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Transcriptional Regulation of the Heat Shock Regulatory Gene *rpoH* in *Escherichia coli*: Involvement of a Novel Catabolite-Sensitive Promoter

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A catabolite-sensitive promoter was found to be involved in transcription of the heat shock regulatory gene *rpoH* encoding the σ^{32} protein. Expression of *lacZ* from the operon fusion, *rpoHp-lacZ*, was partially inhibited by glucose added to the broth medium. Dissection of the *rpoH* promoter region allowed us to localize the glucose-sensitive promoter to the 110-base-pair (bp) segment directly upstream of the *rpoH* coding region. Experiments on *lacZ* expression from the set of fusions in *cya* (adenylate cyclase) and *crp* (cyclic AMP [cAMP] receptor protein) mutants also supported the involvement of a catabolite-sensitive promoter. Analysis of *rpoH* mRNAs by S1 nuclease protection experiments led us to identify a novel promoter, designated P5, that is regulated by cAMP and the cAMP receptor protein. Studies of *rpoH* transcription in vitro demonstrated that RNA polymerase-σ^{70} can transcribe from the P5 promoter only in the presence of cAMP and its receptor protein. The 5′ ends of P5 transcripts obtained in vivo and in vitro were found to be at 61 to 62 bp upstream of the initiation codon, and a putative binding sequence for the cAMP receptor protein was found at 38 to 39 bp further upstream. Transcription from the P5 promoter is increased by the addition of ethanol to the growth medium; however, the increase is greater in the presence of glucose than in its absence. These results add a new dimension to the transcriptional control of *rpoH* and to the regulation of the heat shock response in *Escherichia coli*.

Induction of the heat shock proteins in *Escherichia coli* occurs primarily at the level of transcription (25, 26). The central role of the *rpoH* (*htpR*) gene (14, 26) encoding a minor form of σ factor (σ^{32}) (8, 11, 28) in controlling transcription of heat shock genes is now well established. Not only is σ^{32} essential for transcription from the heat shock promoters (2, 8, 18, 29), but it is actively and directly involved in regulation of the heat shock response (9, 17). Recent evidence indicates that an upshift of temperature from 30 to 42°C causes rapid adjustment of the cellular level of σ^{32} to cope with the higher temperature (12, 16, 17). This appears to be accomplished both by transient induction of σ^{32} synthesis and by transient stabilization of the σ^{32} protein, which is extremely unstable during steady-state growth (9, 17).

As to the induction of σ^{32} synthesis, the nature of the cellular response may depend in part on the kind or severity of environmental conditions to which cells are exposed. In the case of the temperature shift from 30 to 42°C, the response occurs primarily at the level of translation of *rpoH* mRNA (17; H. Nagai, R. Yano, and T. Yura, unpublished results). The overall rate of transcription of *rpoH* hardly increases under these conditions, although *rpoH* transcripts accumulate to moderate extents (5, 6, 19), presumably because of mRNA stabilization brought about by increased translation (see Discussion). When cells are exposed to a lethal temperature (50°C), *rpoH* transcription from one of the promoters (P3) increases markedly (5); it was recently shown to be mediated by RNA polymerase containing a new σ factor called σ^{E} (4) or σ^{24} (24). Transcription from other promoters virtually ceases under such extreme conditions. It remains possible, however, that translational as well as transcriptional regulation is operative under these conditions.

We have previously studied transcription from the heat shock promoters by means of operon fusions that had been inserted into the λpF13 phage vector (27). We made a similar fusion between the *rpoH* promoter (*rpoHp*) region and *lacZ* to study *rpoH* transcription under a variety of conditions. In the latter case, the transcriptional signals (promoters), but not the translational signals, of the *rpoH* gene have been inserted into the cloning site of the λpF13 vector carrying the *ara-trp-lacZ* fusion (10). In the course of analysis of *rpoH* transcription, we observed that *rpoH* transcription, as measured by β-galactosidase activity, is significantly affected by glucose added to the broth medium. This prompted us to examine *rpoH* transcription more closely both in vivo and in vitro, which led to the discovery of a novel promoter whose activity depends on the cellular levels of cyclic AMP (cAMP) and the cAMP receptor protein (CRP) and is presumably sensitive to catabolite repression.

MATERIALS AND METHODS

Bacterial strains, bacteriophage, and plasmids. All bacterial strains used were derivatives of *E. coli* K-12, strain MC4100 (1). Strains KI268 (MC4100 *cya-283 ilv*::Tn*10*) and KY1452 (MC4100 *crp-39 zhd-26*::Tn*10*) were constructed by phage P1-mediated transduction, facilitated by Tn*10* insertions closely linked to *cya* and *crp*, respectively. Plasmid pKV7 used as a source of *rpoH* DNA was constructed by cutting out the *Hpa*I fragment (ca. 2.4 kilobases) from pKV1 (20) and inserting it into the *Pvu*II site of pBR322. The plasmid contains the entire *rpoH* gene (11, 28) with flanking regions on both sides. A set of transcriptional signal detection vectors (10), including both plasmid (pMS436S and

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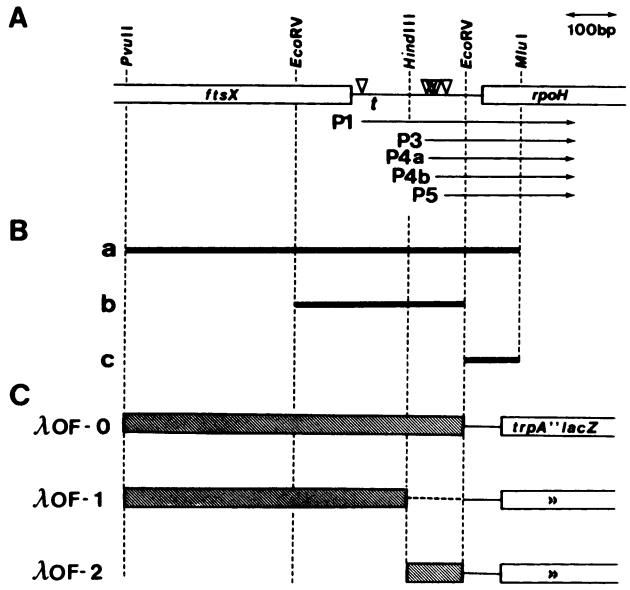

FIG. 1. Structure of the *rpoH* upstream region: promoters, templates (or probes), and operon fusions. (A) Promoters and transcripts. Both the *rpoH* and the upstream *ftsX* genes are transcribed from left to right. Triangles indicate the positions of the 5′ ends of the transcripts initiated from the *rpoH* promoters, P1, P3, P4, and P5. P4a and P4b indicate two different P4 transcripts. *t*, A putative terminator for *ftsX* mRNA. (B) Templates and probes. The segments indicated by a and b were used as templates for in vitro transcription (Fig. 3 and 4); the segment indicated by a was also used as a hybridization probe for S1 mapping of RNAs (Fig. 2, 4, and 5). The segment indicated by c was used as a primer to obtain DNA sequence ladders. (C) *rpoHp-lacZ* operon fusions. Shaded boxes indicate the *rpoH* upstream region inserted into λpF13 to obtain λOF-0, λOF-1, and λOF-2.

pMS434) and phage (λpF13) vectors, were obtained from M. Imai.

Media and chemicals. Polypeptone broth (P broth) has been previously described (20). Minimal medium M9 (13) with 0.2% glucose (or glycerol), thiamine (2 μg/ml), and all amino acids (20 μg of each per ml) was used for ethanol induction experiments. MacConkey-lactose agar (Difco Laboratories) was used for construction of operon fusions.

Construction of λ phage carrying a *rpoHp-lacZ* operon fusion. The *Pvu*II-*Eco*RV segment (659 base pairs [bp]) of the *rpoH* upstream region was cut out from pKV7, ligated with the *Sma*I-digested pMS436S vector, and checked for orientation of the insert. One such plasmid obtained was designated pOF-0 (where OF is operon fusion). Similarly, the *Pvu*II-*Hin*dIII segment with a short vector fragment (*Xho*I-*Sma*I) from pOF-0 DNA was inserted into pMS434 cut with *Xho*I and *Hin*dIII and was designated pOF-1; pOF-0 deleting the same *Pvu*II-*Hin*dIII segment was circularized, yielding pOF-2. These plasmids carrying a *rpoHp-lacZ* operon fusion were transformed into cells lysogenic for λpF13, and each operon fusion was transferred from plasmid to phage vector, yielding λOF phages (designated, respectively, λOF-0, λOF-1, and λOF-2) as described previously (10, 27). Lysogenic derivatives of MC4100, each carrying a single copy of prophage λOF-0, λOF-1 or λOF-2, were obtained as described previously (27).

S1 nuclease mapping of RNAs. S1 nuclease protection experiments were done essentially as described previously (21). Briefly, an excess of probe DNA (*Pvu*II-*Mlu*I fragment; Fig. 1B) end labeled with [γ-^{32}P]ATP was hybridized with RNA at 45°C and digested with S1 nuclease (170 U) for 30 min at the same temperature. RNA was extracted by phenol from cells grown in P broth or M9 medium essentially as described previously (5).

DNA sequence ladders used in mapping 5′ ends of *rpoH* transcripts were generated by the dideoxy-chain termination method. The *Hin*dIII-*Mlu*I segment of *rpoH* DNA obtained from pKV3 (20) was cloned into M13mp11, and the resulting single-stranded DNA was used as a template. The primer was prepared by treating the *Eco*RV-*Mlu*I fragment (Fig. 1B) with exonuclease III, followed by heating to inactivate the enzyme. The resulting DNA was annealed to template DNA, and the polymerization reaction was carried out with [α-^{32}P]dCTP as the labeled substrate.

In vitro transcription. The reaction mixture (50 μl) contained 20 mM Tris hydrochloride (pH 7.9), 150 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 50 μM [α-^{32}P]UTP (5 μCi), 200 μM each ATP, GTP, and CTP, 1 mM dithiothreitol, 2.5 μg of bovine serum albumin, 0.15 to 0.23 pmol of DNA, and 0.45 μg of RNA polymerase-σ^{70} (gift of A. Ishihama). In addition, 0.1 mM cAMP or 0.2 μg of CRP (gift of H. Aiba) or both were added to the reaction mixture as required.

RNA synthesis was carried out by preincubating template DNA with RNA polymerase in buffer for 10 min at 37°C and incubating with substrates for an additional 20 min. The reaction was terminated by adding an equal volume of stop solution (4 M ammonium acetate, 20 mM EDTA, and 1 mg of yeast tRNA per ml). RNAs were isolated by extraction with phenol-chloroform, followed by precipitation with 2-propanol.

RESULTS

Inhibition of *rpoH* transcription by glucose. Transcription of the *rpoH* gene can be monitored by measuring β-galactosidase activity in strains lysogenic for λOF-0, in which *lacZ* expression is directed by the transcriptional signals of *rpoH*. The first evidence for a glucose-sensitive *rpoH* promoter came from the observation that β-galactosidase activity in such lysogens grown in P broth supplemented with glucose (0.2%) was significantly lower than the activity in those grown in P broth without glucose. Since the *rpoH* promoter region (*Pvu*II-*Eco*RV segment of 659 bp) present in the operon fusion contains at least three promoters (P1, P3, and P4) (5, 6), we constructed two additional fusions, λOF-1 and λOF-2, containing the upstream (*Pvu*II-*Hin*dIII) and downstream (*Hin*dIII-*Eco*RV) portions of this region, respectively (Fig. 1). Thus, λOF-1 was expected to contain only promoter P1, whereas λOF-2 should contain promoters P3 and P4.

Derivatives of MC4100 carrying a single copy of prophage λOF-1, λOF-2, or the "parental" λOF-0 were prepared and examined for levels of β-galactosidase in various media at 30°C. It was found that *lacZ* expression from λOF-0 or λOF-2, but not λOF-1, was sensitive to repression by glucose; the extent of repression was about 50% or 70 to 80% for λOF-0 or λOF-2 lysogen, respectively (Table 1). Addition of cAMP to 1 mM (or 10 mM) could hardly reverse the glucose repression observed with either lysogen (data not shown). These results suggested that a glucose-sensitive promoter was located on the 110-bp *Hin*dIII-*Eco*RV segment close to the *rpoH* coding region.

Effects of *cya* and *crp* mutations on *rpoH* transcription. To see whether a putative glucose-sensitive *rpoH* promoter requires cAMP and CRP for activity, the *lacZ* expression

TABLE 1. β-Galactosidase synthesis in strains carrying a *rpoHp-lacZ* operon fusion[a]

Strain	*rpoH* promoters	β-Galactosidase activity (units)[b] in:		
		P broth	P broth + glucose	P broth + cAMP
MC4100(λOF-0)	P1, P3, P4, P5	178	78	170
MC4100(λOF-1)	P1	104	100	
MC4100(λOF-2)	P3, P4, P5	165	33	
KI268 (*cya*)(λOF-0)	P1, P3, P4, P5		39	47
KI268 (*cya*)(λOF-1)	P1		86	96
KI268 (*cya*)(λOF-2)	P3, P4, P5		21	45
KY1452 (*crp*)(λOF-0)	P1, P3, P4, P5		65	
KY1452 (*crp*)(λOF-1)	P1		115	
KY1452 (*crp*)(λOF-2)	P3, P4, P5		20	

[a] Cells were grown at 30°C in P broth with or without added glucose (0.2%) or cAMP (1 mM) as indicated and were assayed for β-galactosidase activity.
[b] Enzyme activities (averages of several experiments) are presented in Miller units (13). The value for strains lysogenic for λpF13 (without promoter inserted) was 0.5 unit under these conditions.

from λOF prophage was examined in *cya* and *crp* mutants deficient in adenylate cyclase and CRP, respectively. These mutants grew poorly in P broth and grew much better if glucose or cAMP (for *cya* mutants) was added to the medium. As expected, β-galactosidase levels in these mutants depended on the kind of supplement added (Table 1). In the case of the *cya* mutant (KI268), the β-galactosidase levels in λOF-0 and λOF-2 lysogens (but not in the λOF-1 lysogen) were much lower than in the *cya*$^+$ strain (MC4100) when grown with glucose but were slightly higher when grown with cAMP. However, the enzyme levels with cAMP were still much lower than those of the wild-type grown without glucose supplement. The enzyme levels for the *crp* mutant (KY1452) grown with glucose were similar to those for the *cya* mutants. These results taken together suggested that one or more promoters located on the *Hin*dIII-*Eco*RV segment were sensitive to glucose (or catabolite) repression and require the cAMP-CRP complex for maximum activity, although glucose repression could not be reversed by exogenous cAMP.

Identification of glucose-sensitive *rpoH* transcripts. RNA was extracted from cells of the wild type, the *cya* mutant, or the *crp* mutant, grown under various conditions, and the *rpoH* transcripts were examined by S1 nuclease protection experiments. In addition to the *rpoH* transcripts initiated from promoters P1, P3, and P4 (5, 6), a novel transcript was found when cells were grown in P broth, and its amount was much reduced when glucose was added to the medium (Fig. 2). Again, addition of cAMP (1 mM) could not reverse the effect of glucose on the transcript level (data not shown). This glucose-sensitive *rpoH* RNA seemed to be initiated from a promoter, designated P5, located downstream of all of the known promoters. When a culture grown at 30°C was shifted to 42°C, the amount of P5 transcript increased severalfold (even with excess glucose), as did the *rpoH* transcripts initiated from other promoters (Fig. 2A and B). The P5 transcript was hardly detected in the *cya* mutants grown with glucose but was found in appreciable amounts when grown with cAMP (Fig. 2C). The enhanced transcription from P5 with cAMP was accompanied by decreased amounts of the P4 transcript, suggesting interference between the promoters. The *crp* mutants produced little or no P5 transcripts but produced normal levels of other *rpoH* transcripts (data not shown). These results indicated that a specific promoter (P5) which is sensitive to glucose repression and which is regulated by cAMP and CRP is involved in the control of *rpoH* transcription in *E. coli*.

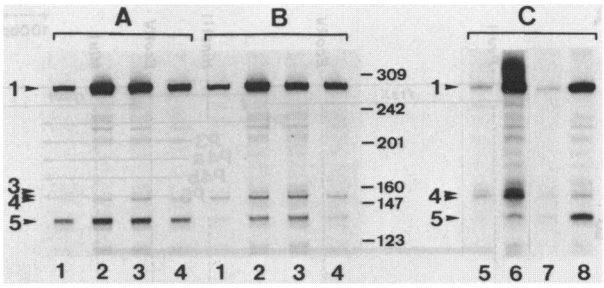

FIG. 2. S1 mapping of in vivo *rpoH* transcripts. RNAs were extracted from log-phase cells grown in P broth with or without supplement at 30°C or after transfer to 42°C. RNA samples (50 μg) were hybridized with a 5′-end-labeled DNA probe and digested with S1 nuclease, and the protected fragments were electrophoresed in 5% polyacrylamide–8 M urea gels. The positions of the fragments protected by the P1, P3, P4, and P5 transcripts are marked by the numbered arrows. Positions (base pairs) of DNA size markers are indicated in the middle. (A) MC4100 grown in P broth. (B) MC4100 grown in P broth with 0.2% glucose. (C) KI268 (*cya-283*) grown in P broth with glucose (0.2%) or cAMP (1 mM). Lanes: 1, 30°C; 2, 5 min at 42°C; 3, 10 min at 42°C; 4, 20 min at 42°C; 5, 30°C (glucose); 6, 5 min at 42°C (glucose); 7, 30°C (cAMP); 8, 5 min at 42°C (cAMP).

Transcription from the *rpoH* P5 promoter in vitro. To directly examine the requirement of cAMP and CRP for transcription from the P5 promoter, *rpoH* transcription was studied in vitro with purified RNA polymerase-σ^{70} holoenzyme and a *rpoH* upstream DNA as templates. The reaction was run at 37°C in the presence or absence of cAMP and purified CRP. Electrophoretic analysis of RNA products obtained in the reactions with two different template DNAs indicated that an RNA of the size expected for the P5 transcript was synthesized, provided that both cAMP and CRP were present (Fig. 3). The P1 and P4 transcripts that are known to be synthesized by RNA polymerase-σ^{70} were produced regardless of the presence or absence of cAMP or CRP; the interference between promoters P4 and P5 observed in vivo was not apparent under the in vitro conditions. No transcription from the P3 promoter was detected, which is in agreement with the results reported previously (4, 5, 24). These results establish that transcription from the P5 promoter is mediated by RNA polymerase-σ^{70} and depends on both cAMP and CRP under the conditions employed in this study.

Determination of the start site of the P5 transcript. To determine the exact start site of the P5 transcript, RNAs synthesized in vivo and in vitro were analyzed by the S1 nuclease mapping procedure. The 5′ ends of RNAs transcribed from the P1 and P4 promoters in vitro were identical to the in vivo 5′ ends (Fig. 4A) and agreed with those reported previously (5, 6, 24). Trace amounts of the P3 transcript obtained in vivo appeared to be initiated from sites previously identified in other strains (4, 5, 6, 24). The start sites for P5 transcripts, both in vivo and in vitro, were found to be at A nucleotides located 61 and 62 bp upstream of the initiation (ATG) codon (Fig. 4A and B). Taken together with the sequence data (11, 28), the −10 region for the P5 promoter appeared to be TAACCT, but there was no obvious −35 region (Fig. 4B). In agreement with the involvement of cAMP and CRP in transcription from P5, a putative CRP binding sequence (TGTGGATAAAATCACG) was found at

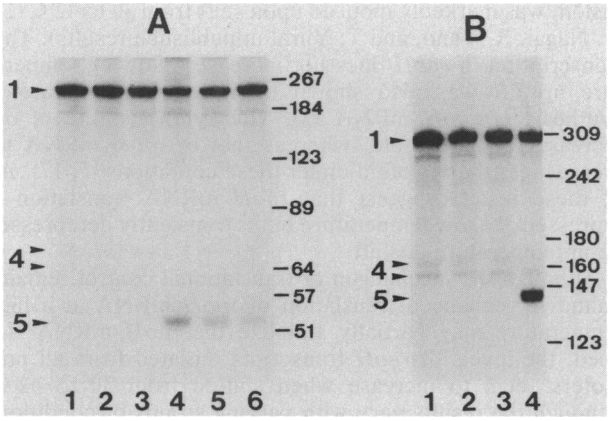

FIG. 3. Analysis of *rpoH* transcripts synthesized in vitro. Transcription reactions were carried out as described in Materials and Methods; cAMP or CRP or both were added as indicated. RNA products were collected and analyzed by 8% (A) or 5% (B) polyacrylamide–8 M urea gel electrophoresis. Transcripts originating from P1 (1), P4 (4), and P5 (5) are indicated by arrows. Positions (base pairs) of DNA size markers are indicated to the right. Template DNAs used were an *Eco*RV-*Eco*RV fragment of 342 bp (A) or a *Pvu*II-*Mlu*I fragment of 752 bp (B) (Fig. 1). Lanes: 1, none (control); 2, 0.1 mM cAMP; 3, 0.2 μg of CRP; 4, 0.1 mM cAMP and 0.2 μg of CRP; 5, 0.1 mM cAMP and 0.1 μg of CRP; 6, 0.1 mM cAMP and 0.05 μg of CRP. The apparent increase of P5 transcripts in panel B versus panel A (lanes 4) reflects a longer transcript which is uniformly labeled.

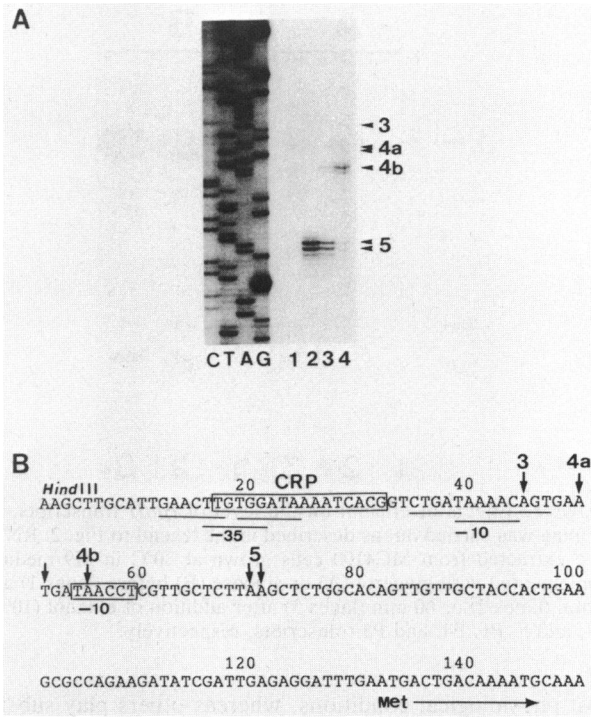

FIG. 4. Start site(s) for the P5 transcript in vivo and in vitro. (A) In vitro transcripts (lanes 1 and 2) were synthesized as shown in Fig. 3B, except that the radioactive UTP was replaced by unlabeled UTP (200 μM). In vivo transcripts (lanes 3 and 4) were extracted from cells of MC4100 5 min after shift from 30 to 42°C. RNAs were hybridized with probe DNA, digested with S1 nuclease as described in the legend to Fig. 2, and analyzed by 6% polyacrylamide–8 M urea gel electrophoresis. DNA sequence ladders were generated as described in Materials and Methods. Lanes: 1, the reaction mixture contained CRP (0.2 μg) but no cAMP; 2, the reaction mixture contained both cAMP (0.1 mM) and CRP (0.2 μg); 3, RNA from cells grown in P broth; 4, RNA from cells grown in P broth with 0.2% glucose. (B) DNA sequence of the P5 promoter region. Only the sequence following the *Hin*dIII site is shown. Arrows indicate the start sites for the P3, P4 (a and b), and P5 transcripts. The −10 and −35 regions for $E\sigma^E$-controlled P3 and $E\sigma^{70}$-controlled P4 promoters are underscored. A putative −10 region for the P5 promoter and a CRP binding sequence are boxed.

38 to 39 bp upstream of the transcription start sites. This overlaps with one of the binding sites for the DnaA protein reported recently (23) and suggests the possible involvement of the DnaA protein in modulating transcription from P5.

Effects of ethanol and starvation for carbon source on *rpoH* transcription. In the course of studies of *rpoH* transcription under various conditions, ethanol (4 to 10%) was found to increase markedly the level of *rpoH* transcripts as detected by S1 nuclease mapping. Such effects of ethanol apparently depend on the kind of carbon source used in the medium. The increase was quite striking in M9 medium with glucose but was observed at a much reduced level with glycerol (Fig. 5). As in the case of temperature upshift, all *rpoH* transcripts seemed to be enhanced.

When cells are starved for carbon source, some of the heat shock proteins, including GroEL and DnaK, are transiently induced (7). We thus examined a possible involvement of P5-mediated *rpoH* transcription under the starvation conditions. However, no significant increase in any of the *rpoH* transcripts could be observed, at least during 3 h of starvation at 30°C (data not shown). Thus, *rpoH* transcription does not seem to be induced during carbon starvation. The result is also consistent with the recent finding that the starvation-induced synthesis of GroEL and DnaK proteins is independent of cAMP and CRP (15).

DISCUSSION

We have identified a novel promoter, *rpoH* P5, located downstream of the several previously identified promoters involved in *rpoH* transcription. Unlike the other promoters, transcription from P5 by RNA polymerase-σ^{70} requires both cAMP and CRP at concentrations comparable to those required for other catabolite-sensitive promoters. Little or no transcription from P5 has been detected in previous studies, presumably because of the experimental conditions employed. In vivo transcripts examined so far have been prepared from cells grown in MOPS (morpholinepropanesulfonic acid) medium (5) or Luria broth medium (6) containing relatively high concentrations of glucose. In in vitro studies, the effects of cAMP and CRP on *rpoH* transcription have not been tested directly (5, 6). The poor reversibility of glucose repression by exogenous cAMP in the wild type and the *cya* mutant may be explained by assuming that the binding of CRP to DNA interferes with transcription from P4 (Fig. 4) and that the strains used are not very permeable to cAMP; cAMP at 10 mM could reverse the glucose repression of P*lac*-directed *lacZ* expression by only 10% (data not shown).

Transcription of *rpoH* appears to be intricately regulated to meet the cellular requirements under various growth conditions. In strain MC4100, used in this study, at least four promoters, P1, P3, P4 and P5, are involved. The most distal (upstream) promoter, P1, seems to play a major role under

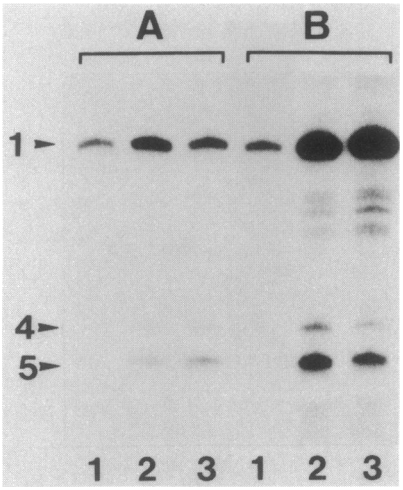

FIG. 5. Effects of ethanol on the level of *rpoH* transcripts. S1 mapping was carried out as described in the legend to Fig. 2. RNAs were extracted from MC4100 cells grown at 30°C in M9 medium supplemented with glycerol (A) or glucose (B) before (lanes 1) and 20 min (lanes 2) or 60 min (lanes 3) after addition of ethanol (10%). 1, 4, and 5, P1, P4, and P5 transcripts, respectively.

most physiological conditions, whereas others play subsidiary but specific roles. P1 is located near the transcription termination region of the adjacent operon (*fts YEX*) known to be involved in cell division (3). Expression of the *fts YEX* operon might therefore interfere with *rpoH* transcription from P1. Furthermore, the −10 region of P1 is located within the sequence highly homologous to the DnaA protein binding sequence. This suggests the possible coupling between *rpoH* transcription and expression of the cell division operon mediated by the DnaA protein (3). Specifically, it appeared likely that the DnaA protein inhibits transcription from P1. In line with such an expectation, β-galactosidase activity in the *dnaA*(λOF-1) mutants is higher (about twofold) than that in the isogenic *dnaA*$^+$(λOF-1) strains (T. Yura, unpublished result).

On the other hand, the transcripts from P3 are most abundant at high temperatures and account for the majority of *rpoH* mRNA at 50°C, where most RNA synthesis ceases. It was recently found that transcription from P3 is mediated by RNA polymerase containing a new σ factor called σ^E (4) or σ^{24} (24). The promoter P4 is recognized by RNA polymerase-σ^{70}, and the level of P4 transcript also increases with increasing temperature (4, 5, 22). Recent evidence indicates that excess DnaA protein inhibits *rpoH* transcription from P3 and P4 both in vivo and in vitro (23). The catabolite-sensitive promoter P5 revealed by the present study adds a new dimension to the transcriptional control of *rpoH* in *E. coli*. Furthermore, these results taken together suggest that the subsidiary proximal promoters, in contrast to the major distal promoter P1, are involved in several elaborate mechanisms acting to maintain the proper level of σ^{32} in a variety of environments and conditions.

In contrast to the transcriptional controls discussed above, σ^{32} synthesis is enhanced primarily at the level of translation when cells are exposed to higher temperature or ethanol. This has been shown by comparing *lacZ* expression from the *rpoH-lacZ* operon fusion used here (λOF-0) and a gene (protein) fusion whose expression depends on both transcriptional and translational signals of the *rpoH* gene; *lacZ* expression from gene fusion, but not from operon fusion, was markedly induced upon shift from 30 to 42°C (17; H. Nagai, R. Yano, and T. Yura, unpublished results). That transcription of *rpoH* does not increase following temperature upshift was also shown by measuring the rate of synthesis of *rpoH* mRNA (5). The synthesis rate of σ^{32} increases about 10-fold, whereas that of *rpoH* mRNA increases less than twofold under these conditions (5, 17). All of these results suggest that *rpoH* mRNA translation is repressed at a low temperature but is transiently derepressed upon temperature upshift.

Although the mechanism of translational control remains unknown, enhanced translation of *rpoH* mRNA at a high temperature may partially stabilize the *rpoH* mRNA. Indeed, the levels of *rpoH* transcripts initiated from all promoters seem to increase when shifted from 30 to 42°C, although the results vary with varying strains or conditions (Fig. 2) (5, 6). Such a stabilization of *rpoH* mRNA must be superimposed on the enhanced transcription from some of the promoters, notably P3. When cells were exposed to 4 to 10% ethanol at 30°C, similar stabilization of *rpoH* mRNA appeared to occur, particularly when glucose was used as the carbon source as compared to when glycerol was used as the carbon source (Fig. 5). In addition, ethanol seems to induce transcription from all promoters by unknown mechanisms.

Thus, transcriptional and translational controls appear to play distinct and perhaps complementary roles in the regulation of *rpoH* expression. Transcriptional control may provide the proper amount of *rpoH* expression under a variety of steady-state conditions, whereas translational control plays the major role in the rapid adjustment of σ^{32} levels that occur upon exposure to higher temperature or other changes in growth conditions. Alternatively, transcriptional control may play a major role in response to certain extreme conditions, whereas translational control plays the predominant role in response to relatively mild conditions.

σ^{32} is an extremely unstable protein and is rapidly degraded during steady-state growth at 30 or 42°C (9, 17). Upon shift from 30 to 42°C or exposure to 4% ethanol, σ^{32} is transiently stabilized, permitting its accumulation (17). Instability of σ^{32} provides the basis for the rapid adjustments in its concentration that regulate the heat shock response (17). Thus, both synthesis and degradation of σ^{32} appear to be intimately regulated so as to provide the proper σ^{32} level required for the synthesis of heat shock proteins in the amounts sufficient to support cell growth under various conditions. Further studies on the mechanisms of these regulatory circuits should contribute to our general understanding of the role of heat shock proteins in cellular adaptation to growth in changing environments.

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LITERATURE CITED

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