A *grpE* mutant of *Escherichia coli* is more resistant to heat than the wild-type

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The *grpE* gene of *Escherichia coli* is essential for bacteriophage λ DNA replication and is also necessary for host RNA and DNA synthesis at high temperature. A *grpE* mutant of *E. coli* was found to be substantially more resistant to 50 °C heat treatment than the wild-type. Upon receiving a 42 °C heat shock for 15 min, both the wild-type and the *grpE* mutant became more resistant to heat (i.e. they became thermotolerant). A *grpE*⁺ revertant behaved similarly to the wild-type in that it was more sensitive to heat than *grpE* cells. In addition, *grpE* cells had the same H₂O₂ and UV sensitivity as the wild-type. This implies that the conditions for which a *grpE* mutation is beneficial are unique to heat exposure and are not caused by H₂O₂ or UV exposure. Furthermore, synthesis of heat-shock proteins occurred sooner in the *grpE* mutant than in the wild-type, indicating that the *grpE* gene of *E. coli* may influence the regulation of the heat-shock response.

Introduction

When *Escherichia coli* is exposed to high temperature, the expression of a small subset of genes is induced. This phenomenon is called the heat-shock response, and is a characteristic of all organisms studied for its presence (Neidhardt *et al.*, 1984; Lindquist, 1986). Expression of these heat-shock genes is positively regulated in *E. coli* by the product of the *htpR* gene (Neidhardt & VanBogelen, 1981; Grossman *et al.*, 1984). The heat-shock response itself appears to be protective since cells exposed to a brief, sub-lethal, dose of heat become resistant to a subsequent, potentially lethal, heat exposure. This increase in heat resistance is called thermotolerance, and has been observed in many organisms (Lindquist, 1986).

Several lines of evidence suggest that thermotolerance is a product of the heat-shock response. For example, an *htpR* mutant of *E. coli*, in which heat-shock protein synthesis is blocked, fails to develop thermotolerance (Yamamori & Yura, 1982). Thermotolerance is induced by agents other than heat, such as H₂O₂ and ethanol, that also induce heat-shock protein synthesis (Lindquist, 1986; VanBogelen *et al.*, 1987a). In addition, when heat-shock protein synthesis is blocked with cycloheximide,

thermotolerance fails to develop (Plesofsky-Vig & Brambl, 1985). Furthermore, the kinetics of thermotolerance induction, and the decay of thermotolerance, coincide with the kinetics of heat-shock protein synthesis and decay (Lindquist, 1986). Interestingly, if the *E. coli* *htpR* gene is fused to an IPTG-inducible promoter, and cells containing such a construct are treated with IPTG, the heat-shock response is induced but thermotolerance does not develop (VanBogelen *et al.*, 1987a). These results imply that, while thermotolerance seems to depend on the proteins of the heat-shock response, some type of signal (e.g. blocked DNA replication) is required to actually trigger the induction of thermotolerance in *E. coli*.

The *grpE* gene of *E. coli* was originally identified because *grpE* mutants failed to support bacteriophage λ DNA replication (Saito & Uchida, 1977). The *grpE* gene product is, in fact, essential for λ DNA replication (Furth & Wickner, 1983). The *grpE* gene product has since been shown to be a heat-shock protein (Ang *et al.*, 1986). In addition, it was shown that bacteria carrying the *grpE280* mutation fail to form colonies above 43.5 °C and do not allow λ replication at any temperature (Ang *et al.*, 1986). Although these characteristics of *grpE* mutants establish the role of the *grpE* gene product in λ DNA replication, no function of the GrpE protein has been correlated to the heat-shock response itself. In this paper, data are presented suggesting that the *E. coli* *grpE* gene product may function as a regulator of the heat-shock response.

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Methods

Bacterial strains. *E. coli* strains used in this study were the generous gift of Debbie Ang (University of Utah, School of Medicine). Strain DA15 served as wild-type. Strain DA16 was derived from DA15 and carries a *grpE280* mis-sense mutation. Both strains have a Tn10 (Tet^r) insertion in the *pheA* gene which is 50–60% cotransducible with *grpE* (D. Ang, personal communication). A *grpE*⁺ revertant derived from DA16 was also used; the isolation of this strain is described in Results.

Heat treatment. Overnight cultures of bacteria were grown at 30 °C in Hershey's broth (Steinberg & Edgar, 1962) prior to each experiment. These cultures were diluted 100-fold into fresh Hershey's broth and incubated with shaking at 30 °C to a concentration of about 1×10^8 cells ml⁻¹. Then, 1 ml of this culture was pipetted into each of seven Eppendorf tubes: one tube for each time point of treatment being studied. These tubes were placed into a 50 °C waterbath until the desired time. At each time point of treatment, one tube was withdrawn from the waterbath. The bacteria were serially diluted in M9 salts solution (Adams, 1959, p. 446) and plated on Hershey's agar plates using the agar overlay method (Adams, 1959, p. 450). Bacterial suspensions at all time points were diluted in duplicate except those at the zero dose time point, which were diluted in triplicate. Plates were incubated at 30 °C for 36–48 h. Survival was determined as the ratio of colony-forming units (c.f.u.) after treatment to the number of c.f.u. at the zero dose time point.

Thermotolerance. Thermotolerance experiments were performed as described above for heat treatment except that prior to 50 °C heat exposure, bacterial suspensions were placed at 42 °C for 15 min. Immediately following this thermotolerance-inducing treatment, the cells were transferred to 50 °C. Survival was determined as above.

H₂O₂ treatment. Bacteria were grown and distributed into Eppendorf tubes as described above. CuSO₄ was added to each tube to a final concentration of 0.1 mM in order to increase the rate of hydroxyl radical formation. H₂O₂ (Mallinckrodt, 30%) was then added to a final concentration of 44 mM. The Eppendorf tubes were kept at room temperature (22 °C) until the desired time point. At that time, 2000 units of catalase (Sigma) were added to the bacterial suspension corresponding to that time point. The bacteria were then serially diluted, plated, and incubated at 30 °C for determination of survival as described above.

UV treatment. Bacteria were grown as described above. When the cell concentration reached about 1×10^8 cells ml⁻¹, the culture was centrifuged at 1000 *g* for 20 min at room temperature. The medium was poured off, and the cell pellet was resuspended in an equal volume of M9 salts solution. This suspension was centrifuged again as above, and the cell pellet was resuspended in an equal volume of M9 salts solution. This bacterial suspension was allowed to stand at room temperature for at least 20 min. Next, 1 ml of the suspension was pipetted into each of seven small dishes (35 × 10 mm), one dish for each time point of UV exposure studied. The dishes were placed, individually, under a UV lamp (General Electric) emitting a dose of 1.0 J m⁻² s⁻¹ for a specific length of time. After exposure, the bacteria were serially diluted, plated, and incubated as described above. Photoreactivation was avoided by maintaining darkened conditions throughout the experiment. Survival was determined as described above.

Labelling and electrophoresis. For labelling of proteins, bacteria were grown as described above, and serially diluted and plated to determine cell concentration. Then, 100 ml of the bacterial culture was pipetted into Eppendorf tubes and placed into a 42 °C waterbath for the desired time. Five minutes prior to each time point, 10 mCi (1100 Ci mmol⁻¹, 40.7 TBq mmol⁻¹) [³⁵S]methionine (ICN) was added to the cells. This allowed 5 min for ³⁵S labelling of proteins. At each time point, the

corresponding tube was withdrawn from the waterbath and rapidly cooled on ice. Cells were collected in a microcentrifuge. The cell pellets were prepared for electrophoresis and the proteins separated by SDS-PAGE (12.5%, w/v, acrylamide) (Laemmli, 1970). Following electrophoresis, the proteins were fixed in a solution containing 45% (v/v) methanol and 10% (v/v) acetic acid. The gel was then dried and exposed to film (Kodak X-OMAT RP) for 48 h prior to development.

Results

The heat sensitivities of the wild-type and the *grpE* mutant are shown in Fig. 1(a). The *grpE* mutant was much more heat resistant than the wild-type. These inactivation curves were replotted with best-fit straight lines obtained by linear regression analysis (not shown). The slope of the killing curve for the wild-type was 2.2-fold greater than that for the *grpE* mutant. Indeed, at the higher dose points, the percentage survival of the mutant was of the order of 100-fold greater than that of the wild-type.

In order to confirm that the greater heat resistance of the *grpE* mutant was associated with the *grpE* mutation itself, a *grpE*⁺ revertant was isolated. This was achieved by taking advantage of the inability of the *grpE280* mutant to form colonies above 43.5 °C and to support λ infection at any temperature. The *grpE* mutant was grown to a cell density of 1×10^8 cells ml⁻¹ in Hershey's broth at 30 °C, plated on Hershey's agar plates, and incubated overnight at 45 °C. Colonies which formed at this temperature were picked and grown again at 30 °C in Hershey's broth. These cultures were then passed through another 45 °C selection as above. Cultures retaining the capacity to form colonies at 45 °C were tested for their ability to support λ replication at 37 °C. Bacterial isolates on which λ plaques could form at this temperature were tested for their ability to support λ growth at 45 °C. The bacterial isolate which had the best efficiency of plating (i.e. that nearest the wild-type) was chosen and used as a *grpE*⁺ revertant. The wild-type strain was quite motile, while the *grpE* mutant was not. The *grpE*⁺ revertant was also motile.

The above revertant was subjected to 50 °C heat treatment and its relative heat sensitivity was determined (Fig. 1b). The *grpE*⁺ revertant was more sensitive to heat than the *grpE* mutant from which it was derived. Comparison of the revertant and *grpE* mutant heat inactivation curves obtained by linear regression analysis revealed that the slope of the revertant inactivation curve was 2.1-fold greater than that of the *grpE* mutant. The slopes of the wild-type and revertant inactivation curves were similar (−0.144 and −0.136 respectively).

To test for thermotolerance, the wild-type, *grpE* and revertant strains were subjected to 50 °C heat treatment following a 15 min exposure to 42 °C. As shown in

Fig. 1

Fig. 1. Sensitivity of wild-type (■), *grpE* (□) and *grpE*⁺ revertant (▲) *E. coli* to 50 °C heat exposure. Each point is a mean value based on determinations from at least four experiments.

Fig. 2

Fig. 2. Sensitivity of wild-type (■), *grpE* (□) and *grpE*⁺ revertant (▲) *E. coli* to 50 °C heat exposure following treatment at 42 °C for 15 min. Each point is a mean value based on determinations from at least four experiments.

Fig. 3. (a) Sensitivity of wild-type (■) and *grpE* (□) *E. coli* to 44 mM-H₂O₂ exposure. Each point is a mean value based on determinations from at least five experiments. (b) Sensitivity of wild-type (■) and *grpE* (□) *E. coli* to UV irradiation. Each point is a mean value based on determinations from at least three experiments.

pronounced increase in heat resistance. The *grpE*⁺ revertant also exhibited thermotolerance, again behaving similarly to the wild-type (Fig. 2b). It appears that *grpE*⁺ and *grpE* cells are similar in their ability to survive high temperature (50 °C) if they receive a prior thermotolerance-inducing treatment. However, if such a treatment is not administered, *grpE* cells are more heat resistant than cells without such a mutation.

The *grpE*⁺ revertant behaved identically to the wild-type in terms of the phenotypic characteristics studied. Therefore, it appears that a single mutation gives rise to all phenotypes associated with the mutant strain. In other words, the *grpE280* mutation not only renders a cell unable to form a colony at high temperature, and unable to support a λ infection, but it also confers heat resistance to that cell.

In order to determine if *grpE* cells are generally resistant to agents which induce the heat-shock response, the wild-type and *grpE* mutant strains were subjected to H₂O₂ and UV treatments, both of which induce the heat-shock response in *E. coli* (VanBogelen *et al.*, 1987b; Krueger & Walker, 1984). In addition, H₂O₂ has been shown to induce thermotolerance in *E. coli* (VanBogelen *et al.*, 1987a). Interestingly, the wild-type and the *grpE* mutant showed equal sensitivities to H₂O₂ and UV (Fig. 3). Therefore, the resistance to heat conferred upon a cell by the virtue of a *grpE280* mutation does not extend to H₂O₂ and UV.

In order to determine if heat-shock protein synthesis is

Fig. 2(a), both the wild-type and the *grpE* mutant exhibited thermotolerance, becoming more resistant to heat following exposure to 42 °C for 15 min (compare with Fig. 1a). The wild-type strain showed the more

Fig. 4. Comparison of heat shock protein synthesis in wild-type and *grpE* *E. coli* treated at 42 °C. Extract from equal numbers of cells was added to each lane. Odd-numbered lanes represent the wild-type; even-numbered lanes represent the *grpE* mutant. Lanes and times of treatment are as follows: lanes 1 and 2, controls (no exposure to 42 °C); lanes 3 and 4, 5 min; lanes 5 and 6, 10 min; lanes 7 and 8, 20 min; lanes 9 and 10, 30 min.

altered in the *grpE* mutant relative to the wild-type, both of these strains were heat-treated at 42 °C and their proteins visualized on a denaturing polyacrylamide gel (Fig. 4). Both strains, as expected, showed induction of heat-shock proteins. However, in the *grpE* mutant, induction seemed to occur earlier than in the wild-type (compare lanes 3 and 4). This early induction was especially pronounced for the DnaK and GroEL proteins and also for a protein of about 84 kDa. After 5 min at 42 °C, these proteins were quite visible in the *grpE* mutant (lane 4). However, in the wild-type strain, the

DnaK and GroEL proteins were not readily apparent until after 10 min of heat treatment (lane 5). This implies that *grpE280* mutants are able to elicit a heat-shock response more quickly than wild-type cells, which may explain the relatively high heat resistance seen in the *grpE* mutant (Fig. 1).

Discussion

In *E. coli*, the heat-shock response is positively regulated by the *htpR* gene (Neidhardt & VanBogelen, 1981). The protein encoded by *htpR* is a sigma factor which directs transcription from heat-shock promoters (Grossman *et al.*, 1984). Synthesis of heat-shock proteins seems to be essential for the development of thermotolerance (Yamamori & Yura, 1982; Plesofsky-Vig & Brambl, 1985). In other words, without heat-shock gene expression a cell cannot become thermotolerant. In this paper, evidence is presented that one of these *E. coli* heat-shock genes, the *grpE* gene, encodes a protein which might influence heat-shock gene expression.

Any hypothetical function of the *grpE* gene product must be reconciled with the previously reported *grpE280* phenotype. The role of the GrpE protein in DNA replication is well documented (Saito & Uchida, 1977; Ang *et al.*, 1986). Therefore, it is likely that DNA replication plays a role in the *grpE280* phenotype described here. The DnaK protein functions at the level of initiation of DNA replication in both bacteriophage λ and *E. coli* (Liberek *et al.*, 1988; Sakakibara, 1988). In addition, it has been shown that purified GrpE protein is able to bind to DnaK *in vitro* (Zylicz *et al.*, 1987). Thus, GrpE may function in the initiation of DNA replication in concert with DnaK, although this has not been directly demonstrated. Further, it has been reported that, in an *in vitro* λ DNA replication system, the presence of GrpE abrogates the requirement for high levels of DnaK protein, possibly because GrpE stabilizes DnaK or enhances its function (Alfano & McMacken, quoted in Zylicz *et al.*, 1987). Additional information implying a functional interaction between GrpE and DnaK comes from the observation that some mutations that suppress *grpE280* map in the region of the *dnaK* gene (Georgopoulos, quoted in Zylicz *et al.*, 1987).

At temperatures lower than 43 °C, the *grpE* and *dnaK* genes are not essential for *E. coli* growth (Ang *et al.*, 1986; Paek & Walker, 1987). At higher temperatures, the GrpE and DnaK proteins might function together, possibly through GrpE stabilization of DnaK at the *E. coli* replication origin, to allow DNA replication to proceed. When the heat stress imposed upon such a cell becomes too great, the heat-shock response would be induced, helping the cell to survive the heat exposure.

One hypothesis to explain the results presented here is that, in a *grpE* mutant, initiation of replication may be blocked at high temperature because the DnaK protein is not stabilized at the origin. This blockage of replication may signal induction of the heat-shock response, as seen in Fig. 4, protecting the cell from the lethal effects of heat until the temperature is lowered. At this point DNA replication could proceed, and in this fashion, a *grpE* mutant could survive heat stress better than wild-type cells because the protective heat-shock response is induced earlier. Following this scheme, the environmental conditions for which the GrpE protein is required (i.e. high temperature) are not present during H₂O₂ or UV exposure. Indeed *grpE280* cells are no more sensitive to H₂O₂ or UV than wild-type cells (Fig. 3).

The heat-shock response of *E. coli* appears to be strongly connected to DNA replication at a fundamental level. Involvement in critical cellular functions such as the initiation of DNA replication could be responsible for the extraordinary evolutionary conservation exhibited by the heat-shock response.

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