σ^{32} synthesis can regulate the synthesis of heat shock proteins in *Escherichia coli*

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The *Escherichia coli rpoH (htpR)* gene product, σ^{32}, is required for the normal expression of heat shock genes and for the heat shock response. We present experiments indicating a direct role for σ^{32} in controlling the heat shock response. Both the induction and decline in the synthesis of heat shock proteins can be controlled by changes in the rate of synthesis of σ^{32}. Specifically, we show that: (1) σ^{32} is an unstable protein, degraded with a half-life of approximately 4 min; (2) increasing the rate of synthesis of σ^{32}, by inducing expression from a P_{lac} or P_{tac}–*rpoH* fusion, is sufficient to increase the rate of synthesis of heat shock proteins; (3) during the shut-off phase of the heat shock response synthesis of σ^{32} is repressed post-transcriptionally, and the *dnaK756* mutation, which causes a defect in the shut-off phase, prevents the post-transcriptional repression of synthesis of σ^{32}. These results serve as a basis for understanding the role of DnaK in the heat shock response, the regulation of σ^{32} synthesis, and the role of σ^{32} in controlling transcription of heat shock genes.

[*Key Words*: Heat shock response; σ^{32}; *dnaK*]

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When cells are shifted from a low- to high-growth temperature, the synthesis of heat shock proteins (hsps) is induced (Schlesinger et al. 1982; Neidhardt et al. 1984; Craig 1985). Synthesis of these proteins is also induced by other forms of stress (for review, see Neidhardt et al. 1984; Grossman et al. 1985). Not only is this response apparently universal, but in addition, the function of some of the hsps might be conserved. *Escherichia coli* hsps DnaK and C62.5 are similar in amino acid sequence to eukaryotic hsp70 and hsp83 (Bardwell and Craig 1984; E. Craig, pers. comm.).

The heat shock response of *E. coli* is characterized by a transient increase in the rate of synthesis of about 20 proteins. The rate of synthesis of each hsp reaches a maximum between 5 and 10 min after temperature upshift and then declines to a new rate of synthesis characteristic of the higher temperature and greater than at the lower temperature (for review, see Neidhardt et al. 1984). The induction of hsp synthesis results from increased transcription of heat shock genes (Yamamori and Yura 1980, 1982; Yamamori et al. 1982; Taylor et al. 1984).

The heat shock response is dependent on the *rpoH* (*htpR*) gene product. Mutations in *rpoH* result in decreased transcription of heat shock genes and prevent induction of hsp synthesis following temperature upshift (Neidhardt and VanBogelen 1981; Yamamori and Yura 1982). The *rpoH* gene product is a 32-kD sigma factor, σ^{32} (Grossman et al. 1984; Bloom et al. 1986), that is homologous to the major sigma factor of *E. coli*, σ^{70} (Landick et al. 1984; Yura et al. 1984), as well as to other bacterial sigma factors (Stragier et al. 1985; Gribskov and Burgess 1986). In vitro, RNA polymerase holoenzyme containing σ^{32} ($E\sigma^{32}$), but not holoenzyme containing σ^{70} ($E\sigma^{70}$), initiates transcription at heat shock promoters (Grossman et al. 1984, 1985; Cowing et al. 1985). A number of heat shock promoters have been characterized, and the consensus sequence derived for them differs from that of promoters utilized by $E\sigma^{70}$ (Cowing et al. 1985).

Previous work has shown that the magnitude of the heat shock response is related to the amount of σ^{32} in the cell. Virtually no heat shock is observed in strains where a nonsense mutation in *rpoH* is inefficiently suppressed by the supC(ts) suppressor. Substitution of more efficient nonsense suppressors, inserting the same amino acid, partially restores the heat shock response (Yamamori and Yura 1982). Furthermore, overexpressing σ^{32} from the λP_L promoter after temperature upshift results in greater than normal synthesis of heat shock proteins (Grossman et al. 1984).

The mechanisms regulating transcription of the heat shock genes are not known. One possibility is that the role of σ^{32} is simply to determine promoter specificity while other transcription factors control the activation or repression of heat shock genes (Briat et al. 1985). Alternatively, as suggested by the experiments discussed above, the rate of transcription initiation at heat shock promoters may be governed by the intracellular concentration of σ^{32}. In this case, σ^{32} would be a direct regulator of heat shock gene expression. This model predicts that the intracellular concentration of σ^{32} must be able to change rapidly in response to stress, and be a limiting

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factor in the expression of hsps. In the present report, we show that σ^{32} is an unstable protein and its intracellular concentration limits the expression of heat shock proteins during steady-state growth. Simply increasing the rate of synthesis of σ^{32}, in the absence of a temperature increase, is sufficient to increase expression of hsps. We also demonstrate that the rate of synthesis of σ^{32} decreases during the shut-off phase of the heat shock response and that a mutant defective in shut-off does not show this decline in σ^{32} synthesis. We discuss these results in terms of a mechanism in which σ^{32} directly regulates heat shock gene expression.

Results

σ^{32} is an unstable protein

Strains carrying plasmids which fuse the P_L promoter of phage λ to *rpoH* oversynthesize σ^{32} following induction of P_L (Grossman et al. 1984). However, there is no noticeable accumulation of σ^{32}, based on staining of gels with Coomassie Blue (data not shown). This observation led us to test whether σ^{32} was unstable. Cells oversynthesizing σ^{32} from P_L after shift to 42°C were labeled with [^{3}H]leucine and [^{3}H]lysine, and then chased with unlabeled leucine and lysine. The amount of labeled σ^{32} remaining at various times after the chase was determined by two-dimensional gel analysis (as described in Materials and methods). σ^{32} was degraded with a half-life ($t_{1/2}$) of approximately 4 min (Fig. 1). Because σ^{32} is unstable, changes in its rate of synthesis will rapidly result in changes in its intracellular concentration.

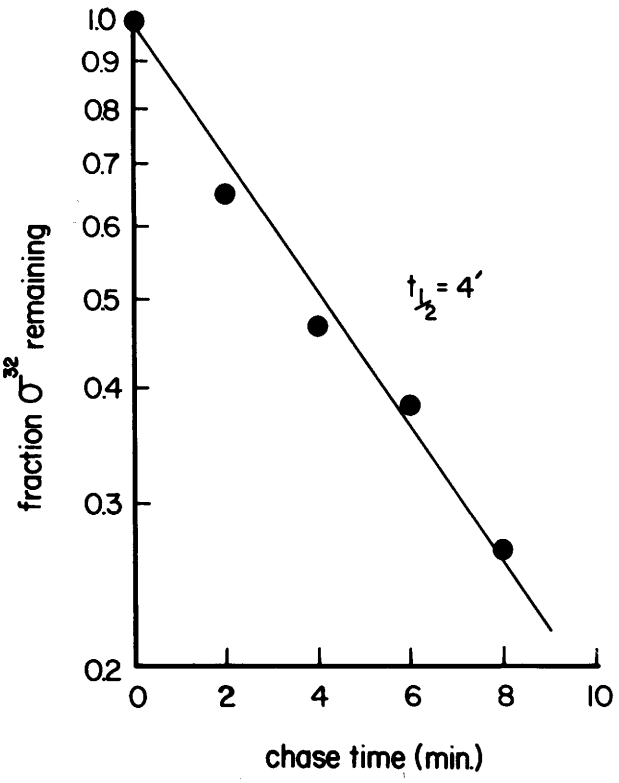

Figure 1 Stability of σ^{32} after shift to 42°C. Strain CAG2167, carrying pAG37 (P_L–*rpoH*), was labeled with [^{3}H]leucine and [^{3}H]lysine from 0 to 5 min following shift from 30° to 42° and then chased with unlabeled leucine and lysine. Aliquots were withdrawn at 2-min intervals, the first being designated as the 0-min time point. The samples were quantitatively analyzed by two-dimensional gel electrophoresis as described in Materials and methods.

Increased synthesis of σ^{32} is sufficient to cause increased synthesis of heat shock proteins

Overproducing σ^{32} from P_L following a temperature upshift causes increased synthesis of heat shock proteins (Grossman et al. 1984). We wondered if overproducing σ^{32} in the absence of a temperature shift would cause increased synthesis of hsps. To test this, two plasmids were constructed, one fusing *rpoH* to the *lac* promoter (pAG41), the other fusing *rpoH* to the stronger *tac* promoter (pDS2). In cells overproducing the *lac* repressor, these promoters are repressed, but they can be induced by the addition of IPTG in the absence of any temperature shift. At 30°C the synthesis of hsps was increased within 5 min after the addition of IPTG to cells carrying a plasmid with *rpoH* under control of P_{lac} (Fig. 2A) or P_{tac} (Fig. 2B). Induction of σ^{32} synthesis from P_{tac} during steady-state growth at 42°C also caused increase in hsp synthesis (Fig. 2C). Thus, an increase in the rate of synthesis of σ^{32} is sufficient to cause an increase in the synthesis of hsps at low or high temperature. No other inducing signal is required and expression of heat shock genes during steady-state growth seems to be limited by the amount of σ^{32} in the cell.

The synthesis of σ^{32} from P_L is regulated post-transcriptionally

Like a normal heat shock response, induction of hsp synthesis was transient when σ^{32} was overproduced from P_L (Fig. 3A and 4A). Initially, hsp synthesis increased after temperature upshift, but then declined following a peak at 10 min. When we measured the rate of synthesis of σ^{32} we found that although σ^{32} was initially overexpressed from P_L its synthesis decreased after 10 min at 42°C (Fig. 4A). This decrease was not due to a drop in transcription initiation from P_L. The strains used for these experiments contain a defective λ prophage which places *galK* under control of P_L. We monitored transcription from P_L by measuring galactokinase activity and found that expression of *galK* continued at a constant differential rate while synthesis of σ^{32} decreased (Fig. 5). Thus, the synthesis of σ^{32} was repressed at some step after transcription initiation, perhaps at the level of translation or of mRNA stability. This post-transcriptional repression of σ^{32} synthesis, coupled with the fact that σ^{32} is degraded with a $t_{1/2}$ of 4 min is sufficient to explain the decline in hsp synthesis following the peak of the heat shock response.

The dnaK756 mutation alters post-transcriptional control of σ^{32} synthesis

The heat shock response is regulated in part by the *dnaK* gene product, itself a heat shock protein. Normally, hsp

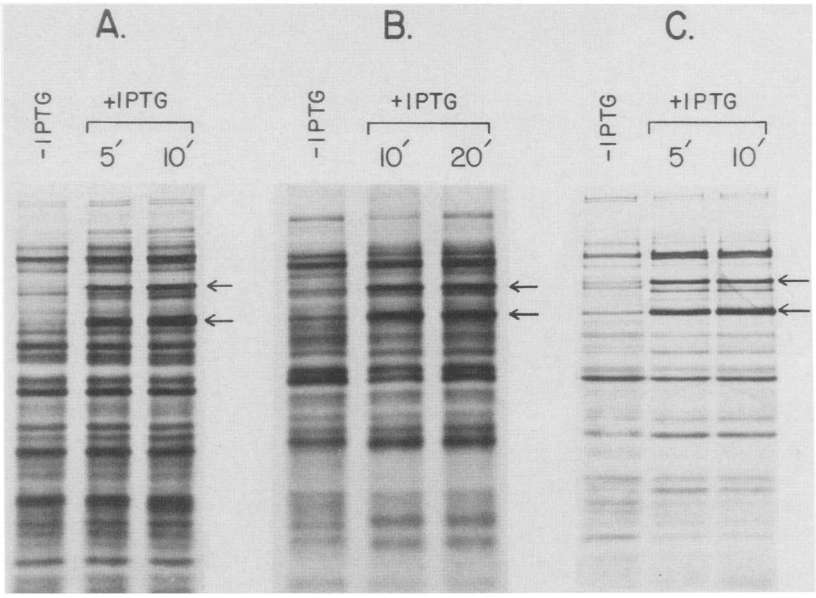

Figure 2 Induction of heat shock protein synthesis following overproduction of σ^{32}. Strains were pulse-labeled with [^{35}S]methionine for 1 min at 30° (*A*,*B*) or 42° (*C*) before, and at the indicated times after addition of IPTG (1 mM) and then chased with unlabeled methionine for 1 min. Samples were analyzed on 8% (*A*) or 10% (*B*,*C*) SDS-polyacrylamide gels. Samples from a given strain contained equal amounts of radioactivity. (*A*) Strain CAG11052, carrying pAG41 (P_{lac}–*rpoH*). (*B*) Strain CAG11037, carrying pDS2 (P_{tac}–*rpoH*). (*C*) Strain CAG2041 carrying pDS2 (P_{tac}–*rpoH*). The arrows indicate the positions of DnaK (top arrow), and GroEL (bottom arrow).

synthesis peaks 5–10 min after temperature upshift and then declines to a new steady-state rate of synthesis. The *dnaK756* mutations causes hsp synthesis to continue at or near the peak rate of synthesis following temperature upshift (Tilly et al. 1983). The *dnaK756* mutation had a similar effect on the heat shock response when σ^{32} was overexpressed from P_L (Fig. 3B and 4B). When we measured the rate of σ^{32} synthesis in a *dnaK756* strain, we found that it remained high at late times after temperature shift (Fig. 4B). It is likely that the continued synthesis of σ^{32} results in the high rate of synthesis of heat shock proteins following the normal peak. Again, the effect on σ^{32} synthesis was not due to altered transcription initiation from P_L, as expression of *galK* from P_L was normal (Fig. 5). Thus, post-transcriptional repression of σ^{32} synthesis is relieved in the *dnaK756* mutant.

Discussion

We have shown that the rate of synthesis of heat shock proteins in *E. coli* can be increased by increasing the rate of synthesis of σ^{32}. When the synthesis of σ^{32} was induced from P_{lac} or P_{tac}–*rpoH* fusions, synthesis of hsps increased, independent of temperature. This suggests that σ^{32} is a limiting factor in the expression of heat shock proteins. The fact that σ^{32} is unstable means that changes in its rate of synthesis will rapidly result in changes in its intracellular concentration. Increased transcription initiation at heat shock promoters could be a direct result of an increase in the amount of σ^{32} in the cell. The signal for increasing hsp synthesis following a temperature upshift could act by increasing the rate of synthesis and/or by decreasing the rate of degradation of σ^{32}.

Our experiments also demonstrate a correlation be-

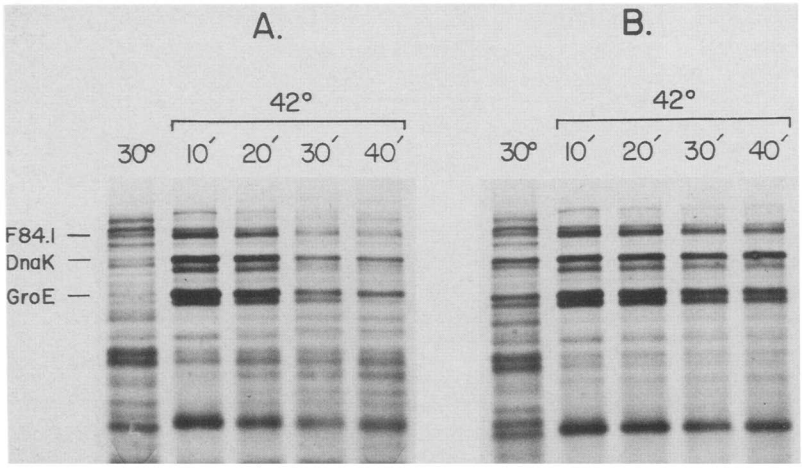

Figure 3 The heat shock response in *dnaK*$^+$ and *dnaK756* strains overexpressing σ^{32}. Strains containing pAG37 (P_L–*rpoH*) were pulse-labeled with [^{35}S]methionine either at 30°C, or at the indicated times following shift to 42°C, and analyzed as in Fig. 2. (*A*) Strain CAG2167 (*dnaK*$^+$); (*B*) CAG2168 (*dnaK756*). The positions of heat shock proteins F84.1, DnaK, and GroEL are shown.

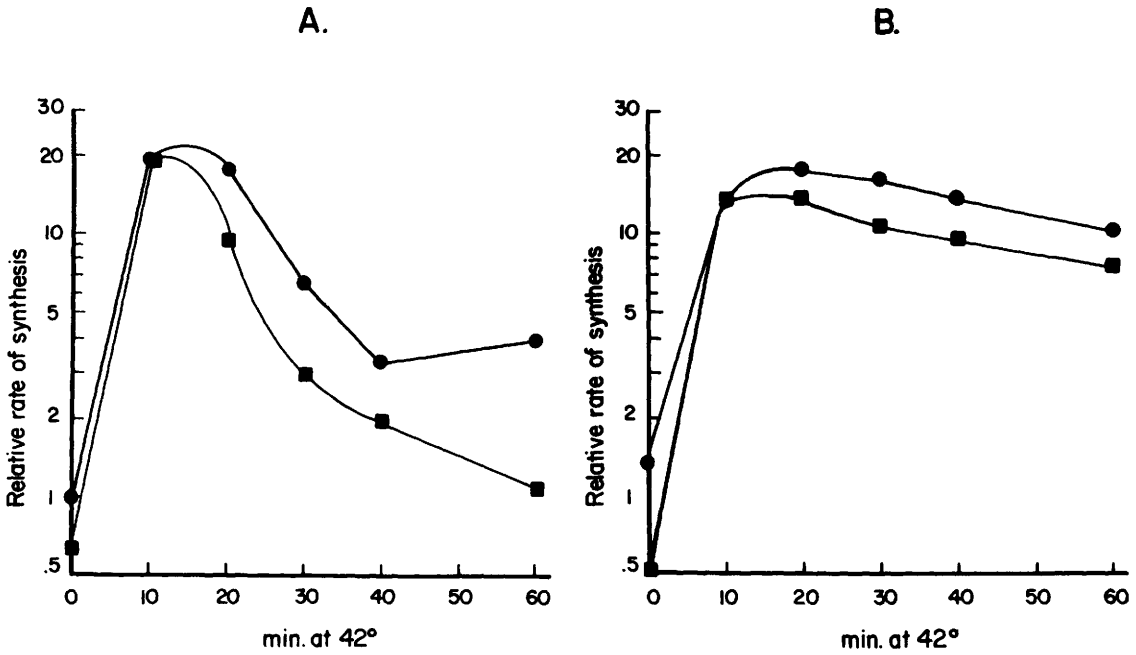

Figure 4 Synthesis of σ^{32} and hsp F84.1 is controlled by *dnaK*. Strains containing pAG37 (P_L–*rpoH*) were pulse-labeled with [^{3}H]leucine and [^{3}H]lysine and 30°C (0 min) and at various times after shift to 42°C. Samples were analyzed quantitatively as described in Materials and methods. (*A*) Strain CAG2167 (*dnaK*$^+$); (*B*) Strain CAG2168 (*dnaK756*). (●) F84.1; (■) σ^{32}. σ^{32} and F84.1 are plotted as equivalent values at 10 min after upshift.

tween a decrease in the rate of synthesis of σ^{32} and a decline in the rate of synthesis of hsps. When σ^{32} is expressed from the P_L promoter, it is synthesized at a high rate immediately after temperature upshift and then its synthesis is repressed. Concomitant with this repression is a decrease in the expression of the hsps. The repression of σ^{32} synthesis is relieved by the *dnaK756* mutation, which also causes a defect in the shut-off phase of the heat shock response. The continued synthesis of σ^{32} in the *dnaK* mutant is sufficient to explain the continued synthesis of hsps in this strain. We suggest that repression of σ^{32} synthesis and degradation of σ^{32} are sufficient to explain the decline in hsp synthesis following the peak of the heat shock response.

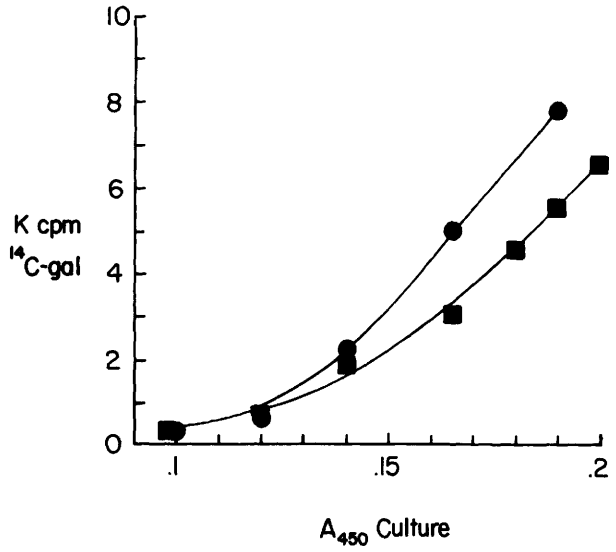

Figure 5. Transcription from the λ P_L promoter continues at a constant rate following induction at 42°C. Cultures of CAG2167 (*dnaK*$^+$)(●) and CAG2168 (*dnaK756*) (■) were grown at 30°C and then shifted to 42°C. The first sample was withdrawn at 30°C and the remainder were taken following shift to 42°C. For each time point the O.D.$_{450}$ and the galactokinase activity of the cultures was determined.

We had previously suggested that the role of DnaK in modulating the heat shock response might be to modify σ^{32}, perhaps by phosphorylation (Grossman et al. 1984). We observed that σ^{32} migrated as two spots in the isoelectric dimension of two-dimensional gels and speculated that one of these forms was phosphorylated. However, we have been unable to detect phosphorylation of σ^{32} (A. Grossman, unpubl.). In addition, the *dnaK756* mutation has no detectable effect on the relative amounts of the two isoelectric forms of σ^{32}.

The present experiments suggest that DnaK is involved in regulating the synthesis of σ^{32}. Repression of σ^{32} synthesis from P_L following temperature upshift occurred post-transcriptionally since transcription initiation, as measured by a P_L–*galK* fusion, continued at a high rate. Post-transcriptional repression is abolished in the *dnaK* mutant strain. We suggest that DnaK, either directly or indirectly, acts to repress translation of *rpoH* mRNA. Induction of the heat-shock response and the corresponding increase in the expression of *dnaK* would cause the translational repression of *rpoH* mRNA. The resulting decrease in σ^{32} synthesis would lead to a rapid decline in the intracellular level of σ^{32} and a decrease in the rate of synthesis of heat shock proteins. Induction of

the heat shock response could occur as a result of derepressing translation of *rpoH* mRNA.

DnaK may have an additional effect on the expression of heat shock genes. The *dnaK756* mutation does partially stabilize σ^{32}, as well as some other unstable proteins (unpublished data). We are investigating the role of *dnaK* and other heat shock genes in protein turnover.

Our experiments do not rule out the existence of other transcriptional regulators of the heat shock response. However, they do indicate a direct role for σ^{32} in controlling expression of heat shock genes. Current experiments are aimed at measuring the intracellular concentration and the rates of synthesis and degradation of σ^{32} before and after induction of heat shock protein synthesis in cells expressing *rpoH* from its normal regulatory region.

Materials and methods

Strains and plasmids

Plasmid pAG41 (P_{lac}–*rpoH*) was constructed by cloning a 1.3-kb *Eco*RV fragment containing the intact *rpoH* gene from pFN97 (Neidhardt et al. 1983) into the *Sma*I site of pUC9 (Vieira and Messing 1982). In this construction expression of the *rpoH* gene is controlled by the *lac* promoter. Plasmid pDS2 (P_{tac}–*rpoH*) was constructed by inserting the same *Eco*RV fragment into the *Sma*I site of the P_{tac} vector, pKK223-3 (Pharmacia). The construction of plasmid pAG37 (P_L–*rpoH*) has been described previously (Grossman et al. 1984). Plasmids pAG41 and pDS2 were carried in the following strains: CAG11037 is MC1061 (Casadaban and Cohen 1980) containing an F′*lacI*^{Q}*lacZ*::Tn*5*; CAG2041 is CSH26 (Miller 1972) containing F′*lacI*^{Q}*lacZ*::Tn*5*; CAG11052 is CSH26 *rpoH165 supC*ts ϕ80 *lac*$^+$/F′*lacI*^{Q} *lacZ*::Tn*5*. pAG37 was carried in strains CAG2167, CAG2168 which are N5242 λcI857 (Gottesman et al. 1980) *dnaK*$^+$ and *dnaK756* respectively.

In vivo labeling

Strains were grown in M9 glucose media supplemented with all amino acids except those used for labeling. Aliquots of exponentially growing cultures were labeled with either 10 μCi/ml [^{35}S]methionine, or 35 μCi/ml each of [^{3}H]leucine and [^{3}H]lysine. Following a labeling period of 1–5 min, cultures were chased with an excess of unlabeled methionine or leucine and lysine (see figure legends for length of chase). Pulse-labeled samples were precipitated on ice for 20 min in 5% TCA. The precipitate was collected by centrifugation, washed with cold 80% acetone, and dried under vacuum. Pellets were resuspended in either SDS sample buffer, for one-dimensional polyacrylamide gels (Laemmli 1970), or isoelectrofocusing buffer for two-dimensional gel analysis (O'Farrell 1975).

Quantitative analysis

For quantitative analysis of individual proteins, samples pulse-labeled with [^{3}H]leucine and [^{3}H]lysine were prepared as above for two-dimensional gel analysis. Aliquots of an extract from the same strain, pulse-labeled with [^{35}S]methionine during a heat shock, were added to the samples and a fraction of each of the combined extracts was TCA precipitated to determine the total ^{3}H-dpm/^{35}S-dpm. A portion of each sample was then subjected to two-dimensional gel analysis according to O'Farrell (1975). Autoradiographs were prepared and individual protein spots were cut from the gels and solubilized in 17% perchloric acid, 21% hydrogen peroxide, at 60°C for 12 hr. The ^{3}H-dpm/^{35}S-dpm in the individual proteins was determined by liquid scintillation counting in Aquasure (Amersham) scintillation fluid. Synthesis of individual proteins is expressed as a fraction of total protein synthesis (relative synthesis rate), corrected for losses during the analysis: (^{3}H-dpm/^{35}S-dpm) in the specific protein/(^{3}H-dpm/^{35}S-dpm) in total protein. Protein stability is expressed as the fraction of the individual protein remaining at various times after the start of the chase and is also corrected for losses during analysis.

Galactokinase assays

Assays were carried out as described by Taylor et al. (1984), except that lysis buffer was modified to have a final concentration of 0.3% Na deoxycholate. Lysis buffer was prepared immediately prior to use to avoid precipitation of the detergent.

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Note added in proof

Recent experiments indicate that the half-life of σ^{32}, in wild-type cells expressing *rpoH* from the chromosome, is approximately 1 min. In addition, the amount of σ^{32} in wild-type cells increases immediately after a shift to a higher temperature.

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Sigma 32 synthesis can regulate the synthesis of heat shock proteins in Escherichia coli.

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