These findings provide us with more specific and closer insight into the possible function of CbpA because the molecular roles of DnaJ in DNA replication have been extensively elucidated through several recent lines of work (35–37). The molecular chaperones, DnaK/DnaJ/GrpE, coordinately function at the λ origin to facilitate the sequential assembly and disassembly of a set of proteins, including λO, λP, and DnaB (35). Similarly, DnaK/DnaJ/GrpE play an essential role in facilitating the functioning of the RepE initiator protein in mini-F replication (36), as proposed for P1 plasmid replication (37). A current model for DnaJ function in DNA replication is that this protein, with GrpE, stimulates the ATPase activity of DnaK (38), thereby catalyzing the assembly and disassembly of certain proteins essential for DNA replication. Thus, a simple explanation of our findings is that CbpA has a DnaJ-like function and that a high concentration of CbpA plays a compensatory role in the *dnaJ* null mutant. More specifically, CbpA may interact directly with DnaK and/or GrpE, thereby stimulating the ATPase activity of DnaK, which is essential for λ phage and mini-F replication. Other explanations can also be envisioned—i.e., that CbpA somehow indirectly compensates for the lack of DnaJ. However, we favor the simple model described above because (*i*) CbpA exhibits a high degree of similarity with DnaJ in primary structure and (*ii*) the defects in both λ phage and mini-F replication, observed also for certain *dnaK* and *grpE* mutations, were not restored on the overexpression of CbpA. To address this issue further, it is of interest to examine *in vitro* whether the purified CbpA protein can stimulate the ATPase activity of DnaK.

Finally, it should be noted that CbpA was first isolated as a DNA-binding protein that preferentially recognizes a curved DNA sequence. Because this study was mainly intended to explore the structural and functional relationship between CbpA and DnaJ, we have not yet characterized the DNA-binding property of CbpA further. Nevertheless, it is of interest to ask whether or not this particular DNA-binding property is relevant to its cellular function. It is also noteworthy that DnaJ has also been characterized as a DNA-binding protein during its purification (39). In any event, further comparative studies on DnaJ and CbpA should shed light on the cellular function of CbpA, which was implicated as a distinctive molecular chaperone. Such studies will also provide an insight into the possible interplay between CbpA and DnaJ in response to environmental stresses.

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