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Cellular Defects Caused by Deletion of the *Escherichia coli dnaK* Gene Indicate Roles for Heat Shock Protein in Normal Metabolism

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DnaK is a major heat shock protein of *Escherichia coli* and has been previously reported to be essential for growth at high temperatures. We systematically investigated the role of DnaK in cellular metabolism at a wide range of growth temperatures by analyzing cellular defects caused by deletion of the *dnaK* gene (Δ*dnaK52*). At intermediate temperatures (30°C), introduction of the Δ*dnaK52* allele into wild-type cells caused severe defects in cell division, slow growth, and poor viability of the cells. Δ*dnaK52* mutants were genetically unstable at 30°C and frequently acquired secondary mutations. At high (42°C) and low (11 and 16°C) temperatures the Δ*dnaK52* allele could only be introduced into the subpopulation of wild-type cells that had duplicated the *dnaK* region of their chromosome. Δ*dnaK52* mutants isolated at 30°C were cold sensitive as well as temperature sensitive for growth. Cell division defects of Δ*dnaK52* mutants at 30°C were largely suppressed by overproduction of the FtsZ protein, which is normally required for septation during cell division; however, slow growth and poor viability at 30°C and cold sensitivity and temperature sensitivity of growth were not suppressed, indicating that Δ*dnaK52* mutants had additional defective cellular functions besides cell division.

The heat shock response is an inducible cellular response to a variety of stresses such as heat, exposure to ethanol, oxidants, and DNA-damaging agents, production of abnormal proteins, viral infections, and starvation for nutrients (6, 28, 35, 36). Not only is the phenomenon of a heat shock response extremely highly conserved during evolution, but individual components of the heat shock response belong to families of proteins which are among the most conserved in nature, which is strong evidence for the biological importance of this system. The Hsp70 and Hsp83 heat shock proteins of humans and *Drosophila melanogaster* share between 50 and 40% amino acid homology to the DnaK and C62.5 heat shock proteins of *Escherichia coli*, respectively (1, 2, 20). Also, mammalian Hsp70 cognate proteins which are not heat inducible share similar homologies to the DnaK heat shock protein of *E. coli* (5). Furthermore, the *E. coli* GroEL heat shock protein shares 46% amino acid homology to the α subunit of Rubisco binding protein of wheat chloroplasts (16), antigenically cross-reacts with mitochondrial proteins of many organisms, and appears to be present in most if not all bacteria (16, 31, 55). The high degree of sequence conservation during the evolution of these proteins indicates that their biological function might also be conserved during evolution. Results of recent studies on the Hsp70 and GroEL proteins suggest that these proteins might act as molecular "chaperones" which trigger the proper folding of other proteins and the assembly and disassembly of oligomeric protein structures (10, 16, 39).

While the idea that Hsp70 and GroEL heat shock proteins act as chaperones is attractive, little is known about their actual biological functions under physiological growth conditions as well as under stress conditions. In the case of Hsp70 proteins of eucaryotes, studies on their in vivo functions are extremely difficult since individual cells encode families of closely related but differently regulated Hsp70 proteins (6, 28, 39). For example, *Saccharomyces cerevisiae* encodes at least eight different Hsp70 proteins which can substitute for each other at least partially (6). In contrast, *E. coli* encodes only one HSP70 protein (1, 38), the DnaK heat shock protein, which makes the *E. coli* DnaK protein an attractive candidate for a genetic analysis of the biological relevance of Hsp70 proteins.

The *E. coli* DnaK protein is encoded for by the *dnaK* gene, which is located at 0.3 min on the genetic map and which forms an operon with the promoter distal *dnaJ* heat shock gene (36). DnaK is induced along with about 19 other heat shock proteins as part of the heat shock response and accounts for as much as 4.3% of total cellular protein after a shift of the cells to 46°C (17). Induction is mediated by the product of the *htpR* gene, σ^{32}, that is required for transcription of heat shock genes (34, 53). DnaK is a 69-kilodalton protein that has an ATPase activity (3, 58), is capable of autophosphorylation at one or more threonine residues (58), and is directly or indirectly involved in phosphorylation of aminoacyl-tRNA synthetases (49). Furthermore, biochemical and genetic evidences suggest that DnaK physically interacts with the DnaJ and GrpE heat shock proteins (57; S. M. Sell, Ph.D. thesis, University of Utah, Salt Lake City, 1987). DnaK is essential for growth of various bacteriophages such as λ. Here, DnaK is required for the initiation of replication of λ DNA and appears to act by dissociating DnaB protein from the λ P protein, thereby allowing the helicase to act (9).

The cellular functions of the DnaK protein are not understood. DnaK is essential for growth of the cells at high temperatures, since most *dnaK* mutants are temperature sensitive for growth (22, 38, 40, 41). A shift of these mutant cells to the nonpermissive temperature causes fast inhibition of synthesis of DNA and RNA (22) and a block in cell division (8, 38, 47). Sakakibara (42) recently found that a conditional mutation in *dnaK* causes inhibition of initiation of chromosome replication at the restrictive 42°C temperature. Furthermore, DnaK plays a role in the regulation of the heat shock response since *dnaK* mutants are slow to turn off the heat shock response after a shift to 42°C (46). DnaK seems to exert this regulatory effect by influencing synthesis and the half-life of σ^{32} (14; D. B. Straus and C. Gross, personal communication). At temperatures below heat

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TABLE 1. Bacterial strains used in this study

Strains	Genetic marker	Source or reference
AB1157	F⁻ *thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 supE37*	Our laboratory collection
GW4813	AB1157, ΔdnaK52::Cm^r	38
GW2727	AB1157, *malE*::Tn*10 lexA3*	Our laboratory collection
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 deoC1 ptsF25 rpsR flbB301*	4
BB1042	MC4100, *thr*::Tn*10* Δ*dnaK52*::Cm^r	This study
BB1372	MC4100, *relA*$^+$	This study
BB1373	MC4100, *relA2*	This study
BB1615	MC4100, *rnh*::Tn*3* pZAQ	This study
CP78	F⁻ *thr-1 leuB6 his-65 argH46 thi-1 ara-13 gal-3 malA1 xyl-7 mtl-2 tonA2*	B. Bachmann
CP79	CP78, *relA2*	B. Bachmann
CSH26	F⁻ *ara* Δ(*lac-pro*) *thi*	J. Yin; (33)
D47.2	CSH26, *rnh*::Tn*3*	J. Yin
D47.22	CSH26, *rnh*::Tn*3 dnaA*::Tn*10*	J. Yin

shock temperatures, the cellular functions of the DnaK protein are even less clear, although the fact that DnaK is expressed at considerable levels below 42°C, e.g., constituting 1.4% of total cellular protein at 37°C (36), suggests that it is also important for growth at lower temperatures.

Our laboratory recently reported the construction and partial characterization of a deletion of the *dnaK* gene (Δ*dnaK52*::Cm^r; referred to as Δ*dnaK52*), which removed the promoter region and 933 base pairs of the coding region of the *dnaK* gene (38). Δ*dnaK52* mutants were not able to grow at 42°C but, unexpectedly, were viable at 30°C. Here we present a more detailed study of Δ*dnaK52* mutants which indicates that DnaK plays important roles in cellular metabolism at a wide range of growth temperatures.

MATERIALS AND METHODS

Bacterial strains, phages, and plasmids. The bacterial strains used in this study are listed in Table 1. λ(*dnaK*$^+$ *dnaJ*$^+$), λ(*dnaK*$^+$), and λ(*dnaJ*$^+$) were obtained from C. Georgopoulos. Plasmids pZAQ, pZAQ (*ftsZ28*::Tn*5*), pZAQ (*ftsQ22*::Tn*5*), and pZAQ (*ftsA5*::Tn*5*) have been described previously (29, 51).

Media, growth conditions, and genetic techniques. Bacteria were grown aerobically at 30°C or at the indicated temperatures in Luria broth (LB) or M9 medium (33) supplemented with carbon sources (0.2%), thiamine (0.001%), and appropriate amino acids (50 μg/ml). The following antibiotics were used at the indicated concentrations: chloramphenicol, 25 μg/ml; tetracycline, 10 μg/ml; ampicillin, 100 μg/ml. λ lysogenizations of cells were done by the method of Silhavy et al. (43). Plasmids were transformed into host cells by the $CaCl_2$ method described previously (30). P1 *vir* transductions were done as described previously (33), but transductions involving the Δ*dnaK52* allele were not performed by that method. This marker was so poorly transducible that the following changes in the experimental conditions were required to increase the transduction frequency to a reasonable level. First, growth of P1 on Δ*dnaK52* mutant cells was so poor that phages were unable to form plaques, and thus, lysates had low titers. The titer of P1 lysates grown on Δ*dnaK52* mutants could be increased considerably by prolonging the infection time to about 12 h at 30°C. Second, the Δ*dnaK52* transduction frequency was increased at least 10-fold when sodium citrate, which was routinely present in the transduction plates to inhibit further lytic growth of P1 on the transduction plate, was omitted.

Strain constructions. λ(*dnaK*$^+$) and λ(*dnaJ*$^+$) lysogens of strain MC4100 contained λ phages that were integrated probably at the *att* site of the chromosome and not by homologous recombination at the chromosomal *dnaK* and *dnaJ* genes. This was verified by showing that none of the lysogens could be crossed out by cotransduction with the *thr*::Tn*10* marker which is linked to the *dnaKJ* operon. After introduction of the Δ*dnaK52* allele into these λ lysogens, the presence of the phages was verified by determining the resistance of the cells to infection with phage λ(*dnaK*$^+$ *dnaJ*$^+$).

relA$^+$ and *relA2* derivatives of strain MC4100 were constructed as follows. First, we transduced cells of strain MC4100 to *argA*::Tn*10* using P1 *vir* lysates of an *argA*::Tn*10* derivative of strain CP78 and obtained *argA*::Tn*10 relA*$^+$ transductants. We tested for the presence of the *relA*$^+$ allele by determining the ability of the cells to grow on M9 agar plates containing glucose, arginine, leucine, threonine, serine, methionine, glycine, and histidine; in contrast, *relA* mutants are unable to grow on these plates (48). Then, this *argA*::Tn*10 relA*$^+$ isolate was transduced to Arg$^+$ by using P1 *vir* lysates grown on strain CP79 (*relA2*), to yield isolates BB1372 (*relA*$^+$) and BB1373 (*relA2*).

DNA manipulations. Chromosomal DNA was prepared as described previously (30). Restriction enzyme digests were performed by using the specifications of the supplier (New England BioLabs, Inc., Beverly, Mass.). ^{32}P labeling of *dnaK*$^+$ probes (pKP31) (38) with a nick-translation kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) and Southern blotting and hybridization (at 65°C) to GeneScreen Plus (Dupont, NEN Research Products, Boston, Mass.) were performed by the instructions of the manufacturers.

Microscopic techniques. For light microscopic analysis, cells from fresh colonies grown on LB agar plates or from exponentially grown liquid LB cultures were prepared on glass microscope slides and observed in a microscope (Axioplan Universal; Zeiss) by using a Plan 100 objective and Nomarsky differential interference contrast optics. Photographs were taken with Tri-X-Pan 400 films (Eastman Kodak Co., Rochester, N.Y.). For electron microscopic analysis, cells were washed in 0.85% NaCl, fixed with OsO_4, and embedded in Epon as described by Kellenberger et al. (25). Thin sections were observed in an electron microscope (100 B; JEOL) and photographed on electron microscope film (4489; Kodak).

RESULTS

DnaK function is required for growth at low as well as at high temperatures. To investigate systematically the cellular requirements for DnaK at various temperatures, we transduced the Δ*dnaK52* allele from strain GW4813 (38) into the wild type and incubated the transduction plates at a series of temperatures from 43 to 11°C. At 30°C the number of Δ*dnaK52* transductants was much higher than it was at all other temperatures tested, but these transductants grew very slowly and formed abnormally flat and translucent colonies (Fig. 1). These phenotypes were likely to have resulted from cellular defects that were present in the Δ*dnaK52* mutants. When kept at 30°C for more than about 7 days, the cells in the flat and translucent Δ*dnaK52* transductant colonies completely lost their viability; they could be kept alive only if

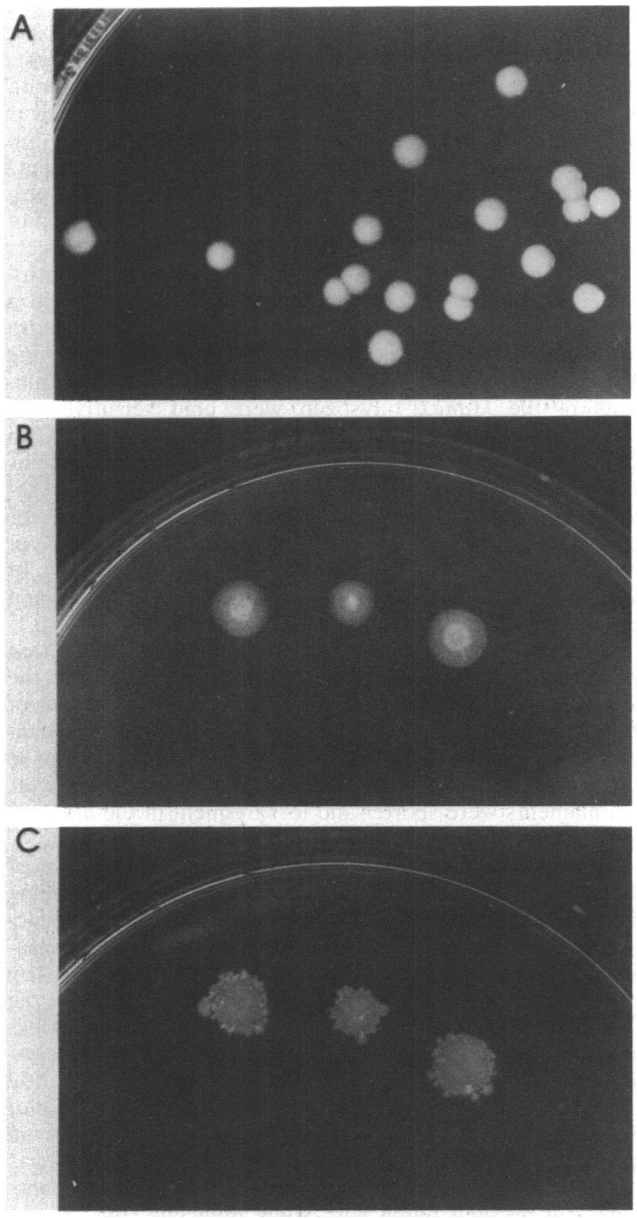

FIG. 1. Abnormal morphology of Δ*dnaK52* transductant colonies at 30°C and appearance of papillae. The Δ*dnaK52* allele of strain GW4813 (38) was transduced into cells of strain MC4100, and the transduction plates (LB-chloramphenicol) were incubated at 30°C. Transductant colonies were photographed after 3 days (B) and 7 days (C). (A) For comparison, colonies formed by wild-type cells (MC4100) after growth on LB agar plates at 30°C are shown.

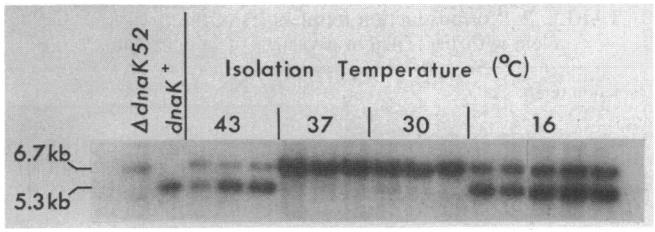

FIG. 2. Presence of *dnaK* gene duplications in Δ*dnaK52* transductants isolated at high and low temperatures. *Hin*dIII-digested chromosomal DNA of Δ*dnaK52* mutants described earlier (38) (GW4813; Δ*dnaK52*), the wild type (MC4100; *dnaK*$^+$), and the Δ*dnaK52* transductants isolated at the indicated temperatures was electrophoresed and hybridized to a ^{32}P-labeled *dnaK*$^+$ probe. The 6.7-kilobase (6.7 kb) upper band corresponds to the *Hin*dIII fragment containing the Δ*dnaK52* allele; the 5.3-kilobase (5.3 kb) lower band corresponds to the fragment containing the *dnaK*$^+$ gene.

they were constantly transferred to fresh agar plates, indicating that Δ*dnaK52* mutants are sensitive to nutrient starvation or drying conditions. Furthermore, prolonged incubation of the transduction plates for about 7 days at 30°C led to the frequent appearance of papillae in the Δ*dnaK52* transductant colonies (Fig. 1). Similarly, after growth of fresh Δ*dnaK52* transductants in liquid medium at 30°C, normally shaped, faster-growing colonies appeared in addition to the flat and translucent colonies. Cells contained within these faster-growing colonies or within papillae contained unlinked secondary mutations which suppressed cellular defects of Δ*dnaK52* transductants at 30°C (unpublished data). We are in the process of genetically characterizing these suppressor mutations. Previously described Δ*dnaK52* strains (38) most likely contained such suppressor mutations, since most of the phenotypes of Δ*dnaK52* transductants described here were absent in these isolates.

At 43°C we obtained a low number of Δ*dnaK52* transductants, despite this being a nonpermissive temperature for Δ*dnaK52* mutants (38). In addition, we obtained a similar low number of Δ*dnaK52* transductants at 16 and 11°C, even after incubation for up to 3 weeks. Furthermore, transductants isolated at high (43°C) as well as at low (11 and 16°C) temperatures differed from the Δ*dnaK52* transductants that were isolated at 30°C in that they all were indistinguishable from wild-type cells with respect to growth rate, colony morphology, temperature range for growth, and sensitivity to bacteriophage λ. We therefore considered the possibility that these transductants contained a second, intact copy of the *dnaK* allele, which resulted from duplication of the chromosomal region containing the *dnaKJ* operon in a subset of the cell population prior to the introduction of the Δ*dnaK52* allele. To test this hypothesis, we carried out Southern hybridizations of *Hin*dIII-digested chromosomal DNA from Δ*dnaK52* transductants isolated at various temperatures using a *dnaK*$^+$ probe. In contrast to Δ*dnaK52* transductants isolated at 30 and 37°C, all transductants isolated at 16 and 43°C contained two copies of the *dnaK* gene, one intact *dnaK*$^+$ allele (Fig. 2, lower band) and one Δ*dnaK52* allele (Fig. 2, upper band). Δ*dnaK52* isolates carrying an additional intact *dnaK*$^+$ allele were genetically unstable. When they were kept on LB-chloramphenicol plates at 30°C, they gave rise to chloramphenicol-resistant, λ-resistant, and temperature-sensitive cells which most likely lost the duplicated *dnaK*$^+$ gene. Thus, it is not possible to introduce the Δ*dnaK52* allele directly into wild-type cells at high (43°C) or low (11 and 16°C) temperatures unless a second, intact copy of the *dnaKJ* operon is present. We further confirmed this result by determining the cotransduction frequency of the Δ*dnaK52* allele with the nearby *thr* marker at various temperatures. While at 30°C the Δ*dnaK52* allele was 41.3% cotransducible with the *thr* marker, it could not be detectably cotransduced at 43, 16, or 11°C and had a low cotransduction frequency at 21°C (Table 2).

Given that the Δ*dnaK52* allele could not be introduced into wild-type cells at high or low temperatures, we expected Δ*dnaK52* transductants isolated at 30°C to be temperature sensitive as well as cold sensitive for growth. Consistently, when streaked onto agar plates which were incubated at a variety of temperatures, Δ*dnaK52* transductants isolated at

TABLE 2. Cotransduction frequencies of the Δ*dnaK52*::Cm^r allele with *thr*::Tn*10* at a variety of temperatures[a]

Isolation temp (°C)	No. of transductants	
	Tc^r	Tc^r Cm^r
11	98	0
16	88	0
21	98	1
30	477	197
43	45	0

[a] Cells of strain MC4100 were transduced to *thr*::Tn*10* by using a P1 lysate grown on a Δ*dnaK52 thr*::Tn*10* strain (BB1042). *thr*::Tn*10* transductants were selected on LB-tetracycline plates which were incubated for up to 2 to 3 weeks (11 and 16°C) at the indicated temperatures. Cotransduction of the Δ*dnaK52* allele was determined by testing the Tc^r transductants for resistance of chloramphenicol. The numbers represent the sum of several experiments.

30°C could not grow at 43 or at 11 and 16°C and grew poorly at 21 and 37°C. Thus, Δ*dnaK52* mutants have a very narrow temperature spectrum for growth (approximately from 21 to 37°C).

Introduction of the Δ*dnaK52* allele into wild-type *E. coli* results in filamentation of the cells at intermediate (30°C) temperatures. To identify cellular defects of Δ*dnaK52* mutants that were isolated at the permissive temperature, we performed a microscopic analysis of cells contained in the flat, translucent Δ*dnaK52* transductant colonies that were isolated at 30°C. The cells formed very long filaments (Fig. 3), and thus were apparently defective in cell division. Δ*dnaK52* cells grown in liquid media at 30°C formed filaments as well. Filamentous growth was also observed with Δ*dnaK52* transductants that were isolated at 21 and 37°C. Defects in cell division at intermediate temperatures turned out to be a general phenotype of Δ*dnaK52* transductants since it occurred in all genetic backgrounds tested. Electron microscopic analysis revealed that the cell filaments were unseptated (Fig. 4), indicating that cell division was blocked at an early stage prior to septum formation. DNA was segregated within the cell filament (Fig. 4), but we cannot exclude the possibility that partitioning of DNA in Δ*dnaK52* mutants may be abnormal. The fact that filamentation of Δ*dnaK52* mutants occurred even at 30°C indicates that the DnaK protein is not only required for cell division after heat shock but also, directly or indirectly, for cell division at intermediate and, possibly, at low temperatures.

Given that defects in cell division leading to the formation of cell filaments usually are lethal (8, 18), it was unexpected that Δ*dnaK52* mutants could grow at 30°C despite their strong filamentation. To test whether newly isolated Δ*dnaK52* transductants are reduced in viability at 30°C, we determined the percentage of living cells among the cells contained in Δ*dnaK52* transductant colonies by counting the cells in a Hausser counting chamber (total cell number) and by determining the plating efficiency (living cell number). The average ratio of living cells to total cells of eight individual Δ*dnaK52* transductant colonies was about 1:7, with considerable variations from colony to colony. In contrast, control cells taken from *recA*::Cm^r transductant colonies yielded a consistent ratio of living cells to total cells of about 1:1. These experiments demonstrate that although Δ*dnaK52* mutants can grow at 30°C, their viability is considerably reduced.

Polar effects of the Δ*dnaK52* mutation on *dnaJ* are not responsible for the defects of Δ*dnaK52* mutants. We were initially concerned about the possibility that the phenotypes of Δ*dnaK52* mutants described above were caused by polar effects of the Δ*dnaK52* mutation on transcription of the promoter distal *dnaJ* gene. There is, in fact, a considerable reduction of the synthesis of DnaJ in Δ*dnaK52* mutants (S. M. Sell and C. Georgopoulos, personal communication), but the amount of the DnaJ protein that is synthesized is sufficient to allow growth of a λ phage carrying *dnaK*$^+$ but not *dnaJ*$^+$ (personal observation). Transcription of the *dnaJ* gene in the Δ*dnaK52* mutant probably is driven by a presumptive promoter that is located in the intergenic region of the *dnaKJ* operon (41). To test whether the cellular defects of Δ*dnaK52* mutants are due to *dnaK* deficiency, we introduced the Δ*dnaK52* allele into cells containing a single extra copy of either the *dnaK*$^+$ gene or the *dnaJ*$^+$ gene. This extra copy was provided by lysogenization with λ phages carrying the *dnaK*$^+$ gene or the *dnaJ*$^+$ gene (41). Δ*dnaK52* transductants of the λ(*dnaJ*$^+$) lysogen were cold sensitive and temperature sensitive for growth and filamented at 30°C to the same extent as Δ*dnaK52* transductants of nonlysogens did. In contrast, Δ*dnaK52* transductants of λ(*dnaK*$^+$) lysogens were not cold sensitive or temperature sensitive for growth and did not filament at 30°C. Thus, filamentation at 30°C, cold sensitivity, and temperature sensitivity of Δ*dnaK52* transductants were due to the lack of DnaK and not to the reduction of expression of DnaJ.

These experiments do not exclude the possibility that mutations in *dnaJ* might cause similar cellular defects as the Δ*dnaK52* mutation. Indeed, we found that introduction of the *dnaJ259* mutation, which was isolated as a λ-resistant mutant of UV-irradiated cells (44), into the wild type caused filamentation of the cells at 30°C (data not shown). However, cell filaments were shorter and less frequent in the *dnaJ259* cell population compared with Δ*dnaK52* transductants.

Overproduction of FtsZ protein suppresses filamentation of Δ*dnaK52* mutants at 30°C. We were interested in establishing the basis for the cell division defects of Δ*dnaK52* transductants at 30°C and therefore attempted to suppress the formation of unseptated filaments of Δ*dnaK52* transductants by increasing the frequency of septation. In wild-type cells, the *ftsZ* gene product is limiting for septation since overproduction of FtsZ causes extra septations, resulting in the formation of minicells (51). We therefore transduced the Δ*dnaK52* allele at 30°C into cells which overproduced FtsZ because of the presence of a plasmid carrying the *ftsQAZ* operon (pZAQ) (51). Filamentation of the transductant cells was largely suppressed, although some relatively short filaments were still present, along with some minicells (Fig. 5). To determine which of the three proteins (FtsZ, FtsA, or FtsQ) was responsible for suppression, we used derivatives of the plasmid, each of which carried a Tn*5* insertion in one of the *ftsQ*, *ftsA*, or *ftsZ* genes (29), and repeated the experiment described above. A Tn*5* insertion in *ftsZ* completely abolished suppression of filamentation of Δ*dnaK52* mutants (Fig. 5). Thus, overproduction of FtsZ is absolutely required for suppression of filamentation of Δ*dnaK52* cells by the *ftsQAZ*-carrying plasmid. Tn*5* insertions in *ftsQ* and *ftsA* slightly and partially reduced suppression of filamentation by the *ftsQAZ*-carrying plasmid, respectively (data not shown). These intermediate suppressive activities are most likely due to polar effects of these Tn*5* insertions on expression of the promoter distal *ftsZ* gene (29). Thus, the defects of Δ*dnaK52* mutants in cell division at 30°C can be largely suppressed by overproduction of FtsZ, a component of the regular cellular cell division machinery. This observation suggests that DnaK plays a role in cell division by affecting the synthesis or action of FtsZ, although more complicated models cannot be excluded.

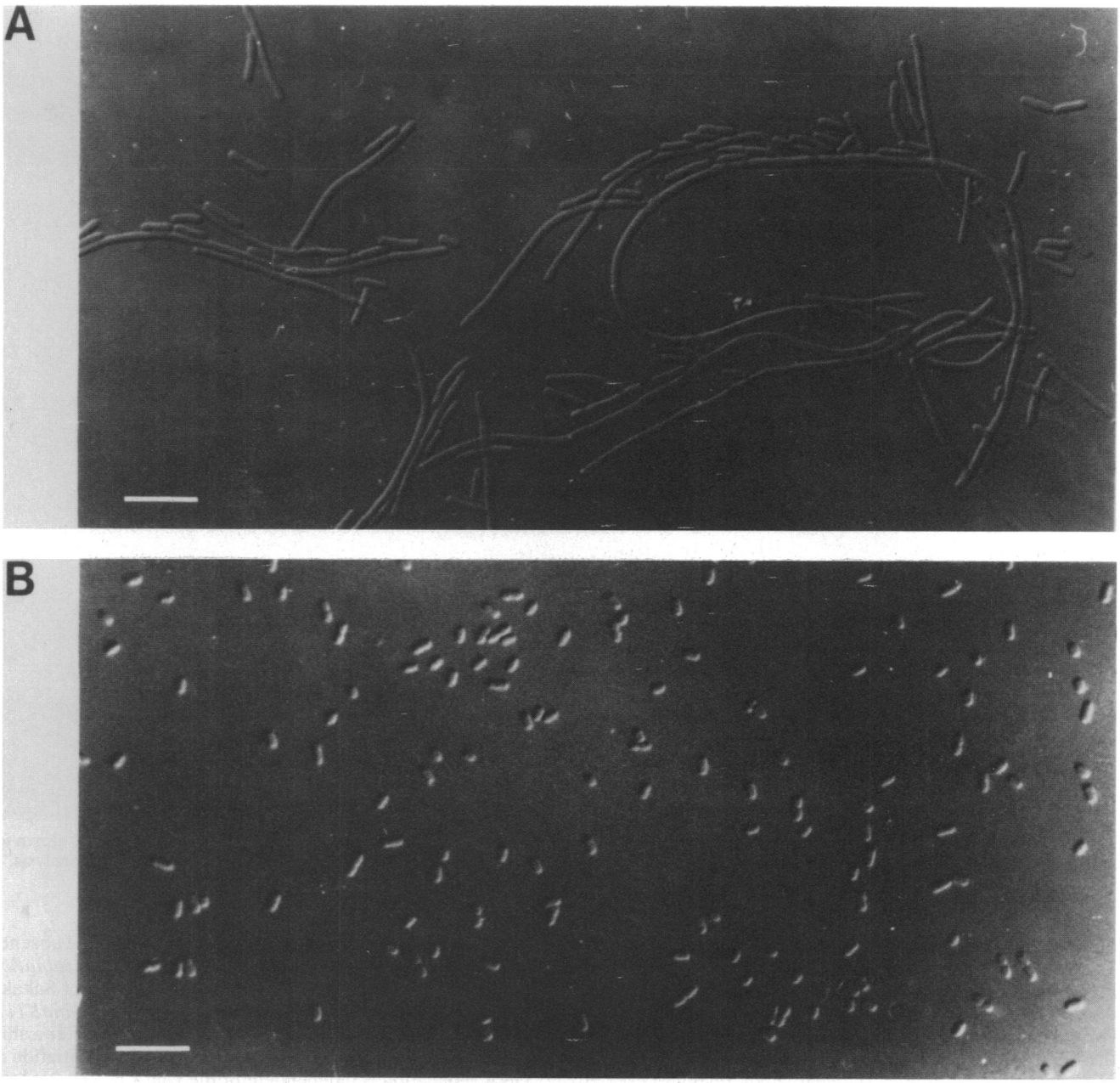

FIG. 3. Formation of cell filaments of Δ*dnaK52* transductants at 30°C. Cells from colonies of Δ*dnaK52* transductants (A) and wild-type cells (MC4100) (B) were subjected to light microscopic analysis after growth for 2 days at 30°C on LB-chloramphenicol or LB agar plates. Bars, 10 μm.

Suppression of filamentation does not restore normal growth of Δ*dnaK52* mutants at 30°C or eliminate their temperature sensitivity and cold sensitivity. Despite efficient suppression of cell division defects of Δ*dnaK52* mutants by overproduction of the FtsQ, FtsA, and FtsZ proteins, these strains still grew slowly and restreaked poorly at 30°C; also, prolonged incubation led to the frequent appearance of faster-growing papillae. These observations indicate that Δ*dnaK52* mutants have additional defects in growth at 30°C besides their defect in cell division and suggest that these other defects can be at least partially suppressed by secondary mutations. Furthermore, when streaked onto agar plates incubated at various temperatures, Δ*dnaK52* mutants carrying the plasmid encoding FtsQ, FtsA, and FtsZ were unable to grow at either high (42°C) or low (11 and 16°C) temperatures. A shift of cultures of these cells from 30 to 42°C led to a slight increase in cell length before cell death (data not shown). Thus, overproduction of FtsZ suppresses cell division defects of Δ*dnaK52* mutants even at 42°C, however to a lesser degree than it does at 30°C. Taken together, these results indicate that the cellular requirements for DnaK at 30°C, and probably at 42°C, are not restricted to a role for DnaK in cell division.

Cellular defects of Δ*dnaK52* mutants are independent of stringent response, SOS response, and the *oriC*/DnaA-dependent initiation of DNA replication. Given that cellular defects of Δ*dnaK52* mutants are not restricted to defects in cell division, we considered two classes of explanations to

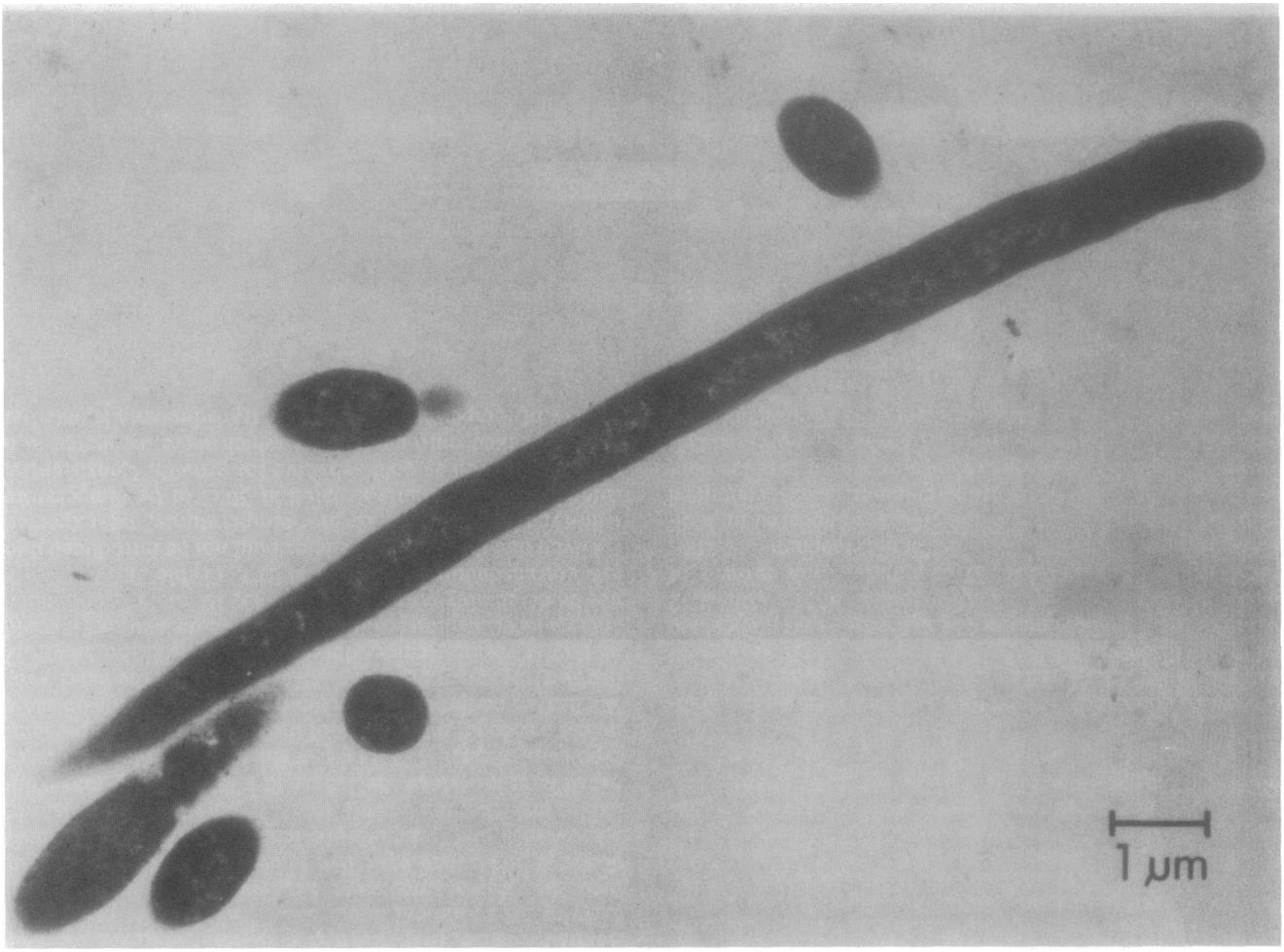

FIG. 4. Cell filaments formed by Δ*dnaK52* transductants at 30°C are unseptated. Δ*dnaK52* transductants of strain MC4100 were grown for 2 days at 30°C on LB-chloramphenicol plates; the transductant colonies were then pooled and prepared for electron microscopic analysis. A typical cell filament is shown.

account for the observed phenotypes of Δ*dnaK52* mutants. The first was that the absence of the DnaK protein led to induction of a cellular response that then caused cellular defects of Δ*dnaK52* mutants. In particular, we considered two inducible cellular responses, the stringent response and the SOS response, since (i) conditions which induce these responses can also induce the heat shock response (15, 26); (ii) induction of the SOS response leads to inhibition of FtsZ activity, leading to filamentation of the cells (50); (iii) conditional mutations in *dnaK* lead to induction of the stringent response at nonpermissive temperatures (21); and (iv) inhibition of the stringent response suppresses inhibition of RNA synthesis of conditional *dnaK* mutants at the nonpermissive temperature (45). These findings made it reasonable to hypothesize a functional relationship between the SOS response, the stringent response, and the DnaK protein; and we therefore tested whether cellular defects of Δ*dnaK52* mutants can be suppressed by inhibition of the SOS response or the stringent response. However, the presence of mutations which do not allow induction of the stringent response (*relA1* and *relA2*) (11) or the SOS response (*lexA3*) (50) did not suppress any of the phenotypes of Δ*dnaK52* transductants at 30°C or their sensitivity to extreme temperatures.

The second explanation we considered was that the DnaK protein normally functions in an *oriC*/DnaA-dependent step of the initiation of DNA replication and that it is the absence of this function that causes the cellular defects of Δ*dnaK52* mutants. This hypothesis is based on the findings of Sakakibara (42) that (i) a specific mutation in *dnaK* (*dnaK111*) causes inhibition of initiation of DNA replication after a shift of the cells to 42°C and (ii) *rnh* mutants, in which initiation of DNA replication is independent of the DnaA protein and the regular origin of replication (*oriC*) (37), continue to synthesize DNA at 42°C even in the presence of the *dnaK* mutation. However, the presence of either the *rnh*::Tn*3* (19) or both the *rnh*::Tn*3* and *dnaA850*::Tn*10* (54) mutations did not suppress any of the described phenotypes of Δ*dnaK52* mutants at 30°C or their temperature sensitivities.

Since these attempts to suppress the cellular defects of Δ*dnaK52* mutants failed, we tested whether the presence of *rnh*::Tn*3* as well as *relA1* mutations in cells which overproduce FtsZ would lead to suppression of cellular defects of Δ*dnaK52* mutants. We therefore introduced, at 30°C, the Δ*dnaK52* allele into cells of *rnh*::Tn*3* *relA1* mutants containing a plasmid carrying the *ftsQAZ* operon. However, while Δ*dnaK52* transductants of this strain did not filament at 30°C, they remained poorly viable at 30°C and remained temperature sensitive and cold sensitive for growth. This result further supports the concept that DnaK plays a complex role in the normal metabolism of *E. coli*.

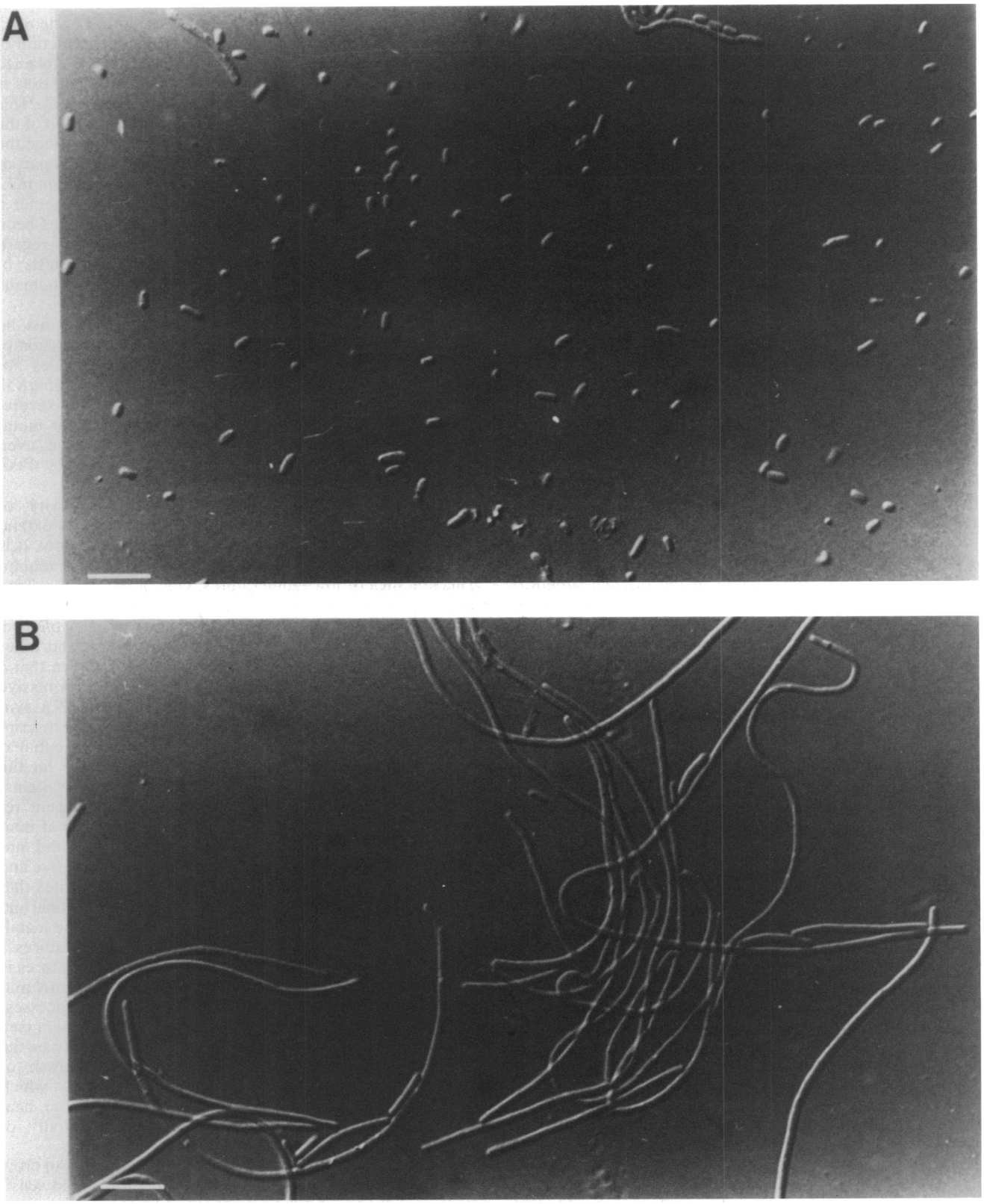

FIG. 5. Overproduction of FtsZ suppresses filamentation of Δ*dnaK52* mutants at 30°C. The Δ*dnaK52* allele was transduced into cells of derivatives of strain MC4100 carrying plasmid pZAQ (A) or pZAQ (*ftsZ28*::Tn*10*) (B). Cells of Δ*dnaK52* transductant colonies grown for 2 days at 30°C were subjected to light microscopic analysis. Bars, 10 μm.

DISCUSSION

We showed that Δ*dnaK52* mutants of *E. coli* possess defects at a wide range of growth temperatures, indicating that the DnaK protein has important functions in cellular metabolism not only during heat shock but also under normal, nonstressed conditions and at low temperatures. The nature of the defects varies with the temperature. At low (11 and 16°C) and high (42°C) temperatures, Δ*dnaK52* mutants were unable to grow. At intermediate (30°C) temperatures they grew slowly, had low viability, and were genetically unstable.

Δ*dnaK52* transductants isolated at 30°C formed flat translucent colonies which frequently acquired papillae on prolonged incubation. We concluded that cells contained in the flat translucent colonies were suppressorless Δ*dnaK52* mutants, in contrast to cells contained in the papillae, which contained suppressors. This conclusion was based on the following arguments. If acquisition of a suppressor mutation is essential for Δ*dnaK52* mutants to grow at 30°C, it must have been acquired during construction of the original Δ*dnaK52* mutant. If this suppressor mutation was linked to *dnaK*, it could be cotransduced with the Δ*dnaK52* allele in subsequent experiments. Construction of the original Δ*dnaK52* mutant isolate involved the transformation of *recBC sbcB* mutants with a linear DNA fragment carrying the Δ*dnaK52* allele (38). This is an extremely inefficient process and routinely yields only 1 to 20 transformants per μg of DNA (52; D. Shevell, personal communication), as was the case in the construction of the original Δ*dnaK52* mutant (K.-H. Paek, personal communication). Thus, if the acquisition of a secondary mutation in a linked gene was essential for Δ*dnaK52* transformants to grow at 30°C, then it would have been practically impossible to cross the Δ*dnaK52* allele into the chromosome. Furthermore, if a suppressor mutation unlinked to *dnaK* was required for Δ*dnaK52* mutants to grow at 30°C, the transduction frequency of the Δ*dnaK52* allele would be considerably reduced, even if the number of target genes for these suppressor mutations was large. However, the Δ*dnaK52* allele was cotransduced with the nearby *thr* marker at close to the expected frequency, indicating that an unlinked suppressor mutation is not a requirement for Δ*dnaK52* mutants to grow. These results and considerations led us to conclude that Δ*dnaK52* mutants are viable at 30°C without the presence of suppressor mutations, although they are genetically unstable and suppressor mutations are frequently acquired during growth.

At the intermediate temperature (30°C), the most striking defect of Δ*dnaK52* mutants was that the cells were inhibited in cell division and hence grew as filaments. It has been reported previously that mutations in *dnaK*, *groE*, and *htpR* genes lead to inhibition of cell division after a shift of the cells to nonpermissive temperatures (8, 12, 38, 47), suggesting that functions of these proteins are required for cell division to occur after heat shock. The present findings indicate that DnaK is required not only for the cell division that occurs after heat shock but also, directly or indirectly, for normal cell division that occurs at a wide range of growth temperatures. We also observed that *dnaJ259* mutants exhibited defects in cell division at 30°C. Since DnaK and DnaJ seem to interact (S. M. Sell and C. Georgopoulos, personal communication), it seems likely that both proteins act together in normal cell division.

The block in cell division of Δ*dnaK52* mutants at 30°C was at or prior to septation. Interestingly, it was largely suppressed by overproducing the FtsZ protein, which was viewed as the key control protein in the division cycle of *E. coli*, being involved in initiation rather than construction of the septum (18). While this suppression might be explained in several ways, one of the most economical hypotheses is that DnaK affects the expression or function of FtsZ. This possible activity of DnaK is not mediated by induction of the SulA protein, which acts as an antagonist of FtsZ, since the *lexA3* mutation which prevents induction of SulA as part of the SOS response did not inhibit filamentation of Δ*dnaK52* mutant cells. It will be interesting to determine whether a requirement for an Hsp70 protein in cell division has been conserved during evolution. It is interesting in this regard that under normal temperature conditions, synthesis of Hsp70 proteins of *Caulobacter crescentus* (13) and humans (24, 32) is cell cycle regulated.

Besides cell division, other cellular functions must be defective at 30°C in Δ*dnaK52* mutants, since suppression of cell division defects by overproduction of FtsZ does not suppress the slow growth and low viability of Δ*dnaK52* mutants at 30°C. These additional defects seem to be severe, since Δ*dnaK52* cells frequently acquired suppressor mutations that provided a considerable growth advantage, even when the cell division defects were suppressed by FtsZ overproduction.

The cold sensitivity and temperature sensitivity of Δ*dnaK52* mutants indicates that DnaK functions to extend the range of temperatures over which *E. coli* can grow. It is not yet clear what the metabolic processes are that require DnaK at high or low temperatures. One of the most striking changes in cellular metabolism which occurs at both extremes of the growth temperature range of *E. coli* is a decrease in cellular levels of ribosomal proteins and other translational and transcriptional proteins (17). Given that a shift of conditional *dnaK* mutants to the nonpermissive temperature leads to an immediate inhibition of RNA synthesis (22), it could be that DnaK has a function in transcriptional and translational processes that becomes essential at low and high temperatures because of limitations in the availability of transcriptional and translational proteins. However, we showed that inhibition of the stringent response, which relaxes inhibition of RNA synthesis at nonpermissive temperatures in *dnaK* mutants (45), did not prevent Δ*dnaK52* mutant cells from being cold sensitive and temperature sensitive for growth. This result indicates that any hypothetical involvement of DnaK in transcriptional and translational processes cannot account solely for the inability of Δ*dnaK52* mutants to grow at extreme temperatures.

Analysis of the functions of DnaK at high temperatures is difficult, since at these temperatures, conditional *dnaK* mutants are inhibited in multiple major biological processes, including synthesis of RNA and DNA and cell division (see above). However, cell division defects are unlikely to be the main reason for temperature sensitivity of growth of Δ*dnaK52* mutants since overproduction of FtsZ, which considerably reduces filamentation of the cells after heat shock, did not suppress the temperature sensitivity of growth of Δ*dnaK52* mutants.

The reasons for the inability of Δ*dnaK52* mutants to grow at low temperatures (11 and 16°C) are unclear. Cold sensitivity of Δ*dnaK52* mutants might result from the absence of a function of DnaK which is conserved during evolution among proteins of the Hsp70 family, since double mutants of *S. cerevisiae* which are mutated in the *YG101* and *YG103* Hsp70 cognate genes also grow increasingly poorly as the temperature is lowered (7). However, cold sensitivity of

Δ*dnaK52* mutants was an unexpected finding since DnaK is not among the *E. coli* proteins induced by cold shock (23); in fact, its steady-state level at 15°C was found to be 89% of its level at 30°C (17). Furthermore, this result is puzzling in view of the recent finding that Δ*htpR* mutants were able to grow only at temperatures below 20°C (27, 56). This apparent paradox might be explained by assuming that the residual synthesis of DnaK which still occurs in Δ*htpR* mutants (56) is sufficient for growth of Δ*htpR* mutants at temperatures below 20°C. Alternatively, cold sensitivity could result indirectly from an elevated basal level of expression of heat shock proteins in Δ*dnaK52* mutants (38). We are investigating these possibilities.

Taken together, our analysis of cellular defects of Δ*dnaK52* mutants provides strong evidence that DnaK has multiple cellular functions which change with growth temperature. This is consistent with the proposed function of DnaK as a chaperone. In such a model, DnaK would have to carry out specific interactions with diverse cellular proteins. At least some of these interactions might not be conserved among the Hsp70 protein family. Such an explanation could account for our unpublished observation that the *dnaK*$^+$ gene from *Mycobacterium tuberculosis* cannot complement Δ*dnaK52* mutations of *E. coli*. It is tempting to speculate that such specific interactions of the Hsp70 proteins might involve the C-terminal domain of these proteins since they have been less conserved during evolution than the amino-terminal domains have.

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LITERATURE CITED

1. **Bardwell, J. C. A., and E. A. Craig.** 1984. Major heat shock gene of *Drosophila* and the *Escherichia coli* heat-inducible *dnaK* gene are homologous. Proc. Natl. Acad. Sci. USA **81**:848–852.
2. **Bardwell, J. C. A., and E. A. Craig.** 1987. Eucaryotic M_r 83,000 heat shock protein has a homologue in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:5177–5181.
3. **Bochner, B. R., M. Zylicz, and C. Georgopoulos.** 1986. *Escherichia coli* DnaK protein possesses a 5′-nucleotidase activity that is inhibited by AppppA. J. Bacteriol. **168**:931–935.
4. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104**:541–555.
5. **Chappell, T. G., W. J. Welch, D. M. Schlossman, K. B. Palter, M. J. Schlesinger, and J. E. Rothman.** 1986. Uncoating ATPase is a member of the 70 kilodalton family of stress proteins. Cell **45**:3–13.
6. **Craig, E. A.** 1985. The heat shock response. Crit. Rev. Biochem. **18**:239–280.
7. **Craig, E. A., and K. Jacobsen.** 1985. Mutations in cognate genes of *Saccharomyces cerevisiae hsp70* result in reduced growth rates at low temperatures. Mol. Cell. Biol. **5**:3517–3524.
8. **Donachie, W. D., K. J. Begg, and N. F. Sullivan.** 1984. Morphogenes of *Escherichia coli*, p. 27–62. *In* R. Losick and L. Shapiro (ed.), Microbial development. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Echols, H.** 1986. Multiple DNA-protein interactions governing high-precision DNA transactions. Science **233**:1050–1056.
10. **Ellis, J.** 1987. Proteins as molecular chaperones. Nature (London) **328**:378–379.
11. **Gallant, J. A.** 1979. Stringent control in *E. coli*. Annu. Rev. Genet. **13**:393–415.
12. **Georgopoulos, C. P., and H. Eisen.** 1974. Bacterial mutants which block phage assembly. J. Supramol. Struct. **2**:349–359.
13. **Gomes, S. L., M. H. Juliani, J. C. C. Maia, and A. M. Silva.** 1986. Heat shock protein synthesis during development in *Caulobacter crescentus*. J. Bacteriol. **168**:923–930.
14. **Grossman, A. D., D. B. Straus, W. A. Walter, and C. A. Gross.** 1987. σ^{32} synthesis can regulate the synthesis of heat shock proteins in *Escherichia coli*. Genes Dev. **1**:179–184.
15. **Grossman, A. D., W. T. Taylor, Z. F. Burton, R. R. Burgess, and C. A. Gross.** 1985. Stringent response in *Escherichia coli* induces expression of heat shock proteins. J. Mol. Biol. **186**:357–365.
16. **Hemmingsen, S. M., C. Woolford, S. M. van der Vies, K. Tilly, D. T. Dennis, C. P. Georgopoulos, R. W. Hendrix, and R. J. Ellis.** 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature (London) **333**:330–334.
17. **Herendeen, S. L., R. A. VanBogelen, and F. C. Neidhardt.** 1979. Levels of major proteins of *Escherichia coli* during growth at different temperatures. J. Bacteriol. **139**:185–194.
18. **Holland, I. B.** 1987. Genetic analysis of the *E. coli* division clock. Cell **48**:361–362.
19. **Horiuchi, T., H. Maki, and M. Sekiguchi.** 1984. RNase H-defective mutants of *Escherichia coli*: a possible discriminatory role of RNase H in initiation of DNA replication. Mol. Gen. Genet. **195**:17–22.
20. **Hunt, C., and R. I. Morimoto.** 1985. Conserved features of eucaryotic *hsp70* genes revealed by comparison with the nucleotide sequence of human hsp70. Proc. Natl. Acad. Sci. USA **82**:6455–6459.
21. **Itikawa, H., H. Fujita, and M. Wada.** 1986. High temperature induction of a stringent response in the *dnaK*(Ts) and *dnaJ*(Ts) mutants of *Escherichia coli*. J. Biochem. **99**:1719–1724.
22. **Itikawa, H., and J.-I. Ryu.** 1979. Isolation and characterization of a temperature-sensitive *dnaK* mutant of *Escherichia coli* B. J. Bacteriol. **138**:339–344.
23. **Jones, P. G., R. A. VanBogelen, and F. C. Neidhardt.** 1987. Induction of proteins in response to low temperature in *Escherichia coli*. J. Bacteriol. **169**:2092–2095.
24. **Kao, H.-T., O. Kapasso, N. Heintz, and J. R. Nevins.** 1985. Cell cycle control of the human HSP70 gene: implications for the role of a cellular E1A-like function. Mol. Cell. Biol. **5**:628–633.
25. **Kellenberger, E., A. Ryter, and J. Sechaud.** 1958. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. J. Biophys. Biochem. Cytol. **4**:671–684.
26. **Krueger, J. H., and G. C. Walker.** 1984. *groEL* and *dnaK* genes of *Escherichia coli* are induced by UV irradiation and nalidixic acid in an *htpR*$^+$-dependent fashion. Proc. Natl. Acad. Sci. USA **81**:1499–1503.
27. **Kusukawa, N., and T. Yura.** 1988. Heat shock protein GroE of *Escherichia coli*: key protective roles against thermal stress. Genes Dev. **2**:874–882.
28. **Lindquist, S.** 1986. The heat-shock response. Annu. Rev. Biochem. **55**:1151–1191.
29. **Lutkenhaus, J., B. Sanjanwala, and M. Lowe.** 1986. Overproduction of FtsZ suppresses sensitivity of *lon* mutants to division inhibition. J. Bacteriol. **166**:756–762.
30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **McMullin, T. W., and R. L. Hallberg.** 1988. A highly evolutionarily conserved mitochondrial protein is structurally related to the protein encoded by the *Escherichia coli groEL* gene. Mol. Cell. Biol. **8**:371–380.
32. **Milarski, K. L., and R. I. Morimoto.** 1986. Expression of human HSP70 during the synthetic phase of the cell cycle. Proc. Natl. Acad. Sci. USA **83**:9517–9521.
33. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold

Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

34. **Neidhardt, F. C., and R. A. VanBogelen.** 1981. Positive regulatory gene for temperature-controlled proteins in *Escherichia coli*. Biochem. Biophys. Res. Commun. **100:**894–900.
35. **Neidhardt, F. C., and R. A. VanBogelen.** 1987. Heat shock response. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella typhimurium*. American Society for Microbiology, Washington, D.C.
36. **Neidhardt, F. C., R. A. VanBogelen, and V. Vaughn.** 1984. The genetics and regulation of heat-shock proteins. Annu. Rev. Genet. **18:**295–329.
37. **Ogawa, T., G. G. Pickett, T. Kogoma, and A. Kornberg.** 1984. RNase H confers specificity in the *dnaA*-dependent initiation of replication at the unique origin of the *Escherichia coli* chromosome *in vivo* and *in vitro*. Proc. Natl. Acad. Sci. USA **81:** 1040–1044.
38. **Paek, K.-H., and G. C. Walker.** 1987. *Escherichia coli dnaK* null mutants are inviable at high temperature. J. Bacteriol. **169:** 283–290.
39. **Pelham, H. R. B.** 1986. Speculations on the functions of the major heat shock and glucose-regulated proteins. Cell **46:**959–961.
40. **Saito, H., and H. Uchida.** 1977. Initiation of the DNA replication of bacteriophage lambda in *Escherichia coli* K12. J. Mol. Biol. **113:**1–25.
41. **Saito, H., and H. Uchida.** 1978. Organization and expression of the *dnaJ* and *dnaK* genes of *Escherichia coli* K12. Mol. Gen. Genet. **164:**1–8.
42. **Sakakibara, Y.** 1988. The *dnaK* gene of *Escherichia coli* functions in initiation of chromosome replication. J. Bacteriol. **170:**972–979.
43. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Sunshine, M., M. Feiss, J. Stuart, and J. Yochem.** 1977. A new host gene (*groPC*) necessary for lambda DNA replication. Mol. Gen. Genet. **151:**27–34.
45. **Tanaka, M., M. Wada, and H. Itikawa.** 1985. Abortive relaxation of RNA synthesis for cellular DNA replication affected by *dnaK*(Ts) and *dnaJ*(Ts) mutations in *Escherichia coli* K-12. Jpn. J. Genet. **60:**27–38.
46. **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.
47. **Tsuchido, T., R. A. VanBogelen, and F. C. Neidhardt.** 1986. Heat shock response in *Escherichia coli* influences cell division. Proc. Natl. Acad. Sci. USA **83:**6959–6963.
48. **Uzan, M., and A. Danchin.** 1976. A rapid test for the *relA* mutation in *E. coli*. Biochem. Biophys. Res. Commun. **69:** 751–758.
49. **Wada, M., K. Sekine, and H. Itikawa.** 1986. Participation of the *dnaK* and *dnaJ* gene products in phosphorylation of glutaminyl-tRNA synthetase and threonyl-tRNA synthetase of *Escherichia coli* K-12. J. Bacteriol. **168:**213–220.
50. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48:**60–93.
51. **Ward, J. E., Jr., and J. Lutkenhaus.** 1985. Overproduction of FtsZ induces minicell formation in *E. coli*. Cell **42:**941–949.
52. **Winans, S. C., S. J. Elledge, J. H. Krueger, and G. C. Walker.** 1985. Site-directed insertion and deletion mutagenesis with cloned fragments in *Escherichia coli*. J. Bacteriol. **161:**1219–1221.
53. **Yamamori, T., and T. Yura.** 1982. Genetic control of heat-shock protein synthesis and its bearing on growth and thermal resistance in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **79:**860–864.
54. **Yin, J. C. P., and W. S. Reznikoff.** 1987. *dnaA*, an essential host gene, and Tn*5* transposition. J. Bacteriol. **169:**4637–4645.
55. **Young, D., R. Lathigra, R. Hendrix, D. Sweetser, and R. A. Young.** 1988. Stress proteins are immune targets in leprosy and tuberculosis. Proc. Natl. Acad. Sci. USA **85:**4267–4270.
56. **Zhou, Y.-N., N. Kusukawa, J. W. Erickson, C. A. Gross, and T. Yura.** 1988. Isolation and characterization of *Escherichia coli* mutants that lack the heat shock sigma factor σ^{32}. J. Bacteriol. **170:**3640–3649.
57. **Zylicz, M., D. Ang, and C. Georgopoulos.** 1987. The *grpE* protein of *Escherichia coli*. J. Biol. Chem. **262:**17437–17442.
58. **Zylicz, M., J. H. LeBowitz, R. McMacken, and C. P. Georgopoulos.** 1983. The *dnaK* protein of *Escherichia coli* possesses an ATPase and autophosphorylating activity and is essential in an *in vitro* DNA replication system. Proc. Natl. Acad. Sci. USA **80:**6431–6435.