DNA bending and expression of the divergent nagE–B operons

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Received November 7, 1997; Revised and Accepted January 14, 1998

ABSTRACT

Repression of the divergent nagE–B operons requires NagC binding to two operators which overlap the nagE and nagB promoters, resulting in formation of a DNA loop. Binding of the cAMP/CAP activator to its site, adjacent to the nagE operator, stabilizes the DNA loop in vitro. The DNA of the nagE–B intergenic region is intrinsically bent, with the bend centred on the CAP site. We show that displacement of the CAP site by 6 bp results in complete derepression of the two operons. This derepression is observed even in the absence of cAMP/CAP binding and despite the fact that the two NagC operators are still in phase, demonstrating that the inherently bent structure of the DNA loop is important for repression. Since no interaction between NagC and CAP has been detected, we propose that the role of CAP in the repression loop is architectural, stabilizing the intrinsic bend. The cAMP/CAP complex is necessary for activation of the nagE–B promoters. In this case protein–protein contacts between CAP and RNA polymerase are necessary for full activation, but at least a part of the activation is likely due to an effect of CAP binding altering DNA structure.

INTRODUCTION

The divergent nagE–BACD operons encode proteins involved in the uptake and degradation of N-acetylgalactosamine (GlcNAc). The nagE gene encodes the N-acetylgalactosamine-specific transporter of the phosphotransferase system (PTS) (reviewed in 1), producing intracellular GlcNAc-6-P. The nagB and nagA genes encode the proteins necessary for catabolism of GlcNAc-6-P, producing fructose-6-P and NH3 (2–4). We have previously shown that expression of the nagE and nagBA genes is co-ordinately regulated (5). The Nag repressor, encoded by the nagC gene, binds to two operators, BoxE and BoxB, which overlap the nagE and nagB promoters and forms a repression loop of DNA. The centres of these two boxes are separated by 93 bp, i.e. by nine turns of B-form DNA double helix, so that the two boxes lie on the same face of the DNA. We have demonstrated the formation of this DNA loop in vitro and in vivo (5,6). In vitro DNase I footprinting shows that NagC binding completely protects the two operators from DNase I attack and, in addition, there is a change in the footprint in the intervening region, with formation of seven hypersensitive DNase I cleavage sites. This is diagnostic of DNA loop formation (7,8). In vivo we have shown that the presence of the two functional sites, situated in phase on the DNA, is necessary for repression of either operon. Thus a mutation which prevents NagC binding to BoxE causes derepression of nagB as well as nagE. Similarly an insertion of 6 bp, corresponding to half a helical turn of DNA, within the loop results in derepression of both operons (5). Although DNA loop formation by several prokaryotic repressors has been detected in vivo (9–12), in certain cases demonstration in vitro needed the use of supercoiled templates or accessory proteins to stabilize the loop (13–15).

Expression of both nagE and nagB is stimulated by the cAMP/CAP complex, the effect being particularly pronounced for nagE. A binding site for cAMP/CAP is located between the two promoters, i.e. it lies within the DNA which forms a loop (6). It is centred at –61.5 bp relative to the nagE transcriptional start site and at –71.5 bp compared with nagB. These two distances are typical for Class I promoters like lac and malT. A Class I promoter is activated only by cAMP/CAP and the site is situated at about –61.5 bp or further upstream of the transcription start site (reviewed in 16). An interesting observation in the case of cAMP/CAP binding to the nagE–BA operators is that binding of cAMP/CAP stabilizes the repression loop in vitro, as demonstrated by the enhanced stability of the ternary complex DNA–NagC–cAMP/CAP compared with the binary complex DNA–NagC on DNA retardation/band shift gels (6).

As CAP is a transcriptional activator protein its presence in a repression loop could appear paradoxical, but in fact it is not a unique situation. For the CytR repressor cAMP/CAP is an obligatory adaptor permitting binding of CytR to its sites and repression of transcription (reviewed in 17). Unlike the CytR case, cAMP/CAP is not essential for repression of the nag genes. In strains where cAMP/CAP cannot bind (e.g. in a Δcyt strain) expression of the nag operons is very low, indeed their expression is minimal, since in the absence of both cAMP/CAP and GlcNAc there is no expression above basal level. When GlcNAc is added to the culture expression rises due to displacement of NagC from its binding sites. When cAMP is added expression increases due to cAMP/CAP stimulation of the basal level and when the two are added together full induction is achieved (5,18).

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CAP binding to DNA produces a bend in the DNA of 90° (19) and a characteristic effect on the DNase I footprint (20). The footprints of the nag intergenic region show that the pattern of DNase I protection and cleavage sites due to CAP binding is in phase with the hypersensitive cleavages produced by NagC and that the two proteins are binding in phase (6). The centre to centre distances between BoxE and CAP and BoxB and CAP are 30.5 and 63.5 bp respectively, i.e. they are separated by three and six turns of a B-form DNA helix. These observations implied that NagC and CAP were binding to the same side of the DNA helix and posed the question as to whether the role of CAP in repression was due to a direct interaction between CAP and NagC, similar to the CytR case, or whether the role of CAP was purely structural, by creating/stabilizing a bend to increase stability of the repression complex. This kind of specific architectural role has been demonstrated for the ubiquitous DNA binding proteins HU and IHF in several nucleoprotein complexes (reviewed in 21). Recently it has been shown that the histone-like binding proteins HU and IHF in several nucleoprotein complexes (23,24), making it analogous to the NagC and CAP effect. Although HU is not a sequence-specific DNA binding protein, it apparently occupies a specific location in the looped nucleoprotein complex with GalR (23,24), making it analogous to the CAP binding site in the Nag repression loop.

It is also possible that the effect of CAP binding on DNA structure has a role in activation. Curved DNA sequences replacing the cAMP/CAP site can enhance transcription at the polymerase binding to non-CAP-dependent promoters (27). In this work we describe two types of experiments to address the question of the role of cAMP/CAP in repression and activation of the nagE–BA operon. The results imply that the role of CAP as a stabilizing influence on formation of the repression loop is purely structural. Moreover, they also show that formation of the repression loop is dependent on the intrinsic structure of the DNA in the loop. For activation of nag expression by CAP part of the activation can probably be assigned to the DNA bending properties of CAP. This is particularly true in the case of nagB, but activation of nagE requires in addition protein–protein interactions between CAP and the RNA polymerase machinery.

MATERIALS AND METHODS

Strains and plasmids

The following strains were used: IBPC5321 (thi-1, his-4, argE3, argG6, xyl-5, mtl-1, lac, X74) and TP2006 (ΔlacX74, Δcya, xyl, gfp-8306) (28). The crp plasmids carrying the pc mutations were pDCRP derivatives carrying H159L, Δ159 or G162C mutations (29–31) and were the generous gift of Steve Busby, pDU9 is the same vector without a 200 bp fragment (Fig. 1) was inserted into pRS415 to make a NagE–lacZ transcriptional fusion and into pRS1274 to make a similar nagE–lacZ fusion (32). The mutations CAP+6 and CAP–6 were made by oligonucleotide mutagenesis, by the method of Kunkel as described (33), on the 200 bp fragment cloned into pTZ18R. The mutation CAP+6 creates an XhoI site, while the CAP–6 deletion creates an SphI site. The double mutation was made by a second round of mutagenesis using a template carrying the CAP+6 mutation. A nagE–lacZ fusion missing the nagB operator (BoxB) and promoter was made by creating a BamHI restriction site by oligonucleotide (Oligo Nag42, Fig. 1) mutagenesis at positions −99 to −104, i.e. in the region of the −35 sequence of the nagB promoter. This truncated BamHI–EcoRI fragment was inserted into pRS1274 to create a nagE–lacZ fusion carrying just the nagE operator (BoxE) and the CAP binding site. The plasmid borne fusions were transferred to bacteriophage λ as described (34) and the resulting lysates used to lysogenize bacteria at low multiplicity and several colonies tested to identify monolysogens. The presence of mutations on the phage was verified by sequencing. β-Galactosidase activities were measured on four aliquots taken during exponential growth as described previously (32,35). Errors between duplicate experiments were generally <15%. The ΔcrpB mutation was introduced into strain IBPC5321 carrying the different fusions by P1 co-transduction with the λhu732::Tn10 transposon (36).

DNA binding assays

NagC and CAP binding to the nagE–B intergenic region were measured by band shift analysis as described previously (6). The buffer was 25 mM HEPES, 100 mM Na glutamate containing 0.5 mg/ml BSA. Labelled DNA fragments were made by PCR using oligonucleotides Nag14E and Nag15B (Fig. 1). One oligo, which near nagE, was 5′-end-labelled by [γ-32P]ATP and polynucleotide kinase. To synthesize fragments carrying just the CAP site and BoxE operator oligonucleotide Nag42B (used to create the internal BamHI site described above) was used. The templates were plasmids carrying the wild-type or mutant intergenic regions. The BoxE super-operator template carried −11G+11C changes in BoxE (32).

RESULTS AND DISCUSSION

Displacement of the CAP site derepresses the nagE–B operons

Three mutations were created in the nagE–B regulatory region by inserting 6 bp between BoxE and the CAP site (mutation CAP+6), by removing 6 bp from between the CAP site and BoxB (mutation CAP–6) and by combining the two to make the double mutation (CAP+6–6) (Fig. 1). These mutations have consequences on both CAP and NagC effects. Insertion or deletion of 6 bp corresponds to half a helical turn of B-form DNA and thus re-orientates the binding site onto the other side of the DNA helix. The mutation CAP+6 puts the CAP site at a non-functional distance for NagE binding site onto the other side of the DNA helix and is easily seen for the fusions where the CAP site is at its normal (functional) location. The CAP–6 mutation puts the CAP site at a non-functional distance for NagB, also expected to be non-functional for cAMP/CAP activation (37,38). Both these single mutations put the two Nag boxes out of phase and, from our previous results (5), would be expected to derepress the two operons. Indeed, this does occur and is easily seen for the fusions where the CAP site is at its normal (functional) location. The CAP–6 mutation results in a 35-fold derepression of nagE (−67.5), while the CAP–6 mutation puts the CAP site at −65.5 for nagB, also expected to be non-functional for cAMP/CAP activation (37,38). Both these single mutations put the two Nag boxes out of phase and, from our previous results (5), would be expected to derepress the two operons. Indeed, this does occur and is easily seen for the fusions where the CAP site is at its normal (functional) location. The CAP–6 mutation results in a 35-fold derepression of nagE and the CAP+6 mutation produces a 35-fold derepression of nagB (Table 1). These values are similar to that produced by growth on GlcNAc for the wild-type construct. The effects of these mutations on fusions where the CAP site is now at a non-functional distance are different.

Considering the effect of the CAP–6 mutation on nagB there is still a large derepression of the fusion (13-fold), showing that, even in the absence of cAMP/CAP activation, the lack of
Figure 1. Organization of the nagE–B intergenic region. The relative positions of the NagC operators and CAP binding site are shown by the hatched and shaded boxes respectively. The sequence is numbered from the nagE transcriptional start site; on this scale the nagB start site is at –133. The –35 and –10 sequences of the two promoters are underlined. The locations of the mutations CAP+6 and CAP–6 are shown, as is the second BamHI site used to make a fragment carrying just the single BoxE operator and the CAP site. The 5′-ends of the three oligonucleotides Nag14E, Nag15B and Nag42B are shown by asterisks.

Repressor binding is sufficient to stimulate nagB expression. The greater expression of nagB in the CAP+6 context than in the CAP–6 case correlates with the fact that activation by CAP is still possible with the CAP+6 mutation (the CAP site is still at –71.5) but not at CAP–6, where the CAP site is now located at –66.5, i.e. on the other side of the DNA helix compared with RNA polymerase (5,18). There is no obvious effect of the CAP+6 mutation on nagE expression but this is presumably due to two opposing effects which are of similar magnitude: an ~10- to 15-fold increase in nagE expression due to loss of repressor binding (equivalent to that seen for the nagB fusion) and a 10- to 15-fold decrease in nagE expression due to a lack of cAMP/CAP activation (5; see also below).

For the double mutation CAP+6–6 the two NagC boxes are now in phase, separated by 93 bp, i.e. by nine turns of the DNA helix, as in the wild-type situation, but unexpectedly there is no re-establishment of the repressed state; the levels of expression are those observed for single mutations with the CAP site at the non-functional distance (Table 1).

Effect of cAMP/CAP binding to the nagE–B intergenic regions with displaced CAP sites

In the CAP+6–6 construct the CAP site, displaced by 6 bp towards nagE, is now out of phase with binding of NagC at both operators and effectively it should mean that the CAP binding site is situated on the outside of the loop rather than within the DNA loop. The derepression observed with the CAP+6–6 mutation could be due to the fact that CAP is permanently bound even during growth on glucose and is bending the DNA in the direction opposite to that required for loop formation. There is circumstantial evidence suggesting that the CAP site in the intergenic nagE–B region is, at least partially, occupied during growth on glucose. The increase in nagE expression between glucose and glycerol is only 3- to 4-fold, while addition of cAMP to the Δcya strain increases expression >10-fold (Table 2; 5,18).

<table>
<thead>
<tr>
<th>Fusion</th>
<th>Loop size</th>
<th>nagE–lacZ</th>
<th>Glc</th>
<th>GlnAc</th>
<th>nagB–lacZ</th>
<th>Glc</th>
<th>GlnAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>9</td>
<td>–61.5</td>
<td>1</td>
<td>24</td>
<td>–71.5</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>CAP+6</td>
<td>9.5</td>
<td>–67.5</td>
<td>1</td>
<td>1.3</td>
<td>–71.5</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>CAP–6</td>
<td>8.5</td>
<td>–61.5</td>
<td>32</td>
<td>43</td>
<td>–65.5</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>CAP+6–6</td>
<td>9</td>
<td>–67.5</td>
<td>1</td>
<td>1.7</td>
<td>–65.5</td>
<td>12</td>
<td>42</td>
</tr>
</tbody>
</table>

β-Galactosidase activities of the nagE–lacZ and nagB–lacZ fusions carrying the different mutations were measured in BP5521R during growth in minimal medium with 0.2% glucose (Glc) or 0.2% GlnAc as carbon source. The relative expression levels in Glc and GlnAc are compared with the basal level for each fusion. For nagE–lacZ the basal level is 21 U and for nagB–lacZ it is 74 U. Loop size gives the distance between the centres of the NagC operators (BoxB and BoxE) in helical turns assuming a pitch of 10.4 bp/turn of B-form DNA. The centre of the CAP site is given relative to the transcriptional start site of the nagE and nagB gene depending on the fusion under study.
Quantitation of CAP binding to the wild-type and mutant DNA regulatory regions by band shift analysis (Fig. 2) showed that the mutations have not altered the binding affinity of CAP. Essentially all the DNA determinants required for maximal CAP affinity are present in a 28 bp fragment with the 22 bp consensus sequence at its centre (39,40). Neither the CAP+6 nor the CAP–6 mutation altered the 22 bp core consensus CAP binding site. The CAP+6 mutation changed the outermost 3 bp of the left flanking sequence of this extended binding region but otherwise it is not surprising that the four sites showed roughly the same affinity for CAP. This means that the lack of repression by NagC binding to the CAP+6–6 regulatory region could be due to the adverse effect of CAP binding to the outside of the loop.

To determine if this was the case the effect of the CAP displacement mutations was measured in the absence of CAP binding in a Δcya background (Table 2). When the CAP site was at a non-functional distance for the promoter of the gene fusion addition of cAMP had no effect on expression. However, cAMP caused a large (15-fold) stimulation of nagE and a 2.5-fold stimulation of nagB when the CAP site was located at its functional distance. For the double mutation the CAP site is wrongly placed for both nagE and nagB expression. The fusions carrying the double mutation were equally derepressed in the absence and in the presence of cAMP (Table 2). Thus it is not binding of cAMP/CAP out of phase with the NagC operators which is causing the derepression but it is an inherent property of the DNA structure in the mutants, which can no longer form a repression loop with NagC. The lack of complex formation is confirmed by band shift analysis of NagC binding to the wild-type and mutant regulatory regions in the presence of cAMP/CAP. For the wild-type there is significant formation of the highly retarded looped compound, as seen previously (6). All three mutations (CAP+6, CAP–6 and CAP+6–6) effectively eliminate the complex, i.e. NagC cannot bind to these DNAs and form a loop (Fig. 3).

The nagE–B intergenic region is intrinsically bent

The above results show that the sequence of the DNA per se affects repressor binding. The nagE–B region DNA shows retarded migration on a native acrylamide gel, implying that it is intrinsically bent. A 232 bp fragment covering the wild-type nag regulatory region migrates like that of a fragment of 281 bp (retardation factor R = 1.21). In contrast, the fragment with the CAP–6 mutation migrates about as expected (234 bp), while the fragments carrying CAP+6 and CAP+6–6 show some intermediate migration (274 and 257 bp respectively). These relative mobilities

### Table 2. Effect of displacing the CAP site on expression of the nagE and nagB genes in a Δcya strain

<table>
<thead>
<tr>
<th>Fusion</th>
<th>Loop size</th>
<th>nagB–lacZ CAP site Gly Gly + cAMP</th>
<th>nagE–lacZ CAP site Gly Gly + cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>9</td>
<td>−61.5</td>
<td>1</td>
</tr>
<tr>
<td>CAP+6</td>
<td>9.5</td>
<td>−67.5</td>
<td>5.9</td>
</tr>
<tr>
<td>CAP–6</td>
<td>8.5</td>
<td>−61.5</td>
<td>13</td>
</tr>
<tr>
<td>CAP+6–6</td>
<td>9</td>
<td>−67.5</td>
<td>6.9</td>
</tr>
</tbody>
</table>

β-Galactosidase activities of the nagE–lacZ and nagB–lacZ fusions carrying the different mutations were measured in TP2006 (Δcya) during growth on glycerol (0.4%) medium, with 2 mM cAMP where indicated. The relative expression levels are compared with the basal value for each fusion, 5.2 U for nagE–lacZ and 56 U for nagB–lacZ. The loop size and location of the CAP site are defined in the legend to Table 1.

![Figure 2](image-url)  
**Figure 2.** CAP binding to wild-type and mutant operators. 5′-End-labelled DNA fragments carrying the wild-type and mutant sequences were made by PCR with oligonucleotides Nag14E and Nag15B. The DNA was incubated with CAP in the presence of 0.2 mM cAMP, 10 mM Mg<sup>2+</sup> and 50 µg/ml poly(dI·dC). Lanes 1, 5, 9 and 13, no CAP; lanes 2, 6, 10 and 14, 1.5 nM CAP; lanes 3, 7, 11 and 15, 15.5 nM CAP; lanes 4, 8, 12 and 16, 15 nM CAP. Complexes were separated on a 6% acrylamide gel. Band F is the uncomplexed DNA and band I is the complex with CAP.

![Figure 3](image-url)  
**Figure 3.** NagC binding to wild-type and mutant operators. DNA as in Figure 2 was mixed with varying amounts of extracts from a NagC overproducing strain in the presence of 1.5 nm CAP and 0.2 mM cAMP. Lane 1, free DNA; lanes 2, 6, 10 and 14, 1.5 nm CAP alone; lanes 3, 7, 11 and 15, 1.5 nm CAP plus 10 µg/ml NagC extract; lanes 4, 8, 12 and 16, 1.5 nM CAP plus 40 µg/ml NagC; lanes 5, 9, 13 and 17, 1.5 nM CAP plus 160 µg/ml NagC. Complexes were separated on a 6% acrylamide gel. Band F is the uncomplexed DNA, band I is the complex with CAP and band II is the looped complex with NagC and CAP which forms on the wild-type fragment.
are seen for the free DNA on the mobility shift gels of Figures 2 and 3. The use of DNA structure prediction programs to look at the form of the regulatory region helped to clarify these observations. Two programs with different parameters (41; and the Bend program of the DNAstar package) were used with similar results. The wild-type fragment (in the absence of cAMP/CAP binding) is predicted to bend through 90° with the centre of curvature at the CAP consensus site. The three mutant forms, with 6 bp of DNA inserted and/or removed on either side of the CAP site are subject to very different constraints and demonstrate a much smaller overall bend angle (Fig. 4). The simple interpretation of the above experimental results taken together with these structural predictions is that NagC can bind to its two operators and interact when the DNA is already bent through 90° but cannot bind co-operatively to a more inherently linear piece of DNA. We propose that binding of CAP to its site enhances the intrinsic bend and thus helps stabilize the NagC–DNA complex in repression of the deoP1 (44; reviewed in 17). It is not easy to measure NagC binding to fragments containing just one functional BoxE site because binding is too weak. However, we recently identified a ‘super-operator’ version of the BoxE operator which has a much higher affinity for NagC and allows detection of a single site BoxE–NagC complex by gel retardation analysis (32). If co-operative binding between CAP and NagC bound to the single BoxE operator exists we reasoned that this should be detected on the super-operator fragment as preferential formation of the ternary DNA–NagC–cAMP/CAP complex, as is observed for the fragment carrying both BoxE and BoxB (Fig. 3; 32). However, the two proteins appear to bind independently and proportionally to their concentration (Fig. 5). At higher concentrations of the two proteins a more highly retarded protein complex is detected, which presumably corresponds to the ternary complex. However, as it is seen in the presence of the two binary complexes NagC–DNA and cAMP/CAP–DNA, it implies that binding is stochastic rather than synergistic.

In conclusion, there is no evidence that the role of the cAMP/CAP complex in repression of the nagE–B operons is anything other than structural. We next tried to see whether DNA bending and/or protein–protein contacts are involved in CAP activation of the nagE–B operons.

**CAP-positive control mutations strongly reduce CAP activation of nagE but do not eliminate CAP activation of nagB**

Positive control mutations in CAP (crp pc mutations) which prevent activation of Class I promoters have been localized to a surface exposed loop comprising residues 156–163 of the CAP polypeptide chain (29–31,45). Several mutations in this region have been described which prevent activation but which still bind normally to DNA. Activation requires that this region of CAP interacts with the C-terminus of the α-subunit of RNA polymerase (reviewed in 46,47). We tested three well-characterized crp pc
For both $nagB$ and $nagE$ in the looped constructs the CAP\(^{pc}\) mutations are apparently more effective at activating transcription in the induced situation the inducer there is effectively no activation of basal $nagB$ lacZ expression (80%) seen with wild-type CAP at the $nagE$ promoter is due to their ability to bend the DNA, while the majority of the activation (80%) seen with wild-type CAP at the $nagE$ promoter is due to direct contact between CAP and RNA polymerase. Since the same level of activation is seen with the truncated single operator site $nagE$–lacZ fusion it implies that no DNA important for CAP stimulation of $nagE$–lacZ expression is situated beyond –100.

The residual activation seen with the CAP\(^{pc}\) mutations varies appreciably with the promoter under test. For the artificial promoters constructed specifically for this type of experiment [the CC (29) and UT series (48)] residual activation with the different $crp^{pc}$ mutations can be very low (<5%), however, on other promoters it is significantly higher. For example, the H159L mutation eliminates activation from the CC+20 promoter (29), allows 17% activation at the $nagE$ promoter (30) and reduces activation by 7- to 12-fold at a series of promoters derived from the $malT$ promoter (29). This last paper demonstrates that residual activation of a $crp^{pc}$ mutation depends upon the DNA sequence and the 20% activation of $nagB$ lacZ observed with the CAP\(^{pc}\) proteins (25,26). The enhanced bending, by whatever means, should facilitate contact between RNA polymerase and the upstream sequences shown to be important for activation at several promoters (reviewed in 46). In particular, at Class I promoters, i.e. where the CAP site is situated at –61.5 or further upstream, which includes both $nagE$ and $nagB$, the

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**Table 3. Effect of CAP\(^{pc}\) mutations on expression of the $nagE$ and $nagB$ genes**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>$nagB$–lacZ</th>
<th>$nagE$–lacZ</th>
<th>$nagE$–lacZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>looped</td>
<td>looped</td>
<td>single site</td>
</tr>
<tr>
<td></td>
<td>Gly Gly</td>
<td>+ GlcNAc</td>
<td>Gly Gly</td>
</tr>
<tr>
<td>pDU9</td>
<td>2.0</td>
<td>0.8</td>
<td>3.5</td>
</tr>
<tr>
<td>pDCRP</td>
<td>7.6</td>
<td>10.0</td>
<td>62.0</td>
</tr>
<tr>
<td>pH159L</td>
<td>3.7</td>
<td>11.0</td>
<td>18.0</td>
</tr>
<tr>
<td>pD159</td>
<td>4.2</td>
<td>10.0</td>
<td>15.0</td>
</tr>
<tr>
<td>pG162C</td>
<td>4.6</td>
<td>11.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

$\beta$-Galactosidase activities of the different fusions were measured in IBPC5321 carrying the $\Delta crp$ mutation. The relative expression levels are given with the maximum value for each fusion, set at 100%. For $nagB$–lacZ this was 1820 U, for $nagE$–lacZ (looped) 680 U and $nagE$–lacZ (single site) 810 U. Cultures were grown in minimal glycerol (0.4%) medium with 0.5% cas amino acids and 0.5 mg/ml ampicillin, with 0.2% GlcNAc where indicated.
C-terminal domain (CTD) of the α-subunit of RNA polymerase contacts not just the CAP protein (16,47) but also appears to directly contact DNA in the region between the –35 and CAP sites (50,51). This is the region of the UP element to which α binds in promoters of the rrrnB type (52). Part of the activity observed at the nagB promoter could be because DNA bending facilitates binding of α-CTD to the region between CAP and the –35 sequence.

CONCLUSIONS

DNA curvature is known to affect the DNA binding properties of numerous proteins and to influence formation of nucleoprotein structures involved in transcription, replication and recombination. Our observations show that NagC and CAP are another example of a multiprotein transcription regulatory system in which DNA structure plays an important role. The nag regulatory region is intrinsically bent and this sequence-directed bend is essential for NagC regulation. NagC binds simultaneously to its two operator structures involved in transcription, replication and recombination. In the wild-type situation, NagC reaches both of its sites on this DNA fragment. This is undoubtedly because the mutations have destroyed the sequence-directed bend.

Roles as accessory factors in various nucleoprotein complexes have been demonstrated for the histone-like proteins (21,24). Although the role of CAP in repression of nagE is likely to be purely architectural, it is clear that a major part of the CAP activation of nagE is due to protein–protein contacts between CAP and RNA polymerase. However, the residual activation of nagE and the majority of that observed at nagB are probably due to effects on DNA structure via DNA bending.

ACKNOWLEDGEMENTS

We thank Steve Busby for the gift of the CAP plasmids and comments on the manuscript and Edouard Yeramian for help with the computer analysis of DNA bending. This work was supported by grants from the CNRS, ARC and the EEC and by the Ministère de l’Education Nationale, de l’Enseignement Supérieur et de la Recherche (ACC-SVS programme).

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