Substitutions in Region 2.4 of $\sigma^{70}$ Allow Recognition of the $\sigma^S$-Dependent aidB Promoter

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The strict dependence of transcription from the aidB promoter (PaidB) on the $\sigma^S$ form of RNA polymerase is because of the presence of a C nucleotide as the first residue of the $-10$ promoter sequence ($-12C$), which does not allow an open complex formation by $\sigma^{70}$. In this report, $\sigma^{70}$ mutants carrying either the Q437H or the T440I single amino acid substitutions, which allow $-12C$ recognition by $\sigma^{70}$, were tested for their ability to carry out transcription from PaidB. The Gln-437 and Thr-440 residues are located in region 2.4 of $\sigma^{70}$ and correspond to Gln-152 and Glu-155 in $\sigma^S$. Interestingly, the Q437H mutant of $\sigma^{70}$, but not T440I, was able to promote an open complex formation and to initiate transcription at PaidB. In contrast to T440I, a T440E mutant was proficient in carrying out transcription from PaidB. No $\sigma^{70}$ mutant displayed significantly increased interaction with a PaidB mutant in which the $-12C$ was substituted by a T (PaidB$^{C\rightarrow T}$), which is also efficiently recognized by wild type $\sigma^{70}$. The effect of the T440E mutation suggests that the corresponding Glu-155 residue in $\sigma^S$ might be involved in $-12C$ recognition. However, substitution to alanine of the Glu-155 residue, as well as Glu-152, in the $\sigma^S$ protein did not significantly affect $\sigma^S$ interaction with PaidB. Our results reiterate the importance of the $-12C$ residue for $\sigma^S$-specific promoter recognition and strongly suggest that interaction with the $-10$ sequence and open complex formation are carried out by different determinants in the two $\sigma$ factors.

The sigma ($\sigma$) subunits are responsible for the sequence-specific binding, correct promoter recognition, and transcription initiation by bacterial RNA polymerase. Seven different $\sigma$ subunits have been identified in Escherichia coli; $\sigma^{70}$ is the main $\sigma$ subunit during active growth and $\sigma^{70}$-associated RNA polymerase ($\sigma^{70}$) carries out transcription from the majority of E. coli promoters. Alternative $\sigma$ subunits (e.g. $\sigma^H$, $\sigma^R$) direct transcription toward specific sets of genes, often in response to cellular stresses, by promoting RNA polymerase binding to promoter sequences that strongly diverge from $\sigma^{70}$ consensus (1–3). However, the alternative $\sigma^S$ subunit, the product of the rpoS gene and mainly expressed in stationary phase of growth, can recognize similar promoter sequences and thus initiate transcription from several $\sigma^{70}$-dependent promoters (4). Recognition of similar promoter sequences by $\sigma^{70}$ and $\sigma^S$ is reflected by their strong similarity in the DNA binding domains (5) and would be consistent with the need of the cell to continue expressing housekeeping genes in physiological conditions that simultaneously result in $\sigma^S$ accumulation and inhibition of $\sigma^{70}$-dependent transcription, such as accumulation of intracellular guanosine tetraphosphate concentrations because of amino acid starvation (6–8). In particular, the alignment of known $\sigma^S$-dependent promoters points to a high similarity of the $-10$ sequence with the canonical TATAAT sequence for $\sigma^{70}$ (9–12), whereas no apparent conservation of the $-35$ sequence is evident. Likewise, the search for an optimal promoter for $\sigma^S$ in vitro using the systematic evolution of ligands by exponential enrichment procedure has led to the proposal of CTATA(c/a)T as the $-10$ consensus sequence for $\sigma^S$ (13). This approach also resulted in the identification of TTGACA, i.e. the consensus $-35$ promoter element for $\sigma^{70}$, as the optimal $-35$ sequence for $\sigma^S$. These results suggest that promoter selectivity between $\sigma^{70}$ and $\sigma^S$ might be determined by different tolerance toward deviations from a common consensus sequence. In addition, factors independent of DNA sequence such as intracellular salt concentration, degree of DNA supercoiling, the guanosine tetraphosphate alarmone, and modulation by transacting regulatory proteins would help determine selective promoter recognition (14–18). A sequence feature important for $\sigma^S$-specific promoter recognition is a C nucleotide immediately upstream of the $-10$ promoter element (CTATA(c/a)T) and conventionally placed at the $-13$ position relative to the transcription start ($-13C$) (13, 19–22). Site-directed mutagenesis of the $\sigma^S$-dependent osmE and csiD promoters have confirmed the importance of the $-13C$ element for efficient $\sigma^S$-dependent transcription (11, 23). Although the $-13C$ nucleotide is an important determinant for $\sigma^S$-dependent transcription at the aidB promoter, (24) the major determinant for $\sigma^S$ selectivity is the presence of a C nucleotide as first nucleotide of the $-10$ hexamer ($-12C$); indeed, its substitution to a T, canonical for the $-10$ element $\sigma^{70}$-dependent promoters, results in loss of specific aidB promoter recognition by $\sigma^S$ (24). The presence of a C as the first nucleotide of the $-10$ sequence is also responsible for specific recognition by $\sigma^S$ at the osmE (23) as well at the csgB and sprE2 promoters (33). Thus, although $\sigma^{70}$ displays a strict requirement for a thymidine as the first nucleotide of the $-10$ sequence, $\sigma^S$ appears to be able to recognize either pyrimidine.

The amino acids contacting the $-10$ promoter element are located in region 2.4 of $\sigma^{70}$ (25–30), and the DNA-protein in-
teraction appears to involve numerous residues between the 425 and 451 positions (31). Suppression genetics studies have identified two mutations, which allow recognition by $\sigma^70$ of a C nucleotide at position 12 at mutant lac and ant promoters (25, 27). Both mutations are single amino acid substitutions at position 437 (a glutamine to a histidine, Q437H) and at position 440 (a threonine to an isoleucine, T440I). In region 2.4 of $\sigma^S$, the amino acids at the corresponding positions are Gln-152 and Glu-155. Interestingly, both these residues have recently been implicated in $\sigma^S$ interaction with the $\sigma^S$ element at the osmE and fic promoters (32).

In this work we have investigated the ability of mutant $\sigma^70$ proteins at residues Gln-437 and Thr-440 to recognize a C as first nucleotide of the −10 element in the context of the strictly $\sigma^S$-dependent aidB promoter. We show that both the Q437H and the T440E mutations allow efficient transcription initiation from the aidB promoter by $\sigma^70$ through improved recognition of the C nucleotide at position −12 (first nucleotide in the −10 promoter element). However, substitutions of the corresponding Gln-152 and Glu-155 amino acids in $\sigma^S$ did not affect aidB promoter recognition, thus suggesting that interaction with the −10 promoter element might be carried out by different determinants in either $\sigma^S$ or $\sigma^70$.

EXPERIMENTAL PROCEDURES

Expression Vectors—The plasmids carrying either the wild type aidB promoter (PaidB) or its PaidB(C12T) derivative were obtained from the pJCD01 vector as described previously (33). To generate 12-histidine-tagged mutants of $\sigma^70$, we performed site-directed mutagenesis of the RpoD allele in the plasmid pET-21 $\sigma^70$ (34) using the QuikChange® XL site-directed mutagenesis kit from Stratagene. For this purpose, we used the following couples of mutagenic primers, Q437H_F, 5'-CCTGAGTGGATCCGTCA-3' and Q437H_R, 5'-GAGCGATCACCCGCTC-3'; T440I_F, 5'-CCGTCAGGCGATCA-3' and T440I_R, 5'-CGCGATAGAGCGG-3'; T440E_F, 5'-CCGTCAGGCGATC-3' and T440E_R, 5'-CCGCGATAGAGCGG-3', to develop the $\sigma^70$(Q437H), $\sigma^70$(T440I), and $\sigma^70$(T440E) mutant proteins, respectively (substitutions are indicated in lowercase italic characters). The correct sequences of the so-obtained plasmids

1 The abbreviations used are: PaidB, aidB promoter; WT, wild type.
otheritol was omitted from the buffer for permanganate (KMnO₄) reaction. Reconstitution concentrations were 0, 7.5, 15, and 50 nM (from left to right), and the σ factors:core RNA polymerase ratio was 2:1. PaidB-RNA polymerase complexes were separated on 5% native polyacrylamide gel and quantitated using a PhosphorImager. B, dashed lines with closed squares (■) = Er₇₀WT; solid lines with open symbols: triangles (△), Er₇₀Q437H; circles (○), Er₇₀T440E; diamonds (◇), Er₇₀T440I.

were verified by sequencing using the primer int-RpoD 5'-GGTTGAA-GCGAACTTACGTCTGG-3'. The Q152A and E155A mutants were verified by sequencing using the primer int-RpoD 5'-AAAACGGTGTGAGTTT-GATAACACACTG-3'.
have shown that the presence of a cytosine (−12C) as the first nucleotide of the $\text{aidB} - 10$ promoter element (CATACT) is the main determinant for specific $\text{aidB}$ promoter recognition by $\sigma^70$-RNA polymerase (Eo70). Substitutions of the −12C to a thymine nucleotide results in a near-consensus −10 sequence for $\sigma^70$ (TATACT) and allows efficient promoter recognition by Eo70 (33). Mutations in $\sigma^70$ have been described that allow transcription initiation from mutant $\text{aidB}$ promoters in which the T at position −12 had been substituted to a C (Plac$_{\text{TT2C}}$, Paut$_{\text{TT2C}}$); these mutant promoters are no longer recognized by wild type $\sigma^70$-RNA polymerase (Eo70) (25, 27). We tested whether these mutations would also confer the ability to carry out transcription at the $\text{aidB}$ promoter, i.e. in a $\sigma^5$-dependent promoter context. In addition to the already characterized Q437H and T440I mutants, we constructed a T440E mutant $\sigma^70$ protein, in which the threonine at position 440 of $\sigma^70$ was substituted to a glutamate, i.e. to the corresponding amino acid residue in $\sigma^5$ (Glu-155). The different forms of RNA polymerase assembled with the three $\sigma^70$ mutants were tested in experiments of in vitro transcription on supercoiled templates and compared with wild type Eo70 and Eo5 (Fig. 1A). Both the Q437H and T440E mutants were more efficient (roughly 4-fold, Fig. 1B) than wild type $\sigma^70$ in carrying out transcription from wild type $\text{PaidB}$; in contrast, the T440I mutation did not improve $\text{aidB}$ transcription significantly (1.2-fold). Stimulation of transcription initiation by the Q437H and T440E mutations appears to specifically depend on increased recognition of the −12C. Indeed, transcription from the mutant $\text{aidB}$ promoter in which the −12C has been substituted to a T (PaidB$_{\text{C12T}}$) was carried out at roughly the same extent by wild type and mutant Eo70’s (Fig. 1). Although the C to T mutation at position −12 also stimulated Eo5$^{\sigma 70}$-dependent transcription, the Eo5$^{\sigma 70}$-Eo70 ratio in transcription initiation was reduced from 9-fold at $\text{PaidB}$ to 3-fold at PaidB$_{\text{C12T}}$ (Fig. 1B), consistent with previous observations (33).

**Gel Retardation and DNase I Protection Experiments**—Transcription initiation is a complex process that takes place in at least three distinct steps, binding of RNA polymerase to the promoter sequence, formation of the so-called “open complex,” and promoter escape. At the $\text{aidB}$ promoter, interaction with $\sigma^{70}$ is limited at the open complex formation step (24, 33). Thus, we investigated the ability of the Q437H, T440E, and T440I $\sigma^{70}$ mutants to form heparin-resistant complexes and to promote open complex formation at $\text{PaidB}$. Fig. 2 shows the results of gel retardation assays performed in the presence of heparin. Both the Eo70$^{\text{Q437H}}$ and the Eo70$^{\text{T440E}}$, forms of RNA...
polymerase were able to promote formation of heparin-resistant complexes with PaidB, in contrast to $\sigma^70_{(WT)}$ and $\sigma^70_{(T440E)}$, and consistent with the results of the in vitro transcription experiments (Fig. 1A). As expected, both wild type and mutant $\sigma^70$s were able to promote efficient heparin-resistant complex formation at the PaidB$_{(C12T)}$ promoter (Fig. 1B).

The heparin-resistant complexes were probed with DNase I to gather more detailed information on the specific RNA polymerase-promoter interactions. Consistent with the results of the previous assays, both the $\sigma^70_{(Q437H)}$ and the $\sigma^70_{(T440E)}$ forms of RNA polymerase, but not $\sigma^70_{(WT)}$ and $\sigma^70_{(T440E)}$, were able to protect the aidB promoter region from DNase I attack (Fig. 3A). The extent and the pattern of protection by $\sigma^70_{(Q437H)}$ and $\sigma^70_{(T440E)}$ were similar to $\sigma^70$; however, subtle differences in the pattern of DNase I-hypersensitive bands by $\sigma^70$ and $\sigma^70$ could be detected in the region around and immediately upstream from the −35 element. Binding by both forms of RNA polymerase results in the appearance of DNase I-hypersensitive bands in both the −35 to −39 and in the −46 to −50 regions of PaidB. However, although binding by $\sigma^70$ results in DNase I hypersensitivity mainly at −36 and −46 positions, binding by $\sigma^70$ results in even stronger hypersensitive bands at −38 and −48 (Fig. 3A, shown by arrows). An identical DNase I-hypersensitivity pattern in the −35 region is induced by both wild type and mutant $\sigma^70$s at the PaidB$_{(C12T)}$ promoter (Fig. 3B), suggesting that both the Q437H and the T440E mutations specifically result in increased interaction with the −10 promoter region, without affecting $\sigma^70$-promoter contacts in the −35 region.

K MnO4 Reactivity Experiments—To directly assess the effect of the $\sigma^70$ mutations on the open complex formation step of transcription initiation, we performed KMnO4 reactivity experiments on the wild type and mutant $\sigma^70$s. The results clearly showed that both the Q437H and T440E mutations, but not T440I, allow $\sigma^70$ to carry out promoter opening at PaidB (Fig. 4). The extension of the KMnO4-reactive region, which corresponds to the single-stranded DNA region induced by open complex formation by RNA polymerase, as well as its intensity (Fig. 4B) is very similar for both the $\sigma^70$ mutants and for $\sigma^70$. The KMnO4 reactivity induced by $\sigma^70$ mutants encompasses the +2 position, which defines an open complex fully competent for transcription initiation at PaidB (24, 33). As expected, both wild type and mutant $\sigma^70$s were fully capable of open complex formation at PaidB$_{(C12T)}$, consistent with the results of the in vitro transcription assays (Fig. 1).

Thus, our results showed that mutations in the Gln-437 and Thr-440 amino acid residues of $\sigma^70$ increase promoter recognition and open complex formation at PaidB, consistent with increased promoter recognition of Plac$_{(T12C)}$ and Pant$_{(T12C)}$ (25, 27). Although Gln-437 also corresponds to a glutamine residue in $\sigma^7$ (Gln-152), the position corresponding to Thr-440 in $\sigma^7$ is a glutamic acid (Glu-155). Thus, the observation that the T440E, but not the T440I, mutation allows PaidB recognition by $\sigma^70$ would suggest that Glu-155 is directly involved in the interaction with the −12C residue by $\sigma^7$. To address this possibility, we performed KMnO4 reactivity experiments with RNA polymerase assembled with a $\sigma^7$ mutant in which the glutamic acid at position 155 had been substituted to an alanine (E155A). In addition, a $\sigma^7$ Q152A mutant was also tested. As shown in Fig. 4, neither $\sigma^7$ mutant was affected in open complex formation at either PaidB or PaidB$_{(C12T)}$. To test whether either the E155A or the Q152A mutations could affect steps in transcription initiation other than open complex formation, we performed in vitro transcription experiments on supercoiled templates. The results clearly showed that both mutant $\sigma^7$ proteins could carry out transcription from the aidB promoter when assembled into RNA polymerase at least as efficiently as wild type $\sigma^7$ (Fig. 5).

**Competition Assays between $\sigma^7$ and Mutant $\sigma^7$**—In a previous report, we have shown that although the substitution of the −12C to a T nucleotide in PaidB restores an almost perfect −10 promoter element sequence for $\sigma^70$ and leads to a dramatic increase of $\sigma^7$ affinity for the aidB$_{(C12T)}$ promoter, $\sigma^70$ is outcompeted by $\sigma^7$ in direct competition assays because of higher affinity by $\sigma^70$ for $\sigma^7$-specific determinants in the aidB promoter outside the −10 sequence (i.e. −13C, displaced TG motif, possibly sequences in the −35 region) (33). Thus, the Q437H and T440E mutations specifically affect interaction with the −10 element of the aidB promoter, we would expect that RNA polymerase assembled with the mutant $\sigma^7$ would still be disfavored in competition assays with $\sigma^70$ for binding to PaidB. In contrast, if the mutations result in a more generalized increase of binding affinity to weak promoters, the mutant forms of $\sigma^70$ might be able to outcompete $\sigma^70$. Thus, we determined relative affinity of the mutant $\sigma^70_{(Q437H)}$ and the $\sigma^70$$_{(T440E)}$ forms of RNA polymerase, as well as of $\sigma^70_{(WT)}$ for wild type PaidB, measured as their ability to compete with $\sigma^70$ for the promoter. Competition experiments were performed as gel retardation assays in the presence of heparin using equal concentrations of $\sigma^7$, $\sigma^7$, and core RNA polymerase mixed together prior to the addition of the promoter DNA. As shown in Fig. 6, the $\sigma^7$ and $\sigma^7$-PaidB complexes run with different electrophoretic mobility in a gel retardation assay. At the concentrations used in our assays, no $\sigma^70$-PaidB complex was detectable (Fig. 6, lanes 2–4); in contrast, both $\sigma^70_{(Q437H)}$ and $\sigma^70_{(T440E)}$ were able to form a complex with PaidB at low RNA polymerase concentrations (Fig. 6, lanes 6 and 10). Apparent higher affinity for the aidB promoter by the $\sigma^70$ mutant forms at low concentrations of RNA polymerase is like to depend on the higher affinity of $\sigma^70$ for the core RNA polymerase (39), which would result in more efficient assembly of $\sigma^70$ than of $\sigma^7$. However, at $\sigma^7$ concentrations allowing efficient binding of core RNA polymerase, the $\sigma^7$ form of RNA polymerase is clearly favored over both $\sigma^70$ mutants (Fig. 6, lanes 8 and 12).

**DISCUSSION**

In this report, we have investigated the interaction between the $\sigma^7$-dependent PaidB and three $\sigma^70$ mutants in region 2.4,
i.e. the \( \sigma^{70} \) protein domain involved in sequence-specific interaction with the \(-10\) promoter element. Two of these mutants, namely \( \sigma^{70}(Q437H) \) and \( \sigma^{70}(T440I) \), had already been described as able to restore transcription initiation from mutants of the \( \mathit{lac} \) and \( \mathit{ant} \) \( \sigma^{70} \)-dependent promoters in which the first nucleotide of the \(-10\) promoter element had been changed from a \( T \) (part of the TATAAT consensus sequence for \( \sigma^{70} \)) to a \( C \) (Plac\( \mathit{ Tic2C} \), and Pant\( \mathit{ Tic2C} \)). The \( T \) to \( C \) substitution at \(-12\) completely abolishes transcription initiation by wild type \( \sigma^{70}(25, 27, 29) \). At the \( \mathit{aidB} \) promoter, selective promoter recognition by \( \sigma^{70} \) depends on the presence of a \( C \) at the \(-12\) position, i.e. as first nucleotide of the \(-10\) promoter element (CATACT), which does not allow open complex formation by \( \sigma^{70} \); substitution of the \(-12C\) to \( T \) results in increased promoter recognition by \( \sigma^{70} \) and loss of \( \sigma^{70} \)-specificity (33). We show that \( \sigma^{70}(Q437H) \), but not \( \sigma^{70}(T440I) \), was able to efficiently initiate transcription at \( \mathit{PaidB} \) (Fig. 1). The Q437H substitution led to an almost 4-fold stimulation in transcription initiation in \( \mathit{in vitro} \), compared with a 1.2-fold stimulation by T440I (Fig. 1). The lack of increase in transcription initiation by the T440I mutant at the \( \mathit{aidB} \) promoter might depend upon its intrinsic lower affinity for promoters carrying at \(-12C\) in comparison to \( \sigma^{70}(Q437H) \); indeed, although the experiments were performed in different genetic systems, the Q437H mutation appears to induce a more drastic stimulation of transcription from both Plac\( \mathit{ Tic2C} \) and Pant\( \mathit{ Tic2C} \), mutant promoters (25, 27). However, substitution of threonine at position 440 to glutamic acid resulted in a clear increase in transcription from \( \mathit{PaidB} \), similar to the effect of the Q437H mutation (Fig. 1). Thus, we could confirm the observations by Waldburger \( \text{et al.} \) (27) and Siegele \( \text{et al.} \) (25) that both Gln-437 and Thr-440 residues of \( \sigma^{70} \) have an inhibitory effect on the recognition of a \( C \) residue as the first nucleotide of the \(-10\) promoter element. The mutants do not appear to be affected in recognition of \( \mathit{PaidB}_{\mathit{C12T}} \), in which the \( C \) at position \(-12\) has been substituted to a \( T \), suggesting that the Gln-437 and Thr-440 residues do not play an essential role in the interaction with the \(-10\) element, consistent with the results of extensive mutagenic analysis of \( \sigma^{70} \) region 2.4 (31). Interestingly, a mutagenic analysis of regions 2.4 and 3.0 has shown that substitution of the glutamine at position 437 to an alanine (Q437A) improves the recognition of mutants in the synthetic extended \(-10\) KAB-TG promoter in which either nucleotide of the TG motif had been changed to a \( C \) (37). Thus, it appears that a possible function of the Gln-437 residue could be to modulate \( \sigma^{70} \) interaction with the \(-10\) promoter region. At the \( \mathit{aidB} \) promoter, both the Q437H and the T440E mutations result in increased formation of a heparin-resistant RNA polymerase-promoter DNA complex, as determined by gel retardation assays (Fig. 2), and in increased open complex formation, as determined with KMnO\(_4\) reactivity assays (Fig. 4). The mutations appear to specifically increase RNA polymerase interaction with the \(-10\) region, without affecting binding to the \(-35\) sequence and to the upstream promoter elements (Fig. 3). Despite their increased ability in transcription initiation and in open complex formation, both \( \sigma^{70}(Q437H) \) and \( \sigma^{70}(T440E) \) show lower relative binding affinity to \( \mathit{PaidB} \) than \( \sigma^{70} \), as determined by direct competition experiments in gel retardation assays (Fig. 6), probably because of the presence of
&specific binding determinants (e.g. –13C, displaced TG motif (33)) that are not recognized by the Eσ70 mutants. Our results confirm the importance of a C nucleotide at the –12 position, i.e. as first nucleotide of the –10 promoter element, for the specific promoter recognition by σ8, at least at a subset of σ8-dependent promoters (roughly 20% of total (33)); both its substitution to a T (Figs. 1–4 (33)) and mutations in σ70 leading to increased recognition of –12C result in a significant loss of specific promoter recognition by Eσ8. Thus, σ8 would appear to be “color blind”, i.e. would not be able to distinguish between C and T at position –12, in contrast to σ70.

The observation that the T440E, but not the T440I, mutation leads to increased transcription initiation and open complex formation at PaidB would suggest that the glutamic acid residue could play a direct role in an interaction with the –12C nucleotide. Indeed, the residue corresponding to Thr-440 in σ8, which is able to recognize a C as first element of the –10 promoter, is a glutamic acid (Glu-155). In contrast, Gln-437 is also conserved in σ8 (Gln-152), despite its apparent inhibitory effect on –12C recognition. However, substitution of the glutamic acid at position 155 of σ8 to an alanine does not affect either transcription initiation or open complex formation by Eσ8 at PaidB (Figs. 4 and 5), strongly suggesting that the Glu-155 residue is not involved in interaction with –12C. Thus, it appears that interaction with the same promoter element might be carried out by different determinants in σ8 and in σ70.

Indeed, a recent report (32) proposes that at the osmE promoter both Gln-152 and Glu-155 residues are involved in the recognition of the –13C, an additional σ8-specific promoter feature present at roughly 70% σ8-dependent promoters (11, 12, 33), rather than the first nucleotide of the –10 promoter element.

At the aidB promoter, the –13C promoter element is necessary for optimal transcription in vivo and contributes to open complex formation by Eσ8 (24, 33). However, neither the Q152A nor the E155A mutations affect transcription initiation at either PaidB or PaidB12C (Figs. 4 and 5) thus suggesting that neither the Glu-152 nor Glu-155 residues play a major role in recognition of the –13C element at these promoters. This observation is in contrast with the role of the Glu-152 and Glu-155 residues in –13C recognition at the osmE (32). Thus, it is possible that different promoter elements and amino acid determinants in σ8 might play different roles at distinct subsets of σ8-dependent promoters. For instance, the –35 promoter element appears to be dispensable at most σ8-dependent promoters, but it is essential at osmE (23, 32). At a subset of σ8-dependent promoters responding to osmotic shock, the so-called GCGG motif in the –35 area acts as an additional promoter determinant for Eσ8 (38). Thus, it could be possible that the promoter context defines the strength of interactions with the different promoter elements (–10 sequence, –13C, TG motif, –35 sequence, GCGG motif, UP element, etc.) and, consequently, the importance of the distinct determinants in the σ8 protein for promoter recognition and transcription initiation.

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