Identification and Characterization of a New Escherichia coli Gene That Is a Dosage-Dependent Suppressor of a dnaK Deletion Mutation

PIL JUNG KANG AND ELIZABETH A. CRAIG*
Department of Physiological Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706

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We report the isolation and characterization of a previously unidentified Escherichia coli gene that suppresses the temperature-sensitive growth and filamentation of a dnaK deletion mutant strain. Introduction of a multicopy plasmid carrying this wild-type gene into a dnaK deletion mutant strain rescued the temperature-sensitive growth of the dnaK deletion mutant strain at 40.5°C and the filamentation, fully at 37°C and partially at 40.5°C. However, the inability of dnaK mutant cells to support bacteriophage λ growth was not suppressed. This gene was also able to suppress the temperature-sensitive growth of a gprE280 mutant strain at 41°C. Filamentation of the gprE280 mutant strain was suppressed at 37°C but not at 41°C. The dnaK suppressor gene, designated dksA, maps near the mrecB gene (3.7 min on the E. coli chromosome). DNA sequence analysis and in vivo experiments showed that dksA encodes a 17,500-M₅ₐ polypeptide. Gene disruption experiments indicated that dksA is not an essential gene.

The heat shock response is one of the most highly conserved biological responses to environmental changes (19, 20, 24). Most organisms so far examined, including many bacterial, plant, and animal species, induce the synthesis of proteins encoded by the HSP60, HSP70, and HSP90 gene families in response to elevated temperatures. Several of the proteins induced by heat are also induced by a variety of other stresses. These heat shock proteins are among the most highly conserved proteins known. DNA sequencing studies have shown that the amino acid sequence of Escherichia coli groEL product is 54% identical to the yeast hsp60 protein (25). E. coli DnaK is about 50% identical to the eucaryotic hsp70 proteins (5), and the E. coli C62.5 heat shock protein is 41% identical to the hsp83 protein of Drosophila melanogaster (4). The universality of the heat shock response and the high degree of conservation of heat shock proteins imply that these proteins have fundamental, common roles in various organisms.

Most eucaryotic organisms contain a family of HSP70-related genes. For example, the Saccharomyces cerevisiae genome contains at least nine genes that are related to HSP70 of higher eucaryotes (19). However, the dnaK gene is the only HSP70-like gene in E. coli (5). The dnaK gene forms an operon with another heat shock gene, dnaJ, with the structure promoter-dnaK-dnaJ.

The dnaK gene was originally identified because it is essential for bacteriophage λ growth (14, 27). Genetic and biochemical studies suggest that DnaK protein is required for the initiation of λ DNA replication by facilitating the dissociation of λ P protein from the λ DNA replication initiation complex, allowing DnaB protein (a helicase) to function (12). DnaK, a 69,000-M₅ₐ protein, is the most extensively characterized HSP70 heat shock protein. Purified DnaK, which binds ATP tightly, has a weak DNA-independent ATPase (more generally, 5'-nucleotidase) activity (6, 37). The purified protein autophosphorylates at one or more threonine residues (37). Although DnaK is biochemically well characterized, little is known about its cellular function.

In vivo analysis of temperature-sensitive dnaK mutants revealed that synthesis of DNA and RNA was rapidly shut off (15) and that cell division was blocked (10, 32) at the nonpermissive temperature. Recent isolation of a new dnaK allele, dnaKI11, showed that dnaKI11 is unable to initiate a new round of DNA replication at the nonpermissive temperature after termination of the round in progress (28). DnaK is also known to be involved directly or indirectly in the phosphorylation of two aminocyl-tRNA synthetases (34). Furthermore, DnaK is a negative modulator of the heat shock response. Cells carrying the dnaK756 mutation showed a high level of synthesis of heat shock proteins even at 30°C and failed to turn off the heat shock response at 42°C (31).

To study the cellular function of DnaK protein, we have constructed a new dnaK deletion mutant strain. Phenotypic analysis of this strain showed that the deletion mutant was temperature and cold sensitive for growth and highly filamentous even at the permissive temperature. As one way to study the cellular function of DnaK protein, we isolated and analyzed suppressors that overcame the dnaK deletion mutant phenotypes. In this paper, we report the isolation of a newly identified gene, dksA, that suppresses the temperature-sensitive growth and filamentation of a dnaK deletion mutant strain when overexpressed. The dksA gene was cloned and sequenced, and its location on the E. coli chromosome was determined.

MATERIALS AND METHODS

Bacterial strains, phages, and media. The bacterial strains and phages used and their genotypes are listed in Table 1. LB broth and M9 minimal medium were prepared as described by Miller (23). When necessary, antibiotics were supplemented to the following final concentrations: ampicillin, 100 μg/ml; tetracycline, 20 μg/ml; and kanamycin, 100 μg/ml. Amino acids were supplemented to 40 μg/ml.

Plasmids. Plasmids obtained in this study are briefly described in Table 2, with further details given in the text.

Genetic manipulations. Linear transformation of DNA into a recB recC strC strain was performed as described by Winans et al. (35). Selections for λ lysogens and P1 transductions were as described by Silhavy et al. (30), except for transductions of the dnaK deletion allele. Growth of phage
TABLE 1. Bacterial and phage strains

<table>
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<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
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<td>MG1655</td>
<td>F-</td>
<td>CGSC*</td>
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<tr>
<td>DS410</td>
<td>F- ara Azl tonA lacY minB minB xyl</td>
<td>C. Grossb</td>
</tr>
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<td>HfrH, nad::Tn10 clp+</td>
<td>C. Gross</td>
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<td>MG1655, zae-502::Tn10</td>
<td>C. Gross</td>
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<td>thi-1 his-4 rpsL136 leuB6 eda</td>
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<tr>
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<td>This study</td>
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<tr>
<td>λ imm11b</td>
<td>dksA+ dksJ+</td>
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<tr>
<td>λ imm11b</td>
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</tr>
<tr>
<td>λ17-181</td>
<td>F. Blattner</td>
<td></td>
</tr>
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</table>

* CGSC, E. coli Genetic Stock Center, Yale University, New Haven, Conn.
* University of Wisconsin-Madison.
* University of Tokyo, Tokyo, Japan.

P1 on the dnaK deletion strain was very poor; the P1 transduction efficiency was extremely low. To increase the P1 transduction efficiency, P1 lysates were prepared on a λ imm11b dnaK+ dnaJ+ lysogen of strain PK101, and the lysates were treated with Lambda Sorb (Promega Biotech) to remove λ phages in the P1 lysate. Transduction efficiency was increased more than 1,000-fold in this way.

Construction of the dnaK, dnaJ, and dksA deletion mutant strains. The basic strategy for construction of a chromosome deletion was to construct deletion mutations of dnaK, dnaJ, and dksA in recombinant plasmids and then to insert these mutations into the E. coli chromosome by linear transformation. The dnaK deletion mutant strain, PK101, carries a deletion of the entire dnaK coding sequence and 155 base pairs (bp) of the 5' end of the dnaJ gene. Since part of the dnaJ gene was also deleted, the resulting construction is DnaK- DNAJ (we named this allele dnaK14 dnaJ14). To compare the mutant phenotypes of DnaK- DNAJ with those of DnaJ-, a dnaJ deletion was also constructed. The dnaJ deletion mutant strain, PK102, carries a deletion of the majority of the dnaJ coding sequence, retaining only the sequences encoding the first 23 and the last 30 amino acids of DnaJ (we named this allele dnaJ15). The details of the construction of the dnaK and dnaJ deletion mutant strains will be described in detail elsewhere. Plasmid pkj21, which carries a deletion of the 408-bp Clal-PvuII fragment of pkj538, was constructed by replacing this region with the kanamycin resistance (kan') cassette from pUC4K (33) to make a dksA deletion mutant strain.

Construction of a genomic library and isolation of suppressor clone. A multiplicity genomic library was constructed by cloning a partial Sau3AI digestion of chromosomal DNA from a spontaneous temperature-resistant suppressor strain, PK113, into the unique BamHI site of pBR322. This DNA was used to transform the dnaK deletion strain, PK101, and the transformants were selected at 42°C on LB-ampicillin plates. Plasmid DNA was isolated from the temperature-resistant clones and used to transform PK101 a second time to confirm suppression of the temperature-sensitive growth of PK101.

Mapping. To map the dksA gene on the E. coli chromosome, the Kan' cassette from pUC4K was inserted into one of the EcoRV sites of PK53. This Kan' marker was introduced into the chromosome by linear transformation and mapped to within a 10-min interval with a set of Hfr strains whose origins are distributed around the chromosome (obtained from C. Gross, University of Wisconsin-Madison). The Kan' marker was then tested for P1 cotransduction with mapped Tn10 insertions in the region as described in the Results. Subsequent transduction analysis was performed by using some nearby selectable markers. Fine mapping was done by Southern hybridization, using 1.2-kilobase-pair (kb)
PvuII fragment of pJK53 as a probe with the λ DNA covering this region (A17-181, obtained from F. Blattner, University of Wisconsin-Madison).

Recombinant DNA techniques. The procedures compiled by Maniatis et al. (22) were generally used with minor modifications. Isolation of λ DNA was carried out by using Lambda Sorb (Promega Biotech).

S1 nuclease mapping. To identify the start site of dskA RNA transcript, S1 nuclease mapping was done as previously described (13). The 5'-end-labeled probe was prepared from pJK53 by cleaving with PvuII, followed by alkaline phosphatase and kinase treatment. The appropriate labeled DNA probe was digested with HincII and purified from an agarose gel. Total RNA was isolated from a mid-log-phase culture of strain MG1655 carrying pJK337 as described by Alba et al. (1). For each S1 digestion reaction, 50 μg of total RNA and 100 pg of labeled probe were hybridized at 55°C, and 200 U of S1 nuclease was treated. A reaction mixture containing 30 μg of yeast tRNA was included as a control for the assay. DNA fragments were electrophoresed on a 6% polyacrylamide gel containing 7 M urea together with a 32P-end-labeled HaeIII digest of pBR322 DNA for marker fragments.

DNA sequencing. DNA sequences were determined by the dideoxy-chain termination method (29), using Sequenase (U.S. Biochemical Corp.) with strain JM109 as the host for M13mp18 and M13mp19 derivatives (36).

Analysis of plasmid-encoded polypeptides. Polypeptides encoded by plasmids were analyzed by using minicells (11). Minicells were prepared from cells carrying plasmids to be tested and labeled with L-[35S]methionine (1.114 Ci/mmol; 30 μCi/ml final concentration; Dupont, NEN Research Products). Labeled protein samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (15% acrylamide, 0.195% bisacrylamide); the gel was stained with Coomassie blue and then treated with 1 M sodium salicylate. The gel was then dried and fluorographed.

Plating efficiency. Cells grown overnight at 30°C were diluted 1:100 into LB broth and grown to an optical density at 600 nm of 0.5. Appropriate dilutions on LB plates were incubated overnight at 30, 37, 40.5, or 41°C, and colonies were counted.

Phase-contrast microscopy. Cells from fresh colonies grown on LB agar plates at 30, 37, 40.5, or 41°C were prepared on glass microscope slides. A Zeiss photomicroscope with ×40 phase objective lens and Kodak PanX film were used in the photography.

RESULTS

Isolation of a suppressor of a dnaK deletion. The dnaK deletion strain PK101, containing the alleles dnaK14 and dnaJ14, is temperature sensitive for growth at 40.5°C. The plating efficiency of PK101 at 40.5°C was only 0.08% of that at 30°C (Table 3). The dnaK deletion strain also showed cold-sensitive growth below 17°C. When P1 transductants at 30°C were replated or when P1 transduction was done below 17°C, few colonies formed, even after 10 days of incubation. Cells were extremely filamentous even at 30°C and seemed to be defective in cell division. As previously mentioned, the dnaK deletion strain PK101 is actually DnaK− DnaJ−. To check the DnaK− phenotype, PK101 was transformed with the DnaJ-expressing plasmid (pJK15). The resulting transformants also showed temperature-sensitive growth and filamentation. Since the DnaJ-expressing plasmid could not be stably maintained in the dnaK deletion strain PK101, PK101 was used in the studies reported here. To genetically identify suppressors of the dnaK deletion, spontaneously arising mutants of PK101 able to grow at 42°C were isolated. The spontaneous reversion frequency was 10−2. One of these strains, PK113, was chosen for further analysis.

Isolation of a temperature-resistant suppressor clone. When we began experiments designed to isolate a suppressor clone, we expected to isolate a mutant gene that could suppress the temperature-sensitive growth of the dnaK deletion strain. However, later results indicated that the isolated suppressor clone described below contained a wild-type gene, not a mutant gene, suggesting that overexpression of this wild-type gene suppressed the temperature-sensitive growth of the dnaK deletion strain PK101.

A multicopy genomic library was constructed by cloning a partial Sau3AI digest of the chromosomal DNA from the spontaneous temperature-resistant suppressor strain, PK113, into the unique BamHI site of pBR322. The temperature-sensitive dnaK deletion strain, PK101, was transformed with the genomic library DNA, and temperature-resistant transformants were selected at 42°C on LB-ampicillin plates. Plasmid DNA was isolated from the temperature-resistant transformants and used to transform the strain PK101 a second time to confirm suppression of the temperature-sensitive growth. After the second screening, five independent clones were isolated. Restriction endonuclease analysis showed that three of them were identical clones and that all the five clones had overlapping DNA segments.

In the initial screening, we isolated dnaK deletion cells transformed with the suppressor plasmids that could grow at 42°C. However, additional studies showed that P1 transduction of the dnaK deletion allele into a wild-type strain carrying the isolated suppressor clone resulted in the formation of viable colonies at temperatures only as high as 40.5°C. This result indicated that the original dnaK deletion mutant strain contained at least one additional mutation which, in combination with the suppressor clone, allowed growth at 42°C.

Mapping of the suppressor locus. To map the suppressor locus on the E. coli chromosome, a Kan' cassette from pUC4K was inserted into one of the EcoRV sites of pJK53. This Kan' marker was introduced into the E. coli chromosome by linear transformation and was mapped between 96 and 6 min with a set of Hfr strains whose origins are distributed around the chromosome. To further delineate the location of the suppressor, a number of strains carrying Tn10 insertions in this region were used. The Kan' marker was tested for P1 cotransduction with mapped Tn10 insertions. One of the strains carrying a Tn10 insertion near 4 min (zae-502::Tn10) showed 80 to 90% cotransduction of the tetracycline resistance (tet') and Kan' markers; a strain carrying a Tn10 insertion near 3 min (zad-220::Tn10) gave 18% cotransduction. Subsequent P1 transduction analysis with nearby selectable markers showed 83% cotransduction of the Kan' marker with the dapD locus (Fig. 1). The relative

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**Table 3. Plating efficiency of the wild type (MG1655) and the dnaK deletion strain (PK101) carrying the dnaK suppressor clone (pJK337) and the control plasmid (pJK333)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plating efficiency*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>37°C</td>
</tr>
<tr>
<td>Wild type</td>
<td>99.4</td>
</tr>
<tr>
<td>PK101(pJK337)</td>
<td>95.6</td>
</tr>
<tr>
<td>PK101(pJK333)</td>
<td>53.7</td>
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</table>

* All strains were considered to have 100% plating efficiency at 30°C.
cotransduction frequencies of the Kan' marker with some other markers suggested that the Kan' marker was located near the mrcB gene.

Comparison of the restriction map of the suppressor clone with the published restriction map of the E. coli chromosome (17) revealed a similar pattern of restriction sites near the mrcB gene. Subsequently, fine mapping was done by Southern hybridization analysis. The 1.2-kb PvuII fragment isolated from a suppressor clone (pJK53) was hybridized to the restriction enzyme-digested DNA of a λ transducing phage (λ17-181) covering this region (Fig. 2). This result indicated that the suppressor was located upstream of the mrcB gene (3.7 min on the E. coli chromosome). The restriction fragments of two other suppressor clones (pJK72 and pJK711) also hybridized with the same probe. This result reconfirmed that all of the originally isolated suppressor clones had overlapping DNA segments.

Fine localization of the suppressor gene. To further define the suppressor gene, sequential subcloning of the originally isolated suppressor clone was done. Each subclone was tested for its ability to allow growth of the dnaK deletion strain PK101 at 40.5°C. The temperature-sensitive growth of strain PK101 was suppressed by plasmids pJK337 and pJK338 but not by pJK330 and pJK333 (Fig. 3). These results suggested that the dnaK suppressor gene was located on the 1.7-kb DNA fragment between one of the PvuII sites and the EcoRV site (*s in Fig. 3).

Overexpression of a wild-type gene suppresses dnaK deletion. Since the suppressor gene was localized upstream of the mrcB gene, the wild-type gene was cloned from a λ transducing phage DNA that covers this region. pJK337, which carried a 1.7-kb PvuII-EcoRV DNA fragment of the phage, was constructed and used to transform the dnaK deletion strain, PK101. PK101 transformed with pJK337 formed colonies at 40.5°C, as did PK101 transformed with pJK337, the plasmid carrying the original suppressor clone. This result implied that the originally isolated suppressor clone was a wild-type gene. Subsequent genetic experiments supported this conclusion. To determine whether a mutation in the isolated gene was responsible for the suppression in the original temperature-resistant suppressor strain, we asked whether the temperature resistance of PK113, the spontaneously isolated suppressor strain, was cotransduced with the Tet' marker present near the suppressor gene. PK113 was cotransduced with P1 vir grown on the strain CAG18436, which contained zae-502::Tn10. Since zae-502::Tn10 was determined to be 80 to 90% cotransduced with the Kan' marker near the suppressor gene, it was expected that if the suppressor gene is the actual site of the mutation, about 70 to 80% of the Tet' transductants should be temperature sensitive. However, more than 95% of the Tet' transductants were still temperature resistant. These results suggested that the originally isolated suppressor clones carried a wild-type gene and that overexpression of this gene suppressed the temperature-sensitive growth of the dnaK deletion strain.

DNA sequence determination. The nucleotide sequence of the 1.7-kb PvuII-EcoRV fragment (*s in Fig. 3) was determined (Fig. 4). Northern (RNA) blot analysis using labeled single-stranded DNA probes showed that transcription occurred in the direction from the PvuII to the EcoRV site. Analysis of the sequence data revealed that there were only two nonoverlapping open reading frames on the sense strand in this region larger than 42 codons. The shorter open
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CHARACTERIZATION OF A dnaK SUPPRESSOR GENE

FIG. 3. Fine localization of the suppressor gene. The originally isolated suppressor clone, pJK53, was subcloned, and mutants were constructed in vitro as described in Materials and Methods. Each clone was tested for suppressing ability by testing for the ability of transformants of PK101 to form colonies at 40.5°C. pJK540 and pJK543 have +1 frameshift mutations. pJK542 has an in-frame deletion in the middle of ORF2. ORF1 and ORF2 are open reading frames determined to be in this region from analysis of DNA sequencing data (Fig. 4).

FIG. 4. Nucleotide sequence of the dksA region. The sequence of the sense strand and the amino acid sequences of the two open reading frames are shown. The sequence is numbered from HincII (left) to the EcoRV site (Fig. 3). The −10 and −35 regions of the possible promoter are indicated. Putative Shine-Dalgarno (S.D) sequences are underlined.
reading frame (ORF1) could encode a polypeptide of 70 amino acids ($M_r$, 7,650), and the longer one (ORF2) could encode a polypeptide of 151 amino acids ($M_r$, 17,480). The predicted ORF2 gene product contains 38% charged residues distributed throughout the length of the protein, with a net charge of $-6$. ORF1 was preceded by a putative ribosome-binding site, GGGAG, located 4 bp upstream of the initiation codon. ORF2 was also preceded by a putative ribosome-binding site, GAGGA, located 6 bp upstream of the initiation codon. A sequence that shares homology to the consensus E. coli promoter sequence (26) was located upstream of ORF1. This organization suggested that ORF1 and ORF2 might comprise an operon.

**Analysis of the RNA transcript start site.** To estimate the size of the mRNA encoded by ORF1 or ORF2, Northern hybridization experiments were done. RNAs isolated from the wild-type strain MG1655 and MG1655 carrying the suppressor clone, pJK537, were separated by electrophoresis and hybridized with the labeled 408-bp Clal-PvuII fragment of pJK538. A major RNA transcript of approximately 500 nucleotides was detected in RNA preparations from the strain without a suppressor clone as well as in preparations from the transformed strain. In addition, a relatively abundant 600-nucleotide RNA was identified in the transformed strain. The largest transcript that hybridized to the probe was 1,000 nucleotides in length (Fig. 5).

To determine the 5' end of RNA transcript, S1 nuclease mapping was carried out. A 5'-end-labeled probe was prepared from the noncoding strand of the 726-bp HincII-PvuII fragment.
fragment (Fig. 6). The probe was hybridized with total RNA isolated from log-phase cells of strain MG1655 carrying the suppressor clone, pJK537. Three major protected fragments with approximate lengths of 560, 255, and 160 nucleotides were observed (Fig. 6). Fragments with the same lengths were also detected by using RNA isolated from untransformed cells (data not shown). In both cases, the 160-nucleotide fragment was the most abundant. The 5' ends of these three major protected fragments approximately mapped to nucleotide positions 166, 501, and 566 in the sequence shown in Fig. 4. Position 166 is located upstream of ORF1 close to a consensus E. coli promoter sequence. Positions 501 and 566, however, are not in close proximity to a consensus E. coli promoter sequence. Although the 160-nucleotide fragment was the most abundant and ORF2 is completely included within pJK530, pJK530 could not suppress the temperature-sensitive phenotype. Therefore, we propose that position 166 is the actual RNA transcription start site and that the longest RNA transcript is processed to give smaller RNA molecules. The 1-kb RNA observed in the analysis of RNA described above is probably the full-length transcript.

**ORF2 encodes the dnaK suppressor gene, dksA, which is a previously unidentified gene.** There are two nonoverlapping open reading frames on the sense strand in the putative dnaK suppressor gene region that are large enough to encode more than 42 amino acids (Fig. 3). Neither pJK530 nor pJK533, which covers the ORF1 or ORF2 region, respectively, had suppressing activity. S1 mapping and DNA sequence analysis suggested that the RNA transcript that starts at position 166 was an original RNA transcript of this region. To identify which open reading frame actually encodes the suppressor of dnaK deletion, mutant clones were constructed in vitro and tested for suppressing activity (Fig. 3). A mutation inactivating only ORF1 (pJK540) did not affect suppressing activity, whereas two mutations inactivating ORF2 (pJK542 and pJK543) lost the ability to suppress. pJK541, which carries the entire ORF2 and part of ORF1, showed no suppressing activity. These results indicated that ORF2 encodes the suppressor of dnaK deletion. We think that pJK541 does not suppress because it lacks a transcription initiation site. Neither nucleotide nor amino acid sequence deduced from ORF2 showed significant homology to any protein sequence in the NBRF data base or any DNA sequence in GenBank. We have designated this newly identified gene dksA (dnaK suppressor).

**Identification of the dksA gene product.** To identify the dksA gene product, the polypeptides encoded by plasmids pJK537 and pJK334 were analyzed in cells carrying the dksA gene on a multicopy plasmid and in minicells (Fig. 7). A strong band at the position expected for an 18,000-Mr polypeptide was detected in MG1655 carrying pJK537 when total cell extracts were analyzed by SDS-PAGE. In contrast, in MG1655 carrying no plasmid or carrying the control plasmid, pJK333, no strong band at this position was observed. The polypeptides encoded by the suppressor clone, pJK537, were also analyzed in minicells. Minicells were prepared from strain DS410 carrying the parental plasmid pBR322, the suppressor clone pJK537, or a control plasmid, pJK334. The minicells were labeled with [35S]methionine for 10 min. Minicells carrying the suppressor clone, pJK537, showed a labeled 18,000-Mr polypeptide that comigrated with the overexpressed 18,000-Mr protein synthesized in whole cells carrying the suppressor clone. The observed molecular weight was consistent with that deduced from the DNA sequence of the dksA gene.

dksA also suppresses the filamentation but not the phage λ growth defect of a dksA deletion mutant. The dksA clone was originally selected for its ability to suppress the temperature-sensitive growth of the dnaK deletion mutant strain PK101. Since the dnaK deletion strain was extremely filamentous even at 30°C, the dksA clone was tested to determine whether it could also suppress the filamentous phenotype. Cells carrying a dksA clone, pJK537, showed greatly reduced cell length at 30 and 37°C and partially reduced filamentation at 40.5°C (Fig. 8). Cells carrying the control plasmid, pJK333, did not show a reduction in filamentation even at 30°C. This result indicated that the dksA clone suppressed not only the temperature-sensitive growth but also the filamentous phenotype of the dnaK deletion strain. Since the dnaK deletion mutant strain was unable to support growth of phage λ, the dksA clone was tested to see whether it could suppress the phage λ growth defect. The dnaK deletion mutant strain PK101, carrying a dksA clone, pJK537, could not support phage λ growth (data not shown). This result suggested that the role of DnaK in λ growth is separable from that in host growth.

dksA also suppresses the grpE280, dnaK756, and dnaJ deletion mutant phenotypes. Since it has been shown both biochemically and genetically that the DnaK and GrpE proteins interact (16), the ability of the dksA clone to suppress the temperature-sensitive growth and the filamentation of grpE280 mutant cells was tested. The grpE280 mutant strain carrying the dksA clone showed about 7,000-fold-higher plating efficiency at 41°C than did a grpE280 strain carrying a control plasmid. The dksA clone suppressed the filamentation of grpE280 at 37°C but not at 41°C (Fig. 9). The phage λ growth defect of the grpE280 mutant was not suppressed by the dksA clone (data not shown).

Since the dnaK deletion strain PK101 used in this study is actually DnaK− DnaJ+, the dnaJ deletion mutant strain PK102 was also tested to see whether the dnaJ deletion mutant phenotypes were suppressed by the dnaK suppressor clone. The dnaJ deletion mutant strain PK102, carrying the dksA clone pJK537, became temperature-resistant even at...
44.5°C, but strain PK102 carrying the control plasmid pJK333 was not viable at that temperature. The dksA clone also suppressed the cell elongation of the dnaJ deletion mutant strain even at 44.5°C (data not shown).

Since the dksA multicopy suppressor was originally isolated on the basis of suppressing the dnaK14 dnaJ14 deletion mutation, the ability of dksA to suppress the dnaK756 missense mutation was tested. As in the case of the dnaK14 dnaJ14 deletion mutation, temperature-sensitive growth and cell elongation caused by the dnaK756 mutation were suppressed by the dksA clone, but the phage λ growth defect was not (data not shown).

To determine whether dksA could suppress other heat shock protein mutant strains, the lon::Tn10, groEL140, and groES30 mutant strains were also tested. The growth phenotypes of these three strains were not suppressed by overexpression of the dksA gene (data not shown). Therefore, dksA does not have the ability to suppress all heat shock mutants and may be specific to the dnaK, dnaJ, and grpE mutant strains.

dksA is not an essential gene. Since dksA is a newly identified gene, we constructed a dksA mutant to determine the effect of the lack of dksA function. A plasmid carrying a deletion in dksA, pJK21, was constructed by removing the 408-bp ClaI-PvuII fragment of pJK538 and replacing it with the Kan' cassette. This in vitro-mutated copy of dksA was integrated into the E. coli chromosome by linear transformation into a strain with Tn10 70 to 80% linked to dksA (zae-502::Tn10). Transformants were selected for Kan' and screened for ampicillin sensitivity (Amp') to confirm recombination into the chromosome. P1 vir was grown on one of the Kan' Tet' Amp' transformants, and the wild-type strain MG1655 was transduced to Kan' Tet'. Since P1 growth in the recB recC sbcB strain was poor, a second P1 lysate was prepared on the one of the Kan' Tet' transductants (PK9), and MG1655 cells were again transduced. When transductants were selected for Kan', 70% were also Tet'. A similar value (77% cotransduction) was obtained when transductants were first selected for Tet'. This result suggested that dksA is not an essential gene. However, we cannot eliminate the possibility of the presence of a closely linked mutation that might suppress dksA loss.

The dksA deletion strain PK201 was found to grow nearly as well as wild-type cells on rich media at 30, 37, and 42°C. However, it grew very poorly on M9 minimal medium supplemented with 20 amino acids and vitamins at all temperatures. This poor growth was not overcome by changing carbon sources. Phage λ growth was normal on the dksA deletion strain. The double mutant of the dnaK deletion and the dksA deletion was also constructed. No phenotypic difference was observed between the dnaK deletion strain and the dnaK dksA double-deletion strain (data not shown).

**DISCUSSION**

We have isolated a previously unidentified E. coli gene, dksA, which can suppress the temperature-sensitive growth of a dnaK deletion mutant in a dosage-dependent manner. The filamentation of a dnaK deletion mutant strain was also suppressed, but the defect of λ growth was not. Although the dksA clone was isolated from a genomic library of DNA from a spontaneous temperature-resistant suppressor strain, the dksA locus was found not to be the original suppressor mutation site, since a dksA clone isolated from a wild-type strain also rescued the temperature-sensitive growth and filamentation of the dnaK deletion strain PK101 when over-
expressed. Examples of dosage-dependent suppression have been reported previously. Kusukawa et al. (18) showed that overproduction of DnaK or GroE protein suppressed temperature-sensitive growth of the rpoH deletion mutant strain. Aldea et al. (2) also cloned a newly identified gene, bolA, which suppressed the wee amber mutation when overexpressed.

Although the dnaK deletion mutant strain formed colonies at 30°C, the original transductants grew extremely slowly and were filamentous. However, cells became much less filamentous after several generations of growth at 30°C. Suppressor mutations appear to accumulate in the dnaK deletion mutant strain even at 30°C. Bukau and Walker (7) have made similar observations. Additional observations from our laboratory also support this idea. In the initial screening of the suppressor clone, we isolated dnaK deletion cells transformed with the suppressor plasmid that could grow at 42°C. However, additional studies have shown that P1 transduction of the dnaK deletion allele into a wild-type strain carrying the isolated suppressor clone resulted in the formation of viable colonies at temperatures only as high as 40.5°C. This failure of transductants to grow at 42°C indicated that there were additional mutations in the dnaK deletion mutant strain that affected growth at high temperatures. In fact, we have genetic evidence that at least three mutations, one of which is tightly linked to dnaK, in the originally isolated spontaneous temperature-resistant suppressor strain PK113 are required for temperature-resistant growth (unpublished result). It is possible that none of these mutations would be sufficient to suppress the temperature-sensitive growth even when present in multiple copies. This would explain why we isolated a wild-type gene and not any mutant genes responsible for growth at high temperatures.

It is not clear why overexpression of dksA suppresses the temperature-sensitive growth and filamentation of the dnaK deletion mutant strain. It is possible that DksA bypasses some of the DnaK protein function(s). Although the dksA gene was found to be a dispensable gene, DksA protein might be important for cell growth at high temperature in the dnaK mutant background. The dksA mRNA level in the dnaK deletion mutant strain PK101 is threefold lower than in the wild-type strain. In addition, the dksA mRNA level in the spontaneously isolated suppressor strain PK113 is fourfold higher than in the deletion strain (unpublished result). Therefore, it is possible that some of the dnaK mutant phenotypes are due to lowered expression of dksA and that the suppression of these phenotypes in the original suppressor strain is in part due to the increased expression of dksA. It is also possible that transcription of certain genes is inhibited in the dnaK mutant strain. Mutation(s) or overexpression of certain proteins that results in an increase in transcription of these RNAs or that bypasses the function of the encoded proteins could suppress the dnaK mutant phenotypes.

It is interesting that overexpression of dksA suppressed only the temperature-sensitive growth of the dnaK deletion mutant strain, not the defect of phage λ growth. This result suggests that the functions of DnaK in host growth and in λ growth are separable. Multiple copies of dksA also suppress some of the phenotypes of a temperature-sensitive grpE mutant, allowing growth of a grpE280 mutant at 41°C and suppressing the filamentation caused by this mutation. This suppression is specific in the sense that some other heat shock genes, groEL, groES, and lon, are not affected. DnaK and GrpE interact both in vivo and in vitro (16). Perhaps dksA bypasses functions carried out by dnaK and grpE needed for growth at high temperatures and normal cell

FIG. 9. Suppression of filamentation of the grpE280 mutant strain by the dnaK suppressor clone (pJK537). pJK333 is a control plasmid.
division. Recently, Ang and Georgopoulos reported that a grpE deletion mutation is viable in some dnaK mutant strains even though grpE is normally an essential gene (3). This viability was due to suppressors in the dnaK mutant strains, not the dnaK mutation itself. These results are consistent with ours, indicating the ability of certain mutations or overproduction of normal proteins to compensate for the absence of both dnaK and grpE.

Genetic mapping and DNA sequencing data indicated that dksA was a previously unidentified gene. Although the dksA deletion strain was viable, it grew very poorly on M9 minimal medium supplemented with 20 amino acids and vitamins. This poor growth was not overcome by changing carbon sources. Therefore, although not essential under all growth conditions, dksA is certainly performing important functions under some growth conditions. Elucidating the function of DksA protein may provide clues about the important cellular functions of DnaK.

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