Defect in export and synthesis of the periplasmic galactose receptor MglB in *dnaK* mutants of *Escherichia coli*, and decreased stability of the *mglB* mRNA

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The high-affinity galactose permease, which comprises the periplasmic galactose receptor MglB, the membrane translocator MglC and the membrane-associated ATPase MglA, displayed a reduced activity in a *dnaK* temperature-sensitive mutant of *Escherichia coli*. This reduced transport activity correlated with a reduction in the quantity of MglB. At 42 °C, an accumulation of pre-MglB in the *dnaK* temperature-sensitive mutant reflected a defect in MglB export. In addition, an accumulation of pre-MglB in *secB*, *secA* and *secY* mutants suggested that SecB and the Sec translocase are also involved in export of the periplasmic galactose receptor. At 30 °C, there was no accumulation of pre-MglB in the *dnaK* mutant, but there was still a decreased amount of MglB in the periplasm. The reduction in MglB expression was not the result of a decrease in its stability, nor was it the result of a general defect in translation or transcription, since the MglA protein (which is expressed from the same operon as MglB) was synthesized in normal amounts. Two mRNAs are implicated in the expression of the *mgl* genes, a polycistronic *mglBAC* mRNA, and a more stable and more abundant *mglB* mRNA, produced by 3'-5' degradation of the *mglBAC* mRNA (R. W. Hogg, C. Voelker & I. von Carlowitz, 1991, *Mol Gen Genet* 229, 453–459). The *mglB* mRNA is protected against exonucleases by a REP (Repetitive Extragenic Palindrome) sequence located at its 3' extremity, which is responsible for the higher expression of MglB compared to MglA and MglC. The decreased MglB expression in the *dnaK* mutant at 30 °C in the present work correlated with a reduced stability of the *mglB* mRNA, which may have resulted from a defective stabilization by the REP sequence, or from a defect in translation of the *mglB* gene.

Keywords: heat shock protein DnaK, protein export, mRNA stability, *Escherichia coli* *dnaK* mutants, periplasmic galactose receptor

INTRODUCTION

Molecular chaperones form a class of polypeptide-binding proteins that are implicated in protein folding, protein targeting to membranes, protein renaturation or degradation after stress, and control of protein–protein interactions (reviewed in Ellis & van der Vies, 1991; Georgopoulos *et al.*, 1994; Hendrick & Hartl, 1993). The major classes of molecular chaperones of *Escherichia coli* comprise DnaK/hsp70 (and its assistants DnaJ and GrpE), GroEL/hsp60 (and its assistant GroES) and

HtpG/hsp90. The heat shock protein DnaK/hsp70 (Georgopoulos, 1977; Bardwell & Craig, 1984; Gross *et al.*, 1990; Bukau & Walker, 1989) plays a role in the maintenance of bacterial viability under stress, in addition to normal cellular functions. DnaK is a cytoplasmic protein, associated in part with the cytoplasmic membrane (Bukau *et al.*, 1993; El Yaagoubi *et al.*, 1994), which behaves as an ATP-dependent chaperone, facilitating the correct assembly or disassembly of certain oligomeric protein complexes, and participating in protein folding and renaturation and in the transmembrane targeting of certain proteins (Wild *et al.*, 1992). DnaK has also been implicated in cell division (Bukau & Walker, 1989), murein synthesis (Wu *et al.*, 1992), flagellar assembly (Shi

Abbreviation: REP, repetitive extragenic palindrome.

et al., 1992) and osmoregulation (Meury & Kohiyama, 1991).

Protein export across the *E. coli* cytoplasmic membrane involves principally the Sec transport apparatus, which is composed of the SecB chaperone, and of a translocase comprising the SecA ATPase and the membrane-embedded SecE/SecY complex (Economou & Wickner, 1994). Whereas SecB is considered to be the main chaperone for protein export, several pre-proteins are maintained in a translocation-competent state by the molecular chaperones DnaK/DnaJ (pre-PhoA) or GroEL/GroES (pre- β -lactamase), and these molecular chaperones probably substitute for SecB under conditions in which SecB becomes limiting (Wild *et al.*, 1992). Furthermore, a signal recognition particle, similar to that found in eukaryotes, and which consists of at least one protein (Ffh) and one RNA molecule (4.5S RNA), functions as a chaperone specific for signal sequences in nascent pre-proteins and maintains their translocation-competent conformation (Luirink & Dobberstein, 1994).

The high-affinity galactose transport system of *E. coli* consists of the periplasmic galactose receptor MglB, an integral membrane protein MglC and the membrane-bound transport ATPase MglA (Harayama *et al.*, 1983). The *mgl* genes are organized in the *mglBAC* operon, which is transcribed into an unstable polycistronic *mglBAC* mRNA. This is degraded in the 3'-5' direction up to a repetitive extragenic palindrome (REP) sequence located between *mglB* and *mglA*, thus leading to the accumulation of a more stable *mglB* mRNA (Newbury *et al.*, 1987a, b; Stern *et al.*, 1988). The stabilization of the *mglB* mRNA by its REP sequence is responsible in part for the higher expression of MglB compared to MglA and MglC (Hogg *et al.*, 1991). In the present report, we show that *dnaK* mutations affect MglB export and expression, with a concomitant decrease in *mglB* mRNA stability.

METHODS

Bacterial strains. *E. coli* K-12 strains C600 (*jenB6 thi-1 thr-1 supE44*), WM1389 [C600 *dnaK756* (ts)], MC4100 (*F' lacU169 araD139 thiA rpsL relA*), MM52 [MC4100 *secA51* (ts)], CK1953 (MC4100 *secB::Tn5*), IQ86 (MC4100, *Tn10*) and IQ85 [IQ86 *secY24* (ts)] were obtained from the laboratories of Drs J. Beckwith, K. Ito, C. Kumamoto, J. C. Walker and C. Georgopoulos. The strains were grown at 30 °C in glycerol minimal medium. The *dnaK*, *secA* and *secY* temperature-sensitive mutants were shifted to 42 °C for several hours (as indicated) before the experiments. The high-affinity galactose transport system was induced with 1 mM fucose.

Transport measurements. The bacteria were washed twice at 0 °C with minimal medium M63 (Miller, 1972) containing 0.4% glycerol and resuspended in the same medium. The washed bacteria were incubated for 10 min at 22 °C before transport measurements. Galactose transport was assayed at 22 °C in a volume of 1 ml containing the equivalent of 50 μ g of cell protein per ml. D-[¹⁴C]galactose (250 μ Ci μ mol⁻¹, Amersham) was added to the bacterial suspension to a concentration of 2 μ M. A 200 μ l sample of the assay mixture was removed from each flask at various times after the addition of the radioactive substrate and filtered through cellulose ester filters (Millipore HAWP, 0.45 μ m

pore size). The filters were washed three times with 1 ml of the transport medium and the radioactivity measured.

Cell fractionation procedure. The periplasmic fraction was prepared by the osmotic shock procedure described by Nossal & Heppel (1966). The bacteria were plasmolysed at 16 °C in 0.03 M Tris/HCl pH 7.3, 20% (w/v) sucrose, 0.2 mM EDTA and osmotically shocked in distilled water containing 0.3 mM MgCl₂ at 0 °C. The shocked cells were separated from the shock fluid (periplasmic fraction) by centrifugation at 10000 *g* for 10 min. The cell lysate was prepared by ultrasonic disruption of bacteria in 50 mM potassium phosphate, pH 6.8 (Branson Sonic Power ultrasonicator, 25 W, five times for 15 sec each), followed by centrifugation of the mixture at 40000 *g* for 15 min.

Immunoblots. Antisera against MglB and MglA were prepared in rabbits by immunization with 50 μ g protein in Freund's complete adjuvant. A booster immunization containing 50 μ g protein in Freund's incomplete adjuvant was administered after 3 weeks. Bleeding was performed 10 d after the injection. SDS-PAGE and immunoblots were carried out according to Towbin *et al.* (1979). The intensity of protein bands was quantified with a Quick Scan densitometer (Helena Laboratories). MglB and MglA were purified as described previously (Richarme, 1983; Richarme *et al.*, 1993).

Radiolabelling, immunoprecipitation and electrophoresis. Samples (1 ml) of cells growing exponentially at 30 °C in glycerol medium were pulse-labelled with 60 μ Ci ml⁻¹ [³⁵S]methionine (Amersham, 1000 Ci mmole⁻¹), chased with nonradioactive L-methionine (200 μ g ml⁻¹) and precipitated with TCA as described by Ito *et al.* (1981). The precursor and mature forms of MglB were immunoprecipitated, resolved by electrophoresis on SDS-polyacrylamide gels [10% (w/v) acrylamide], detected by autoradiography and quantified with an Ambis Scanner interfaced with an IBM computer.

RNA techniques. RNA for Northern blots was isolated by hot SDS/phenol extraction (Miller, 1972). Samples were separated on formaldehyde-agarose gels, transferred to nitrocellulose paper and hybridized to radiolabelled DNA probes in 50% (v/v) formamide as described by Maniatis *et al.* (1982). The size of RNA species was estimated by comparison with rRNA and denatured DNA markers. The probe used was composed of a 22 residue synthetic oligonucleotide complementary to residues 398–419 of the *mglB* mRNA (Hogg *et al.*, 1991). It was labelled with [³²P]ATP by polynucleotide kinase as described by Maniatis *et al.* (1982). Rifampicin was used at 200 μ g ml⁻¹, a high concentration currently used for the inhibition of transcription initiation in *E. coli* *in vivo*.

RESULTS

Decreased galactose transport and decreased amount of the periplasmic galactose receptor in *dnaK* mutants

The *dnaK756* thermosensitive mutant is defective in galactose transport. When transport was measured after growth at the permissive temperature (30 °C), the transport activity was sevenfold lower in the mutant than in the parental strain (Fig. 1a). When transport was measured after growth at 30 °C, followed by a shift to 42 °C for 2 h, the transport activity was tenfold lower in the mutant (Fig. 1a). The defect in galactose transport correlated with a reduction in the amount of periplasmic galactose receptor in the *dnaK* mutant. The amounts of MglB in the periplasm of the *dnaK756* mutant and of the parental strain C600 were measured by immunoblotting the periplasmic

Fig. 1. (a) Decreased galactose transport in the *dnaK756* mutant. Galactose transport was measured as described in Methods at 22 °C in the wild-type strain C600 (□, ■) and in the *dnaK756* mutant (○, ●), grown at 30 °C (□, ○) or grown at 30 °C followed by a shift to 42 °C for 2 h (■, ●). (b) Decreased amount of the MglB protein in the *dnaK756* mutant. Periplasmic fractions from strains C600 and *dnaK756*, grown at 30 °C, were prepared as described in Methods. The fractions were subjected to electrophoresis on a polyacrylamide gel and immunoblotted with anti-MglB antibodies. Column 1 and 2 contain, respectively, 7 and 20 µg of the shock fluid from the parental strain C600. Columns 3 and 4 contain, respectively, 7 and 20 µg of the shock fluid from the *dnaK756* mutant.

fractions from both strains with anti-MglB antibodies. The amounts of MglB were fivefold lower in the *dnaK* mutant than in the parental strain at 30 °C (Fig. 1b) and sixfold lower at 42 °C (not shown). The correlation between reduced transport activity and a reduction in the amount of galactose receptor is consistent with the previous demonstration that when the periplasmic receptor of a binding-protein-dependent transport system is reduced to less than 20% of its wild-type level [while the other transport components remain at the wild-type level (see below)], it becomes limiting for transport (Manson *et al.*, 1985). Similar reductions in galactose transport and in galactose receptor expression were found in the null *dnaK* mutant GW 4813 (Δ *dnaK52*: Cm^r) (Paek & Walker, 1987) (not shown), suggesting that these defects are common to several *dnaK* mutants.

Defect in MglB export in the *dnaK* temperature-sensitive mutant at 42 °C

The efficient export of the galactose receptor to the periplasm of *E. coli* has been recently shown to be dependent on the presence of the SecB chaperone (Powers & Randall, 1995). However, the following results suggest

Fig. 2. (a) MglB precursor in the *dnaK* mutant at 42 °C. Cells growing exponentially in glycerol medium at 30 °C (experiment indicated 30 °C), or growing at 30 °C to OD 0.7 and then transferred to 42 °C for 1 h (experiment indicated 42 °C), were pulse-labelled for 15 s with [³⁵S]methionine, chased with nonradioactive methionine for 30 s and precipitated with TCA. The galactose receptor was prepared from each sample by immunoprecipitation. (b) Kinetics of MglB export. Experiments were carried out as described in the legend to Fig. 2(a). Samples were pulse-labelled for 15 s and chased for the indicated times. The data are the average from two experiments. □, ■, Strain C600 at 30 °C (□) or 42 °C (■); ○, ●, *dnaK756* mutant at 30 °C (○) or 42 °C (●). Since the radiolabel was methionine and three of the nine methionine residues are in the leader sequence, a factor of 1.5 was used to convert the radioactivity recovered in the mature form to the amount of precursor polypeptide from which it was derived.

that *dnaK* mutations also affect the export of MglB. At 42 °C, under conditions in which the parental strain contains only mature galactose receptor (pulse-labelling for 15 sec, chase for 1 min), the *dnaK* temperature-sensitive mutant contains a significant amount of the galactose receptor as the precursor form (Fig. 2a). This secretion defect is not observed at 30 °C, at which temperature the MglB precursor does not accumulate either in the mutant or in the parental strain (Fig. 2a) [it should be noted that at both 30 °C and 42 °C, the amount of MglB synthesized is reduced in the *dnaK* mutant (see below)]. The half-time for complete export of MglB to the periplasm at 42 °C was approximately 1 min in the *dnaK* mutant, compared to less than 10 sec for the parental strain C600 (Fig. 2b), while at 30 °C, the kinetics of MglB export were similar in the mutant and in the parental strain. Thus, *dnaK756* appears to be defective in its secretion of the galactose receptor at the restrictive

Fig. 3. MglB precursor in *secB*, *secA* and *secY* mutants. Cells growing exponentially in glycerol medium at 30 °C (strain CK1953 *secB*:Tn5), or grown at 30 °C to OD 0.7 and transferred to 42 °C for 2 h [strain MC4100 *sec⁺*, strain MM52 *secA51* (ts), strain IQ85 *secY24* (ts)] were pulse-labelled for 15 s with [³⁵S]methionine, chased with nonradioactive methionine for 30 s and precipitated with TCA. The galactose receptor was prepared from each sample by immunoprecipitation.

temperature, but not at 30 °C. While most of the exported proteins of *E. coli* interact with the SecB chaperone (Wild *et al.*, 1992; Kumamoto & Beckwith, 1983), some of them interact alternatively (MalE, LamB) or preferentially (alkaline phosphatase, PhoA) with DnaK (Wild *et al.*, 1992). Since DnaK is involved in many cellular processes, we cannot completely exclude an indirect effect of the *dnaK* mutation on MglB export. However, the delay in the processing of pre-MglB to the mature species was already apparent after a 40 min shift of the thermosensitive mutant at 42 °C (not shown), before the cells stopped growing (growth was severely impaired after 90 min and stopped after 3 h). Furthermore, the SecB-dependent export of several proteins (OmpA, LamB) is not altered in *dnaK* mutants (Wild *et al.*, 1992), suggesting that *dnaK* mutations do not affect the expression or the assembly of the Sec export apparatus.

Involvement of the Sec translocase in MglB export

The results shown in Fig. 3 indicate that, in addition to SecB and DnaK, the SecA and SecY components of the cellular export apparatus are involved in the export of MglB. The MglB precursor accumulates in the *secB* (IQ86), *secA* (MM52) and *secY* (Kumamoto & Beckwith, 1983; Ito *et al.*, 1983; Oliver & Beckwith, 1981) mutants, in conditions where the parental strains do not accumulate significant amounts of the precursor (Fig. 3). This suggests that the role played by DnaK in MglB export could be that of a chaperone functioning in parallel with SecB (or perhaps before SecB), and that membrane translocation of MglB requires the Sec translocase.

Decreased MglB synthesis in the *dnaK* mutant at 30 °C and 42 °C

Further analysis of Fig. 2a shows that MglB expression is decreased in the *dnaK756* temperature-sensitive mutant, as deduced from the amounts of pulse-labelled MglB synthesized in the mutant and in the parental strain. At 42 °C, there is a reduction in the amount of pulse-labelled MglB in the mutant (31 % of the quantity synthesized in the parental strain, in both the precursor and mature form (Fig. 2a, compare lane 1 with lane 2). At 30 °C, in conditions where there is no apparent secretion defect, the amount of pulse-labelled MglB is reduced in the *dnaK* mutant (35 % of the amount synthesized in the parental

Fig. 4. Normal expression of MglA in the *dnaK756* mutant. Cell lysates from the wild-type strain C600 and from the *dnaK756* mutant were prepared as described in Methods and then 20 µg of each lysate was loaded on an SDS-polyacrylamide gel and immunoblotted with anti-MglA antibodies. The band at 51000 Da represents the MglA protein and the band at 38000 Da represents its stable proteolytic product (Richarme *et al.*, 1993).

Fig. 5. Unaltered stability of MglB in the *dnaK756* mutant. Cells growing exponentially in glycerol medium at 30 °C were pulse-labelled for 2 min with [³⁵S]methionine, followed by addition of unlabelled methionine to 0.04 % (w/v). Aliquots were removed at various times after the chase and precipitated with TCA. The galactose receptor was prepared from each sample by immunoprecipitation.

strain, entirely in the mature form, Fig. 2a, compare lane 3 with lane 4). This decreased expression is selective for MglB: the MglA protein (the cytoplasmic galactose-dependent transport ATPase, which is expressed from the same operon, *mglBAC*) is found in similar amounts in the *dnaK* mutant and in the parental strain, as shown in Fig. 4 where crude extracts from both strains are shown immunoblotted with anti-MglA antibodies. We checked, by pulse-labelling of MglA, immunoprecipitation and electrophoresis, that the synthesis of this protein was unaffected in the *dnaK* mutant (not shown). The expression of β -galactosidase was also not affected in the *dnaK* mutant (data not shown).

Unaltered stability of MglB in the *dnaK756* mutant

The different levels of MglB expression in the *dnaK756* mutant and in the parental strain might be due, in part, to different turnover rates. We measured the half-lives of MglB by labelling exponentially growing cells at 30 °C with [³⁵S]methionine for 2 min, followed by a chase with cold methionine. Samples were withdrawn at various times after the initial labelling, MglB was immunoprecipitated and submitted to electrophoresis. The MglB protein was very stable both in the *dnaK* mutant and in the wild-type strain (Fig. 5), suggesting that the *dnaK*

Fig. 6. Decreased stability of the *mglB* mRNA in the *dnaK* mutant. Northern blot of mRNA prepared from strain C600 and the *dnaK756* mutant. Rifampicin was added to growing cells. RNA was extracted at various times after rifampicin addition and analysed by Northern blotting as described in Methods. Thirty micrograms of RNA from the *dnaK756* mutant and 15 μ g of RNA from the parental strain C600 were loaded into each well. Hybridization was to a labelled probe complementary to residues 398–419 of the *mglB* mRNA. Hybridization revealed the 1400 nucleotide *mglB* mRNA. No hybridization was seen when RNA was isolated from cells uninduced for the *mgl* operon (grown in the absence of fucose and in the presence of glucose).

mutation has no significant effect on the stability of the galactose receptor. Similar results concerning the stability of MglB were obtained when labelling was done after a shift to 42 °C for 2 h (not shown).

Decreased stability of the *mglB* mRNA in the *dnaK756* mutant

As described above, the expression of MglB was decreased in the *dnaK756* mutant, and the lower MglB expression resulted from a reduction in MglB synthesis since the stability of the protein was unaffected. Furthermore, the reduction in MglB synthesis contrasted with the normal expression of MglA. The high-affinity galactose permease of *E. coli* is encoded by a three-gene operon containing a large intercistronic region with a REP sequence located between the first gene (*mglB*) and the two distal genes (*mglA* and *mglC*) (Hogg *et al.*, 1991). Previous studies of the *mglBAC* operon have revealed the presence of two mRNA populations (Hogg *et al.*, 1991): one mRNA (*mglB* mRNA) of approximately 1400 nucleotides corresponds to the 5' region of the operon extending to the intergenic space between *mglB* and *mglA*, and a larger mRNA (*mglBAC* mRNA), about 4300 nucleotides long, represents the entire operon and is present at less than 1/10 of the level of the smaller mRNA population. The following results show that the stability of the *mglB* mRNA is decreased in the *dnaK756* mutant. Cells of the *dnaK756* mutant and of the parental strain in exponential phase at 30 °C were treated with rifampicin to inhibit the initiation of RNA synthesis. After appropriate time intervals, samples were taken and the RNA was examined by Northern blotting with a probe complementary to residues 398–419 of *mglB* (Fig. 6). In the parental strain, the 1400 nucleotide *mglB* mRNA displayed a half-life of 6 min, similar to that of other mRNAs stabilized by REP sequences such as the *malE* (Hogg *et al.*, 1991) or the *hisP* mRNAs (Stern *et al.*, 1988), whereas in the *dnaK756* mutant, the half-life of the *mglB* mRNA was reduced to less than 1 min. The decrease in stability of the *mglB*

mRNA correlated with the decrease in galactose transport activity (Fig. 1a), in the level of MglB (Fig. 1b) and in MglB synthesis (Fig. 2a) in the *dnaK* mutant. The normal expression and synthesis of MglA in the *dnaK* mutant suggests that transcription rates of the *mglBAC* operon, stability of the *mglBAC* mRNA and translation of the *mglA* gene are not significantly affected in the mutant [the stability of the *mglBAC* mRNA could not be determined due to a low hybridization signal with the probe (not shown)]. The decreased MglB expression (Fig. 1b) resulted from a decreased synthesis of the protein (Fig. 2a) and correlated with a decreased stability of the *mglB* mRNA (Fig. 6). The lesser stability of the *mglB* mRNA might result from an effect of the *dnaK* mutation on the stability of the *mglB* mRNA, perhaps linked to the presence of the REP sequence at the 3' extremity of this mRNA, or from an effect of the *dnaK* mutation on translation rates of the *mglB* mRNA with a consequent decrease in its stability (see Discussion).

DISCUSSION

In the present report, we show that export and expression of the periplasmic galactose receptor MglB are defective in a *dnaK* mutant. The defect in export is reflected in an accumulation of pre-MglB in the *dnaK756* temperature-sensitive mutant at the restrictive temperature and the defect in expression in reduced MglB synthesis in the mutant (even in conditions where export is not significantly affected). The decreased MglB synthesis correlates with a reduced stability of the *mglB* mRNA.

Several results suggest that heat shock proteins function as chaperones in protein export: the GroEL/GroES complex appears to be the primary chaperone for the export of β -lactamase (Kusukawa *et al.*, 1989). DnaK and DnaJ are involved in the export of alkaline phosphatase, a SecB-independent protein (Wild *et al.*, 1992), and the maltose receptor MalE and the maltoporin LamB in a SecB null mutant (Wild *et al.*, 1992). It has not been determined whether DnaK (like SecB which functions both as a chaperone and as a pilot protein) helps to target the precursor protein to the translocase or functions solely to maintain the precursor in an export-competent conformation. The present results suggest that DnaK does not substitute for SecB in MglB export, and that it does not eliminate the need for the SecA component of the translocase, thus favouring the hypothesis of an early interaction of the DnaK chaperone with nascent pre-MglB and subsequent transfer of the pre-protein to SecB, SecA and SecY/E.

The synthesis of MglB is reduced in the *dnaK* mutant (whereas the synthesis of MglA, which is expressed from the same operon, is not affected) and the lower synthesis of MglB correlates with a decreased stability of the *mglB* mRNA. The *mglB* mRNA derives from the polycistronic *mglBAC* mRNA, and is stabilized against 3'–5' exonucleases by a REP sequence located at its 3' extremity (Hogg *et al.*, 1991; Newbury *et al.*, 1987a, b; Stern *et al.*, 1988). It has been shown previously for the *malEFG*

(maltose transport operon) and *hisP_{JQ}* (histidine transport operon) transcripts of *E. coli*, that the 5' mRNA segment is stabilized against exonucleases by an intercistronic stem-loop REP sequence located at the 3' end of *malE* and *hisP* (Newbury *et al.*, 1987a, b; Stern *et al.*, 1988). Stabilization of the upstream *malE* and *hisP* mRNAs by REP sequences is responsible in part for the high expression of the MalE and HisP proteins, and deletion of these REP sequences destabilizes the upstream *malE* and *hisP* messages and reduces the expression of MalE and HisP. The decreased stability of the *mgIB* mRNA in the *dnaK* mutant might result from a defect in the stabilization of this mRNA by its 3'-located REP sequence. A direct interaction between DnaK and REP sequences seems unlikely, since DnaK does not possess the features of a RNA-binding protein [its ATPase activity is not affected by nucleic acids but is stimulated by peptides and unfolded proteins (reviewed in Georgopoulos *et al.*, 1994)]. The chaperone might, however, be implicated in the expression of proteins involved in the stabilization of mRNAs by REP sequences (Causton *et al.*, 1994), or in the activity of the multiprotein complex involved in mRNA degradation (Causton *et al.*, 1994; Py *et al.*, 1994; Carpoussis *et al.*, 1994), in the same way that it is implicated in the activity of the multiprotein complex involved in the replication of Lambda phages (Georgopoulos *et al.*, 1994). The implication of DnaK in mRNA stability might be restricted to a select subset of mRNAs, since the heat shock response does not appear to affect the stability of total mRNAs (Henry *et al.*, 1992). The chaperone GroEL has been recently identified as a constituent of an mRNA-protection complex in *E. coli* (Georgellis *et al.*, 1995).

Alternatively, the decreased stability of the *mgIB* mRNA in the *dnaK* mutant might be due to a decreased translation rate of the *mgIB* mRNA. It has been frequently observed that translation rates can affect the stability of mRNA molecules (Wagner *et al.*, 1994; Yarchuk *et al.*, 1992; Schneider *et al.*, 1978; reviewed in Petersen, 1993), and that synchronization of transcription and translation is important for the stability of nascent bacterial mRNAs (Wagner *et al.*, 1994; Iost & Dreyfus, 1995). DnaK is known to interact with nascent proteins (Hendrick *et al.*, 1993; Gaitanaris *et al.*, 1994), and the chaperone might interact more specifically with exported proteins (particularly with their signal sequence) than with cytoplasmic proteins. Signal sequence mutations can reduce the synthesis of exported proteins (Hall *et al.*, 1983; Puziss *et al.*, 1989, 1992), and in some cases this reduction occurs independently of an export defect (Puziss *et al.*, 1992). There might be some coupling between a DnaK-dependent folding of MglB and translation rates of the *mgIB* mRNA, leading to a decreased stability of the message in a *dnaK* mutant. A cooperation between the translation machinery and yeast hsp70s of the Ssb class has been reported (Nelson *et al.*, 1992), and DnaK appears to regulate synthesis of the heat shock Sigma factor σ^{32} at the translational level by interacting with a distinct region of the nascent protein (Nagai *et al.*, 1994). Several results suggest that in *E. coli*, 4.5S RNA regulates translation to

allow proteins to fold properly, and that the Ffh/4.5S RNA complex couples translation of exported proteins to membrane translocation (Luirink & Dobberstein, 1994; Brown, 1991; Phillips & Silhavy, 1992). The DnaK/hsp70 chaperones might also be involved in such coupling processes associated with protein folding and protein export.

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