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Isolation and Characterization of *dnaJ* Null Mutants of *Escherichia coli*

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Bacteriophage λ requires the λ O and P proteins for its DNA replication. The rest of the replication proteins are provided by the *Escherichia coli* host. Some of these host proteins, such as DnaK, DnaJ, and GrpE, are heat shock proteins. Certain mutations in the *dnaK*, *dnaJ*, or *grpE* gene block λ growth at all temperatures and *E. coli* growth above 43°C. We have isolated bacterial mutants that were shown by Southern analysis to contain a defective, mini-Tn*10* transposon inserted into either of two locations and in both orientations within the *dnaJ* gene. We have shown that these *dnaJ*-insertion mutants did not grow as well as the wild type at temperatures above 30°C, although they blocked λ DNA replication at all temperatures. The *dnaJ*-insertion mutants formed progressively smaller colonies at higher temperatures, up to 42°C, and did not form colonies at 43°C. The accumulation of frequent, uncharacterized suppressor mutations allowed these insertion mutants to grow better at all temperatures and to form colonies at 43°C. None of these suppressor mutations restored the ability of the host to propagate phage λ. Radioactive labeling of proteins synthesized in vivo followed by immunoprecipitation or immunoblotting with anti-DnaJ antibodies demonstrated that no DnaJ protein could be detected in these mutants. Labeling studies at different temperatures demonstrated that these *dnaJ*-insertion mutations resulted in altered kinetics of heat shock protein synthesis. An additional eight *dnaJ* mutant isolates, selected spontaneously on the basis of blocking phage λ growth at 42°C, were shown not to synthesize DnaJ protein as well. Three of these eight spontaneous mutants had gross DNA alterations in the *dnaJ* gene. Our data provide evidence that the DnaJ protein is not absolutely essential for *E. coli* growth at temperatures up to 42°C under standard laboratory conditions but is essential for growth at 43°C. However, the accumulation of extragenic suppressors is necessary for rapid bacterial growth at higher temperatures.

Georgopoulos and Herskowitz (11) and Saito and Uchida (29) first described a class of mutations in *Escherichia coli*, called *groP* and *grp* mutations, respectively, which block λ DNA synthesis and result in conditionally defective host DNA and RNA syntheses (29, 30, 39). Similarly, Sunshine et al. (33) isolated a bacterial mutation of the *groP* class, *dnaJ259*, which affects the growth of λ and, to a lesser extent, P2 and also results in conditionally defective host DNA and RNA syntheses (39). It was later shown that *dnaJ* is closely linked to another *groP* gene, *dnaK*. The genes constitute an operon, the order being promoter-*dnaK*-*dnaJ* (30, 43). Both genes have been sequenced (2, 3, 26), and the proteins encoded have been purified. DnaK is a 69.5-kilodalton protein that behaves like a monomer at low protein concentrations and possesses in vitro autophosphorylation and 5′-nucleotidase activities (4, 46). DnaJ is a 44-kilodalton protein, behaves as a dimer under nondenaturing conditions, and is a nonspecific DNA-binding protein (47). From in vitro studies, the proteins are thought to act at the same step during the initiation of λ DNA replication (20, 42). DnaK and DnaJ are heat shock proteins, since their rates of synthesis have been shown to increase after a shift to 43°C (3, 12), and the promoters for the operon respond in vitro to the RNA polymerase heat shock sigma factor, σ^{32} (8). The synthesis of DnaK is also induced by a variety of other forms of stress, including infection by bacteriophage λ (9, 17) and exposure to UV light (19). The synthesis of DnaJ should also be induced by these forms of stress, since the *dnaJ* gene is under the control of the *dnaK* promoters (30).

Genetic and biochemical data suggest that the DnaK and DnaJ heat shock proteins may participate in common pathways. For example, certain mutant alleles of the λ *P* gene can suppress the block on phage growth exerted by *dnaK* and *dnaJ* mutations but not by other *groP* mutations (29). Both DnaK and DnaJ act at the same step in in vitro λ O-, λ P-dependent M13 or λ *dv* DNA replication (18, 20, 21, 42). The phosphorylation pattern of *E. coli* proteins is different in *dnaK* and *dnaJ* mutants but not in two other *gro* mutants, *groEL* and *groES* (40). The thermosensitive RNA synthesis of both *dnaK* and *dnaJ* mutants is relaxed in a *relA* background (34). Finally, the DnaK protein has been shown to modulate the heat shock response (35). Synthesis of the heat shock proteins increases at all temperatures in *dnaK* mutants, and the turn-off of the heat shock response is defective. We present evidence for a similar phenotype exhibited by *dnaJ* mutants.

Since the heat shock response is essential for the survival of the organism, it might be expected that the heat shock proteins would be essential at all temperatures. However, it appears that some of the heat shock proteins are essential for *E. coli* growth at all temperatures, whereas others are dispensable under certain conditions or in the presence of suppressor mutations. The *groEL* and *groES* genes are of the first class (10); *grpE*, *dnaK*, *lon*, and *htpG* (C62.5) are of the

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TABLE 1. Bacterial, phage, and plasmid strains

Strain, phage, or plasmid	Genotype or phenotype	Source or reference
Strains		
CG25	B178 *sup*$^+$ *galE*	Our collection; 11
CG26	C600 *thr leu thi supE*	Our collection
CG52	C600 *dnaJ259*	Our collection
CG855	B178 *dnaK*$^+$ *zac*::Kan^r	Our collection
CG990	B178 *dnaJ*::Tn*10*-13	This work
CG992	B178 *dnaJ*::Tn*10*-42	This work
CG995	B178 *dnaJ*::Tn*10*-50	This work
CG997	B178 *dnaJ*::Tn*10*-52	This work
CG1000	B178 *dnaJ*::Tn*10*-55	This work
CG1002	B178 *dnaJ*::Tn*10*-56	This work
CG1014	B178 *dnaJ*::Tn*10*-69	This work
CG1015	B178 *dnaJ*::Tn*10*-71	This work
CG1441	B178 *dnaJ60*	This work
CG1442	B178 *dnaJ61*	This work
CG1443	B178 *dnaJ62*	This work
CG1444	B178 *dnaJ63*	This work
CG1445	B178 *dnaJ80*	This work
CG1446	B178 *dnaJ81*	This work
CG1447	B178 *dnaJ82*	This work
CG1448	B178 *dnaJ83*	This work
Phage		
λ1098	λ::mini-Tn*10*	N. Kleckner; 41
P1L4	Clear plaque former	L. Caro
λ *dnaJ*$^+$Δ135	*imm*$^\lambda$ c1857	30
λ *dnaJ*$^+$	L47.1 *dnaJ*$^+$; *imm*^{434} cI	This work
λ *imm*^{434} cI *h*^{434}	Host range 434	Our collection
λ *imm*^{434} cI *h*$^\lambda$	Host range λ	Our collection
Plasmids		
pCG1	*Hin*dIII *E. coli* DNA *dnaK*$^+$ fragment cloned into pBR322	16
pCG244	*Sal*I-*Pst*I *E. coli* DNA *dnaJ*$^+$ fragment cloned into pTZ18R	Kit Tilly
pNK474	Mini-Tn*10* element	41

second class (1, 23, 28; J. C. A. Bardwell, Ph.D. dissertation, University of Wisconsin, Madison, 1987). The *lon* gene has been shown to be dispensable in the absence of DNA damage (23). The heat-inducible *rpoH* (*htpR*) gene is essential at temperatures above 20°C (44). We have isolated mini-Tn*10* insertions in the *dnaJ* gene, demonstrating that the *dnaJ* gene product is not absolutely essential for bacterial growth at low temperatures. It was shown that the *dnaJ*::mini-Tn*10* insertion mutants grew at low temperatures but accumulated extragenic suppressors at a very high frequency, resulting in rapidly growing bacterial derivatives. Thus, it appears that although the DnaJ protein may not be absolutely essential for *E. coli* growth for temperatures up to 42°C, its presence enables *E. coli* to grow at a faster rate.

MATERIALS AND METHODS

Bacterial and phage strains. All strains used are listed in Table 1. *E. coli* C600 *thr leu thi supE*, from our collection, served as the wild-type strain for our work with the *dnaJ259* mutation. *E. coli* B178 *sup*$^+$ *galE* served as the wild-type strain in the mini-Tn*10* insertion experiments. The λ::mini-Tn*10* vehicle, λ1098, used for the insertion experiments, was a generous gift of Nancy Kleckner (41). The phage used for P1 transductions was P1L4, originally obtained from L. Caro.

Media. The composition of M9 medium for labeling with [^{35}S]methionine has been described previously (35). The medium for the P1 transduction experiments was M9 supplemented with (per liter) 5 g of tryptone, 2.5 g of yeast extract, 2 mg of B1, 25 mg of thymine, and 20 mg of tetracycline.

P1 transduction experiments. Phage P1L4 was grown and used in transduction experiments as described by Miller (24).

Protein labeling. Bacteria were grown and labeled with [^{35}S]methionine (ICN Pharmaceuticals Inc.) essentially as described by Tilly et al. (35). Pellets were lysed and stored frozen in lysis buffer (25).

Immunoprecipitation. Labeled extracts were boiled in sodium dodecyl sulfate (SDS) sample buffer and immunoprecipitated according to Brough et al. (7).

One- and two-dimensional gel electrophoresis. The procedures used have been described previously (25).

Counting of protein samples. The SDS-polyacrylamide gels were dried and exposed to Kodak X-ray film. The positions of the radioactively labeled proteins corresponding to the heat shock proteins of interest were located by superimposing the autoradiograph; the embedded proteins were removed with a mechanical cork borer, solubilized (13), and counted. For all gels, the same control protein, which was not a heat shock protein, was removed to normalize counts between samples.

Western blot (immunoblot) analysis. The Western blots were carried out essentially as described by Towbin et al. (38) except that the gels were soaked for 30 min in 0.1% (wt/vol) SDS–Tris-glycine (pH 9.0). The proteins were electrophoretically transferred to nitrocellulose at 100 V for 2 h at 4°C. After treatment with rabbit antiserum specific for either DnaJ or DnaK and ^{125}I-labeled staphylococcal A

protein (ICN), the nitrocellulose filters were exposed to Kodak X-ray film.

DNA isolation and Southern analysis. The procedure used was as described previously (22). The DNA used for nick translation was as described in the text.

λ hop procedure. The λ hop procedure was as described by Way et al. (41). After transposition, with time allowed for expression, a mini-Tn*10* insertion library consisting of at least 50,000 independent events was obtained.

Isolation of *dnaJ*::mini-Tn*10* insertions. Eight insertion mutants were isolated as colony formers at 30°C after the phage challenge procedure technique described by Georgopoulos and Herskowitz (11).

Isolation of additional *dnaJ* mutants. A B178 (λ *imm*λ *c*I857 *N*am 7) lysogen was grown to 2×10^8 cells per ml in L broth at 30°C. Samples were spread on L plates and incubated at 42°C for 2 days. Bacterial survivors, obtained at $\sim 10^{-5}$, were subsequently screened for inability to support λ *c*I phage growth. Among these survivors, eight were characterized which behaved as *dnaJ* mutants inasmuch as they blocked λ *c*I growth at all temperatures but allowed λ *dnaJ*$^+$ transducing phage growth at all temperatures.

RESULTS

Isolation of mini-Tn*10* insertions in the *dnaJ* gene. A defective Tn*10* transposon, mini-Tn*10*, was used to isolate insertion mutations in the *dnaJ* gene. The defective transposon contains a segment from Tn*10* which consists of the *tet* resistance gene flanked by the outer repeats of the IS*10*-right element and is engineered to stably integrate into the chromosome. Two independent libraries of mini-Tn*10* insertions were made, using the λ hop procedure described by Way et al. (41). Of these isolates, eight were selected because they are able to form colonies at 30°C in the presence of an appropriate amount of lambdoid phages (11). None of the eight isolates plate λ *c*I at any temperature, and all are unable to form colonies at temperatures above 43°C.

Isolation of *dnaJ*$^+$ transducing phages. By simply plating a partial *Sau*3A *E. coli* DNA library prepared in phage λ on the *dnaJ* mutant bacteria, transducing phages that overcame the bacterial block to λ growth were isolated. Upon subsequent testing, it was shown that they were indeed λ *dnaJ*$^+$ transducing phages, since they also propagated on *dnaJ259* mutant bacteria. Bona fide λ *dnaJ*$^+$ transducing phage, from our collection, also grow on the eight *dnaJ*-insertion mutants.

Genetic mapping of the mini-Tn*10* isolates by P1 transduction. Phage P1L4 was grown on each of the eight isolates that were used as donors in transduction analyses designed to map the insertions. The wild-type *E. coli* B178 strain served as the recipient. Transductants were selected for tetracycline resistance at 30°C and tested for temperature sensitivity at 43°C. All eight isolates demonstrated 100% cotransduction of the *tet* marker with the *dnaJ* temperature-sensitive phenotype. The Tet^r colonies grew slower than the isogenic *dnaJ*$^+$ strain, as judged by the fact that they formed slightly smaller colonies at 30°C and very small colonies at 42°C. However, after 48 h, the colonies grown at either 30 or 42°C were shown to contain rapidly growing bacterial derivatives, at a frequency of 10^{-3} to 10^{-4}, as judged by their ability to form larger colonies at 42°C as well as colonies at 43°C. All of the faster-growing derivatives tested were shown to be due to extragenic suppressors, since transducing the Tet^r marker out of these derivatives and into a wild-type background resulted in slow-growing colonies, which again accumulated rapidly growing derivatives. All eight *dnaJ* insertion mutants were shown to filament, although not excessively, at all temperatures tested (from two to five cell lengths). Some of the faster-growing suppressor mutants filamented somewhat less, although others retained the filamentation pattern of the *dnaJ* insertion mutants.

Because of the high frequency of spontaneously occurring extragenic suppressor mutations to a faster growth phenotype, the insertions were further mapped without screening for temperature sensitivity. P1L4 was used to transduce a Kan^r gene 90 to 95% linked to the wild-type *dnaK* and *dnaJ* genes (16) into the eight *dnaJ*::Tn*10* isolates. Kan^r transductants were isolated at 30°C and screened for Tet^r. Only 5 to 10% of the transductants were still Tet^r, confirming that the mini-Tn*10* insertions were indeed in the *dnaK-dnaJ* region. All Tet^r transductants were unable to grow at 43°C, whereas all Tet^s transductants grew well at 43°C.

Southern analysis to determine the position of the mini-Tn*10* insertions. DNA was isolated from each of the eight isolates and digested with *Bgl*II, whose recognition sequence does not occur in either the *dnaJ* gene or the mini-Tn*10* element (Fig. 1A). The DNAs were probed with ^{32}P-labeled DNA specific for the mini-Tn*10* element. All of the isolates exhibited the expected increase in size, from approximately 5 kilobases (kb) to 8 kb, as a result of the presence of the insertion element (Fig. 1B). The DNA from the parent strain was included as a control. Since the mini-Tn*10* preferentially inserts at a 6-base-pair (bp) consensus sequence (15), the sequence of the *dnaJ* gene (3) was searched for the presence of a Tn*10* target sequence (Site Search; J. S. Parkinson, University of Utah). One symmetrical consensus sequence (GCTAAGC) was found at the +4-bp position of the *dnaJ* sequence. Other, degenerate consensus sequences were found 3′ to this site, including one at the +80-bp position (GCCTGGC), into which Tn*10* has been previously shown to insert (15). To localize more precisely the sites of the Tn*10* insertions in the *dnaJ* gene, the DNAs from the isolates were digested with the *Stu*I restriction enzyme and probed with ^{32}P-labeled DNA specific for the *dnaJ* gene. An informative *Stu*I recognition sequence lies between these two potential insertion sites (at +68 bp) (Fig. 1A), such that the *Stu*I restriction fragment, containing the *dnaJ* gene from isolates with an insertion at the carboxy-terminal-coding side of the *Stu*I site, would increase in size. Two of the isolates were found to contain an insertion toward the carboxy-terminal-coding site of the *Stu*I site, whereas the other six contained insertions at the amino-terminal-coding side of the *Stu*I site (Fig. 1C). By digesting the DNA with a restriction enzyme that cuts in the mini-Tn*10* element and using a DNA probe specific for the mini-Tn*10* element, the approximate location and orientation of each insertion were determined (S. M. Sell, Ph.D. thesis, University of Utah, Salt Lake City, 1987; data not shown). The locations were consistent with all of the insertions being at or near the indicated +4-bp and +80-bp positions (Fig. 1A). In addition, each possible orientation was obtained at both insertion sites. The position or orientation of the insertion element in the *dnaJ* gene did not affect the mutant phenotype.

The eight spontaneous *dnaJ* mutants that were isolated at 42°C and blocked λ growth (see Materials and Methods) were also tested to determine whether the *dnaJ* gene or its immediate vicinity had undergone detectable DNA alterations. The rationale behind this experiment was that if the *dnaJ* gene is indeed dispensable for bacterial growth, then deletions or insertions in it could be found. DNA was extracted from these eight isolates as well as from the

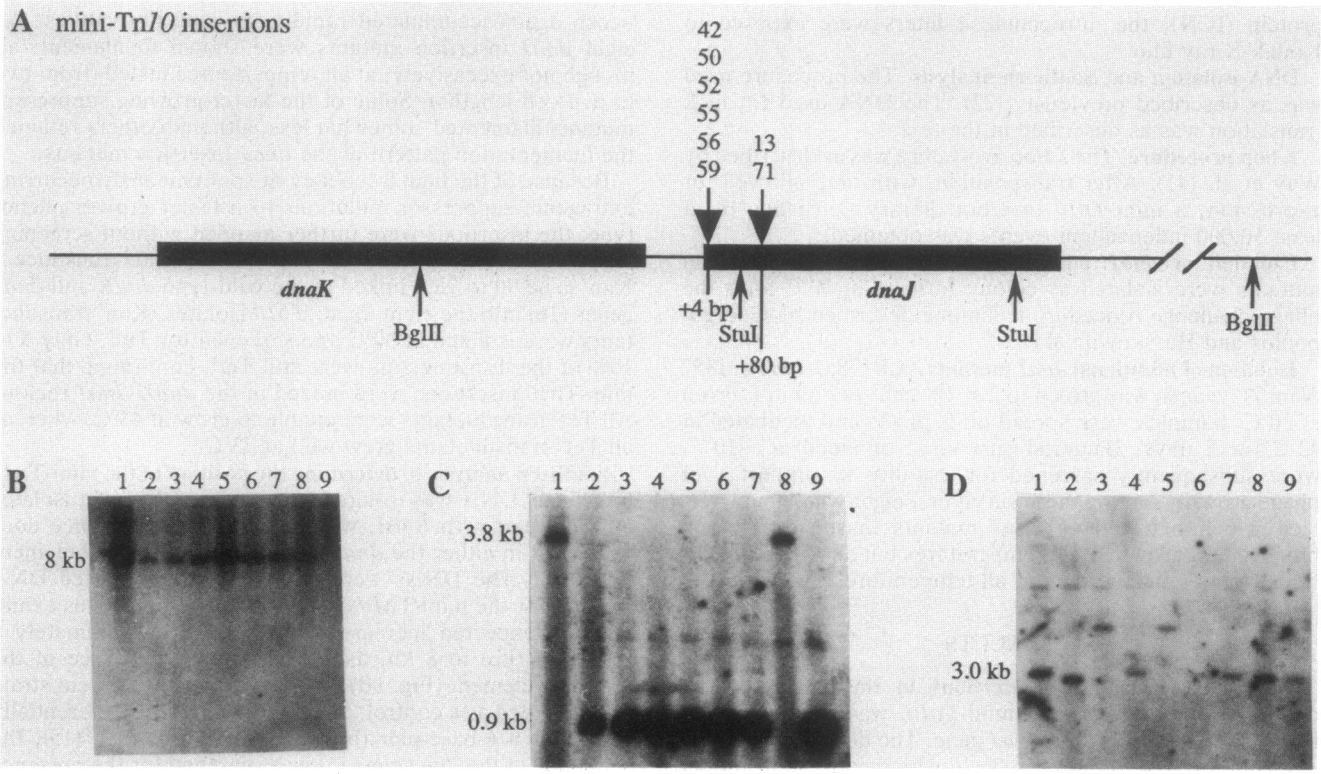

FIG. 1. (A) *Bgl*II-*Stu*I restriction map of the *dnaK-dnaJ* region and mini-Tn*10* element. (B) Southern blot of *Bgl*II-restricted DNA probed with ^{32}P-labeled DNA from a plasmid containing the mini-Tn*10* element. Lanes: 1, *dnaJ*::Tn*10*-13; 2, *dnaJ*::Tn*10*-42; 3, *dnaJ*::Tn*10*-50; 4, *dnaJ*::Tn*10*-52; 5, *dnaJ*::Tn*10*-55; 6, *dnaJ*::Tn*10*-56; 7, *dnaJ*::Tn*10*-69; 8, *dnaJ*::Tn*10*-71; 9, B178 control. (C) Southern blot of *Stu*I-restricted DNA probed with the ^{32}P-labeled 0.9-kb *Stu*I DNA fragment of the *dnaJ* gene. Order of lanes is as in panel B. (D) Southern blot of *Pst*I-digested DNA probed with ^{32}P-labeled pCG244 plasmid DNA. Lanes: 1, B178 control; 2, *dnaJ60*; 3, *dnaJ61*; 4, *dnaJ62*; 5, *dnaJ63*; 6, *dnaJ80*; 7, *dnaJ81*; 8, *dnaJ82*; 9, *dnaJ83*.

parental strain, digested with *Pst*I (which cuts in the *dnaK* structural gene but not in the *dnaJ* gene), and probed with a *dnaJ*-containing, ^{32}P-labeled DNA fragment. It was found that three of the eight isolates possessed gross rearrangements of the *dnaJ* region. One had a smaller *dnaJ*-containing fragment, whereas the other two had fragments larger than that seen in the parental DNA (Fig. 1D).

Western blot analysis. Western blots were performed on extracts from cells grown at 30°C. Since DnaJ protein, which is basic, does not transfer efficiently to nitrocellulose with use of the standard method (38), the proteins were instead transferred by using a high-pH buffer coupled with preequilibration of the gel in SDS. The filters were incubated with rabbit antibodies specific for either DnaJ or DnaK. The immune complexes were visualized by using ^{125}I-staphylococcal A protein, followed by exposure to X-ray film. Bacteria that contained the *dnaJ*::Tn*10*-42-insertion mutation did not synthesize any detectable DnaJ protein (Fig. 2A, lane 4). However, it was observed that expression of DnaK in this mutant was higher than in the wild-type strain (see below). Similarly, extracts from the eight spontaneously occurring *dnaJ* mutants were tested for DnaJ antigen, and

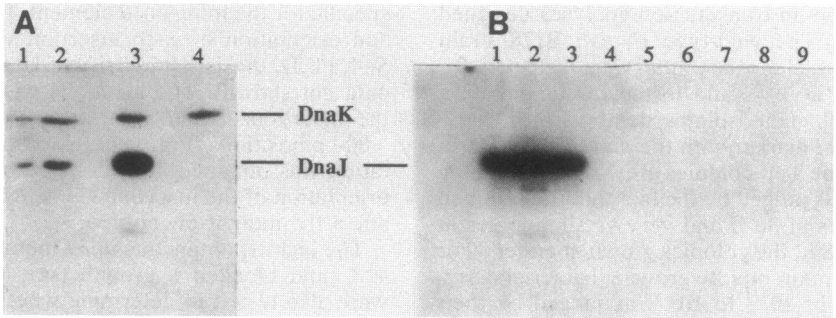

FIG. 2. Western blot analysis of DnaK and DnaJ proteins in cell extracts. Approximately 100 μl of a culture containing 2×10^8 cells per ml, grown at 30°C, was pelleted, and the proteins were solubilized by boiling in SDS sample buffer before loading on a 12.5% SDS-polyacrylamide gel. The gel was transferred to nitrocellulose and probed with DnaK and DnaJ antibodies. (A) Probed with antibodies against both DnaK and DnaJ. Lanes: 1, C600; 2, *dnaJ259*; 3, pure proteins; 4, *dnaJ*::Tn*10*-42. (B) Probed with antibodies against DnaJ only. Lanes: 1, C600; 2, *dnaJ259*; 3, B178; 4, *dnaJ60*; 5, *dnaJ61*; 6, *dnaJ63*; 7, *dnaJ64*; 8, *dnaJ82*; 9, *dnaJ83*.

none were shown to contain any (Fig. 2B). Since three of these mutations were shown to be due to gross DNA rearrangements, the lack of DnaJ antigen in these mutants is most likely due to a rearrangement of the *dnaJ* gene. The other five mutations, which did not result in detectable DNA rearrangements, may be due to small DNA insertions or deletions or to nonsense mutations.

In additional experiments, the eight insertional isolates were labeled with [^{35}S]methionine for 10 min at 30 or 43°C. The cells were lysed by boiling in SDS sample buffer to release the DnaJ protein, and DnaJ was immunoprecipitated. The immunoprecipitated proteins were electrophoresed on an SDS-polyacrylamide gel and exposed to X-ray film. None of the eight isolates contained any detectable DnaJ protein (data not shown).

Kinetics of protein synthesis. Bacteria carrying an insertion in the *dnaJ* gene or carrying the *dnaJ259* mutation were labeled for 10 min with [^{35}S]methionine at 30°C or, after a shift-up to 43°C, were labeled for 10 min beginning at 5 or 60 min after the shift-up. Cell extracts were prepared, and the proteins were separated on two-dimensional gels (Fig. 3 and 4). At 30°C, it was found that both the Tn*10* insertion mutant and the point mutant, *dnaJ259*, showed increased synthesis of heat shock proteins. A similar phenotype has been described for *dnaK* mutants (35). At 43°C, the cells exhibited a higher rate of synthesis of heat shock proteins even after 1 h; synthesis was above that of the isogenic wild-type strain but was lower than that at the early (10-min) time point. This result is quantitatively different from what is observed with *dnaK* mutants, which continue to synthesize high levels of heat shock proteins even after several hours at the higher temperature (35).

To quantitate these differences, the areas of the gels corresponding to the indicated heat shock proteins were solubilized and counted. Both the insertion mutant and the *dnaJ259* mutant exhibited a two- to fivefold increase in the rate of synthesis of the heat shock proteins at 30 and at 43°C compared with the wild-type strain when labeled at 60 min after the temperature shift (Sell, Ph.D. thesis, 1987; data not shown). The rate of synthesis of the heat shock proteins was higher in the *dnaJ259* mutant than in the *dnaJ*-insertion mutants. This result could be due either to the different *E. coli* genetic backgrounds (C600 versus B178) or to the nature and behavior of the DnaJ259 mutant protein.

Steady-state levels of DnaK and DnaJ at 30°C in the *dnaJ259* mutant. Extracts from *dnaJ259* and its isogenic parent, C600, were analyzed by Western blot, using antibodies specific for DnaJ and DnaK (Fig. 2A, lanes 1 and 2). The protein bands corresponding to DnaK and DnaJ were scanned with a 3CS Joyce-Loebl microdensitometer, and the integrated values were compared. The levels of DnaK and DnaJ in the *dnaJ259* mutant were approximately threefold greater than in the *dnaJ*$^+$ parent (Sell, Ph.D. thesis, 1987; data not shown).

DISCUSSION

This report describes the insertional mutagenesis of the *E. coli dnaJ* gene. It demonstrates that *dnaJ*, like *dnaK* (6, 30), is not completely essential for bacterial growth, particularly at temperatures below 42°C. The *dnaJ*-insertion mutants exhibited a temperature-sensitive phenotype for bacterial growth at 43°C and were resistant to phage λ growth at all temperatures. The mapping data, coupled with the fact that the λ *dnaJ*$^+$ transducing phage grows on these mutants, indicated that the insertions were in the region of the *dnaJ* gene. Southern analysis confirmed their location to be near or at two sites in the promoter proximal portion of the *dnaJ* gene (+4 and +80 bp). Analysis of protein by Western blotting and immunoprecipitation with anti-DnaJ antibodies evidenced the absence of the *dnaJ* gene product in extracts of the insertion mutants. The insertion mutants grew less well than the wild type at all temperatures and phenotypically reverted to temperature resistance at a high frequency (10^{-3} to 10^{-4}). None of the revertants, however, allowed growth of phage λ. The kinetics of heat shock protein synthesis was aberrant in these insertion mutants and in the point mutant *dnaJ259*.

The *dnaK* gene, proximal to *dnaJ* in the *dnaK-dnaJ* operon, has been shown by Paek and Walker (28) to be nonessential at 30°C. However, recent data suggest that *dnaK* null mutants accumulate extragenic suppressors rapidly at 30°C (6). The construction of an insertion or deletion in the *dnaK* gene, such as Δ*dnaK52* (28), might be expected to interrupt the transcription of the distal *dnaJ* gene, thus resulting in the absence of both gene products or in the absence of *dnaK* and the reduced synthesis of *dnaJ*. This appears to be the case since (i) DnaJ protein was not detectable by Western analysis in this *dnaK* null mutant (less than 5% of wild-type levels [data not shown]), (ii) the *dnaK* null mutant exhibited a drastically reduced plating efficiency of a λ *dnaK*$^+$ transducing phage, whereas λ *dnaJ*$^+$ *dnaK*$^+$ transducing phage plated efficiently, and (iii) the mutant exhibited a reduced transformation efficiency of a high-copy-number plasmid, pBR322, carrying the *dnaK*$^+$ gene and its corresponding promoter region, a characteristic of *dnaJ* strains (Sell, Ph.D. thesis, 1987). Since the *dnaK* insertion-deletion mutant constructed by Paek and Walker (28) lacks DnaK and appears to possess drastically reduced levels of DnaJ, it is perhaps not surprising that we were able to isolate insertion mutations in the *dnaJ* gene. It would be interesting to assay the phenotype of the *dnaK* insertion-deletion mutation in the presence of wild-type levels of the DnaJ protein, contributed by either a lysogen or a plasmid.

DnaK has been shown to play a role in modulation of the heat shock response in *E. coli* (35). Transcription of the heat shock genes in vitro requires the *rpoH* (*htpR*) gene product, σ^{32}, which confers promoter specificity (8). σ^{32} is an unstable protein with a $t_{1/2}$ of 1 min (32, 36). *dnaK* mutations result in an increase in the half-life of σ^{32} (36) and affect its synthesis (14). Since we have shown that *dnaJ* mutants also affect the kinetics of synthesis of the heat shock proteins, DnaJ may be affecting the stability of σ^{32}, either directly or indirectly through its interaction with other regulatory factors. Ohki et al. (27) have described another regulatory activity for DnaJ which involves the cell-cycle-dependent synthesis of certain membrane proteins and β-galactosidase. However, it is not known what common transcription factor, if any, controls the expression of these genes.

We have recently shown that the DnaK, DnaJ, and GrpE proteins interact in vitro, inasmuch as the simultaneous presence of DnaJ and GrpE proteins results in a 20- to 50-fold stimulation of the ATPase activity of DnaK (unpublished experiments with K. Liberek). We favor a model for these three proteins in *E. coli* physiology in which DnaJ, in conjunction with GrpE, helps release unfolded or misfolded polypeptides bound to DnaK. The stimulation of the ATPase activity of DnaK could help such polypeptides release from the surface of DnaK. This model would explain why mutations in *dnaK*, *dnaJ*, and *grpE* exhibit similar pleiotropic effects on *E. coli* physiology (1, 29, 30, 39). If this model is

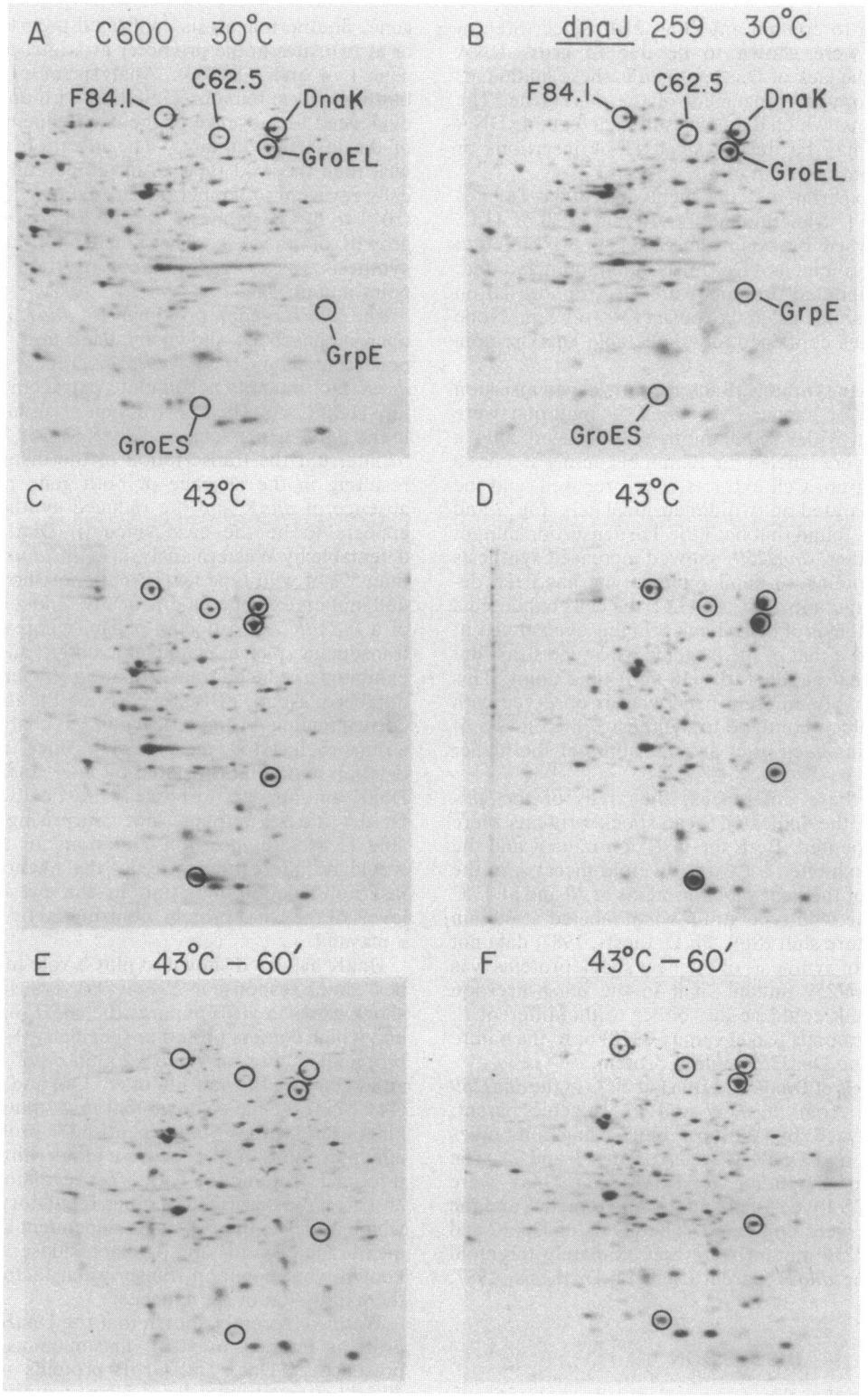

FIG. 3. Two-dimensional gel analysis of labeled *E. coli* proteins. The proteins were labeled for 10 min at the indicated temperatures. (A, C, and E) C600; (B, D, and F), *dnaJ259*. Cells were labeled at 30°C (A and B) or at 5 min (C and D) or 60 min (E and F) after the shift to 43°C.

correct, the role of DnaJ for proper DnaK function would be greater at increased temperatures. In addition, DnaJ may have functions in the cell which are independent of DnaK and GrpE.

The isolation of *dnaJ* insertion mutations suggests the existence of three rather than two classes of heat shock genes: (i) those, like *groES* and *groEL*, which are absolutely essential, (ii) those, like *lon*, which are not essential, and (iii)

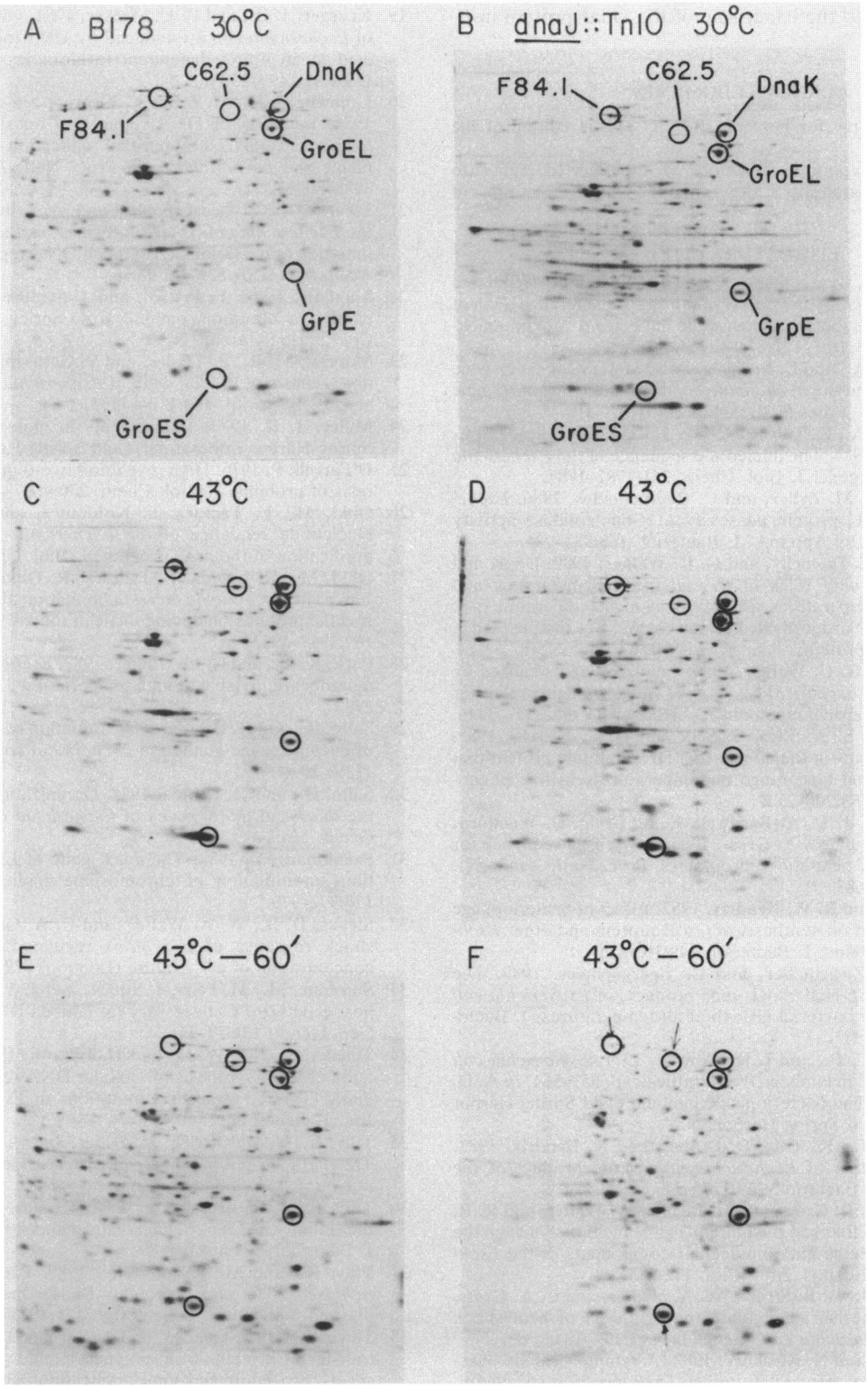

FIG. 4. Two-dimensional gel analysis of labeled *E. coli* proteins. The proteins were labeled as in Fig. 3; (A, C, and E) B178; (B, D, and F) *dnaJ*::Tn*10*-42.

those, like *dnaJ* and *dna*K, which are not absolutely essential under standard laboratory conditions. However, the requirement for DnaK protein appears to be more stringent for bacterial growth than that of DnaJ, inasmuch as *dnaK* null mutants grow extremely slowly and then only in a very restricted 20 to 37°C temperature range (5, 6). The *dnaJ* null mutants grow much better than the *dnaK* null mutants and are able to form colonies at all temperatures except above 42°C. Identification of the extragenic suppressors and their products which allow *dnaJ* null mutants to grow faster may

provide insight into the exact role of the DnaJ protein in *E. coli* growth.

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