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The *cbpA* Chaperone Gene Function Compensates for *dnaJ* in λ Plasmid Replication during Amino Acid Starvation of *Escherichia coli*

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We found previously that λ plasmid DNA replication in amino acid-starved *Escherichia coli relA* mutants (i.e., during the relaxed response), which is carried out by the inherited replication complex, is dependent on functions of DnaK and GrpE molecular chaperones but proceeds in a *dnaJ* mutant at a nonpermissive temperature. Here we demonstrate that this replication is inhibited when functions of both *dnaJ* and *cbpA* are impaired. In complete media, the growth of the λπA66 phage (capable of replicating in *E. coli dnaJ*, *dnaK*, and *grpE* missense mutants at 30°C), as well as efficiency of transformation by the λπA66 plasmid, is significantly decreased in a *dnaJ259 cbpA*::*kan* double mutant. These results strengthen the proposal of other authors (C. Ueguchi, M. Kakeda, H. Yamada, and T. Mizuno, Proc. Natl. Acad. Sci. USA 91:1054–1058, 1994; C. Ueguchi, T. Shiozawa, M. Kakeda, H. Yamada, and T. Mizuno, J. Bacteriol. 177:3894–3896, 1995; and T. Yamashino, M. Kakeda, C. Ueguchi, and T. Mizuno, Mol. Microbiol. 13:475–483, 1994) that the *cbpA* gene product is a functional analog of the DnaJ chaperone in *E. coli*.

In *Escherichia coli*, amino acid starvation provokes many biochemical and physiological changes, together called the stringent response (for a review, see reference 3). The first event of this response is synthesis of relatively large amounts of guanosine 5′-triphosphate-3′-diphosphate (pppGpp) and guanosine 5′-diphosphate-3′-diphosphate (ppGpp) (pppGpp may be converted to ppGpp by the *gpp* gene product). These nucleotides interact with RNA polymerase (9), causing many dramatic changes in expression of different genes. The main protein responsible for (p)ppGpp synthesis is the *relA* gene product, called (p)ppGpp synthetase I. In amino acid-starved *relA* (relaxed) mutants, ppGpp is not accumulated, and such a response to amino acid starvation is called the relaxed response (3).

Replication of plasmids derived from bacteriophage λ (so-called λ plasmids) is inhibited in amino acid-starved wild-type *E. coli* cells because of ppGpp-mediated inhibition of transcriptional activation of *ori*λ (10) but proceeds for several hours in relaxed mutants (16). The replication is carried out by the replication complex inherited by one of two daughter plasmid copies after each replication round (17). Although the functions of the molecular chaperones DnaK and GrpE are necessary for activity of the inherited replication complex, the replication proceeds in amino acid-starved *relA dnaJ259* double mutants at a nonpermissive temperature, 43°C (15). These findings were surprising, as it was demonstrated previously that DnaJ, DnaK, and GrpE proteins cooperate in λ DNA replication in vitro (1, 21). On the other hand, an analog of the DnaJ protein has been recently discovered in *E. coli* (12). A gene coding for this analog, *cbpA*, was cloned and shown to be a suppressor for the Δ*dnaJ* mutation when present on a multicopy plasmid (12). The suppression also concerns the growth of λ phage (12). Therefore, we investigated λ plasmid replication in amino acid-starved *relA2 cbpA*::*kan* and *relA2 dnaJ259 cbpA*::*kan* mutants.

The previously described *cbpA*::*kan* mutant, strain CU245 (13), was used. The *cbpA*::*kan* mutation, as well as *dnaJ259 thr*::Tn*10* from strain BM239 (18), was transferred to strain CP79 carrying the *relA2* allele (4) by P1 transduction. Wild-type λ phage and plasmid cannot replicate in *E. coli dnaJ259*, *dnaK756*, and *grpE280* chaperone mutants at 30°C, but π mutants with mutations in the λ *P* gene can (for recent reviews, see references 8 and 11). The λπA66 phage mutant was isolated previously (7, 15), and the λπA66 plasmid (pAW6) has been constructed (15). The πA66 allele bears a single mutation, a G-to-T alteration at nucleotide position 410, resulting in an arginine-to-leucine exchange at amino acid position 137 of the λ P protein (15). λ phage harboring the πA66 mutation yielded progeny (in one-step growth experiments) at both 30 and 43°C in wild-type cells and only at 30°C in *dnaJ259*, *dnaK756*, and *grpE280* mutants (15). A plasmid derived from λπA66 phage (pAW6) can replicate in the above-mentioned chaperone mutants at 30°C, in contrast to wild-type λ plasmid (15). During the relaxed response, replication of pAW6 was inhibited at 43°C in *dnaK756* and *grpE280* mutants but not in *dnaJ259* bacteria (15).

We investigated the replication of the λπA66 plasmid (pAW6) in *E. coli* strains by estimation of the change in relative plasmid content per bacterial mass with time as previously described (5) and by measurement of incorporation of [^{3}H]thymidine during pulse-labeling according to the method of Herman et al. (6). Amino acid starvation results in an inhibition of bacterial growth. Thus, if plasmid replication were stopped the relative plasmid content per bacterial mass should stay constant. An increase in the plasmid content with time in such an experiment indicates continued plasmid DNA replication. In the second type of experiments, a decrease in [^{3}H]thymidine incorporation during pulse-labeling indicates inhibition of plasmid replication whereas a constant incorporation level re-

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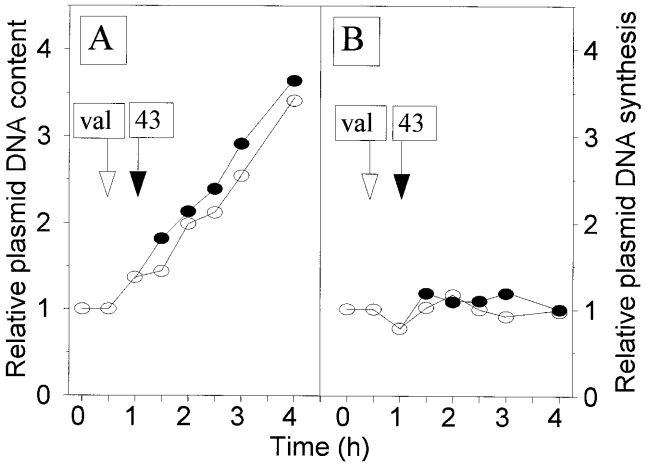

FIG. 1. Relative plasmid DNA content (A) and relative plasmid DNA synthesis (B) per unit of bacterial mass in an isoleucine-starved *E. coli relA2 cbpA*::*kan* strain harboring λ plasmid (pAW6) at 30°C (○) and 43°C (●). Isoleucine starvation was provoked by addition of L-valine (val) to minimal medium up to 1 mg/ml (open arrow). One half of the culture was transferred to 43°C (43) at the time indicated by the filled arrow. Relative plasmid DNA content was estimated by densitometry of appropriate plasmid bands in ethidium bromide-stained agarose gels (5). Relative plasmid DNA synthesis was calculated on the basis of measurement of [^{3}H]thymidine incorporation into plasmid DNA during pulse-labeling (6).

sults from replication carried out by the preassembled replication complexes, whose number does not change over time (17). We found that λ plasmid replication proceeds in *relA2 cbpA*::*kan* bacteria after induction of isoleucine starvation (provoked by addition of an excess of L-valine) at 30 and 43°C (Fig. 1). Similar results were obtained previously with a *relA dnaJ259* mutant (15). However, we found that the replication is inhibited when functions of both *dnaJ* and *cbpA* are abolished at 43°C in the *relA2 dnaJ259 cbpA*::*kan* strain (Fig. 2). These

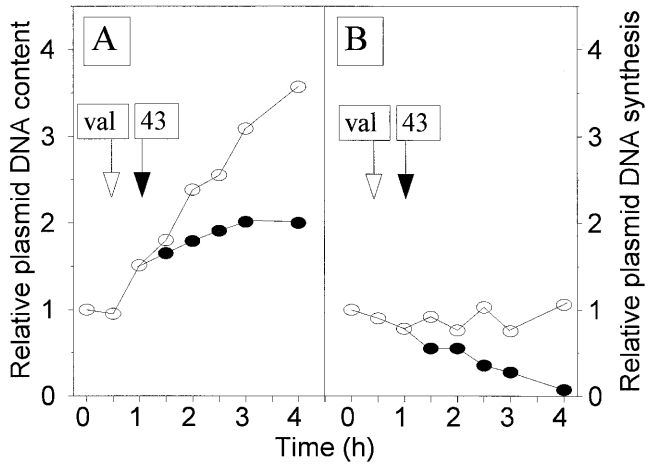

FIG. 2. Relative plasmid DNA content (A) and relative plasmid DNA synthesis (B) per unit of bacterial mass in an isoleucine-starved *E. coli relA2 dnaJ259 cbpA*::*kan* strain harboring λ plasmid (pAW6) at 30°C (○) and 43°C (●). Isoleucine starvation was provoked by addition of L-valine (val) to minimal medium up to 1 mg/ml (open arrow). One half of the culture was transferred to 43°C (43) at the time indicated by the filled arrow. Relative plasmid DNA content was estimated by densitometry of appropriate plasmid bands in ethidium bromide-stained agarose gels (5). Relative plasmid DNA synthesis was calculated on the basis of measurement of [^{3}H]thymidine incorporation into plasmid DNA during pulse-labeling (6).

TABLE 1. Efficiencies of plating and plaque morphologies of λ phages on *E. coli dnaJ* and *cbpA* mutants at 30°C

Relevant genotype of bacteria	Relative efficiency of plating		Morphology of plaques
	λ*cIb2*	λ*cIb2*πA66	
dnaJ$^+$ *cbpA*$^+$	1[a]	1[b]	Normal
dnaJ259 cbpA$^+$	$<10^{-7}$	0.5	Small
dnaJ$^+$ *cbpA*::*kan*	1.4	1.4	Normal
dnaJ259 cbpA::*kan*	$<10^{-7}$	0.00005	Very small

[a] The efficiency-of-plating value of 1 corresponds to 2×10^{10} PFU/ml.
[b] The efficiency-of-plating value of 1 corresponds to 2×10^{11} PFU/ml.

results indicate that *cbpA* function is capable of compensating for *dnaJ* in λ plasmid replication during amino acid starvation. Thus, the replication carried out by the inherited replication complex requires *dnaJ* or *cbpA* function (one of them is enough) as no inhibition in λ plasmid DNA synthesis was observed in the presence of either *dnaJ*$^+$ (Fig. 1) or *cbpA*$^+$ (15). As shown in Fig. 2, λ plasmid DNA replication was significantly decreased but not completely abolished in *relA2 dnaJ259 cbpA*::*kan* mutants after a shift to 43°C. This might result from possible leakage of the *dnaJ259* mutation. In fact, although certain *dnaJ* functions are impaired in the *dnaJ259* strain, our unpublished data indicate that this mutant is able to form colonies at 43°C. One may also speculate that there is still another (as yet undiscovered) DnaJ homolog in *E. coli* that can partially compensate for the loss of both CbpA and DnaJ activity.

Suppression of the *dnaJ* defect in λ phage growth was observed previously only when the *cbpA* gene was present in cells on a multicopy plasmid (12). Our results demonstrate that the chromosomal *cbpA* gene is capable of compensating for *dnaJ* in λ plasmid replication proceeding in amino acid-starved *relA* mutants. Since expression of *cbpA* is increased during the stationary phase of growth (20), one may expect an increased level of the CbpA protein in amino acid-starved bacteria.

We also found *cbpA*-mediated effects on λ phage and λ plasmid replication under normal growth conditions. We investigated the efficiency of plating and plaque morphology of λ phages on different *E. coli* strains. Since strain CP79 is resistant to bacteriophage λ because of the *malA1* mutation (19), a new series of strains was constructed. The *dnaJ259 thr*::Tn*10* strain BM265 (15) and the *cbpA*::*kan* derivative of *E. coli* MC4100, strain CU245 (13), were used as donors in P1 transduction. Next, MC1061-derived and otherwise-isogenic strains containing combinations of the above-described mutations or wild-type *dnaJ* and *cbpA* alleles were constructed. Phages λ*cIb2* and λ*cIb2*πA66 (15) were used. We found that phage λ*cIb2*πA66, able to grow on the *dnaJ259* mutant at 30°C, reveals a significantly lower (more than 4 orders of magnitude) efficiency of plating on the *dnaJ259 cbpA*::*kan* double mutant, forming extremely small plaques (Table 1). We also observed a negative effect of the *cbpA*::*kan* mutation on the efficiency of transformation by a wild-type λ plasmid, pCB104 (2), and its πA66 derivative (15), pAW6 (Table 2). In a control experiment, the efficiency of transformation by a ColE1-type plasmid (pUC19), which does not require DnaJ function, was not significantly affected by *dnaJ259* and *cbpA*::*kan* mutations (Table 2). It is worth mentioning that until now, the effects of the *cbpA* mutation were observed only in a Δ*dnaJ* genetic background (13). Our experiments (Tables 1 and 2) demonstrated for the first time that the absence of *cbpA* function may cause changes in intracellular processes under conditions in which the *dnaJ*$^+$ or *dnaJ259* allele is expressed.

TABLE 2. Transformation efficiency of *dnaJ* and *cbpA* mutants with λ plasmids and a ColE1-type plasmid at 30°C

Relevant genotype of bacteria	Relative transformation efficiency		
	pCB104 (pλ$^+$)	pAW6 (pλπA66)	pUC19 (ColE1)
dnaJ$^+$ *cbpA*$^+$	1[a]	1[b]	1[c]
dnaJ259 cbpA$^+$	$<10^{-4}$	2.3	1.1
dnaJ$^+$ *cbpA*::*kan*	0.11	0.075	0.76
dnaJ259 cbpA::*kan*	$<10^{-4}$	0.070	1.2

[a] The value of 1 corresponds to 1.6×10^5 transformants per 1 μg of plasmid DNA.
[b] The value of 1 corresponds to 1.2×10^5 transformants per 1 μg of plasmid DNA.
[c] The value of 1 corresponds to 3.0×10^6 transformants per 1 μg of plasmid DNA.

In conclusion, the results presented in this work indicate that, contrary to previous suggestions (14, 15), DnaJ function is required for λ plasmid replication carried out by the inherited replication complex in amino acid-starved cells and, when missing, may be replaced by the *cbpA* gene product. This supports the proposal presented by Ueguchi and coworkers (12, 13) and Yamashino et al. (20) that CbpA is a functional analog of DnaJ. Moreover, our results signal a role for *cbpA* in λ DNA replication when the *dnaJ*$^+$ or *dnaJ259* allele is expressed under normal growth conditions. We presume that CbpA may have a role similar to that of DnaJ in the chaperone-mediated insertion of the DnaB helicase-containing structure (preprimosome or inherited replication complex) between the complementary DNA strands, transiently separated through transcriptional activation of *ori*λ, as proposed recently (11, 14, 15, 18).

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