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YbeY, a Heat Shock Protein Involved in Translation in *Escherichia coli*▽

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Here we provide evidence that YbeY, a conserved heat shock protein with unknown function, is involved in the translation process. *ybeY* deletion mutants are temperature sensitive and have a significantly reduced thermotolerance. Nonetheless, there appears to be no damage of the protein quality control of mature polypeptides, as the levels of chaperones and proteases are normal and there is no accumulation of aggregates. Rather, the mutation results in a significant reduction in the level of polysomes, and upon a shift to a restrictive temperature (42°C), there is an immediate and severe slowdown of translation. Taken together, the data indicate that YbeY is an important factor for bacterial translation even at 37°C but becomes essential at high temperatures.

Heat shock results in a dramatic increase in the rate of synthesis of a set of proteins called heat shock proteins (30), many of which are chaperones (3) and proteases (14, 34), protecting cells against damage induced by protein unfolding. Heat shock proteins are also induced by conditions that lead to protein unfolding even at low temperatures, such as exposure to heavy metals, denaturing alcohols (30), or amino acid analogs (12).

Recently, microarray experiments defining the heat shock regulon in *Escherichia coli* (7, 20, 23, 26, 32, 39) revealed the existence of novel heat shock genes. These experiments led to the characterization of heat shock proteins that function in different stages of the translation process (2, 5, 6, 18, 19, 21, 35). As with chaperones and proteases, the function of these genes is important under all growth conditions but is more critical at higher temperatures.

One of the genes identified as a heat shock gene by global transcriptional analysis is *ybeY*, whose function has not yet been determined. YbeY is a 17-kDa protein, highly conserved among bacteria, that belongs to the UPF0054 family. The sequence similarity of YbeY to metal-dependent hydrolases suggests a potential hydrolytic function. The structures of YbeY in *E. coli* (38), *Aquifex aeolicus* (24), and *Haemophilus influenzae* (36) was determined and suggest that YbeY is a metalloprotein with an active site located at the C terminus of the protein. In *A. aeolicus* (24), YbeY structure homology analysis showed similarity to eukaryotic extracelleular proteinases such as colagenase and gelatinase. However, in vitro YbeY did not have colagenease or gelatinase activity, and no other hydrolase activity could be detected.

Recently, a mutant with a deletion of SMc01113, the *Sinorhizobium meliloti* homolog of *ybeY*, was characterized in vivo (9). The SMc01113 protein is required for symbiosis of S. *meliloti* with *Medicago sativa* (alfalfa) and the SMc01113 deletion mutant is sensitive to UV, oxidative stress, and cell wall inhibitors.

Here we show that *ybeY* deletion mutants have a severe growth defect at higher temperatures and essentially no thermotolerance at lethal temperatures. However, the mutants do not appear to be defective in protein quality control of mature polypeptides. Instead, the mutants are impaired in translation, and this phenotype, which exists even at 37°C, is more pronounced as the temperature is increased. Hence, we suggest that the YbeY heat shock protein is involved, directly or indirectly, in the translation process and is particularly important at high temperatures. Moreover, the results provide additional evidence that regulation and control of ribosome activity and maintenance of efficient protein translation at high temperatures are under control of the heat shock response.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* MG1655 (ATCC 47076), a wild-type K-12 strain, was used in all experiments. The λRed system (8) was used for obtaining the *argH*, *ybeY*, and *lacZ* deletions, which were confirmed by PCR. To construct pBAD24*ybeY*, the coding sequence of *ybeY* was PCR amplified using primers containing restriction sites for EcoRI and XbaI. The digested PCR product was ligated into pBAD24 digested with the same restriction enzymes. The construction of pBAD*lacZ* was performed in a similar way.

Growth conditions. Cultures were grown exponentially in salt glycerol minimal medium (10) with aeration at 37°C, unless otherwise stated. For global transcriptional analysis of the heat shock response, wild-type cultures were grown at 30°C to an A_{600} of 0.4 and transferred to 42°C. Samples were taken at 0 and 5 min for RNA isolation. The global transcriptional analysis after canavanine treatment was carried out in a similar way, except that an arginine-requiring mutant (*argH*) was used and the medium was supplemented with arginine (50 μg/ml). The culture was pelleted by centrifugation at room temperature, and the pellet was washed twice with saline solution. Part of the culture was resuspended in the original medium, and the other part was resuspended in medium containing canavanine (100 μg/ml) instead of arginine and incubated for 30 min. The cultures were then centrifuged, washed with saline solution, and resuspended in medium with arginine. Samples were harvested for RNA isolation after 30 min of growth.

Growth measurements. Two milliliters of overnight cultures was used to inoculate 250-ml flasks containing 25 ml of medium (A_{600} = 0.05) and incubated with aeration at 37°C or at 42°C. Turbidity was monitored at 1-h intervals for 15 h.

Thermotolerance assay. Overnight cultures were diluted 1:100, and the cultures were grown to an A_{600} of 0.4 and transferred to a water bath held at 51°C.

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Samples were taken at intervals, diluted, and plated in drops on salt glycerol minimal agar plates. Colonies were counted after incubation at 37°C for 48 h.

Isolation of aggregated proteins. Aggregated proteins were purified as previously described (28, 33). Briefly, cultures were grown to an A_{600} of 0.4 and transferred to 46°C. Ten-milliliter samples were collected from the cultures at 0 and 10 min and immediately centrifuged at 7,600 × *g* and 4°C. The pellets were washed twice with cold TE-PMSF (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, 243 mg of phenylmethylsulfonyl fluoride [PMSF] per liter) and frozen at −70°C in 2-ml tubes. After addition of 0.5 ml of cold TE-PMSF, cells were lysed by sonication, centrifuged at 4°C for 30 min at 20,000 × *g*, and resuspended in 0.5 ml of cold TE-PMSF. Intact cells were removed by 15 min of centrifugation at 2,000 × *g* and 4°C. Aggregates were then pelleted by a 30-min centrifugation at 20,000 × *g*. To lower the concentration of membrane proteins, pellets were resuspended by sonication in 0.5 ml of cold TE-PMSF containing NP-40 (4:1) and centrifuged for 30 min at 20,000 × *g* and 4°C. The aggregates were resuspended in 30 μl of distilled water.

RNA isolation. Total RNA was isolated using the RNA Protect and RNeasy kits (Qiagen) as described by the manufacturer. DNase I (Promega) was used to remove genomic DNA contamination.

Real-time PCR. Two steps of real-time reverse transcription-PCR (RT-PCR) were performed. One microgram of the DNase-treated total RNA was reverse transcribed using random hexamers (Amersham) with ImPromII reverse transcriptase (Promega). To measure *lacZ* transcript levels, the forward primer 5′-GGATT CACTGGCCGTCGTTTTACA-3′ and the reverse primer 5′-GGGATGTGCTG CAAGGCGATTAA-3′ were used. PCRs were performed using 250 nM of each gene-specific primer in a 20-μl volume with 1× Sybr green PCR master mix (Applied Biosystems). Reactions were run on an ABI 7700 instrument (Applied Biosystems) using the following cycling parameters: 95°C for 10 min, 40 cycles of denaturation at 94°C for 15 s, and extension at 60°C for 1 min.

Western blots. Gel electrophoresis was carried out according to published protocols using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Rabbit anti-GroEL (Sigma) and mouse anti-LacZ (Promega) were used as primary antibodies and horseradish peroxidase-conjugated antibodies as secondary antibodies. The EZ-ECL chemiluminescence detection kit (Biological Industries) was used for detection.

Global transcriptional analysis. Reverse transcription and labeling were performed using the CyScribe PostLabeling kit (Amersham) as described by the manufacturer except for the following modifications. Fifteen micrograms of total RNA and 2 μl of the nonamers were used in the reverse transcription. *E. coli*-spotted DNA microarrays were purchased from the Gene Expression Center, University of Wisconsin. Hybridization and washing of the arrays were performed according to the protocols published on the Gene Expression Center website (http://www.biotech.wisc.edu/gec/). The arrays were scanned at a resolution of 10 μm per pixel for fluorescence emission at 532 nm for Cy3 and at 635 nm for Cy5. Scans were preformed using the Affymetrix 428 array scanner (Affymetrix). Image analysis was carried out with the Imagene 5 software (BioDiscovery). For normalization and ratio calculations, we used GeneSight 3.0 (BioDiscovery). The signal intensities were background subtracted and normalized by the total array intensity. Genes were considered upregulated relative to nontreated cultures if they had a 2.5-fold increase in signal intensity.

Polysome profiles. Polysome profiles were prepared essentially as described previously (18, 27). In brief, cultures were grown at 37°C and harvested when the optical density at 600 nm (OD_{600}) reached 0.5. Chloramphenicol was added to a final concentration of 200 μg/ml at 3 min before harvest. The cells were poured onto an approximately equal volume of chipped ice and harvested by centrifugation. The cell pellet was resuspended in 1 ml of cold buffer (20 mM Tris-HCl [pH 7.5], 4 mM β-mercaptoethanol, 6 mM $MgCl_2$, 30 mM NH_4Cl_2) per 100 ml of culture, and lysozyme was added to a final concentration of 0.75 mg/ml. The cells were shock frozen with dry ice and acetone and thawed in an ice-water bath. The freeze-thaw was repeated twice, and then 10% deoxychoalte was added to a final concentration of 0.25% and left for 5 min on ice. The lysate was clarified by 20 min of centrifugation at 22,000 × *g* at 2°C and used immediately. Sucrose gradients were formed by layering of different concentrations of sucrose followed by diffusion. A 1.8-ml aliquot of a 50% sucrose solution (Sigma) and 2.5 ml each of 40/30/20 and 10% sucrose solutions in 20 mM Tris-HCl (pH 7.5)–4 mM β-mercaptoethanol–10 mM $MgCl_2$–100 mM NH_4Cl_2 were layered into Beckman polyallomer SW41 tubes, and diffusion was allowed to proceed overnight in 4°C. Thirteen A_{260} units of the cell lysates was applied on the top of the gradient, and the gradients were centrifuged in an SW41 rotor at 39,000 rpm for 2 h. Two-hundred-microliter fractions were collected on UV-transparent enzyme-linked immunosorbent assay plates (Corning), and absorbance was detected at a wavelength of 260 nm.

TABLE 1. Genes induced by protein unfolding[a]

Gene	Function	Expression ratio	
		Canavanine/arginine	Heat shock/30°C
clpB	Protein disaggregation chaperone	7.0	11.3
dnaJ	Cochaperone with *dnaK*	4.3	8.0
dnaK	Chaperone Hsp70	4.0	8.0
groL	Chaperone hsp60	3.7	7.5
groS	Chaperone hsp10	3.5	13.0
grpE	Protein repair	2.8	8.6
hslU	ATPase component of protease	4.9	7.0
hslV	Peptidase component of protease	2.5	6.5
htpG	Chaperone hsp90	7.5	14.9
ibpA	Small heat shock protein	12.1	59.7
ibpB	Small heat shock protein	14.9	128.0
lon	Protease	8.0	6.1
ybbN	Chaperone and oxidoreductase	3.2	7.0
ybeY	Unknown	3.5	3.0
ybeZ	Unknown	3.7	3.2
yciH	Translation initiation factor	2.5	4.3
ycjF	Unknown	8.6	6.5
ycjX	Unknown	7.0	7.5
yhdN	Unknown	3.5	4.6

[a] Gene expression was determined using microarrays as described in Materials and Methods and represent three microarrays at elevated temperature and two microarrays with canavanine. The expression ratios of the genes listed were verified by real time RT-PCR.

RESULTS

Identification of *E. coli* genes induced by protein unfolding. It is generally accepted that the heat shock response, mediated by σ^{32}, is induced by the presence of unfolded proteins (12, 15, 29). However, the overlap between the elevated temperature and the σ^{32} stimulons is far from complete (39), raising the question which of the genes respond to protein unfolding. In addition, it is conceivable that there are genes that respond to protein unfolding by molecular mechanisms other than by σ^{32} alone.

In order to identify these genes that respond to protein unfolding, we performed transcriptional analysis under conditions that specifically bring about such unfolding. Thus, we performed global expression profiling of *E. coli* following treatment with canavanine and compared it with the transcriptome obtained after 5 min of heat shock at 42°C. Canavanine is an analog of the amino acid arginine and is incorporated into newly synthesized polypeptides instead of arginine. These polypeptides tend to unfold and aggregate (12, 13, 25). Thus, the canavanine treatment leads to the accumulation of unfolded proteins and aggregates at low temperatures. Cultures were treated with canavanine for 30 min to produce proteins containing the analog, washed, and incubated with arginine for an additional 30 min before RNA was extracted.

The results summarized in Table 1 present genes induced following the two treatments, exposure to 42°C and to canavanine. We present the data as the ratio of gene expression after 5 min at 42°C or after 30 min with canavanine to the expression at 30°C. Only genes with an expression ratio of higher than 2.5-fold in both treatments are shown.

As can be seen from the data, most of the known genes encoding chaperones and proteases are within this group, re-

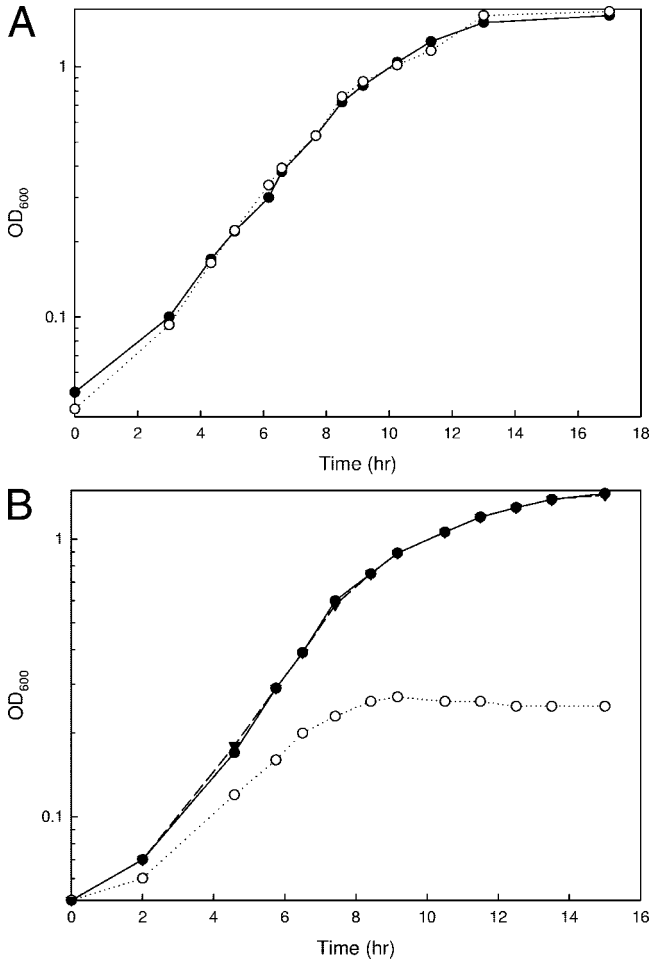

FIG. 1. Effects of the *ybeY* deletion on cell growth. Cultures of the wild type (closed circles), the Δ*ybeY* mutant (open circles), and the Δ*ybeY* mutant carrying pBAD24*ybeY* (closed triangles) were grown in glycerol salt minimal medium at 37°C (A) or 42°C (B).

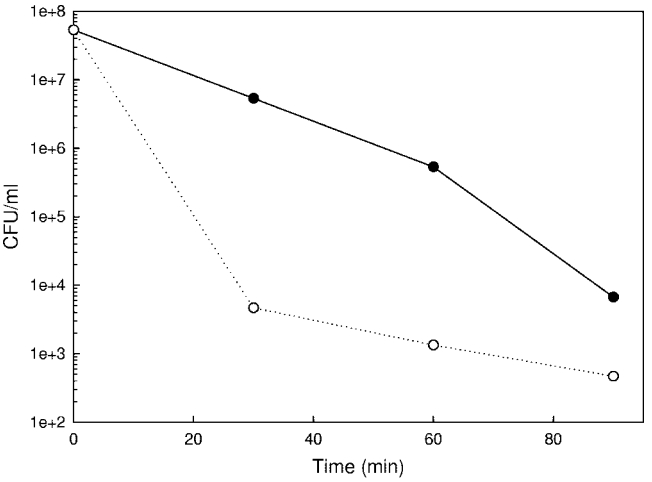

FIG. 2. Effect of the *ybeY* deletion on survival at lethal temperatures. Cultures of the wild type (closed circles) and the Δ*ybeY* mutant (open circles) were grown exponentially at 37°C to and OD_{600} of 0.4 and transferred to 51°C. Samples were removed at intervals, diluted, and plated at 37°C.

inforcing our basic assumption that both treatments result in protein unfolding. Five additional genes encoding proteins with unknown function exhibit the same transcriptional behavior as well. These genes cluster in three operons, *ycjXF*, *ybeZY*, and *yhdN*, all of which were previously reported to be regulated by σ^{32} (23).

YbeY is essential for growth at high temperatures and for survival at lethal temperatures. *ycjX*, *ycjF*, *ybeZ*, *ybeY*, and *yhdN* null mutants were constructed using the λRed gene deletion system (8). No major phenotype was detected in the *ycjX*, *ycjF*, *ybeZ*, and *yhdN* mutants in a set of standard assays. However, the *ybeY* mutant showed severe temperature sensitivity. Although at 37°C the mutant grows as well as the wild type (Fig. 1A), its growth rate at 42°C is substantially decreased, leading to complete inhibition after several hours (Fig. 1B). Growth at 42°C was restored by complementation with the wild-type gene on a plasmid (Fig. 1B), proving that deletion of *ybeY* and not interference with the function of a neighboring gene is responsible for the observed temperature sensitivity.

We next examined the ability of the *ybeY* mutant to recover from exposure to a lethal temperature of 51°C (Fig. 2). After 30 min of incubation at the lethal temperature, the mutation in *ybeY* led to a dramatic increase in mortality, i.e., about 4 orders of magnitude, compared to less than 1 order of magnitude in the wild type, which exhibited a reduction in viability of 4 orders of magnitude only after 90 min at 51°C.

These data indicate that the function of YbeY becomes essential as the growth temperature increases, similar to the function of major heat shock genes.

The levels of total protein aggregates are similar in the *ybeY* mutant and in the wild type. The temperature-dependent and reduced-thermotolerance phenotype of the *ybeY* mutant is similar to the phenotype of chaperone and protease mutants that are involved in protein quality control. This phenotype is due to a decreased ability to resolve protein unfolding, prevent protein aggregation, and dissolve aggregates and leads to accumulation of aggregates at high temperatures (22, 28). Thus, if YbeY functions in the protein quality control system, we would expect an increase in the level of aggregates in *ybeY* null mutants. However, analysis of aggregates in samples taken from the *ybeY* mutant and from the wild type at 37°C and following a 10-min incubation at 46°C indicated that the levels and compositions of total aggregates were essentially similar in the wild type and in the mutant (Fig. 3).

Mutations in heat shock genes encoding major chaperones and proteases also result in higher expression levels of other chaperones and proteases (29). This phenotype was also not observed in *ybeY* mutants, as no difference in the levels of the major chaperone GroEL in the wild type and in the *ybeY* mutant could be detected at 37°C (Fig. 4).

The findings that *ybeY* deletion mutants do not accumulate more aggregates than the wild type, even at 46°C, and do not show higher expression levels of a major chaperone indicate that the mutation is not directly involved in the protein quality control pathway.

YbeY is essential for translation but not for transcription during growth at high temperatures. Recently, several studies

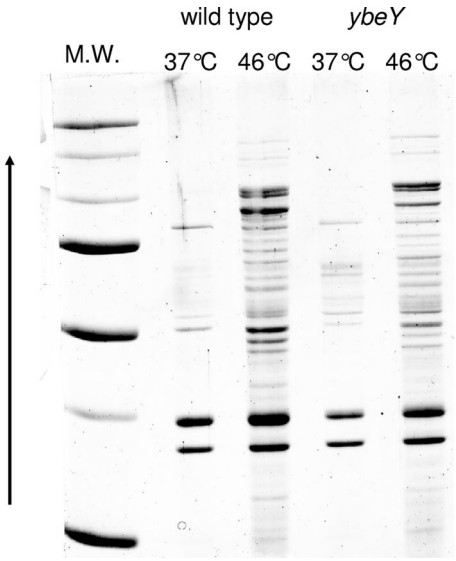

FIG. 3. Effects of the *ybeY* deletion on in vivo protein aggregation. Cultures of the wild-type and Δ*ybeY* strains were grown as described for Fig. 2 and transferred to 46°C for 10 min. Insoluble protein fractions were collected before the temperature upshift and after 10 min and analyzed by SDS-PAGE.

characterized novel heat shock proteins that function throughout different stages of the translation process (2, 5, 6, 18, 19, 21, 35). The function of these heat shock proteins is particularly important during growth at high temperatures. In view of these studies, we examined the ability of the *ybeY* mutant to translate a model protein, LacZ, following a shift from permissive growth conditions to restrictive growth conditions at a high temperature.

Wild-type and *ybeY* mutant cultures, both lacking the endogenous copy of *lacZ*, carrying a plasmid with the *lacZ* gene under control of an inducible arabinose promoter were grown exponentially at 37°C. The cultures were then shifted to 42°C, a restrictive temperature for growth of the *ybeY* mutant, and induced with arabinose, and the levels of LacZ were determined at the indicated time points (Fig. 5). Whereas in the wild-type samples the LacZ protein accumulated to very high levels, the accumulation in the *ybeY* mutant samples was drastically lower.

These results may suggest that YbeY is required for efficient translation. However, this phenotype can be an indirect result of a defect in the transcription of *lacZ* rather than in the translation process itself. To test this possibility, transcript levels of *lacZ* were measured by real-time RT-PCR in parallel with LacZ protein level determination (Fig. 5). The results clearly show that in the *ybeY* mutant the transcript level of *lacZ* following induction with arabinose at 42°C is essentially the same as in the wild type. These results suggest that the lower levels of LacZ at high temperatures in *ybeY* mutants are due to a translational defect and are not an indirect consequence of a transcriptional defect.

Therefore, YbeY is essential for translation of a model protein at high temperatures. Moreover, the translational defect of the *ybeY* mutant is already detectable under normal growth

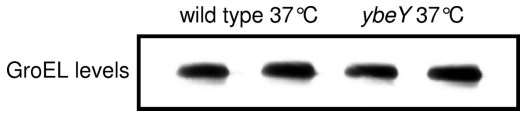

FIG. 4. Effect of the *ybeY* deletion on the level of GroEL. Cultures of the wild-type and Δ*ybeY* strains were grown as described for Fig. 2, and samples were removed, separated by SDS-10% PAGE, and Western blotted with rabbit anti-GroEL antibody.

conditions at 37°C, suggesting that the function of YbeY is important for translation even at 37°C (Fig. 6).

Analyses of ribosome profiles of the *ybeY* mutant point to a global translational defect. The failure of *ybeY* mutants to translate the *lacZ* product at the same rate as the wild type could reflect a global translational defect in the mutant. Ribosome analyses in sucrose gradients indicate that the *ybeY* mutation leads to a major shift from polysomes to single ribosomes and ribosome subunits (Fig. 7). The wild-type strain, growing at 37°C, shows a standard ribosome profile, with most ribosomes present in polysomes and minor amounts of ribosomal subunits (30S and 50S) and 70S complexes. Such profiles indicate that most ribosomes in the cell are engaged in translation. In contrast, the *ybeY* mutants exhibit an altered ribosome profile: there is a substantial accumulation of ribosomal subunits and a very low level of polysomes, suggesting that most ribosomes in the cell are not translating. These results support the assumption that the translational defect in the *ybeY* mutant is global.

The finding that the level of polysomes is drastically reduced in *ybeY* mutants even at 37°C indicates that YbeY participates in the translation process even under non-heat shock conditions (37°C).

DISCUSSION

In this communication, we characterize the highly conserved heat shock protein YbeY and show that it is required in the translation process. This protein is important for translation even at 37°C (Fig. 6 and 7) but is critical at high temperatures, as can be seen from the significantly reduced translation rate (Fig. 5) and the extreme temperature sensitivity of null *ybeY* mutants (Fig. 1B and 2).

YbeY is a heat shock protein that functions in the translation process. As such, it belongs to a growing group of heat shock proteins that do not participate in quality control of mature polypeptides but take part in various stages of the translation process. Recent studies characterized three novel heat shock proteins whose function is important for ribosome assembly/stability (FtsJ) (2, 5, 6), fidelity of translation (YciH and FtsJ) (21, 35), and the recycling of 50S particles following aberrant translation termination events (Hsp15) (18, 19). It has been shown that the function of these heat shock proteins is particularly important under heat shock conditions. *ftsJ* deletion mutants are temperature sensitive (2), and Hsp15 recycles the 50S subunit from an erroneous state that is more prevalent at heat shock temperatures (18). Thus, it appears that in addition to chaperones and proteases, the heat shock regulon also contains several translation factors. These findings indi-

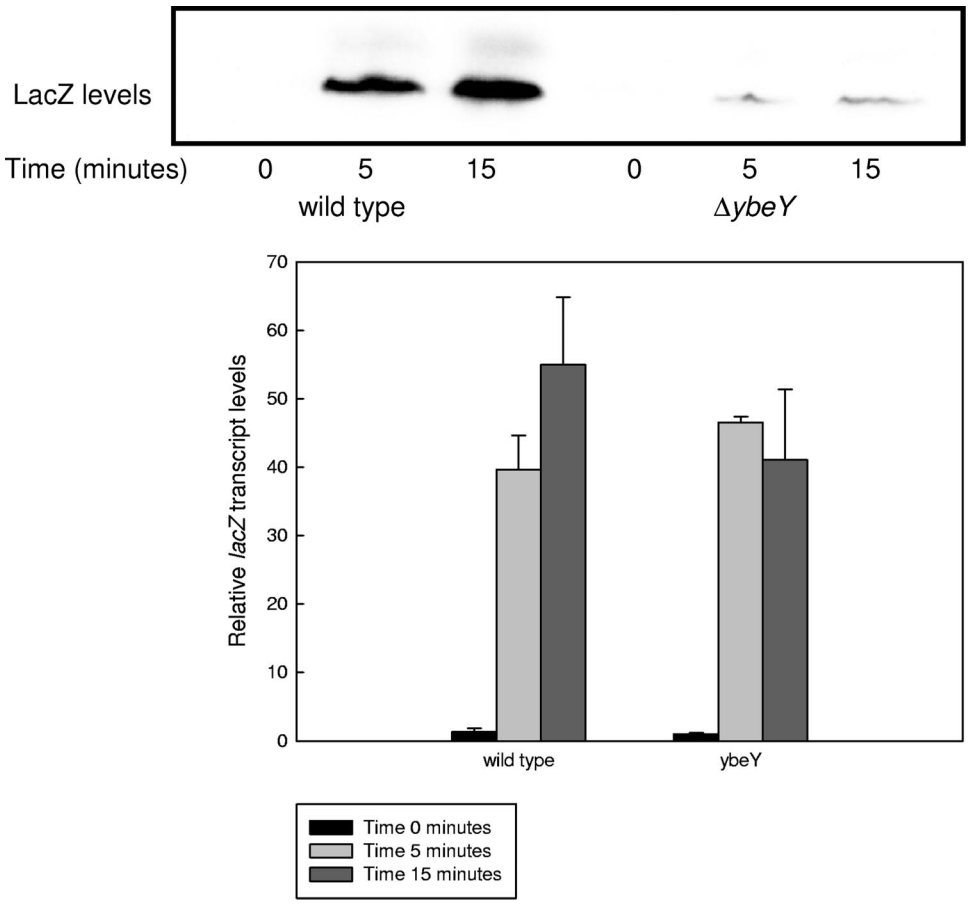

FIG. 5. Effects of the *ybeY* deletion on transcription and translation of *lacZ* at 42°C. Cultures of the wild-type Δ*lacZ* strain carrying pBAD24*lacZ* and of the Δ*ybeY* Δ*lacZ* strain carrying pBAD24*lacZ* were grown exponentially to an OD_{600} of 0.4 at 37°C (time = 0) and induced with arabinose (0.2%) following transfer to 42°C. Samples were collected at the indicated times. LacZ protein levels were determined using SDS-10% PAGE separation, followed by Western blotting with mouse anti-LacZ antibodies (top panel), and *lacZ* transcript levels were measured using real time RT-PCR as described in Materials and Methods (bottom panel). Error bars indicate standard deviations.

cate the importance of the heat shock response in regulating the efficiency of the translation process.

The *ybeY* gene was identified in a screen for genes which are induced by protein unfolding, i.e., in response to elevation of temperature and incorporation of an amino acid analog (canavanine) (Table 1). The induction of *ybeY* is mediated by σ^{32}, as in *rpoH* mutants the transcript levels of *ybeY* do not increase following heat shock (reference 23 and unpublished results). In fact, all the genes that we identified as induced by protein unfolding are regulated by σ^{32} (Table 1). Thus, it seems that σ^{32} is the principal transcriptional regulator which is sensitive to the protein folding status in the cells.

The finding that σ^{32} regulates the expression of proteins that function in the translation process further strengthens the linkage of translation and the heat shock response. The increase in the rate of translation elongation in the 37°C to 42°C temperature range (11) may decrease the fidelity of translation, which is the primary requirement for correct protein folding (13). This increase in translation elongation rate was suggested to compensate for the increased protein degradation rate at heat shock temperatures (11). In addition, it was demonstrated that the state of ribosomes could serve as a signal to induce the heat shock response (31).

Although the growth of *ybeY* mutants is inhibited at 42°C (Fig. 1), it is clear that the function of YbeY is important for translation even at non-heat shock temperatures. Thus, even at 37°C the *ybeY* mutant exhibits reduced LacZ synthesis (Fig. 6) and a completely altered polysome profile (Fig. 7). Apparently, the limitation in ribosome activity is compensated for by an increased synthesis of ribosomes (unpublished data) when the bacteria are growing slowly in minimal salt glycerol medium. However, under growth conditions that support growth at higher rates, such as in rich media, the growth rate of the mutant is lower than that of the wild type even at 37°C (unpublished results). These results indicate that the protein synthesis capacity of the *ybeY* mutant is rate limiting for growth in rich media.

YbeY, a 17-kDa protein, is stable in vivo and belongs to the UPF0054 family. The sequence similarity of YbeY to metal-dependent hydrolases suggests a potential hydrolytic function (1, 36, 37, 38). The assumption that YbeY functions in an enzymatic rather than a stoichiometric manner is also sup-

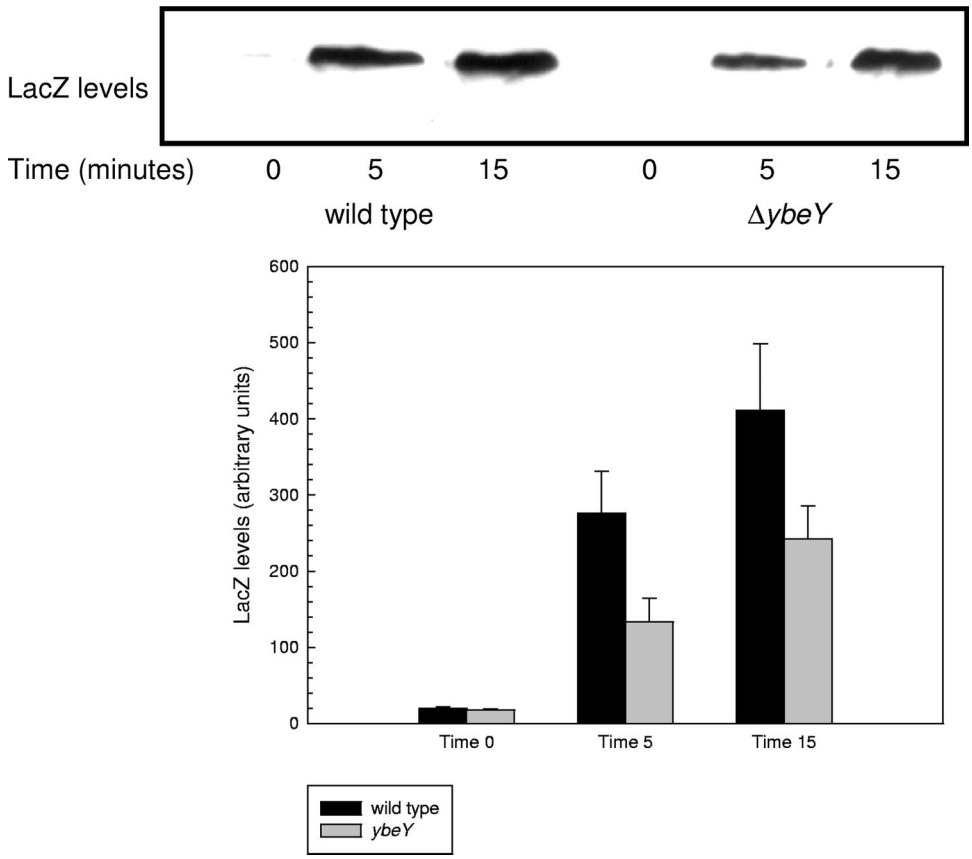

FIG. 6. Effects of the *ybeY* deletion on translation of *lacZ* at 37°C. The experiment was performed as described for Fig. 5 except that cultures were induced with arabinose at 37°C. The Western blot is shown in the top panel, and the densitometric quantification is shown in the bottom panel. Error bars indicate standard deviations.

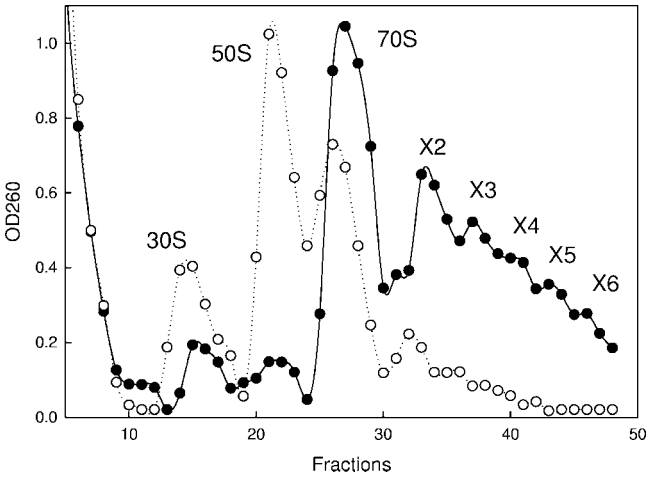

FIG. 7. Effect of YbeY on polysome profile. Cultures of the wild type (closed circles) and the Δ*ybeY* mutant (open circles) were grown as described for Fig. 2. They were treated with chloramphenicol (200 μg/ml) for 3 min, harvested, and lysed as previously described (27). A total of 13 A_{260} units of the lysates was loaded onto a 7 to 47% sucrose gradient, fractionated, and analyzed as described in Materials and Methods.

ported by the finding that its cellular concentration is low and tightly regulated, as its overproduction is toxic at high temperatures.

The *ybeY* gene of *E. coli* is the second gene in the *ybeZY* operon. The first gene, *ybeZ*, is well conserved among bacteria as well. This gene is a homolog of the *Bacillus subtilis phoH* family (16, 17). Positional coupling of this family with neighboring genes points to its involvement in putative metal dependent RNA modification (16). The N terminus of YbeZ has homology to the N-terminal domains of superfamily I RNA helices (17). According to studies of the *E. coli* interactome, YbeZ interacts with YbeY and, in addition, interacts with many ribosomal proteins (4). These data are compatible with the function of the *ybeZY* operon in translation.

One possibility is that the primary function of YbeY is proteolytic. Such a protease could regulate the translation process by degrading an inhibitor or repressor. This possibility is supported by the findings that YbeY is structurally very similar to metalloproteases (24, 38). The fact that its proteolytic activity could not be shown by use of various protease does not rule out this possibility, as YbeY may have narrow specificity or require additional factors (cofactors, other subunits or proteins, etc). As a matter of fact, YbeY also lacks an ATPase domain. However, YbeZ has such an ATPase domain that resembles that of the Clp proteases (A. Rasouly et al., unpublished data). We therefore examined the possibility that YbeZ could inter-

act together with YbeY to produce proteolytic activity, but we could not obtain evidence for such activity. Moreover, a *ybeZ* deletion showed no phenotype in the wild type as well as in the *ybeY* deletion mutant, a result that is difficult to explain if YbeZ is required for YbeY activity.

ybeY deletion mutants show translational defects, indicating that the YbeY protein is involved in translation. This protein could be directly involved in translation, by stimulating or stabilizing a reaction or by acting as a chaperone of one or more proteins involved in translation. However, it is conceivable that YbeY acts indirectly in translation by affecting the activity or concentration of a component of the translation machinery. Clearly, this matter will be resolved when the function of YbeY is fully understood. At any rate, we propose that the highly conserved heat shock protein YbeY is involved in maintaining efficient protein translation. Its activity is required even at non-heat shock temperatures and becomes essential for translation at high temperatures.

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