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Factors Influencing Preferential Utilization of RNA Polymerase Containing Sigma-38 in Stationary-Phase Gene Expression in *Escherichia coli*

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In order to understand the molecular basis of selective expression of stationary-phase genes by RNA polymerase containing σ^{38} ($E\sigma^{38}$) in *Escherichia coli*, we examined transcription from the stationary-phase promoters, *katE*P, *bolA*P, *hdeAB*P, *csgBA*P, and *mcb*P, *in vivo* and *in vitro*. Although these promoters are preferentially recognized *in vivo* by $E\sigma^{38}$, they are transcribed *in vitro* by both $E\sigma^{38}$ and $E\sigma^{70}$ containing the major exponential σ, σ^{70}. In the presence of high concentrations of glutamate salts, however, only $E\sigma^{38}$ was able to efficiently transcribe from these promoters, which supports the concept that the promoter selectivity of σ^{38}-containing RNA polymerase is observed only under specific reaction conditions. The examination of 6S RNA, which is encoded by the *ssrI* gene *in vivo*, showed that it reduced $E\sigma^{70}$ activity during the stationary phase, but this reduction of activity did not result in the elevation of $E\sigma^{38}$ activity. Thus, the preferential expression of stationary-phase genes by $E\sigma^{38}$ is unlikely the consequence of selective inhibition of $E\sigma^{70}$ by 6S RNA.

Key words: RNA polymerase, 6S RNA, *in vitro* transcription, sigma factor

Gram-negative bacteria enter the stationary phase upon nutrint limitation (reviewed in Hengge-Aronis, 1999; Ishihama, 1999). The stationary phase was considered physiologically inert until the discovery of the stationary phase specific sigma factor, σ^{38}, *rpoS* gene product (Mulvey and Loewen, 1989; Lange and Hengge-Aronis, 1991). Since the discovery, an increasing number of genes have been found to be expressed during the stationary phase (Hengge-Aronis, 1996; Zambrano and Kolter, 1996). Stationary phase genes include those that confer resistance to various environmental stresses, such as nutrient starvation, heat, high salt, H_2O_2, UV irradiation, and others presumably to prolong survival during the non-growing stages of bacterial life. These stationary specific genes are typically not expressed during the exponential phase of growth probably to avoid their deleterious effects on rapidly growing bacteria. The stationary phase gene expression is in part explained by the stationary phase sigma factor, σ^{38}. σ^{38} is produced at the onset of the stationary phase (Jishage and Ishihama, 1995) and is required for the expression of a large set of stationary phase genes. It should be noted, however, that many other stationary phase genes are not dependent on σ^{38} (Hengge-Aronis, 1996; Kolter *et al.*, 1993).

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The RNA polymerase holoenzyme of *Escherichia coli* is composed of a core enzyme (α_2, β, β') associated with one of seven σ subunits, which is required for promoter recognition. Among the seven sigma factors present in *E. coli*, σ^{70}, a major σ factor, primarily controls gene transcription during the exponential phase, whereas the rest of σ factors, minor σ factors, regulate the expression of the subset of genes in response to various stresses (Gross *et al.*, 1998; Ishihama, 2000). Different sigma factors compete for core binding. In the regulation of gene expression using minor σ factors, in principle, it is essential that 1) different σ factors recognize different promoter sequences, and 2) minor σ factors should have high affinities for the core to replace σ^{70} in the holoenzyme, if not present in large excess over σ^{70}. Most interestingly, however, in the case of σ^{38}, the affinity for the core is only about a fifth of σ^{70} under the reaction conditions that mimic the physiological conditions in exponentially growing cells (Maeda *et al.*, 2000), while its level is only about a third of σ^{70} at the maximal concentration during the stationary phase (Jishage and Ishihama, 1995; Colland *et al.*, 2002). Thus, an additional factor(s) could be involved in the efficient loading of σ^{38} to the core enzyme at the onset of the stationary phase. Candidates for additional factors include low-molecular weight factors in the cytoplasm, *e.g.*, glutamate salts and trehalose, which have been shown to influence the activity of $E\sigma^{70}$ and $E\sigma^{38}$ holoenzyme in different manners (reviewed in Ishihama, 2000). In addition,

there are two *E. coli* gene products, Rsd, also known as the anti-σ^{70} factor, (Jishage *et al.*, 2001) and 6S RNA (the *ssr1* gene product, Wassarman and Storz, 2000), which selectively inhibits the $E\sigma^{70}$ holoenzyme. The 6S RNA, has been found to be associated with RNA polymerase and directly contacts the σ^{70} and β/β' subunits of RNA polymerase as revealed by UV crosslinking (Wassarman and Storz, 2000). It was proposed that 6S RNA binds to $E\sigma^{70}$ in order to reduce its activity during the stationary phase at which $E\sigma^{38}$ is the predominant form of RNA polymerase. Thus, it is intriguing to speculate that the reduction of $E\sigma^{70}$ by 6S RNA might result in a release of a core enzyme that allows the loading of σ^{38}.

It is generally accepted that the promoter determinant for σ^{38} lies in the sequence near-10 hexamer although other regions in the promoter have also been implicated (Tanaka *et al.*, 1995; Espinosa-Urgel *et al.*, 1996; Wise *et al.*, 1996; Bordes *et al.*, 2000; Lee and Gralla, 2001). Recently, it has been reported that σ^{38} consensus CTATACT (from-13 to-7) differs from the σ^{70} consensus XTATAAT in the two underlined positions (Lee and Gralla, 2001). Using a band shift assay, it was shown that-13C prevented recognition by $E\sigma^{70}$, which suggests that the promoter determinant for $E\sigma^{38}$ is the nucleotide sequence at-13, otherwise two forms of RNA polymerases would recognize the same promoter sequences. However, it should be noted that there are $E\sigma^{70}$-dependent promoters that carry C at this position (Becker and Hengge-Aronis, 2001; Gaal *et al.*, 2001)

In this study, five promoter species from stationary phase-specific genes (*katE*P, *bol*AP, *hdeAB*P, *csgBA*P, and *mcb*P) were examined *in vivo* and *in vitro* in order to understand their selective expression during the stationary phase. The *katE*P (Mulvey *et al.*, 1990) and *bol*AP (Aldea *et al.*, 1989; Aldea *et al.*, 1990) are typical σ^{38}-dependent promoters that are not subject to any other regulation. On the other hand, *hdeAB*P (Yoshida *et al.*, 1993) and *csgBA*P (Hammar *et al.*, 1995) are expressed normally by $E\sigma^{38}$ but also by $E\sigma^{70}$ only when *hns*, encoding histone-like nucleoid structuring protein, is mutated. The *mcb*P (Mao and Siegele, 1998) is a σ^{70}-dependent stationary phase specific promoter. In this study, *in vivo* observations were made which showed that 6S RNA significantly reduced $E\sigma^{70}$ activity during the stationary phase, but the reduction did not lead to enhancing $E\sigma^{38}$ activity. Using *in vitro* transcription analysis, we observed that the selective recognition by $E\sigma^{38}$ holoenzyme increased for these promoters through the addition of high concentrations of glutamate salts.

Materials and Methods

Strains, phages and plasmids

All *E. coli* strains used in this study were derived from the MG1655 background (Table 1). CH1018 was constructed by transducing the *proAB*::*Tn10* mutant strain of MG1655 into Pro^+, Lac^- using a T4 phage grown in MC4100 (Young and Edlin, 1983). The *rpoS* strain was constructed by moving *katF*::*Tn10* from GN124, which was kindly provided by D. Gentry (GlaxoSmithKline, USA), into CH1018 by P1 transduction and subsequently selecting tetracycline sensitive rpoS mutants on Bochner's selection plate containing chlorotetracycline and fusaric acid (Bochner *et al.*, 1980). The rpoS mutants were screened by testing for H_2O_2 sensitivity and glycogen accumulation (Lange and Hengge-Aronis, 1991). λ carrying *bol*AP::*lacZYA*, *katE*P::*lacZYA*, *hdeAB*P::*lacZYA*, *csgBA*P::*lacZYA*, and *mcb*P::*lacZYA* were obtained from the following lysogenic strains: GN207 (D. Gentry), CF5092 (M. Cashel, USA), TY001 (T. Mizuno, Japan), MRH101 (S. Normark, Sweden), and CF5017 (M. Cashel), respectively. The strains that carried *ssr1*::Ap and plasmid pKK*-6S were kindly provided by G. Storz (USA).

The plasmids used in the *in vitro* transcription assay were constructed by cloning DNA fragments that carried promoter sequences into the *Eco*R1 and *Pst*1 sites of the transcriptional vector pSA508 (Choy and Adhya, 1993).

Table 1. Strains and plasmid used in this study

Strains and plasmids	Relevant genotype	Source or reference
GN207	*bolA*::*lacZYA*	D. Gentry
CF5092	*KatE*::*lacZYA*	M. Cashel
CF5017	*mcb*::*lacZYA*	M. Cashel
TY001	*hdeAB*::*lacZYA*	T. Mizuno
MRH101	*csgBA*::*lacZYA*	S. Normark
CH1018	(arg-lac)U169	This work
CH1112	CH1018, ϕ(*bol*AP::*lacZYA*)	This work
CH1114	CH1112, Δ*rpoS*	This work
CH1242	CH1018, ϕ (*KatE*P::*lacZYA*)	This work
CH1243	CH1242, Δ*rpoS*	This work
CH1279	CH1018, ϕ (*csgBA*P::*lacZYA*)	This work
CH1289	CH1279, Δ*rpoS*	This work
CH1281	CH1018, ϕ (*hdeAB*P::*lacZYA*)	This work
CH1282	CH1018, Δ*rpoS*	This work
CH1291	CH1281, Δ*rpoS*	This work
CH1310	CH1018, ϕ (*mcb*P::*lacZYA*)	This work
CH1333	CH1291, *hns*::Km	This work
CH1405	CH1281, *ssr1*::Ap	This work
CH1408	CH1333, *ssr1*::Ap	This work
CH1409	CH1281, pKK*-6S	This work
CH1410	CH1112, pKK*-6S	This work
CH1411	CH1112, *ssr1*::Ap	This work
CH1413	CH1333, pKK*-6S	This work
CH1440	CH1310, *ssr1*::Ap	This work
EY1001	CH1242, *ssr1*::Ap	This work
EY1005	CH1242, pKK*-6S	This work
EY1015	CH1279, *ssr1*::Ap	This work
EY1017	CH1279, pKK*-6S	This work
EY1020	CH1289, *hns*::Km	This work
EY1022	EY1020, *ssr1*::Ap	This work
EY1024	EY1020, pKK*-6S	This work
EY1025	CH1310, pKK*-6S	This work
pKK*-6S	*ssr1* in pKK*	Wassarman and Storz (2000)

Plasmid pGal carries the *gal* promoter sequence between -197 and +91, PbolA carries the *bolA* promoter between 345 and +108, pKatE carries the *katE* promoter between -170 and +49, pHdeAB carries the *hdeAP* promoter between -160 and +98, and pCsgBA carries the *csgBA* promoter between -105 and +52 of each promoters in *E. coli*. The promoter DNA fragments were obtained by PCR amplification using chromosomal DNA as the template with appropriate primers carrying the *EcoR*1 site for top strands and the *Pst*I site for bottom strands.

Growth Condition

Cultures except those carrying λ*csgBA*p::*lacZYA* were grown in Luria-Bertani medium (Difco Laboratories), which contained 1% NaCl, with vigorous aeration at 37°C. For a solid support medium, 1.5%-granulated agar (Difco Laboratories) was included. MacConkey lactose agar was obtained from Difco Laboratories. The strains carrying λ*csgBA*P::*lacZYA* were grown in Cunli Forming Agar (CFA), a low salt medium (100 g casamino acid, 15 g yeast extract, 0.5 g $MgSO_4$, and 0.05 g $MnCl_2$ in 1 l dH_2O, pH 7.4) (Evans *et al.*, 1977). Antibiotics were obtained from Sigma Chemical. The antibiotics were added at the following concentrations: ampicillin for *ssr*1::Ap, 50 μg/ml; chloramphenicol for pKK*-6S, 100 μg/ml; and tetracycline for *rpoS*::Tet, 20 μg/ml. X-gal was from Bachem and was used at 20 μg/ml.

β-Galactosidase assay

β-galactosidase assay was performed as described by Miller (1972), using cells that were permeabilized with Koch's lysis solution (Putnam and Koch, 1975). β-galactosidase specific activity was expressed in Miller units (A_{420}/min/A_{600}). To measure β-galactosidase levels in bacteria at different stages during growth, fresh overnight culture was diluted 1:50 into LB or CFA medium and was grown at 37°C until the cultures reached their stationary phase. Samples were taken for the enzyme assay at regular time intervals. Each strain was assayed in triplicate, and average enzyme activities were plotted as a function of time.

In vitro transcription assay

Transcription reactions were carried out using the procedure described by Choy and Adhya (1993). Briefly, 2 nM DNA template, 1 mM ATP, 0.1 mM GTP, 0.1 mM CTP, 0.01 mM UTP and 10-20 μCi of [α-^{32}P] UTP were preincubated in buffer (20 mM Tris-acetate, pH 7.8, 10 mM magnesium acetate, 100 mM potassium glutamate, 1 mM DTT) at 37°C for 5 min. Transcription was initiated by the addition of RNA polymerase (20 nM) in a total volume of 20 μl and was terminated after 10 min at 37°C by the addition of an equal volume (20 μl) of RNA loading buffer [80% (v/v) deionized formamide/1×TBE (89 mM tris/89 mM boric acid/2 mM EDTA)/0.025% bromophenol blue/0.025% xylene cyanole]. The mixture was heated at 90°C for 2 min and was electrophoresised in 8 M urea /8% polyacrylamide sequencing gels (40 cm×0.4 mm).

Proteins

Sigma-free RNA polymerase core enzyme of *E. coli* W3550 was purified by passing purified RNA polymerase at least three times through phosphocellulose columns while σ^{70} and σ^{38} were purified from over-expressed *E. coli* (Kusano *et al.*, 1996). $E\sigma^{70}$ and $E\sigma^{38}$ holoenzymes were prepared from the purified core enzyme by adding a four-fold molar excess of the respective sigma subunits.

Results

Effect of 6S RNA on RpoS-dependent gene expression

In an attempt to understand the nature of selective gene expression catalyzed by $E\sigma^{38}$ at the onset of the stationary phase, we examined the effect of *ssr1* mutation or over-expression of 6S RNA on the expression from the *rpoS*-dependent promoters, *katE*P, *bolA*P, *csgBA*P, and *hdeAB*P (Fig. 1). The promoter activities were determined using the constructs that carried the test promoters fused to *lacZYA*. Lambda lysogenes carrying these test promoter-*lacZYA* fusions were used in order to eliminate possible changes of the gene copy numbers, if present in the plasmids, during the stationary phase. Bacteria grown in LB (in the case of *bolA*, *katE*, and *hdeAB* fusions) or CFA (in the case of *csgBA* fusion) over night was diluted 50-fold in the respective fresh media and was grown until the cultures entered into the stationary phase. The strain carrying the *lacZYA* that was fused to the *csgBA* promoter was grown in CFA that drives the expression of curli, which was encoded by the *csg* operon (Hammar *et al.*, 1995; Arnqvist *et al.*, 1994). Cell growth was monitored by measuring A_{600} using a spectrophotometer (Fig. 1, left panels), and the *lacZ* expression level was determined by assaying for β-galactosidase activities (Fig. 1, right side panels). The rate of cell growth appeared the same for the three strains grown in LB medium (Fig. 1, A1, B1 and C1) while the growth rate of the strain that carried the *csgBA*P-*lacZ* fusion in CFA medium was slower (Fig. 1, D1). In the wild-type background (open circles), the levels of β-galactosidase activity increased under the control of *bolA*, *katE*, *hdeAB*, and *csgBA* promoters as the culture entered into the stationary phase, and compared to the levels during the exponential phase, they reached the maximum accumulation of about 4-fold (Fig. 1A, *bolA*), 50-fold (Fig. 1B, *katE*), 15-fold (Fig. 1C, *hdeAB*), and 8-fold (Fig. 1D, *csgBA*), respectively. When the rate of increase in *lacZ* expression level parallels the promoter activity, the expression level reached the maximum at the culture time ranging between 3-4 hrs (*bolA* and *hdeAB*), and 4-5 hrs (*katE* and *csgBA*), which suggested that the stage of cell growth to produce the maximum promoter activity is different between the test promoters. This is in good agreement with the finding that the stationary-

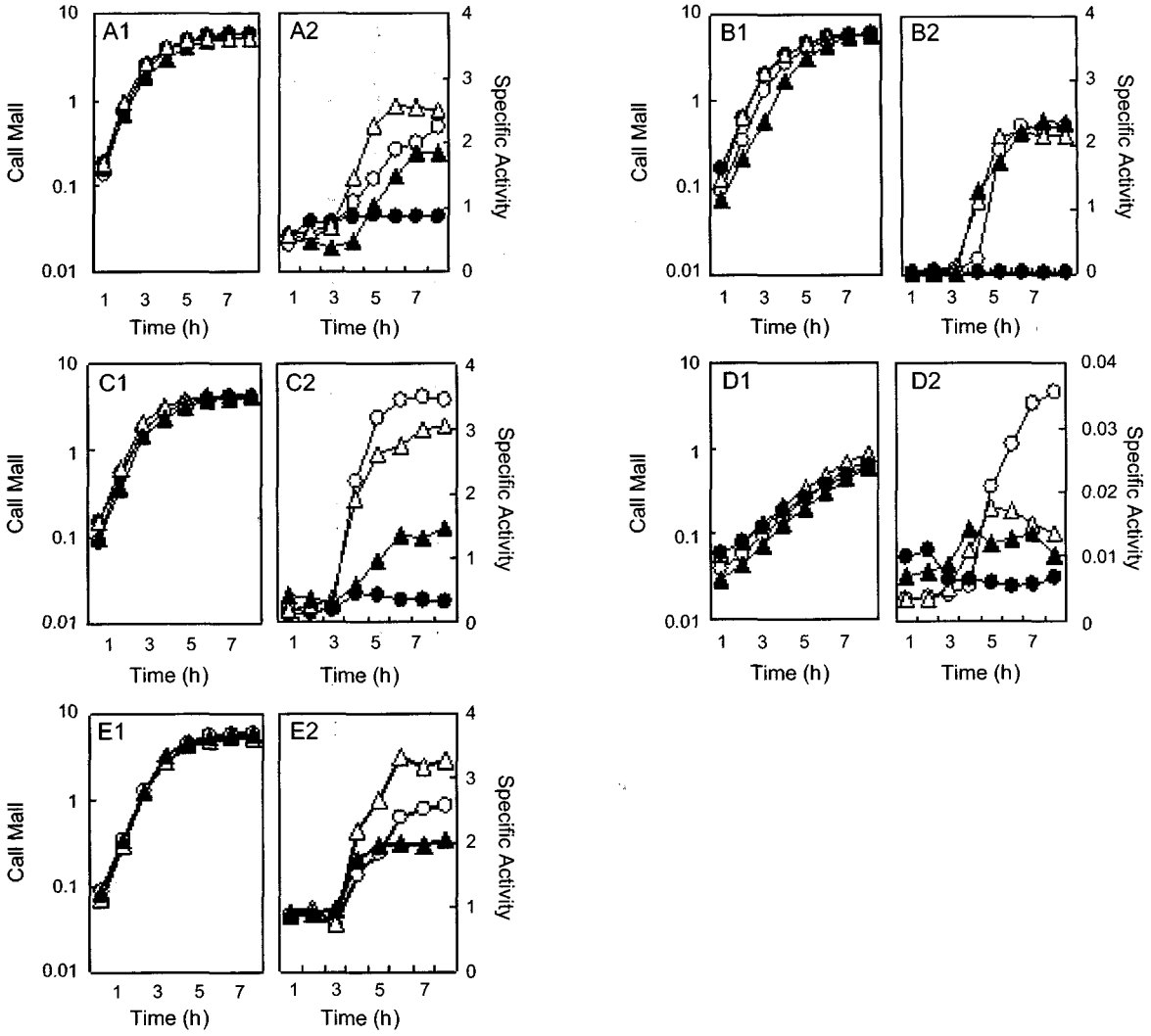

Fig. 1. Expression from various stationary phase promoters, *bolA*P (A), *katE*P (B), *hdeABP* (C), *csgBA*P (D), and *mcb*P (E) that were transcriptionally fused to *lacZYA*. λ lysogens carrying these promoters::*lacZYA* were used for this assay. The bacterial cell mass (A_{600}) and β-galactosidase activity (A_{420} / min / ml / A_{600}) are shown in the left side (series 1) and right side(series 2) panels, respectively. Symbols in panels A, B, C, D, and E are as follows: open circles are the measurements in the wild-type background, closed circles in the RpoS mutant background, open triangles in the Ssr mutant background, and closed triangles in the wild type strain carrying pKK*-6S.

phase genes are expressed sequentially under a defined order (Makinoshima *et al.*, 2002). Induction of these promoter activities are RpoS-dependent since the β-galactosidase activity during the stationary phase in the *rpoS* mutant that is devoid of σ^{38} remained at the basal level even when the culture entered into stationary phase (Fig. 1, right side panels, closed circles).

Subsequently, we examined the role of 6S RNA within the activities of $E\sigma^{38}$. The above σ^{38}-dependent promoter activities were determined in the absence of 6S RNA (Fig. 1, open triangles), in the *ssrI* mutant background, and also in the presence of excess 6S RNA (Fig. 1, closed triangles) that is expressed from the multicopy plasmid, pKK*-6S. It was anticipated that if a reduction of $E\sigma^{70}$ activity due to 6S RNA was responsible for the preferential utilization of $E\sigma^{38}$ during the stationary phase, the σ^{38}-dependent test promoter activities should be reduced in the absence of 6S RNA while being increased in the presence of excess 6S RNA. In the absence of 6S RNA (open triangles), the activities of *hdeAB* (Fig. 1, C2) and *csgBA* promoters (Fig. 1, D2) were more or less reduced in comparison with that of the wild type background (open circles). However, the *katE*P was not changed (Fig. 1, B2). Moreover, the *bolA*P activity was not reduced but was increased in the absence of 6S RNA (Fig. 1, A1). In the presence of excess 6S RNA (closed triangles), all test promoter activities were not increased but were decreased with the exception of *katE*P activity (Fig. 1, B2). The *katE*P activity was essentially the same. Therefore, these results were inconsistent with the predicted role of 6S RNA on $E\sigma^{38}$ activity.

In this study, we also examined the effect of 6S RNA on

the expression from an $E\sigma^{70}$-dependent stationary phase promoter, or *mcb* promoter, which drives the transcription of microcin B17 operon (Mao and Siegele, 1998). Again, a lambda lysogen carrying *mcb*P::*lacZYA* fusion was utilized to measure the activity of *mcb*P. It was predicted that if 6S RNA interferes with $E\sigma^{70}$ activity during the stationary phase, expression from these promoters which are initiated by $E\sigma^{70}$ should increase in the absence of 6S RNA and decrease in the presence of excess 6S RNA. In agreement with this prediction, the expression from *mcb*P (Fig. 1E2) was increased in the strain that lacked 6S RNA (open triangles) while the expression decreased in the strain that over-expressed 6S RNA (closed triangles) in comparison with the expression illustrated in the wild type background (open circles). Taken together, we concluded that 6S RNA reduces $E\sigma^{70}$ activity during the stationary phase. This reduction, however, does not seem to cause an increase in $E\sigma^{38}$ or its activities.

Transcription in vitro from the stationary phase-specific promoters

Transcription from the test promoters was examined *in vitro* using purified components of the reconstituted $E\sigma^{70}$ or $E\sigma^{38}$ holoenzyme. Supercoiled plasmids (Choy and Adhya, 1993), each carrying one of the test promoters, followed by a strong terminator, were used as templates. Fig. 2 shows typical gel patterns of multiple-rounds of transcription. When the pBolA template that carried the bolA promoter was transcribed by $E\sigma^{70}$ (lane 1) or $E\sigma^{38}$ (lane 2), a transcript of expected size (146 nucleotide-long) was generated in addition to the 108 nucleotide-long rna1 (Tomizawa, 1984). Apart from this specific transcript, another transcript, which was approximately 10 nucleotide longer than the bolA transcript, was generated with the use of $E\sigma^{70}$. Although its source is unknown, the generation of this *in vitro* transcript using $E\sigma^{70}$ has been reported (Nguyen *et al.*, 1993). Most importantly, unlike the observation made in vivo where the stationary phase expression of bolA requires RpoS, both the $E\sigma^{70}$ and $E\sigma^{38}$ transcribed from bolAp in vitro. Similar observations were made with the katE, hdeAB, and csgBA promoters, even though the relative level of transcripts by the two holoenzymes was not the same between these promoters.

The *E. coli galE* promoter was also tested, which is expressed *in vivo* by $E\sigma^{70}$ during the exponential phase. The expression of the *gal* operon is driven by two promoters, *P*1 and *P*2 (Choy and Adhya, 1992). The *P*2 RNA is 5-nucleotides longer than *P*1 RNA, which can be deduced by the fact that the *galE*P2 is located 5 bp upstream of *galE*P1. Both $E\sigma^{70}$ and $E\sigma^{38}$ transcribed from *galE*P1 equally well (lanes 9 & 10), although transcription from the *galE*P2 was more efficient with $E\sigma^{70}$ than $E\sigma^{38}$. Thus, despite some difference in the efficiency, purified $E\sigma^{70}$ and $E\sigma^{38}$ transcribed *in vitro* from all promoters that were tested without distinct promoter specificity.

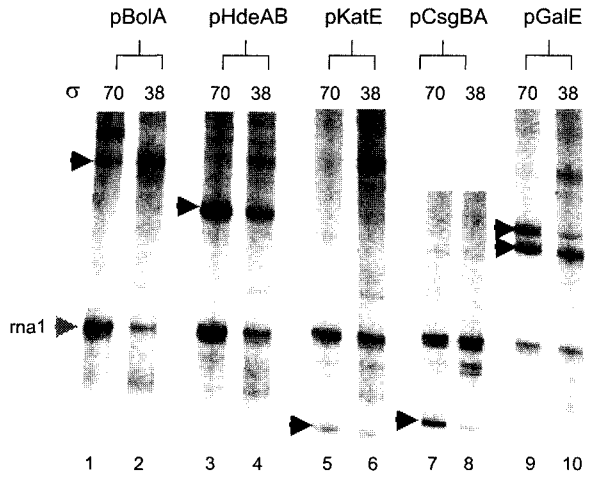

Fig. 2. Autoradiogram from *in vitro* transcription assay using purified $E\sigma^{70}$ (odd number lanes) or $E\sigma^{38}$ (even number lanes). DNA templates were super coiled plasmid DNA that carried *bol*AP (lanes 1 & 2), *hde-AB*P (lanes 3 & 4), *katE*P (lanes 5 & 6), *csgBA*P (lanes 7 & 8), or *galE*P (lanes 9 & 10). The radioactive transcripts were analyzed on a 8% polyacrylamide gel. The black arrows indicate the major transcripts from the test promoters, and the gray arrow indicates *rna*1.

Loss of promoter selectivity for the $E\sigma^{70}$ and $E\sigma^{38}$ holoenzymes has been recognized in transcription *in vitro* using several stationary phase-specific promoters that were under the reaction conditions mimicking the physiological conditions of growing cells (reviewed in Ishihama, 2000). However, the promoter selectivity of $E\sigma^{70}$ and $E\sigma^{38}$ holoenzymes, which mimicked the *in vivo* situations, could be observed when transcription *in vitro* was performed under specific conditions, such as in the presence of high concentrations of glutamate salts (Ding *et al.*, 1995). Then, transcription *in vitro* from the *hdeAB* and *csgBA* promoters was examined in the presence of varying concentrations of potassium glutamate (Fig. 3). The panel on the left and right shows the transcripts generated by $E\sigma^{70}$, and $E\sigma^{38}$, repectively. The transcription from both *hdeAB* and *csgBA* promoters by $E\sigma^{70}$ was found to decrease in the presence of an increasing concentration of K-glutamate while *rna*1 transcript remained more or less the same (Fig. 3, Left). Transcription from the *csgBA* promoter was virtually undetected in the presence of 200 mM potassium glutamate. Conversely, the transcription from both *hdeAB* and *csgBA* promoters by $E\sigma^{38}$ increased in the presence of an increasing concentration of potassium glutamate (Fig. 3, Right). Thus, in the presence of high potassium glutamate in *in vitro* reaction conditions, the transcription of $E\sigma^{38}$-dependent promoters by $E\sigma^{38}$ was enhanced while that by $E\sigma^{70}$ was reduced.

Discussion

The level of σ^{38} in *E. coli* W3110 is much lower than the level of σ^{70} even in the stationary phase (Jishage and Ish-

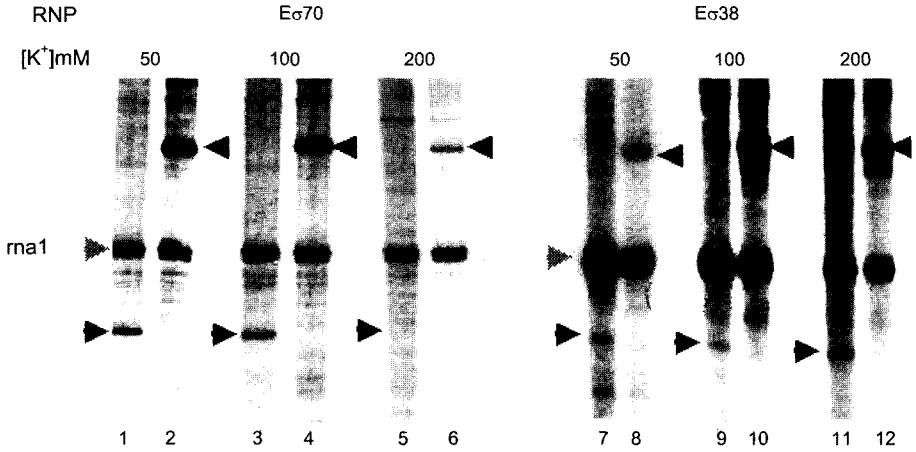

Fig. 3. Potassium glutamate-dependent transcription from *csgBA*p and *hdeAB*p *in vitro* driven by $E\sigma^{70}$ (lanes 1-6) and $E\sigma^{38}$ (lanes 7-12). 50 mM, 100 mM, or 200 mM potassium glutamate was used in the reactions. The transcriptions using the *hdeAB* promoter are shown in the odd number lanes and *csgBA* promoters in even number lanes. The black arrows indicate the major transcripts from the test promoters, and the gray arrow indicates *rna*1.

A

σ^{38}-dependent promoters	Nucleotide Sequence						Reference
		-35	Spacer	-10		+1	
bolA(P1)	TAAG	CTGCAA	TGGAAACGGTAAAAGCG	GCTAGT	ATTT	A	Wise *et al.*, 1996
csgBA	GGGT	GAGTTA	TTAAAAATATTTCCGCAGA	CATACT	TTCCAT	CG	Arnqvist *et al.*, 1994
hdeAB	CAAC	ATGACA	TATACAGAAAACCAGGT	TATAAC	CTCAGT	G	Yoshida *et al.*, 1993
katE	GTCT	CCGAAG	CGGGATCTGGCTGGTGGTC	TATAGT	TAGAGAG	T	Tanaka *et al.*, 1997
gal(P1)	ATGTC	ACACTT	TTCGCATCTTTGTTATGC	TATGGT	TATTTC	A	Lim *et al.*, 2001

B

	Region4,2	Region2,4
σ^{70}	TTLEEVGKQFDVTRERIRQIEAKALRKLR	IRQAITRSIADQARTIRIPVHM
	===* = = * * ==== * === ==* ==	=== = = = = ====*=* = *
σ^{38}	ATLEDVGREIGLTRERVRQIQVEGLRRLR	IRQTIERAIMNQTRTIRLPIHI

Fig. 4. DNA sequences of the promoters used in this study (A) and amino acid sequence in the domain 2.4 and 4.2 of σ^{70} and σ^{38} (B) = Indicates identical amino acids, and * indicates similar amino acids.

ihama, 1995), but the switching of promoters takes place *in vivo* during the growth transition from the exponential phase to the stationary phase. The affinity of σ^{38} for the core enzyme is significantly lower than σ^{70}, as measured under the conditions, that are set up to give the maximum activity for $E\sigma^{70}$ (Maeda *et al.*, 2000). Stationary phase-specific RpoS-dependent promoters are transcribed *in vitro* by both $E\sigma^{70}$ and $E\sigma^{38}$ under these conditions (see Fig. 2; see also Tanaka *et al.*, 1995; Kolb *et al.*, 1995). The DNA sequences at promoter -10 and -35 hexamers in the *rpoS*-dependent promoters tested in this study look very much like a typical σ^{70}-dependent promoter with 16 to 18 bp spacing (Fig 4, panel A). The amino acid sequences at two motives, region 2.4 and region 4.2, each recognizing promoter-10 hexamer and promoter-35 hexamer, respectively, are virtually indistinguishable between σ^{70} and σ^{38}. Such sequences are not conserved in the corresponding regions of other minor σ factors that carry unique motives to recognize specific promoter sequences. Thus, it may not be surprising that both $E\sigma^{70}$ and $E\sigma^{38}$ transcribed from the *rpoS*-dependent promoters *in vitro*. The difference in promoter specificity *in vivo* between the two forms of RNA polymerases may lie in a third element rather than in the promoter determinant itself.

Several osmotically inducible genes are also induced when cultures enter into the stationary phase (Jenkins *et al.*, 1990). Some osmo-regulated promoters, *e.g.*, *osmB* and *osmY*, are known to be under the control of σ^{38} (Yim and Villarejo, 1992). Although the σ^{38} level stays low (Hengge-Aronis *et al.*, 1993), these σ^{38}-dependent genes are induced when exponentially growing bacteria are exposed to osmotic shock. Ding *et al.* (1995) reported that both $E\sigma^{70}$ and $E\sigma^{38}$ transcribed equally well *in vitro* from the osmoregulated promoters under low salt conditions, but in the presence of high concentrations of glutamate salts, $E\sigma^{38}$ exclusively transcribed from these promoters. It was then proposed that the promoter specificity of RNA polymerase is controlled by intracellular concentration of glutamate salts. Here, we also observed that, in high concentrations of potassium glutamate, the σ^{38}-dependent promoters, *csgBA*P and *hdeABP*, were

transcribed preferentially by $E\sigma^{38}$. However, it should be noted that not all RpoS-dependent genes are osmotically inducible (Hengge-Aronis *et al.*, 1993).

Apart from the high glutamate concentration, different requirements have been recognized between $E\sigma^{70}$ and $E\sigma^{38}$ to allow for maximum activity. Trehalose that accumulates in the stationary phase supports the high activity of $E\sigma^{38}$ (Kusano and Ishihama, 1997b). In stationary-phase cells, the accumulation of polyphosphate inhibits transcription *in vitro* by $E\sigma^{70}$, but not by $E\sigma^{38}$ (Kusano and Ishimaha, 1997a). There are global changes that occur within bacteria as culture enters the stationary phase, which may be responsible for the selective enhancement of $E\sigma^{38}$ activity. Upon entry into the stationary phase, DNA superhelicity decreases (Kusano *et al.*, 1996). $E\sigma^{38}$ prefers DNA with a reduced superhelicity as a template. Likewise, the composition of nucleoid-associated proteins is remarkably different between the exponential growth phase and the stationary phase (Ishihama, 1999). The change in nucleoid protein composition is accompanied by the compaction of the chromosomal DNA. The RNA polymerase carrying σ^{38} may be capable of initiating transcription from the genes that are buried in the compacted chromosome. Within this line of idea, the *rpoS*-dependent promoters are possibly associated with nucleoid proteins such that these are transcribed by $E\sigma^{38}$ but not $E\sigma^{70}$.

Preferential and selective utilization of the $E\sigma^{38}$ holoenzyme for the transcription of stationary-phase genes can also be caused by the selective inhibition of $E\sigma^{70}$. Jishage *et al.* (2001) found a stationary phase-specific protein, Rsd (regulator of sigma D), which specifically binds to free σ^{70} to convert it into an inactive stored form. Likewise, 6S RNA interacts directly with $E\sigma^{70}$ to convert it into an inactive form (Wassarman and Storz, 2000). An over-expression of 6S RNA resulted in significant reduction of the expression from σ^{38}-independent stationary promoter by $E\sigma^{70}$ (Fig. 1). However, the observations herein described suggest that 6S RNA is not necessarily implicated in the expression from the *rpoS*-dependent promoters by $E\sigma^{38}$ (see Fig. 1). Thus, 6S RNA, which is produced at the onset of the stationary phase, would be responsible for the reduced $E\sigma^{70}$ activity at the stationary phase as proposed by Wassarman and Storz (2000), but if so, it does not lead to the release of core for the σ^{38} to substitute.

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