Heat shock protein GroE of *Escherichia coli*: key protective roles against thermal stress

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An *Escherichia coli* mutant lacking the heat shock σ -factor (σ^{32}) is defective in transcription from heat shock promoters and cannot grow at temperatures above 20°C. To assess physiological roles of σ^{32} and heat shock proteins, we isolated and characterized a set of temperature-resistant revertants from this deletion ($\Delta rpoH$) mutant. Most of them were found to carry a DNA insertion in the *groE* upstream region, resulting in high-level synthesis of major heat shock proteins GroE (GroES and GroEL). The levels of GroE produced varied in different revertants and correlated well with the maximum permissive temperatures; the highest GroE producers (~10% of total protein) grew up to 40°C but not at 42°C. An additional mutation causing hyperproduction of DnaK (hsp70 homolog) was required for growth at 42°C. Such effects of GroE and DnaK on the σ^{32} -deletion strains were also confirmed by using multicopy plasmids carrying *groE* or *dnaK*. Thus, GroE plays a key protective role in supporting growth at normal physiological temperatures (20–40°C), whereas high levels of DnaK are required primarily at higher temperature.

[**Key Words:** Heat shock response; heat shock protein; σ factor; GroE protein; DnaK protein; bacterial growth]

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A specific set of heat shock proteins (hsps) is markedly induced when cells are exposed to higher temperature. Such induction of hsps has been found virtually in all organisms (Schlesinger et al. 1982). About 20 hsps are known in *Escherichia coli* (Neidhardt et al. 1984); among them DnaK and C62.5 are highly homologous to eukaryotic hsp70 and hsp83, respectively (Bardwell and Craig 1984, 1987). GroEL was recently shown to be structurally related to a mitochondrial hsp58 of several eukaryotic species (McMullin and Hallberg 1988). As in eukaryotes, *E. coli* hsps are induced not only by thermal stress but also by other agents (see Neidhardt et al. 1984; Neidhardt and VanBogelen 1987), implying that hsps play some protective functions. Many of the hsps are implicated in the synthesis, assembly, and turnover of nucleic acids and proteins, but their precise roles in bacterial growth remain unknown.

The heat shock induction in *E. coli* is achieved by increasing transcription of hsp genes (Yamamori and Yura 1980), and is mediated by the product of a regulatory gene (*rpoH*, *htpR*, *hin*) (Neidhardt and VanBogelen 1981; Yamamori and Yura 1982), now identified as a minor σ factor (σ^{32}) (Grossman et al. 1984; Landick et al. 1984; Yura et al. 1984). Transcription initiated at the heat shock promoters is markedly but transiently enhanced by exposure to higher temperature (Grossman et al. 1984; Taylor et al. 1984; Cowing et al. 1985), presumably due

to rapid and transient increase in the σ^{32} level (Grossman et al. 1987; Skelly et al. 1987; Straus et al. 1987).

The σ^{32} -mediated transcription of hsp genes not only plays an important role in the heat shock response but also in normal balanced growth of *E. coli*, because *rpoH* null mutants lacking σ^{32} cannot grow at temperatures above 20°C (Zhou et al. 1988). Analysis of hsp transcripts in these mutants indicated that σ^{32} is solely responsible for transcription from heat shock promoters. Although the physiological significance of the heat shock response is not well understood, the maintenance of steady-state hsp levels and their control upon heat shock is clearly important for normal cell growth, as well as for survival at extreme temperatures.

The isolation and characterization of the temperature-resistant suppressor of the $\Delta rpoH$ strain has allowed us to further dissect the role of hsps during balanced growth. The majority of the single-step revertants exhibited elevated synthesis of the GroES and GroEL proteins. The maximum permissive growth temperature of each revertant correlated well with the amount of GroE proteins produced, suggesting that the enhanced synthesis of two (GroES and GroEL) out of some 20 hsps affected by the $\Delta rpoH$ mutation compensated effectively for the lack of σ^{32} in sustaining cell growth at the normal temperature range (20–40°C). Enhanced synthesis of another hsp (DnaK protein), in addition to GroE, supported growth of the $\Delta rpoH$ strain at temperatures higher than those attained by GroE alone.

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Results

Isolation of temperature-resistant revertants from σ^{32} deletion ($\Delta rpoH$) mutant

As reported elsewhere (Zhou et al. 1988), we have constructed σ^{32} deletion mutants by replacing most of the coding region of the chromosomal *rpoH* by a kanamycin-resistance gene. The deletion ($\Delta rpoH$) strains grow at or below 20°C but not at higher temperatures, and no hsp induction is observed upon temperature upshift. Spontaneous temperature-resistant revertants were obtained from the deletion mutant by one-step selection at 30, 34, 37, and 40°C, but not at 42°C. The reversion frequencies were inversely correlated with the temperature used (10^{-7} to 10^{-9}). Revertants that can grow at 42°C were obtained by a two-step selection at 30°C and then at 42°C; these revertants therefore carry at least two mutations (see below). The revertants were tested for their viability at different temperatures and grouped into four classes (Fig. 1; e.g., R30 designates revertants that grow up to 30°C). R40 revertants exhibited the highest thermoresistance among the single-step revertants and were analyzed most extensively. In peptone (P) broth, R40 grew exponentially at temperatures between 25 and 37°C, and the doubling times were 1.4- to 1.7-fold longer than those for wild type (*rpoH*⁺). At temperatures above 40°C, they grew into long filaments and gradually lost viability (Fig. 2). R40 revertants such as KY1603 exhibited slightly cold-sensitive growth, i.e., very slow growth at 20°C, particularly in minimal medium.

Figure 1. Temperature profile of viability for representative classes of revertants. Exponentially growing cultures at 20°C (for $\Delta rpoH$ and R30) or at 30°C (for R34, R40, and R42) were diluted and plated onto P agar and incubated at the temperatures indicated. The number of colony formers was counted after 24–72 hr (depending on the incubation temperature) and normalized to the value at an optimum temperature for each strain.

Figure 2. Morphology of R40 revertant cells. Cells of KY1603 growing exponentially in L broth at 37°C (A) or after incubation at 42°C for 4 hr (B) were collected, spread onto microslides, and stained with Eosin–Giemsa. Cells of wild-type MC4100 grown at 37 or 42°C were essentially identical to those of R40 at 37°C (not shown).

Constitutive synthesis of GroE proteins

The dramatic suppression of temperature-sensitive growth by R40 reversions suggested that these revertants might also respond to heat shock. Twenty independently isolated R40s were examined for protein synthesis at 30°C and after shift to 42°C. None of the revertants exhibited heat-shock-dependent increase in the synthesis rates of hsps (87K, DnaK, GroEL, etc.). The response of one particular mutant R40-1 to temperature upshift is displayed in Figure 3A. In R40-1, and in all other revertants, the synthesis rate of a 64-kD protein was higher than that in the wild type, both at 30 and 42°C. This protein was identified as one of the major hsps, GroEL, by immunoprecipitation (Fig. 3B).

The enhanced synthesis of GroE in suppressor strains could result from a new *cis*-acting regulatory region located upstream to *groE*. Alternatively, the suppressor could affect a *trans*-acting factor that stimulated the expression of *groE*. Experiments using R40 revertants lysogenic for a recombinant λ phage carrying *lacZ* fused to the *groE* promoter (λ pF13-P*groE*-*lacZ*) ruled out the second possibility, because the levels of β -galactosidase activities, both at 30°C, and after shift to 42°C, were lower than in the wild-type background. If a *trans*-acting factor were affected, the *groE* promoter activity determined by β -galactosidase would have been higher than in the wild type.

Suppressors map at the *groE* region on the chromosome

P1 phage-mediated transduction experiments revealed that the mutation sites in R40 revertants lie near *groE*.

Tetracycline-resistance transposons *zje::Tn10* and *zje1::Tn10*, located on the opposite sides of *groE* (at roughly the same distance from the structural gene), were transduced from an otherwise wild-type donor into three independently isolated R40 revertants. Among the tetracycline-resistant transductants selected at 20°C, ~50% were temperature sensitive (nonviable at or above 30°C), which agreed with the known cotransduction frequencies between *groE* and the Tn10s. Similar experiments with one of the R30 revertants gave identical results. The suppressor locus in the R40 and R30 revertants was designated *suhX* and localized at or around the *groE* operon.

We then cloned the *groE* region from the chromosome of R40s (R40-1, R40-2, and R40-3) and examined the mutation sites more closely. The *groE* operon, located on an 8 kb *EcoRI* fragment, contains two adjacent genes, *groES* and *groEL* (Tilly et al. 1981), that are transcribed from two promoters located upstream of *groES* (Zhou et al. 1988). The two promoters, *groES*, and part of *groEL* are contained in a 2-kb *EcoRV* fragment (Fig. 4A). Chromosomal DNAs from R40s were digested with *EcoRI* or *EcoRV*, joined with pBR322, and recombinant plasmids that complement the temperature-sensitive phenotype of *groES619* or *groEL44* strains were selected. Examination of the cloned DNAs by restriction enzymes revealed that the sizes of the *EcoRI* fragment and the *EcoRV* fragment derived from R40s were 9 and 1.75 kb, respectively, clearly different from those of the respective fragments from wild type. Further experiments established that the chromosomes from all R40s tested

Figure 3. Polyacrylamide gel electrophoresis of proteins. (A) Log-phase cells grown in minimal medium were pulse-labeled with [³⁵S]methionine for 3 min at 30°C or after shift to 42°C for 5, 20, and 60 min. Whole-cell proteins were analyzed by SDS-gel electrophoresis (10⁵ cpm/lane). Arrowheads indicate bands (hsps) that are transiently enhanced by temperature shift in the wild-type MC4100 (WT). (B) Labeled proteins from A were treated with antiserum against GroEL, and the precipitates were electrophoresed as in A.

Figure 4. DNA structure and transcription of the *groE* region. (A) Physical map of the *groE* region. Solid boxes indicate the *groE* genes; the open box represents an insertion on the chromosome of R40-1 (KY1603). Arrows indicate the start points of *groE* transcripts. (A) *AhaII*; (B) *BamHI*; (Bs) *BstEII*; (RI) *EcoRI*; (RV) *EcoRV*; (H) *HindIII*; (S) *SmaI*; (Sa) *SacII*. (B) Nuclease S1 mapping of *groE* mRNAs. RNAs were extracted from log-phase cells grown in L broth at 30°C (20°C for the $\Delta rpoH$ parent) and hybridized with probe DNA derived from the R40-1 chromosome shown in A (5' labeled at the *SacII* site). Arrows indicate positions of messengers transcribed from the major heat shock promoter (1), a minor σ^{70} promoter (2), or a new promoter seen in R40s (3) and undigested probe. The open arrowhead indicates the endpoint of *groE* upstream sequence joined to the insertion on the R40 (and presumably R30 and R42) chromosome, based on the results of Figure 5B. Data for R40-3 and R42-2 are not shown.

contained a 1-kb insertion located upstream of the *groE* heat shock promoter (1) (see Fig. 4A). The *EcoRI* fragment from the R30 revertant was also larger than that from wild type, indicating that a suppressor affected the *groE* region.

groE transcription starts from within the insertion

To examine whether the insertions affected the *groE* transcription, we determined the start points of *groE* mRNA in the revertants by nuclease S1 mapping. The promoter-proximal region (*EcoRV*–*SacII* fragment) of

groE DNA obtained from R40-1 (KY1603) was initially used as a hybridization probe. As seen in Figure 4B, *groE* mRNA in the wild type was mostly transcribed from the heat shock promoter (promoter 1), whereas in the $\Delta rpoH$ revertants it was transcribed from new start points. In the three R40s, a start point (promoter 3) was found within the insertion at about 100 bases upstream from the junction (the junction was verified by S1 mapping using wild-type *groE* DNA as a probe; an example is seen in Fig. 5). Substantial amounts of undigested probe were

seen in R40s suggesting the presence of additional transcript(s) initiated from farther upstream. Thus, the elevated levels of *groE* mRNA seem to be attributable to these multiple transcripts. Three independently isolated R40s were examined and gave virtually identical results. The R30 revertant (KY1615) gave a new start point that probably reflects a structural alteration that is distinct from those seen in R40s. The R42 revertants that were obtained by two-step selections at 30°C and then at 42°C produced moderate amounts of *groE* transcript different from R30 but similar to the transcript from promoter 3 found in R40s.

In $\Delta rpoH$ strains, *groE* transcript initiates at a downstream promoter (promoter 2 in Fig. 4B), known to be transcribed by RNA polymerase containing σ^{70} (Zhou et al. 1988). This is a weak promoter and virtually inactive in R40s or in the *rpoH*⁺ strain, presumably due to interference by strong transcription from upstream promoters.

Suppression of the $\Delta rpoH$ mutant by plasmids carrying *groE*

The above results strongly suggest that the cellular level of GroE protein(s) is critical when cells lacking σ^{32} are exposed to higher temperature. We then introduced multicopy plasmids carrying part or whole of the *groE* operon into the $\Delta rpoH$ mutant. In these strains, *groE* transcription on the plasmid should occur only from promoter 2, but might cause synthesis of GroE proteins sufficient to suppress the temperature-sensitive growth. Indeed, a plasmid carrying both *groES* and *groEL* (*pgroESgroEL*) supported growth of the $\Delta rpoH$ strain up to 37–40°C (Table 1). The plasmid carrying only *groES* was less effective but supported growth up to 34°C. In contrast, a plasmid containing only intact *groEL* was hardly effective, though this could be due to a lower copy number of the plasmid used.

Introduction of *pgroESgroEL* into R40 revertants inhibited rather than stimulated growth at all permissive temperatures (20–40°C) and failed to support growth at 42°C. The growth inhibition was particularly marked at 20°C in the presence of selective antibiotic, and transfer of cells to a nonselective medium caused rapid loss of the plasmid. It should be recalled that R40 itself shows slightly cold-sensitive growth (see above).

The magnitude of GroE production correlates with the upper limit of growth temperature

The correlation between the GroE levels and upper limits of growth temperature was explored by determining both *groE* message and GroEL protein in various strains at different temperatures. The amount of *groE* mRNA was determined by nuclease S1 protection, using excess 5'-labeled promoter-proximal region of wild-type *groE* DNA as a probe (Fig. 5). The *groE* mRNA in R40 at 30°C was very high and comparable to the heat-induced wild type. Much less but appreciable amounts of transcript from promoter 2 are seen in the $\Delta rpoH$ mutant carrying *pgroESgroEL*. The R30 revertant produced still

Figure 5. Quantitation of *groE* mRNA levels. Log-phase cells grown in L broth at the temperature indicated or after transfer to 42°C were used for RNA extraction. RNAs (40 μ g) were hybridized with excess 5'-labeled wild-type *groE* promoter region (*EcoRI*–*SacII* fragment) and subjected to quantitative nuclease S1 protection. Arrows indicate mRNAs initiated from promoter 1 or 2, and the open arrowhead shows the endpoint of the *groE* upstream sequence, as in Figure 4B. (A) Wild-type MC4100 and $\Delta rpoH$ mutant cells at 20°C or after heat shock (HS) at 42°C for 15 min. (B) The $\Delta rpoH/pgroESgroEL$ strain and R30 or R40 revertants grown at 30°C.

Table 1. Growth of $\Delta rpoH$ and R40-1 strains carrying a plasmid

Strain	Plasmid	Growth at temperature (°C)						
		20	25	30	34	37	40	42
$\Delta rpoH$ (KY1612)	—	+	—	—	—	—	—	—
	<i>pgroESgroEL</i>	+	+	+	+	+	±	—
	<i>pgroES</i>	+	—	+	+	—	—	—
	<i>pBR322</i>	+	—	—	—	—	—	—
	<i>p(Ptrp)-dnaK</i> – Trp	+	+	—	—	—	—	—
R40-1 (KY1603)	—	+	—	+	++	++	++	—
	<i>pgroESgroEL</i>	±	—	+	+	+	+	—
	<i>pgroES</i>	+	—	+	++	++	++	—
	<i>pBR322</i>	+	—	+	++	++	++	—
	<i>p(Ptrp)-dnaK</i> – Trp	+	+	+	—	—	+	+
	+ Trp	+	+	+	—	—	+	—

Log-phase cells grown in P broth containing ampicillin at 20°C (34°C for R40-1 derivatives) were streaked onto P agar and incubated for 24–72 hr at the temperatures indicated. Strains carrying *p(Ptrp)-dnaK* were grown in minimal medium containing 0.2% casamino acids and chloramphenicol, diluted 100-fold with fresh medium, with or without L-tryptophan (Trp, 50 µg/ml), and incubated for 24–72 hr. (++) Normal growth; (+ or ±) good or fair growth; (–) little or no growth. Each entry is based on the results for five independent transformants.

less mRNA. Thus, the cellular levels of *groE* mRNA may be lined in the following order: $\Delta rpoH \leq R30 \leq \Delta rpoH$ carrying *pgroES-groEL* $\leq$ R40. This agrees well with the order of the maximum permissive temperature for these strains.

The cellular levels of GroE proteins during steady-state growth were then determined by immunoprecipitation with antiserum against GroEL (Table 2). The GroEL level in the $\Delta rpoH$ mutant is ~50% of the wild type at 20°C. All revertants of $\Delta rpoH$ and the $\Delta rpoH$ mutant carrying *pgroESgroEL* produced significantly more GroEL than the $\Delta rpoH$ parent, and the GroEL levels correlated well with the message levels, as expected. The GroEL level in R40 revertants was extremely high, amounting to 10% of total protein produced at 20°C. Hyperproduction of GroES, as well as GroEL in R40s, has been confirmed by two-dimensional gel analysis of proteins (data not shown). We conclude that an enhanced synthesis of GroE proteins compensates effectively for the lack of σ^{32} in supporting growth at the temperature range of 20–40°C in a dose-dependent fashion.

DnaK protein helps growth at higher temperatures

During analysis of heat shock gene expression in R42 revertants, we found appreciable amounts of *dnaK*

mRNA, in contrast to the $\Delta rpoH$ parent or other revertants. In the two R42 revertants tested, *dnaK* transcripts were apparently initiated from sites that were distinct from those for wild type (Fig. 6). In one strain (lane 6), the start site was located ~10 bp upstream of P1 (a major heat shock promoter), and in the other strain (lane 7), very close to a weak possible promoter (P3) (Cowing et al. 1985). The *dnaK* mRNA levels in R42 revertants at 30°C were comparable to that of wild type that had been heat shocked (lane 9). Enhanced synthesis of DnaK protein was also observed in these strains at 30 or 42°C (data not shown). Furthermore, P1 transduction experiments indicated that the ability of R42 strains to grow at 42°C was due to a mutation at the *dnaK* region (perhaps a large insertion), designated *suhY*. Thus, the R42 revertants carry two suppressor mutations, one elevating *groE* expression (see Fig. 4B) and the other elevating *dnaK* expression by providing new promoters.

We then constructed a plasmid carrying *dnaK* fused to the *trp* promoter and introduced it into the $\Delta rpoH$ mutant and the R40-1 revertant. As expected, R40 strains carrying the plasmid exhibited appreciable growth at 42°C when *dnaK* was derepressed but not when *dnaK* was repressed by tryptophan (Table 1). The production of DnaK regulated by tryptophan was confirmed by immunoprecipitation of labeled DnaK with a specific anti-

Table 2. Cellular contents of GroEL

Strain	Relative content (°C)			Max. permissive temperature in P broth (°C)
	20	30	37	
MC4100 (<i>rpoH</i> ⁺)	0.60 ± 0.08	0.85 ± 0.09	1.00	45
KY1612 ($\Delta rpoH$)	0.31 ± 0.03	—	—	20
KY1615 (R30)	0.58 ± 0.07	0.48 ± 0.14	—	30
KY1612/ <i>pgroESgroEL</i>	0.81 ± 0.09	0.99 ± 0.14	1.07 ± 0.09	37
KY1603 (R40-1)	10.7 ± 0.90	8.41 ± 1.5	3.40 ± 0.49	40
KY1618 (R42-2)	—	—	0.72 ± 0.09	43

Log-phase cells grown in minimal medium at the temperatures indicated were labeled with [³⁵S]methionine for two generations. GroEL was determined by immunoprecipitation, as described in Materials and methods. Values represent averages (± S.D.) of five experiments and have been normalized to those of the wild type at 37°C.

Figure 6. Nuclease S1 mapping of *dnaK* mRNAs. RNAs were extracted from log-phase cells grown in L broth. The probe DNA was 5'-labeled *NruI*–*PvuI* fragment (comprising the *dnaK* promoter region) obtained from pKV101*dnaK*⁺. Hybridizations were carried out at 40°C. (Lane 1) $\Delta rpoH$ KY1612 at 20°C; (lane 2) $\Delta rpoH$, 15 min at 42°C; (lane 3) $\Delta rpoH/pgroESgroEL$ at 30°C; (lane 4) R30 at 30°C; (lane 5) R40-1 at 30°C; (lane 6) R42-1 at 30°C; (lane 7) R42-2 at 30°C; (lane 8) *rpoH*⁺ MC4100 at 30°C; (lane 9) *rpoH*⁺, 15 min at 42°C.

serum. A similar effect of the plasmid was observed when it was introduced into the $\Delta rpoH/pgroESgroEL$ strain (data not shown). In contrast, the same plasmid stimulated growth of the $\Delta rpoH$ mutant only slightly at 20 or 25°C. Thus DnaK is not as effective as GroE either in sustaining growth of the $\Delta rpoH$ mutant at low temperature or in raising the maximum permissive temperature. Rather, DnaK is more effective at higher temperatures (37–42°C) or effective only when a certain threshold level of GroE is present.

Discussion

We have shown that the loss of σ^{32} causing inability to grow at temperatures above 20°C could be effectively compensated with high-level production of a pair of GroE proteins. The ability to grow at temperatures up to 40°C was restored depending on the amounts of GroE synthesized; the higher the GroE level was, the higher the maximum permissive temperature. We thus propose that among the known set of about 20 hsp, GroE plays key protective roles in bacterial survival at a normal

temperature range of 20–40°C. This proposal rests on the assumption that the enhanced GroE level does not increase the synthesis of other hsp, secondarily in the $\Delta rpoH$ -derived strains. Two-dimensional gel analysis of proteins revealed that the levels of many hsp in the deletion mutant were low or hardly detectable at 20°C or after shift to higher temperature and were not increased appreciably in the R40-1 revertant that produces a huge excess of GroE or upon introduction of a *groE* plasmid into the $\Delta rpoH$ mutant (N. Kusakawa, unpubl.). On the other hand, alterations in the levels of several small-molecular-weight proteins that are not hsp were noted in these strains. Hyperproduction of GroE might therefore affect the level of a number of proteins that are as yet poorly defined.

The nature of inserted DNAs causing hyperexpression of the *groE* operon in the revertants remains unknown. Perhaps the best candidate would be insertion sequence (IS) elements, because they often activate a downstream gene(s) due to a promoter within the elements (see Iida et al. 1983). The size of the inserts found in R40s is within the range expected for IS elements known to be present on the *E. coli* chromosome. The fact that several *groE* overexpressing revertants contained identical or nearly identical insertions suggests the involvement of insertion hot spot(s) at the *groE* upstream region. In fact, this region contains a 16-bp AT-rich sequence that may provide a favorable target for IS elements.

It has been reported that mutations in *groEL* or *groES* give similar phenotypes, i.e., defective phage morphogenesis (see Friedman et al. 1984) and defective DNA and RNA synthesis at high temperature (Wada and Ichikawa 1984). GroEL and GroES are also known to interact functionally in vivo and in vitro (see Friedman et al. 1984). Both proteins form oligomers separately and are found mostly in soluble fractions. Thus, the functions of the two GroE proteins are closely related but not interchangeable. In the present study, hyperproduction of GroES alone was partially effective in restoring growth of the $\Delta rpoH$ mutant (Table 1). The effect of excess GroEL alone has not been assessed unequivocally because the *groEL* plasmid used had a much lower copy number than the *groES* plasmid. Thus, the full protection against thermal damage may require interaction between GroEL and GroES, but partial protection appears to be achieved by GroES alone.

Although most GroEL proteins are found in soluble fractions, some GroEL seems to be associated with the 30S ribosomal subunit in amounts that are growth-cycle dependent (Subramanian et al. 1976; Neidhardt et al. 1981). In phage λ morphogenesis, GroEL plays an essential role in helping head assembly by forming an intermediate complex with the λ B protein (Georgopoulos et al. 1983). Multicopy plasmids carrying *groES* and *groEL* were shown to suppress the temperature-sensitive growth of certain *dnaA* mutants defective in chromosomal DNA replication (Fayet et al. 1986; Jenkins et al. 1986). These results implicate structural rather than catalytic roles for GroE proteins in restoring various functions that would require complex macromolecular inter-

actions. The present finding of dose-dependent protective roles for GroE is in good accord with such expectations. It is also consistent with the recently proposed general mechanism for eukaryotic hsp functions (Pelham 1986; Ellis 1987).

Like those in eukaryotes, hsps in *E. coli* are involved in a variety of distinct cellular processes, such as DNA replication, RNA synthesis, and protein synthesis and degradation (see Neidhardt et al. 1984). Although some hsps are known to have enzymatic functions, perhaps the basic mode of action common to many is to ensure that specific target proteins (or nucleic acids) are organized into proper conformations required for whatever reaction(s) that follows. Higher amounts of hsps are required at higher temperature, presumably because some of the hsps, notably GroE, are needed to protect the target macromolecules from forming abnormal or deleterious conformations that would become prevalent under stress conditions. It should be recalled that elevated synthesis of GroE (and DnaK) has not only been observed upon heat shock but also under other stress conditions such as UV irradiation, treatments with nalidixic acid and ethanol (Travers and Mace 1982; Krueger and Walker 1984), deprivation of carbon sources (Groat et al. 1986), and forced production of abnormal proteins (Goff and Goldberg 1985; Ito et al. 1986).

Although GroE and DnaK are the most abundant hsps in *E. coli*, *dnaK* null mutants that can grow at 30°C but not at 42°C have recently been isolated (Paek and Walker 1987). This indicates that DnaK is required for growth only at high temperature, though the possibility that such mutants quickly accumulate extragenic suppressors has not been excluded. Whether GroE is an absolute requirement below 20°C is not known. In the absence of σ^{32} , hyperproduction of GroE but not DnaK can support cell growth at 20–40°C (see Table 1), suggesting that the need for GroE predominates over that for DnaK at the normal physiological temperature range. At 42°C, however, R40 revertants exhibit defective cell division and form filaments unless DnaK is supplied in *trans*, indicating that the cell requires DnaK function that cannot be satisfied by GroE. It is intriguing that GroE and DnaK play distinct roles that appear to be exerted at different stages (severity) of thermal stress.

Proteins homologous to GroEL appear to be present in organisms over a wide range of evolutionary distances. It has been found in bacteria other than *E. coli* (Carrascosa et al. 1982; Reuter and Shapiro 1987); a homopolymeric particle of 14 subunits (67 kD), closely resembling GroEL oligomer, has been found in plant cells (Pushkin et al. 1982); and more recently an antigenically-related protein that can form similar oligomers was found in mitochondria of diverse organisms including *Saccharomyces cerevisiae*, *Tetrahymena thermophila*, *Xenopus laevis*, *Zea mays*, and human cells (McMullin and Halberg 1988). Thus, GroE homologs might be conserved evolutionarily, like hsp70, and further studies on GroE and DnaK functions should provide valuable insights into the role of hsps in general physiology of cell growth and response to environmental stress.

Materials and methods

Strains, phages, and plasmids

The *E. coli* strains used are listed in Table 3. Wild-type MC4100 and the *rpoH* deletion ($\Delta rpoH30::kan$) mutant KY1612 have been described (Zhou et al. 1988). KY1601 ($\Delta rpoH$) is isogenic with KY1612, except for the prophage it carries. The *groE* promoter region carried by λ pF13-(*PgroE-lacZ*) in KY1612 is longer (by 0.7 kb) than that of the similar phage described by Yano et al. (1987). Plasmids pOF12 (*pgroESgroEL*), pJB8*groES*⁺, and pOF14 (*pgroEL*) were obtained from C. Georgopoulos, and pKV101*dnaK*⁺ from R. Yano. p(*Ptrp*)-*dnaK*⁺ (provided by H. Nagai) was constructed by joining the intact *dnaK* and part of *dnaJ* to the *trp* promoter on pSC101 carrying the *cat* gene.

Media

P broth and L broth have been described (Tobe et al. 1984). Minimal medium used was medium E (Vogel and Bonner 1956), supplemented with 0.5% glucose, thiamine (2 μ g/ml), and 16 L-amino acids (20 μ g/ml each), excluding methionine, cysteine, leucine, and lysine. Ampicillin (12 μ g/ml) or chloramphenicol (10 μ g/ml) was added to the medium for growth of strains harboring a plasmid that carries the *bla* or *cat* gene, respectively.

Protein labeling and immunoprecipitation

Cells were grown in minimal medium, pulse-labeled with [³⁵S]methionine (20 μ Ci/ml, 1400 Ci/mmol), and prepared for gel electrophoresis, as described (Yamamori and Yura 1980). Immunoprecipitation of proteins was essentially as described by Ito et al. (1986). In quantitative immunoprecipitation, log-phase cells labeled with [³⁵S]methionine (20 μ Ci/ml, 1 μ g/ml) for two generations were mixed with wild-type cells labeled with [³H]leucine (10 μ Ci/ml, 52 Ci/mmol) and [³H]lysine (10 μ Ci/ml, 88 Ci/mmol) as internal reference to correct for recovery. After electrophoresis on SDS gels (7.5%), bands for individual proteins were cut out, solubilized in NCS (Amersham) at 50°C for 2 hr, and the radioactivity was determined in a liquid scintillation counter. The ratio of ³⁵S/³H radioactivity for each protein was divided by that of total protein to obtain relative content of GroEL or DnaK.

RNA analysis

Bacterial RNAs were extracted and subjected to nuclease S1 protection analysis, essentially as described (Mori and Aiba

Table 3. *E. coli* K-12 strains

Strain	Name used in text	Genetic characters
MC4100	WT	F ⁻ <i>araD</i> Δ (<i>argF-lac</i>)U169 <i>rpsL</i> <i>relA</i> <i>flbB</i> <i>deoC</i> <i>ptsF</i> <i>rbsR</i>
KY1601	$\Delta rpoH$	MC4100 $\Delta rpoH30::kan$ <i>zhf50::Tn10</i> (λ pF13- <i>PrpoD_{hs}-lacZ</i>)
KY1603	R40-1	KY1601 <i>suH</i> X401
KY1606	R40-2	KY1601 <i>suH</i> X402
KY1607	R40-3	KY1601 <i>suH</i> X403
KY1612	$\Delta rpoH$	Same as in KY1601 but (λ pF13- <i>PgroE-lacZ</i>)
KY1615	R30	KY1601 <i>suH</i> X301
KY1616	R34	KY1612 <i>suH</i> X341
KY1617	R42-1	KY1612 <i>sih</i> X302 <i>suH</i> Y421
KY1618	R42-2	KY1612 <i>suH</i> X302 <i>suH</i> Y422

1985). In brief, double-stranded DNA fragments were labeled with [γ - 32 P]ATP by T4 polynucleotide kinase and mixed with RNAs (40 μ g), and hybridizations were performed at 45°C unless stated otherwise. After digestion with nuclease S1 (170 U), the protected DNAs were analyzed by urea (8 M)-gel (5%) electrophoresis.

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Note added in proof

Nucleotide sequence analysis indicated that an IS10-like element has been inserted into the *groE* heat shock promoter (~30 bp upstream of the transcription start site) on the R40-1 chromosome.

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