The chromosomal protein MC1 from the archaebacterium *Methanosarcina* sp. CHTI 55 induces DNA bending and supercoiling

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ABSTRACT

We have investigated the effect on the DNA structure of protein MC1, a basic and small polypeptide (M_r 10700) representing the major chromosomal protein in Methanosarcinaceae. The ability of protein MC1 to strongly favour cyclization upon polymerization of short DNA fragments by T4 DNA ligase indicates that protein MC1 mediates DNA bending. Several negatively supercoiled topoisomers of minicircles were obtained with DNA fragments of 203 and 146 bp, their distribution depends upon the amount of protein MC1 complexed with DNA. In addition, protein MC1 can induce a compaction of a nicked plasmid.

INTRODUCTION

Protein MC1 is the major protein of the deoxyribonucleoprotein complex in Methanosarcinaceae (1, 2). The association of protein MC1 with DNA *in vivo* has been ascertained by the localization of this protein in DNA-rich areas on immunolabelled cryosections of *Methanosarcina barkeri* (3). Protein MC1 is a polypeptide of 93 amino acid residues exhibiting a marked hydrophilic character given by a high amount of basic and acidic residues (24 and 14 respectively) and by the lack of large hydrophobic domains (4). Protein MC1 has been isolated from various strains (1, 2, 5 and 6) and the main features of its amino acid sequence have been reviewed in (6). With respect to the characteristics of its primary and secondary structures (7), protein MC1 differs significantly from eukaryotic histones, from the eubacterial HU-type protein (also called DNA-binding protein II) and from other archaebacterial chromosomal proteins (6).

Protein MC1 does not form stable repetitive globular structures and does not protect DNA from staphylococcal nuclease digestion (3) as do histones which wrap DNA into nucleosomes. On the other hand, protein MC1 can protect DNA against thermal denaturation (2) and modulate transcription *in vitro* (8). These properties shared with chromosomal proteins involved in chromatin structure, suggest that protein MC1 can modify DNA conformation. To explore the possibility that protein MC1 mediates tight DNA curvatures, we have studied its effect on cyclization of short linear DNA fragments in the presence of DNA ligase, taking into account that the natural stiffness of the double helix hinders ring closure of short DNA fragments. The cAMP receptor protein (CRP) in its specific binding mode and protein HU from *E.coli* which have been shown to facilitate DNA ring closure (9–12) were taken as references in our study.

Interaction of DNA with protein HU results in packaging of DNA which is maintained in a state of negative torsional tension (13, 14). Furthermore protein HU serves as an accessory factor in stimulating the interaction between DNA and proteins involved in DNA replication and transcription (for a review see 13).

Our results show that like CRP and protein HU, protein MC1 favours ring formation upon polymerization of fragments of 203 bp, 146 bp and 114 bp. In addition we show that protein MC1 induces a compaction of a nicked plasmid.

MATERIALS AND METHODS

Methanosarcina sp. CHTI 55 (DSM 2902) was provided by J.P.Touzel (Station de Technologie Alimentaire de l'INRA, Villeneuve d'Ascq, France) and was grown as indicated in (2). *E.coli* T4 DNA ligase was from Appligène (France) and DNA topoisomerase I from calf thymus was from BRL (USA).

Preparation of proteins

Protein MC1 was prepared as described in (7) with the following modifications: after elimination of the contaminants by two successive precipitations with ammonium sulphate at 60% and 70% saturation at pH 8.0, protein MC1 was obtained pure by precipitation with ammonium sulphate at 90% saturation at pH 9.0. Protein HU from *E.coli* was prepared as described in (15), CRP was a gift from Dr.B.Blazy (Institut Pasteur, Paris).

Linear DNA fragments

DNA fragments terminated with EcoRI cohesive ends were used. Their length is indicated without considering the cohesive ends.

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The fragment of 203 bp contains the CRP lactose sites I and II, whereas the fragment of 55 bp derives from the 203 bp and contains only the CRP lactose site II. A vector containing the fragment of 146 bp was given by Dr A.Kolb (Institut Pasteur, Paris); this fragment contains the CRP binding site in the gal promoter region.

Ring closure

DNA-protein complexes were prepared by mixing 2×10^{-8} M DNA fragment and proteins (at protein-to-DNA mass ratios ranging from 0.1 to 1.4) in 50 mM Tris (pH 7.6), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP. Complexes made with CRP were prepared in the presence of 20 μM cAMP. Complexes or DNA alone were preincubated for 30 min at 37°C before incubation with T4 DNA ligase (1 unit) for 30 min at 37°C. DNA was deproteinized with phenol/chloroform/isoamyl alcohol (25:24:1, v/v) and precipitated with ethanol.

Analysis of DNA by electrophoresis

DNA was dissolved in 10 mM Tris (pH 8.0), 1 mM EDTA and analysed by electrophoresis on polyacrylamide slab gels (16×14×0.15 cm) in 89 mM Tris, 89 mM boric acid, 2 mM EDTA (pH 8.0) (TBE buffer). Alternatively, electrophoresis was performed in the presence of 0.6 mg/l ethidium bromide (16); in this case a preelectrophoresis was performed overnight at 100 V. The ligation products of the 55 bp DNA fragments were also analysed by electrophoresis on polyacrylamide slab gels in 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA (pH 7.8). Electrophoresis was performed at 110 V until the bromophenol blue reached the bottom except for the products of the 203 bp fragments for which the run was stopped 30 min after the bromophenol blue reached the bottom. Gels were revealed with ethidium bromide (0.5 mg/l).

Analysis of protein MC1-plasmid DNA complexes

0.3 μg of pBR322 plasmid DNA (the nicked and supercoiled forms taken together) or 0.3 μg of pBR322 nicked plasmid DNA were complexed with increasing amounts of protein MC1 in 10 mM Tris (pH 8.0), 1 mM EDTA, 5 mM $MgCl_2$. After incubation for 20 min at room temperature, the complexes were directly loaded onto a 1% agarose gel in 0.5×TBE buffer and run at 100 V.

RESULTS

The postulated ability of protein MC1 to bend DNA was checked by studying the cyclization of small DNA fragments with EcoRI cohesive ends in the presence of T4 DNA ligase. In order to minimize the formation of linear multimers, DNA concentration was set at 2×10^{-8} M as it was in a similar study performed with *E.coli* CRP (10).

Cyclization of the 203 bp DNA fragment

Figure 1 shows the ligation products obtained with the 203 bp DNA fragment. In the presence of T4 DNA ligase alone, the 203 bp DNA yielded a set of multimers of predominantly large sizes (Figure 1, lane 3). In addition to these multimers, a band migrating with an apparent molecular size of about 560 bp corresponds to a circular monomer of the 203 bp fragment as evidenced below. Upon addition of CRP (at a protein-to-DNA mass ratio of 1.2), only faint bands of linear multimers were visible whereas the band of apparent molecular size 560 bp was enhanced. With protein HU and protein MC1 (at a protein-to-DNA mass ratio of 1.0) no linear multimers were visible and nearly all the material migrated as two bands of apparent molecular sizes 560 bp and 520 bp respectively. When the

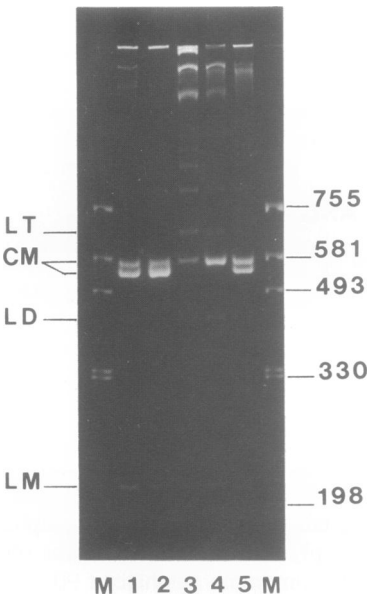

Figure 1. Electrophoretic analysis of ligation products of the 203 bp DNA. Samples were run on a 4% polyacrylamide gel. (1) and (2), DNA complexed with protein MC1 at protein-to-DNA mass ratios (r) equal to 0.4 and 1.0 respectively; (3), DNA alone; (4), DNA complexed with CRP at r = 1.2; (5), DNA complexed with protein HU at r = 1. Letters to the left designate LM, linear monomer; CM, circularized monomer; LT, linear trimer. The linear dimer, LD, is barely detectable. M, size markers derived from hydrolysis of Puc 19 plasmid containing the 203 bp fragment with FnuDII: fragment sizes expressed in bp are indicated on the right.

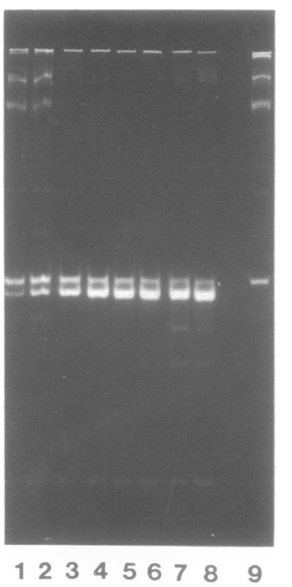

Figure 2. Electrophoretic analysis of ligation products of the 203 bp DNA with increasing amount of protein MC1. Samples were run on a 4% polyacrylamide gel. DNA was complexed with protein MC1 in the following protein-to-DNA mass ratios: (1), 0.1; (2), 0.2; (3), 0.4; (4), 0.6; (5),0.8; (6),1.0; (7),1.2; (8), 1.4. Line 9 corresponds to DNA alone.

deproteinized ligation products were treated with topoisomerase I for 1 h at 37°C, the 520 bp band disappeared and all the DNA migrated as the 560 bp band. This result indicates that the 520 bp band corresponds to topoisomer forms (−1 and −2 as evidenced below) whereas the 560 bp band corresponds to a DNA near to relaxation which is considered as topoisomer 0 in this work.

To confirm that the two ligation products of apparent molecular sizes 560 and 520 bp are circularized monomers of 203 bp, we took advantage of the fact that the 203 bp fragment contains a single HhaI restriction site which divides the molecule into 57 and 144 bp fragments. Treatment with HhaI of the 560 bp and 520 bp bands, extracted from the gel, yielded only fragments of 203 bp without any traces of 118 and 290 bp fragments which would derive from a circularized dimer formed by head-to-head or tail-to-tail bimolecular reactions.

The respective amounts of relaxed and supercoiled DNA depend on the protein-to-DNA mass ratio as shown in Figure 2. At a mass ratio of 0.1, the relaxed minicircle was predominantly obtained together with a small amount of supercoiled minicircle (Figure 2, lane 1). At higher protein-to-DNA ratios, the amount of supercoiled DNA gradually increased at the expense of relaxed DNA and the multimers of high molecular sizes disappeared (Figure 2, lanes 2−8). It is worth pointing out that gel retardation experiments performed in similar conditions of formation of protein-DNA complexes showed that from a protein-to-DNA mass ratio of 0.25 and above, most of the DNA fragments are complexed (F.Culard, personal communication).

When DNA was incubated with T4 DNA ligase alone or together with CRP which introduces very few DNA untwisting if any (10), only relaxed minicircles were obtained as evidenced by electrophoresis performed in the presence of ethidium bromide (Figure 3, lanes 2 and 3). On the other hand, with the complex DNA-protein HU two topoisomers were obtained, the topoisomer 0 and the topoisomer −1 (Figure 3, lanes 4 and 5). Indeed Rouvière-Yaniv *et al.* (17) and Pettijohn (14) showed that protein HU introduces negative superhelical turns in DNA. With the complex DNA-protein MC1, three topoisomers were observed, the topoisomer 0 which appears as a faint band and most probably topoisomers −1 and −2 (Figure 3, lane 1).

Cyclization of shorter DNA fragments

It has previously been shown that the shorter the DNA fragment, the more severely limited the possibility is to form DNA minicircles because the stiffness of the linear DNA hinders contact of its ends (18). To further study the ability of protein MC1 to bend DNA, it was of interest to check whether this protein could mediate cyclization of DNA fragments shorter than 203 bp. Two DNA fragments of 146 bp and 55 bp respectively were used in this study.

The cyclization of the 146 bp fragment by CRP in its specific binding mode was taken as a control in the same experimental conditions used by Dripps and Wartell for a 144 bp fragment (10). Upon addition of CRP (Figure 4, lane 9) a band of circular monomer was observed together with two faint bands of linear dimer which were also obtained with T4 DNA ligase alone (Figure 4, lane 8). The unexpected migration of these bands will be commented on below. It is noteworthy that, as observed by Dripps and Wartell, no trace of circular monomer was obtained with T4 DNA ligase alone which indicates that this short DNA fragment is unable to cyclizise without induction of a bend by a protein. Upon addition of protein MC1 at a protein-to-DNA ratio of 0.1 (Figure 4, lane 1), we observed an intense band of electrophoretic migration identical to that of the circular monomer obtained with CRP. This band became more intense at protein-to-DNA ratios of 0.2 and 0.4 (Figure 4, lanes 2 and 3) and was

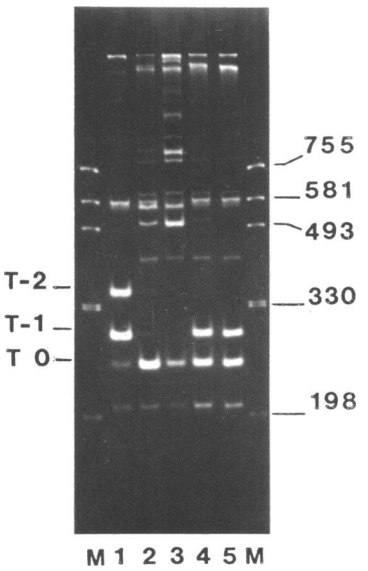

Figure 3. Analysis of ligation products of the 203 bp DNA by electrophoresis in the presence of ethidium bromide. Samples were run on a 4% polyacrylamide gel after an overnight preelectrophoresis. Preelectrophoresis and electrophoresis were performed in the presence of 0.6 mg/l ethidium bromide in the anodic buffer chamber. (1), DNA complexed with protein MC1 at a protein-to-DNA mass ratio (r) of 1.0; (2), DNA complexed with CRP at r = 1.2; (3), DNA alone; (4) and (5), DNA complexed with protein HU at r = 1.0 and 1.5 respectively. M, size markers as in Figure 1. T 0, T −1 and T −2 indicate topoisomers 0, −1 and −2 respectively.

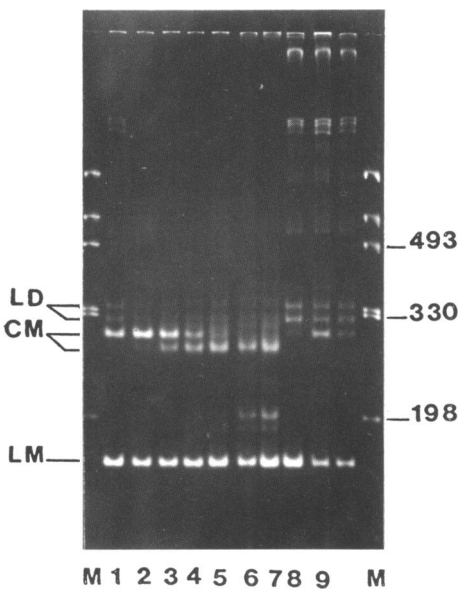

Figure 4. Electrophoretic analysis of ligation products of the 150 bp DNA. Samples were run on a 4% polyacrylamide gel. (1 to 7), DNA-protein MC1 complexes as in Figure 2; (8), DNA alone; (9), DNA complexed with CRP at r = 1.2. M, size markers as in Figure 1.

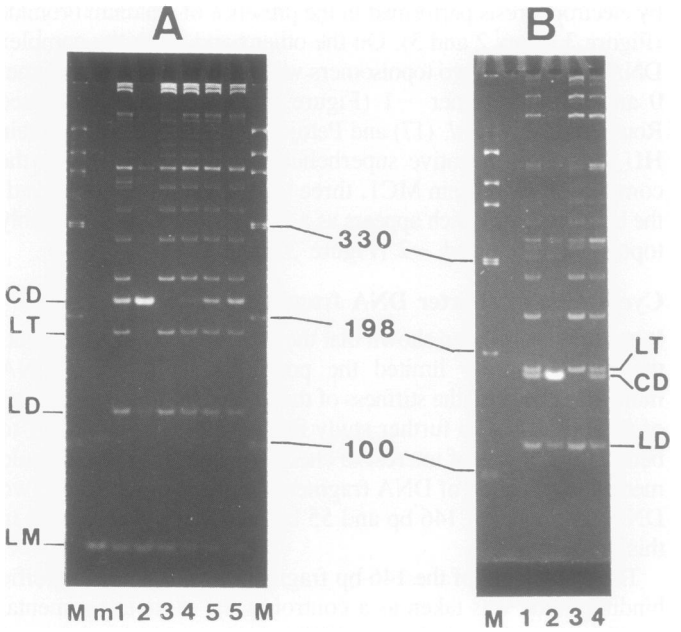

Figure 5. Electrophoretic analysis of ligation products of the 55 bp DNA. A, electrophoresis was performed on a 6% polyacrylamide gel in 89 mM Tris, 89 mM boric acid, 2 mM EDTA (pH 8. 0). B, electrophoresis was performed on a 5% polyacrylamide gel in 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA (pH 7.8). (1) and (2), DNA complexed with protein MC1 at protein-to-DNA mass ratios (r) of 0.4 and 1.0 respectively; (3), DNA alone; (4), DNA complexed with CRP at r = 1.2; (5), DNA complexed with protein HU at r = 1.0. M, size markers as in Figure 1. m, size markers of 103 and 59 bp.

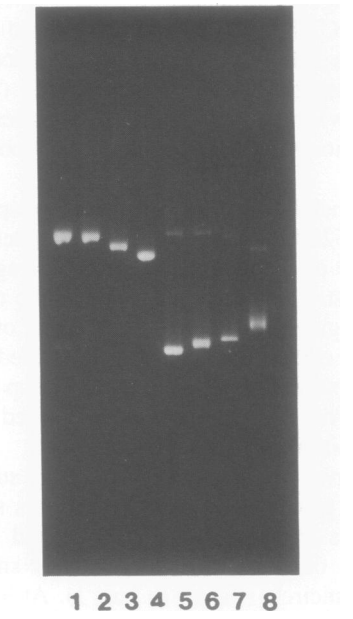

Figure 6. Electrophoretic analysis of protein MC1-plasmid DNA complexes. Electrophoresis was performed on a 1% agarose gel in 0.5×TBE buffer. (1) and (5), nicked and supercoiled pBR322 plasmid respectively. (2), (3) and (4), nicked pBR322 plasmid complexed with protein MC1 at protein-to-DNA mass ratios of 0.04, 0.2 and 0.4 respectively. (6), (7) and (8), supercoiled pBR322 plasmid complexed with protein MC1 at protein-to-DNA mass ratios of 0.04, 0.2 and 0.4 respectively.

progressively replaced by a band of higher electrophoretic mobility when larger amounts of protein MC1 were added to DNA (Figure 4, lanes 4−7). This fast band corresponds to a topoisomer of the circular monomer obtained at low protein-to-DNA ratios (Figure 4, lanes 1−3) since it comigrated with it after treatment with topoisomerase I (data not shown). It has to be emphasized that unexpectedly, two linear dimer forms of apparent molecular sizes 320 bp and 340 bp respectively are generated with the 146 bp DNA. This result may be explained by a natural curvature due to an enrichment in adenine at the 5′ end of the linear monomer. Thus two dimer forms with different electrophoretic mobilities may be generated depending on ends involved in the bimolecular ligation reactions.

Figure 5A show the analytical electrophoresis of the ligation products of the 55 bp fragment in the TBE buffer. After incubation with T4 DNA ligase alone, we can clearly distinguish the monomer and its multimers at least up to the heptamer (lane 3). Upon addition of protein MC1 at a protein-to-DNA ratio of 0.4 (Figure 5A, lane 1), a new DNA band appeared between the trimer and the tetramer: this band corresponds to a DNA ring. When the protein MC1-to-DNA ratio rose to 1, this band significantly increased while the linear multimers became barely visible (Figure 5A, lane 2). There is no simple way to determine whether this DNA ring is a dimer or a trimer of the 55 bp fragment. However the two following observations strongly suggest that this ring is 114 bp in length and is generated after dimerization of the 55 bp fragment. Firstly, this DNA ring migrated faster than the linear trimer in Tris−acetate−EDTA buffer (Figure 5B, lanes 1 and 2) and it is known from previous studies (9, 10 and 12) that a circularized DNA migrates more slowly than its linear counterpart. Secondly, this DNA ring was also obtained with protein HU (Figure 5A, lane 5 and Figure 5B, lane 4) and its size (114 bp) is consistent with the finding of Hodges-Garcia *et al.* who showed that protein HU can mediate ring closure of a 99 bp fragment (12).

Interaction of protein MC1 with a plasmid DNA

The fact that several topoisomers of the minicircles formed with the 203 bp and 146 bp fragments were obtained led us to study the effect of protein MC1 on relaxed (nicked) and supercoiled plasmid DNA in order to get more information on the DNA conformational changes mediated by protein MC1. pBR322 plasmid DNA was incubated with protein MC1 and the complexes were directly analysed by gel electrophoresis (Figure 6). Increasing amounts of protein MC1 induced a gradual increase of the mobility of the nicked plasmid (Figure 6, lanes 1−4) and a gradual decrease of the mobility of the supercoiled plasmid (Figure 6, lanes 5−8).

DISCUSSION

Like the *E.coli* protein HU and CRP, the *Methanosarcina* sp. CHTI 55 protein MC1 strongly favors cyclization upon polymerization of the 203 bp fragment by T4 DNA ligase. Furthermore, the protein MC1 can mediate cyclization of fragments of 146 bp and 114 bp (this latter deriving from dimerization of the 55 bp fragment) which are unable to form ring in the absence of proteins CRP, HU or MC1. These findings indicate that protein MC1 is able to introduce tight DNA curvatures.

The 203 bp DNA fragment yields a relaxed minicircle when complexed with the CRP whereas it gives rise to several topoisomers when complexed with proteins HU and MC1. These

topoisomers are topoisomers 0 and −1 when cyclization is mediated by the protein HU and topoisomers 0, −1 and −2 in the presence of protein MC1. The distribution of topoisomers depends on the amount of protein MC1 added: the amount of topoisomers of lower linking numbers increases upon addition of larger amounts of protein MC1. Moreover, from electrophoretic analysis in presence of ethidium bromide, the topoisomer −2 is obtained at protein-to-DNA ratios of 0.8 and above (data not shown). Introduction of negative superhelical turns in DNA by protein HU was previously evidenced using either the relaxed circular SV40 DNA (17) or the relaxed pMB9 DNA (14). In these experiments, the number of superhelical turns in the DNA also depended on the amount of protein HU added: it was maximum at a protein-to-DNA mass ratio of 1.0. At this ratio, the linking number change is equal to −1 for about 270 bp (14, 17). Our results with protein HU are consistent with these data since topoisomers 0 and −1 were obtained with the fragment of 203 bp. The effect of protein MC1 on the linking number is higher than that of protein HU. In fact, a change in the twist and a change in the writhing in superhelical turns are two mechanisms which can contribute to modify the linking number of a DNA during its covalent closure mediated by a protein. In the case of protein MC1, no information on a putative effect on the double-helix torsion is available yet, therefore the contribution of each mechanism to the MC1-DNA interaction remains to be determined.

On the other hand, we have observed an higher electrophoretic mobility of the nicked plasmid, when associated with the protein MC1. The positive charge and the additional mass provided by the protein should result in a decreased electrophoretic mobility of the protein-DNA complex. The enhanced mobility of this complex likely reflects a folding of the DNA. Mignotte *et al.* have observed a similar effect with a mitochondrial protein which compacts DNA (19). Therefore our data strongly suggest that protein MC1 is involved in DNA packaging.

When associated with protein MC1 at a protein-to-DNA mass ratio of 0.1, which corresponds to one protein molecule for 170 bp of DNA, most of the 203 bp DNA fragment forms DNA rings. Since protein MC1 is present in the deoxyribonucleoprotein complex of the archaebacterium *Methanosarcina barkeri* at a protein-to-DNA mass ratio of 0.11 (3), the amount of protein MC1 appears sufficient to significantly change the chromosomal DNA conformation.

Although the derivative melting profiles of protein MC1-DNA complexes resemble those of nucleosomal particles (2), DNA packaging must result from a mechanism different from the one encountered in eukaryotes since the deoxyribonucleoprotein complex of *M.barkeri* is devoid of stable nucleosomal structure (3). On the other hand, the ability of protein MC1 to induce DNA bending and DNA torsional constraints is likely correlated with the effect of protein MC1 to modulate *in vitro* transcription (8). Together with the eubacterial protein HU and other proteins such as the integration host factor (20, 21), protein MC1 may form a DNA binding protein family which modulates gene regulation, a function achieved through bending.

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