A Novel Factor Required for the Assembly of the DnaK and DnaJ Chaperones of Thermus thermophilus*

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We previously reported the isolation of T.DnaK-DnaJ chaperone complex from Thermus thermophilus. Here, we show that a novel factor is necessary for the assembly of T.DnaK and T.DnaJ into the complex. A dnaK gene cluster of T. thermophilus contained five genes, dnaK-grpE-dnaJ-orf4-clpB. Interestingly, T.DnaJ lacks the whole “cysteine-rich region” that has been postulated to be necessary to bind unfolded proteins. The orf4 gene encodes a novel 78-amino acid protein. Curiously, T.DnaK and T.DnaJ expressed in Escherichia coli did not form the complex. Careful reexamination of the T.DnaK-DnaJ complex revealed the presence of a small protein in the complex, which turned out to be a product of orf4. As expected, expression of three genes, dnaK-dnaJ-orf4, resulted in production of a T.DnaK-DnaJ complex in E. coli that was indistinguishable from the authentic complex in its ability to interact with nucleic acids and denatured proteins. The product of orf4 was also required for in vitro reconstitution of the complex and named T.DafA (T.DnaK-DnaJ assembly factor A). The complex comprises three copies each of T.DnaK, T.DnaJ, and T.DafA. Even though a definite homolog of T.DafA has not been found in the data base, this finding raises a possibility that interaction between DnaK and DnaJ chaperones in other organisms is also mediated by a small protein yet unnoticed.

The ubiquitous Hsp70 stress proteins are major members of the chaperone system, which assists protein folding in the cell (1–4). The Escherichia coli Hsp70 homolog DnaK participates in widely different processes of protein biogenesis such as reactivation of heat-inactivated proteins (5–7), refolding of denatured proteins (8, 9), and degradation of aberrant proteins (10–12). A general feature of this chaperone activity is the ability of DnaK to recognize nonnative conformations of other proteins and to release them in an ATP-regulated manner. The ATPase activity of DnaK is under tight control of cofactors allowing coordination of ATP binding/hydrolysis and substrate protein interaction (8, 13). The weak ATPase of DnaK is stimulated by DnaJ (41 kDa), which accelerates the rate of ATP hydrolysis, and by GrpE (22 kDa), which promotes nucleotides exchange of ATP and ADP (14). DnaJ also possesses a molecular chaperone activity of its own, as revealed by its capacity for binding to denatured proteins to prevent aggregation (9, 15). DnaJ and GrpE are essential for chaperone activity of DnaK in vivo and in vitro. For these cofactors to assist DnaK function, they should physically interact each other and, in fact, GrpE can associate with DnaK to form a relatively stable complex in an ATP-independent manner (16–18). Interaction between DnaK and DnaJ has been also suggested from experiments of glutaraldehyde cross-linking (19) and immunological detection (20); the formation of a high molecular weight ternary complex of DnaK, DnaJ, and a folding intermediate has been reported (8, 15). Nevertheless, attempt to isolate a stable complex of DnaK and DnaJ has been unsuccessful, and physical and chemical aspects of the interaction between DnaK and DnaJ have remained unclear.

DnaK is purified from E. coli cells as monomer with a small amount of dimer or higher oligomer (18, 21, 22). Both DnaJ and GrpE are purified separately as dimer (18, 23). In contrast to the E. coli counterparts, T.Dnak1 from a thermophilic bacterium, Thermus thermophilus, is isolated as a trinlogic ring particle with an approximate molecular size of 300 kDa in which three copies of T.Dnak and T.DnaJ are contained (24). This T.Dnak-DnaJ complex is stable during incubation with MgAT(DP) and can bind reduced carboxymethylated β-lactobumin, a model protein of denatured state. Here, we report that the T.Dnak-DnaJ complex contains additional three copies of a novel small protein whose presence was not noticed previously. This protein is required for the assembly of T.Dnak and T.DnaJ into a complex and is a product of an adjacent gene of dnaJ.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, Media, and Growth Conditions—**T. thermophilus strain HB8 (ATCC 27634) was used as a source of genomic DNA. E. coli strains used here were M1019 for preparation of plasmids (25), CJ 236 for generating uracil-containing single-stranded DNA for site-directed mutagenesis (26) and BL21 (DE3) pLysS for gene expression of plasmids carrying T7 promoter (27). Plasmid pBlue scriptII KS(−) (Stratagene) tailed with dideoxythymidine triphosphate using terminal transferase (28) was used for subcloning of polymerase chain reaction (PCR) products. Plasmid pUC18 (25) was served as a vector for the construction of genomic DNA library and the DNA sequence of T. thermophilus gene. Plasmid pUC118 and helper phage M13K07 (29) were used for generating uracil-containing single-stranded DNA. Plasmids pET3a (Novagen) were used for construction of expression system. T. thermophilus was grown aerobically at 75 °C in a medium containing 10 g of yeast extract, 10 g of polypeptone, and 2 g of NaCl/liter (30). E. coli was grown aerobically at 37 °C in 2 × YT medium. * The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) D84222.

†Recipient of a research fellowship of the Japanese Society for the Promotion of Science for Young Scientists.

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*The abbreviations used are: PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; native-PAGE, polyacrylamide gel electrophoresis in the absence of SDS; Tricine, N-[2-hydroxy-1,1bis(hydroxymethyl)ethyl]glycine; PTH, phenylthiodydantoin; kb, kilobase pair(s); HPLC, high performance liquid chromatography.
media. Ampicillin (50–100 μg/ml) and chloramphenicol (25 μg/ml) were supplemented in the growth media.

Polymerase Chain Reaction—Mixed oligonucleotide primers for PCR amplification were 5'-GCGAAGCTTGGATCCGATATGGGAGC-3' and 5'-ATGGCCGATATGCCGATATGGGAGC-3'. PCR was performed using either 2 μg of chromosomal DNA as a template in a 100 μl of PCR mixture containing 25 pmol Tris-HCl, pH 7.5, 50 mM KCl, 1.5 mM MgCl₂, 0.001% (w/v) gelatin, 200 μM each of dNTP containing 1.0 μM of each primer and 2.5 units of recombinant Taq DNA polymerase (Takara). Samples were subjected to 30 cycles of 1 min of denaturation at 98 °C, 0.5 min of annealing at 55 °C, and 2 min of elongation at 72 °C.

DNA isolation and Cloning and Sequencing of T. dnaK Region—DNA was amplified by standard methods (31) and chromosomal DNA from T. thermophilus was isolated as described previously (32). Southern blot hybridization (33) to total chromosomal DNA from T. thermophilus was performed using the PCR products as probes, and a 5.5-kbp BamHI fragment of the chromosomal DNA was detected. The thermophilus chromosomal DNA was digested with BamHI, DNA fragments with about 5.5 kbp were cut out, and clones containing dnaK gene were selected by colony hybridization using the probe. The probe was labeled and detected by ECL™ random prime labeling and detection system (Amersham Corp.). The total DNA sequences of both strands were determined by the dideoxy chain termination method (34) using a fluorescent DNA sequence analyzer (ALF™ DNA sequencer II, Pharmacia Biotech Inc.).

Construction of Expression System for T. dnaK-DnaJ Complex—The construction for the expression plasmid of the T.DnaK-DnaJ complex was performed as follows. The pMKJ1 containing a� gene, inserted into pUC18, was digested with a restriction enzyme KpnI, and a 2.3-kbp KpnI fragment containing the T. dnaK gene was subcloned into KpnI site of the pUC18 (pMDK1–1). In the case of the dnaJ gene, pMKJ1 was digested with KpnI-HindIII and a 2.2-kbp KpnI-HindIII fragment containing dnaJ gene was subcloned into KpnI-HindIII sites of the pUC18 (pMDJ1-1). In order to construct the expression system, a NdeI site was introduced to the translational initiation region of dnaK and dnaJ genes in pMDK1–1 and pMDJ1–1 by the method of Kunkel et al. (26) using synthetic oligonucleotides 5'-GCGGATCCATATGGGAGC-3' (dnaK) and 5'-GGCCGGCATATGGAATTCCAGGTCCG-3' (dnaJ). Chosen bases are underlined. The dnaK gene carrying a NdeI site in the translational initiation region was digested with NdeI-HindIII and was ligated with the corresponding NdeI-HindIII sites of the pET3a, and it was named the pMDK6 (dnaK). The dnaJ gene carrying a NdeI site in the translational initiation region was digested with NdeI-HindIII and was ligated with the corresponding NdeI-HindIII sites of the pET3a and was named as pMDJ8 (dnaJ), respectively. The dnaK-dnaJ expression system was constructed from pMDK6, pMDJ8, and pMDJ10 (dnaJ) respectively. The T. dnaK-DnaJ-DnaJ-DnaK-DnaJ complex was prepared using the PCR products as probes, and a 5.5-kbp BamHI fragment of the chromosomal DNA was detected.

N-terminal Amino Acid Sequencing—The T. dnaK-DnaJ complex purified from T. thermophilus was loaded onto Tricine-SDS-PAGE (16.5%), and protein bands were blotted to a polyvinylidene difluoride membrane (Bio-Rad). The blotted membrane was stained with Coomassie Brilliant Blue R-250, and a band of the 8-kDa protein was cut out. The cut piece was treated with cyanogen bromide (37) and analyzed with a gas phase peptide sequencer (PI-2020, Tosoh). To estimate the relative molar stoichiometry of T. dnaK, T. dnaJ, and a novel factor (T. DaF) in the T. dnaK-DnaJ complex, the complex was subjected to Edman degradation and PTH-amino acids derived from N termini of each component of the complex were analyzed. The T. dnaK-DnaJ complex (9 μg) in 4 μl guanidine hydrochloride was blotted directly onto a polyvinylidene difluoride membrane. Blotted membrane was dried and stained with Coomassie Brilliant Blue R-250. A protein spot was cut out and analyzed with a gas phase peptide sequencer.

Other Methods—Polyacrylamide gel electrophoresis (PAGE) was carried out on 15% polyacrylamide gel in the presence of 0.1% SDS (SDS-PAGE) (38) or on 7.5% polyacrylamide gel in the absence of SDS (native-PAGE). Tricine-SDS-PAGE (16.5%) was carried out for analysis of low molecular size peptides (39). Gels were stained with Coomassie Brilliant Blue R-250. Protein concentrations were assayed by the method of Bradford with bovine serum albumin as a standard (40).

RESULTS

Cloning of the dnaK Gene Cluster of T. thermophilus—In order to clone the dnaK gene cluster of T. thermophilus using a PCR fragment as a probe, a set of primer oligonucleotides were designed from N-terminal amino acid sequences of T. dnaK and T. dnaJ (24), assuming that, as observed for most bacterial dnaK gene clusters (41), dnaK and dnaJ genes of this bacterium are present in the same gene cluster with dnaK being upstream of dnaJ. A 2.5-kbp DNA fragment was amplified by PCR, sequenced, and confirmed to contain the dnaK gene (data not shown). This DNA fragment was used as a probe to clone a BamHI 5.5-kbp DNA fragment from T. thermophilus that contained dnaK gene, and the DNA sequence was determined for both strands. The region contains at least five genes in the order dnaK-grpE-dnaJ-orf4-dnaF which consisted of T. dnaK-dnaJ complex (45 μg), which consisted of T. dnaK-T. dnaJ and a novel factor (T. DaF), was dissolved in 45 μl of a 8 M urea solution containing 25 μm Tris-HCl, pH 7.5, 3 mM MgCl₂. The 8 M urea solution containing denatured T. dnaK, T. dnaJ, and T. DaF was directly applied to native-PAGE. As a control experiment, a mixture of individually purified T. dnaK (30 μg) and T. dnaJ (15 μg) was treated in the same way and analyzed with native-PAGE.

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Reading frames of dnaJ and orf4 genes overlap 11 bases. dpB gene starts after a 61-base intergenic sequence. With respect of the arrangement of dnaK, dnaJ, and grpE genes, bacterial dnaK gene clusters are grouped into three types: The order observed for T. thermophilus, dnaK-grpE-dnaJ, is the same to that of Streptomyces coelicolor (Fig. 1, second line) (42, 43), but it is different from that of Bacillus subtilis (Fig. 1, third line), where the order of the genes is grpE-dnaK-dnaJ (44). grpE gene of E. coli is not included in the dnaK operon (41) (Fig. 1, fourth line). The presence of dpB gene is a unique feature of the dnaK gene cluster of T. thermophilus. orf4 encodes a novel 78-amino-acid protein (Fig. 2A). No definite homolog of this protein has been found in the GenBank database.

T. dnaJ Lack Whole Cysteine-rich Region—Deduced amino acid sequences of T. dnaK, T. grpE, and T. dnaJ are 53%, 27%, and 34% identical with those of E. coli, respectively (45–48). Their molecular sizes are in general smaller than E. coli homologs. T. dnaK, T. grpE, and T. dnaJ consist of 615, 197, and 280 amino acid residues, respectively, while those of E. coli homologs are 638, 197, and 376 amino acid residues, respectively. Among them, it should be noted that T. dnaJ is 96 amino acid residues shorter than the E. coli homolog. Alignment of T. dnaJ and E. coli dnaJ sequences has revealed that, in addition to the shorter C terminus (24 residues), whole “cysteine-rich region” (69 residues) that contains four repeats of the sequence CXGCGG (49, 50) is lacking in T. dnaJ (Fig. 2B). This is a surprise since the cysteine-rich region has been found in all known bacterial DnaJ molecules functioning in the DnaK chaperone system and most eukaryotic DnaJ homologs. Another feature of T. dnaJ is an unusually high content of proline residues. There are 28 proline residues in a molecule that occupies 10% of total amino acid residues. Particularly, there is a consecutive six-proline sequence from position 75 to 80. E. coli DnaJ contains 15 proline that is 4% of total amino acid residues.

Recombinant T. dnaK and T. dnaJ Cannot Assemble into the Complex by Themselves—We constructed the pMKJ7 plasmid to express recombinant T. dnaK and T. dnaJ in E. coli under the control by the T7 promoter of pET system. Both T. dnaK and T. dnaJ were successfully expressed in E. coli (BL21(DE3)et-lysS) and recovered in a soluble fraction from cell lysate (Fig. 3A, lanes 2 and 3). To remove heat-labile proteins derived from host cells, a soluble fraction of the lysate was treated at 65°C for 10 min. Since the authentic T. dnaK-DnaJ complex isolated from T. thermophilus is stable under the above heat treatment, we expected that expressed T. dnaK and T. dnaJ, if they formed a complex, would have remained soluble after heat treatment. However, most T. dnaJ was precipitated while T. dnaK remained in the soluble fraction after heat treatment (Fig. 3A, lanes 3 and 4). This indicated that the recombinant T. dnaK and T. dnaJ by themselves were unable to assemble into the complex and free T. dnaJ, somehow heat-labile unless it is incorporated into the complex, precipitated during heat treatment. Indeed, the T. dnaK-DnaJ complex band was not seen when soluble fraction after heat treatment was examined with native-PAGE (Fig. 3B, lane 1).

The Authentic T. dnaK-DnaJ Complex Contains a Novel 8-kDa Protein Unnoticed Before—In order to search the reason why the recombinant T. dnaK and T. dnaJ could not form the complex, we carefully repeated analysis of the authentic T. dnaK-DnaJ complex purified from T. thermophilus. Although only DnaK and DnaJ appeared to be components of the T. dnaK-DnaJ complex from SDS-PAGE under the conditions described in the previous paper (24), a small peptide band with molecular size about 8 kDa was clearly seen when the same specimen was analyzed on Tricine-SDS-PAGE (16.5%), which gives better resolution than the usual SDS-PAGE for the analysis of low molecular size peptides (Fig. 4A, lane 1). When the purified T. dnaK-DnaJ complex was subjected to two-dimen-
The N terminus of the 8-kDa protein expressed in E. coli was compared with those of the authentic T.DnaK-DnaJ complex purified from T. thermophilus. Tricine-SDS-PAGE analysis gave an indistinguishable electrophoretic pattern for both complexes (Fig. 4A). The recombinant complex has a weak ATPase activity ($2 \times 10^{-3}$ µmol/min/mg at 80°C), which was exactly the same as measured for the authentic complex. In addition, the ability of the recombinant complex to bind the reduced carboxymethylated a-lactalbumin, which is a model protein of denatured state, was demonstrated with a gel filtration HPLC assay (24). When the mixture of reduced carboxymethylated a-lactalbumin and the complex was applied to a G3000SWXL gel filtration column, reduced carboxymethylated a-lactalbumin was co-eluted with the complex (data not shown). Nucleotide content in the complex differed apparently between the authentic and recombinant complex. The authentic complex has no endogenously bound nucleotide, while the recombinant one has 0.6–1.7 (dependent on preparations) mol of ADP/mol of complex. We have employed hydroxyapatite chromatography as a step of purification of the authentic complex and the proteins were exposed to 10–200 mM sodium phosphate buffer (pH 7.5) during this procedure. Dialysis of the recombinant complex against 10 mM sodium phosphate buffer resulted in loss of all bound ADP from the complex. Therefore, the difference of nucleotide content between authentic and recombinant complexes is attributable to the different purification procedures of the two complexes. When the authentic complex was incubated with MgATP and subjected to a gel filtration column, about 3 mol of ADP/mol of complex remained bound (24). Similarly, when the recombinant complex, previously dialyzed to the phosphate buffer, was incubated with MgATP and gel-filtreted, about 3 mol of ADP/mol of complex remained bound. Altogether, aside from the unblocked N terminus of the 8-kDa protein, the recombinant T.DnaK-DnaJ complex is not distinguishable from the authentic T.DnaK-DnaJ complex purified from T. thermophilus.

The 8-kDa Protein Is Also Required for In Vitro Reconstitution of the Complex—When the complex, either authentic or recombinant, was denatured in 8 M urea and then urea was removed by dialysis, the complex was reconstituted at almost perfect recovery (data not shown). Reconstitution was achieved even when recombinant T.DnaK-DnaJ complex, which contained the 8-kDa protein as a component, was denatured in 8 M urea and proteins were separated from urea by direct loading of the 8 M urea solution on native-PAGE (Fig. 4B, lane 2). However, when a 8 M urea solution containing T.DnaK and T.DnaJ (but not T.DafA) at the same concentrations as the above experiment was applied to native-PAGE, no complex was formed (Fig. 4B, lane 1). Slow removal of urea by dialysis did not change the result (data not shown). Thus, the 8-kDa protein is required for in vitro reconstitution of the complex.

Three Copies of the 8-kDa Protein Are Contained in the Complex—To estimate molar stoichiometry of the 8-kDa protein to T.DnaK and T.DnaJ in the complex, the purified recombinant T.DnaK-DnaJ complex was subjected to Edman degradation and the yield of PTH-amino acids derived from each component of the complex was compared. The amounts of PTH-amino acids averaged for the first four cycles were 34.3 ± 5.1 pmol for T.DnaK (the sequence is AKAV), 33.9 ± 5.8 pmol for T.DnaJ (AAKK), and 32.0 ± 6.9 pmol for the 8-kDa protein (MLAR). Even though the yield of Edman degradation may not always be

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**Tricine-SDS-PAGE and Native-PAGE**

**Fig. 4. Comparison of authentic and recombinant T.DnaK-DnaJ complex and in vitro reconstitution of the T.DnaK-DnaJ complex.**

A. Tricine-SDS-PAGE analyses. Lane 1, authentic (α-) T.DnaK-DnaJ complex purified from T. thermophilus (15 µg). Lane 2, recombinant (α-) T.DnaK-DnaJ complex purified from E. coli harbored by pMKJ8 (15 µg). B, native-PAGE analyses. Lane 1, T.DnaK and T.DnaJ were dissolved in 8 M urea solution and was directly applied on a gel. Lane 2, the mixture of denatured T.DnaK, T.DnaJ, and T.DafA, obtained by dissolving recombinant T.DnaK-DnaJ complex in 8 M urea, was directly applied on a gel. Detailed experimental conditions are described under "Experimental Procedures."
quantitative, the result allowed us to argue that an equal number of copies of T.DnaK and T.DnaJ and the 8-kDa protein is present in the T.DnaK-DnaJ complex. Since the molecular size of the T.DnaK-DnaJ complex has been estimated to be 300 kDa from gel filtration and Ferguson plots and trigonal ring structures were observed with electron microscopy (24), it was concluded that the complex comprises nine polypeptides: three copies of each of the components.

**DISCUSSION**

It is evident from this study that a novel protein factor is necessary for the T.DnaK and T.DnaJ of *T. thermophilus* to assemble into the complex. This protein, now named here as T.DaFA (T.DnaK-DnaJ assembly factor A), is encoded by a gene that is located at immediate downstream of the dnaJ gene in the dnaK gene cluster. T.DnaJ molecule alone is heat-labile, but it becomes heat-stable when assembled into the complex, suggesting the indispensable role of T.DaFA for the growth of this bacterium at optimum temperature (75°C). In addition to T.DnaK, T.DnaJ and T.GrpE, T.DaFA is a fourth member of the DnaK chaperone system of *T. thermophilus*.

The DnaK chaperone system of *T. thermophilus* has a distinct feature from other organisms; T.DnaK and T.DnaJ assemble each other with aid of T.DaFA to form a stable T.DnaK-DnaJ complex. Such a stable complex of DnaK and DnaJ has not been reported for DnaKs from other organisms. However, there are some indications that DnaK (Hsp70) chaperone system functions as a large oligomeric complex. Rodanese or luciferase in nonnative structure forms a large ternary complex of 230–250 kDa with DnaK and DnaJ (8, 15). In reticulocyte lysate from which TRIC (a eukaryotic cytosolic chaperonin) was depleted, nascently synthesized luciferase is bound to Hsp70 and Hsp40 (DnaJ homolog) to form a 230-kDa complex (51). In spite of these works, only little is known about physical and chemical aspects of the interaction of DnaK and DnaJ in chaperone activity. Direct interaction of e. coli DnaK and DnaJ was suggested only from glutaraldehyde cross-linking (19) or immunological detection (20). Interaction of mammalian Hsp70 with Hsp40 has been shown from immunoprecipitation (52). The finding of T.DaFA raises a possibility that an unnoticed factor, possibly a small peptide, is generally required for the stable assembly of DnaK and DnaJ. Since we cannot find a definite homolog of T.DaFA in GenBank, the functional counterpart of T.DaFA in other organisms, if any, might have amino acid sequence with only limited similarity.

As mentioned, organizations of genes in the dnaK gene cluster of *T. thermophilus* and *S. coelicolor* are similar to each other (Fig. 1). Corresponding location of the dafa gene in the gene cluster of *T. thermophilus*, immediate downstream of dnaJ, is occupied by orfX in *S. coelicolor*, which encodes a 151-amino acid protein (43). There is a weak amino acid sequence similarity between T.DaFA and the N-terminal half of the *S. coelicolor* OrfX (21% of amino acid residues in T.DaFA sequence are identical to *S. coelicolor* OrfX). However, since OrfX of *S. coelicolor* has been assigned to be a transcriptional regulator of the dnaK gene cluster (43), it is still undetermined whether *S. coelicolor* OrfX is really related to T.DaFA.

Another finding worth mentioning in this report is that DnaJ of *T. thermophilus* lacks whole cysteine-rich region. All other bacterial DnaJ molecules identified as a member of the DnaK chaperone system so far reported have cysteine-rich region. The T.DaFA does not show amino acid sequence similarity to the cysteine-rich region and functional substitution of the missing cysteine-rich region of T.DnaJ by T.DaFA is impossible. The various DnaJ homologs contain one or more conserved sequences that may correspond to individual functional domains (49, 50). The N-terminal 70 amino acids or “J-region,” conserved among all DnaJ family proteins, is responsible for the interaction with DnaK (Hsp70). The 35-amino acid glycine/phenylalanine-rich region next to the J-region has been proposed to be involved in interaction with DnaK and modulating substrate binding of DnaK (9, 53). The 70-amino acid cysteine-rich region contains four CXXCXXGX sequences, which take two zinc-finger-like structures where two zinc atoms are really bound (9). It has been demonstrated from binding experiments and cross-linking studies using various DnaJ fragments that the cysteine-rich region is required for the DnaJ to specifically recognize and bind to proteins in denatured state (9). The finding of T.DaFA without a cysteine-rich region is in apparent contradiction to the above postulation. There are two possible explanations for the contradiction. We do not know yet whether T.DaFA alone can bind denatured protein. If not under any conditions, it can be due to the lack of the cysteine-rich region. Another explanation assumes that the polypeptide binding domain of DnaK is sufficient for usual chaperone activity and the role of cysteine-rich region of DnaJ to bind denatured protein is only optional. Some of eukaryotic DnaJ homologs such as Saccharomyces cerevisiae Sec63 lack the cysteine-rich region, but they still function as molecular chaperones cooperating with their Hsp70 partners (54, 55). The fact that the DnaJ fragment, containing J-region and glycine/phenylalanine-rich region but not cysteine-rich region, can substitute for intact DnaJ in an assay measuring Λ phage replication (56) also favors the latter explanation. Further analysis of the function of the T.DaFA-DnaK complex may clarify the above argument.

**Acknowledgments**—We thank N. Kato for technical assistance of DNA sequence, Drs. T. Matsui and H. Taguchi for technical instruction and discussion, and Dr. M. Odaka for peptide sequencing.

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