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The Nucleotide Sequence of the *Escherichia coli* K12 *dnaJ*$^+$ Gene

A GENE THAT ENCODES A HEAT SHOCK PROTEIN*

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James C. A. Bardwell‡, Kit Tilly‡, Elizabeth Craig‡, Joshua King§, Maciej Zylicz§¶, and Costa Georgopoulos§

From the ‡Department of Physiological Chemistry, University of Wisconsin Medical School, Madison, Wisconsin 53706 and the §Department of Cellular, Viral, and Molecular Biology, University of Utah School of Medicine, Salt Lake City, Utah 84132

The *Escherichia coli dnaJ* gene product is required for bacteriophage λ DNA replication at all temperatures. It is also essential for bacterial viability in at least some conditions, since mutations in it result in temperature-sensitive bacterial growth. We have previously cloned the *dnaJ* gene and shown that its product migrates as a M_r 37,000 polypeptide under denaturing conditions. Here we present the primary DNA sequence of the *dnaJ* gene. It codes for a processed basic protein (63 basic and 51 acidic amino acids) composed of 375 amino acids totaling M_r 40,973. The predicted NH_2-terminal amino acid sequence, overall amino acid composition, and isoelectric point agree well with those of the purified protein. We present evidence that the rate of expression of the dnaJ protein is increased by heat shock under the control of the *htpR* (*rpoH*) gene product.

Analysis of *Escherichia coli* mutants unable to propagate bacteriophage λ led to the discovery of several bacterial genes (reviewed in Ref. 1). In this group are the *dnaJ* and *dnaK* genes, which are located at 0.3 min on the *E. coli* genetic map and form an operon with the structure promoter *dnaKdnaJ* (2, 3). *dnaJ* and *dnaK* bacterial mutants were shown to block λ propagation at the level of phage DNA replication by specifically interfering with the function of the λ*P* gene product at all temperatures (4 to 6). In addition, these mutants are temperature-sensitive for bacterial growth and exhibit defects in both RNA and DNA syntheses patterns (7, 8). Both the *dnaK* and *dnaJ* genes have been cloned and their products identified and purified (9-12). The *dnaK* gene product is a M_r 70,000 polypeptide whose synthesis has been shown to be induced by heat shock (13). The *dnaK* gene has been sequenced, and the predicted amino acid sequence of the protein is 48% identical to that of the *Drosophila melanogaster* hsp70 gene (14). The M_r 37,000 dnaJ protein has been purified to homogeneity, and both it and the dnaK protein are essential for λ DNA replication *in vitro* (11, 12).

In this paper, we present the primary DNA sequence of the *dnaJ* gene and show at the level of protein synthesis that expression of the *dnaJ* gene is induced by heat shock, as expected by its location in an operon following *dnaK*, a gene encoding another heat shock protein (13).

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¶ Present address: Division of Biophysics, University of Gdansk, 24 Kladki, 80-822 Gdansk, Poland.

MATERIALS AND METHODS

Bacterial and Bacteriophage Strains—Isogenic C600 *dnaJ*$^+$ and *dnaJ*259 bacterial strains have been described previously (10). Strain NM522 71/18 *hsdR*$^-$*hsdM*$^+$ (15), used for growing male-specific phages, was obtained from Dr. Noreen Murray, Department of Molecular Biology, University of Edinburgh, Edinburgh, Scotland. Strain SC122 (16) used in the RNA labeling experiments was obtained from Dr. Fred Neidhardt, Department of Microbiology, The University of Michigan Medical School. The origin of transducing bacteriophage λcI60*dnaJ*$^+$*dnaK*$^+$ has been described (3). The pEMBL8 and pEMBL9 plasmid vectors and phage IR-1 have been described (15).

Media—The media for propagation of bacteria and phage were as described previously (4, 10, 17). Twenty μg/ml tetracycline, ampicillin, or kanamycin were added to media used for selection of the appropriate plasmids. Minimal M9 medium supplemented with 0.3% glucose and all of the L-amino acids except methionine and cysteine was used for protein labeling experiments (18).

Protein Labeling—C600 bacteria were grown exponentially at 30 °C to a concentration of 3×10^8 cells/ml in supplemented M9 medium. One-ml aliquots were transferred to 15-ml Corex tubes prewarmed to the desired temperature. Ten μCi of [^{35}S]Met (Amersham Corp., 800 Ci/mmol) were added for 5 min at the indicated times. The cultures were transferred to microfuge tubes and spun for 30 s in an Eppendorf centrifuge. Cell pellets were resuspended in two-dimensional gel lysis or SDS[1] sample buffer and stored at −20°C until use.

Protein Electrophoresis—One- and two-dimensional gel electrophoreses were carried out as previously described (13). For isoelectric focusing of the dnaJ protein, the samples were run to equilibrium (6400 V-h) using a 2% (w/v) Ampholine mixture (pH 8.0-10.5, Pharmacia) in a 4% (w/v) polyacrylamide gel.

Immunoprecipitations—C600 bacteria (10 ml) were radioactively labeled with [^{35}S]Met for 10 min at 30 °C and between 5 and 15 min after a shift to 43 °C, as described above. The cultures were centrifuged in a Beckman JA-20 rotor at 10,000 rpm for 5 min, and the pellets were resuspended in 10 μl of 30 mM Tris·HCl (pH 8.1) and 20% (w/v) sucrose. The cells were lysed, and cytoplasmic and membrane fractions were prepared according to the procedure described in Ref. 19. Both cytoplasmic and membrane fractions were incubated at 95 °C for 5 min in the presence of 0.5% SDS and 0.5% 2-mercaptoethanol to assure protein solubilization. The extracts were centrifuged for 60 min at 18,000 rpm in a Beckman JA-20 rotor at 4 °C, and the supernatants were diluted 2-fold and adjusted to 1% Triton X-100, 1% deoxycholate, and 100 mM NaCl. Samples were immunoprecipitated with one-tenth volume of anti-dnaJ or control serum for 90 min at 0 °C. IgG-antigen complexes were precipitated with a 10% suspension of formalin-fixed *Staphylococcus aureus* (Cowan I strain) (The Enzyme Center, Inc.) and pelleted at 10,000 rpm for 1 min in a JA-20 rotor. The pellets were washed twice in 50 mM Tris·HCl (pH 7.4), 150 mM NaCl, 1% deoxycholate, 1% Triton X-100, and 0.1% SDS and heated in SDS sample buffer at 95 °C for 2 min. After centrifugation for 10 min at 10,000 rpm in a Beckman JA-20 rotor, supernatants were run on 10% SDS-polyacrylamide gels. The gels were dried and exposed to XAR-5 film (Kodak).

[1] The abbreviation used is: SDS, sodium dodecyl sulfate.

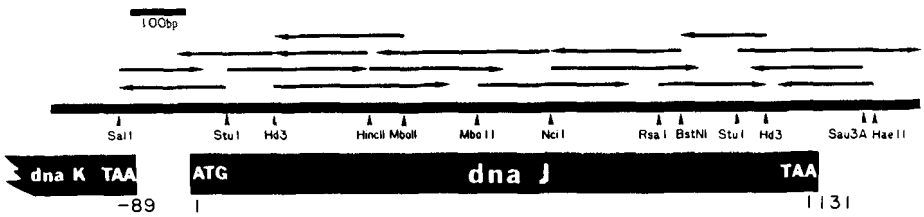

FIG. 1. **Restriction map and strategy used to determine the nucleotide sequence of the *dnaJ* gene.** The *solid bars* below the restriction map indicate the open reading frames corresponding to the coding regions of the *dnaK* and *dnaJ* genes. The lengths of the *arrows* above the restriction map are proportional to the number of nucleotides actually sequenced from each start. Only selected *Sau*3A, *Mbo*II, *Nci*I, *Rsa*I, and *Bst*NI sites are shown. Sequence was determined by the method of Maxam and Gilbert (21) except for the runs going toward the right starting from the *Stu*I and *Hin*dIII sites, in which the chain termination method was used (22).

Plasmid Constructions—All reaction conditions and techniques for restriction enzyme digestion electrophoresis and elution of DNA from agarose gels were as described by Maniatis *et al.* (20) or as specified by the manufacturers. Restriction enzymes and T4 ligase were purchased from New England Biolabs or Bethesda Research Laboratories.

DNA Sequencing—The DNA manipulation procedures in sequencing with the Maxam-Gilbert technique (21) were as previously described (14). For chain-termination method (22), the techniques were as described by Messing (17) except that the pEMBL vector/phage system was used (15). Dideoxynucleotide triphosphates and DNA primers were purchased from Pharmacia/P-L Biochemicals.

RESULTS

Subcloning and Sequencing of dnaJ Gene Fragments—The original λcI857 *dnaK*$^+$*dnaJ*$^+$ transducing phage was derived by the insertion via homologous recombination with the bacterial chromosome of the λ*dnaK*$^+$ transducing phage (which contains a 5.4-kilobase pair *Hin*dIII fragment of *E. coli* DNA), followed by aberrant excision (6). The nonhomologous excision event resulted in the deletion of additional λ DNA sequences and their substitution with the *dnaJ*$^+$ gene and flanking sequences from the bacterial chromosome. Various restriction fragments from the λ*dnaJ*$^+$*dnaK*$^+$ phage were cloned into pBR322 or pEMBL vectors, and their sequences were determined by either the chemical degradation (21) or chain termination (22) methods. The sequencing strategy, including fragments and strands sequenced, is shown in Fig. 1. The DNA sequence of the *dnaJ*$^+$ gene from the *Sal*I site within the published *dnaK* gene sequence (14) 100 base pairs after the putative termination of the predicted protein is shown in Fig. 2. The single long open reading frame begins with an ATG initiation codon at position +1 and terminates with a TAA codon at position 1131. It is composed of 1128 nucleotides, which code for a 376-amino acid protein of M_r 41,104. The sequence of the first eight amino acids matches those published for the purified protein (12) except that the protein lacks the amino-terminal methionine. This modification would decrease the size of the protein to M_r 40,973. In addition, the overall amino acid composition matches closely that of the purified protein (12). The predicted protein contains 63 basic (27 Arg, 26 Lys, and 10 His residues) and 51 acidic (20 Asp and 31 Glu residues) amino acids, consistent with its basic pI of 8.5 (12). The dnaJ sequence was used to search the NBRL protein sequence data base and GenBank™ using the algorithm of Wilbur and Lipman (23). No homologies with previously sequenced proteins or genes were found.

It has been suggested, on the basis of indirect genetic evidence, that there is a weak promoter between the *dnaK* and *dnaJ* genes, in addition to the strong promoter before the whole operon (2). Inspection of the sequence between the two genes using the Targsearch program (24) did not reveal sequences with obvious homology to the consensus −10 and −35 sequences found for *E. coli* promoters. The Perceptron program (25) was used to search for ribosome-binding sites. No sequences with good homology to the Shine-Dalgarno (26) consensus sequence were found. The failure to detect a potential Shine-Dalgarno sequence located 14 bases before the ATG initiation codon for the dnaJ protein is probably due to the fact that the distance is significantly greater than that allowed for optimal translation (27). The poor matches in sequence and spacing may help explain the much lower levels of dnaJ than dnaK protein in the cell. A further possible explanation is the high level of potential secondary structure found between the *dnaK* and *dnaJ* genes (Fig. 3). If such a hairpin structure was to form during transcription, it could prevent ribosomes from binding to the *dnaJ* initiation site, promote attenuation of transcription, or provide sites of cleavage for RNase III, again potentially affecting translation of the mRNA.

There is an additional potential stem-and-loop structure immediately following the dnaJ protein coding region (Fig. 3). This structure resembles a *rho*-dependent terminator and could serve as the transcription termination signal of the *dnaKdnaJ* operon.

DnaJ Is a Heat Shock Protein—The dnaJ protein levels were measured before and after a heat shock to determine whether it is a heat shock protein. Wild-type C600 bacteria were pulse-labeled with [^{35}S]Met at 30 °C and 5 min after a sudden shift to 43 °C, as described under "Materials and Methods." The levels of labeled dnaJ protein in the membrane and soluble fractions were measured by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. Fig. 4 shows that the vast majority of the dnaJ protein is found associated with the membrane fraction. It is not known whether the dnaJ protein is truly found in the membrane or whether its association is fortuitous. The rate of synthesis of dnaJ protein following heat shock is at least 10-fold higher than at 30 °C (Fig. 4). Two-dimensional gel electrophoresis of [^{35}S]Met-labeled extracts verified that the rate of synthesis of the total intracellular *dnaJ* gene product increases after heat shock (data not shown). In agreement with these results, a strain of *E. coli* which overproduces htpR protein,[2] the positive regulator of heat shock gene expression (16, 30), simultaneously overproduces dnaJ protein (data not shown). These results taken together demonstrate that *dnaJ* gene expression is under htpR protein regulation.

DISCUSSION

The isolation and characterization of *E. coli* mutants that interfere with phage λ DNA replication have provided us with information not only about that process but also about the

[2] K. Tilly and C. Georgopoulos, unpublished data.

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                AspAlaGluPheGluGluValLysAspLysLysEnd
-126  TCGACGCTGAATTTGAAGAAGTCAAAGACAAAAAATAATCGCCCTATAAACGGGTAATTA   -67

 -66  TACTGACACGGGCGAAGGGGAATTTCCTCTCCGCCCGTGCATTCATCTAGGGGCAATTTA    -7

                MetAlaLysGlnAspTyrTyrGluIleLeuGlyValSerLysThrAlaGluGlu
  -6  AAAAAGATGGCTAAGCAAGATTATTACGAGATTTTAGGCGTTTCCAAAACAGCGGAAGAG    54

      ArgGluIleArgLysAlaTyrLysArgLeuAlaMetLysTyrHisProAspArgAsnGln
  55  CGTGAAATCAGAAAGGCCTACAAACGCCTGGCCATGAAATACCACCCCGGACCGTAACCAG   114

      GlyAspLysGluAlaGluAlaLysPheLysGluIleLysGluAlaTyrGluValLeuThr
 115  GGTGACAAAGAGGCCGAGGCGAAATTTAAAGAGATCAAGGAAGCTTATGAAGTTCTGACC   174

      AspSerGlnLysArgAlaAlaTyrAspGlnTyrGlyHisAlaAlaPheGluGlnGlyGly
 175  GACTCGCAAAAACGTGCGGCATACGATCAGTATGGTCATGCTGCGTTTGAGCAAGGTGGC   234

      MetGlyGlyGlyGlyPheGlyGlyGlyAlaAspPheSerAspIlePheGlyAspValPhe
 235  ATGGGCGGCGGCGGTTTTGGCGGCGGCGCAGACTTCAGCGATATTTTTGGTGACGTTTTC   294

      GlyAspIlePheGlyGlyGlyArgGlyArgGlnArgAlaAlaArgGlyAlaAspLeuArg
 295  GGCGATATTTTTGGCGGCGGACGTGGTCGTCAACGTGCGGCGCGCGGTGCTGATTTACGC   354

      TyrAsnMetGluLeuThrLeuGluGluAlaValArgGlyValThrLysGluIleArgIle
 355  TATAACATGGAGCTCACCCTCGAAGAAGCTGTACGTGGCGTGACCAAAGAGATCCGCATT   414

      ProThrLeuGluGluCysAspValCysHisGlySerGlyAlaLysProGlyThrGlnPro
 415  CCGACTCTGGAAGAGTGTGACGTTTGCCACGGTAGCGGTGCAAAACCAGGTACACAGCCG   474

      GlnThrCysProThrCysHisGlySerGlyGlnValGlnMetArgGlnGlyPhePheAla
 475  CAGACTTGTCCGACCTGTCATGGTTCTGGTCAGGTGCAGATGCGCCAGGGATTCTTCGCT   534

      ValGlnGlnThrCysProHisCysGlnGlyArgGlyThrLeuIleLysAspProCysAsn
 535  GTACAGCAGACCTGTCCACACTGTCAGGGCCGCGGTACGCTGATCAAAGATCCGTGCAAC   594

      LysCysHisGlyHisGlyArgValGluArgSerLysThrLeuSerValLysIleProAla
 595  AAATGTCATGGTCATGGTCGTGTTGAGCGCAGCAAAACGCTGTCCGTTAAAATCCCGGCA   654

      GlyValAspThrGlyAspArgIleArgLeuAlaGlyGluGlyGluAlaGlyGluHisGly
 655  GGGGTGGACACTGGAGACCGCATCCGTCTTGCGGGCGAAGGTGAAGCGGGCGAGCATGGC   714

      AlaProAlaGlyAspLeuTyrValGlnValGlnValLysGlnHisProIlePheGluArg
 715  GCACCGGCAGGCGATCTGTACGTTCAGGTTCAGGTTAAACAGCACCCGATTTTCGAGCGT   774

      GluGlyAsnAsnLeuTyrCysGluValProIleAsnPheAlaMetAlaAlaLeuGlyGly
 775  GAAGGCAACAACCTGTATTGCGAAGTCCCGATCAACTTCGCTATGGCGGCGCTGGGTGGC   834

      GluIleGluValProThrLeuAspGlyArgValLysLeuLysValProGlyGluThrGln
 835  GAAATCGAAGTACCGACCCTTGATGGTCGCGTCAAACTGAAAGTGCCTGGCGAAACCCAG   894

      ThrGlyLysLeuPheArgMetArgGlyLysGlyValLysSerValArgGlyGlyAlaGln
 895  ACCGGTAAGCTATTCCGTATGCGCGGTAAAGGCGTCAAGTCTGTCCGCGGTGGCGCACAG   954

      GlyAspLeuLeuCysArgValValValGluThrProValGlyLeuAsnGluArgGlnLys
 955  GGTGATTTGCTGTGCCGCGTTGTCGTCGAAACACCGGTAGGCCTGAACGAAAGGCAGAAA  1014

      GlnLeuLeuGlnGluLeuGlnGluSerPheGlyGlyProThrGlyGluHisAsnSerPro
1015  CAGCTGCTGCAAGAGCTGCAAGAAAGCTTCGGTGGCCCAACCGGCGAGCACAACAGCCCG  1074

      ArgSerLysSerPhePheAspGlyValLysLysPhePheAspAspLeuThrArgEnd
1075  CGCTCAAAGAGCTTCTTTGATGGTGTGAAGAAGTTTTTTGACGACCTGACCCGCTAACCT  1134

1135  CCCCAAAAGCCTGCCCGTGGGCAGGCCTGGGTAAAAATAGGGTGCGTTGAAGATATGCGA  1194

1195  GCACCTGTAAAGTGGCGGGGATCACTCCCATAAGCGCT  1232
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FIG. 2. **Nucleotide and predicted amino acid sequence of the 3′ end of the *dnaK* gene and the entire *dnaJ* gene.**

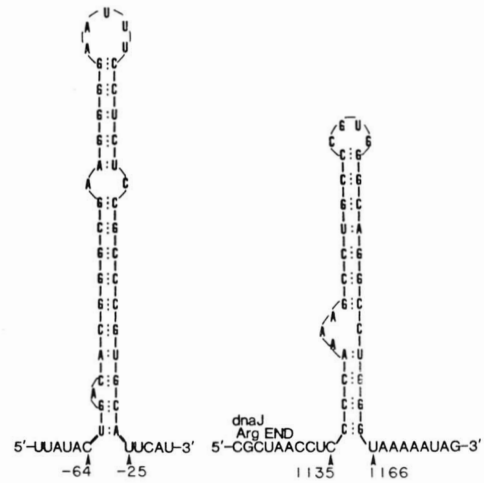

FIG. 3. **Potential secondary structure in the *dnaJ* RNA transcript.** The entire DNA sequence of the *dnaJ* gene and its flanking regions was searched for secondary structure using the computer program "stemloop" (28). Only two stem-loop structures with potential energies of a magnitude greater than 15 kcal were found. One lies between the *dnaK* and *dnaJ* genes (*left*). The other occurs just downstream from *dnaJ*'s termination codon (*right*). These two regions of sequence were manipulated with the computer program "fold" which predicts optimal RNA secondary structures (29). The folding energy of the structure shown in the *left panel* is −32.1 kcal; that in the *right panel* is −25.7 kcal.

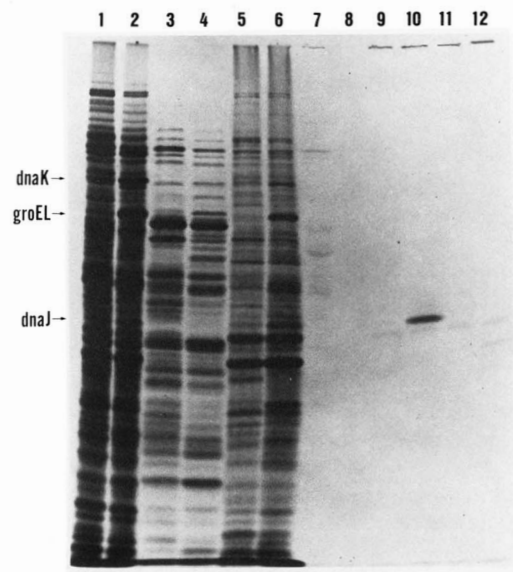

FIG. 4. **Immunoprecipitations of [^{35}S]Met-labeled cultures.** C600 bacteria were labeled at 30 and 43 °C with [^{35}S]Met. Membrane and cytoplasmic fractions were prepared and immunoprecipitated as described under "Materials and Methods." Autoradiogram after SDS-polyacrylamide slab gel electrophoresis (10% acrylamide) of: *lane 1*, total cell extract labeled at 30 °C; *lane 2*, total cell extract labeled at 43 °C; *lane 3*, supernatant fraction of cells labeled at 30 °C, *lane 4*, supernatant fraction of cells labeled at 43 °C, *lane 5*, membrane fraction of cells labeled at 30 °C, *lane 6*, membrane fraction of cells labeled at 43 °C, *lane 7*, immunoprecipitation with anti-dnaJ antibodies of sample shown in *lane 3*; *lane 8*, immunoprecipitation with anti-dnaJ antibodies of sample shown in *lane 4*; *lane 9*, immunoprecipitation with anti-dnaJ antibodies of sample shown in *lane 5*; *lane 10*; immunoprecipitation with anti-dnaJ antibodies of sample shown in *lane 6*; *lane 11*, immunoprecipitation with nonimmune antibodies of sample shown in *lane 5*; *lane 12*, immunoprecipitation with nonimmune antibodies of sample shown in *lane 6*.

roles that the host proteins play in bacterial physiology (reviewed in Ref. 1). The *dnaJ* and *dnaK* gene products are key proteins in both processes. They interact with the λ P and O replication proteins to help the correct assembly and function of the *E. coli* replication proteins at the *ori* region of λ (11, 12, 31, 32). Bacteria with mutations in either the *dnaK* or *dnaJ* gene are unable to grow at high temperatures and have defective RNA, DNA, and protein syntheses patterns (2, 6, 8, 33). In spite of the plethora of information that has accumulated about these proteins, including their purification, properties, and active participation in *in vitro* replication systems, their exact functions in both λ DNA replication and host metabolism remain to be discovered. The sequence of the *dnaJ* gene provides additional information to help in elucidating those functions.

The proof that the DNA sequence presented in this paper truly encodes the dnaJ protein is the following. Besides having the only sizable open reading frame in the DNA segment encoding *dnaJ*$^+$ activity, (*a*) the eight NH_2-terminal amino acids of the purified protein (12) match perfectly those pre-

dicted by the DNA sequence, (*b*) the overall amino acid composition of the protein (12) agrees with that predicted by the sequence, and (*c*) the calculated molecular weight of 40,973 is similar to that estimated by its rate of migration on SDS-polyacrylamide gels (10, 32). Our previous results (12) as well as those shown in Fig. 4 suggest that the dnaJ protein is associated with the *E. coli* membrane fraction. However, a hydropathy plot (34) of the dnaJ amino acid sequence did not reveal any obvious extensive hydrophobic regions. Moreover, not all of the dnaJ protein can be membrane-associated because a Fraction II DNA replication system (35) prepared from wild-type bacteria has dnaJ protein activity (12), although membranes are removed by a centrifugation step in the Fraction II preparation. A fraction of the dnaK protein also appears to be membrane-associated (Fig. 4 and Ref. 11). This association could be by virtue of binding to membrane-bound dnaJ protein, or it is possible that the dnaJ and dnaK proteins, either separately or in combination, form large complexes *in vivo* which are trapped in the membrane fraction.

Although the *dnaJ* and *dnaK* genes are both transcribed from the same strong promoter, which responds to heat shock (30), the levels of the gene products within the cell are disparate. There are about 5000 copies of dnaK protein/cell during steady-state growth at 37 °C (36), yet we find fewer than 500 copies/cell of dnaJ protein. There are several possible explanations for this difference in levels involving premature termination of transcription, RNA processing, or different translational efficiencies. For example, differing translational efficiencies could be due to the fact that the putative *dnaJ* Shine-Dalgarno sequence is located unusually far (14 bases) from the protein initiation codon or that the hairpin loop structure preceding that region (Fig. 3) reduces ribosome binding to that region. Alternatively, this hairpin loop could promote termination of transcription or be a substrate for RNase III activity, resulting in higher levels of translatable *dnaK*, compared to *dnaJ*, transcripts. A further possible source of the discrepancy between dnaJ and dnaK protein levels could be differences in half-lives of the two proteins. The dnaK protein is stable for at least 1 h at 37 °C, but we have not measured the half-life of the dnaJ protein. Whatever the mechanism for obtaining disparate levels of the two proteins, these concentrations reflect the ratio required for maximal *oriλ in vitro* plasmid replication, in which process approximately 20 times fewer dnaJ than dnaK molecules are required (12).

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