JOURNAL OF BACTERIOLOGY, Dec. 1986, p. 1155–1158
0021-9193/86/121155-04$02.00/0
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Vol. 168, No. 3

Heat Shock Regulatory Gene *rpoH* mRNA Level Increases after Heat Shock in *Escherichia coli*

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Received 30 June 1986/Accepted 24 September 1986

The *Escherichia coli rpoH* gene product sigma 32 is essential for the increase in heat shock gene transcription found after exposure of the bacteria to a sudden temperature increase. It is not known how the concentration of active sigma 32 is modulated. We showed that *rpoH* transcript levels increased after heat shock and that the magnitude of the increase in the level of mRNA was correlated with the magnitude of the temperature shift. The increase in the level of *rpoH* mRNA was still found in *rpoH* mutants so the mechanism of induction differed from that of the set of previously identified heat shock genes. The increased concentration of *rpoH* mRNA should result in a higher level of sigma 32, which is likely to be important for increasing heat shock gene transcription.

The bacterium *Escherichia coli* synthesizes a set of proteins at a higher rate after exposure to a sudden temperature increase (called heat shock). A similar response is thought to occur in all organisms (see references 9 and 24 for reviews). At least two of the *E. coli* heat shock proteins, DnaK and C62.5, are related in amino acid sequence to eucaryotic heat shock proteins (2; J. Bardwell and E. Craig, personal communication), suggesting that a heat shock response may have evolved before the divergence of pro- and eucaryotes. Among the 17 or more *E. coli* heat shock proteins are the products of the *dnaK*, *dnaJ*, *groES* (*mopB*), *groEL* (*mopA*), *lysU*, *grpE*, *lon*, and *rpoD* genes (1, 3, 24, and references therein). These proteins are involved in transcription, translation, DNA synthesis, proteolysis, and bacteriophage morphogenesis. One effect of heat shock protein synthesis is to help protect bacteria from being killed by high temperatures (31). It has not been established which heat shock protein(s) contributes to protection from thermal killing, but other stresses, such as bacteriophage infection (10, 17) or treatment with ethanol (29) or DNA-damaging agents (18), induce the synthesis of many of the same proteins, so the proteins involved may protect cells from damage caused by several adverse conditions found in nature.

Several of the steps in the response of *E. coli* to heat shock are now known. Less than 1 min after a temperature upshift, the transcription of heat shock genes begins to increase coordinately (30). The protein synthesis rates and transcript levels increase 5- to 50-fold (8, 26, 31). The synthesis rates peak around 5 to 8 min after the temperature shift and decline to new steady-state levels, which at 42°C are about two times the level at 30°C (15). Heat shock genes are defined by a requirement for the *rpoH* (previously known as *htpR* or *hin*) gene product for their transcription (22, 31). The *rpoH* gene product is called sigma 32 because it acts as an RNA polymerase sigma subunit, directing the enzyme to initiate transcription at heat shock gene promoters (14). The consensus sequence for heat shock promoters differs from that for promoters transcribed by RNA polymerase in combination with sigma 70, the normal sigma subunit (8). Since many, if not all, heat shock proteins are synthesized in unstressed cells (20), sigma 32-RNA polymerase may always be present and active at some level.

The mechanism by which transcription of heat shock genes is reduced to the steady-state level characteristic of the new growth temperature is unknown. Sigma 70 and the *dnaK* gene product seem to be involved, since altering the active levels of either protein alters the kinetics of the shutoff of heat shock gene transcription (13, 27, 31).

How does *E. coli* make the changeover from normal transcription to high-level transcription of the heat shock genes? One possibility is that the amount of sigma 32 increases after heat shock. In support of this model, experiments from our laboratories showed that increasing the amount of sigma 32 by inducing a *tac* promoter-*rpoH* gene fusion is sufficient to turn on heat shock gene transcription at low temperature (C. Georgopoulos and K. Tilly, unpublished results; D. Straus, A. Grossman, and C. Gross, personal communication). To study the in vivo regulation of the *rpoH* gene, *rpoH* transcript levels were measured before and after heat shock. A fivefold increase in *rpoH* mRNA was found, which, if translated, would cause increased sigma 32 concentration and, consequently, increased heat shock gene transcription.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *rpoH*$^+$ strains SC122 and SKB178 were described previously (7, 11). The MC4100 *rpoH6*, *rpoH15*, and *rpoH165* mutants were kindly donated by Takashi Yura and have been previously described (7, 28, 32). The *rpoH* probe was from plasmid pFN97 (23), and the *dnaK* probe was from plasmid pBR322 *dnaK*$^+$ (which contains the 5.3-kilobase-pair *Hin*dIII fragment, including the entire *dnaK* gene, cloned into the *Hin*dIII site of plasmid pBR322; N. McKittrick and C. Georgopoulos, unpublished results).

RNA preparation. RNA was prepared from cells growing in low-phosphate minimal medium by the method of Jinks-Robertson et al. (16), except that the carrier RNA was

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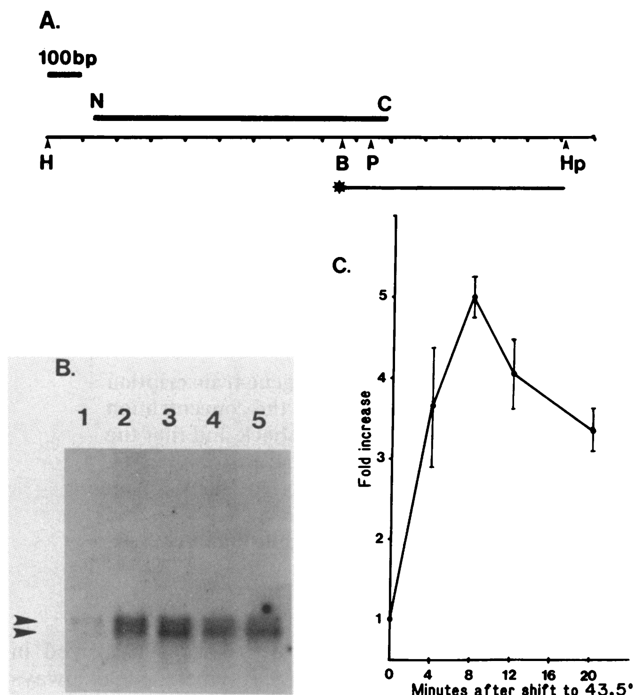

FIG. 1. (A) Restriction map of the *rpoH* gene region, showing restriction sites used for generating probes. The line at the bottom of the figure represents the probe used for S1 mapping, with an asterisk marking the labeled end. The thick line represents *rpoH* coding sequences (N, amino terminus; C, carboxyl terminus); H, *Hin*dIII; P, *Pvu*II; B, *Bss*HII; Hp, *Hpa*I. (B) Northern blot of RNA isolated from strain SC122 during growth at 30°C (lane 1) or at 4, 8, 12, or 20 min after a shift to 43.5°C (lanes 2 through 5, respectively) hybridized with an *rpoH*-specific probe. (C) Quantitation of *rpoH* mRNA by 3′ S1 mapping. The same RNA samples shown in Fig. 1B were analyzed with S1 mapping. The relative levels of *rpoH* mRNA at the indicated times after a shift from 30 to 43.5°C are plotted. The vertical bars at each point indicate the standard deviation for that point.

omitted. The cells were either immediately phenol extracted at 64°C or were placed on ice after lysing, extracted once with phenol, once with phenol-chloroform-isoamyl alcohol (50:50:1), precipitated one or two times with ethanol, and suspended in water or 10 mM Tris (pH 8)–1 mM EDTA.

Northern blots. Northern blots were done essentially as described by Maniatis et al. (21), except that the gels were 1.4% agarose–1% formaldehyde. Hybridizations with nick-translated DNA probes were at 42°C in 50% formamide–1 M NaCl–50 mM Tris (pH 7.5)–10× Denhardt buffer–0.1% sodium pyrophosphate–50 μg of sheared denatured calf thymus DNA per ml. Filters were washed with three changes of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at room temperature and three changes of 0.1× SSC–0.1% sodium dodecyl sulfate at 55°C. The probe for the *rpoH* gene was the 930–base-pair *Hin*dIII-*Pvu*II fragment from pFN97 (Fig. 1; 23), and the probe for the *dnaK* gene was the 1,780-base-pair *Pvu*I-*Sal*I fragment from pBR322 *dnaK*$^+$.

S1 mapping. A 3′-end-labeled *rpoH* probe was prepared by digesting pFN97 (23) with *Bss*HII and by using the Klenow fragment of *E. coli* DNA polymerase I to fill in the end in the presence of [α-^{32}P]dCTP and [α-^{32}P]dGTP. The plasmid was redigested with *Hpa*I, and the *Bss*HII-to-*Hpa*I fragment (Fig. 1A) was electroeluted from an acrylamide gel. Hybridization and S1 digestion conditions were as previously described (4). Experiments were quantitated by cutting the appropriate bands from the analytical gels and measuring the radioactivity in them. The data in Fig. 1C are the averages of four experiments with the same RNA preparation. The magnitude of the *rpoH* mRNA level increase ranged from 5- to 10-fold in other experiments.

RESULTS

***rpoH* transcript.** As a first step in characterizing *rpoH* gene regulation, we visualized the *rpoH* mRNA by using Northern blots. Total *E. coli* RNA was fractionated by electrophoresis through formaldehyde-agarose gels, transferred to nitrocellulose, and hybridized with an *rpoH*-specific probe. We were able to detect two *rpoH* mRNA species of approximately 1,200 and 1,400 nucleotides (Fig. 1B). Given the DNA sequence around the *rpoH* gene (19, 32) and the lengths of the *rpoH* mRNAs, it is likely that the transcripts are monocistronic and are derived from multiple *rpoH* promoters or from posttranscriptional processing of the *rpoH* mRNAs.

Regulation of transcript levels. Since the *rpoH* gene is involved in the regulation of the heat shock response, we examined the relative amounts of *rpoH* transcripts before and after heat shock. RNA was prepared from cells growing at 30°C and at 4, 8, 12, and 20 min after a shift to 43.5°C. The mRNA levels were examined by probing Northern blots with an *rpoH*-specific probe (Fig. 1B). We found a fivefold increase in transcript concentration within 5 min of the shift to the higher temperature. Both *rpoH* transcripts increased by similar amounts. When the magnitude of the increase was measured by quantitative 3′ S1 mapping, it was again found that the *rpoH* mRNA levels increased about fivefold (Fig. 1C).

The maximum level of *rpoH* mRNA was reached between 4 and 10 min after the temperature shift and it declined to about two times the pre-heat shock levels after 30 min at high temperature (Fig. 1B and C, Fig. 2). These kinetics are not substantially different from those of known heat shock gene transcripts. The increases in heat shock protein synthesis

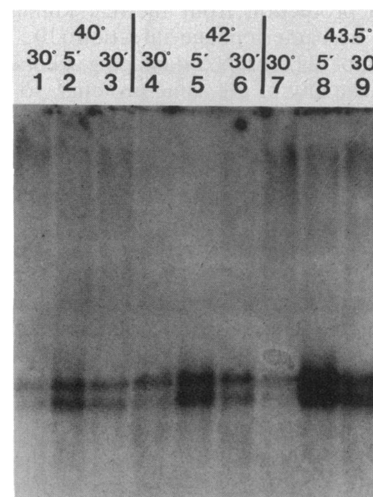

FIG. 2. Effect of temperature on *rpoH* mRNA levels. Northern blot of RNA isolated from strain SKB178 growing at 30°C (lanes 1, 4, and 7) 5 min (5′) after a shift to 40°C (lane 2), 42°C (lane 5), or 43.5°C (lane 8) and 30 min (30′) after a shift to 40°C (lane 3), 42°C (lane 6), or 43.5°C (lane 9). The blot was hybridized with an *rpoH*-specific probe.

rates vary with the temperature to which a culture is shifted (20). We examined the increase in *rpoH* mRNA level after shifts from 30°C to various higher temperatures, to see if the magnitude of the increase would also parallel the magnitude of the shift. We found progressively larger increases in *rpoH* message concentration at temperatures of 40, 42, and 43.5°C (Fig. 2). Perhaps the ultimate temperature to which the culture is shifted or the absolute difference between the pre- and post-heat shock temperatures, or both, are factors determining the induction ratio.

***rpoH* mRNA increases in *rpoH* mutants.** Since the pattern of *rpoH* mRNA induction is similar to those of known heat shock genes (e.g., *rpoD*; 26), we determined whether active sigma 32 is also required for the increase in *rpoH* mRNA concentration. We isolated RNA from three different *rpoH* mutants during growth at 30°C and after shifts to 44°C. The RNA was analyzed on Northern blots (Fig. 3). As expected, mRNA from a heat shock gene (*dnaK*) increased much less in the mutants than in the wild type (Fig. 3). In sharp contrast, we found comparable or slightly greater increases in *rpoH* RNA after heat shock of the *rpoH* mutants (Fig. 3).

DISCUSSION

The identities of the heat shock proteins and events that constitute the response of *E. coli* to stress are starting to be determined. Although the process by which an extracellular stress is sensed and transduced into an intracellular signal is unknown, an essential later step in the response to heat shock is the formation of an active complex between sigma 32 and core RNA polymerase to promote increased transcription of the genes encoding the heat shock proteins. The resultant increased levels of mRNA ultimately result in higher levels of heat shock proteins. The increase in heat shock RNA and protein synthetic rates is transient, and there seems to be feedback inhibition by some of the heat shock gene products (see below). Our finding that levels of *rpoH* mRNA also increase after a temperature increase provides an additional factor to be considered in a description of the response.

Mechanism of *rpoH* mRNA increase. Levels of *rpoH* mRNA increase after heat shock by a novel mechanism, since they still increase in *rpoH* mutants. The increase could be the result of more transcription of the gene, stabilization of the mRNA, or a combination of the two. Transcription could be increased after heat shock by the synthesis or activation of either a new sigma factor or an auxiliary transcription factor. Alternatively, message stabilization, a rare mode of regulation in *E. coli* (6, 12), could lead to an increase in the concentration of *rpoH* mRNA after heat shock. It is also possible that *rpoH* transcripts are translated at a very low level in unstressed cells and that stress increases their translation rate and, concomitantly, their stability. In vitro transcription of the *rpoH* gene, construction of protein fusions between sigma 32 and beta galactosidase, and the isolation of unlinked or *rpoH* mutations affecting *rpoH* gene regulation should help distinguish among these possibilities.

Heat shock response regulation. It will be interesting to determine how the increase in *rpoH* mRNA concentration is involved in the regulation of the heat shock response. The increase could be important for the initiation of the response since it has already been shown that increasing the concentrations of *rpoH* mRNA by inducing *tac* promoter-*rpoH* coding sequence fusions is sufficient to turn on heat shock gene transcription at 30°C (Georgopoulos and Tilly, unpublished results; Straus et al., personal communication). Furthermore, in vitro-synthesized sigma 32 will promote transcription of heat shock genes (5), so no modifications of the protein are required to make it active. When antibodies to sigma 32 become available, it will be interesting to determine whether *rpoH* mRNA and gene product levels vary coordinately.

The level of increase of *rpoH* mRNA could also affect the shutoff of heat shock gene transcription rates. Heat shock gene transcription rates rise rapidly after a temperature increase, peak between 5 and 10 min after the shift, and fall to new steady-state levels by about 30 min after the temperature shift. Since increased levels of *rpoH* RNA should increase the levels of sigma 32, the resultant altered balance between sigmas 32 and 70 could delay the shutoff of heat shock gene transcription, allowing critical levels of heat shock proteins to accumulate. The shutoff of the heat shock response is known to be a complex process. Mutations in the *rpoD* (sigma 70) and *dnaK* genes prolong the high-level synthesis of heat shock proteins after a temperature shift (13, 25, 27). Furthermore, overproduction of wild-type DnaK protein diminishes the heat shock response (25). The sigma 32 concentration could be a critical element in controlling the ratio of heat shock-induced transcription to normal transcription at all stages of the heat shock response. An analysis of *rpoH* mutants that perturb the level of *rpoH* RNA after heat shock should help define the role of sigma 32 in the heat shock response.

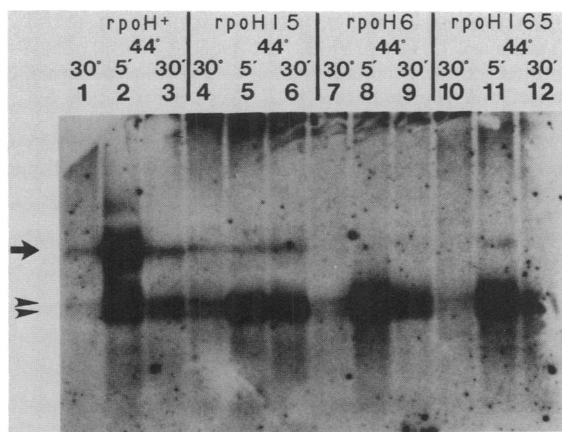

FIG. 3. *rpoH* mRNA increase in *rpoH* mutants. Northern blots of RNA isolated from strain SKB178 (lanes 1 through 3), *rpoH15* (lanes 4 through 6), *rpoH6*(Am) (lanes 7 through 9), and *rpoH165*(Am) (lanes 10 through 12) growing at 30°C (lanes 1, 4, 7, and 10) 5 min (5′) after a shift to 44°C (lanes 2, 5, 8, and 11) or 30 min (30′) after a shift to 44°C (lanes 3, 6, 9, and 12). The blot was hybridized with both *dnaK* (arrow) and *rpoH* (arrowheads) probes.

ACKNOWLEDGMENTS

We thank Karen Palter, Betty Craig, Carol Gross, and Jim Bardwell for constructive criticism of the manuscript. We are grateful to Jim Bardwell, Betty Craig, Dave Straus, Alan Grossman, and Carol Gross for communication of results before publication. These experiments were begun while K.T. was a postdoctoral fellow in the laboratory of Tom Maniatis.

This work was supported by grant DRG 598 from the Damon Runyon-Walter Winchell Cancer Fund and by Public Health Service grants GM10445 from the National Institutes of Health to K.T., AI21029 from the National Institutes of Health to C.G., and GM36278 and AI19635 from the National Institutes of Health to C. A. Gross (for J.E.).

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