Up-promoter mutations in the positively-regulated *mer* promoter of Tn*501*

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Received April 26, 1989; Revised and Accepted June 15, 1989

ABSTRACT

Transcription from the *mer* promoter of transposon Tn*501* is repressed by MerR (the product of the *merR* gene) in the absence of Hg^{2+}, and activated by MerR in the presence of Hg^{2+}. In the absence of MerR, the *mer* promoter has weak constitutive activity. The DNA sequence of the *mer* promoter shows candidate −35 and −10 sequences at the unusually high spacing of 19 base-pairs. We have selected for spontaneous mutations in the *mer* promoter that confer an up-promoter phenotype. Four different mutants have been isolated. Three of these are single base-pair deletions between the −10 and −35 sequences. A fourth removes the −10 sequence entirely, and places a second potential −10 sequence 17 base-pairs from the −35 sequence. None of these mutant promoters are induced by MerR in the presence of Hg^{2+}. Two of them are repressed by MerR irrespective of the presence or absence of Hg^{2+}. Models for the mode of action of the MerR protein are discussed in the light of these results. Our data support a mechanism in which the MerR protein in the presence of Hg^{2+} acts to change the conformation of DNA in the *mer* promoter.

INTRODUCTION.

Comparative studies on large numbers of *E.coli* promoters have revealed a number of conserved features. These include the existence of sequences similar or identical to TTGACA and TATAAT at approximately positions −35 and −10 relative to the transcription start site, and a spacing of 17 +/− 1 base-pairs between these sequences (1,2). Changing the spacing of the −35 and −10 sequences generally gives a down-promoter phenotype (3,4). Conversely, an up-promoter phenotype may be seen if a non-optimal spacing is changed to an optimal one (5). The changes of promoter phenotype with changes toward or away from a consensus is likely to represent differences in the ability of RNA polymerase to recognise the different promoter sequences (6).

Positively-regulated promoters often show an atypical sequence in the −35 region and poor binding of RNA polymerase in the absence of an activator protein (7). It has been suggested (8) that this poor homology to the consensus could be used in searches for positively regulated promoters. Activator proteins are thought to aid in the formation of transcriptionally competent open complexes at the promoter, either by enabling the RNA polymerase to bind to the promoter more rapidly or by speeding up the transition from a closed to an open complex between RNA polymerase and the promoter (6,8). The details of how they do this remain obscure, although there is good evidence that protein-protein contacts are involved (9). Activator binding sites generally overlap with, or are upstream from, the −35 sequence (8).

The *mer* promoter in the transposon Tn*501* is repressed by MerR (the product of the *merR* gene) in the absence of Hg^{2+}, and activated by MerR in the presence of Hg^{2+} (10).

The *mer* promoter contains good potential −35 and −10 sequences (TTGACT and TAAGGT respectively), but with a spacing of 19 base-pairs between them (10). Purified MerR protein has been shown to bind to a region which contains a hyphenated dyad symmetrical sequence, and which overlaps the −35 sequence and the spacer between the −35 and −10 sequences (11). Deletion analysis of the *mer* promoter has shown that the binding of MerR to this region is likely to be responsible both for repression and activation of the *mer* genes (12). The presence of a good −35 sequence, the non-optimal spacing of the −35 and −10 sequences, and the unusual proximity of the regulator protein binding site to the transcription start site, suggest that the mechanism of induction of transcription at the *mer* promoter might be different to other positively regulated promoters studied in *E.coli*.

We report here the isolation of four up-promoter mutations in the *mer* promoter. Three of these were single base-pair deletions in the −35/−10 spacer, while a fourth was an 8 base-pair deletion that removed the −10 sequence and brought a second potential −10 sequence to 17 base-pairs downstream of the −35 sequence. The interactions of these mutant promoters with MerR have been studied. The results of these studies are discussed in terms of models for activation of transcription from the *mer* promoter.

MATERIALS AND METHODS

Bacterial strains.

β-galactosidase assays and routine DNA manipulations were carried out in a RecA derivative of *E. coli* CSH26 (13). Manipulations using M13 or pUC derivatives were in *E.coli* TG1 (14).

Media.

LB agar or LB broth were used for routine growth of bacteria (13). Growth of *E. coli* TG1 for preparations of single-stranded DNA was in 2×YT (1.6% tryptone, 1.0% yeast extract, 1.0% NaCl). For β-galactosidase assays, strains were grown in minimal salts supplemented with 0.2% glucose, 0.2% casamino acids, and 20 μg/ml vitamin B1.

Plasmids.

The plasmids used in this study are listed in Table 1. Details of the construction of those plasmids new to this study are given in the text.

DNA manipulations.

Restriction endonucleases, DNA polymerase I (Klenow fragment), T4 polynucleotide kinase and reverse transcriptase were purchased from Boehringer Mannheim, Anglian Biotechnology, or Amersham International plc and used according to manufacturer's instructions or as described (15). DNA ligase was a gift from L.R. Evans. RNA polymerase was purchased from Northumbrian Biologicals Ltd. DNA sequence analysis was by the dideoxynucleotide chain-termination method.

Determinations of β-galactosidase activity.

Assays were carried out as described (13). Cultures of strains to be assayed were grown overnight in supplemented minimal medium, with selection for plasmid-borne markers, then diluted 1:50 into fresh medium and grown to an A_{600} of 0.4−0.5. For induction with Hg^{2+}, $HgCl_2$ was added to the growing cultures to a final concentration of 0.5 μg/ml 30 mins before starting the assay. The relative copy numbers of plasmids were determined as described elsewhere (17). No significant variation in copy number was detected.

In vivo and in vitro determination of transcription start points.

Transcription start points *in vivo* were determined by extension by reverse transcriptase

of an oligonucleotide, hybridised against total cellular RNA (18). RNA was extracted by the hot acid phenol method (19). The oligonucleotides used for hybridisation and for sequence analysis were synthesised on a Coder 300 DNA synthesiser. The oligonucleotide was end-labelled with [γ-^{32}P]ATP using T4 polynucleotide kinase, and co-precipitated with 25 μg RNA, using an amount of oligonucleotide which was empirically determined to be in excess of the message to which it was complementary. Following resuspension in 50 μl hybridisation buffer (0.4M NaCl, 80% formamide, 20mM HEPES, pH 6.5), hybridisation was for 3 hours at 45°C. The nucleic acids were precipitated and then resuspended in elongation buffer (50mM KCl, 10mM $MgCl_2$, 5mM Tris-Cl pH 8.0, 1mM DTT, 100μM each dNTP), and incubated for 60 mins at 42°C with 20 units reverse transcriptase. The enzyme was then heat inactivated (10 mins, 72°C), and the reaction was treated with 1 mg DNase-free RNase for 30 mins at 37°C. The products were run out on an 8% denaturing acrylamide gel, alongside a nucleotide sequence ladder generated by using the same oligonucleotide as a sequencing primer on template prepared from M13mp9 containing the promoter sequence.

In vitro determination of transcription start points was by run-off transcription of gel purified fragments (20), using purified *E. coli* RNA polymerase.

Selection of up-promoter mutations.

Overnight cultures containing the plasmid pBR-EA-1 (see Results) were spread at a density of about 10^9 cells per plate onto LB plates containing tetracycline (15 μg/ml), and incubated at 37°C for 3 days. Colonies which grew were restreaked on the same medium; only one colony was taken per plate to ensure all mutations were independent. Poorly-growing or mucoid colonies were discarded. DNA was prepared from the remaining colonies (21) and digested with *Eco*RI and *Hin*dIII. DNA preparations which gave a different restriction pattern to pBR-EA-1 were not further investigated. The *Eco*RI-*Hin*dIII fragments from the remaining preparations were isolated and ligated into M13mp10 or −11 and their DNA sequences were determined. Those which showed a mutation in the *mer* promoter were reisolated and cloned into the promoter-probe pRZ5255 for quantitative analysis of promoter strength, as described in Results.

RESULTS

Construction of pBR-EA-1.

The plasmid pBR-EA-1 contains the *mer* promoter from Tn*501* upstream of the tetracycline resistance gene of pBR322. To construct this plasmid, the *mer* promoter was isolated as a 113bp *Eco*RI-*Taq*I fragment from pUC9-H2 (which contains the *mer* promoter on a 200 base-pair *Hae*III fragment in the *Sma*I site of pUC9; 12), and ligated into *Eco*RI-*Acc*I cut pUC9. The resulting plasmid was cut with *Eco*RI and *Hin*dIII and the fragment carrying the promoter was ligated into pBR322 cut with the same enzymes. As *Hin*dIII cleaves between the −35 and −10 sequences for the tetracycline resistance gene promoter,this placed the tetracycline gene under the control of the *mer* promoter. The resulting plasmid was called pBR-EA-1.

Cells containing pBR-EA-1 showed low resistance to tetracycline (m.i.c. 2−3 μg/ml). If a plasmid containing the transposon Tn*501* (pDS6501; 22) was also present, the m.i.c was reduced to 1 μg/ml or lower, presumably due to repression *in trans* of the *mer* promoter in pBR-EA-1 by the MerR protein from Tn*501*. In the presence of 1 μg/ml $HgCl_2$, the m.i.c. was increased to 15 μg/ml. This showed that Hg^{2+} induced transcription from the *mer* promoter could take place in pBR-EA-1 in the presence of MerR.

Table 1: Plasmids used in this study.

Plasmid name	Brief description	Reference
pBR-EA-1	pBR322 derivative with the *tet* gene under transcriptional control of the *mer* promoter	This work
pBR-EA-1 pBR-EA-M3 pBR-EA-M6 pBR-EA-M10	TetR derivatives of pBR-EA-1. containing mutants in P_{mer}	This work
pAC-EH-3	pACYC184 derived plasmid with MerR under control of CAT promoter; $MerR^+$	10.
pAC-EH-1	As pAC-EH-3 but MerR gene in opposite orientation; $MerR^-$	10
pDS6501	pACYC184 with Tn*501* in CAT gene.	22
pRZ5255	Promoter probe plasmid with unique *Eco*RI, *Bam*HI and *Sal*I sites upstream of promoterless lacZ gene	16
pRZ-B-'X'	P_{mer} from pBR-EA-'X' in pRZ5255	This work
pUC-B-'X'	P_{mer} from pBR-EA-'X' in pUC9	This work
pUC9-H2	*Hae*III fragment from positions 500 to 700 in Tn*501* cloned into *Sma*I site of pUC9	Unpublished

Selection and DNA sequence analysis of up-promoter mutants.
Tetracycline resistant mutants of pBR-EA-1 were selected as described in Materials and Methods. After discarding mutants which showed altered restriction patterns (caused presumably by insertions or rearrangements in the plasmid DNA), approximately 50 mutants were left. DNA sequence analysis of these showed that six were mutants in the *mer* promoter, of which two mutants each occurred twice. The nature of the mutations which gave rise to resistance in the remaining isolates was not investigated further. The four mutations in the *mer* promoter were as follows: a deletion of a C at position 560 or 561, the deletion of a T at position 567, an eight base-pair deletion from positions 576−583, and a deletion of a C at position 573. These deletions were designated M1, M3, M6 and M10 respectively, and the pBR-EA-1 derivatives carrying them are referred to as pBR-EA-M1, pBR-EA-M3, pBR-EA-M6 and pBR-EA-M10. The positions of the mutations in the *mer* promoter are shown in Figure 1. The eight base-pair deletion in mutant M6 may have occurred due to the presence of the similar sequences AGTACGG at positions 569−575 and AGTAAGG at positions 577−583.
Assay of promoter strength and MerR regulation of the up-promoter mutations.
To assay the up-promoter mutations, they were transferred to the promoter-probe plasmid pRZ5255 (17). Each pBR-EA derivative was cut with *Hin*dIII, the 5′-terminal extensions

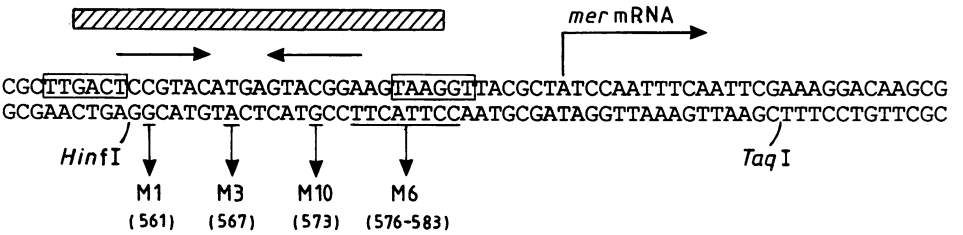

Figure 1: Sequence of the *mer* promoter region in Tn*501*. The −35 and −10 regions are boxed. The arrows above the DNA show the hyphenated dyad symmetry to which MerR binds; the hatched box shows the region protected in DNaseI experiments (11). The lines below the sequence show the deletions which gave an up-promoter phenotype.

were filled-in using Klenow polymerase and the four dNTPs, and *Bam*HI linkers were attached. The plasmids were then cut with *Eco*RI and *Bam*HI and the fragment carrying the mutant promoter was isolated and ligated into *Eco*RI-*Bam*HI cut pRZ5255. The non-mutant *mer* promoter was also cloned into pRZ5255 in the same fashion. The resultant pRZ5255-derived plasmids were called pRZ-B1 (with the wild-type *mer* promoter), pRZ-BM1, pRZ-BM3, pRZ-BM6, and pRZ-BM10. These plasmids were transformed into *E.coli* CSH26*recA* carrying either pAC-EH-3 ($MerR^+$) or pAC-EH-1 ($MerR^-$). β-galactosidase assays were carried out on all of these strains, both in the presence and absence of $HgCl_2$. All determinations were in triplicate. The results of these experiments are shown in Figure 2. The ratios of repressed to derepressed, and induced to repressed activities were calculated for each plasmid. These are shown in Table 2. The results show that all the mutant *mer*

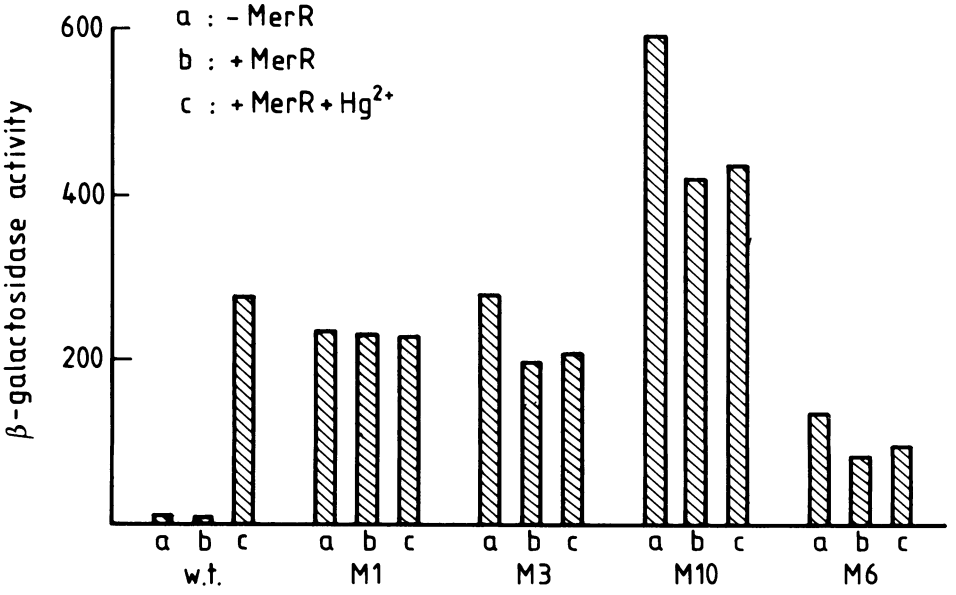

Figure 2: β-galactosidase activities (in Miller units; 13) of the wild-type and the four up-promoter mutants.

Table 2: Ratios of activities of wild-type and mutant promoters

Plasmid:	Repressed/derepressed	Induced/repressed
pRZ-B1	0.76 ± 0.17	32.5 ± 4.10
pRZ-BM1	0.98 ± 0.07	0.99 ± 0.33
pRZ-BM3	0.71 ± 0.05	1.04 ± 0.09
pRZ-BM6	0.62 ± 0.04	1.14 ± 0.02
pRZ-BM10	0.72 ± 0.10	0.96 ± 0.09

Repressed (+MerR), derepressed (−MerR), and induced (+MerR and Hg^{2+}) promoter activities were determined in triplicate for the wild-type and each of the four up-promoter mutants.

promoters with the exception of BM1 are still repressed by MerR. This repression is still seen when Hg^{2+} is present, unlike the wild-type promoter which shows a 32.5-fold induction by MerR in the presence of Hg^{2+}. The mutant promoters BM3 and BM10 in the absence of MerR show levels of expression in excess of the maximum induced (MerR plus Hg^{2+}) level of the wild-type promoter.

Determination of transcription start points.

Transcription start points were determined on RNA isolated from cultures containing each of the pBR-EA derivatives, including pBR-EA-1. RNA was also isolated from CSH26*recA*(pBR-EA-1, pAC-EH-3) which was induced with $HgCl_2$ for 30 mins before harvesting the cells. The oligonucleotide used for primer extension was 5′-ATACACGGTGCCTGACT-3′, which is homologous to the pBR322 sequence (positions 71 to 87) downstream of the *Hin*dIII site. The products of the reverse transcriptase extension reaction were run out on an 8% denaturing polyacrylamide gel next to the DNA sequence of the *mer* promoter determined using the primer 5′-AGGCAGCAAGCCGAGGCGA-3′, which is homologous to positions 696 to 678 in Tn*501*. The results are shown in Figure 3. All single base-pair deletion mutants produce a single transcript which originates at the same base as the induced *mer* transcript, which has previously been determined to be at position 591 (10). No transcript can be detected for the wild-type promoter; this is not surprising as the β-galactosidase assays show that the constitutive strength of this promoter is much weaker than the up-promoter mutants. A similar analysis on a different plasmid containing the wild-type *mer* promoter has confirmed that the sites of initiation of transcription of the derepressed and the induced *mer* promoter are the same (unpublished data). For the mutant M6 (the eight base-pair deletion) no unique transcript is seen, but several bands can be seen on the longer exposure (Figure 3B) some of which are slightly shorter (by 2 to 4 bases) than the wild-type induced transcript, and some of which are centred around a region 13−14 bases 5′ of the start site of the normal *mer* transcript.

To prepare fragments for *in vitro* transcription, the *Eco*RI-*Bam*HI fragments from the pRZ-B series of plasmids were cloned into pUC9. The resulting plasmids are referred to as pUC-B1, pUC-BM1, etc. Preparations of these plasmids purified by CsCl-isopycnic centrifugation were used as a source of *Eco*RI-*Hin*dIII fragments which were transcribed *in vitro* using purified RNA polymerase. An *Eco*RI-*Sal*I fragment of pUC-BM-1 was also transcribed *in vitro* to determine the orientation of any transcript seen (a transcript towards the *Sal*I site would be 14 bases smaller if transcribed from the *Eco*RI-*Sal*I fragment than from the *Eco*RI-*Hin*dIII fragment; a transcript towards the *Eco*RI site would be the same length).

The results of these experiments are shown in Figure 3. All the up-promoter mutants with single base-pair deletions give a transcript of the size predicted for transcription

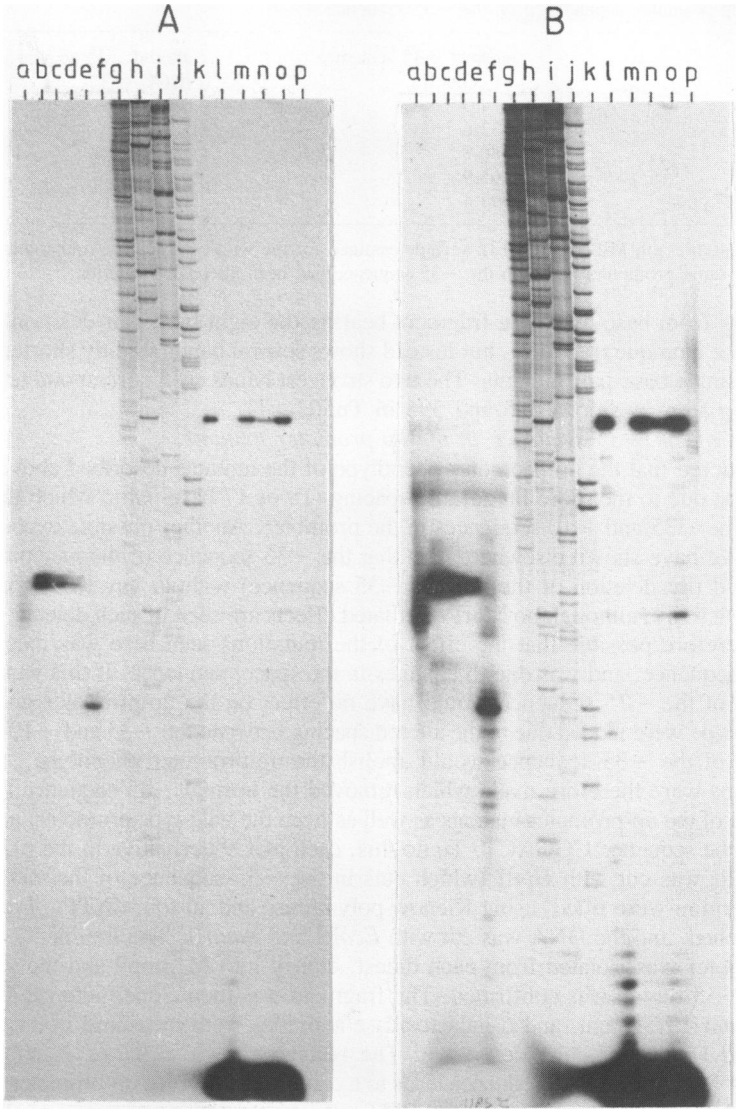

Figure 3: *In vitro* and *in vivo* determined start points of transcription of the wild-type and up-promoter mutants. Tracks (a) to (f) are the result of run-off transcription of DNA fragments, tracks (k) to (p) are the result of primer extensions on RNA. 3A and 3B are different exposures of the same gel.
Tracks: (a) – (d)*Eco*RI-*Hin*dIII fragments from (a) M6, (b) M10, (c) M3, (d) M1; (e) *Eco*RI-*Sal*I fragment M1; (f) *Eco*RI-*Hin*dIII fragment wild-type; (g)–(j) Tn*501* (A,G,C,T) sequence determined as described in text; (k)–(p) RNA from (k) pRZ-EA-1 + pAC-EH-3 + Hg^{2+}; (l) pRZ-EA-1 + pAC-EH-1; (m) pRZ-EA-M1; (n) pRZ-EA-M3; (o) pRZ-EA-M10; (p) pRZ-EA-M6.

Table 3: Up-promoter dependence on the −35 sequence

Plasmid	normal −35 sequence (TTGACT)	altered −35 sequence (CGGACT)
pRZ-B1	4.3	0.9
pRZ-BM1	232.6	3.9
pRZ-BM3	186.8	1.1
pRZ-BM6	165.9	0.8
pRZ-BM10	597.4	1.0

Promoter activities (in Miller units; 13) were determined for the wild-type and the four mutant promoters, and for the same promoters in which the −35 sequence had been altered by deletion.

originating from base 591. The fragment bearing the eight base-pair deletion again does not produce a unique transcript, but instead shows several bands slightly shorter than those from the single base-pair mutants. The two strongest bands seen correspond to transcripts originating from positions 595 and 596 in Tn*501*.

Alteration of the −35 sequence in the up-promoter mutants.

We considered that the up-promoter phenotype of the mutants described above was most likely to be due to the more favourable spacing (18 or 17 base-pairs) which they possess between the −35 and −10 sequences in the promoter. Another possible explanation also existed. We have shown elsewhere (12) that the −35 sequence of the *mer* promoter can be changed (by deletion of the normal −35 sequence) without any loss of constitutive promoter activity, although no MerR-mediated effects are seen in such deleted promoters. It was therefore possible that the effect of the mutations seen here was independent of the −35 sequence, and was due to changes in the spacer sequence. If this were the case, alteration of the −35 sequence would have no effect on the up-promoter phenotype. If the phenotype were indeed due to the altered spacing between the −35 and −10 sequences, alteration of the −35 sequence would abolish the up-promoter phenotype.

Deletions were therefore made which removed the normal −35 sequence (TTGACT) from each of the up-promoter mutants as well as from the wild-type promoter, and replaced this with the sequence CGGACT. To do this, each pUC9 derivative in the pUC-B series of plasmids was cut with *Hin*fI (which cuts in the −35 sequence of the *mer* promoter) and the termini were filled, using Klenow polymerase and all four dNTPs. *Eco*RI linkers were attached, and the DNA was cut with *Eco*RI and *Bam*HI. The fragment carrying the *mer* promoter was isolated from each digest, cloned into M13mp9 and the sequence of the new −35 region was confirmed. The fragment was then cloned into pRZ5255 as an *Eco*RI-*Bam*HI fragment, and β-galactosidase activities were measured in the absence of MerR and $HgCl_2$ for each derivative. The results, shown in Table 3, show that the alteration of part of the −35 sequence leads to a complete loss of the up-promoter phenotype. The −35 sequence of the wild-type and BM1 and BM3 mutant promoters was also changed to ATTCGG, by using S1 nuclease instead of Klenow polymerase to render the *Hin*fI site flush-ended, but otherwise treating as described above. In each case, the activities of these altered promoters were similar to those created from filled-in *Hin*fI sites, the up-promoter phenotype being lost (data not shown).

The decrease in activity seen in these experiments for the wild-type *mer* promoter following alteration of the −35 sequence was not previously observed (12). The fragment used in the experiments which showed that the −35 sequence was not apparently required for *mer* promoter activity contained more of Tn*501* downstream from the transcription

start site than the fragment used in the current experiments. These data suggest that sequences 3′ to the transcription start site may be involved in transcription from the *mer* promoter.

DISCUSSION

The most likely explanation for the phenotype of the three point mutations isolated in this study is that the reduction in spacing between the −35 and −10 sequences of the *mer* promoter changes the interaction between RNA polymerase and the promoter, which leads to an increase in promoter strength. As the *in vivo* and *in vitro* data show that the start sites for transcription in the wild-type and mutant promoters are the same, this change is quantitative rather than qualitative, and probably represents a change in either the rate at which RNA polymerase binds to the promoter, or the rate at which a transcriptionally-competent open complex is formed, or both.

The phenotype of the M6 mutant also appears to be explicable on a similar basis. Here the 8 base-pair deletion creates a new promoter with the *mer* −35 sequence and a new −10 sequence (TACGCT). The spacing between these two sequences in M6 is 17 base-pairs. Transcriptional analysis of this promoter shows several start points for transcription. The most abundant of these originate at positions 595 and 596; both of these are A residues, which accords with the observation that *E.coli* transcripts start preferentially from purines. Both the *in vivo* and the *in vitro* data suggest that several other start points are also used in this promoter. It is not clear why this promoter should be less specific in the start point for transcription initiation than the other *mer* up-promoter mutants. Elsewhere we have found that when the −35 sequence of the *mer* promoter is changed by deletion, the *mer* promoter remains active (as measured by its ability to transcribe the *lacZ* gene) but a specific transcript can no longer be detected *in vivo* or *in vitro* (12). Moreover, in the same study we showed that the −10 sequence can be deleted without loss of promoter activity; only when the second −10 sequence (i.e., the one thought to be active in M6) is deleted is all promoter activity lost (12). These results imply that either potential −10 sequence alone contains enough information to promote transcription, a phenomenon which has been observed elsewhere (23), but not enough information to specify the precise start point for transcription.

The reason for the much higher level of transcription from the M10 promoter compared to the other up-promoter mutants is not known. The deletion removes a base-pair protected against methylation by MerR (24), but so does deletion M1. RNA polymerase does not make specific contacts in the spacer region, but changes in the sequence of the spacer can produce changes in promoter strength (25). Differences in the flexibility of the spacer region may be responsible for the observed differences in the transcriptional efficiency of the up-promoter mutations.

The degree of repression seen in the wild type promoter is rather poor in these experiments (see Table 2). Repression of the *mer* promoter by MerR when the promoter was cloned as a *Hae*III fragment (positions 500 to 700 in Tn*501*) was nearly 90% (12). This suggests that there may be other sites, not present on the *Eco*RI-*Taq*I fragment used in the present study, which are involved in MerR-mediated repression of the *mer* promoter. These sites may include the overlapping *merR* promoter (10,12), part of which lies beyond the *Taq*I site. In the absence of MerR, RNA polymerase occupies the *merR* promoter (24), and the low repression ratio in this study may reflect reduced competition for RNA polymerase between the *mer* and *merR* promoters.

With the exception of BM1, all the up-promoter mutants are repressed by MerR to essentially the same degree as the wild-type promoter. This is somewhat surprising, as the deletions studied all occur within the MerR binding site as defined by DNase I footprinting studies (11). The implication is that the binding of MerR to the mutant promoters BM3, BM6, and BM10, has not been significantly altered with respect to the wild-type promoter. This can be tested *in vitro* by direct measurement of the binding constants in each case. The failure of MerR to induce transcription from the promoters BM3 and BM6 is thus of particular interest (it may also be failing to induce in promoter M10; however, as the constitutive level of M10 is already much greater than the induced level of the wild-type promoter, this cannot be ascertained). There are a number of possible explanations for this. One possibility is that the deletions have changed the relative positions of the binding sites for MerR and RNA polymerase. Thus, RNA polymerase bound to the mutant promoters may no longer be in a position to make a favourable contact with the activator form of MerR. Another possibility is suggested by the atypical spacing of the −35 and −10 sequences in the *mer* promoter. The mechanism of activation by MerR may be the induction of a conformational change in the DNA which enables RNA polymerase to make more favourable contacts with the widely spaced −35 and −10 sequences. In the mutant promoters, this requirement for MerR may be superseded by the altered (and more optimal) length of the spacer. MerR may induce the same conformational change in the DNA of the mutant promoters, but the altered positions of the −35 and −10 sequences would no longer be optimal for RNA polymerase binding. The role of the DNA in the first model is passive, induction being attributed to a protein-protein interaction. In the second model, activation of transcription requires a bend or twist in the DNA. The CAP regulatory protein has been shown to cause a bending the DNA to which it binds (26,27). Recent data on the protection of the *mer* promoter region by RNA polymerase or MerR in the absence of Hg^{2+}, and RNA polymerase and MerR in the presence of Hg^{2+} (24) are consistent with there being local changes in DNA conformation induced by RNA polymerase and MerR. We can speculate that activation of transcription by MerR does indeed involve a conformational change in the DNA that favours either the recognition of the *mer* promoter by RNA polymerase or the formation of the open complex. That expression from the mutant promoters M3 and M10 in the absence of MerR is greater than the fully-induced expression from the wild-type promoter, is good circumstantial evidence that MerR in the presence of Hg^{2+} works by compensating for the unusually long spacer in the *mer* promoter.

ACKNOWLEDGEMENTS.

We thank Steve Busby and Mark Buttner for advice and discussion and Tom O'Halloran for the communication of results prior to publication. This work was supported by MRC Project Grant G.8620891CB and The Royal Society, of which NLB was an EPA Cephalosporin Fund Senior Research Fellow.

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