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Transcriptional Control of the S10 Ribosomal Protein Operon of *Escherichia coli* after a Shift to Higher Temperature

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In the 5 to 10 min immediately following a shift from 30 to 42°C, the differential synthesis rates of ribosomal proteins encoded by the 11-gene S10 operon are transiently decreased. This effect results largely from a two- to threefold decrease in the differential rate of transcription of the operon. The inhibition of mRNA synthesis is apparently due to two types of control: (i) initiation of transcription at the S10 promoter is inhibited and (ii) readthrough at the attenuator in the S10 leader is decreased. Both of these effects on transcription are independent of the heat shock regulatory gene, *htpR*. Furthermore, the inhibition of transcription is observed in both *relA*$^+$ and *relA* cells, suggesting that the temperature-induced repression does not require the *relA*-dependent accumulation of guanosine tetraphosphate (ppGpp). However, recovery from the heat shock was slower in *relA*$^+$ strains than in *relA* strains. None of the other ribosomal protein operons that we analyzed showed such a strong decrease in transcription after the heat shock.

When *Escherichia coli* cells are subjected to an upshift in growth temperature, they respond with a transient and marked increase in the synthesis of a specific set of proteins referred to as the heat shock proteins (14, 22). Much is now known about the molecular processes involved in the induction of heat shock protein synthesis in *E. coli*. The response is dependent on the protein product of the *htpR* (*hin*) gene (18, 23); this protein positively controls transcription of the various, unlinked heat shock genes (23). The *htpR* gene has recently been sequenced and its product has been identified as a sigma factor (designated sigma-32) of RNA polymerase (8, 13). The induction of heat shock protein synthesis thus apparently results from increased initiation of transcription at promoters of heat shock operons, mediated by the *htpR*-encoded sigma factor.

Concurrent with the increased synthesis of heat shock proteins, the synthesis of another set of proteins is transiently decreased (14). Several years ago, Neidhardt and his co-workers showed that the proteins whose synthesis is inhibited after heat shock included several components of the transcription-translation apparatus (14). Since that initial analysis, little attention has been paid to this aspect of the heat shock response. As part of our analysis of the regulation of ribosomal protein (r-protein) synthesis, we were interested in further characterizing the effect of a heat shock on the expression of r-protein genes. Does a temperature upshift result in a significant decrease in the synthesis of r-proteins? If there is an effect of heat shock, is it mediated at the level of translation or transcription? Moreover, is the *htpR* gene product involved in the response?

Our studies have concentrated on the S10 r-protein operon. This operon codes for 11 r-proteins (Fig. 1). The product of the third gene, L4, has a dual function; it serves as a structural component of the 50S subunit, and it also functions as an autogenous regulator of the entire S10 operon (25). We have shown that L4 regulates the S10 operon by regulating the level of readthrough at an attenuator in the leader, located about 30 bases upstream from the first structural gene (15). The attenuation process presumably involves a direct interaction between free L4 and the growing mRNA chain (15). Thus, when L4 accumulates in the cell, attenuation is increased, leading to a decreased expression of the entire S10 operon. On the other hand, attenuation is relieved when the free L4 concentration is reduced by its binding to its 23S rRNA target site during ribosome assembly. In addition to L4-mediated regulation at the attenuator, regulation at the level of the S10 promoter also plays an important role in the overall control of the expression of the S10 operon (L. Lindahl and J. M. Zengel, *Molecular Biology of Bacterial Growth*, in press; L. P. Freedman, J. M. Zengel and L. Lindahl, J. Mol. Biol., in press).

We analyzed here the expression of the S10 operon after a heat shock by measuring both protein synthesis and mRNA synthesis from this operon. Our results indicate that a temperature upshift does indeed result in a strong decrease in the rates of synthesis of proteins encoded by this operon. This response is apparently mediated by *htpR*-independent effects on both transcription initiation and attenuation.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The strains and plasmids used for this study are listed in Table 1. Maps of the *lacZ* fusion plasmids are shown in Fig. 1. Their construction has been described elsewhere (Freedman et al., in press). Cells were grown in AB minimal medium (4) supplemented with thiamine (2.5 μg/ml), 0.2% glucose, and 19 amino acids (minus methionine), each at 40 μg/ml. Cell growth was monitored as the optical density at 450 nm (OD_{450}; OD_{450} = 1 corresponds to ca. 2×10^8 cells per ml).

Protein labeling and gel electrophoresis. Cells were grown exponentially at 30°C. At an OD_{450} of about 0.5, two 100-μl portions were withdrawn and labeled with 2 μCi of [^{35}S]methionine (ca. 1,000 Ci/mmol). After 0.5 min, excess nonradioactive methionine was added (final concentration, 40 μg/ml). After 2 min the cells were harvested in sample buffer (12) at 95°C. For the temperature upshift 12 to 15 ml of the remaining culture was transferred to a 125-ml flask prewarmed to 42°C. At various times thereafter, 100-μl samples were again pulse labeled with [^{35}S]methionine, chased, and harvested as described above. The samples were then fractionated by electrophoresis through a 7.5%

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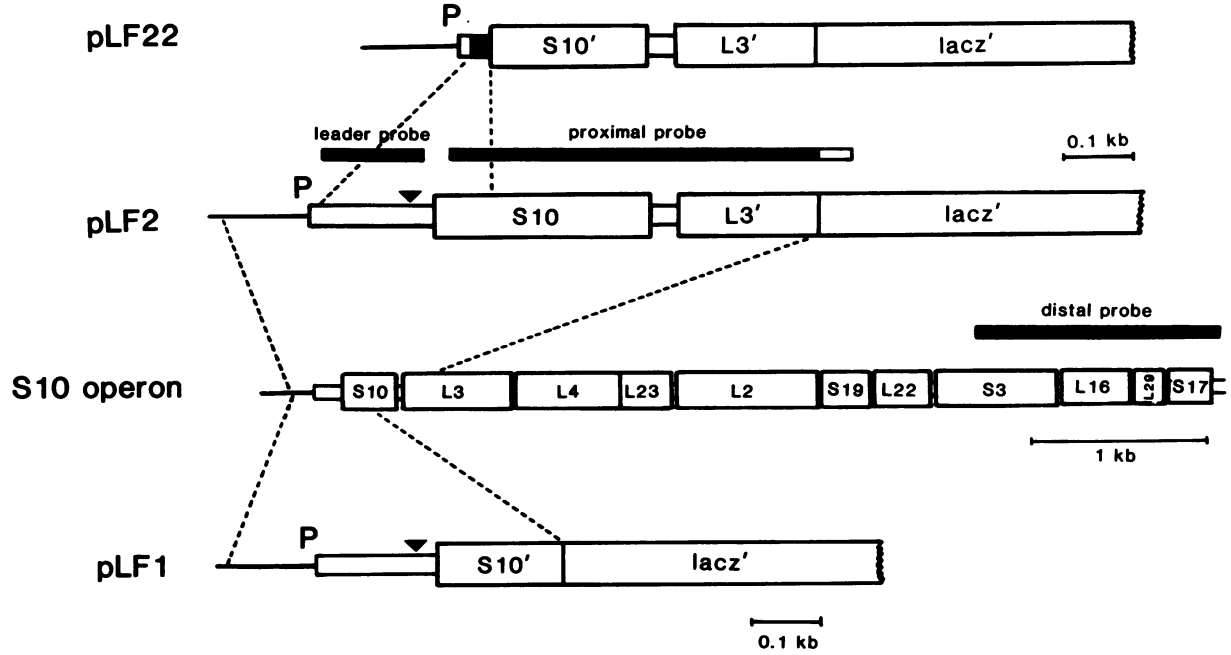

FIG. 1. Map of the S10 operon and *lacZ* fusion plasmids. The entire S10 operon is shown drawn to scale according to the sequence determined by G. Zurawski, DNAX, Palo Alto, Calif. The structure of pLF1 is shown below the map of the S10 operon. The structures of pLF2 and its derivative pLF22 are shown above. The hybridization probes used for these studies are indicated by solid bars above the maps of the S10 operon and pLF2. Note that the proximal probe contains about 50 bases at the 3' end (shown by the open box) which are downstream from the restriction site used to fuse *lacZ'* to the L3 gene. The approximate position of the attenuator in pLF1 and pLF2 is indicated (▼). During the construction of pLF22, the deletion of the leader region was accompanied by an insertion of a short fragment of DNA from the M13 linker region; this sequence is indicated by the solid area.

polyacrylamide-sodium dodecyl sulfate slab gel. Typically, the total trichloroacetic acid-precipitable ^{35}S radioactivity applied to a gel slot was 0.75×10^5 to 1.5×10^5 cpm, as measured by liquid scintillation spectrometry. The appropriate gel bands were cut out, and their radioactivity was determined as described previously (16). The radioactivity in the *lacZ* fusion protein band of preshift samples usually represented 1 to 2% of the input radioactivity for cells carrying pLF2 or pLF22 and 2.5 to 4% of the input radioactivity for cells carrying pLF1. Although these values varied for different hosts, in a given strain and a given labeling experiment the independent duplicate labelings varied less than 15%.

For analysis of r-proteins, 12 ml of SC122 cells were grown exponentially at 30°C in minimal medium supplemented with 17 amino acids (minus leucine, serine, and methionine). At an OD_{450} of about 0.15, the cells were labeled with 0.72 mCi of [^{3}H]leucine (60 Ci/mmol) and 0.72 mCi of [^{3}H]serine (18.3 Ci/mmol). After one doubling, nonradioactive leucine and serine were added (each to 30 μg/ml). Approximately one doubling later, two 2.5-ml portions were transferred to flasks each containing 25 μCi of [^{35}S]methionine (1,000 Ci/mmol). After 0.5 min, the cells were chased for 2 min with nonradioactive methionine (20 μg/ml) and then harvested on ice. For the temperature upshift, 6 ml of the culture was transferred to a 250-ml flask prewarmed to 42°C and incubated with shaking at the same temperature. At 4 min and again at 7 min after the temperature shift, a 2.5-ml portion was transferred to a flask containing [^{35}S]methionine and pulse labeled, chased, and harvested as described above for the preshift samples. Total cell extracts were prepared and fractionated on two-dimensional gels (10) as described previously (16). The radioactivity in protein spots was determined as described previously (16).

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source or reference
Strain		
LL308	F' *pro*$^+$ *lacI*^{q}ZΔM15 *Y*$^+$-Δ(*pro-lac*) *gyrA recA supE thi*	Our strain collection (25)
SC122	*lac*(Am) *trp*(Am) *pho*(Am) *supC*(Ts) *str mal*(Am)	F. C. Neidhardt (5, 18)
K165	*lac*(Am) *trp*(Am) *pho*(Am) *supC*(Ts) *str mal*(Am) *htpR*(Am)	F. C. Neidhardt (5, 18)
CP78-118	*relA*$^+$ *thi leu thr arg his lacZ*-U118	J. Gallant
CP79-118	*relA2 thi leu thr arg his lacZ*-U118	J. Gallant
Plasmid		
pLF1	P_{S10}-leader$_{S10}$-S10'-*lacZ'* on pSC101 vector	(Fig. 1)
pLF2	P_{S10}-leader$_{S10}$-S10-L3'-*lacZ'* on pSC101 vector	(Fig. 1)
pLF22	P_{S10}-Δ(leader$_{S10}$)-'S10-L3'-*lacZ'* on pSC101 vector	(Fig. 1)

For a given gel, the ratio of ^{35}S to ^{3}H for each individual protein spot was divided by the ratio of ^{35}S to ^{3}H of trichloroacetic acid-precipitable material determined for the cell extract loaded on the gel. The values for the postshift proteins were then normalized to the values for the corresponding preshift proteins (determined as the average of the two independently labeled preshift samples).

Hybridization probes. Except for lambda *ilv* (described below), all hybridization probes were single-stranded DNA derived from M13 phages carrying the appropriate inserts. Probes for the S10 leader, the S10-L3 proximal structural genes, and the distal structural genes have been described previously (24) and are shown in Fig. 1. The *lacZ* probe (SUM18) contains a 1.8-kilobase *Hin*cII fragment from the distal half of the *lacZ* gene (1). The alpha operon probe was described previously (15). Three probes for rRNA were used. Total rRNA was measured by using denatured lambda *ilv* DNA (9). A probe specific for the proximal portion of 16S RNA was constructed by cloning a 0.57-kilobase *Hin*dIII fragment from the *rrnB* operon on pKK3535 (2) into the *Hin*dIII site of M13mp10 (17). The 23S rRNA probe was constructed by cloning the 0.50-kilobase *Eco*RI-*Sal*I fragment from pKK3535 into M13mp11 (17) digested with *Eco*RI and *Sal*I.

RNA labelings and hybridizations. Cells were grown exponentially at 30°C. At an OD_{450} of about 0.6, three 2-ml portions were labeled for 0.7 to 1.0 min with 150 μCi of [^{3}H]uridine (40 to 50 Ci/mmol). Approximately 20 ml of the 30°C culture was then transferred to a 500-ml flask prewarmed to 42°C, and the [^{3}H]uridine labelings were repeated at various times after the temperature upshift. The RNA was harvested and hybridized to immobilized DNA on nitrocellulose filters as previously described (15, 25). For r-protein mRNA measurements the input radioactivity was 0.5 × 10^{6} to 2.3 × 10^{6} cpm. For rRNA measurements the input radioactivity was 0.4 × 10^{5} to 1.2 × 10^{5} cpm. Duplicate filters for a given probe were included in each hybridization assay. The radioactivity hybridizing to a specific probe was corrected for unspecific binding of [^{3}H]RNA to the filters (determined as the counts per minute bound to filters loaded with M13 DNA carrying either no insert or an insert in the nonhybridizing orientation). For most experiments, these corrections corresponded to less than 15% of the radioactivity hybridizing to a given probe. One exception was the S10 leader probe in extracts from haploid strains; in these hybridizations the correction was as much as 30%.

RESULTS

Effect of a heat shock on the synthesis of protein from the S10 operon. To facilitate our study of the heat shock response of the S10 operon, we used plasmids carrying the beginning of the S10 operon, including the promoter and leader, and a fusion between the first (S10) or second (L3) structural gene of the operon and the *lacZ* gene coding for β-galactosidase. The structures of these two fusion plasmids, designated pLF1 and pLF2, respectively, are shown in Fig. 1. We have shown previously that these fusion plasmids are regulated in response to L4 oversynthesis, nutritional changes, and amino acid starvation in the same way as the intact chromosomal S10 operon (23; Freedman et al., in press).

We first analyzed the effect of the temperature upshift on the synthesis of the L3′-*lacZ*′ fusion protein encoded by pLF2 (Fig. 1). The strain used for the initial analysis was LL308 (Table 1). Cells were pulse labeled with [^{35}S] methionine either immediately before or at various times after a shift from 30 to 42°C, and total protein extracts were fractionated on a polyacrylamide-sodium dodecyl sulfate gel. To quantitate the heat shock response, we determined the amount of radioactivity in the fusion protein band, as well as in several control bands. The results of this experiment (Fig. 2) indicated that the expression of the partial S10 operon carried on plasmid pLF2 is significantly decreased after a heat shock. The differential rate of synthesis of the L3′-*lacZ*′ fusion protein decreased by about fourfold within the first 5 to 10 min after the temperature shift (Fig. 2a). At the same time synthesis of the heat shock proteins GroEL (Fig. 2d) and DnaK (not shown) was dramatically stimulated. In agreement with the results reported previously (14), synthesis of the beta and beta′ subunits of RNA polymerase was moderately decreased after the temperature shift (Fig. 2b), whereas synthesis of the peptide elongation factor Tu was relatively unaffected (Fig. 2c).

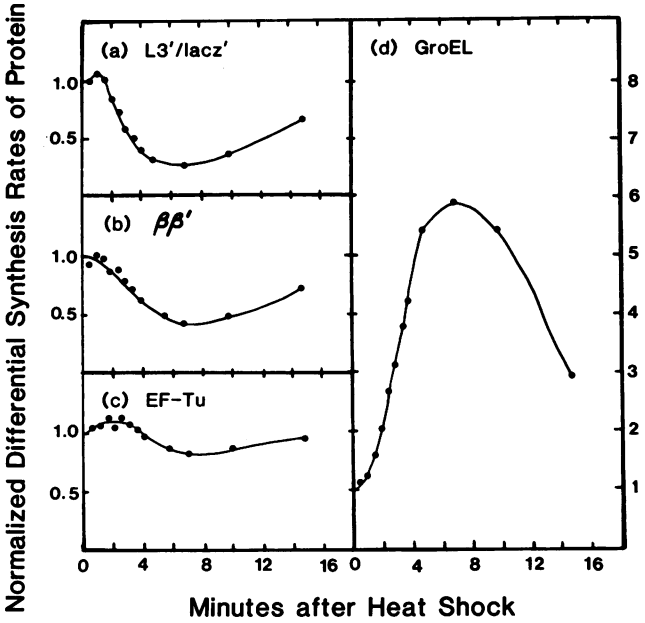

FIG. 2. Effect of a heat shock on synthesis of proteins in LL308. Exponentially growing cells carrying the L3′-*lacZ*′ fusion plasmid pLF2 were pulse labeled with [^{35}S]methionine immediately before and at the indicated times after a shift from 30 to 42°C. Total protein extracts were fractionated on a 7.5% polyacrylamide-sodium dodecyl sulfate gel. The relevant bands were cut out and processed as described previously (16). The radioactivity in a given band was divided by the total trichloroacetic acid-precipitable radioactivity loaded in that lane of the gel. The postshift values for a given protein were then normalized to the average of two preshift determinations for the same protein. For further details, see the text. Panels: a, L3′-*lacZ*′ fusion protein; b, beta and beta′ subunits of RNA polymerase; c, peptide elongation factor Tu (EF-Tu); d, GroEL protein.

To be certain that the effect on L3′-*lacZ*′ fusion protein synthesis reflects a similar response by genuine r-proteins, we directly measured r-protein synthesis from the S10 operon before and immediately after a heat shock. For this experiment we used haploid strain SC122 (Table 1) and fractionated the proteins by two-dimensional gel electrophoresis (10, 16). The results, summarized in Table 2, indicate that the expression of the chromosomal S10 operon is indeed inhibited after a temperature shift. They also suggest that the synthesis of other r-proteins is also inhibited after heat shock, although not as much as proteins encoded by the S10 operon.

TABLE 2. Normalized differential synthesis rates of individual r-proteins after heat shock[a]

r-protein	Synthesis rates after temp shift	
	4 min	7 min
S10 operon		
L3	0.64	0.49
L2	0.65	0.39
S19	0.67	0.50
L22	0.85	0.53
L16	0.99	0.54
spc operon		
L5	0.74	0.79
S14	0.61	0.64
S8	0.65	0.71
L18	0.94	0.76
Alpha operon		
S13	0.78	0.68
S4	0.85	0.68
L17	0.92	0.60
L1-L11 operon L1	0.55	0.77
Other operons		
L13	0.83	0.60
L19	0.83	0.78
L25	0.85	0.37

[a] Strain SC122, prelabeled with [^{3}H]leucine and [^{3}H]serine, was pulse labeled with [^{35}S]methionine immediately before and at the indicated times after a shift from 30 to 42°C. Total cell extracts were processed and fractionated on two-dimensional gels as described previously (16). The values shown are normalized to the preshift values of each protein (see the text for more details).

Effect of the *htpR* allele on the heat shock response of the S10 r-protein operon. The sigma factor encoded by the *htpR* gene (8, 13) is required for the heat induction of most heat shock proteins in *E. coli* (18, 23). Conceivably, RNA polymerase molecules containing the *htpR*-encoded sigma factor could also be responsible for the heat shock response of the S10 operon, either directly by reducing transcription initiation at the S10 promoter or indirectly via its role in the enhanced synthesis of a heat shock protein(s) that might inhibit transcription or translation of the S10 operon. Therefore, to determine whether the inhibition of the S10 operon requires this sigma factor, we measured the effect of a temperature shift in a pair of *htpR*$^+$ and *htpR* strains (Table 1) carrying either pLF1 (P_{S10}-leader$_{S10}$-S10′-*lacZ*′) or pLF2 (P_{S10}-leader$_{S10}$-S10-L3′-*lacZ*′). Our results (Fig. 3) indicate that the product of the *htpR* gene is not essential for the inhibition of protein synthesis from the S10 operon after a heat shock. The synthesis of both the S10′-*lacZ*′ fusion protein from pLF1 (Fig. 3a) and the L3′-*lacZ*′ fusion protein from pLF2 (Fig. 3c) was inhibited to almost the same extent in the *htpR* strain as in the *htpR*$^+$ strain. As expected, the heat induction of the heat shock protein GroEL synthesis was absolutely dependent on the presence of a wild-type *htpR* allele (Fig. 3b and d).

Transcription of the S10 operon after a heat shock of fusion plasmid strains. To determine whether the heat shock response of the S10 operon is exerted at the level of transcription or translation, we measured the rate of mRNA synthesis from the operon after the temperature shift. The *htpR*$^+$ and *htpR* cells carrying pLF2 (P_{S10}-leader$_{S10}$-S10-L3′-*lacZ*′) were pulse labeled with [^{3}H]uridine immediately before and at various times after the heat shock. Radioactive RNA was then hybridized to filters carrying DNA probes specific for various r-protein operons, including the S10 operon, or for the rRNA transcription units. Since all samples were taken within 10 min of the temperature shift, we did not expect the plasmid copy number to change significantly during the experiment. Our results therefore should reflect regulation of the S10 operon.

We observed a 40-to-50% decrease in the differential rate of mRNA synthesis from the leader region of the S10 operon within the first 4 min of the heat shock (Fig. 4a). The effect was essentially the same in both the *htpR*$^+$ and the *htpR* cells. The leader probe measures the 5′ end of the S10 transcript and is almost entirely upstream of the site of attenuation (Fig. 1). Therefore, we interpret these results to mean that a temperature shift induces a reduced rate of initiation at the S10 promoter, independent of the *htpR* allele.

We also measured the differential rates of mRNA synthesis from the structural genes. Using a probe specific for the *lacZ* message transcribed from the fusion plasmid pLF2, we observed a 70-to-80% decrease in transcription, regardless of the *htpR* allele (Fig. 4c). We found similar decreases using a probe for the message of the proximal S10 and L3 structural genes (Fig. 4b) and a probe for the message from the distal

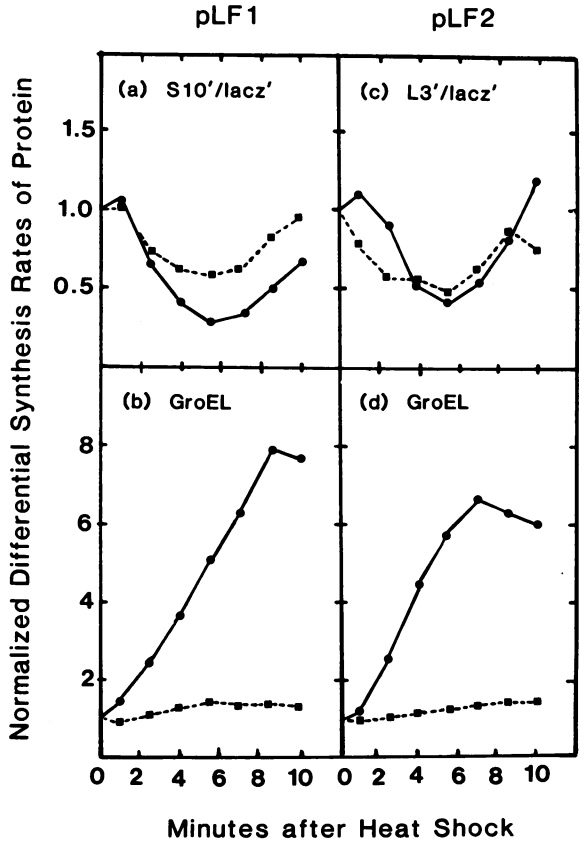

FIG. 3. Effect of a heat shock on synthesis of *lacZ* fusion proteins in *htpR*$^+$ and *htpR* cells. The *htpR*$^+$ strain SC122 (●) and the *htpR* strain K165 (■) carrying either pLF1 (panels a and b) or pLF2 (panels c and d) were pulse labeled with [^{35}S]methionine before and at the indicated times after a shift from 30 to 42°C. Radioactivity in the fusion protein and in the heat shock protein GroEL was determined as described in the legend to Fig. 2 and in the text.

three genes of the operon (Fig. 4d). The effect on structural gene messenger synthesis is somewhat stronger than the effect on leader transcription. We interpret this difference to mean that, in addition to the initiation effect, readthrough at the S10 attenuator is decreased in response to a heat shock (discussed in more detail below). However, we cannot exclude the possibility that part of the difference between leader transcription and structural gene transcription is also due to polarity resulting from heat-induced transcription termination within the sructural genes. In any event, none of these responses is affected by the *htpR* allele.

The strong inhibition of transcription observed for the S10 operon seems to be unique to that ribosomal operon. Transcription of the alpha operon was not significantly affected by the temperature shift (Fig. 4e), nor did any of the other r-protein operons we analyzed, including the *str*, *spc*, and L11-L1 operons, show a strong decrease in transcription (data not shown). We also observed little or no effect on the synthesis of RNA after heat shock when using probes specific for the entire rRNA operon (Fig. 4f) or for the 16S or 23S rRNA species (Fig. 4g and h, respectively).

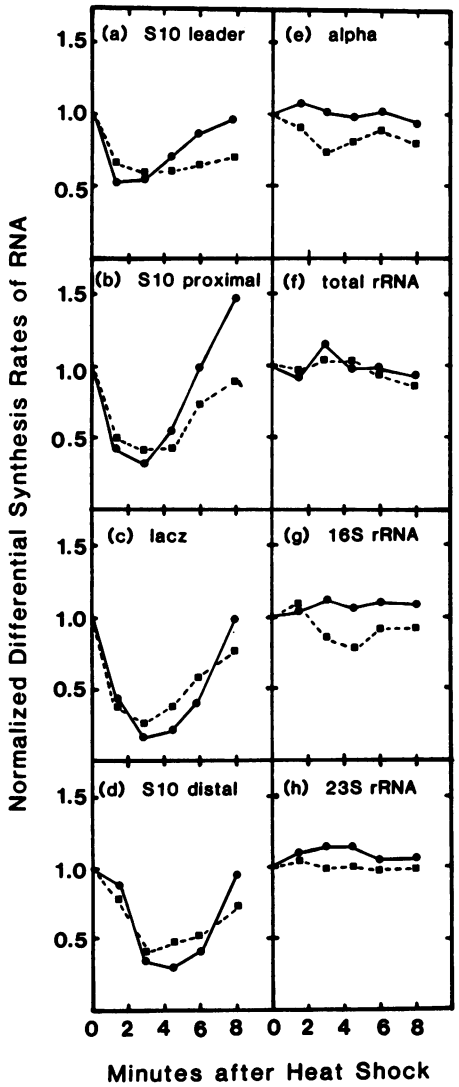

FIG. 4. Transcription of ribosomal operons after a heat shock of *htpR*$^+$ and *htpR* cells. Cells carrying the L3′-*lacZ*′ fusion plasmid pLF2 were pulse labeled for 0.7 min with [^{3}H]uridine before and at the indicated times after a shift from 30 to 42°C. The RNA was extracted and then hybridized to the indicated probes as described in the text. The differential rates of synthesis were calculated as the radioactivity hybridizing to a given probe divided by the total trichloroacetic acid-precipitable radioactivity added to the hybridization assay. These rates were then normalized to the average of three independent determinations of the differential synthesis rates for the same RNA species in preshift cells. Symbols: ●, *htpR*$^+$ cells; ■, *htpR* cells.

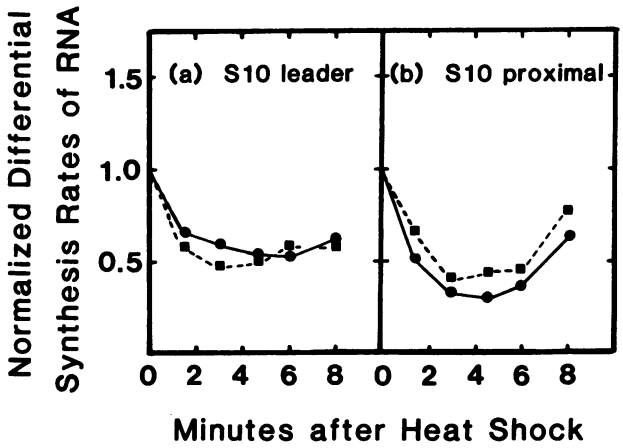

FIG. 5. Transcription of the S10 operon in plasmid-free *htpR*$^+$ and *htpR* cells after a heat shock. RNA was labeled and analyzed as described in the legend to Fig. 4, except the [^{3}H]uridine labeling was for 1.0 min. Symbols: ●, *htpR*$^+$ cells; ■, *htpR* cells.

Transcription of the S10 operon after heat shock of haploid strains. Most of the S10 operon probes used in the experiment just described measure the amount of transcript coming predominantly (S10 leader and S10-L3 proximal structural genes) or exclusively (*lacZ*′) from the medium copy number plasmid pLF2. Although the results with the S10 distal probe, which measures only the chromosomal message, suggested that the inhibition of transcription was occurring on the intact chromosomal S10 operon as well as on the plasmid copies, we were still concerned that the heat-induced decrease in transcription might reflect a plasmid-specific effect. Therefore, we repeated the RNA-labeling experiment using plasmid-free *htpR*$^+$ and *htpR* strains. The results (Fig. 5) confirmed that a heat shock results in an inhibition of transcription in the intact chromosomal S10 operon.

Effect of the *relA* locus on the heat shock response of the S10 operon. Previous experiments have shown that heat shock induces a transient accumulation of ppGpp (3, 6, 14). Whether this increase has a significant effect on rRNA synthesis is somewhat controversial (3, 6, 20). Nevertheless, we had to consider the possibility that the heat shock response of the S10 operon is simply a manifestation of the stringent response characteristic of *relA*$^+$ cells. Several lines of evidence suggest that this is probably not the case. First, it has been shown that the accumulation of ppGpp after a temperature upshift is minimized when the cells are growing in a medium which includes a specific set of amino acids (6). In all of our experiments the cells were grown in minimal medium supplemented with all amino acids except methionine, an amino acid not affecting ppGpp accumulation (6). Second, in a typical stringent response, we would expect to see an inhibition of rRNA synthesis as well as r-protein mRNA synthesis. Yet, in our experiments, transcription of

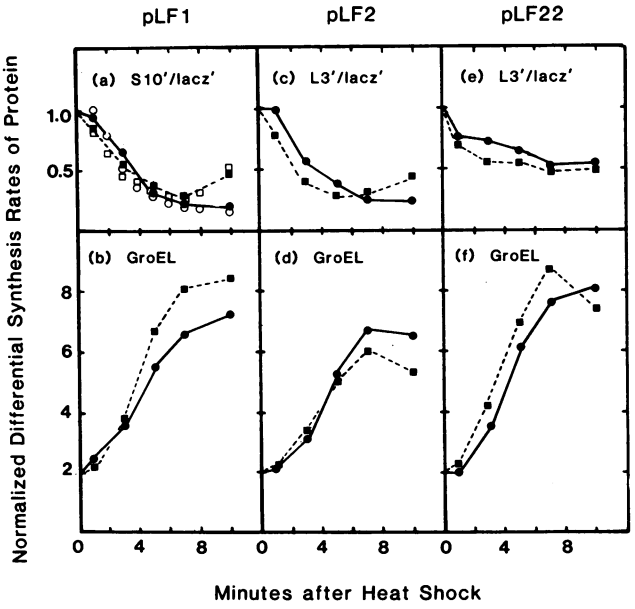

FIG. 6. Effect of a heat shock on fusion protein synthesis in relaxed and stringent cells. Exponentially growing derivatives of the stringent (*relA*$^+$) strain CP78-118 (circles, solid lines) or the relaxed (*relA*) strain CP79-118 (squares, dashed lines) containing pLF1 (panels a and b), pLF2 (panels c and d), or pLF22 (panels e and f) were pulse labeled with [^{35}S]methionine before and at the indicated times after a shift from 30 to 42°C. Total protein extracts were analyzed as described in the legend to Fig. 3. Solid symbols represent data from one labeling experiment; open symbols represent data from a second, independent labeling experiment.

only the S10 operon was significantly decreased after heat shock. Finally, the *htpR* strains used in our experiments have a partially relaxed phenotype, as judged by their ability to accumulate radioactive RNA during valine-induced starvation for isoleucine or lysine hydroxamate-induced starvation for lysine (data not shown).

To assess more precisely the role of the *relA* locus on the heat shock response of the S10 operon, we measured the effect of a temperature upshift in an isogenic pair of *relA*$^+$ and *relA* strains carrying the fusion plasmids pLF1 (P_{S10}-leader$_{S10}$-S10'-*lacZ'*) or pLF2 (P_{S10}-leader$_{S10}$-S10-L3'-*lacZ'*). We first analyzed the effect of a heat shock on fusion protein synthesis in these strains. We found that the synthesis of the *lacZ'* fusion protein from either pLF1 (Fig. 6a) or pLF2 (Fig. 6c) was decreased significantly in both the wild-type (*relA*$^+$) and the relaxed (*relA*) strains. That is, the *relA* locus did not affect the initial response of the S10 operon to a heat shock. However, we did note that the synthesis of *lacZ* fusion protein began to recover by about 6 min in the *relA* strain but not in the *relA*$^+$ strain. We observed the same effect when we repeated the experiment with cells carrying pLF1, this time taking more samples over a longer period of time after the heat shock (Fig. 6a, open symbols).

Our fusion protein synthesis measurements suggested that the *relA*$^+$ allele is not required for the initial response of the S10 operon to a heat shock. However, the *relA* locus may affect the duration of the inhibition. These conclusions were confirmed by direct measurements of transcription of the S10 operon after a heat shock of *relA*$^+$ and *relA* cells carrying the pLF2 fusion plasmid. We found that the synthesis of structural gene message from the S10 operon was significantly inhibited within the first few minutes of heat shock, regardless of the *relA* allele (Fig. 7b, c, and d). However, as predicted by the protein synthesis measurements, transcription of the structural genes in the *relA* cells began to increase again after 3 or 4 min, whereas mRNA synthesis in the *relA*$^+$ cells remained inhibited for at least 8 min.

Contributions of initiation control and attenuation control to the heat shock response of the S10 operon. Transcription of the S10 leader sequence is decreased to about the same extent in the relaxed and stringent strains (Fig. 7a), indicating that the *relA* locus does not influence the inhibition of transcription initiation induced by heat shock. This implies that the *relA* effect on transcription of structural gene message is at the level of attenuation. To quantitate this effect, we determined the level of readthrough at the attenuator (calculated by dividing the radioactivity hybridizing to the proximal structural gene probe by the radioactivity hybridizing to the leader probe). The results (Fig. 8) indicate that there is in fact a stronger decrease in readthrough in the *relA*$^+$ strain than in the *relA* strain. In the *relA* strain, readthrough at the attenuator increases 3 to 4 min after the heat shock; there is no relief of attenuation in the *relA*$^+$ strain (Fig. 8a). We also quantitated the level of readthrough in the pair of *htpR*$^+$ and *htpR* strains (Fig. 8b). These strains exhibited the same small, transient decrease in readthrough observed with the *relA* strain. This result is consistent with

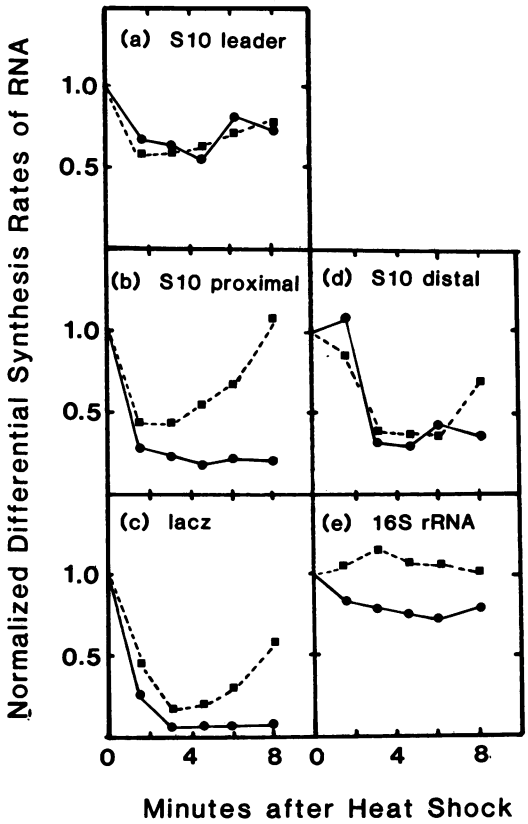

FIG. 7. Transcription of the S10 operon after a heat shock of stringent and relaxed cells. Derivatives of the *relA*$^+$ strain CP78-118 (●) and the *relA* strain CP79-118 (■) carrying the L3'-*lacZ'* fusion plasmid pLF2 were pulse labeled for 0.7 min with [^{3}H]uridine before and at the indicated times after a shift from 30 to 42°C. The RNA was analyzed as described in the legend to Fig. 4.

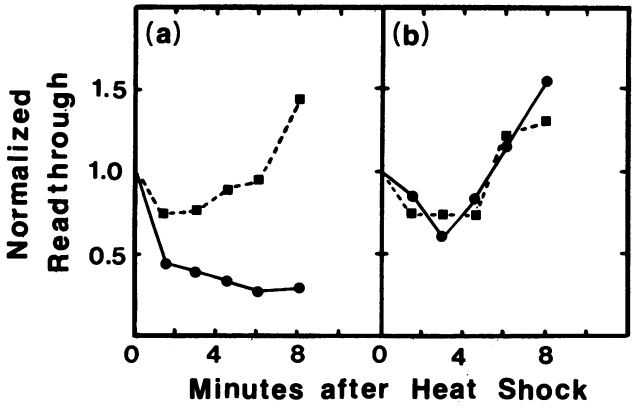

FIG. 8. Readthrough at the S10 attenuator after heat shock. Readthrough was calculated by dividing the radioactivity in the proximal structural gene message by the radioactivity in the leader message (23). The resulting quotients were then normalized to the preshift value. The data shown in panel a are from the experiment described in Fig. 7, measuring transcription of the *relA*$^+$ strain CP78-118 (●) and the *relA* strain CP79-118 (■). The data shown in panel b are from the experiment described in Fig. 4, measuring transcription of the *htpR*$^+$ strain SC122 (●) and the *htpR* strain K165 (■).

our observation that the *htpR*$^+$ and *htpR* strains exhibit a partially relaxed phenotype. The difference in attenuation in stringent and relaxed strains may reflect a difference in the amount of 23S rRNA targets available for L4 binding, since we did observe a slight decrease in the synthesis of rRNA in the stringent strain compared to that of the relaxed strain (Fig. 7e).

Our hybridization data suggested that the decreased expression of the S10 operon after a heat shock results from an effect exerted both at the level of transcription initiation and at the level of attenuation. This conclusion was confirmed by measuring the synthesis of fusion protein from a derivative of pLF2 in which the target for L4-mediated attenuation control has been eliminated by a deletion that removed all of the S10 leader (Freedman et al., in press). The structure of this plasmid, pLF22, is shown in Fig. 1. Both *relA*$^+$ and *relA* cells carrying pLF22 showed a decreased rate of fusion protein synthesis after a heat shock (Fig. 6e). However, the effect was diminished relative to the effect observed in the pLF2 strains (Fig. 6c), resembling the kinetics of leader transcription after heat shock (Fig. 7a). This result is consistent with the hypothesis that the heat shock response of the S10 operon results from the combined effect of initiation regulation and attenuation regulation. In the pLF22-containing cells, only initiation control is contributing to the regulation of fusion protein synthesis after a temperature upshift.

DISCUSSION

Transcription of the 11-gene S10 r-protein operon is inhibited when cells are shifted from 30 to 42°C. This response is independent of the *htpR*-mediated induction of transcription of heat shock operons. The differential synthesis rates of other r-protein operons and rRNA transcription units are also not affected by the *htpR* allele. In light of the recent discovery that the *htpR* gene product is a new sigma factor (8, 13), our results indicate that the synthesis of the modified RNA polymerase after a heat shock does not affect the differential transcription of r-protein or rRNA genes.

It is not clear why transcription of the S10 operon is more sensitive to a heat shock than is transcription of other r-protein or rRNA operons. To some extent this observation can be explained by the fact that the heat shock response in the S10 operon is partly dependent on attenuation control, presumably via the L4-mediated autogenous regulation (15). Autogenous control of other r-protein operons appears to be at the level of translation (19). Therefore, to the extent that autogenous regulation is involved in the heat shock response, the contribution of attenuation control of the S10 operon may be achieved in other r-protein operons by control at the level of translation. However, autogenous regulation cannot account for the effect of heat shock on initiation of transcription at the S10 promoter. The experiments with the *relA*$^+$ and *relA* cells indicate that stringent control is also not responsible for the promoter effect. Since temperature changes can alter the level of supercoiling of DNA (7, 21), one possibility is that the activity of the S10 promoter is more sensitive than the activities of other ribosomal promoters to temperature-induced changes in DNA supercoiling.

Although the *relA* locus does not have an effect on transcription initiation in the S10 operon after a heat shock, it does appear to have an effect on the level of attenuation. The differential synthesis of structural gene message begins to recover several minutes after the heat shock in the *relA* strain but not in the *relA*$^+$ strain, apparently because of increased readthrough at the S10 attenuator. In fact, in the relaxed strains the readthrough rebounds to a level about 50% higher than the preshift value. Since the level of attenuation in the S10 operon presumably reflects the amount of free L4 in the cell (15), this difference in recovery may be due to a relatively lower concentration of free L4 or higher concentration of its 23S target in the *relA* cells compared to the *relA*$^+$ cells. Consistent with this hypothesis, our hybridization results suggest that the differential rate of rRNA synthesis is slightly inhibited in the *relA*$^+$ cells, but not the *relA* cells, after heat shock. Similar observations have been reported by Ryals et al. (20). Thus, the inhibition of rRNA synthesis in the *relA*$^+$ strain probably results in an increase in the amount of free L4, thereby resulting in increased attenuation. Although this model can explain the difference in the recovery in the *relA*$^+$ and *relA* strains, we cannot exclude other possibilities. For example, ppGpp may directly affect the level of attenuation, perhaps by affecting RNA polymerase pausing (11) in the S10 leader. Alternatively, the pair of *relA*$^+$ and *relA* strains used in our experiments may have an unidentified difference at another locus that actually accounts for the disparity in the heat shock response of the two strains.

Although the strongest effect on attenuation was observed in the *relA*$^+$ cells, even *relA* cells exhibit a small, transient decrease in readthrough at the attenuator during their initial response to heat shock. Since we cannot detect any heat-induced effect on the differential synthesis rate of rRNA in the relaxed cells, it is not clear why attenuation is affected. Perhaps, since the pool of free L4 is very small, a very subtle change in the rRNA synthesis rate after heat shock affects the concentration of free L4. Another possibility is that the relative affinities of L4 for rRNA and its own mRNA might change as a result of the temperature increase. This might in turn lead to a temporary imbalance in the L4-mediated regulatory pathways.

Whatever the molecular basis may be for the heat shock response of the S10 operon, the inhibition of transcription is clearly the result of at least two types of control, one regulating the level of transcription initiation, the other

regulating the level of transcription beyond the S10 attenuator. Moreover, still other regulatory pathways are apparently involved in the heat shock response of other r-protein operons. Thus, as we have found from other physiological experiments (24), no simple, single regulatory process can account for the overall regulation of ribosome synthesis. Rather, the regulation appears to result from a complex and dynamic interaction between several different molecular mechanisms.

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