JOURNAL OF BACTERIOLOGY, Aug. 1992, p. 5258–5264
0021-9193/92/165258-07$02.00/0

Vol. 174, No. 16

The Essential *Escherichia coli msgB* Gene, a Multicopy Suppressor of a Temperature-Sensitive Allele of the Heat Shock Gene *grpE*, Is Identical to *dapE*

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Received 2 March 1992/Accepted 27 May 1992

The *grpE* gene product is one of three *Escherichia coli* heat shock proteins (DnaK, DnaJ, and GrpE) that are essential for both bacteriophage λ DNA replication and bacterial growth at all temperatures. In an effort to determine the role of GrpE and to identify other factors that it may interact with, we isolated multicopy suppressors of the *grpE280* point mutation, as judged by their ability to reverse the temperature-sensitive phenotype of *grpE280*. Here we report the characterization of one of them, designated *msgB*. The *msgB* gene maps at approximately 53 min on the *E. coli* chromosome. The minimal gene possesses an open reading frame that encodes a protein with a predicted size of 41,269 M_r. This open reading frame was confirmed the correct one by direct amino-terminal sequence analysis of the overproduced *msgB* gene product. Genetic experiments demonstrated that *msgB* is essential for *E. coli* growth in the temperature range of 22 to 37°C. Through a sequence homology search, MsgB was shown to be identical to *N*-succinyl-L-diaminopimelic acid desuccinylase (the *dapE* gene product), which participates in the diaminopimelic acid-lysine pathway involved in cell wall biosynthesis. Consistent with this finding, the *msgB* null allele mutant is viable only when the growth medium is supplemented with diaminopimelic acid. These results suggest that GrpE may have a previously unsuspected function(s) in cell wall biosynthesis in *E. coli*.

The *Escherichia coli dnaK*, *dnaJ*, and *grpE* gene products were originally identified as host proteins required by bacteriophage λ for the initiation of replication of its DNA (reviewed in reference 5). Subsequently, these three genes were shown to be heat shock regulated; i.e., their expression is hyperinduced under stress conditions, such as a temperature shift, ethanol treatment, and virus infection (reviewed in references 12 and 23). The three proteins cooperate as "molecular chaperones," protecting other proteins under conditions of stress and reactivating those already damaged by stress (5, 36). With respect to λ DNA replication, DnaK, DnaJ, and GrpE are necessary for the dissociation of the λP-DnaB complex localized at *ori*λ, resulting in the activation of DnaB helicase activity and the initiation of λ DNA replication (40). Most likely, the three proteins are also required for housekeeping functions, since they are essential for *E. coli* growth at all temperatures. This conclusion is based on the fact that the corresponding genes cannot be deleted or inactivated, except when extragenic suppressors are present (4, 6, 33). A variety of evidence exists showing that GrpE and DnaJ physically interact with DnaK (5). Most notably, recent work by Liberek et al. (18) showed that the presence of both DnaJ and GrpE greatly stimulates the weak ATPase activity of DnaK. Specifically, DnaJ and GrpE accelerate the rate of hydrolysis of DnaK-bound ATP and the rate of release of bound ATP or ADP, respectively. The hydrolysis of DnaK-bound ATP results in a conformational change in DnaK and the simultaneous release of DnaK-bound polypeptides (19).

In an effort to further understand the cellular function(s) of DnaK, DnaJ, and GrpE and to identify other factors that may interact with them, we searched for and identified genes that suppress mutant phenotypes in *dnaK*, *dnaJ*, or *grpE* bacteria when present in multiple copies. By selecting for these suppressor genes, it may be possible to identify other genes in *E. coli* whose products interact with DnaK, DnaJ, or GrpE directly or indirectly.

E. coli grpE280 cells, which are temperature sensitive, do not have the ability to support λ DNA replication at any temperature (29, 30) and are also defective in host DNA and RNA syntheses at nonpermissive temperatures (2, 3). However, the overproduction of the GrpE280 protein in *E. coli grpE280* cells partially reverses the temperature-sensitive (Ts^-) phenotype, as well as the inability to allow λ growth (1). Unlike the wild-type GrpE protein, the purified GrpE280 protein binds poorly to a DnaK affinity column (1, 39), suggesting that the nature of the GrpE280-DnaK interaction is altered. Here we report the characterization of the *msgB* gene (multicopy suppressor of *grpE*), which suppresses the Ts^- phenotype of *E. coli grpE280* at 42°C when present on a multicopy plasmid.

MATERIALS AND METHODS

Strains and media. The various bacterial strains, plasmids, and bacteriophages used in this work are listed in Table 1. Rich media, L broth, and L agar (22) were used for the growth of the *E. coli* strains and were supplemented with appropriate antibiotics to final concentrations of 100 μg/ml for ampicillin, 20 μg/ml for chloramphenicol, 50 μg/ml for kanamycin, or 20 μg/ml for tetracycline when necessary. P1-mediated transductions were carried out as described by Miller (22).

Isolation of extragenic suppressors of *grpE280*. A library of *E. coli* wild-type DNA was obtained by the method of Groisman et al. with mini-Mu plasmid pEG5294 (11). Strain CG698 was infected with the mini-Mu lysate and plated on L

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TABLE 1. Bacterial strains, bacteriophages, and plasmids

Strain, phage, or plasmid	Genotype or description	Source or reference
Strains		
B178	W3110 *galE sup*$^+$	10
MC4100	*recA*$^+$	7
DH5α	*recA1*	Bethesda Research Laboratories
BL21(DE3)	T7 polymerase under *lac* control	37
CG698	B178 *grpE280 pheA*::Tn*10*	3
JC7623	AB1157 *recB21 recC22 sbcB15 sbcC201*	15
CAG18470	MG1655 *purC80*::Tn*10*	35
BW163	B178 (λRS45 *msgB*$^+$)	This work
BW160	JC7623 (λRS45 *msgB*$^+$) *msgB*::Ω-Cam^r *purC*::Tn*10*	This work
BW161	JC7623 (λRS45 *msgB*$^+$) *msgB*::Ω-Cam^r *purC*::Tn*10*	This work
BW162	JC7623 (λRS45 *msgB*$^+$) *msgB*::Ω-Cam^r *purC*::Tn*10*	This work
KY1621	Δ*rpoH30*::Kan^r	38
SR407	KY1621 *zhf*::Tn*10 rpoH*$^+$	28
Phages		
P1L4	Clear-plaque former	L. Caro
λRS45	*imm*^{21} *ind*$^+$	34
Plasmids		
Mini-Mu	pEG5294	11
pEMBL8(+)	Amp^r Tet^r	8
KS(+) and SK(+)	pBluescript, pUC derivative plasmids with T7 and T3 promoters	Stratagene
pRS550	Amp^r Kan^r	34
pBW7	pEMBL8(+) carrying the 5.7-kbp *Sau*3A fragment from mini-Mu plasmid 52	This work
pBW1	pBluescript SK(+) carrying the *Kpn*I-*Sma*I fragment containing *msgB*$^+$	This work
pBW2 to pBW4	Subclones of pBW1 obtained following restriction digestion	This work (Fig. 1)
pBW13 and pBW44	Derivatives of pBW1 obtained by exonuclease III digestion	This work (Fig. 1)
pBW5 and pBW6	pBW13 and pBW1 into which a 3.7-kbp Ω-Cam^r element had been inserted, respectively	This work

agar-kanamycin plates at 42°C. Mini-Mu plasmids were isolated from temperature-resistant colonies. One of them, 52, was chosen for further study.

Mapping. The map position of *msgB* was determined by hybridization to the *E. coli* library of λ transducing phages carrying ordered overlapping bacterial DNA inserts (14) as described by Lipinska et al. (21).

Subcloning of *msgB* and construction of deletions. All DNA manipulations were performed by standard procedures (31). Sets of overlapping, unidirectional deletions in both directions of plasmid pBW1 were constructed with exonuclease III as described previously (13). Restriction endonuclease digestions and ligations were carried out as recommended by the enzyme manufacturers (Boehringer Mannheim Biochemicals, Bethesda Research Laboratories, and New England BioLabs).

DNA sequencing. The *msgB* gene sequence was determined from the overlapping exonuclease III deletions for both strands by the dideoxy chain termination method (32). The reagents and procedures were provided with the Sequenase system (United States Biochemical Corp.). Comparisons with the GenBank data base were made by use of the FASTA method of Pearson and Lipman (26).

Overexpression of the plasmid-encoded *msgB* gene product and amino acid sequence analysis of the amino-terminal end. Exonuclease III deletion clone pBW13, with *msgB* under the transcriptional regulation of the T7 polymerase promoter, and its *msgB*::Ω-Cam^r derivative, pBW5, were transformed into strain BL21(DE3). Plasmid-encoded proteins were labeled with [^{35}S]methionine following isopropyl-β-D-thiogalactopyranoside (IPTG) induction of the T7 polymerase gene and rifampin treatment as described by Studier and Moffatt (37). Cell extracts were electrophoresed on a 12.5% polyacrylamide–sodium dodecyl sulfate gel by the method of Laemmli (16). For amino-terminal amino acid analysis, the cell lysates were resolved by gel electrophoresis and transferred to an Immobilon polyvinylidene difluoride membrane (Millipore) in accordance with the manufacturer's specifications. The membrane was stained with 0.1% Coomassie brilliant blue R-250 in a 1% acetic acid–40% methanol solution. The corresponding band was excised and used for the analysis of the first seven amino acids with a model 477A sequencer (Applied Biosystems) in accordance with the manufacturer's instructions (analysis was done by R. Schackmann, University of Utah Biotechnology Center). A sample amount of approximately 10 pmol was used.

Construction of an *msgB* diploid strain. An *msgB* diploid strain was made essentially by the method described by Simons et al. (34). The *Sma*I-*Pvu*II fragment from pBW1, containing the *msgB* open reading frame, was cloned into plasmid pRS550, which had been digested with *Bam*HI and treated with the Klenow fragment of polymerase I. MC4100 bacteria bearing the resulting pRS550 *msgB*$^+$ plasmid were infected with phage λRS45. MC4100 bacteria were infected with the resulting lysate and plated on L agar-kanamycin plates to select for λ lysogens in which the prophage had attained a copy of the *msgB*$^+$ gene by homologous recombination. The prophage was induced by UV irradiation, and the resulting lysate was used to infect bacteria of different backgrounds to make them diploid for *msgB*.

Inactivation of the chromosomal *msgB* gene. An *msgB* null allele was constructed in vitro by the insertion of a Cam^r cassette, the Ω-Cam^r element (9, 27), into the *Eco*RI site within the *msgB* coding region of pBW1. The resulting

plasmid, pBW6, was transformed into JC7623 carrying the λ *msgB*$^+$ prophage. Amp^s Cam^r transformants were selected for and obtained (see Results). The chromosomal null allele was transferred by P1 transduction into either an *msgB*$^+$ haploid or an *msgB*$^+$ diploid strain in an otherwise wild-type *E. coli* background. To monitor the cotransduction frequency and also avoid selecting for the null allele directly, we used a Tn*10* insertion (Tet^r) in *purC* (CAG18470) as a nearby marker.

RESULTS

Isolation and mapping of multicopy suppressors of *grpE280*. Initially, 30 mini-Mu plasmids were isolated on the basis of their ability to reverse the Ts$^-$ phenotype of CG698 (*grpE280*) at 42°C. Their ability to suppress the Ts$^-$ phenotype of *grpE280* mutant bacteria was confirmed by retransforming CG698 with the individual purified plasmids and observing similar numbers of transformants at 30 and 42°C. The plasmids fell into at least four complementation groups, as judged by Southern blot analysis (data not shown). One plasmid, 52, was chosen for further study because it is a member of the largest complementation group, consisting of 11 isolates. Because bacteriophage Mu replicates by transposition, all mini-Mu clones are, by necessity, independent isolates.

For determination of the approximate map position of the putative suppressor gene on the *E. coli* chromosome, plasmid 52 DNA was radiolabeled and hybridized to the *E. coli* library of Kohara et al. (14) as described by Lipinska et al. (21). Plasmid 52 DNA hybridized to λ transducing phages 421(4E10), 422(7A8), and 423(4C11) (data not shown), which carry DNA from the 53-min region of the *E. coli* chromosome.

Subcloning of *msgB* and identification of the *msgB* gene product. For subcloning of the putative suppressor gene, plasmid 52 DNA was partially digested with restriction endonuclease *Sau*3A and subsequently ligated into the pEMBL8(+) vector. The ligation reaction was used to transform CG698 directly, selecting for Ts$^+$ transformants at 42°C. Plasmids from these candidate colonies were isolated and tested again for their ability to complement the Ts$^-$ phenotype of CG698, as judged by the numbers and sizes of colonies observed at 30 and 42°C. One of the subclones, pBW7, that complemented the Ts$^-$ phenotype best was subjected to restriction map analysis. The restriction map matched that of *E. coli* DNA carried by λ transducing phages 422 and 423. The *Hin*dIII-*Sma*I fragment containing the approximately 5.7-kbp insert was then recloned into the pBluescript KS(+) and SK(+) vectors for the convenience of later experiments. The resulting high-copy-number recombinant plasmids, when transformed into CG698, complemented the Ts$^-$ phenotype, indicating that at this high copy number the putative suppressor gene is not harmful to cell growth at either 30 or 42°C.

To define the limits of the putative suppressor gene, we constructed additional subclones and nested sets of overlapping exonuclease III clones spanning the 5.7-kbp region from both directions. Each clone was tested for its ability to complement the Ts$^-$ phenotype of CG698 at 42°C, and the results obtained with some representative clones are shown in Fig. 1. pBW13 contains a 1.5-kbp insert that fully retains the ability to rescue the Ts$^-$ phenotype of the *grpE280* mutant strain, in contrast to pBW44, which carries a 1.2-kbp insert that does not complement the Ts$^-$ phenotype (Fig. 2). In addition, a Cam^r cassette was cloned into the *Eco*RI sites of pBW13 and pBW1. Both of the resulting constructs, pBW5 and pBW6, were unable to rescue the Ts$^-$ phenotype. Therefore, it was concluded that the putative suppressor gene, which will be referred to as *msgB* (multicopy suppressor of *grpE*), lies within the 1.5-kbp fragment of pBW13.

The protein encoded by this 1.5-kbp insert in pBW13 was identified in a T7 polymerase expression system (37) and shown by gel electrophoresis to be a unique band with a molecular weight of approximately 40,000 (Fig. 3, lane 2). The same result was obtained with the larger complementing clones, pBW1 and pBW4, whose inserts are in the same orientation as that in pBW13 with respect to the T7 promoter (data not shown). Because of the Cam^r cassette insertion at the *Eco*RI site, the expression of pBW5 in the T7 system resulted in a truncated gene product of approximately 26,000 M_r (Fig. 3, lane 3), instead of the 40,000-M_r protein. The correlation between the disappearance of the 40,000-M_r protein and the inability of pBW5 to rescue the Ts$^-$ phenotype of *grpE280* mutant bacteria strongly suggests that the 40,000-M_r protein encoded by pBW13 is the *msgB* gene product. The orientation of the T7 promoter with respect to the production of the 40,000-M_r protein indicates that the *msgB* gene is transcribed clockwise with respect to the map of the *E. coli* chromosome.

DNA sequencing. The sequence of the *msgB* gene was determined from the set of overlapping exonuclease III deletion clones derived from pBW1. A 1,125-bp open reading frame starting at an ATG initiation codon at nucleotide 326 and terminating at two consecutive TGA codons was found (Fig. 4). The sequence of the open reading frame predicts a 375-amino-acid-residue polypeptide of 41,269 M_r and with an estimated isoelectric point of 5.13. These data are in good agreement with the observed migration of the purified protein on two-dimensional gels (data not shown). A putative Shine-Dalgarno sequence and the −10 and −35 sequences characteristic of σ^{70} promoters are located upstream of the ATG translational initiation codon. In agreement with these data, Northern (RNA) analysis showed that the levels of *msgB* mRNA were similar at both 30 and 42°C, whether in an *rpoH*$^+$ or *rpoH* background, suggesting that *msgB* is not a σ^{32}-regulated heat shock gene (data not shown).

Amino-terminal sequence analysis of the *msgB* gene product (see Materials and Methods) revealed the first seven amino acid residues to be Ser-Cys-Pro-Val-Ile-Glu-Leu (underlined in Fig. 4), indicating that the ATG at nucleotide 326 is indeed the initiation codon for the open reading frame predicted by the DNA sequencing data and that the first methionine is processed.

***msgB* is an essential *E. coli* gene at all temperatures.** To test the essentiality of *msgB*, we used the *E. coli recB21 recC22 sbcB15 sbcC201* strain, JC7623, and plasmid pBW6, carrying the *msgB* null allele construct with the selectable Cam^r marker (Fig. 1). It has been reported that in the *recBC sbcBC* background, gene replacements can be achieved at a comparatively high frequency from double-crossover events involving covalently closed circular plasmid molecules and the genome (24). pBW6 was transformed into strain JC7623, and JC7623 (λ *msgB*$^+$) and Amp^r Cam^r transformants were selected for at 37°C. These transformants were exposed to chloramphenicol alone, prior to screening for Amp^s Cam^r to identify candidates that had lost the plasmid and recombined the Ω-Cam^r insertion into their chromosomes. Amp^s Cam^r transformants were obtained readily in the diploid strain but not in the haploid strain. All Amp^s isolates in the haploid

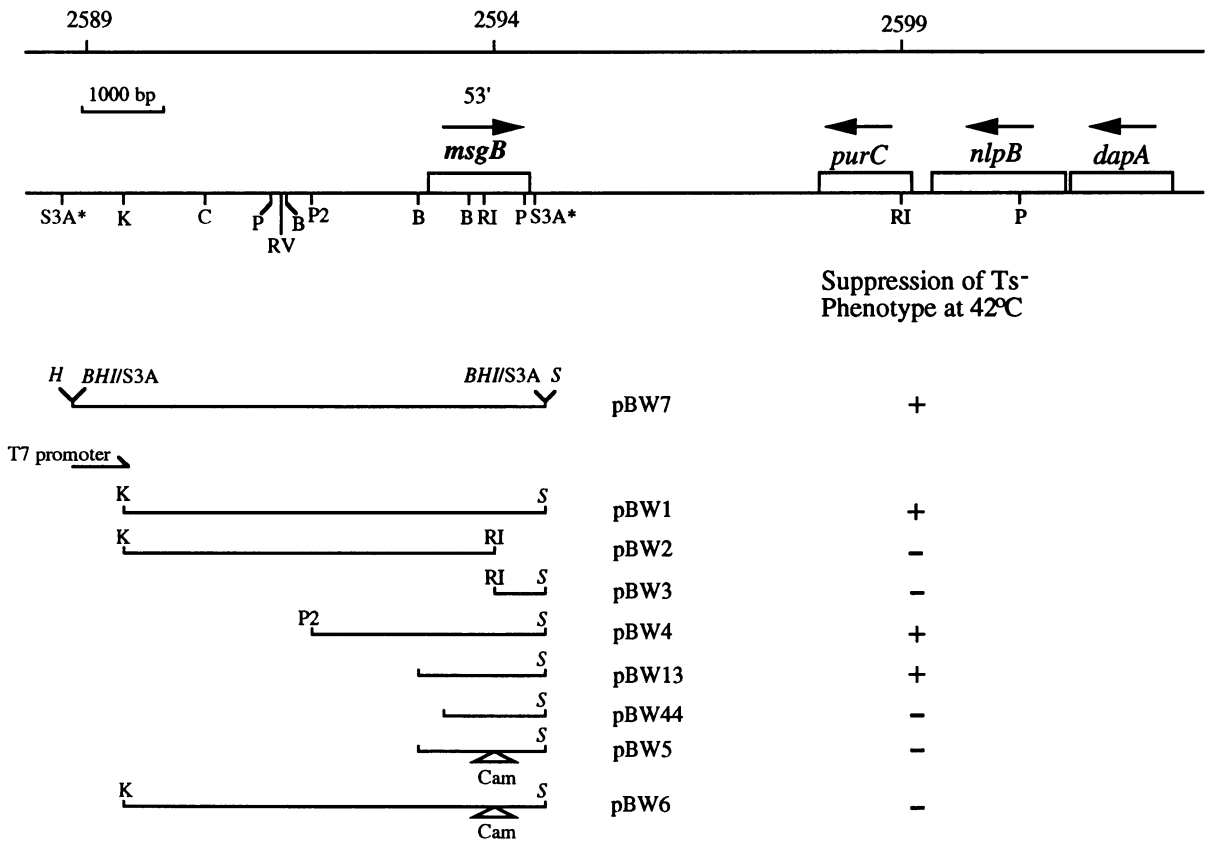

FIG. 1. Subcloning and restriction map of *msgB*. The numbers at the top indicate the corresponding region (unit: kilobase pairs) of the physical map of the *E. coli* chromosome. The genes clockwise from *msgB* (*purC*, *nlpB*, and *dapA*) are drawn to scale. The + and − symbols at the right indicate the ability of each plasmid [cloned in the pBluescript SK(+) vector] to complement the Ts⁻ phenotype of strain CG698. pBW13 and pBW44 are two clones generated by exonuclease III deletions. Abbreviations: B, *Bgl*I; BHI, *Bam*HI; C, *Cla*I; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; P2, *Pvu*II; RI, *Eco*RI; RV, *Eco*RV; S, *Sma*I; S3A, *Sau*3A. Restriction sites in italics (*BHI*, *H*, and *S*) are derived from the vector. The arrows indicate the direction of transcription. The asterisks indicate the two *Sau*3A sites used to subclone *msgB* from plasmid 52 DNA into pEMBL8(+).

strain background were always found to be Cam^s. This result suggested that *msgB* is most likely an essential gene.

The location of the Cam^r cassette on the chromosome in the diploid strain was confirmed by P1 transduction with donor strain CAG18470, which has a mini-Tn*10* insertion in the *purC* gene (~53.25 min). Approximately 40% of the Tet^r transductants remained Cam^r, indicating that the *msgB* gene is approximately 60% cotransducible with the *purC* gene. Southern analysis of one of the Cam^r strains proved that the *msgB* chromosomal locus was indeed replaced by the *msgB*::Ω-Cam^r null allele (data not shown). By P1 transduction, most of the diploid strain transformants were shown to have the *msgB*::Ω-Cam^r null allele at the chromosomal locus and not in the λ *msgB*$^+$ prophage. This result was expected because the copy of the wild-type *msgB* gene in the prophage does not have much homologous chromosomal flanking sequence.

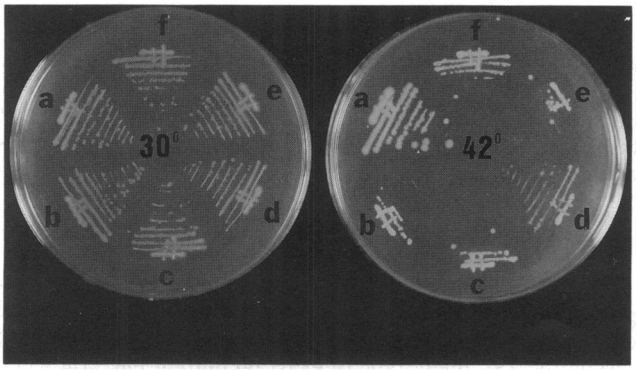

FIG. 2. Suppression of the Ts⁻ phenotype of a *grpE280* mutant strain. CG698 was transformed with various plasmids. Transformants were selected for at 30°C on L agar-ampicillin plates. Individual transformants were streaked on L agar plates and incubated at 30 and 42°C. a, B178; b, CG698; c, CG698[pBluescript SK(+)]; d, CG698(pBW13); e, CG698(pBW44); f, CG698(pBW5).

To confirm the essentiality of the *msgB* gene, we used *purC*::Tn*10* *msgB*::Ω-Cam^r strains BW160, BW161, and BW162 as donors in independent experiments to transduce the *purC*::Tn*10* marker into both *msgB* haploid and *msgB* diploid wild-type backgrounds. The Tet^r transductants were screened for Cam^r. None of the Tet^r transductants was Cam^r when the wild-type haploid strain, B178, was the recipient (Table 2). On the other hand, when the *msgB* diploid strain, BW163, was the recipient, the normal cotransduction frequency of 60% was observed for the two markers. Similar results were obtained when the transductions were carried out at 22, 30, or 37°C (data not shown), demonstrating that the *msgB* gene product is needed for bacterial viability at all temperatures.

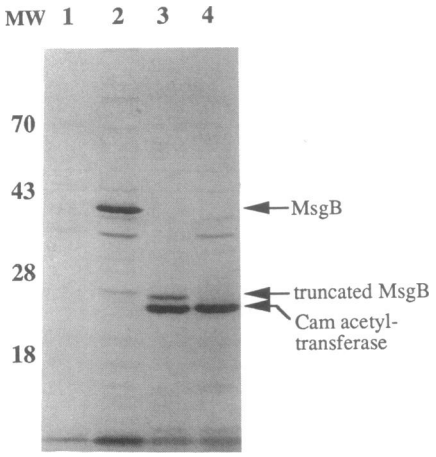

FIG. 3. Identification of the *msgB* gene product. Shown is an autoradiogram of ^{35}S-labeled cell lysates following gel electrophoresis on a 12.5% polyacrylamide–sodium dodecyl sulfate gel. Extracts were obtained from strain BL21(DE3) bearing the pBluescript SK(+) vector (lane 1), pBW13 (lane 2), pBW5 (lane 3), or pBluescript SK(+) carrying the Ω-Cam^r cassette (lane 4) following IPTG induction and rifampin treatment. For further details, see Materials and Methods and Results. Cam, chloramphenicol; MW, molecular weight (in thousands).

The *msgB* gene is identical to *dapE*. Initial computer analysis of the predicted amino acid sequence of the *msgB* gene product provided no information about the possible function of the protein. However, following 1 year of a continued search of the GenBank data base, *msgB* was finally identified as the *E. coli dapE* gene (accession number X57403; 5a, 36a), which codes for *N*-succinyl-L-diaminopimelate desuccinylase (EC 3.5.1.18), an enzyme of the diaminopimelic acid-lysine pathway that catalyzes the hydrolysis of *N*-succinyl-L-diaminopimelic acid to produce L-diaminopimelic acid (L-DAP) and succinate (20). On the basis of this information, transduction experiments were again performed, with selection on media supplemented with L-DAP, the product of the DapE enzyme, to determine whether the *msgB* null mutant is viable under these circumstances. With *msgB* diploid strain BW163 as the recipient, the observed cotransduction frequencies for the Tet^r and Cam^r markers were the same with or without L-DAP. However, when B178 was the recipient, cotransduction of the Cam^r and Tet^r markers was observed only when L-DAP was present in the selection media at concentrations of 0.1 to 10 mM. No Cam^r transductants were observed in the absence of L-DAP. However, even with the addition of 0.5 mM L-DAP, the observed frequency of cotransduction (4 to 5%) into the haploid strain was comparatively lower than the 60% observed with the diploid strain. The reason may be the slow uptake of L-DAP by *E. coli*. When the recipient was preexposed to the appropriate amount of L-DAP prior to transduction, there was a large increase in the number of Cam^r transductants (>25%), although not up to the normal frequency of 60%.

TABLE 2. Percent cotransduction frequency at 37°C for the *purC*::Tn*10* and *msgB*::Ω-Cam^r markers with *E. coli* strains haploid or diploid for the *msgB* gene

Recipient	% Cotransduction frequency (no. of Cam^r transductants/no. of Tet^r transductants) with the following donor:		
	BW160	BW161	BW162
B178 (*msgB*$^+$ haploid)	0 (0/147)	0 (0/99)	0 (0/136)
BW163 (*msgB*$^+$ diploid)	57 (226/396)	66 (229/344)	53 (157/297)

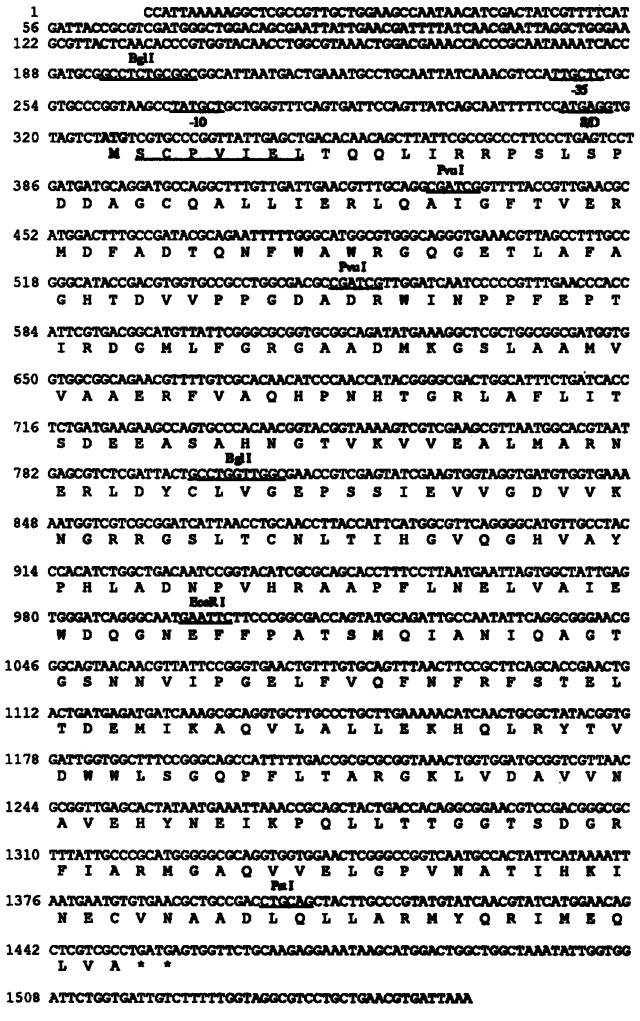

FIG. 4. Nucleotide sequence of the 1.5-kbp DNA fragment containing the *msgB* gene and the deduced amino acid sequence. The putative ribosome-binding site (Shine-Dalgarno sequence [S/D]) and the −10 and −35 promoter consensus sequences are underlined and labeled below the sequence. Selected restriction sites are also underlined and labeled above the sequence. The first seven amino acids (underlined) correlate with the results of the amino-terminal analysis of the overexpressed *msgB* gene product.

DISCUSSION

The *msgB* gene of *E. coli*, an extragenic multicopy suppressor of the *grpE280* point mutation, was cloned and characterized. The *msgB* gene was identified by its ability to rescue the Ts$^-$ phenotype of *grpE280* mutant bacteria when present on multicopy plasmids. Suppression of the Ts$^-$ phenotype was observed when *msgB* was present on high-copy-number plasmids, such as pBluescript, pBR322, or pEMBL, but not low-copy-number plasmids, such as pCL1920 (~5 copies per cell; 17, 37b). Multicopy *msgB* did

not, however, suppress the λ-resistant phenotype of *grpE280* bacteria (unpublished observations). The overproduction of *msgB* did not suppress the Ts^- phenotype of *grpE280* bacteria by simply bypassing the need for GrpE, since it did not suppress the Ts^- phenotype of Δ*grpE* bacteria (4; data not shown).

The *msgB* gene maps at 53 min, corresponding to the 2,593- to 2,595-kbp region of the physical map of the *E. coli* chromosome, with the direction of transcription being clockwise relative to the genetic map. The *msgB* gene was sequenced, and an open reading frame that codes for a protein with a predicted molecular weight of 41,269 was identified. This protein is consistent with the approximately 40,000-M_r protein observed on sodium dodecyl sulfate-polyacrylamide gels following the overexpression and labeling of the *msgB* gene product (Fig. 3). Moreover, the seven amino-terminal residues of the overexpressed protein match perfectly those predicted by the DNA sequence, further confirming the correctness of the open reading frame. The putative promoter sequences as well as the results of Northern analysis indicate that, unlike *grpE*, *msgB* is not a heat shock gene.

During the preparation of this manuscript, we found during a search for amino acid sequence homologies that *msgB* is identical to *dapE* (5a). The *dapE* gene encodes *N*-succinyl-L-diaminopimelate desuccinylase, which produces L-DAP, which is then isomerized to *meso*-diaminopimelic acid, a structural component of the peptidoglycan portion of the bacterial cell wall (25). *N*-Succinyl-L-diaminopimelate desuccinylase has been purified to homogeneity by Lin et al. (20) from cell extracts of *E. coli* carrying a single copy of the gene. The reported monomeric molecular weight of the purified enzyme is approximately 40,000, consistent with our observations. We also purified the MsgB protein from cells overproducing MsgB by using an approach different from the published one. Although we did not attempt to demonstrate an enzymatic activity of the MsgB protein, the facts that (i) the *msgB* and *dapE* genes are identical in sequence, (ii) the MsgB protein and the monomeric form of the purified DapE enzyme are similar in size, and (iii) *msgB* null mutants are viable only in the presence of L-DAP allow us to conclude that *msgB* is the gene encoding *N*-succinyl-L-diaminopimelate desuccinylase.

The puzzling question is why overexpression of this enzyme is able to suppress the Ts^- phenotype of *grpE280* mutant bacteria. One possibility is that in *grpE280* bacteria, under nonpermissive conditions, the MsgB function directly or indirectly is limiting for bacterial growth. This effect could be at the level of transcription, translation, protein assembly, or a combination of any of these. We previously showed that total RNA synthesis is severely affected in *grpE280* mutant bacteria at the nonpermissive temperature (3). However, preliminary Northern blot analyses showed that *msgB* transcription is largely unaffected in a *grpE280* background (data not shown).

Another explanation is that the GrpE protein may be a limiting factor in the production of a cell wall component(s). For example, in the *grpE280* mutant at 42°C, the lack of a fully functional GrpE protein may lead to a decrease in the level of *N*-succinyl-L-diaminopimelate desuccinylase present in cells, eventually affecting cell wall synthesis and subsequent elongation and septation during cell growth. Therefore, the overexpression of the MsgB protein at 42°C may bypass the impaired function of the GrpE280 mutant protein. Alternatively, the effective enzymatic activity of the MsgB protein may depend on the correct conformation or oligomerization state of the MsgB subunit. The MsgB protein is present in low levels in bacteria and exists as a mixture of dimeric and tetrameric species of identical subunits (20). At 42°C, the formation of the effective MsgB protein dimer or tetramer may be inefficient, requiring the aid of chaperone proteins, such as DnaK, DnaJ, and GrpE. However, in the *grpE280* mutant background at a high temperature, the formation of MsgB oligomers may be compromised. Increasing the concentration of MsgB by overexpression may push the equilibrium towards the formation of more MsgB oligomers, allowing sufficient L-DAP for cell growth and division. Consistent with MsgB being one of the key, limiting components under conditions compromising DnaK, DnaJ, or GrpE activity are the following observations: (i) high MsgB levels improve the growth of *dnaJ* mutant bacteria at 43°C (37a) and (ii) high MsgB levels allow *dnaK* mutant bacteria to survive the transition from poor to rich media (10a). Whatever the mechanism of suppression by MsgB, this study suggests that GrpE may have some interesting properties not previously suspected and perhaps worth studying further.

ACKNOWLEDGMENTS

We thank Satish Raina and Margaret Karow for helpful discussions and encouragement during the course of this work. We also thank Patrick Stragier for communicating his results with *dapE* prior to publication.

This work was supported by grants NIH AI21029 and FNS 31-31129.91.

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