JOURNAL OF BACTERIOLOGY, July 1995, p. 3894–3896 Vol. 177, No. 13
0021-9193/95/$04.00+0

A Study of the Double Mutation of *dnaJ* and *cbpA*, Whose Gene Products Function as Molecular Chaperones in *Escherichia coli*

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The CbpA protein is an analog of the DnaJ molecular chaperone of *Escherichia coli*. To gain insight into the function of CbpA, we examined the nature of a *cbpA* null mutation with special reference to those of *dnaK* and *dnaJ* null mutations. In particular, the *cbpA dnaJ* double-null mutant was found to exhibit severe defects in cell growth, namely, a very narrow temperature range for growth, a defect in cell division, and susceptibility to killing by carbon starvation. These phenotypes are very similar to those reported for *dnaK* null mutants but not to those of *dnaJ* null mutants. Our results are best interpreted by assuming that CbpA is capable of compensating for DnaJ for cell growth and thus that the function(s) of CbpA 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 it results in the induction of a subset of proteins called heat shock proteins (HSPs). This response to temperature is nearly universal among prokaryotes and eukaryotes. HSPs have recently become central to the study of the correct folding of nascent polypeptides, assembly of protein complexes, and uptake of proteins into organelles (3). Some HSPs are currently recognized as molecular chaperones that play crucial roles even under nonstressed physiological conditions (2).

For *Escherichia coli*, the HSP70 class of chaperones, represented by the HSP DnaK, is one of the best characterized examples. DnaK has been shown to function together with other HSPs, i.e., DnaJ and GrpE. These proteins are known to modulate interactions between proteins involved in the initiation of DNA replication of phage λ, phage P1, and the mini-F plasmid (5, 12, 16). DnaK, DnaJ, and GrpE also coordinately regulate HSP synthesis and contribute to the translocation of some proteins across the cytoplasmic membrane (11, 14). In vitro analyses have shown that DnaK requires ATP for activity and that its ATPase activity is stimulated by the presence of DnaJ and GrpE (6). These results indicate that DnaK, DnaJ, and GrpE function as typical molecular chaperones in intimate coordination with each other in a variety of cellular processes.

CbpA is an analog of DnaJ. DnaJ homologs have been discovered in a number of organisms, including higher eukaryotes (9). We recently demonstrated that *E. coli* itself possesses an analog of DnaJ (13). This DnaJ analog, named CbpA, consists of 297 amino acids and exhibits 39% amino acid identity plus 17% conserved substitutions with respect to DnaJ. Several lines of genetic evidence have also led us to suggest that CbpA is an analog of DnaJ from the functional point of view (13). For example, the corresponding gene, *cbpA*, functions as a multicopy suppressor of *dnaJ* mutations. Mutational lesions characteristic of *dnaJ* null mutants, i.e., temperature sensitivity for growth and defects in λ phage and mini-F plasmid DNA replication, are all restored upon introduction of the *cbpA* gene on a multicopy plasmid. At present, however, little is known about the physiological importance of CbpA. To gain insight into the cellular function of CbpA, in this study we examined the nature of a *cbpA* null mutation, with special reference to those of *dnaK* and *dnaJ* null mutations, by specifically addressing the issues described below.

Nature of a *cbpA* null mutant in comparison with that of a *dnaJ* or *dnaK* null mutant. Walker and his colleagues have reported an analysis of cellular defects at a variety of growth temperatures for a *dnaK* null mutant (named the Δ*dnaK52* mutant) (1, 7). Interestingly, this *dnaK* null mutant is cold sensitive as well as heat sensitive and thus possesses a very narrow temperature range for growth. This *dnaK* null mutant has multiple cellular defects that lead to poor viability and to a severe defect in cell division (e.g., filamentation). Sell et al. have characterized *dnaJ* null mutations (*dnaJ*::mini-Tn*10* insertions) and have shown that DnaJ is not absolutely essential for growth at temperatures of up to 42°C (8). Although *dnaJ* null mutations also cause growth defects, they are considerably less severe. In particular, the *dnaJ* null mutant does not exhibit cold sensitivity, and its filamentation is not as excessive as that observed for the *dnaK* null mutant. As mentioned above, DnaK and DnaJ are both required for propagation of phages λ and P1 and the mini-F plasmid (5, 12, 16). In fact, mutation of either of these genes blocks propagation of the replicons equally. In this sense, one can suppose that DnaJ functions in intimate coordination with DnaK. Nevertheless, the question arises as to why the *dnaJ* null mutant exhibits defects in cell growth that are less severe than those of the *dnaK* null mutant. In this study, we hypothesized that the *cbpA* gene on the chromosome may be capable of compensating for the *dnaJ* gene in some cellular processes, particularly for cell growth. Here we provide evidence that this is most likely the case.

The *cbpA dnaJ* double-null mutant exhibits a very narrow temperature range for growth. We previously constructed an insertional inactivation mutation of *cbpA*, in which the *cbpA* coding sequence was replaced by the kanamycin resistance gene (*kan*) on the chromosome (13). The resultant *cbpA*::*kan*-carrying strain was not noticeably changed in phenotype under the laboratory conditions tested, particularly with regard to temperature sensitivity for growth. To address the issues described above, we prepared a set of mutants, namely, the *dnaJ*::Tn*10-42* (strain KY1456), *cbpA*::*kan* (strain CU245), and *dnaJ*::Tn*10-42 cbpA*::*kan* (strain CU247) mutants. These are otherwise isogenic derivatives of MC4100. These strains were allowed to grow on Luria agar plates at various temperatures (Fig. 1A). The *cbpA* null mutant and the wild type grew normally at a wide range of temperatures. The *dnaJ* null mutant did not grow at temperatures above 42°C, while it was able to

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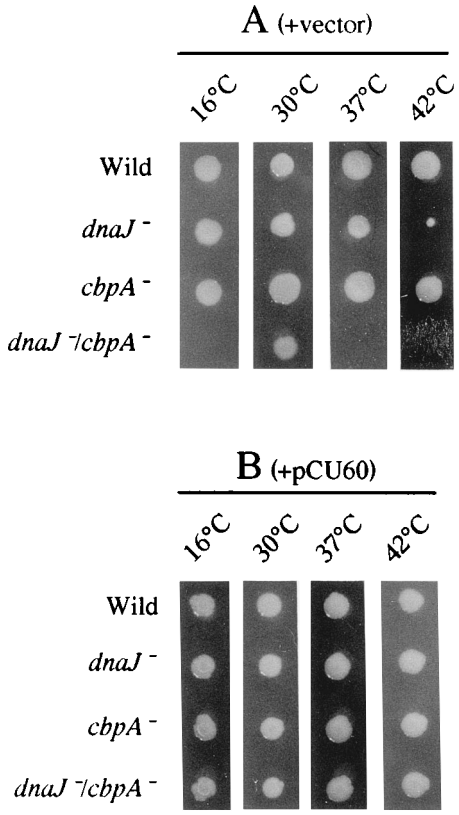

FIG. 1. Temperature sensitivity for growth. Strains MC4100 (Wild), KY1456 (*dnaJ*::Tn*10-42* [DnaJ$^-$]), CU245 (*cbpA*::*kan* [CbpA$^-$]), and CU247 (*dnaJ*::Tn*10-42 cbpA*::*kan* [DnaJ$^-$ CbpA$^-$]) were grown in Luria broth at 30°C overnight. After appropriate dilution, portions (about the same numbers of cells for each strain) were spotted on Luria agar plates and then incubated at the indicated temperatures. (A) Results for strains carrying a control plasmid vector. (B) Results for strains carrying a multicopy plasmid (pCU60) containing the *cbpA* gene.

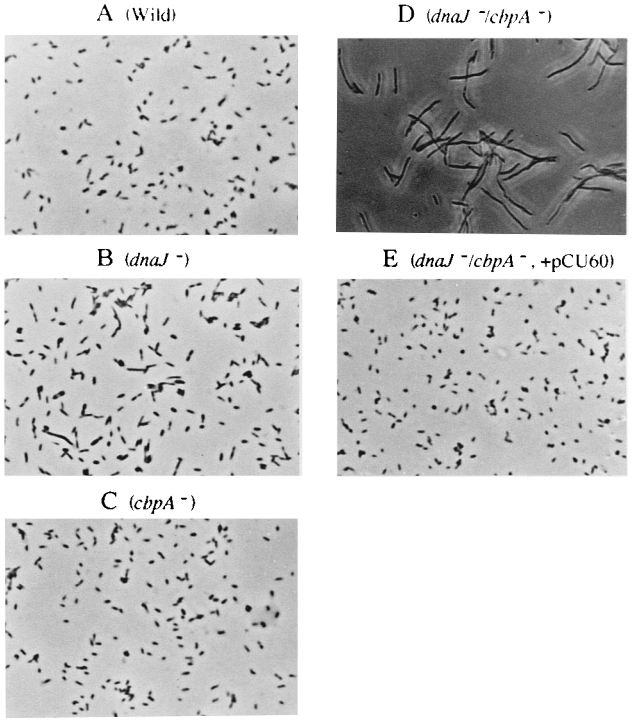

FIG. 2. Microscopic observation of cell morphology. The same set of strains as for Fig. 1 was grown in Luria broth at 30°C overnight. After being inoculated into fresh medium, they were incubated further for 6 h at 37°C. A drop of culture was placed on a glass slide and then examined in a microscope (Olympus model BH-2) with a DPlanApo 100UV objective and phase-contrast optics. Photographs of representative cells from multiple cultures were taken with Fujichrome 100 film. Scale bars are not indicated because the wild-type cells were found to have ordinary sizes (i.e., 2 to 3 μm in length), and all micrographs were prepared with the same magnification as that for the wild type.

grow at lower temperatures. These observations are consistent with those reported previously (8, 13). However, the *dnaJ cbpA* mutant exhibited a very narrow temperature range for growth. It was sensitive for growth not only at high temperatures (>37°C), as noted previously (13), but also at low temperatures (<16°C). The temperature-dependent defect for growth was relieved upon introduction of the *cbpA* gene on a multicopy plasmid in both the *dnaJ* and *dnaJ cbpA* null mutants (Fig. 1B). The very narrow temperature range for growth observed here for the *dnaJ cbpA* null mutant but not for the *dnaJ* null mutant was found to be very similar to that reported for the *dnaK* null mutant (1, 7, 8).

The *cbpA dnaJ* double-null mutant exhibits a defect in cell division. Next, to examine a possible defect in cell division, these strains were grown in Luria broth at 30°C and then incubated at 37°C for 6 h. These cells were observed microscopically (Fig. 2). The wild-type and *cbpA* null mutant strains appeared to be normal (Fig. 2A and C, respectively), while the *dnaJ* null mutant exhibited an abnormal morphology with a slightly longer cell length in a subpopulation (Fig. 2B). However, the *dnaJ cbpA* null mutant produced many more filaments (Fig. 2D). Although the *dnaJ cbpA* null mutant cells were filamentous even when growing at 30°C (data not shown), they formed more filaments after the temperature shift to 37°C. Furthermore, this defect in cell division was complemented by introduction of the *cbpA* gene (Fig. 2E). The filamentous cells of the *dnaJ cbpA* null mutant were examined by means of fluorescence microscopy after DAPI (4′,6-diamidino-2-phenylindol) staining in order to see if the chromosomal DNA segregation was also defective (Fig. 3). The chromosome segregation appeared to occur normally, and each filamentous cell therefore contained multiple nuclei positioning regularly in the compartment (Fig. 3B). However, electron microscopic observation of a thin section of the *dnaJ cbpA* mutant cells revealed no septum in the filament (Fig. 3C). Thus, the excessive defect in cell division (e.g., filamentation without septation) observed for the *dnaJ cbpA* null mutant, but not for the *dnaJ* null mutant, was again found to be very similar to that reported for the *dnaK* null mutant (1, 7, 8).

The *cbpA dnaJ* double-null mutant is sensitive to carbon starvation. Another characteristic property reported for a *dnaK* mutation (*dnaK103* allele) but not for *dnaJ* null mutations is that the *dnaK* mutant is highly susceptible to killing by starvation for carbon, as reported by Spence et al. (10). The set of strains used in this study was grown on Luria agar plates at 30°C overnight, and then washed cells were spotted onto MOPS (morpholinepropanesulfonic acid) plates which lacked carbon (glucose). It was found that the *dnaJ cbpA* null mutant was unable to survive with the starvation for carbon, while other strains, including the *dnaJ* null mutant, were able to do so (data not shown).

Implications. In this study, we have demonstrated that the *cbpA* null mutation exhibits synthetic phenotypes, particularly when combined with the *dnaJ* null mutation. In particular,

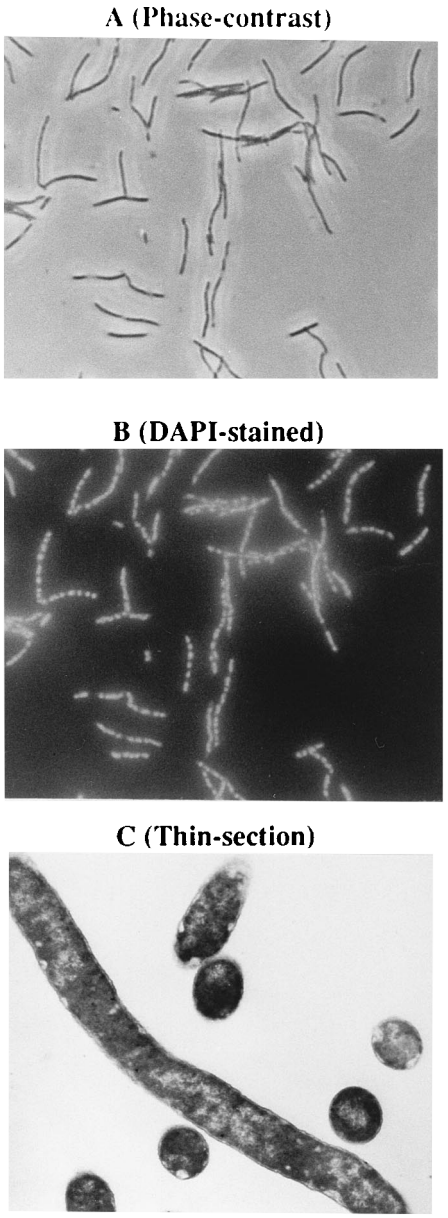

FIG. 3. Microscopic observation of DAPI-stained cells. Strain CU247 (*dnaJ cbpA*) was grown under the conditions described in the legends to Fig. 1 and 2. The cells were stained with DAPI and then examined by phase-contrast microscopy (A) or fluorescence microscopy (B). Thin sections of the cells were also subjected to electron microscopic observation (C). For thin sectioning, samples were fixed overnight with 1% OsO_4 in acetate-Veronal buffer, dehydrated with ethanol, and embedded in Epon. Sections were stained with uranyl acetate and lead citrate. Observation was carried out with a Hitachi model HS-9 electron microscope.

these synthetic phenotypes (i.e., a very narrow temperature range for growth, a defect in cell division with filamentation, and a susceptibility to killing by carbon starvation) are all very similar to those characteristic for *dnaK* mutations. Together with the fact that *dnaJ* mutations alone do not vigorously exhibit such phenotypes, our results are compatible with the idea that the *cbpA* gene product from the chromosome is capable of compensating for the *dnaJ* gene product in at least some cellular processes even under physiological conditions. As has been postulated for DnaJ, CbpA may directly interact with DnaK and/or GrpE. It would thus be of interest to examine this possibility in vitro. It is also worth mentioning that we recently demonstrated that the expression of *cbpA* is largely dependent on the alternative sigma factor, σ^S (15), whereas that of *dnaJ* is well known to be controlled by σ^{32} (4). This fact may suggest that DnaJ and CbpA display similar (or overlapping) activities but function preferentially under different circumstances. In short, on the basis of these results, we propose that the function(s) of CbpA in *E. coli* is closely related to that of DnaJ.

We thank C. Wada (Institute for Virus Research, Kyoto University) for the gift of the set of mutant heat shock genes.

This study was supported by a Grant-in-Aid for Scientific Research on a Priority Area (no. 06261217) from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. **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.
2. **Ellis, R. J., and S. M. van der Vies.** 1991. Molecular chaperones. Annu. Rev. Biochem. **60:**321–347.
3. **Georgopoulos, C.** 1992. The emergence of the chaperone machines. Trends Biochem. Sci. **17:**295–299.
4. **Grossman, A. D., J. W. Erickson, and C. A. Gross.** 1984. The *htpR* gene of *E. coli* is a sigma factor for heat-shock promoters. Cell **38:**383–390.
5. **Kawasaki, Y., C. Wada, and T. Yura.** 1990. Roles of *Escherichia coli* heat shock proteins DnaK, DnaJ and GrpE in mini-F plasmid replication. Mol. Gen. Genet. **220:**277–282.
6. **Liberek, K., J. Marszalek, D. Ang, C. Georgopoulos, and M. Zylicz.** 1991. *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc. Natl. Acad. Sci. USA **88:**2874–2878.
7. **McCarty, J. S., and G. C. Walker.** 1994. DnaK mutants defective in ATPase activity are defective in negative regulation of the heat shock response: expression of mutant DnaK proteins results in filamentation. J. Bacteriol. **176:**764–780.
8. **Sell, S. M., C. Eisen, D. Ang, M. Zylicz, and C. Georgopoulos.** 1990. Isolation and characterization of *dnaJ* null mutants of *Escherichia coli*. J. Bacteriol. **172:**4827–4835.
9. **Silver, P. A., and J. C. Way.** 1993. Eukaryotic DnaJ homologs and the specificity of Hsp70 activity. Cell **74:**5–6.
10. **Spence, J., A. Cegielska, and C. Georgopoulos.** 1990. Role of *Escherichia coli* heat shock proteins DnaK and HtpG (C62.5) in response to nutritional deprivation. J. Bacteriol. **172:**7157–7166.
11. **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.
12. **Tilly, K., and M. Yarmolinsky.** 1989. Participation of *Escherichia coli* heat shock proteins DnaJ, DnaK and GrpE in P1 plasmid replication. J. Bacteriol. **171:**6025–6029.
13. **Ueguchi, C., M. Kakeda, H. Yamada, and T. Mizuno.** 1994. An analogue of the DnaJ molecular chaperone in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **91:**1054–1058.
14. **Wild, J., E. Altman, T. Yura, and C. A. Gross.** 1992. DnaK and DnaJ heat shock proteins participate in protein export in *Escherichia coli*. Genes Dev. **6:**1165–1172.
15. **Yamashino, T., M. Kakeda, C. Ueguchi, and T. Mizuno.** 1994. An analogue of the DnaJ molecular chaperone whose expression is controlled by σ^S during the stationary phase and phosphate starvation in *Escherichia coli*. Mol. Microbiol. **13:**475–483.
16. **Zylicz, M., D. Ang, K. Liberek, and C. Georgopoulos.** 1989. Initiation of λ DNA replication with purified host- and bacteriophage-encoded proteins: the role of the *dnaK*, *dnaJ* and *grpE* heat shock proteins. EMBO J. **8:**1601–1608.