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Protein–protein interactions during transcription activation: the case of the *Escherichia coli* cyclic AMP receptor protein

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SUMMARY

The *Escherichia coli* cyclic AMP receptor protein (CRP) is a homodimeric transcription activator triggered by cyclic AMP. *Escherichia coli* contains more than 100 different promoters that can be activated by CRP: in most cases the CRP acts by making direct contact with RNA polymerase. Remarkably, there is considerable variation in the location of the DNA site for CRP from one CRP-dependent promoter to another. Genetic methods have been used to locate the activating regions of CRP that make contact with RNA polymerase at promoters of different architectures. At promoters where the DNA site for CRP is centred near to positions -61, -71 or -81 (i.e. 61, 71 or 81 base pairs upstream of the transcript start-point, respectively), a single surface-exposed loop (Activating Region 1) in the downstream subunit of the CRP dimer makes contact with RNA polymerase. The contact site in RNA polymerase is located in one of the C-terminal domains of two RNA polymerase alpha subunits. At promoters where the DNA site for CRP is centred near to position -41, both subunits of the CRP dimer make contact with RNA polymerase via three separate surface exposed regions (Activating Regions 1, 2 and 3). At these promoters, where bound CRP overlaps with RNA polymerase-binding elements, the C-terminal domains of the polymerase alpha subunits are displaced and bind upstream of CRP.

Activation at a number of *E. coli* promoters is dependent on binding of two CRP dimers, with one dimer bound near to position -41 and the other dimer bound further upstream. In these cases, both bound CRP dimers contact RNA polymerase. The CRP dimer bound around position -41 contacts RNA polymerase via Activating Regions 1, 2 and 3, whereas the upstream bound CRP dimer contacts one of the displaced alpha C-terminal domains via Activating Region 1 in the downstream CRP subunit. Thus in these cases, codependence on two activators is due to simultaneous contacts between separate activators and RNA polymerase. This mechanism allows great flexibility, as any activator that can contact the C-terminal domain of the RNA polymerase alpha subunits can act cooperatively with CRP.

1. INTRODUCTION

Although the majority of transcription activators function by making direct contacts with the transcription apparatus, our knowledge of the details of these contacts is sketchy in most cases. Here, we review a series of studies on the *Escherichia coli* cyclic AMP receptor protein (CRP) that identify the regions of CRP making contact with RNA polymerase (RNAP) during transcription activation, and suggest models for the organization of transcriptionally competent ternary CRP-RNAP-promoter complexes.

CRP activates expression from more than 100 different promoters in *E. coli* (reviewed by Kolb *et al.* 1993*a*; Busby & Kolb 1996). CRP binding to target promoters is dependent on cyclic AMP (cAMP), and increases in both cAMP levels and CRP levels are triggered by a variety of stresses (in particular, glucose starvation). CRP binds as a dimer to 22 base pair (b.p.) target sequences: the consensus 22 b.p. sequence is a perfect inverted repeat. The structure of CRP bound to a synthetic DNA target has been determined using X-ray methods. Each CRP subunit consists of two domains, an N-terminal cAMP-binding domain and a C-terminal DNA-binding domain: each subunit of the CRP dimer binds to one half of the target sequence via a helix-turn-helix structure in the C-terminal domain (Schultz *et al.* 1991). Upon binding to target sites, CRP causes a sharp bend (greater than 90°) in the DNA.

A striking feature of *E. coli* CRP-dependent promoters is that the location of the DNA site for CRP differs from one case to another (Busby & Kolb 1996). The most commonly found location is -41.5 (i.e. the centre of the 22 b.p. sequence is located between base pairs 41 and 42 upstream from the transcript start, e.g. at the *galP1* and *melR* promoters), but there are many cases where the CRP site is around -61.5 (e.g. at the *lac* promoter), and several cases where CRP binds further upstream. Additionally, multiple CRP binding sites are found at some target promoters. In this article, we consider promoters where CRP binds at -61.5, pro-

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moters where CRP binds at -41.5 and, finally, promoters where two CRP dimers bind in tandem.

2. PROMOTERS WHERE CRP BINDS AT -61.5

Footprinting studies at promoters activated by CRP binding at -61.5 show that RNAP and CRP bind adjacently and cooperatively, and interact directly (e.g. at the *lac* promoter, Spassky *et al.* 1984). The activating region (AR) on CRP that makes contact with RNAP was identified from the location of positive control mutants in CRP: these are single amino acid substitutions that interfere with transcription activation at promoters like the *lac* promoter, but do not affect DNA binding or triggering by cAMP (Bell *et al.* 1990; Eschenlauer *et al.* 1991; Zhou *et al.* 1993*a*). These substitutions all fall in a single surface-exposed β turn in the small DNA-binding domain of CRP (AR1, see figure 1). Saturation mutagenesis of *crp* proved that AR1 is the only functional activating region at promoters where CRP binds at -61.5, and alanine scanning showed that T158 is the most crucial residue of AR1 (Niu *et al.* 1994). Furthermore, although CRP is a dimer carrying a potentially functional AR1 on both subunits, only the activating region in the downstream subunit of the dimer is functional. This was demonstrated using the technique of oriented heterodimers in which CRP dimers carrying one active AR1 and one inactive AR1 were made (Zhou *et al.* 1993*b*). These heterodimers were then oriented with active AR1 either upstream or downstream (this was done using single amino acid substitutions in the CRP helix-turn-helix that alter DNA binding preference): CRP dimers carrying a single functional AR1 in the downstream subunit are active at the *lac* promoter, whereas dimers carrying the functional AR1 in the upstream subunit are inactive.

E. coli RNAP is an assembly of five subunits β, β′, σ and α_2. The β and β′ subunits carry the active centre of the enzyme, whereas the role of the σ subunit is to recognize promoter -10 and -35 elements. Each α subunit consists of two independently folding domains, an N-terminal domain (αNTD) essential for the assembly of RNAP and a C-terminal domain (αCTD)(Blatter *et al.* 1994; Kimura *et al.* 1994). Several pieces of evidence show that the contact site for CRP on RNAP is located in αCTD. In ternary CRP-RNAP-*lac* promoter complexes, AR1 and αCTD can be efficiently photo-crosslinked so must be in close proximity (Chen *et al.* 1994). Purified RNAP α subunits interact with CRP and this interaction is dependent on AR1 (Attey *et al.* 1994; Savery *et al.* 1995). CRP-dependent activation of the *lac* promoter can be suppressed by single amino acid substitutions or deletions in αCTD (Igarashi & Ishihama 1991; Zou *et al.* 1992, Tang *et al.* 1994). Additionally, footprint analysis shows that αCTD footprints *lac* promoter DNA immediately downstream of bound CRP (Kolb *et al.* 1993*b*). This suggests a simple model for CRP-dependent activation in which the downstream subunit of the bound CRP dimer contacts αCTD directly, thereby recruiting αCTD to the promoter DNA and

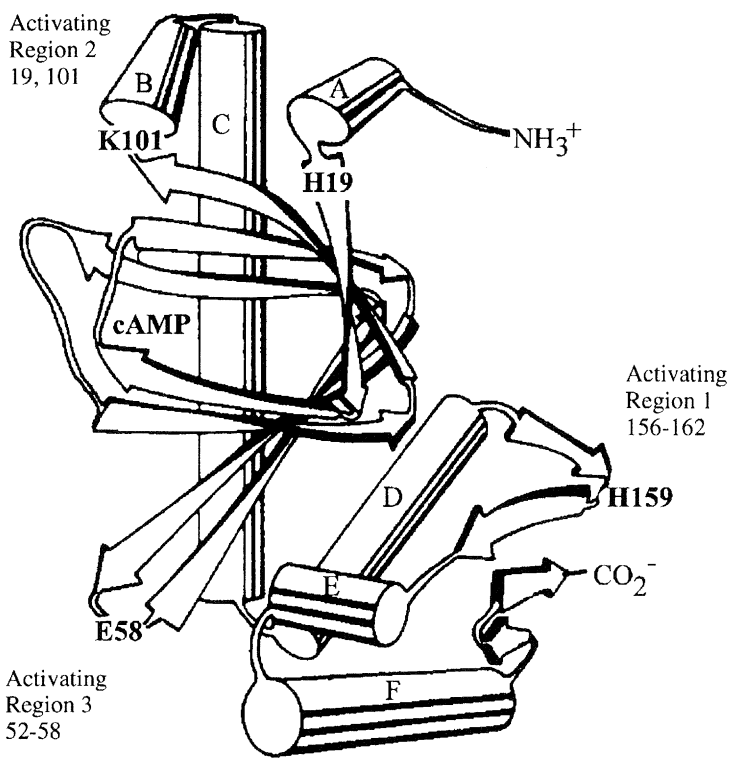

Figure 1. The activating regions of CRP. The figure shows a ribbon diagram of a CRP monomer with α helices shown as cylinders and β sheets shown as arrows, taken from the work of McKay *et al.* (1982). The figure shows the large N-terminal cAMP-binding domain and the C-terminal DNA-binding domain (helix F is the recognition helix that penetrates the major groove at target promoters). The figure shows the location of Activating Regions 1, 2 & 3 as defined by the location of positive control mutants that interfere with transcription activation at different promoters. The locations of several crucial amino acids, H159 in AR1, H19 & K101 in AR2 and E58 in AR3 are shown.

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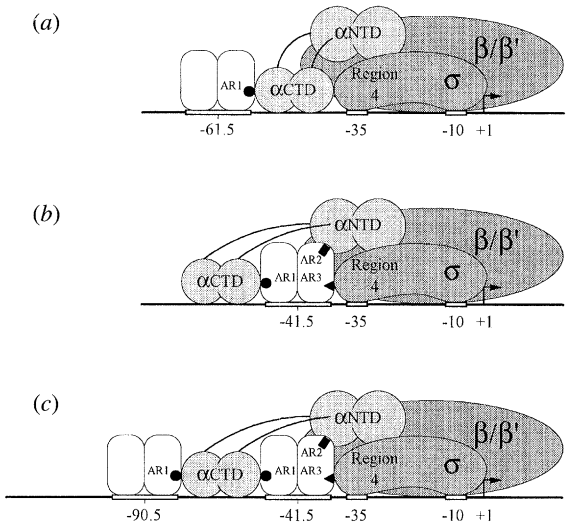

Figure 2. Organization of CRP and RNAP subunits at different promoters. (*a*) Promoter with a single DNA site for CRP at -61.5 (e.g. the *E. coli lac* promoter). A contact between AR1 in the downstream subunit of the CRP dimer and αCTD promotes RNAP binding. (*b*) Promoter with a single DNA site for CRP at -41.5 (e.g. the *E. coli melR* promoter). AR1 in the upstream subunit and AR2 & AR3 in the downstream subunit of the CRP dimer contact RNAP and promote binding. AR1 contacts αCTD and AR3 contacts Region 4 of σ. The contact site for AR2 is currently unknown. (*c*) Promoter with tandem DNA sites for CRP at -41.5 and -90.5 (e.g. the *E. coli cdd* promoter). Both the downstream and upstream CRP dimers contact RNAP. Contacts involving the downstream CRP dimer are as in (*b*). A contact between AR1 in the downstream subunit of the upstream CRP dimer and the second αCTD subunit promotes RNAP binding and is responsible for synergy.

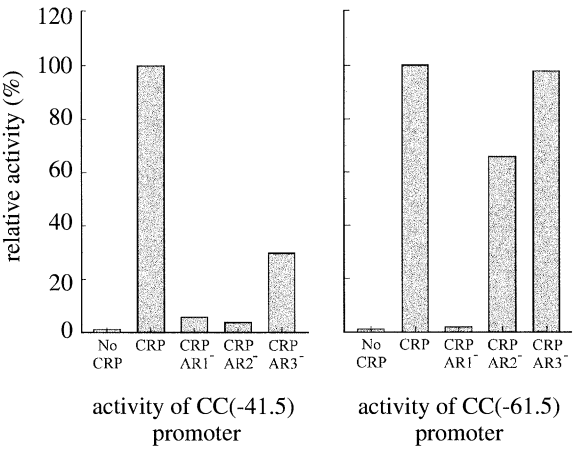

Figure 3. Activation of CC(-41.5) and CC(-61.5) promoters by different CRP mutants. The CC(-41.5) and CC(-61.5) promoters are derivatives of the *E. coli melR* promoter carrying CRP sites either at -41.5 or -61.5. These promoters were cloned in a *lac* expression plasmid, transformed into Δ*lac* Δ*crp E. coli* cells, and plasmids carrying different mutant *crp* genes were introduced. The figure shows the resulting β-galactosidase levels, measured in exponentially growing cells. The different plasmids encoding CRP were: pDU9, control encoding no *crp*, pDCRP carrying wild type *crp*, pDCRP carrying *crp* with the HL159 (AR1⁻); KE101 (AR2⁻) or EH58 (AR3⁻) substitutions.

improving the binding affinity of RNAP (figure 2*a*). The model proposes that the two αCTDs of RNAP fit into the gap between the DNA target for CRP and the -35 hexamer (the target for the C-terminal region, Region 4, of the RNAP σ subunit), most likely making simultaneous contact with CRP and Region 4 of σ.

3. PROMOTERS WHERE CRP BINDS AT -41.5

RNAP and CRP interact cooperatively at promoters dependent on CRP binding to sites around -41.5 (e.g. at the *galP1* promoter, Spassky *et al.* 1984) and footprint analysis shows that RNAP binds both upstream and downstream of CRP. The upstream protection is due to the αCTD dimer (Attey *et al.* 1994), the linker joining αCTD to αNTD being sufficiently flexible to permit αCTD to stretch around bound CRP (Blatter *et al.* 1994) (figure 2*b*). The effects of many different single amino acid substitutions in CRP have been examined, to identify the activating regions that contact RNAP. For example, the substitutions in AR1 that suppress activation at the *lac* promoter also interfere with activation at this group of promoters (Bell *et al.* 1990, Williams *et al.* 1991; Zhou *et al.* 1994*a*). However, in many instances, the effects of these substitutions are smaller than at the *lac* promoter, suggesting that CRP makes supplementary contacts with RNAP when it is bound at -41.5 (West *et al.* 1993). Screening of CRP mutant libraries for further positive control mutants suggests that two supplementary activating regions (AR2 and AR3) play a role (R. Ebright, personal communication; R. Williams, V. Rhodius & S. Busby, unpublished results). AR2 is a patch made up of several positively charged amino acids in the CRP N-terminal domain, whereas AR3 is another surface-exposed β turn on the opposite face of CRP to AR1 (figure 1). Substitutions in AR1, AR2 and AR3 reduce CRP-dependent expression at promoters where CRP binds at around -41.5, although the effects of substitutions in AR3 are less than those due to changes in AR1 and AR2 (figure 3). Strikingly, substitutions in AR2 and AR3 have little or no effect at promoters where CRP binds at around -61.5. Thus we conclude that whereas CRP contacts RNAP solely via AR1 at promoters when it binds at -61.5, CRP can contact RNAP via AR1, AR2 and AR3 at promoters where it binds around -41.5. It is likely that these three contacts are additive and defects in one activating region can be compensated for by improving the other regions. For example, the effects of the TA158 or HL159 substitutions that inactivate AR1 can be reversed by the EG96 or KN52 substitutions that improve AR2 or AR3 respectively (Williams *et al.* 1991; West *et al.* 1993). Interestingly, the effects of different substitutions in the three activating regions differ from one promoter to another, suggesting that contribution of the three contacts varies from case to case.

Inspection of the CRP structure shows that AR1, AR2 and AR3 are located on opposite faces of the CRP monomer such that it would be difficult for RNAP to contact each activating region simultaneously (figure 1). However, in the CRP dimer, AR1 in one subunit is

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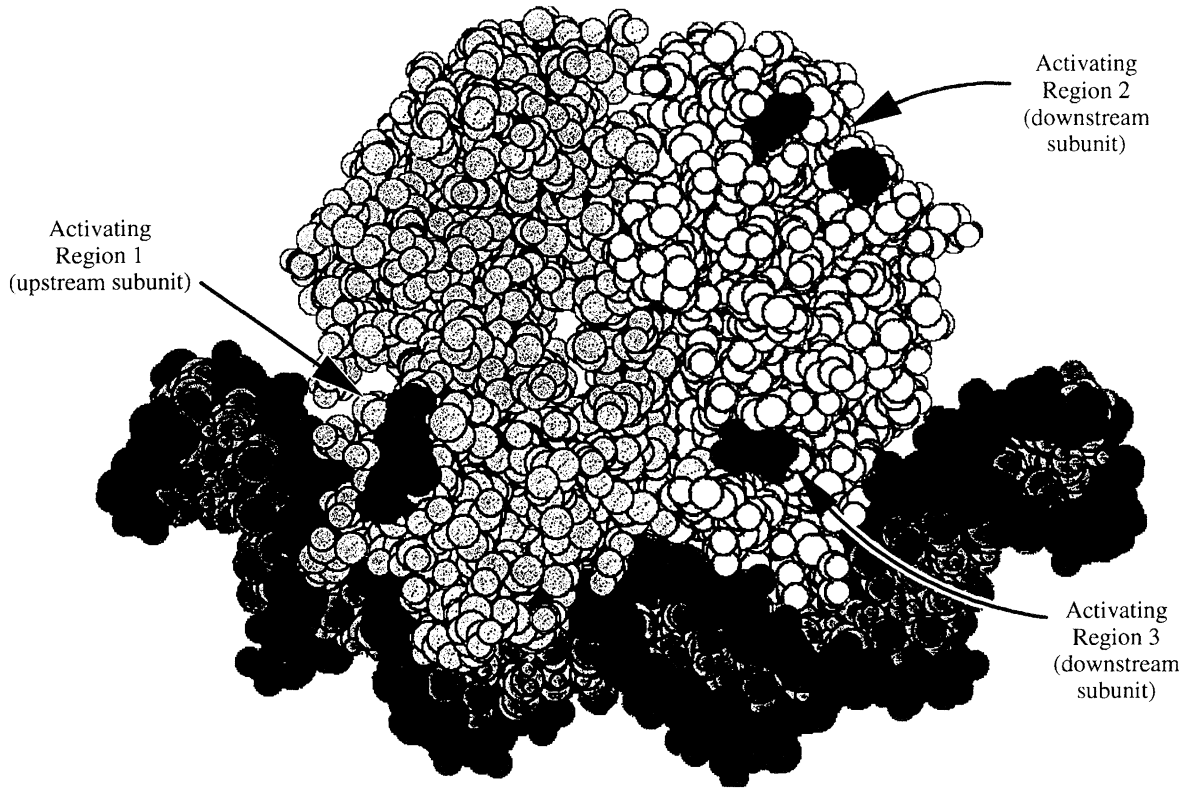

Figure 4. Model of CRP dimer bound to DNA. The model, taken from Schultz *et al.* (1991), shows that adjacent faces of the CRP dimer carry different surfaces. Thus AR1 in one subunit is displayed adjacent to AR2 and AR3 in the neighbouring subunit.

displayed on the same face of the dimer as AR2 and AR3 in the adjacent subunit (figure 4). This suggests a simple model (figure 2*b*) in which AR1, AR2 and AR3, displayed on adjacent faces of the CRP dimer, are contacted by different parts of RNAP as it binds to promoter elements both upstream and downstream of CRP bound at -41.5. Substantial evidence for this model comes from oriented heterodimer experiments, which show that although the CRP dimer carries two copies of each activating region, AR1 is functional only in the upstream subunit (Zhou *et al.* 1994*b*); whereas AR2 and AR3 are functional only in the downstream subunit (Williams *et al.* 1996).

The AR1 heterodimer experiment (illustrated in figure 5*a*) was performed with CRP heterodimers carrying a functional AR1 in one subunit and an inactive AR1 (due to either the TA158 or HL159 substitution) in the other subunit. At a model promoter dependent on CRP binding at -41.5 (derived from the *melR* promoter), these heterodimers are active when the functional AR1 is located in the upstream subunit, but are inactive when the functional AR1 is located in the downstream subunit. Thus AR1 is functional only in the upstream CRP subunit, where it docks with αCTD which is located upstream of bound CRP (figure 2*b*). Further evidence for this comes from footprint analysis of ternary CRP-RNAP-promoter complexes: disruption of AR1 interferes with protection only in the zone between bound CRP and upstream-bound αCTD (Attey *et al.* 1994).

The AR2/AR3 heterodimer experiment (illustrated in figure 5*b*, *c* & *d*) was performed by exploiting the EG96 and KN52 substitutions that improve AR2 and AR3, respectively. Starting with CRP carrying the HL159 substitution which inactivates AR1, heterodimers were made carrying wild type AR2/AR3 in one subunit and improved AR2 (due to the EG96 substitution) or improved AR3 (due to the KN52 substitution) in the other subunit. These heterodimers are active only when the improved AR2 or AR3 is located in the downstream subunit of the CRP dimer, showing that AR2 and AR3 interact with RNAP via the downstream CRP subunit. As yet, the contact sites in RNAP for AR2 and AR3 have not been unambiguously identified, although several lines of evidence suggest that AR3 is likely to contact Region 4 of σ. First, AR3 on the downstream subunit of CRP bound at -41.5 is ideally placed to contact σ Region 4 (recall, that Region 4 binds to the -35 hexamer at bacterial promoters). Second, substitutions in Region 4 of σ interfere with activation by a number of factors that bind to sites overlapping the -35 region (Ishihama 1993). Finally, deletions in Region 4 of σ prevent activation of the *galP1* promoter by CRP (Kumar *et al.* 1994).

4. PROMOTERS CARRYING TANDEM CRP-BINDING SITES

A number of *E. coli* promoters contain two DNA sites for CRP, occupation of both sites being essential for optimal transcription activation (Busby & Kolb 1996). A good example is the *cdd* promoter which carries sites centred at -41.5 and -90.5 (Holst *et al.* 1992): deletion of the upstream CRP-binding site results in a fivefold reduction in promoter strength. The mechanism of

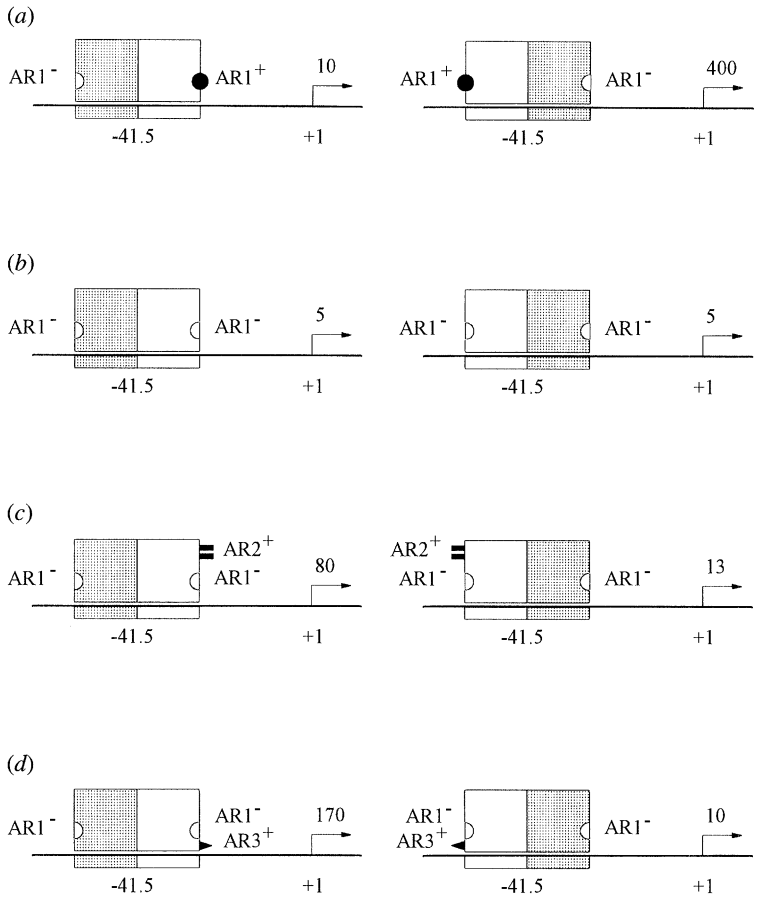

Figure 5. Oriented heterodimer experiments. The experiments illustrated in this figure were performed using CRP heterodimers with one subunit carrying a wild type DNA binding specificity (filled squares) and one subunit carrying an altered DNA binding preference due to the EV181 substitution in the recognition helix (open squares). The horizontal lines represent the promoters used, each promoter carrying a 22 b.p. DNA site for CRP centred 41.5 b.p. upstream from the transcription start at +1. In each case, one half of the DNA site for CRP contains the consensus sequence for binding wild type CRP (filled rectangles) and the other half contains a mutant binding sequence for CRP carrying the EV181 substitution (open rectangles). The use of hybrid DNA sites for CRP results in the heterodimer binding in an oriented manner, either with the subunit carrying wild type DNA binding specificity upstream (left hand half of the figure) or downstream (right hand half of the figure). The activity of each heterodimer combination at each promoter was measured: the activity is shown next to the transcript start in each case (arbitrary units). (*a*) CRP subunit with altered DNA binding preference (open square) has a functional AR1 (filled circle) and CRP subunit with wild type DNA binding specificity (filled square) has a non-functional AR1 due to the TA158 or HL159 substitutions (open semicircle). (*b*) Both CRP subunits with altered DNA binding preference (open square) and wild type DNA binding specificity (filled square) have non-functional AR1 due to the TA158 or HL159 substitutions (open semicircles). (*c*) Both CRP subunits with altered DNA binding preference (open square) and wild type DNA binding specificity (filled square) have non-functional AR1 (open semicircles). CRP with altered DNA binding preference (open square) carries EG96 substitution that improves AR2 (two small filled rectangles). (*d*) Both CRP subunits with altered DNA binding preference (open square) and wild type DNA binding specificity (filled square) have non-functional AR1 (open semicircles). CRP with altered DNA binding preference (open square) carries KN52 substitution that improves AR3 (filled triangle).

synergy between tandem-bound CRP dimers has been investigated using a set of engineered promoters based on the *melR* promoter: starting with a derivative carrying a single DNA site for CRP centred at -41.5, recombinant DNA techniques were used to insert a second DNA site for CRP at different distances upstream (Busby *et al.* 1994). Synergic CRP-dependent activation of transcription was found when the upstream CRP was bound around -74.5, -83.5, -93.5 and -102.5, but not at intermediate distances (figure 6). One possible explanation for synergy is that CRP can bind cooperatively to tandem-bound sites when they are spaced at particular distances: however, *in vitro* studies failed to find evidence for such cooperative interactions. The alternative explanation is that upstream-bound CRP stimulates transcription by making an independent contact with RNAP. Evidence for this comes from the observation that synergic activation by tandem bound CRP requires AR1 in the upstream-bound CRP (Busby *et al.* 1994). This result implies that upstream-bound CRP must make contact with αCTD. Figure 2*c* shows the likely arrangement of RNAP and CRP subunits: a contact between αCTD and upstream-bound CRP is clearly possible because the αCTD dimer is positioned just upstream of downstream-bound CRP. It seems likely that the

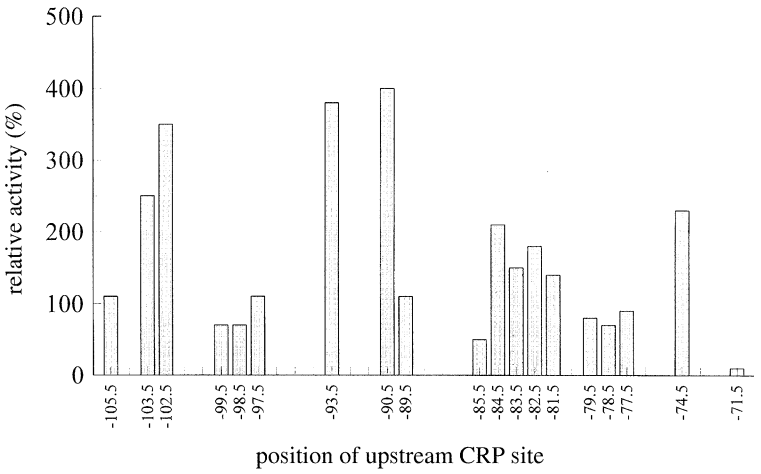

Figure 6. Activity of promoters carrying tandem DNA sites for CRP. The different promoters are derived from the *E. coli melR* promoter and carry tandem DNA sites for CRP, with one site centred at -41.5 and the second centred at different upstream locations as shown on the x-axis. These promoters were cloned in a *lac* expression plasmid, transformed into Δ*lac crp*$^+$ *E. coli* cells and β-galactosidase levels were measured in exponentially growing cells. The histogram shows relative levels of β-galactosidase expression for the different promoters: 100% is the value obtained with promoters carrying solely the downstream DNA site for CRP.

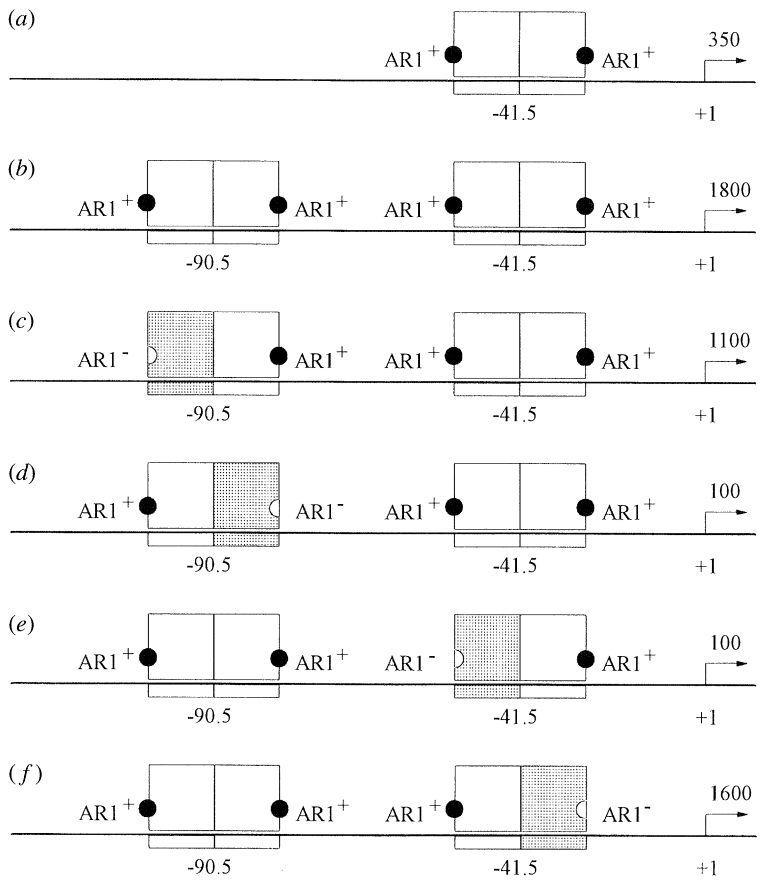

Figure 7. Location of functional AR1 at promoters with tandem-bound CRP. The figure shows a series of promoters derived from the *E. coli melR* promoter with one DNA site for CRP centred at -41.5 and a second site centred at -90.5 (shown as horizontal rectangles). In this experiment the CRP-binding sites carried a mutant binding sequence (open rectangles) that is recognized by CRP containing the EV181 substitution in the recognition helix (open squares). CRP carrying EV181 has a functional AR1, shown by filled circles, as in figure 5. Promoters C-F were constructed so as to contain one CRP-binding half site carrying the binding sequence for wild type CRP (filled rectangles). This half site could be filled with CRP carrying a wild type binding specificity (filled squares) but with an inactive AR1 due to either the TA158 or HL159 substitutions (open semicircles). The activity of the different promoters was measured in cells carrying CRP Val181 AR1$^+$ (denoted as open squares) and CRP Glu181 AR1$^-$ (denoted as filled squares). The activity of each promoter is shown by the transcript start in each case. The upstream CRP site in promoter A was destroyed by multiple base substitutions.

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flexibility of the linker joining αCTD and αNTD permits contact with upstream-bound CRP located at a variety of locations, provided that the upstream-bound CRP is on the same face of the DNA helix (figure 6).

Figure 7 illustrates an experiment to investigate the location of αCTD-CRP contacts in ternary CRP-RNAP-promoter complexes at a promoter carrying tandem DNA sites for CRP. A series of promoters was constructed, each containing a target site for a single CRP monomer carrying inactive AR1. Introduction of CRP carrying an inactive AR1 causes a sharp reduction in promoter activity when positioned at the upstream subunit of the downstream-bound CRP dimer or when positioned at the downstream subunit of the upstream-bound CRP dimer (figure 7*d* & *e*). This result suggests that the two αCTD domains of RNAP contact the upstream subunit of downstream CRP at -41.5 and the downstream subunit of upstream CRP (at -90.5 in this case), and confirms that both bound CRP dimers contact RNAP during synergic activation of transcription.

5. CONCLUSIONS

CRP is a compact molecule consisting of two domains (figure 1). The activating regions that make contact with RNAP during transcription are clearly integral parts of the structure, and are not located in separable domains as in some eukaryotic factors. Two of the regions (AR1 and AR3) are found in β turn regions containing several surface-exposed amino acid sidechains which are available for heterologous contacts. The other region is a basic patch made up of at least two separate elements. The activating regions of CRP are approximately the size of epitopes and they have most likely evolved 'merely' as contact points for RNAP. As yet, we are ignorant of the molecular details of the interactions between each AR and the cognate contact site in RNAP: progress is contingent on further genetic analysis or, preferably, structural analysis of complexes between CRP and different components of RNAP.

The diversity in the structures of CRP-dependent promoters is a long-standing puzzle. The existence of different activating regions, the observation that they can function in either of the CRP subunits, and the discovery that αCTD (the target for AR1) is tethered flexibly to the rest of RNAP, allows us to understand how CRP can function from a series of different locations. In cases such as the *lac* promoter, CRP makes just one significant contact with RNAP, whereas at the *melR* promoter it touches three different parts of RNAP. At the *lac* promoter only one CRP subunit makes contact, although both subunits are involved at the *melR* promoter. The existence of multiple contact sites for RNAP also can explain the molecular basis of synergic activation by tandem bound CRP dimers: the two CRP dimers independently touch different contact sites on RNAP. It is important to note that the various CRP-RNAP contacts at different promoters are likely to be facilitated by CRP-induced DNA bending, but bending alone is insufficient to activate transcription. CRP mutants carrying inactive AR1 and AR2 can bind and bend DNA normally but are unable to activate transcription at most CRP-dependent promoters (the exceptions are complex promoters where CRP acts by displacing a repressor or repositioning an activator).

Finally, it is interesting to note that CRP belongs to a family of related transcription activators, found in a wide range of microorganisms (Spiro 1994). It will be informative to investigate whether homologues from other microorganisms carry similar activating regions, and whether they too are able to function at a range of promoters with differing architectures. A number of recent studies have focused on the *E. coli* FNR protein, a transcription activator that is essential for adaptation to anaerobic conditions: the primary sequences of FNR and CRP are sufficiently related for us to be sure that the two proteins adopt similar structures (reviewed by Spiro & Guest 1990). Recent work has identified AR1 and AR3 homologues in FNR (Bell & Busby 1994), shown that FNR can activate transcription at promoters of differing architectures (Wing *et al.* 1995), and demonstrated that tandem bound FNR dimers can activate transcription synergically (Scott *et al.* 1995).

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REFERENCES

Attey, A., Belyaeva, T., Savery, N., Hoggett, J., Fujita, N., Ishihama, A. & Busby, S. 1994 Interactions between the cyclic AMP receptor protein and the alpha subunit of RNA polymerase at the *Escherichia coli* galactose operon *P1* promoter. *Nucl. Acids Res.* **22**, 4375–4380.

Bell, A. & Busby, S. 1994 Location and Orientation of an Activating Region in the *Escherichia coli* transcription factor, FNR. *Molec. Microbiol.* **11**, 383–390.

Bell, A., Gaston, K., Williams, R., Chapman, K., Kolb, A., Buc, H., Minchin, S., Williams, J. & Busby S. 1990 Mutations that affect the ability of the *Escherichia coli* cyclic AMP receptor protein to activate transcription. *Nucl. Acids Res.* **17**, 3865–3874.

Blatter, E., Ross, W., Tang, H., Gourse, R. & Ebright, R. 1994 Domain organization of RNA polymerase α subunit: C-terminal 85 amino acids constitute a domain capable of dimerization and DNA binding. *Cell* **78**, 889–896.

Busby S. & Kolb, A. 1996 The CAP modulon. In *Regulation of gene expression in* Escherichia coli (ed. E. C. C. Lin), chpt. 12, pp. 255–279. Georgetown, Texas: R. G. Landes Company.

Busby, S., West, D., Lawes, M., Webster, C., Ishihama, A. & Kolb, A. 1994 Transcription activation by the *Escherichia coli* cyclic AMP receptor protein: receptors bound in tandem can interact synergistically. *J. Molec. Biol.* **242**, 341–352.

Chen, Y., Ebright Y. & Ebright R. 1994 Identification of the target of a transcription activator protein by protein-protein photocrosslinking. *Science, Wash.* **265**, 90–92.

Eschenlauer, A. & Reznikoff, W. 1991 *Escherichia coli* catabolite gene activator protein mutants defective in positive control of *lac* operon transcription. *J. Bacteriol.* **173**, 5024–5029.

Holst, B., Sogaard-Andersen, L., Pedersen, H. & Valentin-Hansen, P. 1992 The cAMP-CRP/CytR nucleoprotein

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

complex in *Escherichia coli*: two pairs of closely linked binding sites for the cAMP-CRP activator complex are involved in combinatorial regulation of the *cdd* promoter. *EMBO J.* **11**, 3635–3643.

Igarashi, K. & Ishihama, A. 1991 Bipartite functional map of the *E. coli* RNA polymerase alpha subunit: involvement of the C-terminal region in transcription activation by cAMP-CRP. *Cell* **65**, 1015–1022.

Ishihama, A. 1993 Protein-protein communication within the transcription apparatus. *J. Bacteriol.* **175**, 2483–2489.

Kimura, M., Fujita, N. & Ishihama, A. 1994 Functional map of the alpha subunit of *Escherichia coli* RNA polymerase. *J. Molec. Biol.* **242**, 107–115.

Kolb, A., Busby, S., Buc, H., Garges, S. & Adhya, S. 1993*a* Transcriptional regulation by cAMP and its receptor protein. *A. Rev. Biochem.* **62**, 749–795.

Kolb, A., Igarashi, K., Ishihama, A., Lavigne, M., Buckle, M. & Buc, H. 1993*b* *E. coli* RNA polymerase deleted in the C-terminal part of its alpha subunit, interacts differently with the cAMP-CRP complex at the *lacP1* and *galP1* promoters. *Nucl. Acids Res.* **21**, 319–326.

Kumar, A., Grimes, B., Fujita, N., Makino, K., Malloch, R., Hayward, R. & Ishihama, A. 1994 Role of the Sigma^{70} subunit of *Escherichia coli* RNA polymerase in transcription activation. *J. Molec. Biol.* **235**, 405–413.

McKay, D., Weber, I. & Steitz, T. 1982 Structure of catabolite gene activator protein at 2.9 A resolution. *J. biol. Chem.* **257**, 9518–9524.

Niu, W., Zhou, Y., Dong, Q., Ebright, Y. & Ebright, R. 1994 Characterization of the activating region of *Escherichia coli* catabolite gene activator protein (CAP). 1. Saturation and alanine-scanning mutagenesis. *J. Molec. Biol.* **243**, 595–602.

Savery, N., Rhodius, V., Wing, H. & Busby, S. 1995 Transcription activation at *Escherichia coli* promoters dependent on the cyclic AMP receptor protein: effects of binding sequences for the RNA polymerase α-subunit. *Biochem. J.* **309**, 77–83.

Schultz, S., Shields, G. & Steitz, T. 1991 Crystal structure of a CAP-DNA complex: the DNA is bent by 90°. *Science, Wash.* **253**, 1001–1007.

Scott, S., Busby, S. & Beacham, I. 1995 Transcriptional coactivation at the *ansB* promoters: involvement of the activating regions of CRP and FNR when bound in tandem. *Molec. Microbiol.* **18**, 521–531.

Spassky, A., Busby, S. & Buc, H. 1984 On the action of the cyclic AMP-cyclic AMP receptor protein complex at the *Escherichia coli* lactose and galactose promoter regions. *EMBO J.* **3**, 43–50.

Spiro, S. 1994 The FNR family of transcriptional regulators. *Antonie van Leuwenhoek* **66**, 23–36.

Spiro, S. & Guest, J. 1990 FNR and its role in oxygen-regulated gene expression in *Escherichia coli*. *FEMS Microbiol. Rev.* **75**, 399–428.

Tang, H., Severinov, K., Goldfarb, A., Fenyo, D., Chait, B. & Ebright, R. 1994 Location, structure and function of the target of a transcriptional activator protein. *Genes Dev.* **8**, 3058–3067.

West, D., Williams, R., Rhodius, V., Bell, A., Sharma, N., Zou, C., Fujita, N., Ishihama, A. & Busby, S. 1993 Interactions between the *Escherichia coli* cyclic AMP receptor protein and RNA polymerase at Class II promoters. *Molec. Microbiol.* **10**, 789–797.

Williams, R., Bell, A., Sims, G. & Busby, S. 1991 The role of two surface-exposed loops in transcription activation by the *Escherichia coli* CRP and FNR proteins. *Nucl. Acids Res.* **19**, 6705–6712.

Williams, R., Rhodius, V., Bell, A., Kolb, A. & Busby, S. 1996 Orientation of functional activating regions in the *Escherichia coli* CRP protein during transcription activation at class II promoters. *Nucl. Acids Res.* **24**. (In the press.)

Wing, H., Williams, S. & Busby, S. 1995 Spacing requirements for transcription activation by *Escherichia coli* FNR protein. *J. Bacteriol.* **177**, 6704–6710.

Zhou, Y., Zhang, X. & Ebright, R. 1993*a* Identification of the activating region of CAP: isolation and characterization of mutants of CAP specifically defective in transcription activation. *Proc. natn Acad. Sci. U.S.A.* **90**, 6081–6085.

Zhou, Y., Busby, S. & Ebright, R. 1993*b* Identification of the functional subunit of a dimeric transcription activator protein by use of oriented heterodimers. *Cell* **73**, 375–379.

Zhou, Y., Merkel, T. & Ebright, R. 1994*a* Characterization of the activating region of *Escherichia coli* catabolite gene activator protein (CAP). 2. Role at Class I and Class II CAP-dependent promoters. *J. Molec. Biol.* **243**, 603–610.

Zhou, Y., Pendergrast, P., Bell, A., Williams, R., Busby, S. & Ebright, R. 1994*b* The functional subunit of a dimeric transcription activator protein depends on promoter architecture. *EMBO J.* **13**, 4549–4557.

Zou, C., Fujita, N., Igarashi, K. & Ishihama, A. 1992 Mapping the cAMP receptor protein contact site on the a subunit of *Escherichia coli* RNA polymerase. *Molec. Microbiol.* **6**, 2599–2605.

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