Location of essential sequence elements at the *Escherichia coli melAB* promoter

Jennifer KEEN, Jacqueline WILLIAMS* and Stephen BUSBY†

School of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

The *Escherichia coli melAB* promoter has been cloned on a short DNA fragment and subjected to deletion mutagenesis, random mutagenesis and site-directed mutagenesis. In previous work we had shown that expression from the *melAB* promoter is triggered by melibiose and that this requires the MelR transcription activator. Melibiose-dependent expression is suppressed by deletions that remove both DNA-binding sites for MelR and by point mutations in the –10 hexamer, the –35 hexamer and the

region just upstream of the –35 hexamer. The point mutations identify promoter elements that are essential for triggering the *melAB* promoter. The importance of these elements was confirmed by site-directed mutagenesis. The results show that the organization of the *melAB* promoter is fundamentally different from the organization of other bacterial promoters controlled by homologues of MelR.

INTRODUCTION

The activity of most *Escherichia coli* promoters is dependent on two hexamer sequence elements, the –10 and –35 hexamers located around 10 and 35 bp respectively upstream from the transcript start point, that are recognized by the RNA polymerase σ subunit during transcription initiation [1]. At promoters dependent on a transcription activator, one of these elements is usually defective, and the promoter contains a binding site for the activator, in most cases located upstream of the –35 hexamer [2]. In many cases, activators function by interacting directly with contact sites in either the σ or α subunits of RNA polymerase, thereby recruiting the polymerase to the promoter [3].

In previous work, we investigated the *E. coli melAB* promoter (*pmelAB*) [4–6]. This promoter controls expression of the *melAB* operon which encodes proteins essential for melibiose metabolism. Transcription initiation at *pmelAB* is completely dependent on MelR, a transcription activator that is triggered by melibiose. Thus expression of the *E. coli melAB* operon is coupled to the availability of melibiose in the bacterial growth medium. Scrutiny of the base sequence of *pmelAB* reveals a plausible –10 hexamer, 5' CATGAT 3', but there is no hexamer in the –35 region that resembles the consensus. Upstream, *pmelAB* contains two identical 18 bp elements that are organized as an inverted repeat from positions –109 to –92 and from positions –54 to –71. Our previous footprinting and mutational studies have shown that these are the binding sites for MelR [6–8]. In the work described here, we have mutagenized *pmelAB* and selected for mutations that suppress melibiose-dependent induction. The locations of single base pair changes that interfere with induction confirm the importance of the –10 hexamer and show that the base sequence in and just upstream of the –35 region plays an important role. The organization of *pmelAB* is compared with that of other promoters that are dependent on activators related to MelR.

EXPERIMENTAL

Strains, plasmids, promoter constructions and mutagenesis

The bacterial strains, plasmids and DNA fragments used in this work are listed in Table 1. For most experiments, we used M182

carrying plasmid KK3/2501 as the host strain. These cells were grown in media containing 35 μ g/ml tetracycline to maintain the plasmid. KK3/2501 was constructed by cloning the KK3 *Hind*III fragment carrying the *E. coli melR* gene [4] into broad-host-range plasmid pRK2501 [12].

Standard methods for manipulating recombinant DNA, performing site-directed mutagenesis (using an Amersham Kit; cat. no. RPN1523) and PCR (using a Techne thermal cycler) were used throughout. The KK43 *Eco*RI–*Hind*III fragment carrying *pmelAB* was obtained from our previous work [8]. This fragment carries a unique *Bgl*II site just upstream of MelR-binding site 2 [8]. To facilitate further manipulation of *pmelAB*, we made a derivative, KK63, carrying a *Bam*HI site immediately downstream of MelR-binding site 2. The KK63 fragment was obtained by site-directed mutagenesis of the KK43 fragment. The function of *pmelAB* and melibiose induction is unaffected by the *Bam*HI site (J. Williams, unpublished work). The KK67 fragment was derived from the KK63 fragment by PCR that relocated the downstream *Hind*III site to position +8 with respect to the transcript start site.

Plasmid pAA224 was derived from pAA204 [11] by replacing the *Eco*RI–*Hind*III fragment carrying the *gal* promoter with the *Eco*RI–*Hind*III polylinker from plasmid pUC18 (Pharmacia). The KK67 fragment was cloned in pAA224 to give a plasmid encoding a *pmelAB*–*lacZ* fusion. This recombinant was used as the starting point for both random and site-directed mutagenesis of *pmelAB*. Random mutagenesis was performed by PCR synthesis of the KK67 insert cloned in pAA224, using the flanking oligonucleotides 5' ACCTGACGTCTAAGAAACC 3' and 5' CCATTAAGCTTCTTGAATAACTTC 3' as primers, using the strategy and techniques described by Zhou and co-workers [13]. The PCR product was restricted with *Eco*RI and *Hind*III and the product was purified and recloned into pAA224. Recombinants were screened for the Lac phenotype on MacConkey Lactose indicator plates containing 80 μ g/ml ampicillin and 35 μ g/ml tetracycline using M182 carrying KK3/2501 as a tester strain. Colonies displaying a Lac[–] phenotype due to mutations in the KK67 *pmelAB* fragment were retained and the KK67 insert was sequenced. Candidates carrying single base substitutions were

Abbreviation used: *pmelAB*, *melAB* promoter.

* Present address: Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France.

† To whom correspondence should be addressed.

Table 1 Bacterial strains, plasmids and promoter fragments used in the present study

Name	Brief description	Origin and references
Bacterial strains		
M182(KK3/2501)	Δ <i>lac mel</i> <i>E. coli</i> K12 strain containing broad-host-range plasmid encoding MelR	[9]; present work
WAM105	<i>E. coli</i> K12 strain carrying <i>rpoA341</i> (<i>phs</i>) mutation	[10]
WAM106	<i>E. coli</i> K12 strain carrying <i>rpoA</i> ⁺ (parent to WAM105)	[10]
Bacterial plasmids		
pAA224	pBR322-derived vector for cloning <i>EcoRI</i> – <i>HindIII</i> promoter fragments; encodes resistance to 80 μ g/ml ampicillin	[11]; present work
KK67/224	KK67 <i>EcoRI</i> – <i>HindIII</i> fragment carrying <i>pmelAB</i> cloned in pAA224	Present work
KK3/2501	Fragment encoding MelR cloned into broad-host-range plasmid pRK2501; encodes resistance to 35 μ g/ml tetracycline	Present work
Promoter fragments (all <i>EcoRI</i>–<i>HindIII</i> fragments)		
KK43	Fragment carrying <i>pmelAB</i> sequences from –136 to +21 with an <i>EcoRI</i> linker upstream and a <i>HindIII</i> linker downstream with a unique <i>BglII</i> site between MelR-binding sites 1 and 2 (located from positions –73 to –68)	[8]
KK63	Derivative of KK43 carrying five base changes that create a unique <i>BamHI</i> site downstream of MelR-binding site 2 (located from positions –52 to –47)	Present work
KK67	Derivative of KK63 with downstream <i>HindIII</i> linker located at +8; lacks the <i>melA</i> translation initiation region	Present work

retained for further study, whereas a small number of candidates carrying more than one substitution were discarded. Site-directed mutagenesis of the –35 region of the KK67 insert cloned in pAA224 was performed by PCR using primer oligonucleotides carrying different substitutions. A second set of site-directed KK67 mutant derivatives was generated by moving the upstream *EcoRI* linker to different positions closer to the transcript start site. The entire base sequence of the KK67 fragment was checked for each mutant using the flanking primer 5' ACCTGACGTC-TAAGAAACC 3' and double-stranded sequencing reactions. By convention, promoter sequences are numbered with the *pmelAB* start site taken as +1, with upstream and downstream positions given negative and positive co-ordinates respectively. Point mutations in *pmelAB* are denoted by the letter p followed by the location of the mutation and the nature of the new base on the upper strand. Deletions are denoted by Δ followed by the location of the upstream limit of the *pmelAB* sequence.

Assays of promoter activity *in vivo*

To assay the activities of different promoters, pAA224 derivatives carrying the KK67 *EcoRI*–*HindIII* fragment with wild-type and mutant *pmelAB* sequences were transformed into M182 cells carrying KK3/2501, encoding the transcription activator MelR. Transformants were grown in minimal medium either with or without added melibiose, exactly as previously described [14], and β -galactosidase expression was measured using the Miller method [15] as in our previous work [7,8,14]. β -Galactosidase activities are taken to be proportional to the activity of the cloned promoter, and so the melibiose-dependent triggering of *pmelAB* can be quantified.

RESULTS AND DISCUSSION

Construction and use of a new *pmelAB*–*lacZ* fusion

In our previous work we constructed a number of plasmid-borne *pmelAB*–*lacZ* fusions that were used to study the regulation of *pmelAB* [4,7,8,14]. We showed that expression from *pmelAB* is dependent on MelR and is triggered by the presence of melibiose in the growth medium. Our aim in the present work was to create random substitutions in *pmelAB* and select those that resulted in reduced *pmelAB* expression, in order to identify the essential

sequence elements. To do this we needed a simple system in which the *pmelAB*–*lacZ* fusion conferred a clear MelR-dependent Lac⁺ phenotype on host cells. Because none of our previously characterized *pmelAB*–*lacZ* fusions gave a sufficiently clear phenotype, we developed a new fusion. In our previous studies we worked with *pmelAB* on an *EcoRI*–*HindIII* fragment carrying sequences from –136 to +21, which includes the *melA* translation initiation signal. The starting point of the present work was the removal of this signal to give the KK67 fragment carrying *pmelAB* sequences from –136 to +7 (Figure 1 and Table 1). We reasoned that we could produce a strong MelR-dependent Lac⁺ phenotype if we cloned this fragment immediately upstream of the *lacZ* gene that carried a strong translation start signal. To do this we exploited the plasmid pAA224, which carries the *lacZ* gene fused to the strong *galE* translation start and translation initiation signal (Figure 2 and Table 1). In our previous work we had introduced *EcoRI* and *HindIII* sites immediately upstream of the translation initiation signal that controls expression of the *galE*–*lacZ* fusion [11]. By cloning the KK67 *EcoRI*–*HindIII* *pmelAB* fragment into pAA224, we created a plasmid (KK67/224) in which the *lacZ* gene carrying a strong translation start signal was located immediately downstream of the *melAB* transcription start site. M182 Δ *lac* cells carrying plasmid KK3/2501 as a source of *melR* appear strongly Lac⁺ on Macconkey indicator plates when transformed with KK67/224. Preliminary studies showed that the Lac⁺ phenotype is totally dependent on *pmelAB* and the *melR* gene supplied by KK3/2501. Subsequent assays showed that this expression was induced by melibiose (see below).

Deletion analysis of *pmelAB*

We made a series of derivatives of the KK67 *pmelAB* fragment in which the upstream *EcoRI* linker was moved from position –136 to positions –125 (Δ 125), –94 (Δ 094), –72 (Δ 072) and –51 (Δ 051), thereby removing increasing amounts of base sequence upstream of the *pmelAB* transcript start site (Figure 1). The data in Table 2 show that β -galactosidase expression in M182 cells carrying plasmids KK67/224 (encoding the *pmelAB*–*lacZ* fusion) and KK3/2501 (encoding *melR*) was induced by melibiose. Melibiose-dependent induction was completely suppressed by the Δ 051 deletion, which removes both MelR-binding sites 1 and 2, but only partially suppressed by the Δ 072

Figure 1 Base sequence of the KK67 *EcoRI*–*HindIII* fragment carrying *pmelAB*

The base sequence of the upper strand of the KK67 fragment carrying *pmelAB* is shown. The promoter sequence from -136 to $+7$ is enclosed by square brackets and the *EcoRI* and *HindIII* linker sequences are shown outside the brackets. The *pmelAB* transcript start site at $+1$ is indicated by the broken arrow, and the -10 and -35 hexamers are boxed. The MelR-binding sites 1 and 2 are indicated by horizontal arrows. Vertical or curved arrows indicate the positions of six single base substitutions discussed in the text. Vertical lines indicate the positions of the upstream ends of the *pmelAB* sequence in the $\Delta 125$, $\Delta 094$, $\Delta 072$ and $\Delta 051$ constructions that move the *EcoRI* linker to these locations.

Figure 2 The *lac* expression vector pAA224

The figure (not to scale) illustrates the expression vector used in this work, showing the location of the *bla* gene encoding resistance to ampicillin, the pBR322 replication origin *ori*, and the *lacZ*, *lacY* and *lacA* genes. The *lacZ* gene is fused to the N-terminal region of *galE* carrying the *galE* translation initiation region (TIR). The locations of the unique *EcoRI* and *HindIII* sites are indicated. In pAA224 these sites are separated by a polylinker, but this can be replaced by any *EcoRI*–*HindIII* fragment carrying a promoter. The upper part of the figure shows the base sequence just downstream of the *HindIII* site carrying the *galE* TIR with the Shine–Dalgarno sequence (GGAG) underlined, and the *galE* N-terminal coding region, starting at the initiator ATG, fused to the eighth codon of *lacZ*.

and $\Delta 094$ deletions, which remove only the upstream site 1. Surprisingly the $\Delta 125$ deletion, which removes sequence upstream of site 1, led to a small but definite reduction in expression. These results show that *pmelAB* sequences downstream of -51 have little or no activity, that MelR binding to the downstream site 2 is sufficient for some (but not for optimal) melibiose-triggered expression, and that sequences upstream of site 1 contribute to *pmelAB* activity.

Random mutagenesis of *pmelAB*

To identify sequence elements that are essential for *pmelAB* activity, we created a library of random point mutations in the KK67 *EcoRI*–*HindIII* fragment by synthesizing the fragment using PCR in conditions designed to create, on average, one base change per molecule. A total of 40 independent PCR reactions were performed, and the product from each reaction was

Table 2 Activity of *pmelAB* in different KK67 derivatives

Shown are β -galactosidase levels in M182[KK3/2501] cells containing different *pmelAB* derivatives (shown in Figure 1) cloned in pAA224. Cells were grown aerobically in minimal medium containing fructose as a carbon source, containing 35 μ g/ml tetracycline and 80 μ g/ml ampicillin, and either with or without 0.05% (w/v) melibiose. Cells were harvested during exponential growth and β -galactosidase levels were assayed. Levels are expressed in standard Miller units [15]. Each assay was performed independently three or more times; the values shown are the means of these determinations, which varied by less than 10%.

		β -Galactosidase level (units)		
Promoter		— Melibiose	+ Melibiose	Induction factor (fold)
Deletions of upstream sequences				
	KK67	73	1135	15.5
	KK67 Δ 125	108	926	8.6
	KK67 Δ 094	163	720	4.4
	KK67 Δ 072	170	770	4.5
	KK67 Δ 051	153	135	0.9
	No promoter (pAA224 alone)	120	110	0.9
Single base changes				
	KK67 p11G	48	66	1.4
	KK67 p35G	53	197	3.7
	KK67 p36A	49	99	2.0
	KK67 p37A	51	177	3.5
	KK67 p41G	62	564	9.1
	KK67 p69T	60	310	5.2

restricted with *EcoRI* and *HindIII* and cloned into pAA224. The resulting recombinants were transformed into M182[KK3/2501] cells and the transformants were plated on MacConkey Lactose indicator plates. After screening 20000 colonies (from the 40 transformations), we picked six colonies that displayed a Lac[–] phenotype due to a single mutation in the KK67 *EcoRI*–*HindIII* fragment. As a control for the mutagenesis we also picked a number of Lac⁺ colonies and sequenced the KK67 insert to show that these inserts contained mutations (that had no apparent effect on *pmelAB* activity according to the indicator plates). Figure 1 shows the locations of the six single base pair substitutions in the KK67 *pmelAB* fragment that resulted in reduced expression. The mutations are p11G, p35G, p36A, p37A, p41G and p69T; the effects of these substitutions on the melibiose-dependent induction of *pmelAB* are listed in Table 2. One of the substitutions is located in MelR-binding site 2, one is in the –10 hexamer and one is in the –35 hexamer. Surprisingly, the other three substitutions fell in the region just upstream of the –35 hexamer.

Site-directed mutagenesis of *pmelAB*

The –35 hexamer of *pmelAB*, 5'AGGTTT 3', accords with the consensus sequence 5'TTGACA 3' at only one out of six positions. Equally, other neighbouring hexamers bear little relation to the consensus. However, a single base pair substitution at the first position of the hexamer (p35G) and other substitutions just upstream lead to sharp reductions in *pmelAB* activity, suggesting that this region does contain an important promoter element. To check this conclusion we used site-directed mutagenesis to make a series of mutant derivatives of KK67 (M1–M7; Figure 3) carrying multiple substitutions in different 'windows' across the –35 region. The effects of the different substitutions were quantified and the data are presented in Table 3. Substitutions of both the upstream and downstream halves of the –35 hexamer suppressed the melibiose-dependent triggering of *pmelAB* (constructs M4 and M5). Similarly, substitutions in the 4 bp window from positions –39 to –42 (construct M1) and

the 3 bp window from positions –36 to –38 (construct M2) also reduced expression, showing that both the –35 hexamer and the region immediately upstream of it are essential. As controls, we replaced all six base pairs of the *pmelAB* –35 hexamer with unrelated sequence (construct M6) or with the –35 consensus hexamer (construct M7). The data in Table 3 show that the *pmelAB* M6 derivative was inactive whereas the M7 derivative gave higher levels of melibiose-independent expression.

Effect of the *rpoA341* mutation on *pmelAB* activity

A previous report [10] has shown that the *phs* mutant, which creates a single amino acid substitution in the C-terminus of the RNA polymerase α subunit, results in cells displaying a Mel[–] phenotype, and has suggested that the C-terminal domain of the α subunit may be essential for transcription activation by MelR. To confirm this, we measured melibiose-dependent expression from *pmelAB* in the *phs* strain WAM105 and in the parent strain WAM106. The data in Table 4 show that the *phs* mutation caused a reduction in melibiose-dependent transcription activation at *pmelAB*. Control experiments with the KK67 M7 mutant (Figure 3) showed that the *phs* mutation had no effect on melibiose-independent expression from this derivative carrying a consensus –35 hexamer element.

Conclusions

Expression from *pmelAB* is completely dependent on the MelR protein, which binds to two 18 bp upstream elements organized as an inverted repeat. The deletion analysis reported here shows that *melAB* promoter sequences downstream of these two elements have little or no activity in isolation. In the present study we used random mutagenesis to identify DNA sequence elements that are essential for *pmelAB* function. At first sight, it is surprising that more single mutations were not isolated in MelR binding sites 1 and 2. However, in our previous studies [7,8], we found that *pmelAB* activity was unaffected by most single-site substitutions in site 1; presumably this is because

Figure 3 Site-directed mutations in the -35 region of *pmelAB*

The figure shows the base sequence of the upper strand of *pmelAB* around the -35 hexamer (boxed). The multiple base substitutions in seven mutants, M1–M7, discussed in the text are indicated by vertical arrows enclosed with braces.

recognition is due to multiple MelR–DNA interactions spanning 18 bp, and single changes fail to produce sufficiently strong effects.

One of the single base substitutions that reduces *pmelAB* activity (p11G) falls in the -10 hexamer 5' CATGAT 3', previously identified as being similar to the consensus -10 hexamer sequence 5' TATAAT 3' (Figure 1). Another of the substitutions (p35G) falls at the first base of the -35 hexamer 5' AGGTTT 3'. This hexamer bears little relation to the consensus, 5' TTGACA 3', which is not surprising since *pmelAB* is dependent on an activator. Two previous studies of -35 hexamer sequences concluded that an A or a G at position 1 is equally deleterious for promoter function [16,17]. The strong effect of the p35G substitution must mean that the base sequence in the *pmelAB* -35 region plays an important role. This suggests that the way in which RNA polymerase recognizes the *pmelAB* -35 region is distinct from the way that consensus promoters are recognized. This conclusion is supported by our observation that single base changes in the region immediately upstream of the -35 hexamer result in substantial decreases in promoter activity. At most *E. coli* promoters, such 'down' mutations are restricted to the -10 and -35 hexamers and activator-binding sites [18]. In the case of *pmelAB*, the region immediately upstream of the -35 hexamer is clearly crucial.

Consideration of the organization of other bacterial promoters activated by transcription factors related to MelR also suggests that *pmelAB* is an atypical promoter. MelR belongs to a family of related activators and, to date, more than 27 members of this family have been identified [19]. Members of this family all share a common modular structure, all are activated by ligands and all bind to ~ 18 bp target sites that are organized as either inverted

Table 3 Site-directed mutants in the -35 region of *pmelAB*

Shown are β -galactosidase levels in M182[KK3/2501] cells containing different *pmelAB* derivatives (shown in Figure 3) cloned in pAA224. Cells were grown aerobically in minimal medium containing fructose as a carbon source, containing 35 μ g/ml tetracycline and 80 μ g/ml ampicillin, and either with or without 0.05% (w/v) melibiose. Cells were harvested during exponential growth and β -galactosidase levels were assayed. Levels are expressed in standard Miller units [15]. Each assay was performed independently three or more times; the values shown are the means of these determinations, which varied by less than 10%.

Promoter	β -Galactosidase level (units)		Induction factor (fold)
	– Melibiose	+ Melibiose	
KK67 (wild type)	73	1135	15.5
KK67 M1 (p39T + 40T + 41G + 42T)	59	112	1.9
KK67 M2 (p36A + 37A + 38A)	47	84	1.8
KK67 M3 (p37C)	38	68	1.8
KK67 M4 (p33A + 34T + 35G)	100	146	1.5
KK67 M5 (p30A + 31G + 32G)	90	107	1.2
KK67 M6 (p30A + 31G + 32G + 33A + 34T + 35G)	45	50	1.1
KK67 M7 (p30A + 31C + 32A + 33G + 34T + 35T)	11740	9600	0.8

Table 4 Induction of *pmelAB* in a *phs* background

WAM105 or WAM106 cells containing different *pmelAB* derivatives cloned in pAA224 were grown aerobically in minimal medium containing fructose as a carbon source and 80 μ g/ml ampicillin, either with or without 0.05% (w/v) melibiose. Cells were harvested during exponential growth and β -galactosidase levels were assayed. Values shown are the induction factors due to melibiose, derived by calculating the ratio of activities in cultures grown with and without melibiose. Each assay was performed independently three or more times and the values shown are the means of these determinations. Note that the induction factor in WAM106 cells was lower than that in the M182[KK3/2501] host. Expression of *melR* is unaffected in the *phs* mutant strain (J. Keen, unpublished work).

Promoter	Induction factor (fold)	
	WAM106 (<i>rpoA</i> ⁺)	WAM105 [<i>rpoA341</i> (<i>phs</i>)]
KK67 (wild type)	13.0	3.4
KK67 M7 (p30A + 31C + 32A + 33G + 34T + 35T)	0.8	0.9
No promoter (pAA224 alone)	1.0	0.9

Figure 4 Organization of binding sites for AraC-class activators at target promoters

The figure shows sketches illustrating the location of binding sites for MelR at *pmelAB* [4], AraC at *paraBAD* (and *paraE* and *paraJ* [20,24]), XylS at *Pm* [25], RhaR at *prhaSR* [26] and RhaS at *prhaBAD* (and *prhaT*) [27,28]. At each promoter the transcript start site at +1 and the -10 and -35 hexamers are identified. The locations of activator binding sites are indicated by the horizontal arrows that show the polarity of the sites.

or direct repeats [19]. Figure 4 illustrates the organization of binding sites for such activators in the five cases where the organization of target promoters has been studied. In the cases of AraC binding to *paraBAD* (and *paraE* and *paraJ*), XylS binding to *Pm*, RhaR binding to *prhaSR* and RhaS binding to *prhaBAD* (and *prhaT*), the downstream activator sites overlap with the -35 hexamer. In the case of *paraBAD* it has been shown that this overlap is essential for transcription activation, most likely because AraC (and presumably XylS, RhaR and RhaS at their targets) makes direct contact with the RNA polymerase σ subunit that is docking to the -35 hexamer [20].

Figure 4 shows that the situation is radically different for MelR binding at *pmelAB*; in this case, MelR binds clearly upstream of the -35 hexamer, with an 18 bp gap between the downstream MelR-binding site and position -35. We can suggest four possible simple explanations to account for this, and for the importance of base sequences upstream of the -35 hexamer at *pmelAB*. The first possibility is that the 18 bp of sequence upstream of the -35 hexamer comprises a third MelR-binding site and that MelR bound to this third site contacts the RNA polymerase σ subunit. Although this is a formal possibility, there is no direct evidence for such binding, the base sequence of this region bears no resemblance to a MelR-binding site, and cloning of MelR-binding sequences into this region results in inactivation rather than activation of *pmelAB* (J. Williams, unpublished work; [21]). The second possibility is that a second transcription factor binds between MelR and RNA polymerase, forming a 'bridge'. Again, there is no direct evidence for this, and genetic analysis of *E. coli* Mel⁻ mutants mapped all the mutations to the *melR-melAB* locus [22]. However, the binding of such a factor might explain the unexpected effects of the p41G, p37A and p36A substitutions, and the sequence element 5' GGAAGNNGGAGG 3' from positions -44 to -33 would be a plausible target. The third possibility is that no protein binds between -36 and -54 at *pmelAB* but that the promoter DNA adopts a bent or distorted conformation such that bound MelR can contact the RNA polymerase σ subunit docking at the -35

hexamer. The final possibility is that MelR activates transcription by contacting the C-terminal domain of the RNA polymerase α subunit and that the sequence from -36 to -54 acts as a docking site for this domain. Support for this comes from the observation that many upstream-bound transcription activators act by recruiting the RNA polymerase α subunit C-terminal domain to promoter DNA [3,23]. In addition, the *phs* mutation, which causes a lysine for glutamic acid substitution at position 271 in the α subunit, interferes with *pmelAB* activity. However, there is no direct evidence for this and, to our knowledge, the *pmelAB* sequence from -36 to -54 does not resemble any known α -subunit-binding element. Thus the organization of the *melAB* promoter poses a problem that we cannot resolve. We have established that the region upstream of the -35 hexamer plays a crucial role, but we cannot say why this is. Our current studies are directed towards finding and identifying any supplementary factor essential for *pmelAB* activation, and setting up an *in vitro* system to study transcription initiation at *pmelAB*.

This work was funded by the U.K. BBSRC with a project grant and a research studentship. We are grateful to Carmen Michan and Eva Hyde for stimulating discussions, to Christine Webster for technical assistance, and to Mark Thomas for providing the *E. coli phs* strain.

REFERENCES

- Gross, C., Lonetto, M. and Losick, R. (1992) in *Transcription Regulation* (McKnight, S. and Yamamoto, K., eds.), vol. 1, pp. 129–178, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Raibaud, O. and Schwartz, M. (1984) *Annu. Rev. Genet.* **18**, 173–206.
- Busby, S. and Ebright, R. (1994) *Cell* **79**, 743–746.
- Webster, C., Kempell, K., Booth, I. and Busby, S. (1987) *Gene* **59**, 253–263.
- Webster, C., Gaston, K. and Busby, S. (1988) *Gene* **68**, 297–305.
- Webster, C., Gardner, L. and Busby, S. (1989) *Gene* **83**, 207–213.
- Caswell, R., Webster, C. and Busby, S. (1992) *Biochem. J.* **287**, 501–508.
- Williams, J., Michan, C., Webster, C. and Busby, S. (1994) *Biochem. J.* **300**, 757–763.
- Casadaban, M. and Cohen, S. (1980) *J. Mol. Biol.* **138**, 179–207.
- Thomas, M. and Glass, R. (1991) *Mol. Microbiol.* **5**, 2719–2725.
- Bingham, A. and Busby, S. (1987) *Mol. Microbiol.* **1**, 117–124.

-
- 12 Lodge, J., Williams, R., Bell, A., Chan, B. and Busby, S. (1990) *FEMS Lett.* **67**, 221–226
 - 13 Zhou, Y., Zhang, X. and Ebright, R. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6081–6085
 - 14 Caswell, R., Williams, J., Lyddiatt, A. and Busby, S. (1992) *Biochem. J.* **287**, 493–499
 - 15 Miller, J. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
 - 16 Kobayashi, K., Nagata, K. and Ishihama, A. (1990) *Nucleic Acids Res.* **18**, 7367–7372
 - 17 Moyle, H., Waldberger, C. and Susskind, M. (1991) *J. Bacteriol.* **173**, 1944–1950
 - 18 McClure, W. (1985) *Annu. Rev. Biochem.* **54**, 171–204
 - 19 Gallegos, M.-T., Michan, C. and Ramos, J.-L. (1993) *Nucleic Acids Res.* **21**, 807–810
 - 20 Reeder, T. and Schleif, R. (1993) *J. Mol. Biol.* **231**, 205–218
 - 21 Williams, J. (1993) Ph.D. Thesis, University of Birmingham
 - 22 Schmitt, R. (1968) *J. Bacteriol.* **96**, 462–471
 - 23 Ishihama, A. (1993) *J. Bacteriol.* **175**, 2483–2489
 - 24 Hendrickson, W., Stoner, C. and Schleif, R. (1990) *J. Mol. Biol.* **215**, 497–510
 - 25 Kessler, B., de Lorenzo, V. and Timmis, K. (1993) *J. Mol. Biol.* **230**, 699–703
 - 26 Tobin, J. and Schleif, R. (1990) *J. Mol. Biol.* **211**, 75–89
 - 27 Egan, S. and Schleif, R. (1994) *J. Mol. Biol.* **243**, 821–829
 - 28 Moralejo, P., Egan, S., Hidalgo, E. and Aguilar, J. (1993) *J. Bacteriol.* **175**, 5585–5594
-

Received 12 February 1996/18 April 1996; accepted 7 May 1996