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The *Escherichia coli* regulatory protein OxyR discriminates between methylated and unmethylated states of the phage Mu *mom* promoter

Michael Bölker and Regine Kahmann

Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, D-1000 Berlin 33, FRG

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Expression of the phage Mu *mom* gene is transcriptionally regulated by DNA methylation. Three GATC sites upstream of the *mom* promoter have to be methylated by the *Escherichia coli* deoxyadenosine methylase (Dam) to allow initiation of transcription. An *E.coli dam* strain was mutagenized with Tn*5* in an attempt to isolate mutants which allow *mom* gene expression. Three independent Tn*5* mutants were isolated, each mapped to a gene at 89.6 min which we designate *momR*. The wildtype gene was cloned and sequenced, it encodes a protein of 305 amino acids. The protein belongs to a group of related bacterial activators recently identified as the LysR family (Henikoff *et al.*, 1988). MomR protein was overproduced and purified. Expression of *momR* is autoregulated; MomR binds to a 43 bp region upstream of its coding sequence. In the *mom* promoter MomR protects a 43 bp region containing the three GATC sites. Specific binding to these sequences was oberved only with unmethylated DNA. Fortuitously, we learned that MomR is identical to OxyR, a regulatory protein responding to oxidative stress. We discuss the implications of this control for Mu development.

Key words: transcription regulation/DNA modification/methylation dependent gene expression/repressor/LysR family

Introduction

The *mom* gene of phage Mu encodes a DNA modification function which converts adenine to acetamido adenine in a sequence-specific manner (Swinton *et al.*, 1983; Kahmann, 1984). The gene is subject to complex transcriptional as well as post-transcriptional control (see review by Kahmann and Hattman, 1987), presumably to ensure that *mom* is expressed very late in the phage life cycle to minimize detrimental effects of this modification on phage development. Phage Mu has an exceptionally broad host range and the *mom* specific modification serves to protect Mu DNA from a variety of host controlled restriction systems (Toussaint, 1976). One of the intriguing facets of *mom* gene expression is its positive regulation by DNA methylation. A cluster of three GATC sites (termed region I, see Figure 1) upstream of the promoter has to be methylated by the *E.coli* Dam function (Marinus and Morris, 1973), only then is transcription of *mom* initiated (Hattman, 1982). Promoter activity in wildtype and *dam* strains differs by at least a factor of 200 (M.Bölker, unpublished). A methylation requirement for promoter activity in prokaryotes is highly unusual, in most other cases where Dam methylation affects gene expression, e.g. the transposase promoter of Tn*10* and IS*10* (Roberts *et al.*, 1985), the *sulA* promoter (Peterson *et al.*, 1985), the *trpA* promoter (Marinus, 1985) and the *glnS* promoter (Plumbridge and Söll, 1987), activity is enhanced in *dam* strains. Aside from *mom*, only two genes, *dnaA* and *mioC*, are positively regulated by Dam, their products are involved in DNA replication (Kücherer *et al.*, 1986; Braun and Wright, 1986; Schauzu *et al.*, 1987). In all these cases the Dam sites are located within the −10 or −35 regions and presumably influence directly the interaction of RNA polymerase with the promoter. In contrast, the Dam sites affecting *mom* promoter activity are located further upstream in a region extending from position −55 to −87 (Kahmann, 1983; Plasterk *et al.*, 1983). In addition to DNA methylation, the *mom* promoter is positively regulated by the Mu gene *C* product (Hattman *et al.*, 1985; Heisig and Kahmann, 1986) which binds to a site located upstream of the −35 region. Binding of C is not affected by Dam-methylation, although the C footprint extends close to GATC site III (Bölker *et al.*, 1989; and see Figure 1). The Dam requirement for *mom* gene expression can be alleviated when at least two of the three GATC sites in region I are eliminated by point mutation, when parts of region I are deleted or when the spacing of the GATC sites is altered (Seiler *et al.*, 1986). In all cases the requirement for activation of the promoter by C protein is maintained. These features have led to the proposal of a repressor model (Hattman and Ives, 1984), which assumes that there is a cellular repressor protein for the *mom* gene which binds to region I in its un- or hemimethylated state and prevents, by steric hindrance, access to the promoter by C protein. Support for the existence of such a cellular repressor came from our observation that a *dam* strain which carries a large deletion encompassing *mutH* allowed expression of the *mom* gene (Seiler *et al.*, 1986). MutH is a component of the methyl-directed mismatch repair system and is responsible for recognition of the methylation status of the DNA (Kramer *et al.*, 1984). Subsequent experiments (S.Hattman, personal communication and M.Bölker, unpublished) convinced us, however, that this effect could not be attributed to the lack of *mutH* alone, since expression of a cloned *mutH* gene did not restore repression of the *mom* gene in the *dam*Δ*mutH* strain. This result prompted us to undertake a new search for the host function which can discriminate between unmethylated and methylated states of the Mu *mom* promoter.

Results

Isolation of mutants in the mom repressor gene

If there is a gene encoding a repressor for the *mom* operon and the gene is nonessential it should be possible to isolate mutants that allow expression of a cloned *mom* gene in the absence of Dam.

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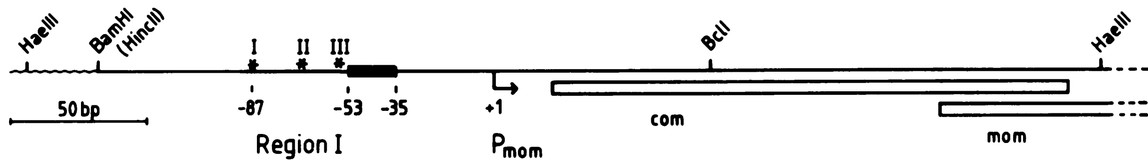

Fig. 1. Schematic overview of the organization of the phage Mu *mom* operon. Part of the *mom* operon as it is cloned in pMuASIC1 (Seiler *et al.*, 1986) is shown. The solid line represents Mu DNA, the wavy line indicates flanking pBR322 sequences. Only restriction sites used for preparation of fragments are indicated. The reading frames for *com* and *mom* are represented by open bars. The +1 position is the transcriptional start determined by primer extension (Bölker *et al.*, 1989), the arrow indicates the direction of transcription. The solid bar symbolizes the binding site for the Mu C protein (Bölker *et al.*, 1989). Asterisks mark the GATC sites, their numbering is according to Seiler *et al.* (1986). Numbers give positions relative to the start of transcription.

Table I. Phage Mu *mom* gene expression in various CSH50(Mucts62) derivatives

Relevant markers	*mom* gene expression (efficiency of plating[a]) in strains harbouring	
	no plasmid	pMomR1200
dam13 :: Tn9 momR$^+$	5.0×10^{-5}	n.t.
dam13 :: Tn9 momR1 :: Tn5	7.5×10^{-1}	n.t.
dam13 :: Tn9 momR2 :: Tn5	7.8×10^{-1}	n.t.
dam13 :: Tn9 momR3 :: Tn5	1.0	1.1×10^{-4}
dam$^+$ momR$^+$	5.4×10^{-1}	7.7×10^{-3}
dam$^+$ momR1 :: Tn5	1.0	n.t.
dam$^+$ momR3 :: Tn5	9.7×10^{-1}	n.t.

[a]The efficiency of plating was determined as described in Materials and methods.

The *dam* strain CSH50*dam*13::Tn*9* was mutagenized with Tn*5* (see Materials and methods). A pool of approximately 3×10^4 independent mutants was transformed with the test plasmid pMCCL. In pMCCL the *mom* promoter including region I directs synthesis of a LacZ fusion protein. The *C* gene encoding the transcriptional activator for *mom* is cloned on the same plasmid (for details see Materials and methods). In *dam* strains the *lac*Z fusion gene on pMCCL is poorly expressed and yields pale blue colonies on X-Gal indicator plates. After transformation of the Tn*5* mutant pool we scored clones which were dark blue on X-Gal plates at a frequency of ~0.1%. These strains were lysogenized with Mucts62 and assayed for *mom* gene expression (see Materials and methods). Of 30 strains tested, three allowed full expression of the *mom* gene (data not shown). The Tn*5* insertions from these strains were transduced (Materials and methods) to CSH50*dam13*::Tn*9* (Mucts62) to verify that the mutation was linked to Tn*5*. The respective strains are designated CSH50*dam13*::Tn*9* *momR1-3*::Tn*5*(Mucts62). In all three cases transductants were obtained in which *mom* expression was as high as in the Dam$^+$ control strain CSH50(Mucts62) (Table I), which is about three orders of magnitude higher than in the parent strain CSH50*dam13*::Tn*9*(Mucts62). The successful isolation of host mutants that allow Dam independent *mom* expression strongly supports the repressor hypothesis.

To investigate the mutants further, their Tn*5* insertions, including flanking sequences, were cloned as *Eco*RI fragments into the respective site of pTZ18R. Tn*5* does not contain cleavage sites for *Eco*RI (Jorgensen *et al.*, 1979). All three Km^R recombinant plasmids contained *Eco*RI fragments of ~25 kb in length. Subsequent restriction analysis revealed that the same *Eco*RI fragment had been cloned from all three mutant strains, only the location of the Tn*5* insertion was different in each clone. The Tn*5* insertions were mapped to a region comprising ~500 bp (Figure 2). We refer to this locus as *momR*. The restriction map, furthermore, allowed an unambiguous alignment with the physical map of the *E.coli* chromosome established by Kohara *et al.* (1987) and placed the *momR* locus just downstream of the *argECBH* genes around position 89.6 min. This gene cluster has already been located on a 17.3 kb *Eco*RI fragment (Devine *et al.*, 1977) and we expected the *momR* locus to reside on the same fragment.

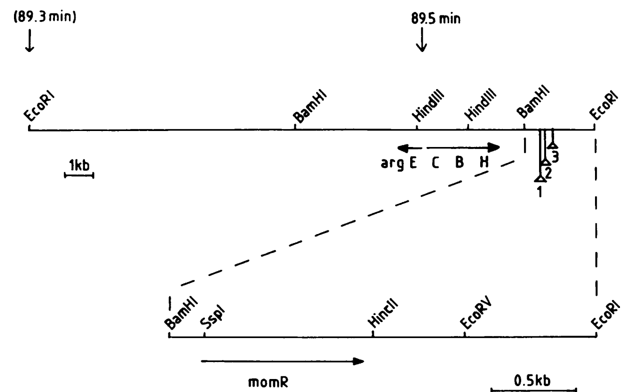

Fig. 2. Restriction map around the *momR* locus. The upper line represents the 20 kb *Eco*RI fragment cloned from strains carrying Tn*5* insertions in *momR*, the locations of the Tn*5* insertions are indicated by open triangles. The positioning of the *argECBH* gene cluster has been published (Bachmann, 1987). Only restriction sites used to identify the chromosomal location of this fragment are indicated. Numbers are minutes on the standard *E.coli* map, parentheses indicate that this number has been calculated by assuming that 0.1 min represents 5 kb. The lower part shows the fragment cloned in pMomR2300, only restriction sites used to generate subclones are indicated. The transcripts of known genes are indicated by arrows.

Cloning and characterization of the wildtype mom repressor gene, momR

To isolate the *momR* wildtype locus, *Eco*RI fragments of strain CSH50 were cloned in pBR322. A plasmid complementing the *argE* mutation was identified after transformation of the *argE* strain CP78. This plasmid, pBRargE, contained a 20 kb *Eco*RI fragment homologous in restriction pattern to the Tn*5* containing fragments cloned from the mutants strains. From pBRargE we subcloned a 2.3 kb *Bam*HI–*Eco*RI fragment since all three Tn*5* insertions had occurred in this fragment. As a functional assay we tested the ability of this plasmid, pMomR2300, to repress the *mom* gene in CSH50*dam13*::Tn*9momR* 3::Tn*5*(Mucts62). *mom* expression was lowered to the level observed in CSH50*dam13*::Tn*9*(Mucts62). The same assay was employed to define the functional limits of the *momR* locus more precisely. Both a 1.5 kb *Bam*HI–*Eco*RV

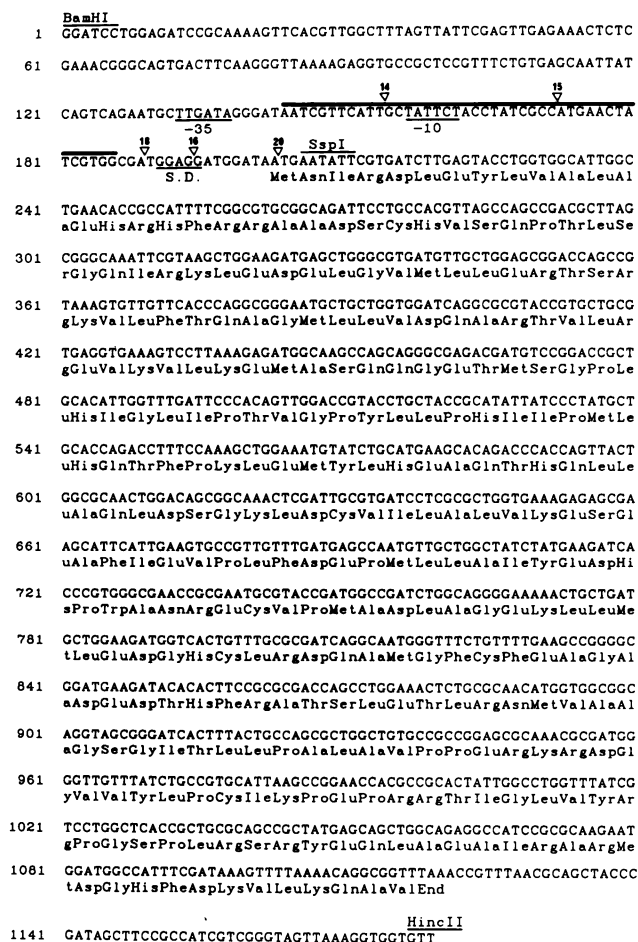

Fig. 3. Nucleotide sequence and deduced amino acid sequence of the *momR* gene. −10 and −35 mark matches to the *E.coli* consensus promoter. The positioning of this putative promoter is in accordance with S1 mapping data of Christman *et al.* (1989). The Shine−Dalgarno (S-D) sequence is underlined. The heavy line indicates the region protected by MomR in MPE·Fe(II) footprints. Deletion endpoints are indicated by open triangles. The nucleotide sequence is identical to the sequence of *oxyR* now published by Christman *et al.* (1989).

fragment (pMomR1500) and a 1.2 kb *Bam*HI−*Hin*cII fragment (pMomR1200) expressed a functional repressor for *mom*. The value for pMomR1200 is shown in Table I.

The 1.2 kb *Bam*HI−*Hin*cII fragment of pMomR1200 was sequenced (Figure 3). This fragment contains a single open reading frame (ORF) spanning 915 bp which could code for a protein of 305 amino acid residues having a predicted mol. wt of 34.4 kd. Since all three Tn*5* insertions which destroy repressor function map in this ORF (Figure 2), we designate the corresponding gene *momR*. Transcription of *momR* is clockwise in the same direction as *argCBH* (Bachmann, 1987). The ATG start is preceded by a Shine−Dalgarno sequence, a putative *E.coli* consensus promoter sequence is located 40 bp upstream of the translational start (Figure 3). The *Bam*HI−*Hin*cII fragment complements the *momR*::Tn*5* mutation when cloned in pTZ18R and pTZ19R in either orientation (data not shown). Expression of *mom* is thus independent of plasmid promoters, making it likely that a promoter for *momR* is contained on this fragment.

MomR affects mom expression in Dam$^+$ strains

To investigate the role of *momR* in Dam$^+$ strains, the natural hosts for Mu, we asked whether the copy number of *momR* would affect the level of *mom* gene expression. pMomR1200 was introduced to CSH50(Mu*c*ts62). Phage progeny from this strain were about 70-fold less modified than progeny from CSH50(Mu*c*ts62) (Table I). We conclude that MomR must interact with the *mom* promoter even in Dam$^+$ strains, where fully unmethylated DNA does not exist. This indicates that hemimethylated DNA as it is generated transiently after passage of the replication fork through region I, is a substrate for MomR. Furthermore, the degree of repression appears to be limited by the amount of MomR in the cell.

We next examined the effects of a *momR* mutation in Dam$^+$ conditions. The *momR1*::Tn*5* mutation was transduced in CSH50(Mu*c*ts62). Phage lysates were prepared and assayed for mom specific modification (Table I). Neither the phage burst (not shown), nor the degree of modification showed pronounced differences compared to phage progeny from CSH50(Mu*c*ts62). The degree of modification in the mutant strains appeared to be slightly higher than in the wildtype strain (Table I). A reason why this effect is not more pronounced could be that in the wildtype strain *mom* expression already leads to nearly complete modification of DNA. The biological assay for *mom* thus cannot detect any further increases in promoter activity.

Overexpression and purification of the momR gene product

Initial attempts to overexpress the *momR* gene by fusing the 1.2 kb *Bam*HI−*Hin*cII fragment to either the λpL or the T7ϕ10 promoter were unsuccessful. A gene fusion plasmid, in which the *Bam*HI−*Ssp*I fragment containing just the first two codons of *momR* and upstream sequences (see Figure 3) was linked with *lacZ*, in pMLB1034, showed only very low levels of β-galactosidase expression when introduced into CSH50*dam13*::Tn*9* but higher levels of expression in CSH50*dam13*::Tn*9momR*::Tn*5* (data not shown). This might

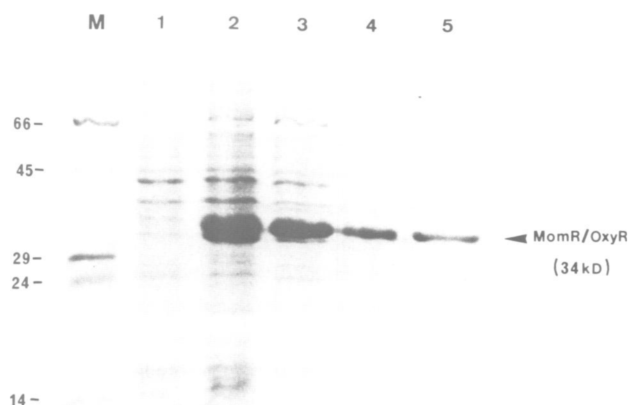

Fig. 4. Overproduction and purification of MomR protein. Extracts were prepared from DH5/pJLmomRΔ15: (1) before heat induction, (2) 2 h after heat induction, (3) crude extract, (4) fraction P, (5) fraction S, see Materials and methods. In lanes 3−5, 5 μl of the respective fractions were loaded. Separation was on a 10% SDS−polyacrylamide gel. Bands were visualized after staining with Coomassie brilliant blue. The band corresponding to MomR is indicated. Lane M, mol. wt markers: BSA, 66 000; ovalbumin 45 000; carbonic anhydrase, 29 000; trypsinogen, 24 000; lysozyme, 14 000.

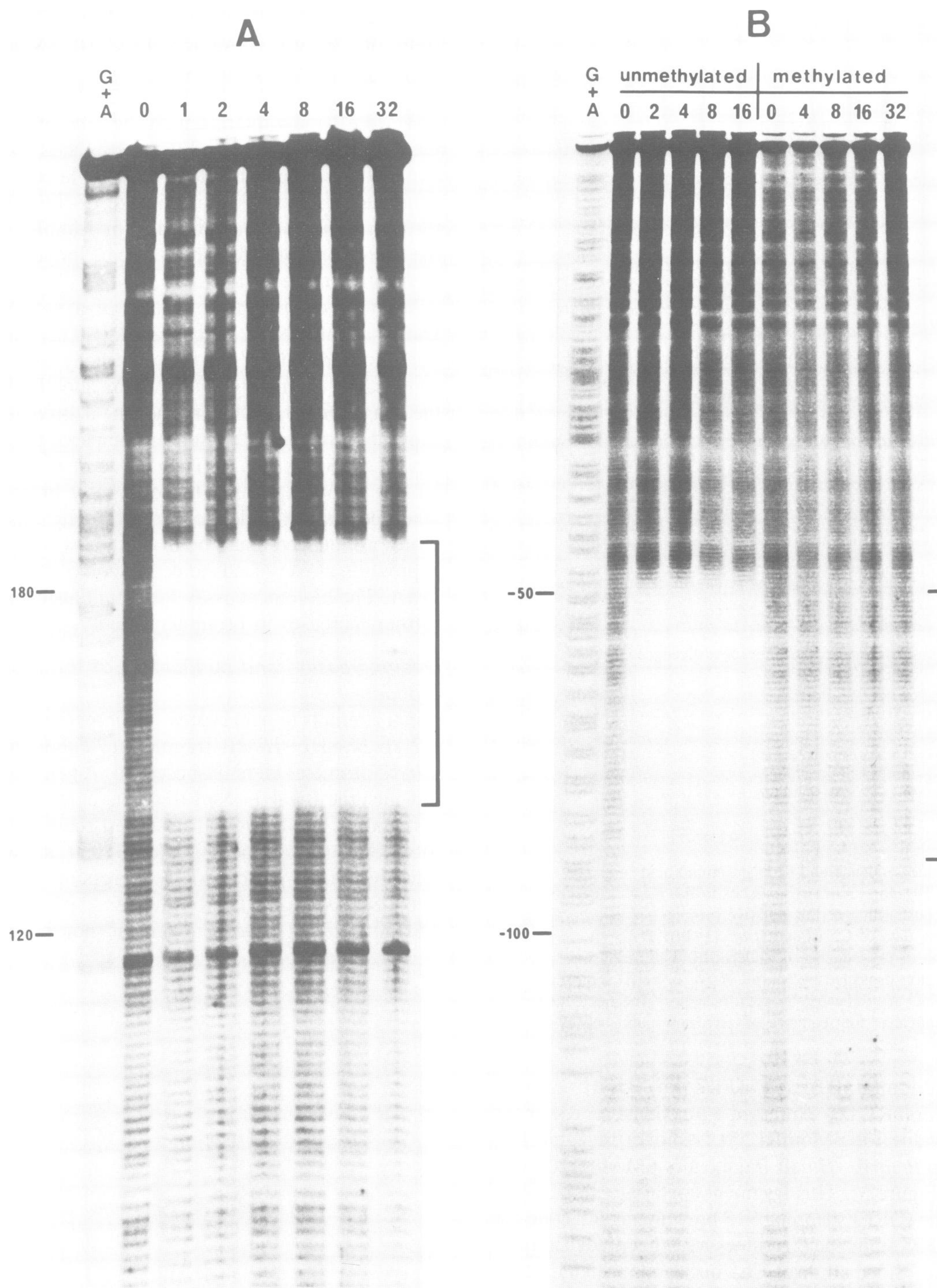

Fig. 5. Binding of MomR to its own promoter and to the regulatory region of the *mom* gene. MPE·Fe(II) footprinting reactions were performed as described in Materials and methods. Footprints are indicated by brackets. (**A**) Binding of MomR to its own promoter. A 316 bp *Bam*HI–*Alu*I fragment from pMomR1200 containing the *momR* promoter and the beginning of the *momR* ORF was 5′-end ^{32}P-labelled at the *Bam*HI end. Reactions contained 10 ng labelled DNA; in lane 0 no MomR protein was added; lanes 1, 2, 4, 8, 16 and 32 contain 25 ng, 50 ng, 100 ng, 200 ng, 400 ng and 800 ng MomR protein respectively. A Maxam–Gilbert G+A sequencing reaction performed on the same fragment serves as size marker (G+A). (**B**) Binding of MomR protein to the *mom* promoter. A 407 bp *Hin*dIII–*Xba*I fragment from pTZH380a containing the *mom* promoter was 5′-end ^{32}P-labelled at the *Hin*dIII site. Unmethylated DNA isolated from a *dam* strain and methylated DNA from a Dam$^+$ strain were used as indicated. Reactions contained 50 ng labelled DNA. In lanes 0 no MomR protein was added; lanes 2, 4, 8, 16 and 32 contain 50 ng, 100 ng, 200 ng, 400 ng and 800 ng MomR protein respectively. A Maxam–Gilbert G+A sequencing reaction performed on the same fragment serves as size marker (G+A).

indicate that *momR* autoregulates its own synthesis. As this might interfere with overproduction of MomR we reduced the size of untranslated leader sequences. To this end a set of *Bal*31 deletions starting at the *Bam*HI site and extending towards the ATG start codon (for details see Materials and methods) were generated. Shortened fragments of the appropriate size were inserted downstream of the λP_L-P_R tandem promoter in the expression vector pJLA502

(Schauder *et al.*, 1987) to generate plasmids pJLmomRΔ14, Δ15, Δ18, Δ16 and Δ20 respectively. The deletion endpoints were sequenced and are indicated in Figure 3. Expression of *momR* was monitored in DH5 after induction of the λ promoter. Whole cell lysates were analysed by SDS−PAGE. Only strains harbouring pJLmomRΔ15 and Δ18 showed strong overexpression of a 34 kd protein, the size expected for MomR from the nucleotide sequence (data are shown for pJLmomRΔ15 in Figure 4). The amount of MomR in this case was estimated to be 30% of total protein. The lack of *momR* expression in pJLmomRΔ16 and pJLmomRΔ20 is readily explained as the Δ16 deletion destroys the Shine−Dalgarno sequence and Δ20 eliminates the putative translational start. The behaviour of the other deletion derivatives suggests that the 18 bp region flanked by deletion endpoints Δ14 and Δ15 interferes with overexpression of MomR.
(Figure 4). MomR could be precipitated from the crude extract by low speed centrifugation, indicating that the overproduced protein might form inclusion bodies inside the cell. The precipitated material could be partially solubilized by high salt (see Materials and methods). Such preparations of MomR were ~90% pure (Figure 4).

DNA binding studies with purified MomR protein

A region containing the putative autoregulatory site of *momR* was analysed for binding of MomR by MPE·Fe(II) footprinting assays (Materials and methods). A 316 bp *Bam*HI−*Alu*I fragment extending from position 1−316 (Figure 3) was 5′ labelled at the *Bam*HI end and incubated with different amounts of MomR protein prior to the addition of MPE·Fe(II). The molar ratios of protein to DNA ranged between 15:1 and 480:1 in lanes 2−32 respectively (Figure 5A). The region between position 144 and 186 showed specific protection from MPE·Fe(II) digestion at all protein concentrations used. This result unambiguously showed that MomR is a specific DNA binding protein.

Is MomR also able to bind to the *mom* promoter? To this end we performed footprinting assays on a fragment containing the *mom* promoter and adjacent region I sequences. Such a fragment was excised from pTZH380a and specifically labelled at the *Hin*dIII end to yield protection patterns for the upper DNA strand. Identical fragments were prepared from plasmids propagated in wildtype and *dam* strains and subjected to footprinting analyses (Figure 5B). In *dam* DNA a protected region extending from position −92 to −50 is observed (Figure 5B/unmethylated). The protected region coincides with region I and contains all three Dam sites. In Dam$^+$ DNA a MomR footprint is not detectable even at higher MomR concentrations (Figure 5B/methylated). We have also footprinted the lower strands of the same DNA fragments, essentially yielding identical protection patterns (data not shown). These results demonstrate that MomR binds to the *mom* promoter when region I is unmethylated but not if region I is fully methylated. MomR hence fulfills all properties proposed for the *mom* gene repressor.

Discussion

In our search for the *mom* gene repressor we have shown that (i) three independent Tn*5* insertions allowing *mom* gene expression in *dam* strains affect the same gene, (ii) purified MomR protein binds specifically to region I of the *mom* promoter and (iii) the binding of MomR is affected by Dam-methylation. Taken together these results are in favour of MomR being the repressor itself rather than MomR being a positive regulator for a repressor gene. The results contrast our previous assertion that MutH represses the *mom* gene in *dam* strains (Seiler *et al.*, 1986). *mutH* maps at 61 min (Bachmann, 1987) which is far removed from the position determined for *momR* at 89.6 min. Since the particular *mutH* mutation giving the highest level of *mom* expression in *dam* strains carries a large deletion we entertain, in retrospect, the possibility that a gene from this area may be involved in regulating *momR* expression. The reason why such a gene was not picked up in the mutational analysis might be that the screening for full levels of *mom* expression diminished the chance of finding mutants in which *mom* expression is only enhanced. It will be a rewarding task to study *momR* expression in the *mutH* strains which gave elevated levels of *mom* expression.

A protein database search with the MomR sequence picked up significant homology to *E.coli* IlvY and LysR (not shown), two regulatory proteins recently identified as members of a large family of bacterial activators termed the LysR family (Henikoff *et al.*, 1988; Chang *et al.*, 1989). Other members of this group are *E.coli* CysB, *Salmonella typhimurium* MetR, *Rhizobium* NodD and *Enterobacter cloacae* AmpR (Henikoff *et al.*, 1988). Proteins in the LysR family share several features. They are all inducible positive activators for transcription with different small molecules acting as inducers. They are presumed to be DNA binding proteins since they contain a helix−turn−helix motif (Pabo and Sauer, 1984) near the N-terminus. MomR matches the derived LysR consensus in this region in 9 of 20 positions (amino acids 18−37; not shown), making it likely that MomR has a regulatory function in the cell.

At the time the momR gene was mapped and sequenced the genetic maps of *E.coli* and *S.typhimurium* gave no hints for a gene at position 89.6 min. It was G.Christie who called our attention to the *oxyR* gene first described in *S.typhimurium* (Christman *et al.*, 1985). G.Storz kindly supplied her then unpublished sequence of *oxyR* which unambiguously showed that *momR* is identical to *oxyR* (now published in Christman *et al.*, 1989). *oxyR* encodes a regulatory protein which, in response to oxidative stress, induces a set of at least nine genes involved in stress tolerance. Among these are the genes encoding catalase and an alkyl hydroperoxide reductase (Christman *et al.*, 1985; Jacobson *et al.*, 1989). Strains in which *oxyR* is deleted do not show the adaptive response to hydrogen peroxide and are hypersensitive to a variety of oxidizing agents (Christman *et al.*, 1985). In accordance with this *oxyR* phenotype, all three *momR*::Tn*5* insertion mutants are hypersensitive to hydrogen peroxide in the filter-disc inhibition assay (see Christman *et al.*, 1985; data not shown).

We have shown that MomR binds to a 43 bp region upstream of its own coding sequence. A similar result has been obtained by Christman *et al.* (1989) using extracts from an OxyR overproducing strain in DNase I footprinting experiments. The same authors have determined that the binding site covers the −10 region of the *oxyR* promoter. Binding of OxyR to the promoter region provides an explanation of how OxyR can autoregulate its own synthesis.

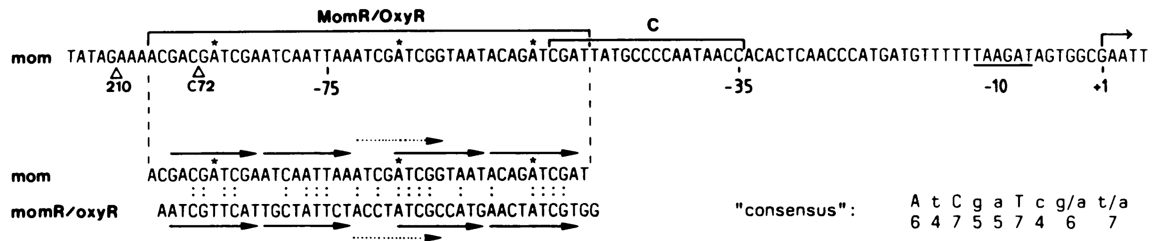

Fig. 6. Location and alignment of MomR/OxyR binding sites. The upper part shows the nucleotide sequence of the regulatory region of *mom*. MPE·Fe(II) footprints of MomR/OxyR and C are indicated by brackets. The numbering follows Figure 1. The extent of the C-binding site has been determined by Bölker *et al.* (1989). Open triangles mark deletion endpoints referred to in Results. Asterisks mark Dam sites. The lower part shows an alignment of sequences bound by MomR/OxyR. Only the regions protected from MPE·Fe(II) cleavage are shown (see Figure 3 for location of the autoregulatory site, here designated as momR/*oxyR*). Dots indicate homologous positions. The eight nonamer repeats used to derive the 'consensus' on the right are indicated (——>), an additional nonamer motif is indicated (···>). Numbers below the consensus sequence represent the number of identical residues at a position.

Our failure to overexpress the gene when this region is present might indicate that OxyR binding to its own promoter may also interfere with transcription from a promoter further upstream. Autoregulation has been reported for at least four members of the LysR family (for reference see Henikoff *et al.*, 1988).

In the regulatory region of the *mom* operon OxyR protects all three Dam sites (Figure 6) when they are unmethylated. By deletion analysis it has been demonstrated that the region upstream of position −95 is not necessary for methylation dependent *mom* expression (Plasterk *et al.*, 1983) while a deletion extending up to GATC site I renders *mom* expression Dam-independent (A.Seiler and R.Kahmann, unpublished). These deletion endpoints are indicated in Figure 6. In conjunction with the MomR/OxyR footprint, the behaviour of these mutants suggests that essential protein−DNA contacts are made within the first 5 bp of the protected region. At the right border of the region conferring methylation dependence the regions protected by MomR/OxyR and by C overlap by only 4 bp (see Figure 6), leaving at least two possibilities to achieve repression: OxyR binding could block access of C protein to its binding site, alternatively, both proteins could bind simultaneously. In the latter case, due to interaction with OxyR, C might have lost its capability to activate transcription.

It is peculiar that OxyR which normally acts as an activator represses transcription from the *mom* promoter, although the binding site is located in a region where binding could potentially stimulate transcription. At present there are no data avialable pertaining to the question whether hydrogen peroxide might not, for example, convert OxyR to an inducer for *mom* in the absence of C; *mom* expression has always been assayed under conditions where oxidative stress was not applied. In this respect it should also be most interesting to learn where OxyR binding sites are located in promoters that are activated and how the constitutive mutant *oxy*R2 (Christman *et al.*, 1985), behaves with respect to *mom* gene expression.

The two MomR/OxyR binding sites we have analysed share several features; they both comprise 43 bp, of which 20 bp are identical. Conserved positions are scattered over the entire binding region (Figure 6). Each site contains four repeats of a nonamer sequence; the arrangement of this motif in the two sites is identical (Figure 6). Dyad symmetry elements are scarce and do not occur at the same position in both sites (not shown). The size of the protected regions and the arrangement of repeated motifs suggest, that a multimeric form of MomR/OxyR may be the active DNA binding species. For another member of the Lys family, CysB, it has been reported that the protein exists as a tetramer in solution (Miller and Kredich, 1987). Since the autoregulatory binding site does not contain any Dam sites, it is obvious that the ability of MomR/OxyR to discriminate between methylated and unmethylated DNA, as in the *mom* promoter, appears not to be needed for its cellular function.

We have previously assumed that the methylation dependent regulation of the *mom* gene delays the onset of *mom* expression to a phase where Mom-specific DNA modification does not interfere with phage development (Kahmann *et al.*, 1985). The result that the phage burst is not drastically changed in MomR/OxyR mutant strains, however, leads us to consider that repression of the *mom* gene by OxyR may be important at some other stages, e.g. lysogenization, prophage stability or lytic phage development which we have not yet analysed.

The whole scenario of having an operator-like sequence distal to the binding site for a positive regulator is in itself quite unusual. Even if it turns out that it is just a coincidence that OxyR binds to this operator and that this binding is affected by Dam methylation, it is fascinating to see how a phage has recruited a cellular protein and provided it with a novel function.

Materials and methods

Bacterial strains and phages

The following bacterial strains were used: DH5 (Hanahan, 1985), C600 is F$^-$, *thr, leu, lac, ton*B, SuII (Appleyard, 1954), C600(P1Cm) is C600 lysogenic for P1Cm (Toussaint, 1976), CSH50 is F$^-$, *ara*, Δ[*lac pro*], *str*A, thi (Miller, 1972). CSH50*dam*13::Tn*9* was generated by P1 transduction from GM2199 (Marinus *et al.*, 1973), LE392 is F$^-$, *sup*E44, *sup*F58, *lac*IY1 or Δ[*lac*IZY]6, *trp*R55, *gal*K2, *gal*T22, *met*B1, *hsd*R514 and was used to propagate the Tn*5* containing λ phage. CP78 is *arg*E, *thr*1, *leu*B6, *his*65, *gal*3, *thi*1, *xyl*7, *mal*A1, *mtl*2, *ara*13, *ton*A2 (Dabbs, 1980) and was kindly provided by C.Weigelt. NM522 is Δ(*lac−pro*AB], *thi*, *hsd*Δ5, *sup*E, [F′, *pro*AB, *lac*IqZΔM15].

The Tn*5* containing phage λ467 carries b221, *rex*::Tn*5*, *c*I857, *O*am29, *P*am80 and was used to generate a random pool of Tn*5* insertions. P1vir was used in all transductions (Miller, 1972). Mucts62 has a Mom$^+$ phenotype (Howe, 1973). M13K07 (Vieira and Messing, 1987) was used as helper phage for the preparation of single stranded DNA templates.

The Dam$^-$ phenotype of respective strains was verified by isolating chromosomal DNA and restricting it with *Mbo*I.

Plasmids

The following plasmids have been used: pBR322 (Bolivar *et al.*, 1977), pTZ18R and pTZ19R (Mead *et al.*, 1986). pMuAS1C1 contains the rightmost 1110 bp of Mu DNA including the regulatory region of the *mom* operon and intact *com* and *mom* genes (Seiler *et al.*, 1986). pMuPH6R contains the Mu *C* gene including its own promoter on a 2.1 kb *Taq*I fragment cloned into pBR322 (Heisig and Kahmann, 1986). pJLA502 is an inducible

expression vector, carrying the λ P_L and P_R promoters in tandem orientation and the λcI857 repressor gene (Schauder *et al.*, 1987). pMLB1034 contains a truncated *lacZ* gene; the first eight codons, promoter and ribosome binding site are missing (Silhavy *et al.*, 1984).

The tester plasmid pMCCL (8.9 kb) contains a *com–lacZ* fusion gene under control of the *mom* promoter and the Mu *C* gene expressed by its own promoter. The plasmid was constructed by cloning the *Hin*cII–*Bcl*I fragment encompassing region I, the *mom* promoter, and the N-terminal part of the *com* gene from pMuAS1C1 into the *Sma*I and *Bam*HI sites of pMLB1034, thus creating a *com–lacZ* fusion and introducing a *Bam*HI site. In a second step the *C* gene on an *Eco*RI–*Bam*HI fragment from pMuPH6R was inserted into the respective sites of the plasmid generated in the first step.

For the construction of the *momR* overproducing plasmids pJLmomRΔ14–20, the vector pMomR1200 (see results) was linearized with *Bam*HI, treated with *Bal*31 and cut with *Hin*cII. Fragments carrying deletions ending in the 5′ untranslated region of the *momR* gene were subcloned into the *Sma*I site of pTZ18R, excised as *Sal*I–*Eco*RI fragments and cloned into the *Xho*I and *Eco*RI sites of the expression vector pJLA502. To facilitate the labelling of mom promoter containing fragments, the plasmid pTZH380a was constructed by cloning of a 380 bp *Hae*III fragment from pMuAS1C1 encompassing region I and the *mom* promoter (see Figure 1), into the *Sma*I site of pTZ18R. The *mom* promoter reads in the opposite orientation to the *lac* promoter of pTZ18R.

In vitro DNA manipulations

Conditions for enzymic reactions were those described by the suppliers. Other protocols were essentially as described in Maniatis *et al.* (1982). DNA sequence analysis was performed on single and double stranded DNA templates by the chain termination technique of Sanger *et al.* (1977) after subcloning fragments in pTZ18R and pTZ19R.

Tn5 transposon mutagenesis

1 ml of log-phase culture of CSH50*dam13*::Tn9 in dYT containing 0.2% maltose was infected with λ 467 at a multiplicity of infection of 1. The mixture was allowed to stand for 2 h at 28°C. Cells were spread onto YT plates containing 40 μg/ml kanamycin. After overnight incubation at 37°C 30 000 kanamycin-resistant colonies were pooled, grown to OD_{560} = 0.6 in dYT kanamycin and transformed with the plasmid pMCCL. Transformants were selected on YT plates containing 40 μg/ml kanamycin, 100 μg/ml ampicillin and 50 μg/ml X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside). Dark blue colonies were purified and lysogenized with Mucts62. Phage lysates were prepared for determination of the Mom phenotype.

Determination of the Mom phenotype

Mu phage lysates were prepared by thermal induction as described previously (Bukhari and Ljungquist, 1977). Phage titres were determined on C600 and C600(P1Cm). The P1 restriction system restricts unmodified Mu phage while Mom-modified phage is resistant. An efficiency of plating (EOP) [titre on C600(P1Cm)/titre on C600] of $<10^{-4}$ indicates a Mom^-, and an EOP of $>10^{-2}$ indicates a Mom^+ phenotype (Toussaint, 1976). Values in between are considered to indicate partial expression of the *mom* gene.

Overexpression and purification of the momR gene product

DH5 harbouring the overproducing plasmid pJLmomRΔ15 was grown at 28°C in 300 ml dYT containing 60 μg/ml ampicillin to an OD_{550} of 0.8. The culture was shifted to 42°C, and incubation continued for 2 h. The cells were harvested by centrifugation, washed in 15 ml of buffer 1 (10% glycerol, 20 mM Tris hydrochloride, pH 7.5, 1 mM EDTA, 1 mM DTT) containing 100 mM NaCl, resuspended in 15 ml of the same buffer and frozen at −80°C. After thawing, phenylmethylsulphonyl fluoride was added to a concentration of 0.2 mg/ml and the cells were disrupted in a french pressure cell (15 000 p.s.i.) (Figure 4, crude extract). The crude extract was centrifuged at 2000 *g* for 10 min at 4°C. The pellet was resuspended in 30 ml of buffer 1 containing 1 M NaCl; this fraction was highly enriched in MomR protein (Figure 4, fraction P). Partial solubilization was achieved by vigorous shaking for 30 min at 4°C. The suspension was cleared by centrifugation at 18 000 *g* for 30 min at 4°C. The supernatant (Figure 4, fraction S) was stored at −80°C and used in the DNA footprinting experiments. The purification was monitored by SDS–PAGE, using the procedure of Laemmli (1970). Total protein concentrations were determined according to Bradford (1976) using BSA as standard. Total yield of MomR protein was ~10 mg.

MPE·Fe(II) footprinting

Between 10 and 50 ng of 5′-end ^{32}P-labelled restriction fragment was incubated with 25–800 ng of MomR protein in a total volume of 20 μl containing 100 mM NaCl, 25 mM Tris–HCl, pH 7.9, 1.2 mM EDTA, 1 μg BSA and 1 μg sonicated calf thymus DNA for 20 min at 25°C. Cleavage reagent methidiumpropyl-EDTA (Hertzberg and Dervan, 1984) (MPE, kindly provided by P.Dervan) at a concentration of 1.2 mM was mixed with an equal volume of 1.2 mM $Fe(II)(NH_4)_2(SO_4)_2$ and immediately diluted 5-fold with H_2O. 2 μl of this mixture was added, and the reaction started by addition of 2 μl DTT (10 mM), kept for 10 min at 37°C and stopped by adding 50 μl of a solution containing 0.5 M sodium acetate and 20 μg tRNA per ml. DNA was extracted once with phenol and once with phenol:chloroform:isoamylalcohol (25:24:1), precipitated with ethanol and redissolved in loading dye (95% formamide, 10 mM EDTA, 0.1% xylene cyanol, 0.1% bromphenolblue). After incubation for 2 min at 90°C samples were loaded on 0.2 mm thick 6% sequencing gels.

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