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ΔdnaK52 Mutants of *Escherichia coli* Have Defects in Chromosome Segregation and Plasmid Maintenance at Normal Growth Temperatures

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Major heat shock proteins, such as the *Escherichia coli* DnaK protein, not only are required for cell growth after heat shock but seem to possess important functions in cellular metabolism at normal growth temperatures as well. *E. coli* Δ*dnaK52* mutants have severe cellular defects at 30°C, one of which is in cell division (B. Bukau and G. C. Walker, J. Bacteriol. 171:2337–2346, 1989). Here we show that at 30°C, Δ*dnaK52* mutants have defects in chromosome segregation and in maintenance of low-copy-number plasmids. Fluorescence microscopic analysis revealed that chromosomes were frequently lacking at peripheries of cell filaments of Δ*dnaK52* mutants and clustered at other locations. In other parts of the cell filaments, chromosomes were apparently normally distributed and they were also present in most of the small cells found in populations of Δ*dnaK52* cells. These defects might be at the level of DNA replication, since Δ*dnaK52* mutants have a threefold lower rate of DNA synthesis than wild-type cells. Chromosome segregation defects of Δ*dnaK52* mutants were also observed in an *rnh dnaA* mutant background, in which initiation of DNA replication is DnaA-*oriC* independent. We also found that low-copy-number P1 miniplasmids could not be stably maintained in Δ*dnaK52* mutants at 30°C. Δ*par* P1 miniplasmids that carry the P1-encoded *rep* functions required for their replication but lack the P1-encoded *par* functions required for faithful partitioning of the plasmids during cell division were also unstable in Δ*dnaK52* mutants. Taken together, our results indicate important, although not absolutely essential, functions for DnaK at 30°C in one or more processes necessary for correct replication and/or partitioning of chromosomes and P1 miniplasmids. Furthermore, we found that P1 miniplasmids were also highly unstable in *dnaJ259* mutants, indicating a role for the DnaJ heat shock protein in maintenance of these plasmids.

Recent genetic evidence suggests important cellular functions for major heat shock proteins, not only under stress conditions such as heat shock but under physiological conditions at lower temperatures as well. Thus, *Escherichia coli* mutants deleted for the *rpoH* (*htpR*) gene, which encodes the alternative sigma subunit for heat shock genes, σ^{32} (29), are unable to grow at temperatures higher than 20°C (22, 52). Deletions of the *groEL groES* operon or the *grpE* gene, which encode the GroEL-GroES and GrpE heat shock proteins, respectively, are lethal at all growth temperatures tested (2, 9). Deletion of the *dnaK* gene (Δ*dnaK52*) (31), which encodes the DnaK heat shock protein, causes cold sensitivity as well as temperature sensitivity for cell growth (4, 5). Within the narrow permissive temperature range around 30°C, Δ*dnaK52* mutants grew slowly, were poorly viable, and were genetically unstable. Biochemical data suggest that major heat shock proteins, such as DnaK and GroEL, act as macromolecular detergents or chaperonins which mediate folding and unfolding of other proteins and assembly and disassembly of oligomeric protein structures (15, 24, 33). However, the biological processes which involve major heat shock proteins are still largely unknown.

We are interested in identifying cellular functions of the *E. coli* DnaK heat shock protein. DnaK is a member of the highly conserved Hsp70 protein family and shares about 50% amino acid homology with the Hsp70 proteins of eucaryotes such as *Drosophila melanogaster* and humans (24). DnaK is a 69-kilodalton protein which has ATPase activity (3, 54), is capable of autophosphorylation (54), and physically interacts with the DnaJ and GrpE heat shock proteins (19, 53; S. M. Sell, Ph.D. thesis, University of Utah, Salt Lake City, 1987). *dnaK* forms an operon together with the promoter-distal *dnaJ* heat shock gene (29, 30). *dnaK* is expressed at 37°C at a high level such that DnaK protein constitutes 1.4% of the total cellular protein at that temperature (30). Heat shock and other stresses induce further expression of *dnaK* as part of the heat shock response.

Only the function for DnaK in replication of lambda DNA has been clearly established. There, DnaK is required for initiation of replication and appears to act by dissociating DnaB protein from the λ P protein, thereby allowing the helicase to act (8, 10, 23). Most information about the cellular functions of DnaK has originated from mutant analysis. *dnaK* mutants described earlier were isolated either at 30°C as λ-resistant clones (13, 34) which often were unable to grow at high temperatures (13) or as conditional mutants unable to grow at high temperatures (18). A shift of temperature-sensitive *dnaK* mutants to nonpermissive (43°C) temperatures leads to inhibition of synthesis of RNA and DNA (18, 34) and to a block in cell division (34, 45). λ-resistant, temperature-sensitive *dnaK756* mutants were also shown to be defective at low temperatures in degradation of abnormal proteins (40) and of the normally very unstable σ^{32} protein (D. B. Straus and C. Gross, personal communication; 43). Partial stabilization of σ^{32} in *dnaK756* mutants leads to defects in recovery from the heat shock response (42). Using selection for conditional mutants defective at high temperatures in normal DnaA protein and *oriC*-dependent initiation of chromosomal DNA replication,

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TABLE 1. Bacterial strains

Strains	Genetic markers	Reference or source
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 deoC1 ptsF25 rpsR flbB301*	6
BB1042	MC4100 *thr*::Tn*10* Δ*dnaK52*::Cm^r	5
BB1176	MC4100(pZAQ)	5, 49
CSH26	F⁻ *ara* Δ(*lac-pro*) *thi*	J. Yin; 28
D47.2	CSH26 *rnh*::Tn*3*	J. Yin
D47.22	CSH26 *rnh*::Tn*3 dnaA*::Tn*10*	J. Yin
JFL100	*ftsZ84*	J. Park

Sakakibara recently isolated a new allele of *dnaK*, *dnaK111* (36).

Although these particular *dnaK* alleles have proved useful for revealing cellular functions for DnaK after heat shock, they do not provide clear information about functions for DnaK in cellular metabolism at lower temperatures. To elucidate these functions, we are performing a detailed analysis of the cellular defects at 30°C of Δ*dnaK52* mutants which completely lack the DnaK protein (31). We previously reported that Δ*dnaK52* mutants possess multiple cellular defects at 30°C, one of which was shown to be in cell division (4, 5), suggesting that DnaK is directly or indirectly involved in the regular division process. The present study was undertaken to identify other unknown cellular defects of Δ*dnaK52* mutants. Since previous reports had indicated involvement of DnaK in DNA replication after heat shock (see above), we specifically asked whether DNA metabolism is also altered in these mutants at 30°C. Our results indicate that DnaK plays an important role at low temperatures in one or more processes necessary for correct segregation of chromosomes. Furthermore, DnaK, as well as the DnaJ heat shock protein, is involved in replication of low-copy-number P1 miniplasmids at 30°C. Functions for heat shock proteins in plasmid replication have been independently identified by Tilly and Yarmolinsky and are described in the accompanying report (44).

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The bacterial strains used are listed in Table 1. λ(*dnaK*⁺) was obtained from C. Georgopoulos. λ *kan* cI857–P1:R5-3 (λ–mini-P1) and the spectinomycin resistance plasmid pSP102 were described before (11, 39, 44).

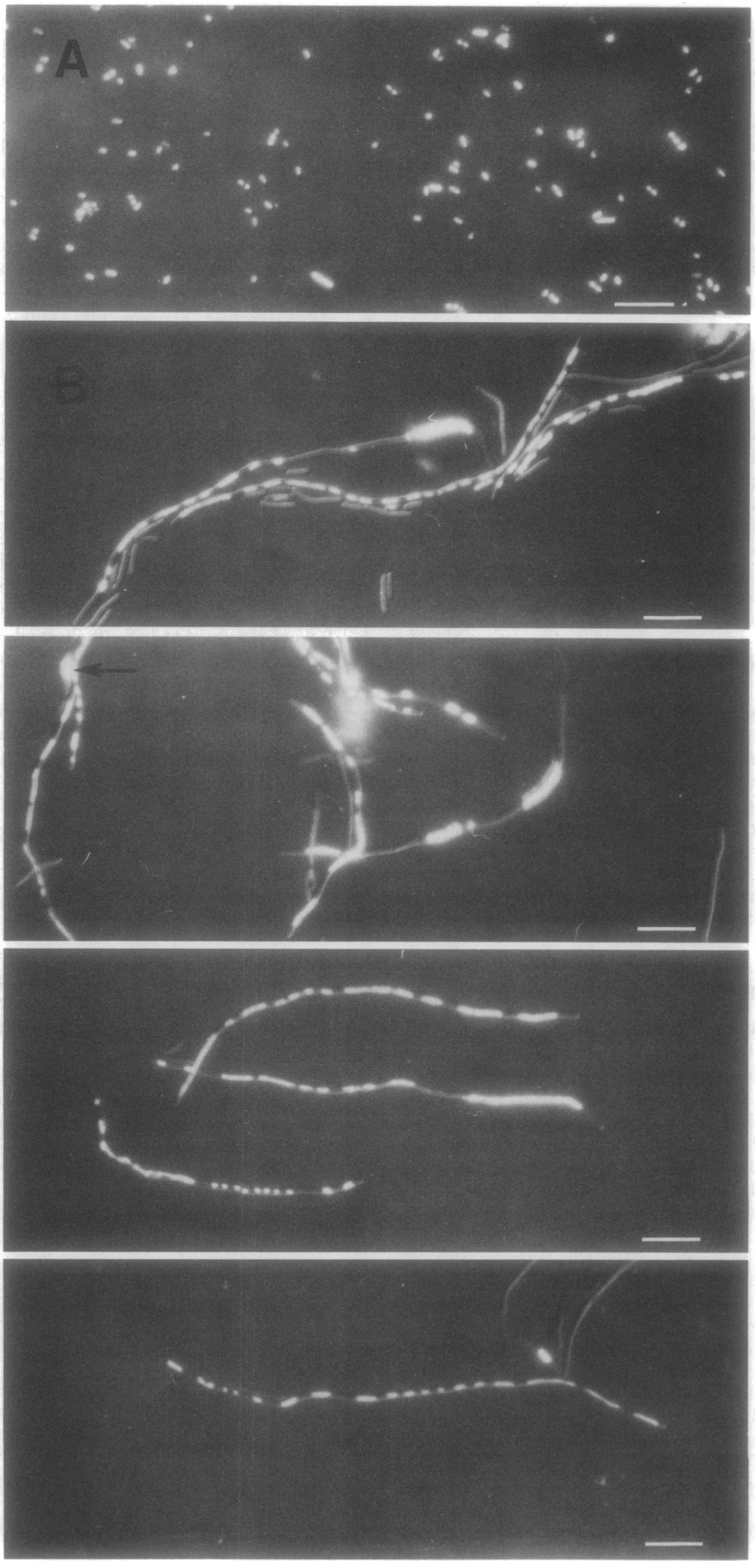
Media and growth conditions. Bacteria were grown aerobically at 30°C in Luria broth (LB) (28). Chloramphenicol, tetracycline, kanamycin, and spectinomycin were used at final concentrations of 25, 10, 75, and 100 μg/ml, respectively.

Genetic manipulations. P1 *vir* transductions, transformations and, lysogenizations were done as described earlier (25, 28, 37). The presence of plasmids in cells lysogenized with λ–mini-P1 or transformed with pSP102 was verified by agarose electrophoresis of DNA preparations of the cells.

Plasmid stability measurements. For plasmid stability tests, wild-type cells (MC4100) carrying λ–mini-P1 or pSP102 were transduced at 30°C to *thr*::Tn*10* by using a P1 lysate grown on a *thr*::Tn*10* Δ*dnaK52* strain (BB1042). Some of the resulting Tc^r transductants obtained the linked Δ*dnaK52* allele by cotransduction. Colonies formed by these *thr*::Tn*10* Δ*dnaK52* transductants are morphologically distinct from colonies formed by *thr*::Tn*10* *dnaK*⁺ transductants in that they are flat and translucent (5). Cells from Tc^r Δ*dnaK52* and *dnaK*⁺ transductant colonies were suspended in LB-tetracycline medium containing the appropriate antibiotic for selection of the plasmid and grown at 30°C to mid- or late-logarithmic phase. The cells were then diluted 1:1,000 in LB-tetracycline medium lacking the selective antibiotic and grown at 30°C for about 10 generations. Samples were taken at various times, and appropriate dilutions were plated on LB-tetracycline agar plates. Loss of λ–mini-P1 and pSP102 was determined by replica plating of the resulting colonies onto LB-tetracycline plates containing the selecting antibiotic.

DNA synthesis experiments. Cells of strain MC4100 were transduced at 30°C to *thr*::Tn*10* with a P1 *vir* lysate grown on a *thr*::Tn*10* Δ*dnaK52* strain (BB1042). Cells of Tc^r transductant colonies of normal morphology (*thr*::Tn*10* *dnaK*⁺) and cells of flat, translucent Tc^r transductant colonies (*thr*::Tn*10* Δ*dnaK52*) were picked and grown overnight in LB-tetracycline at 30°C. The cultures were then diluted in the same medium to an optical density at 600 nm of about 0.05 and further grown to an optical density at 600 nm of 0.5. One-milliliter portions of the cultures were distributed in prewarmed tubes, and 20 μCi of [^{3}H]thymidine (72 Ci/mmol; Amersham Corp.) with 0.1 μg of unlabeled carrier thymidine and 200 μg of 2'-deoxyadenosine was added. Samples (150 μl) were removed at 1, 5, 10, 20, and 30 min after addition of the radioactive label and added to cold trichloroacetic acid (final concentration, 10%) to precipitate macromolecules. Trichloroacetic acid-precipitated samples were collected on Whatman GF/C filters which were then washed three times with 10% trichloroacetic acid and once with ethanol and dried. Radioactivity was determined by scintillation counting, and incorporation of [^{3}H]thymidine into DNA was normalized to milligrams of total cell protein. Cells from the cultures used in these experiments were plated on LB-tetracycline and LB-chloramphenicol plates and grown at 30 and 42°C to determine whether the Δ*dnaK52* allele was present in the transductant cells and to determine the frequency of acquisition by Δ*dnaK52* transductants of secondary mutations which might alter their growth behavior (faster growth, formation of colonies of normal morphology, and suppression of filamentous growth at 30°C [5]). Data from DNA synthesis experiments were only used when the number of suppressor-carrying Δ*dnaK52* mutant cells in the culture by the above-described criteria was less than 4% of the total number of cells. In one experiment (see Fig. 5), less than 1% of the Δ*dnaK52* mutants seemed to have acquired secondary mutations.

Fluorescence microscopy. Cells (i) collected from fresh colonies grown on LB agar plates or (ii) grown logarithmically in LB liquid medium were washed in Veronal-acetate buffer (pH 7.5) and fixed for 1 h at 4°C in the same buffer containing 0.1% OsO_4. The fixed cells were pelleted and suspended in Veronal-acetate buffer containing Fluorochrome 33342 (Hoechst-Roussel Pharmaceuticals, Inc.) at 20 μM. The cells were immediately analyzed in a Zeiss Axioplan Universal microscope using a Plan 100 objective and Nomarsky differential interference contrast optics and appropriate filters to detect fluorescence. Bacteria were photographed with Tri-X-Pan 400 films (Eastman Kodak Co.). Untreated samples of the cell cultures of Δ*dnaK52* mutants used for microscopy were tested for the presence of suppressor mutations as described for DNA and protein synthesis experiments.


A
B

RESULTS

Δ*dnaK52* mutants are defective in chromosome segregation at 30°C. To determine whether DnaK has a role in replication of cellular DNA in vivo at low temperatures as well as in cell division, we asked whether Δ*dnaK52* mutants have defects in chromosome segregation. Defects in DNA replication typically cause formation of cell filaments with centrally located chromosomes (17). This morphological phenotype is useful for detecting defects in synthesis of chromosomal DNA in *dna* mutants and for distinguishing *dna* mutants from *fts* mutants which are defective in cell division but not in DNA synthesis (7, 16, 17, 21). We therefore determined the pattern of segregation of chromosomes within cell filaments of Δ*dnaK52* mutants at 30°C. The Δ*dnaK52* allele was transduced into wild-type bacteria, and cells from Δ*dnaK52* transductant colonies were stained, either directly or after growth to mid-log phase in selective liquid medium, with a fluorescent dye that binds to DNA. They were then subjected to fluorescence microscopic analysis. This approach largely avoided the complication of suppressor mutations, which might accumulate in the genetically unstable Δ*dnaK52* mutants (5).

We found that cell filaments of Δ*dnaK52* mutants had abnormally segregated chromosomes (Fig. 1). Frequently, large parts of the cell filaments lacked chromosomes, while other parts contained chromosomes that were often unevenly distributed. The parts of the cells lacking chromosomes were frequently delimited by large, strongly fluorescent nucleoids, each presumably containing several unsegregated chromosomes. Some cells contained only one, centrally located nucleoid that exhibited unusually bright fluorescence (Fig. 1B, arrows), also suggesting that they contained several unsegregated chromosomes. These cells usually formed filaments significantly shorter (four to eight times the length of wild-type cells) than the typically long cell filaments formed by Δ*dnaK52* mutants (more than 20 times the length of wild-type cells), suggesting arrest of cell growth at an early stage. In yet other cases, the whole Δ*dnaK52* mutant cell filament was fluorescent, suggesting destruction of the morphological integrity of the chromosomes. It is possible that this phenotype results from breakage and nucleolytic degradation of chromosomes during cell death. However, removal of a major cellular nuclease, exonuclease V, by mutation of the *recBC* genes (38) did not eliminate this phenotype (data not shown).

While many cell filaments exhibited chromosome segregation defects, as described above, some cell filaments, as well as many short cells, did not exhibit detectable defects. The partial nature of these chromosome segregation defects might explain why populations of Δ*dnaK52* mutants are able to grow at 30°C. It is the subpopulation of cells with little or no chromosome segregation defects that is likely to be proficient for further growth.

The defects in chromosome segregation of Δ*dnaK52* mutants at 30°C are due to lack of DnaK. To show this, we transduced the Δ*dnaK52* allele into wild-type cells carrying one extra copy of the *dnaK*$^+$ gene on a lysogenized λ(*dnaK*$^+$) phage. Δ*dnaK52*[λ(*dnaK*$^+$)] lysogens did not form cell filaments at 30°C and did not possess defects in chromosome segregation (data not shown). The most economical explanation of the phenotypes observed is that segregation of chromosomes is occasionally, eventually irreversibly, blocked in Δ*dnaK52* mutants at 30°C, indicating an important, although not essential, role for DnaK in one or more processes necessary for correct chromosome segregation.

Abnormal segregation of chromosomes of Δ*dnaK52* mutants is unlikely to result from defects in cell division. In *E. coli*, cell division and DNA replication are coregulated processes (7, 46). It was therefore possible that the observed defects in chromosome segregation of Δ*dnaK52* mutant cells resulted from defects in cell division. We have previously shown that cell division defects of Δ*dnaK52* mutants can be largely suppressed by overproduction of the FtsZ protein (5), indicating that DnaK is directly or indirectly involved in normal cell division, possibly in septation at the level of FtsZ action. To investigate whether the observed defects in chromosome segregation of Δ*dnaK52* mutants were caused by reduced FtsZ activity, we determined whether conditional *ftsZ84* mutants, which are deficient in septum formation at nonpermissive temperatures (17), exhibit phenotypes similar to those of Δ*dnaK52* mutants. After growth of *ftsZ84* mutant cells for 2 h at a nonpermissive temperature, the cells formed filaments containing extremely well-separated and regularly distributed chromosomes (Fig. 2). Even after incubation of the cells for 14 h at a nonpermissive temperature, chromosomes were regularly distributed over the entire cell body (data not shown). This phenotype is consistent with previously published results (17) and is in clear contrast to the phenotype of Δ*dnaK52* mutants described above, suggesting that abnormal chromosome segregation in Δ*dnaK52* mutants did not result from lack of FtsZ activity.

We also analyzed the pattern of chromosome segregation in Δ*dnaK52* mutants that carry a high-copy-number plasmid encoding the *ftsZ* gene (pZAQ) (49). Presence of this plasmid causes overproduction of the FtsZ protein and, as a consequence, leads to suppression of cell division defects in Δ*dnaK52* mutants (5). Although many cells with lengths similar to that of the wild type possessed chromosomes, some cells of this class, as well as most of the minicells present in the cell population, lacked DNA. Furthermore, short cell filaments present in the cell population had abnormally distributed chromosomes. Thus, chromosome segregation seemed to be abnormal in Δ*dnaK52* mutants with suppressed cell division defects as well, although we found it difficult to evaluate whether these defects were as severe as in the cell filaments of Δ*dnaK52* mutants. Taken together, these experiments further support the hypothesis that Δ*dnaK52* mutants possess defects in chromosome segregation in addition to defects in septation.

Defects in segregation of chromosomes of Δ*dnaK52* mutants are independent of DnaA and *oriC*. The fact that DnaK is essential for initiation of replication of λ DNA (8, 10, 23) led us to consider the possibility that the observed defects in chromosome segregation were due to defects of Δ*dnaK52* mutants in initiation of chromosomal DNA replication under non-heat shock conditions. Furthermore, since Sakakibara had reported evidence suggesting that DnaK is involved in

FIG. 1. Abnormal segregation of chromosomes in cell filaments of Δ*dnaK52* mutants at 30°C. Wild-type cells (MC4100) were transduced at 30°C to *thr*::Tn*10* Δ*dnaK52* as described for DNA synthesis experiments in Materials and Methods. Fresh *thr*::Tn*10* Δ*dnaK52* transductants (B) and *thr*::Tn*10* *dnaK*$^+$ control cells (A) were grown at 30°C in LB-tetracycline medium to the logarithmic phase and subjected to fluorescence microscopic analysis. Arrows indicate short filaments with centrally located nucleoids. Bars, 10 μm.

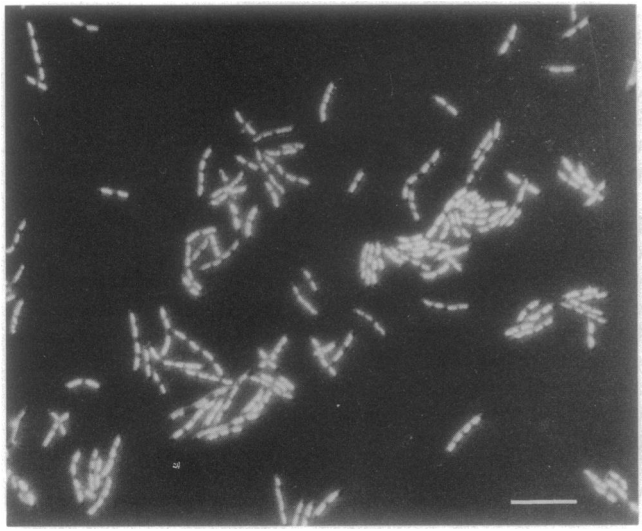


FIG. 2. Regular segregation of chromosomes of *ftsZ84* mutants at nonpermissive temperatures. *ftsZ84* mutant cells (strain JFL100) were grown at 30°C in LB medium to the logarithmic phase, shifted to 43°C for 2 h, and subjected to fluorescence microscopic analysis. Bar, 10 μm.

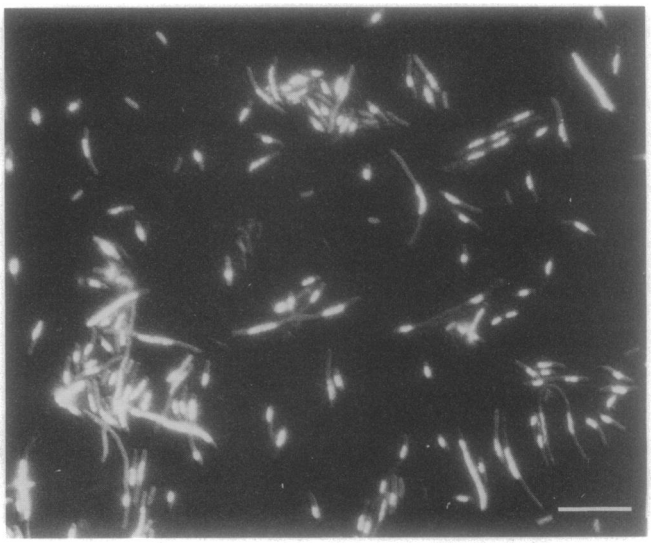


FIG. 3. Abnormal segregation of chromosomes in *dnaA850*::Tn*10* *rnh*::Tn*3* Δ*dnaK52* mutants. *dnaA850*::Tn*10* *rnh*::Tn*3* mutant cells were transduced to Δ*dnaK52*, and fresh Δ*dnaK52* *dnaA850*::Tn*10* *rnh*::Tn*3* transductants were grown at 30°C in LB-chloramphenicol medium to the logarithmic phase and subjected to fluorescence microscopic analysis. Bar, 10 μm.

DnaA-*oriC*-dependent initiation of chromosomal DNA replication after heat shock (36), it seemed possible that DnaK was likewise involved in DnaA-*oriC*-dependent initiation of chromosomal DNA replication at lower temperatures.

In his studies of initiation of chromosomal replication after heat shock, Sakakibara had reported that a specific mutation in *dnaK* (*dnaK111*) causes inhibition of initiation of DNA replication after shift of the cells to 42.5°C (36). Furthermore, he found that this inhibition could be suppressed by introduction of an *rnh* mutation, which allows initiation of DNA replication to occur in a DnaA-*oriC*-independent fashion (26). In a similar fashion, we therefore examined whether the presence of an *rnh*::Tn*3* mutation suppressed the abnormalities in segregation of chromosomes that we observed in Δ*dnaK52* mutants at 30°C. However, chromosome segregation defects were present in Δ*dnaK52* *rnh*::Tn*3* mutants (data not shown), indicating that the *rnh* mutation did not suppress the defects in Δ*dnaK52* mutants that lead to abnormal chromosome segregation. Chromosomes in *rnh*::Tn*3* *dnaK*$^+$ cells were mostly normally segregated. Furthermore, introduction of the Δ*dnaK52* allele into *rnh*::Tn*3* *dnaA850*::Tn*10* double mutants that completely lack DnaA activity also caused abnormal segregation of chromosomes (Fig. 3). Cell filaments were, on the average, shorter than filaments formed by *rnh*$^+$ *dnaA*$^+$ Δ*dnaK52* mutants, and they frequently contained only one, centrally located nucleoid. This phenotype suggests that segregation of chromosomes is more frequently inhibited in *rnh dnaA* Δ*dnaK52* strains, thus preventing further cell growth. The two simplest models to explain these findings are that (i) DnaK affects DnaA-*oriC*-dependent initiation of replication in *rnh*$^+$ strains and DnaA-*oriC*-independent initiation of replication in *rnh* strains and (ii) DnaK affects one or more postinitiation steps necessary for correct segregation of chromosomes in both *rnh*$^+$ and *rnh* strains. More complicated models cannot be excluded.

Reduced rate of DNA synthesis at 30°C in Δ*dnaK52* mutants. Involvement of DnaK in chromosomal DNA replication should lead to decreased rates of DNA synthesis in Δ*dnaK52* mutants compared with those of the wild type. We therefore determined the incorporation of radioactively labeled thymidine into DNA of wild-type cells and Δ*dnaK52* mutant cells at 30°C (Fig. 4). The rate of DNA synthesis was about threefold lower in Δ*dnaK52* mutants than in wild-type cells. This finding suggests a role for DnaK in DNA replication that might cause chromosome segregation defects of Δ*dnaK52* mutants. We cannot exclude the possibility, however, that this role for DnaK is only indirect and that defects of Δ*dnaK52* mutants in processes other than DNA synthesis

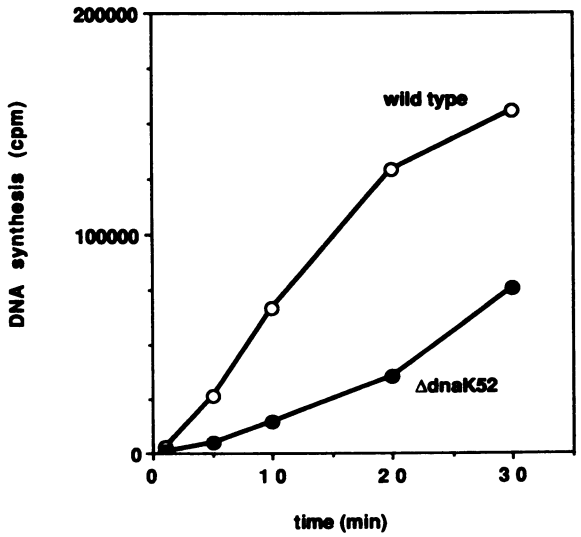


FIG. 4. DNA synthesis of Δ*dnaK52* mutants at 30°C. Incorporation of [^{3}H]thymidine at 30°C into DNA of *thr*::Tn*10* Δ*dnaK52* transductants (●) and *thr*::Tn*10* *dnaK*$^+$ transductants (○) was determined as described in Materials and Methods.

TABLE 2. Effects of *dnaK* and *dnaJ* mutations on stability of λ–mini-P1 and pSP102 plasmids

Plasmid and strain	% Plasmid loss or generation
λ–mini-P1	
MC4100	0.25
MC4100 Δ*dnaK52*	4.57
MC4100[λ(*dnaK*$^+$)]	0.39
MC4100[λ(*dnaK*$^+$)] Δ*dnaK52*	0.15
MC4100 *dnaK756*	0.66
MC4100(pZAQ)	0.4
MC4100(pZAQ) Δ*dnaK52*	5.38
MC4100 *dnaJ259*	4.14
pSP102	
MC4100	0.008
MC4100 Δ*dnaK52*	1.1

cause abnormal chromosome segregation and a lower rate of DNA synthesis. One such process might be the rate of synthesis of proteins, which was also found to be reduced in Δ*dnaK52* mutants (about 1.5- to 3-fold) compared with wild-type cells.

Defect of Δ*dnaK52* mutants in stable maintenance of λ–mini-P1 plasmids. As shown above, the presence of multiple cellular defects at 30°C renders identification of defects in chromosomal DNA synthesis of Δ*dnaK52* mutants difficult. As an alternative approach to identifying a role for DnaK in DNA synthesis at 30°C, we analyzed the stability of low-copy-number plasmids in Δ*dnaK52* mutants. Low-copy-number plasmids are experimentally easily accessible and are widely used as model systems to study replication as well as partitioning of DNA to daughter cells during cell division. We used a derivative of bacteriophage P1, λ *kan* cI857–P1:R5-3 (λ–mini-P1) (11, 39), which replicates as a plasmid with a copy number similar to that of the host chromosome (39). λ–mini-P1 consists of a λ vector containing a 7-kilobase insert that encodes the *rep* and *par* functions of P1 required for replication and partitioning, respectively, of this plasmid (39, 51). We introduced the Δ*dnaK52* allele at 30°C into wild-type cells carrying λ–mini-P1 as a lysogen and determined its stability. In these experiments, fresh Δ*dnaK52* transductants were immediately tested for plasmid stability without further purification of the colonies to avoid accumulation of suppressor mutations. In Δ*dnaK52* mutants, λ–mini-P1 plasmids were lost at a rate about 18 times higher than in *dnaK*$^+$ cells (Table 2).

Involvement of DnaK heat shock protein in stable maintenance of λ–mini-P1 plasmids. The inability of Δ*dnaK52* mutants to maintain λ–mini-P1 is due to loss of DnaK. This was shown by determining the loss of λ–mini-P1 in Δ*dnaK52* mutants which contained one extra copy of the *dnaK*$^+$ gene provided by a lysogenic λ(*dnaK*$^+$) phage. Presence of this extra copy of *dnaK*$^+$ fully restored the ability of Δ*dnaK52* mutants to stably maintain the λ–mini-P1 plasmid (Table 2).

We also examined the stability of λ–mini-P1 in mutants that carry the conditional *dnaK756* mutation and found that the presence of this mutation caused only a slight decrease (less than threefold) in the stability of λ–mini-P1 compared with wild-type cells (Table 2). This indicates that the DnaK756 mutant protein possesses at least partial activity at 30°C, allowing the λ–mini-P1 plasmid to be maintained fairly stably.

These results show that the DnaK protein plays an important role in stable maintenance of λ–mini-P1 plasmids. However, this function of DnaK is not essential for maintenance of λ–mini-P1, since these plasmids could be maintained in Δ*dnaK52* mutants with selective pressure.

Defects in maintenance of λ–mini-P1 are not caused by cell division defects of Δ*dnaK52* mutants. We considered the possibility that instability of λ–mini-P1 in Δ*dnaK52* mutants resulted from cell division defects of these mutants. We therefore asked whether suppression of cell division defects of Δ*dnaK52* mutants by overproduction of FtsZ can suppress the instability of λ–mini-P1. However, while presence in Δ*dnaK52* mutants of a high-copy-number plasmid encoding FtsZ (pZAQ) did suppress cell division defects of these cells, it did not improve the stability of λ–mini-P1 plasmids (Table 2). Thus, plasmid maintenance defects of Δ*dnaK52* mutants are not caused by cell division defects.

Defect of Δ*dnaK52* mutants in replication of Δ*par* mini-P1 plasmids. Stable maintenance of λ–mini-P1 depends on replication and partition functions of P1 that are encoded by adjacent but genetically separable *rep* and *par* regions present on these plasmids (39, 51). Instability of λ–mini-P1 in Δ*dnaK52* mutants might a priori be due to functions of DnaK in replication or partitioning of the plasmid. To distinguish between these two possibilities, we determined the effect of the Δ*dnaK52* mutation on the stability of plasmids (pSP102) which carry the essential *rep* functions but lack the *par* functions (44). In wild-type cells, pSP102 plasmids have increased copy numbers due to deletion of the copy number control region and are partitioned statistically during cell division (32). Introduction of the Δ*dnaK52* allele into cells that carry pSP102 plasmids caused a greater-than-100-fold increase in the rate of loss of this plasmid (Table 2). This result indicates that DnaK is involved at the level of replication of these plasmids and excludes the possibility that DnaK is required only for partitioning of P1-derived plasmids.

Defect of *dnaJ259* mutants in stable maintenance of P1 miniplasmids. The DnaK protein physically interacts with the DnaJ heat shock protein (Sell, Ph.D. thesis). This interaction might have functional significance, since mutations in *dnaJ* cause cellular defects similar to those caused by mutations in *dnaK*, such as defects in DNA synthesis after heat shock (29). We therefore considered the possibility that not only DnaK but also DnaJ is required for stable maintenance of λ–mini-P1 plasmids and determined the stability of these plasmids in *dnaJ259* mutants (41). We found that λ–mini-P1 was lost at the same high rate in *dnaJ259* mutants as in Δ*dnaK52* mutants (Table 2). These results indicate that the DnaK and DnaJ heat shock proteins are both required for stable maintenance of λ–mini-P1 plasmids.

DISCUSSION

In this study, we demonstrated that at low temperature (30°C), Δ*dnaK52* mutants have defects that lead to abnormal segregation of chromosomes and instability of low-copy-number plasmids derived from P1. Defects in chromosome segregation and P1 plasmid stability result from defects in replication and/or partitioning of DNA (16, 17, 21, 51), and therefore, these phenotypes of Δ*dnaK52* mutants indicate important, although not absolutely essential, functions for DnaK in replication and/or partitioning of chromosomes and P1 miniplasmids at 30°C.

It is not clear which of the processes required for replication and for partitioning of chromosomes and P1 plasmids involve DnaK. In view of the mechanistic similarities of at

least some of the processes leading to correct segregation of chromosomes and those leading to stable maintenance of P1 plasmids, it might be that DnaK affects the same process(es) in each case. Our finding that the rate of synthesis of chromosomal DNA was decreased about threefold in Δ*dnaK52* mutants at 30°C compared with wild-type cells is consistent with a role for DnaK in regular chromosomal DNA synthesis at 30°C and with its apparent role in DNA synthesis after heat shock. The defects of Δ*dnaK52* mutants in chromosome segregation did not depend exclusively on regular, DnaA-*oriC*-dependent initiation of replication, since they also occurred in Δ*dnaK52 rnh dnaA* mutants which use an alternative, *oriK*-dependent pathway for initiation of replication (26). The two simplest models to explain our findings are that (i) DnaK affects DnaA-*oriC*-dependent initiation of chromosome replication in *rnh*$^+$ strains and DnaA-*oriC*-independent initiation of chromosome replication in *rnh* strains or (ii) DnaK affects postinitiation steps necessary for correct segregation of chromosomes, such as elongation or termination of replication, or partitioning of chromosomal DNA in both *rnh*$^+$ and *rnh* strains. The defects of Δ*dnaK52* mutants in P1 miniplasmid maintenance were also observed with Δ*par* P1 miniplasmids which carry the *rep* functions of P1 required for their replication but lack the *par* functions of P1 required for their faithful partitioning during host cell division. This result indicates a role for DnaK in plasmid replication that is sufficient to explain the instability of P1 miniplasmids in Δ*dnaK52* mutants, although we cannot rule out a role for DnaK in partitioning as well. Thus, as for chromosomal DNA, DnaK might affect (i) initiation of plasmid replication or (ii) a postinitiation process necessary for stable maintenance of the plasmids.

A role for DnaK in normal initiation of replication, as suggested by the first model, is consistent with a number of other findings. After heat shock, DNA synthesis of conditional *dnaK* mutants is slowly inhibited (18, 34, 36), which suggests a defect in initiation of DNA replication rather than in elongation (21). Moreover, at nonpermissive temperatures, the *dnaK111* mutation was found to inhibit DnaA-*oriC*-dependent initiation of replication specifically (36). In addition, the transient increase in the rate of initiation of DNA replication observed after heat shock is *htpR*$^+$ dependent, indicating that heat shock proteins may be involved (14). Finally, DnaK has a known role in the initiation of λ replication (8, 10, 23). There is no evidence supporting the second model, which suggests a role for DnaK in a postinitiation process.

At first glance, involvement of DnaK in replication of chromosomes and plasmids might seem surprising, since it is dispensable under most conditions for reconstituted in vitro replication systems (12, 26). However, a requirement for DnaK might have been missed because it is not absolutely essential for replication of chromosomes and P1 miniplasmids at 30°C or because the reconstituted in vitro replication systems used do not fully reflect in vivo conditions. In fact, preliminary experiments of Sakakibara indicated that *oriC*-dependent plasmid replication is lower with a crude enzyme system prepared from *dnaK111* cells than with a crude enzyme system prepared from *dnaK*$^+$ cells (36). Also, the activity of fraction II extracts prepared from *dnaK* mutants in *oriC*-dependent replication was stimulated upon addition of purified DnaK protein (55). Subsequent addition of antibodies to DnaK inhibited the replication activity of these extracts.

Besides DnaK, the DnaJ heat shock protein was also found to be an important host cell factor required for stable maintenance of P1 miniplasmids. In addition, Tilly and Yarmolinsky show in the accompanying report (44) that λ–mini-P1 plasmids were unstable in *grpE* heat shock mutants, and they present data indicating that the *grpE* mutation, as well as *dnaJ* mutations, affects replication of P1 plasmids. Since the DnaK, DnaJ, and GrpE heat shock proteins interact in vivo (19, 53; Sell, Ph.D. thesis), they might act together in plasmid replication, possibly in a functionally active complex.

Taken together, our findings and previously reported results from other laboratories indicate functions for major heat shock proteins of *E. coli*, including DnaK, DnaJ, GrpE, GroEL, and GroES, as well as the activator of the heat shock response, σ^{32}, in normal replication of DNA of phages and plasmids and in processes required for proper segregation of chromosomal DNA (this study; 1, 18, 23, 34, 35, 44, 47, 48). It is interesting in this regard that under normal temperature conditions, expression of Hsp70 proteins of humans is induced and Hsp70 proteins migrate to the nucleus upon entry of the cells into the S phase (20, 27). Furthermore, cells treated with a DNA synthesis inhibitor are blocked in induction of Hsp70 expression following serum stimulation (50). These results might indicate a function of Hsp70 proteins in DNA synthesis. Functions of heat shock proteins in DNA synthesis might be one of the reasons for their ubiquity and extreme conservation in nature.

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