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Comparison of the binding sites for the *Escherichia coli* cAMP receptor protein at the lactose and galactose promoters

Annie Kolb, Stephen Busby, Michel Herbert, Denise Kotlarz and Henri Buc*

Institut Pasteur, Département de Biologie Moléculaire, 25, rue du Docteur Roux, 75724 Paris Cedex 15, France

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Polyacrylamide gel electrophoresis has been used to visualise and quantitate complexes between the *Escherichia coli* cyclic AMP receptor protein (CRP) and DNA fragments containing the promoter region of either the *E. coli* galactose or lactose operons. We show that, although CRP binding to the *gal* fragment is weaker than binding to the *lac* fragment, in each case, stable complexes are formed between one dimer of CRP and one molecule of DNA. We have examined the effects of a series of deletions and point mutations in the *gal* promoter region on CRP binding. From the position of deletions and mutations which prevent the formation of stable complexes, we deduce the location and extent of the sequence at the CRP binding site. We show that it covers approximately the same length of sequence as the binding site at the *lac* promoter. Unlike the *lac* site, the *gal* site contains no palindromic sequence. We discuss the importance of symmetry in the sequence at CRP binding sites and the validity of CRP binding consensus sequences which have been proposed.

Key words: Escherichia coli/cAMP receptor protein/*lac* and *gal* promoters/palindromic sequences

Introduction

The *Escherichia coli* cyclic AMP receptor protein (CRP) is a dimer which, when activated by cAMP, binds specifically to the promoter region of a large number of operons and regulates the initiation of transcription by RNA polymerase (reviewed by Ullmann and Danchin, 1982). Protection studies have shown that, at different promoters, CRP fixes at different positions with respect to the transcription start point (e.g., Majors, 1977; Schmitz, 1981; Taniguchi *et al.*, 1979; Queen and Rosenberg, 1981; Valentin-Hansen, 1982; Legrice *et al.*, 1982). For example, at the *lac* promoter, CRP binds to sequences between −47 and −72, although in some conditions weaker binding to a site around +12 can be detected (Schmitz, 1981). In contrast, at the *gal* promoter, CRP protects sequences between −25 and −50 (Taniguchi *et al.*, 1979).

At the *lac* promoter, the DNA sequence at the CRP binding site contains a palindrome: two closely related sequences occur disposed about a 2-fold axis between base pair −61 and −62. In contrast, at the *gal* promoter, the sequence at the CRP binding site exhibits no such symmetry. As most regulatory proteins are multimeric and bind to sites containing symmetry in the DNA sequence (reviewed by Gicquel-Sanzey and Cossart, 1982), this raises the question of whether the same number of CRP molecules bind at the two promoters and of how the CRP subunits are arranged at the *gal* promoter. To answer these points we have exploited a recently described method whereby, using polyacrylamide gel electrophoresis, it is possible to visualise and quantify stable complexes between regulatory proteins, including CRP, and short DNA fragments (Garner and Revzin, 1981; Fried and Crothers, 1981). We used this method firstly to compare the stoichiometry and affinity of CRP binding to the *gal* and *lac* operon promoter regions. Secondly, we examined the effect of a series of deletions and point mutations at the *gal* promoter. The location of deletions and mutations which prevent the formation of stable complexes allows us to define the sequences important for CRP binding at the *gal* promoter.

Results

The stability and stoichiometry of complexes between CRP and promoter DNA

Polyacrylamide gel electrophoresis can be used to detect stable complexes between DNA and proteins (Garner and Revzin, 1981; Fried and Crothers, 1981). Increasing concentrations of CRP were mixed with a ^{32}P-labelled 203-bp fragment covering the wild-type *lac* promoter region prior to loading the sample on a gel. Figure 1 a–d shows the effect of CRP on the migration of the DNA fragment. Addition of CRP causes the disappearance of the band due to the free

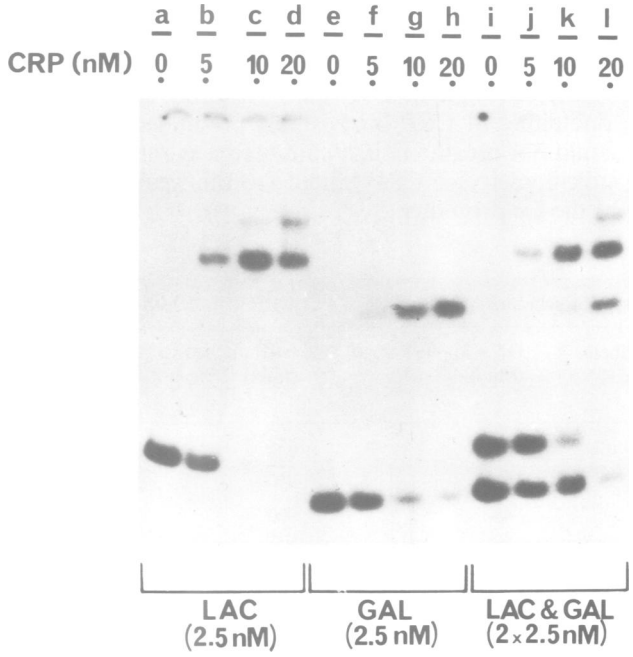

Fig. 1. Visualisation of complexes between DNA and CRP using gel electrophoresis. 16 μl samples containing ^{32}P-labelled DNA fragments and different concentrations of CRP were applied to a polyacrylamide gel run in buffer containing 200 μM cAMP. The figure shows an autoradiogram of the gel after electrophoresis. The samples in **lanes a–d** contained 2.5 nM of a 203-bp *lac* promoter fragment, in **lanes e–h** 2.5 nM of a 144-bp *gal* promoter fragment and in **lanes i–l** 2.5 nM of each fragment. The concentration of CRP was 0 nM (**lanes a, e, i**), 5 nM (**lanes b, f, j**), 10 nM (**lanes c, g, k**) or 20 nM (**lanes d, h, l**).

*Reprint requests can be sent to any author.

fragment and the appearance of a new less mobile band corresponding to the formation of the CRP-DNA complex (Figure 1 a–d). At higher concentrations of CRP (Figure 1d), a second less mobile band appears corresponding to the CRP binding to the secondary site around +12 (Crothers and Fried, 1982; A. Spassky and A. Kolb, unpublished data).

Figure 1 (e–h) shows a similar experiment with a 144-bp fragment covering the *gal* promoter region. A new band appears corresponding to the complex, but even at higher CRP concentrations, no second band is observed. This experiment shows that CRP binding at the *gal* promoter is weaker than at the *lac* promoter, as it is necessary to add more CRP to displace totally the free DNA band with the *gal* fragment than with the *lac* fragment. To compare directly the affinity of CRP for the two fragments we repeated the experiment by adding increasing concentrations of CRP to an equimolar mixture of the 203-bp *lac* promoter fragment and the 144-bp *gal* promoter fragment (Figure 1, i–l). The result confirms that the initial binding of CRP to the *lac* fragment is tighter than the binding of the *gal* fragment which in turn is tighter than the binding to the second site on the *lac* fragment. From the relative intensity of the bands we can estimate that the affinity of CRP for the *gal* site is 5–10 times less than the affinity for the *lac* site, and that the fixation of CRP at the second *lac* site is 20–30 times weaker than the fixation at the first *lac* site.

We have exploited the fact that the CRP-DNA complexes migrate as discrete complexes to determine the stoichiometry of CRP binding at each promoter. ^{125}I-labelled CRP was mixed with ^{32}P-labelled *gal* or *lac* promoter fragments. After loading on a gel and electrophoresis, as in Figure 1, we cut out the bands containing either the complex between *gal* DNA and CRP or the major complex between *lac* DNA and CRP. From the number of ^{125}I and ^{32}P counts in each band, and from the known specific activity of the CRP and DNA, we could deduce the amount of both DNA and protein in each complex (Table I). The results show that at both promoters one dimer of CRP binds stably per molecule of DNA. We could not obtain enough counts to accurately determine the stoichiometry of CRP binding to the weaker secondary site at the *lac* promoter.

Deletions and mutations which prevent CRP binding at the gal promoter

We have recently isolated and sequenced a series of deletions starting either upstream or downstream of the *gal* promoter region, which removed progressively longer tracts of *gal* sequence (Busby *et al.*, in preparation). Using the gel electrophoresis method we have examined the binding of CRP to several fragments containing such deletions to try to define the limits of the sequence required for stable CRP binding. Figure 2A (lanes e–h) illustrates the effect of CRP binding on the migration of a ^{32}P-labelled 855-bp fragment containing the intact *gal* promoter region (see Materials and methods). The result in lanes i–l shows that an internal deletion which removes all the *gal* sequence downstream of −26 does not affect CRP binding. In contrast, the result in lanes a–d shows that binding is prevented by a longer deletion which removes all the *gal* sequence downstream of −100.

Stable CRP binding is seen by the disappearance of the band corresponding to free DNA and the appearance of a less mobile band corresponding to the CRP-DNA complex. However, independently of the formation of this band, we always find, at higher concentrations of CRP, that the sharp bands are replaced by a smear and, eventually, by a band which remains at the origin of the gel. As this arises with all fragments, whether or not specific CRP-DNA interactions take place, we attribute this effect to the weaker non-specific binding of CRP described by many authors (Saxe and Revzin, 1979; Takahashi *et al.*, 1979).

The results in Figure 2A demonstrate that the specific CRP binding site at the *gal* promoter is located upstream of −26. To find the other limit of the site, we examined the effect of a series of deletions originating upstream of the *gal* promoter region. Figure 2B, lanes e–h, shows the effect of CRP binding on migration of a 248-bp fragment containing the intact *gal* promoter region. The results in lanes a–d and i–l demonstrate that internal deletions which remove the *gal* sequence upstream of either −45 or −29 prevent stable CRP binding. In contrast, a deletion which removes the *gal* sequence upstream of −56 has no effect on CRP binding (not shown).

Figure 3 illustrates the extent of each deletion and sum-

Table I. Stoichiometry of binding of [^{125}I]CRP to a 203-bp *lac* promoter fragment or a 270- or 144-bp *gal* promoter fragment

Fragment	DNA applied to gel (fmol)	CRP applied to gel (fmol)	^{32}P counts in the complex	^{125}I counts in the complex	DNA bound in complex (fmol)	CRP bound in complex (fmol)	CRP/DNA ratio
203-bp *lac*	303	635	3812	13 653	125	111	0.89
	313	950	4183	21 156	184	172	0.94
	480	960	6475	18 845	206	227	1.10
270-bp *gal*	1000	635	806	7500	65	61	0.94
	378	840	11 061	13 377	170	161	0.94
144-bp *gal*	624	950	485	7890	105	64	0.60
	300	840	12 283	13 904	180	167	0.93
	480	960	6172	14 050	163	168	1.03

CRP-DNA complexes were formed and visualised using the gel electrophoresis technique described in Figure 1. Columns 2 and 3 indicate, respectively, the amounts of DNA (in fmol of promoter) and of CRP (in fmol of dimeric protein) applied to the gel. After electrophoresis and autoradiography, bands corresponding to the CRP-DNA complexes were cut out and counted. Columns 4 and 5 show the ^{32}P and ^{125}I counts found in the complex in each experiment and columns 6 and 7 show the molar quantities of DNA and CRP calculated from these counts. In the last column the protein/DNA ratio is calculated for the complex in each experiment.

marises the results of the CRP binding tests. From this we deduce that the CRP binding site is located in the sequence from −27 to −56. The effect of the R503 deletion implies that an element in the sequence from −46 to −56 must be crucial. To define the other important sequences we examined the effects of a number of point mutations on CRP binding.

Figure 2C shows the fixation of CRP to a 144-bp fragment containing the wild-type *gal* promoter region (lanes e−h) or point mutations at −37 (lanes a−d) or −38 (lanes i−l). Both point mutations prevent the formation of stable complexes. In this experiment the non-specific complexes appear as a smear in the gel rather than at the origin. Figure 2D

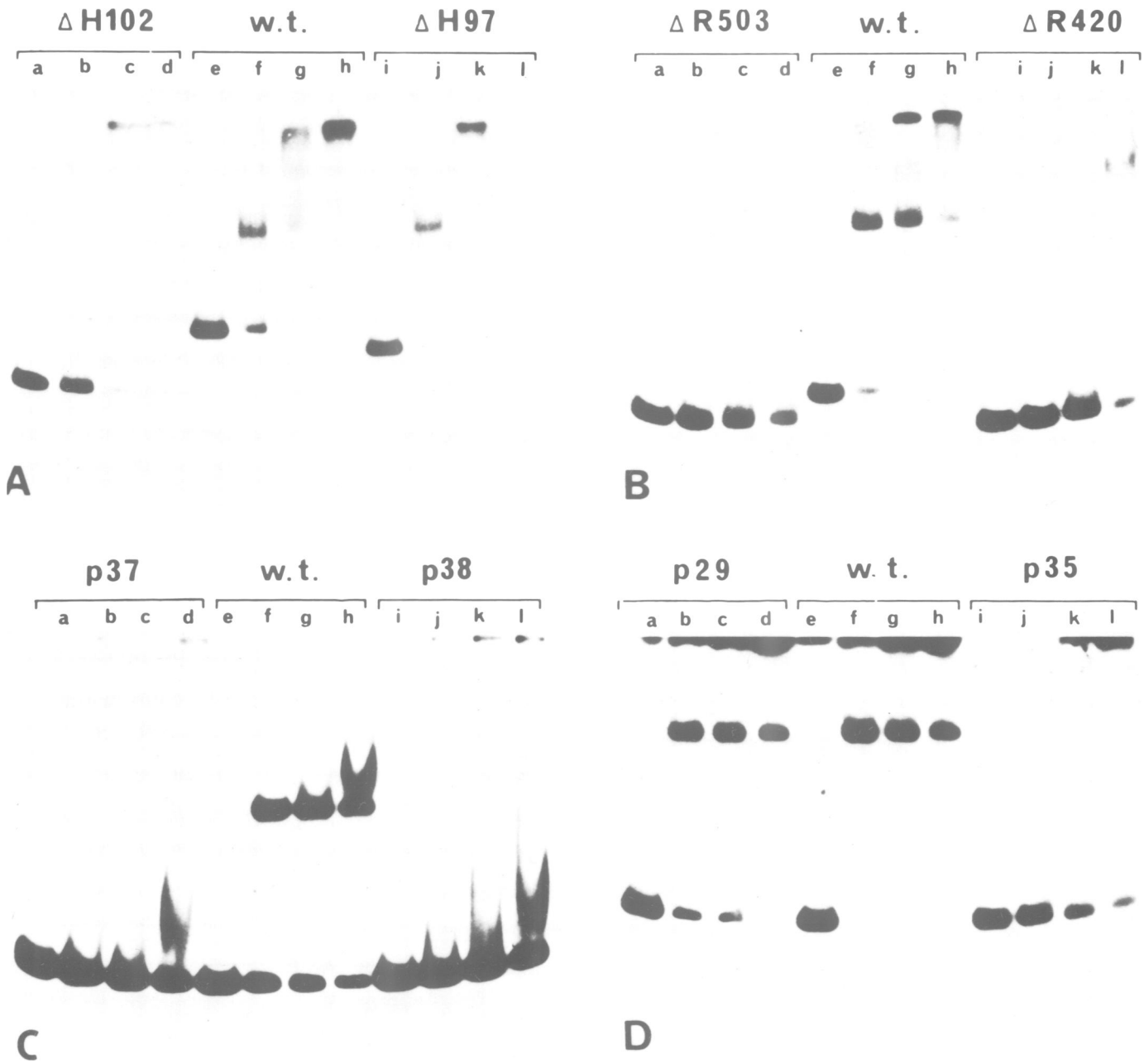

Fig. 2. The effect of mutations and deletions on CRP binding to the *gal* promoter region. Each photograph shows an autoradiogram of a gel in which different ^{32}P-labelled *gal* promoter fragments were incubated with different concentrations of CRP before loading on the gel and electrophoresis as in Figure 1. **A.** 2 nM of *gal* promoter fragment was incubated alone (**lanes a, e, i**), with 75 nM CRP (**lanes b, f, j**), with 250 nM CRP (**lanes c, g,k**) or with 500 nM CRP (**lanes d, h, l**) and then applied on a 5% gel. In **lanes e−h**, the fragment was a 855-bp *Eco*RI-*Pvu*II fragment containing the 270-bp *gal* promoter fragment. In **lanes a−d** we used a fragment containing the H102 deletion of the *gal* sequence downstream of −100. In **lanes i−l** we used a similar fragment containing the H97 deletion of the *gal* sequence downstream of −27. **B.** 4 nM of *gal* promoter fragment was incubated alone (**lanes a, e, i**), with 75 nM CRP (**lanes b, f, j**), with 250 nM CRP (**lanes c, g, k**) or 500 nM CRP (**lanes d, h, l**) and then applied to a 7.5% gel. In **lanes e−h**, we used a 248-bp *gal* promoter fragment. In **lanes a−d** we used the same fragment but containing a deletion (△R503) of the *gal* sequence upstream of −29. In **lanes i−l**, we used a similar fragment containing a deletion of the *gal* sequence upstream of −45 (△R420). **C.** 4 nM of a 144-bp *gal* promoter fragment was incubated alone (**lanes a, e, i**), with 75 nM CRP (**lanes b, f, j**), with 150 nM CRP (**lanes c, g, k**) or with 250 nM CRP (**lanes d, h, l**) and then loaded on 7.5% gel. The fragment contained the wild-type *gal* promoter (**lanes e−h**), the P37 mutation (**lanes a−d**) with a CG to TA transition at −37 or the P38 mutation (**lanes i−l**) with a TA to AT transversion at −38. **D.** 0.3 nM of a 270-bp *gal* promoter fragment was incubated alone (**lanes a, e, i**), with 50 nM CRP (**lanes b, f, j**), with 100 nM CRP (**lanes c, g, k**) or with 150 nM CRP (**lanes d, h, l**) and then loaded on a gel. The fragment contained with wild-type *gal* promoter region (**lanes e−h**), a GC to CG transversion at −35 (**lanes i−l**) or an AT to GC transition at −29 (**lanes a−d**).

shows the effects of point mutations at −35 (lanes i–l) or −29 (lanes a–d) on the binding of CRP to a longer 270-bp *gal* promoter fragment (lanes e–h). Whilst the mutation at −35 prevents stable complex formation, the mutation at −29 has no effect on binding. The positions of the point mutations and their effects are also summarised in Figure 3.

Discussion

The gel electrophoresis method provides a simple, rapid assay for detecting the formation of complexes between DNA fragments and CRP. We have exploited this to compare the relative affinity of CRP binding to the *lac* and *gal* promoter regions and to determine the stoichiometry of binding. Further, we used this method to rapidly determine the effects of a series of point mutations and deletions on CRP binding at the *gal* promoter. In the gel system, DNA fragments complexed to CRP migrate as discrete bands separate from the free DNA, provided that the rate of dissociation of the binary

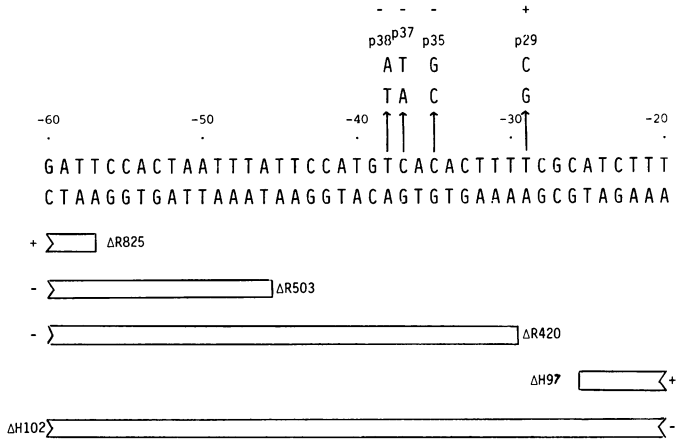

Fig. 3. Sequence around the CRP binding site of the *gal* promoter. The amount of sequence removed by each deletion is indicated by a horizontal bar and the position of each of the point mutations is marked with an arrow. Mutations and deletions which prevent stable CRP binding are marked '−' and those which have no effect on binding are marked '+'.

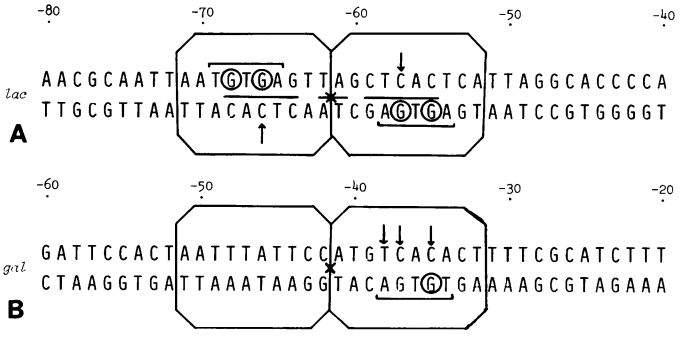

Fig. 4. Comparison of the sequence at the CRP binding sites at the *gal* and *lac* promoters. The figure compares the sequence between −40 and −80 at the *lac* promoter with the sequence between −20 and −60 at the *gal* promoter. The sequences cover the region protected from DNase attack in footprinting experiments (see text). The positions where point mutations prevent stable CRP binding (see text) are indicated with arrows and the guanines which are protected from methylation with dimethylsulphate are encircled (Majors, 1977; Taniguchi *et al.*, 1979). The lines between the two strands in the *lac* sequence show the palindrome centered around a point between −61 and −62. The brackets (⌐——⌙) drawn above and below the sequences correspond to the consensus 5′TGTGA3′ motif. The two ovals sketched around the sequence represent, schematically, the position of the two CRP subunits at each promoter.

complex is slower than the rate of entry of the complex into the gel. The crucial limitation of the method is that only stable complexes can be detected.

For the formation of complexes between CRP and DNA fragments, we chose the conditions in which specific effects on *gal* and *lac* transcription had been demonstrated (Herbert, 1982). The results show that, in these conditions, the affinity of CRP for the principal site at the *lac* promoter is higher (by 5- to 10-fold) than the affinity for the site at the *gal* promoter, and is also higher (by 20- to 30-fold) than the affinity for the second site at the *lac* promoter. At the main site at the *lac* promoter we have found, in agreement with Crothers and Fried (1982), that one molecule of CRP dimer binds to form a stable complex. How is this dimer arranged on the DNA? Footprinting and protection studies have shown that CRP binds to the sequence between −47 and −72, a region which contains a palindrome, consisting of two related sequences arranged around an axis of symmetry between −61 and −62 (Figure 4A) (Majors, 1977; Simpson, 1980; Schmitz, 1981). The distribution of the groups necessary for CRP binding and the bases protected by CRP, as probed by a variety of chemical agents, indicates that binding is symmetric about this axis (reviewed by Simpson, 1980). Moreover the importance of the palindrome has been demonstrated genetically by the fact that mutations which prevent CRP binding fall in these sequences at one of two symmetrically related positions (Dickson *et al.*, 1977). The present evidence suggests, therefore, that at this site the two subunits of the CRP dimer each interact with one of the two symmetrically disposed sequences (Figure 4A).

At the *gal* promoter, CRP protects sequences between −25 and −50 from attack by DNase (Taniguchi *et al.*, 1979). The present observation that sequences downstream of −26 and upstream of −56 can be deleted without affecting stable CRP binding shows that the sequences essential for binding are in the protected zone. Furthermore, it demonstrates that no neighbouring sequence is required by CRP for entry to its binding site. However, although this site is about the same size as the CRP binding region at the *lac* promoter it contains no palindromic sequence. The fact that one dimer of CRP binds to the *gal* site, as to the *lac* site, shows that sequence symmetry is not a prerequisite for stable binding. A similar conclusion was reached by Ebright (1982) from a survey of the DNA sequence at seven different CRP binding sites.

What are the sequences within the protected region necessary for stable CRP binding at the *gal* promoter? On selecting mutations which suppressed the stimulation of promoter expression by cAMP *in vivo*, we repeatedly obtained mutations at −35, −37 or −38 (Busby *et al.*, 1982b; Busby and Dreyfus, 1983). Here we have shown that each of these simple mutations prevent stable CRP binding to the promoter region. These changes fall in the sequence 5′TGTGA3′ between −34 and −38, a sequence which appears once at the *gal* CRP site and at nearly all the other CRP binding sites which have been characterised to date (reviewed by Ebright, 1982). At the *lac* CRP site this motif is found in the palindrome: it appears between −69 and −65, together with the closely related sequence, 5′AGTGA3′, symmetrically disposed between −54 and −58 (see Figure 4A). The location of mutations demonstrates that this 'consensus' sequence is as important for CRP binding at the *gal* promoter as at the *lac* promoter. However, it is clear that the number of CRP subunits bound is not determined by the number of consensus sequences at the binding site. If we suppose that for *gal*, as for

lac, one CRP subunit binds to one consensus sequence, we are left with the question of where the other subunit binds. The fact that replacement of the sequence upstream of −45 also prevents stable CRP binding, shows that this zone, which contains nothing resembling the apparent consensus sequence, 5′TGTGA3′, must also be important. If we suppose that the spacing between the sequences recognised by the CRP subunits at the *lac* and *gal* promoters is the same (as originally suggested by Queen and Rosenberg, 1981), the second subunit must recognise the sequence around −45 to −49. Gicquel-Sanzey and Cossart (1982) recently suggested that the second subunit recognises the sequence between −25 and −28, whilst O'Neill *et al.* (1981) proposed that it bound to the sequence between −40 and −36. Clearly neither of these suggestions accounts for the effects of the point mutations and deletions which we have described here (Figure 3). The former is not consistent with the lack of effect of the H97 deletion whilst the latter does not explain the effect of the R503 deletion.

In Figure 4, we have sketched a plausible arrangement of the CRP subunits at the *gal* and *lac* promoters, which is consistent with the effects of the point mutations and deletions. At the *lac* promoter, binding of the CRP dimer is stabilised by interaction with two symmetrically disposed versions of the consensus CRP binding sequence. At the *gal* promoter, as only one consensus sequence is present, the second subunit must interact with a second sequence. As discussed above, the region removed by the R503 deletion must contain this sequence (see Figure 3). However, the present lack of point mutations in the *gal* promoter upstream of −45 prevents us from defining more explicitly the sequence important for the binding of the second CRP subunit. Nevertheless, comparison of the sequence in this region with the sequence at the CRP binding sites of the *ara*BAD (Ogden *et al.*, 1980; Lee *et al.*, 1981) and *mal*T (Chapon, 1982) promoters reveals, in each case, the presence of the sequence 5′ AAAT3′ eight base pairs from the 5′TGTGA3′ motif, on the same strand. This may well be the second sequence responsible for the formation of stable complexes with CRP dimer at these promoters.

The affinity of CRP for the *gal* promoter is 5−10 times less than that for the principal site at the *lac* promoter. This corresponds to a difference in binding energies between the two complexes of ~0.6−1.2 kcal/mol, roughly equivalent to the formation of a single additional hydrogen bond. At both promoters one CRP subunit interacts with the sequence 5′TGTGA3′. We suggest that the difference in the binding affinity of CRP at the two promoters arises from the variation in the sequence seen by the second subunit of the CRP dimer.

Materials and methods

By convention, *lac* and *gal* sequences are numbered from the transcription start point of the cAMP-CRP dependent promoter. Sequences downstream of this point are labelled with a '+' prefix and sequences upstream are labelled with a '−' prefix.

DNA fragments

The 203-bp *lac* fragment containing the *lac* promoter sequence from −140 to + 63 was isolated from a pBR322 derivative (Schaeffer *et al.*, 1982).

The *gal* promoter region was isolated on either a 144-bp fragment covering the promoter sequence from −92 to +45 (isolated from pAA187, Busby and Dreyfus, 1983) or on a 270-bp fragment covering the promoter sequence from −190 to +45 (isolated from pAA195, Busby *et al.*, 1982a). The isolation of the various point mutations in these fragments has been previously described (Busby *et al.*, 1982b; Busby and Dreyfus, 1983).

The 855-bp *Eco*RI-*Pvu*II fragment used in Figure 2A was isolated from pAA196 (Busby *et al.*, in preparation) and consists of the 270-bp *gal* promoter fragment upstream of 585 bp of *trpB* sequence. The H97 and H102 deletions remove all the *gal* sequence downstream of −26 and −100, respectively and result in a shortened fragment in which the *gal* sequence from −190 to either −100 or −26 is cloned upstream of the same *trpB* sequence (Busby *et al.*, in preparation). The 248-bp *Hha*I-*Hind*III fragment used in Figure 2B was isolated from pAA187 and consists of the 144-bp *gal* promoter fragment downstream of 100 bp of pBR322 sequence. The R503 and R420 deletions remove all the *gal* sequence upstream of −45 and −29, respectively, and result in a shortened fragment in which the *gal* sequence from +45 to either −29 or −45 is cloned downstream of the same 100-bp pBR322 sequence (Busby *et al.*, in preparation).

DNA fragments were isolated by electroelution and labelled with [α-^{32}P]dATP (Amersham) using Klenow fragment given by W. McClure. Labelled fragments were diluted with excess unlabelled fragment of known concentration. We used fragments with a specific activity of 5−70 c.p.m./fmol.

CRP

Purified CRP was kindly given by Bernadette Blazy. It was >98% pure as judged by SDS-polyacrylamide gel electrophoresis. Its concentration was determined spectrophotometrically using ϵ_{278} = 4.1 x 10^4/M/cm per CRP dimer (Takahashi *et al.*, 1982). CRP protein was labelled with ^{125}I using Bolton and Hunter reagent (Bolton and Hunter, 1973). The specific activity of the labelled CRP was determined after running the sample on an SDS-polyacrylamide gel, calibrated with unlabelled CRP of known concentration, stained by the silver nitrate procedure (Ansorge, 1982). After determination of the counts in the [^{125}I]CRP band cut from the gel, we corrected the radioactivity for quenching due to the stain and deduced the protein concentration from the calibration curve. We used iodinated protein with a specific activity of 83 or 123 c.p.m./fmol. The labelled protein was as active as unlabelled CRP of the same concentration in binding to the *lac* and *gal* promoters at 1 mM cAMP.

Polyacrylamide gel electrophoresis

Samples (16 μl) containing different concentrations of CRP and labelled DNA were incubated for 30 min at 37°C in 40 mM Tris pH 8, 10 mM $MgCl_2$, 100 mM KCl, 1 mM dithiothreitol, 100 μg/ml bovine serum albumin. Immediately before loading on the gel 3 μl of 50% glycerol, 0.01% xylene cyanol blue in the same buffer was added. Electrophoresis was performed at room temperature in 7.5% (or 5%) polyacrylamide slab gels (150 x 105 x 1.5 mm), equilibrated with a buffer consisting of 90 mM Tris, 90 mM borate, 2.5 mM EDTA pH 8.2 containing 200 μM cAMP, at 17 V/cm. After 2 h of electrophoresis, the position of the bands were revealed by autoradiography.

To quantitate the radioactivity in the bands the gel was sliced up; ^{32}P counts were determined by Cerenkov counting, and ^{125}I radioactivity was determined on a gamma counter.

Acknowledgements

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References

Ansorge,W. (1982) *Anal. Biochem.*, in press.
Bolton,A.E. and Hunter,W.M. (1973) *Biochem. J.*, **133**, 529-539.
Busby,S., Irani,M. and de Crombrugghe,B. (1982a) *J. Mol. Biol.*, **154**, 197-209.
Busby,S., Aiba,H. and de Crombrugghe,B. (1982b) *J. Mol. Biol.*, **154**, 211-227.
Busby,S. and Dreyfus,M. (1983) *Gene*, **21**, 123-133.
Chapon,C. (1982) *EMBO J.*, **1**, 369-374.
Crothers,D. and Fried,M. (1982) *Cold Spring Harbor Symposium on DNA Structure*, published by Cold Spring Harbor Laboratory Press, NY, in press.
Dickson,R., Abelson,J., Johnson,P., Reznikoff,W.S. and Barnes,W.M. (1977) *J. Mol. Biol.*, **111**, 65-75.
Ebright,R. (1982) in Griffin,J. and Duax,W. (eds.), *Molecular Structure and Biological Activity*, North Holland-Elsevier, NY, pp. 91-100.
Fried,M. and Crothers,D. (1981) *Nucleic Acids Res.*, **9**, 6505-6525.
Garner,M. and Revzin,A. (1981) *Nucleic Acids Res.*, **9**, 3047-3060.
Gicquel-Sanzey,B. and Cossart,P. (1982) *EMBO J.*, **1**, 591-595.
Herbert,M. (1982) DEA Thesis, Paris-VI University.
Lee,N., Gielow,W.O. and Wallace,R.G. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 752-756.

Legrice,S., Matzura,H., Marcoli,R., Iida,S. and Bickle,T. (1982) *J. Bacteriol.*, **150**, 312-318.
Majors,J. (1977) Ph.D. Thesis, Harvard University.
Ogden,S., Haggerty,D., Stoner,C., Kolodrubetz,D. and Schleif,R. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 3346-3350.
O'Neill,M., Amas,K. and de Crombrugghe,B. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 2213-2217.
Queen,C. and Rosenberg,M. (1981) *Nucleic Acids Res.*, **9**, 3365-3377.
Saxe,S. and Revzin,A. (1979) *Biochemistry (Wash.)*, **18**, 255-263.
Schaeffer,F., Kolb,A. and Buc,H. (1982) *EMBO J.*, **1**, 99-105.
Schmitz,A. (1981) *Nucleic Acids Res.*, **9**, 277-292.'
Simpson,R. (1980) *Nucleic Acids Res.*, **8**, 759-766.
Takahashi,M., Blazy,B. and Baudras,A. (1979) *Nucleic Acids Res.*, **7**, 1699-1712.
Takahashi,M., Gronenborn,A.M., Clore,G.M., Blazy,B. and Baudras,A. (1982) *FEBS Lett.*, **139**, 37-40.
Taniguchi,T., O'Neill,M. and de Crombrugghe,B. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 5090-5094.
Ullmann,A. and Danchin,A. (1982) *Adv. Cyclic Nucleotide Res.*, **15**, in press.
Valentin-Hansen,P. (1982) *EMBO J.*, **1**, 1049-1054.

Note added in proof

Garner and Revzin (*Biochemistry (Wash.) 1982*, **21**, 6032-6035) have recently reported a similar stoichiometry at the *lac* promoter. They have also shown that a single molecule of cAMP is found in the complex.