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An Essential Role for the *Escherichia coli* DnaK Protein in Starvation-Induced Thermotolerance, H_2O_2 Resistance, and Reductive Division

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During a 3-day period, glucose starvation of wild-type *Escherichia coli* produced thermotolerant, H_2O_2-resistant, small cells with a round morphology. These cells contained elevated levels of the DnaK protein, adjusted either for total protein or on a per-cell basis. Immunoprecipitation of [^{35}S]methionine-labeled protein produced by such starving cells demonstrated that DnaK underwent continuous synthesis but at decreasing rates throughout this time. Glucose resupplementation of starving cells resulted in rapid loss of thermotolerance, H_2O_2 resistance, and the elevated DnaK levels. A *dnaK* deletion mutant, but not an otherwise isogenic wild-type strain, failed to develop starvation-induced thermotolerance or H_2O_2 resistance. The filamentous phenotype associated with DnaK deficiency was suppressed by cultivation in a defined glucose medium. When starved for glucose, the nonfilamentous and rod-shaped *dnaK* mutant strain failed to convert into the small spherical form typical of starving wild-type cells. The *dnaK* mutant retained the ability to develop adaptive H_2O_2 resistance during growth but not adaptive resistance to heat. Complementation of DnaK deficiency by using P_{tac}-regulated *dnaK*$^+$ and *dnaK*$^+$*J*$^+$ expression plasmids confirmed a specific role for the DnaK molecular chaperone in these starvation-induced phenotypes.

Several features of nonsporulating bacteria, including cell morphology and resistance to killing, can depend upon growth state. Stationary-phase cells produced by nutrient deprivation (25) or nutrient excess (12) exhibit a distinctive rounded cell shape of reduced volume (33, 37, 45, 46) and a generalized resistance to extremes of heat, oxidizing agents such as hydrogen peroxide (H_2O_2), and sodium chloride (for reviews, see references 28 and 38). Starvation-induced resistance is dependent upon protein synthesis (25) and the regulons controlled by the alternative sigma factors σ^{32} (24) and σ^S (39), encoded by the *rpoH* and *rpoS* (*katF*) genes, respectively (for a review, see reference 36). A requirement for σ^S in the development of stress resistance and reductive division has also been demonstrated in stationary-phase cells under conditions of nutrient excess (32, 33). Since stress resistance in starving cells is significantly greater than that produced by the adaptive treatment of growing cells (25), unique mechanisms may be employed in nongrowing cells to create the stress-resistant state (34). For example, an important role for trehalose synthesis in nutrient excess stationary-phase thermotolerance has been reported (20). In addition, expression of the *htrE* operon, which is required for growth above 43.5°C and is controlled by σ^E (13), and *rpoS* (49) may play some role in stationary-phase thermotolerance.

Stationary-phase cells assume a characteristic small and spherical cell morphology in a process termed reductive division (45). This morphological change is likely to involve the morphogene *bolA* (1) and *ficA* (26). Stationary-phase cells are also typified by an extreme resistance to oxidants such as H_2O_2. An important role for *rpoS* (32, 39) and the *rpoS*-controlled gene *dps* (2) or *pexB* (35) in this unique physiologic condition is supported by the apparent H_2O_2-sensitive and stationary phase-specific phenotypes of mutants in these genes. RpoS is also important for the expression of *katE*, encoding the hydroperoxidase II catalase, and *xthA*, encoding exonuclease III (36). In contrast, distinct regulatory systems appear to control H_2O_2 resistance in growing cells (for a review, see reference 11).

The ability of bacteria to withstand thermal stress depends upon the synthesis of heat shock proteins (HSPs) (for reviews, see references 14, 17, and 56). A critical role for HSPs in thermotolerance is supported by the thermosensitive phenotype of HSP mutants. In addition, mutants lacking σ^{32}, which are deficient in or have reduced expression of most HSPs, express a severe temperature growth-restricted phenotype (57) which can be suppressed by plasmid-mediated HSP overproduction (30). HSPs are preferentially produced in nutrient-starved *Escherichia coli* during the first several hours of starvation (16, 52). This is likely to reflect the ongoing presence of σ^{32} during the starvation period examined (24). Since the development of the starvation-induced stress-resistant state is sensitive to a block in protein synthesis imposed at the onset of starvation (25), it is likely that synthesis of particular HSPs may be important in starvation-induced changes.

The HSP and molecular chaperone DnaK is one of the starvation-induced HSPs. DnaK controls protein folding in an ATP-dependent process involving the cochaperones DnaJ and GrpE (for a review, see reference 14). Mutations in *dnaK* exhibit several common phenotypes, including heat-sensitive growth, an inability to support bacteriophage growth, and a defect in the attenuation of the heat shock response (17, 56). The deletion allele Δ*dnaK52* (47) results in severe filamentation, defective chromosome segregation, and a more restricted range of growth temperatures than observed with previously described *dnaK* mutant alleles (5, 6). Several phenotypes also suggest a role for DnaK in carbon metabolism. The *dnaK103* mutation (22, 52) reduces cell survival on a carbon-deficient solid medium as well as the synthesis of a subset of starvation proteins (52). Another mutation, *dnaK25*, inhibits mannose

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and sorbitol metabolism (8). The *dnaK* gene is highly expressed under normal growth conditions (48) but retains the capacity for further induction in response to heat (55), oxidation (44), osmotic stress (41), and starvation (16). In *E. coli*, the concentration and rate of synthesis of this protein have been shown to correlate directly with increasing growth rate and are therefore at the lowest levels in slowly dividing cells (48). Growth limitation elevates DnaK levels in starving *Vibrio* species (21) and in sodium chloride-stressed *E. coli* (41). Artificially elevated DnaK levels are also preferentially toxic in stationary phase *E. coli* in a *dnaJ*-dependent manner (3). In the studies described here, the absolute levels of DnaK and the consequence of DnaK deficiency were examined to explore the role of DnaK in the starvation-induced stationary phase.

MATERIALS AND METHODS

Bacterial strains and cultivation. *E. coli* K-12 strains used were PBL500 (*lacZ*::Tn*5* *lacI*^{q1}), a *recA*$^+$ homolog of PBL325 (3, 29); GW4813 (Δ*dnaK52*::Cm^r), kindly provided by G. Walker (47); and PBL501 (Δ*dnaK52*::Cm^r *lacZ*::Tn*5* *lacI*^{q1}), a prototrophic *dnaK* mutant derivative of PBL500. Plasmids used in these studies were pBN13 (P$_{tac}$::*dnaK*$^+$ *lacI*$^+$ *bla*$^+$), pBN15 (P$_{tac}$:: *dnaK*$^+$ *dnaJ*$^+$ *lacI*$^+$ *bla*$^+$), pBN16 (P$_{tac}$::*dnaJ*$^+$ *lacI*$^+$ *bla*$^+$), and pBN8 (*lacI*$^+$ *bla*$^+$) (3). These plasmids utilize a ColE2 replicon and are therefore DnaK independent for replication (15, 40). Plasmid-bearing derivatives of PBL500 or PBL501 were constructed by transformation at 30°C. Complementation of the chromosomal Δ*dnaK52* mutation was required for efficient transduction of the Δ*dnaK52* mutant allele (40). A pBN15 transformant of GW4813 was used as the donor to transfer the Δ*dnaK52*::Cm^r mutation into the PBL500 background by phage P1 *vir* transduction. Cell densities in both growing and starving cultures were monitored spectrophotometrically at a wavelength of 600 nm. The medium used was either M9 minimal medium (42) containing glucose at the concentration indicated or LB rich medium (42). Chloramphenicol, kanamycin, and ampicillin were added as needed at final concentrations of 25, 75, and 100 μg/ml, respectively.

Starvation protocols. Starvation cultures were maintained at either 30 or 37°C as indicated in 500-ml volumes of minimal medium containing 0.025% (wt/vol) glucose (4). Cultures were inoculated with exponential-phase cells pregrown in minimal medium at a cell density of 6.0×10^6/ml. Samples were removed for analysis at mid-exponential phase, at the onset of starvation when the absorbance of the culture ceased to increase, and then after the onset of starvation at the times indicated. Mid-exponential phase was equivalent to 5.6×10^7 cells per ml, and starvation ensued at a cell density of 1.5×10^8/ml. Starving cultures were maintained with gentle agitation at the temperatures indicated. Dehydration of the cultures due to evaporation was minimized by resupplying distilled water at regular intervals based on changes in observed weight of the culture vessel. Glucose resupplementation of starving cultures was performed by the addition of glucose to a final concentration of 0.4% (wt/vol). Samples were removed for analysis every 12 min for the first hour and then every 24 min for an additional hour. After 2 h, the cells were subcultured into fresh prewarmed medium containing excess glucose (0.4% [wt/vol]) and maintained in exponential phase for an additional 10 generations prior to analysis. Viable cell numbers were determined by plating diluted culture samples on LB plates in duplicate.

Thermotolerance measurements were performed essentially as described previously (25). Samples in 2-ml volumes were subjected to heating at 57°C in a prewarmed heating block. Heated samples were then diluted and spread onto LB plates in duplicate and incubated overnight at the temperature indicated. H_2O_2 (15 mM) was used to assess the extent of oxidant resistance as described previously (25). All samples were adjusted to achieve a cell density of 1.5×10^8/ml prior to exposure to heat or H_2O_2. Cell morphology was examined by phase-contrast microscopy. Cell samples used for microscopy were from liquid cultures which were starved for glucose or were in exponential phase and growing in a rich or minimal medium. All cultures used for microscopy were grown at 30°C and were concentrated by centrifugation to a cell density of 5×10^{10}/ml prior to examination. Cell size was determined by comparison with 0.8-μm-diameter Bactolatex beads (Difco). Photomicrographs were prepared with a Nikon Microphot microscope and then scanned with a GDS CCCD video camera. Scanned images were analyzed with Image-1 software (Universal Imaging Inc.).

In vivo labeling of proteins and immunoprecipitation. In vivo labeling of cellular protein was performed as described previously (29), using [^{35}S]methionine (Trans label; NEN) with a specific activity of 1,000 Ci/mmol. To achieve sufficient incorporation of [^{35}S]methionine, samples were incubated in the presence of label for progressively longer times: 6 min for exponential phase and starvation onset, 1 h for 4 h of starvation, and 12 h for all subsequent starvation samples. The cells contained in the following culture samples were used for in vivo labeling: 18 ml for exponential phase; 4 ml for starvation onset, 4 h of starvation, and 24 h of starvation; and 36 ml for 3 days of starvation. To minimize cellular perturbations, [^{35}S]methionine was added to concentrated cell suspensions resuspended in clarified culture supernatants from starving cell cultures. The quantity of label was also adjusted to ensure sufficient labeling of cell protein. The quantities were 10 μCi for exponential phase, starvation onset, and 4 h starvation and 25 μCi for all other samples. All samples were chased with a 1,000-fold molar excess of nonradioactive methionine for 10 min. Following labeling, cells were pelleted by centrifugation and washed twice in cold 20 mM sodium phosphate–150 mM sodium chloride (pH 7.4) (phosphate-buffered saline [PBS]), and the cell pellets were stored at −20°C.

Immunoprecipitation of DnaK from in vivo-labeled cells was performed as described previously (51), with the following modifications. Cell pellets representing 1 ml of a cell suspension with an A_{600} of 1 were resuspended in PBS containing 1% (vol/vol) Triton X-100 and 5 mM magnesium chloride. Samples were sonicated at 4°C by three 30-s pulses in a Braun sonicator equipped with a microtip probe at a power setting of 30%. The samples were then centrifuged for 5 min at 13,000 × *g* at 4°C, and the supernatants were transferred to a new tube. The solutions were adjusted to 1% (vol/vol) Triton X-100, 1% (wt/vol) sodium deoxycholate, and 0.1% (wt/vol) sodium dodecyl sulfate (SDS). Thirteen micrograms of purified anti-DnaK monoclonal antibody (29) was added per ml of the cell sonic extract, and then the mixture was incubated at 4°C for 1 h. Protein A-agarose (Bethesda Research Laboratories) was added at 70 μg of protein A per reaction, and the solution was incubated for 90 min at 4°C with occasional mixing. Samples were centrifuged at 13,000 × *g* for 10 min, and the pellet was washed three times with PBS containing the same concentrations of detergents. The washed pellets were then processed for SDS-polyacrylamide gel electrophoresis (PAGE). Proteins were detected by autoradiography using Kodak XAR film.

Western blot (immunoblot) and DnaK analysis. To quantitate total DnaK levels by Western blot analysis, cells from cultures grown at 37°C were concentrated by centrifugation at 13,000 × *g*, and the resulting cell pellets were frozen at −20°C. The cell pellets were then resuspended in 25 mM Tris-hydrochloride–40 mM glycine at 4°C and sonicated to achieve a minimum of 90% cell lysis. Samples were removed to determine protein concentrations by using the bicinchoninic acid protein assay (Pierce). The remaining samples were adjusted to 0.1% (wt/vol) SDS and frozen at −20°C. Proteins were resolved by SDS-PAGE, using 12.5% (wt/vol) acrylamide separating gels and 10% (wt/vol) acrylamide stacking gels as described previously (3, 31) and prestained low-molecular-weight markers (Bio-Rad). Western blots were prepared essentially as described previously (19, 29). Western blots were processed and developed with disodium 3-(4-methoxyspiro[1,2-dioxetane-3,2′-(5′-chloro)tricyclo[3.3.1.1^{3,7}]decan]-4-yl)phenyl phosphate (Tropix) as described by the manufacturer. Western blots were probed with the monospecific anti-DnaK monoclonal antibody 2G5 (29) and then a goat anti-mouse immunoglobulin G-alkaline phosphatase conjugate (Gibco BRL). The quantity of monoclonal antibody was kept constant at 37 μg/100 cm^2 of membrane. DnaK standards (3) were included in all blots at 0.75 to 6.0 ng. The regression coefficient for the DnaK standard curve used to determine the levels of DnaK in cell extracts was always greater than 0.98. Cell extracts were diluted to ensure that DnaK levels were within the linear range of the standard curve. Chemiluminescence was measured by autoradiography using Kodak XAR film. Autoradiograms were analyzed by densitometry using a GDS 7500 densitometer and Gel base/gel blot software (Ultraviolet Products).

RESULTS

DnaK levels, thermotolerance, and H_2O_2 resistance during glucose starvation and glucose resupplementation. Strain PBL500 was subjected to glucose starvation by allowing cells to deplete the culture medium of growth-limiting levels of glucose, resulting in a final cell density of 1.5×10^8 CFU/ml. After 3 days of starvation, cultures were resupplemented with an excess (0.4% [wt/vol]) of glucose. Throughout the starvation time period, there was a 25% increase in viable cell counts. Thermotolerance and H_2O_2 resistance were monitored during both the onset and exit of cells from the starvation state by determination of viable cell counts of culture samples treated at 57°C or with 15 mM H_2O_2. As reported previously (25), a rapid increase in thermotolerance and H_2O_2 resistance was observed as starvation proceeded (Fig. 1). Maximum resistance to both stresses was reached after 48 h of starvation. However, following glucose resupplementation of starving cell cultures, there was an immediate and rapid reduction of thermotolerance and H_2O_2 resistance to prestarvation, dividing cell levels (Fig. 1, arrow).

In the absence of further cell division resulting from glucose starvation, continued DnaK synthesis could result in the accumulation of this protein. To examine this possibility, an anti-DnaK monoclonal antibody (2G5) was used to quantitate DnaK levels in culture extracts by Western blot analysis.

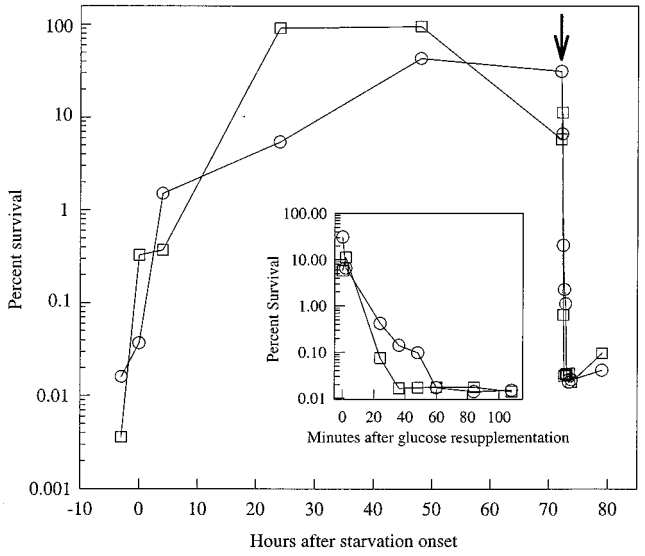

FIG. 1. Thermotolerance and H_2O_2 resistance in glucose-starved and resupplemented *E. coli*. Thermotolerance of PBL500 (wild-type) cell samples was determined at 57°C after 4 min (circles) of heat exposure. H_2O_2 resistance was determined by exposure to 15 mM H_2O_2 for 30 min (squares). The number of surviving cells was normalized to the number of viable cells in an untreated but otherwise identical sample. The values shown are the averages of duplicate samples. The onset of glucose starvation is indicated as time zero, and the time at which cultures were resupplemented with glucose is indicated by the arrow. The insert shows cell survival frequencies for glucose-resupplemented cells on an expanded time scale. The insert *x*-axis value for time zero corresponds to 72 h of glucose starvation.

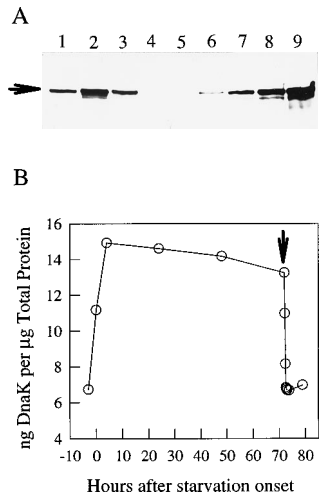

FIG. 2. Total levels of DnaK in glucose-starved and glucose-resupplemented *E. coli*. (A) Western blot of starvation samples. Lanes: 1, PBL500 (wild type), mid-log phase; 2, PBL500, 3 days after the onset of starvation; 3, PBL500, 36 min following glucose resupplementation; 4, PBL501 (Δ*dnaK52*), mid-log phase; 5, PBL501, 3 days of starvation; 6, 0.75 ng of DnaK; 7, 1.5 ng of DnaK; 8, 3 ng of DnaK; 9, 6 ng of DnaK. The blot was probed with the anti-DnaK monoclonal antibody 2G5. (B) DnaK per total cell protein. DnaK values shown are normalized to the total cell protein content of each sample examined. The onset of glucose starvation is indicated as time zero, and the addition of glucose is indicated by the arrow. The values shown are the averages of duplicate samples, and variation between duplicate samples was less than 5%.

Monoclonal antibody 2G5 detects an N-terminal DnaK epitope located at residues 288 to 310 (29). Total DnaK levels of either growing, glucose-starved, or glucose-resupplemented cells indicated there were significant changes in absolute DnaK levels during this period (Fig. 2A, lanes 1 to 3). No other proteins of similar size were detected in extracts of an otherwise isogenic Δ*dnaK52* mutant strain, PBL501, during growth or starvation under the same conditions (Fig. 2A, lanes 4 and 5). Quantitation of DnaK levels in samples from the starving PBL500 culture obtained throughout this time period indicated that DnaK levels, when adjusted for the quantity of protein in each sample, rapidly increased after entry into stationary phase (Fig. 2B). Levels in exponentially growing cells were 6.72 ng/μg and increased to a maximum level of 14.93 ng/μg after 4 h of glucose starvation. The elevation in DnaK levels was maintained at 89% of maximum amounts for the duration of the starvation period. However, immediately following glucose resupplementation, elevated DnaK levels rapidly decreased to prestarvation levels (Fig. 2B, arrow). During the first hour following glucose resupplementation, the absorbance of the culture increased slightly from an initial value of 0.16 to a final value of 0.21, although there was no detectable change in viable counts. The rapid decrease in DnaK levels closely parallels the observed changes in thermotolerance and H_2O_2 resistance resulting from glucose resupplementation of starving cells (Fig. 1). Levels of DnaK in glucose-resupplemented cells remained at this lower level following an additional 10 generations of division.

De novo DnaK synthesis during prolonged carbon starvation. To determine if continued DnaK synthesis was at least in part responsible for the starvation-induced increase in total DnaK levels, starving cells were subjected to in vivo protein labeling with [^{35}S]methionine. To compensate for reduced rates of bulk protein synthesis, culture samples were labeled for increasing times and in increasing amounts as starvation proceeded (2). Starving cell extracts were then analyzed by SDS-PAGE and examined by autoradiography (Fig. 3A). A large number of changes in newly synthesized proteins were seen which ranged widely in size. Variation in the apparent levels of labeled protein observed between samples is most likely a reflection of differences in sample specific activities.

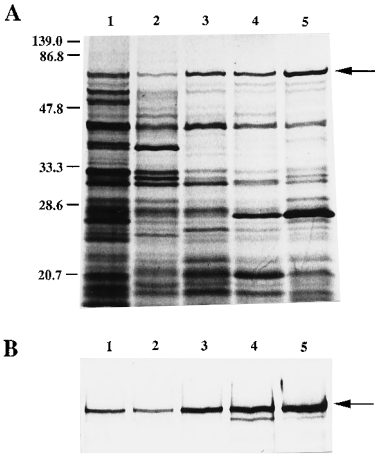

FIG. 3. Prolonged DnaK synthesis during glucose starvation. (A) Total de novo protein synthesis. Strain PBL500 was labeled with [^{35}S]methionine during growth or at various times after the onset of starvation. Lanes: 1, exponential-phase cells; 2, onset of starvation; 3, 4 h of starvation; 4, 24 h of starvation; 5, 72 h of starvation. Equal volumes of labeled cell extracts were loaded in all lanes. (B) Autoradiogram of DnaK immunoprecipitates. [^{35}S]methionine-labeled PBL500 cell extracts were treated with the anti-DnaK monoclonal antibody 2G5 and immunoprecipitated with protein A-agarose beads. Equal volumes of resuspended immunoprecipitates were loaded per lane. The samples shown were prepared at the same time intervals as those in panel A.

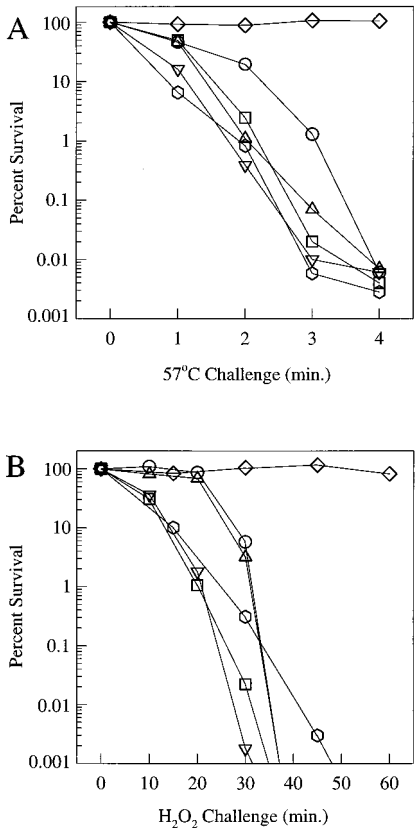

FIG. 4. Stress resistance of wild-type and *dnaK* mutant strains. (A) Thermotolerance of wild-type and *dnaK* mutant strains. Cells were examined following 3 days of carbon starvation, or during growth and with or without heat adaptation, for resistance to killing at 57°C for the times indicated. The strains were PBL500 (wild type) and PBL501 (Δ*dnaK52*). The growth conditions prior to heat challenge were as follows: PBL500, glucose starved (diamonds); PBL500, heat adapted (circles); PBL500, growing (squares); PBL501, glucose starved (hexagons); PBL501, heat adapted (triangles); and PBL501, growing (inverted triangles). (B) H_2O_2 resistance of wild-type and *dnaK* mutant strains. Cultures were exposed to 15 mM H_2O_2 for the times indicated and then plated for survivors. The strains and culture conditions are as indicated for panel A.

This is a consequence of the exponential decrease in rates of bulk protein synthesis which occurs during the starvation regimen. A protein with an apparent mass of 70 kDa was continually produced throughout the course of the experiments (Fig. 3A, arrow). To determine if this was the DnaK protein, autoradiographic analysis was performed on [^{35}S]methionine-labeled DnaK immunoprecipitates prepared with the anti-DnaK monoclonal antibody 2G5 (29). This antibody recognizes a DnaK epitope accessible in both native and denatured DnaK protein states; consequently, mildly denaturing conditions were used during the immunoprecipitation (51). Using this approach, we recovered newly synthesized DnaK by immunoprecipitation throughout the 3-day period (Fig. 3B). Some DnaK degradation products were evident, particularly in later samples, reflecting the extended labeling period and the increase in proteolytic activity of the starving cell extracts.

Starvation-induced stress resistance in wild-type and *dnaK* mutant strains. The absolute requirement for DnaK protein in starvation-induced thermotolerance and H_2O_2 resistance was examined by testing the abilities of freshly constructed and otherwise isogenic wild-type and loss-of-function Δ*dnaK52* mutant strains to resist heat killing by treatment for various times at 57°C (Fig. 4A). As reported previously, strains harboring the Δ*dnaK52* mutation can accumulate second-site suppressors, most of which result in loss of the heat-sensitive *dnaK* mutant phenotype due to mutations in *rpoH* (7, 47). Consequently, efforts were made in the experiments described here to avoid *dnaK* mutant storage and unnecessary cultivation. The percentage of the mutant population that lost the heat-sensitive growth phenotype was monitored throughout these experiments by direct plating at 30 and 42°C and by the screening of colonies grown at 30°C for the ability to form colonies at 42°C. The frequency of heat-resistant derivatives never exceeded 1% of the population. In addition, no reduction in *dnaK* mutant viability was observed during the course of these experiments. Viability counts at the onset of glucose starvation were 2.73×10^7 and 2.53×10^7 CFU/ml for strains PBL500 and PBL501, respectively. After 3 days of glucose starvation, the viabilities of strains PBL500 and PBL501 were 3.85×10^7 and 3.30×10^7 CFU/ml, respectively.

Strains PBL500 (wild type) and PBL501 (Δ*dnaK52*) were subjected to glucose starvation for 3 days. Following this starvation period, the degree of thermotolerance was determined by heating cells at 57°C and plating to determine viable counts. In addition, the ability of each of these strains to adapt to heat stress during growth was examined. This was performed by shifting cells growing at 30°C to 42°C, waiting for 30 min, and then again shifting the cells to 57°C. The wild-type strain was most resistant to heating following starvation, and there was also a small increase in thermotolerance in heat-adapted wild-type cells (Fig. 4A). The magnitude of adaptive heat resistance of the wild-type strain during growth was significantly less than exhibited during starvation, as reported previously (25). However, the *dnaK* mutant was unable to develop either starvation-induced thermotolerance or adaptive thermotolerance during growth (Fig. 4A).

Resistance to H_2O_2 is another distinguishing phenotype of starving *E. coli*. The role of DnaK in starvation-induced oxidant tolerance was therefore examined by determining the sensitivity of strains PBL500 and PBL501 to H_2O_2 killing following 3 days of glucose starvation. Cells were exposed to 15 mM H_2O_2 for 60 min and periodically sampled for viable counts by plating on LB plates. Again, 3-day-starved wild-type cells were the most resistant to H_2O_2; the Δ*dnaK52* mutant, however, was incapable of developing starvation-induced H_2O_2 resistance (Fig. 4B). Since treatment with sublethal levels of H_2O_2 can also elicit increased or adaptive resistance in growing cells (44), it was of interest as well to examine if DnaK played a role in this process. Wild-type and *dnaK* mutant cells from mid-exponential-phase cultures were pretreated with 60 μM H_2O_2 for 1 h and then treated with a lethal concentration of H_2O_2. Equivalent extents of adaptive H_2O_2 resistance were observed in the wild-type and *dnaK* mutant strains (Fig. 4B), indicating that *dnaK* is not required for adaptive H_2O_2 resistance. The *dnaK* mutant effect was specific for starvation-induced H_2O_2 resistance, since growing cultures of the wild-type and *dnaK* mutant strains exhibited equal H_2O_2 sensitivities. Thus, the lack of DnaK did not increase the sensitivity of growing cells to H_2O_2.

Restoration of stress resistance by *dnaK* complementation. The Δ*dnaK52* mutation was constructed by the removal of approximately 1.7 kb of chromosomal DNA from the *dnaK* locus (47). The deleted region included all of the *dnaK* promoters and 933 bp of sequences encompassing approximately one-third of the 5′-terminal *dnaK* coding sequence. In addition, this deletion removed 800 bp of DNA upstream of the *dnaK* promoter. This upstream region encodes a locus called *htgA* (9, 23) or *htpY* (43) shown previously to play a role in the control of the heat shock response. Removal of the *dnaK*

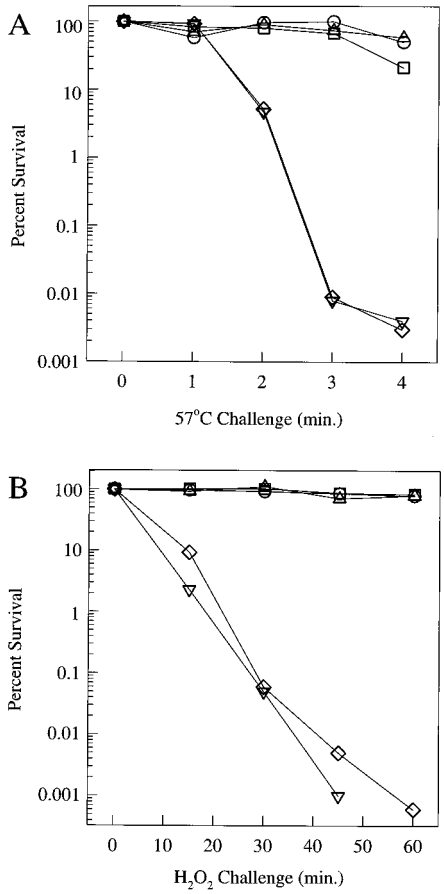

FIG. 5. Restoration of starvation-induced stress resistance by *dnaK* complementation. (A) Restoration of starvation-induced thermotolerance in strains PBL501/pBN13 (squares), PBL501/pBN15 (triangles), PBL501/pBN16 (inverted triangles), PBL501/pBN8 (diamonds), and PBL500/pBN8 (circles). (B) Restoration of H_2O_2 resistance. Cells were examined following 3 days of carbon starvation for resistance to treatment with 15 mM H_2O_2 for the times indicated. The results shown are for the same strains as indicated for panel A.

promoter(s) may also be responsible for a reduction in DnaJ levels in strains containing the Δ*dnaK52* mutation (40). It was therefore necessary to determine to what extent DnaK deficiency specifically was responsible for the starvation-related Δ*dnaK52* mutant phenotypes. A complementation analysis was performed with a series of expression plasmids to better define the role of DnaK in the development of starvation-induced stress resistance. The plasmids employed a P_{tac} promoter to express either $dnaK^+$ (pBN13), $dnaK^+J^+$ (pBN15), or $dnaJ^+$ (pBN16). In addition, the effect of the expression plasmid alone (pBN8) on the starvation-induced behavior of both PBL501 and PBL500 was determined. Presence of the plasmid-encoded *lacI* gene and the chromosomally encoded *lacI* mutation $lacI^{q1}$ ensured that P_{tac} promoter expression was largely repressed. Repressed (uninduced) levels of plasmid-encoded *dnaK* expression were sufficient to approximate the levels produced in wild-type cells (3). Presence of the $dnaK^+$ (pBN13) or $dnaK^+J^+$ plasmid (pBN15) but not the $dnaJ^+$ plasmid (pBN16) restored wild-type levels of starvation-induced thermotolerance (Fig. 5A) and starvation-induced H_2O_2 resistance (Fig. 5B). No effect of the control plasmid (pBN8) on the resistance of the wild-type strain or on the sensitivity of the Δ*dnaK52* mutant strain to either heating or H_2O_2 treatment was observed (Fig. 5).

Role of DnaK in cell morphology and starvation-induced reductive division. In addition to the induction of stress resistance, glucose starvation also precipitates a morphological change in cell shape by a process of reductive division. The role of DnaK in this morphological change was examined initially by comparing the shapes of growing and starving wild-type and *dnaK* mutant strains. Phase-contrast micrographs of PBL500 (wild type) and PBL501 (Δ*dnaK52*) were prepared by using either cells growing in minimal medium or a rich medium or cells that had been glucose starved for 3 days. Cell surface areas and volumes were determined from video scans of photomicrographs for 20 individual cells for each strain under the different culture conditions. As reported previously, the wild-type strain underwent normal reductive division, thus changing in shape from a rod to a sphere with a final cell volume 30% of that of cells growing in a minimal medium (Fig. 6A and C). The filamentous phenotype of the Δ*dnaK52* mutant (6) was also readily apparent during growth in a rich medium. Surprisingly, however, the Δ*dnaK52*-dependent filamentous phenotype was largely absent in cells of this strain, PBL501, growing in a minimal glucose medium (Fig. 6D). Such *dnaK* mutant cells were only slightly larger (43%) than the wild-type cells. The filamentous phenotype was readily apparent, however, when PBL501 was subcultured from minimal glucose medium, during exponential growth or following 3 days of glucose starvation, back into a rich medium (Fig. 6E). This finding excluded the possibility that PBL501 had sustained a mutation which resulted in loss of the Δ*dnaK52* mutant filamentous phenotype. In contrast to the spherical morphology assumed by starving wild-type cells, the *dnaK* mutant failed to complete reductive division and convert from its rod form into the small and rounded starvation form (Fig. 6F). However, a decrease of 45% in cell volume was observed in the *dnaK* mutant, resulting in slightly smaller rods. This finding indicates that the *dnaK* mutant could respond at least in part to starvation but was unable to complete the morphologic transition into the spherical cell form.

To better understand if the inability of the *dnaK* mutant to complete reductive division was due to the specific loss of *dnaK*, a complementation analysis using the $dnaK^+$, $dnaK^+J^+$, and $dnaJ^+$ expression plasmids was performed. Strains containing these plasmids were examined microscopically during exponential growth in minimal medium and in rich medium and following 3 days of glucose starvation (Fig. 7). Presence of the $dnaK^+$ (pBN13) or $dnaK^+J^+$ (pBN15) plasmid (Fig. 7F and I) but not the $dnaJ^+$ (pBN16) or control (pBN8) plasmid (Fig. 7L and O) restored the ability of the *dnaK* mutant to undergo reductive division during glucose starvation. A small percentage (27%) of the cells of strains containing either pBN13 or pBN15 failed to complete reductive division and instead remained rod shaped (Fig. 7F and I). Such cells are likely to have lost their complementing plasmids. This possibility was supported by the observation that 28% of the starving cells were ampicillin sensitive and thus were plasmid segregants. Complementation of the Δ*dnaK52* mutant filamentous phenotype was also observed as expected with the *dnaK* plasmid-bearing strains during growth in rich medium (Fig. 7E and H). We observed no effect on cell morphology by the presence of the control plasmid, pBN8, in either the wild-type strain (Fig. 7A to C) or the *dnaK* mutant (Fig. 7M to O). Cell surface areas, volumes, and surface area-to-volume ratios were determined to more accurately assess the consequences of the *dnaK* mutation and its complementation on cell morphology. Twenty cells from cultures of each of the plasmid-bearing strains, either growing in minimal medium or after 3 days of glucose starvation, were analyzed by computer-assisted microscopic

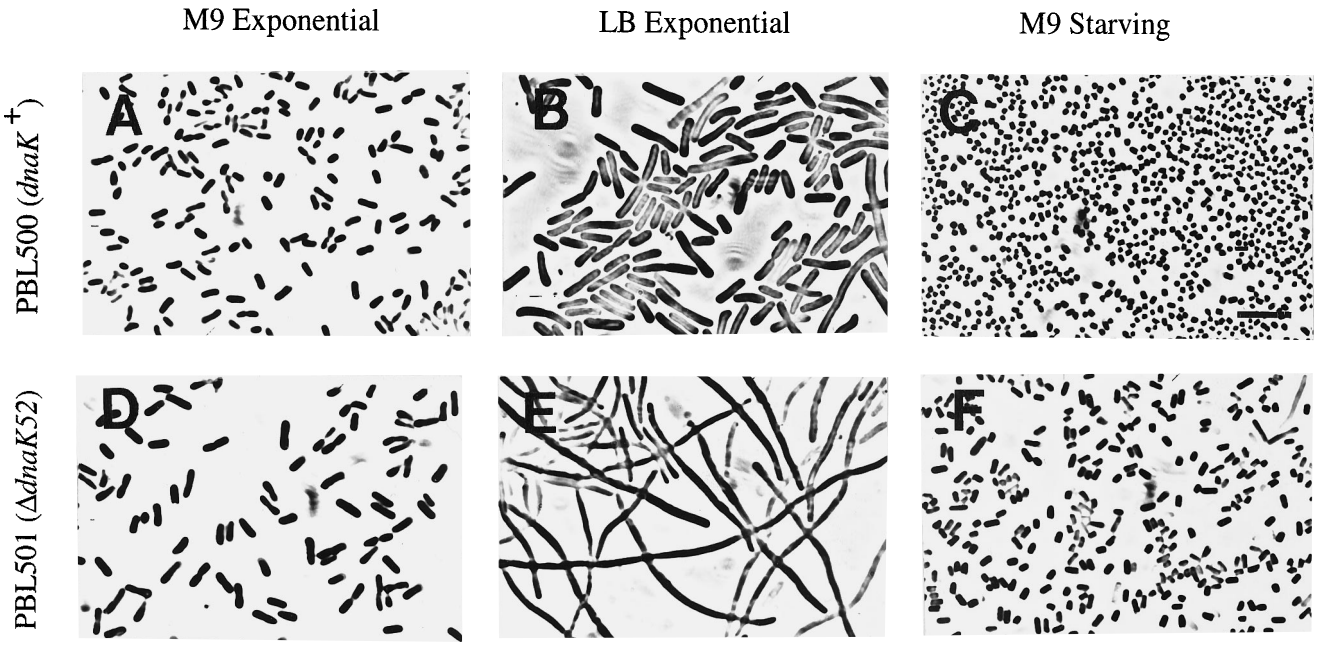

FIG. 6. Cell morphology of growing and starving wild-type and *dnaK* mutant strains. Phase-contrast photomicrographs are shown. Magnification was ×1,880 for all panels. A bar representing 5 μm is indicated in panel C. The cells shown were either growing in minimal glucose medium (A and D) or rich medium (B and E) or starved for glucose for 3 days (C and F). (A to C) PBL500 (wild type); (D to F) PBL501 (Δ*dnaK52*).

examination. The averages of these values and their standard deviations are shown in Table 1. The most striking difference noted in cell morphology was observed with starving cells. Cell volume and surface area-to-volume ratios were most distinct between glucose-starved *dnaK* mutant strains containing *dnaK*$^+$ plasmids and those lacking it. The wild-type strain containing the control plasmid, pBN8, and the *dnaK* mutant strain containing either the *dnaK*$^+$ or the *dnaK*$^+$*J*$^+$ plasmid (Table 1) had significantly smaller volumes and larger surface area-to-volume ratios during starvation than did the *dnaK* mutant derivatives containing either the *dnaJ*$^+$ plasmid or the control plasmid (Table 1). A small but significant reduction in cell volume was also noted with the *dnaJ*$^+$ plasmid-bearing *dnaK* mutant strain. These results indicate that *dnaK* deficiency results in larger starving cells with greater volumes and that DnaK is therefore required to complete the starvation-induced morphologic transition into spherical cells.

DISCUSSION

The induction of stress resistance in carbon-starved *E. coli* requires protein synthesis (25). The events necessary to create the resistant state are completed within the first several hours following the onset of starvation and coincide with a drop in the remaining protein synthetic capacity to 1% of growing cell levels. Over a more prolonged period of several days, there is an accompanying morphological change from a rod to a small and rounded cell shape. During the 3-day period examined in this study, wild-type cells underwent a 3.3-fold reduction in cell volume and a 2.2-fold increase in DnaK bulk levels, adjusting either for total cell protein or on a per-cell basis. Elevated DnaK levels, thermotolerance, and H_2O_2 resistance rapidly readjusted to prestarvation levels following glucose resupplementation, while conversion into the rod cell form was more gradual. It is likely that the maintenance or removal of cell protein is critical for starvation-induced stress resistance and changes in cell morphology. If the role of DnaK in the starvation-induced stationary phase is to control protein folding, then starvation may increase the capacity of such cells to tolerate or process misfolded protein in a DnaK-dependent manner. Despite the observed correlation between the changes in the degree of thermotolerance, H_2O_2 resistance, and DnaK levels accompanying starvation and the resumption of growth, it has been shown that the mere overproduction of HSPs without cell heating fails to elicit thermotolerance (53). However, the analysis in this case was of growing cells. It is possible that in nongrowing cells, in which protein synthesis is curtailed and protein dilution by ongoing cell division has ceased, DnaK can function more effectively to modify existing substrates.

A specific role for DnaK was observed in three of the distinguishing features of starving *E. coli*: thermotolerance, H_2O_2 resistance, and reductive division. Interestingly, all three of the *dnaK* mutant starvation phenotypes are shared by *rpoS* mutants (32, 33, 39); however, the interrelations between DnaK and RpoS are unclear. The apparent requirement for a functional *dnaK* gene for the development of starvation-induced stress resistance and reductive division indicates that other genes such as *rpoS* are necessary but not sufficient for these stationary-phase processes. Defects in starvation survival on a solid medium (52) and reduced viability of *dnaK* mutant transductant colonies on LB (rich) plates (6) indicate that *dnaK* plays a role in stationary-phase survival. However, no reduction in culture viability was observed in the studies reported here for the *dnaK* mutant strain after 3 days of starvation in a liquid minimal glucose medium. Previous reports have also indicated that the Δ*dnaK52* mutation resulted in an increase in sensitivity to heat and H_2O_2 (10). However, the comparisons were between nonisogenic strains and used chilled cell suspensions. In addition, the antecedent growth state of the cells used was not described; therefore, it is difficult to compare these results with those presented here. It was observed in the studies reported here, however, that the filamentous *dnaK* mutant phenotype (6) could be suppressed by cultivation in a defined

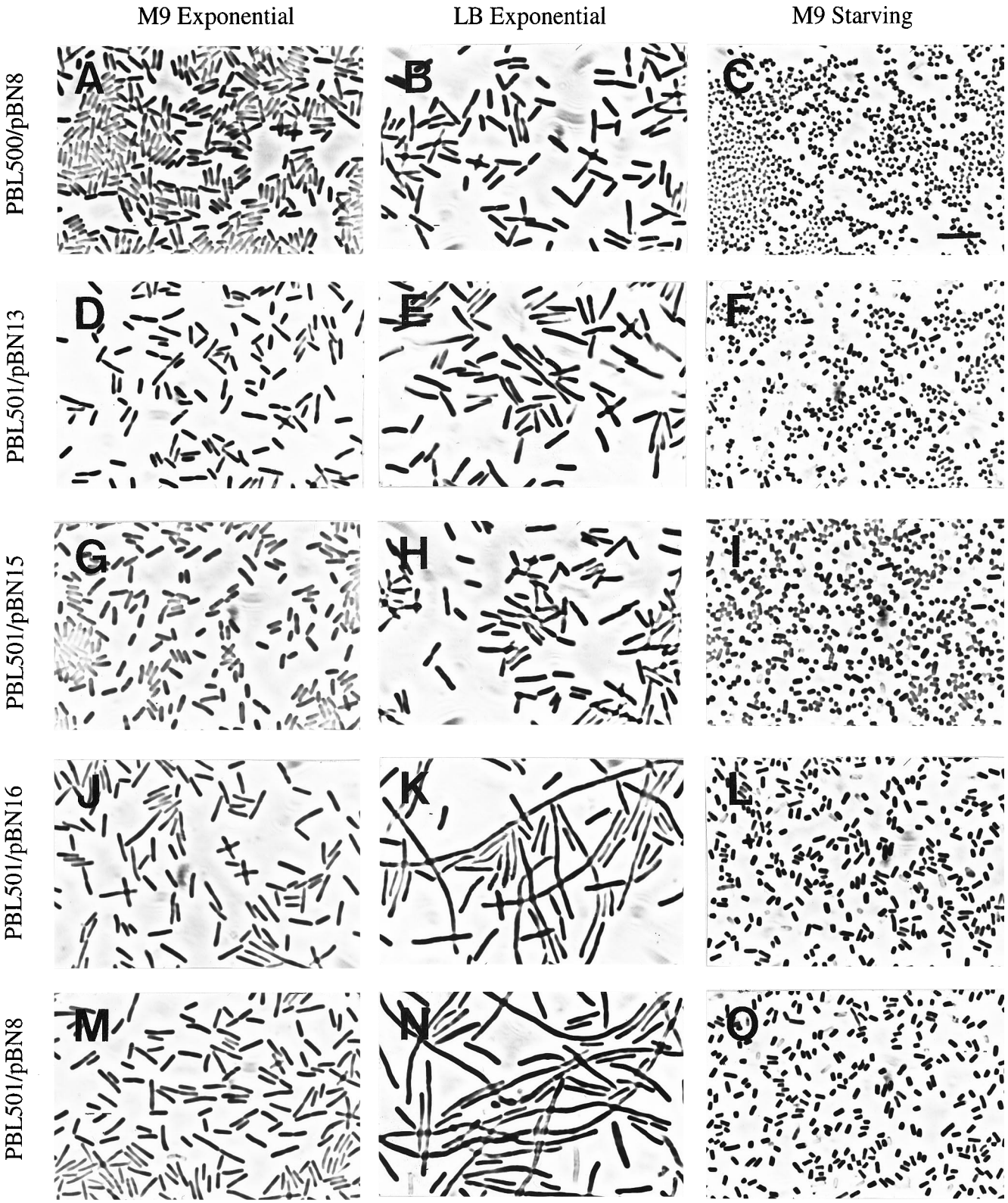

FIG. 7. Cell morphology of growing and starving plasmid-bearing strains. Phase-contrast micrographs were prepared by using cells either growing in minimal glucose medium (A, D, G, J, and M) or rich medium (B, E, H, K, and N) or starved for glucose for 3 days (C, F, I, L, and O). (A to C) PBL500/pBN8; (D to F) PBL501/pBN13; (G to I) PBL501/pBN15; (J to L) PBL501/pBN16; (M to O) PBL501/pBN8. A bar representing 5 μm is included in panel C. Magnification was ×2,000 for all panels.

TABLE 1. Surface areas and volumes of growing and starving plasmid-bearing wild-type and *dnaK* mutant strains[a]

Strain	Avg of 20 cells ± SD					
	Exponential phase			Starvation phase		
	SA (μm^2)	V (μm^3)	SA/V	SA (μm^2)	V (μm^3)	SA/V
PBL500/pBN8	3.04 ± 0.74	0.42 ± 0.16	7.29	1.11 ± 0.05	0.11 ± 0.01	10.31
PBL501/pBN13	3.49 ± 0.47	0.51 ± 0.09	6.87	1.07 ± 0.12	0.10 ± 0.02	10.41
PBL501/pBN15	3.60 ± 0.69	0.53 ± 0.12	6.73	1.22 ± 0.21	0.13 ± 0.04	9.52
PBL501/pBN16	4.31 ± 1.27	0.63 ± 0.29	6.88	2.35 ± 0.57	0.30 ± 0.12	7.77
PBL501/pBN8	4.60 ± 1.00	0.75 ± 0.23	6.15	3.03 ± 0.61	0.46 ± 0.14	6.64

[a] Surface area (SA) was calculated by using the formula $(4/3\pi b)(2a+b)$ for the rod-shaped cells and the formula $4\pi ab$ for the spherical cells. Volume (V) was calculated by the formula $(\pi ab)(4/3b)$ for the rod-shaped cells and the formula $(4/3\pi)(ab)^{3/2}$ for the spherical cells. Value *a* is equal to cell length in micrometers on the major semiaxis, and value *b* is equal to the cell length in micrometers on the minor semiaxis.

medium. Since both filamentation and reduced viability were not apparent during carbon starvation, it is possible that reduced *dnaK* mutant viability and the filamentous phenotype are related. Alternatively, the differences in viability may be related to the mode of cultivation, e.g., solid and liquid media. As the mutation frequency of PBL501 (Δ*dnaK52*) to growth at 42°C was below 1% of the population throughout these experiments, it seems unlikely that compensatory mutations such as *sid* mutants (7) contribute significantly to the results shown. The occurrence of suppressors or revertants in these studies is further excluded by the ability of the *dnaK* mutant strain following 3 days of carbon starvation to morphologically change from small rods to filaments upon subculture from a minimal medium to a rich medium. However, the starvation-induced transition between short rod and round cell morphology was blocked by DnaK deficiency. The defect in reductive division observed in the *dnaK* mutant identifies another morphological mutant phenotype for this gene and further supports a role for DnaK in the process of cell septation (6).

Previous estimates of the abundance of this protein in *E. coli* B/r during exponential-phase growth in a minimal glucose medium were 9.33 ng/μg of total protein (48, 54). Levels were determined by steady-state in vivo labeling using a double-labeling procedure and two-dimensional PAGE to identify and recover proteins. The levels for DnaK in exponential-phase cells reported here for *E. coli* K-12 are in close agreement at 7.0 ng/μg of total protein. The anti-DnaK monospecific antibody 2G5 can therefore be used as an alternative and specific method for the quantitation of DnaK. The increase in bulk DnaK levels upon carbon starvation was 2.2-fold, which indicates that DnaK varies inversely with growth state, exhibiting maximum levels in nongrowing cells and minimum levels in dividing cells. In addition, DnaK synthesis has also been shown to vary directly with growth rate (48) and to increase following amino acid limitation (18). The consequences of the increase in DnaK concentration in starving cells is unknown but might result in increased protein refolding capacity or play a role in the increase in thermotolerance and H_2O_2 resistance observed upon starvation. The increase in absolute DnaK levels seen here further extends the range of prokaryotes that exhibit this compositional change in response to starvation (21).

A *dnaK* gene homolog termed *hsc* was recently detected in *E. coli* (27, 50). It shares DNA sequence and certain antigenic epitopes with DnaK and may also be expressed during growth (40). However, monoclonal antibody 2G5 did not detect any proteins, such as Hsc, in the Δ*dnaK52* mutant during either growth or starvation. This is consistent with the divergence in sequence at the 2G5 DnaK epitope located at amino acids 288 to 310 (29) with the corresponding positions in Hsc. The contribution of this DnaK homolog to the processes examined here are unknown, but the locus can be activated by mutation (27). Complementation analysis of the Δ*dnaK52* mutant with a *dnaJ*$^+$ plasmid did not indicate a role for DnaJ in the starvation-induced *dnaK* mutant phenotypes other than a modest effect on reductive division. However, the effect of DnaJ deficiency on the starvation-induced phenotypes studied here may not be as readily apparent at the reduced levels reported to be present in Δ*dnaK52* mutant strains (40). A requirement for DnaK during carbon starvation is also consistent with previous reports on the inability of an *htpR* mutant to develop starvation-induced thermotolerance or H_2O_2 resistance (24). The specific role for DnaK in the starvation response as shown here may in part be responsible for this *htpR* starvation-induced mutant phenotype. Thus, in addition to its important functions in growing cells, the data presented here identify a major and specific role for DnaK in the physiology of the starvation-induced stationary phase of *E. coli.*

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REFERENCES

1. **Aldea, M., T. Garrido, C. Hernandez-Chico, M. Vincente, and S. R. Kushner.** 1989. Induction of a growth-phase-dependent promoter triggers transcription of *bolA*, an *Escherichia coli* morphogene. EMBO J. **8:**3923–3931.
2. **Almiron, M., A. J. Link, D. Furlong, and R. Kolter.** 1992. A novel DNA-binding protein with regulatory and protective role in starved *Escherichia coli*. Genes Dev. **6:**2646–2654.
3. **Blum, P., J. Ory, J. Bauernfeind, and J. Krska.** 1992. Physiological consequences of DnaK and DnaJ overproduction in *Escherichia coli*. J. Bacteriol. **174:**7436–7444.
4. **Blum, P. H., S. B. Jovanovich, M. P. McCann, J. E. Schultz, S. A. Lesley, R. R. Burgess, and A. Matin.** 1990. Cloning and in vivo and in vitro regulation of cyclic AMP-dependent carbon starvation genes from *Escherichia coli*. J. Bacteriol. **172:**3813–3820.
5. **Bukau, B., and G. C. Walker.** 1989. Δ*dnaK52* mutants of *Escherichia coli* have defects in chromosome segregation and plasmid maintenance at normal growth temperatures. J. Bacteriol. **171:**6030–6038.
6. **Bukau, B., and G. C. Walker.** 1989. Cellular defects caused by deletion of the *Escherichia coli dnaK* gene indicate roles for heat shock protein in normal metabolism. J. Bacteriol. **171:**2337–2346.
7. **Bukau, B., and G. C. Walker.** 1990. Mutations altering heat shock specific subunit of RNA polymerase suppress major cellular defects of *E. coli* mutants lacking the DnaK chaperone. EMBO J. **9:**4027–4036.
8. **Burkholder, W. F., C. A. Panagiotidis, S. J. Silverstein, A. Cegielska, M. E. Gottesman, and G. A. Gaitanaris.** 1994. Isolation and characterization of an *Escherichia coli* DnaK mutant with impaired ATPase activity. J. Mol. Biol. **242:**364–377.
9. **Dean, D. O., and R. James.** 1991. Identification of a gene, closely linked to *dnaK*, which is required for high-temperature growth of *Escherichia coli*. J. Gen. Microbiol. **137:**1271–1277.
10. **Delaney, J. M.** 1990. Requirement of the *Escherichia coli dnaK* gene for thermotolerance and protection against H_2O_2. J. Gen. Microbiol. **136:**2113–2118.

11. **Demple, B.** 1991. Regulation of bacterial oxidative stress genes. Annu. Rev. Genet. **25:**315–337.
12. **Elliker, P. R., and W. C. Frazier.** 1938. Influence of time and temperature of incubation on heat resistance of *Escherichia coli*. J. Bacteriol. **36:**83–97.
13. **Erickson, J. W., and C. A. Gross.** 1989. Identification of the σ^E subunit of *Escherichia coli* RNA polymerase: a second alternate σ factor involved in high-temperature gene expression. Genes Dev. **3:**1462–1471.
14. **Georgopoulos, C.** 1992. The emergence of the chaperone machines. Trends Biochem. Genet. **17:**295–299.
15. **Giraldo-Suarez, R., E. Fernandez-Tresquerres, R. Diaz-Orejas, A. Malki, and M. Kohiyama.** 1993. The heat-shock DnaK protein is required for plasmid R1 replication and it is dispensable for plasmid ColEI replication. Nucleic Acids Res. **21:**5495–5499.
16. **Groat, R. G., J. E. Schultz, E. Zychlinsky, A. Bockman, and A. Matin.** 1986. Starvation proteins in *Escherichia coli*: kinetics of synthesis and role in starvation survival. J. Bacteriol. **168:**486–493.
17. **Gross, C. A., D. B. Straus, J. W. Erickson, and T. Yura.** 1990. The function and regulation of heat shock proteins in *Escherichia coli*, p. 167–189. *In* R. I. Morimoto, A. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
18. **Grossman, A. D., W. E. Taylor, S. 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.
19. **Harlow, E., and D. Lane.** 1988. Antibodies, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Hengge-Aronis, R., W. Klein, R. Lange, M. Rimmele, and W. Boos.** 1991. Trehalose synthesis genes are controlled by the putative σ factor encoded by *rpoS* and are involved in stationary-phase thermotolerance in *Escherichia coli*. J. Bacteriol. **173:**7918–7924.
21. **Holmquist, L., A. Jouper-Jaan, D. Weichart, D. R. Nelson, and S. Kjelleberg.** 1993. The induction of stress proteins in three marine Vibrio during carbon starvation. FEMS Microbiol. Ecol. **12:**185–194.
22. **Jacobson, A., and D. Gillespie.** 1970. An RNA polymerase mutant defective in ATP initiations. Cold Spring Harbor Symp. Quant. Biol. **35:**85–93.
23. **James, R., D. O. Dean, and J. Debbage.** 1993. Five open reading frames upstream of the *dnaK* gene of *E. coli*. DNA Seq.-J. **3:**327–332.
24. **Jenkins, D. E., E. A. Auger, and A. Matin.** 1991. Role of RpoH, a heat shock regulator protein, in *Escherichia coli* carbon starvation protein synthesis and survival. J. Bacteriol. **173:**1992–1996.
25. **Jenkins, D. E., J. E. Schultz, and A. Matin.** 1988. Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli*. J. Bacteriol. **170:**3910–3914.
26. **Kawamukai, M., H. Matsuda, W. Fujii, R. Utsumi, and T. Komano.** 1989. Nucleotide sequences of *fic* and *fic-1* genes involved in cell filamentation induced by cyclic AMP in *Escherichia coli*. J. Bacteriol. **171:**4525–4529.
27. **Kawula, T. H., and M. J. Kelivelt.** 1994. Mutations in a gene encoding a new Hsp70 suppress rapid DNA inversion in *bgl* activation, but not *proU* derepression, in *hns-1* mutant *Escherichia coli*. J. Bacteriol. **176:**610–619.
28. **Kolter, R., D. A. Siegele, and A. Tormo.** 1993. The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. **47:**855–874.
29. **Krska, J., T. Elthon, and P. Blum.** 1993. Monoclonal antibody recognition and function of a DnaK (HSP70) epitope found in gram-negative bacteria. J. Bacteriol. **175:**6433–6440.
30. **Kusukawa, N., and T. Yura.** 1988. Heat shock protein GroE of *Escherichia coli*: key protective roles against thermal stress. Genes Dev. **2:**874–882.
31. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
32. **Lange, R., and R. Hengge-Aronis.** 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. Mol. Microbiol. **5:**49–59.
33. **Lange, R., and R. Hengge-Aronis.** 1991. Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor σ^S. J. Bacteriol. **173:**4474–4481.
34. **Li, C., and S. Clarke.** 1992. A protein methyltransferase specific for altered aspartyl residues is important in *Escherichia coli* stationary-phase survival and heat-shock resistance. Proc. Natl. Acad. Sci. USA **89:**9885–9889.
35. **Lomovskaya, O. L., J. P. Kidwell, and A. Matin.** 1994. Characterization of the σ^{38}-dependent expression of a core *Escherichia coli* starvation gene, *pexB*. J. Bacteriol. **176:**3928–3935.
36. **Lowen, P. C., and R. Hengge-Aronis.** 1994. The role of the sigma factor σ^S (*katF*) in bacterial global regulation. Annu. Rev. Microbiol. **48:**53–80.
37. **Maaloe, O., and N. O. Kjeldgaard.** 1966. Control of macromolecular synthesis. W. A. Benjamin, Inc., New York.
38. **Matin, A., E. A. Auger, P. H. Blum, and J. E. Schultz.** 1989. Genetic basis of starvation survival in nondifferentiating bacteria. Annu. Rev. Microbiol. **43:**293–316.
39. **McCann, M. P., J. P. Kidwell, and A. Matin.** 1991. The putative σ factor *katF* has a central role in development of starvation-mediated general resistance in *Escherichia coli*. J. Bacteriol. **173:**4188–4194.
40. **McCartry, J. S., and G. C. Walker.** 1994. DnaK mutants defective in ATPase activity are defective in negative regulation of the heat shock response: expression of mutant DnaK protein results in filamentation. J. Bacteriol. **176:**764–780.
41. **Meury, J., and M. Kohiyama.** 1991. Role of heat shock protein DnaK in osmotic adaptation of *Escherichia coli*. J. Bacteriol. **173:**4404–4410.
42. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
43. **Missiakas, D., C. Georgopoulos, and S. Raina.** 1993. The *Escherichia coli* heat shock gene *htpY*: mutational analysis, cloning, sequencing, and transcriptional regulation. J. Bacteriol. **175:**2613–2624.
44. **Morgan, R. W., M. F. Christman, F. S. Jacobson, G. Storz, and B. N. Ames.** 1986. Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA **83:**8059–8063.
45. **Morita, R. Y.** 1993. Bioavailability of energy and the starvation state, p. 1–23. *In* S. Kjelleberg (ed.), Starvation in bacteria. Plenum, New York.
46. **Ostlung, J., L. Holmquist, K. Flardh, B. Svenblad, A. Jouper-Jaan, and S. Kjelleberg.** 1994. Starvation and recovery of vibrio, p. 103–127. *In* S. Kjelleberg (ed.), Starvation in bacteria. Plenum, New York.
47. **Paek, K.-H., and G. C. Walker.** 1987. *Escherichia coli dnaK* null mutants are inviable at high temperature. J. Bacteriol. **169:**283–290.
48. **Pederson, S., P. L. Bloch, S. Reeh, and F. C. Neidhardt.** 1978. Patterns of protein synthesis in *E. coli*: a catalog of the amount of 140 individual proteins at different growth rates. Cell **14:**179–190.
49. **Raina, S., D. Missiakas, L. Baird, S. Kumar, and C. Georgopoulos.** 1993. Identification and transcriptional analysis of the *Escherichia coli htrE* operon which is homologous to *pap* and related pilin operons. J. Bacteriol. **175:**5009–5021.
50. **Seaton, B. L., and L. E. Vickery.** 1994. A gene encoding a DnaK/hsp70 homolog in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **91:**2066–2070.
51. **Shand, R. F., L. J. W. Miercke, A. K. Mitra, S. K. Fong, R. M. Stroud, and M. C. Betlach.** 1991. Wild-type and mutant bacterioopsins D85N, D96N, and R82Q: high-level expression in *Escherichia coli*. Biochemistry **30:**3082–3088.
52. **Spence, J., A. Cegielska, and C. Georgopolous.** 1990. Role of *Escherichia coli* heat shock proteins DnaK and HtpG (C62.5) in response to nutritional deprivation. J. Bacteriol. **172:**7157–7166.
53. **VanBogelen, R. A., M. A. Acton, and F. C. Neidhardt.** 1987. Induction of the heat shock regulon does not produce thermotolerance in *Escherichia coli*. Genes Dev. **1:**525–531.
54. **VanBogelen, R. A., P. Sankar, R. L. Clark, J. A. Bogan, and F. C. Neidhardt.** 1992. The gene-protein database of *Escherichia coli*: edition 5. Electrophoresis **13:**1014–1054.
55. **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.
56. **Yura, T., H. Nagai, and H. Mori.** 1993. Regulation of the heat-shock response in bacteria. Annu. Rev. Microbiol. **47:**321–350.
57. **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.