AraC–DNA Looping: Orientation and Distance-dependent
Loop Breaking by the Cyclic AMP Receptor Protein

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The arabinose operon promoter, pBAD, is negatively regulated in the absence of arabinose by AraC protein, which forms a DNA loop by binding to two sites separated by 210 base-pairs, araO2 and araI. pBAD is also positively regulated by AraC–arabinose and the cyclic AMP receptor protein, CRP. We provide evidence that CRP breaks the araO2–araI repression loop in vitro. The ability of CRP to break the loop in vitro and to activate pBAD in vivo is dependent upon the orientation and distance of the CRP binding site relative to araI. An insertion of one DNA helical turn, 11 base-pairs, between CRP and araI only partially inhibits CRP loop breaking and activation of pBAD, while an insertion of less than one DNA helical turn, 4 base-pairs, not only abolishes CRP activation and loop breaking, but actually causes CRP to stabilize the loop and increases the araO2-mediated repression of pBAD. Both integral and non-integral insertions of greater than one helical turn completely abolish CRP activation and loop breaking in vitro.

1. Introduction

Many transcription, recombination and replication systems are regulated by the interaction between proteins bound at distantly spaced sites along the DNA, a process known as DNA looping (for an example of each, see Dunn et al., 1984; Moitoso De Vargas et al., 1989; Mukherjee et al., 1988). The formation of a DNA loop requires the two sites to be brought into close proximity by both bending and twisting the DNA, processes that can cost considerable energy. Factors that affect DNA topology, such as supercoiling and bending, should therefore affect DNA looping; and, indeed, this has been found to be so. Looping in the ara and lac operons is affected by supercoiling (Hahn et al., 1986; Whitson et al., 1987; Krämer et al., 1987). Also, the integration of phage lambda into the Escherichia coli chromosome involves DNA loop formation by Int protein, IHF protein, a protein that bends DNA, facilitates this looping interaction (Moitoso De Vargas et al., 1989), and can be replaced by either a naturally bent DNA sequence or another protein that bends DNA, the cyclic AMP receptor protein, CRP (Goodman & Nash, 1989).

In this paper, we demonstrate that DNA looping can also be negatively affected by a protein that bends DNA. CRP prevents formation of the repression DNA loop in the araBAD operon, a phenomenon that could contribute to the transcriptional activation of the operon by CRP.

A complex regulatory system controls the expression of the araBAD operon promoter, pBAD. The promoter is regulated both positively and negatively by AraC (Sheppard & Englert, 1967; Greenblatt & Schleif, 1971; Schleif & Lis, 1975; Dunn et al., 1984), a protein that binds to three operator sites upstream from pBAD (see Fig. 1) (Ogden et al., 1980; Lee et al., 1981; Dunn et al., 1984). Positive regulation requires the interaction of AraC–arabinose at araI (Ogden et al., 1980), a site immediately adjacent to the RNA polymerase site.
of $\psi_{BAD}$. Negative regulation requires a DNA loop between AraC bound at araO$_2$ and araI (Dunn et al., 1984; Martin et al., 1986). The DNA loop also represses the promoter of the AraC-encoding gene, $P_C$ (Huo et al., 1988; Hamilton & Lee, 1988), which is transcribed divergently in relation to $\psi_{BAD}$.

The araBAD genes are also regulated by the cyclic AMP receptor protein, CRP, which mediates catabolite repression in operons that control the metabolism of a variety of sugars. CRP activates both the $\psi_{BAD}$ and $P_C$ promoters from a single site located between araO$_2$/P$_C$ and araI (Lee et al., 1981; Dunn & Schleif, 1984; Stolzfus & Wilcox, 1989). The mechanism by which CRP activates $\psi_{BAD}$ is of interest for two reasons. First, the mechanism of the protein's action is not known for any system, and second in the ara system AraC protein lies between CRP and RNA polymerase on the DNA, whereas in most operons CRP binds at a site adjacent to RNA polymerase. The mechanism of activation of $\psi_{BAD}$ by CRP apparently does not involve stimulation or stabilization of AraC binding to araI, since no cooperativity is observed between the binding of these proteins to their sites in vitro (Hendrickson & Schleif, 1984).

Two findings suggest that part of the CRP activation of $\psi_{BAD}$ involves opening the araO$_2$-mediated repression loop. Hahn et al. (1984) found that $\psi_{BAD}$ is inducible in cells that are deficient in CRP binding due to a lack of cyclic AMP synthesis, but becomes inducible when araO$_2$ is deleted, and almost full induction of $\psi_{BAD}$ is achieved when AraC is overexpressed. The idea that CRP activates $\psi_{BAD}$ by opening the repression loop was further supported by an in vitro study that showed that the requirement for CRP in the transscription of $\psi_{BAD}$ from supercoiled templates is greatly reduced by either deleting araO$_2$ or by misorienting the araO$_2$ and araI sites (Hahn et al., 1986).

CRP can also activate $\psi_{BAD}$ by an araO$_2$-independent mechanism. Both in vivo and in vitro, CRP can stimulate transcription when looping is impossible due to the absence of the araO$_2$ site (Hahn et al., 1986; Lichenstein et al., 1987; Stolzfus & Wilcox, 1989). As is the case in the araO$_2$-dependent activation mechanism, the amount of the CRP stimulation is affected by the concentration of AraC.

DNA looping by AraC in vitro requires supercoiled DNA templates (Hahn et al., 1986). Recently, we utilized an electrophoretic technique to study AraC DNA looping on small supercoiled DNA molecules, or minicircles, and showed that the DNA repression loop in the araCBA operon is maintained by a single AraC dimer bound between araO$_2$ and half of the araI site, araI$_1$ (Lobell & Schleif, 1990). Additionally, we showed that when arabinose is added to the looped complex, the loop breaks, and the AraC dimer remains bound to araI while shifting its contacts from the distal araO$_2$ site to the previously unoccupied half of araI. araI$_1$. In this paper, we extend our study of the regulation of araO$_2$-araI looping on minicircles, and explore the effect of CRP on this process.

We find that CRP increases the dissociation rate of AraC from araI$_1$ on minicircles from a slow rate characteristic of the looped state to a much faster rate characteristic of the unlooped state. This result shows that CRP breaks the araO$_2$-araI repression loop in vitro. Insertions of DNA between the CRP binding site and araI have similar effects on the ability of CRP to break the loop in vitro and to activate $\psi_{BAD}$ in vivo. CRP can activate $\psi_{BAD}$ and break the loop only when its binding site is positioned on the same face of the DNA as in the wild-type, and when the distance between the CRP and araI sites is not increased by more than one DNA helical turn. When the CRP binding site is mis-oriented on the opposite face of the DNA helix relative to its wild-type position, CRP actually stabilizes the loop in vivo and enhances araO$_2$-mediated repression of $\psi_{BAD}$ in vivo. These results support the hypothesis that part of the activation of $\psi_{BAD}$ by CRP occurs through CRP-mediated loop breaking.

2. Materials and Methods

(a) General methods and materials

DNA manipulations, growth of cells and other general methods were performed as described by Schleif & Wensink (1981) and Maniatis et al. (1982). Sequencing was performed as described by U.S. Biochemical Co. for use with their modified phage T4 DNA polymerase, Sequenase. Restriction endonucleases were obtained from New England Biolabs. Phage T4 DNA ligase and T4 DNA kinase were obtained from U.S. Biochemical Co. [gamma-32P]ATP used for DNA end-labeling was obtained from New England Nuclear. All other reagents were obtained from Sigma Chemical Co., Fisher Scientific or Biorad Laboratories. beta-Galactosidase assays were performed as described (Miller, 1972).

(b) Plasmid constructs of minicircles

A 404 bp HindIII DNA fragment containing araO$_2$ and araI separated by 160 bp was isolated from pRL516 and
Minicircles plus AraC

unlabeled aral DNA (x) minutes

araO2

ara0 ara1 ara2

arabinose 1 minute

araO2

ara0 ara1 ara2

restriction enzyme
4 minutes

non-specific DNA
1 minute

load gel

Figure 2. The looping-restriction cutting assay shows that araO2 stabilizes binding of AraC to ara0. A flow chart of the looping-restriction cutting assay is shown on the left. Three relevant states of AraC binding are depicted, a looped state with AraC binding to ara0 and ara1, an unlooped state with AraC-arabinose binding to ara0 and ara1, and AraC-arabinose binding to ara0 on the linearized DNA. The dissociation rate of AraC from ara0 on minicircles containing either the wild-type araO2 site, or a point mutation at position -271 within araO2, was measured by the looping-restriction cutting assay; the data are shown in the gel on the right side of the Figure.

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used to generate "wild-type" minicircles. pRL515 is a derivative of pES27, which is similar to pTD3 (Dunn & Schleif, 1984), except that it contains unique restriction sites created by the following mutations in positions outside regulatory sites: G to C at -76, T to G at -77, T to C at -107, C to G at -162, A to C at -166. pRL515 was derived from ES27 by making the following modifications. A fragment containing araO1 was deleted by filling in the ends with the Klenow fragment of DNA polymerase at StyI (-166) and ClaI (-110) followed by ligation. pRL515 contains a HindIII site at position +50, and a second HindIII site at position -409, introduced by filling in with Klenow at the EcoRI site (-399) and ligation of the sequence: -410 AAGCTTGAGTC -400.

Minicircles containing an araO2 mutant site were generated from a 404 bp HindIII fragment, which was isolated from pRL526, a derivative of pRL515 containing a C to T change at position -271 (Martin et al., 1986).

A 408 bp HindIII fragment containing araI, with both araO2 and araO1 deleted, was isolated from pRL518 and used in the experiment shown in Fig. 2(b). pRL518 was derived from pES27 as follows. The SspI site (-606) within the pBR322 sequences of pES27 was filled in with the Klenow fragment of DNA polymerase, and a HindIII linker (5' AAGCTTGAGTC 3') was ligated in. The region containing the araO2 site was deleted by filling in with Klenow at EcoRI (-400) and BstEII (-203) followed by ligation. The region containing araO2 was deleted by filling in with Klenow at StyI (-166) and ClaI (-110) followed by ligation.

(c) Plasmid constructs for CRP–araI spacing mutations

pRL41, a derivative of pDL3, which contains 440 bp of the pBAD upstream region fused to the galK leader region
and the lacZ structural gene (Lee & Schleif, 1989), was the starting plasmid used in the creation of plasmids containing different spacings between the CRP site and araI. pRL41 contains the following point mutations outside regulatory sites, which create unique restriction sites: G to C at -76, T to G at -77, T to C at -107, C to G at -162 and A to C at -166. The spacing between the CRP site and araI was varied by inserting the following DNA sequences between positions -80 and -81 of araBAD:

+4 bp CTATCTAGGGCTACTAGCTGAGGCGGGTTGTCAGG
+4 bp CTATCTAGGGCTACTAGCTGAGGCGGGTTGTCAGG
+33 bp CTATCTAGGGCTACTAGCTGAGGCGGGTTG
+26 bp CTATCTAGGGCTACTAGCTG
+15 bp CTATCTAGGGG
+11 bp CTATCTAG
+4 bp CTAT

The constructs containing insertions of 4, 15 and 26 bp between CRP and araI also contain an insertion of 7 bp (5' GATCAT 3') at the ClaI site (-110), such that a total of an integral number of DNA helical turns is inserted between araO2 and araI. The CRP mutant binding site constructions contain a C to T mutation at -101, which eliminates the presence of cyclic AMP-CRP, a complex is shown in Figure 2 and described below. The cyclic AMP-CRP eliminates the occupancy of araI in the loop. Since all samples are incubated for a fixed length of time after arabinose addition, the amount of linear DNA–AraC complex at each time point reflects the occupancy of araI in the loop.

We compared the dissociation rate from araI, in minicircles containing either wild-type araO2 or a point mutation in this site by the looping–restriction cutting assay (Fig. 2). The data show that the dissociation rate from araI, is eight-fold slower on minicircles containing wild-type araO2 compared to that from the mutant araO2 site, demonstrating that araO2 stabilizes AraC binding to araI.

The following demonstrates that cyclic AMP-CRP eliminates the araO2-mediated stabilization of AraC binding to araI. Cyclic AMP-CRP was added to a reaction containing looped AraC–minicircle complexes, and then the dissociation of AraC was examined by the looping–restriction cutting assay. The data (Fig. 3(a)) show that AraC dissociates 16-fold faster in the presence of cyclic AMP-CRP. In the absence of CRP, AraC dissociates from the minicircles with a half-time of 40 minutes. In the presence of cyclic AMP-CRP, a complex is...
formed that contains CRP and AraC bound to the DNA; AraC dissociates from this complex with a half-time of 2.5 minutes, resulting in a complex that contains only CRP bound to the DNA. In the absence of arabinose and CRP, AraC binds to araI in an unlooped state on minicircles containing a deletion of araO2, and dissociates from the DNA with a half-time of two minutes (Fig. 3(b)). Thus, the presence of CRP shifts the dissociation rate of AraC from araI on minicircles to a value characteristic of unlooped DNA. It is most likely, therefore, that CRP breaks the loop, and forces AraC to bind to araI in the unlooped state.

When CRP is added to looped complexes in the absence of cyclic AMP, only AraC–DNA complexes are observed, and these dissociate with a half-time, 40 minutes, characteristic of the looped state (Fig. 3). This shows that the loop-breaking effect of CRP requires binding to the DNA, since CRP binding requires cyclic AMP. Additionally, we found that CRP has only a small, about a twofold, negative effect on the binding of AraC–arabinose to araI in the unlooped state (Fig. 4). This suggests that the mechanism of loop breaking is only partially due to a direct, negative interaction of CRP with AraC bound at araI; the majority of the
Figure 4. CRP has only a small effect on AraC binding to araI in the unlooped state. A limiting concentration of AraC was incubated with araO2-araI minicircles in the presence of arabinose and cyclic AMP such that araO2 is not occupied. CRP was added where indicated, and the occupancy of AraC at araI was examined as a function of the dissociation time by the restriction-cutting assay.

(b) CRP-induced loop breaking in vitro is dependent upon the orientation and distances of the CRP binding site relative to araI.

CRP bends the DNA when it binds to lac operon DNA (Wu & Crothers, 1984), and to other operons that it regulates, including araBAD (Lichenstein et al., 1987). araO2-araI loop breaking by CRP could be due to bending of the DNA by CRP in a manner that inhibits the formation of the repression loop. We reasoned that if a CRP-induced bend is responsible for loop breaking, then CRP might break the loop from other positions between araO2 and araI. The following data, however, show that CRP can break the loop only when its binding site is located close to and oriented properly to araI. We varied the location of the CRP binding site by inserting either 4, 11 or 40 base-pairs between the CRP and araI sites. The dissociation rate of AraC from minicircles containing these different spacings was measured in the presence or absence of CRP by the looping-restriction cutting assay. The data from these spacing mutations are shown in Figures 5 and 6, and summarized in Table 1, along with in vivo pBAD-lacZ expression measurements (see section (c), below). CRP-induced loop breaking is dependent upon the orientation between the CRP and araI sites. When four base-pairs, nearly half a DNA helical turn, are inserted between CRP and araI, CRP not only fails to break the loop but appears to stabilize it; AraC dissociates three times more slowly in the presence of CRP in this spacing variant (Fig. 5(a) and Table 1). However, when the distance between CRP and araI is increased by 11 base-pairs, i.e. one complete DNA helical turn, CRP breaks the loop, although less effectively than when it is at the wild-type spacing. With the 11 base-pair insert, the half-time for dissociation of AraC from araI decreases from 40 minutes to 4.5 minutes in the presence of CRP (Fig. 5(b)). The CRP-induced loop-breaking effect, that is, the amount of the increase in the AraC dissociation rate, is reduced from a 16-fold to a ninefold effect by inserting one DNA helical turn between the CRP and araI sites.

CRP-induced loop breaking is not only dependent upon the relative orientation between the CRP and araI sites, but also on the absolute distance between the sites. Thus, when the CRP-araI spacing is increased by 40 base-pairs, there is no observable effect of CRP on the dissociation rate of AraC (Fig. 6). The relatively slow dissociation of AraC in this construct shows that looping is still occurring, as expected. We conclude that CRP-induced loop breaking is dependent upon the distance and orientation of CRP relative to araI. When the CRP site is misoriented relative to its wild-type position within araBAD, CRP can actually enhance the looping interaction.

(c) Activation of pBAD by CRP in vivo is dependent upon the orientation and distance of the CRP binding site relative to araI.

Loop breaking by CRP could function in the activation of pBAD. We would therefore expect that
Figure 5. Loop breaking and loop stabilization by CRP in the +4 bp and +11 bp CRP-araI spacing mutants. (a) The dissociation rates from araI in a spacing mutant containing 4 bp inserted between CRP and araI were compared in the presence and absence of cyclic AMP-CRP by the looping-restriction cutting assay. (b) Same as in (a) except a construct containing an 11 bp insertion between CRP and araI was assayed.

activation of $p_{BAD}$ by CRP would depend on the distance and orientation of the CRP binding site relative to araI, as is seen with the CRP-induced loop breaking in vitro. The following shows that CRP activation in vivo is also dependent upon the distance and orientation between the CRP and araI sites.

We varied the orientation and distance between the araI and CRP sites in a $p_{BAD}$-lacZ fusion construct by inserting either integral or non-integral numbers of DNA helical turns between the sites. Where non-integral numbers of turns were inserted, a compensatory insert between the CRP binding site and araO2 was introduced so that the total insertion between araO2 and araI was an integral number of DNA helical turns; this maintains the
Figure 6. CRP does not affect looping when the CRP-aral spacing is increased by 40 bp. The dissociation rates of AraC from aral, in the presence and absence of CRP were compared in constructs containing either the wild-type CRP-aral spacing or a 40 bp insert between CRP and aral by the looping-restriction cutting assay.

proper orientation between arao and aral and allows for the repression of p_{BAD} by arao in vivo (Dunn et al., 1984).

The insertions might alter the expression of the p_{BAD}-lacZ fusion not only by affecting the ability of CRP to activate, but also by affecting formation of the repression loop. To detect any effects of this nature we created identical spacing inserts that also contained point mutations in the CRP binding site.

<table>
<thead>
<tr>
<th>CRP-aral spacing</th>
<th>β-Galactosidase units</th>
<th>Factor stimulation of p_{BAD} by CRP</th>
<th>Half-time of AraC on aral</th>
<th>Factor of CRP stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRP⁺</td>
<td>CRP⁻</td>
<td></td>
<td>+CRP</td>
</tr>
<tr>
<td>+0 (WT)</td>
<td>36,000</td>
<td>3500</td>
<td>10.3</td>
<td>25</td>
</tr>
<tr>
<td>+4</td>
<td>3200</td>
<td>9100</td>
<td>0.4</td>
<td>50</td>
</tr>
<tr>
<td>+11</td>
<td>20,000</td>
<td>3200</td>
<td>0.1</td>
<td>4.5</td>
</tr>
<tr>
<td>+15</td>
<td>6000</td>
<td>6800</td>
<td>0.9</td>
<td>17</td>
</tr>
<tr>
<td>+22</td>
<td>7700</td>
<td>6100</td>
<td>1.3</td>
<td>40</td>
</tr>
<tr>
<td>+26</td>
<td>7400</td>
<td>9500</td>
<td>0.8</td>
<td>16</td>
</tr>
<tr>
<td>+33</td>
<td>8200</td>
<td>8800</td>
<td>0.9</td>
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<tr>
<td>+40</td>
<td>9400</td>
<td>8400</td>
<td>1.1</td>
<td>17</td>
</tr>
</tbody>
</table>

Expression of the p_{BAD}-lacZ fusion from a multicopy plasmid containing the CRP⁺ and CRP⁻ constructs and the various CRP-aral spacing mutations. The CRP mutations are C to T and G to A conversions at positions -89 and -98, respectively. Promoter activity is expressed as β-galactosidase units, and is an average of at least 3 independent measurements. The standard deviations of the measurements ranged from 10 to 20% of the total units. Shown also is the effect of CRP on the dissociation rate of AraC from aral in several of the spacing mutants. Dissociation rate data are averages of 3 independent measurements. Dissociation half-times are reported as averages of 2 independent experiments, derived from autoradiograms that were scanned and quantified using a Biorad 620 densitometer. The dissociation half-times of complexes were determined relative to the 15-s time point. Typically between 45 and 65%, and not 100% of the DNA, is bound as AraC-DNA or AraC-CRP-DNA complexes at the 15-s time point. The lack of complete binding is due to dissociation of complexes after arabinose addition. Since the dissociation of complexes after arabinose addition is fixed for all of the time points, the dissociation half-time relative to the 15-s time point reflects the dissociation rate prior to arabinose addition. WT, wild-type.
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spacing inserts containing the wild-type CRP binding site with the same spacing variants containing the mutant CRP binding site.

The data in Table 1 show that, like CRP-induced loop breaking, activation of $p_{BAD}$ by CRP is dependent upon its orientation and distance from ara1. $p_{BAD}$ expression decreases tenfold when the CRP binding site contains point mutations at positions −89 and −98 (Table 1); the same order of decrease is seen in a deletion that removes the entire CRP binding site while maintaining the araO2–araI looping interaction (data not shown). Thus, CRP containing the mutant CRP binding site.

whether the destabilization is due to the bend introduction by the binding of CRP or the mere presence of the protein was not determined by the experiments reported here.

Could CRP-induced loop breaking exist in other systems? As in araCBAD, the promoters of the lac and deo operons are repressed via DNA loop formation (Eismann et al., 1987; Krämer et al., 1987; Dandanell & Hammer, 1985; Amouyal et al., 1989), and positively activated by CRP. Additionally, as in araCBAD, CRP binds adjacent to the promoter-proximal operator site within the DNA loop both in lac and deo. Therefore, as in araCBAD, CRP might also break the loop in lac and deo, and thereby indirectly activate their respective promoters.

The mechanism by which CRP activates independently of repression looping in araCBAD (Hahn et al., 1986; Lichenstein et al., 1987; Stolzus & Wilcox, 1989), is likely to be different from that in other systems. In the lac operon, CRP binds cooperatively with RNA polymerase and enhances the isomerization rate of RNA polymerase from the closed to the open complex, from a site approximately 15 base-pairs from the lac P1 promoter (Straney et al., 1989). This mechanism may not be possible in the araO2-independent activation of $p_{BAD}$ by CRP, considering that AraC is bound between CRP and RNA polymerase when the operon is both repressed and induced (Martin et al., 1986; Lobell & Schlief, 1990).

The finding that CRP has a small, twofold, negative effect on AraC–arabinose binding to araI in the unlooped state (Fig. 4) suggests a mechanism that could account for the araO2-independent activation of $p_{BAD}$ by CRP. AraC binds to two half-sites within araI (Lee et al., 1987), making strong contacts to araI and weak contacts to araI2 (Brunelle & Schlief, 1989). AraC binding to araI involves significant DNA bending (Lobell, 1990). Since both AraC and CRP bend the DNA and bind close to each other along the DNA, the proteins could interfere with each other’s binding to the DNA. Thus, CRP might force AraC to lose some of its contacts with araI and to “roll over” more onto araI2, where AraC would be in closer proximity to contact and activate RNA polymerase. Since araI2 is apparently a weaker binding site than araI, due to several positions of non-homology with the AraC direct repeat consensus binding sequence (Brunelle & Schlief, 1989), by forcing AraC onto araI2, CRP would lower the affinity of AraC binding to araI, as we observed (Fig. 4). This model makes a testable prediction: the contacts made by AraC at araI2 should be affected by CRP.

As suggested above, CRP-induced loop breaking could occur in lac and other operons. Additionally, the activation of the lac promoter and araCBAD by CRP is similar in that CRP shows the same orientation dependence in both operons; as in araCBAD, CRP can partially activate the lac promoter when its distance from the promoter is increased by 11 base-pairs, but not by five base-pairs, both in vivo (Mandecki & Caruthers, 1984) and in vitro (Straney et al., 1989). CRP-induced DNA bending could be involved in the mechanism of CRP activation; the orientation dependence could be due to the requirement that the CRP-induced DNA bend be positioned correctly relative to the promoter.
Our finding that CRP breaks the araO2-araI DNA loop demonstrates a second mechanism, in addition to that of arabinose-mediated loop breaking (Lobell & Schleif, 1990), by which this looping interaction can be abolished. Conversely, Int-mediated DNA looping is enhanced by two proteins that bind DNA, THF and CRP, as well as by naturally bent DNA (Moitoso de Vargas et al., 1989; Goodman & Nash, 1989). We expect that DNA looping in other systems will be either positively or negatively affected by other proteins that bind the DNA, or otherwise effect the topology of a DNA loop.

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References


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Note added in proof: We wish also to bring to the reader’s attention the following two recent papers, which investigate stimulation by CRP in the lac and melR promoters in which the location of the CRP binding site is systematically varied: Stringent spacing requirements for transcription activation by CRP, Gaston, K., Bell, A., Kolb, A., Buc, H. & Busby, S. (1990). Cell, 62, 733–743; Helical phase dependent action of CRP: effect of the distance between the CRP site and the –35 region on promoter activity. Ushida, C. & Aiba, H. (1990). Nucl. Acids Res., 18, 6325–6330. In contrast to the present study, CRP stimulation in these systems appears not to be predominantly assisting the breaking of a DNA loop. Nonetheless, CRP stimulation in these systems as well is confined to spacings in which CRP occupies one face of the DNA helix.