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# Participation of *Escherichia coli* Heat Shock Proteins DnaJ, DnaK, and GrpE in P1 Plasmid Replication

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**Low-copy-number plasmids, such as P1 prophage and the fertility factor F, require a plasmid-encoded replication protein and several host products for replication. Stable maintenance also depends on active partitioning of plasmids into daughter cells. Mini-P1 *par*$^+$ and *par* plasmids were found to be destabilized by mutations in the *dnaJ*, *dnaK*, and *grpE* genes of *Escherichia coli*. The transformation efficiency and stability of mini-F plasmids were also reduced in the mutant strains. These results indicate that heat shock proteins DnaJ, DnaK, and GrpE play roles in the replication of plasmid P1 and probably also in that of F.**

Bacteriophage P1 is a temperate coliphage whose prophage exists as a plasmid with a copy number approximately that of the host chromosome (32). Several mechanisms ensure stable retention of P1 and other low-copy-number plasmids. They include replication systems that control plasmid copy number and a partition apparatus that directs faithful segregation of plasmids to daughter cells (see reference 50 for a review).

The plasmid replicon of P1 is organized similarly to several other replicons, including two in fertility factor F (21, 36). Each consists of an origin of replication and an adjacent gene (*repA* in P1) that encodes an essential initiator protein. Immediately downstream of the initiator gene is a regulatory site (*incA* in P1) that exerts copy number control. Functions essential for plasmid partitioning are located adjacent to copy number control regions in P1 and F (2, 7).

Several *Escherichia coli* proteins are known to participate in the replication of these low-copy-number plasmids. In vivo, P1 replication requires genes *dnaA* (17), *dnaB*, *dnaC*, and *dnaG* (38). In vitro experiments indicate that DNA gyrase, RNA polymerase, and a fraction that contains DNA polymerase III are also required (49; S. Wickner, personal communication). Plasmid F requires many of the same proteins for its replication (15, 16, 20, 27, 44, 45). Mutations in several genes that destabilize F-derived plasmids have defined other functions, such as HU, that are required for F (and potentially P1) replication (29, 47, 48). Unlike F replication from *ori-2*, efficient P1 plasmid replication depends on adenine methylation at GATC sites within the origin (1). Unlike P1, F depends on the heat shock σ factor for transcription of a gene that encodes an initiator protein for plasmid replication (46; unpublished data).

As part of an effort to identify host proteins required for faithful plasmid maintenance, we tested *E. coli* mutants with defects in heat shock genes *dnaJ*, *dnaK*, and *grpE*. The products of these genes are necessary at elevated temperatures for *E. coli* growth (5, 10, 13, 34, 42) and at all temperatures for replication of bacteriophage λ (13, 33, 42). During λ DNA replication, the three proteins participate in the same step of initiation (25, 52). There is both genetic and biochemical evidence of pairwise interaction of DnaK with the other two proteins (S. Sell, Ph.D. thesis, University of Utah, Salt Lake City, 1987; D. Ang, Ph.D. thesis, University of Utah, Salt Lake City, 1988; 19, 51). The experiments described here provide genetic evidence for the involvement of the DnaK, DnaJ, and GrpE proteins in both mini-P1 (*oriR*) and mini-F (RepFIA *ori-2*) plasmid replication. The proteins probably play the same roles in the replication of complete P1 and F plasmids. B. Bukau and G. C. Walker independently found that mini-P1 plasmids are unstable in *dnaJ* and *dnaK* mutant strains (see reference 11).

## MATERIALS AND METHODS

**Media.** L broth (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl per liter; Quality Biological, Inc.) was used for growth of bacteria in liquid. Antibiotics were used at the following concentrations: spectinomycin, 40 μg/ml; ampicillin, 100 μg/ml; chloramphenicol, 20 μg/ml.

**Bacterial strains.** The *E. coli* K-12 strains used in this study are described in Table 1. Transductions and transformations were performed as described in reference 39.

**Plasmid and phage constructions.** The cloning procedures used were as described in reference 23. The spectinomycin-resistant version of λ–mini-P1 used in all λ–mini-P1 experiments (unless otherwise indicated) was derived from λ–P1:5R-3 (41). The plasmid was constructed by first ligating the P1 *Sal*I-*Bam*HI *loxP*-containing fragment of pRH43 (4) with the *Bam*HI-*Hin*dIII *aadA*$^+$ fragment of pDPT270 (43), which confers spectinomycin resistance, and *Hin*dIII-*Sal*I-digested pMC9 DNA, which contains *lacI*$^q$ (26). The *lacI*$^q$ *aadA loxP* part of this plasmid was crossed into λ–P1:5R-3 *lacI*$^q$ *tet* (constructed by E. B. Hansen) by homologous recombination with the *lacI*$^q$ gene and the remaining segment of the pMC9 *tet* gene. The λ–mini-F used was constructed by crossing λ-F585 (7) with a similarly constructed chloramphenicol-resistant (Cm$^r$) *lacI*$^q$ *loxP* λ–mini-P1 and selecting for phage that made bacteria Cm$^r$ but were not destabilized by P1 *incA* cloned on a mini-F vector. Mini-P1 plasmids pSP102, pSP108 (30), and pSP152 contain, respectively, none, one, and three of the nine RepA-binding sites that make up plasmid replication regulatory region *incA*. The binding site in pSP108 is in its natural location (downstream of the *repA* gene), but the three binding sites present in pSP152 were placed in a *Pvu*I site at a comparable distance to the other side of the plasmid origin (D. Chattoraj, personal communication). Spectinomycin-resistant versions of pSP102 and pSP108 were constructed by replacing the *Bam*HI-*Pst*I fragment containing the chloramphenicol acetyltransferase gene (*cat*) in each plasmid with a *Bam*HI-*Pst*I *aadA*$^+$ fragment containing the *Bam*HI-*Hin*dIII fragment

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TABLE 1. Bacterial strains

| Strain | Genotype | Source or reference |
|---|---|---|
| MF670 | W3110 *trpR thr* | 14 |
| JCB113 | C600 *thr*$^+$ *dnaJ259* | 42 |
| BR3671 | MF670 *thr*$^+$ *dnaJ259* | This work |
| PK102 | MG1655 Δ*dnaJ*::*kan* | P. J. Kang |
| BR3673 | MF670 *thr*$^+$ Δ*dnaJ*::*kan* | This work |
| RB85 | *thr leu supE thi lacY rpsL* | C600 of reference 18 |
| RB851 | RB85 *thr*$^+$ *dnaK7 tonA* | 18 |
| DA15 | B178 *pheA*::Tn*10* | 19 |
| DA16 | DA15 *grpE280* | 19 |
| DA258 | CG799 *thr*::Tn*10* Ω-*cat*, mini-Tn*5* near *grpE*$^+$ | 6 |
| DA259 | DA258 Δ*grpE*::Ω-*cat* | 6 |

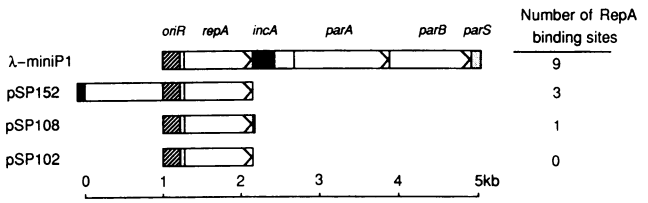


FIG. 1. Mini-P1 plasmid structures. The large arrows represent the open reading frames for the indicated genes. The filled boxes represent the indicated sites. The segment of *incA* present in pSP152, RepA-binding sites 7 to 9 (3), is located to the opposite side of *oriR* from that found in pSP108, which has binding site 9 in its natural location. Only the RepA-binding sites derived from *incA* are enumerated. kb, Kilobase pairs.

from pDPT270 (43) and pUC18 polylinker sequences (from a plasmid constructed by S. Elledge, Stanford University, Stanford, Calif.). The plasmids and transducing phages used to complement the mutant strains have been previously described (λ *dnaK*$^+$ and λ *dnaK*$^+$J$^+$ [34], pBR322 *dnaK*$^+$ is p*dnaK*$^+$ [8], and pBR322 *grpE*$^+$ is pDA1 [6]).

**Plasmid stability assay.** Plasmid stability was measured by plasmid loss during nonselective growth at 32°C. Strains were transformed or lysogenized with the plasmid or phage to be assayed, purified, and grown with the appropriate antibiotic to ensure plasmid retention. The cells were diluted into nonselective medium, and the percentage of plasmid-free cells was measured by plating a sample of the culture on L plates and assaying the fraction of drug-sensitive cells by replica plating cells onto plates containing the appropriate antibiotic. Less than 1% of the cells were plasmid free, except when otherwise indicated. After growth for a time, the proportion of plasmid-free cells was again measured and the number of generations elapsed was calculated. At least 100 colonies were tested for each time point. Logarithmic loss was observed and used for extrapolation to 20 generations, and only cultures that had grown nonselectively for more than 15 generations were used as a basis for extrapolations. All experiments were repeated at least twice with independent transformants or lysogens. In all comparisons among strains, the relative instability of plasmids remained similar.

**Copy number determination by slot blot hybridization.** Total *E. coli* DNA was extracted from selectively grown log-phase cultures by the method of reference 39. Denaturation of DNA and application to nitrocellulose in a Schleicher & Schuell Minifold II slot blotter were as recommended by the manufacturer. Hybridizations with nick-translated probes were performed as described in reference 23. The probe for pSP102 and pSP108 was a 1.9-kilobase-pair fragment containing the *cat* gene (40). For the spectinomycin-resistant versions of pSP108 and pSP102, the probe was a 2-kilobase-pair *Bam*HI fragment that contains the *aadA* gene and pUC18 polylinker sequences (S. Elledge, personal communication). The *oriC* probe was a 1.3-kilobase-pair *Hin*cII fragment from pUA6 (a gift from A. Leonard, Roswell Park Memorial Institute) which contains the *oriC* region. The fragments were isolated from minigels with NA45 membrane (Schleicher & Schuell) as recommended by the manufacturer.

## RESULTS

**Instability of λ–mini-P1 in *dnaJ* mutant bacteria.** λ–mini-P1 is a hybrid containing the plasmid replication and partition regions of P1 cloned into an integration-defective bacteriophage λ vector (Fig. 1). It replicates as a unit copy plasmid in the prophage state, driven by the P1 plasmid replication system and partitioned by the P1 partition system. λ–mini-P1 was significantly less stable in the *dnaJ259* (point mutant) and Δ*dnaJ*::*kan* (substitution mutant, abbreviated Δ*dnaJ*) strains than in the wild-type strain (Table 2), suggesting that the *dnaJ* gene product plays a role in mini-P1 plasmid replication or partitioning. The requirement for DnaJ protein was not absolute, since it was possible to construct Δ*dnaJ* mutant strains containing λ–mini-P1 plasmids.

The *dnaJ* gene is located in an operon downstream of the *dnaK* gene (34), and there is evidence that the two proteins interact (Sell, Ph.D. thesis). Complementation experiments were performed to determine whether or not the *dnaJ* mutation was directly responsible for the plasmid instability phenotype. The presence of a λ *dnaK*$^+$ transducing phage did not increase the stability of λ–mini-P1 in *dnaJ* mutants, whereas the presence of both the *dnaJ*$^+$ and *dnaK*$^+$ genes (provided by λ *dnaK*$^+$ *dnaJ*$^+$) stabilized the plasmid (Table 2). These results indicate that the plasmid instability phenotype was not an indirect effect on *dnaK* gene expression or DnaK protein activity. We also found that transduction of a *dnaJ* strain to *dnaJ*$^+$ (in which case any unlinked compensatory mutations would remain) restored the ability of the

TABLE 2. Instability of mini-P1 in *dnaJ* mutant strains

| Plasmid and host genotype | % Loss in 20 generations[a] |
|---|---|
| λ–mini-P1 | |
| *dnaJ*$^+$ | 1 |
| *dnaJ259* | 31 |
| Δ*dnaJ* | 99 |
| *dnaJ259*(λ *dnaK*$^+$) | 47 |
| *dnaJ259*(λ *dnaK*$^+$*J*$^+$) | 2 |
| pSP102 | |
| *dnaJ*$^+$ | <0.5 |
| *dnaJ259* | 1 |
| Δ*dnaJ* | 5 |
| pSP108 | |
| *dnaJ*$^+$ | <0.5 |
| *dnaJ259* | 3 |
| pSP152 | |
| *dnaJ*$^+$ | 2 |
| *dnaJ259*[b] | 55 |

[a] More than 500 colonies were counted to obtain these numbers.
[b] When grown with selection, 28% of the cells were plasmid free.

strain to maintain λ–mini-P1 stably. Therefore, the defect in plasmid maintenance was due to the *dnaJ* mutation.

**Instability of mini-P1 Δ*par* plasmids in *dnaJ* mutant bacteria.** λ–mini-P1 requires both replication and partition functions to be stably retained during growth of a bacterial culture. To distinguish host defects that affect replication from those that affect partitioning, we tested the effects of the *dnaJ* mutations on the stability of mini-P1 Δ*par* plasmids that are maintained independently of the P1 partitioning system. Host mutations that affect only plasmid partitioning would have no effect on such plasmids, but mutations that affect plasmid replication should alter their stability. The *par*-deleted plasmids pSP108, pSP102, and pSP152 (Fig. 1) have higher copy numbers than λ–mini-P1 because of partial (pSP108 and pSP152) or complete (pSP102) deletion of copy number control region *incA*. Their increased copy numbers allow the plasmids to be reasonably stable despite being partitioned passively.

The Δ*dnaJ* mutation destabilized pSP102 (which normally has a copy number of about eight per host chromosome; 30). Although the *dnaJ259* mutation had no detectable effect on pSP102 stability (Table 2), it did destabilize lower-copy-number plasmid pSP108. This plasmid has a copy number of about four per host chromosome in wild-type cells (30), and its stability should, therefore, be more sensitive to a replication defect. The copy number of pSP152 should be lower than that of pSP108 because it bears three of the nine replication regulatory repeats that make up *incA* (Fig. 1). The stability of pSP152 is even more sensitive to the *dnaJ* defect (Table 2). These results suggest that mini-P1 plasmid replication is defective in *dnaJ* mutant strains.

A further indication of a plasmid replication defect in *dnaJ* mutant strains was provided by measurements of mini-P1 plasmid copy numbers. The copy number of pSP102 was reduced by 25% in the *dnaJ259* strain and was fourfold lower in the Δ*dnaJ* strain. These results are consistent with the stability measurements and confirm that the *dnaJ* gene product participates directly or indirectly in P1 plasmid replication.

**Instability of mini-P1 Δ*par* plasmids in *dnaK* mutant bacteria.** The knowledge that the DnaK and DnaJ proteins participate in the same stage of λ DNA replication (25, 52) prompted us to test whether DnaK protein also participates in P1 plasmid replication. It was not possible to test λ–mini-P1 stability in the *dnaK7* mutant, because λ–mini-P1 did not lysogenize the strain at an observable frequency and the mutation was never successfully transduced into a strain containing λ–mini-P1. Plasmids pSP108 and pSP152 (Fig. 1) transformed the *dnaK7* mutant strain and were destabilized by the mutation. These results indicate that the DnaK protein also participates in normal mini-P1 replication (Table 3). The presence of a *dnaK*$^+$ plasmid increased the stability of pSP108, whereas the presence of the vector pBR322 did not (Table 3), indicating that the *dnaK* mutation was responsible for the defect in mini-P1 plasmid replication.

**Instability of λ–mini-P1 in *grpE* mutant bacteria.** Knowing that the GrpE protein also interacts with DnaK protein (19) and participates in λ DNA replication (33), we measured λ–mini-P1 stability in *grpE* mutants. λ–mini-P1 was sufficiently unstable in both *grpE* point and deletion mutant strains (Table 4) to cause the *grpE* λ–mini-P1 lysogens to make tiny colonies. λ–mini-P1 was stable in Δ*grpE* strains containing a *grpE*$^+$ plasmid (Table 4), showing that the defect in plasmid retention resulted from the *grpE* mutation and not from any second-site suppressors. Also, the λ–mini-P1 lysogens in the complemented *grpE* deletion mutant made normal-size colonies. These findings indicate that the *grpE* gene product is also normally required for mini-P1 maintenance.

TABLE 3. Instability of mini-P1 Δ*par* plasmids in a *dnaK* mutant strain

| Plasmid and host genotype | % Loss in 20 generations[a] |
|---|---|
| pSP108 | |
| *dnaK*$^+$ | <1 |
| *dnaK7* | 5 |
| *dnaK7*(pBR322) | 8 |
| *dnaK7*(pBR322 *dnaK*$^+$) | 1 |
| pSP152 | |
| *dnaK*$^+$ | 3 |
| *dnaK7*[b] | 53 |

[a] More than 200 colonies were counted to obtain these numbers.
[b] When grown with selection, 26% of the cells were plasmid free.

**Instability of mini-P1 Δ*par* plasmids in the *grpE280* strain.** To examine the effects of *grpE* mutations on replication alone, we assayed the stability of mini-P1 Δ*par* plasmids in the *grpE280*-carrying strain. We found that mini-P1 Δ*par* plasmid pSP152, which presumably has a lower copy number than either pSP102 or pSP108, was unstable in the *grpE280* mutant strain (Table 4), suggesting that the GrpE protein plays a role in P1 plasmid replication. There was no observable effect on the stability of higher-copy-number mini-P1 Δ*par* plasmid pSP108, unless the *grpE* mutation was introduced into a strain that already contained the plasmid. When MF670 carrying pSP108 was transduced to tetracycline resistance by a transducing phage stock grown on a strain in which the *grpE280* mutation is linked to *pheA*::Tn*10*, about 20% of the *grpE280* transductants had lost pSP108 upon retesting, whereas no more than 2% of the *grpE*$^+$ transductants had lost the plasmid. In this experiment, about 40 generations of nonselective growth were allowed after transduction and there was no selection for plasmid retention, which could enrich for suppressors of the GrpE$^-$ phenotype. It is therefore likely that the GrpE protein also participates in P1 plasmid replication.

**Participation of heat shock proteins in mini-F maintenance.** Three experiments suggest that mini-F replication from the RepFIA *ori-2* origin also relies on the *dnaJ*, *dnaK*, and *grpE* gene products. We measured the transformation frequency of mini-F *rep*$^+$ *par*$^+$ plasmid pMF3 (24) relative to the frequency of transformation of pBR322 into various strains. The mini-F transformation frequency was reduced at least

TABLE 4. Instability of mini-P1 plasmids in *grpE* mutant strains

| Plasmid and host genotype[a] | % Loss in 20 generations |
|---|---|
| λ–mini-P1 | |
| *grpE*$^+$ | 4 |
| *grpE280* | 20 |
| *grpE*$^+$ | 9 |
| Δ*grpE* | 86 |
| Δ*grpE*(pBR322) | 56 |
| Δ*grpE*(pBR322 *grpE*$^+$) | 2 |
| pSP152 | |
| *grpE*$^+$ | 1 |
| *grpE280*[b] | 9 |

[a] The isogenic *grpE*$^+$ and *grpE280* strains were DA15 and DA16; the isogenic *grpE*$^+$ and Δ*grpE* strains were DA258 and DA259.
[b] When grown with selection, 5% of the cells were plasmid free.

20-fold by *dnaJ*, *dnaK*, and *grpE* mutations. We also found that a λ–mini-F hybrid (Materials and Methods) was lost from about 65% of the cells in a *dnaJ259* culture after overnight nonselective growth (approximately 18 generations), whereas the loss from isogenic wild-type cells was less than 5%. In addition, we found that transduction of *pheA*::Tn*10* linked to *grpE280* into a wild-type strain (MF670) containing pMF3 led to loss of the plasmid in nine of nine *grpE* transductants and none of five $grpE^+$ transductants. Therefore, it is likely that heat shock proteins DnaK, DnaJ, and GrpE are required for stable mini-F maintenance and, by analogy with P1, for F plasmid replication.

## DISCUSSION

The *E. coli* heat shock proteins comprise a group of at least 17 proteins whose synthesis rises dramatically after a sudden increase in growth temperature (see reference 28 for a review). Heat shock-regulated proteins have been found in all organisms tested (22, 37), and the amino acid sequences of at least some of the heat shock proteins have been remarkably conserved throughout evolution (8, 9, 17). These results suggest that heat shock proteins are of fundamental importance.

The DnaJ, DnaK, and GrpE heat shock proteins exhibit several pairwise interactions (Sell, Ph.D. thesis; Ang, Ph.D. thesis; 19, 52) and act together in bacteriophage λ DNA replication. The experiments described in this report provide the first evidence that these three proteins participate in plasmid replication. Normal mini-P1 replication and mini-F plasmid maintenance were found to be dependent on them. Involvement in mini-F replication, as opposed to partitioning, has not been proven. These experiments distinguish a new class of replicon, the members of which require DnaA protein (as *E. coli* does and λ does not) and also depend on the DnaJ, DnaK, and GrpE heat shock proteins (as λ does and *E. coli* apparently does not). Although there is genetic evidence that DnaK protein participates in normal *E. coli* replication (35), biochemical confirmation has not been obtained and there is neither in vivo nor in vitro evidence that DnaJ and GrpE proteins are directly involved in bacterial replication.

The requirements for replication may reflect the competitive disadvantage of low-copy-number plasmids. Heat shock proteins are thought to facilitate proper assembly and disassembly of macromolecular structures (12, 31). Low-copy-number plasmids may use heat shock proteins to promote proper assembly and disassembly of replication complexes and increase their ability to compete with other replicons. The DnaK, DnaJ, and GrpE proteins help dissociate the bacteriophage λ P protein from *E. coli* DnaB protein at the λ origin so that DNA replication can proceed (25, 52). As in λ, the origin-specific proteins of low-copy-number plasmids may attract a component of the bacterial replication machinery to the origin, but the affinity required to compete effectively with the bacterial origin may be so great that facilitation of subsequent dissociation is required.

The *dnaJ*, *dnaK*, and *grpE* mutations were found to have various effects on the stability of λ–mini-P1 and mini-P1 Δ*par* plasmids. In all cases, λ–mini-P1 was less stable than mini-P1 Δ*par* plasmids in heat shock gene mutants. Possible explanations for this difference include the following. (i) Mutations that affect replication have a greater effect on λ–mini-P1 because the copy number of λ–mini-P1 is lower than that of any of the mini-P1 Δ*par* plasmids (each of which lacks at least part of *incA*). (ii) λ–mini-P1 is more sensitive to small changes in replication efficiency because its partition region causes the variation in copy number per cell to be less than that of mini-P1 Δ*par* plasmids. A small change in average copy number could have a large effect on the stability of λ–mini-P1 by reducing the number of plasmids in every cell below a threshold value required for stable maintenance. A comparable shift in the average copy number of mini-P1 Δ*par* plasmids might have less of an effect on stability, because the proportion of bacteria whose plasmid copy number would dip below the threshold value would be relatively small. (iii) There may be uncontrolled differences in the accumulation of suppressor mutations because of differences in the selection pressure exerted in the construction of λ–mini-P1 lysogens and the strains containing mini-P1 Δ*par* plasmids. The Δ*grpE* strain must carry a suppressor mutation because the deletion could be transferred only into strains that had previously contained *dnaK* mutations (as is true for DA258; 6).

Although the *dnaJ*, *dnaK*, and *grpE* mutations destabilize mini-P1 plasmids, the inhibition of replication is not complete, even in the deletion mutants tested. In contrast, S. Wickner (personal communication) has demonstrated that in vitro P1 plasmid replication (49) is completely dependent on the DnaJ, DnaK, and GrpE proteins. The differences between in vivo and in vitro dependence on heat shock proteins may be due to the absence from the extracts of proteins active in vivo or the possible acquisition of suppressors in the strains used in the in vivo experiments. Further in vitro experiments should help define the steps at which the heat shock proteins act, clarify the nature of their roles in plasmid and λ DNA replication, and shed light on their functions in *E. coli* growth.

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