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Consensus sequence for *Escherichia coli* heat shock gene promoters

(transcription initiation/σ factors/*dnaK* regulon)

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ABSTRACT **We have identified promoters for the *Escherichia coli* heat shock operons *dnaK* and *groE* and the gene encoding heat shock protein C62.5. Transcription from each promoter is heat-inducible *in vivo*, and each is recognized *in vitro* by RNA polymerase containing σ^{32}, the σ factor encoded by *rpoH* (*htpR*) but not by RNA polymerase containing σ^{70}. We compared the sequences of the heat shock promoters and propose a consensus promoter sequence, having T-N-t-C-N-C-c-C-T-T-G-A-A in the −35 region and C-C-C-C-A-T-t-T-a in the −10 region. These sequences differ from the consensus sequence recognized by holoenzyme containing σ^{70}, the major σ in *E. coli*. We suggest that the accumulated consensus sequences of promoters recognized by alternate forms of holoenzyme are compatible with a model in which σ recognizes only the −10 region of the promoter.**

When cells are shifted from low to high temperature, the synthesis of the heat shock proteins increases transiently (1). The heat shock response is universal (2) and the function of heat shock proteins may be conserved since the 70-kDa heat shock proteins of *Drosophila*, yeast, and *Escherichia coli* are homologous (3). In *E. coli* the heat shock response is regulated by the *rpoH* (*htpR*) gene product. When the nonsense mutation *rpoH165* is suppressed by a temperature-sensitive tRNA, the synthesis of heat shock proteins does not increase after shift from low to high temperature (4–7). The *rpoH* gene product is a 32-kDa σ factor (σ^{32}) that stimulates transcription initiation from heat shock promoters (8, 9). We report the identification and the nucleotide sequence of promoter regions upstream of the heat shock genes *dnaK* and *groE* and the C62.5 gene (5). RNA polymerase containing σ^{32} ($E\sigma^{32}$) initiates transcription from these promoters *in vitro*.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. CAG440 is SC122 (4), CAG456 is SC122 *rpoH165* (10), CAG1831 is SC122 *galK2*, and CAG1832 is SC122 *galK2 rpoH165* (11).

Plasmids pK01 and pKG1900 (12), pKWT5 (11) and the *dnaK* plasmid (3) have been described. pDC401 and pDC403 were made by ligation of the fragments shown in Fig. 1*D* into the *Sma* I site of pK01. The C62.5 gene was cloned into pBR322 as a 6-kilobase *Eco*RI fragment (unpublished data). *groE* plasmids pS2 and pS4 were from C. Georgopoulos.

Biochemical Techniques. Standard methods were used for DNA sequencing (13) and nuclease S1 mapping (14, 15). DNA end-labeling (3), *in vitro* transcriptions (8), and galactokinase assays (11) were as described. *E. coli* RNA was isolated (16, 17) from CAG440 and CAG456. S1 hybridization was at 45°C unless otherwise noted. Transcription reactions were at 37°C in 100 mM NaCl, except when stated otherwise.

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RESULTS

Strategy for Identification of Heat Shock Promoters. We determined the positions of the 5′ ends of the *in vivo* transcripts of *dnaK*, *groE*, and the C62.5 gene by S1 mapping. To test whether these 5′ ends corresponded to heat-inducible RNAs, we compared the amount of labeled fragment protected by RNA isolated at low temperature with that protected by RNA isolated 5–9 min after a shift to 42°C. To determine whether these corresponded to transcripts from $E\sigma^{32}$-specific promoters we compared the 5′ ends observed *in vivo* with the 5′ ends of transcripts initiated by $E\sigma^{32}$ *in vitro*.

***groE* Promoter.** S1 mapping of *groE* mRNA detected one heat-inducible RNA (Fig. 1*A*), with the 5′ end 72 bases upstream of the initiating AUG for GroES. RNA transcribed from *groE in vitro* by $E\sigma^{32}$ protected the same DNA sequence from S1 digestion as did *in vivo* RNA (Fig. 1*B*, lanes 1–3). The locations of the *groE* promoter and the 5′ end of the mRNA in the DNA sequence upstream of *groE* are shown in Fig. 1*D*.

Promoter Region of the C62.5 Gene. S1 mapping of the C62.5 gene mRNA identified two heat-inducible RNAs (Fig. 2*A*, lanes 3–5). The 5′ ends are at −43 and −34 from the initiating AUG (Fig. 2*C*) and are shown by arrows in Fig. 2*E*, with the potential promoters, P1 and P2. Induction of these RNAs after heat shock was eliminated or decreased in an *rpoH165* strain (not shown). S1 mapping transcripts initiated by $E\sigma^{32}$ *in vitro* showed that the *in vivo* and the *in vitro* starts were the same (Fig. 2*B*, lanes 2 and 3), although the RNA initiating from P2 was transcribed poorly *in vitro* compared with that initiating from P1. Since we have not separated the two potential promoters, we do not know if P2 is an independent promoter.

***dnaK* Promoters.** Three RNAs, all of which increased in abundance during the heat shock response, were observed by S1 mapping of *dnaK* mRNA (Fig. 3*A*, lanes 3 and 4). The 5′ ends of the transcripts are −115, −40, and −19 nucleotides from the initiating AUG (Fig. 3*C*) and are indicated on the sequence with arrows (Fig. 3*E*). We designate the potential promoters corresponding to these 5′ ends P1, P2, and P3.

P1 and P2 are promoters based on both *in vitro* transcription by $E\sigma^{32}$ and promoter cloning. Transcripts from P1 and P2 were the major products of *in vitro* transcription of *dnaK* by $E\sigma^{32}$ (Fig. 3*B*, lanes 1 and 2). Fragments containing either P1 or P2 alone (Fig. 3*D*) were inserted upstream of the promoterless *galK* gene in pK01. Both had promoter activity at low temperature, were heat-inducible, and were under the control of the *rpoH* gene (Table 1). We do not know if P3 is a promoter or if the third transcript results from processing. $E\sigma^{32}$ transcribes P3 poorly, if at all, *in vitro* from either supercoiled or linear templates. Because P2 and P3 overlap, a clone containing only P3 has not yet been made to test the *in vivo* promoter activity of P3.

We compared transcription of *dnaK* in the *rpoH* and the *rpoH165* strains both by quantitative S1 mapping and by galactokinase assays using the promoter–*galK* fusions. For

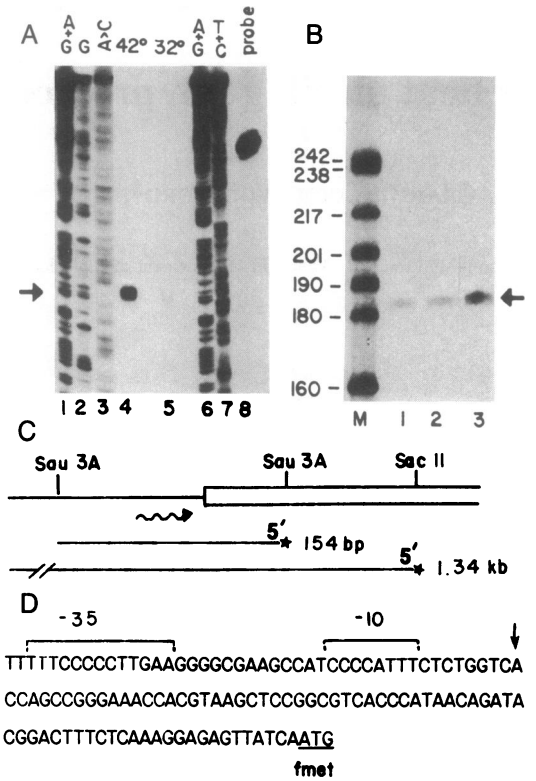

FIG. 1. S1 mapping of the 5′ end of the *groE* transcript. Probes were DNA fragments 5′ end-labeled at *Sau*3A (+21 of the coding sequence) or *Sac* II (+111). The protected fragments are indicated by arrows. (*A*) *In vivo* RNA. The *Sau*3A probe was hybridized at 55°C to 20 μg of heat shock RNA (lane 4) or 75 μg of 32°C RNA (lane 5). A fragment of known sequence was used as a size standard (lanes 1–3, 6, and 7). Lane 8, untreated probe. (*B*) *In vitro* transcription. The *Sac* II probe was hybridized to 50 μg of 25°C RNA (lane 1), 10 μg of heat shock RNA (lane 2), or RNA transcribed by Eσ^{32} *in vitro* (lane 3). (*C*) Schematic. The open bar represents the coding region. The second line shows the probe, with a star at the position of the ^{32}P label. The wavy line represents the RNA 5′ end. kb, Kilobases. (*D*) *groE* promoter region sequence. The arrow indicates the RNA 5′ end. We have adjusted the position of the 5′ end based on the difference in mobility between fragments generated by sequencing reactions and those generated by S1 digestion (18). Brackets indicate the −35 and −10 regions of the promoter.

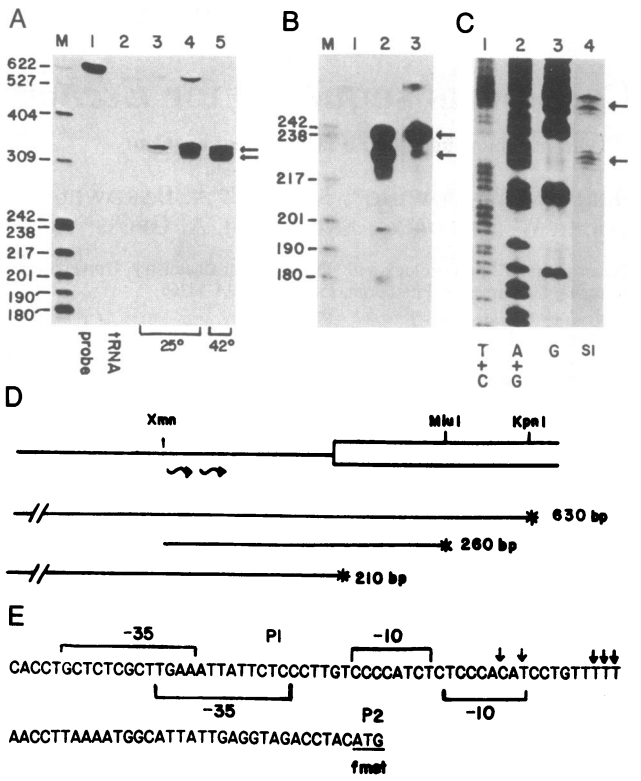

FIG. 2. S1 mapping of the 5′ ends of the C62.5 gene transcripts. Probes were DNA fragments labeled at *Kpn* I (+300 of the coding sequence), *Mlu* I (+185), or *Alu* I (+45). (*A*) *In vivo* RNA. The *Kpn* I probe was hybridized at 50°C to 100 μg of tRNA (lane 2), 10 μg of 25°C RNA (lane 3), 100 μg of 25°C RNA (lane 4), or 10 μg of heat shock RNA (lane 5). Lane 1, untreated probe. (*B*) *In vitro* transcription. The *Mlu* I probe was hybridized to tRNA (lane 1), 20 μg of heat shock RNA (lane 2), or RNA transcribed *in vitro* by Eσ^{32} (lane 3). Lane 2, a lighter exposure than the rest of the autoradiogram. (*C*) High-resolution S1 mapping. Lane 4, 10 μg of heat shock RNA was hybridized at 37°C to the *Alu* I probe. Lanes 1–3, Maxam–Gilbert sequencing of the *Alu* I fragment. Lane 4, a darker exposure than lanes 1–3. (*D*) Schematic. Symbols as in Fig. 1*C*. bp, Base pairs. (*E*) C62.5 gene promoter region sequence. Symbols as in Fig. 1*D*.

P1, induction after heat shock is eliminated by the *rpoH165* mutation, and for P2, the degree of induction is reduced (Fig. 3*A*, Tables 1 and 2). Expression of both *dnaK* promoters is reduced by about 50% at 25°C by the poorly suppressed *rpoH165* nonsense mutation (Tables 1 and 2). A similar 50% reduction in the *rpoH165* strain has been observed for the *lon* heat shock promoter (19). These results indicate that at least 50% of the transcription of these promoters is dependent upon Eσ^{32} at low temperature and suggest that Eσ^{32} is responsible for steady-state as well as heat-inducible transcription from Eσ^{32} promoters.

Eσ^{70} Does Not Transcribe Heat Shock Promoters. We assayed transcription from the *dnaK* and *rpoD* P_{HS} promoter regions with Eσ^{70} reconstituted from core RNA polymerase and σ^{70}. Although Eσ^{70} transcribed RNA-I of ColE1 plasmids, it did not initiate at either Eσ^{32} promoter (Fig. 4). We have seen no Eσ^{70} transcription from these promoters on either linear or supercoiled templates, using a range of salt

Table 1. Rates of galactokinase expression from *dnaK* promoter–*galK* fusion plasmids

Strain	Relevant genotype	Plasmid	Promoter	Promoter activity* 25°C	42°C	42°C/25°C
CAG1831	*rpoH*$^+$	pDC401	*dnaK* P1	4.5	30	6.7
		pDC403	*dnaK* P2	1	40	40
		pKG1900	Pgal	1.4	1.4	1
CAG1832	*rpoH165*	pDC401	*dnaK* P1	2.1	2.1	1
		pDC403	*dnaK* P2	0.4	3.2	8
		pKG1900	Pgal	1.8	1.8	1

Data are normalized to the rate of galactokinase synthesis from pDC403 in *rpoH*$^+$ at 25°C. Data for a representative experiment are shown; similar results were obtained in two to four independent experiments.
*Rate of galactokinase expression.

Table 2. Quantitative S1 mapping of *dnaK* mRNA

Relevant genotype	Promoter	Relative amount of RNA 25°C	42°C	42°C/25°C
rpoH$^+$	*dnaK* P1	2.8	14.8	5.2
	dnaK P2	1	8.4	8.4
	dnaK P3	0.8	6	7
rpoH165	*dnaK* P1	1.6	0.7	0.4
	dnaK P2	0.3	0.8	2.4
	dnaK P3	0.3	0.4	1.3

An S1 experiment was quantitated by densitometric scanning of the autoradiograms. Data are normalized to the P2 band at 25°C in *rpoH*$^+$.

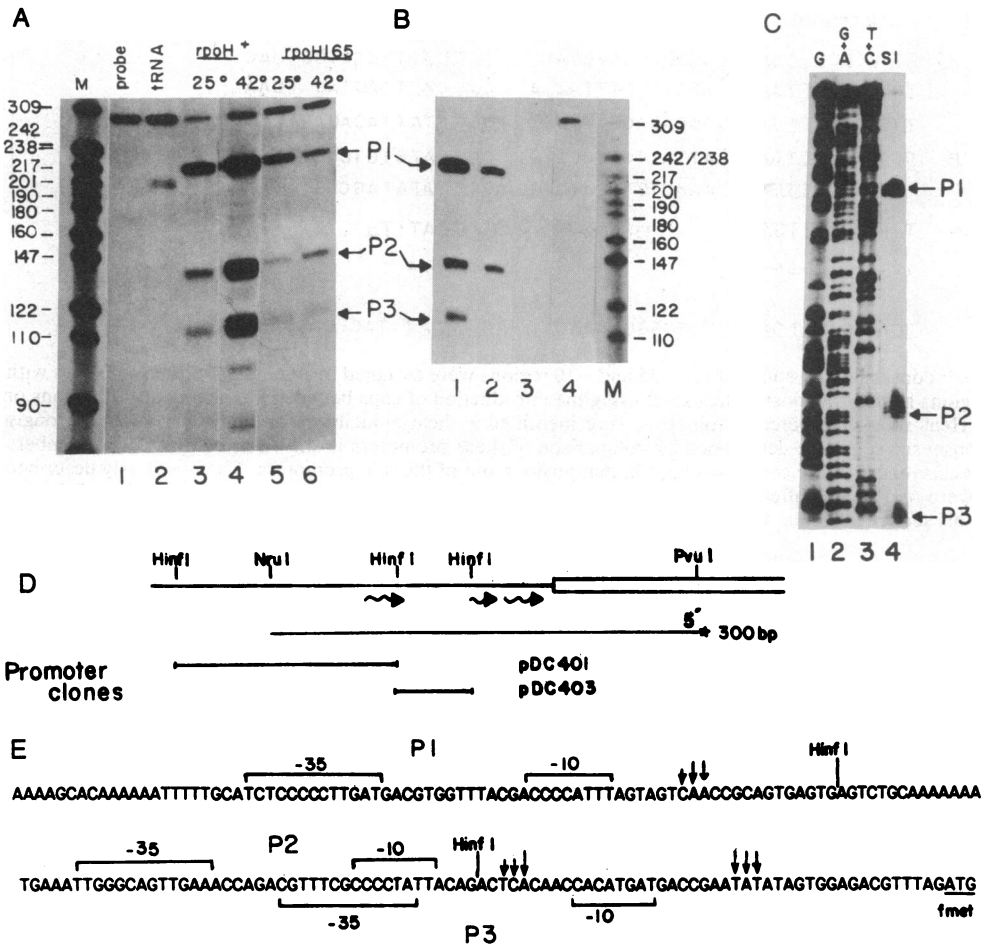

FIG. 3. S1 mapping of the 5' ends of *in vivo* and *in vitro dnaK* transcripts. A DNA fragment 5' end-labeled at the *Pvu* I site 100 base pairs (bp) into the coding region of *dnaK* was used as probe. (*A*) *In vivo* RNA. RNAs hybridized to the probe were tRNA (lane 2), 10 μg of *rpoH*$^+$ 25°C RNA (lane 3), 10 μg of *rpoH*$^+$ heat shock RNA (lane 4), 10 μg of *rpoH165* 25°C RNA (lane 5), or 10 μg of *rpoH165* heat shock RNA (lane 6). Lane 1, untreated probe. We believe the band comigrating with full-length probe in lanes 3–6 is caused by DNA re-annealing; other experiments have shown no full-length probe protection. (*B*) *In vitro* transcription. Probe was hybridized to 20 μg of *rpoH*$^+$ heat shock RNA (lane 1), RNA transcribed *in vitro* by Eσ^{32} (lane 2), or tRNA (lane 3). Lane 4, untreated probe. (*C*) High-resolution S1 mapping. Lanes 1–3, sequence reactions of the *Pvu* I fragment. Lane 4, the fragments protected from S1 by 10 μg of *rpoH*$^+$ heat shock RNA. (*D*) Schematic. Symbols are as in Fig. 1*C*. The lower part of the schematic shows the two fragments that are cloned into pK01 to make pDC401 and pDC403. pDC401 contains a 150-bp fragment including P1, and pDC403 contains a 50-bp fragment including P2. (*E*) *dnaK* promoter region sequence. Symbols as in Fig. 1*D*.

concentrations from 50 to 200 mM NaCl. Similar results were obtained by using the *groE* or C62.5 promoter regions (not shown).

DISCUSSION

We have identified heat-inducible promoters for the operons containing heat shock genes *dnaK* and *dnaJ* (the *dnaK* operon) and *groES* and *groEL* (the *groE* operon) and for heat shock gene C62.5. Along with the *rpoD* heat shock promoter P_{HS} (11), these promoters control the production of 6 of the 17 known heat shock proteins. These promoters are regulated by *rpoH* (*htpR*) *in vivo* and recognized by Eσ^{32} but not by Eσ^{70} *in vitro*.

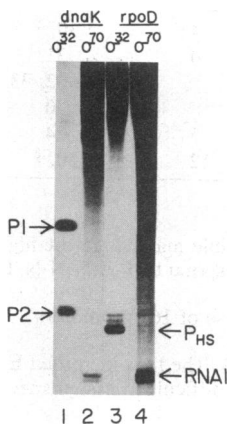

FIG. 4. *In vitro* run-off transcription of Eσ^{32} promoters. *Pvu* I-cut pdnaK (lanes 1 and 2) or *Sac* I-cut pKWT5 (lanes 3 and 4) was transcribed by Eσ^{32} in 200 mM NaCl (lanes 1 and 3) or by Eσ^{70} in 50 mM NaCl (lanes 2 and 4). The ColE1 RNA-I transcript and run-off transcripts initiating at *dnaK* P1 and P2 and at *rpoD* P_{HS} are indicated. The minor bands in lane 4 that comigrate with the P_{HS} transcript do not originate from P_{HS}, as determined by S1 mapping (data not shown).

Our data indicate that transcription of *groE*, *dnaK*, and C62.5 gene originates only from Eσ^{32} promoters both at low temperature and during heat shock. Since the *rpoH165* mutation reduces transcription from the *dnaK* promoters by 50% at low temperature and since Eσ^{70} does not recognize heat shock promoters *in vitro*, it would appear that Eσ^{32} is responsible for transcription of these genes under all conditions. However, Yura *et al.* (20) report that strains with the *rpoH165* nonsense mutation are viable at low temperature and synthesize heat shock proteins in the absence of suppressor tRNAs. There are several possible explanations for the transcription of heat shock genes in the *rpoH* amber strains: (*i*) Eσ^{70} may recognize Eσ^{32} promoters *in vivo* with a positive activator; (*ii*) another, unidentified, σ factor may recognize these promoters; (*iii*) a compensating non-tRNA suppressor mutation may be linked to *rpoH* in the *rpoH* amber strains.

Four of the heat-inducible RNAs identified *in vivo* corresponded to promoters that were strongly transcribed *in vitro* by Eσ^{32}:P1 and P2 of *dnaK*, the *groE* promoter, and P1 of the C62.5 gene. In addition, we used S1 mapping to show that the *rpoD* P_{HS} promoter used *in vitro* is identical to that used *in vivo* (not shown). Based on a comparison of these promoters, we propose a consensus sequence for Eσ^{32} promoters having T-N-t-C-N-C-c-C-T-T-G-A-A in the −35 region and C-C-C-C-A-T-t-T-a in the −10 region (Fig. 5*A*). A sequence similar to the Eσ^{32} consensus is found upstream of the heat-inducible gene *lon* (21) and may be another Eσ^{32} promoter (A. Markowitz, personal communication) (Fig. 5*B*). The consensus −35 region of Eσ^{32} promoters shares the sequence T-T-G-A with the consensus −35 region of Eσ^{70} promoters, T-T-G-A-C-A (22), while the consensus −10 region of Eσ^{32} promoters has little similarity to the Eσ^{70} −10

	Promoter	-35 region		-10 region	+1
A	groE	TTTCCCCTTGAA	GGGGCGAAGCCAT	CCCCATTTCTCTGGTCAC	
	dnaK P1	TCTCCCCTTGAT	GACGTGGTTTACGA	CCCCATTTAGTAG TCAA	
	dnaK P2	TTGGGCAGTTGAA	ACCAGACGTTTCG	CCCCTATTACAGACTCAC	
	C62.5 gene P1	GCTCTCGCTTGAA	ATTATTCTCCCTTGT	CCCCATCTCTCCCACATC	
	rpoD P_{HS}	TGCCACCCTTGAA	AAACTGTCGATGTGG	GACGATATAGCAG ATAA	
	σ^{32} consensus	T tC CcCTTGAA	13-15 bp	CCCCATtTa	
		4 4 5 455554		445444 5	
B	lon	TCTCGGCGTTGAA	TGTGGGGGAAACAT	CCCCATATACTGACGTAC	

FIG. 5. Heat shock promoters and Eσ^{32} consensus. Positions of the −35 and −10 regions were assigned by alignment of the promoters with one another and determination of the regions having the most matches, allowing the introduction of gaps between the −35 and −10 regions or between the −10 and +1 regions. (*A*) Heat shock promoters. Promoters were identified as heat-inducible promoters *in vivo* and strongly transcribed by Eσ^{32} *in vitro*. The σ^{32} consensus sequence determined by comparison of these promoters is shown in boldface. The numbers below the consensus are the number of occurrences of the consensus base in that position out of the five promoters. The previously described −35 and −10 regions of *rpoD* P_{HS} (11) were reevaluated after comparison with these other heat shock promoters. (*B*) The position of the *lon* promoter has not been determined, but this sequence similar to the σ^{32} consensus is found 5′ to the *lon*-coding region (A. Markowitz, personal communication).

consensus, T-A-T-A-A-T. The identification of a consensus sequence for Eσ^{32} promoters that differs from the consensus for Eσ^{70} promoters and the demonstration that Eσ^{70} does not transcribe these promoters contribute to the evidence that changing the σ subunit of RNA polymerase changes the promoter recognition properties of holoenzyme (23).

Regulation of gene expression by alternate σ factors was first described by Lee and Pero, who observed that when *Bacillus subtilis* σ^{43} was replaced with a phage-encoded σ, the new form of holoenzyme recognized promoters that were not transcribed by Eσ^{43} (24). The new promoters differed from Eσ^{43} promoters at both the −10 and the −35 regions. To explain how the different forms of holoenzyme discriminated between promoter sequences, Losick, Pero, and co-workers (23–25) suggested that each σ confers specificity by making contacts with both regions of the promoter. An alternative, that σ factors make contacts only in the −10 region and affect the recognition of the −35 region indirectly by inducing a specific conformation in core RNA polymerase, was thought less likely. It was recently determined that RNA polymerase containing the T4^{gp55} σ (26) recognizes T4 late promoters containing an invariant −10 region but lacking specific sequences in the −35 region (27). Thus, the gp55 σ appears to make specific contacts with DNA only in the −10 region of the promoter. This led us to consider the possibility that other σ factors contact only the −10 region. We propose another version of the class of models in which −35 region contacts are made by holoenzyme subunits other than σ.

We suggest that each σ confers specificity to holoenzyme by interacting directly with the −10 region of the promoter. In addition, the different size and shape of each σ factor could alter the precise region of holoenzyme that contacts the −35 region. This altered geometry of the holoenzyme–DNA complex could lead to differences in the spacing between the conserved sequences and in the sequence in the −35 region recognized by holoenzyme. According to our model, the −10 regions of consensus promoters should be sufficiently different to account for the discrimination by various forms of holoenzyme. The −35 regions, recognized by subunits common to each holoenzyme, could be more similar than the −10 regions, recognized by different σs, but such a similarity would not be required to be consistent with the model. The consensus sequence for Eσ^{32} promoters is consistent with such a model, as its −10 region is quite different from the Eσ^{70}

Table 3. Conserved regions of promoters

Holoenzyme*	−35†	Spacing, bp	−10†	No.‡	Refs. or source
E. coli					
Eσ^{70} (168)	**T-T-G-A-C-A**	16–18	**T-A-T-A-A-T**	136	22
Eσ^{32} (6)	T-N-t-C-N-C-c-C-**T-T-G-A-A**	13–15	C-C-**C-C-A-T**-t-T-a	0	This work
T4 Eσ^{gp55} (4)	—		**T-A-T-A-A-A-T-A**	1	27, 28
B. subtilis					
Eσ^{43} (9)	**T-T-G-A-C-A**	17–18	**T-A-T-A-A-T**	136	29
Eσ^{29} (4)	**T-T-N-A-A-A**	14–17	**C-A-T-A-T-T**	14	30, §
Eσ^{28} (2)	**C-T-A-A-A**	16	**C-C-G-A-T-A-T**	0	31
Eσ^{37} (4)¶	A-G-N-N-T-T	13–16	G-G-N-A-T-T-N-T-T		30, 32, 33
Eσ^{32} (2)¶	A-A-A-T-C	14, 15	T-A-N-T-G-N-T-T-N-T-A		30
SP01 Eσ^{gp28} (5)	T-N-A-G-G-A-G-A-N-N-A-N-T-T	12–13	T-T-T-N-T-T-T	5	24
SP01 E$\sigma^{gp33-34}$ (5)	C-G-T-T-A-G-A	17–19	G-A-T-A-T-T	12	30, §

References for conserved sequences are given. bp, Base pairs.

*The total number of sequences analyzed for each class of promoters is shown in parentheses.

†The most highly conserved bases are shown in boldface in the Eσ^{70} consensus. In the other −35 sequences, possible matches to the highly conserved bases of the Eσ^{70} −35 are shown in boldface. In the other −10 sequences, the positions that could correspond to T-A-N-N-N-T of the Eσ^{70} −10 are shown in boldface.

‡The number of times the boldface doublet in the −10 region of an alternate promoter appears in the Eσ^{70} data base of 168 promoters.

§R. Losick and J. Pero, personal communication.

¶We believe it premature to consider the *B. subtilis* Eσ^{37} and Eσ^{32} promoters in comparison of consensus sequences. The four identified Eσ^{37} promoters are quite variable in sequence. The two *B. subtilis* Eσ^{32} promoters overlap Eσ^{37} promoters, making it difficult to assign specific sequences required for Eσ^{32} promoter function. In addition, one of the two is a weak promoter (34).

−10 region, its −35 region sequence includes the most conserved bases of the $E\sigma^{70}$ −35 region, and the spacing differs from that of $E\sigma^{70}$ promoters.

We have also examined the consensus sequences and spacing of *B. subtilis* promoters recognized by alternate forms of holoenzyme (Table 3) and believe the data are consistent with our model as well as with the model proposed by Losick and Pero. In almost every case, the −10 region appears to lack the requirements for interaction with $E\sigma^{70}$. In the $E\sigma^{70}$ −10 region the most conserved bases are T-A-N-N-N-T (22). In promoters recognized by alternate forms of holoenzyme, T-A is not present in this position relative to any thymidine in the conserved sequence. The sequences found instead of T-A are present rarely in $E\sigma^{70}$ promoters and, with the exception of the G-A (present in *B. subtilis* $E\sigma^{gp33\text{-}34}$ promoters), they differ in their hydrogen bond donor–acceptor patterns (35). The −35 regions, on the other hand, appear to fall within the range of what could be recognized by $E\sigma^{70}$. The most conserved bases in the −35 region of $E\sigma^{70}$ promoters are T-T-G-A (22). Many of the *B. subtilis* promoter consensus sequences contain two or three of the four bases (Table 3). *B subtilis* $E\sigma^{28}$ promoters have only a 2/4 match to the T-T-G-A sequence, but $E\sigma^{28}$ also recognizes the *E. coli* $E\sigma^{32}$ promoter *rpoD* P_{HS} (36), which contains T-T-G-A, and so apparently does not depend on the −35 consensus sequences identified from the small existing data base. The variability found in consensus −35 sequences is explained, according to our model, by altered geometry of the holoenzyme causing slightly different regions, or additional regions, of core RNA polymerase to make −35 region contacts (resulting in different sequences recognized) or eliminating such contacts (as in the case of T4 $E\sigma^{gp55}$). The spacing between conserved regions of promoters recognized by alternate forms of holoenzyme differs from that of $E\sigma^{70}$ promoters, which is also consistent with our model.

Analysis of the promoters recognized by different RNA polymerase holoenzymes indicates that it is reasonable to consider that the σ subunit recognizes only the −10 region directly and indirectly affects recognition at the −35 region by altering the geometry of the holoenzyme–DNA complex. We wish to emphasize, however, that sequence comparisons cannot distinguish between the models proposed for promoter recognition and hope that the current interest in analyzing promoters recognized by different holoenzymes will lead to experiments able to resolve this issue.

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