Mutations that alter the ability of the *Escherichia coli* cyclic AMP receptor protein to activate transcription

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Received October 2, 1990; Revised and Accepted November 23, 1990

ABSTRACT

The effects of a number of mutations in the *E. coli* cyclic AMP receptor protein (CRP) have been determined by monitoring the *in vivo* expression and *in vitro* open complex formation at two semi-synthetic promoters that are totally CRP-dependent. At one promoter the CRP-binding site is centred around 41.5 base pairs upstream from the transcription start whilst at the other promoter it is 61.5 base pairs upstream. The CRP mutation E171K reduces expression from both promoters whilst H159L renders CRP totally inactive: neither mutation stops CRP binding at either promoter. The mutations K52N and K52Q reverse the effect of H159L and 'reeducate' CRP to activate transcription. CRP carrying both H159L and K52N activates transcription from the promoter with the CRP site at -41.5 better than wild type CRP. In sharp contrast, this doubly changed CRP is totally inactive with respect to the activation of transcription from the promoter carrying the CRP site at -61.5. Our results suggest that CRP can use different contacts and/or conformations during transcription activation at promoters with different architectures.

INTRODUCTION

The *E. coli* cyclic AMP receptor protein (CRP, also known as CAP) is a transcription activator protein important for bacterial adaptation during growth in conditions of glucose starvation (1). CRP is, perhaps, the best understood of all transcription activators: in response to increases in cellular cyclic AMP levels, dimeric CRP binds to 22 base pair operator sequences located at target promoters and can accelerate the initiation of transcription at these promoters (2). Much is understood about how CRP recognises the 22 base pair target, mainly due to our knowledge of the structure of CRP from X-ray diffraction analysis (3). In contrast, our understanding of the molecular mechanisms by which CRP promotes transcription is poor, although most of

the available evidence, summarised by Ushida and Aiba (4), suggests that CRP must contact RNA polymerase. To attempt to locate sites on CRP that are involved directly in this interaction, positive control mutations have been sought by a number of groups. These are mutations in CRP that prevent transcription activation whilst not affecting CRP binding to target sites: some of these mutations may be located at CRP side chains that make direct contact with RNA polymerase during the acceleration of transcription initiation. Here we present experiments with CRP containing the positive control mutation, E171K, previously described by Irwin and Ptashne (5), together with a study of a second mutation, H159L, which totally destroys the transcription activation function of CRP. This second mutation was chosen after local mutagenesis of the *crp* gene and selection for positive control alleles (Karen Chapman, unpublished results). Starting with CRP H159L, we have searched for second site revertants which restore the ability of CRP to activate transcription, reasoning that, if the H159L mutation removed a group that contacts RNA polymerase directly, revertants may create a 'patch' on CRP that restores this interaction. We show that these second site mutations occur at residue 52, a position located on the surface of CRP but not adjacent to residue 159. We suggest that it is unlikely that the changes at position 52 directly recreate a contact destroyed by H159L.

One of the most intriguing features of CRP-dependent promoters is that, in different cases, the CRP-binding sequence is located at different positions with respect to the transcription start site (2). We recently described a series of semi-synthetic promoters in which a CRP-binding sequence resembling the consensus was cloned at different positions upstream of the same -10 sequence, derived from the *melR* promoter (6). We showed that CRP-dependent transcription was optimal when the centre of the CRP-binding sequence was located 41.5 or 61.5 base pairs upstream from the transcription start. Interestingly, these distances correspond precisely to the positions of the CRP-binding sites at the *E. coli gal* and *lac* promoters respectively. Here we report that CRP carrying both the positive control mutation,

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H159L, and the second site revertant, K52N, can activate transcription at the promoter with the binding site centred at -41.5 , but not with the site at -61.5 . The mutations have thus created an allele of *crp* with dramatically altered ability to activate transcription at different promoters.

MATERIALS AND METHODS

Bacterial strains, plasmids and constructions

The host strain M182 *crp lac* (7) was used in these studies. Table I lists the plasmids used throughout this work.

To clone *crp*, the *SalI*-*EcoRV* fragment carrying the structural gene and promoter (Fig.1A) was inserted into pBR322 to give pDCRP (Fig.1B). To make this plasmid, *SalI*-*HindIII* and *HindIII*-*EcoRV* fragments, isolated from plasmids made by Karen Chapman (unpublished) and Hiroji Aiba (8) respectively, were ligated, together with the *EcoRI*-*HincII* linker from M13mp8 (which includes a *Bam*HI site), between the *EcoRI* and *SalI* sites of pBR322. Using site directed mutagenesis, a unique *SphI* site was introduced by changing *crp* codon 162 from GGT to GGC (Fig.3) and further mutations were made as outlined below. *EcoRI*-*HindIII* fragments carrying the *crp* structural gene and derivatives were transferred to pHA7 (supplied by Hiroji Aiba: Fig.1B), placing the expression of *crp* under the control of the pBR322 P1 promoter (8,27). *crp* derivatives were also 'shuttled' on *Bam*HI-*SalI* fragments between pDCRP and the low copy number plasmid, pLG339 (supplied by Ian Roberts) conferring host resistance to kanamycin (9).

The nucleotide sequences of 4 'test' promoters used in this work are shown in Fig.2. Each promoter was cloned on an *EcoRI*-*HindIII* fragment either in the broad host range *lac* expression vector, pRW2 (10), conferring resistance to tetracycline or in the *cat* expression vector pKK232.8 (11), supplied by Pharmacia, conferring host resistance to ampicillin.

M182 *crp* transformants were grown on plates or liquid media containing 80 μ g/ml ampicillin to maintain derivatives of pDCRP or pKK232.8, 35 μ g/ml tetracycline to maintain derivatives of pRW2 and 25 μ g/ml kanamycin for pLG339 derivatives. MacConkey medium bought from Difco, was used throughout this work and the culture medium for β -galactosidase assays was the fructose-containing minimal medium described by Busby and Dreyfus (12).

Recombinant DNA methods

Standard methods were used for plasmid isolation, restriction, fragment purification and cloning (13). Oligonucleotides for site directed mutagenesis were designed to effect sequence changes at positions 159, 162 and 171 (shown in Fig.3) and were bought from Alta Bioscience at the University of Birmingham. To make sequence changes, the *EcoRI*-*HindIII* fragment including *crp* (Fig.1B) was first cloned into M13mp18 or mp19 (Pharmacia) and single stranded DNA was made. The Amersham *in vitro* mutagenesis kit (code RPN 1523) was then used following the manufacturer's protocol booklet. Single stranded DNA was made from candidates and checked for the presence of the desired base changes using the Pharmacia T7 sequencing kit (code 27-1680-01). In each case the total *crp* sequence was checked prior to transferring mutated *crp* genes back into pDCRP.

Physical methods

Mutant CRP protein was purified from M182*crp* cells carrying pDCRP derivatives according to the Ghosaini method (14). Protein concentrations were estimated from gels calibrated with

known concentrations of CRP: typically we obtained around 600 μ l of 2 μ M CRP from 1 litre of culture grown overnight in L broth. DNase I footprints and transcription run off experiments using purified *EcoRI*-*HindIII* fragments carrying the *CCpmelR* and *CC+20pmelR* promoters, purified CRP and purified *E.coli* RNA polymerase were performed precisely as before (6). Gel binding assays were made as described by Gaston *et al.*(15).

RESULTS

Positive control mutations in *crp*

The *crp* gene under the control of the *crp* promoter was cloned in pDCRP, illustrated in Fig.1B: in this plasmid the *crp* gene is cloned between a unique *HindIII* site just upstream of the translation start, and an *EcoRI*/*Bam*HI linker added at the *EcoRV* site located downstream of the translation stop codon. Base

Figure 1. DNA and plasmids used in this work. **A:** Schematic representation of the *E. coli* chromosome around the *crp* gene. The black bar represents the *crp* structural gene and the promoter and direction of transcription is indicated by an arrow. Restriction sites exploited in this work are indicated. **B:** Schematic representation of two recombinant pBR322 derivatives, pDCRP and pHA7, carrying *crp*. The *crp* structural gene, the fragment carrying the *crp* promoter and vector DNA are filled-in black, cross-hatched and hatched respectively. The location of the *crp* promoter in pDCRP and the pBR322 *P1* promoter in pHA7 are shown together with the restriction sites exploited in this work. The position at which an *SphI* site was introduced into the *crp* gene is shown bracketed. **C:** Schematic representation of the broad host range *lac* expression vector, pRW2, showing the location of the *lac* genes and the *EcoRI*-*HindIII* linker from M13mp8 (shaded black).

changes were introduced into *crp* in order to create the known positive control mutation E171K and the possible positive control mutation H159L: the location of these changes and new sequences are illustrated in Fig.3.

To determine the effects of the E171K and H159L substitutions in CRP, we measured transcription initiation at four 'model' promoters, illustrated in Fig.2. These promoters were cloned on

EcoRI-HindIII fragments into the broad host range *lac* vector pRW2 (Fig.1C) such that *lac* expression was dependent on promoter activity. The promoters *CCpmelR* and *CC+20pmelR* contain synthetic CRP-binding sequences positioned 41.5 and 61.5 base pairs upstream of the *melR* transcription start: we previously showed that transcription from these two promoters is totally dependent on CRP, and that these positions are optimal

Table I. Plasmids used in this work

Name	Rep. Origin	Antibiotic used for maintenance	Special features	Source
pDCRP	colE1	ampicillin	<i>crp</i> cloned under the control of <i>crp</i> promoter	This work
pDU9	colE1	ampicillin	pDCRP with <i>crp</i> gene replaced by M13mp8 polylinker	This work
pHA7	colE1	ampicillin	<i>crp</i> cloned under pBR322 promoter	Aiba (8)
pRW2	RK2	tetracycline	broad host range <i>lac</i> expression vector with no (10) inserted promoter	Williams
pRW2 <i>CCpmelR</i>	RK2	tetracycline	pRW2A with semisynthetic CRP-dependent promoter controlling <i>lac</i> expression	Williams (10)
pKK232.8	colE1	ampicillin	<i>cat</i> expression vector	Pharmacia (11)
pKK232.8 <i>CCpmelR</i>	colE1	ampicillin	pKK232.8 with <i>CCpmelR</i> promoter controlling <i>cat</i>	This work
pLG339	pSC101	kanamycin	low copy number cloning vector compatible with colE1 and RK2 based plasmids	I.Roberts (9)
pLG339 /CRP	pSC105	kanamycin	pLG339 carrying <i>crp</i> under the control of <i>crp</i> promoter	This work

Figure 2. Nucleotide sequence of test promoters used in this work. Each sequence is numbered with the transcription start point taken as +1: in each case the -10 hexamer sequence is identified by a squared bracket. The promoters were cloned with an upstream *EcoRI* site and a downstream *HindIII* site. The location of the 22 base pair CRP binding sequence in each case is indicated by underlining with a dot to show the centre of symmetry of the site. The construction of the derivatives carrying *CCpmelR*, *CC+20pmelR*, *galP1* are described in references 24,6 and 25. The 20 base pair linker that was inserted into *CCpmelR* to make *CC+20pmelR* is boxed. The *galP1Δ4* promoter was made by removing 4 base pairs from the synthetic *SphI* site (5' GCATGC 3' at -31) that had been introduced into *galP1*.

Figure 3. Sequence alterations in CRP mutants. CRP is represented as a bar running from position 1–209 with the positions of α helices A–F and regions of β sheet 1–12 shown by boxes and solid lines respectively. Amino acid sequences corresponding to residues 51–54, 158–163 and 170–172 are shown expanded at the bottom of the figure together with sequence changes responsible for changes discussed in the text. In this figure we have deliberately copied the style of Fig.2 from reference 20.

Figure 4. Activation of different promoters by CRP and derivatives. The bars in the figure represent β -galactosidase expression (measured in Miller (26) units) by pRW2 and derivatives in M182*crp* cells. The pRW2 plasmids contained no promoter (0) or the *CCpmelR*, *CC+20pmelR*, *galP1* or *galP1 Δ 4* promoters as shown on the abscissa. pDCRP derivatives carrying wild type CRP or the H159L or E171K changes were introduced as indicated. pDU9 is a derivative of pDCRP in which the *EcoRI-HindIII* fragment carrying *crp* was replaced by a polylinker and is used as a control.

for CRP-induced transcription initiation (6). Expression from the third promoter, *galP1*, is stimulated by CRP binding 41.5 base pairs upstream of the transcription start but the promoter can function in the absence of CRP (16). The fourth promoter, *galP1 Δ 4*, is derived from *galP1* by the deletion of 4 base pairs at the *SphI* site between the CRP-binding site and the transcription start. Like *galP1*, this promoter is active in the absence of CRP, but activity is sharply inhibited by the binding of CRP: CRP-dependent inhibition of expression from this promoter can, therefore, be used to monitor CRP binding to target sites *in vivo*,

independently of any transcription activation. Since the plasmids, pDCRP and pRW2 are compatible, and encode resistance to different antibiotics, they can be introduced into the same *crp* strain in order to measure the activation of expression from the test promoters by different *crp* alleles cloned in pDCRP.

The data in Fig.4A show the activation of transcription at the promoters *CCpmelR* and *CC+20pmelR* by CRP E171K, H159L, or wild type CRP. The different *crp* alleles were carried on pDCRP, and a derivative, pDU9, deleted for the *crp* gene was used as a control. The data show that expression from the

promoters *CCpmelR* and *CC+20pmelR* is totally dependent on CRP. The E171K substitution reduces expression by different amounts from the two promoters, whilst H159L totally abolishes CRP-dependent activation at both promoters. To check that the mutations did not stop binding to operator sequences, the effects of CRP E171K, H159L, or wild type CRP on expression from the *pgalP1δ4* promoter were determined. The different *crp* alleles were introduced in *trans* to the *pgalP1δ4* promoter cloned in pRW2 in M182*crp*: in each case the CRP-independent expression from this promoter was totally blocked by CRP suggesting that the two mutations in *crp* have little or no effect on binding to DNA. From this, it is apparent that H159L is a 'strong' positive control mutation whilst E171K has less clear-cut effects.

Second site revertants of *crp* H159L

In order to select for mutations that restored activity to CRP H159L, the *Bam*HI-*Sal*I fragment carrying this allele was transferred to plasmid pLG339 to give pLG339/CRP H159L. This plasmid, encoding resistance to kanamycin, was introduced into strain M182*crp* carrying two compatible plasmids: the broad host range plasmid pRW2/*CCpmelR*, containing the *lac* genes under the control of the *CCpmelR* promoter, and pKK232.8/*CCpmelR* containing the genes encoding resistance to chloramphenicol under the control of the same *CCpmelR* promoter. Since CRP H159L cannot activate transcription from the *CCpmelR* promoter, transformants carrying all three plasmids are Lac⁻ and Cm^s. Lac⁺ revertant colonies were then selected from plates containing chloramphenicol. In four independent colonies we found that the mutation causing *lac* expression and resistance to chloramphenicol was located on the pLG339 derivative encoding CRP H159L. In these four cases, the *Bam*HI-*Sal*I fragment carrying *crp* was transferred from the pLG339 derivative back to pDCRP. The mutation(s) were located between the *Sph*I and *Hind*III sites (Fig. 1B) and the entire 513 base pair nucleotide sequence of the *Sph*I-*Hind*III fragment from each of the four candidates was determined. In each case the mutations causing the H159L mutation were present (Figs 3 and 5): additionally in three cases the *crp* codon corresponding to lysine 52 was changed from AAA to AAC to give CRP K52N, whilst in the fourth case the change was to CAA to give CRP K52Q. Since both new mutations created similar phenotypes CRP H159L K52N was retained for further study and the K52N mutation was separated from H159L by exploiting the unique *Sfa*NI site in *crp* between codons 52 and 159.

The effects of the new mutations on CRP activity at the *CCpmelR* and *CC+20pmelR* promoters (cloned in the broad host range plasmid, pRW2) were quantified by measuring *lac* expression in M182*crp* cells carrying different pDCRP derivatives. The results in Fig. 6 show that CRP-dependent expression from *CCpmelR*, which is totally suppressed by H159L, is restored by the double change, H159L plus K52N. Interestingly, CRP containing the double change, or K52N alone, stimulates transcription from *CCpmelR* better than the wild type. In sharp contrast, the K52N substitution fails to reverse the knock-out effect of H159L on expression from the *CC+20pmelR* promoter. However, CRP carrying K52N alone, activates transcription at the *CC+20pmelR* promoter to 70% of the level with wild type CRP. Thus in the presence of H159L, the K52N mutation gives a form of CRP that can activate transcription from a promoter with the CRP-binding site located at -41.5 but not from the promoter with the site at -61.5.

Because expression of *crp* in pDCRP is from a promoter that

Figure 5. Sequence analysis of CRP substitutions H159L, K52N and K52Q. Sequence reactions were performed on single stranded DNA from M13mp18/19 carrying different *crp* alleles. Relevant parts of the sequence gels are shown. For H159L the sequence of the non-coding strand is shown together with the complement and the translated amino acid sequence. Base substitutions responsible for the change H159L, together with the change that introduced an *Sph*I site are encircled. For K52N and K52Q the sequence shown corresponds to the coding strand. Base changes are circled and the amino acid change at position 52 is underlined.

Figure 6. Activation of *CCpmelR* and *CC+20pmelR* by CRP and derivatives. The bars represent β -galactosidase expression controlled by the *CCpmelR* and *CC+20pmelR* promoters cloned in pRW2, determined as in Fig. 4. pDCRP derivatives encoding wild type *crp*, or *crp* carrying either the H159L or K52N changes or both, were introduced as indicated. pDU9 is a control from which *crp* was removed.

is autoregulated by CRP (17), the new *crp* alleles were transferred to pHA7 such that expression would be under the control of a CRP-independent pBR322 promoter (P1: see ref. 27). The experiment described in Fig. 6 was then repeated using pHA7 derivatives carrying each of the mutated *crp* genes. The results (not shown) were almost identical showing that the conclusions are not biased by variations in CRP expression due to the nature of the promoter.

In vitro studies with mutant CRP protein

Small quantities of CRP carrying the single substitutions H159L or K52N, together with wild type CRP and protein carrying both changes, were prepared. Gel binding assays showed that all four proteins bound to DNA fragments carrying CRP-binding sequences (Fig. 7 lanes a–e). Titrations showed that the H159L mutation had little effect on the affinity of CRP binding to DNA fragments, whilst the K52N mutation weakens binding by 3–8 fold (A. Kolb, unpublished). Further experiments (Fig. 7 lanes f–i) demonstrated that the purified CRP carrying K52N (with or without H159L) still required cAMP for binding to the target site.

Next we determined the ability of the four different preparations of CRP to promote transcription at the *CCpmelR* and *CC+20pmelR* promoters. Purified CRP and RNA polymerase were incubated with DNA fragments and open complexes were probed by DNase I: the resulting footprints are shown in Fig. 8. In parallel to the footprinting, 32 P-labelled nucleoside triphosphates were added to aliquots of the protein-DNA mixes and single-round run-off transcripts were made. After gel electrophoresis these transcripts were quantified and the relative amounts of transcript in each case are shown in Fig. 8 under the appropriate lane.

With *CCpmelR* (Fig. 8 lanes a–g), in the presence of RNA polymerase and wild type CRP, open complexes form which give a large footprint (lane c) from positions –65 to +20 (with respect to the transcription start point). Similar but stronger footprints are seen with mutant CRP carrying K52N (lane f) or the double change K52N, H159L (lane e). Interestingly, the amount of

Figure 7. Binding of different CRP derivatives to DNA fragments. The figure shows autoradiograms of gel binding assays run to detect the binding of purified CRP to 32 P-labelled fragments containing the *CC* CRP binding site (24). The experiment in lanes a–e was performed with gels and all buffers containing 100 μ M cAMP. In the gel shown in lanes f–i, cAMP (200 μ M) was included only in the incubation buffer of samples applied to lanes g and i. Lane a shows the migration of the labelled fragment alone whilst in the other lanes the fragment (1 nM) was incubated with 50 nM purified CRP prior to electrophoresis. The CRP derivatives used were as follows: wild type (lane b), H159L (lane c), H159L plus K52N (lanes d, f and g) or K52N (lanes e, h and i).

transcript increases from 10 units with wild type CRP to 35 units with CRP carrying K52N. In contrast with CRP H159L very little open complex forms *in vitro* and very little transcript is seen even though the footprint of CRP alone around –40 is clearly seen (lane d). Hence with *CCpmelR* the *in vitro* results (Fig. 8) agree well with the *in vivo* data (Fig. 6): H159L stops the activation of transcription but this effect is reversed by K52N. CRP containing both H159L and K52N or K52N alone promote open complex formation and transcription activation more effectively than wild type CRP.

With *CC+20pmelR* (Fig. 8 lanes h–n), again, in the presence of RNA polymerase and wild type CRP, open complexes form which give a large footprint and which are competent in transcription initiation (lane j). With CRP H159L these open complexes do not form although a clear CRP footprint is seen (lane k) confirming that CRP carrying this mutation is unable to activate transcription. Strikingly, in contrast to the situation with *CCpmelR*, the K52N mutation does not reverse the effect of H159L (lane l). Thus *in vitro*, as *in vivo*, CRP carrying both the H159L and K52N changes can activate transcription initiation at *CCpmelR* but not at *CC+20pmelR*. Interestingly RNA polymerase and CRP carrying K52N alone (lane m) gave a weak footprint at *CC+20pmelR*, again demonstrating agreement between the findings *in vitro* and *in vivo* (compare results in Fig. 6 with Fig. 8).

DISCUSSION

The changes E171K and H159L in CRP are clear examples of positive control mutations that reduce the ability of CRP to activate transcription without stopping recognition and binding to operator sites. E171K has received particular attention since it alters an acidic amino acid from helix E of the helix-turn-helix motif (5). However, in our hands, the effects of H159L are much clearer: the E171K substitution causes different effects at different promoters whilst the H159L change results in totally inactive

Figure 8. DNase I Footprint analysis of open complex formation. *EcoRI-HindIII* fragments carrying the *CCpmeIR* (lanes a–g) or *CC+20pmeIR* (lanes h–n) promoters were labelled on the 'upper' strand at the *EcoRI* site (Fig.2) and incubated with different combinations of CRP and/or RNA polymerase prior to treatment with DNase I. The figure shows autoradiograms of gels on which the resulting fragments were analysed. The gels were calibrated with G sequence reactions of the two labelled fragments: the calibration for each promoter is shown alongside the gel, numbered with respect to the transcription start point. The proteins incubated with the labelled DNA fragments before DNase I treatment were as follows: lanes a,h, no protein; lanes b,i, wild type CRP; lanes c,j, wild type CRP plus RNA polymerase; lanes d,k, CRP H159L plus RNA polymerase; lanes e,l, CRP H159L/K52N plus RNA polymerase; lanes f,m, CRP K52N plus RNA polymerase; lanes g,n, RNA polymerase only. The extent of footprints due to CRP and RNA polymerase alone are indicated by vertical arrows. The numbers under each lane estimate the amount of transcript initiating at +1 when the 4 nucleoside triphosphates were added to each mixture instead of DNase I: to measure this transcripts were labelled, separated by electrophoresis and estimated, in arbitrary units, after scanning autoradiograms of the gels. More details of this protocol and representative gels showing transcripts are in Fig.2 of reference 6.

CRP. Since it is widely believed that one way by which CRP activates transcription is to contact RNA polymerase directly, it is pertinent to ask whether E171 and H159 are amongst the residues that make this specific contact. To attempt to find evidence to support this proposition for H159L, we searched for second site mutations, reversing the deleterious effect of H159L, and thus 'reeducating' CRP as a transcription activator. However the location of the second site mutations at position 52 sheds little light on the exact nature of the defect in CRP carrying H159L. The fact that position 52 is situated far from position 159 (18) argues that the change K52N cannot directly recreate the contact lost by the H159L substitution. One possibility is that K52N sets up a new additional interaction, say with RNA polymerase, which is productive only at *CCpmelR*, with the CRP site centred at -41.5. This would explain the effects of the K52N change alone, increasing expression from *CCpmelR* but hardly affecting expression from *CC+20pmelR*. Alternatively, the effect of the K52N substitution may be relayed by a conformational change through the protein. Interestingly both K52 and H159 are located in β hairpins, the conformation of which may be particularly sensitive to sequence changes (19). Aiba *et al.* (20) reported a change at position 53 (D53H) which creates a *crp** phenotype i.e. it allows CRP to adopt a conformation that activates transcription in the absence of cyclic AMP. We obtained *PHA7* carrying this and other *crp** mutations from Hiroji Aiba and compared their behaviour *in vivo* with CRP carrying K52N. Whilst the *crp** derivatives from Aiba checked out exactly as previously described (20), we could find no evidence *in vivo* that the change at position 52 relaxed the requirement for cyclic AMP for transcription activation (S.B. and C.Webster, unpublished).

The striking result from this work is that CRP carrying the double mutation H159L, K52N can activate transcription from a model promoter with the CRP site centred at 41.5 base pairs upstream of the transcription start (*CCpmelR*), but is totally inactive at a similar promoter with the site centred 61.5 base pairs upstream (*CC+20pmelR*). In recent analogous experiments we have investigated the activity of CRP carrying H159L and K52N at the *E. coli gal* and *lac* promoters: CRP H159L, K52N activates expression at the *gal* promoter but is inactive at *lac* promoter (R. Williams, unpublished data). The clear implication of this is that different CRP elements can be used at promoters with different geometry. Perhaps K52N creates a contact that stimulates open complex formation only at promoters where CRP binds around -41.5. We recently reported 'subtle' differences in the kinetics of CRP-dependent transcription activation at *CCpmelR* and *CC+20pmelR*: in the former, CRP primarily accelerates isomerisation from the closed to open complex, whilst in the latter, it aids initial binding of RNA polymerase (6). Presumably these differences are a reflection of different contacts being made and broken at promoters of different architecture. All surveys have concluded that CRP-dependent promoters can be organised in many different ways (2,4,6,21-23) and it now appears that CRP must be capable of activating transcription by a variety of mechanisms. To our knowledge this is the first report of a *crp* allele which has a clear-cut differential effect at two well-characterised promoters. It remains to be seen whether further genetic analysis will allow us to define clear-cut 'patches' on the CRP molecule which are essential for stimulating activity at promoters with particular geometries.

ACKNOWLEDGEMENTS

We thank Hiroji Aiba for communicating results prior to publication and generously supplying many of his CRP plasmid

constructions. This work was funded by the U.K. Science and Engineering Research Council (SERC) and the Wolfson Foundation. KG was supported by a Wellcome Travelling Research Fellowship and AB by an SERC studentship.

REFERENCES

1. Ullmann, A. and Danchin, A. (1983) *Adv. Cyclic Nuc. Res.* **15** 2-53.
2. de Crombrughe, B., Busby, S. and Buc, H. (1984) *Science* **224** 831-838.
3. Weber, I. and Steitz, T. (1984) *Proc. Natl. Acad. Sci. USA* **81** 3973-3977.
4. Ushida, C. and Aiba, H. (1990) *Nucl. Acids Res.* **18** 6325-6330.
5. Irwin, N. and Ptashne, M. (1987) *Proc. Natl. Acad. Sci. USA* **84** 8315-8319.
6. Gaston, K., Bell, A., Kolb, A., Buc, H. and Busby, S. (1990) *Cell* **62** 733-743.
7. Busby, S., Kotlarz, D. and Buc, H. (1983) *J. Mol. Biol.* **167** 259-274.
8. Aiba, H., Fujimoto, S. and Ozaki, N. (1982) *Nucl. Acids Res.* **10** 1345-1361.
9. Stoker, N., Fairweather, N. and Spratt, B. (1982) *Gene* **18** 335-341.
10. Lodge, J., Williams, R., Bell, A., Chan, B. and Busby, S. (1990) *FEMS Lett.* **67** 221-226.
11. Brosius, J. (1984) *Gene* **27** 151-160.
12. Busby, S. and Dreyfus, M. (1983) *Gene* **21** 121-131.
13. Maniatis, T., Fritsch, E. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
14. Ghosaini, L., Brown, A. and Sturtevant, J. (1988) *Biochemistry* **27** 5257-5261.
15. Gaston, K., Chan, B., Fox, J., Kolb, A. and Busby, S. (1988) *Biochem. J.* **252** 809-818.
16. Chan, B. and Busby, S. (1989) *Gene* **84** 227-236.
17. Morita, T., Shigesada, K., Kimizuka, F. and Aiba, H. (1988) *Nucl. Acids Res.* **16**, 7315-7332.
18. Weber, I. T. and Steitz, T. (1987) *J. Mol. Biol.* **198** 3121-326.
19. Sibanda, B. L., Blundell, T. L. and Thornton, J. M. (1989) *J. Mol. Biol.* **206**, 759-777.
20. Aiba, H., Nakamura, T., Mitani, H. and Mori, H. (1985) *EMBO J.* **4** 3329-3332.
21. Sogaard-Andersen, L., Mollegaard, N., Dowthwaite, S. and Valentin-Hansen, P. (1990) *Mol. Microbiol.* **4** 1595-1602.
22. Aiba, H., Hanamura, A. and Tobe, T. (1989) *Gene* **85** 91-97.
23. Goransson, M., Forsman, K., Nilsson, P. and Uhlin, B. (1989) *Mol. Microbiol.* **3** 1557-1565.
24. Bell, A., Gaston, K., Cole, J. and Busby, S. (1989) *Nucl. Acids Res.* **17** 2865-2874.
25. Gaston, K., Kolb, A. and Busby, S. (1989) *Biochem. J.* **261** 649-653.
26. Miller, J. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
27. Balbas, P., Soberon, X., Merino, E., Zurita, M., Lomeli, H., Valle, F., Flores, N. and Bolivar, F. (1986) *Gene* **50** 3-40.