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TRANSIENT SHUT OFF OF *ESCHERICHIA COLI* HEAT SHOCK PROTEIN SYNTHESIS UPON TEMPERATURE SHIFT DOWN

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SUMMARY: A moderate downward shift in growth temperature (37 to 30°C in strain B/r and 37 to 24°C in strain K-12) was found to depress markedly the synthesis of major heat shock proteins GroEL and DnaK in *E. coli*. The depression was transient and canceled gradually to a new steady state level, taking 60-80 min. The synthesis of β-galactosidase directed by transcription initiated at the *groE* promoter behaved similarly, suggesting that this regulation, termed "reverse heat shock response", occurs at the transcriptional level.

E. coli cells respond to a sudden elevation in temperature (e.g. 30 to 42°C) by inducing the synthesis of a set of heat shock proteins (Hsps), which reaches a maximum level within 5-10 min followed by a decline to a new steady state level (1,2). A key element in this control is the gene *rpoH* (*htpR*)(3,4) which codes for a specificity factor of RNA polymerase, called σ^{32} (5). A transient increase in the cellular content of σ^{32} that occurs immediately after up-shift appears to be responsible for the enhanced transcription of the Hsp genes (6), although the molecular mechanisms by which the cell senses the temperature change and promptly accumulates σ^{32} are only poorly understood.

We now show that a regulation in the opposite direction also exists. During the course of our experiments involving filtration of *E. coli* cultures, we noticed a marked inhibition in the synthesis of GroE protein shortly after filtration. This response was shown to be ascribable at least partly to a lowered temperature during filtration. Prompted by this observation, we have looked more closely into the effects of temperature down-shift on the synthesis of Hsps, the subject of the present communication.

MATERIALS AND METHODS

The *Escherichia coli* strains used were B/r and K-12 (strain MC4100, ref. 7). MC4100 lysogenic for λ pF13-(P*groE-lacZ*) was described previously (8). Cells were grown in M9 medium (7) supplemented with thiamine (2 μg/ml) and glycerol (0.4%) with a shaking water bath. After temperature shift, samples

Abbreviation: Hsp, heat shock protein.

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of 0.1 ml were removed and labeled with [^{35}S]methionine (1,000 Ci/mmol, American Radiolabeled Chemicals) for 3 min, and the labeling was terminated by mixing with an equal volume of cold 10% trichloroacetic acid. Samples were processed for either direct SDS-polyacrylamide gel electrophoresis or immunoprecipitation before electrophoresis, as described previously (9). Samples for two-dimensional gel electrophoresis (10) were prepared as described previously (11). Intensities of the labeled proteins were quantitated by tracing autoradiograms with a Biomed Lasar Scanning Densitometer.

RESULTS

Effects of temperature shift down on the synthesis of the GroEL and the DnaK proteins in E. coli strains B/r and K-12

Within the physiological temperature range (between 23 and 37°C), the steady state amounts of GroEL vary less than 2-fold (12). We confirmed by pulse-labeling experiments that the differential synthesis rate of GroE at 30°C was 75-100% the value at 37°C (see Fig. 1, lanes 1 and 2). However, the GroE synthesis in strain B/r was found to be markedly depressed transiently upon temperature shift from 37 to 30°C. Shown in Fig. 1 are patterns of the whole cell proteins of B/r pulse-labeled with [^{35}S]methionine at different time points after a 37 to 30°C temperature shift. Labeling of a major band with electrophoretic mobility identical to GroEL was evidently reduced at 5 min (lane 4). The identity of the band as GroEL was confirmed by immunoprecipitation (gel patterns not shown), and densitometric quantitations of radioactivities associated with the immunoprecipitated GroEL showed about 5 fold reduction within 5 min after the shift. Recovery to the steady state level required about 60 min (Figs. 1 and 2).

Two dimensional gel electrophoresis of the labeled proteins showed that the spots of GroEL and DnaK were both diminished after temperature shift (Fig.

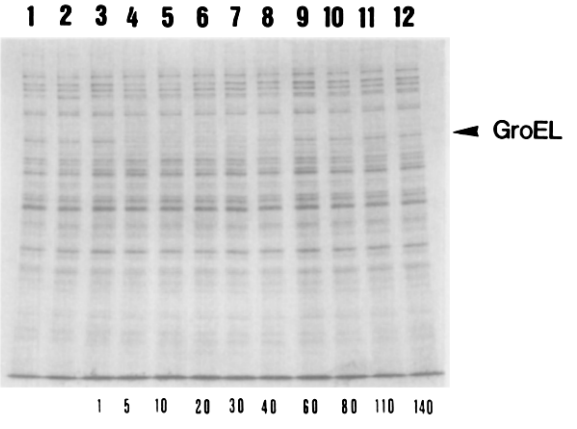

Fig. 1. Total cell proteins synthesized after a 37 to 30°C shift in strain B/r. Cells were grown at 37°C (lane 1), shifted to 30°C (lanes 3-12), and, at the indicated time points (min), pulse-labeled with [^{35}S]methionine. Lane 2 was from a steady state culture at 30°C.

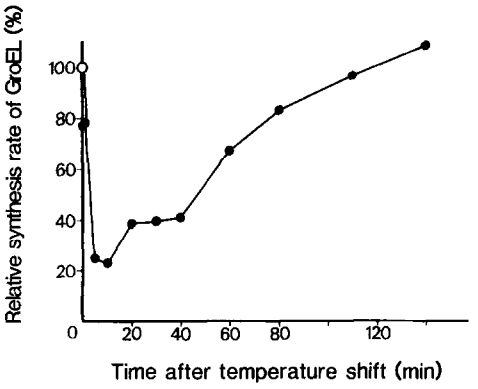

Fig. 2. Synthesis of GroEL following temperature shift. The B/r strain was temperature shifted and pulse-labeled as in Fig. 1, followed by immunoprecipitation of the GroEL protein, gel electrophoresis and quantitation of the autoradiogram. Equal radioactivities were used for immunoprecipitations. Shown are values relative to the steady state level at 30°C (○). The steady state value at 37°C (● at time zero) in this particular experiment was lower than expected presumably due to some sample loss.

3C). Among the 17 Hsps reported to be identifiable by the two dimensional gel analysis (2), we could identify putative spots for 7 of them, among which GroEL and DnaK were the two most clearly affected followed by HtpM (Fig. 3). The other Hsp spots were affected only marginally under these conditions (data not shown). Because the response observed may be regarded as a mirror image of the heat shock response, we propose to call it "reverse heat shock response" which should be distinguished from the previously proposed terminology, "cold shock" (13) for the bacterial response upon exposure to even lower temperature (10°C).

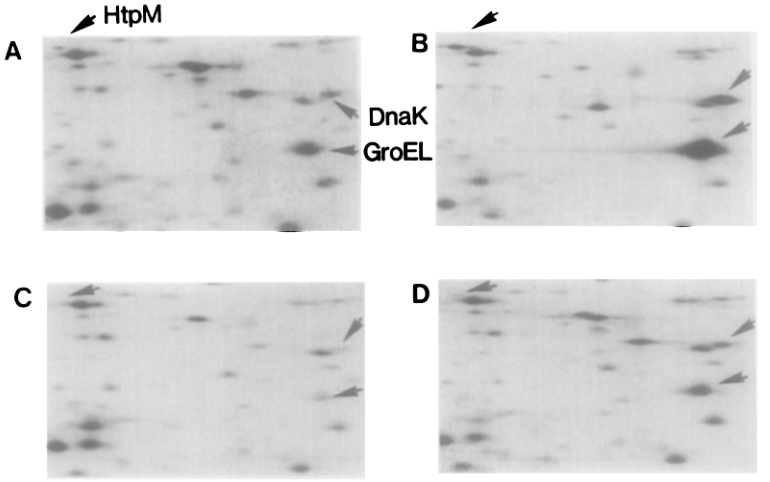

Fig. 3. Two dimensional gel patterns of the GroEL-DnaK region. Pulse-labeled samples of steady state growth at 37°C (A) and 30°C (D), as well as those 10 min after temperature shift from 30 to 42°C (B), or from 37 to 30°C (C) were subjected to the two-dimensional gel system of O'Farrel with a pI range of 5-7. The acidic end is toward the right.

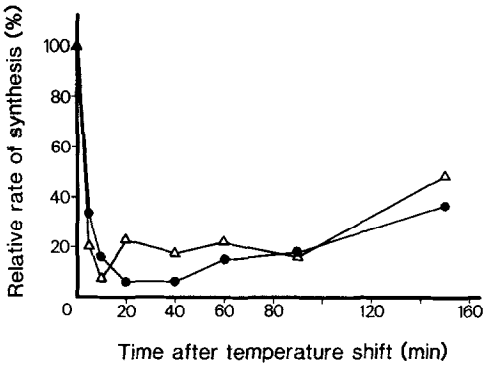

Fig. 4. Reverse heat shock in strain K-12 carrying a *groE-lacZ* operon fusion. MC4100[λ pF13(P*groE-lacZ*)] were temperature shifted (37 to 24°C), and, at intervals indicated, pulse-labeled with [^{35}S]methionine for 5 min. Equal radioactivities of samples were immunoprecipitated with a combination of anti-GroEL and anti-LacZ sera, electrophoresed and autoradiographed. Values relative to the preshift rate are shown (●, GroE; △, LacZ).

Similar reduction in the synthesis of GroEL and DnaK was observed also with a K-12 strain, MC4100, but to a less extent upon a 37 to 30°C shift (data not shown) than that observed in B/r. A clear reverse heat shock response was observed when the K-12 strain was shifted down to 24°C (see Fig. 4); the reduced GroEL synthesis persisted for about 80 min.

Reverse heat shock regulation occurs at the level of transcription

To examine whether the reduced GroEL synthesis was manifested through a transcriptional event or a post-transcriptional event, we examined the synthesis of β-galactosidase (LacZ) from a *groE-lacZ* operon fusion. In this construction, the *lacZ* transcription is under the control of the *groE* promoter, whereas its translation is initiated at the translation initiation site of the *trpB* fragment to which *lacZ* has been fused in frame (8). The LacZ synthesis from the fused gene was found to be subject to reverse heat shock regulation; it declined in parallel with GroEL after shift from 37 to 24°C (Fig. 4). Thus, the reverse heat shock regulation appears to occur primarily at the transcription level.

To examine whether a decrease in σ^{32} accompanies the reverse heat shock response, crude cell extracts were examined semi-quantitatively for their σ^{32} content by immunoblotting experiments. No significant difference was observed between the samples from temperature down-shifted cells and the steady state cells either at 30 or 37°C (data not shown). Thus, the reduced Hsp synthesis after temperature shift down is caused by mechanisms other than a simple decrease in the cellular amount of the σ^{32} protein.

DISCUSSION

The major Hsps, GroEL and DnaK are essential for viability of the *E. coli* cell at any temperature (14,15), and their increased abundance plays an important role in protecting the cell from thermal stress (16). Their cellular abundance is controlled accordingly to the cell's needs, such that cells growing at higher temperature contain more of these proteins. However, in the "normal" temperature range (23-37°C), the variation in the steady state levels of GroEL is not extensive, being 0.61 at 23°C and 0.76 at 30°C relative to the level at 37°C (12). These small variations may nevertheless be important for cell physiology, and the transient adjustment of the synthesis rate in response to a sudden temperature change should assure the rapid achievement of the steady state level. However, the downward adjustment by the cessation of synthesis will be achieved only after growth-associated dilution of pre-existing (and stable) Hsp molecules, and we indeed observed a long recovery time in the GroEL synthesis following a shift down.

Lemaux *et al* (17) described that shifting the culture of a B/r strain NC3 from 36 to 28°C resulted in transient decrease in the synthesis of several proteins. However, the extents of the downfalls were small (in most cases within about 20%) and canceled within 12 min or so, with an exception of protein F84.1 (presumably the HtpM protein) which showed a more prominent response. Neidhardt *et al* (18) also presented data (Fig. 2 of ref. 18) showing a transient decrease in GroEL synthesis upon shift from 36 to 28°C in the same strain, but again the decrease observed was not extensive and was canceled within 15 min. The difference between our observations and those of Neidhardt *et al* with respect to the extent and the duration of the synthesis down regulation could be due to the temperature, media, or strain difference. Jones *et al.* (13) regarded regulation that occurs after more extensive temperature shift (from 37 to 10°C) as analogous to heat shock, and termed it "cold shock", in which the synthesis of many proteins are depressed, whereas several "cold-shock proteins" are induced. The cold-shock regulation is different from the regulation we have described and proposed to term "reverse heat shock".

The B/r and the K-12 strains were somewhat different with respect to the effective temperature range that caused reverse heat shock response. The basis for this difference is not known.

Several possibilities can be considered as the molecular mechanisms of the reverse heat shock regulation, that occurs at the transcription level without involving a loss of σ^{32}. A factor could be considered which inactivates σ^{32}, which dissociates σ^{32} from the core RNA polymerase, or which directly binds to the control regions of the Hsp genes. For instance, one (or several) Hsp could act as the negative regulator at lower temperature. In the

two dimensional gel analysis, we preliminarily identified a B/r protein with a basic isoelectric point and an apparent molecular weight of 42,000, that was specifically and transiently synthesized after reverse heat shock (data not shown). Such a protein could have a role in the regulation. After completion of this work, we exchanged information with Carol Gross, who reached a conclusion that the inhibition of Hsp synthesis that could be observed after a 42 to 30°C temperature shift (19) is probably due to some inactivation of σ^{32} (D.B. Straus, W.A. Walter and C.A. Gross, manuscript submitted). The reverse heat shock system should provide new opportunities to investigate the mechanisms of gene regulation that involves different σ factors in *E. coli* at both the molecular and cellular levels.

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