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cAMP Receptor Protein from *Escherichia coli* as a Model of Signal Transduction in Proteins – A Review

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Abstract
In *Escherichia coli*, cyclic AMP receptor protein (CRP) is known to regulate the transcription of about 100 genes. The signal to activate CRP is the binding of cyclic AMP. It has been suggested that binding of cAMP to CRP leads to a long-distance signal transduction from the N-terminal cAMP-binding domain to the C-terminal domain of the protein, which is responsible for interaction with specific sequences of DNA. The signal transduction plays a crucial role in the activation of the protein. The most sophisticated spectroscopic techniques, other techniques frequently used in structural biochemistry, and site-directed mutagenesis have been used to investigate the details of cAMP-mediated allosteric control over CRP conformation and activity as a transcription factor. The aim of this review is to summarize recent works and developments pertaining to cAMP-dependent CRP signal transduction in *E. coli*.

AR = activating region; cAMP = 3′,5′-cyclic adenosine monophosphate; CAP = catabolite gene activator protein from *Escherichia coli*; cGMP = 3′,5′-cyclic guanosine monophosphate; CRP = cyclic AMP receptor protein from *Escherichia coli*; CRP wt = CRP wild type; CRPW85-CRP = CRP without tryptophan residues in one subunit and without Trp13 in the second subunit; CRPD138A = CRP mutant with the aspartic acid residue in position 138 replaced by alanine in both subunits; CRPD138K = CRP mutant with the aspartic acid residue in position 138 replaced by lysine in both subunits; CRPD138F = CRP mutant with the aspartic acid residue in position 138 replaced by phenylalanine in both subunits; CRPT127I = CRP mutant with the threonine residue in position 127 replaced by isoleucine in both subunits; CytR = cytidine repressor; DLS = dynamic light scattering; gal = fragment of DNA sequence recognized by CRP in the galP1 promoter; HTH = helix-turn-helix motif; lac = fragment of DNA sequence recognized by CRP in the lacP1 promoter; RNAP = RNA polymerase from *Escherichia coli*.

Introduction

The best known transcription activator protein from *Escherichia coli*, which interacts with the α subunit of RNA polymerase, is cAMP receptor protein, CRP, also called catabolite gene activator protein, CAP [Busby and Ebright, 1999; Harman, 2001; Kolb et al., 1993, Krueger et al., 2003]. CRP was the first purified transcriptional activator [Zubay et al., 1970], and it was the first from this group of proteins for which the three-dimensional structure was determined [McKay et al., 1982]. It can act as an activator, a repressor, a coactivator or a corepressor [Kolb et al., 1993]. Following the binding of cAMP (an allosteric

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effector), the protein binds to specific DNA sites in or near the promoter sequences, enabling the recruitment of RNA polymerase to DNA and activating transcription [Busby and Ebright, 1999].

With regard to the mechanism of signal transduction, which is connected to CRP activation, it is often perceived as a prokaryotic protein model. The experiments performed so far allow for partial understanding of the proposed mechanisms of signal transduction of CRP. Elucidation of the mechanisms involved in CRP activation will allow for an explanation of the functions of other regulator proteins, both prokaryotic and eukaryotic, that are similar in structure and function to CRP.

The aim of the present paper is to summarize the results of experiments carried out during the last few years that have improved the understanding of CRP structure and function.

CRP Structure

Through work done with X-ray crystallography, the structures of CRP complexed with cyclic AMP [McKay et al., 1982; Passner et al., 2000; Weber and Steitz, 1987] or complexed with cAMP and DNA [Parkinson et al., 1996; Passner and Steitz, 1997; Schultz et al., 1991] have been determined. Unfortunately, the crystallization of unligated CRP has not been successful so far.

CRP is a homodimer, and each subunit consists of 209 amino acids. The molecular weight of the protein is 47,238 Da, which was calculated on the basis of amino acid composition [Aiba et al., 1982; Anderson et al., 1971]. CRP is a basic protein, and the value (measured experimentally) of its isoelectric point is 9.12 [Harman, 2001].

The CRP subunit consists of two domains. The N-terminal domain (larger) is composed of amino acids 1–133 and is responsible for subunit dimerization and cAMP binding in the *anti* conformation. As far as secondary structure is concerned, it is known that this domain is formed by three α helices (A, B, C) and eight β strands, which are located in the antiparallel orientation to create a β barrel where the first cAMP-binding site is situated [Weber and Steitz, 1987]. This domain shows homology in amino acid sequence to ion channels ligated by cyclic nucleotides and protein kinases that depend on cAMP, which occur in mammals [Shabb and Corbin, 1992]. The C-terminal domain (smaller) is composed of amino acids 139–209. In its secondary structure, three dominant α helices (D, E, F) and four β strands (9–12) have been observed [Weber and Steitz, 1987]. It is involved in the interaction with cAMP and is responsible for binding to DNA, in which the characteristic motif HTH [Brennan and Matthews, 1989] is involved. The motif consists of helices E and F [Passner and Steitz, 1997]. The second ligand-binding site in the *syn* conformation is situated between the domains and is created by the motif HTH and the loop between two antiparallel β strands (from the larger domain) and a fragment of helix C [Passner and Steitz, 1997]. The C-terminal domain shows similarity in amino acid sequence to the domains of the other regulatory proteins, like the *cro* repressor [Ebright et al., 1992].

CRP subunits associate with a dimerization constant in range of 10^9–10^{10} M^{-1} [Brown and Crothers, 1989]. They interact with each other mainly via helix C, which is composed of amino acids 112–133 and is the largest helix in the N-terminal domain [Weber and Steitz, 1987]. Experiments with protein mutants indicated that the tyrosine residue in position 99 and serine residue in position 128 are involved in the stabilization of the protein quaternary structure [Baker et al., 2001; Malecki and Wasylewski, 1998]. The C-terminal domain participates slightly in the interaction between subunits [Weber and Steitz, 1987].

The crystallographic data indicated that the CRP-$(cAMP)_2$ complex is characterized by asymmetry: one subunit is in the open conformation and the second one in the closed conformation [Weber and Steitz, 1987]. The complex composed of CRP, two cAMP molecules in the *anti* conformation and DNA does not demonstrate asymmetry: both subunits are in the closed conformation [Schultz et al., 1991]. A molecular dynamics simulation showed that CRP is a very dynamic molecule, and the transition from 'open-closed' to 'closed-closed' conformations can take place very fast [Garcia and Harman, 1996]. In accordance with those experiments and other results obtained using the same method [Berrera et al., 2007], the predicted CRP structure in solution (with cAMP and without DNA) shows symmetry (both subunits are in the closed conformation), whereas the asymmetry obtained in the crystallographic measurements most probably is the result of stabilization by crystal lattice interactions. The results discussed above show the great dynamics and flexibility of CRP. Those properties can play a crucial role in the structural changes of the protein after cAMP and DNA binding [Berrera et al., 2007; Spolar and Record, 1994].

Amino acid residues 134–138 form an elastic hinge that links both of the domains mentioned above [Ryu et al., 1993]. The conformational change of hinge region allows a rotation of the smaller domain of 25° about an axis that

is slightly offset from the E helix axis, and this triggers the transition of the subunit from the open to the closed conformation [Passner et al., 2000]. The subunit in the open conformation is characterized by easier accessibility to solvent molecules. Additionally, in the open conformation, residue Arg142 does not form a hydrogen bond with the carbonyl group from helix E [Passner et al., 2000].

Measurements of the thermal denaturation of CRP proved that it is an irreversible process. The isolated N-terminal domain is characterized by greater structural stability than that of the native protein [Blaszczyk and Wasylewski, 2003]. In contrast, the denaturation conducted using chemical compounds like guanidine hydrochloride is fully reversible. The results of CRP denaturation indicate that the transition can be described by a three-state model: $(CRP_{native})_2 \leftrightarrow 2\ (CRP_{native}) \leftrightarrow 2\ (CRP_{denatured})$. The faster process, characterized by the relaxation time $\tau_2 = 80 \pm 3$ ms, corresponds to the dissociation of the CRP dimer into monomers. The slower process has a relaxation time $\tau_1 = 1.9 \pm 0.1$ s and corresponds to the cooperative unfolding of the CRP monomer. The free energy change in the absence of a denaturant of CRP dissociation is $\Delta G°_{dis} = 46.9 \pm 2.5$ kJ/mol, and for monomer unfolding $\Delta G°_{unf} = 30.9 \pm 1.3$ kJ/mol [Malecki and Wasylewski, 1997].

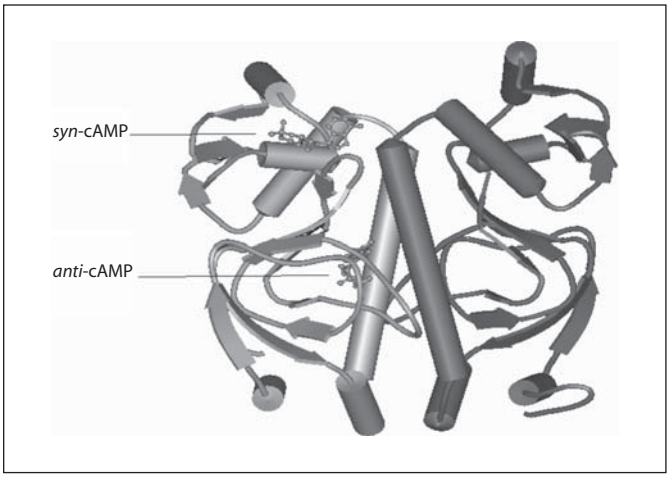

Fig. 1. Structure of CRP dimer with the cAMP molecules in the *anti* and *syn* conformation bound to the one subunit of CRP. The figure was generated with WebLab ViewerPro (version 3.7) using atomic coordinates obtained from the Protein Data Bank (entry 1HW5).

Cyclic AMP Binding by CRP

The binding of cyclic AMP to CRP causes conformational changes that enable the protein to bind to specific DNA sequences and interact with RNA polymerase. The CRP molecule has four sites for cyclic nucleotides. In 1997, the crystallographic structure of the CRP protein complexed with DNA, two molecules of cAMP in the *anti* conformation and two molecules of cAMP in the *syn* conformation was presented [Passner and Steitz, 1997] (fig. 1).

The results obtained so far indicate that CRP can exist in three forms: apo-CRP, which dominates in solutions devoid of cAMP; CRP-$(cAMP)_2$, the form of protein that dominates in solutions with micromolar concentrations of the ligand and is responsible for transcription activation, and CRP-$(cAMP)_4$, the form of protein that dominates in solutions with millimolar concentrations of cAMP [Harman, 2001]. The latest papers indicated that the active form of the protein is the form with only one molecule of cAMP and the binding of the other cAMP molecules reduced the ability of CRP to interact with DNA [Tutar, 2008a, b].

Apo-CRP binds DNA but with low affinity and without specificity for the CRP-DNA-binding site. The presence of a micromolar concentration of cAMP induces a strong affinity for specific DNA sequences and also interaction with RNA polymerase. The addition of cAMP in millimolar concentrations prevents binding of CRP to DNA and transcription activation [Mukhopadhyay et al., 1999].

The binding of cAMP to CRP takes place in a certain order of events: first of all, the ligand binds to the sites located in the N-terminal domain of CRP (which are saturated with micromolar concentrations of cAMP) and afterwards it binds to the sites located in the C-terminal domain of CRP (which are saturated with millimolar concentrations of cAMP). Therefore, it can be concluded that CRP uses the binding energy of cyclic AMP molecules to induce changes in its structure that increase the affinity for specific DNA sequences [Passner et al., 2000].

The average dissociation constant of the complex from two cAMP molecules is $27.5 \times 10^{-6}\ M^{-1}$ and from the next two cAMP molecules is $2.0 \times 10^{-3}\ M^{-1}$ [Malecki et al., 2000]. Taking into account that the intracellular concentration of cAMP fluctuates in the range of 0–10 μM and the ionic strength equals about 200 mM [Epstein et al., 1975], it seems that apo-CRP and the CRP-$(cAMP)_2$ complexes are biologically important protein forms [Harman, 2001]. The amino acids involved in the interaction with

cAMP molecules in the *anti* conformation are Gly71, Glu72, Arg82, Ser83 and Thr127 from the same subunit and Ser128 from the other subunit of CRP [Weber and Steitz, 1987]. Arg82 and Ser83 interact with the phosphate group, Gly71 and Glu72 form bonds with the ribose of cAMP and Thr172 from the same subunit and Ser128 from the other subunit interact with adenine of cAMP. In addition, water molecules are involved in the cAMP-CRP interaction. Residues Glu72 (which forms a hydrogen bond with ribose) and Arg82 (which forms an ionic bond with a phosphate group) are fundamental for cAMP binding. Their substitutions lead to significant decreases in CRP affinity for cyclic AMP [Passner et al., 2000]. CRP uses the unique structure of phosphoribose from cAMP to recognize the proper cyclic nucleotide. Furthermore, the ribose from the ligand is responsible for the signal transmission to the DNA-binding domain [Tutar, 2008a].

The fast kinetics measurements of binding of the first two cAMP molecules to CRPT127I proved that the introduced mutation does not affect cyclic nucleotide binding. This has been confirmed by measuring the dissociation constant of the CRPT127I–$(cAMP)_2$ complex, which is about 20.1 μM [Polit, unpubl. data]. However, the mutation disturbs the conformational changes in the protein that follow cAMP binding, which in wild-type CRP cause an increase in affinity for DNA. The determined isomerization equilibrium constant between the two conformational states of CRPT127I complexed with *anti*-cAMP is 0.860, about 20-fold higher in comparison to the same constant for wild type. As a consequence, there is a significant reduction in the affinity of the binding sites localized between the protein domains, and the dissociation constant for these sites is about 8.5 mM. The parameters of cyclic AMP binding to both binding sites determined by kinetic experiments unambiguously show that the point mutation perturbs the communication between the protein domains [Polit et al., 2003b].

The binding of the first cAMP molecule to CRP is exothermic with an enthalpy change of $\Delta H_1 = -6.5$ kJ mol^{-1}, while the binding of the second cAMP molecule is endothermic with an enthalpy change of $\Delta H_2 = 31.5$ kJ mol^{-1}. The entropy changes in both cases are positive and equal to $\Delta S_1 = 71$ J mol^{-1} K^{-1} and $\Delta S_2 = 208$ J mol^{-1} K^{-1}, respectively [Gorshkova et al., 1995]. Similarly, for the two primary binding sites for cAMP, the binding parameters for two *syn*-cAMP were also determined using isothermal titration calorimetry. The experiments were conducted in Tris buffer pH 7.8 (with 500 mM KCl) at 35°C. The average association constant for *syn*-cAMP is much lower than for *anti*-cAMP and is equal to $K = 2.3 \times 10^2$ M^{-1}. The binding process is exothermic with an enthalpy change of $\Delta H = -63.3$ kJ mol^{-1} and an entropy change of $\Delta S = 105$ J mol^{-1} K^{-1} [Lin and Lee, 2002].

As mentioned above, the domain that binds cAMP in the *anti* conformation showed similarity to domains of protein kinases that depend on cAMP and ion channels gated by cyclic nucleotides. In particular, the presence of six amino acid residues in the cyclic nucleotide's binding pocket is characteristic: Gly33, Gly45, Gly71, Glu72, Arg82 and Ala84 [Shabb and Corbin, 1992]. The main structural elements that are significantly different in the various proteins include loop 3 (formed by amino acids 50–61) and loop 4 (formed by amino acids 73–81) [Scott et al., 1996]. On the basis of screening mutations in the loops (deletion in loop 3, insertion in loop 4 and the combined mutations), it has been found that these loops can play important roles in the function of CRP [Chen and Ching Lee, 2003]. Loop 3 is involved in intersubunit signal communication, whereas loop 4 is involved in the cAMP-binding pocket and interdomain signal transmission [Shen et al., 2008].

The cAMP molecules in the *syn* conformation bind between the HTH motif formed with helices E and F of the smaller domain and the loop formed by the two antiparallel β strands, and they interact indirectly (through water molecules) with DNA. The adenine of cAMP forms a hydrogen bond with Ala135 from the second subunit of the protein. The ribose of cAMP interacts with Glu58 and Arg180 using hydrogen bonds with the oxygen of the phosphate group. Glu181 (which undergoes protonation) is involved in the interaction with water molecules and takes part in the interaction with DNA [Passner and Steitz, 1997].

Cys178 is probably involved in the interaction with cAMP molecules in the *syn* conformation, because the reactivity of Cys178 decreases with millimolar concentrations of cAMP [Passner and Steitz, 1997]. The additional sites for the ligand in the *syn* conformation are formed as a result of conformational changes in the protein after cAMP binding in the *anti* conformation to the N-terminal domain [Won et al., 2002].

Millimolar concentrations of cAMP are not found in physiological conditions [Epstein et al., 1975], so there are a few suppositions for the function of cAMP molecules in the *syn* conformation. It may be that CRP complexed with four cAMP molecules is stabilized in the presence of RNAP or CRP could associate with adenylate cyclase, which causes the increase in the cAMP concentration to millimolar values in the local environment [Passner and Steitz, 1997].

The presence or lack of the ligand in the case of CRP is also linked with its susceptibility to the protease digestion. Apo-CRP is resistant to proteases; the addition of cAMP in micromolar concentrations sensitizes the protein to digestion, whereas the increase of cAMP to millimolar concentrations makes CRP resistant to proteolytic enzymes again [Harman, 2001]. CRP can also bind two molecules of cGMP with affinity similar to two molecules of cAMP, but cGMP does not activate the protein [Won et al., 2002].

The results of circular dichroism measurements indicated that structural changes associated with cAMP binding result from small changes that take place in the secondary structure of CRP. The binding of cAMP in the *anti* conformation causes about a 4% increase in the α helical structure within the N-terminal domain of the examined protein. Moreover, the formation of the CRP-$(cAMP)_2$ complex changes the tertiary structure of CRP, which was shown with rotational correlation times of the CRP molecule determined by fluorescence decay anisotropy of the fluorescent probe 1.5-I-AEDANS covalently attached to Cys178 of the protein. The rotational correlation time of apo-CRP is equal to about 24 ns. Binding of cAMP in the binding pocket localized in the N-terminal domain causes a significant elongation of the rotational correlation time of the overall molecule [Blaszczyk et al., 2001]. The binding of two additional molecules of cyclic AMP does not cause any considerable changes in the tertiary structure of CRP, which confirms the rotational correlation time values determined in the measurements of fluorescence decay anisotropy of the covalently attached fluorescent probe 1.5-I-AEDANS. The rotational correlation time of the CRP-$(cAMP)_4$ complex is equal to about 32 ns and is about 1 ns longer than the time determined for CRP-$(cAMP)_2$ [Blaszczyk et al., 2001]. In order to study whether global conformational changes accompany cAMP binding, DLS measurements were performed. Those experiments show that the hydrodynamic radius of CRP in the complex with the ligand (*anti*-cAMP) is reduced, which can indicate the appearance of conformational changes within the protein that lead to more a compact structure in the sense of hydrodynamic properties or to an increase in its symmetry. A small increase in the compactness of structure was also observed in the case of cAMP binding to the dimer of N-terminal domains [Blaszczyk et al., 2001]. The structural changes observed in the DLS measurements, which indicated a reduction of the hydrodynamic radius, were also confirmed by Forster resonance energy transfer studies. They showed that, after cAMP and DNA binding, there is a decrease in the distance between residue Trp85, localized in the N-terminal domain, and residue Cys178, localized within the HTH motif of the C-terminal domain. Those results proved the appearance of structural changes resulting probably from the rearrangement of the N- and C-terminal domains. Presumably, in solution both CRP subunits exist in the open conformation and after cAMP binding, there is the possibility of conversion to the closed conformation, in which both domains that form the subunit lie closer to each other [Fic et al., 2006; Polit et al., 2003a].

The kinetic measurements conducted on homodimeric CRP have shown that the formation of the CRP-$(cAMP)_4$ complex can be described by the equilibrium between two forms of the CRP-$(cAMP)_2$ complex, where only one form is able to bind cAMP in the *syn* conformation. This process can be observed by the conformational changes of CRP that surround the tryptophan residue in position 85 present in both subunits. Recently, the kinetics of cAMP binding to a CRP heterodimer mutant, in which a single Trp85 residue was present in only one subunit of the protein (CRPW85-CRP), were measured under pseudo-first-order conditions and the experimental data could be fitted to single-exponential curves [Fic et al., 2006]. The observed rate constant for the CRPW85-CRP heterodimer decreased with an increase in cAMP concentration, as observed in the case of wild-type CRP [Malecki et al., 2000]. Therefore, it seems that two conformational states of CRP exist and only one of those states is able to bind the ligand. The obtained results suggest that the kinetic mechanism of binding of cAMP to both *syn* sites of CRP is very similar [Fic et al., 2006].

The kinetic experiments of cAMP binding to CRP mutants in position 138 (in the hinge region) allowed the observation of extra stages of conformational changes for wild-type CRP, which could not be determined for the unaltered protein because the rate constants are on the same time scale. The scheme of the ligand-binding reaction proved to be a five-stage process, where after binding of two ligand molecules, three sequential conformational changes occur. It has been concluded that the two ligands are bound independently to the subunits with the same rate constants, which were determined by means of kinetic measurements based on natural fluorophores. The experiments conducted with the use of labeled proteins showed the appearance of significant conformational changes just after the binding of two ligand molecules. In addition, the strong dependence between the isomerization constant of the conformational changes and the affinities of the mutants for promoter sequences has been shown [Gorecki, unpubl. data].

CRP Activation Induced by cAMP Binding

The binding sites of the first two molecules of the ligand (cAMP) are too far from the DNA-binding site (10 Å) [Passner et al., 2000] to directly influence the interaction of the protein with nucleic acids. So two questions remain: how is the information about the binding of cyclic AMP transferred from the N-terminal domain to the C-terminal domain and in what way do the elements of the C-terminal domain obtain the ability to recognize the specific DNA sequences? Because so far the crystallographic structure of CRP without ligands has not been resolved, there is no possible way to compare the structure of apo-CRP and the CRP-$(cAMP)_2$ complex.

cAMP binding causes the reorientation of protein subunits and the reorientation of domains within each of the subunits. The transmission of the allosteric signal is possible in two ways: (1) from the N-terminal (cAMP binding) domain via the hinge region to the C-terminal DNA-binding domain, and (2) from the N-terminal domain via changes in the interactions between the domains after cAMP binding.

When the structures of apo-CRP (using NMR spectroscopy) and the CRP complex with two molecules of cAMP (obtained using X-ray crystallography) were compared, it was found that the main differences in the secondary structure concern the following residues: Gly71, Glu72, Arg82, Ser83, Arg123, Thr127 and Ser128. In contrast to the CRP-$(cAMP)_2$ complex, apo-CRP does not show the presence of any β structure in this region. Rather, the NMR measurements suggest the presence of an α helix or turn structure. The results of NMR studies confirm that after ligand binding, helix F protrudes, although its size is the same for apo-CRP and CRP-$(cAMP)_2$ [Won et al., 2000]. cAMP binding to the protein causes changes in the subunit structure: the conformation of the DNA-binding domain (C-terminal domain) becomes more flexible and the conformation of the N-terminal domain becomes more compact and rigid and there are changes in the interactions between the subunits. There are also changes in the α helical structure on the surface of both subunits and changes in the DNA-binding domain (helix F is more exposed on the protein surface, as mentioned above) [Dong et al., 2002].

The changes following ligand binding can be observed using the presence of natural fluorophores, like tryptophan residues in positions 13 and 85, both situated in the N-terminal domain of the protein [Weber and Steitz, 1987]. In apo-CRP, the Trp13 residue is more exposed to solvent and accessible for quenchers, but the Trp85 residue in the protein without ligand is inaccessible for quenchers, because it is located in the cleft between the domains [Wasylewski et al., 1995]. In the presence of 100 μM cAMP, both of the Trp residues are quenched, which can indicate that after ligand binding, the surface between the larger and smaller domains opens to solvent or that the dynamics of the protein molecule increases.

Interestingly, cGMP binding leads to similar changes in the N-terminal domain conformation as does cAMP binding, but there is no relaxation effect in the case of the DNA-binding domain [Dong et al., 2002].

The studies using mutants with substituted amino acids in positions 128 and 141 have shown that residue 128, in spite of its participation in cAMP binding, when replaced by alanine does not have any effect on the ability of the mutated protein to distinguish between cAMP and cGMP. On the other hand, the substitution of glycine in position 141 (with glutamine) causes the loss of CRP's ability to discriminate between cAMP and other cyclic nucleotides, although Gly141 is not involved in the interaction with cAMP and in the interaction between subunits [Cheng and Ching Lee, 1998].

Probably the activation of CRP by cyclic nucleotides is connected with the energetically reasonable possibility that the nucleotides help to overcome unfavorable structures inside the subunits. The mutation in position 141 lowers this energetic barrier.

Various experiments with many CRP mutants have shown that despite amino acid substitutions in positions 53, 62, 127, 141 and 148, cAMP still remains the strongest CRP activator [Dai et al., 2004]. Small changes in the local structure of the protein caused by amino acid substitutions are significantly amplified in the general dynamics of CRP and they indicate that structural resilience plays an important role in the modulation of the allosteric behavior of CRP [Gekko et al., 2004]. The ligand induces conformational changes in CRP through the influence on its dynamics.

In the cases of the CRPD138A, CRPD138K and CRP-D138F mutants (mutations that are introduced in the hinge region of the protein), the connection between the changes in the hinge region and the N-terminal domain structure was observed. The comparison of emission fluorescence spectra and quenching parameters obtained for apo-CRP and the mutants responsible for cAMP binding confirms the earlier observations [Gorecki, unpubl. data] that mutations in position 138 of CRP differentially disturb the adoption of the proper conformation by CRP for interaction with DNA. Unfortunately, the complex type of fluorescence emission of the two-trypto-

phan mutants does not allow any unambiguous definition of the type of changes that occur near the Trp13 and Trp85 residues [Tworzydlo, unpubl. data].

CRP Interaction with DNA

The characteristic motif HTH is involved in the CRP-DNA interaction. The HTH motif is composed of helices E and F from the C-terminal domain of the protein [Passner and Steitz, 1997]. Following cyclic AMP binding, the recognition helices of the HTH motif undergo insertion into two adjacent DNA major grooves [Kim et al., 1992], because helices F of both subunits are in the distance of 34 Å, the value of a complete turn of the DNA double helix (fig. 2).

In crystallographic structures obtained so far, CRP is present as a complex with cAMP molecules and synthetic palindromic DNA sequences, which contain the sequence of five nucleotides 5′-TGTGA-3′ [Parkinson et al., 1996; Passner and Steitz, 1997].

In the CRP-nucleic acid interaction, the amino acid residues in positions 180, 181, and 185 are mainly involved. The side chain of Arg180 forms hydrogen bonds with the oxygen in position 6 and the nitrogen atom in position 7 of guanine 5. Glu181 forms a hydrogen bond with the nitrogen atom in position 4 of cytosine 7′. In the second subunit, Arg180 and Glu181 form symmetric hydrogen bonds with guanine 18′ and cytosine 16, respectively.

The side chain of Arg185 forms hydrogen bonds with the oxygen in position 6 and the nitrogen atom in position 7 of guanine 7, a hydrogen bond (by means of water molecules) with the nitrogen atom in position 7 of guanine 9′ and another hydrogen bond (also by means of water molecules) with thymine 6 [Parkinson et al., 1996]. The most important bases involved in the CRP-DNA interaction are guanines in positions 5 and 7.

Concomitant double mutation of positions 138 and 141 has allowed the determination that the interactions between residues Asp138 and Gly141 are necessary to obtain the proper conformation by the DNA-binding domain [Ryu et al., 1993].

When CRP binds DNA, it makes the nucleic acid bend about 87° in the case of consensus sequence [Parkinson et al., 1996]. The change of the nucleic acid sequence to the one that introduces the natural bend abrogates the requirement for transcription activation. Also, the use of a protein that by itself introduces a DNA bend, like integration host factor, can replace CRP in transcription activation [Dethiollaz et al., 1996]. In the case of the *lac* promoter, the introduced bend is symmetric [Pyles et al., 1998] and for the *gal* promoter the bend is asymmetric [Pyles and Lee, 1998]. This is a result of the fact that in the *lac* promoter, the DNA-binding sites are symmetric and the flanking sequences are almost identical mirror images, whereas the *gal* promoter sequence does not have such properties [Lin and Lee, 2003]. Because the geometry of the CRP-DNA complex depends on the kind of promoter and the location of the DNA site for CRP, this could be the main factors that determine the molecular mechanism of gene expression.

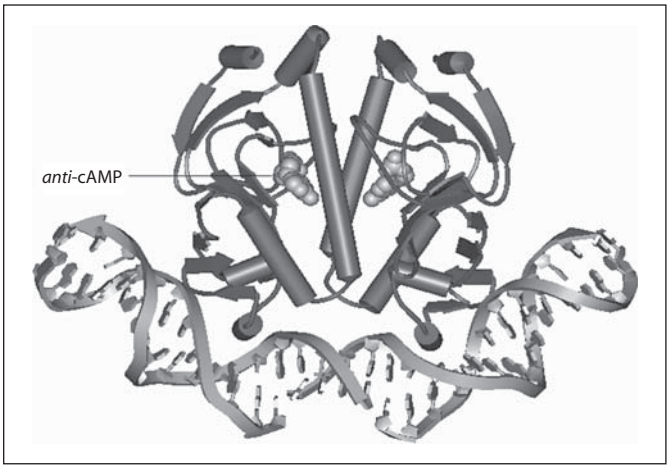

Fig. 2. Structure of CRP-$(cAMP)_2$-DNA complex. The figure was generated with WebLab ViewerPro (version 3.7) using atomic coordinates obtained from the Protein Data Bank (entry 1J59).

The hydrodynamic properties of CRP complexes with three DNA sequences were investigated using DLS [Blaszczyk et al., 2001]. The obtained results pointed to differences in the shapes of the complexes, probably resulting from the various ways that the DNA double helix can be deformed in the studied complexes. The result of fluorescence quenching of CRP complexes with three promoter sequences proved that there is transmission of a signal concerning the conformational changes from the C-terminal DNA-binding domain to the N-terminal domain of the protein. Additionally, those studies showed that the differences in the sequences of promoter fragments influence the protein conformation. The observed changes, monitored using fluorescence of the Trp13 residue, take place in the direct vicinity of sites responsible for the CRP-RNAP and the regulator protein CytR interactions [Tworzydlo et al., 2005].

Binding of DNA promoter sequences to the C-terminal domain causes changes in the overall protein structure, both in the presence and in the absence of the ligand. It can be concluded that signal transmission from the C-terminal domain to the N-terminal domain may play a crucial role in gene expression [Fic et al., 2007]. Moreover, CRP binding to the specific DNA sequences depends not only on the presence of the consensus sequence but also on the presence of proper flanking sequences of DNA [Dai et al., 2004].

The interactions between CRP and DNA have an electrostatic character. In their complex, the positively charged protein fragments, from both the C- as well as the N-terminal domain of CRP, are involved [Katouzian-Safadi et al., 1993].

CRP can bind DNA in a non-specific way in the absence of cyclic AMP, because there is a possibility that interactions between positively charged amino acid residues and the negatively charged phosphate groups of DNA occur [Salemme, 1982]. In this non-specific interaction, the two domains of the CRP subunit are involved [Katouzian-Safadi et al., 1993]. However, the association constant without the ligand is equal to about 10^4 M^{-1} [Giraud-Panis et al., 1994]. After the addition of cAMP (in micromolar concentrations), the DNA association constant increases from 10^7 to 10^{10} M^{-1} depending on the specific DNA sequence with which CRP interacts [Harman, 2001].

The observed reduction in the affinity of CRP for DNA, which takes place in the millimolar concentration range of cAMP, can be a result of competition between the ligand molecule and DNA for the Arg180 residue, which is responsible for the specificity of nucleic acid binding, as has been shown by means of site-directed mutagenesis [Zhang and Ebright, 1990].

DNA binding by the protein can induce changes in the regions of CRP involved in the interaction with other proteins, for example RNAP [Bonarek, unpubl. data]. The information is probably transmitted from the DNA-binding site to helix B, which causes changes in helix C that are involved in the interaction between CRP subunits and linking the DNA-binding site with helix B [Baichoo and Heyduk, 1999].

On the other hand, it has been also shown that CRP and RNAP interact with each other in solution without the presence of a DNA promoter fragment with the association constant 3.6×10^6 M^{-1}, which could have physiological meaning and constitute strong evidence that transcription activation by CRP requires a direct protein-protein interaction with RNA polymerase [Heyduk et al., 1993]. According to the results of crystallographic analysis obtained by Passner and Steitz [1997] for the complex composed of CRP, two cAMP molecules and a palindromic DNA fragment (46 base pairs), both CRP subunits are in the closed conformation.

CRP-Dependent Promoters

CRP-dependent promoters show a lot of deviations from the consensus sequence of the strong promoter of *E. coli*. None of them contains a –35 sequence close to the consensus sequence and some of them also do not contain the classic 'TATA-box' sequence. CRP-dependent promoters can be divided into three classes, depending on the location of the CRP DNA-binding site and the number of protein molecules involved in the initiation of transcription [Busby and Ebright, 1997, 1999; Ebright, 1993; Zhou et al., 1994].

Class I promoters require only CRP to transcription activation and have a single site on DNA for CRP, localized before the site to which RNA polymerase binds. The CRP-binding site can be situated in the various distances from the transcription start point, at positions: –93, –83, –72, –62. The best known promoter belonging to class I is *lac* promoter, which is the part of lactose operon [Jacob and Monod, 1961]. For this promoter the CRP-binding site on DNA is situated at position –61.5 [Niu et al., 1994]. The CRP amino acid residues from 156 to 164 (activating region 1 – AR1) are involved in the process of transcription activation at *lac* promoter. They are localized within C-terminal domain of CRP, directly preceding HTH motif. When the site-directed mutagenesis was used, it has been shown that the side chain of Thr158 plays a crucial role in AR1.

Class II CRP-dependent promoters require also CRP for the transcription activation and have a single site on DNA for CRP, which is the same as the binding site for RNA polymerase (generally it is the –35 promoter sequence). The promoter belonging to this class is *gal P1*, its binding site of CRP to DNA is at position –41.5 [Busby and Ebright, 1997]. AR2, characteristic for class II CRP-dependent promoters, is composed of four amino acid residues: His19, His21, Glu96 and Lys101, localized in N-terminal domain (responsible for the binding of cAMP molecules in *anti* conformation) of CRP. AR2 carries a net positive charge of +2 and it is thought that the presence of this charge is critical for AR2 function [Niu et al., 1996]. For class II promoters, the overlapping of CRP-binding site with –35 sequence is characteristic and it ex-

cludes the presence of consensus sequence in this site [Rhodius et al., 1997]. In case of class II promoters, the presence of both AR1 and AR2 is required for the transcription activation.

AR1 and AR2 can be replaced by a third non-native activating region 3 (AR3) [Niu et al., 1996]. It is composed of amino acid residues 52–58. AR3 is formed by substitution of Lys52 by a neutral or negatively charged residue. The substitution of Lys52 significantly increases the transcription activation at class II but not at class I promoters, and partly it can overcome the substitution effects in AR1, AR2 or both of them.

In case of some CRP-dependent promoters, two or more protein molecules are involved in the transcription activation and also in some cases there are other activator or repressor proteins [Merkel et al., 1995; Weyand et al., 2001; Zhang and Schleif, 1998]. Such promoters are characterized by diverse structures and various distances between the DNA sites for CRP and the DNA sites for RNA polymerase. The mechanisms of transcription activation at these promoters are usually a combination of elements and processes known for class I and II CRP-dependent promoters [Ebright and Busby, 1999]. One of the proteins acting together with CRP in the transcription regulation is CytR. The binding regions of both proteins overlap, therefore there is the possibility of direct interaction between them [Søgaard-Andersen and Valentin-Hansen, 1993]. The surface of CRP interaction with CytR is formed by the following amino acid residues: Glu12, Trp13, His17, Leu105, Val108 and Pro110 – it is a repressor region [Søgaard-Andersen et al., 1991]. The cooperation of CRP and CytR might cause the activation as well as the repression of genes transcription.

Conclusions

The CRP is thought of as a model protein for at least two reasons. It functions as a transcriptional regulator in prokaryotic *E. coli* cells, where it participates or co-participates in the control of expression of over 100 genes [Gutierrez-Ríos et al., 2007; Semsey et al., 2007; Zheng et al., 2004]. A relatively simple expression system exists in these bacteria, while at the same time being significantly similar to the systems that operate in higher organisms. Additionally, the mechanism of CRP action is relatively easy to study. This protein therefore became a model in the research of signal transduction. A characteristic feature of CRP is that it reveals its function only in the presence of the ligand-cyclic AMP.

The present review, however, indicates that CRP, a relatively simple transcriptional regulator, functions in a very complex way.

The most sophisticated spectroscopic techniques and others frequently used in structural biochemistry as well as site-directed mutagenesis were used in order to investigate the conformational changes within the protein molecule induced by the binding of the physiological ligand and the effect of these changes on the interaction with DNA and RNAP. Only from such advanced experiments does an overall picture of CRP function appear. It seems that these studies will be helpful in the understanding of the mechanism of action of eukaryotic transcription factors.

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References

Aiba H, Fujimoto S, Ozaki N: Molecular cloning and nucleotide sequencing of the gene for *Escherichia coli* cAMP receptor protein. Nucleic Acids Res 1982;10:1345–1362.

Anderson WB, Schneider AB, Emmer M, Perlman RL, Pastan I: Purification of and properties of the cyclic adenosine 3′,5′-monophosphate receptor protein which mediates cyclic adenosine 3′,5′-monophosphate-dependent gene transcription in *Escherichia coli*. J Biol Chem 1971;246:5929–5937.

Baichoo N, Heyduk T: DNA-induced conformational changes in cyclic AMP receptor protein: detection and mapping by a protein footprinting technique using multiple chemical proteases. J Mol Biol 1999;290:37–48.

Baker CH, Tomlinson SR, Garcia AE, Harman JG: Amino acid substitution at position 99 affects the rate of CRP subunit exchange. Biochemistry 2001;40:12329–12338.

Berrera M, Pantano S, Carloni P: Catabolite activator protein in aqueous solution: a molecular simulation study. J Phys Chem B 2007; 111:1496–1501.

Blaszczyk U, Polit A, Guz A, Wasylewski Z: Interaction of cAMP receptor protein from *Escherichia coli* with cAMP and DNA studied by dynamic light scattering and time-resolved fluorescence anisotropy methods. J Protein Chem 2001;20:601–610.

Blaszczyk U, Wasylewski Z: Interaction of cAMP receptor protein from *Escherichia coli* with cAMP and DNA studied by differential scanning calorimetry. J Protein Chem 2003; 22:285–293.

Brennan RG, Matthews BW: The helix-turn-helix DNA-binding motif. J Biol Chem 1989; 264:1903–1906.

Brown AM, Crothers DM: Modulation of the stability of a gene-regulatory protein dimer by DNA and cAMP. Proc Natl Acad Sci USA 1989;86:7387–7391.

Busby S, Ebright RH: Transcription activation at class II CAP-dependent promoters. Mol Microbiol 1997;23:853–859.

Busby S, Ebright RH: Transcription activation by catabolite activator protein. J Mol Biol 1999;293:199–213.
Chen R, Ching Lee J: Functional roles of loops 3 and 4 in the cyclic nucleotide-binding domain of cyclic AMP receptor protein from *Escherichia coli*. J Biol Chem 2003;278: 13235–13243.
Cheng X, Ching Lee J: Interactive and dominant effects of residues 128 and 141 on cyclic nucleotide and DNA bindings in *Escherichia coli* cAMP receptor protein. J Biol Chem 1998;273:705–712.
Dai J, Lin SH, Kemmis C, Chin AJ, Lee JC: Interplay between site-specific mutations and cyclic nucleotides in modulating DNA recognition by *Escherichia coli* cyclic AMP receptor protein. Biochemistry 2004;43:8901–8910.
Dethiollaz S, Eichenberger P, Geiselmann J: Influence of DNA geometry on transcriptional activation in *Escherichia coli*. EMBO J 1996; 15:5449–5458.
Dong A, Malecki JM, Lee L, Carpenter JF, Ching Lee J: Ligand-induced conformational and structural dynamics changes in *Escherichia coli* cyclic AMP receptor protein. Biochemistry 2002;41:6660–6667.
Ebright RH: Transcription activation at class I CAP-dependent promoters. Mol Microbiol 1993;8:797–802.
Ebright YW, Chen Y, Pendergrast PS, Ebright RH: Incorporation of an EDTA-metal complex at a rationally selected site within a protein: application to EDTA-iron DNA affinity cleaving with catabolite gene activator protein (CAP) and Cro. Biochemistry 1992;31: 10664–10670.
Epstein W, Rothman-Denes LB, Hesse J: Adenosine 3′,5′-cyclic monophosphate as mediator of catabolite repression in *Escherichia coli*. Proc Natl Acad Sci USA 1975;72:2300–2304.
Fic E, Gorecki A, Wasylewski Z: Fluorescence quenching studies of conformational changes induced by cAMP and DNA binding to heterodimer of cyclic AMP receptor protein from *Escherichia coli*. Protein J 2007;26:457–466.
Fic E, Polit A, Wasylewski Z: Kinetic and structural studies of the allosteric conformational changes induced by binding of cAMP to the cAMP receptor protein from *Escherichia coli*. Biochemistry 2006;45:373–380.
Garcia AE, Harman JG: Simulations of CRP: $(cAMP)_2$ in non-crystalline environments show a subunit transition from the open to the closed conformation. Protein Sci 1996;5: 62–71.
Gekko K, Obu N, Li J, Lee JC: A linear correlation between the energetics of allosteric communication and protein flexibility in the *Escherichia coli* cyclic AMP receptor protein revealed by mutation-induced changes in compressibility and amide hydrogen-deuterium exchange. Biochemistry 2004;43:3844–3852.
Giraud-Panis MJ, Toulme F, Blazy B, Maurizot JC, Culard F: Fluorescence study on the nonspecific binding of cyclic-AMP receptor protein to DNA: effect of pH. Biochimie 1994; 76:133–139.
Gorshkova I, Moore JL, McKenney KH, Schwarz FP: Thermodynamics of cyclic nucleotide binding to the cAMP receptor protein and its T127L mutant. J Biol Chem 1995;270:21679–21683.
Gutierrez-Ríos RM, Freyre-Gonzalez JA, Resendis O, Collado-Vides J, Saier M, Gosset G: Identification of regulatory network topological units coordinating the genome-wide transcriptional response to glucose in *Escherichia coli*. BMC Microbiol 2007;DOI: 10.1186/147-2180-7-53.
Harman JG: Allosteric regulation of the cAMP receptor protein. Biochim Biophys Acta 2001;1547:1–17.
Heyduk T, Lee JC, Ebright YW, Blatter EE, Zhou Y, Ebright RH: CAP interacts with RNA polymerase in solution in the absence of promoter DNA. Nature 1993;364:548–549.
Jacob F, Monod J: Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol 1961;3:318–356.
Katouzian-Safadi M, Blazy B, Cremet JY, Le Caer JP, Rossier J, Charlier M: Photo-cross-linking of CRP to nonspecific DNA in the absence of cAMP. DNA interacts with both the N- and C-terminal parts of the protein. Biochemistry 1993;32:1770–1773.
Kim J, Adhya S, Garges S: Allosteric changes in the cAMP receptor protein of *Escherichia coli*: hinge reorientation. Proc Natl Acad Sci USA 1992;89:9700–9704.
Kolb A, Busby S, Buc H, Garges S, Adhya S: Transcriptional regulation by cAMP and its receptor protein. Annu Rev Biochem 1993;62: 749–795.
Krueger S, Gregurick S, Shi Y, Wang S, Wladkowski BD, Schwarz FP: Entropic nature of the interaction between promoter bound CRP mutants and RNA polymerase. Biochemistry 2003;42:1958–1968.
Lin SH, Lee JC: Communications between the high-affinity cyclic nucleotide-binding sites in *Escherichia coli* cyclic AMP receptor protein: effect of single site mutations. Biochemistry 2002;41:11857–11867.
Lin SH, Lee JC: Determinants of DNA bending in the DNA-cyclic AMP receptor protein complexes in *Escherichia coli*. Biochemistry 2003;42:4809–4818.
Malecki J, Wasylewski Z: Stability and kinetics of unfolding and refolding of cAMP receptor protein from *Escherichia coli*. Eur J Biochem 1997;243:660–669.
Malecki J, Wasylewski Z: The effect of Ser 128 substitution on the structure and stability of cAMP receptor protein from *Escherichia coli*. J Protein Chem 1998;17:219–228.
Malecki J, Polit A, Wasylewski Z: Kinetic studies of cAMP-induced allosteric changes in cyclic AMP receptor protein from *Escherichia coli*. J Biol Chem 2000;275:8480–8486.
McKay DB, Weber IT, Steitz TA: Structure of catabolite gene activator protein at 2.9-Å resolution. J Biol Chem 1982;257:9518–9524.
Merkel TJ, Dahl JL, Ebright RH, Kadner RJ: Transcription activation at the *Escherichia coli uhpT* promoter by the catabolite gene activator protein. J Bacteriol 1995;177:1712–1718.
Mukhopadhyay J, Sur R, Parrack P: Functional roles of the two cyclic AMP-dependent forms of cyclic AMP receptor protein from *Escherichia coli*. FEBS Lett 1999;453:215–218.
Niu W, Kim Y, Tau G, Heyduk T, Ebright RH: Transcription activation at class II CAP-dependent promoters: two interactions between CAP and RNA polymerase. Cell 1996; 87:1123–1134.
Niu W, Zhou Y, Dong Q, Ebright YW, Ebright RH: Characterization of the activating region of *Escherichia coli* catabolite gene activator protein (CAP). I. Saturation and alanine-scanning mutagenesis. J Mol Biol 1994; 243:595–602.
Parkinson G, Wilson C, Gunasekera A, Ebright YW, Ebright RE, Berman HM: Structure of the CAP-DNA complex at 2.5-Å resolution: a complete picture of the protein-DNA interface. J Mol Biol 1996;260:395–408.
Passner JM, Schultz SC, Steitz TA: Modeling the cAMP-induced allosteric transition using the crystal structure of CAP-cAMP at 2.1-Å resolution. J Mol Biol 2000;304:847–859.
Passner JM, Steitz TA: The structure of a CAP-DNA complex having two cAMP molecules bound to each monomer. Proc Natl Acad Sci USA 1997;94:2843–2847.
Polit A, Blaszczyk U, Wasylewski Z: Steady-state and time-resolved fluorescence studies of conformational changes induced by cyclic AMP and DNA binding to cyclic AMP receptor protein from *Escherichia coli*. Eur J Biochem 2003a;270:1413–1423.
Polit A, Bonarek P, Kępys B, Kedracka-Krok S, Gorecki A, Wasylewski Z: Kinetic studies of cAMP-induced allosteric changes in mutants T127I, S128A, and T127I/S128A of the cAMP receptor protein from *Escherichia coli*. J Biol Chem 2003b;278:43020–43026.
Pyles EA, Chin AJ, Lee JC: *Escherichia coli* cAMP receptor protein-DNA complexes. 1. Energetic contributions of half-sites and flanking sequences in DNA recognition. Biochemistry 1998;37:5194–5200.
Pyles EA, Lee JC: *Escherichia coli* cAMP receptor protein-DNA complexes. 2. Structural asymmetry of DNA bending. Biochemistry 1998; 37:5201–5210.
Rhodius VA, West DM, Webster CL, Busby SJ, Savery NJ: Transcription activation at class II CRP-dependent promoters: the role of different activating regions. Nucleic Acids Res 1997;25:326–332.
Ryu S, Kim J, Adhya S, Garges S: Pivotal role of amino acid at position 138 in the allosteric hinge reorientation of cAMP receptor protein. Proc Natl Acad Sci USA 1993;90:75–79.

Salemme FR: A model for catabolite activator protein binding to supercoiled DNA. Proc Natl Acad Sci USA 1982;79:5263–5267.
Schultz SC, Shields GC, Steitz TA: Crystal structure of a CAP-DNA complex: the DNA is bent by 90 degrees. Science 1991;253:1001–1007.
Scott SP, Harrison RW, Weber IT, Tanaka JC: Predicted ligand interactions of 3′5′-cyclic nucleotide-gated channel-binding sites: comparison of retina and olfactory binding site models. Protein Eng 1996;9:333–344.
Semsey S, Krishna S, Sneppen K, Adhya S: Signal integration in the galactose network of *Escherichia coli*. Mol Microbiol 2007;65:465–476.
Shabb JB, Corbin JD: Cyclic nucleotide-binding domains in proteins having diverse functions. J Biol Chem 1992;267:5723–5726.
Shen X, Tao W, Kong J, Yu S: The role of loops 3 and 4 in the interdomains and intersubunits communication of *Escherichia coli* cAMP receptor protein. Int J Biol Macromol 2008;42:372–379.
Søgaard-Andersen L, Mironov AS, Pedersen H, Sukhodelets VV, Valentin-Hansen P: Single amino acid substitutions in the cAMP receptor protein specifically abolish regulation by the CytR repressor in *Escherichia coli*. Proc Natl Acad Sci USA 1991;88:4921–4925.
Søgaard-Andersen L, Valentin-Hansen P: Protein-protein interactions in gene regulation: the cAMP-CRP complex sets the specificity of a second DNA-binding protein, the CytR repressor. Cell 1993;75:557–566.
Spolar RS, Record MT Jr: Coupling of local folding to site-specific binding of proteins to DNA. Science 1994;263:777–784.
Tutar Y: Chemical linkage at allosteric activation of *Escherichia coli* cAMP receptor protein. Protein J 2008a;27:21–29.
Tutar Y: Syn, anti, and finally both conformations of cyclic AMP are involved in the CRP-dependent transcription initiation mechanism in *Escherichia coli* lac operon. Cell Biochem Funct 2008b;26:399–405.
Tworzydlo M, Polit A, Mikolajczak J, Wasylewski Z: Fluorescence quenching and kinetic studies of conformational changes induced by DNA and cAMP binding to cAMP receptor protein from *Escherichia coli*. FEBS J 2005;272:1103–1116.
Wasylewski M, Malecki J, Wasylewski Z: Fluorescence study of *Escherichia coli* cyclic AMP receptor protein. J Protein Chem 1995;14:299–308.
Weber IT, Steitz TA: Structure of a complex of catabolite gene activator protein and cyclic AMP refined at 2.5-Å resolution. J Mol Biol 1987;198:311–326.
Weyand NJ, Braaten BA, van der Woude M, Tucker J, Low DA: The essential role of the promoter-proximal subunit of CAP in *pap* phase variation: *Lrp*- and helical phase-dependent activation of *papBA* transcription by CAP from –215. Mol Microbiol 2001;39:1504–1522.
Won HS, Lee TW, Park SH, Lee BJ: Stoichiometry and structural effect of the cyclic nucleotide binding to cyclic AMP receptor protein. J Biol Chem 2002;277:11450–11455.
Won HS, Yamazaki T, Lee TW, Yoon MK, Park SH, Kyogoku Y, Lee BJ: Structural understanding of the allosteric conformational change of cyclic AMP receptor protein by cyclic AMP binding. Biochemistry 2000;39:13953–13962.
Zhang X, Ebright R: Identification of a contact between arginine-180 of catabolite gene activator protein (CAP) and base pair 5 of the DNA site in the CAP-DNA complex. Proc Natl Acad Sci USA 1990;87:4717–4721.
Zhang X, Schleif R: Catabolite gene activator protein mutations affecting activity of the *araBAD* promoter. J Bacteriol 1998;180:195–200.
Zheng D, Constantinidou C, Hobman JL, Minchin SD: Identification of the CRP regulon using in vitro and in vivo transcriptional profiling. Nucleic Acids Res 2004;32:5874–5893.
Zhou Y, Merkel TJ, Ebright RH: Characterization of the activating region of *Escherichia coli* catabolite gene activator protein (CAP). II. Role at class I and class II CAP-dependent promoters. J Mol Biol 1994;243:603–610.
Zubay G, Schwartz D, Beckwith J: Mechanism of activation of catabolite-sensitive genes: a positive control system. Proc Natl Acad Sci USA 1970;66:104–110.