The *Escherichia coli* gapA Gene Is Transcribed by the Vegetative RNA Polymerase Holoenzyme Eσ^70^ and by the Heat Shock RNA Polymerase Eσ^32^

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The NAD^+^-dependent phosphorylating 1,3-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which converts 1,3-glyceraldehyde-3-phosphate into 1,3-diphosphoglycerate (22), is a key enzyme of the glycolytic and glucogenogenesis pathways. In eubacteria (5, 14, 17, 46) and archaeabacteria (4), the GAPDH gene (gap) belongs to a cluster of genes coding for several glycolytic enzymes: 3-phosphoglycerate kinase (pgk), class II fructose 1,6-bisphosphate aldolase (fda), and triosephosphate isomerase (tpi). A peculiar situation is observed in *Escherichia coli*, in which the gapB gene, located in this cluster, does not produce GAPDH activity (2, 25). The active gapA gene and the pgk gene are 22.6 min apart on the *E. coli* chromosome (7, 50). In addition, the gapA gene codes for a protein which has stronger homology with mammalian GAPDHs than with bacterial enzymes (6). A eukaryotic origin has thus been proposed for the gapA gene: enterobacteria would have acquired the gapA gene through a horizontal transmission of genetic information, concomitant with the loss of activity of the gapB gene (2, 16, 36). On the basis of their observation of a gene similar to the *E. coli* gapA gene in a cyanobacterium, Martin et al. (35) suggested an alternative explanation: the presence of both gapA and gapB genes in ancestor bacteria. In order to further address this problem, it was of interest to us which kind of transcriptional promoter controls the gapA gene. The exceptionally high expression of the cloned gapA gene (7) suggested that the gapA promoter was very efficient. Therefore, a study of the gapA promoter was also expected to yield some information on the structural parameters responsible for this high efficiency.

The majority of the *E. coli* transcriptional promoters are recognized by the RNA polymerase holoenzyme Eσ^70^, which is formed by association of the holoenzyme with the major sigma factor, σ^70^ (24). A few subsets of genes that respond to various environmental or nutritional changes require specific recognition by RNA polymerase associated with the alternative sigma factors σ^32^ (21), σ^E^ (18, 52), σ^24^ (26), or σ^5^ (39). The heat shock response mediated by the Eσ^32^ RNA polymerase holoenzyme has been the subject of several studies (for reviews, see references 8 and 40). When *E. coli* cells growing at 30°C are shifted to 42°C, the rate of synthesis of the so-called heat shock proteins increases 5- to 20-fold, reaching a peak within 5 to 15 min; synthesis then declines until it reaches a new rate, characteristic of high temperatures. Seventeen proteins meeting these criteria were detected in *E. coli* (40). The rpoH gene, which codes for σ^32^, is controlled by a multiple promoter system containing four different polymerase-binding sites (8). One, recognized by the Eσ^E^ holoenzyme, ensures heat inductibility; the three others are recognized by Eσ^70^ and are differentially regulated (8). A consensus sequence for recognition by the Eσ^32^ RNA polymerase has been proposed (15). An extensive analysis of heat shock transcriptional induction in *E. coli* revealed the presence of at least 36 genes whose expression is induced by heat shock (12).

In this paper, we demonstrate that the gapA gene of *E. coli* is controlled by a multiple promoter system consisting of four different RNA polymerase recognition sites; one of them has the characteristic features of Eσ^32^ promoters. Transcription initiation at this site was found to be increased when cells were subjected to heat shock. In this way, the transcription level of the gapA gene is preserved during heat shock. Using the GAPDH mutant strain DF221 (25), we showed that expression of a cloned GAPDH gene in this strain increases its capacity to grow at 43°C on succinate glycerol medium. Hence, in addition to genes whose transcription is increased upon heat shock, the heat shock regulon probably contains genes whose expression during heat shock is kept constant or at a minimal
level by Es32 RNA polymerase transcription. These results are discussed in view of the present knowledge of the highly efficient E. coli rnrB promoter region (41), which has strong homology with the gapA promoter region.

MATERIALS AND METHODS

Bacterial strains and plasmids. The relevant genotypes of the E. coli strains used in this study are listed in Table 1. These strains were transformed with the plasmid pBS::EcogapA. This plasmid was constructed by cutting out the HindIII-BamHI 2,149-nucleotide fragment from phage M13mp9::EcogapA (38) and inserting it into plasmid pBluescript II K（Stratagene). The plasmid pBS::EcogapA contains the entire gapA gene with a 346-nucleotide upstream flanking region. E. coli DF221 was transformed with both plasmids pBR322 and pBR322::EcogAPDH (7).

Media. The rich medium used was L broth (34). The defined medium was M63 (19) supplemented with thiamine (1 μg/ml) and Casamino Acids (0.1%, wt/vol). The specific carbon source was either a mixture of succinate (0.4%, wt/vol) and glycerol (0.08%, vol/vol) or glycerol (0.2%, vol/vol). Strains containing plasmids were grown in the presence of ampicillin (100 μg/ml). The effect of cyclic AMP (cAMP) in the medium was tested with cAMP at 1 mM.

Cell cultivation and viability experiments. In routine experiments cells were grown aerobically at 37°C. Growth was monitored by measuring the optical density at 600 nm (OD600). For temperature shift experiments, cultures grown at 30°C in a gyratory water bath were split in two in the middle of the exponential growth phase (OD600 = 0.5). One half was cultivated at 30°C for 10 min, and the other half was cultivated at 42°C for 10 min. A gyratory water bath was used in both cases. To test for the effect of cAMP in the medium, cells were grown at 37°C, to an OD600 of 0.2, in supplemented M63 minimal medium with 0.2% glycerol as the carbon source. The culture was split in two, and one half was supplemented with cAMP at a concentration of 1 mM. For both cultures, growth was continued at 37°C to an OD600 of 0.4.

To test for the effect of temperature on cell growth, cells were grown with shaking at 30°C to an OD600 of 0.2 or 0.3; 100-ml fractions of the culture were then placed in a gyratory water bath at 30, 43, or 53°C, and growth was monitored by OD600 measurements at various times following the temperature shift. The 53°C culture was also used for cell viability experiments. Aliquots were periodically plated on M63-sucinate-glycerol agar plus ampicillin and incubated at 37°C for 24 h.

RNA isolation. Total RNA was isolated by the hot-phenol extraction procedure (1); extractions were always performed with the same amount of cells, corresponding to an OD600 of 6. These cells were collected at various times during the culture or after temperature shifts or tests of cAMP effect. In the latter cases, RNA was also extracted from control cells which were not subjected to the temperature shift or incubated in the presence of cAMP. The total amount of extracted RNA was determined by OD260 measurement.

In vitro transcription. In vitro transcription assays were performed with the commercial E. coli Ex70 RNA polymerase holoenzyme (Boehringer) with the supercoiled plasmid pBS::EcogapA as template. The reaction mixture contained 40 mM Tris hydrochloride (pH 8), 30 mM KCl, 10 mM MgCl2, 1 mM dithiothreitol, 200 μM (each) ATP, CTP, GTP, and UTP, 2 to 3 mM DNA, and 50 nM RNA polymerase. Incubation was for 25 min at 37°C. The reaction was stopped by phenol-chloroform extraction, which was followed by precipitation with alcohol. The precipitated RNA was analyzed by primer extension, as described below.

Primer extension of in vitro-synthesized RNA transcripts and mRNAs. Two synthetic oligonucleotides, OG1 (5’-TTTCTGAGCAGCAGCGGAA3’) and OG2 (5’-ATAGTCATATGTTCCTCA3’), complementary to nucleotide positions 52 to 68 and 8 to –11 of the gapA gene, respectively (6), were 5’-end labeled with [γ-32P]ATP (3,000 Ci/mmol) (Amersham) and T4 polynucleotide kinase (Boehringer) and used as primers. In standard experiments, 2 μg of RNA isolated from bacterial strains containing plasmid pBS::EcogapA, 20 μg of RNA isolated from bacterial strains without plasmid, or all transcripts obtained in an in vitro transcription assay were annealed with 5 ng of 5’-end-labeled primer. An exception was the experiment to study the evolution of gapA mRNA composition in the course of a fermentation. In this case, each primer extension was performed with an amount of total RNA corresponding to 6 × 106 cells. For annealing, the RNA and the labeled oligonucleotide were heated to 70°C for 5 min in 10 μl of 50 mM Tris hydrochloride (pH 8.3)-40 mM KCl-6 mM MgCl2 and then cooled slowly to room temperature (20 min). cDNA synthesis was performed in the same solution for 30 min at 45°C in the presence of the nonradioactive deoxyribonucleoside triphosphates (dNTPs) at a concentration of 225 μM each and 1 U of Rav-2 reverse transcriptase (Amersham). The reaction was stopped by addition of 100 μl of 0.3 M sodium acetate and 500 μl of ethanol, and precipitation was done at –80°C. The precipitated material was dissolved in a formamide dye solution (34). The sample was electrophoresed on a 7% polyacrylamide–8 M urea sequencing gel and compared with dideoxy sequencing reaction products (48) obtained with the same primer and the single-stranded form of phage M13mp9::EcogapA as the template.

Reverse transcript quantification. Reverse transcript quantification was achieved with a Molecular Dynamics Phosphorimagier 410 system. The sensitivity and linearity of the Molecular Dynamics signal was demonstrated on model systems (32). For quantification the reverse transcripts were fractionated on a 7% polyacrylamide–8 M urea sequencing gel. The gel was scanned for radioactivity for 10 min to 12 h, depending on the amount of radioactivity loaded. The results obtained were quantified by using the Molecular Dynamics Imagequant software. We proceeded as follows. A rectangular surface was designed so that it could include any of the bands to be quantified. It was then successively fitted with each of the bands, and the grey levels in the fitted rectangular surfaces were registered. The values obtained are expressed in Phosphorimagier signal units. The background was automatically subtracted by this software.

mRNA sequencing with reverse transcriptase. Direct sequencing of mRNA was done as described by Hu Qu et al. (29) with oligonucleotide OG1 as the primer. Annealing between the gapA mRNAs and the 5’-end-labeled oligonucleotide used as the primer was performed as described above, with 25 ng of primer and 10 μg of RNA, except for the presence of
RESULTS

Identification of the gapA gene transcription start sites in vivo and in vitro. We previously observed two putative overlapping RNA polymerase recognition sites in the upstream noncoding region of the gapA gene (6). They are denoted P1 and P’1 in Fig. 1A. To identify the transcriptional start sites used in vivo, E. coli XL1-Blue with or without the pBS::Eco
gapA overproducing plasmid was grown in L broth at 37°C, and total RNA was isolated from exponentially growing cells (OD600 of 0.5). The gapA mRNAs were analyzed by primer extension with oligonucleotide OGI or OG2 (Fig. 1A) as the primer. To get quantitative results, reverse transcriptase and all substrates, except the template mRNA, were used in excess. Under these conditions, the amount of reverse transcript obtained was found to be proportional to the amount of template transcript (43a). Similar patterns of fractionation of the reverse transcripts were obtained for the host strain and the transformed strain, no matter which oligonucleotide was used as the primer (Fig. 2). A major reverse transcript (band P1), whose 3’ end maps to the expected initiation site of the putative P1 promoter (Fig. 1), was always present, as were other reverse transcripts. None of them corresponded to an initiation at the putative P’1 promoter. The minor products, with high electrophoretic mobilities, were probably due either to degradation of mRNA initiating from P1 or to incomplete reverse transcription of P1 transcripts. The only explanation for the observation of reverse transcripts P2, P3, and P4, which had low electrophoretic mobilities, is the presence of at least one upstream promoter. These reverse transcripts were not generated by a nonspecific hybridization of the primer oligonucleotide with other mRNAs, since two different primers gave the same result and since direct sequencing of the P2, P3, and P4 transcripts demonstrated that they are transcripts from the gapA gene (Fig. 3). To determine whether the four mRNAs observed in vivo can be produced by in vitro transcription, the supercoiled plasmid pBS::Eco
gapA was transcribed in vitro with the E. coli Eo70 RNA polymerase. Only the P1 site and, to a very small extent, a new site denoted P5 were used under these conditions (Fig. 4).

To find an explanation for these results, the DNA sequences that preceded the ends of the various transcripts, detected in vivo and in vitro, were compared with the consensus promoter sequences of E. coli RNA polymerases Eo70 (−35, TTGACA, and −10, TATAAT, separated by 17 bases) (23) and Eo32 (−35, CCTTGGAA, and −10, CCCCATNTT, separated by 13 to 15 bases) (15) (Fig. 1B). We also looked for the presence of possible cAMP/cAMP receptor protein (CRP) binding sites on the basis of the consensus sequence AAATGTCATCTA GTACACATT proposed for the most frequently observed cAMP-CRP binding sites (3) (Fig. 1C). Upstream from the P1 site, the sequences TTGACG and TAATTT, separated by 16 bases (Fig. 1A), were previously found as −35 and −10 potential promoter sequences, respectively, of the E. coli Eo70 RNA polymerase (6). For P2, the sequences CCCCCAAA and CCCCCATGT, separated by 15 bases (Fig. 1A and B), were found as potential promoter sequences recognized by the E. coli Eo32 RNA polymerase. This was surprising, since bacterial GAPDH had not been described as a heat shock protein previously. For P3 site, the sequence TAAAT was found as a potential −10 promoter sequence of the RNA polymerase Eo70, and instead of a −35 sequence we observed the presence of a potential cAMP-CRP binding site (AAATCGTGAT GAAAATCAGATT) (Fig. 1A). The organization of the P3 upstream sequence is very similar to that of the gapA P1 promoter (Fig. 1C). For both sites P4 and P5, a potential −10 promoter sequence of Eo70 was found (TAAACT and TATCGT, respectively), but no potential −35 promoter sequence was found. The results of this DNA comparison were in agreement with our experimental data. The presence of other potential promoters in addition to the potential P1 promoter was an explanation for the presence of mRNAs initiated at various sites. The fact that among the P1, P2, P3, P4, and P5 sites, only P1 was preceded by the −10 and −35 sequences required for in vitro transcription by the E. coli Eo70 RNA polymerase explained the strong in vitro utilization of this promoter. The presence of very small amounts of transcripts initiated at the P5 sites in vitro may be an artifact or could correspond to the presence of an RNA polymerase binding site with very low transcriptional activity.

Effect of heat shock on gapA mRNA composition. To verify that initiation at the P2 site was generated by the Eo32 RNA polymerase, heat shock experiments were performed with E. coli XL1-Blue, with or without plasmid pBS::Eco
gapA, grown to mid-logarithmic phase at 30°C in L broth. We compared the relative abundances of gapA transcripts after 10 min of growth at 30°C and after 10 min of growth at 42°C. The gapA transcripts were analyzed by primer extension under conditions allowing their quantification (Fig. 5). After a 10-min shock at 42°C, either in the presence or in the absence of the overproducing plasmid, an increase in transcripts initiated at the P2 site, concomitant with a decrease in transcripts initiated at the P1 site, was observed (Fig. 5). This observation and the strong homology of the sequence upstream of the P2 site with heat shock promoters are strong indications that initiation at the P2 site is generated by the Eo32 RNA polymerase. We noticed stronger utilization of the P3 site at 37°C (Fig. 5C).

Effect of gapA gene expression on cell growth and viability at high temperatures. Several previously characterized E. coli heat shock proteins were found to increase growth and viability of cells at high temperatures (40, 53). To test whether GAPDH has the same effect, the growth of the GAPDH− E. coli strain DF221 transformed with plasmid pBR322 in M63-succinate-
glycerol at 30, 43, and 53°C was compared with that of the same strain transformed with plasmid pBR::EcoGAPDH, which overproduces the GAPDH (Fig. 6). The growth of the two transformed strains is identical at 30°C, whereas cells transformed with plasmid pBR::EcoGAPDH continue to grow at the rate seen at 30°C for 4 h when the culture is shifted to 43°C, cells transformed with plasmid pBR322 grow at a slower rate after the temperature shift (Fig. 6B). Nevertheless, no significant positive effect of the expression of GAPDH on cell growth and viability at 53°C was observed (Fig. 6C and D). Thus, in E. coli DF221, the expression of the gapA gene has a positive effect on cell growth at 43°C.

Effect of cAMP and CRP on initiation at the P3 site. To determine whether utilization of the P3 site was subject to catabolic repression, E. coli TP006 (which is deficient in cAMP production) and E. coli M182Δcrp (which is deficient in CRP production) and its isogenic strain M182 were transformed with plasmid pUC19 grown at 37°C in M63-glycerol medium to mid-logarithmic phase. The gapA mRNAs were analyzed by primer extension (Fig. 7). No transcription took place at the P3 site in the absence of cAMP (Fig. 7A) or CRP (Fig. 7B). With strain TP006, initiation at the P3 site was restored when the medium was supplemented with cAMP (Fig. 7A). Thus, utilization of the P3 site is subject to catabolic repression.

Evolution of gapA mRNA composition in the course of
E. COLI gapA GENE REGULATION

A.

**DNA sequence of the regulatory region of the E. coli gapA gene.**

The sequence is from reference 6. The first nucleotide of the ATG initiation codon is numbered 1; the preceding one is numbered −1. The transcription initiation sites observed in vivo and in vitro are marked with numbered arrows. The −35 and −10 regions of the box-controlled gapA promoters are boxed. The −35 and −10 regions of the σ32-controlled P2 promoter are overlined. The −35 and −10 regions of the previously proposed P1 promoter, which according to the results of this study are not used, are underlined. The cAMP-CRP binding site upstream of the P5 initiation start site is indicated by horizontal arrows in opposite directions. The horizontal arrows labeled OG1 and OG2 show the sequences which are complementary to the primer oligonucleotides. The putative binding sites for the FIS protein are indicated; positions in agreement with the FIS binding site consensus sequence GT1-YR-A/T-YR-C/A (27) are marked by filled circles. (B) Comparison of the DNA sequences of the gapA promoter with those of other promoters which are recognized by the Eσ32 RNA polymerase (groE, dnaK P1, dnaK P2, htpG P1, rpoD Phe, lon, and C62,5 P1) (40) and with the consensus sequence established for the σ32-controlled promoter (15). Matches to the consensus sequence are underlined in the gapA P2 promoter. The initiation start sites are indicated by boldface. (C) Comparison of the DNA sequence of the gapA P3 promoter with that of the gal P1 promoter (33) and with the consensus sequence established for the cAMP-CRP binding sites (3). The −10 region is boxed, the cAMP-CRP binding sites are indicated by horizontal arrows in opposite directions, and the initiation start sites are boldfaced.

**fermentation.** To determine whether the relative amounts of the four gapA mRNAs were constant throughout the culture time or whether they varied, E. coli XL1-Blue transformed with plasmid pBS:EcogapA was grown at 37°C in 1 liter of L broth. Samples were collected at regular time intervals, and mRNAs were analyzed by primer extension (Fig. 8). For quantitative comparison of the results obtained at different times during the culture, all primer extension assays were performed with a total RNA amount corresponding to the same number of cells. All of the reverse transcription reaction...
mixture was fractionated on a polyacrylamide gel, and the relative amounts of reverse transcripts were estimated with a Molecular Dynamics Phosphorimager system. The values obtained (Fig. 8C) reflect variations in the total amounts of transcript. Figure 8D is another representation of the results, showing the variations of the amount of each transcript in a given amount of total RNA extract during cultivation (relative abundance). Both the total amount and the relative abundance of transcripts initiated at the P1 site were very high during the exponential phase and decreased in the stationary phase. The relative abundance of transcripts initiated at the P2 site was high during the exponential phase, decreased abruptly at the beginning of the stationary phase, and increased again later in the stationary phase. The transcripts initiated at the P3 site peaked during the beginning of the stationary phase and were present in very small amounts during the late stationary phase. The transcripts initiated at the P4 site were found in very small amounts from the beginning of the culture to the late stationary phase, when they disappeared. Longer transcripts were detected in this late phase. The experiment was repeated several times and the same results were observed.

FIG. 2. Determination of the in vivo start sites of the gap4 gene. Primer extension was performed with oligonucleotides OG1 (A) and OG2 (B) with total RNA extracted from E. coli XL1-Blue cells grown on L broth at 37°C to mid-log phase (OD$_{600}$ = 0.5) in the presence (lane 1) or absence (lane 2) of plasmid pBS::EcogapA. Total RNA extraction and primer extension were performed as described in Materials and Methods. In this case 2 µg of total RNA was used for both transformed and untransformed cells. Markers (lanes A, C, G, and T) were made by sequencing phage M13::EcogapA with both primers and T7 DNA polymerase. The positions of the four initiation sites P1, P2, P3, and P4 are indicated, and the nucleotide sequences around the different start sites are given on the left.

FIG. 3. Direct RNA sequencing of the gap4 transcripts produced in vivo. The gap4 mRNAs contained in 10 µg of total RNA extract from E. coli XL1-Blue cells transformed with plasmid pBS::EcogapA and grown on L broth at 37°C to mid-log phase (OD$_{600}$ = 0.5) were sequenced by the reverse transcriptase method as described in Materials and Methods, with oligonucleotide OG1 as the primer. The positions of the P1, P2, and P3 start sites are indicated.

DISCUSSION

On the basis of their homology with promoter sequences of the Eor$^{39}$E. coli RNA polymerase, the existence of two putative overlapping promoter sequences upstream of the gap4 coding region was previously reported (6). The promoter sequence denoted P1 in this paper (Fig. 1) is used in vitro as well as in vivo. The other one, P1', was never found to be used in any of the experiments performed. This inefficiency is probably due to an unfavorable distance between the −10 and −35 sequences (19 bases) (Fig. 1A). The P1 promoter utilization is very strong at the beginning of the exponential phase and seems to depend upon growth phase (Fig. 8), a situation which is very similar to that found for promoters required for transcription of stable RNAs (42). The strong activation of the rRNA P1 promoter at the beginning of the exponential phase was found to be a positive regulation mediated by the binding of the FIS protein to an upstream DNA element (42, 44). The dependence of the rRNA P1 promoter upon growth rate is, in contrast, a negative regulation (20). The question is whether the gap4 P1 promoter is subject to the same kind of regulation. In this respect, we observed several putative FIS binding sites in the upstream noncoding region of the gap4 gene (Fig. 1).

In addition to the P1 promoter, we found that three other promoters were used in vivo. The P4 promoter was used to a very low extent under all fermentation conditions used and whatever the genetic context. The absence of a −35 promoter
sequence and the inefficiency of this promoter in vitro suggest that either its utilization requires transcriptional activators or it represents a secondary RNA polymerase binding site. Indeed, secondary RNA polymerase binding sites, without efficient transcriptional activity and located upstream of the efficient promoters, have been observed in many *E. coli* genes, particularly those encoding stable RNA species or ribosomal proteins (51). It was proposed that their role is to recruit RNA polymerase and thus contribute to the efficiency of the strong downstream promoter (51). The P4 promoter, used to a low extent in vivo, as well as the P5 promoter, used to a low extent in vitro, may represent such secondary binding sites.

Utilization of the P3 start site is subject to catabolic repression. This may seem surprising for an enzyme involved in glucose metabolism. But as we mentioned above, the activity of the P1 promoter is growth phase dependent. Under conditions causing an intermediate or low growth rate, cells may need GAPDH activity to metabolize glucose which has been synthesized by gluconeogenesis or which is produced by hydrolysis of another carbon source, for instance, lactose or saccharose. Under such conditions, the existence of a transcript induced by the cAMP-CRP complex may give a selective advantage to the cells. Another possibility is that transcripts initiated at the P3 site serve to produce GAPDH for gluconeogenesis. An illustration of the P3 site utilization was given by our study of the variation of gapA transcript composition in the course of an *E. coli* growth.
coli XL1-Blue fermentation in L broth (Fig. 8). Between 4 and 10 h of incubation, the contribution of the P3 promoter to the production of gapA mRNAs was important. This is probably the result of a high concentration of cAMP-CRP complexes in the cells during this fermentation period.

The recognition of the P2 promoter by the heat shock RNA polymerase Eσ32 was unexpected. GAPDH was not found to be a protein overexpressed during heat shock (12, 40), and the transcription of the gapA gene was not found to be induced by heat shock (12, 13), probably because the sole effect of transcription of the P2 promoter by Eσ32 during heat shock is to maintain the gapA gene transcription level. The recent extensive study by Chuang and Blattner (12) of the E. coli genes which are induced by heat shock showed that at least 36 genes fit these criteria. It now appears that other genes, like the GAPDH gene, may be transcribed during heat shock by Eσ32 without an increase in their transcription yield and thus would not be detected by the method of Chuang and Blattner (12). The heat shock regulon of E. coli is considerably larger than is indicated by the number of E. coli heat shock proteins characterized on the basis of their increased synthesis upon heat shock (40). We have not tested whether the transcriptional activity of the gapA gene during heat shock resulted in GAPDH production. This is likely, however, and we observed an increased capacity of the E. coli GAPDH mutant DF221 transformed with a plasmid overproducing GAPDH to grow at 43°C. This observation indicates a positive effect of GAPDH on the growth of E. coli at high temperatures. In our experiments, the effect may have been reinforced by the fact that plasmid pBR322::EcoGAPDH overproduced GAPDH in very large quantities (7). An additional control will be to compare the abilities of the DF221 mutant and its isogenic wild-type strain to grow at high temperatures.

The production of 11 E. coli heat shock proteins is induced upon carbon starvation (31), and the level of σ32 was found to be increased upon starvation (30). Thus, our observation of a reactivation of the P2 promoter, late in the stationary phase when the medium is exhausted (Fig. 8), is likely explained by an increase of Eσ32 RNA polymerase activity late in the fermentation. This is a strong indication that the P2 promoter allows expression of the gapA gene, under conditions both of heat shock and of starvation, and that these observations suggest that in addition to glycolysis, GAPDH may have other biological roles in bacteria. In light of this hypothesis, it should be pointed out that several roles have been proposed for eukaryotic GAPDH (28, 37, 47).

An intriguing point in our observations is the strong utilization of the P2 site during the exponential phase of growth, with an abrupt decrease of utilization at the beginning of the stationary phase. Since the concentration of σ32 was not reported to be high in the exponential phase (8), one explana-
FIG. 8. Variation of gapA mRNA composition in the course of a fermentation. A 1-liter culture of E. coli XL1-Blue transformed with plasmid pBS::EcogapA was grown in L broth at 37°C. The growth curve is shown in panel A. Fractions corresponding to $6 \times 10^8$ cells were collected at regular time intervals, and total RNA was extracted and analyzed by primer extension, with oligonucleotide OG1 as the primer. All of the reverse transcription reaction mixture was fractionated on a polyacrylamide gel (B). Lanes: A, C, G, and T, sequencing markers; 1, 2 h; 2, 4 h; 3, 5.5 h; 4, 6.5 h; 5, 7.5 h; 6, 9.5 h; 7, 10.7 h; 8, 24 h. Products corresponding to initiation at the P1, P2, P3, and P4 sites are indicated. The amounts of the reverse transcripts were estimated with a Molecular Dynamics Phosphorimager system, and the results obtained are presented in panel C. For each reverse transcript, the value obtained by Phosphorimager analysis are plotted against the time at which the culture sample was collected. Panel D represents the variation of the quantities of transcripts P1, P2, P3, and P4 in a given amount of total RNA (1 μg) during the fermentation; for each reverse transcript the Molecular Dynamics signal units (C) were divided by the amount of total RNA recovered in the corresponding RNA extraction. The values obtained were plotted against the times at which the bacterial samples were collected.
tion could be the presence during this phase of protein factors reinforcing the utilization of the P2 promoter by Eσ2 RNA polymerase. The rmnA operon was recently shown to be transcribed by the E. coli RNA polymerase Eσ2 in vitro, and the FIS protein was found to increase the yield of in vitro transcription (41). As mentioned above, we observed the presence of several putative binding sites for the FIS protein upstream of the P4 start site, and experiments are under way to test for their possible roles.

The gapA gene and the rmn operons have several characteristic features in common. But whereas in the case of the rmn operons, transcription by Eσ2 and Eσ3 polymerases initiates at exactly the same position, mRNAs with S′ untranslated regions of different lengths are produced from the gapA gene. This situation offers the additional possibility of regulation at the translational level or by modulation of mRNA stability. It should also be noted that the complexity of the gapA transcriptional regulatory region is greater than that of the rmn operons. It is very similar to that found for the rpoH and rpoD genes, which produce σ22 (8) and σ70 (49), respectively, and for the glnALG operon (43).

We previously observed that the codon usage of the gapA gene was perfectly adapted to E. coli and corresponds to that found for highly expressed E. coli proteins (6). Now we show that the transcriptional regulatory region of the gapA gene belongs to the small family of highly sophisticated E. coli promoters which include several of the general E. coli transcriptional regulation strategies. This may seem to contradict the idea that the gapA gene was inherited by horizontal transmission of the genetic information. Nevertheless, very little is known about the rate at which promoters evolve, and an already optimized promoter region may have been fused to the eukaryotic GAPDH coding region. Thus, the hypothesis of horizontal transmission of the gapA gene cannot be excluded by our observation. Nevertheless, both the transcriptional characteristics of gapA and the recent observation of a gapA-like gene in a cyanobacterium (35) again raise the debate.

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