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Promoter paper

Identification and analysis of the *rpoS*-dependent promoter of *katE*, encoding catalase HPII in *Escherichia coli*

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Abstract

The *rpoS* gene of *Escherichia coli* encodes an alternative sigma factor of RNA polymerase σ^{38} (or σ^{S}) that is required for transcription of *katE* encoding catalase HPII. The transcription start site of the single *katE* transcript identified by ribonuclease protection has been determined by primer extension analysis to be either 53 or 54 bp (depending on the strain used) upstream of the open reading frame. A series of promoter fragments were constructed and fused to *lacZ* to confirm the start site location. A -10 sequence similar to that found in other σ^{70}- and σ^{38}-dependent *E. coli* promoters was identified 8 or 7 bp upstream of the start site but a σ^{70}-dependent -35 sequence was not evident.

Keywords: Catalase; Stationary phase; *Escherichia coli*; *rpoS*; Promoter region

The genome of the enteric bacterium *Escherichia coli* contains the genes for two different catalases; *katE* encodes HPII and *katG* encodes HPI [1–4]. HPI is an adaptive enzyme regulated by OxyR, a major regulatory transcription factor responding to oxidative stress [5,6]. HPII is expressed maximally in stationary phase, and its expression is completely dependent on the product of *katF* [2,7]. Although *katF* was first identified as a positive regulator of HPII [2,7], it has been found to control the expression of many stationary phase specific and osmotically regulated genes [8]. Subsequently, the *katF* gene product was shown to function as a sigma transcription factor for RNA polymerase [9,10] resulting in it being renamed *rpoS* for stationary phase or starvation sigma factor [11,12]. The protein is referred to variously as RpoS, σ^{38} and σ^{S}. Analysis of a number of promoters controlled by *rpoS* had suggested that the -10 sequence preferred by σ^{38} was similar to the sequence recognized by the major σ factor, σ^{70}, TATAAT [10]. However, the start site originally identified upstream of *katE* [7] at -126 relative to the first base of the open reading frame did not contain such a sequence raising doubt as to the accuracy of that site [13]. In order to clarify this question and understand better the σ^{38}-dependent promoter structure, we have re-characterized the *katE* promoter.

The multiplicity of *katE* transcripts was first determined by ribonuclease protection analysis [14,15]. The 3,466 bp *Pst*I-*Cla*I fragment containing the *katE* gene [7] (GenBank accession number M55161) was

Abbreviations: HP, hydroperoxidase; Eσ^{38}, RNA polymerase holoenzyme containing σ^{38} (or σ^{S}); Eσ^{70}, RNA polymerase holoenzyme containing σ^{70}; bp, base pair(s); nt, nucleotide(s).

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first cloned in pBluescriptM13$^+$KS using complementation of catalase activity in a *katE*-deficient strain for selection to generate pKTE1. From this clone, the 1251 bp *Pst*I-*Hin*dIII fragment containing the *katE* promoter was cloned in pTZ19R to generate pKTE20 which was linearized with either *Pst*I or *Nsp*I. The linearized pKTE20 was used as a template in an in vitro transcription reaction using T7 RNA polymerase (Takara Shuzo) in the presence of [α-^{32}P]UTP (Amersham) to prepare single-strand RNA probes complementary to the *katE* promoter region. Total RNA to be probed was prepared from JM103 (F′ *traD36 lacI*^{q} *Δ(lacZ)M15 proAB / endA1 supE sbcBC thi-1 rpsL*(Str^r) *Δ(lac-pro)*) as described [16]. As shown in Fig. 1, a unique transcription product was identified using both probes (lanes 1 and 4), and this signal was not detected with an RNA preparation from an *rpoS*-deficient strain JM103F (JM103 but *rpoS*::Tn10) (lanes 2 and 5). This suggested that there was a single transcription start site and a single *rpoS*-dependent promoter.

Having confirmed the presence of a single transcription start site, its location was then identified by primer extension analysis [16,17]. The oligonucleo-

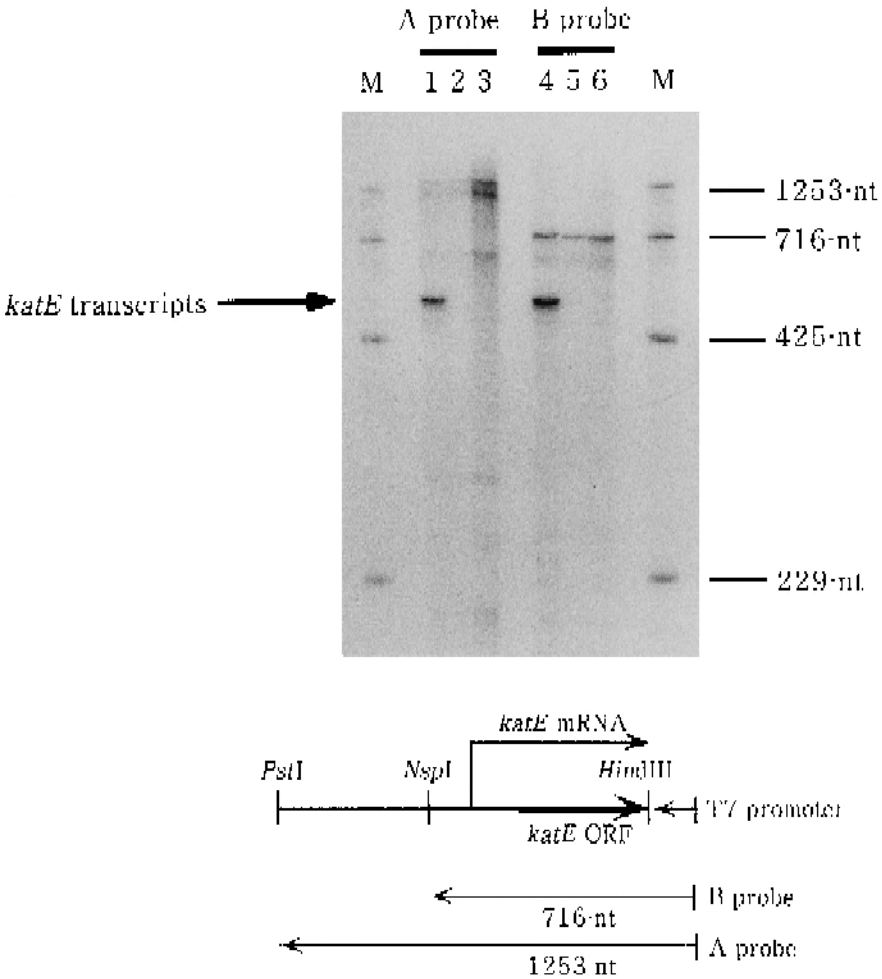

Fig. 1. Ribonuclease protection assay of the *katE* promoter region. pKTE20 was digested with *Pst*I for probe A and *Nsp*I for probe B, and single strand RNA probes were prepared by in vitro transcription reactions with T7 RNA polymerase as described [15]. The lengths of the resultant probes are 1253 nt for probe A and 716 nt for probe B. 40 μg of RNA preparations (lanes 1 and 4, JM103; lanes 2 and 5, JM103F; and lanes 3 and 6, no RNA) were hybridized with either probe A (lanes 1–3) or probe B (lanes 4–6), digested with RNase, and the protected fragments were analyzed by polyacrylamide gel electrophoresis containing 8 M urea. Lanes M contain molecular weight marker.

tide primer complementary to the 5′ end of the *katE* open reading frame (5′-TGTAGTGGTGACTG-GTGCTGATGTGGGTTC, shown as an arrow in Fig. 2) was end-labeled with [γ-^{32}P]ATP (Amersham), and elongated by reverse transcriptase using RNA extracted from JM103 and JM103F as template. As shown in Fig. 3, a unique 5′ end was identified at the U of position −53 relative to the translational start site at +1 using RNA from JM103, whereas no corresponding signal was detected using RNA from JM103F. A minor strain-specific variation was noted when the start site in transcripts isolated from NM522 [18] was found to be G at −54. Immediately upstream of the identified start site is a putative −10 sequence TATAGT, consistent with Eσ^{38} recognizing a TATAAT-type −10 sequence [19,20].

Because this site at −53/−54 was different from the previously identified site at −126 [7], we wanted to corroborate the result independently. To do this a series of promoter fragments were amplified by PCR using primers listed in Table 1 and cloned upstream of *lacZ* in the operon fusion plasmid pRS415 [21]. The *Eco*RI, *Sma*I and *Bam*HI sites in each primer allowed directed cloning into the multiple cloning site of the vector. β-Galactosidase levels were assayed in both *rpoS*$^+$ (NM522 [18]) and *rpoS*$^-$ (UM315 [22]) strains as shown in Table 2 revealing that fragments containing the region from −165 to −77 (pEP2 and pEP5) did not promote *lacZ* expression, whereas fragments containing the region from −77 to +6 (pEP9 and pEP13) did promote *rpoS*-dependent expression. Further truncation of the upstream end of the promoter to −70 (pEP31) or −39 (pEP15) resulted in significantly less or no expression. This confirmed that the transcript could not be initiated at −126 as previously reported, but was starting between −77 and −44 consistent with the primer extension results in Fig. 3. The previously reported result [7] may be due to a non-specific annealing of the synthetic primer. With transcription being initiated at −53/−54 and the active *rpoS*-dependent promoter extending only to −77, it appeared that a −35 sequence was not necessary for expression. The reduced expression from pEP31 may indicate the presence of an unidentified sequence element between the −10 and −35 regions.

Further confirmation of the *rpoS*-dependence of the promoter initiating at −53/−54 was obtained by assaying the promoter activity of a fragment extending from −125 to +6 during growth through exponential phase into stationary phase. The fragment was amplified by PCR using primers KTE5 and KTE3 (Table 1) and cloned into the operon fusion plasmid pRS551 [21] using the *Eco*RI and *Bam*HI sites in the primers. Subsequent cloning into λRS45 [21] allowed the lysogenization of KT1008 (MC4100 but *relA*$^+$) and KT1005 (KT1008 but *rpoS*::Tn10) to form strains KT1008EL and KT1005EL, respectively, containing a single copy of the *katE*::*lacZ* fusion. β-Galactosidase activity was assayed as shown in Fig. 4, revealing that *rpoS*-dependent expression (only in KT1008EL) commenced in late exponential phase and peaked in stationary phase consistent with earlier reports [4,22].

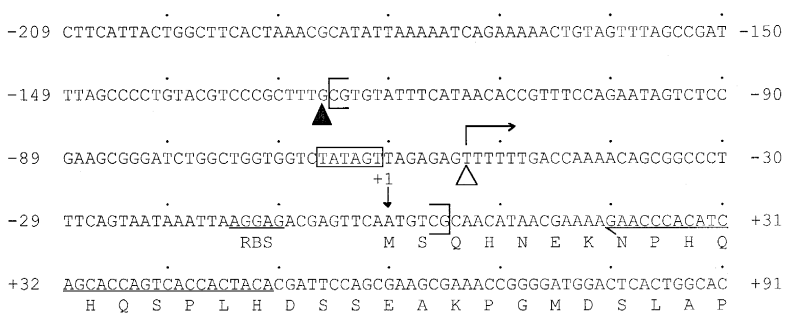

Fig. 2. Nucleotide sequence of the *katE* promoter region. Nucleotide sequence from −209 to +91 with respect to the translational start site of the *katE* open reading frame is shown. The suggested ribosome binding site (RBS) is underlined. The arrow under the sequence indicates the position of the antisense primer used for the primer extension analysis. The open triangle denotes the transcriptional start site determined in this study, and the closed triangle shows the previously reported start site [7]. The boxed sequence indicates the predicted −10 sequence. A DNA region used for the *lacZ* expression analysis described in Fig. 4 is shown in brackets.

In a previous in vitro study, no transcripts corresponding to the −126 start site was detected using $E\sigma^{38}$ [10]. However, recent results by Kusano et al. [23] demonstrated that the promoter element identified in this study was recognized in vitro by $E\sigma^{38}$, also supporting the present result.

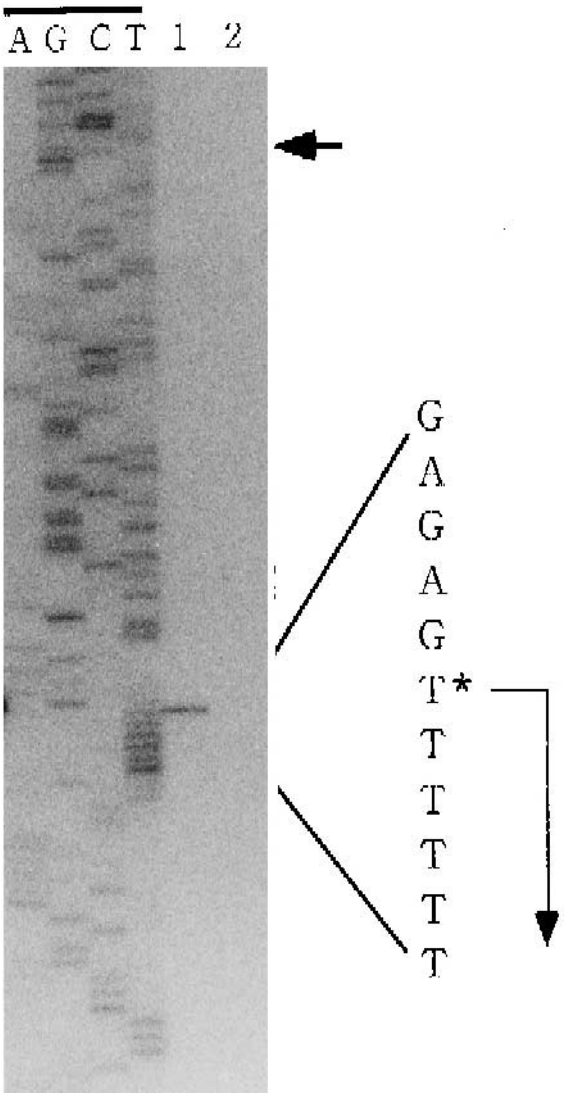

Fig. 3. Primer extension mapping of the 5′ termini of the *rpoS*-dependent *katE* transcripts. The primer oligonucleotide was end-labeled with [γ-^{32}P]ATP, and annealed to 40 μg of RNA isolated from JM103 (lane 1) or JM103F (lane 2). Primer extension reactions using RAV-2 reverse transcriptase (Takara Shuzo) were performed as described [17], and the reaction mixture was fractionated on a 6% polyacrylamide sequencing gel. Dideoxy sequence reactions using the same primer are also shown (lanes A, G, C, and T). The sequence shown is the sense strand around the transcription start site. The position of the previously reported start site [7] is indicated by the horizontal arrow.

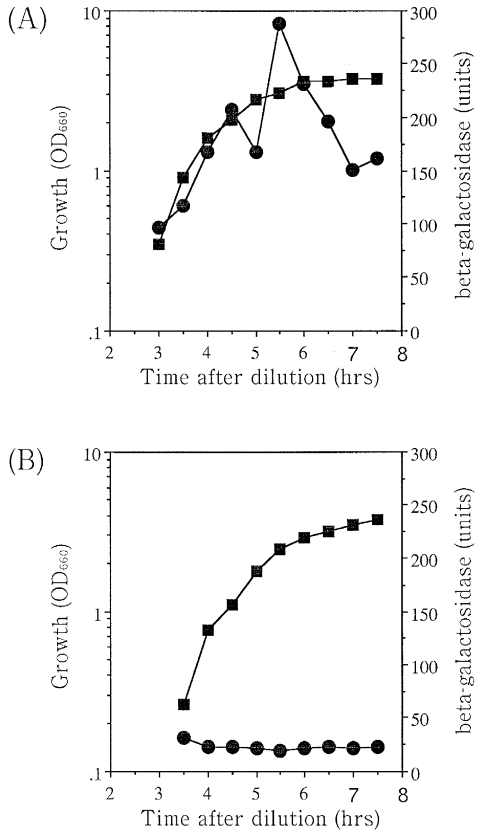

Fig. 4. Dependence of the *katE* promoter defined in this work on *rpoS*. KT1008 (*rpoS*$^+$) (a) and KT1005 (*rpoS13*::Tn10) (B) lysogenized with λKTE (*katE*::*lacZ*) were cultured in LB medium at 37°C overnight. The overnight cultures were diluted 1000-fold, and incubations continued at 37°C. The β-galactosidase activity [28] was measured periodically and growth was monitored from exponential to stationary phase. Circles and squares indicate the β-galactosidase units and the OD_{660}, respectively.

Whereas almost all $E\sigma^{38}$ promoters share the TATAAT-type −10 sequence [10,19], there is still not a clear consensus as to the identity of the −35 element required for $E\sigma^{38}$ recognition. In the case of the *fic* promoter, for example, the downstream of the position −17 appeared to be sufficient for recognition [19]. In accordance with this result, base changes in the −10 element affected $E\sigma^{38}$ transcription [19,20], while base changes in the −35 element did not [24]. On the other hand, DNA sequence around the −35 region has been shown to affect transcrip-

Table 1
Oligonucleotide primers used in the PCR amplification of *katE* promoter fragments

Designation	Sequence	Location [a]
KTE5	GGGCCGAATTCGTGTATTTCATAACACCGTT	−125 to −105
EP12	AAATCAGGAATTCTGTAGTTT	−165 to −157
EP14	CCGAAGCGGAATTCGGCTGGTGGT	−77 to −68
EP15	TTTTTTGACGAATTCAGCGGCCC	−39 to −31
EP21	GGATCTGGCCCGGGGTCTATAGT	−70 to −61
KTE3	GGCCGGATCCGACATTGAACTCGTCTCCT	+6 to −14
EP10	TTTTCGTTAGGATCCGACATTGA	+6 to −3
EP18	ATAGACCAGGATCCAGATCCCG	−76 to −85
EP19	GTGTTATGGGATCCACGCAAA	−122 to −129

[a] The positions indicated are relative to the first nucleotide in the *katE* open reading frame as shown in Fig. 2.

Table 2
β-Galactosidase levels in expressed in pRS415-clones containing various fragments from the region upstream of *katE*

Plasmid	Primers	Promoter Segment [a]	β-Galactosidase	
			NM522 (*rpoS*$^+$)	UM315 (*rpoS*$^-$)
pRS415	–	–	2	0
pRSkatE16	–	complete promoter	6250	190
pEP2	EP12 + EP19	−165 to −122	175	187
pEP5	EP12 + EP18	−165 to −76	110	136
pEP9	EP12 + EP10	−165 to +6	4166	250
pEP13	EP14 + EP10	−77 to +6	5599	869
pEP15	EP15 + EP10	−39 to 6	32	0
pEP31	EP21 + EP10	−70 to +6	1622	193

[a] The positions indicated are relative to the first nucleotide in the *katE* open reading frame as shown in Fig. 2.

tion in some promoters [25]. Recently, Espinosa-Urgel et al. proposed a σ^{38} promoter consensus consisting of the −10 sequence CTATACT and an upstream region with intrinsic DNA curvature, which may act as an ancillary element compensating for the absence of a −35 sequence [26]. The −10 sequence CTATAGT identified in this study coincides well with the proposed consensus sequence. However, it would require further experiments to demonstrate the involvement of the upstream DNA curvature.

It should be also noted that the *katE* transcripts initiated with either UUUUUU (U_6) or GUUUUUU (GU_6), depending on the strains used. Whether these initiation sites are subjected to the regulation by reiterative transcription [27] would be another interesting question to address.

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